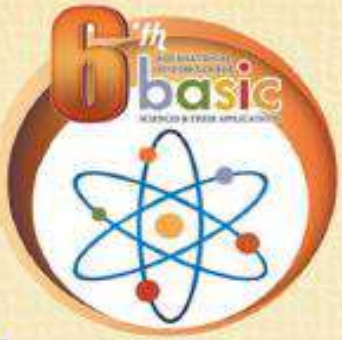


جامعة عمر المختار

OMAR AL-MUKHTAR UNIVERSITY

كلية العلوم

FACULTY OF SCIENCE

المؤتمر الدولي السادس للعلوم الأساسية وتطبيقاتها

6th International Conference of Basic Sciences and Their Applications

6th ICBSA

2nd December 2023

El–Beida – Libya

برعاية

NCB

المصرف التجاري الوطني

National Commercial Bank

Email science.conference2023@omu.edu.ly

AlQalam Journal of Medical and Applied Sciences
Special Issue for 6th International Conference in Basic Sciences and Their Applications (6th ICBSTA, 2023), *https://journal.utripoli.edu.ly/index.php/Alqalam* ***eISSN 2707-7179***

Addressing the Missing Data Challenge in Time Series Analysis: Strategies and Implications

Suaad O. Ben-Farag and Intesar N. El-Saeiti
Statistics Department, Faculty of Science, University of Benghazi
Correspondence authors: *suaad.benfarag@uob.edu.ly*

Abstract:

Missing data is a common and challenging issue in time series analysis, which can significantly affect the accuracy and reliability of the results. This article explores the problem of missing data in time series analysis and investigates data with all values and same data with randomly missing value to address this challenge effectively. We examine the implications of missing data on statistical estimation, forecasting, and the interpretation of time series behavior patterns. We provide a time series data of different percentage of randomly missing for handling missing data in time series, the randomly missing will be varied between 5 and 50 percentage of all data. The advantages and limitations of each percentage missing are discussed, along with statistical estimation, forecasting, and the interpretation of time series behavior patterns. Furthermore, we highlight the importance of understanding the mechanisms leading to effect of missing data and the potential biases that can arise if not properly handled. This article aims to provide researchers and practitioners in time series analysis with valuable insights and guidance in effectively dealing with the missing data challenge, ultimately improving the robustness and validity of their analyses.

Keywords: Missing data; Time Series Model; Stationary, Autocorrelation; imputation methods.

Introduction:

Time series data analysis plays a crucial role in various fields, including economics, finance, climate science, and signal processing. However, one common challenge encountered in time series analysis is the presence of missing data. Missing data can arise due to various reasons, such as equipment malfunction, data transmission errors, or incomplete data collection processes. Ignoring or mishandling missing data can lead to biased estimates, incorrect inferences, and compromised forecasting accuracy

(Rubin, 1976). When this occurs, it is necessary to obtain estimates of the missing value in order to gain a better understanding of the nature of the data and make a more accurate forecast possible (Little & Rubin, 2019) and (Howell, 2007). To address this challenge, researchers have developed numerous techniques for handling missing data in time series analysis. These techniques aim to impute or estimate the missing values, allowing for the utilization of the complete data set and ensuring the integrity of the analysis. However, the performance and efficacy of these techniques may vary depending on the percentage of missing data. There are some researchers have explored relevant research articles, studies, and scholarly works that delve into this topic. Some authors used techniques to dealing with missing value; (Rankin & Marsh, 1985) explores the impact of missing data on the statistical analysis of clinical time series, and focused on the effects of missing data on the statistical analysis of clinical time series. It aimed to investigate how the presence of missing data can affect the validity and reliability of statistical analyses conducted on time series data in clinical settings. Velicer & Colby (2005) focuses on the comparison of different methods for handling missing data in ARIMA (Autoregressive Integrated Moving Average) time-series analysis, their study employ Monte Carlo simulation techniques to assess the performance of four different missing-data procedures: listwise deletion (LD), pairwise deletion (PD), mean substitution (MS), and multiple imputation (MI). They examined the effects of different levels of missingness, time-series length, and autocorrelation structure on the performance of these procedures. In this article, we focus on investigating the impact of different percentages of randomly missing data in time series analysis. Specifically, we will consider six scenarios: 5%, 10%, 20%, 30%, 40% and 50% randomly missing data percentages. We aim to provide insights into the behavior and performance of various missing data handling techniques under these different scenarios. By providing time series data with varying percentages of missing values, we enable researchers to evaluate and compare the effectiveness of different missing data handling methods. This empirical analysis will offer valuable insights into the strengths and limitations of existing techniques, allowing for informed decision-making when dealing with missing data in time series analysis. In addition, we will look at some missing data handling strategies, such as Listwise deletion (LD) and imputation methods like mean imputation (MS). For comparison purposes, the entire dataset will be used to compare these missing data handling approaches. Through this comprehensive examination, we aim to provide a practical understanding of the implications

of randomly missing data at different percentages in time series analysis. The findings of this study will contribute to the development of best practices and guidelines for handling missing data in time series analysis, improving the accuracy and reliability of future research and applications in this field. This paper proceeds as follows: In the next section the univariate statistical time series model is defined in basic terms and introduces the datasets used in analysis with the mechanism of missing data. The succeeding section explains and discusses the results of analysis. The paper ends with a short summary of the insights in the final section.

Statistical Methodology:

Statistical model: The autoregressive model is one of the simplest and most useful model to study time series data. In this model the current value X_t of the time series is expressed linearly in terms of its previous values and error terms ε_t. The error terms ε_t are generally assumed to be independent, identically distributed random variables (white noise) from a normal distribution with a mean zero and constant variance σ^2. The notation AR(p) refers to a model with p order at terms and is written as:

$$X_t = \sum_{i=1}^{p} \varphi_i X_{t-i} + \varepsilon_t$$

where $X_{t-1}, X_{t-2}, \cdots, X_{t-p}$, are previous series values (lags) and $\varphi_1, \varphi_2, \cdots, \varphi_p$ are the corresponding parameters, which are estimated using the maximum likelihood approach.

Data analysis: The data used for this study is generated time-series data. This data were extracted from (Priestley, 1981), which was generated from AR(1), it is defined as : $X_t = 0.6X_{t-1} + \varepsilon_t$. The dataset consists of 500 observations. From the complete time series dataset generated from the AR(1) model, randomly simulated missing data patterns with six different percentages were deleted, namely : 5, 10, 20, 30, 40, and 50 percent. These missing data were produced to evaluate the accuracy of imputation techniques. Furthermore, the complete case of time series data (0% missing) was also included to provide a validity check. Analysis software R, version 4.1.1, is used

for all analysis. The "forecast" package is used to fit the AR(1) time series model.

A variety of approaches to dealing with missing data have been used, with varying degrees of success depending on the nature of the data and the nature of the missingness pattern. Two different missing-data approaches were used to conduct this study: (a) The Listwise Deletion (LD) approach is one of the most common traditional missing data techniques, involving no estimation of missing data and condensing and analyzing the time series as a shorter series. In other words, it simply excludes observations with missing data and performs analyses on the remaining cases. (b) Mean Substitution (MS), the most basic and well-known technique for studying missing data. It consists of replacing each missing value in the series with the mean of the observed values, or whereby the mean of the time series is imputed in place of the missing observations.

Evaluation Criteria:

Model selection criteria are used to compare statistical models. Several evaluation criteria are frequently used in the selection of the time series model, including mean absolute error (MAE), root mean square error (RMSE), multiple R square ($\boldsymbol{R^2}$), Ljung-Box test, AIC, AICC, and BIC.

1. The mean absolute error is the average difference between predicted and actual data values, and is given by :

$$MAE = \sum_{i=1}^{n} \frac{|Y_i - Y_{imputed}|}{n}$$

2. The root mean squared error is one of the most commonly used measures of success for numerical prediction. Its value is computed by :

$$RMSE = \sqrt{\sum_{i=1}^{n} \frac{MSE}{n}} = \sqrt{\sum_{i=1}^{n} \frac{(Y_i - Y_{imputed})^2}{n}}$$

3. Multiple R square R^2 explains how much of the variability in the imputed data can be explained by the fact that they are related to the observed values. It is given by

$$R^2 = 1 - \frac{SSE}{SST}$$

4. The Ljung-Box test, or $Q(r)$ statistic suggested by (Ljung & Box, 1978), is a portmanteau lack of fit test for checking the independently assumption. The $Q(r)$ statistic is calculated by the following equation:

$$Q(r) = n(n+2)\frac{\sum_{k=1}^{h} r_k^2(a)}{n-k}$$

where n is the sample size, $r_k^2(a)$ is the residual autocorrelation of order k and h is the number of autocorrelation lags being tested. The null hypothesis of the Ljung-Box test is that Residual is white noise. If the value of $Q(r)$ exceeds the critical value of a chi-square distribution with h degrees of freedom at the specified significance level, then at least one value of $r_k^2(a)$ is statistically different from zero.

5. Information criteria: Akaike Information Criterion (AIC) (Akaike, 1974); Corrected Akaike Information Criterion (AICc) (Sugiura, 1978); and Schwarz Information Bayesian (BIC) (Schwarz, 1978). The estimated model with the minimum of these criteria is assumed to describe the data series. A brief description of the criteria for selecting the best model are given by:

$$AIC = -2\ln(L) + 2K$$
$$AIC_C = AIC + \frac{2K(K+1)}{n-K-1}$$
$$BIC = -2\ln(L) + K\ln(n)$$

where K is the number of parameters to be estimated and L_{max} is the maximized value of the likelihood function of the fitted model. The minimum value of these criteria is desirable for the better performance of the model.

RESULTS AND DISCUSSION

In this section, we will discuss briefly the results of the statistical analysis. To obtain a statistically valid evaluation of the efficiency of approaches to missing data in the univariate time series data, we first have determined whether the time series data is stationary or not by considering the scatter plot of dataset. A stationery series is characterized by a constant mean and variance over time, a flat appearance, and no trend. **Figure 1** displays the data's scatter plot. The series does not appear to show any discernible variations over time, with the lower values showing nearly the same variation as the higher values. We claim that the stationarity behavior of the series and

a stationary model seem to be reasonable. Two interpolation approaches have been tested on six different percentages: 5%, 10%, 20%, 30%, 40%, and 50%. The results of the overall performance of these approaches are reported in Table 1 and Table 2

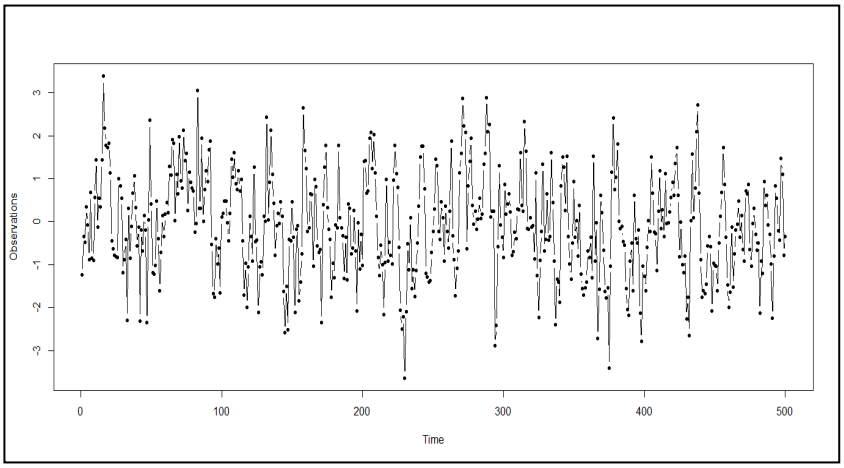


Figure 1 : The time series data generated from AR (1)

Table 1 displays the estimated parameter, the standard error of the autoregressive parameter using varying percentages of missing data for two estimation approaches, and the bias of the parameter estimate from the true parameter from the original data. In comparison to the actual values of the autoregressive parameter for complete data (0% of missing data), the results demonstrate a significant difference in parameter estimate and standard error estimate for both listwise deletion and mean substitution approaches at various percentages of missing data. It is evident that when the percentage of missing data rises from 5 to 50 percent, the parameter estimate's bias gets worse. It is evident that when missing data percentages are up to 10%, parameter bias is smaller; in contrast, when missing data percentages are 25%, parameter bias is larger. From Table 1, it is necessary to notice for all percentages of missing data that both approaches underestimate the parameter of the AR(1) model. When missing data occurs in time series data, up to 5 percentages of missing values, listwise deletion (LD) seems to be a better approach to deal with missing data. It provides the least biased parameter estimate. Also, the bias is smaller for listwise deletion (LD) than for mean substitution (MS), up to 20 percent of missing values. Overall, the mean substitution (MS) is not good enough to handle a large percentage of missing data since it will produce a large bias in the autoregressive parameter of the

AR(1) model compared to the listwise deletion (LD) approach. Table 1 also displays that, the mean substitution (MS) approach follows the trends as listwise deletion (LD) approach that the standard error of autoregressive parameter estimate is increased as percentages of missing values increases in the time series data.

Table 1 : Estimate and standard error of the parameter for missing data approaches and for each percent of missing data.

Missing data (%)	Missing Data Estimation Method							
	Listwise Deletion (LD)				Mean Substitution (MS)			
	Estimate (Bias)	SD	ML	R^2	Estimate(Bias)	SD	ML	R^2
Complete (0%)	0.5526(0.000)	0.037	-704.01	0.302	0.5526(0.000)	0.037	-704.01	0.302
5	0.542(-0.010)	0.039	-674.717	0.316	0.531(-0.021)	0.038	-701.548	0.279
10	0.522(-0.030)	0.040	-641.160	0.359	0.494(-0.059)	0.039	-696.040	0.236
20	0.519(-0.034)	0.043	-578.280	0.395	0.432(-0.121)	0.040	-694.326	0.180
30	0.430(-0.123)	0.048	-528.268	0.428	0.367(-0.186)	0.042	-680.855	0.130
40	0.355(-0.198)	0.054	-469.625	0.446	0.313(-0.239)	0.042	-663.284	0.094
50	0.352(-0.200)	0.059	-374.118	0.520	0.268(-0.285)	0.043	-589.826	0.069

ML: Model LogLikelihood

Using the listwise deletion (LD) approach, as shown in Table 1, the log likelihood function values increase significantly for all missing percentages of data; consequently, a significant reduction in the number of values in the time series to be calculated for the log likelihood function occurs. Therefore, it is not reasonable to assume that the fitted time series AR(1) model is better after listwise deletion (LD). RMSE and mean absolute errors (MAE) are also calculated Table 2 and plotted as functions of various percentages of missing values for the dataset Figure 2 Based on these results, we observe that the RMSE and mean absolute error MAE values are greater for the mean substitution method than for the listwise deletion (LD) approach. It is clearly evident that, among all percentages of missing data, the listwise deletion (LD) method gives the best result (smallest error) (Error! Reference source not found.). For 5% missing values, both approaches (listwise deletion and mean substitution) give approximately the same values of RMSE and mean absolute errors (MAE) of the complete data (0%). Overall, it seems that the mean substitution (MS) method of these performance indicators got substantially worse at each increasing level of missing data (over 10%). Then these results clearly show that, for up to 10 percent of missing data, the listwise deletion (LD) method is preferred to the mean substitution (MS) method investigated in this work. It gives the best performance for solving the missing values problem in terms of the accuracy measures using RMSE and MAE.

Table 2 : Comparison of estimation methods based on RMSE, MAE, AIC, AICc, BIC and Test with four different percentages of missing values of the AR(1) data.

		Missing data (%)						
Method		0%	5	10	20	30	40	50
LD	RMSE	0.989	1.001	1.006	1.027	1.094	1.157	1.080
	MAE	0.788	0.794	0.797	0.807	0.867	0.929	0.871
	AIC	1412.01	1353.434	1286.320	1160.560	1060.535	943.251	752.235
	AICc	1412.03	1353.460	1286.347	1160.590	1060.570	943.291	752.284
	BIC	1420.44	1361.761	1294.538	1168.543	1068.251	950.65	759.278
	Q(18)	23.83	18.994	15.076	15.692	19.137	17.351	39.010
MS	RMSE	0.989	1.010	1.026	1.084	1.129	1.177	1.113
	MAE	0.788	0.818	0.843	0.912	1.001	1.078	1.017
	AIC	1412.01	1407.095	1396.081	1392.651	1365.710	1330.568	1183.653
	AICc	1412.03	1407.119	1396.105	1392.675	1365.734	1330.592	1183.677
	BIC	1420.44	1415.524	1404.510	1401.080	1374.139	1338.997	1192.082
	Q(18)	23.83	17.110	15.758	27.179	19.113	15.160	9.114

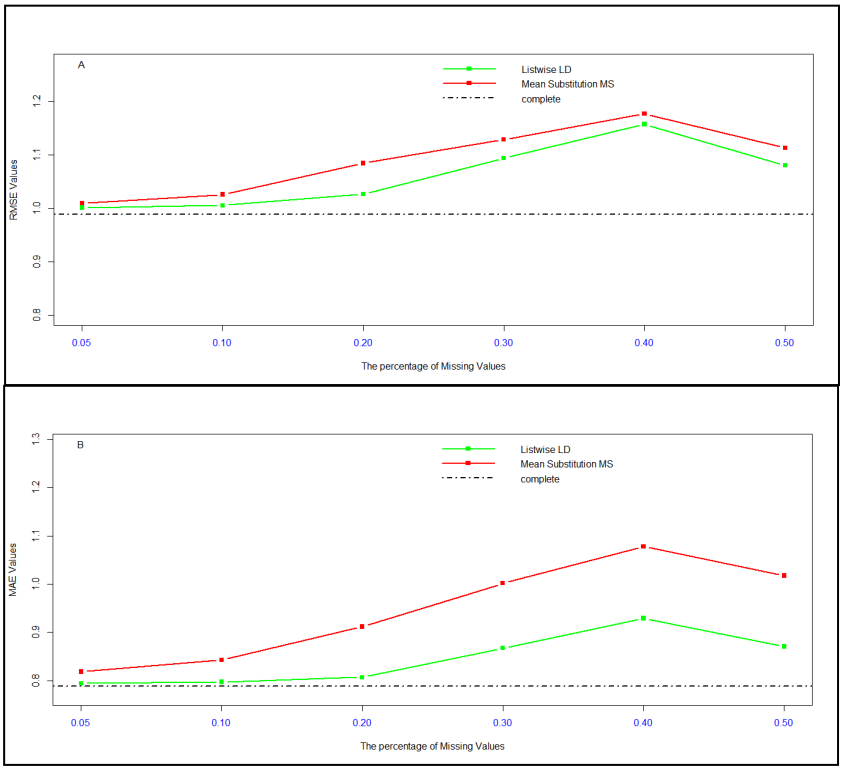


Figure 2: Comparison of RMSE and MAE for various percentages of missing values by LD and MS. Black horizontal. line marks RMSE (A) and MES (B) for the complete dataset.

The AIC, AICc and BIC (Error! Reference source not found.) that were computed from autoregressive model AR(1) are also plotted as functions of missing data percentages. Based on Table 2 and Error! Reference source not found., display the performance of the best estimation approach for missing

values in time series data based on the three (AIC, AICc and BIC) indicators with various percentages of missing values. These results show that, in general, the mean substitution (MS) approach is suitable for estimating missing data in time series data. These information criteria indicate that there are large differences between the listwise deletion (LD) and mean substitution (MS) of missing data imputation approaches. When missing values up to 5%, both the listwise deletion (LD) and mean substitution (MS) give approximately same results of the information criteria (AIC, AICc and BIC) indicators. Over 10% of the missing value results of both approaches in terms of AIC and BIC are less than those from the complete dataset (0.0%). In addition, above 5 percent of missing data, listwise deletion (LD) underestimates the results. When missing data ranges from 5 percent to 10 percent, the mean substitution approach gives efficient results.

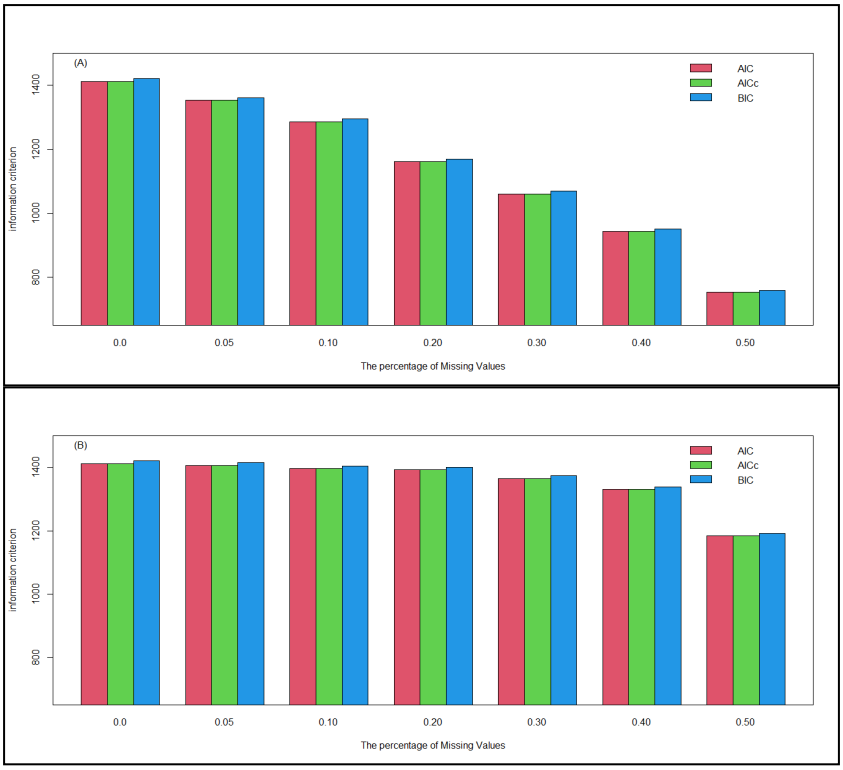


Figure 3: Comparison of AIC, AICc and BIC for various percentages of missing values by LD (A) and MS (B).

As it is clearly seen from the results, up to 5 percent of missing data, both imputation approaches, listwise (LD) and mean substitution (MS), give an efficient estimate for multiple R-square (Error! Reference source not found.).

Over 10 percent of the missing data, the listwise deletion (LD) approach overestimates the multiple R square statistic from the complete dataset but the method of mean substitution (MS) underestimates it. The mean substitution (MS) results in Multiple R-square are highly accurate up to 5 percent of missing data and less over 5 percent of missing values than multiple R-square of the complete data. The mean substitution (MS) has an advantage over the listwise (LD) in terms of AIC, AICc and BIC while the listwise (LD) produces the most accurate results in terms of multiple R-square. Overall, from these results, if there is over 10 percent of missing data, the mean substitution (MS) approach is the last choice to estimate missing time series data for the AR(1) model because it gave high values for RMSE and MAE, and low value for the multiple R-square. Based on the Ljung-Box test (lag 18) described above (Error! Reference source not found.), the Ljung-Box statistic of the residual for the fitting AR(1) model is not significantly different from zero with an associated p-value > 0.05. Thus, for both estimation methods, the fitted AR(1) models their residuals have no serial correlation. This means the assumption of white noise in residuals was fulfilled by all the autoregression AR(1) models using both listwise (LD) and mean substitution (MS) for solving missing data in time series analysis. The time series model that will satisfy the requirement of no serial correlation in the residuals, it will be considered for further analysis and forecasting.

Conclusion:

In this paper, we focus on missing data problems and approaches to addressing them for time series data. We study approaches for dealing with missing data on a complete dataset generated from an autoregressive time series model. Our results show that when the percentage of missing data increases to 50%, the listwise deletion method is the least effective for dealing with problem. Up to 5 percentage of missing data, the approach of mean substitution (MS) is efficient procedure according to AIC, AICc and BIC compared with the LD. Nevertheless, the mean substitution (MS) approach is the most inaccurate compare to listwise deletion (LD) when considering the RMSE and MAE criteria and Multipe R^2, this approach will introduce bias in the estimated of parameter since all the imputed values are the same. But it yields better results because it keeps the number of values in the data set is constant during the statistical analysis. In view of this, in time series analysis, it is recommended that the mean substitution (MS) be used in estimating missing values when is the percentage of missing values up to 5, until further

studies proves otherwise. In this paper, we have only examined two statistical approaches, namely, listwise deletion (LD) and mean substitution (MS), to treat missing values problems in time series data. In order to gain a better understanding and be able to estimate missing values more accurately, further research needs to be undertaken on time series data. It is also recommended that this study be extended to study of other imputation approaches at various percentages of missing data that have to be taken into account in terms of their efficiency and accuracy. Furthermore, it is recommended that this study be extended to use these approaches with real-life time series data. Finally, there is a need to examine the efficiency and accuracy of the estimation of missing values for each imputation approach with other time series models for further research by studying the autoregressive and moving average ARMA model.

References:

Akaike, H. (1974). A new look at the statistical model identification. *IEEE transactions on automatic control*, *19*(6), 716-723.

Howell, D. C. (2007). The treatment of missing data.

Little, R. J., & Rubin, D. B. (2019). *Statistical analysis with missing data.* John Wiley & Sons.

Ljung, G. M., & Box, G. E. (1978). On a measure of lack of fit in time series models. *Biometrika*, pp. 297–303.

Priestley, M. B. (1981). *Spectral analysis and time series.*

Rankin, E. D., & Marsh, J. C. (1985, July). Effects of missing data on the statistical analysis of clinical time series. *Oxford University Press*, pp. 13-16.

Rubin, D. B. (1976). Inference and missing data. *Biometrika*, pp. 581-592.

Schwarz, G. (1978). Estimating the dimension of a model. *Annals of Statistics*, pp. 461-464.

Sugiura, N. (1978). Further analysis of the data by Akaike's information criterion and the finite corrections: further analysis of the data by Akaike's. *Communications in Statistics-theory and Methods*, pp. 13-26.

Velicer, W. F., & Colby, S. M. (2005). A comparison of missing-data procedures for ARIMA time-series analysis. *Educational and Psychological Measurement*, pp. 596-615.

AlQalam Journal of Medical and Applied Sciences
Special Issue for 6th International Conference in Basic Sciences and Their Applications
(6th ICBSTA, 2023), https://journal.utripoli.edu.ly/index.php/Alqalam eISSN 2707-7179

Correlation between Diameter and Quality Control Parameters of Folic Acid Tablets from Different Brands Available on the Libyan Market

Azah M. Ali[1]*, Osama I. G. Khreit[2], Aida B. Abdulkarim[3], Alaa Y. Alsadiq[3] and Eman S. Almahdi[3]

[1]*Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Omar AL-Mukhtar University, El-Beida, Libya;*
[2]*Department of Basic Medical Veterinary Sciences, Faculty of Veterinary Medicine, Omar Al-Mukhtar University, El-Beida, Libya;*
[3]*Laborator of faculty of pharmacy, faculty of pharmacy, Omar Al-Mukhtar University, El-Beida, Libya.*
Correspondence authors: azza.manbi@omu.edu.ly

Abstract:

The aim of this study was to assess the quality control parameters of folic acid tablets (5 mg) using diameter measurements. Eight different brands of folic acid tablets with varying diameters were purchased from different private pharmacies in El-Beida City, Libya. The tablets' quality control parameters were tested in accordance with the United States Pharmacopoeia (USP) guidelines using in-vitro tests. The tests included uniformity of weight, thickness, friability, hardness, disintegration time, and assay content using the high-performance liquid chromatography (HPLC) method. The diameter measurements were taken for all eight brands of folic acid tablets, and statistical analysis was performed using Graph Pad InStat, version 3.10. The results showed that all eight brands of folic acid tablets met the USP standards for quality in the prescribed in-vitro quality control tests. Furthermore, all brands also met the diameter specifications. The study found a strong correlation ($r = 0.9766$) between the diameter and weight of folic acid tablets. Additionally, the diameter had weak to moderate correlations with other parameters, such as thickness, hardness, friability, disintegration time, and the state of the content of folic acid tablets. This study shows that the diameter can be used to detect the weight of a tablet. An increase in tablet diameter was found to be an indication of a significant correlation ($p < 0.0001$) with weight increase.

Keywords: Quality control, Folic acid, Tablet, Hardness, Friability, Weight variation, Thickness, Diameter.

Introduction:

Folic acid, commonly known as pteroyl-L-glutamic acid, is a component of the vitamin B group (specifically vitamin B9) found in dietary supplements and processed foods (Mohammed, 2014). It has the chemical formula N-[4-[[(2-amino-1, 4-dihydro-4-oxo 6-pteridinyl) methyl] amino] benzoyl]-L glutamic acid (Đuriš *et al.,* 2017). Folic acid and folate are frequently used interchangeably (Arcot *et al.,* 2002). The term "folic acid" refers to the fully oxidized synthetic compound available in dietary supplements, while "folate" refers to the several tetrahydrofolate derivatives naturally present in foods. However, there is no distinction between "natural" and "synthetic" folic acid. It is the same molecule (Gazzali *et al.,* 2016). It is a water-soluble vitamin that has been used for a long time to treat macrocytic anemia. It gained popularity in the 1990s due to its disease-prevention capabilities. Taking the vitamin during pregnancy lowers the possibility of neural tube abnormalities (NTDs) such as spina bifida. Folic acid has also been associated with a lower incidence of cardiovascular disease and psychiatric illnesses, including dementia. In the future, folic acid is predicted to play an essential role in the prevention and treatment of various diseases (Arcot *et al.,* 2002; Younis, 2003). It is available in multivitamins, prenatal vitamins, supplements that combine other B-complex vitamins and/or minerals, and as a single supplement (Đuriš *et al.,* 2017). Folic acid is commonly taken orally as a tablet supplement. The dosage typically depends on the specific medical condition. Adults should consume 400 µg of folic acid per day (Mohan & Madhulika, 2016). The quality requirements for dietary supplements are not yet globally harmonized, which means that products available on the market may contain insufficient levels of the active component, potentially resulting in a lack of the desired consequence (Đuriš *et al.,* 2017). Tablet quality control parameters are valuable tools for ensuring uniformity in batch-to-batch manufacturing, and they should be utilized for all drug products (Arcot *et al.,* 2002). This type of dosage form is available in many shapes, sizes, and colours. This considerably helps in distinguishing between medications and is also effective for product branding (Ahmat *et al.,* 2014). The tablets shape and size are based on the amount of medicinal substances and the desired route of administration (Kumar *et al.,* 2016). Regarding the quality of pharmaceutical tablets, various pharmacopoeias have established numerous standards. These characteristics include shape, thickness, weight, hardness, friability,

disintegration, and dissolution (Ahmat *et al.*, 2014). USP recommends several tests on finished tablets to ensure their suitability for therapeutic use. These tests are essential to ensuring that several batches of the same product consistently meet these standards (Al-Achi, 2019). Some of the tests performed include physical examination, thickness testing, weight variation testing, hardness testing, friability testing, disintegration testing, drug assays, and dissolution testing (Đuriš *et al.*, 2017; Gupta *et al.*, 2020). Manufacturers must ensure that the final product meets acceptable quality standards (Kumar *et al.*, 2016). The consistency of tablet diameter is crucial for preventing patient confusion resulting from variable tablet sizes and enhancing patient compliance. Moreover, varying sizes can lead to patient uncertainty regarding the active substance content of the tablets (Al Ali *et al.*, 2022). The current study aims to assess the feasibility of using tablet diameter as an offline indicator of several tablet quality control parameters. Consequently, this research will evaluate the current quality status of marketed folic acid tablets available in Libya's El-Beida city, determine whether different brands of folic acid preparations meet USP specifications for various quality control parameters, and measure the diameter of these different brands. Finally, the study will correlate diameter data with quality control parameters for various folic acid brands.

Materials and Methods:

Eight commercially available single-component folic acid supplements were investigated, as shown in Table 1. Different brands were purchased from various private pharmacies in El-Beida City, Libya. All the brands of folic acid were labelled to contain 5 mg of folic acid per tablet. The samples were randomly coded as FA n, where n = 1, 2, 3, 4, 5, 6, 7, and 8, and all samples were assessed within their valid shelf lives. A folic acid standard (98–101% purity) was purchased from Alpha Chemika, India. Distilled water was used throughout the work. Potassium phosphate monobasic (Sigma, USA), potassium hydroxide pellets (Riedel-de Haën, Czech Republic), sodium perchlorate (BDH Laboratory Supplies, England), and methanol HPLC grade (Scharlau, Spain) were used without further purification.

Thickness: Ten tablets were randomly selected, and the thicknesses were measured individually using a digital calliper (PowerfixProfi, Neckarsulm, Germany). The average thickness of the ten tablets and the percentage

deviations of the tablet thickness from the average thickness were calculated (Wahed *et al.*, 2021).

Table 1: Detail information on folic acid tablets included in the study

Code	Manufacturer	Country Origin	Strength	Batch number
FA 1	Wochardt Laboratories	United Kingdom	5mg	XV10033
FA 2	Accord	United Kingdom	5mg	HU183
FA 3	Crescent pharma limited	United Kingdom	5mg	63383
FA 4	Dalkeith laboratories	United Kingdom	5mg	61741
FA 5	Replek farm	Macedonia	5mg	A9687
FA 6	Itqan pharmaceutical industries	Jordan	5mg	0452
FA 7	El-Nile Co.	Egypt	5mg	T210629
FA 8	Julphar	United Arab Emirates	5mg	0095

Weight variation: Twenty tablets of each brand were weighted individually using an analytical balance (Sartorius, Mettler Toledo, Germany). The average weight of the twenty tablets and the percentage deviations of individual weights from the average weight were calculated (Đuriš *et al.*, 2017).

Hardness: Tablet hardness was measured for ten tablets of each brand on an Erweka tablet hardness tester TBH 220 D (Erweka GmbH, Heusenstamm, Germany) (Đuriš *et al.*, 2017).

Friability: Twenty tablets of each investigated brand were weighed and rotated for 4 minutes at 25±1 rpm in an Erweka TAR 220 friability tester (Erweka GmbH, Heusenstamm, Germany). Thereafter, the tablets were weighed, and friability (F %) was calculated (Đuriš *et al.*, 2017).

Disintegration test: Disintegration time was determined on the QC-21 laboratory tablet disintegration tester (Hanson Research, Chatsworth, USA). Six tablets of each investigated brand were placed in the tubes of the apparatus basket, and the discs were added. The disintegration medium should be 900 ml of distilled water at 37±2°C (Đuriš *et al.*, 2017).

Assay: The assay of tablets containing only folic acid as an active ingredient was performed using the standard testing protocol following the procedure described in the USP monograph of folic acid (USP 29-NF 24, 2007). An assay of folic acid is performed using HPLC (Thermo Fisher Scientific, UK), equipped with a UV detector. The column used in the analysis was packing L1 (4.6 mm × 25 cm). The mobile phase consisted of methanol and phosphate

buffer solution, and the flow rate was 1 ml/min. The column temperature was 25°C, and detection was performed at 254 nm.

Diameter: Ten tablets were randomly selected, and the diameter was measured individually using a digital caliper (PowerfixProfi, Neckarsulm, Germany). The average diameter of the ten tablets and the percentage deviations of the tablet diameter from the average diameter were calculated (Wahed *et al.*, 2021).

Statistical analysis was performed using Graph Pad InStat, version 3.10 (Graph Pad Software, USA), where Pearson's correlation (r) was used to perform the correlation, and a one-way ANOVA statistical analysis was performed, with p-values < 0.05 considered significant.

Results and Discussion:

Thickness: The thickness of each tablet should not exceed ± 5% of the average thickness (Chavan *et al.*, 2018). The result for tablet thickness is shown in Table 2. Brand FA6 had the highest average thickness of 3.151 mm, while brand FA7 had the lowest average thickness of 2.201 mm, and the thickness deviation did not exceed 5%. All of the tablets met the standard tablet thickness specification.

Table 2: The thickness variation of eight different brands of folic acid tablets

Brand Code	FA 1	FA 2	FA 3	FA 4	FA 5	FA 6	FA 7	FA 8
Average thickness per tablet (mm) ± SD	2.572 ± 0.015	2.447 ± 0.027	2.665 ± 0.046	2.687 ± 0.045	6.053 ± 0.909	3.151 ± 0.017	2.201 ± 0.021	2.288 ± 0.021
Range of % thickness variation (n=10)	-0.855 to 0.700	-1.921 to 1.757	-3.189 to 1.689	-2.854 to 2.357	-0.649 to 0.649	-0.666 to 0.920	-1.408 to 1.772	-1.661 to 1.399

Weight variation: Table 3 shows the uniformity of weight determination for the eight brands of folic acid tablets. The average weight of brands FA1, FA2, FA3, FA4, FA5, FA6, and FA8 is less than 130 mg, while brand FA7's average weight is between 130 and 324 mg. The USP specification states that the limit of deviation is 10% for tablets weighing 130 mg or less, 7.5% for tablets weighing more than 130 mg to 324 mg, and 5% for tablets weighing more than 324 mg (Prakash *et al.*, 2020). The maximum percent deviation of

all eight brands was 4.565, and the minimum was -5.808, indicating that the tablets passed the weight variation test.

Table 3: The weight variation of eight different brands of folic acid tablets

Brand Code	Average weight per tablet (mg) ± SD	% of weight variation (n=20)
FA 1	65.478 ± 0.663	-1.982 to 2.539
FA 2	61.742 ±1.164	-2.464 to 4.565
FA 3	69.656 ± 0.665	-1.372 to 2.361
FA 4	70.453 ± 1.566	-4.760 to 4.083
FA 5	82.244 ± 0.909	-1.536 to 2.987
FA 6	126.778 ± 1.479	-1.907 to 2.132
FA 7	130.277 ± 3.305	-5.808 to 4.001
FA 8	115.246 ± 1.877	-3.450 to 3.397

Hardness: according to Table 4, brand FA6 had the highest hardness of 7.576 kg, while brand FA3 had the lowest hardness of 4.007 kg. The hardness of uncoated oral tablets ranges between 4 and 10 kg (Karmoker *et al.*, 2016). All the brands had acceptable hardness values.

Table 4: Average hardness of eight different brands of folic acid tablets

Brand Code	FA 1	FA 2	FA 3	FA 4	FA 5	FA 6	FA 7	FA 8
Average Hardness (Kg) ± SD	5.425 ± 0.681	5.476 ± 1.564	4.007 ± 0.221	4.701 ± 0.551	4.446 ± 0.327	7.576 ± 1.053	4.721 ± 0.347	4.038 ± 0.575

Friability: tablet friability should be less than 1%, according to USP specifications (Farhana *et al.*, 2018). Table 5 shows the friability of the eight different brands of folic acid tablets tested. Friability ranged between 0.056% for brand FA5 and 0.864% for brand FA3. The eight different brands of folic acid tablets met USP guidelines, indicating that the tablets had good mechanical strength (Đuriš *et al.*, 2017).

Table 5: Friability of eight different brands of folic acid tablets

Brand Code	FA 1	FA 2	FA 3	FA 4	FA 5	FA 6	FA 7	FA 8
Friability (%)	0.551	0.405	0.864	0.596	0.056	0.169	0.364	0.455

Disintegration test: Uncoated tablets should disintegrate within 15 minutes, according to BP, which is 30 minutes in the case of USP (Karmoker *et al.*, 2016). Table 6 shows the disintegration times of the eight different brands of folic acid tablets evaluated. The disintegration time of all folic acid tablet

brands ranges from 0.2 minutes for brand FA5 to 3.26 minutes for brand FA1, which is acceptable, as uncoated USP tablets have disintegration time standards as low as 5 minutes.

Table 6: Disintegration time (min) of eight different brands of folic acid tablets

Brand Code	FA 1	FA 2	FA 3	FA 4	FA 5	FA 6	FA 7	FA 8
Disintegration time (min)	3.26	0.25	1.54	2.43	0.2	1.45	2.09	1.19

Assay: The USP specifications for the content of folic acid tablets are no less than 90.0% and no more than 115.0% (USP 29-NF 24, 2007). Table 7 shows the percentage drug content for the various brands of folic acid tablets evaluated. The results of the drug content assay of folic acid tablets revealed that the active content of all brands ranged between 90% brand FA4 and 106% brand FA1 of the labelled amount specified for folic acid tablets. According to the USP specification, all brands complied with the USP specification.

Table 7: Assay of eight different brands of folic acid tablets

Brand Code	FA 1	FA 2	FA 3	FA 4	FA 5	FA 6	FA 7	FA 8
Average quantity in each tablet (%)	106	101	95	90	100	101	92	98

Diameter of tablets: The diameter test revealed that all of the brands of folic acid tablets tested met the specifications; the deviation in the mean diameter should not exceed 3% for tablets with a diameter of 12.5 mm or more and 5% for tablets with a diameter of less than 12.5 mm (Chowdhur *et al.*, 2015). According to the data in Table 8, the FA8 brand had the largest average diameter of 7.279 mm, while the FA1 brand had the smallest average diameter of 5.522 mm. All brands' percentage deviations were within specification (not exceeding ±5% of the mean diameter), as both the deviations for the maximum and minimum diameter values were within specification. Most of the time, deviations from the standard specification are rare when determining the diameter of tablets because the diameter size is usually determined by the dimensions of the die and punches used to make the tablets (Salako *et al.*, 2020). The potential problems with tablet weight and thus content uniformity can be detected at an early stage by monitoring the diameter of the tablets at regular intervals (Karmoker *et al.*, 2016).

Table 8: The diameter variations of eight different brands of folic acid tablets

Brand Code	Average diameter per tablet (mm)± SD	Range of % diameter variation (n=10)
FA 1	5.522 ± 0.015	-0.398 to 0.507
FA 2	5.531 ± 0.051	-1.464 to 0.886
FA 3	5.542 ± 0.015	-0.397 to 0.505
FA 4	5.567 ± 0.012	-0.305 to 0.413
FA 5	6.053 ± 0.023	-0.710 to 0.611
FA 6	7.047 ± 0.024	-0.383 to 0.610
FA 7	7.220 ± 0.005	-0.139 to 0.139
FA 8	7.279 ±0. 008	-0.397 to 0.153

The correlation between the diameter and thickness of folic acid tablets: The correlation between the diameter and thickness of folic acid tablets is represented in Figure 1. From the graph, it is obvious that the thickness of folic acid tablets has a weakly negative correlation (r = - 0.1688) with the diameter of the tablets, and statistically, it is not considered a significant correlation (P- value = 0.6895).

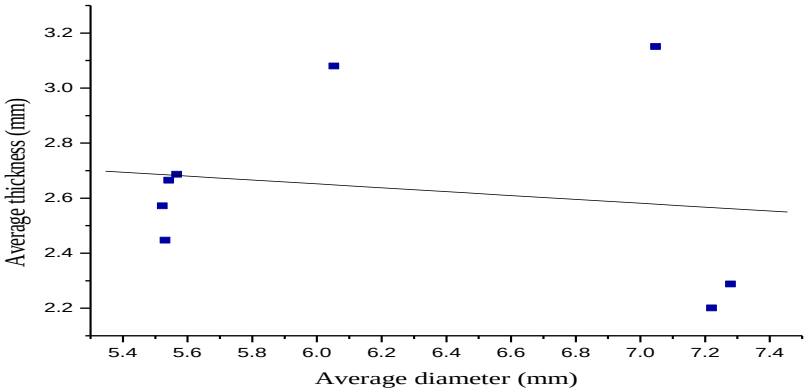


Figure 1: Average thickness as function of diameter

The correlation between the diameter and weight of folic acid tablets: Figure 2 shows the plot of the diameter against the weight of folic acid tablets. A strongly positive correlation (r = 0.9766) is found between the diameter and weight of folic acid tablets, and statistically, it is considered extremely significant ($p < 0.0001$).

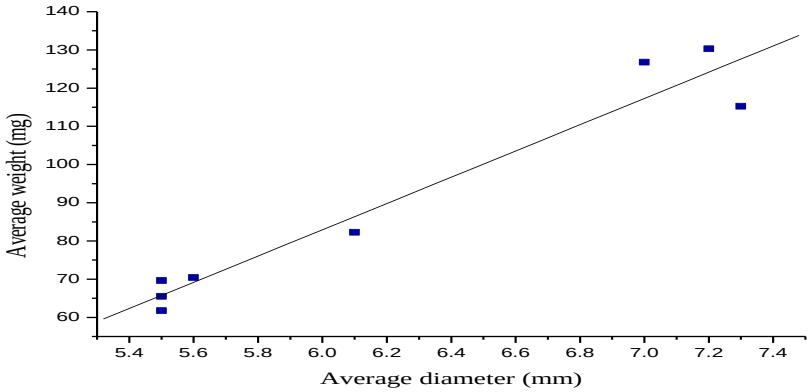


Figure 2: Average weight as function of diameter

The correlation between the diameter and hardness of folic acid tablets: The correlation between the diameter and hardness of folic acid tablets is represented in Figure 3. From the graph, it is obvious that the correlation between hardness and diameter of folic acid tablets is a weakly positive correlation (r = 0.1761), and statistically, it is not considered a significant correlation (P_value = 0.6765).

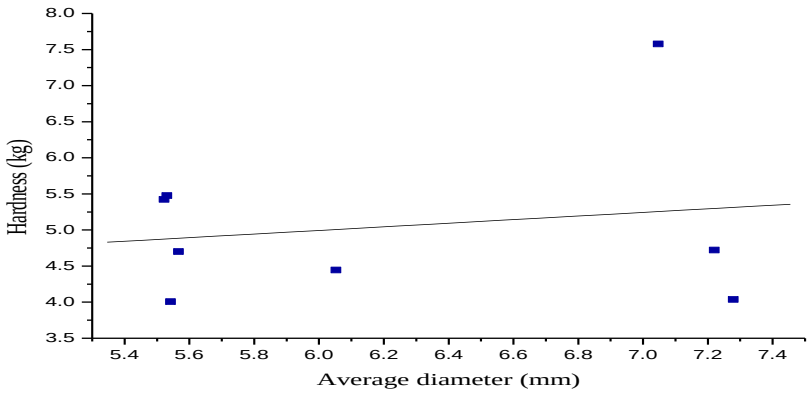


Figure 3: Hardness as function of diameter

The correlation between the diameter and friability of folic acid tablets: The correlation between the diameter and friability of folic acid tablets is represented in Figure 4. From the graph, it is obvious that the correlation between the friability and diameter of folic acid tablets is a moderately

negative correlation (r = -0.4597), and statistically, it is not considered a significant correlation (P- value = 0.2518).

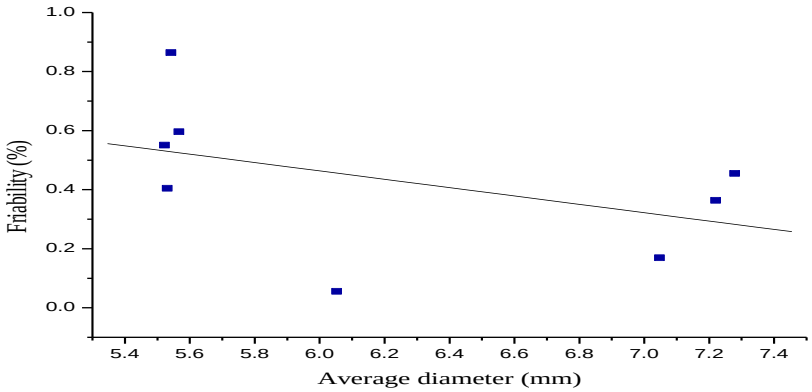


Figure 4: Friability as a function of diameter

The correlation between the diameter and disintegration time of folic acid tablets: Figure 5 shows a weakly negative correlation (r =-0.09433) between the disintegration time and diameter of folic acid tablets, which is not statistically significant (P- value = 0.8242).

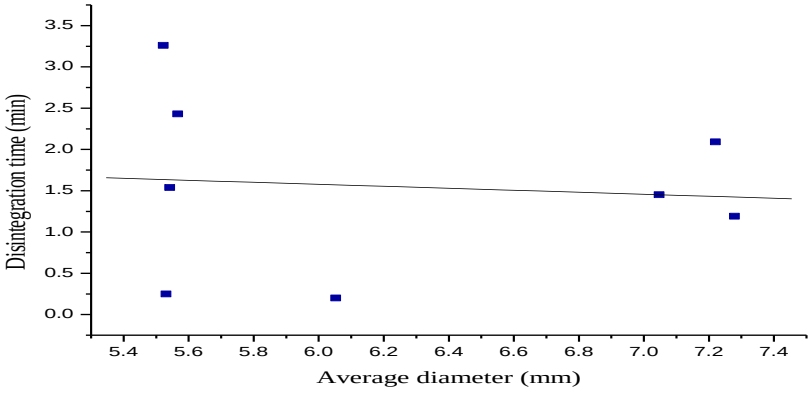


Figure 5: Disintegration time as a function of diameter

The correlation between the diameter and drug content of folic acid tablets: The correlation between the diameter and drug content of folic acid tablets is represented in Figure 6. From the graph, it is obvious that the correlation between the drug content and diameter of folic acid tablets is a

weakly negative correlation (r = -0.1145), and statistically, it is not considered a significant correlation (P- value = 0.7381).

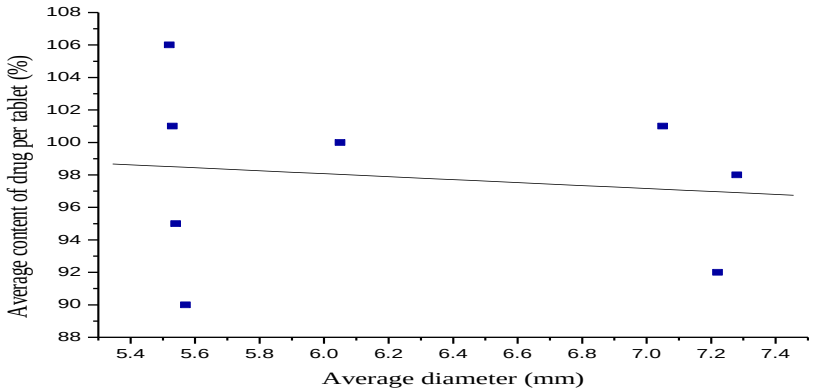


Figure 6: Average content of drug per tablet as function of diameter

Conclusion:

Tablets are a preferred dosage form for both patients and practitioners due to the ease of self-administration. In this project, we aimed to evaluate the suitability of tablet diameter as an offline indicator of various quality control parameters of tablets. Eight brands of folic acid tablets were tested for thickness, weight variation, hardness, friability, disintegration, and drug content. The study concluded that all eight brands were within the specified limits as mentioned in the USP. Additionally, we found that all brands of were within the limit when tested for diameter. This study showed that the diameter of a tablet was well correlated with its weight. Therefore, weight can be characterized in terms of tablet diameter, and it has a moderate to weak correlation with thickness, hardness, friability, disintegration, and drug content. To strengthen the scientific aspects of this project, future studies should include the dissolution test of tablets, which is a crucial quality control test for tablets. Furthermore, conducting the same tests on different drugs available in various tablet sizes can further improve the results of this project.

Ethics:

The products tested in this study are widely and frequently used in our country and the area of study. There is no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for litigation but for the advancement of knowledge. Also, the research

was not funded by the producing company but rather by the personal efforts of the authors.

References:

Ahmat, Norhayati, Castro, Gabriela González, & Ugail, Hassan. (2014). Automatic shape optimisation of pharmaceutical tablets using Partial Differential Equations. Computers & Structures, 130, 1-9.

Al-Achi, AJ. (2019). Tablets: a brief overview. Journal of Pharmacy Practice and Pharmaceutical Sciences(1), 50.

AlAli, Samer Hasan Hussein, Rasha Khaled Ali Abuthawabeh, Nour Sadeq Ali, Hmaidan, &, Hanadi Abdel Ibrahim Efseseh. (2022). Formulation and evaluation tablets of perindopril erbumine-layered double hydroxides prepared by direct compression method. Australian Journal of Science and Technology, 6(1), 12-18.

Arcot, Jayashree, Shrestha, Ashok K, & Gusanov, Uta. (2002). Enzyme protein binding assay for determining folic acid in fortified cereal foods and stability of folic acid under different extraction conditions. Food Control, 13(4-5), 245-252.

Chavan, Harishchandra, Chhabra, Gurmet, Gujarathi, Nayan, & Jadhav, Anil. (2018). Comparative study of In-process and finished products quality control test for tablet and capsules according to pharmacopoeias. Asian Journal of Pharmaceutical Research and Development, 6(3), 60-68.

Chowdhury, Md Mazharul Islam, Tajrin, Mohonoor, & Kabir, Nafisa(2015). Statistical Evaluation of In-vitro Analysis of Different Brands of Metronidazole Tablets Available in Bangladesh. *ARC Journal of Pharmaceutical Sciences*, 1(2), 6-10

Đuriš, Jelena, Čalija, Bojan, Vidović, Bojana, Dobričić, Vladimir, Milić, Jela, & Ibrić, Svetlana. (2017). Comparative analysis of mechanical and dissolution properties of single-and multicomponent folic acid supplements. Journal of Food Composition and Analysis, 60, 17-24.

Farhana, Jannatul, Uddin, Md Najem, & Islam, Md Robiul. (2018). Comparative in vitro quality evaluation of some paracetamol tablets, commercially available in Bangladesh drug market. Indo American journal of pharmaceutical sciences, 5(1), 527-533.

Gazzali, Amirah Mohd, Lobry, Mathilde, Colombeau, Ludovic, Acherar, Samir, Azaïs, Henri, Mordon, Serge, . . . Frochot, Céline. (2016).

Stability of folic acid under several parameters. European Journal of Pharmaceutical Sciences, 93, 419-430.

Gupta, Madan Mohan Khoorban, Avinash Ali, Ahamad Ramlogan, Ornella Talukdar, & Debjyoti. (2020). Comparative quality control study of different brands of diclofenac sodium tablet available in local and government pharmacies by in-vitro testing. Cureus, 12(11).

Karmoker, James Regun, Joydhar, Prince, Sarkar, Shuvro, & Rahman, Mostaqur. (2016). Comparative in vitro evaluation of various commercial brands of amlodipine besylate tablets marketed in Bangladesh. Asian Journal of Pharmaceutical and Health Sciences, 6(1).

Kumar, Davinder, Singh, Jasbir, Antil, Mamta, & Kumar, Virender. (2016). Quality control of tablets: a review. International Journal of Universal Pharmacy and Bio Sciences, 5(4), 53-167.

Mohammed, BB. (2014). Co-processing effect of microcrystalline cellulose/pre-gelatinized maize starch on properties of directly compressed folic acid tablets. World Journal of Pharmaceutical Research, 3(7), 24-32.

Mohan, Gupta Madan, & Madhulika, Gupta. (2016). Comparative pharmaceutical quality control testing of different brands of Paracetamol tablet available in Trinidad & Tobago, West Indies. Int J Pharm Sci Res, 7(7), 2830-2836.

Prakash, Ghimire, Chandra, Shrestha Abinash, Sandhya, Pandey, Bidur, Chapagain, & Samir, Dhakal. (2020). Pharmacopoeial comparison of in-process and finished product quality control test for pharmaceutical tablets. GSC Biological and Pharmaceutical Sciences, 11(3), 155-165.

Salako, KS, Adepoju-Bello, AA, & Silva, BO. (2020). Quality assessment of fifteen brands of artemether-lumefantrine tablets in Lagos metropolis, Nigeria. West African Journal of Pharmacy 31 (1): 111–122.

USP 29-NF 24. United States Pharmacopeial Convention, 2007. The United States Pharmacopeia and National Formulary. Rockville: United States Pharmacopeial Convention.

Wahed, Mir Imam Ibne, Lahiry, Amitav, Shorna, Jinat Fatema, Rahman, Masudur, Akter, Khurshida, & Rahman, Mst. (2021). Comparative Analysis of In Vitro Quality Parameters of Different Brands of

Marketed Ciprofloxacin Tablets Available in Bangladesh. Journal of Advances in Medical and Pharmaceutical Sciences, 29-38.

Younis, Islam Rasem. (2003). Pharmaceutical quality performance of folic acid supplements: West Virginia University.

AlQalam Journal of Medical and Applied Sciences
Special Issue for 6th International Conference in Basic Sciences and Their Applications (6th ICBSTA, 2023), *https://journal.utripoli.edu.ly/index.php/Alqalam* ***eISSN 2707-7179***

Distribution of *Thapsia garganica* in the Jabal Al Akhdar (The Green Mountain) area at Libya

Muoftah A. Bataw
Department of Natural Resources, Faculty of Natural Resources and Environmental Science, Omer Al-Mukhtar University, El-Beida, Libya
Correspondence authors: *mbataw@hotmail.co.uk*

Abstract:

Jabal Al Akhdar region is located in north-eastern of Libya, Dryas (*Thapsia garganica*) is toxic to cattle, camels and sheep when dried as fodder. Dryas seem to grow in wooded areas, areas prepared for cultivation, and rocky areas, plants can grow in a variety of different combinations of soil and also in different areas of shade and light it also spreads in urban area (in cultivated and uncultivated lands and between roads). The aim of this study is to identify the factors that promote its growth. (such as temperature, precipitation, and soil type). That may affect plant growth and has been found to occupy very diverse areas. The majority of the plants are distributed between Derna, and Taknis and the Mediterranean coast. Usually the temperature is a major factor in the growth of the plant, however the changes in temperature did not seem to affect the plant (Dryas) and the range of temperatures recorded in Al-Jabal Al-Akhdar did not appear to be inconsistent with sustainable growth in which temperature, day length, and light levels are important regulators of plant growth. It can also be found in regions with different climatic conditions, Just as dracaena plants grow individually and in groups. Many other plants were found around it. Some of these plants are in different quantities and juniper is considered. Juniperus phonicea is most closely related to the Dryas plants.

Keywords: *Thapsia garganica*, Al-Jabal Al-Akhdar, Libya.

Introduction:

Thapsia garganica found in more easterly regions compared with *Thapsia. transtagana* could be found in Western Mediterranean areas (Smitt, *et. al.*, 1995). *Thapsia garganica* is a herbaceous perennial in the Apiaceae, and traditionally used in North Africa as a remedy for arthritis, herpes, hair-fall, hypertension, rheumatic, eczema and scabies. (Abd El-Nasser *et.al.* 2020). These plants are toxic for the grazing livestock specially sheep, and other

animals which do not appear to be able to identify the association of this plant with toxicity. On some grazing pastures, regular removal of the plants shoots is carried out during growth season. The plants don't seem to have an effect on feral sheep because they either distinguish the plants, so that they avoid them, or they eat the young plants and develop tolerance. The plant doesn't have a great effect on goats, cattle, camels and wild animals because they are continuously moving around and that means they can eat the young plants and gain tolerance (Bataw, 2006). Al-Jabal Al-Akhdar which is true to its name, green and also the most vegetated part of the country (Johnson, 1973). It has considerable potential for agricultural activities since the natural resources are promising. Out of the diverse flora of Jabal Al-Akhdar ecosystem, the actual area of Jabal Al-Akhdar forests which is used productively (Al-Idrissi *et al.*, 1996). These natural forests degraded result of various reasons that leading to a serious disruption in the fragile ecosystem balance especially in Jabal Al-Akhdar (Eldoumi *et al.*, 2002). Jabal Al-Akhdar region is a typical mountainous ecosystem, North East of Libya characterized by unique environment in the Middle East. (Mohamed *et al.*, 2021). The grazing value of some plants, the locals graze their livestock (sheep and goats) using wild pants like *Pistacia lentiscus* and *Arbutus pavarii*. In addition, extra trampling of animals might cause damage of ecosystem in the area. Consequently, the vegetation could not tolerate this disturbance or recover again (Elshatshat & Mansour, 2014). Although this region is one of the important areas of wildlife in Libya, it suffers from extreme biodiversity destruction and degradation (El-Barasi & Manam, 2013). The pastures in and around the Jabal Al Akhdar area have sustained tribes of pastoral peoples for many hundreds of years. The majority of the northern tribes move only a few kilometres between the coastal terraces and the higher ridges of the Jabal. One factor which contributed to the restricted movement was the occurrence over much of the north of a plant, known Derias, which, when dried for fodder, is poisonous to cattle, camels and sheep. Animals had to stay in the one region during winter and spring, and could be moved in from pastures summer in south of Jabal Al Akhdar to north (Fisher, 1978). The Eastern Zone, sometimes known as the Benghazi region, lies between the Gulf of Sidra shoreline in the west to the Libyan frontier in east. The area was traditionally known as Barquah, or Cyrenaica in Europe (Benzabih, 1985). Jabal Al Akhdar (the Green Mountain) which lies at the center of this region has acquired this name from its evergreen forest. The area

surrounding the Green Mountain is one of the richest distributions of *Thapsia garganica* plants which in Libya found only in The Jabal Al Akhdar region. Jabal Al-Akhdar upland represents a plateau from tectonic lifting up of a primary plain of marine deposits, with the maximum altitude of the upland being 855.5 m in the Sidi Al-Hemery region. It also contains coastal hills, coastal plain, inland plateau and valleys (Azzawam, 1984; Zunni *et al.*, 1996. Al-Sodany *et al.*, 2003).

Materials and Methods:

Several studies and tours were carried out to find out the locations of the existence plant in the Green Mountain region, its density and its disappearance in the area between the eastern city of Derna (Al-Fatyeh agricultural area) to Taknes region and the Mediterranean coast to south of Jabal Al-Akhdar. As well as study the predominant species and companion species of *Thapsia* in the same areas as *Thapsia*. Went to the meteorological stations located in the region, such as those in Al-Fataeh project, Al-Marj agricultural project, and the Shahat meteorological station, to know the weather elements represented in heat, rain, humidity, day length, and other weather elements. Soil samples were also taken Shahhat and Al-Hamama regions in forested, and nonforested areas. Physical properties and chemical analysis of these samples was conducted.

Results:

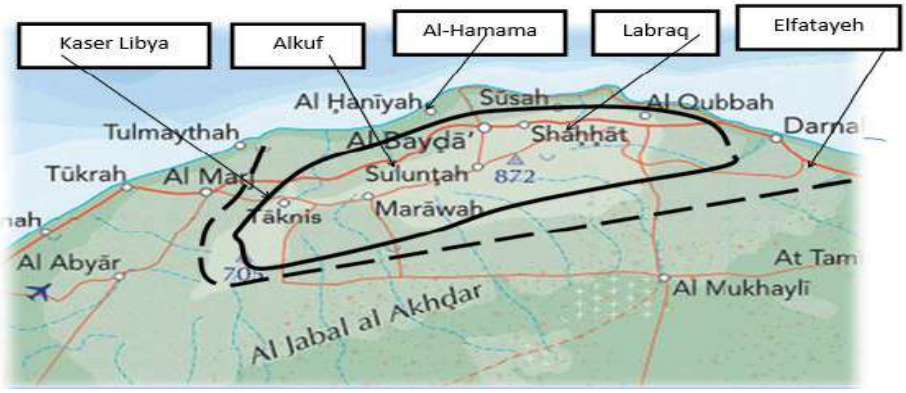


Figure 1: shows the distribution of *Thapsia garganica* in the Cyrenaica Region of Libya. Areas enclosed by ▬▬ are areas of high plant density while areas enclosed by ▬ ▬ are areas of low abundance of *Thapsia*. (Bataw, 2006).

Table 1: Predominant plant species and their frequency associated with *Thapsia garganica*.

