

Isolation, Identification, and Pathogenicity of *Alternaria alternata* Associated with Apple Branch Canker in Al-Jabal Al-Akhdar Region, Libya

Gazala Saad* 

Department of Plant Protection, Faculty of Agriculture, Omar Al-Mukhtar University, Al-Bayda, Libya

Email: gazala.ibrahim@omu.edu.ly

Abstract

Apple (*Malus domestica* Borkh.) represents a major fruit crop of global economic importance. Despite its widespread cultivation, fungal diseases infecting branches and vegetative tissues continue to be a major concern, adversely affecting orchard productivity and compromising fruit quality. The present study investigated the fungal pathogen associated with branch canker symptoms observed in apple orchards located in the Al-Jabal Al-Akhdar region, Libya. Symptomatic branch samples exhibiting bark cracking, canker formation, and progressive dieback were collected and subjected to fungal isolation under laboratory conditions. Isolates were purified on potato dextrose agar (PDA) medium and identified according to morphological and microscopic characteristics using standard taxonomic keys. Pathogenicity assays were conducted on healthy apple seedlings to verify the causal relationship between the isolated fungus and disease development. Morphological observations demonstrated that the recovered isolate belonged to *Alternaria alternata* (Fr.) Keissl., characterized by dark olivaceous to brown colonies and multicellular conidia arranged singly or in short chains. Conidial dimensions ranged from 14.05–14.38 μm in length and 8.07–9.81 μm in width, with 2–8 transverse septa. Artificial inoculation induced typical symptoms, including bark necrosis, branch canker formation, chlorotic lesions, and progressive tissue collapse. Re-isolation from infected tissues successfully fulfilled Koch's postulates. The findings confirm the pathogenic role of *A. alternata* in apple branch canker development under local environmental conditions. This study represents one of the first detailed reports concerning the occurrence and pathogenicity of *A. alternata* associated with apple branch canker in eastern Libya and provides baseline information for future epidemiological and disease management studies

Keywords. *Alternaria Alternata*, Apple Branch Canker, Pathogenicity, Fungal Morphology, Apple Diseases, Libya.

Introduction

Apple (*Malus domestica* Borkh.) is among the most widely cultivated and economically significant deciduous fruit crops worldwide. Its fruits are highly valued for their nutritional composition, serving as an important source of carbohydrates, dietary fiber, vitamins, antioxidants, and diverse phenolic compounds that contribute to human health and food security. Owing to its economic importance and broad consumer demand, apple production plays a vital role in the horticultural sector across many regions of the world. (1,2). In Mediterranean and semi-arid agroecosystems, apple cultivation constitutes a key component of agricultural development, providing substantial economic benefits and supporting the livelihoods of farming communities.

Despite its economic importance, apple production is severely constrained by several fungal diseases that affect leaves, fruits, shoots, and branches. Among these diseases, branch cankers and dieback symptoms are particularly destructive because they reduce vegetative growth, weaken tree vigor, predispose trees to secondary infections, and ultimately reduce productivity and orchard longevity. (3). Several fungal genera have been associated with canker development on apple trees, including *Botryosphaeria*, *Neofusicoccum*, *Cytospora*, and *Alternaria* species (4). Species belonging to the genus *Alternaria* are among the most widespread fungal pathogens affecting horticultural crops. These fungi are capable of surviving as saprophytes, opportunistic pathogens, or aggressive necrotrophs, depending on environmental conditions and host susceptibility (5). *Alternaria alternata* is recognized as an important phytopathogenic fungus with a global distribution across various agricultural production systems. It has been implicated in several economically significant diseases, including leaf blotch, fruit spotting, postharvest decay, and branch lesions in a wide range of fruit crops, resulting in considerable yield and quality losses. (6).

