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Original article

Assessing the Efficacy of Plant Extracts in Managing Postharvest Fungi (*Alternaria alternata* and *Penicillium expansum*) on Apple Fruits

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Abstract
The efficacy of wormwood, thyme, and camphor extracts on fungal growth and spore formation was evaluated using potato dextrose agar (PDA) medium, with each extract dissolved separately in water, alcohol, and hexane. The study results showed that aqueous and alcoholic camphor extracts most significantly inhibited fungal growth, with reductions to 0.87 cm for *Penicillium. expansum* and 0.8 cm and 1.08 cm, respectively, for *Alternaria. alternata*. Hexane and aqueous thyme extracts were most effective in preventing *P. expansum* spore formation, at 1.49% and 1.97%, respectively, while hexane wormwood extract most inhibited *A. alternata* spore formation at 1.46%. These extracts were also tested for their efficacy in controlling apple fruit rot induced by *P. expansum* and *A. alternata* under storage conditions in Red Delicious and Golden Delicious cultivars. Notably, the aqueous wormwood extracts limited *P. expansum* infection in Red Delicious apples to 7%. The study revealed that certain plant extracts effectively prevented fungal infection in apple fruits. Specifically, no infection was detected in fruits treated with alcoholic and hexane wormwood extracts, alcoholic thyme, or hexane camphor. Among Golden Delicious apples, the hexane camphor extract treatment resulted in the lowest *P. expansum* infection rate (3.5%), whereas the aqueous wormwood extract treatment led to the highest infection rate (65%). In Red Delicious apples, the highest *A. alternata* infection rate (2.1%) was observed with alcoholic thyme extract treatment. Conversely, no infection was observed in fruits treated with wormwood (alcoholic and hexane), aqueous thyme, or alcoholic and hexane camphor extracts. Additionally, Golden Delicious apples treated with alcoholic wormwood extract exhibited complete resistance to *A. alternata* infection.
Keywords. *A. alternate*, *Penicillium. expansum*, People Plants Extracts, Apple Fruits.

Introduction

Apple trees worldwide are exposed to numerous fungal and bacterial pathogens, as well as viral diseases. Root rot and leaf spot are common fungal diseases, wilt (leaf and flower), and fruit issues like decay and spotting, leaf drop, and cankers of trunks, branches, and twigs [1]. However, it is worth noting that physiological disorders and pathogenic diseases may plague the storage period following harvest, consequently compromising both the quality and quantity of the crop. This unfortunate outcome translates into notable economic losses [2,3].

Plant pathogens pose a significant economic threat to fruit production by reducing availability and storage life [4]. They contain various biologically active compounds and specific phenolic compounds known for their antioxidant properties. The main fungal pathogens responsible for apple fruit losses are major postharvest fungal pathogens, including *Fusarium expansum*, *B. cinerea*, and *Mucor piriformis*. Notably, *P. expansum* is a significant concern due to its role in spoilage and patulin production, a mycotoxin found in rotting apples and apple juice [6]. These pathogens, particularly Botrytis cinerea, are responsible for substantial postharvest losses [7]. *Botrytis* species pose a significant threat to various crops, including ornamentals and field crops [8, 9, 10].

Fruit is susceptible to fungal infection during handling, transportation, and storage due to these pathogens. Recent studies have shown that waste from total fruit and vegetable production can be as high as 33% and the Annual losses range from 5 to 35% [11-13]. Apart from their economic consequences, these fungi are also recognized as strong producers of toxic secondary metabolites, including a variety of mycotoxins (e.g., chaetoglobosins and patulin), allergens, and antibiotics, which can exhibit carcinogenic and mutagenic effects, posing serious health risks [14].

While plants are generally shielded from fungal diseases by utilising synthetic fungicides, their excessive use has raised concerns due to high costs, residue issues, and the development of resistance, ultimately harming human health and the environment. Alternatively, extracts from medicinal plants with antibacterial properties offer promising benefits. These plants contain secondary metabolites like alkaloids, tannins, saponins, glycosides, and flavonoids, which have shown potent antifungal effects [15]. Notably, Alternaria, a widespread fungal disease affecting various fruits, requires attention. The research aims to test the efficiency of some natural plant extracts in controlling postharvest fungi (*Alternaria alternata* and *Penicillium expansum*) on apple fruits.

