Al-Mukhtar Journal of Agricultural, Veterinary and Environmental Science 03 (3): 248-258, 2025
Doi: DOI: https://doi.org/10.54172/7pgt3861

Research Article **Open Access**

Relevance of gray mold disease on grapes in different regions of Al-Jabal Al-Akhdar, Libya.

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Received:
13.07.2025
Accepted:
09.09.2025
Publish online:
09.09.2025

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Abstract

The abstract is a digest of the entire paper and should be given the same consideration as the main text. It does not normally include any reference to the literature. Abbreviations or acronyms must be preceded by the full term at the first use. An abstract should be between 150-200 words. It includes a brief statement of problem, a concise description of the research method and design, a summary of major findings, including their significance or lack of it, and conclusions.

Keywords: Component; Formatting; Style; Styling; Insert (6-8 words)

أهمية مرض العفن الرمادي على العنب في مناطق مختلفة من الجبل الاخضر

المستخلص: أجريت الدراسة الحالية من شهر مايو حتى سبتمبر موسم 2023 وتهدف إلى حصر مرض العفن الرمادي على العنب في بعض مناطق الجبل الأخضر وهي الوسيطة ومسة والفايدية والعويلية وبطة. تم جمع وفحص العينات من الأوراق المصابة، بينت نتائج العزل والتعريف بعد تحضير شرائح في النمو الفطري ووصف المسبب المرضي *Botrytis cinerea* وأخذ قياساته تحت المجهر الضوئي، حيث سُجل سمك الميسيليوم القديم المسجل 5–12.5 ميكرون بمتوسط ، وتراوح سمك الميسيليوم الحديث 3–7.5 ميكرون بمتوسط ، طول الجراثيم 7.5–12.5 ، عرض الجراثيم 5 – 7.5 ميكرون وطول الحامل الجرثومي 15–25 ميكرون. وكما أشارت نتائج التحليل الإحصائي إلى وجود فروق معنوية بين مناطق الدراسة، فكانت منطقة العويلة أعلى نسبة إصابة 54% خلال شهر مايو، بينما منطقة مسه الأدنى معدل إصابة فقد بلغت في شهر سبتمبر نسبة 0.22%. وتراوحت شدة الإصابة بين 0.06 إلى 13.67% في نفس الظروف.

الكلمات المفتاحية: مرض العفن الرمادي، فطر *Botrytis cinerea*، العنب، منطقة الجبل الأخضر.

INTRODUCTION

Grapes are a globally significant crop, for both local consumption and exportation. They belong to the Vitaceae family, which includes about 16 genera and 900 species (Wang *et al.,* 2018; Liu *et al.,* 2016). The genus *Vitis* **L.**, a woody climber, is native to the temperate regions of Northern hemisphere and is divided into three main groups: American, East Asian, and European/West Asian. The last group contains the single species *Vitis vinifera* **L.**, from which most high-quality grape cultivars are derived (Riaz *et al.,* 2018; Sargolzaei *et al.,* 2021). in 2022 grapevines are grown on approximately 7.5 million hectares worldwide, global production reached about 75 million tons (FOA, 2024), cementing their place among the top five fruit crops. Grapes are valued for their rich nutritional profile, numerous health benefits, and versatility. Grape yield has increased during the last five years, and they are currently consistently placed among the top five fruit crops globally (USDA, 2023). Grapes (*Vitis vinifera* L.) are among the most valuable fruits globally, renowned for their rich nutritional profile and numerous health benefits. Due to their exceptional flavor and versatility, grapes are cultivated widely across different regions. (Chen *et al.,* 2018). A key focus of the expanding grape industry is the advancement of innovative processing, storage, and marketing technologies to enhance postharvest shelf life and product quality (Elsayed *et al.*, 2022).

Gray mold, caused by the fungus *Botrytis cinerea* Pers. Fr. (teleomorph: *Botryotinia fuckeliana* (de Bary) Whetzel), is one of the major diseases affecting grapevines (Elad and Fillinger, 2016; (Youssef *et al.,* 215). Although *B. cinerea* can adopt multiple infection strategies—acting as a saprophyte, necrotroph, or parasite on various grapevine tissues (Elad and Fillinger, 2016; Elmer and Michailides, 2007), its most detrimental impact on grape yield and quality typically occurs during the berry ripening stage (Elad and Fillinger, 2016). Environmental conditions are often conducive to *B. cinerea* development throughout the late growth phases, particularly from growth stage (GS) 81 to GS89 as defined by Lorenz *et al.* (1995). During this period, biochemical and structural changes in the berries make them especially susceptible to direct infection. Additionally, berries previously exposed at earlier stages may harbor latent infections, further increasing the risk of visible mold development as they ripening (Kosuge and Hewitt, 1964; Tyson *et al.,* 2022).