Historically, the apple pathotype of *Alternaria* was described as *Alternaria mali*, a host-selective toxin-producing pathogen capable of causing severe defoliation and fruit damage in susceptible cultivars. Recent phylogenetic and genomic investigations, however, demonstrated that the apple pathotype belongs to the *A. alternata* species complex. These pathogenic strains produce host-selective AM toxins encoded by genes located on conditionally dispensable chromosomes, which contribute directly to virulence and host specificity. (7). In recent years, diseases caused by *Alternaria* spp. on apple trees have increased in incidence and severity in several countries, including China, France, Italy, Israel, and the United States. Environmental conditions characterized by moderate temperatures, prolonged humidity, rainfall events, and mechanical injuries greatly enhance disease establishment and fungal colonization. (8).

Furthermore, climate variability and intensified orchard practices may increase disease pressure in apple-producing regions (9). In Libya, particularly within the Al-Jabal Al-Akhdar region, apple cultivation represents an important agricultural activity because of the region's relatively favorable climatic conditions. However, information regarding fungal pathogens associated with apple branch canker remains extremely limited. To date, no comprehensive morphological and pathogenicity-based investigation has been conducted on *Alternaria alternata* associated with branch canker symptoms in Libyan apple orchards. To improve understanding of the etiology of apple branch canker in Al-Jabal Al-Akhdar, Libya, the

present study aimed to investigate the fungal pathogen associated with symptomatic branches. Particular emphasis was placed on the isolation and identification of the pathogen, the characterization of its morphological attributes, and the evaluation of its pathogenicity through controlled inoculation assays.

Taxonomic Overview of *Alternaria* Species

The genus *Alternaria* comprises more than 300 described species distributed across diverse ecological habitats worldwide. Members of this genus are characterized by darkly pigmented septate hyphae and multicellular conidia that vary considerably in shape, septation, and chain formation. Traditional taxonomy relied primarily on morphological characteristics; however, modern phylogenetic analyses using multilocus sequence data have substantially revised species boundaries within the genus. (5,10). According to recent molecular studies, the *Alternaria alternata* species complex includes numerous morphologically similar taxa that are difficult to distinguish based solely on microscopic features. Consequently, integrated taxonomic approaches combining morphology, molecular phylogeny, pathogenicity, and secondary metabolite profiling are increasingly recommended for accurate species delimitation. (11) .

***Alternaria*-Associated Diseases of Apple**

Alternaria diseases of apple are commonly expressed as *Alternaria* leaf blotch (ALB), fruit spot, and occasionally branch canker or shoot blight. Symptoms generally begin as small necrotic lesions surrounded by chlorotic halos that expand progressively under favorable environmental conditions. Severe infections may lead to premature defoliation, fruit blemishes, reduced marketability, and branch dieback. Among the diseases caused by *Alternaria* spp., *Alternaria* leaf blotch has emerged as a significant constraint to apple production in susceptible cultivars worldwide (12). Previous studies have demonstrated that severe epidemics may lead to extensive premature defoliation, reaching up to 80% in highly susceptible genotypes. Consequently, affected trees exhibit reduced vigor and diminished fruit quality, resulting in substantial economic losses and decreased orchard performance.

Pathogenicity and Virulence Mechanisms

The virulence of *A. alternata* is closely linked to the synthesis of host-selective toxins and extracellular enzymes capable of degrading plant cell walls, thereby promoting tissue invasion and symptom expression. (7). In apple pathotypes, AM toxins interfere with membrane integrity and induce necrosis in susceptible tissues. In addition, the pathogen can penetrate wounded tissues, making mechanical injuries important infection sites during orchard operations. Environmental factors such as temperature, humidity, leaf wetness duration, and pH greatly influence fungal growth and disease development. Several studies demonstrated that optimum growth of *A. alternata* occurs at temperatures ranging from 25–30°C under humid conditions.

Global and Regional Studies

Recent studies conducted in China identified *A. alternata* as the dominant pathogen associated with black spot disease of bagged apple fruits. (13). Similar investigations in France and Israel confirmed the widespread occurrence of *Alternaria* spp. in commercial apple orchards and emphasized the importance of integrated disease management strategies. (14). Although several international reports have documented the epidemiology and pathogenicity of *Alternaria* species on apple, information from North African countries remains scarce (15). Consequently, additional regional investigations are required to understand disease distribution, pathogen variability, and environmental adaptation under Mediterranean climatic conditions. (11).