. Recently, research has focused on utilizing plant-derived products as innovative fungicides for managing post-harvest diseases. Considerable investigations have highlighted the significance of using plant extracts

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and volatile oils as natural fungicides, such as thyme, cloves, cinnamon, lemon, and basil. Some studies have also indicated that essential oils extracted from thyme can significantly decrease the occurrence of blue mold and green mold induced by *Penicillium* species on harvested citrus fruits, and also control other fungal diseases [16, 17].

Multiple experiments have been conducted on new plant extracts with a potent fungicidal nature around the world, and studies have shown that plant extracts, prepared with water or organic solvents, exhibit antifungal properties against multiple pathogens in fruits like citrus, apples, and pears [18, 19].

Alternaria fungi cause several types of apple rots. These rots appear on the fruit as irregular, dark brown spots that grow into black spots [20]. Other studies have also shown that treating apples with thyme essential oils effectively reduces damage caused by *Penicillium* fungi. The findings showed that 14% of apples stored under control conditions were free of Penicillium disease, while 39-42% of the thyme-treated apples had no fungal contamination [21]. Certain fungi, for instance, P. expansum, present a significant health risk due to their production of the mycotoxin patulin, citrinin, and chitoglobulin, which are carcinogenic [22].

Several studies have indicated the mechanism of volatile oils in inhibiting or stopping the growth of fungi, which includes several assumptions, including (a) the presence of the OH group and multiple hydrogen bonds formed by essential oils leads to harmful changes in cellular enzyme activity and the functions of intracellular organelles. These effects include: (a) morphological alterations due to the breakdown of cell cohesion and integrity, (b) membrane instability, (c) disruption of membrane permeability, and (d) cytoplasmic granulation. Many plant extracts contain various antimicrobial compounds, and the benefits of plant-derived fungicides are their biodegradability and their non-toxic nature to plants. Thus, Higher plant-derived fungicides could serve as substitutes for synthetic fungicides [26]. In addition to these substances, other antimicrobial agents comprise acetaldehyde, allicin, and various phenolic compounds, among others [27, 28]. A study showed that phenolic compounds like ferulic acid and resveratrol reduced blue mold in apples, with quercetin and umbelliferone being the most effective at 86-92% inhibition [29].

Nevertheless, phenolics and certain plant-derived bioactive compounds with antimicrobial properties, such as nonanone acetaldehyde, citral, cinnamaldehyde, and nerolidol, were also found to be efficacious in rescuing citrus fruits from damage caused by the pathogen *P. digitatum* [30].

Various isothiocyanates have been shown to reduce the rate of infection from *Botrytis Cinerea* and *Penicillium expansum* in apples by up to 85% [31]. In general, the method/solvent used in extracting plant extracts affects their antifungal effect, which may be linked to the solvent's polarity in the extraction process. Using methanol as a polar solvent facilitates the derivation of plant compounds like phenolics and terpenes, which have been shown to possess antimicrobial properties due to their biologically active constituents [32]. Various studies have shown that certain methanol extracts from Acacia seyal and Withania somnifera plants can effectively manage green mold disease by enhancing the plant's innate defence mechanisms. [33, 34]. Several investigations have indicated the effectiveness of camphor extracts in treatment and beauty products [35]. The effectiveness of camphor extracts in killing and repelling insects has also been supported [36, 37]. Some results have shown that camphor extract may exhibit significant inhibitory activity against plant pathogenic fungi, most notably *Fusarium* spp. This is attributed to the fact that the cell membrane may be a target for camphor extract, as it can disrupt the fungal cell membrane, improving its permeability and potentially releasing large molecules, such as nucleic acids and proteins, into fungal cells [38].