Botrytis fungus is facultative, so it contributes to cell death, causing gradual decomposition of the infected tissues. *Botrytis* overwinters in the soil as mycelium in decaying plant debris and as sclerotia, melanized mycelial survival structures (Staats *et al.,* 2005). *Botrytis* rot occurs in vineyards all over the word, but is most common in regions with cool to moderate temperatures during the pre-harvest period. (Wilcox, 2005).The following infection pathways can cause gray mold on ripening berries: (i) latent infections that develop during flowering and manifest as rotted berries; (ii) berry infection brought on by conidia generated by the mycelium colonizing the bunch trash (such as calyptras, dead stamens, aborted flowers and berries, and tendrils); (iii) direct berry infection brought on by conidia dispersed by the wind; and (iv) berry-to-berry infection brought on nearby infected berries within the cluster. (Elmer and Michailides, 2007). As the disease progresses, the skin becomes sunken and water-soaked around the infection region, with dense gray mycelial growth and conidial masses visible. Similar early-stage lesions were observed on grapevine leaves after six days of inoculation (Jayawardena *et al.,* 2018).

The genus *Botrytis* includes the well-known generalist species *B. cinerea*, which infects over 200 eudicot hosts—particularly those that are senescing, stressed, or wounded (MacFar-

lane, 1968). *Botrytis*, along with its sexual form *Botryotinia* Whetzel, comprises 22 species and one hybrid (Hennebert, 1973; Yohalem *et al.,* 2003), and belongs to the family Sclerotiniaceae Whetzel (In-operculate Discomycetes). Species identification has traditionally relied on morphological characteristics, especially macroconidium ontogeny, and species have often been named based on host association. Most *Botrytis* species have a global distribution or occur wherever their host crops are cultivated (Jarvis, 1977).The aim of this study was to survey of grape gray mold disease in some Al-Jabal Al-Khdar regions. Such as Wesita, Masa, Al-Faidia, Al-Aoilia and Batta. And identification the cause of this disease.

MATERIALS AND METHODS

The studied regions included Al-wasita, Massa, Batta, Al-aoilia and Al-faidia. During the seasons 2023, three farms were inspected from one location, five trees per farm and thirteen leaves per tree. The Infection percent was estimated, and disease density estimated by calculating spott on the leaves according to scale (Ciliberti, *et al.,* 2015), as follows: (0 = no symptoms, 1 = 0.1-5%, 2 = 5.1-15%, 3 = 15.1-30%, 4 = 30.1-45%, 5 = 4.1-65%, 6 = 65.1-85%, 7 = 85.1- 100%). The following equation was applied: Disease density = [(injury degree * Number of plant leaves) / total number of plant leaves * highest injury degree)] * 100 (McKinney, 1923; Chiang *et al.,* 2017).

The pathogen was identified based on morphological characteristics and pathogenicity tests (Jayawardena *et al.,* 2018). Diseased grapevine samples were collected and placed in separate plastic bags with sterilized tissues dipped in distilled water to maintain humidity. The pathogen is isolated from the infected leaves cut into parts of 1 cm ^{2}, and after sterilizing it with sodium hypochlorite, 10% for two minutes, then washed with sterile water three times, dried up with filtration paper and transferred to the dishes of Petri Potatoes, PDA, added to it the antibiotic streptomycin 50µg/ ML), The pure isolates obtained were cultured on potato dextrose agar (PDA) plates with sterilized filter paper pieces and incubated for 7–10 days at 20 °C. The morphology of fungal colonies was recorded following the method of Hennebert (1963), Zhang et al. (2010 a, b) and Zhou et al. (2014). The fungal were examined under the microscope to study the vegetative and reproductive structures was measured and shape, and the mycelium thickness measurements were taken as a result.