Materials and Methods

Sample Collection

Symptomatic apple branches exhibiting characteristic canker symptoms, including bark fissuring, tissue necrosis, and shoot dieback, were collected from orchards located in the Masa district, Al-Jabal Al-Akhdar, Libya, throughout the 2024–2025 growing seasons. The collected specimens were individually placed in sterile polyethylene bags and transported to the laboratory under hygienic conditions. Upon receipt, samples were processed without delay to ensure the integrity of the associated fungal pathogens and to facilitate accurate isolation procedures.

Surface Sterilization and Isolation

Small tissue pieces (approximately 5–10 mm in length) were aseptically excised from the interface between healthy and diseased tissues using a sterile scalpel. The excised segments were surface-disinfected by immersion in a 10% sodium hypochlorite solution for 2–3 min, followed by three successive rinses with sterile distilled water. The sterilized tissues were then blotted dry on sterile filter paper to remove excess moisture. Subsequently, the tissue segments were transferred onto potato dextrose agar (PDA) plates and incubated at 25 ± 2°C for 5–7 days under laboratory conditions. Fungal colonies developing from the plated tissues were periodically examined, and representative colonies were subcultured onto fresh PDA medium to obtain pure cultures for further characterization.



Figure 1. Symptoms of the disease on the branches

Morphological Identification

The fungal isolate was identified based on standard taxonomic and morphological criteria. Colony characteristics, including color, texture, growth pattern, and sporulation, were recorded. Microscopic features were examined and documented using a Leica DM1000 LED microscope fitted with a digital imaging system. A total of 20–25 conidia and conidiophores were measured to determine conidial dimensions, septation patterns, and morphological variability.

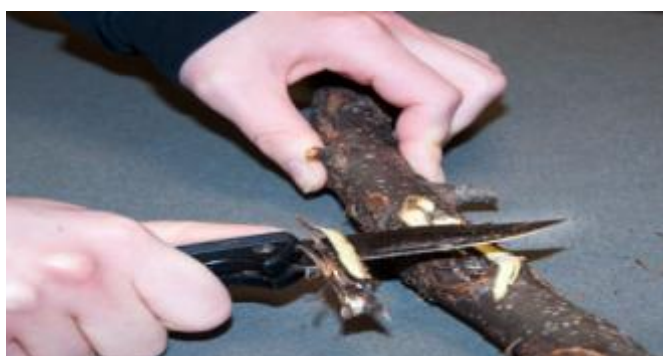


Figure 2. Treatment of infected branches in the laboratory

Pathogenicity Test

Pathogenicity assays were conducted on healthy apple seedlings under controlled conditions. Small wounds were made on stems using a sterile needle, and fungal inoculum obtained from actively growing colonies was applied to the wounded sites. Control plants received sterile PDA plugs only. Each treatment consisted of three replicates. Inoculated plants were maintained under humid conditions and monitored regularly for symptom development. Re-isolation of the fungus from symptomatic tissues was conducted to verify Koch's postulates.



Figure 3. Pathological ability test according to (6)

Statistical Analysis

A Completely Randomized Design (CRD) was employed for all experimental procedures, with three independent replicates per treatment. Pathogenicity-related parameters, including lesion length, disease incidence, and disease severity, were statistically evaluated to determine the impact of *Alternaria alternata* inoculation on apple seedlings. The obtained data were subjected to one-way analysis of variance (ANOVA), and significant differences among treatment means were determined using Tukey's honestly significant difference (HSD) test at the 5% probability level ($P \leq 0.05$). (16).

$F = \text{MS}_{\text{between}} / \text{MS}_{\text{within}}$,

Where:/.n

MS between=Mean square between treatments/n

MS within=Mean square within treatments

When significant differences were detected, treatment means were separated using Tukey's Honestly Significant Difference (HSD) test at $P \leq 0.05$.