Several experiments have indicated the biological activity of camphor extract as an antimicrobial against several plant fungi, for instance, (*Fusarium oxysporum, Fusarium solani, and Alternaria alternata*). The bioactive metabolites are the cause of this effect. The antimicrobial effect of camphor substances may be attributed to the extract containing several phenolic and flavonoid compounds, including catechins and gallic acid [39].

Methods

Study design and setting

The was carried out in 2022 at the Department of Plant Protection, Faculty of Agriculture. Omar Al-Mukhtar University, Al Bayda, Libya, for the efficiency evaluation of three plant extracts on fungal growth postharvest apple fruits.

Plant Extracts

Three plant extracts were used for antifungal activity studies. The tested plants were presented in Table 1. Multiple prior investigations have elucidated the significance of these extracts in impeding the expansion of mycelium and the initiation of spore germination in various pathogenic fungi [40].

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Table 1. The tested plants for antifungal activity studies.

English name	Scientific Name
Artemisia	*Artemisia*
Thyme	*Thymus Vulgaris*
Camphor	*Cinnamomum camphora*

Plant extracts preparation

Plant extraction using methanol, hexane, and water was used as the extraction system. A total of 100 grams of the respective collected botanical extraction was subjected to disinfection using a 2% sodium hypochlorite solution for 15 minutes. After disinfection, the material was flushed twice with purified water for 15 minutes each and then allowed to air dry. at 26 C° for 3 to 4 hours, according to the type of plant infection. Subsequently, the material was carefully placed in polyethylene bags and frozen at a temperature of -20 degrees Celsius for a minimum duration of 12 hours. Upon thawing at room temperature for 1 hour, the plant materials were delicately wrapped in a fine cotton fabric and subjected to pressing to obtain the desired extracts. This process of freezing and thawing effectively ruptured the plant cells, thereby facilitating the collection of fluid extracts devoid of any tissue. The extracts were then subjected to centrifugation using a refrigerated RC-3B centrifuge from Sorvall Instruments, at a force of 4500xg for a period ranging from 30 to 60 minutes, while maintaining a temperature of 19 degrees Celsius. The resulting supernatants were subsequently subjected to filter sterilization using a 0.22 µm Millipore filter from Massachusetts, USA. These sterilized extracts were then utilized as test extracts, as indicated by [41]. Finally, the extracts were stored in sterile amber bottles at a temperature of 4 degrees Celsius, with a maximum storage period of 60 hours.

In vitro antifungal assay of plant extracts

Mycelial inhibition was quantified as the percentage of in vivo assays conducted on wounded apples. In each trial, fresh samples of a pathogen and an antagonist were defrosted and diluted to concentrations of 105 CFU/ml for the pathogens, 107 CFU/ml for the antagonistic fungal strains [42].

In vivo antifungal activity of plant extracts

For laboratory tests, Golden Delicious apples that had not undergone late-season fungicide treatments were utilised. These apples were stored for a maximum of 8 weeks at a temperature of 2°C and a relative humidity of 95%. Before testing, the apples were subjected to surface sterilization by being soaked in 70% ethanol for 3 minutes. Then, the method indicated by [43] was followed to inject apple fruits with a fungal spore suspension**.**

Statistical analysis

Involved the conversion of the estimated area of Inhibition of fungal proliferation, expressed relative to the control percentage, through arcsine transformation to ensure the homogeneity of variance. The subsequent analysis of the transformed data was conducted using SPSS version 22. The major separation was achieved by employing Duncan's multiple range tests at a significance level of $p<0.05$.

Results

The objective of the experiment was to investigate the Impact of plant-derived extracts on the growth of mycelium in two fungal species, namely *Penicillium expansum* and *Alternaria alternata*. Regarding the growth of *P. expansum*, the outcomes depicted in Figure 1 exemplified the efficacy of plant-derived extracts in impeding the growth of the fungus relative to the control group. The treatment involving aqueous and alcoholic camphor extract exhibited the highest efficiency in inhibiting fungal mycelial growth, followed by the alcoholic Artemisia extract, resulting in respective fungal growth measurements of (0.87, 0.87, and 1.66 cm).