Plant species	Frequency	Abundance (%)
Juniperus phonicea	27	40.92
Haloxylon raetem	7	10.6
Artmisia herba-alba	5	7.59
Calicotoma rigida	4	6.06
Sarcopterium spinosum	4	6.06
Cupressus sempervirens	4	6.06
Ziziphus lotus	3	4.55
Rhus tripartita	2	3.03
Lycium arabicum	2	3.03
Pistacia litiscus	1	1.51

Table 2: Outcome species found as companion plants where *Thapsia* is most abundant.

Plant species	Frequency	Abundance (%)
Uriginea maritime	25	14.04
Asphodelus microcarpus	18	10.11
Ceratonia siliqua	13	7.3
Calicotoma rigida	12	6.74
Pistacia litiscus	11	6.18
Thuymus capitatus	10	5.61
Cistus sp.	8	4.49
Rhamanus oleiodes	7	3.93
Phomis floccosa	6	3.38
Arbutus pavarii	6	3.38
Quercus coccifera	5	2.81
Olea europea	4	2.25
Atriplex halimus	4	2.25

Table 3: Annual rainfall in weather stations in the study area.

Rainfall (mm)	Albayda	Kaser Libya	Alkuf	Shahhat	Alkoba	labraq	Martuba
200	81	92	95	99	98	99	62
250	66	86	88	99	92	38	38
300	50	72	78	99	86	86	18
350	34	58	66	98	58	58	6
400	18	46	50	84	42	38	1
450	8	30	34	50	21	15.9	0
500	3	18	21	18	8	5.5	0

Table 4: Mean day length in meteorological stations.

Station	Lowest Day Length	Highest Day Length	Winter (hours)	Spring (hours)	Summer (hours)	Autumn (hours)	Annual (hours)
Darnah	4.9	10.3	5.2	7.8	10.1	7.2	7.5
Shahhat	4.8	11.4	5.1	8	11.2	7	7.9
Bartamid	4.3	10	4.3	7	9.6	6.7	6.9
Alkuf	4.2	10.9	4.7	5.4	10.2	8	7.1

Table 5: Soil collected from different depths in the forested and non-forested areas of Shahhat.

Depth	Soil type	Forested (%)	Non-forested (%)	Mean (%)
Shallow Soil	Clay	42.66	46.82	44.74
	Silt	34.53	31.06	32.79
	Sand	22.81	22.11	22.46
Deeper Soil	Clay	44.87	46.28	45.58
	Silt	34.24	30.25	32.25
	Sand	20.89	23.47	22.18

Table 6: Soil collected from different depths in the forested and non-forested areas of Al-Hamama.

Depth	Soil type	Forested (%)	Non-forested (%)	Mean (%)
Shallow Soil	Clay	41.51	48.58	45.05
	Silt	47.26	38.98	43.12
	Sand	11.23	12.44	11.84
Deeper Soil	Clay	45.43	45.06	45.25
	Silt	41.84	37.20	39.52
	Sand	12.73	17.74	15.24

Table7: Primary soil humidity from soil collected in forested and non-forested areas of Shahhat and Al-Hamama.

Depth	Location	Forested (1° soil humidity %)	Non-forested (1° soil humidity %)	Mean (1° soil humidity %)
Shallow Soil	Shahhat	5.15	2.55	3.85
	Al-Hamama	6.90	5.13	6.02
Deeper Soil	Shahhat	5.50	5.06	5.28
	Al-Hamama	7.78	7.28	7.53

Table 8: pH of soil retrieved from Shahhat (600 m above sea level) and Al-Hamama (10 m above sea level).

Depth	Location	Forested pH	Non-forested pH
Shallow Soil	Shahhat	7.65	7.87
	Al-Hamama	7.70	7.98
Deeper Soil	Shahhat	7.88	7.90
	Al-Hamama	7.72	8.07

Table 9: Electrical conductivity (EC) from soil taken from Shahhat and Al-Hamama.

Depth	Location	Forested EC	Non-forested EC
Shallow Soil	Shahhat	0.77	0.35
	Al-Hamama	0.43	0.16
Deeper Soil	Shahhat	0.70	0.42
	Al-Hamama	0.15	0.29

Table 10: $CaCO_3$ % of soil collected in Shahhat and Al-Hamama

Depth	Location	Forested $CaCO_3$ %	Non-forested $CaCO_3$ %
Shallow Soil	Shahhat	36.90	41.37
	Al-Hamama	3.56	5.37
Deeper Soil	Shahhat	44.73	51.43
	Al-Hamama	3.13	16.53

Table 11: Organic composition of soil collected in Shahhat and Al-Hamama.

Depth	Location	Forested areas	Non-forested areas
Shallow Soil	Shahhat	7.30	2.29
	Al-Hamama	6.59	4.65
Deeper Soil	Shahhat	2.16	1.77
	Al-Hamama	4.24	4.01

Discussion:

The area between Shahhat and Alkuf has the highest rainfall, 400-600 mm during the rainy season (Binkhial and Bukhechiem 1989). Maximum rainfall can reach 500 to 600 mm per year (Schliephake 1980). Average annual rainfall for the country is 26 mm, whereas it reaches 250-600 mm in the green mountain (Al-Idrissi et al. 1996). Most precipitation is rain; snow is rare and the soils are mainly heavy clay (El-Tantawi, 2005). In Shahhat; the mean number of rainy days is 78 days/year, most of these days during October to February meanwhile the highest rainfall during December and January with a duration of 7-24 days of rain. In spring, the rainfall lasts between 4-22 days/month. Alkuf, has slightly less rainfall averaging 77.3 days/year and Kaser Libya has a rainfall mean of 49 days/year (Nuh, 1998). Table 3 shows rainfall levels indicating the percentage of time that the rainfall reaches the level indicated in column one. The temperature ranges annually from a maximum of 30oC in July to a minimum of 7oC or 8oC in December or January (Farley, 1971). In winter, northern Cyrenaica has low temperatures and is subject to winter sleet, and for short periods, the higher hills may carry snow cover. The temperatures are distinctly low in summer on Al-Jabal Al-Akhdar (Fisher, 1978). Overall, the temperature is quite variable, suggesting that *Thapsia garganica* is not particularly sensitive to extreme temperatures. Table 4 shows the mean day lengths at different times of the year in the coastal zone of adjacent to the Green Mountain. Average values (hours) are given in columns "Winter" – "Annual" while the minimum and maximum

values for the entire years are given in columns 2 and 3. Tables 5, 6 show respectively the soil composition in Shahhat and Al-Hamama regions. The main soil type in Shahhat is a clay. Shahhat is in the heart of the area in which Thapsia is commonly found. In Al-Hamama area, where Thapsia is much less common, the predominant soil type is still clay based. In the mountain zone are larger pockets of good soils such as the brown Mediterranean soil in the Labraq area (inside the Thapsia zone) and in the El Marj and El Fatayah areas (outside the Thapsia zone). Under arid conditions, soils are generally shallow and less developed with inadequate vegetative cover as a result of low annual precipitation with high temperature, however due to the lack of enough water for leaching excessive salts from the soil slump; the chance of salinity or alkalinity is high (Cerda, 1998). Plants are often highly sensitive to soil pH and other chemical characteristics, data on the local soil characteristics of the zones where Thapsia grows have been investigated. General measure of the ion content is a complicated measure that depends on a number of factors, it is used as an assessment of the salinity of a soil sample presented. Table 9 shows that the soil in Shahhat has higher conductivity than that found in Al-Hamama but both have extremely low conductivity values suggesting that these differences are probably insignificant. Calcium carbonate levels in soil gives an indication of the exposure of the soil to limestone rock types and can affect the pH (usually raises the pH). Considerable differences were observed between the calcium carbonate content of soils from Shahhat and Al-Hamama zones. Little difference was observed between Shahhat and Al-Hamama areas whereas. More difference was seen between the forested (higher organic levels) and non-forested areas (lower organic levels).

Conclusion:

The majority of the plants are distributed between Darna and Taknis, the greatest abundance of Thapsia garganica has been found in the middle zone of this area, it also spreads in urban rigions. Thapsia appears to grow in forested areas, areas cleared for planting and stony areas which means that Thapsia can grow in a variety of different types of soil and also at different shade and light areas. Temperature is a major factor in plant growth. Changes in temperature did not appear to affect survivability and the range of temperatures recorded in the Thapsia zone of the Jabal did not seem inconsistent with sustained growth at any of the temperatures tried. Day length and light levels are important

regulators of plant growth. Thus, it appears that Thapsia can exist in areas of quite differing climatic conditions. A wide range of other plant species also exists in the location occupied by Thapsia. Tables show the results of some studies of these companion plants. Overall, the results show that there is a wide diversity of plants found in the same locations as Thapsia suggesting that these regions are not highly restrictive to plant growth such that dominance is seen. Many other plants are found around Thapsia garganica.

References:

Abd El-Nasser S. Al Borki, Abdulhamid K. Alzerbi, Hanan F. Kabiel, Ahmad K. Hegazy (2020) Variations in phenological and functional traits in *Thapsia garganica* populations in Al Jebel Al Akhdar, Libya' African journal of ecology p 639-648.

Al-Idrissi, M., Sbeita, A., Jebriel, S., Zintani, A., Shreidi, A., Ghawawi, H., Tazi, M. (1996) Libya: Country Report to the FAO International Technical Conference on Plant Genetic Resources. Leipzig, Germany. FAO.

Al-Sodany, Y.M., Shehata, M.N., Shaltout, K.H. (2003) Vegetation along an elevation gradient of the Jabal Akhdar, Libya. Ecologia Mediterranea, 29, 35-47.

Azzawam, S. (1984) "Al Jabal Al Akhdar: A Natural Geography Study". Garyounis University, Benghazi, Libya.

Bataw,M.(2006) An investigation of phytotoxicity and phylogeny of *Thapsia garganica.* Biomedical Science Research Institute School of Environment and Life Science, The University of Salford.UK *(Unpublished phD. Thesis*).

Benkhial, A.S and Bukechiem, A.A. (1989) Irrigated farming in the Jebel el Akhdar: Prospects and problems. Libya: state and Region: A study of Regional Evolution. *Centre of Near and Middle Eastern studies London*73- 81.

Benzabih, H.A. (1985) Developing trends of farm/rangeland resources in the eastern zone of Libya. in Baru, M. M., Ghanem, S. M and Mchhachlan, K. S. Planning and Development in Modern Libya. *The society for*

Libyan studies and Middle East and North African studies press limited, London 130-137.

Cerda, A. (1998) Soil aggregate stability under different Mediterranean vegetation types. Catena, 32, 73-86.

Eldoumi, F., Elsaedi, O., Zunni, S. (2002) Study of Aljabal Alakhdar's plant cover. Final report in Arabic. MSc Thesis. University of Omar Al mokhtar, El-Bieda, Libya.

Elshatshat, S., Mansour, A. (2014) Disturbance of flora and vegetation composition of Libya by human impacts: Costal region of Al-Jabal Al-Akhdar area as model. Advances in Applied Science Research, 5(5), 286-292.

El-Barasi, Y.M., Manam, W.S. (2013) Threats to plant diversity in the North Eastern part of Libya (El-Jabal El-Akahdar and Marmarica Plateau). Journal of Environmental Science and Engineering, 2, 41-58.

Farley, R. (1971) Planning for development in Libya: the Exceptional Economy in the developing world. *Praeger Publisher, New York*.

Fisher, W.B. (1978) The Middle East. *A physical, social and regional geography, Methuen and Cotted, Cambridge.*

IUCN (2013) International Union for Conservation of Nature (IUCN) Red List of Threatened Species, Version. 1, 2013, www.iucnredlist.org .

Johnson, D.L. (1973) Jabal Al-Akhdar, Cyrenaica: An historical geography of settlement and livelihood. Research paper 48, Department of Geography, University of Chicago.

Mohamed H. A., Mosallam A. H., Sergwa S. S., Gibreel A. M., (2021) Impact of Edaphic Factors on Vegetation Composition in Al-Jabal Al-Akhdar, Libya . Egypt. J. Bot., Vol. 61, No. 1, pp. 191-201.

Nuh, S. (1998) Climate of Aljabel Al Akhder. *University of Omer Almukhtar, Elbida, Libya.*

Schliephake, K. (1986) Tunisia &Libya-Diversity of Economic Strategies & similarities of Geological constraints. *The Maghreb Review*, 11: 121-130. Smitt, U.W. (1995) Chemotaxonomic Investigation of *T. villosa* L.

Apiaceae (Umbelliferae). *Botanical Journal of the Linnean Society*, 118: 367-377.

Zunni, S.A., Saadi, P.T., Bayoumi, M.P. (1996) The impact of environmental factors on natural pastures. Al-Marj, Libya, Science Magazine, (3).

AlQalam Journal of Medical and Applied Sciences
Special Issue for 6th International Conference in Basic Sciences and Their Applications
(6th ICBSTA, 2023), https://journal.utripoli.edu.ly/index.php/Alqalam eISSN 2707-7179

Evaluation of the Activity of Alcoholic Extract from The Leaves of *Salvia officinalis* and *Mentha longifolia* against Some Fungi Isolated from Soil

Fatma B. Abuaziza* and Ibtisam M. Ahamdi
Department Botany, Arts and Science Faculty, Al-Abyar, University of Benghazi, Benghazi, Libya
Correspondence authors: *Fatma.aboaziza@uob.edu.ly, ibtisam.ahmadi@uob.edu.ly

Abstract:

The inhibitory effect of the alcoholic extracts of *Salvia officinalis* and *Mentha longifolia* was studied. Five concentrations for each type of these extracts (5%, 10%, 15%, 20%, 25%) were tested by their effect in inhibiting the fungal growth of four types of filament fungi isolated from the soil (*Aspregillus niger, Penicillium chrysogenum, Fusarium solani, Alternaria alternata*) on the SDA food medium in the laboratory, the results showed that the ethanol extracts Of both plants had an inhibitory effect on the growth of fungi on the food medium, measured by the Abbott's formula equation, where the alcohol extract of *M. longifolia* plant at a concentration of 25% gave the highest inhibition rate for the fungus *A. niger* with a inhibition of 61.09%, and The inhibitory effect of both plant extracts increased with increasing concentration, and chemical detection tests showed that they contain active anti-fungal and anti-suppressive substances to the growth of fungi such as flavonoids, glycosides, and volatile oils. Therefore, we recommend utilizing these botanical extracts as an alternative to chemical fungicides due to their cost-effectiveness, safe application, and absence of harmful effects on the environment.

Keywords: Lamiaceae, *Salvia officinalis, Mentha longifolia*, Soilborne fungi.

Introduction:

The Lamiaceae family spreads mainly in the Mediterranean region with about 180 genera and 3,500 plant species of which it was mentioned in the Libyan Flora that there are 22 genera and 56 species (El-Gadi, 1989). The species belonging to this family are distinguished by that essential oils are extracted which source of antioxidants and antimicrobials (Skendi et al., 2020). There are many microorganisms in soil which are soil-borne pathogens that infect many crops and causes significant damage leading to a severe shortage in

production and a decrease in its quality (Agrios 2005; Babalola & Glick, 2012). The most important of which is fusarium wilt disease which causes many agricultural losses all over the world (Khan & Khan, 2002). Although the importance of using chemical pesticides in controlling plant pests, it has harmful effects on the environment and humans (Kumar et al., 2014) including the tendency of fungi to develop chemical resistance necessitating a higher dose and non-biodegradable nature (Lanciotti et al., 2004; Anjum & Shafique, 2006). As the indiscriminate use of many fungicides led to the emergence of many harmful biological effects especially cancer (Shafique et al., 2007). So, the world has turned to clean agriculture which contributes to protecting plants without affecting the ecobalance by using biological agents that do not have a negative impact on humans or their environment (Pieta & Pastucha, 2004; Srinivasan & Shanmugam, 2004), such as the use of plant extracts whose active constituents affect plant-pathogenic organisms moreover being safe and less cost (Agrios, 2005). The World Health Organization has paid increasing attention to finding alternatives to medications that cause side effects. The first of these alternatives was medicinal plants (Cowan, 1999) Therefore, many extracts must be examined for their ability to inhibit fungi and toxins produced. For phenols, flavonoids and tannins have antifungal properties (El-Desouky, 2021). Flavonoids are also one of the second essential metabolites of plants which have multiple roles in plant defense mechanism and antifungal properties. due flavonoids exhibit antimicrobial effects through virulence factors, membrane rupture, cell and nuclear envelope formation (Biharee et al., 2020). Saponin is also considered an antifungal that by testing plant extracts rich -saponin against common fungi (Chapagain et al., 2007). The species, which contain volatile oils, are also considered anti-inflammatory and fungicidal candidates (Angioni et al., 2006; Kumar et al., 2014). Thus, Aromatic plants like *Salvia officinalis* have been utilized as antibacterial agents since ancient times (Lima et al., 2004), which is considered one of the oldest medicinal herbs and is mentioned in all medical books in ancient Rome. The name *Salvare* came from Latin which means to save, meaning to preserve life and *officinalis*, which means medicinal referring to the medicinal properties possessed by this plant. The height of *Salvia officinalis* can reach about 60 centimeters and has leaves that are silvery-green in color. Its flowers are of different colors ranging between white, blue, and rose. It is found wild and farmed (Dordevic et al., 2000). Reports have been published on the composition of essential oils of this genre

by several authors (Santos-Gomes & Fernandes-Ferreira, 2001; Velickovic et al., 2002; Radulescu et al., 2004). *Mentha longifolia* is considered a perennial plant which height ranges between 40 and 120 cm. It is characterized by its strong smell, oval leaves and a stem covered with white or gray hairs. It shows activity in the field of anti-fungal and anti-oxidation (Dzamicetal, 2010). The objective of this paper was study the ability of different concentrations of alcoholic extracts of *Salvia officinalis* and *Mentha longifolia* to inhibit the growth of some plant-pathogenic fungi.

Materials and Methods:

Sample Collection

Plant samples were collected from Derna region in Northeast of Libya. They were identified according to (El-Gadi & Jafri 1986). *Salvia Officinalis* L belongs to the Lamiaceae family and locally name is (*Teffah eshahi*). It is widely used in folk medicine. As for the *Mentha longifolia* Huds (L), it also belongs to the Lamiaceae family and is locally name as '*Vicks*' due to its pungent smell. It is used locally in folk medicine to treat colds. The plants are then air-dried away from the sun to be later grinded by mortar and pestle and stored at 4°C until extraction.

Preparation of plant extracts

The alcoholic extract was prepared according to the method of (Hernandezi et al., 1994), with a weight of 50 grams of plant powder, 150 ml of 99% ethanol was added to it to be after placed in an opaque flask for 48 hours. Next, the solution was filtered by Whattman No.1 filter paper and placed into the centrifuge at 2000 cycles per minute. The pasteurization process took place at a temperature of 64 °C in a water bath for 15 minutes and then concentrations were prepared.

The phytochemical screening

The detection of *Salvia officinalis* and *Mentha longifolia* extract was carried out to test the presence of the most significant active substances such as flavonoids according to the method of (Harbon, 1998), glycosides by GlycosidesFehling Test (Fahmy, 1933) as well as volatile oils according to the method of (Geismann, 1962) and saponins by Frothing Test (Trease & Evans, 1989). The detection of tatins was carried out according to the method referred to by (Geismann, 1962).

Isolation of fungi

The fungal species were used *Fusarium solani*, *Penicillium chrysogenum*, *Aspregillus niger* and *Alternaria alternata* which were isolated from the soil and cultured on Sabouraud Dextrose Agar (SDA), incubated at a temperature of 27°C for 5 days. At the end of the incubation period, fungal isolates were transferred to Petri dishes containing the same media for the purpose of purification (Atlas, 2010). Pure mold isolates were examined and identified microscopically according to the morphology and the nature of growth in the dishes and compared to identification books and references and the use of classification keys (Campbell et al., 2013; Leslie & Summerell, 2006; Watanabe, 2010).

The Effect of Extracts in Inhibiting the Growth of Pathogenic Mites

The Poisoned Food Technique was used as five concentrations of the two extracts were taken separately which were (5%, 10%, 15%, 20%, 25%) based on the volume of the extract to the volume of the medium (SDA), and the concentrations of sterile extracts were added to Sabouraud Dextrose Agar before mixing well and pouring onto the dishes. After solidification, a disk of 0.5 cm diameter was transferred from the tested fungal cultures which were five days old using a cork puncture. Each disk was placed in the middle of the dish under sterile conditions with three replications for each treatment and for each fungus. Under the study, the comparison treatment was used in which an untreated nutrient medium with concentrations of plant extracts was used then the dishes were incubated at 27 °C. After that, the growth rate of each fungus was measured in the different treatments after five days of fungal growth, by measuring the growth rate of two diameters perpendicular to the growth of the colony passing through the center of the dish, after which the percentage of inhibition was measured according to the calculation of (Abbot, 1925).

Results and Discussion

1. Detection of Active Substances

Table 1: Result of photochemical test

Scientific name	Glycosides	Flavonoid	Volataile olis	Tannin	Saponins
Salvia officnalis	++	++	++	++	++
Mentha longifolia	+	++	++	+	+

(++) Highly present, (+) low, (-) absent

The results of chemical detection showed that the alcoholic extract of the two plants contained Volatile oils, Flavonoids, Glycosides, Saponins, and Tannin as the results were positive for all tests (Tab. 1), and were consistent with (Adham, 2015; Omran, 2018), but there was a discrepancy in the results. (Fig. 1) shows all tests for the active substances that had a clear appearance in the *S. officinalis*, while in (Fig. 2), saponins, tannins, and glucosides had a lesser appearance in the *M. longifolia*. However, in general, both plants were rich in flavonoids.

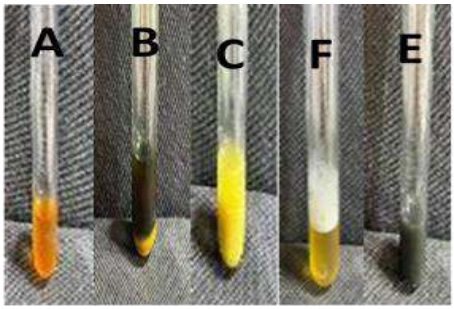


Figure 1: Result of the phytochemical Test of *S. officinalis* (A) Extract (B) Glucosides (C) Flavonoids (D) Saponins (F) Tannin

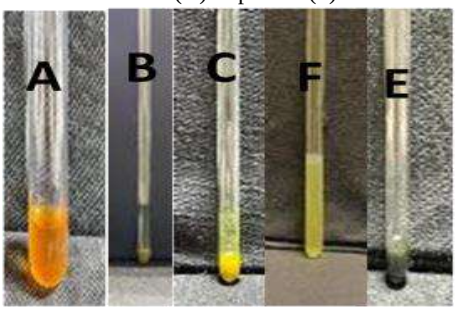


Figure 2: Result of the phytochemical Test of *M. longifolia* (A) Extract (B) Glucosides (C) Flavonoids (D) Saponins (F) Tannin

2. Effect of Alcoholic Extract of *Salvia officinalis* on Tested Fungi

Table 2. Effect of concentrations of *S. officinalis* on the growth of the tested fungi

Extract	Fungi	Control	Concentrations				
			5%	10%	15%	20%	25%
Salvia officnalis	*Alternaria alternata*	**3.65***	17.53% **3.01**	30.68 **2.53**	24.38 **2.76**	30.14 **2.55**	33.42 **2.43**
	Aspegillus niger	**4.56**	13.38 **3.95**	17.11 **3.78**	53.54 **2.30**	48.10 **2.05**	60.76 **1.55**
	Fusarium solani	**5.61**	23.71 **4.28**	24.96 **4.21**	32.62 **3.78**	43.85 **3.15**	54.01 **2.58**
	Penicillium chrysogenum	**3.16**	8.86 **2.88**	25.63 **2.35**	34.18 **2.08**	49.37 **1.60**	60.44 **1.25**

*Average of three repetitions in cm; % percentage

Results showed a significant decrease in the growth diameter of *Alternaria alternata* (Tab. 2) of the *S. officinalis* extract on the growth of the tested fungi. The average diameter of the control treatment (proof) was 3.65 cm, which gave fairly close percentages of inhibition that were 17.53%, 30.18%, 24.38%, 30.14%, 33.42% for the studied concentrations respectively (Fig. 3) which had been the variable effect on fungus growth based on changing the concentrations of the extract. This varying effect reflects complex interactions between the components of the extract, which is consistent with previous research that show variation in the effects of extracts plants on this fungus (Martinez et al., 2018; Nikolova et al., 2006). A decrease in the growth diameter of the *Aspergillus niger* (Tab. 2) was observed with an increase in the concentration of the alcoholic extract. When the alcoholic extract was treated at two concentrations of 5% and 10%, the inhibition percentage was 13.38% and 17.11%, respectively. The inhibition percentage increased with an increase in the concentration of the alcoholic extract of the plant, reaching 53.54%, 48.10% for the concentration of 15%, 20% respectively while the concentration of 25% reached 66.76%, with an average growth diameter of 1.55 cm compared to the control of 4.56 cm in (Fig. 3). This effect indicates that the extract carries compounds that possess an inhibitory effect that can be enhanced by increasing the concentration of the extract and is consistent with previous research that reported similar effects (Johnson et al., 2019). Multiple studies affirm the presence of terpenes in *S. officinal* is extract (Horuchi et al., 2001; Miura, 2007). Moreover, Aspergillus fungus threatens society, due to its resistance to antibiotics (Tan et al., 2022). As *Fusarium solani*, (Tab. 2), the average growth diameter was 5.61 cm and the rates of inhibition were very close, reaching 23.71%, 24.96%, 32.62%, 43.85%, and 54.01% for the concentrations of the alcoholic extract of the *S.officinalis*, (Fig. 3). This inhibitory effect on the growth of fungi indicates that *Salvia officinalis* extract can be a powerful source of inhibitory for this fungus, and this aligning with previous study (Gupta et al., 2020). *Penicillium chrysogenum*, (Tab. 2) by a percentage up to 60.40% using a concentration of 25% of the alcoholic extract which had an average growth diameter of 1.25 cm compared to a control treatment of 3.16 cm, with the inhibition rate range of 8.86%, 25.63%, 34.18%, 49.37%. (Fig. 3) shows the ability of the extract to inhibit the growth of this fungus and is consistent with previous studies that indicated inhibitory effects of plants on similar species of fungi (Smith et al., 2017). The reason for the effect of the alcoholic extract is that it contains various effective

compounds such as chlorides which have an inhibitory effect on the growth of many pathogenic microorganisms including the fungi under study. Several studies have shown the ability of *S. offcinals* extract to inhibit of Saccharomyces cerevisae (Farcasanu & Oprea, 2006) whose reflects the effects of chemical compounds present in *S. officinalis*, which may have antimicrobial effects (Rios & Recio, 2005). Such as, Theanines, Flavonoids, and Saponins are these compounds to which active effects is attributed as these results are consistent with (El-Desouky, 2021) where flavonoids are believed to possess antimicrobial properties and inhibitory effects on the growth of fungi thanks to their interference within microbial cells in the oxidation systems (Cushnie & Lamb, 2005). In addition, tannins can play a role in inhibiting the growth of fungi by interfering with protein interactions and inhibiting the activity of enzymes necessary for the growth of microbial cells (Haslam, 1998). And they act as antioxidants, that is, protect important vital compounds and have a toxic on fungi (Cowan, 1999). The presence of saponins in these extracts has an inhibitory effect on the growth of fungi (Srimal, 1997). The mechanism of action of the saponins depends on the formation of sterolites complexes in the fungal cell membrane which leads to the membrane losing its function (Keukens, 1995).

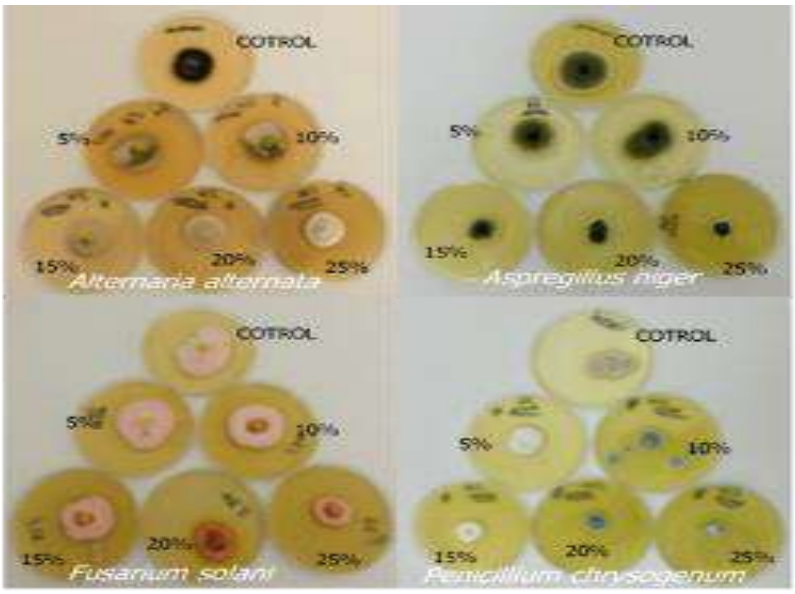


Figure 3: The effect of *S. officinalis* plant concentrations on the tested fungi

4. Effect of Alcoholic Extract of *Mentha longifolia* on Tested Fungi

Table 3: The effect of concentrations of *Mentha longifolia* on the tested fungi

Extract	Fungi	Control	Concentrations				
			5%	10%	15%	20%	25%
Mentha longifolia	*Alternaria alternata*	**3.51***	11.68% **3.1**	17.38 **2.9**	23.65 **2.68**	32.19 **2.38**	48.43 **1.81**
	Aspegillus niger	**4.63**	24.84 **3.48**	43.20 **2.63**	49.24 **2.35**	48.16 **2.4**	61.99 **1.76**
	Fusarium solani	**4**	8 **3.66**	34.25 **2.63**	26 **2.96**	37.25 **2.51**	44.25 **2.23**
	Penicillium chrysogenum	**3.11**	12.22 **2.73**	22.51 **2.41**	37.30 **1.95**	40.51 **1.85**	47.59 **1.63**

*Average of three repetitions in cm; % percentage

Results varied between the species studied as the *Alternaria alternata* showed variation in its response to the concentrations of the alcoholic extract of the *M. longifolia* at the different concentrations of 5%, 10%, 15%, 20%, and 25% (Tab. 3). We observed an increased inhibition in the growth. In fact, the inhibition rates ranged between 11.68% and 48.43%, with a noticeable reduction in the growth diameter at the highest concentration, reaching 1.81 cm compared to the control treatment of 3.51 cm, (Fig. 4). This indicates a direct effect of the concentration of the alcoholic extract on fungal growth inhibition. As for *Aspergillus niger*, the inhibition percentages were similar when using different concentrations of the extract. The inhibition percentages at the tested concentrations were 24.84%, 43.20%, 49.24%, and 48.16%, respectively, (Tab. 3) and the inhibition percentage reached only 61.99% at concentration 25. %, and an average growth diameter of 1.76 cm was recorded compared to the average growth diameter of the control, which was 4.63 cm, (Fig. 4). These results show a similar effect with increasing concentration of the extract which indicates an increasing effectiveness of the alcoholic extract in inhibiting the growth of this fungus. The effectiveness of the extracts of *M. Longifolia* used in this study are distinctive and highly effective against different fungi. In a study of *A. niger*, it was completely inhibited by a 100%. The high fungi inhibitor effectiveness is due to it containing essential oils such as Menthon, Menthol, Menthofuranem, and Menthe acetate, as it is known that essential oils whether in mint or other plants have anti-fungal activity (Sitara et al., 2008). The *Fusarium solani* showed variation in the effect of the extract in (Tab. 3). The lowest percentage of inhibition was recorded at the concentration of 5% and reached 8% with an average growth diameter of 3.66 cm compared to the control which was 4 cm. On the other hand, a high inhibition rate of 34.25% was recorded at a concentration of

10%, an inhibition rate of 26% at a concentration of 15%, and an inhibition rate of 37.25% at a concentration of 20 %. The maximum inhibition rate reached 44.25% at a concentration of 25%, and the average growth diameter reached 2.23 cm, (Fig. 4). This variable effect shows complex interactions between the extract and the fungus which is similar to previous reports that dealt with the effect of plant extracts on this fungus. Inhibition rates for *Penicillium chrysogenum* were studied in (Tab. 3), after measuring the average growth diameter of the control, which reached 3.11 cm. Inhibition rates were calculated at the concentration of 5% and found to be about 12.22%. As for the concentrations of 10%, 15%, 20%, and 25%, the inhibition rates were fairly close and respectively at 22.51%, 37.30%, 40.51%, and 47.59% (Fig. 4). These results indicate the effect of the extract on the growth of the *P. chrysogenum* in which the inhibition rates increased with increasing concentrations used. This is an indication of the effectiveness of the active substances in suppressing the growth of this fungus.

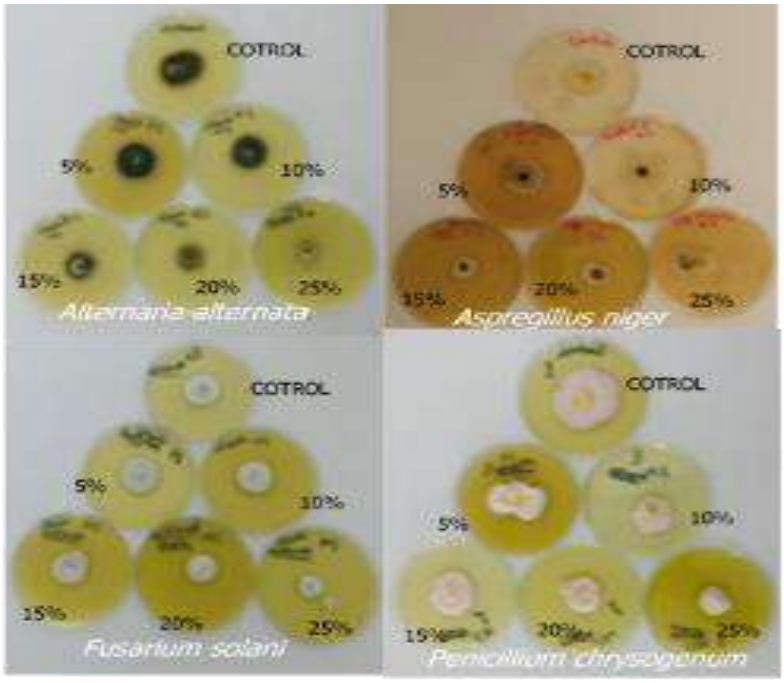


Figure 4: The effect of *M. longifolia* concentrations on the tested fungi

Conclusion:

The study demonstrates the inhibitory effects of *S. officinalis* and *M. longifolia* extracts on fungi, suggesting their potential as natural fungicides in agriculture and environmental health. Variability in effects among fungi species calls for further research on active compounds and mechanisms for effective application. This promising trend in plant extract-based fungicide control requires more investigations for identifying active compounds and precise application methods in agriculture and the environment.

References:

Abbott, W.S. (1987). A Method of computing the Effectiveness of an Insecticide. *Journal of The American Mosquito Control Association*, *3*(2), 302-303.

Adham, A. N. (2015). Comparative extraction methods, phytochemical constituents, fluorescence analysis and HPLC validation of rosmarinic acid content in Mentha piperita, *Mentha longifolia* and *Osimum basilicum*. *Journal of Pharmacognosy and Phytochemistry*, *3*(6), 130-139.

Agrios, G.N. (2005). *Plant Pathology*. Academic Press, New York.

Angioni, A., Barra, A., Coroneo, V., Dessi, S., & Cabras, P. (2006). Chemical composition, seasonal variability, and antifungal activity of *Lavandula stoechas* L. ssp. stoechas essential oils from stem/leaves and flowers. *Journal of agricultural and food chemistry*, *54*(12), 4364-4370.

Anjum, B., & Shafique, S. (2006). Evaluation of antifungal activity of Cicer arictinum L. *Pak. J. Bot*, *38*, 175-184.

Atlas, R. (2010). *Handbook of Microbiology Media*. 4th Ed. Taylor and Francis Group LLC.

Babalola, O. O., & Glick, B. R. (2012). Indigenous African agriculture and plant associated microbes: Current practice and future transgenic prospects. *Sci. Res. Essays*, *7*(28), 2431-2439.

Biharee, A., Sharma, A., Kumar, A., & Jaitak, V. (2020). Antimicrobial flavonoids as a potential substitute for overcoming antimicrobial resistance. *Fitoterapia*, *146*, 104720.

Campbell, C. K., Johnson, E. M., & Warnock, D. W. (2013). *Identification of pathogenic fungi* .2nd Ed. WILEY-BLACKWELL.

Chapagain, B. P., Wiesman, Z., & Tsror, L. (2007). In vitro study of the antifungal activity of saponin-rich extracts against prevalent phytopathogenic fungi. *Industrial Crops and Products*, *26*(2), 109-115.

Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical microbiology reviews*, *12*(4), 564-582. Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical microbiology reviews*, *12*(4), 564-582.

Cushnie, T. T., & Lamb, A. J. (2005). Antimicrobial activity of flavonoids. *International journal of antimicrobial agents*, *26*(5), 343-356.

Dordevic, S., Cakic, M., & Amr, S. (2000). The extraction of apigenin and luteolin from the sage *Salvia officinalis* L. from Jordan. *Facta universitatis-series: Working and Living Environmental Protection*, *1*(5), 87-93.

Dzamic, A. M., Sokovic, M. D., Ristic, M. S., Novakovic, M., Grujic-Jovanovic, S., Tesevic, V., & Marin, P. D. (2010). Antifungal And Antioxidant Activity of *Mentha Longifolia* (L.) Hudson (Lamiaceae) essential oil. *Botanica serbica*, *34*(1), 57-61.

El-Desouky, T. A. (2021). Evaluation of effectiveness aqueous extract for some leaves of wild edible plants in Egypt as anti-fungal and anti-toxigenic. *Heliyon*, *7*(2).

El-Gadi, A., (1989). Flora of Libya. Vols. 145-147. Department of Botany, Al-Faateh University, Tripoli, Libya.

Fahmy, I. R. (1933). Constituents of plant crude drugs. *Poul Barby. Cario. Egypt*.

Farcasanu, I. C., & Oprea, E. (2006). Ethanol extracts of *Salvia officinalis* exhibit antifungal properties against *Saccharomyces cerevisiae* cells. *Analele Universitanii din Bucuresti–Chimie, Anul XV (serie noua)*, *1*, 51-55.

Geismann, T, A. (1962). *The Chemistry of flavonoid compounds*. The Macmillan CO., New York.

Gupta, S., Patel, D., & Thompson, M. A. (2020). Inhibition of *Fusarium solani* by *Salvia officinalis* Extracts. *Plant Health Progress*, *21*(1), 56-64.

Harborne, A. J. (1998). *Phytochemical methods a guide to modern techniques of plant analysis*. springer science & business media.

Haslam, E. (1998). *Practical polyphenolics: from structure to molecular recognition and physiological action*. Cambridge University Press.

Hernandez-Perez, M., Lopez-Garcia, R. E., Rabanal, R. M., Darias, V., & Arias, A. (1994). Antimicrobial activity of *Visnea mocanera* leaf extracts. *Journal of ethnopharmacology*, *41*(1-2), 115-119.

Horiuchi, K., Shiota, S., Hatano, T., Yoshida, T., Kuroda, T., & Tsuchiya, T. (2007). Antimicrobial activity of oleanolic acid from *Salvia officinalis* and related compounds on vancomycin-resistant enterococci (VRE). *Biological and Pharmaceutical Bulletin*, *30*(6), 1147-1149.

Jafri, S.M.H., & El-Gadi, A., (1986). Flora of Libya. Vols. 25-144. Department of Botany, Al-Faateh University, Tripoli, Libya.

Johnson, M. L., Martinez, R. E., & Williams, S. P. (2019). Antifungal Activities of Plant Extracts against *Aspergillus niger*. *Plant Science Today*, *6*(2), 123-130.

Keukens, E., De Vrije, T., van den Boom, C., De Waard, P., Plasman, H., Thiel, F., Chupin, V., Jongen, W. & De Kruijff, B. (1995). Molecular basis of glycoalkaloid induced membrane disruption. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, *1240*(2), 216-228.

Khan M. R., & Khan S. M., (2002). Effects of root-dip treatment with certain phosphate solubilizing microorganisms on the fusarial wilt of tomato. *Bioresource Technol*, *85*(2), 213-215.

Kumar, V., Mathela, C. S., Tewari, A. K., & Bisht, K. S. (2014). In vitro inhibition activity of essential oils from some Lamiaceae species against phytopathogenic fungi. P*esticide biochemistry and physiology*, *114*, 67-71.

Lanciotti, R., Gianotti, A., Patrignani, F., Belletti, N., Guerzoni, M.E. & Gardini, F. (2004). Use of natural aroma compounds to improve shelf-

life and safety of minimally processed fruits. *Trends in Food Science & Technology*, *15*(3-4), 201–208.

Leslie, J. F., & Summerell, B. A. (2006). *The Fusarium laboratory manual*. Blackwell Publishing Ltd.

Lima, C.F., Carvalho, F., Fernandes, E., Bastos, M.L., Santos-Gomes, P.C., Fernandes-Ferreira, M., & Pereira-Wilson, C. (2004). Evaluation of toxic/protective effects of the essential oil of *Salvia officinalis* on freshly isolated rat hepatocytes. *Toxicology in vitro*, *18*(4), 457– 465.

Martinez, E. F., Gupta, R., & Davis, L. W. (2018). Impact of Plant Extracts on *Alternaria alternata* Growth. *Mycology Research Journal*, *42*(4), 320-328.

Miura, K., Kikuzaki, H., & Nakatani, N. (2001). Apianane terpenoids from *Salvia officinalis*. *Phytochemistry*, *58*(8), 1171-1175.

Nikolova, M., Janicsak, G., Genova, E., & Mathe, I. (2006). Comparative analysis of external flavonoids of Bulgarian and Hungarian samples of *Salvia* species. *Acta Botanica Hungarica*, *48*(3-4), 361-367.

Omran, A. M. (2018). Antimicrobial and phytochemical study of *Matricaria chamomilla* L., *Menthalongi folia* L. and *Salvia officinalis* L. *Plant Archives*, *18*(1), 387-397.

Pieta, D., & Pastucha, A. (2004). Biological methods of protecting common bean (*Phaseolus vulgaris* L.). *Folia Universitaris Agriculturae Stetinensis Agricultura*, *95*, 301-305.

Radulescu, V., Chiliment, S., Oprea, E. (2004). Capillary gas chromatography–mass spectrometry of volatile and semi-volatile compounds of *Salvia officinalis*. *Journal of chromatography A*, *1027*(1-2), 121– 126.

Rios, J. L., & Recio, M. C. (2005). Medicinal plants and antimicrobial activity. *Journal of ethnopharmacology*, *100*(1-2), 80-84.

Santos-Gomes, P.C., & Fernandes-Ferreira, M. (2001). Organ-and season-dependent variation in the essential oil composition of *Salvia officinalis* L. cultivated at two different sites. *Journal of agricultural and food chemistry*, *49*(6), 2908-2916.

Shafique, S., Javaid, A., Bajwa, R., & Shafique, S. (2007). Effect of aqueous leaf extracts of allelopathic trees on germination and seed-borne mycoflora of wheat. *Pakistan Journal of Botany*, *39*(7), 2619-2624.

Sitara, U., Niaz, I., Naseem, J., & Sultana, N. (2008). Antifungal effect of essential oils on in vitro growth of pathogenic fungi. *Pakistan Journal of Botany*, *40*(1), 409.

Skendi, A., Katsantonis, D. N., Chatzopoulou, P., Irakli, M., & Papageorgiou, M. (2020). Antifungal activity of aromatic plants of the Lamiaceae family in bread. *Foods*, *9*(11), 1642.

Smith, J. K., Johnson, A. B., & Brown, C. D. (2017). Inhibitory Effects of Plant Extracts on *Penicillium chrysogenum* Growth. *Journal of Agricultural Science*, *25*(3), 145-152.

Srimal, R. C. (1997). Turmeric: a brief review of medicinal properties. *Fitoterapia (Milano)*, *68*(6), 483-493.

Srinivasan, R., & Shanmugam, V. (2004). Post-harvest management of onion bulb rot by botanicals and antagonists. *Annals of Plant Protection Sciences*, *12*(2), 310-313.

Tan, L. F., Yap, V. L., Rajagopal, M., Wiart, C., Selvaraja, M., Leong, M. Y., & Tan, P. L. (2022). Plant as an Alternative Source of Antifungals against *Aspergillus* Infections: A Review. *Plants*, *11*(22), 3009.

Trease, G.E., & Evans, W.C. (1989). *Pharmacognosy*, 11th Ed., Bailliere Tindall, London, pp. 45-50.

Velickovic, D. T., Ristic, M. S., Randjelovic, N. V., & Smelcerovic, A. A. (2002). Chemical composition and antimicrobial characteristic of the essential oils obtained from the flower, leaf and stem of *Salvia officinalis* L. originating from Southeast Serbia. *Journal of Essential Oil Research*, *14*(6), 453-458.

Watanabe, T. (2010). *Pictorial Atlas of Soil and Seed Fungi* .3rd Ed. Taylor and Francis Group LLC.

AlQalam Journal of Medical and Applied Sciences
Special Issue for 6th International Conference in Basic Sciences and Their Applications (6th ICBSTA, 2023), *https://journal.utripoli.edu.ly/index.php/Alqalam* ***eISSN 2707-7179***

Investigate the Prevalence of Pathogenic Bacteria at Random Selected surfaces and preventive practices among working staff at the Neonate Care Unit in Benghazi Medical Center

Salah A. Hamad Gadalla[1]*, Mohamed A. Alhammad[2] and Mailud El-amari[3]
[1]Department of Environmental Health, Faculty of Public Health, University of Benghazi, Benghazi, Libya
[2]Medical Laboratory Technology, Faculty of Public Health, University of Benghazi, Benghazi, Libya
[3]Department of Health Education, Faculty of Public Health, University of Benghazi, Benghazi, Libya
* **Corresponding author**: *Salah.gadalla@uob.edu.ly*

Abstract:

Neonates admitted to hospitals in a neonatal care unit (NCU) have host determinants that not only make them more susceptible to the acquisition of nosocomial infections but also increases their risk of developing more serious diseases. This study aims to investigate some items at NCU which may act as reservoirs of pathogens of nosocomial infections and investigate if the workers are aware of the importance of preventive measures practices. A mixed-method descriptive cross-sectional study, combining structured self-administered questions, with bacteriological sampling of selected environmental surfaces. This study was conducted from Jan 18 to Aug. 18, 2022, in the Neonate Unit of the Benghazi Medical Center located in Benghazi City, Libya. Out of 31 samples, bacterial growth was found in 4 samples representing 13% of the samples; their contamination rate was 13%**.** A total of 21 workers responded to questions measuring working experience, specialty, practices, and measures of infection prevention. The results revealed that alcohol is the main disinfectant applied in NCU with a percentage of 61.5% of the responses 67% of the workers had not attended any training before.

Keywords: Nosocomial Infection, Neonatal Care Unit**,** preventive measures, awareness.

Introduction:

Pollution in health care institutes environment causes bacterial colonization and nosocomial infection as well (Lemiech-Mirowska, *et al.,* 2021). Nosocomial infections; they are infections acquired in health care units while

receiving care and treatment of other health problems. The health care institutes acquired infections are common among patients of all ages and are estimated to lead 2 million infections, 90,000 deaths, and cost 28$ to 45$ billion for additional care of these infections annually (Polin, *et al.,* 2012). Neonates admitted to hospitals in neonatal intensive care unit have host determinants which not only make the more susceptible to acquisition of nosocomial infections but also increases their risk of developing more serious diseases. Either the newborn is born preterm or at term, many factors of their innate and immune defense mechanism expose reduced function in comparison with children and adults (Polin, *et al.,* 2012). Nosocomial infection are considered hazardous factors of death for neonates because of immature immunity and vulnerability to disease. Therefore, the surfaces in the health care setting and cleaning these surfaces has significant role in interrupting outbreaks of specific organisms in NICUs and in eliminating the environmental reservoirs of potential pathogenic microorganisms (Bokulich, *et al.,* 2013). In particular, infections associated with newborn care make public health issue which causes excessive mortality and morbidity rates among neonates (Chiguer, *et al.,* 2019). Intensive care units (ICU), are considered main elemental service in health care setting for care of life threatening circumstances. Underweight newborns who are admitted in neonatal intensive care unit (NICU) are critically low immune-compromised and vulnerable to hospital acquired infections (HAIs) (Bhatta, *et al.,* 2021). Neonates are admitting to the NCU, known as highly vulnerable to health care acquired infections; because, they have impaired defense system, prolonged hospital stay, long lasting of application of medical care equipments, and associated medical health problems (Brodie, *et al.,* 2000). New born often undergo invasive measures and are lay on central catheters to feed and ventilators to breath (Ramasethu, 2017). Hospital acquired infections among neonates are related to prolonged hospital stay, neurodevelopmental problems and high death rates. During the previous decade many protective strategies have led to significant reductions in HCAIs (Jansen, *et al.,* 2021). Neonates as they present health care environment in which antibiotics are applied invasively and frequently; for this reason, pathogenic micro-organisms to cause nosocomial infections among admitted neonates (Lam, *et al.,* 2004). However, uninfected neonates compared to those developed one or more nosocomial infections during hospital stay are significantly high to die (Stoll,

et al., 2004). Pathogenic micro-organisms which are responsible for HIs are most commonly transmitted health care staffs including nurses, physicians, physiotherapists by their hands (Lam, *et al.,* 2004). Nosocomial present many environments for contamination by bacteria. In the health care environments, intensive care units, use exceptional medical devices, and have patients with infectious diseases, more antibiotics use, and patients with minimized immunity. However, neonatal intensive care units exhibit further worries (Auriti, *et al.,* 2003). Inappropriate utilizing and giving antibiotic drugs can result in resistance and outbreak of infections (Abolhasan, *et al.,* 2020). This study was aimed to investigate some items at NCU which may act as reservoirs of pathogens of nosocomial infections and investigate if the workers are aware of importance of preventive measures practices.

Materials and Methods:

Study Area and Design

A mixed-method descriptive cross-sectional study, combining structured self-administered questionnaire, with bacteriological sampling of selected environmental surfaces. This study was conducted from Jan 18 to Aug. 18, 2022 in Neonate Unit of the Benghazi Medical Center which is located at Benghazi city, Libya; the largest referral medical institute in eastern part of the nation; that providing tertiary services and training for medical staff and students as well.

Participants

A total of 21 medical staff have been interviewed and responded to questions that evaluated demographic data, educational level, working experience, specialty, and practices and measures of infections prevention and control, training, number of staffs every shift, times of disinfections and routine cleaning, mattresses, blankets, and incubators' occupancy in the Neonate Care Unit (NCU). Despite total number of staff. All medical workers in both day and night shifts during the study were involved and recruit. The responses were analyzed by using Micro-Soft Excel.

Environment reservoirs evaluation

A total of 31 random samples were collected from different surfaces, frequently touched by caregivers, including cots, door handles, charts,

incubators' surfaces, the floor of the unit, air ducts, feeding tubes, basins, desks, note sheet, sterilizing handle, cabinet drawers, suction cups and tubes, and faucets at NCU in Benghazi Medical Center. Sterile swabs were used; one for each item by rolling the swabs on the surfaces. A part of times of routine cleaning and disinfection, samples had been collected during day and night shifts at different time points. The swabs were immediately cultivated on already prepared standardized media. All plates were incubated at 37°C for 48 hours and then were bacteriologically investigated to identify the contamination rate of selected surfaces.

Results:

Questions:

As concluded in Table 1, the study focus mainly on preventive measures and the effectiveness of disinfection. All participants were included; who were working during the study in NCU at both day and night shifts. Researchers interviewed participants and submitted questionnaire. Later on the responses to questions had been collected and analyzed. The analysis revealed that all the subjects are female; working in NCU. More than half the subject age from 30 to 39 years. Majority of the subjects are high diploma holders. Specialty of the selected participants is nursing; made 52% of study population. Only 33% of them attended at least one training program in infection protection and control (IPC). According to responses of participants; number of working staff in each shift mainly ranged from 11-20 members. The results revealed that from 1-10 neonates are incubated on daily basis; made 61.9% of the responses. 85.7% of the subjects are working more than 8 hours. Disinfectants were mainly applied twice every day with percentage 95%. Alcohol is the most common disinfectant applied in NCU as presented in Figure 1 with percentage 61.5% of the responses . All the working staff in the study responded positively regarding wearing personal protective equipment including gloves and mask; and apply hand washing Figure 2. And 90.5% of the responses discovered that tools used in NCU have been sending to Central Sterilization Service Center on daily basis as shown in Figure 3.

Table 1: Demographic characterisitics of selected participants and questions which determine some practices of IPC

Demographic characteristics		Frequency	Percentage (%)
Age group	20-29 yrs	9	42.9
	30-39 yrs	11	52.4
	≥ 40 yrs	1	4.7
	Total	21	100
Educational level	Mid diploma	4	19
	High diploma	15	71.5
	University	2	9.5
	Total	21	100
Nature of working	Nursing	11	52.5
	Anesthesia	1	4.5
	Intensive care	2	9.5
	Missing	7	33.5
	Total	21	100
Question			
Did you Attend any training on infection control and prevention?	Attended	7	33
	Not attended	14	67
	Total	21	100
How many staff members do work in shift?	1-10	7	33
	11-20	12	57
	Missing	2	10
	Total	21	100
How many neonates usually admitted to incubators on day basis?	Range Number of Neonates	Response of subject	Percentage
	1-10 neonates	13	61.9
	10-20 neonates	3	14.3
	> 20 neonates	3	14.3
	unknown	2	9.5
How many hours do you work in shift?	Total	**21**	**100**
	8 Hours	1	4.8
	Less than 8 hours	2	9.5
	More than 8 hours	18	85.7
	Total	21	100.0
How many times items and surfaces have been disinfecting per day ?	2 times	20	95
	1 time	0	0
	Never	1	5
	Total	21	100

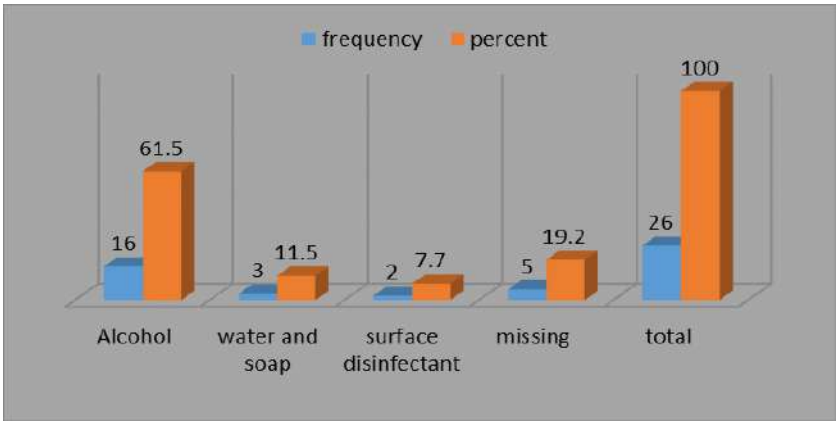


Figure 1: Disinfecting agents used in NCU according to participants

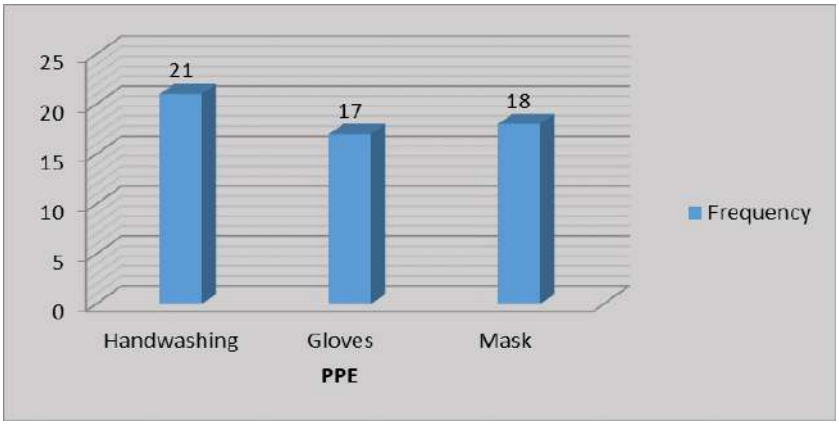


Figure 2: Personal protective equipment used by medical staff in NCU

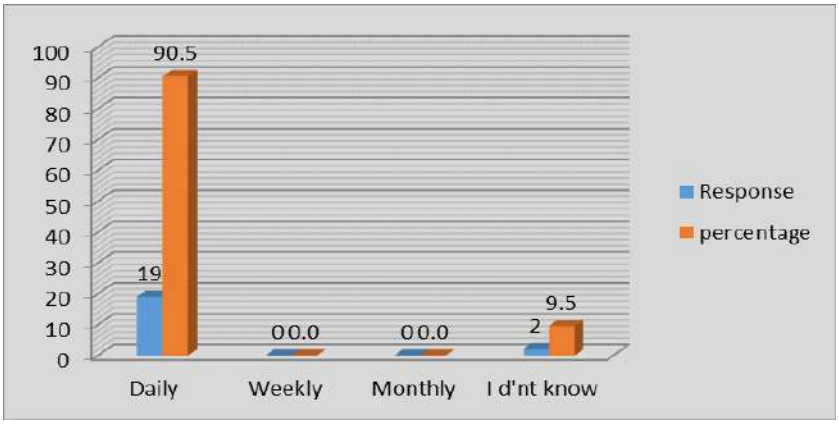


Figure 3: Times of sending tools to central sterilization service center (CSSC)

Lab analysis:

A total of 31 swaps were collected from many environmental surfaces. Out of 31 samples, bacterial growth was found in 4 samples represented 13% of the samples; contamination rate was 13%. Species which were isolated included *Acinetobacter baummanii, Bacillus cereus, Staphylococcus aureus, and Alcaligenes* Table 2; majority of them showed resistance on anti-biotic sensitivity scale as shown in

Table 3.

Table 2: Isolate bacteria

No	Detected bacteria	Resistance
	Acinetobacter baummanii	
	Bacillus cereus	
	Staphylococcus aureus	MRSA
	Alcaligenes	

Table 3: Lab analysis results

CODE	Sample Type	Bacterial Results	Sensitivity																										
			AN	CIP	OFX	CXM	CN	CT	CAZ	AMC	SXT	AMZ	AMC	SAM	DOE	OCR	IPM	AK	ETP	MEM	XNL	CTM	ATM	LEV	PY	VA	OX	NV	OP	B
2676	1	N.G																												
2677	2	NG																												
2678	3	NG																												
2679	4	NG																												
2683	5	1 Acinobacter spp	-	R	-	R	R	S	R	-	-	-	R	S	-	R	R	R	R	R	R	-	I	R	-	-	-	-	-

		B. cereus	-	S	-	R	S	-	R	-	-	-	S	S	S	R	S	R	R	S	R	-	S	-	S	R	-	-	-
2684	6	NG																							S				
2686	8	S aureus	-	R	-	R	R	S	R	-	R	R	-	S	R	R	R	R	R	-	S	-	S	R	-	-	-	-	-	-
2692	13	A calgenes	-	S	-	R	R	S	S	-	-	-	S	R	R	S	R	R	S	R	R	S	-	-	R	-	-	-	-
2685	7	NG																											
2687	9	NG																											
2688	10	NG																											
2690	11	NG																											
2691	12	NG																											
2693	14	NG																											
2695	15	NG																											
2696	16	NG																											
3961	17	NG																											
3962	18	NG																											
3979	19	NG																											
3980	20	NG																											
3982	21	NG																											

3983	22	NG																									
3983	23	NG																									
3986	24	NG																									
3987	25	NG																									
3988	26	NG																									
3989	27	NG																									
3990	28	NG																									
3991	29	NG																									
4008	30	NG																									
4010	31	NG																									

NG: no growth, R; resistant, S: sensitive; I: Intermediate; NA: Nalidixic acid; CIP: Ciprofloxacin; PFX: Ofloxacin; CXM: Cefuroxime; CN: Cefoxitin; CL: Colistin; AMC: Amoxicillin; SXT: Sulfamethoxazole; AZM: Azithromyci;, AM: Ampicillin; SAM: Ampicillin-Sulbactam; DO: Doxycyclinex; E: Erythromycin; CRO: Ceftriaxone; IPM: Imipenem; AK: Amikacin; ETP: Ertapenem; MEM: Meropenem; CTX: Cefotaxim; ATM: Aztreonam; LEV: Levofloxacin; PY: Piperacillin; VA: Vancomycin; OX: Oxacillin; NV: Novobiocin; TPN: Teicoplanin; B: Bacitracin.

Discussion:

Questions:

The results collected from the responses of subjects showed low percentage of working staff (33%) who attended training programs in infection control. Since they are from mid-aged group and had no specialty in IPC and most of them had diploma in general nursing; therefore, the training programs will be effective in control of infections. In addition, nosocomial infections were prevented by establishment of training programs. But health care institutes which do not have effective preventive programs; the rates of nosocomial infections getting higher. A study was conducted by Farotimi and associates concluded that structured training program was effective in enhancement of

nurses' knowledge and perception regarding control of infections; the study showed that a training program is very effective and all nurses should be trained and aware and skills in regarding control of infections in the hospitals (Farotimi, *et al.,* 2018). Micro-organisms which are responsible for health care acquired infections are mainly transmitted by hands of health care workers including physicians, nurses and other working persons (Lam, *et al.,* 2004). However, hand hygiene practices are considered as the most effective preventive measure of health care acquired infections (Lam, *et al.,* 2004). Yet, compliance with guidelines and standards recommendation is not easy to be achieved during deal with critical cases of neonates (Pessoa-Silva, *et al.,* 2007).

Lab analysis

One of the isolated bacteria is *Acinetobacter baumanni* , it is multidrug-resistant bacteria, which showed resistance to many antibiotics including CIP, CXM, CN, AMC, SAM, CRO, IPM, AK, ETP, MEM, CTX, and PY as presented in Table 3. This pathogenic microbe, has big concern regarding threatening to public health and one of those responsible for serious infections in health care institutes (Nguyen & Joshi, 2021); this type of bacteria can cause infections such as pneumonia , wounds infections and blood. In addition, *Acinetobacter baumanni* can form colonies in different parts of the body of the patient without any symptom of infection (CDC, 2021). As shown in Tables1, 2; the results reveal colonization by *staphycoccus aureus* which is MRSA. Many studies of staphylococcal infections in ICUs confirm necessity for care of both patients and financial cost. Nosocomial infections by *S. aureus* in ICUs may be causing agent for as many as 200,000 cases per year in the United States (Gidengil, *et al.,* 2015). Our findings are demonstrate staphylococcal contamination which means direct causing neonatal care related infections in Benghazi Medical Center; similar finding demonstrate by (Keilman, *et al.,* 2021). In addition, we have demonstrated the presence of viable *Bacillus cereus* in our sample as presented in table 3. Which exhibit resistance to many antibiosis (Table 3). *Bacillus cereus* is a foodborne pathogenic microorganism by own toxin causing gastrointestinal problems including vomiting and diarrhea; these illnesses occur following ingestion contaminated food with toxin of the bacteria (cereulide) (CDC, 2021). *Alcaligenes spp.* were identified in our sample as shown in table1; on

sensitivity test exhibit resistance to many antibiotics. Sub groups of alcaligenes such as *alcaligenes faecalis* is associated with health care associated infections among hospitals admitted patients (Hasan, *et al.*, 2019).