Performed on the young ,leaves surface sterile *'Alatica'* grapevine plants. A pure culture of *Botrytis cinerea* was obtained from earlier infected specimens, and a spore suspension was prepared at a concentration of 10^6 spores/ml. For inoculation, making small wounds on the leaves using sterilized needles, and droplets of the spore suspension were applied. The inoculated leaves were then placed in 9 cm petri dishes containing water agar (WA) and maintained at 20 ± 1°C. The experiment included four replicates per treatment, and disease progression was assessed 11 days post-inoculation.

Statistical analysis: Complete Randomized Design (CRD) was used in this study. The statistical analysis process was conducted using SPSS Ver. 23.0 (ANOVA analysis).

RESULTS and DISCUSSION

Figure 1 illustrates the symptoms of gray mold disease on infected leaves and grapefruit in the study region, which appear as large, irregular, brown spots located primarily near the edges of the leaf blades or along the main veins. Mold growth was also observed on grape berries, which initially softened before rotting. A grayish fungal mycelium and a dense mass of gray conidia developed on the infected fruits. These symptoms align with the findings of Wilcox (2005), Jayawardena et al.

(2018), and Zhou et al. (2014), who also identified *Botrytis cinerea* as the cause of this disease in grapevines.

Figure: (1). The symptoms of gray mold disease on the leaves and grape fruit.

Based on Table 1, which shows the climatic conditions of the study regions, the highest recorded temperature was 40.54C° in June in the Al-Wesita zone. The lowest temperature was in May in both Massa and Al-Faidia. Relative humidity ranged between 58.17% and 79.15% during the study period. Al-Wesita recorded the highest humidity, and June had the highest percentages comparison all regions.

The results of the study, shown in Table 2, indicated a high incidence of gray mold disease in May in the Aoilia and Al-Wesita regions (54.0% and 46.0%, respectively). The lowest incidence was recorded in August and September in the Batta and Massa regions (2.66% and 6.44%, respectively). This decrease was even more pronounced in September (2.22% and 0.22%) for both regions, respectively.

Based on Table 3, May shown the highest density of gray mold disease on the studied grapevines. In contrast, August and September had the lowest density in all regions except for Al-Faidia and Al-Aoilia, which showed an increase in density throughout the study months. In these two regions, reached 7.4% and 8.6%, respectively, in September. Overall, the highest infection densities were recorded in Al-Aoilia and Al-Wesita at 13.7% and 11.5%, respectively, while the lowest densities were observed in the Massa and Batta regions throughout all the study months. The results showed that grape trees in all study regions were infected with gray mold disease. This may be due to the difference in weather conditions between May and September, where the disease was suitable moderate and humid weather, as confirmed by (Wilcox, 2005).

Table:(1). Climatic condition not illustrated in materials and methods in the study zones during May to September 2023.

Months	Temperature (C)		
	Zone 1	Zone 2	Zone 3
May	28.47	30.75	31.69
June	31.41	33.44	33.63
July	32.96	40.54	39.79
August	33.75	38.43	37.44
September	31.43	33.65	33.35
	Relative Humidity (%)		
May	66.16	72.95	65.67
June	69.89	79.15	72.64
July	59.11	76.91	65.21
August	58.17	71.66	61.75
September	74.21	73.45	73.81

Zone 1: Masa and Al-Faidia
Zone 2:: Al-Wesita
Zone 3: Al-Aoilia and Batta
https://power.larc.nasa.gov/data-access-viewer

Table:(2). The infection rate of gray mold disease in study regions during the 2023.

Months	Al-Jabal Al-Khdar regions				
	Al-Wesita	Masa	Al-Faidia	Al-Aoilia	Batta
May	46.0 ab	17.3 defgh	34.7 bc	54.7 a	18.7 cdefgh
June	24.7 cdef	18.0 cdefgh	33.6 bcd	28.6 cde	22.6 cdefg
July	14.9 efghi	9.4 fghi	20.3 cdefg	25.0 cdef	12.7 efghi
August	8.4 fghi	6.4 ghi	11.8 efghi	14.4 efghi	2.7 hi
September	13.1 efghi	0.22 i	23.8 cdef	23.8 cdef	2.2 hi
Average	21.43	10.28	24.81	29.16	11.36
Min	46.00	17.97	34.67	54.00	22.56
Max	13.11	0.22	11.77	14.44	2.22

Table:(3). The density of gray mold disease infection in the study regions during the 2023.