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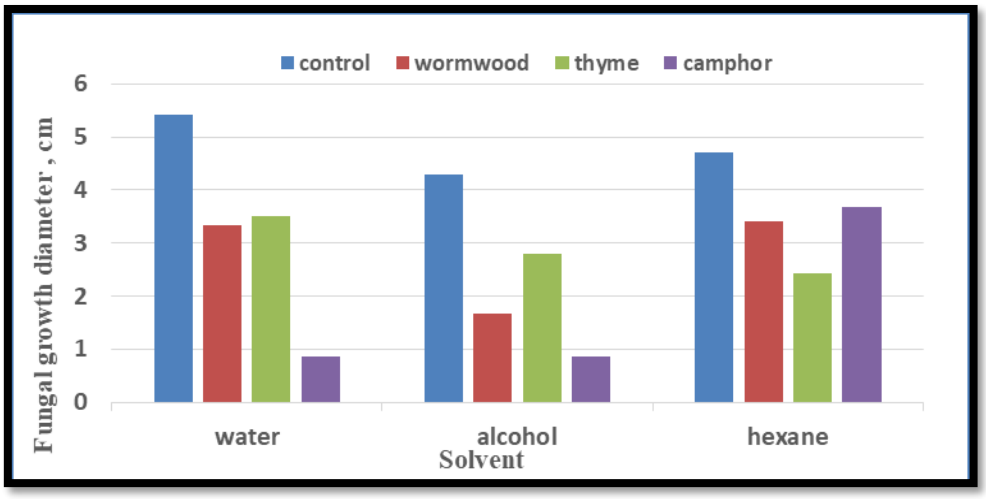

Figure 1. The fungal growth diameter of the fungus Penicillium expansum on potato agar medium, supported by a 10% concentration of some plant extracts

Figure 2 demonstrates the ability of natural plant extracts to suppress the growth of *Alternaria alternata* relative to the control group. The treatment involving aqueous and alcoholic camphor extract was most efficacious in impeding the mycelium growth, followed by the alcoholic thyme extract. The respective fungal growth measurements were (0.80, 1.08, and 1.17 cm).

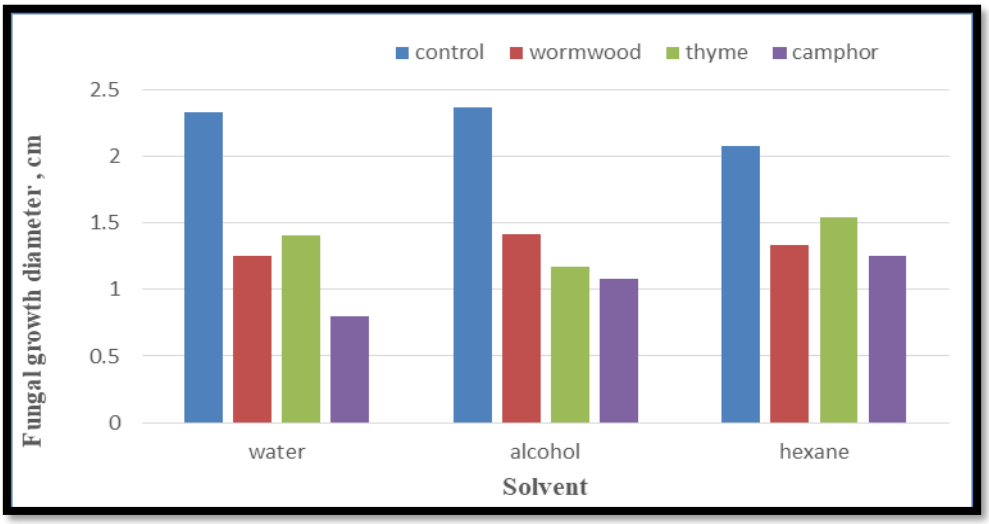

Figure 2. The fungal growth diameter of the fungus Alternaria alternata on potato agar medium supported by a 10% concentration of some plant extracts