Conclusion:

We conclude from our study that practices of IPC are moderately satisfied concerning to disinfection, personal protective devices, and sending to central sterilization service center and the contamination rate of selected surfaces was low. but we recommend to:

- To establish national surveillance system for health care acquired infections.
- To establish infection control teams at secondary and tertiary institutes.
- To make environmental monitoring to evaluate the effectiveness of preventive and control measures at Neonatal Care Units.
- To establish educational and training programs for working staffs at NCU on regular basis in control health care acquired infections.

References:

Abolhasan Choobdar, F., Khosravi Rostami, N., Khalesi, N., Haghighi Aski, B., Behdadmehr, S., Safari, A., & Kalantar, S. (2020). Determination of Antibiotic Resistance Pattern of Bacteria Isolated from Blood, Cerebrospinal Fluid, and Urine Samples in Neonatal Intensive Care Unit of Ali Asghar Hospital, Iran during 2013-15. *Iranian Journal of Neonatology IJN*, *11*(3), 38-43.

Auriti, C., Maccallini, A., Di Liso, G., Di Ciommo, V., Ronchetti, M. P., & Orzalesi, M. (2003). Risk factors for nosocomial infections in a neonatal intensive-care unit. *Journal of Hospital Infection*, *53*(1), 25-30.

Bhatta, D. R., Hosuru Subramanya, S., Hamal, D., Shrestha, R., Gauchan, E., Basnet, S., & Gokhale, S. (2021). Bacterial contamination of neonatal intensive care units: How safe are the neonates?. *Antimicrobial Resistance & Infection Control*, *10*, 1-6.

Bokulich, N. A., Mills, D. A., & Underwood, M. A. (2013). Surface microbes in the neonatal intensive care unit: changes with routine cleaning and over time. *Journal of clinical microbiology*, *51*(8), 2617-2624.

Brodie, S. B., Sands, K. E., Gray, J. E., Parker, R. A., Goldmann, D. A., Davis, R. B., & Richardson, D. K. (2000). Occurrence of nosocomial bloodstream infections in six neonatal intensive care units. *The Pediatric infectious disease journal*, *19*(1), 56-65.

Chiguer, M., Maleb, A., Amrani, R., Abda, N., & Alami, Z. (2019). Assessment of surface cleaning and disinfection in neonatal intensive care unit. *Heliyon*, *5*(12). 1-5.

Farotimi, A. A., Ajao, E. O., Nwozichi, C. U., & Ademuyiwa, I. Y. (2018). Effect of training on knowledge, perception and risk reduction regarding infection control among nurses in selected teaching hospitals in Nigeria. *Iranian Journal of Nursing and Midwifery Research*, *23*(6), 471.

Gidengil, C. A., Gay, C., Huang, S. S., Platt, R., Yokoe, D., & Lee, G. M. (2015). Cost-effectiveness of strategies to prevent methicillin-resistant Staphylococcus aureus transmission and infection in an intensive care unit. *infection control & hospital epidemiology*, *36*(1), 17-27.

Hasan, M. J., Nizhu, L. N., & Rabbani, R. (2019). Bloodstream infection with pandrug-resistant Alcaligenes faecalis treated with double-dose of tigecycline. *IDCases*, *18*, e00600.

Jansen, S. J., Lopriore, E., van Der Beek, M. T., Veldkamp, K. E., Steggerda, S. J., & Bekker, V. (2021). The road to zero nosocomial infections in neonates—a narrative review. *Acta Paediatrica*, *110*(8), 2326-2335.

Keilman, R., Harding, S., Rowin, M., Reade, E., Klingborg, P., Levine, D., & Spratt Jr, H. (2021). Investigations of Staphylococcal contamination on environmental surfaces of a neonatal intensive care unit of a children's hospital. *American Journal of Infection Control*, *49*(11), 1450-1453.

Lam, B. C., Lee, J., & Lau, Y. L. (2004). Hand hygiene practices in a neonatal intensive care unit: a multimodal intervention and impact on nosocomial infection. *Pediatrics*, *114*(5), e565-e571.

Lemiech-Mirowska, E., Kiersnowska, Z. M., Michałkiewicz, M., Depta, A., & Marczak, M. (2021). Nosocomial infections as one of the most important problems of healthcare system. *Annals of Agricultural and Environmental medicine*, 28(3) , 1-10.

National Healthcare Safety Network. CDC/NHSN surveillance definitions for specific types of infections. Centers for Disease Control and Prevention

website. https://www.cdc.gov/nhsn/pdfs/pscmanual/17pscnosinfdef_current.pdf. Published 2021. Assessed November 30, 20 21.

Nguyen, M., & Joshi, S. G. (2021). Carbapenem resistance in Acinetobacter baumannii, and their importance in hospital-acquired infections: a scientific review. *Journal of applied microbiology*, *131*(6), 2715-2738.

Pessoa-Silva, C. L., Hugonnet, S., Pfister, R., Touveneau, S., Dharan, S., Posfay-Barbe, K., & Pittet, D. (2007). Reduction of health care–associated infection risk in neonates by successful hand hygiene promotion. *Pediatrics*, *120*(2), e382-e390.

Polin, R. A., Denson, S., Brady, M. T., Committee on Fetus and Newborn, Committee on Infectious Diseases, Papile, L. A., ... & Zaoutis, T. E. (2012). Epidemiology and diagnosis of health care–associated infections in the NICU. Pediatrics, 129(4), e1104-e1109.

Ramasethu, J. (2017). Prevention and treatment of neonatal nosocomial infections. *Maternal health, neonatology and perinatology*, *3*, 1-11.

Stoll, B. J., Hansen, N. I., Adams-Chapman, I., Fanaroff, A. A., Hintz, S. R., Vohr, B & National Institute of Child Health and Human Development Neonatal Research Network. (2004). Neurodevelopmental and growth impairment among extremely low-birth-weight infants with neonatal infection. *Jama*, *292*(19), 2357-2365.

AlQalam Journal of Medical and Applied Sciences
Special Issue for 6th International Conference in Basic Sciences and Their Applications (6th ICBSTA, 2023), ***https://journal.utripoli.edu.ly/index.php/Alqalam*** ***eISSN 2707-7179***

Checklist of Medicinal Plants of Ibrak Nouta, Soussa, Al-Jabal Al-Akhdar, Libya

Adam S. Adam, Rania F. M. Ali, Hana Y. O. Shoaib and Sabah H. Lamlom
Department of Botany, Faculty of Science, University of Omar AlMukhtar, Albaida, Libya.
Correspondence authors: adam.shouab@omu.edu.ly

Abstract:

Al-Jabal Al-Akhdar area in Libya has high plant diversity. The aim of this study was to produce an inventory of all medicinal plants of Ibrak Nouta, Soussa – Al-Jabal Al-Akhdar, Libya. This study was conducted for four seasons during the years 2021/2022, Medicinal plants in the study area were identified and classified .The results indicated the presence of a total of 102 medicinal plants. These taxa are belonging to 43 families. Two families of Gymnosperms are represented by 3 species, 3 genera and the remaining 41 families are belonging to Angiosperms. Dicotyledons are represented by 81 species and 66 genera, and 32 families Monocotyledons are represented by 18 species, 17 genera, and 9 families. Two families were Parasite represented by 7 species 2 on the stems *Cuscuta epithymum subsp. Epithymum*, *Cuscuta planiflora Ten*, and the remaining 5 on the roots *Orobanche amethystea* Thuill. *Orobanche amethystea* Thuill. *subsp. amethystea Orobanche cyrenaica* Beck ex E.A.Durand & Barratte. *Orobanche pubescens* d'Urv. *Phelipanche mutelii* (F.W.Schultz) Pomel. Furthermore, the findings revealed that most represented life-forms of the medicinal plants in Ibrak Nouta were Therophytes (Th ,36%) 34 species, Chamaephytes (Ch,16%), 17 species, 15 species, followed by Geophytes (G ,15%) 15 species, Nano-Phanerophytes (N.Ph ,12%) 12 species, Hemicryptophytes (H,7%) 7 species, Parasite (Pa ,7%) 7 species, Phanerophytes (Ph ,5%) 5 species, and Lianas (Li ,2%) represented by 2 species. Also on the list is an endemic medicinal plant represented by the species *Onopordum cyrenaicum*, *Teucrium* apollinis.

Keywords: Ibrak Nouta, Al-Jabal Al-Akhdar, Medicinal plants, Soussa

Introduction:

There are over 1,825 vascular plant species in Libya; some of them are endemic, making it one of the countries that has a wide variety of medicinal plant species. According to literature review, about 450 species have

medicinal value (Auzi, 1999). Apiaceae, Asteraceae, Lamiaceae, Poaceae, Fabaceae, and Brassicaceae families are the most significant. All around the country, there are medicinal plants, but they are more distributed in the regions; Al-Jabal Al-Akhdar, Ghadames, Gharian, Awbari, and Tarhona (Guenther, 1972; Rateeb *et al* 1986). More than 100 species are extensively used by Bedouins and local people in folk medicine drinks, or chewed fresh or dry. They are used to cure dermal diseases, viral or bacterial infections, insect bites, burns and sometimes to treat hair problems. Medicinal plants are the foundation of herbal medicine, aromatherapy, homoeopathy and ayurvedic medicine. Herbal medicine is defined as the use of plant material or plant extract to cure illness, relieve pain or boost overall health (White & Foster, 2000). Also they are the basic constituents of some conventional pharmaceuticals, certain chemical constituents. Ibrak Nouta, Soussa consists of two lakes. It is located about 20 km west of the city of Soussa. Ibrak Nouta is another face of the beauty of nature in Libya. One of these lake is considered the largest, with a diameter of about 350 meters, and a depth of 60 meters, its shape appears as a funnel that narrows as we go down to the bottom. It is noteworthy that the distance between the Ibrak Nouta lakes and the see does not exceed 300 meters, as they are separated by the western coast road between Soussa in the east and El Haniyeh in the west. In the lakes, there are rare types of diverse marine and wild life, such as sea snakes and small fish that feed on water weeds. The water of the lake mix between fresh and salty, and many types of wild birds live within its rocky gaps, and migratory wild geese take it as a station. Floristic investigations provide reliable information about the nomenclature, distribution, ecology and utility of various medicinal plant species. Besides, the study may provide valuable information for sustainable management and conservation of plant resources. The study area is located on the western coast of Soussa. It lies around the cross point of Latitude 32° 54. 555 ′ N, and Longitude 21° 48. 842′ E. on the North East region, Al-Jabal Al-Akhdar.

Materials and Methods:

The study area is located on the western coast of Soussa. It lies between Latitude 32° 54. 555 ′ N, and Longitude 021° 48. 842′, E on the North East region, Al-Jabal Al- Akhdar**.**

Figure 1: Illustrated the study area is located on the western coast of Soussa

Specimen Collection and Identification

Frequent weekly visits to the study area conducted during the four seasons (autumn, winter, spring and summer) of the years (2021-2022).Plant samples photographed with a camera before they were collected. Plants were collected during the flowering and fruiting period, and annual plants were collected by taking an entire plant, and for trees and shrubs by taking a branch about 25cm with leaves, fruits or flowers. Samples dried for two weeks by the press and papers to dry, taking into account the change of papers daily, the plant specimens kept in herbarium sheet by glue. Plant samples dissected in the Sylphium herbarium with the help of the herbarium team, using dissection tools and placed in a dissection microscope and identified using Flora Libya books. The plant samples kept in the Sylphium herbarium.

Results:

Floristic and Ecological study of Ibrak Nouta, Soussa was carried out during the four seasons from 2021 to 2022. 102 species were medicinal. The results revealed that, these taxa are belonging to 43 families. Two families of Gymnosperms are represented by 3 species, 3 genera and the remaining 41 families are belonging to Angiosperms. Dicotyledons are represented by 81 species and 66 genera, and 32 families Monocotyledons are represented by 18 species, 17 genera, and 9 families. Two families were Parasite represented by 7 species 2 on the stem *Cuscuta epithymum subsp. Epithymum, Cuscuta planiflora Ten*, and the remaining 5 on the roots *Orobanche amethystea*

Thuill. *Orobanche amethystea* Thuill. *subsp. amethystea Orobanche cyrenaica* Beck ex E. A. Durand & Barratte. *Orobanche pubescens* d'Urv. *Phelipanche mutelii* (F. W. Schultz) Pomel. Table (1) shows the medicinal plants in the study area. Table (2) showed that most represented life-forms of the medicinal plants in Ibrak Nouta were Therophytes (Th, 36%) 34 species, Chamaephytes (Ch, 16%), 17 species, 15 species, followed by Geophytes (G, 15%), Nano-Phanerophytes (N.Ph, 12%) 12 species, Hemicryptophytes (H, 7%) 7 species, Parasite (Pa, 7%) 7 species, Phanerophytes (Ph, 5%) 5 species, as for Lianas (Li, 2%) represented by 2 species. Also on the list is an endemic medicinal plant represented by the species *Onopordum cyrenaicum*, *Teucrium apollinis*, major life form was Therophytes while leaf spectrum shows highest percentage of Nanophylls.

Table 1: Scientific name, Vernacular name, Life form, Family, Used part for medicinal plant in Ibrak Nouta, Soussa

No.	*Scientific name*	Vernacular name	Life form	Family	Used part
1	*Mesembryanthemum nodiflorum* L.	Ghassoul Kebir	Th	Aizoaceae	**Leaves**
2	*Beta vulgaris subsp.maritima* (L.) Arcang.	Selqe	H	Amaranthaceae	**Leaves**
3	*Allium roseum* L.	Ghazul	G	Amaryllidaceae	**Leaves, Flower and Bulbs.**
4	*Narcissus tazetta* L.	Nargis	G		**Rhizomes**
5	*Pistacia lentiscus* L.	Battoom	Ph	Anacardiaceae	**Leaves, seeds, fruits and the resinous juice known as mastic obtained from the inner bark.**
6	*Searsia tripartite (Ucria.) Moffett Rhus tripartite* (Ucria) Grande	Ijdari	N.Ph		**Bark**
7	*Eryngium compestre* L.	Shook El-Jamel	Ch	Apiaceae	**Roots, Leaves**
8	*Thapsia garganica* L.	Deryas	Ch		**Roots, Gum Oil**
9	*Arisarum vulgare* O.Targ.Tozz.	Weden Essalogi	G	Araceae	**Rhizomes.**
10	*Arum cyrenaicum* Hruby	Renish	G		**Rhizomes**
11	*Nerium oleander* L.	Defla	N.Ph	Apocynaceae	**The glycosides obtained from the whole plant.**
12	*Periploca angustifolia* Labill	Hallab	N.Ph	Apocynaceae	**Leaves, Roots, resin**
13	*Asparagus aphyllus* L.	Sakkoom	G	Asparagaceae	**Roots, Young shoots**
14	*Asparagus horridus* L	Sakoom	G		**Roots, Young shoots**
15	*Anthemis pseudocotula* Boiss	--	Th	Asteraceae	**The aerial parts**
16	*Carthamus lanatus* L.	Goos	Ch		**Entire plant**
17	*Centaurea alexandrina* Delile	Mrrier	Th		**Entire plant**
18	*Glebionis coronaria* (L.) Cass.ex Spach	Gahwan	Th		**Leaves, flowers, seeds.**
19	*Cichorium pumilum* Jacq.	Moagdah, Shecoria	Th		**Leaves**
20	*Cichorium spinosum* L.	Shecoria	Ch		**Leaves**
21	*Cynara cardunculus* L.	Zarzor, shouk el bill	Ch		**Leaves, Stems, Fruits**
22	*Helichrysum stoechas* (L)Moench	Emsyka, Eshbet Iarnib	Ch		**The whole herb**
23	*Phagnalon rupestre* (L)DC.	Eshbet Iarnib	Ch		**The whole herb**
24	*Onopordum cyrenaicum* Maire & Weiller	Elberof	Ch		**Entire plant**
25	*Silybum marianum* (L.)Gaertn	Ergytat hanesh, Shobrum	Th		**The whole herb**
26	*Sonchus oleraceus* L.	Tefaf	Th		**Entire plant**
27	*Matricaria aurea* (Loefl.)Sch. Bip.	Egmyla	Th		**Florescence**
28	*Matricaria chamomilla* L.	--	Th		**Florescence**
29	*Echium angustifolium* Mill	Henna alagrab	Ch	Boraginaceae	**Aerial parts**
30	*Capsella bursa-pastoris* (L.) Medik	--	Th	Brassicaceae	**Entire plant**

31	*Diplotaxis harra* (Forsk)Boiss	Harra	H		**Leaves**
32	*Diplotaxis muralis* (L.)DC.	--	Th		**Leaves**
33	*Campanula erinus* L.	--	Th	Campanulaceae	**Leaves and Roots.**
34	*Capparis spinosa* L.	Kabbar	H	Capparaceae	**Over – ground parts**
35	*Paronychia arabica* (L) Dc	Tifun, Gefet El-abed	Th	Caryophyllaceae	**All part of plant**
36	*Gypsophila vaccaria* (L.)Sm.	Ful-el Arab	Th	Caryophyllaceae	**Roots**
37	*Silene gallica* L.	--	Th	Caryophyllaceae	
38	*Colchicum palaestinum* (Baker) C.Archer	Garshod	G	Colchicaceae	**Corms**
39	*Convolvulus althaeoides* L.	Ullag	H	Convolvulaceae	**Leaves, Flowers**
40	*Cuscuta epithymum* (L.) L.	Harir Ezzaater	Pa		**Entire dried plant**
41	*Cuscuta planiflora* Ten	Harir Ezzaater	Pa		**Entire dried plant**
42	*Cupressus sempervirens* L.	Srow	Ph	Cupressaceae	**Cones, wood and Fruits.**
43	*Juniperus Phoenicea ssp. turbinata* (Guss.)Nyman	Arar	Ph	Cupressaceae	**Leaves, Fruits**
44	*Ephedra alata* Decne.	Alanda	Ch	Ephedraceae	**The entire herb.**
45	*Euphorbia helioscopia* L.	Lebbana	Th	Euphorbiaceae	**Aerial parts**
46	*Euphorbia dendroides* L.	Taghma	N.Ph	Euphorbiaceae	**Aerial parts**
47	*Euphorbia peplus* L.	Lebbana	Th	Euphorbiaceae	**Latex**
48	*Mercurialis annua* L.	Halboob	Th	Euphorbiaceae	**Herb**
49	*Calicotome villosa* (Poir.)Link	Gandol	N.Ph	Fabaceae	**Leaves, Fruits**
50	*Ceratonia siliqua* L.	Kharob	Ph		**Fruits known as pods and gum obtained from the endosperm of the seed.**
51	*Lathyrus aphaca* L.	--	Th	Fabaceae	**Ripe seeds.**
52	*Lotus corniculatus* L	Goret	H	Fabaceae	**Entire plant**
53	*Vicia sativa* L.	Jilban	Th	Fabaceae	**Entire plant**
54	*Erodium cicutarium* (L.)L'Her	Dahmiyet El-Ghazl	Th	Geraniaceae	**Roots, Leaves, Seeds**
55	*Erodium moschatum* (L.)L'Her	Mseka	Th	Geraniaceae	**Entire plant**
56	*Moraea sisyrinchium* (L)Ker_ Gawler	Kheta, Sawsan	G	Iridaceae	**Rhizomes**
57	*Juncus acutus* L.	Dees	G	Juncaceae	**Roots, Fruits, Flowers**
58	*Marrubium vulgare* L.	Robia	G	Lamiaceae	**Entire vegetative plant without roots**
59	*Micromeria nervosa* (Desf.) Benth.	Eshbet Ishahy	Ch	Lamiaceae	**Flowering plant.**
60	*Phlomis floccosa* D. Don	Zahira	N.Ph	Lamiaceae	**Flowering plant.**
61	*Pseudodictamnus mediterraneus* Salmaki &Siadati	Inmyla	Ch	Lamiaceae	**Shoot**
62	*Teucrium apollinis* Maire Schreber	Jaada	H	Lamiaceae	**Leaves**
63	*Malva aegyptia* L.	Khobaiz	Th	Malvaceae	**Leaves, Stem, Flowers**
64	*Malva sylvestris* L.	Khobaiz	Ch	Malvaceae	**Entire plant Leaves, Stem, Flowers**
65	*Neurada procumbens* L.	Saadan	Th	Neuradaceae	**Leaves, Fruits**
66	*Olea europaea ssp., cuspidate* (Wall. & G.Don)Cif.	Zayton bare	Ph	Oleaceae	**Fruits, Oil, Leaves Bark**
67	*Orobanche amethystea* Thuill.	--	Pa	Orobanchaceae	**Young shoots, Seeds, Flowers**
68	*Orobanche amethystea* Thuill. *subsp. amethystea*	--	Pa	Orobanchaceae	**Young shoots, Seeds, Flowers**
69	*Orobanche cyrenaica* Beck ex E.A.Durand & Barratte	--	Pa	Orobanchaceae	**Young shoots, Seeds, Flowers**
70	*Orobanche pubescens* d'Urv.	Enum	Pa	Orobanchaceae	**Young shoots, Seeds, Flowers**
71	*Phelipanche mutelii* (F.W.Schultz) Pomel.	--	Pa	Orobanchaceae	**Young shoots, Seeds, Flowers**
72	*Papaver dubium* L.	Eslya, Bugraum, Talma	Th	Papaveraceae	**Fruits, Leaves, Seeds, Petals**
73	*Papaver rhoeas* L.	Eslya, Garun Bugraum	Th	Papaveraceae	**Fruits, Leaves, Seeds, Petals**
74	*Plantago albicans* L.	Henam	H	Plantaginaceae	**Seeds, Leaves**
75	*Plantago coronopus* L .	Krah-digiagia	Th	Plantaginaceae	**Seeds, Leaves**
76	*Plantago ovata* Forsk	Halma	Th	Plantaginaceae	**Seeds, Leaves**
77	*Veronica arvensis* L.	--	Th	Plantaginaceae	**Infusions of dried flowering plant**
78	*Arundo donax* L.	Gassba	G	Poaceae	**Leaves, Rhizomes**

79	*Avena fatua* L.	Khafor	Th	Poaceae	**Seeds**
80	*Cynodon dactylon*(L.) Pers	Najem	G		**Leaves, Rhizomes**
81	*Dactylis glomerata* L.	--	G		**Entire plant**
82	*Hordeum murinum* L.	Zewan	Th		**Grain**
83	*Lolium rigidum Gaudin*	Sammah	Th		**Leaves**
84	*Stipellula capensis* (Thunb.) Roser & Hamasha	Behma	Th		**Entire plant**
85	*Emex spinosus* L.	Henzab	Th	Polygonaceae	**Entire plant**
86	*Cyclamen rohlfsiaanum* Aschers	Rakaf	G	Myrsinaceae	**Leaves, Tuberous**
87	*Lysimachia arvensis* (L.) U.Manns & Anderb.	Eshbet Iraay, Ain-Al-Gatuus	Th	Myrsinaceae	**Entire plant**
88	*Adonis aestivalis* L.	--	Th	Ranunculaceae	**Entire plant except roots**
89	*Rhamnus oleoides* L.	Sellouf	N.Ph	Rhamnaceae	**Dried Leaves, Bark**
90	*Ziziphus lotus* (L.)Lam.	Sedr	N.Ph	Rhamnaceae	**Leaves,**
91	*Sarcopoterium spinosum* (L.) Spach	Shebreg	Ch	Rosaceae	**Leaves**
92	*Rubia tinctorum* L.	Fuwah	Li	Rubiaceae	**Rhizomes, Leaves bases, Roots**
93	*Smilax aspera* L.	Rough	Li	Smilacaceae	
94	*Datura innoxia* Mill.	Fadda	Ch	Solanaceae	**Dried leaves, flowers**
95	*Lycium europaeum* L.	Ausej	N.Ph		**Leaves**
96	*Nicotiana glauca* R.C. Graham	Akkuze Musas	N.Ph		**Leaves and over ground parts.**
97	*Daphne jasminea* Sibth. et Sm.	--	N.Ph	Thymelaeaceae	**Leaves, Bark**
98	*Thymelaea hirsuta* (L.) Endl	Metnan	N.Ph	Thymelaeaceae	**Leaves, Bark**
99	*Urtica pilulifera* L.	Horeg	Th	Urticaceae	**Entire plant before flowering the whole fresh plant without roots**
100	*Zygophyllum album* L.f	Belbal	Ch	Zygophyllaceae	**Leaves**
101	*Zygophyllum creticum* (L.) Christenh.& Byng	Taleha	Ch		**Leaves**
102	*Asphodelus ramosus* L.	Onsail	G	Xanthorrhoaceae	**Tuber roots Seeds**

Table (2): Life- form spectrum of Medicinal plants in Ibrak Nouta, Soussa

Life form	No. of species
Therophytes	**37**
Chamaephytes	**17**
Geophytes	**15**
Nano-Phanerophytes	**12**
Hemicryptophytes	**7**
Parasite	**7**
Phanerophytes	**5**
Lianas	**2**

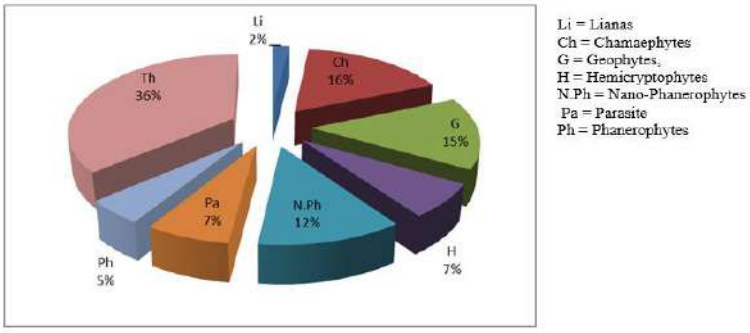


Figure (2): Medicinal plant life form percentages in Ibrak Nouta, Soussa.

Discussion:

The results from this study were consistent with several prior studies (Khan *et al.*, 2014). The analysis of the distribution of the species showed that Asteraceae, represented with 14 species, followed by the Apiaceae family, present 7 species, Fabaceae, Lamiaceae, and Orobanchaceae, 5 species, and Plantaginaceae, 4 species, the Solanaceae and Papaveraceae family, represented by 3 species for each them, and the rest of the families are present with one or two plants. Fabaceae and Boraginaceae attained the highest number of genera recorded in the study area with 3 genera and 3 species, followed by Apiaceae, Liliaceae and Primulaceae with 2 genera and 2 species. There is more than one family that was represented by one genus and one species. Similar findings were reported in prior reports investigating the vegetation of Al-Jabal Al-Akhdar's valleys; Asteraceae was the most dominant family in Wadi Al-Ager, with 46 species (Alaib *et al.*, 2017). Asteraceae was also the largest family in the Sedy Boras region, represented by 130 species (Alzerbi & Alaib, 2016). This study is consistent with earlier studies carried out in different locations of Al-Jabal Al-Akhdar area. It should be noted that Therophytes, the most dominant life-form, of the existing plants are annual plants with the capacity to adapt to the high temperatures in the summer. They represent the Mediterranean region (Archibold, 2012; El-Mokasabi, 2014).

Conclusion:

Al-Jabal Al-Akhdar is considered one of the important areas in Libya due to the abundance of vegetation in the Al-Jabal Al-Akhdar due to the availability of rain. There are many medicinal plants that the Al-Jabal Al-Akhdar is famous for, which must be preserved because many plants are exposed to extinction due to urban expansion and the random cutting of plants, as well as overgrazing. We also need for further studies in many areas of Al Jabal Al Akhdar to determine the plants in this region, which is rich in its distinctive vegetation.

References:

Alaib, M., El-Sherif, I., & Al-Hamedi, R. (2017). Floristic and ecological investigation of Wadi Al-Agar in Al-Jabal Al-Akhdar, Libya. *Libyan Journal of Science &Technology*, *5*(1), 57–61.

Alzerbi, A. & Alaib, M. (2016). Study of vegetation in Sedy Boras region in Al-Jabal Al-Akhdar, Libya. *Journal of Environmental Science and Engineering*, *1*(1), 67–72.

Archibold, O. W. (2012). *Ecology of world vegetation*. Springer Science & Business Media.

Auzi, A. (1999). Medicinal plants in Libya, Paper presented in "First Conference on Natural Resources. Sert, Libya.

El-Mokasabi, F. M. (2014).Floristic composition and traditional uses of plant species at Wadi Alkuf, Al-Jabal Al-Akhdar, Libya. *American Eurasian Journal of Agricultural and Environmental Sciences, 14*(8), 685-697.

Guenther, E. (1972). *The Essential Oils, Robert E. Krieger Publishing Company*, New York, USA.

Khan, M. A., Ullah, A., Rashid, A., Shah, S. M., & Fida, S. (2014). Floristic leaf-size and life form spectra of Asshab Baba Graveyard Chaghar Matti, district Peshawar, Khyber Pakhtoonkhwah, Pakistan. *Int. J. Biol. Biotechnol*, *11*(1), 167-171.

Rateeb, F., F. Adurahaman and A. Auzi. (1986). IUCN, program for conservation and sustainable use of medicinal plants. Libya.

White, L. & Foster, S. (2000). *The herbal drugstore*. Rodale Inc. London.

AlQalam Journal of Medical and Applied Sciences
Special Issue for 6th International Conference in Basic Sciences and Their Applications (6th ICBSTA, 2023), https://journal.utripoli.edu.ly/index.php/Alqalam eISSN 2707-7179

Removal of Toxic Lead Ions from their Aqueous Solutions Using Fava Beans Phytoadsorption Technique

Salaheddin A. Sharif[1,3*], Widad Saed El-Mugrbi[2,3], Hameda Ali Mohamed Naser El-Moghrabi[2,3], Amal Ramadan Elarfy[3] and Noura Atiah Alshahopy[3]
[1]*Mercury Research Group, Department of Chemistry, Faculty of Arts and Science, University of Benghazi, Ghemines, Libya*
[2]*Department of Botany, Faculty of Arts and Science, University of Benghazi, Ghemines, Libya*
[3]*Department of Biology, Faculty of Education, University of Benghazi, Ghemines, Libya*
***Corresponding author:** s.sharif@uob.edu.ly*

Abstract:

Toxic heavy metal contamination of soil and water ecosystems is a significant concern worldwide and dangerous issues to be urgently solved. This pollution is mainly caused by human's activities. Exposure to any level of these contaminating elements lead to enormous diseases and disorders to human, animals and marines. Cleaning up the environment from these pollutants require the development of simple, green and sustainable techniques. Biosorption is one of the most benign and cheap bioremediation processes for the removal of the toxic heavy metal from environment such as lead, arsenic, and cadmium. We have developed a green and simple method for the removal of lead heavy metal from its aquatic system. Heavy metal phytoadsorption methodology using different amounts of fava beans-dead biomass (0.5, 1.0, and 2.0 g) was able to remove up to 83% of Pb^{2+} ions from their 100-mL aqueous solutions at room temperature and neutral *p*H. The results of these instrument-shaking experiments were compared with hand-shaking experiments and showed similar efficiency. It was observed that the fava beans (*Vicia faba* L.) can be used as a potential adsorbent for the removal of heavy metals from contaminated water.

Keywords: Biosorption, phytosorption, phytoadsorption, fava beans, lead ions, toxic heavy metal, pollution.

Introduction:

Contamination of ecosystems represents a globally significant concern. Anthropogenic activities and natural sources are the main sources of environmental pollution. Heavy metals consist the most important pollutants to all types of the ecosystems. There are two types of heavy metals; essential heavy metals, such as Fe, Cu, Zn, Ni and Mn, and non-essential heavy metals,

such as As, Pb, Cd, Hg, Cr, and Co. The non-essential heavy metals, which do not show any role in the life, are classified as toxic elements because they can cause a wide spectrum of dangerous diseases and disorders (Khalid *et al.*, 2017; Yan *et al.*, 2020; Bhat *et al.*, 2022; Mitra *et al.*, 2022; Zaynab *et al.*, 2022). Intensity of heavy metal toxicity depends on many factors such as (1) type, speciation and solubility of heavy metal, (2) dosage, duration, and method of exposure of organisms to heavy metal, (3) bioaccumulation and biomagnification, and (4) resistance to biological and chemical degradation, detoxification, and elimination (Caito and Aschner ,2015; Zaynab *et al.*, 2022; Sharif *et al.*, 2023). One of the most toxic pollutants is the lead heavy metal (Pb). Exposure to this toxic metal can cause damage to kidneys, reproductive system, central and peripheral nervous systems, gastrointestinal tract, skeletal system, and hematopoietic system. (Clarkson, 1987; Tangahu *et al.*, 2011; Pandey and Madhuri, 2014; Caito and Aschner, 2015; Sumiahadi and Acar, 2018). Removal of toxic heavy metals from environment systems is still a challenge worldwide. There are many successful physical, mechanical, chemical, and biological approaches are used for these purposes (Karman *et al.*, 2015; Khalid *et al.*, 2017; Wołowiec *et al.*, 2019; Ali Redha, 2020; Kumar *et al.*, 2021). The most interesting method is called bioremediation process. In this technique, natural sources can be applied. There are three types of the bioremediation processes; zooremediation, microbial remediation, and phytoremediation. In the phytoremediation (plant-base bioremediation) method, a wide range of plants were used for the removal of the toxic heavy metals from polluted environment. This green, and sustainable approach is a cheap and highly efficient method for the cleaning up and decontamination of soil and water (Prasad and Freitas, 2000; Sekhar *et al.*, 2003; Suman *et al.*, 2018; Al-Khazan and Al-Zlabani, 2019; Nedjimi, 2021; Sabreena *et al.*, 2022; Sharma *et al.*, 2023). According to contaminated ecosystem, type of contaminant, part of plant, state of biomass (living or dead), and applicability, the phytoremediation technology is classified into seven mechanisms: (1) phytoextraction (phytoaccumulation, phytoabsorption, or phytosequestration), (2) phytostabilization (phytoimmobilization), (3) phytodegradation (phytotransformation), (4) phytovolatilization, (5) phytostimulation (rhizodegradation), (6) phytofiltration (rhizofiltration), and (7) phytosorption. Phytosorption is sub-classified into two mechanisms; phytoadsorption or phytoabsorption. The living cell biomass of plant can be used for the removal of contaminants using any mechanism depending on the used plant part. The

uptake of pollutants using the dead cell biomass can be described by the phytoadsorption term (Tangahu *et al*., 2011; Karman *et al*., 2015; Al-Khazan and Al-Zlabani, 2019). Phytoadsorption mechanism, is a biological physico-chemical interaction between the dead biomass of plant and contaminants to uptake them from ecosystems by physical adsorption or chemical adsorption. In the physical adsorption, the dead cell biomass uses the physical attractions such as hydrogen bonding or Van der Waals attractions to bind the pollutants on their cell surface. On the other hand, the chemical adsorption approach is referred to the adhesion of pollutants on the biomass surface due to the formation of chemical bonds between the biological material and that contaminant. The phytoadsorption is affected by *p*H, temperature, contact time, biomass dosage, initial metal concentration, presence of another cations, and chemical modifications of biosorbents. Phytosorption of heavy metals from the aquatic systems was performed by the adsorption of metal ions onto the surface of dead biomass particles of the plant (Al Saharty, 2014; Dubey *et al*., 2014; Etorki *et al*., 2014; Ali Redha, 2020; Kumar *et al*., 2021; Sharif *et al*., 2023). Many plant families were used in the phytoremediation processes and evaluation of their potential for the removal of the toxic heavy metals (Prasad and Freitas, 2000; Sekhar *et al*., 2003; Srivastava *et al*., 2005; McGrath *et al*., 2006; Niu *et al*., 2007; Probst *et al*., 2009; Dubey *et al*., 2014; Waoo *et al*., 2014; Dube and Chingoma, 2016; Tang *et al*., 2019; Huynh *et al*., 2021; Ramadan and Balah, 2022). *Vicia faba* L. which is known as fava beans or broad beans has showed a high potential in the removal of the toxic heavy metals such as lead, cadmium, zinc and nickel from soil and aquatic ecosystems. It is also used for the toxicity monitoring and environmental pollution (Srivastava *et al*., 2005; Probst *et al*., 2009; Al Saharty, 2014; Etorki *et al*., 2014; Iqbal, 2016; Tang *et al*., 2019; Alobaidi *et al*., 2020; Sharif *et al*., 2023). More recently, we have reported an efficient, simple and low-cost hand-shaking phytosorption method for the removal of the lead ions from their aqueous solutions (Sharif *et al*., 2023). In this work, we will study the removal of Pb^{2+} from the aquatic system taking in our account the dead-biomass amount of fava beans loaded and the type of shaking.

Materials and Methods:

Sample Preparation: Lead Metal (Pb^{2+})

Different concentrations of the lead metal ion solutions were prepared (50, 100, 200, 500 and 1000 ppm) by dissolving of lead nitrate, $Pb(NO_3)_2$, in distilled water which were utilized later for the designed experiments.

Biomass

The biomass of plant fava bean (*Vicia faba* L.) was selected from the local market as dry seeds. The seed coats (testa) were removed and the seed beans were ground into a powder form. Next, they were stored in bottles which were used as absorbents for the removal of lead metal from its aqueous solutions as in the designed experiments. The species of the fava beans was determined in the laboratories of the Department of Botany, Faculty of Arts and Science – Ghemines, University of Benghazi.

Experiment and Sample Analysis

A specific amount (0.5, 1.0, and 2.0 g) of plant biomass was added to each a 100 mL lead metal solution separately in a 500-mL container (polyethylene bottle) and then they were shaken well at room temperature for 30 minutes using the Orbital Incubator Shaker at speed of 200 rpm. All experiments were carried out at room temperature and a neutral *p*H. On the other hand, a 2.0 g fava beans biomass was used for the removal of lead metal by manual shaking under the same conditions of the instrumental shaking method. All lead-beans mixture bottles were left for 24 hours to allow the solid matter to precipitate. Next, the precipitated mixtures were filtered using the Whatman filter papers No 1. The filtrates were then stored in refrigerator for the next step. Concentration of all Pb-beans solutions were detected using the flame atomic absorption spectroscopic (FAAS) instrument (Perkin Elemer 500).

Results and Discussion:

Having all samples of lead metal-filtrates in hand, detection of the remained metal ion concentrations was performed using a flame atomic absorption spectroscopic (FAAs) instrument (Model: Perkin Elemer 500) at a room temperature of 24°C and a *p*H of solutions at 3.79-5.11. The obtained results of all samples showed that all Pb^{2+} concentrations have remarkably decreased. This decrease was as a result of the adsorption effect of fava bean biomass on lead ions through the high affinity of metal cations and the negative charges of nucleophilic atoms such as oxygen and nitrogen on the protein chains. The amounts of metal ions removed from lead metal solutions by the beans-biomass were gradually increased with the increasing of the used biomass in all samples. It was found that the biomass factor is very important for the designed experiments. For example, in the case of 0.5 g fava beans-biomass array, the detected amount of Pb^{2+} ions in its 50-ppm solution was 42 ppm,

84%, using the shaker after 30 minutes (Table 1 and Figure 1). This means that the removed amount of lead ions from the original solution was 8 ppm or 16% (entry 1). The amount of remained lead metal ions were gradually reduced via phytoadsorption with the increased concentrations of the metal solutions used in the next experiments at the same consumed time. For example, the detected amount of the remained lead ions by AAS instrument of the 100, 200, 500, and 1000 ppm metal solutions after shaking were 79, 128, 245, and 210 ppm (or a 79, 64, 49, and 21%), respectively (entries 2-5). These findings showed a gradual increase in the biosorption of the fava-beans biomass particles towards the lead ions in the solutions. These results can be represented as metal-removal ratios of 21, 36, 51, and 79%, respectively (Figure 2).

Table 1: The detected amount of Pb^{2+} after an instrumental shaking using 0.5 g fava beans.

Entry	Pb-Solution Conc. (ppm)	Pb^{2+} Remained (ppm)	Pb^{2+} Removed (ppm)	Pb^{2+} Remained (%)	Pb^{2+} Removed (%)
1	50	42	8	84	16
2	100	79	21	79	21
3	200	128	72	64	36
4	500	245	255	49	51
5	1000	210	790	21	79

*All analysis experiments were carried out using the flame atomic absorption spectroscopic (FAAS) instrument (Perkin Elemer 500).

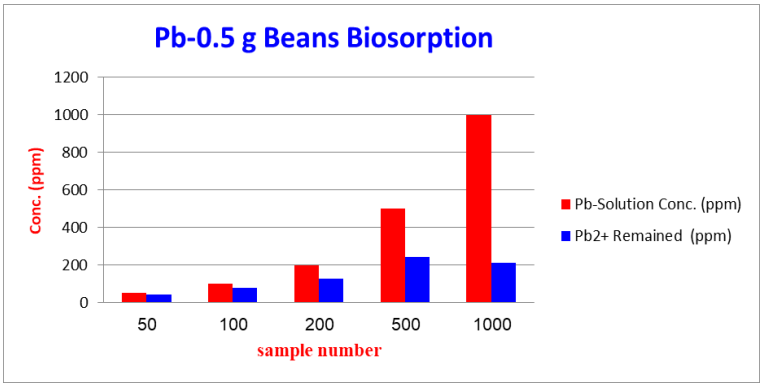


Figure 1: Concentrations of Pb^{2+} (ppm) before and after a 0.5 g fava beans-biosorption using an instrumental shaking.

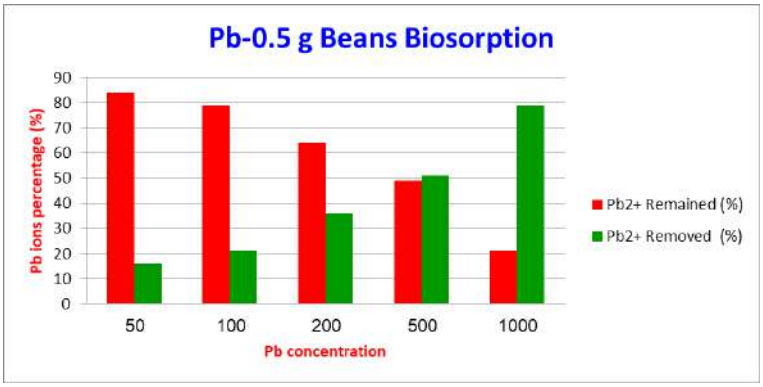


Figure 2: Percentages of remained and removed Pb^{2+} (%) after a 0.5 g fava beans-biosorption using an instrumental shaking.

For the 1.0 g fava beans biomass, as expected, it was found that this biosorbent showed a higher up take of the lead metal ions from their solutions except of the lower concentration of metal solution; 50 ppm, using the same period of time (30 minutes) (entry 1, Table 2 and Figure 3). For instant, a 76 ppm was the detected concentration of Pb^{2+} ions from the 100 ppm-Pb solution after biosorption process using the same shaker in a ratio of 76% of the remained metal ions. Similarly, the higher concentrations showed a gradual decrease in the remained of Pb ions in their solutions after each experiment in 118, 230, and 210 ppm (59, 46, and 21%), respectively (entries 2–5). This means that the percentages of the removed Pb-ions form their solutions were 41, 54, and 79%, respectively (Figure 4).

Table 2: The detected amount of Pb^{2+} after an instrumental shaking using a 1.0 g fava beans.

Entry	Pb-Solution Conc. (ppm)	Pb^{2+} Remained (ppm)	Pb^{2+} Removed (ppm)	Pb^{2+} Remained (%)	Pb^{2+} Removed (%)
1	50	42	8	84	16
2	100	76	24	76	24
3	200	118	82	59	41
4	500	230	270	46	54
5	1000	210	790	21	79

*All analysis experiments were carried out using the flame atomic absorption spectroscopic (FAAS) instrument (Perkin Elemer 500).

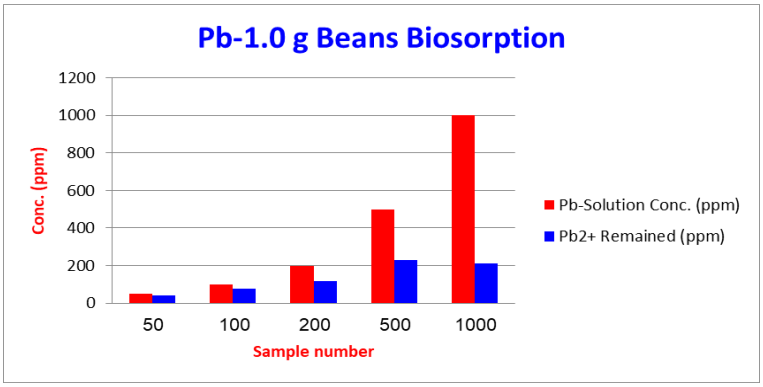


Figure 3: Concentrations of Pb^{2+} (ppm) before and after a 1.0 g fava beans-biosorption using an instrumental shaking.

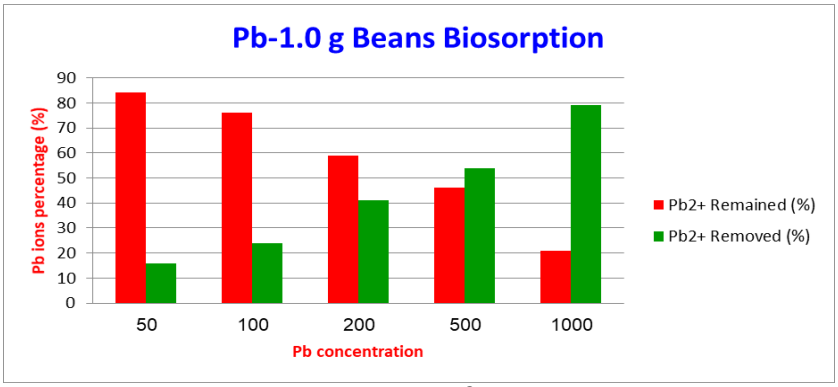


Figure 4: Percentages of remained and removed Pb^{2+} (%) after a 1.0 g fava beans-biosorption using an instrumental shaking.

In the same pattern for the 2.0 g fava beans biomass, the excess amount of biomass increased the removal of the lead metal ions from their solutions after the instrumental shaking. For example, the detected amount of lead ions from the 50 ppm-solutions was 40 ppm as 80%, which means a 20% of the removed ions (entry 1, Table 3, Figure 5). On the same way, the detected concentrations of 100, 200, 500, and 1000 ppm lead ion solutions were 64, 112, 205, and 200 ppm (as 64, 56, 31, and 20%), respectively (entries 2-5). Therefore, the percentages of the removed metal ions from their solutions were 20, 36, 44, 69, and 80%, respectively (Figure 6). In this stage, the removed amount of toxic metal ions increased compared to the less loads of fava beans biomass that were used in the previous experiments.

Table 3: The detected amount of Pb^{2+} after an instrumental shaking using 2.0 g fava beans.

Entry	Pb-Solution Conc. (ppm)	Pb^{2+} Remained (ppm)	Pb^{2+} Removed (ppm)	Pb^{2+} Remained (%)	Pb^{2+} Removed (%)
1	50	40	10	80	20
2	100	64	36	64	36
3	200	112	88	56	44
4	500	205	295	31	69
5	1000	200	800	20	80

*All analysis experiments were carried out using the flame atomic absorption spectroscopic (FAAS) instrument (Perkin Elemer 500).

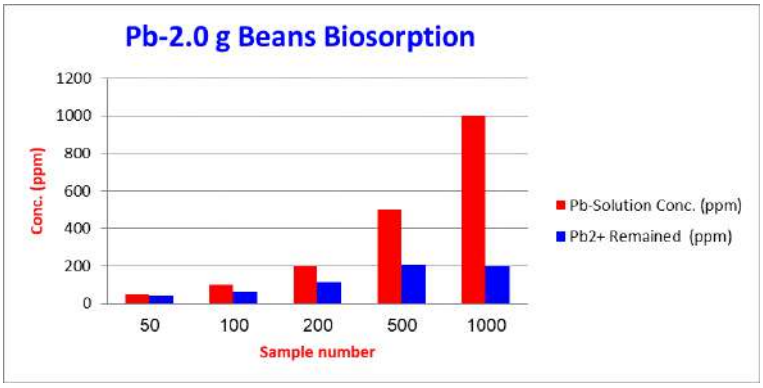


Figure 5: Concentrations of Pb^{2+} (ppm) before and after a 2.0 g fava beans-biosorption using an instrumental shaking.

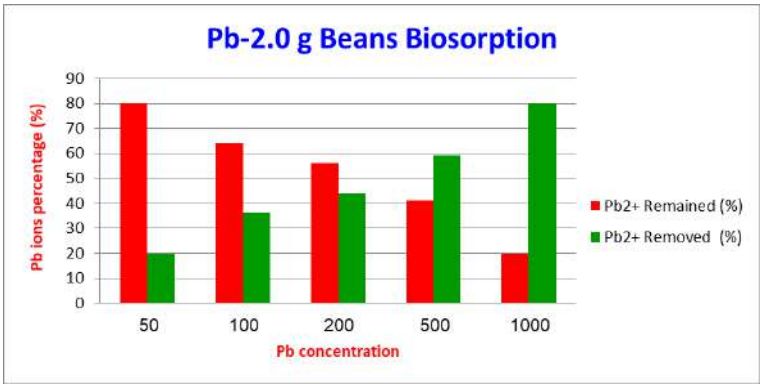


Figure 6. Percentage of remained and removed Pb^{2+} (%) after a 2.0 g fava beans-biosorption using an instrumental shaking.

Interestingly, investigation of another approach for the removal of the lead heavy metal was required. It was found that an interesting relationship between the instrumental shaking and the manual shaking of Pb-contaminated samples. However, the hand-shaking of the Pb-fava beans biomass mixtures for the 2.0 g-biomass were carried out. It was found that the hand-shaking

method is significantly better than the instrument-shaking method at the same experimental time. For example, the amount of the detected lead metal ions after 30 min of hand-shaking of the Pb-beans mixture was 39 ppm, which represent a 78% of the original Pb-solution (entry 1, Table 4, Figure 7). This means that the removed Pd-ions were 22% of the original concentration. Using the same pattern of the other experiments, it was found that the detected concentrations of Pb metal ions in the sample concentrations of 100, 200, 500, and 1000 ppm were 60, 91, 175, and 170 ppm, respectively (entries 2-5). In other words, the removed amounts of lead metal ions from these samples represent a 40, 54, 65, and 83%, respectively (Figure 8).

Table 4: The detected amount of Pb^{2+} after a manual shaking using 2.0 g fava beans.

Entry	Pb-Solution Conc. (ppm)	Pb^{2+} Remained (ppm)	Pb^{2+} Removed (ppm)	Pb^{2+} Remained (%)	Pb^{2+} Removed (%)
1	50	39	11	78	22
2	100	60	40	60	40
3	200	91	109	45.5	54.5
4	500	175	325	35	65
5	1000	170	830	17	83

*All analysis experiments were carried out using the flame atomic absorption spectroscopic (FAAS) instrument (Perkin Elemer 500).

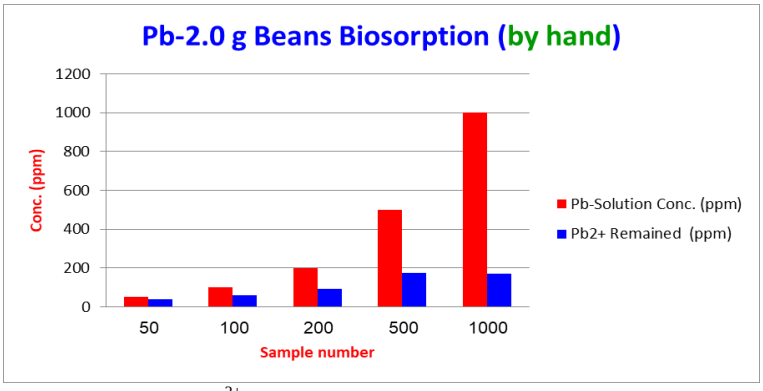


Figure 7: Concentrations of Pb^{2+} (ppm) before and after a 2.0 g fava beans-biosorption using a manual shaking.

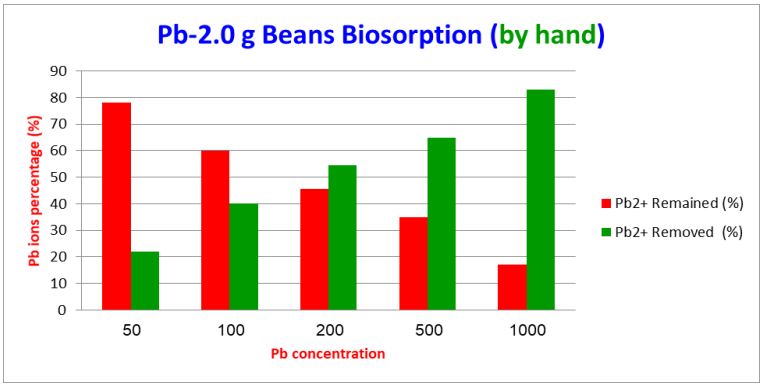


Figure 8: Percentage of remained and removed Pb^{2+} (%) after a 2.0 g fava beans-biosorption using a manual shaking.

Results of this study showed that the *Vicia faba* L. dead-biomass can be applied as biosorbent for the removal of the toxic heavy metals from the aquatic ecosystems. These results were in consistence with the previous studies (Al Saharty, 2014; Etorki *et al.*, 2014; Sharif *et al.*, 2023). Finally, and according to Tables 5, Figures 9-11, we found that the 2.0 g fava beans phytoadsorption of the toxic heavy metal lead ions from their solutions using the hand-shaking method gave better results than the instrument-shaking method under the described conditions. This observation might be due to the efficient mixing of biomass particles inside the bottles and the circulation pattern of shaking of the used instrument.

Table 5: Comparison between the instrumental and manual shaking methods for the removal of Pb^{2+} using a 2.0 g fava beans biomass.

Entry	Pb-Solution Conc. (ppm)	Pb^{2+} Remained (ppm)		Pb^{2+} Remained (%)		Pb^{2+} Removed (%)	
		Instrument Shaking	Manual Shaking	Instrument Shaking	Manual Shaking	Instrument Shaking	Manual Shaking
1	50	40	39	80	78	20	22
2	100	64	60	64	60	36	40
3	200	112	91	56	45.5	44	54.5
4	500	205	175	41	35	59	65
5	1000	200	170	20	17	80	83

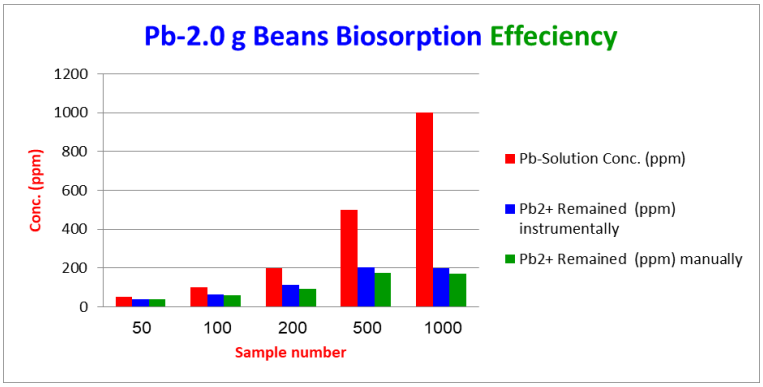


Figure 9: Comparison between the instrumental and manual shaking methods for the removal of Pb^{2+} ions showed the remained ions in ppm.

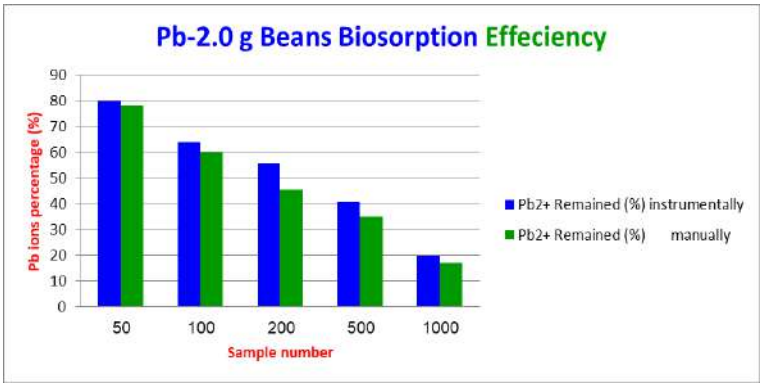


Figure 10: Comparison between the instrumental and manual shaking methods for the removal of Pb^{2+} ions showed in the percentage of remained ions.

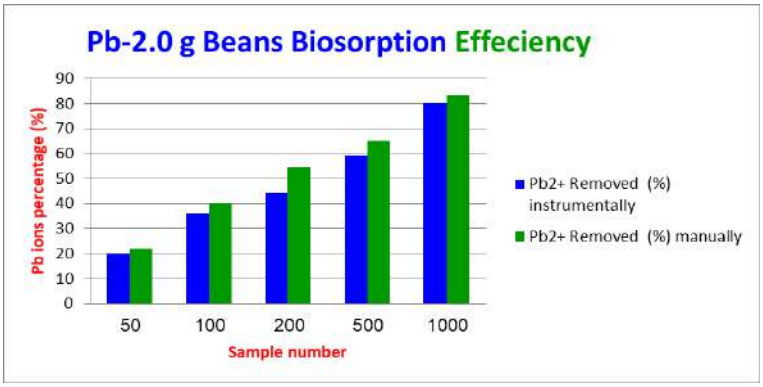


Figure 11. Comparison between the instrumental and manual shaking methods for the removal of Pb^{2+} ions showed in the percentage of removed ions.

Conclusion:

Heavy metals constitute a primary concern for all ecosystems than other environmental pollutants. Necessity for the bioremediation processes is demanded for the removal of toxic heavy metals from contaminated areas. Phytosorption mechanisms, which represents one of the green, sustainable, and simple phytoremediation techniques, have a high potential for the removal of the toxic heavy metals from their ecosystems. The resulting data from the conducted phytoadsorption experiments showed that the fava beans dead-biomass can be used as an excellent biosorbent for the removal of toxic heavy metal lead ions (Pb^{2+}) from their aqueous solutions up to 83% of the original solution. This observation was due to the high empathy of fava beans-protein functional groups to the metal cations. Therefore, the developed approach can be applied for the cleaning up of the heavy metal-contaminated aquatic environmental systems.

Acknowledgment:

The authors are grateful for Dr Fawzia Muftah Aljazwi, Genetics Research Laboratory, Department of Botany, Faculty of Science, University of Benghazi, for providing the Orbital Incubator Shaker. Authors are also thankful to Khaled Ali Jhawi, Major Technician, Faculty of Arts and Science - Ghemines, and Salh Ahmed Alsunousi, Major Technician, Faculty of Science - Benghazi, University of Benghazi, for their help and providing all necessary facilities to complete this work. Authors appreciate Farag M. Ali, Quality Control Specialist, Water Quality Control Department, Man-Made River Project, Benghazi–Libya, for conducting the atomic absorption analysis.

References:

Al Saharty, A. (2014). Phytoremediation of Heavy Metals from Wastewater Using Bean Plants. *Biosciences, Biotechnology Research Asia*, *11*(3), 1373-1380.

Ali Redha, A. (2020). Removal of heavy metals from aqueous media by biosorption. *Arab Journal of basic and applied sciences*, *27*(1), 183-193.

Al-Khazan, M. M., & Al-Zlabani, R. M. (2019). Toxic materials phytoremediation potential of four common trees in Saudi Arabia: A review. *Egypt. J. Exp. Biol. (Bot.)*, *15*, 87-97.

Alobaidi, K. H., Oraibi, A. G., Yahya, H. N., & Al-Obaidi, J. R. (2020). Phytoremediation of zinc ion using faba bean *Vicia faba* plant in vitro. *Indian Journal of Ecology*, *47*(4), 965-968.

Bhat, S.A., Bashir, O., Haq, S. A. U., Amin, T., Rafiq, A., Ali, M., Américo-Pinheiro, J. H. P. & Sher, F. (2022). Phytoremediation of heavy metals in soil and water: An eco-friendly, sustainable and multidisciplinary approach. *Chemosphere*, *303*, 134788.

Caito, S., & Aschner, M. (2015). Neurotoxicity of metals. *Handbook of Clinical Neurology*, *131*, 169-189.

Clarkson, T. W. (1987). Metal toxicity in the central nervous system. *Environmental Health Perspectives*, *75*, 59-64.

Dube, D., & Chingoma, C. (2016). Removal of heavy metal ions from household drinking water using *Acacia galpinii* seeds and seed pods. *Journal of Health and Pollution*, *6*(12), 7-14.

Dubey, A. M. S. S. A., Mishra, A., & Singhal, S. (2014). Application of dried plant biomass as novel low-cost adsorbent for removal of cadmium from aqueous solution. *International Journal of Environmental Science and Technology*, *11*, 1043-1050.

Etorki, A. M., El-Rais, M., Mahabbis, M. T., & Moussa, N. M. (2014). Removal of some heavy metals from wastewater by using of fava beans. *American Journal of Analytical Chemistry*, *5*, 225-234.

Huynh, A. T., Chen, Y. C., & Tran, B. N. T. (2021). A small-scale study on removal of heavy metals from contaminated water using water hyacinth. *Processes*, *9*(10), 1802.

Iqbal, M. (2016). *Vicia faba* bioassay for environmental toxicity monitoring: a review. *Chemosphere*, *144*, 785-802.

Karman, S. B., Diah, S. Z. M., & Gebeshuber, I. C. (2015). Raw materials synthesis from heavy metal industry effluents with bioremediation and phytomining: a biomimetic resource management approach. *Advances in Materials Science and Engineering*, *2015*.

Khalid, S., Shahid, M., Niazi, N. K., Murtaza, B., Bibi, I., & Dumat, C. (2017). A comparison of technologies for remediation of heavy metal contaminated soils. *Journal of Geochemical Exploration*, *182*, 247-268.

Kumar, M., Seth, A., Singh, A. K., Rajput, M. S., & Sikandar, M. (2021). Remediation strategies for heavy metals contaminated ecosystem: A review. *Environmental and Sustainability Indicators*, *12*, 100155.

McGrath, S. P., Lombi, E., Gray, C. W., Caille, N., Dunham, S. J., & Zhao, F. J. (2006). Field evaluation of Cd and Zn phytoextraction potential by the hyperaccumulators *Thlaspi caerulescens* and *Arabidopsis halleri*. *Environmental Pollution*, *141*(1), 115-125.

Mitra, S., Chakraborty, A. J., Tareq, A. M., Emran, T. B., Nainu, F., Khusro, A., Idris, A. M., Khandaker, M. U., Osman, H., Alhumaydhi, F. A. & Simal-Gandara, J. (2022). Impact of heavy metals on the environment and human health: Novel therapeutic insights to counter the toxicity. *Journal of King Saud University-Science*, *34*(3), 101865.

Nedjimi, B. (2021). Phytoremediation: a sustainable environmental technology for heavy metals decontamination. *SN Applied Sciences*, *3*(3), 286.

Niu, Z. X., Sun, L. N., Sun, T. H., Li, Y. S., & Hong, W. A. N. G. (2007). Evaluation of phytoextracting cadmium and lead by sunflower, ricinus, alfalfa and mustard in hydroponic culture. *Journal of Environmental Sciences*, *19*(8), 961-967.