Months	Al-jabal Al-khdar regions				
	Al-Wesita	Masa	Al-Faidia	Al-Aoilia	Batta
May	11.5 ab	4.3 cdefgh	8.7 bc	13.7 a	4.7 cdefgh
June	6.3 cdefg	4.5 cdefgh	8.4 bcde	7.1 bcdef	5.7 cdefg
July	4.2 cdefgh	2.6 fgh	6.4 cdefg	7.2 bcdef	3.6 efgh
August	2.7 fgh	1.7 gh	3.66efgh	3.9 cdefgh	0.7 h
September	3.7 defgh	0.1 h	8.6 bcd	7.4 bcdef	0.6 h
Average	5.61	2.26	7.12	7.83	3.03
Min	2.67	0.06	3.55	3.89	0.56
Max	11.50	4.33	8.67	13.67	5.66

Figure 3 described the structures of the fungus isolated from grape leaves after being purified on a culture medium PDA. (Figure 2a) shown a dark gray fungal colony. Sclerotia are hardened, black survival structures that developed under specific conditions, enabling the fungus to endure adverse environmental situations. while Figure 2b shows mycelium initially hyaline (clear) (figure 2b), becoming grey with age, and typically branched, septate (divided by cross-walls) (figure 2c,d), and branched, bearing clusters of conidia at their tips. These branching patterns are often described as tree-like. Conidia: that is typically oval or round, single-celled, and greyish in mass, giving the characteristic "grey mold" appearance. the morphological characteristics, on the PDA medium showed a cottony growth with a white color that quickly turned gray, as mentioned by (Khazaeli *et al.,* 2010). Upon examination with a light microscope of the morphological structures, the results showed that mycelium was branched and gray in color. The figure shows that the fungus has the ability to infect the leaves and cause disease after injecting them, as the symptoms of the disease appear as shown in Figure (3).

Based on Table 4, the fungus *B. cinerea* is classified according to its morphological characteristics. A noticeable variation was observed in mycelial thickness. The thickness of the young mycelium was between 3-7.5 µm with an average of (4.55 µm), while the thickness of the old mycelium ranged between 5-12.5 µm with an average of (9 µm. The conidiophores were straight or curved and branched at the top in a dendritic shape and their length ranged between 15-25 µm with an average of (18.77 µm). The conidiospores were spherical to oval, gray in shape, and their length ranged between 7.5-12.5 µm with an average of (11.25 µm) and their width ranged between 5-7.5 µm with an average of (5.5 µm). These findings are consistent with those reported by Elad (2007) and Khazaeli *et al.* (2010). The morphological characteristics of the fungus isolated from gray mold-infected grape leaves corresponded closely with those of *Botrytis cinerea*, as described by Hennebert (1973) and Jarvis (1977), (1980) and Farr et al (1989) and Muñoz (2016).