Enumeration of fungal spores

Regarding the formation of germs, the findings demonstrated the impact of extracts from plants on their formation of the fungus *Penicillium expansum*. Their number decreased in all treatments except for the treatment with Artemisia extract (dissolved in hexane) relative to the control group Figure 3. The most effective of them were the aqueous thyme (dissolved in hexane and water) extract and the hexane camphor extract, and the average number of germs in the dishes was 1.49, 1.97, and 2.21, respectively. Regarding the formation of germs, the outcomes demonstrated the impact of plant-derived extracts on their formation of the fungus *Alternaria alternata*. Their number decreased in all treatments except for the treatment with Artemisia extract (dissolved in alcohol) relative to the control Figure 4. The most impact of them were the aqueous hexane Artemisia extracts and camphor (dissolved in alcohol and hexane) extract, and the average number of germs in the dishes was (1.46, 2.24, and 2.24), respectively.

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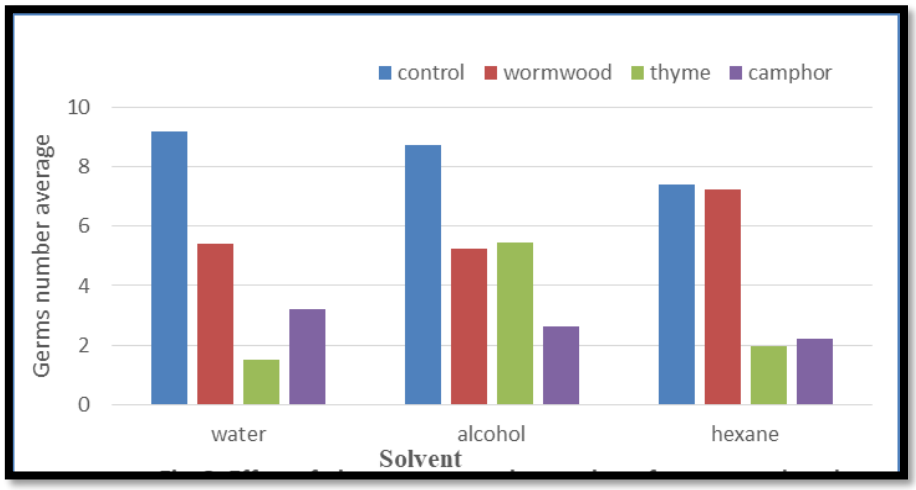

Fig. 3: Impact of plant extract on the spore production by the fungus Penicillium expansum

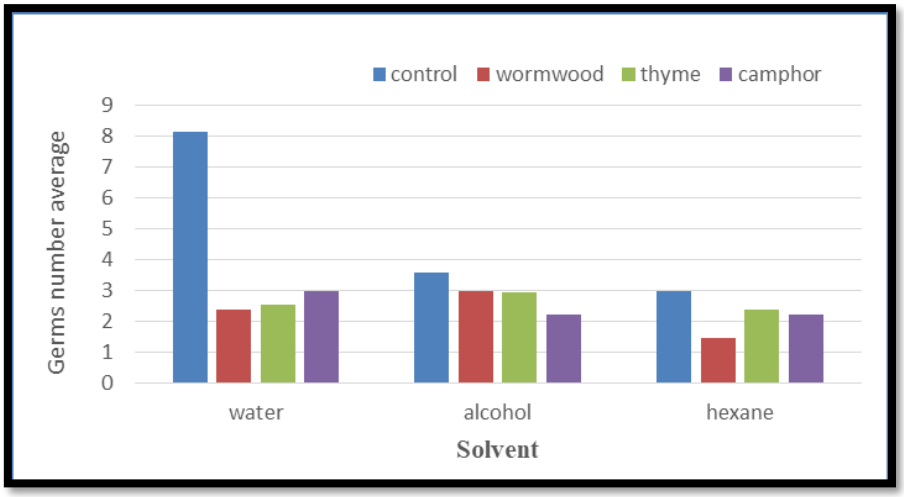

Fig. 4: Influence of plant extract on spore number produced by the fungus Alternaria alternata

Percentage of fungal infection

The results in figure (5) showed the percentage of *Penicillium expansum* infection on apple fruits (Red Delicious variety) treated with In Vivo plant extracts. The results found that, the value of maximum infection percentage was 7 % for water *Artemisia*, followed by hexane thyme and water thyme of 5.5 and 3.1 %, respectively. In contrast, there was no infection for Artemisia (dissolved in alcohol and hexane) and hexane camphor.