Pandey, G., & Madhuri, S. (2014). Heavy metals causing toxicity in animals and fishes. *Research Journal of Animal, Veterinary and Fishery Sciences*, *2*(2), 17-23.

Prasad, M. N. V., & Freitas, H. (2000). Removal of toxic metals from solution by leaf, stem and root phytomass of *Quercus ilex* L. (holly oak). *Environmental Pollution*, *110*(2), 277-283.

Probst, A., Liu, H., Fanjul, M., Liao, B., & Hollande, E. (2009). Response of *Vicia faba* L. to metal toxicity on mine tailing substrate: geochemical and morphological changes in leaf and root. *Environmental and Experimental Botany*, *66*(2), 297-308.

Ramadan, W. F., & Balah, M. A. (2022). The use of some weeds type in the disposal of heavy metals in contaminated soil. *Journal of the Saudi Society of Agricultural Sciences*, *21*(5), 289-295.

Sabreena, Hassan, S., Bhat, S. A., Kumar, V., Ganai, B. A., & Ameen, F. (2022). Phytoremediation of heavy metals: An indispensable contrivance in green remediation technology. *Plants*, *11*(9), 1255.

Sekhar, K. C., Kamala, C. T., Chary, N. S., & Anjaneyulu, Y. (2003). Removal of heavy metals using a plant biomass with reference to environmental control. *International Journal of Mineral Processing*, *68*(1-4), 37-45.

Sharif, S. A., El-Moghrabi, H. A. M. N., El-Mugrbi, W. S., Alhddad, A. I. (2023). Fava Beans (*Vicia faba* L.) Phytosorption of Pb^{2+} Ions from its Aqueous Solutions. *Asian Journal of Green Chemistry*, 7, 85-90.

Sharma, J. K., Kumar, N., Singh, N. P., & Santal, A. R. (2023). Phytoremediation technologies and their mechanism for removal of heavy metal from contaminated soil: An approach for a sustainable environment. *Frontiers in Plant Science*, *14*, 1076876.

Srivastava, S., Mishra, S., Dwivedi, S., Baghel, V. S., Verma, S., Tandon, P. K., Rai, U. N. & Tripathi, R. D. (2005). Nickel phytoremediation potential of broad bean, *Vicia faba* L., and its biochemical responses. *Bulletin of Environmental Contamination and Toxicology*, *74*, 715-724.

Suman, J., Uhlik, O., Viktorova, J., & Macek, T. (2018). Phytoextraction of heavy metals: a promising tool for clean-up of polluted environment?. *Frontiers in Plant Science*, *9*, 1476.

Sumiahadi, A., & Acar, R. (2018). A review of phytoremediation technology: heavy metals uptake by plants. In *IOP Conference Series: Earth and Environmental Science* (Vol. 142, p. 012023). IOP Publishing.

Tang, L., Hamid, Y., Zehra, A., Sahito, Z. A., He, Z., Hussain, B., Gurajala, H. K. & Yang, X. (2019). Characterization of fava bean (*Vicia faba* L.) genotypes for phytoremediation of cadmium and lead co-contaminated soils coupled with agro-production. *Ecotoxicology and Environmental Safety*, *171*, 190-198.

Tangahu, B. V., Sheikh Abdullah, S. R., Basri, H., Idris, M., Anuar, N., & Mukhlisin, M. (2011). A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. *International Journal of Chemical Engineering*, *2011*.

Waoo, A. A., Khare, S., & Ganguli, S. (2014). Comparative tissue culture studies on Lantana Camara and Datura Inoxia at heavy metal. *International Journal of Advances in Biology (IJAB)*, *1*(1).

Wołowiec, M., Komorowska-Kaufman, M., Pruss, A., Rzepa, G., & Bajda, T. (2019). Removal of heavy metals and metalloids from water using drinking water treatment residuals as adsorbents: A review. *Minerals*, *9*(8), 487.

Yan, A., Wang, Y., Tan, S. N., Mohd Yusof, M. L., Ghosh, S., & Chen, Z. (2020). Phytoremediation: a promising approach for revegetation of heavy metal-polluted land. *Frontiers in Plant Science*, *11*, 359.

Zaynab, M., Al-Yahyai, R., Ameen, A., Sharif, Y., Ali, L., Fatima, M., Khan, K. A. & Li, S. (2022). Health and environmental effects of heavy metals. *Journal of King Saud University-Science*, *34*(1), 101653.

AlQalam Journal of Medical and Applied Sciences
Special Issue for 6th International Conference in Basic Sciences and Their Applications (6th ICBSTA, 2023), *https://journal.utripoli.edu.ly/index.php/Alqalam* ***eISSN 2707-7179***

Curcumin Attenuated Inflammation on Hepatic Induced of by tramadol in male rabbits

Fayrouz. A. khaled*[1], Marfoua. S. Ali[2], Faraj. A. Hussin[2]
[1]*Chemistry Department, Faculty of Science, Omar El-Mokhtar University, El -Beyda-Libya*
[2]*Zoology Department, Faculty of Science, Omar El-Mokhtar University, El - Beyda-Libya*
**Corresponded authors: fayalzobair@yahoo.com*

Abstract:

Worldwide, people use tramadol. It has certain negative reactions and side effects, and it can lead to physical and psychological addiction just like other opiates do. Turmeric's main ingredient, curcumin, is recognized for a number of biological functions, chiefly related to its antioxidant capacity. Thus, the goal of the current experiment is to ascertain whether curcumin can reduce the toxic effects of tramadol on male rabbits' hepatotoxicity. A total of twenty rabbits were split into four equal groups, each consisting of five rabbits. As a control, the first group was given an equal amount of distill water. A second group was used to examine the effects of 50 mg/kg body weight of tramadol. The effects of 10 mg/kg body weight of curcumin were investigated in the third group. The effects of 10 mg/kg body weight of curcumin were investigated in the third group. Tramadol and curcumin were combined, and the results were examined in the fourth group. For six weeks, the rabbits received their prescribed dosages orally every day. Tramadol was found to significantly ($P < 0.05$) raise the levels of alkaline phosphatase (AlP), total bilirubin, alanine transaminase (ALT), and aspartate transaminase (AST). All parameter levels were significantly reduced by curcumin alone. Also, the presence of curcumin with tramadol alleviated its harmful effects on most of the tested parameters. Consequently, the current findings suggested that curcumin therapy could reduce tramadol's harmful effects.

Keywords: Rabbits; Tramadol ; Curcumin; liver

Introduction:

Tramadol is a centrally acting analgesic with efficacy and potency ranging between weak opioids and morphine (Raffa *et al.*, 1992)**.** The Food and Drug Administration only gave tramadol approval in 1995 to treat and manage moderate-to-severe pain conditions, despite the fact that it had been around since the 1970s (Bloor *et al.*, 2012, Eassa and El-Shazly, 2013)**.** The drug is structurally related to codeine and morphine, but it is 6000-times less potent than morphine and 10-times less potent than codeine (Lavasani *et al.*, 2013).

Tramadol is metabolized and eliminated by the liver and kidneys (Milne et al., 1997). During its metabolism, tramadol has the potential to be nephrotoxic and hepatotoxic. Alkaline phosphatase (ALP), aspartate aminotransferase (AST), creatinine, and urea were found to have significantly increased in plasma. Chronic heroin users have been found to have elevated levels of lipid peroxides, lactate dehydrogenase (LDH), and alanine transaminase (ALT) (Panchenko et al., 1999). Medicinal plants are generally contain constituents that found useful for the treatment and management of both animal and human diseases (Okolie and Abu, 2019). In experimental animal models, several traditional remedies with plant origins, like curcumin, are tested for their potential antioxidant properties. *Curcuma longa*, an Indian rhizomatous herbal plant belonging to the Zingiberaceae family, is a well-known source of medicinal benefits (Panpatil *et al.*, 2013, Pawar *et al.*, 2014). The active ingredients in turmeric known as curcumin oids may be responsible for its therapeutic effects. Oid is one of curcumin's most intriguing ingredients. It is a lipophillic polyphenolic compound with a small molecular weight that is soluble in ethanol, dimethyl sulfoxide, and other organic solvents but insoluble in water or ether (Aggarwal *et al.*, 2003). Curcumin is also stable at the acidic pH of the stomach (Kharat *et al.*, 2017). According to Pawar *et al.* (2014), there are additional ingredients such as sugars, proteins, and resins along with volatile oils like tumerone, atlantone, and zingiberone. Curcumin, the active ingredient in turmeric, is extracted from Curcuma longa and gives turmeric its color. Numerous studies have been conducted on this type of bioactive component (Hewlings and Kalman, 2017). Using a variety of animal models, researchers examine the protective effects of curcumin *in vitro* and discover that it induces hepatotoxicity and cardiotoxicity at biochemical parameters such as serum marker enzymes and antioxidants in target tissues. The increased relative weight of liver and heart, liver injury and isoproterenol induced cardiac necrosis were also reduced by curcumin treatment (Quiles *et al.*, 2002). The study examined elevated levels of serum marker enzymes, namely AST, ALT, and ALP, in the tissues of the granulomatus, liver, and heart, respectively, during liver injury and cardiac necrosis (Scannell *et al.*, 2012). The study demonstrated that protective effect of curcumin with experimentally drugs that induced hepatotoxicity in male rabbits**.**

Materials and Methods:

In this study tramadol and curcumin were used. Tramadol was purchased from pharmacy Al-Salam hospital in El –Bayda Libya. Curcumin was purchased from public market for medicinal herbs in El-Bayda City. Mature male New Zealand White rabbits age of 6 -weeks and initial weight of (1892± 50.79g) were used. Animals were individually housed in cages and weighed weekly

throughout six weeks experimental period. Twenty mature male rabbits were randomly divided into four equal groups (each five rabbits) as follows -:Group I: Rabbits were used as control treated with distul water. Groups II: Rabbites were treated with curcumin 15 mg/kg body weight (Somsak *et al.,* 2016) Group III: Rabbites were treated with tramadol by gavage at a dose of 50 mg/kg body weight per day (1/50 of tramadol) lethal dose (Ahmad *et al.,* 2019)**.** Group VI: Rabbites were treated with combination of tramadol and curcumin. All groups were treated for 6 successive weeks. Blood samples were collected from the ear vein of all animals every other week throughout the 6-week experimental period. Blood samples were obtained in the morning before accesses to feed and water and placed immediately on ice. The blood samples were collected in tube containing heparin was used to obtain plasma. The activities of plasma aspartate transaminase (AST; EC 2.6.1.1) and alanine transaminase (ALT; EC 2.6.1.2) were assayed by the method of Reitman and Frankel (1957). Alkaline phosphatase (AlP; EC 3.1.3.1) activity was determined in plasma according to the method of (Principato *et al.*, 1985). Total bilirubin was measured using the method of (Walters and Gerarde, 1970).

Statistical analysis

Where applicable, statistical analysis was carried out in Minitab software (version 17). Statistical significance was assessed using ANOVA analysis with Tukey multiple comparison test after detection normal distribution to the data and appropriate $P < 0.05$ consider significant.

Results:

Table(1) and Figures 1-4 were represented the mean values of activities of AST, ALT, ALP and total bilirubin in serum of male rabbits treated with curcumin, tramadol and their combination. Treatment with tramadol alone caused a significant (*P<0.05*) increased in activity of AST, ALT and non-significant increase of ALP activity. At same time it caused decreases in level of total bilirubin. While, treatment with curcumin alone caused significantly (*P<0.05*) decreased in activates of AST, ALT, non-significant ALP and increased level of total bilirubin compared to control group. The presence of curcumin with tramadol minimized its effect of these parameters that reach to control levels.

Table 1: The activities of AST, ALT, ALP and total bilirubin in serum of male rabbits treated with curcumin, tramadol and their combination.

Parameters	*Experimental groups*			
	Control ***Mean± SEM***	**Curcumin** ***Mean± SEM***	**Tramadol** ***Mean± SEM***	**Curcumin + Tramadol** ***Mean± SEM***
AST (U/L)	41.84 ± 2.655[a]	30.89± 2.75[b]	46.79 ±3.030[a]	40.85 ±1.135[a]
ALT (U/L)	43.16 ±1.21[b]	37.76 ±1.180[b]	60.51± 1.682[a]	43.92 ± 1.92[b]
ALP (IU/L)	44.30 ±2.413[ab]	41.04± 2.06[b]	51.43± 1.93[a]	42.22 ± 1.38[b]
Bilirubin (mg/dL)	1.030± 0.189[b]	1.736± 0.039[a]	0.856± 0.047[b]	1.025± 0.109[b]

Values are expressed as means ± SEM; n = 5 for each treatment group.Mean values within a row not sharing a common superscript letters (a or b) were significantly different (P<0.05).

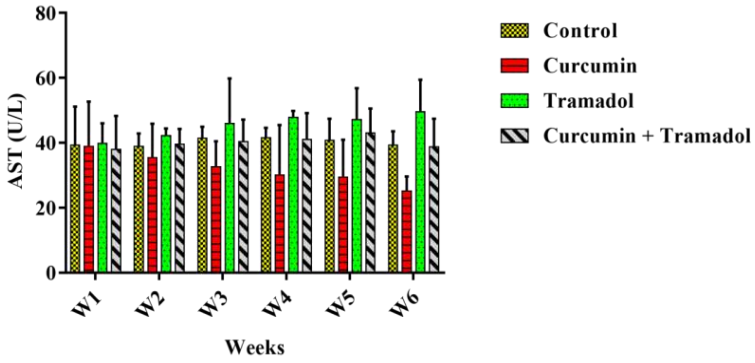


Figure 1: Changes in aspartate transaminase (AST) throughout treatment of male rabbits with curcumin, tramadol and their combination.

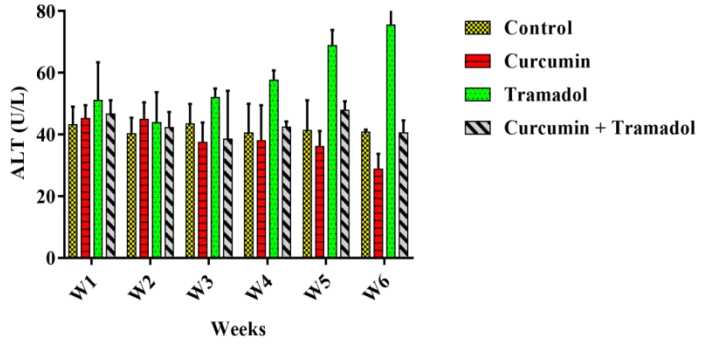


Figure 2: Changes in alanine transaminase (ALT) throughout treatment of male rabbits with curcumin, tramadol and their combination.

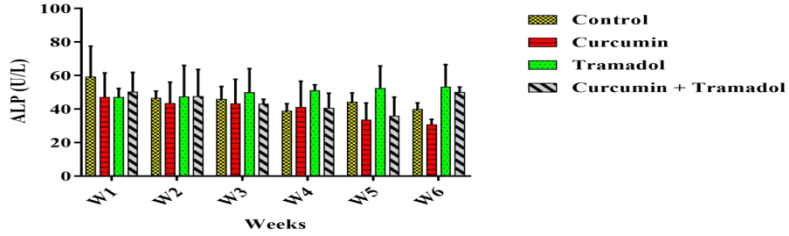


Figure 3: Changes in alkaline phosphatase (ALP) throughout treatment of male rabbits with curcumin, tramadol and their combination.

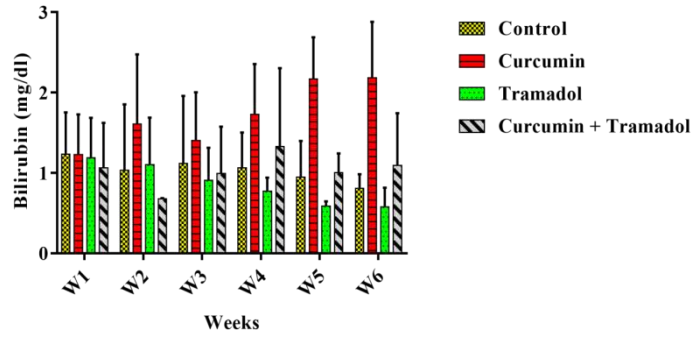


Figure 4: Changes in total bilirubin throughout treatment of male rabbits with curcumin, tramadol and their combination.

Discussion:

In the current investigation, tramadol treatment by itself resulted in a considerable drop in total bilirubin levels and an increase in AST, ALT, and ALP activity. These findings verify that the administration of tramadol boosted the activities of the ALT and AST liver function enzymes. The severity of the liver damage was indicated by the high levels of liver enzymes in the blood (Simeon and Abbey, 2018). Borzelleca *et al*. (1994) observed elevated ALT and AST activities in rats following extended use of levo-alpha-acetylmethadol HCl (LAAM), a morphine-like substance. Analogously, prior research on rabbits (Panchenko *et al*., 1999) and rats (Owoade *et al*., 2019, Sheweita et al., 2018). Previous study revealed a noteworthy rise in the activations of ALT, AST, and ALP by tramadol. Tramadol also results in severe cellular toxicity and liver failure. (Elmanama *et al.*, 2015). *Aldalou et al.* (2014) observed that sildenafil and tramadol administration might be responsible for impaired liver function. Tramadol administration was associated with hepatic congestion, hemorrhage and necrosis in the male rats (Loughrey *et al.*, 2003). Impaired secretion of hepatic ALP may be accompanied by acute cell necrosis, so liberation of ALP in the circulation is elevated. The increased activities of AST, ALT, ALP, and bilirubin may indicate that the cellular injury is still present. The results of this study were consistent with those of Sayed and Zidan (2016), who found that rats exposed to acute and gradually increasing doses of tramadol until reaching dependency had significantly higher activities of ALT, AST, ALP, and bilirubin when compared to the control group. The elevated plasma level of these enzymes in rabbits treated with tramadol may be caused by necrosis or damage to the liver cell membrane, which leaks the enzymes. Increased secretion of these liver enzymes may be accompanied by acute cell necrosis (Loughrey *et al*., 2003). Nna *et al*. (2015) also observed that the hepatotoxicity of tramadol (2 mg/100 g BW for 8 weeks) is reversed after withdrawal it for 8 weeks. Another study indicate that long term tramadol abuse cause decreased activity of AST, ALT, ALP and serum bilirubin (Sayed and Zidan, 2016). This is in line with the findings of El-Agamy (2010) who found that curcumin acted as an antioxidant and raised levels of GSH, or non-enzymatic antioxidant.

Also, other investigators Kowluru and Kanwar (2007) have considered that the beneficial effects of curcumin are mediated by its antioxidant defense ability and the scavenging of free radicals; furthermore, they stated that curcumin is 10 times more active as an antioxidant compared with vitamin E as it has potent anti-inflammatory property. Because curcumin neutralizes free radicals, which are extremely unstable molecules that can damage cellular structures through abnormal oxidative reactions, it is suggested that

curcumin's antioxidant and anti-inflammatory properties are responsible for the amelioration of tramadol-induced liver damage seen in the current study. These findings support the findings before that administering curcumin prevented increases in ALT, AST, and ALP and enhanced liver function (Hemeida and Mohafez, 2008, Sanaa *et al.*, 2014)

In conclusion, the data indicated that pretreatment of rabbits with curcumin, prior to administration of tramadol alleviated the changes in the liver marker enzymes.

References:

Aggrawal, B. B., Kumar, A., & Phatric, A. C. (2003). Anticancer potential of curcumin. *Anticancer Res*, *23*(1A), 366-378.

Ahmad, R. M., AL-Hubaity, A. Y., & Alazow, N. S. (2019). The role of Vitamin C on the structural changes of male Albino rats kidney induced by tramadol. *Annals of the College of Medicine, Mosul*, *41*(1), 57-62.

Aldalou, A. R., Abdel-Aziz, I., & Shahwan, O. (2014). Impact of giving sildenafil (viagra)/tramadol (tramal) combination on the blood of domestic rabbits. *Journal of Science*, *4*(3), 162-169.

Bloor, M., Paech, M. & Kaye, R. (2012). Tramadol in pregnancy and lactation. *International journal of obstetric anesthesia*, 21, 163-167.

Borzelleca, J. F., Egle Jr, J. L., Harris, L. S., Johnson, D. N., Terrill, J. B., & Belleville, J. A. N. (1994). Toxicological evaluation of μ-agonists part I: Assessment of toxicity following 30 days of repeated oral dosing of male and female rats with levo-alpha-acetylmethadol HCl (LAAM). *Journal of Applied Toxicology*, *14*(6), 435-446.

Eassa, B. I., & El-Shazly, M. A. (2013). Safety and efficacy of tramadol hydrochloride on treatment of premature ejaculation. *Asian journal of andrology*, *15*(1), 138.

El-Agamy, D. S. (2010). Comparative effects of curcumin and resveratrol on aflatoxin B 1-induced liver injury in rats. *Archives of toxicology*, *84*, 389-396.

Elmanama, A. A., Abu Tayyem, N. E., Essawaf, H. N., & Hmaid, I. M. (2015). Tramadol-induced liver and kidney toxicity among abusers in Gaza Strip, Palestine. *Jordan Journal of Biological Sciences*, *147*(3427), 1-5.

Hemeida, R. A., & Mohafez, O. M. (2008). Curcumin attenuates methotraxate-induced hepatic oxidative damage in rats. *Journal of the Egyptian National Cancer Institute*, *20*(2), 141-148.

Hewlings, S. J., & Kalman, D. S. (2017). Curcumin: A review of its effects on human health. *Foods*, *6*(10), 92.

Kharat, M., Du, Z., Zhang, G., & McClements, D. J. (2017). Physical and chemical stability of curcumin in aqueous solutions and emulsions: Impact of pH, temperature, and molecular environment. *Journal of agricultural and food chemistry*, *65*(8), 1525-1532.

Kowluru, R. A., & Kanwar, M. (2007). Effects of curcumin on retinal oxidative stress and inflammation in diabetes. *Nutrition & metabolism*, *4*(1), 1-8.

Lavasani, H., Sheikholeslami, B., Ardakani, Y. H., Abdollahi, M., Hakemi, L., & Rouini, M. R. (2013). Study of the pharmacokinetic changes of Tramadol in diabetic rats. *DARU Journal of Pharmaceutical Sciences*, *21*, 1-9.

Loughrey, M. B., Loughrey, C. M., Johnston, S., & O'Rourke, D. (2003). Fatal hepatic failure following accidental tramadol overdose. *Forensic science international*, *134*(2-3), 232-233.

Milne, R. W., McLean, C. F., Mather, L. E., Nation, R. L., Runciman, W. B., Rutten, A. J., & Somogyi, A. A. (1997). Influence of renal failure on the disposition of morphine, morphine-3-glucuronide and morphine-6-glucuronide in sheep during intravenous infusion with morphine. *Journal of Pharmacology and Experimental Therapeutics*, *282*(2), 779-786.

Nna, V. U., Akpan, U. P., Okon, V. E., & Atangwho, I. J. (2015). Hepatotoxicity following separate administration of two phosphodiesterase-5 inhibitors (sildenafil & tadalafil) and opioid (tramadol); evaluation of possible reversal following their withdrawal. *Journal of Applied Pharmaceutical Science*, *5*(8), 105-113.

Okolie, N. P., & Abu, O. D. (2019). Effects of Aqueous Extract of Annona muricata Leaves on Cyanide–induced Toxicity in New Zealand Rabbits. *NISEB Journal*, *16*(4).

Owoade, A., Adetutu, A. & Olorunnisola, O. (2019). Hematological and biochemical changes in blood, liver and kidney tissues under the effect of tramadol treatment. *J Alcohol Drug Depend*, 7, 2-10.

Panchenko, L. F., Pirozhkov, S. V., Nadezhdin, A. V., VIu, B., & Usmanova, N. N. (1999). Lipid peroxidation, peroxyl radical-scavenging system of plasma and liver and heart pathology in adolescence heroin users. *Voprosy meditsinskoi khimii*, *45*(6), 501-506.

Principato, G. B., Aisa, M. C., Talesa, V., Rosi, G., & Giovannini, E. (1985). Characterization of the soluble alkaline phosphatase from hepatopancreas of Squilla mantis L. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry, 80(4), 801-804.

Pawar, H., Karde, M., Mundle, N., Jadhav, P., & Mehra, K. J. M. C. (2014). Phytochemical evaluation and curcumin content determination of turmeric rhizomes collected from Bhandara District of Maharashtra (India). *Med. Chem*, *4*(8), 588-591.

Quiles, J. L., Mesa, M. D., Ramírez-Tortosa, C. L., Aguilera, C. M., Battino, M., Gil, Á., & Ramírez-Tortosa, M. C. (2002). Curcuma longa extract supplementation reduces oxidative stress and attenuates aortic fatty streak development in rabbits. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *22*(7), 1225-1231.

Raffa, R. B., Buschmann, H., Christoph, T., Eichenbaum, G., Englberger, W., Flores, C. M., ... & Tzschentke, T. M. (2012). Mechanistic and functional differentiation of tapentadol and tramadol. *Expert opinion on pharmacotherapy*, *13*(10), 1437-1449.

Reitman, S., & Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American journal of clinical pathology*, *28*(1), 56-63.

Sanaa, A., Boulila, A., Boussaid, M., & Fadhel, N. B. (2014). Pancratium maritimum L. in Tunisia: Genetic and chemical studies among the threatened populations. *Industrial Crops and Products*, *60*, 75-78.

Sayed, H. Y., & Zidan, A. H. (2016). Histopathological and biochemical effects of acute and chronic tramadol drug toxicity on liver, kidney, and testicular function in adult male albino rats. *J Forensic*, *1*(2), 41.

Scannell, J. W., Blanckley, A., Boldon, H., & Warrington, B. (2012). Diagnosing the decline in pharmaceutical R&D efficiency. *Nature reviews Drug discovery*, *11*(3), 191-200.

Sheweita, S. A., Almasmari, A. A., & El-Banna, S. G. (2018). Tramadol-induced hepato-and nephrotoxicity in rats: Role of Curcumin and Gallic acid as antioxidants. *PLoS One*, *13*(8), e0202110.

Simeon, G. G., & Abbey, S. T. (2018). Some marker enzymes and histological alteration on the administration of tramadol hydrochloride on rat liver. *Modern Research in Inflammation*, *7*(1), 9-20.

Somsak, V., Polwiang, N., & Chachiyo, S. (2016). In vivo antimalarial activity of Annona muricata leaf extract in mice infected with Plasmodium berghei. *Journal of pathogens*, *2016*.

Walters, M. I., & Gerarde, H. W. (1970). An ultramicromethod for the determination of conjugated and total bilirubin in serum or plasma. *Microchemical Journal*, *15*(2), 231-243.

AlQalam Journal of Medical and Applied Sciences
Special Issue for 6th International Conference in Basic Sciences and Their Applications
(6th ICBSTA, 2023), https://journal.utripoli.edu.ly/index.php/Alqalam eISSN 2707-7179

Juniperus turbinata dieback in El-Jabal El-Akhdar Mountain, northeast Libya: insights from dendrochronological analysis

Ahmed A. Al-Shaikhy[1], Manam W. B. Saaed[2*] and Yacoub M. El-Barasi[3]
[1]*Department of Environmental Sciences and Engineering, Libyan Academy, Benghazi.*
[2]*Department of Botany, Faculty of Arts and Sciences-Al-Abyar, Benghazi University.*
[3]*Department of Botany, Faculty of Sciences, Benghazi University.*
***Corresponding author:** manam.saaed@uob.edu.ly

Abstract:

Juniperus turbinata Guss. plays a vital role in the ecosystems of El-Jabal El-Akhdar Mountain, comprising an essential component of its climax community. However, it is susceptible to significant deterioration due to various factors, including tree dieback. The current work aims to understand this phenomenon and examine its interaction with drought events by utilizing dendrochronological analysis. The study was conducted at 14 study sites across the entire El-Jabal El-Akhdar Mountain, and trunk core samples from 4-6 trees at each site were collected and investigated. The examined trees have an age range of 79-269 years, and the region has experienced successive cycles of drought. A significant 85% of the study sites have encountered notable water stress caused by the ongoing drought cycle, which has exceeded expectations in terms of duration and impact. The effects of the drought vary across different areas due to local site characteristics and various anthropogenic activities. The findings suggested that the drought cycles are an integral component of the ecosystems and have a prolonged period of drought surpassing common perceptions, and the tree dieback appears to be an adaptive response to these drought cycles, achieved by reducing the overall green biomass. The proliferation of dense epiphytes is thought to occur as a consequence of their capacity to exploit weakened trees and capitalize on available sunlight following leaf shedding and branch exposure. Increased future drought intensity and duration will likely increase juniper dieback rates, extending its prevalence to various other tree species in the study area.

Keywords: El-Jabal El-Akhdar, dendrochronology, dieback, drought, juniper trees.

Introduction:

This work marked the second phase of a series of studies focused on understanding the phenomenon of *Juniperus turbinata* Guss. tree dieback in El-Jabal El-Akhdar Mountain (EEM). The first study (Saaed *et al.*, 2021) focused on identifying the epiphytes growth associated with this phenomenon in the aforementioned tree species. The EEM encompasses a distinctive ecosystem renowned for its exceptional environmental characteristics and unique ecosystems, rendering it one of Libyan's most significant vegetated zones. Of particular importance within this ecosystem is the *J. turbinata* tree (Henceforth, it is referred to as the juniper tree), which is deemed a key component of the climax community coexisting with numerous other species. The juniper tree exhibits remarkable ubiquity throughout the entire altitudinal range of the EEM ranging between 0-884 m above sea level (a.s.l.) and is distributed across all its areas, accounting for approximately 70% of the vegetation cover (Saaed *et al.*, 2021). However, there is a growing concern that the widespread dieback of this tree in this mountainous area could lead to a significant reduction in the green areas within a country where approximately 95% of the land comprises hot desert regions (Sahara). The *J. turbinata* tree possesses a broad geographical distribution and is not currently classified as globally threatened. However, its range within Libya is experiencing a declining trend due to the combined effects of climate change, anthropogenic pressures, and the dieback phenomenon (Saaed *et al.*, 2021). This evergreen coniferous tree belongs to the *Cupressaceae* family. The currently accepted taxonomic nomenclature for this taxon is *Juniperus turbinata* Guss. [=*Juniperus phoenicea* subsp. *turbinata* (Guss.) Nyman], previously recognized as *Juniperus phoenicea* L. (Pavon, 2020; APD, 2023; POWO, 2023). In recent decades, there has been a notable occurrence of extensive forest mortality documented globally (Allen *et al.*, 2010). However, the initial documentation of tree dieback within the EEM can be traced back to the late 1980s when local residents, residing around El-Marj city northwest of the EEM, submitted a report to the relevant authorities, highlighting the occurrence of this phenomenon. Tree dieback signifies a highly critical form of degradation that poses a severe threat to forest ecosystems in the EEM. The phenomenon observed in the EEM is characterized by a decline in tree vigour and growth, accompanied by a significant increase in crown mortality rates. This leads to the eventual demise of foliage, twigs, and branches, typically

starting from the outermost parts and rarely spreading throughout the entire tree (Fig. 1). Notably, it is associated with a profuse proliferation of epiphytes. However, widespread and complete tree mortality has not been observed on a large scale. Based on previous studies conducted elsewhere (e.g., Rice *et al.*, 2004; Linaldeddu, 2011; El-Juhany, 2015; Camarero *et al.*, 2020), the causes of juniper tree mortality or weakening have been attributed to various factors such as specific types of fungi, bacteria, or water stress. In the EEM, previous investigations (e.g., Sa'id and Al-Maswari, 1990; Zaid *et al.*, 2005; Ali and El Shatshat, 2015) have not definitively determined the cause of juniper dieback. The ongoing and inconclusive debate surrounding this phenomenon and its underlying causes, whether it relates to biotic agents (e.g., insects and pathogens), hydraulic failure, or carbon starvation, hampers our ability to model and predict the risk of forest dieback and alterations in species distribution ranges. The lack of a clear understanding coupled with the escalating rates of forest dieback served as the primary motivation for conducting this series of studies. The present work specifically focuses on investigating the growth rings of juniper trees with the aim of addressing the fundamental question: Do the growth rings of this tree species provide an explanation for the dieback phenomenon observed in the EEM?

Figure 1. The dieback of *Juniperus turbinata* trees is characterized by branch mortality and the shedding of leaves, starting from the upper canopy and outermost sections of the main branches, concomitant with a profuse proliferation of epiphytes.

Materials and Methods:

Study area

The EEM is a low- to medium-mountainous region located in the northeastern part of Libya along the Mediterranean Sea (Figure 2). It is distinguished by a predominantly Mediterranean climate, featuring short warm and wet winters, and long hot and dry summers. Annual rainfall in the northern areas ranges from 400 to 600mm, while the evapotranspiration rate is approximately 2000mm per year. Conversely, the southern parts exhibit a semi-desert climate, with low and erratic rainfall typically below 200mm per year, and high evaporation rates exceeding 2000mm annually (El-Barasi *et al.*, 2011). Covering an approximate area of 8000 km^2, the EEM encompasses dense vegetation spanning around 5000 km^2. The vegetation mostly consists of Mediterranean Garigues, characterized by short trees, shrubs, sub-shrubs, and ephemerals, mainly on shallow and alkaline soils. The region has been inhabited since ancient times, and the main anthropogenic activities include firewood collection, charcoal production, harvesting of medicinal and aromatic species, vegetation clearance for agricultural practices, overgrazing, and wildlife overhunting (El-Barasi *et al.*, 2011). In recent decades, substantial urbanization has encroached upon natural habitats.

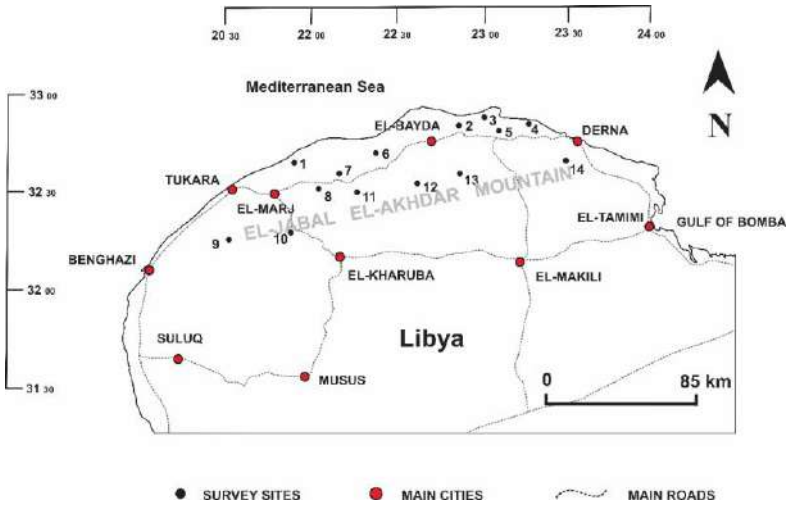


Figure 2. The geographical location of the study area, El-Jabal El-Akhdar Mountain, illustrates the spatial distribution of the different survey sites across the entire region.

Plot establishment and data collection

The study area (EEM) was divided into three sections: northern, middle, and southern, based on the distance from the coast and altitude (m a.s.l.). To ensure comprehensive coverage of the juniper tree distribution, 14 survey sites were carefully selected; four in the northern section, five in the middle section, and five in the southern section (Figure 2). Fieldwork was carried out between 2020 and 2021, during which comprehensive data at each survey site were collected, including coordinates, altitude, slope %, soil surface condition, vegetation description, and anthropogenic activities. Greater emphasis was placed on the evaluation of juniper trees regarding their height, vigour, and extent and severity of dieback. Additionally, the intensity of epiphytes on the juniper trees was assessed. To investigate the tree age and the duration and influence of dry and wet years, dendrochronological samples were collected and processed (Fritts, 1976; Maxwell *et al.*, 2011). An increment borer was used, consisting of a hollow cylindrical metal drill, two rotating arms, and a metal tongue. At each survey site, four to six trees were chosen based on their suitability for trunk core sampling (trunk height, diameter, and shape). Careful considerations were made to ensure coring was done at a right angle on a section of the tree trunk without distorted growth (Stokes and Smiley, 1968). The aim was to target the pith of the tree to include all growth rings and facilitate subsequent analysis. The core samples were carefully collected and stored in thick-wall paper cylinders, transferred to the laboratory, air dried, and mounted onto special wooden platforms to ensure stability. Underwent progressive sanding with different types of sandpaper until a smooth polish was achieved, allowing for clear visibility of the tree rings. The growth rings are distinguished by a clear contrast between the final few rows of flattened and thick-walled late-wood tracheid and the larger and thin-walled early-wood tracheid of the subsequent growth ring. This distinct difference in cell characteristics marked the boundaries of each growth ring, with every ring representing a year of the tree's life. A binocular microscope was used to observe the growth rings and absolute dates have been assigned to each ring in the samples (Maxwell *et al.*, 2011), and ring thickness was measured with a precision of 0.1 mm. Trends in ring thickness were examined over 10-year intervals to facilitate efficient data analysis and the generation of relevant graphs.

Figure 3. An increment borer was used to extract the trunk corer from the *Juniperus turbinata* tree at the study area (El-Jabal El-Akhdar Mountain)

Figure 4. An increment core was extracted from a *Juniperus turbinata* tree at the study area (El-Jabal El-Akhdar Mountain).

Statistical analyses

After the completion of data collection, a thorough review and validation process was conducted. Subsequently, data was organized, tabulated, systematically arranged, and entering it into the Microsoft Excel 2019 spreadsheet program. To determine dry and wet years, the overall mean value of all samples was computed and used as the baseline for the normal thickness of growth rings. Values above this baseline were considered wet years, while values below were classified as dry years. Utilizing the statistical software IBM SPSS version 26, descriptive analyses were performed, followed by an assessment of data distribution to ascertain its normal or non-normal distribution. In instances where the data exhibited a normal distribution, an analysis of variance (ANOVA) was conducted. Conversely, for non-normally distributed data, a Kruskal-Wallis one-way ANOVA was employed to detect any significant variation among the data sets. A correlation test was examined to identify potential relationships between the variables under investigation. The time series analysis for growth ring values and general polynomial trend were performed using the Excel program.

Results:

Field observations

The survey sites examined in this study encompassed a broad range of altitudes, spanning from 29 to 785 m a.s.l (mean = 383.71±18.010 SE). The slope degree varied between 2 and 69% (mean = 17.81±1.521 SE). The stone

and rock cover on the soil surface ranged from 8 to 100% (mean = 39.63±1.898 SE (Table 1). The vegetation cover percentage, considering all species, varied between 40 and 85% (mean = 68.33%±0.958 SE). For juniper trees, the percentage cover ranged from 38.6 to 83.9% (mean = 62.31%±1.240 SE). Tree trunk diameter varied between 5 and 90 cm (mean = 27.72±1.189 SE), and total tree height ranged from 0.5 to 12 m (mean = 3.64 m±0.161 SE). The percentage of juniper trees affected by dieback fluctuated between 0 and 85% (mean = 36.21%±7.973 SE), and the percentage of dieback observed in other tree species ranged from 0 to 60% (mean = 11.14%±4.837 SE).

Table 1. The Minimum, Maximum, Mean, and Standard Error values for the investigated parameters at the various survey sites in El-Jabal El-Akhdar Mountain

Parameters	Minimum	Maximum	Mean	Std. Error
Altitude (m a.s.l.)	29	785	383.71	18.010
Slope %	2	69	17.81	1.521
Stone and rock %	8	100	39.63	1.898
Total vegetation cover %	40	85	68.33	0.958
J. turbinata trees cover %	38.6	83.9	62.31	1.240
J. turbinata tree trunk diameter (cm)	5	90	27.72	1.189
J. turbinata trees height (m)	0.5	12	3.64	0.161
Affected *J. turbinata* trees %	0	85	36.21	7.973
Affected other trees species %	0	60	11.14	4.837

Dendrochronological analysis

The results offered valuable insights into the characteristics of drought cycles in the EEM. They revealed the presence of periodic drought events, which indicated that water stress periods (drought cycle) are an integral component of the ecosystems in the EEM and have a prolonged range of drought than expected. About 85.7% of the survey sites illustrated that the area now is experiencing a drought period (from 30 to 90 years), the duration differs from one site to another due to the variation in rainfall and site characteristics within the EEM (Figs. 5-18). The longest water stress period was noticed in survey site 3 (Fig. 7), and the least was observed in survey site 10 (Fig. 14) and 12 (Fig. 16). According to the general historical trend covered by the dendrochronological analysis (Fig. 19), a previous wet period extended for several decades and ended around the end of the 1770s, followed by a drought

period that lasted for eight decades and ended around the end of the 1850s. This was followed by a wet period that lasted for ten decades and ended around the end of the1950s, and then a drought period that continues to this day, which seems to be more profound and impactful than the previous cycles. The results revealed a significant difference (*p*-value = 0.000) in tree age among the various geographical locations (Northern, Middle, and Southern sections).

The trees in the southern section exhibited the oldest age ranging between 129-269 years. In the northern section ranging between 119-179 years. While the middle section was characterized by the presence of comparatively younger trees ranging between 79-129 years. The results showed no statistically significant correlation between tree age versus site altitude, surface slope, and epiphytes density. However, there was a different response to the drought periods among the various geographical locations. Unexpectedly, the trees in the southern section of the EEM exhibited a lesser degree of vulnerability to drought periods, consequently displaying longer lifespans. In contrast, the trees in the northern section displayed greater susceptibility to drought and shorter lifespans. The trees in the middle section recorded the highest rate of vulnerability to drought periods, accompanied by the shortest lifespan (Fig. 20).

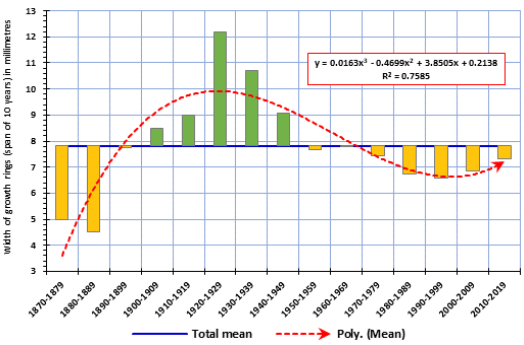


Figure 5. Time series analysis of the dendrochronological results obtained from survey site 1, computed at 10-year intervals.

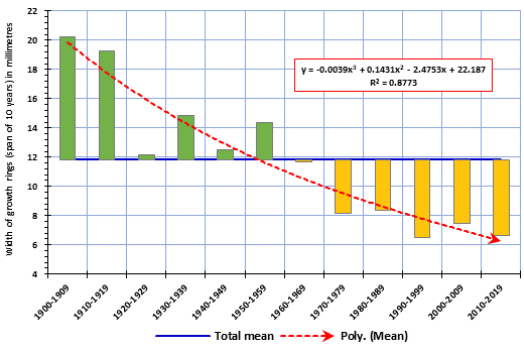


Figure 6. Time series analysis of the dendrochronological results obtained from survey site 2, computed at 10-year intervals.

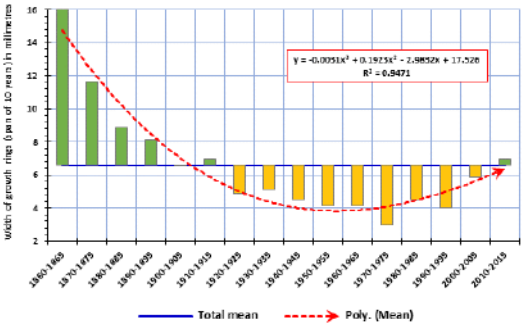


Figure 7. Time series analysis of the dendrochronological results obtained from survey site 3, computed at 10-year intervals.

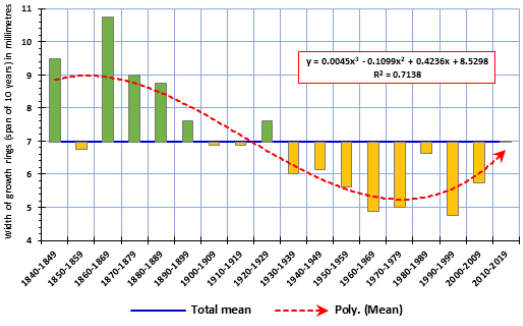


Figure 8. Time series analysis of the dendrochronological results obtained from survey site 4, computed at 10-year intervals.

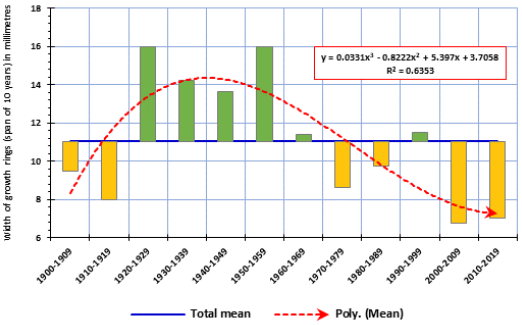


Figure 9. Time series analysis of the dendrochronological results obtained from survey site 5, computed at 10-year intervals.

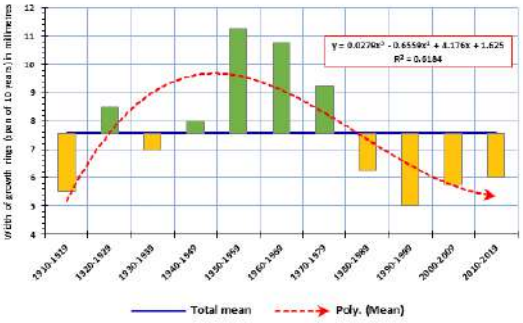


Figure 10. Time series analysis of the dendrochronological results obtained from survey site 6, computed at 10-year intervals.

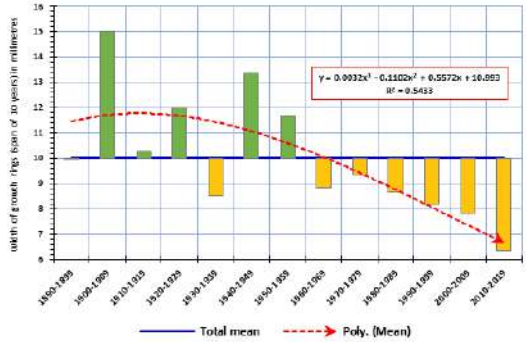


Figure 11. Time series analysis of the dendrochronological results obtained from survey site 7, computed at 10-year intervals.

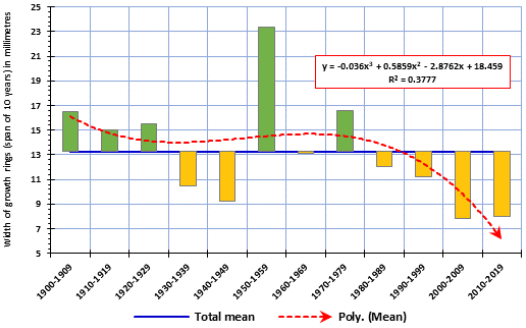


Figure 12. Time series analysis of the dendrochronological results obtained from survey site 8, computed at 10-year intervals.

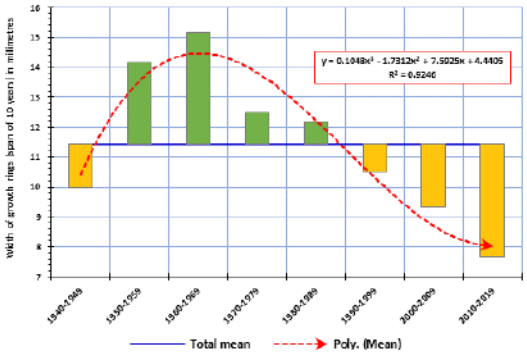


Figure 13. Time series analysis of the dendrochronological results obtained from survey site 9, computed at 10-year intervals.

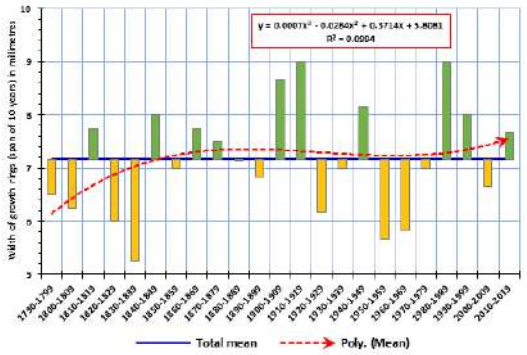


Figure 14. Time series analysis of the dendrochronological results obtained from survey site 10, computed at 10-year intervals.

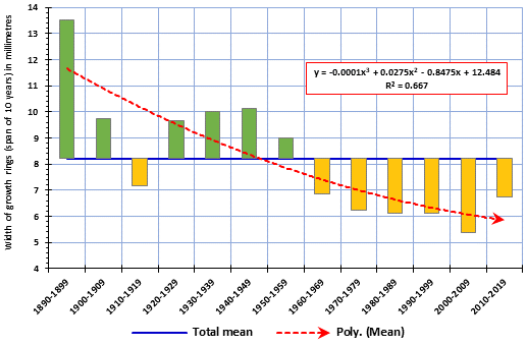


Figure 15. Time series analysis of the dendrochronological results obtained from survey site 11, computed at 10-year intervals.

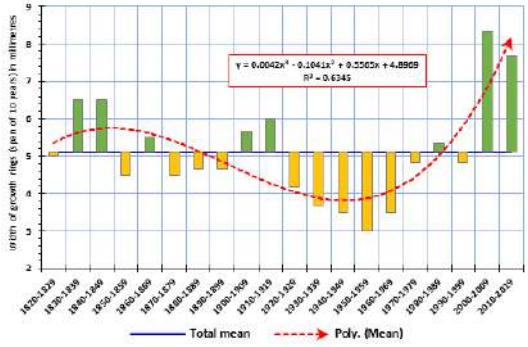


Figure 16. Time series analysis of the dendrochronological results obtained from survey site 12, computed at 10-year intervals.

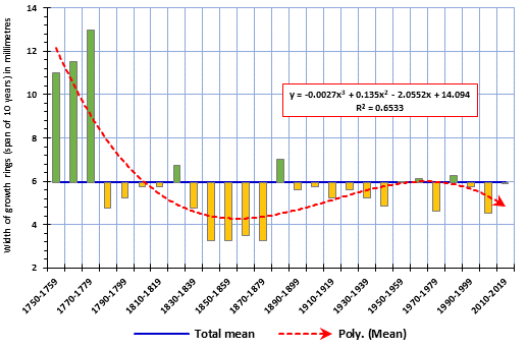


Figure 17. Time series analysis of the dendrochronological results obtained from survey site 13, computed at 10-year intervals.

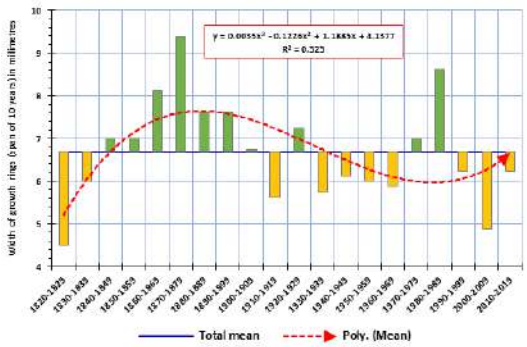


Figure 18. Time series analysis of the dendrochronological results obtained from survey site 14, computed at 10-year intervals.

Figure 19. The general time series analysis illustrates the prolonged sequences of drought cycles in El-Jabal El-Akhdar Mountain.

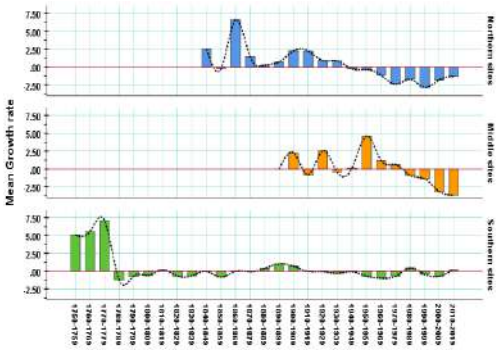


Figure 20. Time series analysis illustrates the different responses to drought cycles among the various geographical locations in El-Jabal El-Akhdar Mountain (Northern, Middle, and Southern sections).

Discussion:

While the study area has not witnessed widespread and complete tree mortality on a large scale, similar to what has been observed in various regions worldwide (Matusick *et al.*, 2013), instances of partial death among individual juniper and other tree species are evident. In times of severe or prolonged drought, crown defoliation seems to function as a survival mechanism (Koepke *et al.*, 2010; DeSoto *et al.*, 2020). The growth rings analysis of juniper trees in this study reveals a reduction in their growth rate below mean levels, in recent decades, as a result of a prolonged drought period that affected the EEM. The southern section of the EEM has been the least affected by drought cycles, with trees in this area exhibiting the longest lifespan. The northern section shows a more severe impact on trees compared to the south, resulting in a shorter lifespan. The middle section exhibits the most profound impact of drought, with trees having the shortest lifespan. Previous findings (e.g., Gazol *et al.*, 2020) has shown that negative growth trends in trees often occur long before visible signs of dieback become apparent. Climate change is expected to exacerbate regional mortality events; however, prediction remains challenging because the physiological mechanisms underlying drought survival and mortality are poorly understood (McDowell *et al.*, 2008). Drought events, in such arid and semi-arid ecosystems, do not necessarily mean climate change. Therefore, it is crucial to focus not only on total annual rainfall and mean temperature but also on the rainfall patterns and the intensity and distribution of heat events. Heatwaves have been observed to increase the risk of mortality under drought conditions

(Cochard, 2019). While a temperature-driven carbon starvation mechanism has been proposed as a cause of tree dieback during hot droughts (Cochard, 2019). Differences in the alignment of wet and dry periods among the various survey sites in the EEM suggested that water stress does not solely result from changes in climate factors such as rainfall and temperature. Instead, it is likely attributed to changes in landscape functionality and disruptions in the ecosystems' balance caused by unsustainable anthropogenic activities over history. These activities have been intensified during the 20th century, leading to the profound impact of the current drought cycle. Severe droughts can reduce ecosystem resilience and trigger tree mortality. Resilience to dry conditions is crucial for the long-term survival of trees, as they often face multiple drought events throughout their lifespan (DeSoto *et al.,* 2020). Forest damage arises when the resilience threshold is breached as a result of severe drought and heat events, a phenomenon that has been observed in numerous instances worldwide. These occurrences underscore the susceptibility of forests to extreme climatic conditions and their limited capacity to withstand such stressors (Allen *et al.,* 2010). Prolonged drought periods in the EEM caused chronic stress, leading to growth reductions, crown dieback, and forest decline. Water deficit due to prolonged drought periods is widely recognized as the primary constraint on plant establishment, survival, and growth, particularly in water-scarce ecosystems. This limitation significantly affects plant physiology, reducing their ability to carry out essential functions like photosynthesis and nutrient uptake, resulting in stunted growth, wilted leaves, and potentially death under severe conditions. However, in addition to drought, other secondary factors such as unsustainable anthropogenic activities have contributed to the increased severity of the dieback phenomenon and amplified the drought effects and water budget deficit through substantial shifts in ecosystem structure and landscape functionality. The unfavourable anthropogenic activities in the EEM, have fragmented the ecosystem of the juniper forests and reduced its resilience to drought events. Ecosystem fragmentation has a great impact on the vegetation, soil microbial mass, and disturbs ecosystem balance, and profoundly alters the microclimate. This effect requires further attention and understanding in the EEM (El-Barasi & Saaed 2013). The current study suggested that the dieback in the EEM is primarily caused by prolonged drought periods. According to Mcdowell *et al.* (2008), when trees are exposed to dry years, they undergo a mechanical

process of reducing green mass to minimize water loss through transpiration. This reduction starts with the secondary terminal twigs and continues to the main branches, ultimately leading to the loss of resilience and may the death of the trees. This process, to a large extent, explains the appearance of the dieback in the EEM. To some extent, the findings of Zaid *et al.* (2005) and Ali & El Shatshat (2015) support this assumption, suggesting that drought is the main reason for tree dieback in the EEM. On the other hand, the study by Sa'id and Al-Maswari (1990) proposed bacteria causing coral knot disease as the main reason, however, it conflicts with the observation that most trees with dieback do not exhibit coral knot disease. Also, the present findings do not align with other studies that have investigated dieback on juniper trees elsewhere, which suggested that the main reason is a biotic agent (e.g., Rice *et al.*, 2004; Linaldeddu, 2011; El-Juhany, 2015; Camarero *et al.*, 2020). The vulnerability to drought-induced mortality varies between gymnosperms (e.g., juniper trees) and angiosperms. Gymnosperms are more susceptible to reduced recovery after drought, while angiosperms are more prone to lower resistance during the initial drought (DeSoto *et al.*, 2020). Gymnosperms may experience long-term legacy effects from hydraulic failure, and those with less recovery are more likely to die one or more decades after severe droughts, as their internal carbon reserves may be severely impacted (carbon starvation) under extreme hydraulic failure (DeSoto *et al.*, 2020).

Drought can lead to xylem cavitation or embolism, where the water column breaks and air and water vapor enter the xylem conduits. This hydraulic disconnection between the leaves and roots can occur due to climate change, drought, and other environmental stressors. The risk of hydraulic failure, characterized by cavitation events and water transport disruption, is closely linked to tree mortality caused by drought. As observed in the study area, mature trees, with larger volumes of aboveground woody tissues, including a thicker trunk and more abundant foliage, are more vulnerable to dieback incidents compared to younger ones due to their complex structure, which leads to increased carbon consumption and higher respiration needs, to fulfill the demands of regular life activities (Wang and Ljungqvist, 2019). The ongoing debate surrounding tree dieback is centered around three hypotheses: biotic agents, hydraulic failure, and carbon starvation (McDowell *et al.*, 2008). However, to date, there is insufficient robust evidence in the EEM to support the biotic cause hypothesis. Hydraulic failure occurs when reduced

soil water supply and high evaporative demand lead to xylem cavitation. This can occur due to various reasons, including climate change, drought, and other environmental stressors that increase transpiration rates and reduce soil moisture content (Vilagrosa *et al.,* 2012). The hydraulic architecture of the xylem network is associated with crown design and branch distribution (Vilagrosa *et al.,* 2012). Carbon starvation, on the other hand, results from stomatal closure, reducing carbon uptake for photosynthesis and leading to a decrease in carbohydrate availability. Both hypotheses offer different explanations for plant responses to water scarcity, and the relative importance of each mechanism is still under discussion in the EEM. The presence of dense epiphyte growth on the juniper trees is often associated with poor plant health due to various reasons such as prolonged drought, which is the case in the EEM (Saaed *et al.,* 2021). This tree's weak condition encourages dense epiphyte growth on bare and weak branches, utilizing available sunlight after leaf shedding. Notably, similar dense growth was observed also on other plant species in the area, albeit less commonly than in juniper trees, such as *Pistacia lentiscus* L., *Olea europaea* L., *Rosmarinus officinalis* L., *Phillyrea angustifolia* L, and *Rhamnus oleoides* L.

Conclusion:

The findings of this study offer significant insights that challenge previous beliefs regarding tree lifespan, duration of drought cycles, and causes of dieback in juniper trees. The dieback observed in the EEM most likely is an adaptive response to extreme heat and drought stresses, which was amplified in recent decades by ecosystem fragmentation and a decline in landscape functionality. This response may involve a substantial reduction in the tree's hydraulic safety margin in such arid conditions. The relationship between juniper trees' exposure to decades of drought and the dieback phenomenon is that prolonged drought causes a mechanical process of reducing green mass to minimize water loss. This process leads to the death of parts of the trees starting with leaf shedding and death of terminal twigs, in severe cases, it extends to the main branches and may cause a complete tree death. Juniper trees as a gymnosperm species, exhibit higher susceptibility to limited recovery following drought periods. The recent drought-related mortality events that have been observed worldwide (Suarez *et al.,* 2004; Allen & Breshears, 2007) have the potential to become more frequent and exert an

impact on larger areas (Jentsch *et al.*, 2007). The EEM is no exception, therefore, increased intensity and duration of future droughts will increase rates of juniper dieback, which is expected to be more common on many other tree and shrub species in the area. More detailed investigations are needed to explore the impact of drought cycles and their association with other factors, e.g., anthropogenic activities, biotic agents, hydraulic failure, and carbon starvation.

Acknowledgment:

We would like to express our sincere gratitude to Professor Shyane M. Jacobs from the Department of Conservation Ecology and Entomology at Stellenbosch University in South Africa. Professor Jacobs generously provided some tools and equipment used in the collection of tree growth ring samples. Additionally, he kindly offered valuable insights and guidance during our discussions on the study's findings. Unfortunately, Professor Jacobs passed away before publishing this work.

References:

Ali, M. and El Shatshat, S. (2015). Ecological study of *Juniperus phoenicea* L. in EL-Jabal El-Akhdar area, Libya. European Journal of Experimental Biology. 5(7):71–76.

Allen C.D., Breshears D.D. (2007). Climate-induced Forest dieback as an emergent global phenomenon. Eos, Transactions American Geophysical Union 88: 504–505.

Allen C.D., Macalady A.K., Chenchouni H., Bachelet D., McDowell N., Vennetier M., Kitzberger T., Rigling A., Breshears D.D., Hogg E.H., Gonzalez P., Fensham R., Zhang Z., Castro J., Demidova N., Lim J.H., Allard G., Running S.W., Semerci A., Cobb N. (2010). A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. For Ecol Manag 259:660–684.

APD (2023). The African Plant Database. Facilitated by the South African National Biodiversity Institute, the Conservatoire et Jardin Botaniques de la Ville de Genève, Tela Botanica, and the Missouri Botanical Garden. Available from: https://africanplantdatabase.ch/en (accessed 02 August 2023).

Camarero J. Julio, Gazol Antonio, Sánchez-Salguero Raúl, Sangüesa-Barreda Gabriel, Delgado Ricardo Díaz-, Casals Pere (2020). Dieback and mortality of junipers caused by drought: Dissimilar growth and wood isotope patterns preceding shrub death, Agricultural and Forest Meteorology, 291, 108078.

Cochard Hervé. (2019). A new mechanism for tree mortality due to drought and heatwaves. BioRxiv, pp.531632. 10.1101/531632. hal-02273372

DeSoto L., Cailleret M., Sterck F., Jansen S., Kramer K., Robert E., Aakala T., Amoroso M., Bigler C., Camarero J.J., Čufar K., Gea-Izquierdo G., Gillner S., Haavik L., Hereş Ana-Maria, Kane J., Kharuk V., Kitzberger T., Klein T., Levanič T., Linares J.C., Mäkinen H., Oberhuber W., Papadopoulos A., Rohner B., Sangüesa-B.G., Stojanovic D.B., Suárez M.L., Villalba R., and Martínez-Vilalta J. (2020). Low growth resilience to drought is related to future mortality risk in trees. Nature Communications, https://doi.org/10.1038/s41467-020-14300-5

El-Barasi, Y.M., Saaed M.W., El-Amronuni A.O. and Mohamad N.F. (2011). Checklist of flora and vegetation in south El-Marj Zone: South El-Jabal El-Akhadar–Libya. Annals of Faculty Engineering Hunedoara, Tome IX (Fascicule 3) 141-146.

El-Barasi, Y.M. and Saaed, M.W.B. (2013). Threats to plant diversity in the Northeastern part of Libya (El-Jabal El- Akhdar and Marmarica Plateau). Journal of Environmental Science and Engineering 2:41-58.