Disease Severity Assessment

Disease severity was evaluated using the Disease Severity Index (DSI) according to the following formula:

$$DSI(\%) = \sum d \times f / N \times D \times 100$$

where:

DSI = Disease Severity Index (%)

d = Disease rating scale

f = Frequency (number) of infected plants in each disease category

N = Total number of examined plants

D = Maximum disease rating scale

Statistical analyses were conducted using IBM SPSS Statistics software (Version 25.0; IBM Corp., Armonk, NY, USA).

Disease severity data were calculated according to the method of (15). All experimental data are presented as mean values $\pm$ standard error (SE). Differences among treatments were evaluated using the appropriate statistical tests, and treatment means were considered significantly different at a probability level of $P \leq 0.05$.

Results

Symptom Description in the Field

Naturally infected apple branches exhibited typical canker symptoms characterized by bark cracking, tissue discoloration, branch necrosis, and progressive dieback. In advanced stages, infected tissues became dark brown and partially collapsed, while some branches showed peeling bark and drying of terminal shoots.

Morphological Characteristics of the Isolated Fungus

The fungal isolate produced rapidly growing colonies on PDA medium with dark olive-brown to black pigmentation. The mycelium appeared compact and partially submerged within the medium. The septate conidiophores were observed under a microscope to produce the conidia singly or in short chains. The conidia exhibited a considerable morphological variability, ranging from oval and ellipsoid to obclavate and inversely pear-shaped forms. They were darkly pigmented with smooth to slightly roughened surfaces. 2–8 transverse septa occasionally accompanied by longitudinal septa. The measurements showed that the mean conidial length ranged from 14.05 to 14.38 μm , and the mean conidial width varied from 8.07 to 9.81 μm . The fungal isolate was identified as *Alternaria alternata* using the morphological characteristics of the fungal isolate and taxonomic criteria.

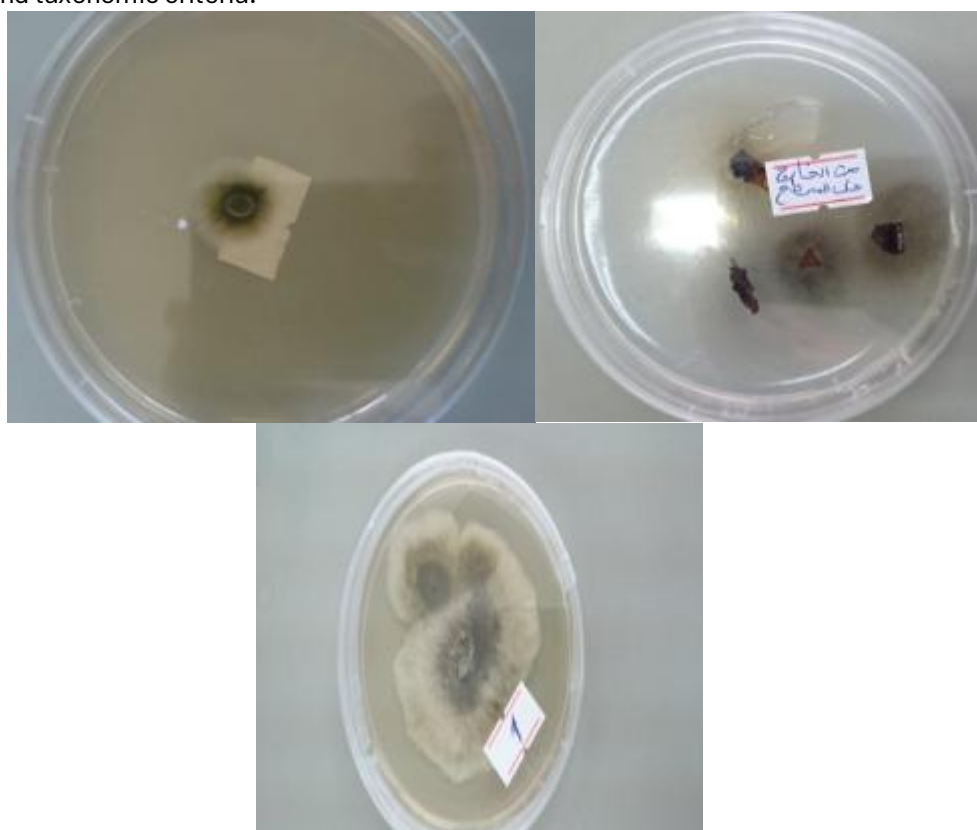
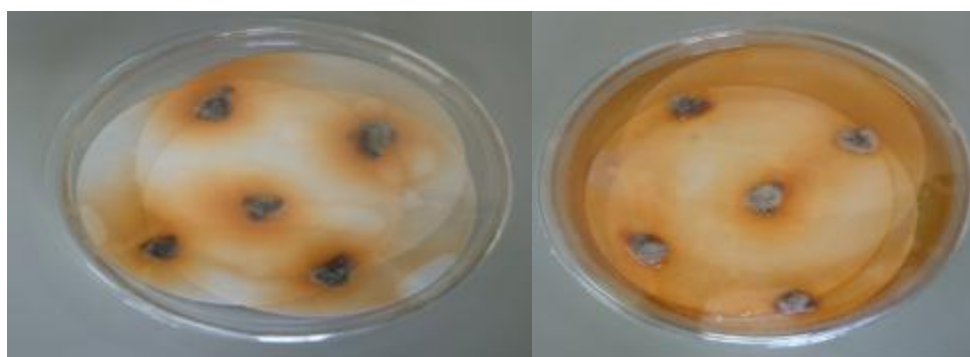