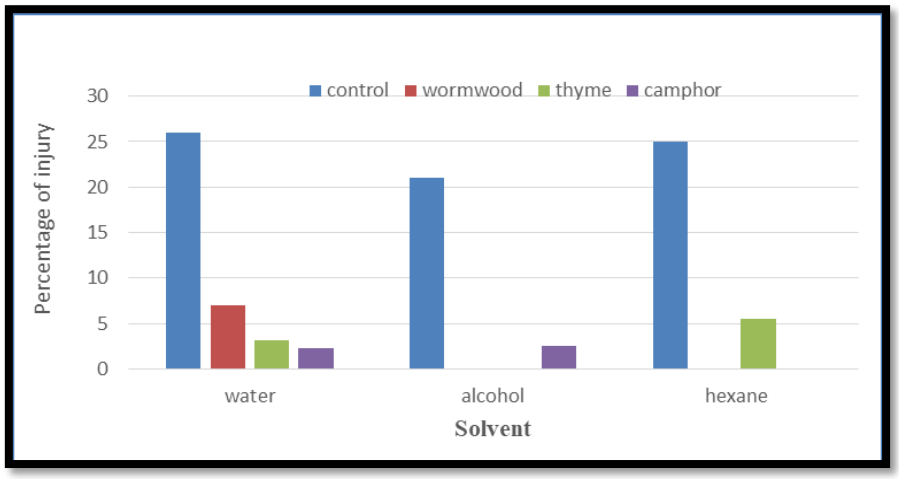

Fig. 5: Percentage of Penicillium expansum infection on apple fruits of (Red Delicious) variety treated with in vivo plant extracts

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The results in figure (6) showed the percentage of *Penicillium expansum* infection on apple fruits (Golden Delicious variety) treated with In Vivo plant extracts. The results found that, the highest infection percentage of Artemisia followed by thyme and camphor, where the infection rate was highest (65 %) of water Artemisia. While, the lowest infection percentage was 3.5 % of hexane camphor.

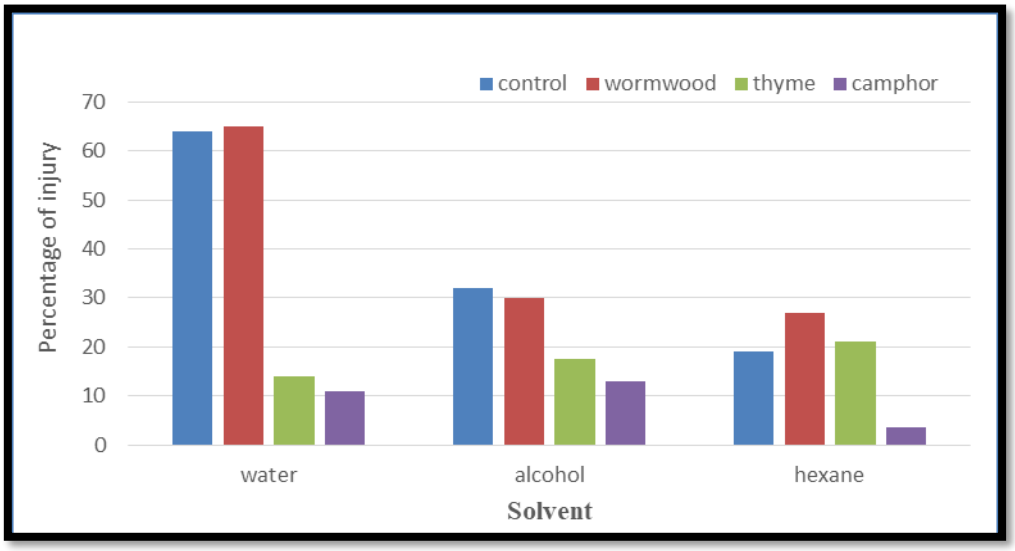

Fig. 6: Percentage of Penicillium expansum infection on apple fruits (Golden Delicious) treated with invivo plant extracts

The results in figure (7) showed the percentage of *Alternaria alternata* infection on apple fruits (Red Delicious variety) treated with In Vivo plant extracts. The results found that, the value of maximum infection percentage was 2.1 % for alcohol thyme, followed by water Artemisia and hexane thyme of 1.6 and 0.5 %, respectively.