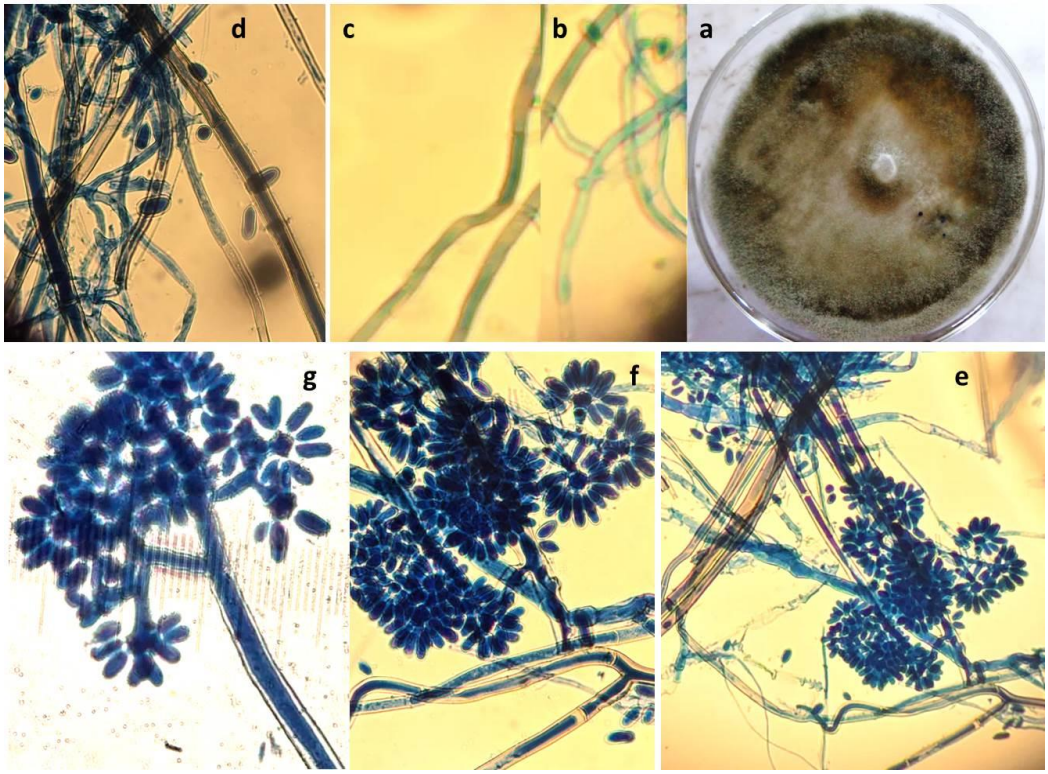

Figure: (2). Description of the fungal. [a: colony, along with the vegetative and reproductive structures of *Botrytis cinerea*, b: young mycelium observed under a light microscope at (20x). c-d: old mycelium observed under a light microscope at (20x). e-g: reproductive structures of *Botrytis cinerea* e: (10x). f: (20x). g: (40x).]

Figure: (3). The pathogenicity of *B. cinerea* on fresh grape leaves. [a: Leaves not injected with fungus b: Leaves injected with fungus.]

Table:(4). Characteristics of the fungus *Botrytis cinerea*

Morphological characteristics		Measurements (µm)
Young mycelium	Thick	3-7.5 (4.55*)
Mature mycelium		5-12.5 (9)
Conidiophore		15-25 (18.77)
Conidiospore	Length	7.5-12.5 (11.25)
	Width	5-7.5 (5.5)

(*) average

The study successfully isolated and identified the fungus *Botrytis cinerea* from grapevine leaves, confirming the presence of gray mold disease in all of the regions surveyed. The researchers attributed the variations in the incidence and density of the disease to differences in climatic conditions and the susceptibility of the grape cultivars to the causal fungus. The fungus was identified based on its morphological characteristics after being isolated from infected leaves. Additionally, it was proven to be capable of causing infection on new leaves of the *'Alatica'* cultivar.

B. cinerea has been identified as the world's second most significant plant pathogenic fungal species (Dean *et al*., 2012). The primary source of grapevine infection in the spring is the conidia—spores produced in late winter and early spring from overwintering fungal mycelium on host tissues (Elmer & Michailides, 2004). Under humid conditions, gray mold infection leads to serious losses in the yield and quality of numerous crops (Vail *et al*., 1998; Leroch *et al*., 2012, González-Domínguez *et al*., 2015).

Rising Precent and density of the disease are attributed to the quantity of antifungal compounds, such as phenolic substances. The 'Alatica' cultivar showed high susceptibility to *B. cinerea* infection. According to a study by (Mohamed *et al*., 2007) an increase in disease to a decrease in the thickness of the leaf's cuticle or changes in the antifungal chemical compounds present in grape leaves, such as phytoalexins, which increase in response to *B. cinerea* infection.

CONCLUSION

The results indicated that through the symptoms and morphological characteristics of the fungus and the plant host, the fungus causing the disease is *Botrytis cinerea*, and that the fungus is a major causative agent on grapes and it causes significant economic losses to leaves, flowers and fruits in both the field and in storage, particularly under conditions of high humidity combined with low to moderate temperature.

RECOMMENDATIONS

Based on the results of this study, the following recommendations are proposed to manage and limit the impact of gray mold disease on grapes in Al-Jabal Al-Akhdar region: Integrated Disease Management (IDM), Apply preventive fungicides during critical growth stages, especially in when environmental conditions are favorable for *B. cinerea*.Adopt modern molecular diagnostic tools such as PCR or qPCR for detection this fungus.

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