El-Juhany L. (2015). The Magnitude of Dieback on *Juniperus procera* trees in the Natural Forests in the Southwestern Region of Saudi Arabia. Biosciences Biotechnology Research Asia, 12(1), 219-230.

Fritts, H. C. (1976). Tree Rings and Climate. London: Academic Press.

Gazol A., Sangüesa-Barreda G., and J. Julio C. (2020). Forecasting forest vulnerability to drought in Pyrenean Silver Fir Forests Showing Dieback. Front. For. Glob. Change 3:36. doi: 10.3389/ffgc.2020.00036

Jentsch A., Kreyling J., Beierkuhnlein C. (2007). A new generation of climate-change experiments: events, not trends. Frontiers in Ecology and the Environment 5: 365–374.

Koepke D.F., Kolb T.E., and Adams H.D. (2010). Variation in woody plant mortality and dieback from severe drought among soils, plant groups, and species within a northern Arizona ecotone. Oecologia, 163:1079–1090.

Linaldeddu B.T., Scanu B., Maddau L., and Franceschini A. (2011). *Diplodia africana* causing dieback on *Juniperus phoenicea*: a new host and first report in the northern hemisphere. Phytopathol. Mediterr, 50, 473−477.

Matusick G., Ruthrof K.X., Brouwers N.C., Dell B., Hardy G. (2013). Sudden forest canopy collapse corresponding with extreme drought and heat in a Mediterranean-type Eucalypt Forest in southwestern Australia. Eur J Forest Res, DOI 10.1007/s10342-013-0690-5

Maxwell R.S., Wixom J.A., and Hessl A.E. (2011). A comparison of two techniques for measuring and cross-dating tree rings. Dendrochronologia 29: 237-243.

McDowell N., Pockman W.T., Allen C.D., Breshears D.D., Cobb N., Kolb T., Plaut J., Sperry J., West A., Williams D.G., and Yepez E.A. (2008). Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? New Phytologist, 178: 719–739.

Pavon D., Véla E., and Médail f. (2020). Are Mediterranean trees well known? "*Juniperus turbinata*" (*Cupressaceae*), a common but misunderstood taxon. Ecologia Mediterranea, 46 (2): 77-104.

POWO (2023). Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. Available from: http://www.plantsoftheworldonline.org (accessed 02 August 2023).

Rice K.J., Matzner S.L., Byer W., and Brown J.R. (2004). Patterns of tree dieback in Queensland, Australia: the importance of drought stress and the role of resistance to cavitation. Oecologia, 139: 190–198 DOI 10.1007/s00442-004-1503-9

Saaed M.W.B, EL-Barasi Y.M, El-Shaikhy A.A. (2021). Noteworthy records of the lichens associated with *Juniperus phoenicea* L. dieback in El-Jabal El-Akhdar region, NE Libya. Ecologia Mediterranea, 47 (2):41-49.

Sa'id M.A., and Al-Maswari M.A. (1990). Isolation and characterization of the causal organism of bacterial knot disease of *junipers phoenicea* L. Arab J. Pl. Prot., 8(2):131-132.

Stokes M.A., Smiley T.L. (1968). An introduction to tree-ring dating. University of Chicago Press, Chicago, IL.

Suarez M.L., Ghermandi L., Kitzberger T. (2004). Factors predisposing episodic drought-induced tree mortality in Nothofagus–site, climatic sensitivity, and growth trends. Journal of Ecology 92: 954–966.

Vilagrosa A., Chirino E., Peguero-Pina J.J., Barigah T.S., Cochard H., and Gil-Pelegrín E. (2012). Xylem Cavitation and Embolism in Plants Living in Water-Limited Ecosystems. In Aroca R. (ed.), Plant Responses to Drought Stress, Springer-Verlag Berlin Heidelberg. DOI: 10.1007/978-3-642-32653-0_3

Wang X., Yang B., and Ljungqvist F.C. (2019). The vulnerability of Qilian Juniper to extreme drought events. Front. Plant Sci. 10:1191. doi: 10.3389/fpls.2019.01191

Zaid, A.A., Aldomi F.M., Bataw A.A., Al-Saadi O.R., Mustafa A.M., Ammar A.A., et al. (2005). Study and evaluation of natural vegetation cover in the Green Mountain region, Southern Green Mountain Project, Second periodic report, Omar Al-Mukhtar University, Al-Bayda, Libya.

AlQalam Journal of Medical and Applied Sciences
Special Issue for 6th International Conference in Basic Sciences and Their Applications
(6th ICBSTA, 2023), https://journal.utripoli.edu.ly/index.php/Alqalam *eISSN 2707-7179*

The Connection between Regular Relation and Subcanonical Hypergroup of Canonical Hypergroup

Yasser A. AbouElwan* and Maher A. Alderawe
[1]*Department of Mathematics, Faculty of Science, University of Omar Al-Mukhtar, Libya*
Correspondence authors: yasser.abouelwan@omu.edu.ly

Abstract:

For various hyperstructures, such as semihypergroups, hypergroups, and canonical hypergroups, we specifically examine the concept of quotient. In order to understand the concepts of regular relations, the canonical hypergroup is used as the basis for their introduction and analysis. This allows us to connect the subcanonical hypergroup and the regular relation which defined on the canonical hypergroup.

Keywords: Canonical hypergroup, subcanonical hypergroup, normal subcanonical hypergroup, regular relation, and quotient of canonical hypergroup.

Introduction:

The concept of hyperstructure, especially hypergroup, was introduced in 1934 by the French mathematician (Marty, 1934). Basic definitions and propositions about the hyperstructures are found in Burris and Sankappanavar (1981); Corsini (1993); Corsini and Leoreanu (2003); Davvaz and Leoreanu-Fotea (2007); Davvaz (2013); Davvaz (2022); AbouElwan *et al.* (2019 a); AbouElwan *et al.* (2019 b), AbouElwan *et al.* (2022). Hyperstructures have many applications to other areas of various sciences. Many books and papers have been published related to the applications of hyperstructures in the fields of geometry, hypergraphs, binary relations, lattices, fuzzy sets, etc. (Vougiouklis; 1994; Davvaz, 2013). Canonical hypergroup as a special kind of hypergroups is indeed a natural generalization of the concept of abelian group. This kind of hypergroup is a basic additive hyperstructure of many hyperstructures. Burris and Sankappanavar (1981) established connection between congruence on group G (ring R) and normul subgroup of G (ideal of ring R). By using a certain type of equivalence relations, semihypergroup can be connected to semigroup, hypergroup to group and canonical hypergroup to abelian group. These equivalence relations are called regular relations. More

exactly, by given (a semihypergroup, a hypergroup, and a canonical hypergroup) and by using a regular relation on them, (a semigroup, a group, and an abelian group, respectively) can be constructed from their quotient hyperstructures.

1. Preliminaries

Firstly, let me introduce some preliminary concepts:

Definitions 1.1.

1. A hyperoperation o on a non-empty set H is a mapping $o: H \times H \to P^*(H)$, where $P^*(H)$ is the set of all non-empty subsets of H. Moreover, the couple (H, o) is called a hyper-groupoid. For any two non-empty subsets A and B of H and $x \in H$, the sets $A\ o\ B$, $A\ o\ x$ and $x\ o\ A$ are defined by $A\ o\ B = \bigcup\{a\ o\ b \mid a \in A, b \in B\}$, $A\ o\ x = A\ o\ \{x\}$ and $x\ o\ A = \{x\}\ o\ A$.
2. A hypergroupoid (H, o) is called a semihypergroup if for all a, b, c of H, we have
   $a\ o\ (b\ o\ c) = (a\ o\ b)\ o\ c$, this means that $\bigcup_{u \in b\ o\ c} a\ o\ u = \bigcup_{v \in a\ o\ b} v\ o\ c$.
3. A semihypergroup (H, o) is called a hypergroup if for all $a \in H$, we have $aoH = Hoa = H$, that is called the reproduction axiom. A hypergroup (H, o) is called a commutative hypergroup if for all $a, b \in H$, we have $a\ o\ b = b\ o\ a$.
4. A non-empty subset K of a hypergroup (H, o) is called a subhypergroup of H if K is a hypergroup under the hyperoperation o. In other words, it is a hypergroup according to the hyperoperation on H. Several books have been written on hyperstructure theory (Corsini, 1993; Corsini and Leoreanu, 2003).

Definitions 1.2. Let (H, o) be a semihypergroup and R be an equivalence relation on H. If A, B are non-empty subsets of H, then $A\bar{R}B$ means that $\forall a \in A, \exists b \in B$ such that aRb, and $\forall b` \in B, \exists a` \in A$ such that $a`Rb`$.

Furthermore, $A\bar{\bar{R}}B$ means that $\forall a \in A, \forall b \in B$, we have aRb.

In addition, the equivalence relation R on H is said to be:

1) Regular on the right (on the left) if for all x of H, from aRb, it follows that $(a\ o\ x)\bar{R}(b\ o\ x)$ $((x\ o\ a)\bar{R}(x\ o\ b)$ respectively).
2) Strongly regular on the right (on the left) if for all x of H, from aRb, it follows that $(a\ o\ x)\bar{\bar{R}}(b\ o\ x)$ $((x\ o\ a)\bar{\bar{R}}(x\ o\ b)$ respectively).

3) Regular (strongly regular) if it is regular (strongly regular) on the right and on the left (AbouElwan, 2022).

Let (H, o) be a hypergroup, for an equivalence relation R on H, we use $R(x)$ to denote the equivalence class of x with respect to R and use H/R to denote the family of equivalence classes $\{R(x) \mid x \in H\}$ of R. The reader can find the proofs of the following two theorems in Davvaz and Leoreanu-Fotea (2007); Davvaz (2022).

Theorem 1.3. If (H, o) is a semihypergroup (respectively, a hypergroup) and R is a regular relation on H, then the quotient H/R is a semihypergroup (respectively, a hypergroup) under the operation defined by

$$R(x)\otimes R(y) = \{R(z) \mid z \in x\ o\ y\}.$$

Theorem 1.4. If (H, o) is a semihypergroup (respectively, a hypergroup) and R is a strongly regular relation on H, then the quotient H/R is a semigroup (respectively, a group) under the operation defined by

$$R(x)\otimes R(y) = R(z), \text{ for all } z \in x\ o\ y.$$

Definition 1.5. A canonical hypergroup $(M, +)$ is a non-empty set M together with a hyper-operation $+$ which satisfies the following axioms:

i. for every $x, y \in M$, $x + y = y + x$,

ii. for every $x, y, z \in M$, $x + (y + z) = (x + y) + z$,

iii. there exists $0 \in M$ (called neutral element of M) such that $0 + x = \{x\} = x + 0$, for all $x \in M$,

iv. for every $x \in M$, there exists a unique element denoted by $-x \in M$ such that $0 \in x + (-x) \cap (-x) + x$,

v. for every $x, y, z \in M$, $z \in x + y$ implies $y \in -x + z$ and $x \in z + (-y)$, that is called the reversibility axiom.

A non-empty subset N of a canonical hypergroup $(M, +)$ is called a subcanonical hypergroup of M if $(N, +)$ is a canonical hypergroup itself. Equivalently, $x - y \subseteq N$, for every $x, y \in N$. In particular, for any $x \in N$, $x - x \subseteq N$. Since $0 \in x - x$, it follows that $0 \in N$. Moreover, N is said to be normal if $x + N - x \subseteq N$, for all $x \in M$. In addition, a subcanonical hypergroup N of M is called a subgroup of M if $(N, +)$ is a group, that is, if $x + y$ is a singleton set for all $x, y \in N$.

Example 1.6. Consider the set $M = \{0, a, b\}$. Define a hyperaddition + on M as in the following table

+	0	a	b	c
0	$\{0\}$	$\{a\}$	$\{b\}$	$\{c\}$
a	$\{a\}$	$\{0, b\}$	$\{a, c\}$	$\{b\}$
b	$\{b\}$	$\{a, c\}$	$\{0, b\}$	$\{a\}$
c	$\{c\}$	$\{b\}$	$\{a\}$	$\{0\}$

Then, $(M, +)$ is a canonical hypergroup, $\{0, b\}$ is a subcanonical hypergroup of M, and $\{0, c\}$ is a subgroup of M.

Remark 1.7. Let $(M, +)$ be a canonical hypergroup, and N be a subcanonical hypergroup of M, the quotient $M/N = \{x + N \mid x \in M\}$, where $x + N = \{x + n \mid n \in N\}$, we will use $\bar{x}$ instead of $x + N$.

Theorem 1.8. (Velrajan and Asokkumar, 2010) Let $(M, +)$ be a canonical hypergroup, and let N be a subcanonical hypergroup of M. Then M/N is a canonical hypergroup with respect to the following hyperoperation

$$(x + N)\oplus(y + N) = \{z + N \mid z \in x + y\}, \text{ for all } x + N,\ y + N \in M/N.$$

Proof. Let $x_1, y_1, x_2, y_2 \in M$ such that $\bar{x}_1 = \bar{x}_2$ and $\bar{y}_1 = \bar{y}_2$ then $x_2 \in x_1 + N$ and $y_2 \in y_1 + N$. Let $z_2 \in x_2 + y_2 \subseteq (x_1 + N) + (y_1 + N)$. Since M is commutative, $z_2 \in z_1 + n$ for some $z_1 \in x_1 + y_1$ and for some $n \in N$. That is, $z_2 + N = z_1 + N$. Hence,

$$\bar{x}_2\oplus\bar{y}_2 \subseteq \bar{x}_1\oplus\bar{y}_1.$$

Also, since $x_1 \in x_2 + N$ and $y_1 \in y_2 + N$, by a similar argument, we get,

$\bar{x}_1\oplus\bar{y}_1 \subseteq \bar{x}_2\oplus\bar{y}_2$. Hence,

$\bar{x}_1\oplus\bar{y}_1 = \bar{x}_2\oplus\bar{y}_2$. Thus, $\oplus$ is well defined.

Let $\bar{x}, \bar{y}, \bar{z} \in M/N$. If $\bar{u} \in (\bar{x}\oplus\bar{y})\oplus\bar{z}$, then $\bar{u} \in \bar{p}\oplus\bar{z}$ for some $\bar{p} \in \bar{x}\oplus\bar{y}$. That is, $\bar{u} = \bar{a}$ for some $a \in p + z$. Also $\bar{p} = \bar{b}$ for some $b \in x + y$.

Now, $a \in p + z \subseteq b + N + z = b + z + N$. That is, $a \in v + N$ for some $v \in b + z \subseteq (x + y) + z = x + (y + z)$. So, $v \in x + t$ for some $t \in y + z$. This means that, $\bar{a} = \bar{v}$ and $\bar{v} \in \bar{x}\oplus\bar{t}$. Since $\bar{t} \in \bar{y}\oplus\bar{z}$, we have

$$\bar{u} = \bar{a} = \bar{v} \in \bar{x}\oplus\bar{t} \subseteq \bar{x}\oplus(\bar{y}\oplus\bar{z}).$$

This means that, $\bar{u} \in \bar{x}\oplus(\bar{y}\oplus\bar{z})$. Hence,

$$(\bar{x}\oplus\bar{y})\oplus\bar{z} \subseteq \bar{x}\oplus(\bar{y}\oplus\bar{z}).$$

Similarly, we get

$$\bar{x}\oplus(\bar{y}\oplus\bar{z}) \subseteq (\bar{x}\oplus\bar{y})\oplus\bar{z}. \text{ Hence, } (\bar{x}\oplus\bar{y})\oplus\bar{z} = \bar{x}\oplus(\bar{y}\oplus\bar{z}).$$

Thus, the hyperoperation $\oplus$ is associative.

Consider the element $\bar{0} = 0 + N \in M/N$. Now, for any $x \in M$, we have

$$\bar{x}\oplus\bar{0} = \{\bar{z} \mid z \in x + 0\} = \bar{x}.$$

Similarly, $\bar{0}\oplus\bar{x} = \bar{x}$. Thus, $\bar{0}$ is the zero element of M/N.

Let $x \in M$, then $\bar{x}\oplus(-\bar{x}) = \{\bar{z} \mid z \in x + (-x) = x - x\}$. Since $\bar{0} \in x - x$, we get, $\bar{0} \in \bar{x}\oplus(-\bar{x})$. Similarly, $\bar{0} \in (-\bar{x})\oplus\bar{x}$. Let $\bar{x} \in M/N$, and suppose that $\bar{y} \in M/N$ such that $\bar{0} \in \bar{y}\oplus\bar{x}$, then $\bar{0} = \bar{a}$, where $a \in y + x$. That is, $y \in a - x \subseteq N - x$, and hence $\bar{y} = -\bar{x}$. Thus, the element $\bar{x} \in M/N$ has a unique inverse $-\bar{x} \in M/N$. Suppose that $\bar{z} \in \bar{x}\oplus\bar{y}$, then $\bar{z} = \bar{a}$, where $a \in x + y$. This implies, $x \in a - y \subseteq z + N - y$. That is, $x \in r + N$, where $r \in z - y$. Thus, $\bar{x} = \bar{r} \in \bar{z}\oplus(-\bar{y})$. Similarly, we can show that $\bar{y} \in (-\bar{x})\oplus\bar{z}$. Since M is commutative, it is obvious that M/N is also commutative. Thus, M/N is a canonical hypergroup. ■

Theorem 1.9. (Velrajan and Asokkumar, 2010) Let $(M, +)$ be a canonical hypergroup, and let N be a normal subcanonical hypergroup of M. Then, $(M/N, \oplus)$ is an abelian group.

2. MAIN RESULTS

Definition 2.1. Let $(M, +)$ be a canonical hypergroup, and ρ be an equivalence relation on M, then ρ is called:

1) Regular if for all $a, b \in M$, $a\rho b$ implies that for every $x \in M$, for every $u \in a + x$ there exists $v \in b + x$ such that $u\rho v$ and for every $v' \in b + x$ there exists $u' \in a + x$ such that $u'\rho v'$.
2) Strongly regular if for all $a, b \in M$, $a\rho b$ implies that for every $x \in M$, for every
   $u \in a + x$ and for every $v \in b + x$ one has $u\rho v$.

Theorem 2.2. Let $(M, +)$ be a canonical hypergroup, and let N be a subcanonical hypergroup of M. If a, b are elements in M, then the binary relation ρ defined on M by:

$$a\rho b \quad \text{iff} \quad a \in b + N,$$

is a regular on M with $\rho(0) = N$.

Proof. Let $a, b, c \in M$. Clearly $a \in a + 0 \subseteq a + N$, so ρ is reflexive. If $a \in b + N$ then $a \in b + n$ for some $n \in N$. That is, $b \in a - n \subseteq a + N$, so ρ is symmetric. For transtivity, if $a \in b + N$ and $b \in c + N$ then $a \in b + n$ and $b \in c + m$ for some $n, m \in N$, imply that $a \in c + m + n \subseteq c + N$. Thus ρ is an equivalence relation on M.

Next, for regularity of ρ, suppose that $a\rho b$ then $a \in b + N$, this means that $a \in b + n$ for some $n \in N$. Let $x \in M$, to prove that $(a + x)\bar{\rho}(b + x)$. If $u \in a + x$ then

$$u \in b + n + x = b + x + n \subseteq b + x + N.$$

Therefore, there exists $v \in b + x$ such that $u \in v + N$, so $u\rho v$. On the other hand, if $v' \in b + x$ and by reversibility of M then

$$v' \in a - n + x = a + x - n \subseteq a + x + N.$$

Therefore, there exists $u' \in a + x$ such that $v' \in u' + N$, so $v'\rho u'$. Thus

$$(a + x)\bar{\rho}(b + x).$$

Hence, ρ is a regular relation on M.

Now, to prove that the equivalence class $\rho(0)$ is a subcanonical hypergroup of M. Clearly, $\rho(0) = \{a \in M \mid a\rho 0\}$ and $0 \in \rho(0)$, so $\rho(0)$ is a non-empty subset of H. Let $a, b \in \rho(0)$ then $a\rho 0$ and $b\rho 0$, by regularity of ρ we have

$$(a + b)\bar{\rho}(0 + 0),$$

This means that for all $u \in a + b$ there exists $v \in 0 + 0$ which is 0 such that $u\rho 0$, so $u \in \rho(0)$ and so $a + b \subseteq \rho(0)$, since $a \in \rho(0)$ it follows that $-a \in \rho(0)$. Therefore $\rho(0)$ is a subcanonical hypergroup of M.

Finally, to prove that $\rho(0) = N$, if $a \in \rho(0)$ then $a\rho 0$ implies $a \in 0 + N$, so $a \in N$, thus $\rho(0) \subseteq N$. Conversly, if $a \in N$ then $a \in 0 + N$, it follows that $a\rho 0$ implies $a \in \rho(0)$, thus $N \subseteq \rho(0)$. Hence, $\rho(0) = N$. ■

Theorem 2.3. Let θ and ρ are two regular relations on a canonical hypergroup $(M, +)$ such that $\theta(0) = \rho(0)$ then $\theta = \rho$.

Proof. It is enough to show that

$$x\theta y \quad \text{iff} \quad x\rho y \quad \text{for all } x, y \in M.$$

Let $x, y \in M$ and suppose that $x\theta y$, since θ is regular, then $(x + x)\bar{\theta}(x + y)$, and since $0 \in x + x$, then there exists $t \in x + y$ such that $0\theta t$, so $t \in \theta(0)$. Thus, $t \in \rho(0)$ and so $0\rho t$, since ρ is also regular on M, it follows that $(x + y)\bar{\rho}\{0\}$. Since ρ is regular, then $x\rho 0$ and $y\rho 0$ and by symmetry of ρ, then we have $x\rho 0$ and $0\rho y$ imply that $x\rho y$.

Conversely, suppose that $x\rho y$, since ρ is regular on M, then $(x + x)\bar{\rho}(x + y)$, this means, there exist $0 \in x + x$ and $r \in x + y$ such that $0\rho r$, so $r \in \rho(0)$. Thus, $r \in \theta(0)$ and so $0\theta r$, but θ is also regular on M, it follows that $(x + y)\bar{\theta}\{0\}$. Since θ is regular, then $x\theta 0$ and $y\theta 0$ and by symmetry of θ, then we have $x\theta 0$ and $0\theta y$ imply that $x\theta y$. ■

Conclusion

There is a unique regular relation defined on a canonical hypergroup, such that the equivalence class $\rho(0)$ is a subcanonical hypergroup of That canonical hypergroup.

References

AbouElwan, Y., Fanoush, M. and Elmabrouk, T. (2019 b). On Hyper Ideals of Γ – hypernear-ring. *Global Libyan Journal*, 47, 01-12.

AbouElwan, Y., Samah Qadous, Sanaa Qadous. (2022). On Canonical Hypergroup and Congruence of Semihypergroup. *Global Libyan Journal*, 56, 01-07.

AbouElwan,Y., Fanoush, M. and Aldaikh,A. (2019 a). Ideals of Quotient Nearring, (JLBS), 8, 80-88.

AbouElwan, Y., Alderawe, M. (2023). On Homomorphisms and Congruences of Canonical Hypergroups. *Global Libyan Journal*, 59, 01-06.

Burris, S., & Sankappanavar, H. P. (1981). *A course in universal algebra* (Vol. 78, pp. xvi+-276). New York: Springer.

Corsini, P. (1993). Prolegomena of hypergroup theory. *Aviani editore*.

Corsini, P., & Leoreanu, V. (2013). *Applications of hyperstructure theory* (Vol. 5). Springer Science & Business Media.

Davvaz, B. (2013). *Polygroup Theory and Related Systems*, World Scientific Publishing Co. Pte. Ltd.

Davvaz, B. (2022). Hypergroup Theory, World Scientific Publishing Co. Pte. Ltd.

Davvaz, B., & Leoreanu-Fotea, V. (2007). *Hyperring theory and applications* (Vol. 347). International Academic Press, USA.

Marty, F. (1934). Sur une generalization de la notion de groups. In *8th congress Math. Scandinaves, Stockholm, (1934)*.

Velrajan, M., & Asokkumar, A. (2010). Note on isomorphism theorems of hyperrings. *International Journal of Mathematics and Mathematical Sciences, 2010*.

Vougiouklis, T. (1994). *Hyperstructures and their representations*. Hadronic Press.

AlQalam Journal of Medical and Applied Sciences
Special Issue for 6th International Conference in Basic Sciences and Their Applications (6th ICBSTA, 2023), https://journal.utripoli.edu.ly/index.php/Alqalam eISSN 2707-7179

Staphylococcus xylosus isolated from surface plants in the El-Beyda Medical Center garden and their antibiotics sensitivity

Alzubair A. R. Alhasni[1] and Nagah S. A. Abubaker[2]
[1]*Faculty of medical technology, University Darnah, Libya*
[2]*Botany Department, Faculty of Sciences, Omar AL-Mukhtar University, El-Beyda, Libya*
Correspondence authors: nagah.abubaker@omu.edu.ly

Abstract

The present study collected isolates from surfaces of plants surrounding the Al-Beida medical center were subject to bacteriological examination. Further, biochemical characteristics by use Phoenix system. Results found that the bacterial isolates were identified as: *Staphylococcus xylosus* were gram positive cocci clusters, catalase positive, coagulase negative, and for its resistance to antibiotics. All isolates were resistant to Imipenem, Cefotaxime, Ampicillin, Penicillin, Oxacillin, Amoxicillin_clavulanate and sensitive to Gentamycin, Daptomycin, Trimethoprim- sulphamethoxazole, Teicoplanin, Vancomycin, Clindamycin, Erythromycin, Linezolid, Mupirocin, Nitrofurantion, Ciprofloxacin, Moxifloxacin, Rifampin, and Tetracycline respectively. *S. xylosus* type, as pathogenic and causes urinary tract infections as these bacteria have never been isolated from the vicinity of hospitals this indicates the leakage of bacteria from inside the hospital to the surrounding area, which is dangerous for visitors and also for patients while they are inside the garden. And was noticed during the collection of samples the presence of a sewage leak inside the hospital garden, which is considered the main cause of the leakage of bacteria in the hospital environment.

Keywords: *Staphylococcus xylosus*, isolation, surface plants, El-Beida medical center, antibiotics sensitivity.

Introduction:

The main characters for Staphylococcus xylosus is a gram positive, coagulase negative, novobiocin resistant (Holt, *et al.*, 1994). To distinguished their ability to ferment carbohydrate the reaction pattern was used for the separation of *staphylococcus* species. Therefore, *S. xylosus* strains usually ferment xylose, arabinose, and β-gentiobiose in difference to other

staphylococci Schleifer and Kloos (1975). Grow at 15 - 45 °C, and at high concentration of NaCl 10 % and 15 % in growth media, possessing protease and haemolysin (Al-Mathkhury *et al*., 2008). Produce extracellular lipases (Bouaziz *et al.*, 2011). Collected from nosocomial infections, and were multi-resistant to various antibiotics (Barreire, *et al*., 2001). It is causing agent of urinary tract infections (UTI) in women (Rupp, *et al.*, 1992 and Tselenis-Kotsowilis *et al.*, 1982). And cutaneous lesion (Thornton, *et al*., 2003). After recorded high percentage (25%) from UTI patients, attention was paid to *S. xylosus* as causing agent of UTI (Al-heety, 2005). Although these organisms are part of the normal flora, they appeared to be associated with a variety of human and animal infections, and they have established themselves as The clinically significant pathogen showing increasing trends towards antibiotic resistance, which have led to rise strains that are multi resistant (WHO, 2020). Where a bacteria is capable to survive exposure to an antibiotic (Abadi *et al*., 2019). Slaughter *et al.* (2001) reported that all isolates were identify as coagulase-negative *staphylococci* with *S. sciuri* and *S. xylosus* were the most dominant. The results indicated that 99% of all isolates were resistant to *penicillin G* and 59% of the isolates were resistant to *oxacillin*. *S. xylosus* isolates were susceptibility to *ciprofloxacin* and resistant to *erythromycin* (Al-Mathkhury *et al*., 2008). Five out of 11 staphylococci isolates were resistant to penicillin and ampicillin, while all isolates were susceptibility to trimethoprim-sulfamethoxazole, vancomycin, and tetracycline (Pamela *et al.*, 2011).

Aim of the study:

- Identification of isolated bacteria and testing their sensitivity to antibiotics using the Phoenix device.

- To determine the extent of the spread of bacteria in the garden of Al-Bayda medical Center as a source of transmission of infection.

Materials and Methods:

Collection of sample

Samples were collected by swab from the surface plants in the hospital garden (El-Beyda Medical Center) placed on a selective media of mannitol salt agar (MSA) and were incubated at 37C° for 24 to 48 hours. The bacteria were purified for identification. The bacteria suspicious grown colonies was based

on gram staining and standard biochemical reactions, including catalase, coagulase and antibiotic susceptibility test (konuku *et al*., 2012).

Gram stain

The most common and useful staining procedure is the gram stain which separates bacteria into two groups according to the composition of their cell wall and was done as describe (Collee *et al*., 1996).

Catalase test

Transfer a small amount of bacterial colony to a surface of clean, dry glass slide using a sterile loop, Place a drop of 3% H_2O_2 on to the slide and mix. A positive result is the rapid evolution of oxygen (within 5-10 sec.) as evidenced by bubbling, negative result is no bubbles or only a few scattered bubbles (Collee *et al*., 1996).

Coagulase test

Divide the slide into two sections with grease pencil. One should be labeled as test and the other as control. Place a small drop of distilled water on each area. Emulsify one or two colonies of *Staphylococcus* on mannitol salt agar plate on each drop to make a smooth suspension. The test suspension is treated with a drop of citrated plasma and mixed well with a needle. Do not put anything in the other drop that serves as control. The control suspension serves (Collee *et al*., 1996).

Identification of isolates bacteria

Identify isolated bacteria by BD Phoenix automated microbiology system. (Liu, Ling and Cheng, 2005).

Results and Discussions

Identification of bacterial isolates

As the present study collected samples from the hospital garden were subject to bacteriological examination. Further, morphological and biochemical characteristics by use Phoenix system. Results as shown at tables (1and 2) found that the bacterial isolates were identified as: *S. xylosus* were gram positive cocci clusters, catalase positive, coagulase negative and fermenter of manitol. as *S.xylosus*, is present in the natural environment surrounding of humans and is classified as the cause of urinary tract infections, *S. xylosus* type, as pathogenic and causes urinary tract infections has been isolated from

the surfaces of the garden plants surrounding the hospital as these bacteria have never been isolated from the vicinity of hospitals from this indicates the leakage of bacteria from inside the hospital to the surrounding area, which is dangerous for visitors and also for patients while they are inside the garden, and noticed during the collection of samples the presence of a sewage leak inside the hospital garden, which is considered the main cause of the leakage of bacteria in the hospital environment. and for its resistance to antibiotics, was resistant to Imipenem, Cefotaxime, Ampicillin, Penicillin, Oxacillin, Amoxicillin, clavulanate sensitive to Gentamycin, Daptomycin, Trimethoprim- sulphamethoxazole, Teicoplanin, Vancomycin, Clindamycin, Erythromycin, Linezolid, Mupirocin, Nitrofurantion, Ciprofloxacin, Moxifloxacin, Rifampin, and Tetracycline respectively. The highest efficacy against *S. xylosus* was revealed for Cefacetrile and Cefoperazone 80.1% and 76.2% respectively. A significant quantity of isolates were resistant to Streptomycin, Linkomycin, Penicillin and Neomycin (Bochniarz *et al*., 2014). Moreover *S. xylosus* isolates exposed high susceptibility to Ciprofloxacin and high resistant to Erythromycin (Al-Mathkhury *et al*., 2008). Methicillin-resistant CoNS strains were 67.5%. Resistance rates of methicillin-resistant strains to the antibiotics agents, were: gentamicin 90% and 17%, erythromycin 80% and 37%, clindamycin 72% and 18%, trimethoprim-sulfamethoxazole 68% and 38%, ciprofloxacin 67% and 23%, tetracycline 60% and 45%, chloramphenicol 56% and 13% and fusidic acid 25% and 15%. None of the strains were resistant to vancomycin and teicoplanin (Koksal *et al.*, 2009).

Table 1: Identification of bacterial isolates by using biochemical test and confirm the identification by Phoenix system

NO	Gram stain and shape	Catalase Test	Coagulase Test	Fermentation manitol	Result of identification
1	Gram positive cluster cocci	+	-	+	*Staphylococcus xylosus*
2	Gram positive cluster cocci	+	-	+	*Staphylococcus xylosus*
3	Gram positive cluster cocci	+	-	+	*Staphylococcus xylosus*
4	Gram positive cluster cocci	+	-	+	*Staphylococcus xylosus*
5	Gram positive cluster cocci	+	-	+	*Staphylococcus xylosus*

Table 2: Effect different type of antibiotics by use Phoenix system on *S. xylosus*

Antibiotic	*Staph xylosus* (1)	*Staph xylosus* (2)	*Staph xylosus* (3)	*Staph xylosus* (4)	*Staph xylosus* (5)
Gentamicin	S	S	S	S	S
Imipenem	R	R	R	R	R
Cefotaxime	R	R	R	R	R
Ampicillin	R	R	R	R	R
Penicillin	R	R	R	R	R
Oxacillin	R	R	R	R	R
Amoxicillin_clavulanate	R	R	R	R	R
Daptomycin	S	S	S	S	S
Trimethoprim-sulphamethoxazole	S	S	S	S	S
Teicoplanin	S	S	S	S	S
Vancomycin	S	S	S	S	S
Clindamycin	S	S	S	S	S
Erythromycin	S	S	S	S	S
Fusidic acid	-	-	-	-	-
Linezolid	S	S	S	S	S
Mupirocin	S	S	S	S	S
Nitrofurantion	S	S	S	S	S
Ciprofloxacin	S	S	S	S	S
Moxifloxacin	S	S	S	S	S
Rifampin	S	S	S	S	S
Tetracycline	S	S	S	S	S
Resistance of antibiotics %	30%	30%	30%	30%	30%

Conclusion:

S. xylosus causes urinary tract infections as these bacteria have never been isolated from the vicinity of hospitals this indicates the leakage of bacteria from inside the hospital to the surrounding area, which is dangerous for visitors and also for patients while they are inside the garden. Overuse of antibiotics leads to an acceleration of its resistance along with a deterioration in infection prevention and control. The antibiotic steps can be taken at all levels of society to reduce the impact of that resistance and restrict its spread. The best result of antibiotic testing were that Gentamycin, Daptomycin, Trimethoprim- sulphamethoxazole, Teicoplanin, Vancomycin, Clindamycin, Erythromycin, Linezolid, Mupirocin, Nitrofurantion, Ciprofloxacin, Moxifloxacin, Rifampin, and Tetracycline respectively against all isolates of *S. xylosus*.

References

Abadi, A.T.B. Rizvanov, A.A. Haertlé, T. and Blatt, N.L.(2019) World Health Organization Report: Current Crisis of Antibiotic Resistance. Bionanotecnology of Science Journal. (9):778-788.

Al-heety, A. (2005) Role of peptidoglycan in pathogenicity of Staphylococcus saprophyticus. MSc. thesis. University of Baghdad. College of Science.

Al-Mathkhury, H.J.F., Flaih, M.T. and Al-Ghrairy, Z.K.A. (2008) Pathological Study on *Staphylococcus xylosus* Isolated From Patients With Urinary Tract Infections. Journal of Al-Nahrain University. 11 (2):123-130.

Barreire, C. Bruncker, R. and Talon, R.(2001) Characterization of single superoxide dismuatse of *Staphylococcus xylosus*. Journal Applied and Enviromental Microiology. 67 (9):4096-4104.

Bochniarz,M. Wawron,W. Szczubiał,M. Brodzki,P. Piech,T. and Kusy,R. (2014) Characteristics of *Staphylococcus xylosus* isolated from subclinical mastitis in cows. De Gruyter, Annals of Animal Science. 14 (4):859-867.

Bouaziz, A., Horchani, H., Ben Salem, N., Gargouri ,Y., Sayari, A. (2011). Expression, purification of a novel alkaline *Staphylococcus xylosus* lipase acting at high temperature, Biochemical Engineering Journal 54:93–102.

Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney (1996). Practical Medical Microbiology. 14th Edition, Churchill Livingstone.

Holt, J. Kreig, N. Sneath, P. Staley, J. and Williams, S. (1994). Bergey's manual of determinative bacteriology 9th ed. Williams and Willikins Maryland, USA.

Koksal, T. , Yasar, H. , Samasti, M. (2009).Antibiotic resistance patterns of coagulase-negative staphylococcus strains isolated from blood cultures of septicemic patients in Turke. Microbiology research. 164(4):404-410.

Konuku, S. Rajan, M.M. and Muruhan, S. (2012). Morphological and Biochemical Characteristics and Antibiotic Resistance Pattern of *Staphylococcus aureus* Isolated from Grapes. International Journal of Nutrition Pharmacology Neurological Diseases. 2 (1):70-73.

Kotsowilis, A.D.T. Koliomichalis, M.P. and Papvassiliou, J.T. (1982). Acute pyelonephritis caused by *Staphylococcus xylosus*. Journal of Clinical Micorbiology. 16(3):593-594.

Liu , Z.K. Ling, T.K. and Cheng, A.F. (2005). Evaluation of the BD Phoenix Automated Microbiology System for Identification and Antimicrobial Susceptibility Testing of Common Clinical Isolates. medical principles and practice, Medical Principles and Practice 14 (4):250-254.

Pamela J. Y., Dawn M. Simon , Jess A. Millar, Forrest Alexander H., Darleen Franklin (2011)A diversity of Antibiotic-resistant Staphylococcus spp. in a Public Transportation System. public health and Research perspectives 2(3):202-209.

Rupp, M. E. Soper, D.E. and Archer, G.L. (1992). Colonization of the female genital tract with *Staphylococcus saprophyticus*. Journal of Clinical Microbiology. 30(11):2975-2979.

Schleifer, K.H and Kloos, W.E.(1975). Isolation and characterization of *Staphylococci* from human skin I. amended descriptions of *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* and descriptions of three new species: *Staphylococcus cohnii*, *Staphylococcus haemolyticus*, and *Staphylococcus xylosus*. International Journal of Systematic Bacteriology. 25 (1):50-61.

Slaughter, D.M. Patton, T.G. Sievert, G. Sobieski, R.J. and Crupper, S.S.(2001). Antibiotic resistance in *coagulase-negative Staphylococci* isolated from Cope's gray tree frogs (Hyla chrysoscelis). Journal of the federation of european microbiological societies microbiology letters.205 (2): 265-270.

Thornton, V.B., Dovis, J.A. , Clair, M.B. S. and Cole, M.N.(2003). Inoculation of *Staphylococcus xylosus* in JL/J mice to determine pathogenicity. Animal Science. 42(4):49-52.

Tselenis-Kotsowilis, A. D. , Koliomichalis, M.P. and Papavassiliou (1982) Acute Pyelonephritis Caused by *Staphylococcus xylosus*. Journal of clinical microbiology, 16(3):593-594

WHO (World Health Organization Technical Series), (2020). Antibiotic Resistant.

AlQalam Journal of Medical and Applied Sciences
Special Issue for 6th International Conference in Basic Sciences and Their Applications
(6th ICBSTA, 2023), https://journal.utripoli.edu.ly/index.php/Alqalam eISSN 2707-7179

The Prevalence and Morphology of Toxoplasmosis in Domestic and Semi-domestic Cat in El-Beyda City, Libya

Ahmed M. Ekhnefer[1], Amal S. Hamd[2], Abdulwahed B. Hador[1], Salem Mohamed Bowashia[1]
[1]*Zoology Department, Faculty of Science, Omar Al-Mukhtar University, El-Beyda, Libya.*
[2]*Department of Biology, Faculty of Education, Derna University, Derna Libya.*
Corresponding author: *Salem.bowashia@omu.edu.ly*

Abstract:

This study was the first study for determine the prevalence and morphology of toxoplasmosis in domestic and semi domestic cat of Elbayda city, Libya. The total of 306 domestic and semi-monastic cat fecal samples were collected from 11 regions of the city. The fecal samples were divided according to sex, age and bread of the cats. And using modified Sheather's Sugar Floatation Technique. The 90 samples were infected, Where the result showed The Description of morphology *Toxoplasma gondii* was observed Pri-oocyst the smallest size of the Oocyst, and the basis of the dimensions, the length was 7-9µm and the width was 6-9µm while the fertilized was larger and its color was 7-12µm and display 7-10µm, and the prevalence of infection according to the sex was 77.78% (n=70) and 22.22% (n=20) in female and male cats respectively. The African breed of experimented cat was more infected by toxoplasma parasite with prevalence of 35.60%, the bread of Siamese cat was the lowest rate of infection, 4.40%. The study showed that the seventh line and Al-Gharigah of the study areas were the most prevalent site of infection with the *Toxoplasma* parasite (47.05%), and the lowest prevalence of toxoplasmosis infection in Al-300 Villat and The new El-Bayda (4.40%).

Keywords:. Cat, toxoplasmosis, *Toxoplasma gondii* El-Bayda, Libya

Introduction:

A third of all people on the earth are infected with *Toxoplasma*, an internal protozoan parasite that can survive in several hosts. a parasite that infects both people and animals and causes toxoplasmosis; it is a member of the family Sarcocystidae and the phylum Apicomplexa subclass Coccidiasina; its definitive host is the Felidea family; its intermediate hosts include many mammalian and bird species (Smith & Rebuck, 2000). Humans infect

Toxoplasma gondii mostly through eating raw meat contaminated with viable tissue cysts or drinking water contaminated with oocysts from cat feces (J. P. Dubey & Beattie, 1988). After consuming prey or tainted water, domestic cats and other members of the Felids shed enormous numbers of *Toxoplasma* oocysts. These oocysts grow in the environment and spread via rain and surface water, causing extensive environmental contamination (J. Dubey, 2001; Hill & Dubey, 2002). Kittens are probably the major source of contamination as they are common and produce large numbers of oocysts and Tachyzoites transmitted through the placenta from infected pregnant mother to her fetus (J. Dubey & Carpenter, 1993; Meireles *et al.*, 2008). *T. gondii* The disease has received significant medical attention due to its widespread distribution throughout the world and has been shown to have serious effects on humans, especially pregnant women and newborn children, due to the high rate of miscarriage or childbirth, which is transmitted to the fetus from the mother during pregnancy. Even if a child is born after the months of pregnancy are complete, it still exhibits symptoms of serious mental retardation and epilepsy, as well as the effects of eye and brain sprain and skull hypertrophy where the head is stuffed or the fetus is smaller than normal size (Akyar, 2011; J. Dubey *et al.*, 2009). The first known instance of congenital toxoplasmosis in 1939 was found in a 3-day-old kid who experienced seizures (Wolf, Cowen, & Paige, 1939). After the baby's postmortem, brain calcification, retinochoroiditis, and hydrocephalus were noted. The infant only lived for one month. This is now recognized as the classic triad of congenital toxoplasmosis symptoms (SABIN, 1942). When *T. gondii* parasites were first found in enucleated eyes in the 1950s, it was assumed that this type of ocular toxoplasmosis resulted from congenital transmission of the parasite (WILDER, 1952). However, more recent studies have described an unexpectedly high number of cases of ocular toxoplasmosis caused by postnatal acquired infection (Burnett *et al.*, 1998; Gilbert *et al.*, 1999; Montoya & Remington, 1996). Reason for choosing the topic is that cats are common in households where they are considered the final host for the transmission of toxoplasmosis. This study was aims to determine the prevalence of the disease among cats in the EL- Bayda City of Libya and studies of some morphological criteria for some phases of a parasite *Toxoplasma* found in cat feces.

Materials and Methods:

Study location

The study was conducted in the city of Al-Bayda, the city of Al-Bayda has a moderate temperatures and cold in winter, with an average annual precipitation of 400mm, al-Bayda is located at-32-45'59 N 21-44'30 E and has an area of 11.429 Km². The monthly temperature ranges from 5Cº to 36Cº, must update this information, 2012 very old information (Fig. 1).

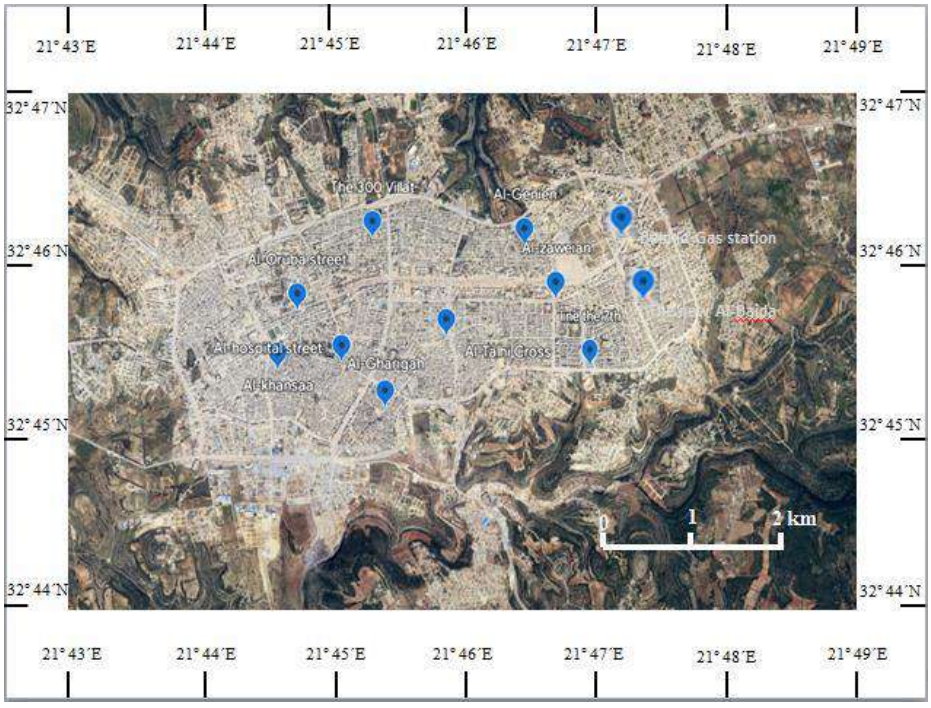


Figure 1: The sampling locations of Al-Bayda

Samples Collection:

From April 2018 to December 2018,cats fecal samples (n=306) were collected directly from the environment (*i.e.* public streets, squares and backyards) in the AL Bayda city Libya, The collected samples were put in plastic vials, identified and stored in isothermal boxes at 4˚C until laboratory processing.

Data on the animals, such as name, species, breed, age, and sex, were obtained from the Parasitological Examination Requisition forms. Age-related data were grouped and defined as follows: young animals <1 year old, adult animals 1–8 years old, and senior animals >8 years old.

Laboratorial Procedures:

Sheather's Sugar Floatation Technique:

Sugar floatation technique (Sheather, 1923) was performed by using 5 g of faces mixed with 45ml of sugar solution (50%) (density 1.208) and centrifuged at 1,000×*g* for 10 minutes, and the suspension was transferred to a slide and observed for presence of 10µm thick walled unstained Oocysts under microscope (1000×) (Bowman, 2022; de Santana, da Silva, Ramos, Alves, & de Carvalho, 2015; Norhamizah *et al.*, 2016; Rey, 2002).

Morphology:

The prepared microscopic slid is examined using this method Sheather's Sugar Floatation Technique and The identification of *T. gondii* oocyst and Pri-oocysts were by using the lica microscope system and according to (Norhamizah *et al.*, 2016) *T. gondii* oocycts appear as red cystic structures, 10µm in diameter.

The questionnaire

1- Age According month. 2- Sex, male or female. 3- Life style ,confined, semi-confined or free. 4- Feeding commercial food, Homemade food, Raw food. 5-Hunting yes, no. 6- Breed of cats. 7- Study area of AL Bayda.

Statistical Data Analysis:

The collected data were analyzed using descriptive statistics, with simple frequencies for qualitative variables and measures of central tendencies for quantitative variables. age, sex and parasitism was evaluated using the chi-square test for two variables or more variables. All statistical analyses were performed using the Mini tab 2014 program using ANOVA computer program with a 95% confidence

Results:

The study examined total of 306 Cat fecal material obtained from the eleven different locations of the study area (nearly 28 samples every locations).

The Description of morphology *T. gondii* was observed that Pri-oocyst were the smallest size of the Oocysts, and the eggs were not enriched by red color using the 10% Iocene dye. On the basis of the dimensions, the length was 7-9µm and the width was 6-9µm while the fertilized was larger and its color was 7-12µm and display 7-10µm as shown in the table (1).

Table (1): The measurement of the dimensions of Pri-oocysts and oocysts

Diameter	Pri- oocysts	Oocysts	Mean ±SE
Length	7-9 µm	7-12 µm	9.44±3.5
Width	6-9µm	7-10µm	7.33±3

Figure (2): Pri-oocysts of *Toxoplasma* 1000 X by lica system microscopy.

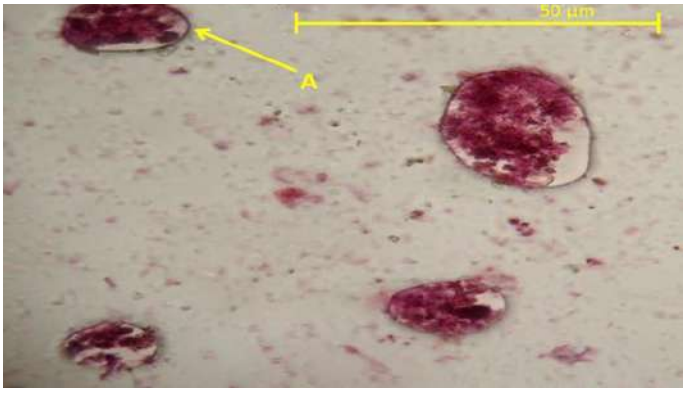


Figure (3): Oocysts mature of *Toxoplasma* 1000X by lica system microscopy

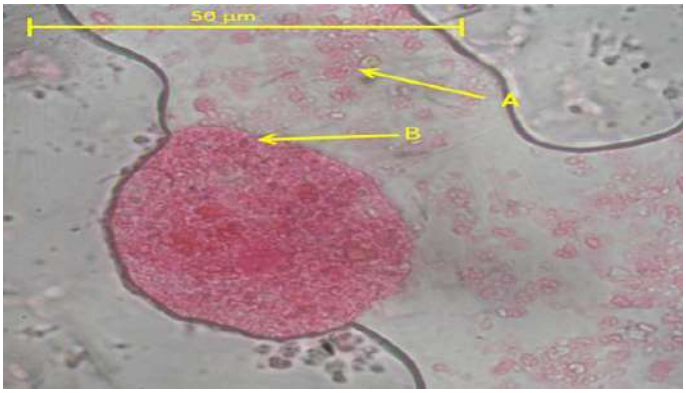


Fig.(4): *Toxoplasma gondii*: (A) Tachyzoites Free, (B) Oocyte filed with many Tachyzoites 1000X, by lica system microscopy

Analysis of 306 fasel samples cats from domestic and semi-domestic cats using the Sheather's Sugar Floatation Technique show 90 infection female samples cats and where she was the prevalence of cat toxoplasmosis infection Al-Bayda city libya were 29.41%

Table (2): Infection rate between sex

The total number of infected samples	Total number of male infected samples	%	Total number of female infected samples	%
90	20	22.22	70	77.78

The percentage of infection in females was higher than in males (77.78% and 22.22%, respectively). and there were significant differences at p= 0.005 (Table 2).

The infection rate was highest in the age group 25-36 months, where it was 44.40% and the lowest percentage of infected cats in the age group of -48 months, where it was 5.60% (Table 3).

Table (3): Infection rate by age

Age	Number	%	Sex and Infection			
			Male	%	Female	%
from one to 24 months	10	11.1	3	15	7	10
from 25 to 36 months	40	44.4	4	20	36	51.4
from 37 to 48 months	5	5.6	2	10	3	4.3
from 49 to 60 months	20	22.2	5	25	15	21.4
from 61 to 72 months	15	16.7	6	30	9	12.9
Total	90	100	20	100	70	100

P=0.005 and Chi-sq.=53.880

The percentage of infection according to the reigns of the study area was high in Al-Gharigah and The line7th where it was 14.40% and the lowest percentage in Al-300 Villat and The new AL-Bayda where it was 4.40% (Table4).

Table (4): Infection rate in the reigns of the study area

Study area	Number	%	Infection			
			Male	%	Female	%
The seventh line	13	14.4	3	15	10	14.3
Al-hospital street	10	11.1	3	15	7	10
The new Al-Bayda	4	4.4	1	5	3	4.3
Behind Gas station	6	6.7	2	10	4	5.7
Al-Genien	5	5.6	1	5	4	5.7
Al-Talhi Cross	12	13.4	2	10	10	14.3
Al-Gharigah	13	14.4	2	10	11	15.7
The 300 Villat	4	4.4	-	-	4	5.7
Al- zaweian	5	5.6	3	15	2	2.9
Al- khansaa	8	8.9	2	10	6	8.6
Al- Oruba street	10	11.1	1	5	9	12.8
Total	90		20		70	

P=0.005 and Chi-sq.=7.554

According to the information found in the questionnaire, our results showed that the fields that were less infected with parasite were cats fed on conned or stewed food and their livelihood was not free to live, while the most affected fields with parasite wear cats fed on raw food or fed from the street and their livelihood was free.

The rate of infection was high in the African cat type, which is the common type in Libya, where it was 35.6% and the lowest rate of infection in the Siamese 4.4% (Table5).

Table (5): Infection rate by Breed of cats

Breed of cats	Number	%	Infection			
			Males	%	Females	%
African cat	32	35.6	8	40	24	34.3
Shirazi cat	5	5.6	2	10	3	4.3
Persian cat	19	21.1	5	25	14	20
Siamese cat	4	4.4	-	-	4	5.71
Persian hybrid cat	6	6.7	-	-	6	8.57
Shirazi hybrid cat	24	26.7	5	25	19	27.14
Total	90					

P = 0.005and Chi-sq.=4.125.

Discussion:

This study shows the sporozoite is the stage of the parasite present in the sporulated Pri-oocysts and Oocysts where she was that Pri-oocyst were the smallest size of the Oocysts, and the eggs were not enriched by red color using the 10% Iocene dye. On the basis of the dimensions, the length was 7-9µm and the width was 6-9µm while the fertilized was larger and its color was 7-12µm and display 7-10µm reported that oocysts 10µm, Meireles et al. (2008) has been described the morphological stage of Toxoplasma where Pri-oocysts 8µm in length and orange-red and oocysts are 10µm and dark red and as inexperienced anyone can distinguish Toxoplasma in cat feces (Norhamizah *et al.*, 2016), in study conducted by Ferguson (2009) in Tokyo it was found that oocysts are 5µm in length and Hartmann et al. (2013) was found the oocysts is round to oval and has a length of 10-12µm and where he indicated (Ygor Henrique 2023) Unsporulated oocyst of *Toxoplasma gondii is 41-32* µm Our results are therefore consistent with Isaac-Renton et al. (1998), Meireles et al. (2008), Norhamizah et al. (2016) and Hartmann et al. (2013), In contrast, our study differed with Ferguson (2009) and Ygor Henrique 2023 In this study show a total infection rate for cats was 29.411% This result was comparable to a study conducted by Zhang et al. (2009) and Kulasena,

Rajapakse, Dubey, Dayawansa, and Premawansa (2011). In contrast, our study differed with (Campos *et al.*, 2016; de Quadros, Trevisani, de Moura, & Ramos, 2021; Lima, Rezende, Rocha, & Castro, 2018); Nabi et al. (2018); (Silva *et al.*, 2023), and Hartmann et al. (2013), Al-Kappany et al. (2011), Afonso, Thulliez, and Gilot-Fromont (2010), Millán, Cabezón, Pabón, Dubey, and Almería (2009) and Michalski, Platt-Samoraj, and Mikulska-Skupien (2010) as the prevalence in recent coproparasitological studies in cats in the country varied between 17% and 75.26% (Campos *et al.*, 2016; Lima *et al.*, 2018; Quadros *et al.*, 2021). The infection rate is high compared to other research, possibly as a result lack of awareness of the disease. Availability of suitable conditions for proliferation due to lack of control over slaughterhouses. In our study, the incidence of females was higher than that of males where there were significant differences compared to previous studies and we not found that there is study that is consistent with our results where all studies indicate that there is no relationship between disease and sex (Leguía, 2002; Troncoso Toro, Uribe Henríquez, Arrué Brenet, Valenzuela Contreras, & Fischer Wiethuchter, 2015). It may be the result of physiological changes that occur to females during periods of covert menstruation, pregnancy or lactation. Perhaps the periods of pregnancy and lactation in females are more voracious and therefore looking for food soon and away become more susceptible to infection (Leguía, 2002; Miró *et al.*, 2004; Troncoso Toro *et al.*, 2015). According to age, our results indicate that there are significant differences between the age groups studied where the age group 2 to 3 years was larger than the other groups, where we did not find any study agreed with our results and may be the cause of the high incidence of this age group being the most active period and search for food and other partner and therefore move from one place to another and travel great distances and therefore be move vulnerable to injury (Leguía, 2002; Troncoso Toro *et al.*, 2015). According to the fields of the study, our results showed that there are high moral differences between the different fields of study, where the infection rate was very high in the Al-Gharigah and the seventh line at a rate of 14.4%. where the questionnaire showed us that new villas and white areas, where most cat car owners use special food either canned or cooked and living cats confined inside the house, while other use raw food kindle let cats roam the streets and thus consistent with our results with Afonso, Thulliez, and Gilot-Fromont (2006) and Woods, McDonald, and Harris (2003).

According to the of cats our study recorded that there are no significant differences between the types of cats studied where the highest infection rate in the African cat rate 35.6% and the lowest was Siamese cat rate of 4.4% and therefore that does not distinguish any type of cats where our study is consistent with Leguía (2002) and Troncoso Toro et al. (2015).

Reference

Afonso, Eve, Thulliez, P, & Gilot-Fromont, Emmanuelle. (2010). Local meteorological conditions, dynamics of seroconversion to Toxoplasma gondii in cats (Felis catus) and oocyst burden in a rural environment. Epidemiology & Infection, 138(8), 1105-1113.

Afonso, Eve, Thulliez, Philippe, & Gilot-Fromont, Emmanuelle. (2006). Transmission of Toxoplasma gondii in an urban population of domestic cats (Felis catus). International journal for parasitology, 36(13), 1373-1382.

Akyar, I. (2011). Seroprevalence and coinfections of Toxoplasma gondii in childbearing age women in Turkey. Iranian journal of public health, 40(1), 63.

Al-Kappany, YM, Lappin, MR, Kwok, OCH, Abu-Elwafa, SA, Hilali, M, & Dubey, JP. (2011). Seroprevalence of Toxoplasma gondii and concurrent Bartonella spp., feline immunodeficiency virus, feline leukemia virus, and Dirofilaria immitis infections in Egyptian cats. The Journal of parasitology, 97(2), 256-258.

Bowman, Dwight D. (2022). Georgi. Parasitología para veterinarios: Elsevier Health Sciences.

Burnett, Andrew J, Shortt, Stanley G, Isaac-Renton, Judith, King, Arlene, Werker, Denise, & Bowie, William R. (1998). Multiple cases of acquired toxoplasmosis retinitis presenting in an outbreak. Ophthalmology, 105(6), 1032-1037.

Campos, Diefrey Ribeiro, Oliveira, Luanna Castro, de Siqueira, Daniele Fassina, Perin, Lívia Reisen, Campos, Nayara Camatta, Aptekmann, Karina Preising, & Martins, Isabella Vilhena Freire. (2016). Prevalence and risk factors associated with endoparasitosis of dogs and cats in Espírito Santo, Brazil. Acta Parasitologica, 61(3), 544-548.

de Quadros, Rosiléia Marinho, Trevisani, Natascha, de Moura, Anderson Barbosa, & Ramos, Carlos José Raupp. (2021). Helmintofauna parasitária em gatos errantes de Lages, Santa Catarina, Brasil. Revista Brasileira de Higiene e Sanidade Animal: RBHSA, 15(1), 10.

de Santana, Breno Barros, da Silva, Tallys Leandro Barbosa, Ramos, Rafael Antonio Nascimento, Alves, Leucio Câmara, & de Carvalho, Gílcia

Aparecida. (2015). Evaluation of different parasitological techniques for diagnosing intestinal parasites in dogs. Open Journal of Veterinary Medicine, 5(02), 19.

Dubey, Jitender Prakask, & Beattie, CP. (1988). Toxoplasmosis of animals and man: CRC Press, Inc.

Dubey, JP. (2001). Oocyst shedding by cats fed isolated bradyzoites and comparison of infectivity of bradyzoites of the VEG strain Toxoplasma gondii to cats and mice. Journal of Parasitology, 87(1), 215-219.

Dubey, JP, Bhatia, CR, Lappin, MR, Ferreira, LR, Thorn, A, & Kwok, OCH. (2009). Seroprevalence of Toxoplasma gondii and Bartonella spp. antibodies in cats from Pennsylvania. Journal of Parasitology, 95(3), 578-580.

Dubey, JP, & Carpenter, JL. (1993). Neonatal toxoplasmosis in littermate cats. Journal of the American Veterinary Medical Association, 203(11), 1546-1549.

Ferguson, David JP. (2009). Toxoplasma gondii: 1908-2008, homage to Nicolle, Manceaux and Splendore. Memorias do Instituto Oswaldo Cruz, 104, 133-148.

Gilbert, RE, Dunn, DT, Lightman, S, Murray, PI, Pavesio, CE, Gormley, PD, . . . Stanford, MR. (1999). Incidence of symptomatic toxoplasma eye disease: aetiology and public health implications. Epidemiology & Infection, 123(2), 283-289.

Hartmann, K, Lloret, A, Ferrer, L, Addie, Diane, Belak, Sándor, Boucraut-Baralon, Corine, . . . Hosie, Margaret J. (2013). Cryptococcosis in cats: ABCD guidelines on prevention and management. Journal of Feline Medicine and Surgery, 15.

Hill, Dolores, & Dubey, Jitender P. (2002). Toxoplasma gondii: transmission, diagnosis and prevention. Clinical microbiology and infection, 8(10), 634-640.

Isaac-Renton, Judith, Bowie, William R, King, Arlene, Irwin, G Stewart, Ong, Corinne S, Fung, CP, . . . Dubey, 0JP. (1998). Detection of Toxoplasma gondii oocysts in drinking water. Applied and environmental microbiology, 64(6), 2278-2280.

Kulasena, VA, Rajapakse, RPVJ, Dubey, JP, Dayawansa, PN, & Premawansa, S. (2011). Seroprevalence of Toxoplasma gondii in cats from Colombo, Sri Lanka. The Journal of parasitology, 97(1), 152-152.

Leguía, Guillermo. (2002). Enfermedades parasitarias de perros y gatos. Epidemiología y control, 2, 150-155.

Lima, Jaqueline Ataíde Silva, Rezende, Hanstter Hallison Alves, Rocha, Tamires Mariana Dias Damas, & Castro, Ana Maria de. (2018). Analysis of the accuracy of different laboratory methods for the

diagnosis of intestinal parasites from stray and domiciled cats (Felis catus domesticus) in Goiânia, Goiás, Brazil. Revista Brasileira de Parasitologia Veterinária, 27, 94-97.