Figure 4. Mycelium illustrates the role of the food web in the environment

Taxonomic Classification of the Isolated Fungus

Taxonomic Rank	Classification
Kingdom	Fungi
Phylum	Ascomycota
Class	Dothideomycetes
Order	Pleosporales
Family	Pleosporaceae
Genus	<i>Alternaria</i>
Species	<i>Alternaria alternata</i>

**Figure 5. Conidia forms****Figure 6. Incubation in a humid chamber**

Microscopic examination revealed that the conidiophores were either simple or branched, occurring singly or in small clusters. They were straight to flexuous in shape, pale brown to brown in color, and possessed smooth walls, with dimensions ranging from 20–50 µm in length and 3–5 µm in width. Conidia were formed predominantly in acropetal chains consisting of 2–7 spores, although solitary conidia were occasionally observed. The conidia displayed considerable variation in morphology, including obovoid, ellipsoid, obclavate, and obpyriform forms. They were dark brown to olivaceous-brown in color, with surfaces varying from smooth to verruculose. The conidia contained 2–8 transverse septa together with one or several longitudinal or oblique septa.

Conidial dimensions measured from 20–25 randomly selected conidia were:

- Length: 14.05–14.38 µm (mean: 14.22 ± 0.17 µm)
- Width: 8.07–9.81 µm (mean: 8.94 ± 0.87 µm)
- Length/width ratio: 1.59–1.78 (mean: 1.69)

These measurements are consistent with published descriptions of *A. alternata* (7, 8; 5; 16).

Pathogenicity Assay

Artificial inoculation of healthy apple seedlings with the isolated *Alternaria alternata* resulted in the development of characteristic disease symptoms within 7–10 days after inoculation. The earliest visible symptoms appeared as small water-soaked lesions at the inoculation sites, which gradually enlarged and developed into well-defined cankers. These cankers were associated with bark discoloration and cracking, followed by peeling and sloughing of the affected bark tissues. As disease progression continued, dieback extended from the point of inoculation, accompanied by yellowing and browning of leaves on infected branches. In severe cases, branch girdling was observed, resulting in the death of distal tissues beyond the affected area. In contrast, control seedlings inoculated with sterile PDA plugs showed no visible symptoms throughout the experimental period. Re-isolation of the pathogen from the advancing margins of the cankers consistently recovered the same fungal species, exhibiting morphological characteristics identical to those of the original

isolate. These findings fulfilled Koch's postulates and confirmed *A. alternata* as the causal agent of branch canker in the investigated apple orchard.