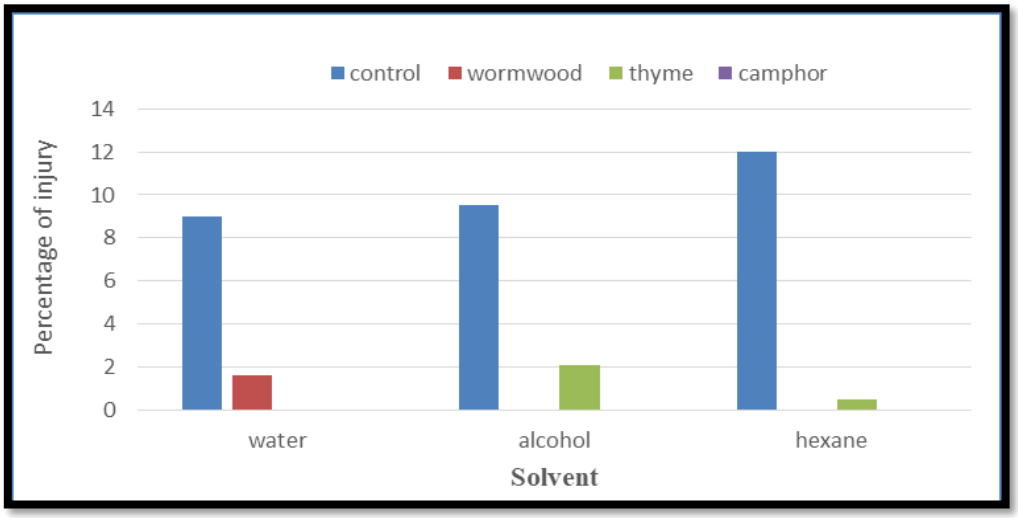

Fig. 7: Percentage of Alternaria alternata infection on apple fruits of (Red Delicious) cultivar treated with in vivo plant extracts

In contrast, there was no infection for Artemisia (dissolved in alcohol and hexane), water thyme, and camphor (dissolved in water, alcohol, and hexane). The results in Figure 8 showed the percentage of *Alternaria alternata* infection on apple fruits (Golden Delicious variety) treated with In Vivo plant extracts. The results found that the highest infection percentage of hexane Artemisia was 10.5 %, whereas the lowest infection percentage was 1.5 % of alcohol camphor. In contrast, there was no infection for alcohol Artemisia. The statistical analysis indicated significant variations between the plant extracts tested.

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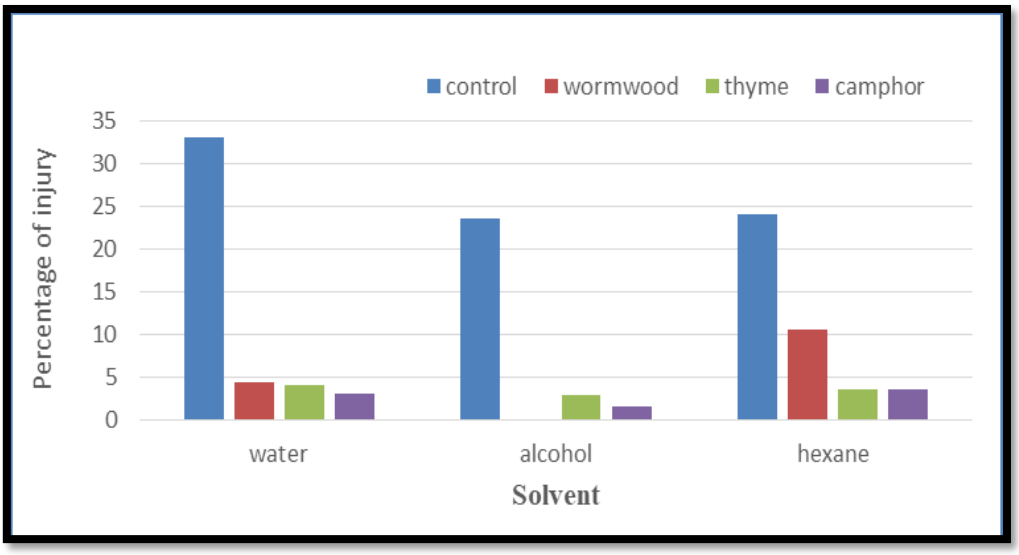