Meireles, Luciana Regina, Tsutsui, Vinícius Suehiro, Carmo, Claudia Villano do, Galisteo Jr, Andrés Jimenez, Hiramoto, Roberto Mitsuyoshi, Terentowicz, Henrique César Katsumi, & Andrade Júnior, Heitor Franco de. (2008). Quantitative Toxoplasma gondii oocyst detection by a modified Kato Katz test using Kinyoun staining (KKK) in ME49 strain experimentally infected cats. Revista do Instituto de Medicina Tropical de São Paulo, 50, 187-190.

Michalski, Mirosław M, Platt-Samoraj, Aleksandra, & Mikulska-Skupien, E. (2010). Toxoplasma gondii antibodies in domestic cats in Olsztyn urban area, Poland. Wiadomości Parazytologiczne, 56(3).

Millán, Javier, Cabezón, Oscar, Pabón, Marcela, Dubey, JP, & Almería, Sonia. (2009). Seroprevalence of Toxoplasma gondii and Neospora caninum in feral cats (Felis silvestris catus) in Majorca, Balearic Islands, Spain. Veterinary Parasitology, 165(3-4), 323-326.

Miró, Guadalupe, Montoya, Ana, Jiménez, Santos, Frisuelos, Carolina, Mateo, Marta, & Fuentes, Isabel. (2004). Prevalence of antibodies to Toxoplasma gondii and intestinal parasites in stray, farm and household cats in Spain. Veterinary Parasitology, 126(3), 249-255.

Montoya, Jose G, & Remington, Jack S. (1996). Toxoplasmic chorioretinitis in the setting of acute acquired toxoplasmosis. Clinical infectious diseases, 23(2), 277-282.

Nabi, Habibun, Rashid, Muhammad Imran, Islam, Saher, Bajwa, Amna Arshad, Gul, Rahim, Shehzad, Wasim, . . . Waqas, Muhammad. (2018). Prevalence of Toxoplasma gondii oocysts through Copro-PCR in cats at Pet Center (UVAS), Lahore, Pakistan. J Pak Med Assoc, 68(1), 115-118.

Norhamizah, A, Norina, L, Rashidah, C, Norsharina, A, Hanafi, H, & AR, SAIPUL BAHARI. (2016). Detection of Toxoplasma gondii oocyst in cats using Modified Kato-Katz and Sheather's sugar methods. Malays. J. Vet. Res, 7(2), 85-89.

Rey, Luís. (2002). Bases da Parasitologia Médica. 2ª edição. Rio de Janeiro Editora: Guanaba Koogan.

SABIN, ALBERT B. (1942). Toxoplasmosis: a recently recognized disease of human beings. Advances in Pediatrics, 1(1), 1-56.

Sheather, A Leslie. (1923). The detection of intestinal protozoa and mange parasites by a flotation technique. J Comp Pathol Ther, 36, 266-275.

Silva, Ygor Henrique da, Campos, Diefrey Ribeiro, Lima, Gabriel Alcides Capucho, Quintal, Janaína Pires, Guimarães, Brena Gava, Rêgo, Guilherme Mota Maciel do, . . . Scott, Fabio Barbour. (2023).

Prevalence of gastrointestinal parasites in domestic cats (Felis catus) diagnosed by different coproparasitological techniques in the municipality of Seropédica, Rio de Janeiro. Revista Brasileira de Parasitologia Veterinária, 32, e006223.

Smith, JE, & Rebuck, N. (2000). Toxoplasma gondii strain variation and pathogenicity. Microbial foodborne diseases: mechanisms of pathogenesis and toxin synthesis., 405-431.

Troncoso Toro, Ignacio Eduardo, Uribe Henríquez, Paola Alejandra, Arrué Brenet, Karen Constanza, Valenzuela Contreras, Adela Antonieta, & Fischer Wiethuchter, Christof. (2015). Soroprevalência de Toxoplasma gondii em gatos (Felis catus, Linnaeus 1758) residentes em Sam Carlos, Chile. Revista de Medicina Veterinaria(29), 23-31.

Wilder, Helenor Campbell. (1952). Toxoplasma chorioretinitis in adults. AMA archives of ophthalmology, 48(2), 127-136.

Wolf, Abner, Cowen, David, & Paige, Beryl. (1939). Human toxoplasmosis: occurrence in infants as an encephalomyelitis verification by transmission to animals. Science, 89(2306), 226-227.

Woods, Michael, McDonald, Robbie A, & Harris, Stephen. (2003). Predation of wildlife by domestic cats Felis catus in Great Britain. Mammal review, 33(2), 174-188.

Zhang, H, Zhou, DH, Zhou, P, Lun, ZR, Chen, XG, Lin, RQ, . . . Zhu, XQ. (2009). Seroprevalence of Toxoplasma gondii infection in stray and household cats in Guangzhou, China. Zoonoses and public health, 56(9-10), 502-505.

AlQalam Journal of Medical and Applied Sciences
Special Issue for 6th International Conference in Basic Sciences and Their Applications (6th ICBSTA, 2023), *https://journal.utripoli.edu.ly/index.php/Alqalam* ***eISSN 2707-7179***

Effect of Caffeine on the embryonic brain development in chick

Kawakeb A. O. Saad and Rema F. M. El mabrouk
Zoology Department, Faculty of Science, Omar Al Mukhtar University, El-Beyda, Libya
Correspondence author: *kawakeb.ali@omu.edu.ly*

Abstract:

Caffeine is a natural ingredient found in coffee, tea and cocoa, it is added to some soft drinks, energy drinks, and to some over counter medications, pregnant women consume caffeine during pregnancy, which leads to a teratogenic effect on embryos; were it passes easily from a mother's blood to embryos through a placenta. this study examined the effect of caffeine on morphological abnormalities in a brain of chicken embryos, fertilize eggs were incubated at temperature ranging from 37-38°C, and embryos were injected with caffeine at concentrations 0.5 mg/ml in the second day of incubation at stage HH8,. Embryos were collected later at stages HH17, HH21, HH29 and morphological changes were observed. The results showed abnormalities in the brain, where a small size of brain and sometimes a fusion of the brain in the cranium caffeine could be affected the neural tube parting through signaling inhibition.

Keywords: Caffeine, Brain, Chicken embryos development.

Introduction:

It is believed that 95% of pregnant women use caffeine as a beverage or in combination with drugs. Structure of caffeine closely resembles DNA components; researchers have been concerned about a drug's potential teratogenic and mutagenic effects (Mulvihill, 1973; Thayer *et al.*, 1975). Caffeine freely travels from a mother to embryos across a placental barrier (Weathersbee & Lodge, 1977), and a main caffeine metabolism enzyme, cytochrome (CYP1A2), which is lacking in a placenta and embryos, maternal caffeine intake during pregnancy has a direct impact on fetus depending on caffeine exposure levels (Aldridge *et al.*, 1979). Caffeine has a much longer half-life during pregnancy; because it cannot be digested by embryos or a

placenta (Aldridge *et al.*, 1981). Caffeine intake during pregnancy raises circulating catecholamine levels, which could contribute to fetoplacental vasoconstriction and hypoxia, affecting fetal growth and development (Kirkinen *et al.*, 1983), because the enzymes in a human liver do not exist until the 8th month of life, a half-life is 10 hours in a first trimester and 18 hours in a third trimester (Grosso & Bracken, 2005). Caffeine consumption during pregnancy has also been linked to an increased risk of miscarriage and fetal death, according to past studies (Weng *et al.*, 2008). In the present study, we studied the effects of caffeine on morphological and histological features of brain chicken embryos, in stage HH8 chick embryos and found the caffeine has an effect on them.

Materials and Methods:

Fertilized chicken eggs *Gallus gallus*, obtained from the farm of Mr. Abdul Sayed Al-Dakmak, in the Ras Al-Turab area in eastern Libya, and were incubated.

Before incubation, eggs were sterilized by 70% ethanol alcohol, and then incubated at 37-38°C, and 60% humidity. In second day of incubationeggs were candled and embryos locations were labeled and staged according to the Hamilton & Hamburger (1951).

Eggs were divided into three groups as follow, control group without injection, group injected with distilled water, and an experimental group injected with 0.5 mg/ml caffeine, after determined an air sac we made an opening in it with a special sterile needle with a diameter of20 gauge (G). Two ml of albumin was pulled. Moreover, a new needle was used to pull out 0.5 mg/ml of caffeine and injected it through an air sac, and then we closed the hole with a scotch tape. After that, eggs returned to incubator until it reached required stages HH17, HH21 and HH29 according to the Hamilton & Hamburger (1959). After embryos reached to required stages, they have been collected by opening a top of eggs, a place where we revealed an embryo by a candling. Then embryos were taken out with spoon and put them in PBS. Extra membranes were removed, and then embryos kept in Ependorph tubes containing 10% formalin. Embryos were photographed using dissected microscope and a canon IXUS 125 HS digital camera 16.1 mega pixels, whole mount surface area of each embryo were measured using software “Image J”.

Results:

As shown in figure 1, the control group was normal and 100% alive, while fertility ranged from 90% to 100%, as the percentage of abnormalities was 0%, for all stages HH17, HH21and HH 29. The same result was found in the embryos that have been injected with distilled water. As for the embryos that were injected with 0.5 mg\ml, it was noted that the group collected at stage HH17 had a survival rate of 80%, death 20%, deformities 100%, and fertility 90%. As for the embryos collected at stage HH21, the survival rate was 90%, death was 10%, malformations were also 100%, and fertility was 100%. As for the embryos collected at stage HH29, the percentage of survival was 80%, death was 20%, and the percentage of both malformations and fertility was 90%.

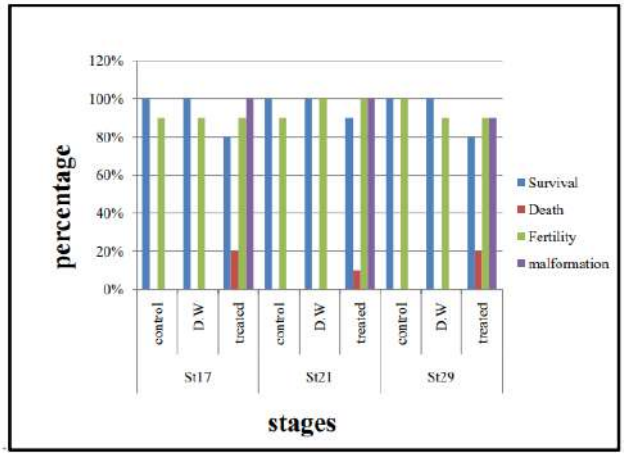


Figure 1: Histogram showing the Percentage of survival, death, fertility, and malformation of embryos injected with 0.5 mg/ml(caffeine) at HH17,HH21,HH29.

The bar chart in figure 2 illustrates the whole embryo surface area for different stages of embryos stages HH17, HH21 and HH29 between control groups, distil water and embryos treated by 0.5 mg/ml of caffeine. Figure2,showsthat there were no marked changes in the mean for whole surface area in mm for control and distilled water group of chicken embryo.On the other hand, the mean for whole surface area in mm, showed A decrease in the embryos that treated by 0.5 mg\ml. compared to the control group and distilled water

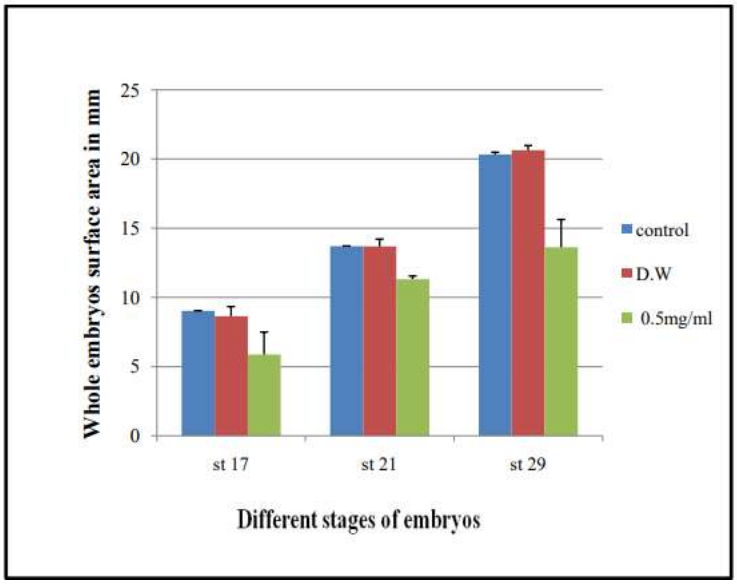


Figure 2: Histogram showing the whole embryos surface area in mm. between controls, distil water and concentration 0.5 mg/ml of caffeine. Error bar presented by standard deviation.

Figure 3 shows the effect of caffeine on chicken embryos at different stages, in the figures A1(control group), and B1(distilled water group)shows there is no difference, as find the embryos has grown organs normally, as the somites were extended to the tail and eyes were unpainted. In addition, the brain was complete, and the heart was normal. As for the caffeine-treated form C1, the embryos suffered from retardation and deficiency in growth and the weight of the embryos was lower compared to the control group and distilled water, as well as a microcephaly, and craniofacial dysmorphisms. For stage HH21: both the control (A2) and the distilled water group (B2), we note the embryos developing normally. The head was naturally divided, and we notice the presence of pigmentation in the eyes, Moreover, the heart was developing normally, the tail was towards the head, and the limb buds were wide. While the group treated with caffeine C2, had decreased growth, smaller size and less weight compared to the control group and distilled water as well as the microcephaly, and the heart had deformities, and the pigment of the eyes did not appear, as well as we note that the trunk and tail did not curve normally, and as we note micromelia. For stage HH29: the normal growth in both control group (B3) and distilled water group (A3) Where the brain was developing normally, as well as the normal growth of the eye and the appearance of the front and hind limbs naturally where it was the embryos has three toes in addition to the groove marks between the digits, as well as the

beginning of the formation of the beak. In the group treated with caffeine C3, we also note the small size of the embryos and a decrease in its growth compared to the control group and distilled water, as well as a small head size or what is known as microcephaly and deformation in the cranium, also the lack of normal growth of the eyes.

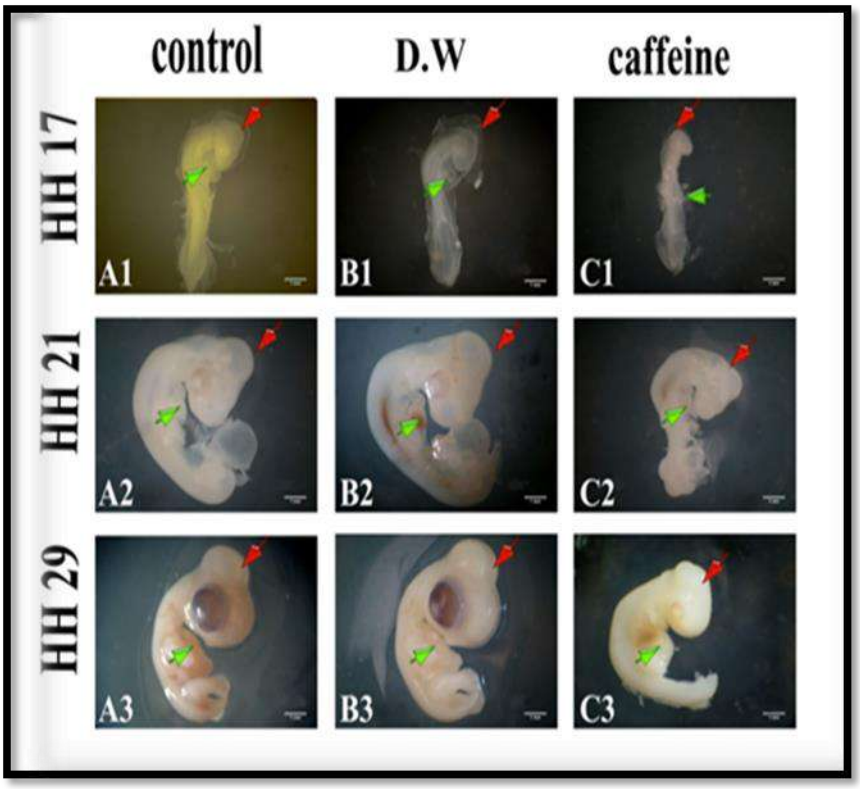


Figure 3: shows the effect of caffeine at a concentration of 0.5 mg / ml on embryo growth at different stages. Control (A1 HH17, A2 HH21, A3 HH29), distil water (B1 HH17, B2 HH21, B3 HH29), treated by caffeine (C1 HH17, C2 HH21, C3 HH29, red arrows point to the brain and green arrows indicate the heart.

Histological section results:

Figure 4 illustrated a cross section of chick embryos at stage HH29 at the heart and trunk levels. Figure 4-A showed a control, where a normal shape of a spinal cord was indicated by a red arrow, a notochord indicated by a yellow arrow, and blood vessels indicated by a black arrow, whereas figure 4-B shows embryos treated with 0.5 mg/ml, where was a neural tube not closed, a

layer of spinal cord cells that was thin compared to a control, and an abnormal shape of a notochord region where an elongation of this region was observed.

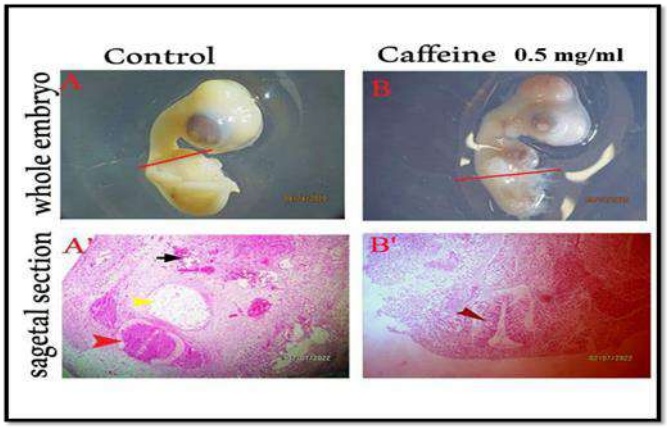


Figure 4: Transverse section at a heart and trunk levels. A represents a control, B represents an embryo treated with 0.5 mg/ml of caffeine. Where red arrows indicate to spinal cord, yellow arrows indicate to notochord, and black arrows indicate to blood vessels.

Discussion:

Caffeine is prohibited or advised in low doses during pregnancy due to their ability to cross a placental membrane and accumulate in an embryo's body. In several studies caffeine consumption has been linked to lower rates of fertilization, embryonic implantation, changes in placental structure, low fetal weight and an increased risk of intrauterine growth restriction (IUGR) (Bakker *et al.*, 2010; Bracken *et al.*, 2003; Eskenazi *et al.*, 1999; Fenster *et al.*, 1991; Sengpiel *et al.*, 2013), as shown in results that were presented in figures 2, Caffeine caused a reduction in a size of embryos, as well as abortion, which is represented in a death of embryos, according to results presented in this research, where at a concentration of 0.5 mg/ml, a rate of fetal death was about 20% and a percentage of malformations ranged between 90% - 100%, as shown in figure 1.

Furthermore, caffeine consumption during critical periods of pregnancy can produce epigenetic changes in a developing embryos or even germ cells, which can lead to adult onset illnesses in future generations(Qian *et al.*, 2020). Caffeine is using as a " legal drug ", it is known to have negative health effects, most notably disrupting proper fetal development in a case of high maternal ingestion, but this precise processes are unknown (Ma *et al.*, 2012).

It was clear through results of our research; exposure to caffeine showed alters brain development. Moreover, a concentration of 0.5 mg/ml noticed

significant abnormalities in a brain, as shown in figure 3 - C1 (small size of a brain and atrophy in a midbrain). In figure 3 - C2, there was a deformation in a midbrain, and figure 3 - C3 showed atrophy of a midbrain. All these changes indicated by red arrow.

Abnormalities that occurred in brain regions can be discussed as follows: caffeine may be inhibit both Wnt and BMP morphogens signalling located in a dorsal region, and thus led to an inhibition of Pax7 and Pax6 morphogens signalling located in the same region. In addition, caffeine inhibited the Shh morphogen located in a ventral region, which in turn led to an inhibition of Nkx6.1morphogen located in the ventral region. Also caffeine works on inhibit the action of adenosine receptors, which in turn maintains a rate of oxygen in embryos, thus caffeine works to disrupt a normal oxygenation process inside cells, and also due to a similarity between a caffeine molecule and adenosine, which is an important in the process of DNA methylation. In addition, caffeine may lead to Mutations in the process of DNA methylation.

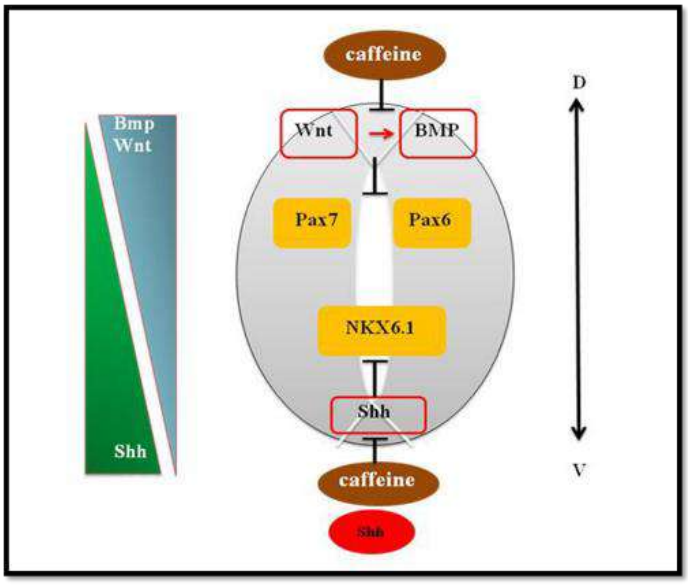


Figure 5: Diagram showed caffeine inhibition of signalling in a brain.

Conclusion

In this research, chicken embryos used to study an effect of caffeine on the brain of embryos in stages HH17, HH21 and HH29. Embryos treated with caffeine showed various abnormalities such as small head size, abnormalities in a brain, these abnormalities are represented in a microcephaly of the brain with a cranium and its absence in some cases, when compared with control

also. it should be warn the public to be wary about caffeine use during pregnancy and, if possible, avoid high amounts of caffeine consumption.

References:

Aldridge, A., Aranda, J.-V., & Neims, A. H. (1979). Caffeine metabolism in the newborn. *Clinical Pharmacology & Therapeutics*, *25*(4), 447–453.

Aldridge, A., Bailey, J., & Neims, A. H. (1981). The disposition of caffeine during and after pregnancy. *Seminars in Perinatology*, *5*(4), 310–314.

Bakker, R., Steegers, E. A. P., Obradov, A., Raat, H., Hofman, A., & Jaddoe, V. W. V. (2010). Maternal caffeine intake from coffee and tea, fetal growth, and the risks of adverse birth outcomes: The Generation R Study. *American Journal of Clinical Nutrition*, *91*(6), 1691–1698. https://doi.org/10.3945/ajcn.2009.28792

Bracken, M. B., Triche, E. W., Belanger, K., Hellenbrand, K., & Leaderer, B. P. (2003). Association of maternal caffeine consumption with decrements in fetal growth. *American Journal of Epidemiology*, *157*(5), 456–466.

Eskenazi, B., Stapleton, A. L., Kharrazi, M., & Chee, W.-Y. (1999). Associations between maternal decaffeinated and caffeinated coffee consumption and fetal growth and gestational duration. *Epidemiology*, 242–249.

Fenster, L., Eskenazi, B., Windham, G. C., & Swan, S. H. (1991). Caffeine consumption during pregnancy and fetal growth. *American Journal of Public Health*, *81*(4), 458–461. https://doi.org/10.2105/AJPH.81.4.458

Grosso, L. M., & Bracken, M. B. (2005). Caffeine metabolism, genetics, and perinatal outcomes: a review of exposure assessment considerations during pregnancy. *Annals of Epidemiology*, *15*(6), 460–466.

Hamburger, V., & Hamilton, H. L. (1951). A series of normal stages in the development of the chick embryo. *Journal of Morphology*, *88*(1), 49–92.

Kirkinen, P., Jouppila, P., Koivula, A., Vuori, J., & Puukka, M. (1983). The effect of caffeine on placental and fetal blood flow in human pregnancy. *American Journal of Obstetrics and Gynecology*, *147*(8), 939–942.

Ma, Z. lai, Qin, Y., Wang, G., Li, X. di, He, R. rong, Chuai, M., Kurihara, H., & Yang, X. (2012). Exploring the caffeine-induced teratogenicity on neurodevelopment using early chick embryo. *PLoS ONE*, *7*(3). https://doi.org/10.1371/journal.pone.0034278

Mulvihill, J. J. (1973). Caffeine as teratogen and mutagen. *Teratology*, *8*(1), 69–72.

Qian, J., Chen, Q., Ward, S. M., Duan, E., & Zhang, Y. (2020). Impacts of Caffeine during Pregnancy. *Trends in Endocrinology and Metabolism*, *31*(3), 218–227. https://doi.org/10.1016/j.tem.2019.11.004

Sengpiel, V., Elind, E., Bacelis, J., Nilsson, S., Grove, J., Myhre, R., Haugen, M., Meltzer, H. M., Alexander, J., Jacobsson, B., & Brantsæter, A. L.

(2013). Maternal caffeine intake during pregnancy is associated with birth weight but not with gestational length: Results from a large prospective observational cohort study. *BMC Medicine*, *11*(1). https://doi.org/10.1186/1741-7015-11-42

Thayer, P. S., Palm, P. E., & Flamm, G. (1975). A current assessment of the mutagenic and teratogenic effects of caffeine. *CRC Critical Reviews in Toxicology*, *3*(3), 345–369.

Weathersbee, P. S., & Lodge, J. R. (1977). Caffeine: its direct and indirect influence on reproduction. *The Journal of Reproductive Medicine*, *19*(2), 55–63.

Weng, X., *American Journal of Obstetrics and Gynecology*, *198*(3), 279-e1.Odouli, R., & Li, D.-K. (2008). Maternal caffeine consumption during pregnancy and the risk of miscarriage: a prospective cohort study.

AlQalam Journal of Medical and Applied Sciences
Special Issue for 6th International Conference in Basic Sciences and Their Applications (6th ICBSTA, 2023), *https://journal.utripoli.edu.ly/index.php/Alqalam* ***eISSN 2707-7179***

The Prospective Use of Locally Produced Borate Glass as Windows and Shields in Imaging Photon and Neutron Environments

Salima A. Abdelrahman[a], Rasha A. Mansouri[a], Seham Y. M. Dogharsham[c], Abdulwahab A. M. Adam[a,b], Nagi A. Hussein[a,*]
[a]*Department of Physics, Faculty of Science, University of Benghazi, Benghazi, Libya*
[b]*Department of Physics, Faculty of Education, University of Benghazi, Marij, Libya*
[c]*Department of Engineering Sciences, Faculty of Engineering, Ajdabiya University, Ajdabiya, Libya*
Correspondence authors: nagi.hussein@uob.edu.ly

Abstract:

The potential use of borate glass as windows and shields in imaging photon environment as well as a shield for neutrons has been investigated in this research. Borate glass was produced locally in four different compositions that contain Bi_2O_3, PbO, Al_2O_3, Na_2O and Tl_2O_3 oxides. Their densities were measured and compared with empirically calculated values which showed reasonable correlation and accuracy that might be improved. Imaging photons attenuation parameters: LAC, HVL and TVL, at the energy range 15 -150 keV, were calculated using *Phy-X* and *WinXcom* codes. Sample S4 showed the best attenuation properties followed by S1 then S3 and S2 due to their high lead and bismuth contents. Good accuracy was obtained in LAC calculations using the two codes. Neutron attenuation was conducted by comparing the samples' FNRCS values which showed that sample S2 was the best neutron attenuator due to its high boron content compared with the others.

Keywords: Attenuation coefficient, Borate glass, FNRCS, Phy-X/PSD, WinXcom, Radiation shield, radiation window

Introduction:

There is a major consensus among scientists on the significance of protection from ionizing radiation being photons such as imaging diagnostic x-rays used in hospitals or particulate radiation such as neutrons used in nuclear and industrial facilities. For many years, the field of radiation protection has been evolving and number of policies and procedures have been adopted by international and national scientific bodies and organizations to regulate the

safety aspects related to ionizing radiation such as ICRP and IAEA. Extensive research papers, books, reports and documents are written on the subject and can be found in the literature. Principles and concepts of radiation protection are established and recognized globally such as the famous ALARA principle which affirms the aspects regarding lower exposure from ionizing radiation of short time, large distance and shielding (ICRP, 1977),(OECD, 2011).

X-ray imaging is a universal practice in hospitals and clinics and x-ray machines have become familiar equipment within these facilities where patients get imaged for diagnosis purposes. The existence of x-ray machines in these places make them an ionizing radiation environment that need to be protected. Other than the patients who by default must receive exposure dose there are radiologists, radiographers and medical physicists in this environment (x-ray room) who supervisor the imaging process and must be protected also. These personnel make the imaging process from a control room in short time from some distance and behind a shield to comply with rules while observing the patient. This observation must be done through a window made of glass which is transparent and should have attenuation properties to the x-ray photons. The shielding material that is commonly used in the diagnosis facilities is lead. Leaded aprons and screens as well as leaded doors and leaded glass for windows are employed. Lead is heavy element and highly dense which are the properties that required for photon attenuation. However, lead is highly toxic and dangerous pollutant to the environment in addition to the economic burden of purchasing large amount of the material (Agar, 2018). Many researchers, started looking for other materials, mainly glass (Sharma *et al.*, 2005: Kaur *et al.*, 2016; Bagheri & Adeli, 2020; Al-Ghamdi, 2022), with the benefit of x-ray photon attenuation and transparency to be used as windows (Celikbilek *et al.*, 2019; kityk *et al.*, 2017; Kumar *et el.*, 2021). In this paper, a proposal of such glass material in the form of borate glass was made that might fulfill the above benefits. Therefore, Borate glass of various compositions were produced locally in the laboratories of the physics department, Faculty of science, University of Benghazi. The paper will explain some of their physical and radiation attenuation properties for x-ray photons and neutrons where the latter was considered to be one of the main source of radiation in nuclear facilities.

Materials and Methods:

A description of the production procedures of borate glass samples were discussed below as well as measurement and calculation of densities of glass samples. Finally, the methods which were used to calculate radiation attenuation parameters were also discussed.

i. Borate Glass Production

Four glass sample composed of four compounds of miscellaneous concentrations as shown in Table 1 were manufactured. Each one of the four compounds was selected from a series of compositions of varying percentages that have been prepared in the lab.

The only common compound between these samples was Borate oxide (B_2O_3) and therefore the type of glass was named Borate glass. The basic proportion of powder materials were weighed accurately, mixed thoroughly and melted in high temperature furnace then quenched rapidly in preheated disk shaped engraves in aluminum mold and finally annealed in 400°C oven and left to cool slowly to room temperature.

ii. Glass Physical Properties

Density, the most important physical property of the produced borate glass samples, was measured experimentally and calculated theoretically. The experimental densities of the prepared borate glass samples were measured by the Archimedes method in various liquids using a specially fabricated device designed for that purpose and their averages were taken. The theoretical densities of borate glass samples were calculated using the empirical formula of Inaba and Fujino (2010):

$$(1)\rho = 0.53\ \frac{\sum(M_i \cdot x_i)}{\sum(V_i \cdot x_i)}$$

Where M_i is molar weight (kg/mol), x_i is molar fraction (mol%) and V_i is the packing density parameter (m^3/mol).

Transparency and coloration of the borate glass samples can be seen clearly by visual inspection and pictures of the samples are displayed in Figure 1.

Table 1: composition of borate glass samples

Sample Code	Composition of Glass Mixtures
S1	**20%**PbO - **10%** Bi_2O_3 - **20%**Na_2O - **50%**B_2O_3
S2	**10%**Al_2O_3 - **10%**Bi_2O_3 -**10%**Tl_2O_3 - **70%** B_2O_3
S3	**25%**PbO - **5%** Tl_2O_3 - **20** Na_2O - **50%** B_2O_3
S4	**30%**PbO - **10%**Bi_2O_3-**10%** Na_2O - **50%** B_2O_3

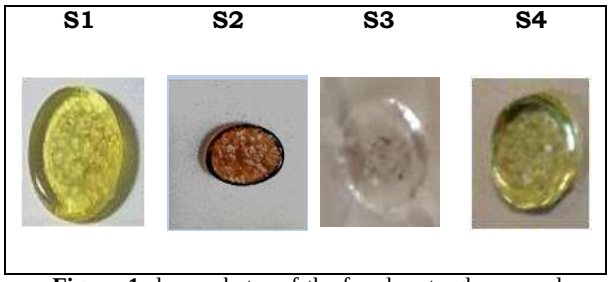


Figure 1 shows photos of the four borate glass samples

iii. Attenuation Parameters

There are many photon attenuation parameters that can be either measured or calculated and of interest in field of x-ray diagnostic radiation protection. The energy range of the x-ray imaging photons selected for this research was 15 - 150 keV which covers all the diagnostic applications such as mammography, conventional radiography and CT. This paper dealt with only three radiation parameters: linear attenuation coefficient(LAC), half value layer (HVL) and tenth value layer(TVL). These parameters were calculated based on the extensive Hubble tabulations (Hubble, 1982) and using Phy-X/PSD (Sakar *et al.,* 2020) and WinXcom (Gerward *et al.,* 2004) codes

LAC (cm^{-1}) is defined as the probability of interaction of the photon as they pass through the glassy material which equal the sum of coherent scattering photoelectric absorption and Compton scattering interaction cross section processes. LAC depends on the photon energy and the atomic number of the material and is given by

$$LAC = -\frac{1}{x}\ln\left(\frac{I}{I_0}\right) \qquad (2)$$

Where x is sample thickness, I and I_0 are the incident and transmuted intensities respectively.

HVL(cm) is defined as the thickness required to reduce the intensity by 50%. HVL depends also on the photon energy and the atomic number of the material whereas TVL(cm) is defined as the thickness required to reduce the intensity by 10%. HVL and TVL are given by

$$HVL = \frac{0.693}{LAC} \qquad (3)$$

$$TVL = \frac{2.302}{LAC} \qquad (4)$$

Neutron attenuation parameter is given by fast neutron removal cross section (FNRCS) term which defined as effective or sum neutron removal cross section of elements in glass composition and was calculated by Phy-X/PSD code. The neutron removal cross section of an element with atomic number Z in the glass composition, ΣR_z was based on the equations (Malidarre *et al.,* 2021):

$$\Sigma R_z(cm^2 g^{-1}) = 0.190\, Z^{-0.743} \quad for \quad Z \leq 8 \qquad (5)$$

$$\Sigma R_z(cm^2 g^{-1}) = 0.125\, Z^{-0.565} \quad for \quad Z > 8 \qquad (6)$$

Results and Discussion:

1. Density

The results of the experimentally measured and the theoretically calculated densities of the four borate glass samples are presented in Table 2 and plotted in Figure 2. It can be noticed that the two typed of density are close to each other with deviation error from 1.5% to about 4.5% for sample S1, S3 and S4 with the exception of sample S2 which has deviational error of more than 18%. This large error may be attributed to the configuration of this particular sample as it contained Tl in the composition as well as Al which is an element of lower atomic number compared with Pb in the other samples. The empirical formula used did not contain the element Tl for its formulation and may have to be adjusted to include such element in the model. A correlation between the experimental and empirical densities is made and presented in Figure 3 which reveals a modest correlation between the two densities; correlation coefficient, R = 0.86 and coefficient of determination R^2 = 0.74. This correlation will definitely improve once the empirical formula was modified. Figure 3 also reveals the linear relationship that exist between the measured and calculated densities.

Table 2: density of borate samples

Sample code	ρ_{emp} (g/cm^3)	ρ_{exp} (g/cm^3)	RD%
S1	4.14	4.20	1.45
S2	3.46	4.10	18.50
S3	3.80	3.96	4.21
S4	4.65	4.55	2.15

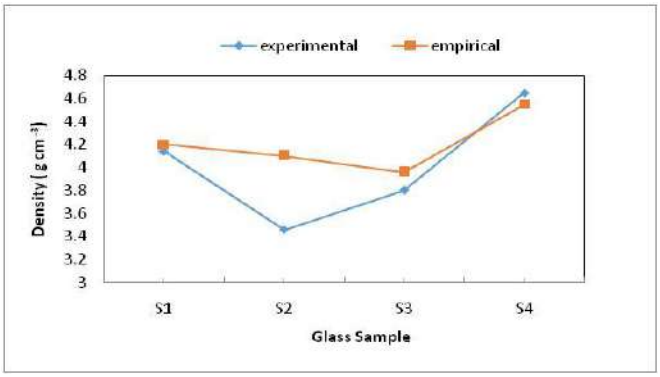


Figure 2 shows experimental and empirical densities of borate glass samples.

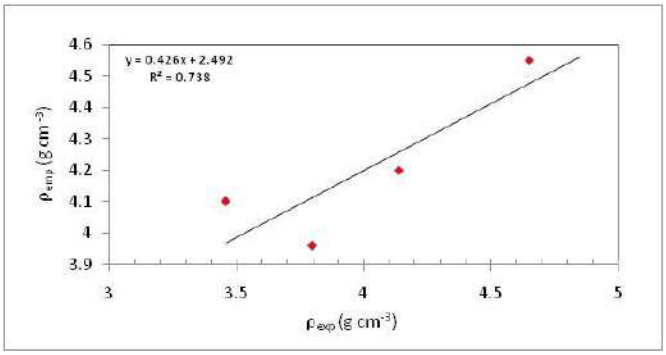


Figure 3 shows the correlation between experimental and empirical densities of borate glass samples.

2. Attenuation Parameters

The results for LAC, HVL and TVL are shown in Figure 4, Figure 5 and Figure 6 respectively for the four borate glass samples and at the imaging photon energy range 15-150 keV. The linear attenuation coefficients in Figure 4 for all the sample shows the familiar decreasing exponential behavior with photon energies. The dominance process is for photoelectric effect at low energies then Compton scattering process takes its place at higher energy. At the low photon energy range between 15-40 keV used for dental and mammography images the glass sample with better attenuation was S4 followed by S1 while samples S2 and S3 showed slight weaker attenuating power. At the radiographic imaging range of 40-80 keV, the Compton effect process started to take over from photoelectric process and the same pattern of attenuation between the samples was maintained with S4, S1 were first and second followed by S2 and S1. At higher photon energy range of 80 to 150 keV dedicated mainly for CT imaging, the dominant process was the Compton scattering process. Glass samples showed slight rise of LAC at 80 to 100 keV then decreased gradually after that. The usual pattern of attenuating power was repeated: S4, S1, S3 and S2. HVL and TVL parameters both had inverse relationship with LAC and therefore showed proportional relationship with photon energy and this can be observed in Figure 5 and Figure 6 respectively. Higher values of HVL can be seen for sample S2 and S3 than S1 and S4 which meant a greater thickness was required from sample S2 and S3 to attenuate photons compared with sample S3 while S4 was the sample with the least thickness to do the same attenuating job.

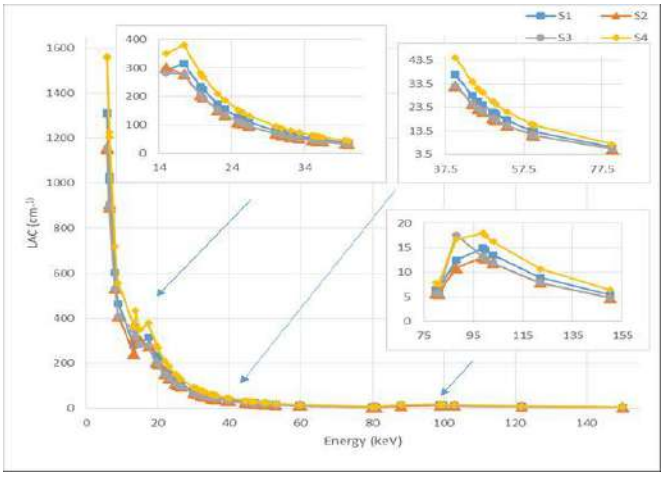


Figure 4: LAC for borate glass samples in the 15-150 keV photon energy range.

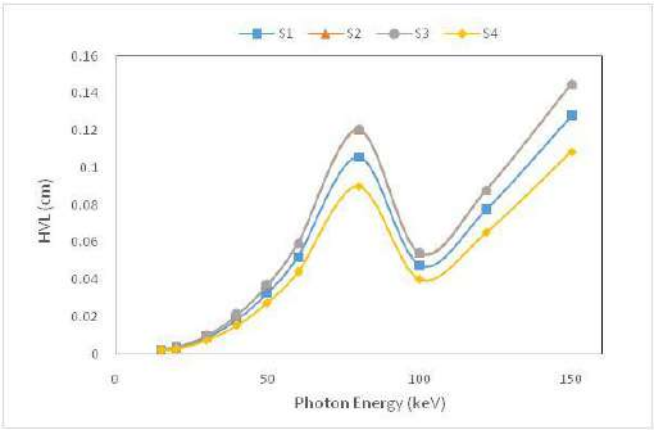


Figure 5: HVL for the borate glass samples in the 15 -150 keV photon energy range.

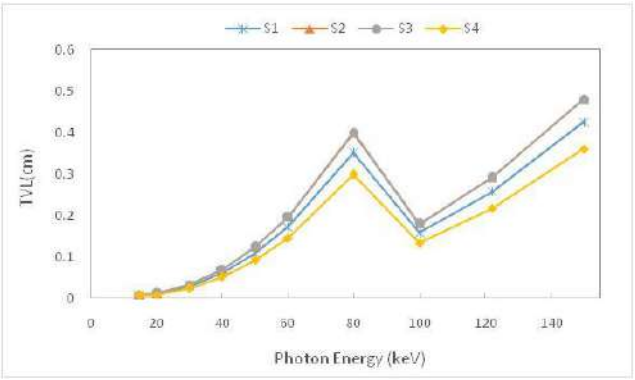


Figure 6: TVL for the borate glass samples in the 15 -150 keV photon energy range.

A comparison of LAC values, calculated by Phy-X/PSD and WinXcom codes, were conducted, as shown in Figure 7, to ensure accurate confirmation of results. It can be noted that similar results were obtained by both codes for all borate glass samples at this energy range which confirm the reliability of both codes in the calculation of attenuation coefficients in research work. The better attenuation properties of Sample S4 may be due to its large contents of high atomic number Pb and Bi elements compared with the others while sample S2 did not contain any Pb at all and had low content of Bi. However, researches are moving now towards the use of lead free material as Pb is considered to be environment pollutants and materials that do not contain lead, as in sample S2, or heavy metals for that matter, are most demanded. Neutrons can be attenuated by glass and windows made of such glass is needed, Borate glass samples were examined for neutron attenuation by calculating fast neutron removal cross section parameter (FNRCS) using Phy-X/PSD code that relies on equations 5 and 6. The results are shown in Figure 8 which indicate that sample S2 was the best attenuator of neutrons followed by S1, S3 and S4 respectively in contrast to photons. This can be explained by the fact that S2 contained the highest contents of Boron which is good neutron absorber, compared with the other samples.

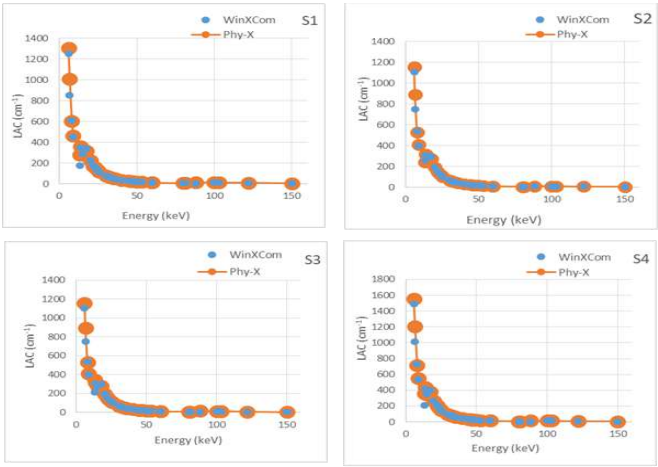


Figure 7 shows LAC parameter calculated by Phy-X/PSD and WinXcom software for the borate glass samples.

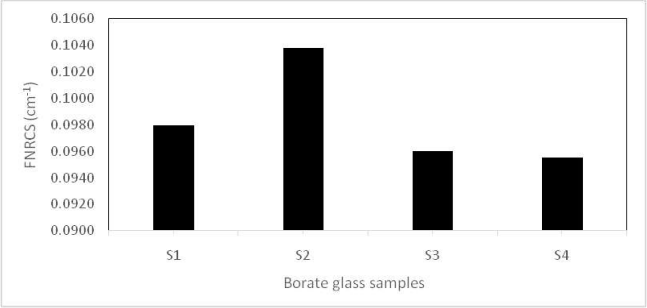


Figure 8: FNRCS for the borate glass samples.

Conclusion:

Successful production of borate glass samples at the local level has been established by the researchers. A great deal of experience in preparation, measurement and calculation has been gained as well as knowledge in radiation and glass physics. Borate glass in its various compositions showed its good attenuation properties for imaging photons and neutrons. It promises to be a good window and shield material as far as radiation properties are concerned. More theoretical and experimental research are required in this interesting glassy material field.

Acknowledgement:

This research work has been conducted by researchers working for scientific projects supervised by *Physics Research Group*, PRG, founded at the Department of Physics, Faculty of Science, University of Benghazi.

References:

Agar,O., (2018),Investigation on Gamma Radiation Shielding Behaviour of $Cd–WO_3–TeO_2$ Glasses from 0.015 to 10 MeV, Cumhuriyet Science Journal, 39,4, 983-990.

Al-Ghamdi, H.; Sayyed,M.I.; Kumar, A.; Yasmin, S.; Elbashir, B.O.; Almuqrin, A.H. (2022), Effect of PbO and B2O3 on the Physical, Structural,and Radiation Shielding Properties of PbO-TeO2-MgO-Na2O-B2O3 Glasses, Sustainability, 14,9695. DOI: https://doi.org/10.3390/su14159695. Academic Editor: Michael I. Ojovan

Bagheri, R., and Adeli, R., (2020),Gamma-ray shielding properties of phosphate glasses containing Bi_2O_3, PbO, and BaO in different rates, Journal of Radiation Physics and Chemistry, 174, 108-118.

Celikbilek E., M., Ersundua, A., E., Gedikoğlua, N., Şakarb, E., Buyukyıldızc, M., Kurudirekb, M., (2019), Physical, mechanical and gamma-ray

shielding properties of highly transparent ZnO-MoO3-TeO2 glasses, Journal of Non-Crystalline Solids, 524, 119648.

Kaur, P., Singh,D., and Singh, T., (2016),Heavy metal oxide glasses as gamma rays shielding material, NuclearEngineering and Design,307, 364–376.

Gerward, L., Guilbert, N., Jensen, K. B.,and Levring, H., (2004),WinXcom a program for calculating x-ray attenuation coefficients, Rad. Phys. and Chem.,71, 653-654.

Hubble, J., H., (1982), Photon mass attenuation and energy absorption coefficients, Int. J. App. Rad. Isot., 33,1269-90.

ICRP, (1977), Recommendation of ICRP, ICRP publication 26, Ann. ICRP 1(3).

Inaba, S., and Fujino, S., (2010),Empirical Equation for Calculating the Density of Oxide Glasses, Journal of the American Ceramic Society,93, 1, 217–220.

kityk, I., V.,Lakshminarayanaa,G., Baki, S., O., Lira, A., Mahdi, M., Kaky, K., M., Caldino, U., and Kitykd, I., V., (2017),Structural, thermal and optical investigations of Dy3+-doped B_2O_3-WO_3-ZnO-Li_2O-Na_2O glasses for warm white light emitting applications, Journal of Luminescence, 186, 283- 300. .

Kumar, A., Jain, A., Sayyed, M., I., Laariedh, F., Mahmoud, K., A., Nebhen, J., Mayeen Uddin Khandaker, M., U., and Faruque, M., R., I., (2021),Tailoring bismuth borate glasses by incorporating PbO/GeO2 for protection against nuclear radiation, Scientific Reports 11:7784.

Malidarre, R. B., Akkurt, I., and Kavas,T., (2021),Monte Carlo simulation on shielding properties of neutron-gamma from ^{252}Cf source for Alumino-Boro-Silicate glasses, Rad. Phys. And Chem., 186.

OECD, (2011), Evolution of ICRP Recommendations 1977, 1990, 2007, NEA No.6920.

Sakar, E., Ozpolat, O. F., Alim, B., Sayyed, M. I., and Kurudirek, M., (2020), Phy-X/PSD: Development of a user friendly online software for calculation of parameters relevant to radiation shielding and Dosimetry, Rad. Phys. and Chem.,166, 108496.

Sharma, G., Singh, K.,Gerward, L., Khanna, A., Kumar, R., Nathuram, R., Singh, H., and Sahota, H., S.,(2005), Gamma-ray shielding properties of CaO-SrO-B_2O_3 glasses, Rad. Phys. and Chem., 72,2-3, 225–228.

AlQalam Journal of Medical and Applied Sciences
Special Issue for 6th International Conference in Basic Sciences and Their Applications (6th ICBSTA, 2023), https://journal.utripoli.edu.ly/index.php/Alqalam eISSN 2707-7179

Using Sulfuric Acid to Produce Bioethanol from Fruit Waste: Banana Peels

Dalal M. Ibrahim*, Azza H. Mohammed and Mastora A. Moamen
Department of Chemistry, Faculty of Science, Omar Al-Mukhtar University, Ei-Beida, Libya
***Correspondence authors:** dalal.m.ibrahim@omu.edu.ly

Abstract:

Agricultural waste made of lignocellulosic materials, such as banana peels, has the potential to yield bioethanol, a sustainable energy source. Our investigation verified the effectiveness of the acidic pretreatment method as a pretreatment for bioethanol production. We used the same H_2SO_4 hydrolysis method for the pretreatment method. Our findings indicated that pretreating banana peels with acid reduced sugar content the most. The reducing sugars were fermented for three days using an active strain of *S. cerevisiae* to produce bioethanol. The maximum absorption spectrum for the bioethanol was found at 280 nm using the potassium dichromate technique. The band in FT-IR spectra confirmed the υ(OH) stretching vibration of ethanol.

Keywords: Bioethanol; Waste Banana peels; UV; IR.

Introduction:

The primary energy source in the world is fossil fuels, which are linked to of energy-related issues, including climate change, global warming, uneven distribution within countries, and non-renewability. Bioethanol, a clean fuel for combustion engines that is derived from plant materials, is a readily available substitute; in 2008, its total consumption was over 65,000 million liters, and its use is growing rapidly, as it replaced 5.4 % of gasoline consumption in 2013 (El-Zawawy *et al.*, 2011). However, deriving bioethanol from food sources is not a viable alternative, because we have to choose between food and ethanol (Sakai *et al.*, 2007). The chemical formula of ethanol, generally known as alcohol, is C_2H_6O. It is a colorless, flammable, volatile liquid. Its density is 0.789 g/cm^3, its melting point is -114°C, its boiling point is 78.37°C, and its molar mass is 46.07 g/mole. It finds extensive usage in the industry as a fuel, solvent, and raw material for the synthesis of other beneficial compounds. In addition, it is used as an antiseptic, heated in homes, and drunk as an alcoholic beverage (Curreli *et al.*, 1997; Yang and

Wyman, 2004). It is an effective, economical, and secure food substitute. Dedicated energy crops, industrial and municipal wastes, and agricultural or forestry leftovers are examples of this type of biomass. The items that have been utilized earlier to create bioethanol include sugarcane bagasse, corn curb, newspapers, sawdust, rice straw, wood, wheat, etc. One of the key characteristics of lignocellulosic biomass is its carbohydrate content, which is necessary for the manufacture of ethanol. Cellulose (40-50 %), hemicellulose (25-35 %), and lignin (15-20 %) make up the biomass (Gray *et al.*, 2006). Out of the three components, cellulose is the predominant polymer. Effective lignocellulosic biomass conversion to fermentable sugars is the rate-limiting stage in the generation of ethanol ((Farrell *et al.*, 2006; Gray *et al.*, 2006). The kind of plant substantially determines the quantity of hemicellulose to be detected. Lignin, a complex polymer of aromatic alcohols known as monolignols that sustain the plants, is the final part of the lignocellulosic biomass. The ultimate goal of all pretreatments and hydrolysis procedures is to overcome the strength and compactness of the lignocellulosic biomass in the ethanol production process (Wyman, 2003). There are two distinct steps involved in turning cellulose polymer into ethanol. **1.** Hydrolyzing cellulose to create glucose units; **2.** Fermenting the sugars obtained from the hydrolysis process with *Saccharomyces cerevisiae* to make ethanol. To produce the sugars required for the hydrolysis and fermentation processes, pre-treatments are often used to separate the mixed polymers of cellulose, hemicellulose, and lignin (Endo *et al.*, 2008). 15 billion metric tons of fossil fuels are used annually. The three nations that utilize the most fossil fuels are China, the United States, and India. According to the Global Material Flow Database (developed by UN Environment Program) by weight, these nations use 54 % of the fossil fuel produced worldwide. Bioethanol may be made inexpensively available to consumers, boosting rural jobs and farmer income while lowering carbon emissions (Hendriks and Zeeman, 2009). We attempted to produce bioethanol from banana peels by pulping them using diluted sodium hydroxide, hydrolysis, and acidic pretreatment with sulfuric acid. We chose banana waste because it was easily accessible and readily available, but we only used acid hydrolysis for the hydrolysis, which was then followed by fermentation with *S. cerevisiae*. Banana peels are a great source of bioethanol, although the yield depends on pre-treatment. This was determined by estimating the sugar content of the peels using Benedict's solution (Cardona *et al.*, 2010).

Materials and Methods:

Banana peel waste is gathered from the neighbourhood fruit juice facility. The fruit skins were chopped into little pieces to make it simpler to handle. They were exposed to both solar and overnight 60ºC oven drying. For further examination, the dry substrate was pulverized, placed in a polyethylene bag, and kept at 0°C.

Pretreatment of waste banana peel

Pretreatment aims to lessen the fiber's crystal nature, compactness, and strength (cellulose). A 200 ml solution of banana peel powder was steeped for 30 to 180 minutes, and the mixture was then autoclaved for 15 minutes at 120°C. The pretreated waste was filtered and the filtrate was btained.

Hydrolysis

Hydrolysis aims to further decompose polysaccharides into constituents of monosaccharides. A solution that had been pretreated was combined with 10 % sulfuric acid. The filtrate was mixed with sulfuric acid in a 6:1 ratio, and the mixture was heated to 120°C for approximately 6 hours before being allowed to cool. Before the fermentation process begins, the Benedict test is used to check the presence of reducing and no reducing sugars.

Benedict test

It saves a great deal of time and work to confirm whether sugar is present before fermentation. The Benedict test was used to figure out how much sugar was produced. 1 ml of the sample solution and 2 ml of Benedict reagents were completely mixed in the test tube, and it was left in a boiling water bath for about 15 minutes. The presence of decreasing sugar is shown by the color changing from blue to green. The last step in the synthesis of bioethanol is fermentation. With the aid of the enzymes zymase and invertase, saccharomyces cerevisiae was utilized to hydrolyze the mono- and disaccharides generated into bioethanol. It has been discovered that Saccharomyces cerevisiae has these enzymes. The hydrolyzed solution is supplemented with yeast cells containing glucose. After the sample was centrifuged and the fermentation process was allowed to continue for eight or nine days, the fermented bioethanol was distilled.

Sample characterization

To determine its unique qualities, the produced bioethanol was subjected to characterization procedures. To determine the surface plasmon resonance (SPR) band for optical qualities, the absorption spectra was recorded using a UV-Vis spectrometer. The method of FTIR Spectroscopy involves determining how an infrared radiation interacts with a sample, which may be solid, liquid, or gaseous. It detects the strengths of these absorptions as well as the frequencies at which the sample absorbs. Because various frequencies of radiation are absorbed by chemical functional groups, the frequencies may be used to determine the chemical composition of the sample.

Results and Discussion:

The investigation's findings demonstrated that a sizable amount of ethanol was created by the fermented banana peels. To producing ethanol, the solution was stored for three to four days. Regularly record any changes. As the quantity of yeast increases, the amount needed for the fermentation process drastically lowers. The Bioethanol estimation done by potassium dichromate method (Five different test tubes were taken and to each test tube ethanol is added in the increasing concentration and then 2 ml of potassium dichromate solution is added to each test tube and shaked well. UV-Vis Spectroscopy. the absorption peak or biodiesel is around 280 nm this is the value of λ max of bioethanol as shown in figure 1.

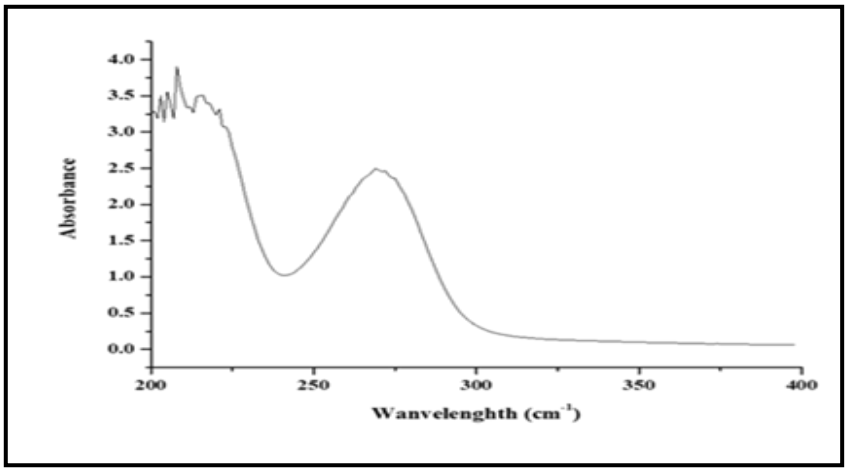


Figure 1. The absorption peaks for bioethanol

Infrared spectral analysis

The infrared absorption spectra of the investigated bioethanol are shown in figure 2. Inspection of the IR spectra and the bands frequencies data shows the presence of a broad band at 3498-3496 cm^{-1} which corresponds to the stretching vibration of the OH group. The broad shape is usually attributed to the fact that the molecules containing the OH group. The bands in the 2821-2819 cm^{-1} region are caused by the stretching vibration of aliphatic C–H bonds (alkyl group) (Phwan *et al.,* 2019).

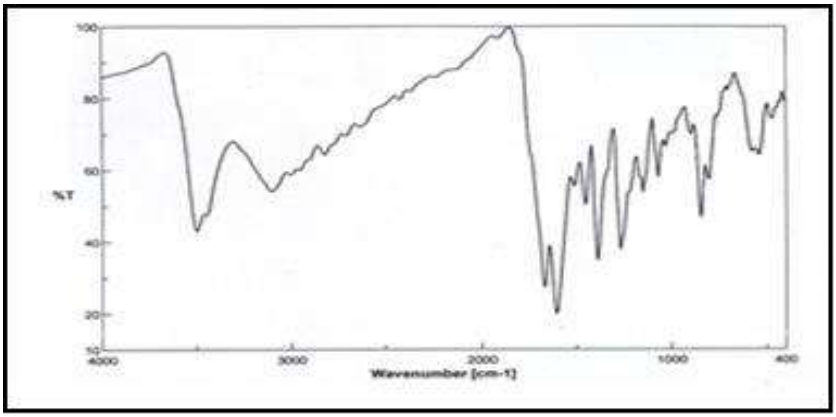


Figure 2: The Infrared of bioethanol

Conclusion:

To determine whether it would be feasible to produce bioethanol from banana peels as a source of lignocellulosic biomass, this study used three different pretreatment techniques as the first step in the experimental design: acidic, alkaline, and water pretreatments. These techniques produced varying concentrations of reducing sugar after hydrolysis with sulfuric acid; Benedict's test confirmed that pretreatment with acid produced the highest reducing sugar concentration. However, alkaline pretreatment achieved the best conversion of reducing sugars to ethanol. The outcome unequivocally shows that every pretreatment method has benefits of its own. Pretreatment with water alone, without any chemical augmentation, often produced poor results in the glucose and ethanol studies for Bioethanol Production from Banana Peels. This study has established that the most effective way to make ethanol from banana waste is to use an alkaline pretreatment, followed by acid hydrolysis, and then *S. cerevisiae* for the fermentation process. In general, additional enzymatic approaches should be applied for the hydrolysis because

acid hydrolysis is quite extremely costly. More particular ways to increase the production of bioethanol are therefore necessary.

References:

Cardona, C. A., Quintero, J. A., & Paz, I. C. (2010). Production of bioethanol from sugarcane bagasse: status and perspectives. *Bioresource Technology*, *101*(13), 4754-4766.

Curreli, N., Fadda, M. B., Rescigno, A., Rinaldi, A. C., Soddu, G., Sollai, F., Vaccargiu, S., Sanjust, E. & Rinaldi, A. (1997). Mild alkaline/oxidative pretreatment of wheat straw. *Process Biochemistry*, *32*(8), 665-670.

El-Zawawy, W. K., Ibrahim, M. M., Abdel-Fattah, Y. R., Soliman, N. A., & Mahmoud, M. M. (2011). Acid and enzyme hydrolysis to convert pretreated lignocellulosic materials into glucose for ethanol production. *Carbohydrate Polymers*, *84*(3), 865-871.

Endo, A., Nakamura, T., Ando, A., Tokuyasu, K., & Shima, J. (2008). Genome-wide screening of the genes required for tolerance to vanillin, which is a potential inhibitor of bioethanol fermentation, in Saccharomyces cerevisiae. *Biotechnology for Biofuels*, *1*, 1-6.

Farrell, A. E., Plevin, R. J., Turner, B. T., Jones, A. D., O'hare, M., & Kammen, D. M. (2006). Ethanol can contribute to energy and environmental goals. *Science*, *311*(5760), 506-508.

Gray, K. A., Zhao, L., & Emptage, M. (2006). Bioethanol. *Current Opinion in Chemical Biology*, *10*(2), 141-146.

Hendriks, A. T. W. M., & Zeeman, G. (2009). Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresource Technology*, *100*(1), 10-18.

Phwan, C. K., Chew, K. W., Sebayang, A. H., Ong, H. C., Ling, T. C., Malek, M. A., Ho, Y. C. & Show, P. L. (2019). Effects of acids pre-treatment on the microbial fermentation process for bioethanol production from microalgae. *Biotechnology for Biofuels*, *12*(1), 1-8.

Sakai, S., Tsuchida, Y., Okino, S., Ichihashi, O., Kawaguchi, H., Watanabe, T., Inui, M. & Yukawa, H. (2007). Effect of lignocellulose-derived inhibitors on growth of and ethanol production by growth-arrested Corynebacterium glutamicum R. *Applied and Environmental Microbiology*, *73*(7), 2349-2353.

Wyman, C. E. (2003). Potential synergies and challenges in refining cellulosic biomass to fuels, chemicals, and power. *Biotechnology Progress*, *19*(2), 254-262.

Yang, B., & Wyman, C. E. (2004). Effect of xylan and lignin removal by batch and flowthrough pretreatment on the enzymatic digestibility of corn stover cellulose. *Biotechnology and Bioengineering*, *86*(1), 88-98.