Symptom Progression

Disease progression followed a characteristic pattern:

- Days 1–3: No visible symptoms
- Days 4–7: Appearance of small, water-soaked lesions at the wound site
- Days 8–14: Lesion expansion, bark discoloration (brown to dark brown), initial peeling
- Days 15–21: Canker development, extensive bark peeling, dieback of affected branches, leaf yellowing, and browning

The highest disease severity was observed at 25–30°C, which is in agreement with the optimal temperature range previously reported for the growth and development of *Alternaria alternata* (13).



Figure 7. Pathological ability test

Statistical Analysis of Disease Parameters

The results of the statistical analysis indicated significant differences ($P \leq 0.05$) between inoculated and non-inoculated seedlings for all evaluated disease parameters. Apple seedlings inoculated with the pathogen exhibited a mean lesion length of 4.82 ± 0.31 cm, while no lesions were detected in the control treatment. Furthermore, inoculated seedlings showed significantly greater infection incidence and disease severity, with mean values of $86.7 \pm 3.2\%$ and $78.4 \pm 2.5\%$, respectively. By comparison, control seedlings remained symptomless throughout the experiment and recorded 0.0% infection incidence and disease severity.

Table 2. Effect of *Alternaria alternata* inoculation on disease development in apple seedlings

Treatment	Lesion Length(cm)	Infection Percentage (%)	Disease severity (%)
<i>A.alternata</i> inoculation	$4.82 \pm 0.31a$	$86.7 \pm 3.2a$	$78.4 \pm 2.5a$
Control	$0.00 \pm 0.00b$	$0.0 \pm 0.0b$	$0.0 \pm 0.0b$

Mean values sharing different letters within a column were considered significantly different based on Tukey's HSD multiple comparison test at $P \leq 0.05$. The ANOVA results confirmed that fungal inoculation exerted a statistically significant influence on the assessed disease development parameters, indicating a clear effect of the pathogen on disease progression.

Table 2. One-way ANOVA for disease severity parameters

Source of Variation	df	Mean	F-value	P-value
Treatment	1	42.61	58.73	0.001
Error	4	0.73		
Total	5			

The disease Severity Index (DSI) further confirmed the aggressiveness of the pathogen under artificial inoculation conditions. Disease Severity (%) = $\frac{\sum(\text{rating} \times \text{number of infected plants})}{\text{maximum rating} \times \text{total number of plants}} \times 100$ Where:

r = disease rating score

n = number of infected plants at each rating

R = maximum disease rating

N = total number of assessed plants

The average DSI value recorded for inoculated seedlings was 78.8%, indicating high pathogenic virulence of the isolated *A.alternata* strain on apple branches under favorable environmental conditions.

Discussion

The present study confirmed the association of *Alternaria alternata* with branch canker symptoms affecting apple trees in the Al-Jabal Al-Akhdar region of Libya. The morphological characteristics observed for the recovered isolate were

consistent with previously published descriptions of *A. alternata* obtained from apple and other woody hosts, supporting its taxonomic identification. Pathogenicity tests further demonstrated the ability of the isolate to induce necrotic lesions and branch dieback under artificial inoculation conditions. The successful reproduction of disease symptoms and subsequent re-isolation of the pathogen provide strong evidence of its pathogenic role. These findings are in agreement with previous reports indicating that *A. alternata* can act as an opportunistic pathogen, readily colonizing wounded tissues and initiating disease development when environmental conditions are favorable (11).

Environmental factors characteristic of the Al-Jabal Al-Akhdar region, including moderate temperatures and periods of elevated seasonal humidity, may play an important role in pathogen establishment and disease progression (12, 15). Previous studies have shown that the optimum growth temperature for *A. alternata* ranges between 25 and 30°C, which closely corresponds to the climatic conditions prevailing during the apple growing season in the study area. The variability observed in conidial morphology and septation patterns agrees with the well-documented phenotypic plasticity of the *A. alternata* species complex. Although morphological characterization remains a valuable tool for preliminary identification, recent phylogenetic studies have highlighted the importance of molecular approaches for accurately delimiting species boundaries and distinguishing pathogenic lineages within the genus (15).