Fig. 8: Percentage of Alternaria alternata infection on apple fruits of (Golden Delicious) treated with in vivo plant extracts.

Discussion

The results obtained in this research can be discussed as follows: The results revealed a discrepancy in the antifungal effects of plant extracts. The results indicated that aqueous and alcoholic camphor extracts were the most effective in inhibiting fungal growth, followed by alcoholic wormwood extract, this may be due to the ability of the active ingredients in camphor extract to disrupt the cell membrane and potentially release large molecules, such as nucleic acids and proteins, into fungal cells. This is consistent with the experimental results in [38]. The inhibitory effect of camphor extracts may be attributed to the extract containing numerous phenolic and flavonoid compounds, including catechins and gallic acid [39]. In general, alcoholic plant extracts were the most effective in inhibiting fungal growth in the PDA environment. This is due to it containing many compounds, for instance, Alkaloids, tannins, saponins, which are potent Secondary metabolites may be present in these plants and possess antifungal properties [15]. Also, they contain various biologically active compounds and specific phenolic compounds known for their antioxidant properties. *Penicillium expansum i*s a meaningful fungal species that poses a health risk arising from its production of the carcinogenic toxins patulin, citrinin, and chitoglobulin [22].

The findings of this research indicated the effectiveness of thyme and wormwood extracts in preventing the growth of *Alternaria alternata* and *Penicillium expansum*. The effectiveness of these extracts is attributed to their volatile oil content, which inhibits or stops fungal growth. This effectiveness is attributed to several hypotheses, including: (a) the occurrence of some mutations in cellular enzymes and their functions; (b) the appearance of changes in cell cohesion and integrity; (c) the occurrence of a disturbance in the stability of plasma membranes; and (d) disturbances in membrane permeability. findings align with previous studies [22, 25]. The outcomes of the study are also in agreement with [26], showing that numerous plant extracts, such as thyme and wormwood, contain various antimicrobial compounds, with essential oils among them, flavonoids, quinones, alkaloids, and tannins. Other compounds exhibiting antimicrobial activities contain allicin, isothiocyanates, hexanol, methyl salicylate, lipoxygenase, and phenolic compounds. [26, 28]. Similarly, research by [26] showed that phenolic compounds, including resveratrol, scopoletin, and umbelliferone, effectively controlled *P. expansum* infection on Granny Smith and Golden Delicious apples. These activities have been documented as one of the antimicrobial processes and efficacy of raw plant extracts; yet, in-depth evaluation of their biological impacts, distribution within plant tissues, and the advancement of accurate formulations and detection techniques." of active ingredients requires extensive scientific interests for their use as economical plant fungicides.

Conclusion

Through numerous studies and research into new alternatives to chemicals used to combat fungal plant pathogens, new methods were developed in the 1980s. These methods rely on extracting active ingredients found in considerable natural plant species. To date, extensive research has been conducted on these plant extracts; further discoveries have been made about their active ingredients, and their effectiveness in eliminating or reducing fungal diseases has been established. Most of this research has indicated the high effectiveness of these plant extracts and the potential for their use as a successful substitute to synthetic fungicides.

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Conflicts of Interest
The authors confirm that this research is free from any conflicts of interest.

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