AlQalam Journal of Medical and Applied Sciences
Special Issue for 6th International Conference in Basic Sciences and Their Applications (6th ICBSTA, 2023), ***https://journal.utripoli.edu.ly/index.php/Alqalam*** ***eISSN 2707-7179***

Effects of ginger and garlic on semen quality in male rabbits

Amani M. M. Salem
Department of Biomedical Science, Faculty of Pharmacy, Omar Al-Mukhtar University, El-Beida, Libya.
Correspondence authors: *amani.elshohopy@omu.edu.ly*

Abstract:

Ginger powder and extricates have been considered for their antioxidant and antimicrobial properties both in dietary supplementation and in nourishment conservation. Analysts have demonstrated that garlic has restorative, antimicrobial properties, speeds up assimilation and broadly utilized as additives, flavor and condiment in numerous homes. This think about was pointed to ponder the impacts of these cancer prevention agents on levels of semen quality in male rabbits. Creatures were relegated to one of three treatment bunches control bunch, 40 mg A garlic or 100 mg ginger/kg BW. In general, the two plants appear comparative impact on diverse measured parameters. Comes about gotten appeared that treatment with garlic or ginger caused significant ($P<0.05$) increment in BW compared to control creatures. In same table, comes about were found altogether increment ejaculate volume (EV), sperm concentration and total sperm output (TSO), Packed sperm volume PSV sperm motility (%), total motile sperm (TMS), total functional sperm fraction (TFSF), normal sperm, initial fructose (In case) and significant ($P<0.05$) diminish in pH, dead sperm and reaction time (RT) compared to control gather.

Keywords: Rabbits; garlic; ginger; semen.

Introduction:

Ginger (Zingiber officinale Roscoe, Zingiberaceae) contains naturally dynamic substances such as gingerol, shogaols, gingerdiol, and gingerdione (Banihani, 2019). In expansion, ginger rhizome has been found to contain wide run of antioxidant compounds, such as vitamin E, ascorbic acid, pyridoxine, beta-carotenes, quercetin, lutein, lycopene, tannin, and genistein (de Lima *et al.*, 2018). Restorative characteristics of ginger are inferable to antioxidant (Danwilai *et al.*, 2017), immunomodulatory (Ali *et al.*, 2008), anti-

inflammatory (Jeena *et al.,* 2013), antimicrobial (Park *et al.,* 2008), antitumorigenic (Akhlaghi *et al.,* 2014), and antiapoptotic properties (Ali *et al.,* 2008). The utilize of ginger (in several ways), for progressing semen quality, has been already explored in a few tests on male people and animals (Hosseini *et al.,* 2016). Amin and Hamza (2006) and Amin *et al.* (2008) detailed changes in sperm reasonability, sperm motility, and sperm variation from the norm in male pale skinned person rats encouraged orally 1 g/kg/day with ethanol extricate of ginger for 26 days. It has been illustrated that the verbal utilization of 250 mg ginger powder (GP) in infertile men a twice a day diminished sperm DNA fragmentation, in any case, it had no impact on sperm number and sperm mortality (Hosseini *et al.,* 2016). In expansion, ginger contains an adequate sum of gingerol, gingerdiol, and ginerdione, related with the enactment of the digestive enzyme action (Dieumou *et al.,* 2009). Undoubtedly, ginger extracts has been appeared to enhance the growth performance, resistance, and antioxidative capacity of rabbits (Ogbuewu and Mbajiorgu, 2022). Ginger is additionally utilized to upgrade the carcass composition and filet quality in rabbit meat (Mancini *et al.,* 2018). The admissions of ginger (*Zingiber officinale* Roscoe) altogether diminished the concentration of thiobarbituric acid-reactive substances (TBARS), lipid peroxidation and the arrangement of malonaldehyde in rats (Ippoushi *et al.,* 2007). Garlic (Gar) increases antioxidant defense component in animals (Ouarda and Abdennour, 2011). Supplementation of garlic oil at 0.5 g/kg of slim down reveals a positive impact on testicles weight, antioxidant status, and testosterone hormone in rabbits (El-Gogary *et al.,* 2018). Alagawany *et al.* (2016) appeared that garlic enhanced the immunity responses and dropped the lipid profile in blood, lipid peroxidation in liver, and expanded hepatic antioxidant action in treated rabbits. The major bioactive components in garlic such as Allicin are mostly dependable for the positive impacts of garlic (Santhosha *et al.,* 2013). Fertile and regenerative exhibitions as well as physiological parameters were made strides essentially by expansion of garlic powder to rabbit slim down (Onu and Aja, 2011).

Materials and Methods:

Ginger was gotten from Transcendent Food and Enumerating by Jarrow Conditions, Los Angeles, USA. Garlic oil was obtained from open advertise for restorative herbs in El-Beida city. Create male white rabbits (6 months ancient) and beginning weight of (1.892±27.8 Kg) were utilized. Animals

were separately housed in cages and weighed week by week all through 6-weeks. The group begin with gather was utilized as control. Whereas, groups 2 and 3 were treated with garlic 40 mg/kg and ginger 100 mg/kg BW separately (El-Speiy *et al.*, 2017). Rabbits were orally managed their particular measurements for 6-weeks. At the conclusion of the exploratory period, body weight of rabbits was recorded. Semen collection was done week after week and proceeded all through the 6-weeks test period, so 60 ejaculates gotten per treatment. Ejaculates were collected utilizing a counterfeit vagina and a secret doe. The volume of each ejaculate was recorded (employing a graduated collection tube) after evacuation of the gel mass. A frail eosin arrangement (Smith and Mayer, 1955) was utilized for assessment of sperm concentration by the progressed Neubauer haemocytometer slide (GmbH+Co., Brand stwiete 4, 2000 Hamburg 11, and Germany). Total sperm output calculated by increasing semen ejaculate volume and semen concentration. Resolution of initial fructose concentration in seminal plasma was decided promptly after semen collection agreeing to Mann (1948). Evaluation of dead and typical spermatozoa were performed utilizing an eosin-nigrosine blue staining combination (Blom, 1950). The proportion of motile sperm were assessed by visual examination under low-power amplification (10×) utilizing light magnifying lens. Whole number of motile sperm was calculated by duplicating the proportion of motile sperm and entire sperm output. Reaction time was resolved as the time of subjecting a doe to the buck until the completion of erection; it was measured in seconds. Starting hydrogen particle concentration (pH) was decided instantly after collection utilizing pH agreeable paper (Universal indicator pH 0-14 Merck, Merck Kga A, 64271 Darmstadt, Germany). Packed sperm volume (PSV) was recorded. Total functional sperm fraction (TFSF) was calculated as the result of add up total sperm output, motility (%), and normal morphology (%) (Correa and Zavos, 1996). Where appropriate, factual investigation was carried out in Minitab computer program (form 17). Measurable noteworthiness was surveyed utilizing ANOVA investigation with Tukey numerous comparison test after location typical dispersion to the information and suitable $P<0.05$ consider critical.

Results:

Table 1 representing body weight (BW) of male rabbits (control and treated with garlic and ginger). The current results indicated that treatment with garlic

and ginger caused significant (P<0.05) increase in body weight compared to control animals. In same table, results were found significantly increase ejaculate volume (EV), sperm concentration, total sperm output (TSO), packed sperm volume (PSV) sperm motility (SM), total motile sperm (TMS) , total functional sperm fraction (TFSF) , normal sperm, initial fructose IF and significant (P<0.05) decrease in pH, dead sperm and reaction time RT compared to control group.

Table 1. The overall means (±SE) of BW, EV, pH, RT, PSV, SC, TSO, SM, TMS, DS, TFSF,NS and IF during treatment of male rabbits with ginger, /or garlic.

Parameter	Groups		
	Control	Ginger	Garlic
BW (g)	1892±50.79^{a}	3648±42.8^{b}	1918±39.84^{a}
EV (ml)	0.74±0.017^{b}	0.82±0.018^{a}	0.80±0.022^{a}
PH	7.83±0.022^{b}	7.69±0.038^{a}	7.75±0.054^{b}
RT (sec.)	4.05±0.099^{b}	3.23±0.145^{a}	3.68±0.17^{a}
PSV (%)	15.3±0.16^{b}	17.6±0.38^{a}	15.4±0.35^{a}
SC	263±4.5^{b}	319±7.0^{a}	278±6.4^{a}
TSO (×10^{6})	195±5.1^{b}	265±9.5^{a}	222±5.3^{a}
SM (%)	68.2±0.7^{b}	73.3±0.9^{a}	70.3±1.3^{a}
TMS (×10^{6})	133±4.1^{b}	197±8.4^{a}	157±4.9^{a}
DS (%)	26.6±0.82^{b}	22.0±0.63^{a}	23.0±0.99^{a}
TFSF (×10^{6})	108±3.4^{b}	168±7.9^{a}	131±4.3^{a}
IF (mg/dl)	257±3.9^{b}	276±3.7^{a}	271±5.4^{a}
NS (%)	80.1±0.3^{b}	84.7±0.4^{a}	83±0.4^{a}

Values are expressed as means ± SE; n=5 for each treatment group. Mean values within a row not sharing a common superscript letter (a, b, c, d) were significantly different, p< 0.05.

Discussion:

The increment body weight detected within the current study due to treatment with ginger is accordance with Celia *et al.* (2016) and Omage *et al.* (2007). Too, Ademola *et al.* (2009) described remarkable increment in body weight gain (14.4 %) of broilers nourished ginger. They described that increment in body weight gain of the broilers nourished ginger demonstrates the positive nutritive impacts of this natural bolster additive. The consequence of the current study about showed that *Zingiber officinale* has an advantageous impact on male regenerative functions and semen characteristics in rabbits. According to the reports, ginger's antioxidant and androgenic properties were able for expanding fertility potential following its administration (Gholami-Ahangaran *et al.*, 2021; Akhlaghi *et al.*, 2014). Moreover, it has been indicated that the high sperm count within the sperm storage tubules in

inseminated hens was the result of feeding aged chickens with an antioxidant source (Borghei-Rad *et al.,* 2017). This development in growth rate of rabbits bolstered garlic supplemented diets compared to the control is in line with the discoveries of Ademola *et al.* (2009) who detailed increment in weight gain of rabbits and broilers nourished garlic supplemented diets consecutively. Moreover, the current study too demonstrated that ginger alone caused remarkable increments in ejaculate volume, sperm concentration, total sperm output, sperm motility, normal sperm, total motile sperm, total functional sperm fraction and semen packed sperm volume. Whereas, remarkable diminishes dead sperm, semen initial fructose, semen initial hydrogen ions concentration (pH) and reaction time. These results are in considerable agreement with the results of El-Amary and Abou-Warda (2007) who found that quality of semen parameters was essentially higher after including garlic to male rabbit diets. Moreover, the current study are compatible with the results of Ouarda and Abd-Ennour (2011) who found that treatment of rabbits with row garlic reestablished lead-induced diminish in sperm speed, motility and practicality.

Conclusion: Treatment with ginger and garlic caused an upgrade within the productive and regenerative execution of male rabbits.

References:

Ademola, S. G., Farinu, G. O., & Babatunde, G. M. (2009). Serum lipid, growth and haematological parameters of broilers fed garlic, ginger and their mixtures. World J. Agric. Sci, 5(1), 99-104.

Akhlaghi, A., Ahangari, Y. J., Navidshad, B., Pirsaraei, Z. A., Zhandi, M., Deldar, H., ... & Peebles, E. D. (2014). Improvements in semen quality, sperm fatty acids, and reproductive performance in aged Cobb 500 breeder roosters fed diets containing dried ginger rhizomes (Zingiber officinale). *Poultry Science*, *93*(5), 1236-1244.

Alagawany, M., Ashour, E. A., & Reda, F. M. (2016). Effect of dietary supplementation of garlic (Allium sativum) and turmeric (Curcuma longa) on growth performance, carcass traits, blood profile and oxidative status in growing rabbits. *Annals of Animal Science*, *16*(2), 489-505.

Ali, B. H., Blunden, G., Tanira, M. O., & Nemmar, A. (2008). Some phytochemical, pharmacological and toxicological properties of ginger (Zingiber officinale Roscoe): a review of recent research. *Food and chemical Toxicology*, *46*(2), 409-420.

Amin, A., & Hamza, A. A. (2006). Effects of Roselle and Ginger on cisplatin-induced reproductive toxicity in rats. Asian journal of andrology, 8(5), 607-612.

Amin, A., Hamza, A. A., Kambal, A., & Daoud, S. (2008). Herbal extracts counteract cisplatin-mediated cell death in rat testis. Asian journal of andrology, 10(2), 291-297.

Banihani, S. A. (2019). Effect of ginger (Zingiber officinale) on semen quality. Andrologia, 51(6), e13296.

Blom, E. (1950). A one-minute live-dead sperm stain by means of eosin-nigrosin. *Fertility and sterility*, *1*, 176-177.

Borghei-Rad, S. M., Zeinoaldini, S., Zhandi, M., Moravej, H., & Ansari, M. (2017). Feeding rosemary leaves powder ameliorates rooster age-related subfertility. *Theriogenology*, *101*, 35-43.

Celia, C., Cullere, M., Gerencsér, Z., Matics, Z., Giaccone, V., Kovács, M., ... & Dalle Zotte, A. (2016). Dietary supplementation of Digestarom® herbal formulation: effect on apparent digestibility, faecal and caecal microbial counts and live performance of growing rabbits. *World Rabbit Science*, *24*(2), 95-105.

Correa, J. R., & Zavos, P. M. (1996). Preparation and recovery of frozen-thawed bovine spermatozoa via various sperm selection techniques employed in assisted reproductive technologies. *Theriogenology*, *46*(7), 1225-123

Danwilai, K., Konmun, J., Sripanidkulchai, B. O., & Subongkot, S. (2017). Antioxidant activity of ginger extract as a daily supplement in cancer patients receiving adjuvant chemotherapy: a pilot study. *Cancer management and research*, 11-18.

de Lima, R. M. T., Dos Reis, A. C., de Menezes, A. A. P. M., Santos, J. V. D. O., Filho, J. W. G. D. O., Ferreira & Melo-Cavalcante (2018). Protective and therapeutic potential of ginger (Zingiber officinale) extract and [6]-gingerol in cancer: A comprehensive review. *Phytotherapy research* 32(10), 1885-1907.

Dieumou, F. E., Teguia, A., Kuiate, J. R., Tamokou, J. D., Fonge, N. B., & Dongmo, M. C. (2009). Effects of ginger (Zingiber officinale) and garlic (Allium sativum) essential oils on growth performance and gut microbial population of broiler chickens. *Livestock research for rural development*, *21*(8), 23-32.

El-Amary, H. H., & Abou-Warda, M. A. (2007). Effect of different levels of garlic and leek as additives to rabbit rations on production and reproductive performance. *J. Agric. Sci. Mansoura Univ*, *32*(12), 9832-9843.

El-Gogary, M. R., Mansour, A. M., & El-Said, E. A. (2018). Blood biochemical and immunological responses to garlic oil administration

in growing rabbits diet. *Journal of Agricultural Science*, *10*(1), 217-224.

El-Speiy, M. E., Khaled, F. A., & El-Hanoun, A. M. (2017). Effect of ginger supplementation on reproductive performance of male rabbits. *Glob Sci J Biol*, *2*(2), 26-31.

Gholami-Ahangaran, M., Karimi-Dehkordi, M., Akbari Javar, A., Haj Salehi, M., & Ostadpoor, M. (2021). A systematic review on the effect of Ginger (Zingiber officinale) on improvement of biological and fertility indices of sperm in laboratory animals, poultry and humans. Veterinary medicine and science, 7(5), 1959-1969.

Hosseini, J., Mamaghani, A. M., Hosseinifar, H., Gilani, M. A. S., Dadkhah, F., & Sepidarkish, M. (2016). The influence of ginger (Zingiber officinale) on human sperm quality and DNA fragmentation: A double-blind randomized clinical trial. *International Journal of Reproductive BioMedicine*, *14*(8), 533.

Ippoushi, K., Takeuchi, A., Ito, H., Horie, H., & Azuma, K. (2007). Antioxidative effects of daikon sprout (Raphanus sativus L.) and ginger (Zingiber officinale Roscoe) in rats. *Food Chemistry*, *102*(1), 237-242.

Jeena, K., Liju, V. B., & Kuttan, R. (2013). Antioxidant, anti-inflammatory and antinociceptive activities of essential oil from ginger. *Indian J Physiol Pharmacol*, *57*(1), 51-62.

Mancini, S., Secci, G., Preziuso, G., Parisi, G., & Paci, G. (2018). Ginger (Zingiber officinale Roscoe) powder as dietary supplementation in rabbit: life performances, carcass characteristics and meat quality. *Italian Journal of Animal Science*, *17*(4), 867-872.

Mann, T. (1948). Fructose content and fructolysis in semen. Practical application in the evaluation of semen quality. *The Journal of Agricultural Science*, *38*(3), 323-331.

Ogbuewu, I. P., & Mbajiorgu, C. A. (2022). META-ANALYSIS OF THE POSITIVE EFFECT OF GINGER FEED ADDITIVE ON HEALTH AND PRODUCTION INDICES OF LAYING HENS. Applied Ecology & Environmental Research, 20(2).

Omage, J. J., Onimisi, P. A., Adegbite, E. K., & Agunbiade, M. O. (2007). The effect of ginger (Zingiber officinale Roscoe) waste meal on growth performance, carcass characteristics, serum lipid and serum cholesterol profiles of rabbit. Pakistan Journal of Nutrition, 6(4), 359-362.

Onu, P. N., & Aja, P. M. (2011). Growth performance and haematological indices of weaned rabbits fed garlic (Allium sativum) and ginger (Zingiber officinale) supplemented diets. *International Journal of Food, Agriculture and Veterinary Sciences*, *1*(1), 51-59.

Ouarda, Abdennour, C. (2011). Evaluation of the therapeutic efficiency of raw garlic on reproduction of domestic rabbits under lead induced toxicity. *Ann Biol Res*, *2*(3), 389-393.

Park, M., Bae, J., & Lee, D. S. (2008). Antibacterial activity of [10]-gingerol and [12]-gingerol isolated from ginger rhizome against periodontal bacteria. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 22(11), 1446-1449.

Santhosha, S. G., Jamuna, P., & Prabhavathi, S. N. (2013). Bioactive components of garlic and their physiological role in health maintenance: A review. *Food Bioscience*, *3*, 59-74.

Smith, J. T., & Mayer, D. T. (1955). Evaluation of sperm concentration by the hemacytometer method: Comparison of four counting fluids. *Fertility and Sterility*, *6*(3), 271-275.

AlQalam Journal of Medical and Applied Sciences
Special Issue for 6th International Conference in Basic Sciences and Their Applications (6th ICBSTA, 2023), https://journal.utripoli.edu.ly/index.php/Alqalam eISSN 2707-7179

A New Study Stable Symmetric Families of Exponential Distribution

Naeima N. Abd elati[1], Kamilah A. Othman[2], Jalila Alfirjany[3] and Rabeia N. Elfargane[4]
[1]Department of Statistic, Faculty of Science, University of Benghazi, Benghazi, Libya.
[2]Department of Statistic, Faculty of Arts and Science Al-Marj, University of Benghazi, Benghazi, Libya
[3]Department of Electrical and Electronic, Faculty of Engineering, Benghazi University, Benghazi, Libya.
[4]Department of computer science, Benghazi University, Benghazi, Libya.
Correspondence author: alfrjanynymh25@gmail.com

Abstract

In this study, we aim to introduce and explore a new category of density functions that requires the addition of two parameters through a family of distributions. The stable symmetric family of distribution functions (cdf's) includes the reverse of every cdf that belongs to it and the combination of the baseline distribution and its inverse can be stable when multiplied and divided with the same parameter. The objective of this technique is to produce a new three-parameter extension of the exponential cdf that can be symmetric and have a function of non-constant hazard rate. The properties of this category of density functions are investigated.

Keywords: Stable symmetric family, Mixture distribution, Parametric family, Exponential function, Skewness, Kurtosis.

1. Introduction:

Adding parameters (usually, adding one or more) shape parameters to a well-known distribution which, is called from now on the baseline distribution, to reshape this baseline distribution. The primary goal of this method is to obtain a distribution family that has additional statistical characteristics that are preferable, such as higher values of range for skewness or kurtosis coefficients or its failure rate function (or risk function) has a certain mathematical form differently than its baseline distribution. Existing work has explored extended families of distribution such as the one proposed by Marshall (2014). These families achieve a unique stability property: adding their defining shape parameter twice to the baseline distribution results in further expansion of the family. This indicates a well-defined and non-redundant parameterization, making them suitable for modeling diverse data. In addition, the power

distribution family, a well-known distribution family, is achieved by increasing the baseline distribution to a positive power. A generalization of the beta distribution is a significant extended family that has been studied by Jones *et al*. (2004). To obtain this family, substitute the independent variable in the partial beta function for the distribution of the baseline. Also, the skew-normal distribution suggested by Azzalini (1985) is used to construct Azzalini's family, which is considered one of the most significant extended families. This family has been generalized by Arellano-Valle *et al*. (2004) through the addition of more shape parameters. The Kumaraswamy family is a more adaptable extended family than the beta-distribution family that was proposed by Cordeiro and de Castro (2011). This family has nearly identical mathematical characteristics to the beta distribution family, but unlike it, it does not rely on any particular function, which makes it more manageable than the beta distribution family. Alzaatreh (2011) employed the gamma distribution through an expanded distribution family, while Lee *et al*. (2013) developed a general method to expand any starting distribution into a distribution family including its baseline. The literature now covers dozens of distribution families created from numerous baseline distributions, with their characteristics, advantages, and disadvantages being studied. Barakat (2015) introduced a new distribution family (A symmetric family that is additive and stable, abbreviated by ASS) the combination of the baseline distribution makes it simpler than the most common families. After adding to it a positive location parameter, and its reverse distribution subtracting the same location (distribution of negative random variable), thus the used location parameter revolved to a parameter of shape and developed an extended distribution family with the mixture parameter and this location parameter. This family possesses a single property, on which it is built on the base, and if the family involves any distribution, it should include the reverse distribution, which the author calls a stable symmetrical family. In addition, Barakat (2015) proved that these families could describe several forms of statistical data by using standard normal distribution or df of exponential as baseline distribution. Moreover, The distribution function should be a part of one or more the following nine types : (00,0+,0-,+0,++,+-,-0,-+,--) where, the first side of sign refer to the coefficient of skewness (symmetric '0', positive symmetric'+', negative symmetric'-') and the second side of sign denote to the coefficient of kurtosis (mesokurtic '0', leptokurtic'+', platykurtic'-').

2. Material and Method:

There are many methods of generalized families (extended families) of distributions such as using a differential equation to generate, adding shape parameters, composition, transformation, compounding, and finite mixture. We focus on the generalization based on a finite mixture with an exponential distribution function. In this study, we present and explore a new stable family that uses the distribution function of exponential mixture and its inverse, by multiplying and dividing scale parameters, correspondingly to provide a new three-parameter expansion of exponential cdf. In subsection 2.1 we show a short view of finite mixture generalization and in subsection 2.2 displays that the stability of any baseline df and its inverse combination when perform multiplying and dividing to the same scale parameter remains constant. Moreover, we study the mathematical characteristics of the symmetrical family that is stable of df's. In section 3, we call that the proposed family (a new stable family), the properties of this family are studied; also the new proposed family, which is multiplicative and stable, is known as the MSSE family.

2. 1. Finite Mixture Generalization

This method also defined as the mixture model, that is a convex grouping of two or more, pdf's is a significant and suitable tool for modeling difficult data since it associations the characteristics of the individual pdf's. In addition to these advantages, mixture models are utilized in numerous statistical analysis and machine learning applications for instance: modeling, clustering, classifications, latent class and survival analysis. The term mixture employed to describe the pdf of a random observation is a mixing of various different element density functions of the form.

$f(x) = \sum_{i=n}^{m} a_i f(x_i)$, with $\sum_{i=n}^{m} a_i = 1, a_i > 0$, for all $i = 1,2, \dots, m,$ and $f(x_i), i = 1,2, \dots, m,$ are diverse densities through identified form. Moreover, individually one of these densities is determined by one or more unidentified parameters. Lastly, every observation originates from one of the m (diverse) element distributions with unidentified membership status.

2. 2. The stable symmetric family of distribution function

To every continuous df F_X, use the combination of df's F_X and F_{-X} to build a new extended family – stable symmetric family with two extra parameters, $c > 0$ and $0 \le \alpha \le 1$, as follows:

$$G_Y(x;\alpha,c) = S^{(*)}_{\alpha,C}(F_X(x)) = \alpha F_X\left(\frac{x}{c}\right) + \bar{\alpha}\,\bar{F}_X(-cx) \qquad (2.1)$$

The $\bar{\alpha}$ is define as $\bar{\alpha} = 1 - \alpha$. In particular, Definition 2.1 provides an explanation for these designations. The df F_X is referred to as the base or reference df and $S^{(*)}_{\alpha,C}$ is known as the operation of symmetric parameterization. In certain situations, such as when discussing the G moments, it is more appropriate to assign the df G through its random variable (let's assume Y) and write G_Y. It is simple to calculate the survival function and pdf of the function of new distribution df $G_Y(x;\alpha,c)$ are, separately:

$$g_y(x;\alpha,c) = \frac{d}{dx}\left(G_Y(x;\alpha,c)\right) = \frac{\alpha}{c} f_X\left(\frac{x}{c}\right) + c\bar{\alpha} f_X(-cx) \qquad (2.2)$$

$$\bar{G}_Y(x;\alpha,c) = 1 - G_Y(x;\alpha,c) = \alpha\left(1 - F_X\left(\frac{x}{c}\right)\right) + \bar{\alpha}\left(1 - \bar{F}_X(-cx)\right)$$

$$= \alpha\bar{F}_X\left(\frac{x}{c}\right) + \bar{\alpha}\,F_X(-cx) \qquad (2.3)$$

Definition 2.1 if $G_Y \in SS$ indicates that $G_{-Y} \in SS$, then we refer to a category SS of df's stable symmetric.

It is evident that, for all continuous df's F_X, then

$$k_F = \left\{G_Y : S^{(*)}_{\alpha,C}(F_X) = G, 0 < \alpha < 1, c > 0\right\} = SS.$$

(Note that $S^{(*)}_{\alpha,C}(F_X) = G_Y(\alpha,c) \in K_F$ then $S_{\bar{\alpha},\frac{1}{c}}(F_X) = G_{-Y}(\bar{\alpha},\frac{1}{c}) \in K_F$.

Proposition 2.1 it is simple to verify for every base df F_X

1. $S^{(*)}_{\alpha,c}(F_X(x)) = G_Y(x;\alpha,c)$ is symmetric about zero for every $0 \le \alpha \le 1$ and every $c > 0$ in the case of the base df F_X is symmetric around zero (i.e. $G_Y(x;\alpha,c) = \bar{G}_Y(-x;\alpha,c)$).
2. $S^{(*)}_{1,1}(F_X(x)) = F_X(x)$, That is, as a particular case, its base is involved in the newly formed G. Moreover, for $\alpha \neq 1$, $S^{(*)}_{\alpha,1}(F_X(x)) = F_X(x)$, if and only if F_X is symmetric about zero.

3. $S^{(*)}_{\alpha,\frac{1}{c}}\left(F_{-X}(x)\right) = G_{-Y}(x;\alpha,c)$, (that is, the (2.1) family has symmetric stability), then given (2.3), where

$$G_{-Y}(x;\alpha,c) = \bar{G}_Y(-x;\alpha,c) = \alpha F_{-X}\left(\frac{x}{c}\right) + \bar{\alpha}\,\bar{F}_{-X}(-cx) = S^{(*)}_{\alpha,\frac{1}{c}}\left(F_{-X}(x)\right) \qquad (2.4)$$

4. $S^{*}_{\vartheta,1}\left(S^{*}_{\alpha,1}\left(F_X(x)\right)\right) = \vartheta G_Y(x;\alpha,1) + \bar{\vartheta}\,\bar{G}_Y(-x;\alpha,1) = (\alpha\vartheta + \bar{\alpha}\,\bar{\vartheta})F_X(x) + (1-\alpha\vartheta-\bar{\alpha}\,\bar{\vartheta})\bar{F}_X(-cx) = S^{*}_{\alpha\vartheta+\bar{\alpha}\,\bar{\vartheta},1}\left(F_X(x)\right)$

The process of univariate stable symmetric parameterization is stable according to Marshall(2014).

5. The application of operator $S^{*}_{\alpha,c}$ to the multivariate df 's, is evident for example, for all baseline bivariate df F_{X_1,X_2} , we get

$$G_{Y1,Y2}(x_1,x_2;\alpha,c) = S^{(*)}_{\alpha,C}\left(F_{X_1,X_2}(x_1,x_2)\right) = \alpha F_{X_1,X_2}\left(\frac{x_1}{c},\frac{x_2}{c}\right) + \bar{\alpha}\,\bar{F}_{X_1,X_2}(-cx_1,-cx_2) \qquad (2.5)$$

The survival function and probability density function of the multiple df 's:
Where $\bar{F}_{X_1,X_2}(-cx_1,-cx_2) = 1 - F_{X1}(x_1) - F_{X2}(x_2) + F_{X_1,X_2}(x_1,x_2)$

$$\bar{G}_{Y1,Y2}(x_1,x_2;\alpha,c) = 1 - G_{Y1}(x;\alpha,c) - G_{Y2}(x;\alpha,c) + G_{Y1,Y2}(x_1,x_2;\alpha,c) = \alpha\bar{F}_{X_1,X_2}\left(\frac{x_1}{c},\frac{x_2}{c}\right) + \bar{\alpha}\,F_{X_1,X_2}(-cx_1,-cx_2) \qquad (2.6)$$

$$g_{Y1,Y2}(x_1,x_2;\alpha,c) = \alpha f_{X_1,X_2}\left(\frac{x_1}{c},\frac{x_2}{c}\right) + \bar{\alpha}\,f_{X_1,X_2}(-cx_1,-cx_2) \qquad (2.7)$$

Theorem 2.1 Alfarageny (2019). If we have rv X, let $M_X^{[K]} = E(x^k)$ and $C_X^{[K]} = E(X - M_X)^K$ is the kth moment of X, correspondingly, when $M_X = E(x)$ also $k = 1,2,\ldots$. Then,

$$M_Y^{[K]} = [\,\alpha c^k + (-1)^K\bar{\alpha}\,c^{-k}]M_X^{[K]} \qquad (2.8)$$

And

$$C_Y^{[k]} = \sum_{j=0}^{k}\binom{k}{j}C_X^{[j]}\left(\frac{1}{c}\right)^k[(1+c^2)M_X]^{k-j}\left(\alpha\bar{\alpha}^{k-j}c^{2j} + (-1)^k\bar{\alpha}\alpha^{k-j}\right) \qquad (2.9)$$

3. The Multiplicative Stable Symmetric Exponential Family (The MSSE Family)

Let $F_X(x;\theta) = \left(1 - e^{-\frac{1}{\theta}x}\right)I_{(0,\infty)}(x)$, $\theta > 0$, represents the df of exponential with parameter θ. When $I_A(x) = 0,1$ if $x \notin A$, $x \in A$,

respectively by take $F_X(x;\theta)$ as a baseline df, the proposed new family, indicated by family of MSSE, is described through the mixture of the $F_X(x;\theta)$ and its reverse $\bar{F}_X(-x,\theta)$ inside the equation (2.1) in subsection (2.2), by:

$$G_Y(x;\alpha,c,\theta) = S^{(*)}_{\alpha,C}(F_X(x,\theta)) = \alpha\left(1 - e^{\frac{-x}{\theta c}}\right) I_{(0,\infty)}(x) + \bar{\alpha}\, e^{\frac{\alpha x}{\theta}} I_{(-\infty,0)}(x) + \bar{\alpha} I_{(0,\infty)}(x) \qquad (3.1)$$

The survivor function and the pdf are, respectively

$$\bar{G}_Y(x;\alpha,c,\theta) = 1 - G_Y(x;\alpha,c,\theta)$$
$$= \alpha\, e^{\frac{-x}{\theta c}} I_{(0,\infty)}(x) + \bar{\alpha}\left(1 - e^{\frac{cx}{\theta}}\right) I_{(-\infty,0)}(x) + \alpha I_{(-\infty,0)}(x) \qquad (3.2)$$

And $$g_Y(x;\alpha,c,\theta) = \frac{\alpha}{\theta c}\, e^{\frac{-x}{\theta c}} I_{(0,\infty)}(x) + \frac{\bar{\alpha} c}{\theta} e^{\frac{cx}{\theta}} I_{(-\infty,0)}(x) \qquad (3.3)$$

Theorem 3.1, dedicated to the family of MSSE, we obtain

1. The mean is $M_Y = \left(\alpha c - \frac{\bar{\alpha}}{c}\right)\theta$
2. The variance is $\delta_Y^2 = C_Y^2 = \frac{\theta^2}{C^2}\left((1 + C^2)^2 \alpha\bar{\alpha} + (\alpha c^4 + \bar{\alpha})\right)$
3. The skewness coefficient is

$$\gamma_Y^{[1]} = \frac{C_Y^3}{(\delta_Y)^{3/2}} = \frac{(1-c^2)^3 \alpha\bar{\alpha}(1-2\alpha) + 3(1+c^2)^2(c^2-1)\alpha\bar{\alpha} + 2(\alpha c^6 - \bar{\alpha})}{((1+C^2)^2\alpha\bar{\alpha} + (\alpha c^4 + \bar{\alpha}))^{3/2}}$$

where

$$\gamma_Y^{[1]} = \begin{cases} = 0, & \text{if } \alpha = \frac{1}{2}, c = 1 \\ > 0, & \text{if } \begin{cases} \alpha > \frac{1}{2}, c \geq 1 \\ \alpha \leq \frac{1}{2}, c > 1 \end{cases} \\ < 0, & \text{if } \begin{cases} \alpha < \frac{1}{2}, c \leq 1 \\ \alpha \geq \frac{1}{2}, c < 1 \end{cases} \end{cases}$$

4. The kurtosis coefficient is

$$\gamma_Y^{[2]} = \frac{C_Y^4}{(\delta_Y^2)^2} = \frac{(1+c^2)^4 \alpha\bar{\alpha}(\bar{\alpha}^3 + \alpha^3) + 6(1+c^2)^2 \alpha\bar{\alpha}(\bar{\alpha} c^4 + \alpha) + 8(1+c^2)(c^6+1)\alpha\bar{\alpha} + 9(\alpha c^8 + \bar{\alpha})}{((1+C^2)^2\alpha\bar{\alpha} + (\alpha c^4 + \bar{\alpha}))^2}$$

Proof. Theorem 2.1 is immediately applied to the proof, using standard computations.

Proposition 3.1 (the hazard rate function). By using (3.3) and (3.2), the hazard rate function of the MSSE family is:

$$h_y(x) = \frac{g_Y(x;\alpha,c,\theta)}{G_Y(x;\alpha,c,\theta)} = \frac{\frac{\alpha}{\theta c} e^{\frac{-x}{\theta c}} I_{(0,\infty)}(x) + \frac{\bar{\alpha}c}{\theta} e^{\frac{cx}{\theta}} I_{(-\infty,0)}(x)}{\alpha e^{\frac{-x}{\theta c}} I_{(0,\infty)}(x) + \bar{\alpha}(1-e^{\frac{cx}{\theta}}) I_{(-\infty,0)}(x) + \alpha I_{(-\infty,0)}(x)}$$

$$= \frac{\bar{\alpha}c e^{\frac{cx}{\theta}}}{\theta\left(1-\bar{\alpha} e^{\frac{cx}{\theta}}\right)} I_{(-\infty,0)}(x) + \frac{1}{\theta c} I_{(0,\infty)} .$$

Then, the subsequent characteristic could be directly proved

1. $h_y(x)$ increases on $x \in (-\infty, 0)$ and has a constant value on $\in (0, \infty)$.
2. $\lim_{x\to-\infty} h_y(x) = 0$, $\lim_{x\to 0^-} h_y(x) = \frac{\bar{\alpha}c}{\alpha\theta}$, $\lim_{x\to\infty} h_y(x) = \frac{1}{\theta c}$

Table 3.1: Some selected values of $\gamma_Y^{[1]}$ and $\gamma_Y^{[2]}$

Types	$0 \le \alpha \le 1$	$c > 0$	$\theta > 0$	$\gamma_Y^{[1]}$	$\gamma_Y^{[2]}$	δ_Y^2	M_Y
0+	0.5	1	1	0	6	2	0
0+	0.5	1	3	0	6	18	0
-+	0.9	0.5	1	-3.1074	31.4377	1.1875	0.25
-+	0	2	3	-2	9	2.25	-1.5
-+	0.4	0.5	3	-2.2293	9	36	-3
++	0.1	2	3	6.489	31.4377	10.6875	-0.75
++	1	2	3	2	9	36	6
++	0.2	3	3	8.9438	25.9256	33	1

Table 3.1 presents some values of M_Y , δ_Y^2 , $\gamma_Y^{[1]}$ and $\gamma_Y^{[2]}$ that contains three types (0+, -+, ++) of statistical data of df 's, whereas the exponential distribution contains one type of statistical data (++). To calculating these moments, we generated a simple algorithm by Matlab 8.2 on a laptop Intel 1.8GHZ computer.

We include the graph of the pdf (Figure 3.1) of the second one of every type in Table 3.1.

Proposition 3.2. From the density function of the MSS-exponential we have

1. The explanation of the nonlinear equation identifies the mode m
   $\frac{\alpha}{c^2} e^{\frac{-m}{\theta c}} - \bar{\alpha} c^2 e^{\frac{cm}{\theta}} = 0$, $0 < \alpha < 1$
   Moreover, $m = \infty$, if $\alpha = 0$, while $m = -\infty$, , if $\alpha = 1$.
2. The median M is determined by the solving the nonlinear equation
   $\alpha\left(1 - e^{\frac{-M}{c\theta}}\right) + \bar{\alpha} e^{\frac{cM}{\theta}} = 0.5$, $0 < \alpha < 1$

Moreover, $M = \frac{\theta \log 0.5}{c} < 0$, if $\alpha = 0$, while $= -c\theta \log 0.5 > 0$, $\alpha = 1$

3. The moment generation function (mgf) is given by

$$\Upsilon_Y(t) = E(e^{tx}) = \alpha(1 - \theta ct)^{-1} + \bar{\alpha}c(c + \theta t)^{-1}$$

Proof. The proof is elementary. Moreover, we note that if $= \frac{1}{2}, c = 1$, we get the mean equal to the median and the mode, i.e., $\mu_Y = M = m$.

4. Result:

- The MSSE family contains many forms of statistical data, and has a remarkably diverse range of indexes of skewness and kurtosis. For example, $\gamma_Y^{[2]}$ reached 31.4377, however the maximum amount of the kurtosis coefficient of Azzalini's family is 3.869. In contrast, $-3.1074 \leq \gamma_Y^{[1]} \leq 8.9438$, whereas for the skew-normal distribution, we have $-0.995 \leq$ the skewness coefficient ≤ 0.995.
- The hazard rate function is increases (i.e., the new family has non–fixed function of hazard rate).
- The MSS family is symmetric when $\alpha = \frac{1}{2}, c = 1$, also it can be shown that $\gamma_Y^{[2]} = \gamma_X^{[2]}$ if $\alpha = 0$ or $\alpha = 1$ and $G_Y(x; \alpha, c, \theta)$ is leptokurtic for any $c > 0$ and $0 \leq \alpha \leq 1$.

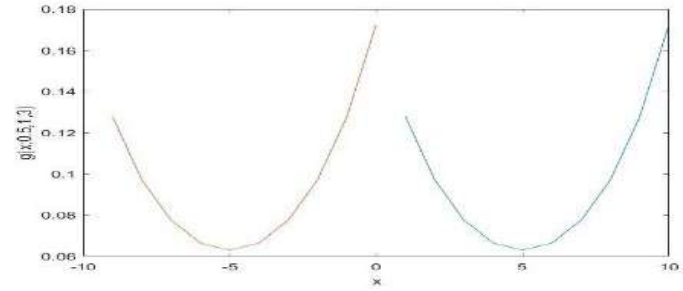
5. Conclusion:

We studied a class of distribution referred to as the multiplicative stable symmetric exponential family (MSSE Family), we have consequent several characteristics of the MSSE family, containing the mean, variance, skewness, kurtosis, mode, median, the moment generating function, and the hazard rate function. We have that the MSSE family contains many varieties of statistical data, and possesses a very remarkably varied range of indexes of skewness and kurtosis. Moreover, the family is symmetrical and has a non-fixed function of hazard rate.

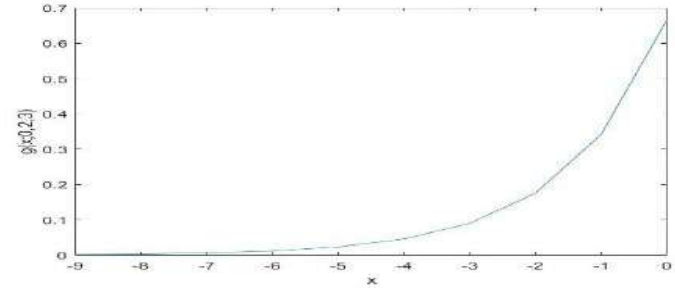
6. Future work:

Performing simulation studies with various different sample sizes and various parameter values of the MSS exponential family can result in statistical inferences for the MSSE family (3.1). Furthermore, The EM algorithm can be utilized to obtain the standard maximum likelihood estimation (MLE) of the family parameters by applying iterative approaches to approximate the maximum likelihood function. Additionally, apply the

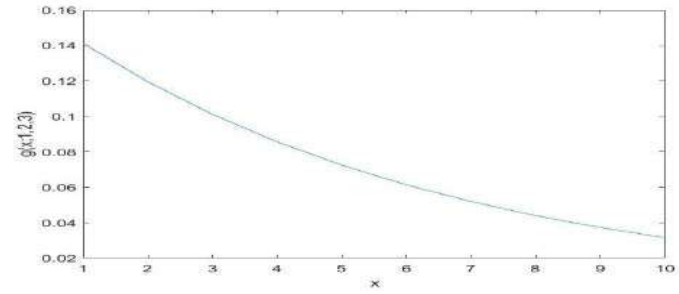
real data, and compare them with other distributions for instance (Weibull distribution, Exponential distribution, Normal distribution, Laplace distribution).



Type (0+)



Type (-+)



Type (++)

Figure 3.1 The pdf of the MSSE family

References

Arellano-Valle, R. B., Gómez, H. W., & Quintana, F. A. (2004). A new class of skew-normal distributions. *Communications in Statistics - Theory*

and Methods, *33*(7), 1465–1480. https://doi.org/10.1081/STA-120037254.

Azzalini, A. (1985). A Class of Distributions Which Includes the Normal Ones. *Scandinavian Journal of Statistics*, *12*(2), 171–178. http://www.jstor.org/stable/4615982.

Alzaatreh, A. (2011). *A new method for generating families of continuous distributions*. Ph.D. thesis, Central Michigan University Mount Pleasant, Michigan.

Alfarageny, N. (2019). *Study of Some Stable Symmetric Families of Distributions*. Ph.D. thesis, Faculty of Science.Alexandria University. Alexandria Egypt.

Barakat, H. M. (2015). A new method for adding two parameters to a family of distributions with application to the normal and exponential families. *Statistical Methods and Applications*, *24*(3), 359–372. https://doi.org/10.1007/s10260-014-0265-8.

Cordeiro, G. M., & de Castro, M. (2011). A new family of generalized distributions. *Journal of Statistical Computation and Simulation*, *81*(7), 883–898. https://doi.org/10.1080/00949650903530745.

Jones, M. C., Arnold, B. C., David, H. A., Kent, J. T., Nagaraja, H. N., Ferreira, J. T. A. S., & Steel, M. F. J. (2004). Families of distributions arising from distributions of order statistics. *Test*, *13*(1), 1–43. https://doi.org/10.1007/BF02602999

Lee, C., Famoye, F., & Alzaatreh, A. Y. (2013). *Methods for generating families of univariate continuous distributions in the recent decades*. https://doi.org/10.1002/wics.1255.

Marshall, B. Y. A. W. (2014). *A new method a parameter to a family for adding with to the exponential and application of distributions Weibull families*. *84*(3), 641–652.

AlQalam Journal of Medical and Applied Sciences
Special Issue for 6th International Conference in Basic Sciences and Their Applications (6th ICBSTA, 2023), *https://journal.utripoli.edu.ly/index.php/Alqalam* ***eISSN 2707-7179***

Chemical and Microbiological Analysis of Groundwater after Major Flood Incident in Derna City

Nuri M. Abduali* and Nawal A. Rajab
Department of Chemistry, Faculty of Science, Derna University, Al-Gubba, Libya
Correspondence authors: * *nuri.masoud@uod.edu.ly*

Abstract:

During and after flooding, human exposure to bacteria, microorganisms and chemical pollutants through contaminated water causes epidemic disease outbreaks. Unprecedented torrential rainfall with high intensity and dam failure created major floods, inundating the wells and allowing sewage-contaminated water to enter the wells. In this study, groundwater samples were collected immediately after the flood stage (September 2023) from seven different regions in Derna city to determine heavy metals concentration, microbiological and physico-chemical properties. The results showded that the concentrations of the studied heavy metals ranged between (0.0010–0.070µg/g), (0.0001–0.0012µg/g), (0.002–0.09µg/g) and (0.003–0.012µg/g) for Pb, Cd, Fe, and Cu, respectively. The concentrations of metals were very much below the permissible limits defined by WHO. Additionally, a chemical analysis of the samples revealed that the water varied from neutral to slightly alkaline (pH 7.3 – 7.8) and (E.C) ranged between (926 –2056 µs/cm), which indicates little increase in the proportion of water salinity. The results also showed the values of parameters; most of them exceeded the maximum allowed limit in drinking water: TDS, TH, TA, and Cl^- ranged (463–1028) (250–760), (228.4–380.3), and (290–406), whereas other parameters were within the limits allowed: TOC, Ca^{2+}, Na^+, and NO_3^- ranged (12.9–15.8), (63–235), (21.38–42.19) and (1.04–2.86), parameters expressed in mg/L,. respectively. With regard to microbiological characteristics, the total bactria count was determined by microbiological examination to be (>100 cfu/100ml). As well as, *Escherichia coli*, a hazardous bacteria pathogen, which was found in some of the samples analyzed.

Keywords: Derna, Groundwater, Heavy metals, Chemical/Microbiological, Contamination.

Introduction:

Environmental factors such as geology and geochemical processes may have an impact on groundwater quality. Geogenic sources are one of the causes of the fluctuation in the chemical composition of groundwater, which varies with time and place. It is determined by the parent rock, the degree of weathering, the length of residency, and external elements such as precipitation, temperature, and so on. Weathering, dissolving, mixing, ion exchange and other hydrogeochemical processes influence the concentration of main and minor ions in groundwater (Zahid *et al.*, 2008). Pathogenic microorganisms in soil and groundwater can affect human, animal and plant health. Extensive cultivation increases salinization risk, while fertilizers and pesticides pollute groundwater. Industrial areas often experience heavy metal pollution from complex effluents (Abdelrahman and Eltahir, 2010).

Microbial Contamination and Chemical Changes

Microbial contamination can have several negative effects on water quality can cause waterborne diseases. These diseases can range from mild gastrointestinal illnesses to more severe conditions sush as cholera, typhoid fever, or hepatitis. Also, Microbes can contribute to the turbidity or cloudiness of water. This reduces its aesthetic appeal and can make it less suitable for certain uses like swimming or fishing (Edberg *et al.*, 2000). Likewise, physicochemical properties of drinking water can impact its quality, potentially causing corrosion, scaling, taste issues, and affecting the growth and survival of aquatic organisms. Water with higher EC levels may have a distinct taste or odor due to the presence of minerals, salts, or other dissolved solids. It can give a "metallic" or "salty" taste to the water, which may not be preferred by everyone (WHO, 2011).

Heavy Metals: Sources of Pollution and Effects on Human Health

In context, the term heavy metal refers to any metallic chemical element that has a relatively high density (greater than 5 g/cm^3), a high atomic weight, and is hazardous or poisonous at low concentrations. They include transition metals, metalloids, lanthanides, and actinides (Akan *et al.*, 2010). In comparison to their physical features, the chemical properties of heavy metals are the most useful. Environmental toxicity that exceeds established maximum residue limits (MRL) has attracted increased attention from think tanks around the world. Mercury, cadmium and lead generate a frightening combination of

environmental and health issues (Storelli, 2009). Copper, iron, manganese, and zinc, among other metals, are essential micro-nutrients for humans involved in important biological processes. These elements prevailingly plays functional and structural role in human body (Jaishankar *et al* 2014). Scientists categorize heavy metal sources into anthropogenic and natural sources. Natural sources include sedimentary rocks, volcanic eruptions, soil formation, rock weathering and carbonate rocks, while anthropogenic sources include mining, agriculture, industries, and home wastewater (Izah *et al* 2016). Diabetes, Alzheimer's disease, and various types of cancer are just a few of the illnesses that are becoming more prevalent as a result of heavy metal pollution. Acute metal poisoning results in substantial abnormalities in the nervous, reproductive, and renal systems in people. In addition to the specific symptoms of metal toxicity, chronic exposures to copper, lead, mercury, cadmium, and arsenic have been linked to gastrointestinal (GI) dysfunctions, diarrhea, stomatitis, shivering, and hemoglobinuria that causes rust-red depression. Heavy metals can be harmful to human health even when present in small amounts in the environment (Appenroth, 2010). Generally, the aim of the study was to examine the impacts of flood events on water quality. As shown in Figure (1), pollution and factors affecting water quality are identified, such as physical-chemical parameters that control the chemistry of water in this area, essential and toxic heavy metal content, and microbial contamination, to assess the suitability of groundwater for human consumption.

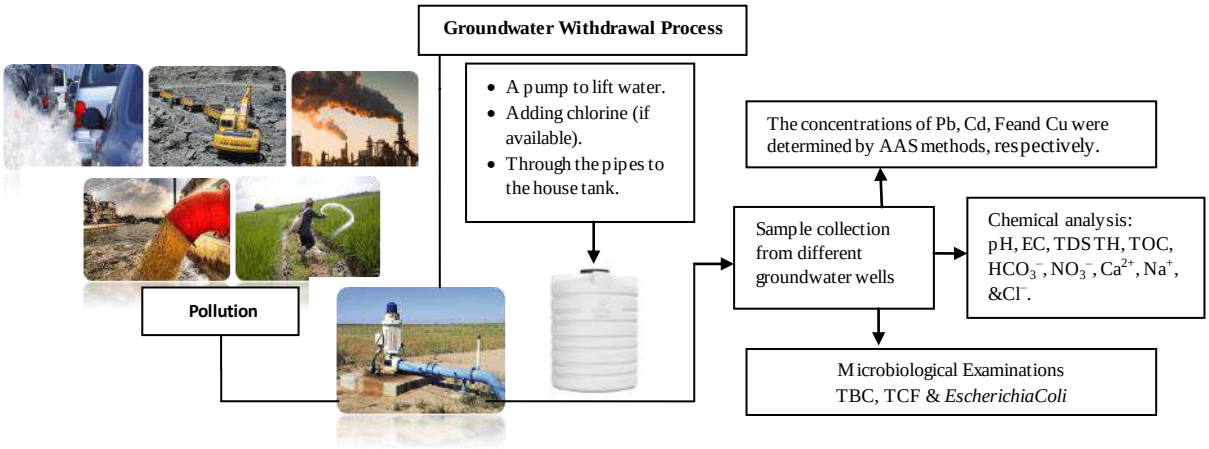


Figure (1): Scheme of the process followed for detection of heavy metals, microbiological and physico-chemical characteristics of groundwater samples.

Materials and Methods:

Description of the Study Area

The area of study is located in the north-east of Libya in an important locality on the eastern coast of Libya, which is also part of the south shoreline of the Mediterranean basin and its geographical coordinates are located between the latitudes of 32°37' and 32°48' north and the longitudes of 22°30' and 22°55' east. The farms are spread out over a large region of the city, and the zone has been heavily impacted by pollutants from a cement factory, a water desalination plant, agricultural chemicals, and recently, floods. The soil of Derna is classified as Rendolls, which is rich in calcium carbonate and is juvenile, and it belongs to the order Mollisols, which is frequent in arid and sub-humid locations (Zurqani *et al*., 2018). Groundwater wells are the primary water source in Libya, including the city of Derna; seven sites were selected at city is a coastal city with a population of (170,289) as the city is boarded from the south by a chain of rocky hills the city has expanded along the shore line Figure (2).

Figure (2): Map of the locations of the studied area (Google Earth)

Sampling

Seven groundwater samples (two replicates from groundwater wells) were collected in pre-washed bottles (1.5 litres) from a representative wells from a flood-inundated region and a similar wells a few hundred meters distant to represent the non-inundated area. The sampling bottles were cleaned with detergent and rinsed with the groundwater to be analyzed. They were transported to the laboratory for relevant chemical and microbiological analysis within 72 hours after sample collection given in Table (1).

Table (1): Description of groundwater well samples.

S. no.	Wells Sites	Wells Depth (M)	Coordinates	
			"N	"E
1	Shehha El-Gharbia	200	32°45'36.1	22°37'17.1
2	Shehha El-Sharqia	150	32°45'41.3	22°37'54.2
3	Alsahil El-Sharqi	350	32°44'56.5	22°39'26.7
4	Alsahil El-Gharbi	300	32°44'55.8	22°39'09.4
5	Bab -Tubraq	200	32°45'25.8	22°38'41.0
6	Al-Maghar	350	32°45'43.6	22°38'01.0
7	Embekh	250	32°46'11.8	22°35'14.9

Chemical Analysis

Measurement of pH: The pH-value of water sample was measured in the site immediately after collection using Bench type (HANNA Digital, HI98107 pH-Meter).

Measurement of Electrical Conductivity and Total Dissolved Solids: EC and TDS of the samples were measured on the spot itself using (HANNA Autoranging Microprocessor Meter).

Measurement of Total hardness and Calcium: The concentration of Ca^{2+} and TH in water samples were determined by Complexometric titration with (EDTA) according to the procedure used by (APHA, 2017).

Measurement of Total Alkalinity: A titration of standard solution (HCl) and a suitable indicator (methyl orange and phenolphthalein) were used to estimate the TA of a water sample experimentally, as described by (Dhoke, 2023).

Measurement of Total Organic Carbon and Nitrate: TOC and NO_3^- were determined using spectroscope analytical techniques (UV/Vis Spectrophotometery – JENWAY™, 6305) at a wavelength of (220 - 280 nm) according to standard methods for (Eaton *et al.*, 2005).

Measurement of Sodium: A flame photometer (JENWAY - CLINICAL, pfp7) was used to measured the concentration of Na^+.

Measurement of Chloride: The chlorine ion-specific electrode (HANNA instruments, HI93711) is used to measure the concentration of Chloride (Cl^-) in water samples.

Heavy Metals Analysis

Water samples were digested using concentrated HNO_3/HCl, diluted with distilled water, and analyzed for heavy metal concentration using an Atomic Absorption Spectrophotometery (AAS) according to the procedure used by (APHA, 2017).

Microbiological Examination

Total bacterial count (TBC) was determined using nutrient agar according to the method described by (Cabral, 2010).Total coliform counts by the five – tube most probable number (MPN) method and *Escherichia. coli* determined on Macconkey agar according to (Figueras and Borrego, 2007).

Results and Discussion:

Physico – Chemical Properties

Results given in Table (2) and Figure (3) indicated that the pH values showed no significant difference between groundwater wells that were within the normal range and leaned toward alkalinity, which may be attributed to basic ions. The pH level of drinking water can vary depending on the source. According to WHO and Libyan standards, the recommended pH range of drinking water is between (6.5 - 8.5). The pH range of water is safe for human consumption, avoiding adverse health effects. Too acidic or too alkaline water can affect taste, smell, treatment effectiveness, tooth erosion, plumbing corrosion, and household appliance damage (Murhekar Gopalkrushna, 2011). Moreover, EC and TDS values displayed a significant increase in some groundwater samples. The EC values vary between 926 and 2056 µS/cm. The highest value of 2056 µS/cm was recorded at the site of (Alsahil El-Gharbi) and the lowest value of 926 µs/cm was recorded at the site of (Embekh). while the values of TDS in this study varied between 463 and 1028 mg/L. The highest value is 1028 mg/L, recorded at location (Alsahil El-Gharbi), while the lowest value was recorded at location (Embekh), which is 463 mg/L. These values are above the permissible level of the WHO, which is recommending TDS values less than 1000 mg/L. These values indicate that the water samples are rich in dissolved materials (ions), designate the water as saline water. Consuming water with high electrical conductivity can upset the balance of electrolytes in the body, leading to electrolyte imbalances. Electrolytes are essential for various bodily functions, including muscle contractions, nerve signaling, kidney Function and maintaining fluid balance

(Batool *et al*., 2018). Concerning the accumulation of TH, TA and Ca^{2+} content in groundwater wells, which ranged from 250 to 390 mg/L, 228.4 to 343.5 mg/L, and 63 to 235 mg/L, respectively, it has been found that values of TH were relatively higher in the Alsahil El-Gharbi region (390 mg/L) than in the Shehha El-Sharqia and Al-Maghar regions (280 and 283 mg/L) as shown in Table 2. Total hardness is a measure of the concentration of certain minerals in water, primarily calcium and magnesium ions. These minerals contribute to the formation of scale in water pipes and appliances, and can also affect the taste and appearance of water. It's important to note that while hard water is not harmful to human health, it can have several adverse effects. It can cause mineral deposits in pipes and appliances, reduce the effectiveness of soaps and detergents, and lead to the formation of scale on faucets and fixtures (Schwartz *et al*., 2002). Furthermore, it was found that the World Health Organization's (WHO) maximum allowable level for total alkalinity in drinking water is 200 mg/L. Upon comparing this figure with the total alkalinity (TA) readings of groundwater samples from the Derna regions, which vary from 228.4 to 343.5 mg/L, we discover that all sample total alkalinity values exceeded the WHO's permissible limit. On the other hand, in compared to the WHO and Libyan standards recommended limits for drinking water (75 mg/L), excessive amounts of Ca^{2+} were found in all groundwater samples except the Alsahil El-Sharqi groundwater well, as indicated in Table 2. Underground water sources, such as wells or aquifers, may naturally contain high levels of calcium due to the mineral content of the surrounding rocks or soil.Regarding estimated TOC and NO_3^- values in the groundwater samples, the highest value of TOC was 15.8 mg/L in (Alsahil El-Sharqi), and the lowest value was 13.7 mg/L in Shehha El-Gharbia wells. Monitoring TOC in water is important because organic carbon can react with disinfectants, such as chlorine, to form potentially harmful disinfection byproducts. High levels of organic carbon in water can also lead to taste, odor, and color issues. While nitrate values were higher value of NO_3^- was 2.86 mg/L in (Bab - Tubraq), and the lowest value was 1.04 mg/L in Shehha El-Gharbia wells. Although nitrate ions by themselves pose no health risks to humans, they can give an indication of potentially harmful bacterial populations. Moreover, methemoglobinemia, or "blue baby" sickness, can be brought on by excessive nitrate ion levels (Pennino *et al*., 2017). Therefore, all values fall within the permissible limits According to WHO specifications and Libyan standards. Meanwhile, the values of sodium content were the highest in Al-Maghar and

Alsahil El-Gharbi district (42.19 and 30 mg/L, respectively) compared other regions. The water of the studied wells was also classified as being mostly contaminated with chlorides, as all of them exceeded the maximum permissible limit in drinking water. Drinking highly concentrated chloride water can lead to dehydration as it can draw water from the cells and cause imbalances in electrolyte levels. Also, consuming too much salt (chloride) can elevate blood pressure levels, especially in individuals who are already predisposed to hypertension (Wu *et al*., 2021).

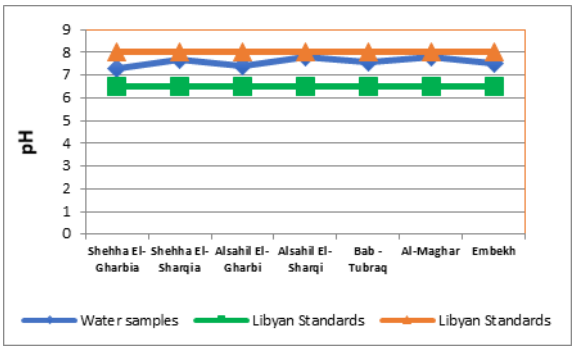
Table (2): The values of physico-chemical parameters of the studied groundwater samples.

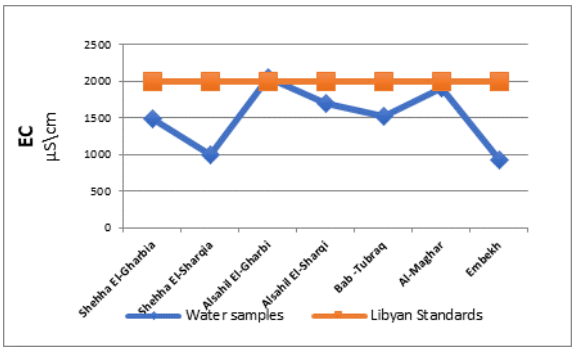
Groundwater Wells Sites	pH	E.C (µs/cm)	T.D.S (mg/L)	TH (mg/L)	TA (mg/L)	TOC (mg/L)	Ca^{2+} (mg/L)	Na^{+} (mg/L)	NO_3^- (mg/L)	Cl^- (mg/L)
Shehha El-Gharbia	7.3	1486	734	382	280.3	13.7	186	30	1.04	326
Shehha El-Sharqia	7.7	990	463	250	314.2	14.1	115	27	1.15	290
Alsahil El-Gharbi	7.4	2056	1028	760	330.8	14.9	128	38	2.56	389
Alsahil El-Sharqi	7.8	1697	848	390	335.5	15.8	63	24.18	2.75	397
Bab -Tubraq	7.6	1530	765	320	320.6	13.8	235	35	2.86	406
Al-Maghar	7.8	1912	956	380	343.5	15.2	90	42.19	2.10	390
Embekh	7.5	926	495	325	228.4	12.9	198	21.38	1.24	377
Max	7.8	2056	1028	760	343.5	15.8	235	42.19	2.86	406
Average	7.58	1514	756	401	308	14.3	145	31.1	1.95	368
SD	0.19	429.5	214.8	166	40.4	1.00	62.6	7.5	0.74	43

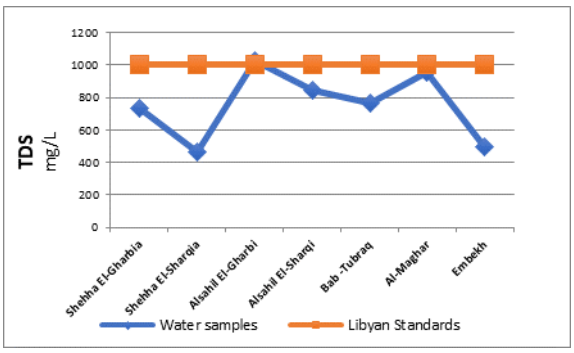
Libyan standards and WHO values for groundwater were used as standards (LNCSM, 2015), as shown in Table (3).