In addition, *A. alternata* has been reported to survive for prolonged periods on infected plant debris, serving as a persistent source of inoculum within orchard ecosystems. Such survival capacity contributes to disease recurrence and may complicate management strategies, particularly where sanitation measures are insufficient (13). Overall, the results of the present study provide the first baseline information on the occurrence and pathogenicity of *A. alternata* associated with apple branch canker in Libyan orchards. Nevertheless, further research involving molecular characterization, mycotoxin profiling, evaluation of cultivar susceptibility, and epidemiological investigations is required to improve our understanding of pathogen diversity and disease dynamics under local environmental conditions.

Conclusions

The results of the present study demonstrated a strong association between *Alternaria alternata* and branch canker symptoms observed on apple trees in the Al-Jabal Al-Akhdar region of Libya. Morphological characterization, together with pathogenicity testing, confirmed the identity and pathogenic potential of the isolated fungus, which was capable of inducing necrotic lesions, bark peeling, and branch dieback in inoculated plants. The findings also suggest that mechanical wounds may facilitate fungal entry and promote disease establishment, particularly under environmental conditions characterized by moderate temperatures and high humidity that favor fungal growth and development. Overall, this study provides valuable baseline information on fungal diseases affecting apple orchards in Libya and underscores the importance of implementing integrated disease management strategies, as well as conducting further molecular investigations to improve understanding of pathogen diversity and epidemiology.

Recommendations

In the management of *Alternaria* branch canker in apple orchards, regular monitoring is essential to enable the early detection of symptoms and thereby reduce the risk of widespread infection. Preventive measures should focus on minimizing mechanical injuries during pruning, transportation, and other orchard management practices, as such wounds often serve as entry points for fungal pathogens. The removal and destruction of infected branches and plant debris are critical steps in reducing inoculum sources and limiting disease transmission within the orchard environment. Beyond immediate orchard practices, further molecular investigations employing ITS and multilocus sequencing are recommended to confirm the identity of the causal species and to assess genetic diversity, which will provide a stronger foundation for targeted control strategies. Screening of locally cultivated apple cultivars for resistance to *Alternaria* infection is also necessary to identify and promote varieties with enhanced resilience. Finally, integrated disease management approaches that combine sanitation measures, cultural practices, and fungicide application should be systematically evaluated under Libyan field conditions to establish effective, context-specific protocols for sustainable disease control.

Conflicts of Interest

The author declares no conflicts of interest.

References

1. Al-Naeemi, F. 1983. Nutritional value of fruits and vegetables. [In Arabic]. University Publications, Libya.
2. Simmons EG. 2007. *Alternaria: An Identification Manual*. CBS Fungal Biodiversity Centre.
3. Agrios, G.N. 2005. *Plant Pathology* (5th ed.). Elsevier Academic Press, San Diego, CA, USA.
4. Timmer, L.W., Garnsey, S.M., and Graham, J.H. 2003. *Compendium of Citrus Diseases* (2nd ed.). APS Press, St. Paul, MN, USA.
5. Armitage A.D., Cockerton HM, Sreenivasaprasad S, Woodhall J, Lane CR, Harrison RJ, Clarkson JP. 2020. Genomics evolutionary history and diagnostics of the *Alternaria alternata* species group including apple and Asian pear pathotypes. *Front Microbiol.* 10:3124.

6. Tsuge T, Harimoto Y, Akimitsu K, Ohtani K, Kodama M, Akagi Y, Egusa M, Yamamoto M, Otani H. 2013. Host-selective toxins produced by the plant pathogenic fungus *Alternaria alternata*. FEMS Microbiol Rev. 37(1):44-66.
7. Harimoto Y, Hatta R, Kodama M, Yamamoto M, Otani H, Tsuge T. 2007. Expression profiles of genes encoded by the supernumerary chromosome controlling AM-toxin biosynthesis and pathogenicity in the apple pathotype of *Alternaria alternata*. Mol Plant Microbe Interact. 20(12):1463-1476.
8. Thomma BPHJ. 2003. *Alternaria* spp.: from general saprophyte to specific parasite. Mol Plant Pathol. 4(4):225-236.
9. Garrett, K.A., Dendy, S.P., Frank, E.E., Rouse, M.N., & Travers, S.E. 2006. Climate change effects on plant disease: genomes to ecosystems. Annual Review of Phytopathology, 44, 489–509.
10. Woudenberg JHC, Seidl MF, Groenewald JZ, de Vries M, Stielow JB, Thomma BPHJ, Crous PW. 2015. *Alternaria* section *Alternaria*: species formation patterns in *Alternaria* and allied genera. Stud Mycol. 82:1-21.
11. Rotondo F, Collina M, Brunelli A, Pryor BM. 2012. Comparison of *Alternaria* spp. collected in Italy from apple with *Alternaria mali* and other AM-toxin-producing strains. Phytopathology. 102(11):1130-1142.
12. Filajdić N, Sutton TB. 1991. Identification and distribution of *Alternaria mali* on apples in North Carolina and susceptibility of different apple varieties to *Alternaria* blotch. Plant Dis. 75(10):1045-1048.
13. Zhang Y, Shao Y, Zhao J, Lin J, Zhang X, Wang X, Xu X, Xu C. 2024. Identification and pathogenicity of *Alternaria* and *Fusarium* species associated with bagged apple black spot disease in Shaanxi, China. Front Microbiol. 15:1457315.
14. Gur L, Reuveni M, Cohen Y. 2017. Occurrence and etiology of *Alternaria* leaf blotch and fruit spot of apple caused by *Alternaria alternata* f. sp. *mali* on cv. Pink Lady in Israel. Eur J Plant Pathol. 147(3):695-708.
15. Armitage, A.D., Bäck, M., Taylor, A., and van den Berg, F. (2021). Identification and pathogenicity of *Alternaria* species associated with apple tree defoliation in France. Frontiers in Plant Science, 12, 757–772.
16. Tukey JW. 1953. The Problem of Multiple Comparisons. Princeton University, Department of Statistics.
17. Horsfall JG, Barratt RW. 1945. An improved grading system for measuring plant diseases. Conn Agric Exp Stn.
18. Andrew, M., Peever, T. L., and Pryor, B. M. 2009. An expanded multilocus phylogeny does not resolve morphological species within the small-spored *Alternaria* species complex. Mycologia, 101(1), 95–109.
19. Al-Waeli ADS. 1988. Practical Plant Diseases: Laboratory and Field Manual. College of Agriculture, University of Baghdad, Ministry of Higher Education and Scientific Research.
20. Ellis MB. 1971. Dematiaceous Hyphomycetes. Commonwealth Mycological Institute; Kew, Surrey, UK.
21. Ellis MB. 1976. More Dematiaceous Hyphomycetes. Commonwealth Mycological Institute; Kew, Surrey, UK.
22. Fontaine K, Fourrier-Jeandel C, Armitage AD, Boutigny AL, Crépet M, Caffier V, Gride DC, Shiller J, Le Cam B, Giraud M, Iloos R, Aguayo J. 2021. Identification and pathogenicity of *Alternaria* species associated with leaf blotch disease and premature defoliation in French apple orchards. PeerJ. 9:e12496.
23. Gilman JC. 1957. A Manual of Soil Fungi. Iowa State College Press.
24. Hubballi, M., Nakkeeran, S., Raguchander, T., Anand, T., & Samiyappan, R. 2011. Effect of environmental conditions on growth of *Alternaria alternata* causing leaf blight of noni. World Journal of Agricultural Sciences, 7(2), 171–177.
25. Johnson RD, Johnson L, Kohmoto K, Otani H, Lane CR, Kodama M. 2000. A polymerase chain reaction-based method to specifically detect *Alternaria alternata* apple pathotype (*Alternaria mali*). Phytopathology. 90(9):973-976.
26. Li Y, Hu XL, Trigliano RN, Aldwinckle H, Cheng ZM. 2019. Evaluation of 110 apple cultivars for resistance to *Alternaria* blotch caused by *Alternaria alternata* apple pathotype. HortScience. 54(8):1268-1274.