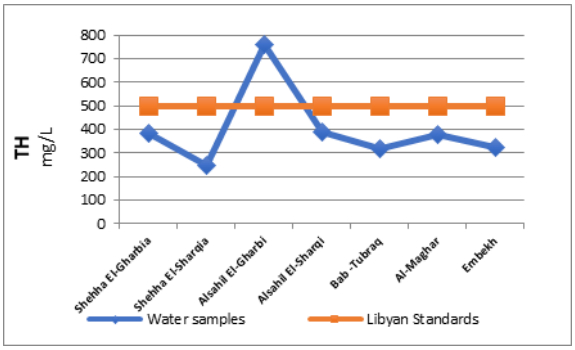
Table (3): Libyan and WHO standards values of drinkinng water

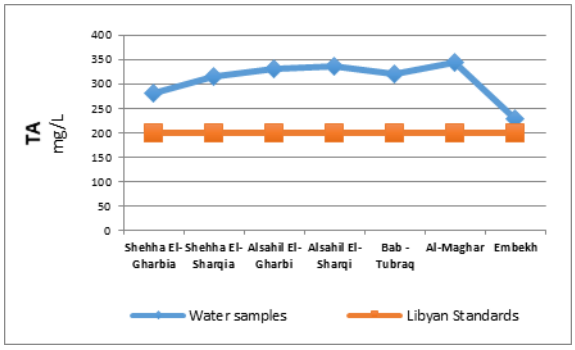
Parameters	Units	Standard values	
		Libyan Standards	WHO Standards
pH	-	6.5 – 8	6.5 – 8
EC	(µs/cm)	2000	2000
TDS	(mg/L)	1000	1000
TH	(mg/L)	500	500
TA	(mg/L)	200	200
TOC	(mg/L)	100	100
Ca^{2+}	(mg/L)	200	200
Na^{+}	(mg/L)	200	200
NO_3^-	(mg/L)	45	50
Cl^-	(mg/L)	250	250

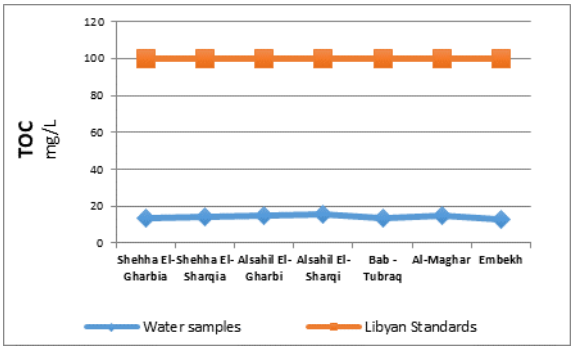

pH
9
8
7
6
5
4
3
2
1
0
Shehha El-Gharbia
Shehha El-Sharqia
Alsahil El-Gharbi
Alsahil El-Sharqi
Bab - Tubraq
Al-Maghar
Embekh
Water samples
Libyan Standards
Libyan Standards

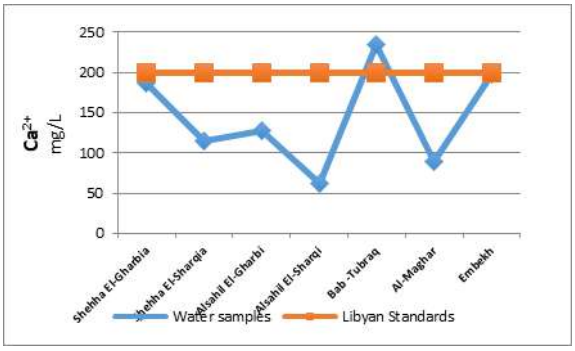

EC
µS\cm
2500
2000
1500
1000
500
0
Shehha El-Gharbia
Shehha El-Sharqia
Alsahil El-Gharbi
Alsahil El-Sharqi
Bab -Tubraq
Al-Maghar
Embekh
Water samples
Libyan Standards

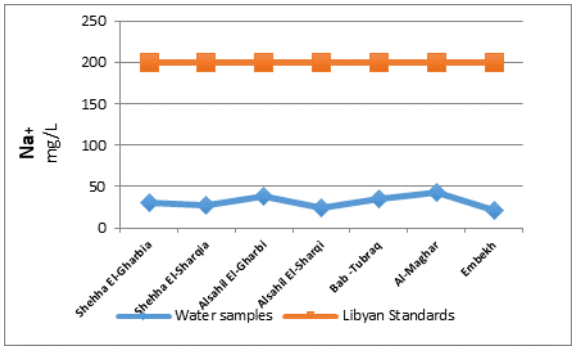

TDS
mg/L
1200
1000
800
600
400
200
0
Shehha El-Gharbia
Shehha El-Sharqia
Alsahil El-Gharbi
Alsahil El-Sharqi
Bab -Tubraq
Al-Maghar
Embekh
Water samples
Libyan Standards


TH
mg/L
800
700
600
500
400
300
200
100
0
Shehha El-Gharbia
Shehha El-Sharqia
Alsahil El-Gharbi
Alsahil El-Sharqi
Bab -Tubraq
Al-Maghar
Embekh
Water samples
Libyan Standards


TA
mg/L
400
350
300
250
200
150
100
50
0
Shehha El-Gharbia
Shehha El-Sharqia
Alsahil El-Gharbi
Alsahil El-Sharqi
Bab - Tubraq
Al-Maghar
Embekh
Water samples
Libyan Standards


TOC
mg/L
120
100
80
60
40
20
0
Shehha El-Gharbia
Shehha El-Sharqia
Alsahil El-Gharbi
Alsahil El-Sharqi
Bab - Tubraq
Al-Maghar
Embekh
Water samples
Libyan Standards


Ca2+
mg/L
250
200
150
100
50
0
Shehha El-Gharbia
Shehha El-Sharqia
Alsahil El-Gharbi
Alsahil El-Sharqi
Bab -Tubraq
Al-Maghar
Embekh
Water samples
Libyan Standards


Na+
mg/L
250
200
150
100
50
0
Shehha El-Gharbia
Shehha El-Sharqia
Alsahil El-Gharbi
Alsahil El-Sharqi
Bab -Tubraq
Al-Maghar
Embekh
Water samples
Libyan Standards

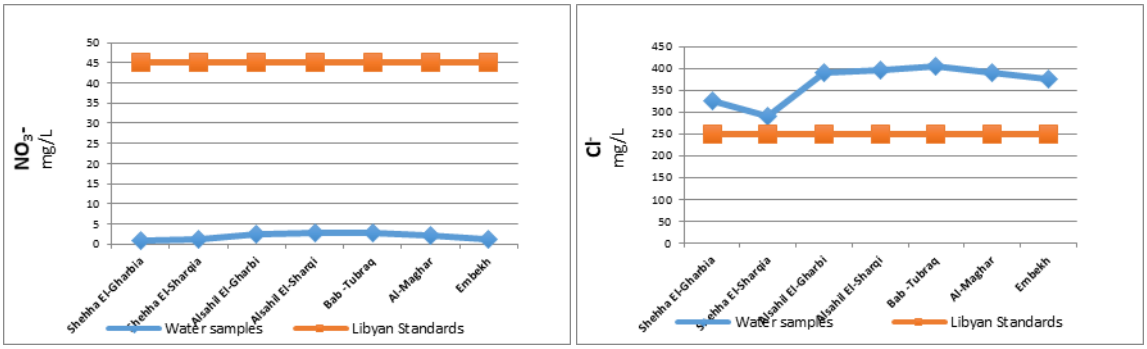


Figure (3): Comparison of physico-chemical parameters values of the water sample and Libyan standard of drinking water

Heavy Metals Content

In this study, the concentrations of heavy metal in groundwater samples collected from Derna city were determined. (Pb, Cd, Cu, and Fe) were measured. The analyzed results were compared to the limits established by the Libyan specification standards and the World Health Organization (WHO) for heavy metal concentration in drinking water to determine whether any metals in the study area had values that exceeded the acceptable limits Table (4). Chemical analysis shows the presence of small percentages of heavy metals; the lead (Pb) concentration ranged from 0.0010 to 0.070 µg/g, while cadmium (Cd) concentrations in all samples ranged from 0.0001 to 0.0012 µg/g. On the other hand, the iron (Fe) contents ranged from 0.0001–0.0012 µg/g, whereas the copper (Cu) concentration varied from 0.003 – 0.012 µg/g.

Table (4): The concentrations (µg/g) of heavy metals different grounwater samples

Groundwater Wells Sites	Pb	Cd	Fe	Cu
Shehha El-Sharqia	0.0011	0.0001	0.002	0.003
Shehha El-Gharbia	0.023	0.0002	0.007	0.008
Alsahil El-Sharqi	0.0010	0.0005	0.03	0.007
Alsahil El-Gharbi	0.0015	0.0001	0.01	0.004
Bab –Tubraq	0.0019	0.0012	0.09	0.012
Al-Maghar	0.070	0.0003	0.04	0.010
Embekh	0.0018	0.0007	0.05	0.002
WHO Standards	0.05	0.005	0.3	1.5
Libyan Standards	0.01	0.003	0.3	1.0

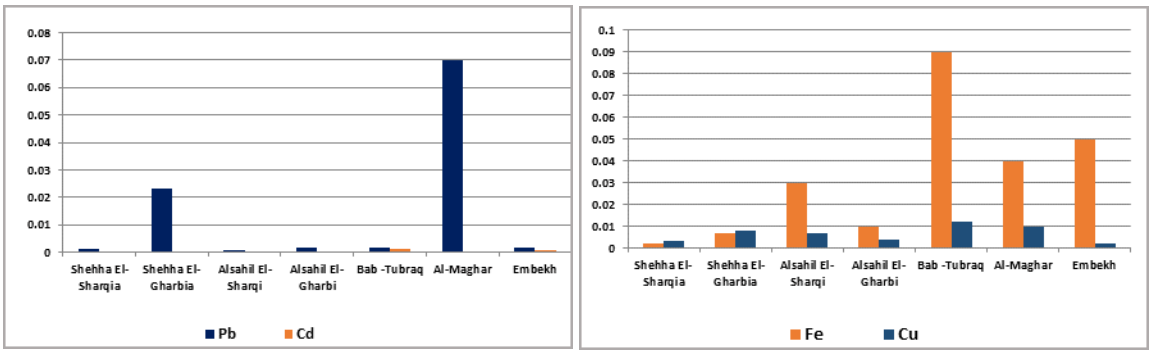


Figure (4): Comparative levels of selected toxic (Pb, Cd) and essential (Fe, Cu) metals in grounwater samples

Microbiological Characteristics

The microbial examinations indicated that grounwater samples had the lowest value of total viable bacterial count. But three groundwater wells in the following reagions (Alsahil El-Sharqi, Bab-Tubraq, and Maghar) were contaminated, with the highest count being > 100 cfu/100ml. The WHO recommends that the total bacterial count in drinking water should not exceed 0 cfu/100ml. We noticed that the wells in the previously mentioned reagions tested positive for the presence of *Escherichia coli* bacteria, while the rest of the wells had a negative result. The occurrence of *E. coli* in groundwater wells is a major public health problem. In addition, total coliforms were found in three groundwater wells with varying numbers of propagations depending on their location in the research region; however, they reach the WHO's recommended safe level for drinking water, as shown in Table (5).

Table (5): Microbiological analysis of groundwater samples.

Groundwater Wells Sites	TBC (cfu/100ml)	TCF (MPN/ 100ml)	*Escherichia coli*
Shehha El-Sharqia	- ve	- ve	- ve
Shehha El-Gharbia	- ve	- ve	- ve
Alsahil El-Sharqi	260	32	+ ve
Alsahil El-Gharbi	80	- ve	- ve
Bab –Tubraq	320	37	+ ve
Al-Maghar	340	30	+ ve
Embekh	- ve	- ve	- ve

* TBC = Total bacterial count TCF = Total coliforms - ve = Negative result +ve = positive result

Conclusion

Based on the results of this study, laboratory analysis shows signs of microbial contamination in some groundwater wells in the city of Derna affected by the floods and contaminant sources might differ from one house to the next or from one region to the next. As a result, the samples were unfit for drinking or other domestic usage. As for the chemical analysis, it showed the presence of certain heavy metals for analysis was proven in samples selected from different districts in relatively good concentrations compared to the WHO standard specifications for drinking water. Because these concentrations were below permissible levels and international standards, restricting their intake would not be harmful for human consumption. Additionally, the study relied on assessing a variety of physical and chemical parameters to establish the appropriateness of water samples for drinking and domestic usage. Some physical and chemical values measured for these samples were found to be higher than the allowable limits under Libyan drinking water standard regulation, such as alkalinity and Cl. To ensure the safety of groundwater, various treatment techniques are used to remove microbiological contaminants and chemical contaminants. These include filtration, disinfection with chlorine, and adsorption of organic compounds by activated carbon. Finally, the government and local authorities are supposed to carry out (ecological restoration) in the event of disturbances in the area. We suggest that future studies test for both pathogens and fecal indicator bacteria in light of these findings. Additionally, research into mitigating strategies, including holding dams, altered dam release schedules, or structural solutions to shield water sites from flood water incursion, should be continued.

Acknowledgment:

The authors gratefully acknowledge the Alqumma Libya Al-Oula Laboratory, Food and Drugs Control Center and Environmental Sanitation for all branches of Al-Bayda, Tripoli, and Zliten for providing lab facilities and supporting our research.

References

Abdelrahman, A. A., & Eltahir, Y. M. (2011). Bacteriological quality of drinking water in Nyala, South Darfur, Sudan. *Environmental Monitoring and Assessment*, *175*, 37-43.

Akan, J. C., Abdulrahman, F. I., Sodipo, O. A., & Chiroma, Y. A. (2010). Distribution of heavy metals in the liver, kidney and meat of beef, mutton, caprine and chicken from Kasuwan Shanu market in Maiduguri Metropolis, Borno State, Nigeria. *Research Journal of Applied Sciences, Engineering and Technology*, *2*(8), 743-748.

APHA, (American Public Health Association). (2017). Standard methods for the examination of waters and wastewaters. APHAAWWA- WEF, Washington, DC. Murhekar, G. H. physicochemical status of ground water samples in *Akot city. Res. J. Chem. Sci.*, *1*(4), 117-124.

Appenroth, K. J. (2010). Definition of "heavy metals" and their role in biological systems. *Soil Heavy Metals*, 19-29.

Batool, A., Aziz, S., Imad, S., Kazmi, S. S., Shafqat, M., & Ghufran, M. A. (2018). Physico-chemical quality of drinking water and human health: a study of Salt Range Pakistan. *International Journal of Hydrology*, *2*(6), 668-677.

Cabral, J. P. (2010). Water microbiology. Bacterial pathogens and water. *International Journal of Environmental Research and Public Health*, *7*(10), 3657-3703.

Dhoke, S. K. (2023). Determination of alkalinity in the water sample: a theoretical approach. *Chemistry Teacher International*, *5*(3), 283-290.

Eaton A. D., Clesceri L. S., Rice E. W., Greenberg A. E. & Franson M. A. (2005). Standard methods for the examination of water and wastewater, 21st ed., American Public Health Association, American Water-works Association, Water Environmental Federation.

Edberg, S. C. L., Rice, E. W., Karlin, R. J., & Allen, M. J. (2000). *Escherichia coli*: the best biological drinking water indicator for public health protection. *Journal of Applied Microbiology*, *88*(S1), 106S-116S.

Figueras, M. J., & Borrego, J. J. (2010). New perspectives in monitoring drinking water microbial quality. *International Journal of Environmental Research and Public Health*, *7*(12), 4179-4202.

Izah, S. C., Chakrabarty, N., & Srivastav, A. L. (2016). A review on heavy metal concentration in potable water sources in Nigeria: human health effects and mitigating measures. *Exposure and Health*, *8*, 285-304.

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B. B., & Beeregowda, K. N. (2014). Toxicity, mechanism and health effects of some heavy metals. *Interdisciplinary Toxicology*, *7*(2), 60.

LNCSM (Libyan National Center for Standardization and Metrology). (2015). Drinking Water. LNS 82, Second edition. Tripoli-Libya.

Murhekar Gopalkrushna, H. (2011). Assessment of physico-chemical status of ground water samples in Akot city. *Research Journal of Chemical Sciences*, *1*(4), 117-124.

Pennino, M. J., Compton, J. E., & Leibowitz, S. G. (2017). Trends in drinking water nitrate violations across the United States. *Environmental Science & Technology*, *51*(22), 13450-13460.

Schwartz, B. F., Schenkman, N. S., Bruce, J. E., Leslie, S. W., & Stoller, M. L. (2002). Calcium nephrolithiasis: effect of water hardness on urinary electrolytes. *Urology*, *60*(1), 23-27.

Storelli, M. M. (2008). Potential human health risks from metals (Hg, Cd, and Pb) and polychlorinated biphenyls (PCBs) via seafood consumption: estimation of target hazard quotients (THQs) and toxic equivalents (TEQs). *Food and Chemical Toxicology*, *46*(8), 2782-2788.

WHO (World Organization Health). (2011). Guidelines for drinking water quality. 4th ed.,Geneva.

Wu, D., Hu, Y., Liu, Y., & Zhang, R. (2021). Review of chloride ion detection technology in water. *Applied Sciences*, *11*(23), 11137.

Zahid, A., Hassan, M. Q., Balke, K. D., Flegr, M., & Clark, D. W. (2008). Groundwater chemistry and occurrence of arsenic in the Meghna floodplain aquifer, southeastern Bangladesh. *Environmental Geology*, *54*, 1247-1260.

Zurqani, H. A., Post, C. J., Mikhailova, E. A., Schlottman, M. A. and Al-Hawaij, A. R. (2018). Reviewing Libyan soil databases for use within the framework of ecosystem services. *Int. J. Ob. Appl. Earth. Geoinf.*, *69*, 175-185.

AlQalam Journal of Medical and Applied Sciences
Special Issue for 6th International Conference in Basic Sciences and Their Applications (6th ICBSTA, 2023), https://journal.utripoli.edu.ly/index.php/Alqalam eISSN 2707-7179

Studying the bacterial species that cause urinary tract diseases and their resistance to some antibiotics in laboratories of Al-Marj city

Asma youssif ali alorfy[1] and Rafaa hamed bogreen[2]
[1]*Faculty of Arts and Sciences of Elmarj. Benghazi University. Libya*
[2]*Faculty of Arts and Sciences of Alabyar. Benghazi University. Libya*
Correspondence authors: asma.essa@uob.edu.ly

Abstract:

The current study included a study and knowledge of some types of bacteria that cause urinary tract infection, as this disease is one of the most serious health problems facing large numbers of people annually. To the analysis, laboratories inside the city of AL-Marj, and the samples taken were of different ages and for both sexes.The results showed that from these pathological samples, (36) isolates belonging to *Escherichia coli* (45%) were diagnosed, *Staphylococcus aureus* 19 isolates (23%), *Proteus spp* 4 isolates (5%), *Pseudomonas spp* 9 isolates (11%). *Streptococcus pyogenes* were 12 isolates (15%), as shown in Table (2).It was higher in females than in males, as it reached 65% in females, while the incidence of male infection was 35%. A drug sensitivity test was conduct on bacterial samples diagnosed using antibiotics. *E.coli* and Staphylococcus bacteria showed the highest percentage of resistant strains.

Keywords: Urinary tract infection, *Escherichia coli*, *Staphylococcus* bacteria, Drug sensitivity.

Introduction:

Urinary tract infections (UTIs) are an interesting health problem because they affect millions of people annually, and they constitute the second most important infection that affects the body. There are three types of UTIs, including what occurs in the bladder and was called cystitis. And in the kidneys it is called Pyelonephritis and the third occurs in the urethra and it is called (Urethritis). The risk of UTIs increases when there is any obstruction that prevents the smooth and easy flow of urine through the urinary tract (Gupta and Trautner, 2012). The urinary system, consisting of the kidneys, ureters, and urethra, is one of the important organs in the human body due to

the nature of the work that it performs in purifying the blood from harmful substances and materials that are surplus to the body's need and disposing of them in the form of urine. The urine and its characteristics and contents are good indicators that reflect the normal or pathological physiological state. In addition to other functions of the kidneys in maintaining the natural balance of body fluids. The widespread and ill-conceived use of antibiotics has increased the problems of urinary tract infection (UTI), as this may be due to the continued use of these antibiotics. In addition, for long periods, it led to the emergence of resistance shown by microorganisms and the emergence of strains with high tolerance to antibiotics. Most UTIs was caused by *E. coli*, which causes more than 90% of all UTIs. *Staphylococcus* saprophyticus causes (10-20%) and some *Klebsiella* species (6-15%). In addition to other types of bacteria such as Chlamydia and Mycoplasma (Ramos *et al.*, 2012; Ejrnaes, 2011). *E. coli* bacteria are found naturally in the gastrointestinal tract, and may result in many different infections, causing about (90%) of UTIs. It is called [(UPEC) Uropathogenic] *E. coli*, as it resides, colonizes and multiplies in the gastrointestinal tract, excrement, skin, around the outlet and vagina, and is transmitted through the blood and lymphatic system or directly to the urinary tract (Bien *et al*., 2012; Schwan, 2008). The study aims to identify the different bacterial species that cause urinary tract infection in men and women in the city of AL-Marj.

Materials & Methods

The study sample:

A total of 80 analysis samples were collected from laboratories inside the city of Al-Marj, during the period between (1-4-2023) to (1-6-2023).

Statistical analysis:

The data obtained were analyzed using the SPSS statistical program, and the results of the research were obtained.

Obtaining bacteria:

Where *Staphylococcus* is a spherical bacterium in clusters belonging to the family Micrococcaceae, non-motile, positive for the catalase test. Highly virulent opportunistic pathogen in patients with weak defense mechanisms, causing bacteremia, and eye and wound infections. An aerobic gram-positive

bacterium is not bactericidal and has the ability to produce toxins. While *E.coli* bacteria are motile bacilli that are Gram-negative and facultatively anaerobic, positive for the methyl red test and the indole test. It lives in the digestive tract of humans and animals and causes human diseases. It belongs to the Enterobacteriaceae family. The bacterial genus *Proteus* Spp. This bacterial genus is a Gram-negative rod-shaped (polymorphic) cell that does not form a capsule. It is characterized by its active movement by means of flagella surrounding the entire bacterial cell at a temperature of 20-28 degrees Celsius. The bacterial species *proteus mirabilis* and the bacterial species *proteus* vulgaris are considered among the most important. Species that belong to this bacterial genus. The bacterial genus *Proteus Spp* does not have the ability to ferment lactose, but it has the ability to secrete the urease enzyme, which breaks down urea within 4 hours. *Pseudomonas* spp the cells of this bacterial genus are aerobic bacillus, 0.6 in width and 2 µm in length, which are Gram-negative and non-spore-forming. It is mobile by flagella (one or two flagella) located at the poles of the cell, and some strains of the bacterial type *Pseudomnas aeruginosa* ,may be preservative and is considered one of the most important pathogenic bacterial genera out of 200 species of this genus and has the ability to grow at a temperature of 42-37 degrees Celsius .It secretes two soluble dyes: pyocyanin, which gives it a blue color when grown in the laboratory, and pyoverdin, which gives it a greenish-yellow color. This dye is abundantly secreted in the food medium. *Streptococcus pyogenes* is a type of gram-positive bacteria. It has an important role in diseases that affect humans. It is usually pathogenic,although it is present as part of the skin flora. Its spherical shape, with a size of approximately 0.5-1.2 micrometers, characterizes it. The most important characteristic of this type of bacteria is its inability to move, but it does not produce spores.

Results:

In this research, a number of cases were studied that frequented analysis laboratories within the city of Al-Marj, The results of the current study were shown in (Table 1, Figure 1) which includes 80 patients, of which 52 patients were female, and 28 patients were male.

Table 1: Number of patients and percentages of patients by sex

Sex	Number of patients	percentages of patients
Male	28	35%
Female	52	65%

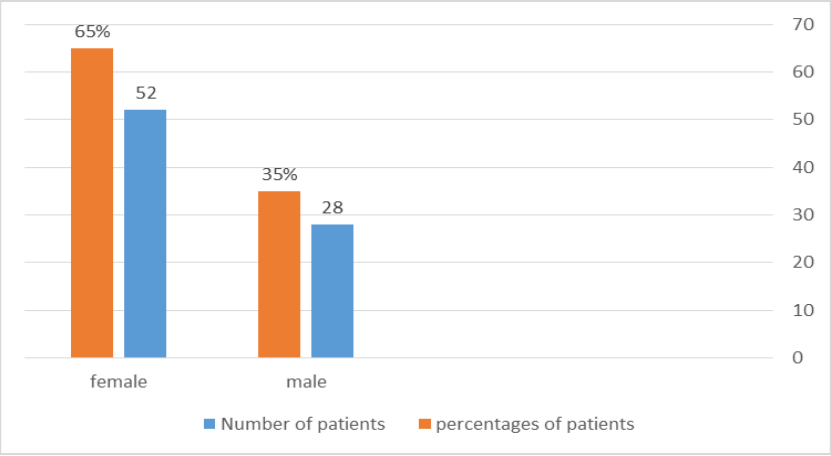


Figure 1: Number of patients and percentages of patients by sex

Through the samples, it became clear that there are five types of bacteria that cause urinary tract infection, and the bacteria are *E. coli*, *Staphylococcus aureus, Proteus spp., Pseudomonas spp., and Streptococcus pyogenes*.

Table 2: Type and percentage of bacteria

Types of microbes	Repetition	percentages
E. coli	36	45%
Staphylococcus aureus	19	23%
Proteus spp.	4	5%
Pseudomonas spp.	9	11%
Streptococcus pyogenes	12	15%
	80	100

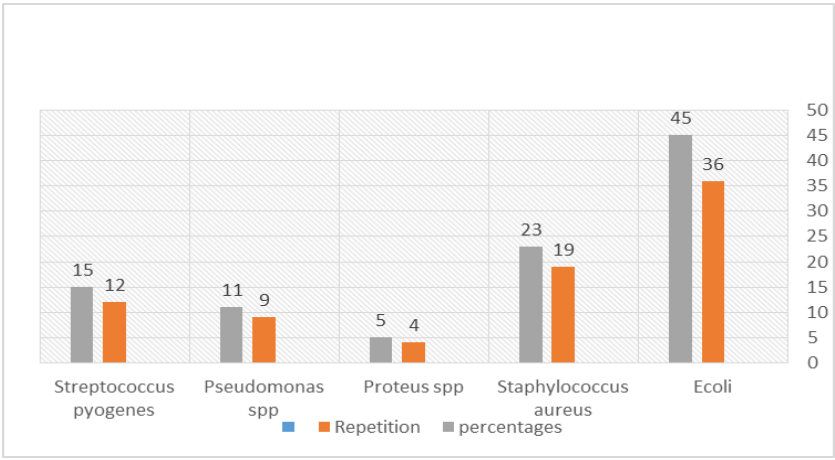


Figure 2: Type and percentage of bacteria

Table 3: Types of pathogenic bacteria and the number of infected people distributed according to the sex of the infected person

Types of pathogenic bacteria	Number of infected males	Number of infected females
Escherichia coli	14	22
Staphylococcus aureus	11	8
Proteus spp.	1	3
Pseudomonas spp.	5	4
Streptococcus pyogenes	7	5
Account	28	52

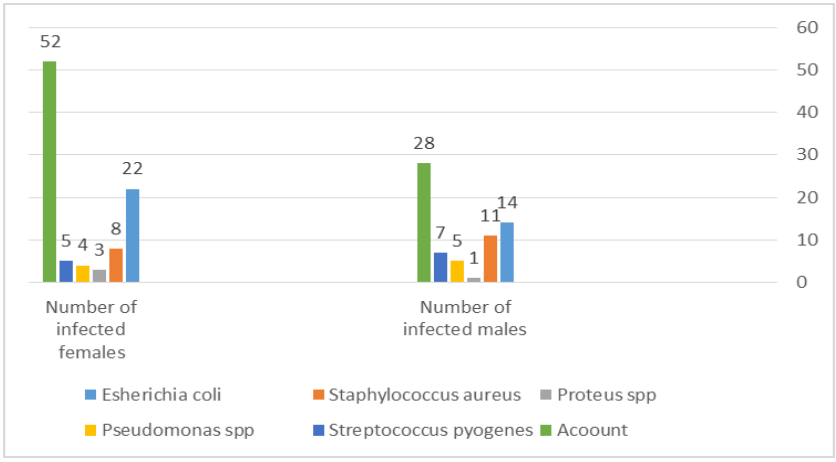


Figure 3: Types of pathogenic bacteria and the number of infected people distributed according to the sex of the infected person

Discussion:

The laboratory diagnosis of UTI was based on microscopic examination and bacteriological culture. Accordingly, the patients were classified into infected and uninfected. Females are more susceptible to the disease than males (Table 1), and the reason for this may be the proximity of the opening of the urinary tract to the anus in females, which facilitates upward infection more than males, and these results are consistent with what he found (Larabi *et al*., 2003), and agreed with (Abu Daia *et al*., 2000). The results of the research showed that from these pathological samples, (36) isolates belonging to *Escherichia coli* (45%) were diagnosed, *Staphylococcus aureus* 19 isolates (23%), *Proteus spp.* 4 isolates (5%), *Pseudomonas spp.* 9 isolates (11%). *Streptococcus pyogenes* were 12 isolates (15%). As shown in Table (2). It was noted that *E. coli* was one of the most common causes of UTI, and it came in agreement with the results obtained by the researcher Ali (2011) and (Wullt *et al.*, 2003; Akbar *et al*., 2001), as it was the most common cause, and it also agreed with the researcher (Wajeha, 2007). In addition to other bacteria that were isolated as pathogens, there were also Gram-positive bacteria associated with inflammatory conditions, such as *Staphlococcus, Streptococcus*. Isolates of *E. coli* have appeared resistant to four antibiotics, or multi-resistant, and these names include Cephalothin (CEP), Ciproflaxiam (CIP), Cefrazidime (CEF), and Levofloxacin. Below are some important points, including Gentamicin, Ampicillin, and Amoxicillin- Cavulanate. As for *Staphylococcus aureus* bacteria, it showed resistance to amoxicillin and sensitivity to tetracycline and cephalexin antibiotics. While *Streptococcus pyogenes* bacteria are resistant to gentamicin and sensitive to amoxicillin-cavulanate. *Pseudomonas* spp. bacteria are resistant to the antibiotics gentamicin and ciprofloxacin, As for *Proteus* spp. bacteria, they showed resistance to the antibiotic azithromycin and sensitive to the antibiotic gentamicin and ciprofloxacin.

	Proteus spp.	*Pseudomonas spp.*	*Streptococcus pyogenes*	*Staphylococcus aureus*	*E. coli*
Azithromycine	-	+	+	-	-
Gentamicin	-	-	-	+	-
Nitroforantion	-	-	-	-	+
Cefotaxima	+	-	+	-	+
Ciproflaxiam	-	+	+	-	+
Erythromycin	-	-	-	+	-
Augmentin	-	-	-	-	-

(+) Resistance (-) Sensitive

Recommendations:

Emphasizing the awareness and health education of the community and urging patients with urinary tract infection to receive appropriate treatment to prevent them from becoming a hotbed of infection transmission. Directing them to complete the treatment course for the purpose of eradicating the bacteria and preventing the transformation of acute cases into chronic cases, i.e. preventing the transformation of sensitive bacteria isolates into antibiotic-resistant bacteria, which complicates healing process and increases other complications.

References:

Abu Daia, J. M.; Al-Aaly, M. and De Castro, R. (2000). Urinary tract infection in childhood. Saudi Medical Journal, 21(8): 711-714.

Akbar, D. H. (2001). Diabetics and non-diabetics patients. Saudi Medical Journal 22(4):326-329.

Ali, M. M.(2011). Evaluation of antimicrobial susceptibility &rapid urine screening tests in asymptomatic urinary tract infection in pregnant women in Karbala, Kerbala Journal of pharmaceutical science. (2):23-34.

Bien, U., Sokolova, O. and Bozko, P. (2012). "Role of Uropathogenic *Escherichia coli* Virulence Factors in Development of Urinary Tract Infection and Kidney Damage," International Journal of Nephrology, 15.

Ejrnaes, K. (2011). Bacterial characteristics of importance for recurrent urinary tract infections caused by Escherichia coli. Thesis, Danish, Med. Bullltin.

Gupta, K. and Trautner, B. (2012). Urinary tract infections, pyelonephritis, and prostatitis. In: Harrison's principles of internal medicine. McGraw Hill Medical, 2387–2395.

Larabi, K., Masmoudi, A. and Fendri, C. (2003). Bacteriological and susceptibility study of 1.930 strains isolated from UTIs in Tunis

university hospital.Me'denice ET Maladies Infectiousness, 33(7): 348-352.

Ramos, N.L., Sekikubo, M., Dzung, D.T., Kosnopfel, C., Kironde, F., Mirembe, F. and Brauner, A. (2012). Uropathogenic Escherichia coli isolates from pregnant women in different countries. J. Clin. Microbiol. 50: 3569-3574.

Schwan, W. R. (2008). "Flagella allow uropathogenic Escherichia coli ascension into murine kidneys," International Journal of Medical Microbiology, 298(5): 441–447.

Wullt, B.; Bergsten, G.; Samuelesson, M. and Svanborg, C. (2003). The role of P fimbriae for *Escherichia coli* establishment and mucosal inflammation in the human urinary tract. International Journal of Antimicrobial Agents 19: 522-538.

AlQalam Journal of Medical and Applied Sciences
Special Issue for 6th International Conference in Basic Sciences and Their Applications (6th ICBSTA, 2023), https://journal.utripoli.edu.ly/index.php/Alqalam eISSN 2707-7179

Epidemiology of Cattle Gastrointestinal Helminthes in Almarj District, Libya

Abdelhakim A. Eljaki[1,4*], Munthir S. Alasbali[2], Farag A. Bleiblo[1], Souad A. Moftah[3], and Madiha W. Elawamie[1]

[1]*Department of Microbiology, Faculty of Science, University of Benghazi, Benghazi, Libya*
[2]*Department of Zoology, Faculty of Art and Science, University of Benghazi, Almarj Campus, Libya*
[3]*Department of Physiology, Faculty of Medicine-Al-Marj Campus, University of Benghazi, Benghazi, Libya.*
[4]*Departments of Biology & Microbiology and Veterinary & Biomedical Sciences, South Dakota State University, Brookings, SD 57007, United States*
* Corresponding author: abdelhakim.eljaki@uob.edu.ly

Abstract:

Almarj is one of the most important areas of agriculture and animal production in Libya. It is a source for producing meat and milk. This study shows the prevalence and intensity of cattle gastrointestinal helminth. 406 cattle fecal samples from different sexes, ages, and breeds have been examined by the Modified Wisconsin Sugar Fecal Worm Egg Flotation Method. The prevalence of infection was 65.27%. In males, the infection was 70.45%, and in females, it was 62.64%. Age has no significant effect on the infection. The prevalence rate ranged from 65% to 68.94% among young and old calves as well as adults. The prevalence of weaned cattle was 47.46%. Santa was more infected, with a prevalence rate of 81.82%. The intensity ranged between minor infection and medium. The fecal samples contained eggs of *Taenia*, *Moniezia*, *Toxocara*, *Trichuris*, *Enterobius*, and roundworms. The infection by only one type of worm egg is 50.94%, and samples infected by two types of parasites were 36.23%. Samples that contained three types of eggs were 10.19%, while samples that contained four types of eggs were 2.26%. 47.54% of samples were infected with roundworms. 28.57% of samples were infected by Toxocara. Taenia was found in 88 samples. Moniezia was 4.19%, and the prevalence of *Enterobius* was 3.94%. The lowest percentage was for two samples that contained *Trichuris*, with a rate of 0.49%. The southwest of Almarj was the most likely site for the cattle gastrointestinal helminths, where the infection rate was 78.44%.

Keywords: Almarj, Cattle, Gastrointestinal helminthes.

Introduction:

Cattle form a part of the livestock industry in Libya for the purpose of mainly beef and, in some cases, milk production. In Libya, during the 1970s, the number of cattle, particularly dairy cattle, increased, as did milk and meat production. By 1985, there were nearly 209,000 head of cattle in the countryside of Almarj. In 2015, there were 9720 head of cattle in the countryside of Almarj (Libyan Ministry of Agriculture, 2018). The parasitic infections of production animals have a great economic impact, especially in developing countries. Cattle act as the final or intermediate host for many helminths. This type of parasitism is regarded as the most important cause of economic loss, as it is a flock or herd problem (Ndarathi, *et. al.*, 1989; Cobon and O'Sullivan 1992, Suttle 1994). Infections with parasites, especially those of the gastrointestinal tract, can and, in some circumstances, do cause substantial losses to bovine owners. (Maina, 1986; Kudi and Kalla, 2001). Eradication of these parasites is impossible, especially in a degrading environment (Ohaeri, 2011). Gastrointestinal parasite infections have numerous negative impacts on the productivity and fertility of herds (Elsa *et. al.*, 2012). Gastrointestinal helminthesis is a common veterinary disease in many countries around the world. The Food and Agriculture Organization (FAO) reported that this type of disease is a cosmopolitan veterinary disease. This disease affects many types of domestic and wild animals, especially farm animals like sheep, goats, cattle, and camels. (FAO, 2009). A marked growth in cattle numbers and currently contains the largest number of commercial herds in Libya. Considerable information on cattle helminths all over the world is present, but little is known on the overall prevalence and intensity rate of gastrointestinal helminth infection in Libya. Up-to-date information is lacking on the magnitude of gastrointestinal tract parasites in livestock maintained in traditional grazing systems in Almarj, Libya. Detailed information about helminthes in cattle of Almarj is still not scanty; for example, epidemiology, prevalence, and pathogenicity of helminthes.

Materials and Methods:

Study Area:

Almarj is a city located in the northeastern part of Libya, figure 1. It is situated on the plateau at the western edge of the Aljabal Alakdar Mount, and it is near the Mediterranean coast with a shoreline on the north. Almarj is

about 106 km west of Albayda city and 95 km east of Benghazi city. According to latitude and longitude coordinates, Almarj is located at 32°29'16.73" N and 20°49'54.26" E, with an area of 10,000 km2, figure 2. The elevation of the geographical location of the study area is 333 meters. The monthly mean minimum and maximum temperatures range from 6 °C to 35 °C. Almarj is agricultural land. Its population in 2006 was 185,848; this was reported to the Ministry of Housing and Utilities of the Libyan government in 2019.

Figure (1): The study area (Almarj district) location *Source of map: https://www.scirp.org/journal/paperinformation.aspx?paperid=101999

The study area, Almarj, was divided into four main regions: A, B, C, and D. Region A is in the northwest of Almarj, Region B is in the northeast, Region B is in the southwest, and Region D is in the southeast region of Almarj, figure 2.

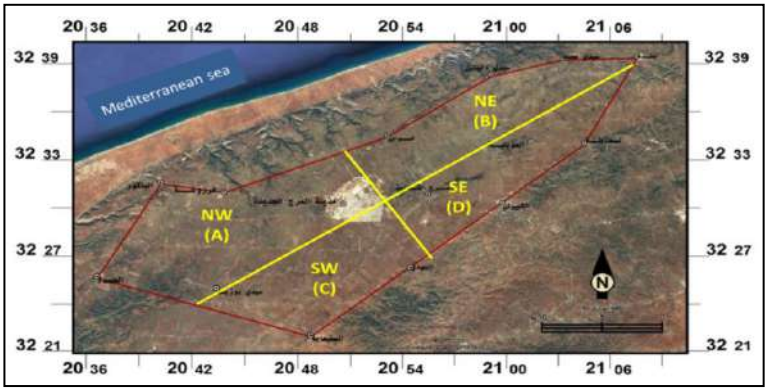


Figure (2): Map of the study area, Almarj district, where Location A is the Northwest region (NW), Location B is the Northeast region (NE), Location C is the Southwest region (SW).

Animal Sampling and Data Collection:

In the Almarj countryside, there are four types of cattle bread: Friesian, Jersey, Santa, and local (the breads of the experimental animals). The samples were collected from all four breads of the four experimental locations. The animals were divided according to sex and age; the age of the animal depends on the cattle owner's information. The age classification of cattle is shown in Table 1, where the animals are divided into four age categories: less than 6 months old (young calves), 7–12 months old (old calves), 13–24 months old (weaners), and over 24 months old (adults).

Table 1: Age groupings of the experimental animals

No.	Age (months)	Categories
1	Less 6	Young Calves
2	7 – 12	Old Calves
3	13 – 24	Weaners
4	Over 24	Adults

The examined animals were also categorized into three body condition groups, involving poor, medium, and good body condition, based on the description of Nicholson and Butterworth (1986). Poor-body-condition cattle had prominent dorsal spines pointed to the touch and individual visible transverse processes. Cattle with usually visible ribs with little fat cover and barely visible dorsal spines were considered to have a medium body condition score. A good body condition score was given for cattle when fat cover was easily seen in critical areas and the transverse processes were not felt (Turton, 1999).

Fecal Samples Collection:

During November 2016 to January 2018, fresh fecal samples of 406 animals were collected in the morning from the four different locations of the study area. The fecal samples were collected directly from the ground after animal defecation by using sterile disposable plastic gloves. The fecal samples were directly put in plastic bags consisting of polythene. All information about the animal, like sex, age, and bread, was recorded on the bag, in addition to the place and date of the collection. The samples were transported in an icebox to the laboratory and kept at 4 °C for further examination.

Fecal Samples Examinations:

The examination of the fecal samples depends on two styles: macroscopic examination and microscopic examination. The macroscopic examination is by naked eyes to determine the color, consistency, and presence of blood, mucous, larvae, tapeworm segments, and dead worms. The microscopic examination was done by direct smears (fecal wet mount) and by sucrose flotation technique. The eggs of parasites were identified by their morphological characters. Eggs per gram (EPG) of infection were determined by the modified McMaster technique (Todd, 1972).

Statistical Analysis:

The data collected from the study was first entered into the MS Excel program (Microsoft Corporation, USA) and screened for errors that might have occurred during the entry. Any error detected was corrected by rechecking against the original data forms. The collected data were tabulated and analyzed by descriptive statistics such as average, percentage, etc. using the software Microsoft Excel 2019.

Results:

The result shows that the southwestern location of Almarj district was the most cattle breeding area and the percentage of the fecal samples was 41.13% (n = 167). Table 2 shows the number and percentage of samples according to the four locations of the Almarj district.

Table (2): Number of fecal samples from the four study locations

Location of Study	Number of Samples	Percentage
Northeast (NE)	80	19.70
Northwest (NW)	75	18.47
Southeast (SE)	84	20.69
Southwest (SW)	167	41.13

The jersey was the most common breed in Almarj district (204 samples), followed by Friesian (132 samples), Local (37 samples), and Santa (32 samples). The numbers of samples that were collected from each breed according to location and their percentage are in Table 3.

Table (3): Samples number and their percentage of each bread according to the study locations

Location	Jersey	Friesian	Local	Santa	Total	%
NE	30	17	33	0	80	19.70
NW	43	27	4	1	75	18.47
SE	71	13	0	0	84	20.69
SW	60	75	0	32	167	41.13
Total	204	132	37	33	406	
%	50.25	32.51	9.11	8.13		

Female cattle fecal samples were more frequent, with a percentage of 89.16% (n=362), than male cattle fecal samples, which were 10.84% (n=44). Table 4 shows the numbers of samples obtained from both sexes of experimental animals according to the location of the study area.

Table (4): Number of fecal samples according to the cattle sex in each study location

Location	Fecal Samples of Female Cattles	Fecal Samples of Male Cattles
Northeast (NE)	74 (18.23%)	6 (1.48%)
Northwest (NW)	70 (17.24%)	5 (1.23%)
Southeast (SE)	79 (19.46%)	5 (1.23%)
Southwest (SW)	139 (34.24%)	28 (6.90%)
Total	362 (89.16%)	44 (10.84%)

The study of the body condition of the experimental animals was dependent on the external features of the animal, according to Nicholson and Butterworth (1986). This part of the study is based on only 270 animals from the total of 406 animals, which were 58, 180, and 32 with poor, medium, and good body conditions, respectively.

The consistency of the fecal samples was formed, loose, soft, and watery. There were 221, 34, 73, and 78 samples, respectively, figure 2.

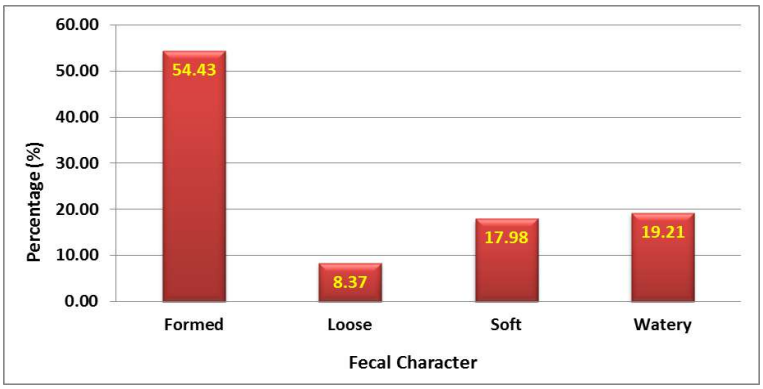


Figure (3): Percentage of fecal samples according to their characteristics

All-fecal samples were negative according to the macroscopic examination, but according to the microscopic examination using the Modified Wisconsin Sugar Fecal Worm Egg Flotation Method, the prevalence of infection was 65.27% (n = 406). Table 5 shows the number of infected samples according to study locations.

Table (5): Number of infected fecal samples according to the study location

Location	NE	NW	SE	SW	Total
Total of sample	80	75	84	167	406
Infected sample	53	46	35	131	265
Prevalence	66.25%	61.33%	41.67%	78.44%	65.27%

* NE: North East, NW: North West, SE: South East and SW: South West

31 cattle male fecal samples were positive with a percentage of 70.75% (n=44), while the infection in the female samples was 64.64% (n=362). According to the age of the animals, the prevalence of infection was as shown in Table 6.

Table (6): The prevalence of intestinal helminthes according to the age group of the experimental animals

Age of Animal	Young Calf	Old Calf	Weaner	Adult
Number of Samples	63	20	59	264
Infected Samples	42	13	28	182
Prevalence	66.67%	65.00%	47.46%	68.94%

Within the age groups of the Friesian, young calves were more infected by intestinal helminths than other age groups, their prevalence was 81.48 %. The weaner age of Santa and the old calves age of Local bread were more infected by 100%, than the other ages of these two breads. The Santa breed was highly infected among the other cattle breeds by a percentage of 81.82%, followed by the local cattle by 75.68%. Numbers of infective Friesian and Jersey were 132 and 204, respectively. In general, the prevalence of infection is shown in figure 4, where more than 50% of the four types of cattle breeds were infected by intestinal parasitic helminthes.

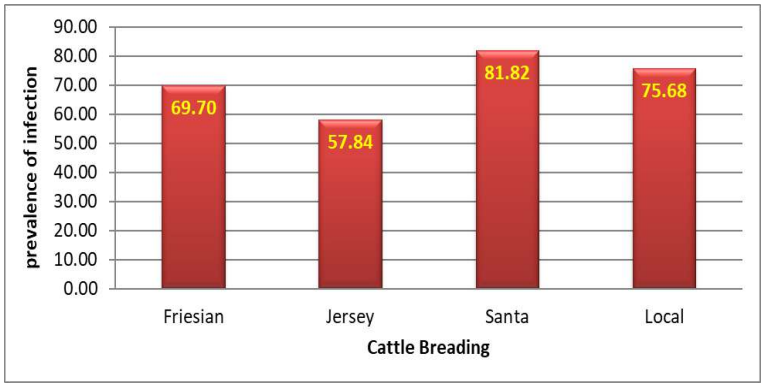


Figure (4): The Prevalence of intestinal parasitic helminthes in the experimental animals

The microscopic examination showed that the infected samples contained eggs of many types of intestinal Nematoda and Cestoda. The microscopic examination showed six different types of roundworm eggs, in addition to the eggs of *Toxocara*, *Trichuris*, and *Enterobius*. The eggs of Cestoda were for *Taenia* and *Moniezia.* The eggs of the six types of round worms may belong to one of the following genera: *Bunostomum*, *Cooperia*, *Haemonchus*, *Oesophagostomum*, *Ostertagia*, *Trichostrongylus*, and *Strongyloides.* The most samples were infected with roundworms, (47.54%, n=193). The *Toxocara* came in second rank of infection and was found in 116 samples of the total 406 samples, 28.57%. The *Taenia* eggs were found in 88 fecal samples (21.67%), the *Moniezia* eggs were found in 17 samples (4.19%), and the *Enterobius* eggs were found in 16 samples (3.94%), followed by the *Trichuris* eggs in 2 samples (0.49%). Figure 5 shows the number of fecal samples that were infected by the intestinal helminthes. Some fecal samples were infected by more than one type of parasite. 2.26% (n = 6) of the samples were infected by 4 types of helminthes. Also, 10.19% (n = 27) of the samples were infected by three different types of helminthes. In addition to this, 36.23% (n = 96) of the fecal samples were infected by 2 types of parasitic helminthes, and the most fecal samples (n = 135) were infected by only one type of parasitic helminthes, with a percentage of 50.04%.

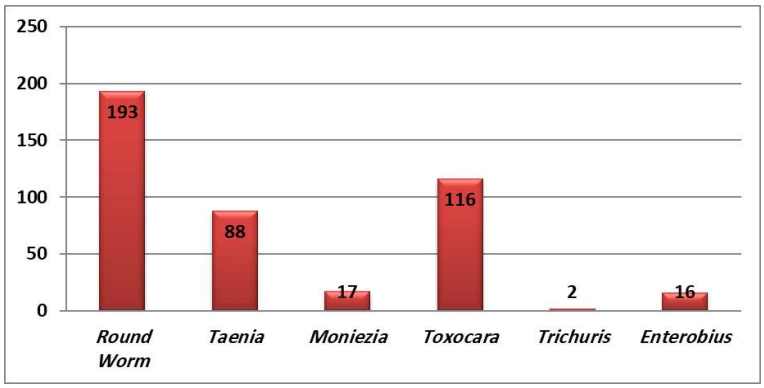


Figure (5): Number of infected animals according to the type of parasitic worm

The intensity of infection was divided into three categories: low (1–10 eggs per gram), medium (11–20 eggs per gram), and high intensity (more than 20 eggs per gram). Table 7 shows the infection intensity in the experimental animals. In general, the intensity of taenia was low in the most infected cattle. 185 fecal samples were infected by many types of round worms: *Bunostomum*, *Cooperia*, *Haemonchus*, *Oesophagos-tomum*, *Ostertagia*, *Trichostrongylus*, and *Strongyloides*, with low intensity, and 8 samples had medium intensity. Only one sample had high intensity with the round worms. There was no significant difference of prevalence and intensity between the samples that were collected during the cold and hot seasons.

Table (7): Number of the infected cattle according to the worm types and the intensity

Type of Worm	Intensity		
	Low (1-10 eggs/gram)	Medium (11-20 eggs/gram)	High (> 20 eggs/gram)
Taenia	82	6	1
Moniezia	17	0	0
Round Worm *	185	8	1
Toxocara	109	2	2
Trichuris	2	0	0
Enterobius	15	1	0

* The round worms are included *Bunostomum, Cooperia, Haemonchus, Oesophagos-tomum, Ostertagia, Trichostrongylus and Strongyloides.*

Discussion:

This study found that most breeders in the four different study locations were following the same method and system for cattle breeding. They were depending on the breed of animals on closed farms, especially during the fall and winter seasons. They were feeding the animals in closed barns, which

lacked the most health care requirements for animals. The immunization and preventive treatments were given to animals without carrying out veterinary examinations or laboratory tests. According to this study, the prevalence of cattle intestinal helminth infection in Almarj, Libya, was 65.27% (n = 406). This prevalence is higher than the prevalence of infection in some African countries, such as Soudan, Nigeria, and South Africa, for example, which was between 34.38% and 60.4% (Lemy and Egwunyenga, 2017, Usman *et. al*., 2016, Oyedeji and Nike, 2016, Yahaya and Tyav, 2014). The prevalence in Kenya was higher than in Almaraj; that was 69.2% to 85.5% (Maichomo *et. al.*,2004). In Tanzania in 2006, Keyyu et. al.,reported that the prevalence of cattle intestinal helminthes infection was 63.8%, and this prevalence was close to the prevalence of infection in Almarj. In some Asian countries, like India, Bangladesh, and Pakistan, the prevalence of infection with cattle intestinal helminthes ranged from 11% to 76%. This is according to Swarnakar *et. al.*,2015, Patel *et. al*.,2015, Muzaffar 2018, Wadhwa *et al.*, 2011, Vanisri *et. al.*,2016, Marskole *et al.*, 2016, Nath *et. al.*, 2013, Ilyas *et. al.*, 2016, Raza *et. al.*, 2007, Zaman *et. al.*, 2014, and Bhutto *et. al.*,2002. And this was close to the prevalence of infections in Almarj. The overall prevalence of helminthiasis was higher in young animals compared with adult animals. This high rate of prevalence of intestinal parasites infections if compared to some African and Asian countries may be due to the moderate temperature of the study area (Almarj). in addition to the low of veterinary care and the breeding of animals in the closed areas for long periods of the year. This led to the animal will get more infected stages than in the open place as which in the other African countries. The all samples were collected and examined for gastrointestinal parasitic helminthes infection using standard coprological methods. This microsocial examination revealed six types of parasitic helminthes that included round worms, *Taenia*, *Moniezia*, *Toxocara*, *Trichuris*, and *Enterobius*. The eggs of the round worms may belong to one of the following genera: *Bunostomum, Cooperia, Haemonchus, Oesophagostomum, Ostertagia, Trichostrongylus,* or *Strongyloides*. Determining the genus of the round worm depends on the molecular technique (Eljaki, *et al.*, 2016, Boufana, *et al.*, 2015, Eljaki, *et al.*, 2011, Grosz, *et al.*, 2012, Eljaki, *et al.*, 2010, Tashani, *et al.*, 2002). PCR is the best and perfect method to determine the genus of all organisms because the eggs of the roundworm have the same morphological characteristics. The size, shape, and color of the eggs may be similar in the roundest worms. For that reason, this

study did not give the scientific name of the roundest worm that was detected in the fecal samples.

Conclusion:

This study showed that there was no effect of age on the prevalence of gastrointestinal helminthes. All the age groups had the same rate of prevalence, and this effect of age had an exception, where the infection by Toxocara was very high at the young age group (young and old calves) more than the other age categories. The diversity in genera of intestinal worms may be due to the unavailability of final and intermediate hosts for the parasitic worms that are necessary to complete their life cycle. This limits the presence of certain types of worms in Libya. In addition, these genera of gastrointestinal parasitic worms may be found in other farm animals like sheep, goats, and camels. That helps in the distribution of the parasite in Libya.

Acknowledgements:

This work was supported by the Department of Zoology, Faculty of Science, Omar Al-Mukhtar University, and the Department of Zoology, Faculty of Art and Science, University of Benghazi, Branch of Almarj. We happily acknowledge them for their support and for giving us the opportunity to complete this work.

References:

Bachal, B., Sharif, P., Rahamatullah, R., & Aijaz, H. S. (2002). Prevalence of gastro-intestinal helminths in Buffalo calves. Journal of Biological Sciences, 2(1), 43-45.

Boufana, B., Lett, W., Lahmar, S., Griffiths, A., Jenkins, D. J., Buishi, I., ... & Craig, P. S. (2015). Canine echinococcosis: genetic diversity of Echinococcus granulosus sensu stricto (ss) from definitive hosts. Journal of helminthology, 89(6), 689-698.

Cobon, D. H., & O'Sullivan, B. M. (1992). Effect of Haemonchus contortus on productivity of ewes, lambs and weaners in a semi-arid environment. *The Journal of Agricultural Science*, *118*(2), 245-248.

Eljaki, A. A., Al Kappany, Y. M., Grosz, D. D., Smart, A. J., & Hildreth, M. B. (2016). Molecular survey of trichostrongyle nematodes in a Bison bison herd experiencing clinical parasitism, and effects of avermectin treatment. *Veterinary Parasitology*, 227, 48-55.

Eljaki, A. A., Grosz, D. D. and Hildreth, M. B. PCR survey of trichostrongyle genera present in a heavily infected bison herd from eastern South

Dakota. *56th Annual Meeting of the American Association of Veterinary Parasitologists, St. Louis, MO*, July 16-19, 2011. Science Annual Meeting. Vermillion, South Dakota.

Eljaki, A.A., Knutsen, J.A., and Hildreth, M.B. Absence of Haemonchus contortus and other abomasal nematodes in eastern South Dakota whitetail deer during the 2010 winter season. *94th Annual Meeting of the South Dakota Academy of Science Annual Meeting. Spearfish, SD.* April 10, 2010.

FAO (Food and Agriculture Organization). (2009). Production Yearbook. Food and Agricultural Organization. Rome, Italy.

Grosz, D. D., Eljaki, A. A., Holler, L. D., Petersen, D. J., Holler, S. W., & Hildreth, M. B. (2013). Overwintering strategies of a population of anthelmintic-resistant Haemonchus contortus within a sheep flock from the United States Northern Great Plains. *Veterinary parasitology*, *196*(1-2), 143-152.

Ilyas, N., Hossain, M. M., Bhuyan, M. J. U., & Khan, M. M. H. (2016). Prevalence of Gastro-intestinal Nematodes Infection of Cattle in Bangladesh. *AJPCT*, *4*(03), 091-097.

Iqbal, Z., Jabbar, A., & Yaseen, M. (2007). Point prevalence of gastrointestinal helminthiasis in ruminants in southern Punjab, Pakistan. *Journal of Helminthology*, *81*(3), 323-328.

Keyyu, J. D., Kassuku, A. A., Msalilwa, L. P., Monrad, J., & Kyvsgaard, N. C. (2006). Cross-sectional prevalence of helminth infections in cattle on traditional, small-scale and large-scale dairy farms in Iringa district, Tanzania. *Veterinary research communications*, *30*, 45-55.

Kudi, A. C., & Kalla, D. J. U. (2001). Prevalence and effect of parasitic infections on cycling in traditionally managed cattle herds in Bauchi, Nigeria. *PERTANIKA JOURNAL OF TROPICAL AGRICULTURAL SCIENCE*, *24*(2), 87-94.

Lamy, E., van Harten, S., Sales-Baptista, E., Guerra, M. M. M., & de Almeida, A. M. (2012). Factors influencing livestock productivity. *Environmental stress and amelioration in livestock production*, 19-51.

Lemy, E. E. and Egwunyenga, A. O. (2017). Prevalence of parasitic helminthes from feacal samples of cattle at various abattoirs in Abraka, Delta State, Nigeria. *Journal of Animal Health and Behavioural Science* 1, 1-4.

Libyan ministry of agriculture, https://www.agriculture.gov.ly(2018)

Maichomo, M. W., Kagira, J. M., & Walker, T. (2004). The point prevalence of gastro-intestinal parasites in calves, sheep and goats in Magadi division, south-western Kenya. *Onderstepoort Journal of Veterinary Research*, *71*(4), 257-261.

Maina, J. A. (1986). Animal Health in sub-humid Nigeria. In Livestock System Research in Nigeria's Sub-Humid Zone, eds. *Von Kaufmann, R, Charter, S. and Blench, R.* Pp 167-188.

Marskole, P., Verma, Y., Dixit, A. K., & Swamy, M. (2016). Prevalence and burden of gastrointestinal parasites in cattle and buffaloes in Jabalpur, India. *Veterinary World*, *9*(11), 1214.

Ministry of Housing and Utilities of Libyan government. (2018). /http://www.mofp-ly.com.

Mir, M. R., Chishti, M. Z., Rashid, M., Dar, S. A., Katoch, R., Kuchay, J. A., & Dar, J. A. (2013). Point prevalence of gastrointestinal helminthiaisis in large ruminants of Jammu, India. *International Journal of Scientific and Research Publications*, *3*(3), 5-8.

Nath, T. C., Bhuiyan, M. J. U., & Alam, M. S. (2013). Prevalence of gastro-intestinal parasites of calves in Mirsarai Upazilla of Chittagong district of Bangladesh. *Bangladesh Journal of Animal Science*, *42*(2), 139-142.

Ndarathi, C. M., Waghela, S., & Semenye, P. P. (1989). Helminthiasis in Maasai ranches in Kenya. *Bulletin of animal health and production in Africa*.

Nicholson, M. J., & Butterworth, M. H. (1986). *A guide to condition scoring of zebu cattle*. ILRI (aka ILCA and ILRAD).

Ohaeri, C. C. (2011). The Human Environment, Associated Risks and Remedy for Health. *International Journal of Biological Science* 3, 76-82.

Oyedeji, F. N. (2016). Intestinal helminth parasites of cattle slaughtered in abattoirs in Gwagwalada. *Wld Rural Obser*, *8*(1), 23-6.

Patel, H. C., Hasnani, J. J., Patel, P. V., Pandya, S. S., Solanki, J. B., & Jadav, S. J. (2015). A study on helminth parasites of buffaloes brought to Ahmedabad slaughter house, Gujarat, India. *Life*, *50*, 20.

Suttle, N. F. (1994). Seasonal infections and nutritional status. *Proceedings of the Nutrition Society*, *53*(3), 545-555.

Swarnakar, G., Bhardawaj, B., Sanger, B., & Roat, K. (2015). Prevalence of gastrointestinal parasites in cow and buffalo of Udaipur district, India. *Int. J. Curr. Microbiol. App. Sci*, *4*(6), 897-902.

Tashani, O. A., Zhang, L. H., Boufana, B. A., Jegi, A., & McManus, D. P. (2002). Epidemiology and strain characteristics of Echinococcus granulosus in the Benghazi area of eastern Libya. *Annals of Tropical Medicine & Parasitology*, *96*(4), 369-381.

Todd, A. C., Myers, G. H., Bliss, D., & Cox, D. D. (1972). Milk production in Wisconsin dairy cattle after anthelmintic treatment. *Veterinary medicine, small animal clinician: VM, SAC*, *67*(11), 1233-1236.

Turton, J. (1999). Condition Scoring of Cattle. National Department of Agriculture and Obtainable From, National Department of Agriculture

in Cooperation with ARC-Onderstepoort Veterinary Institute, Pretoria, South Africa.

Usman, A. M., Malann, Y. D., & Babeker, E. A. (2016). Prevalence of Gastrointestinal parasitic infections among ruminants animals slaughtered in Katagum abattoir of Bauchi state, Nigeria. *Int J Innov Res Adv Stud*, *3*, 167-70.

Vanisri, V., Subramanian, N., & Muthu, M. (2016). Prevalence of gastrointestinal parasites in cattle in and around Cheyyar taluk, Thiruvannamalai district. *International Journal of Information Research and Review*, *3*(11), 3282-3294.

Wadhwa, A., Tanwar, R. K., Singla, L. D., Eda, S., Kumar, N., & Kumar, Y. (2011). Prevalence of gastrointestinal helminthes in Cattle and buffaloes in Bikaner, Rajasthan, India. *Veterinary World*, *4*(9), 417.

Yahaya, A., & Tyav, Y. (2014). A survey of gastrointestinal parasitic helminthes of bovine slaughtered in abattoir, Wudil Local Government Area, Kano state, Nigeria. *Greener J Biol Sci*, *4*(4), 128-34.

Zaman, M. A., Sajid, M., Sikandar, A., & Awais, M. M. (2014). Point Prevalence of Gastrointestinal Helminths and their Association with Sex and Age of Buffaloes in Lower Punjab, Pakistan. *International Journal of Agriculture & Biology*, *16*(6).