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RESEARCH ARTICLE

Pathogenicity and chemical control of *Alternaria* sp. on date palm (*Phoenix dactylifera* L.)

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ABSTRACT

Recently, a wide range of symptoms including light yellow lesions gradually turning into brown stripes were noticed on date palm leaves in Iraq. In this context, the aim of this study were to isolate the phytopathogens associated with these symptoms, evaluate their pathogenicity and assess the efficacy of two fungicides (Score and Pentanol) under *in vitro* and *in vivo* conditions. Two fungal species (*Alternaria* sp. and *Fusarium* sp.) were isolated from the symptomatic leaves of date palm. The results of pathogenicity tested proved the ability of *Alternaria* sp. inoculated separately or in combination with *Fusarium* sp. to infect the leaves of date palm trees with disease severity index (DSI) values of 67.33% and 65.99%, respectively. The effect of Score (88.76%) and Pentanol (82.91%) against *Alternaria* sp. was examined by poisoned food technique, which leads a significant increase in mycelial growth inhibition (for 300% of commercial recommended dose of fungicide). Test results indicate that prophylactic spraying of date palm leaves with Score or Pentanol effectively controlled *Alternaria* sp. with DSI values of 22.65% and 17.87%, respectively. To control *Alternaria* sp. in field within integrated pest management strategies, chemical control using Score or Pentanol should be taken in consideration.

Introduction

Phoenix dactylifera L. (Arecaceae) is thought to be the oldest fruit tree grown in Iraq and is an important crop in term of the number of trees and their distribution. Date palm is widely considered as a strategic source of food security and an essential crop in the Iraqi economy (1). Date palm represents a source of income to many farmers in large parts of Iraq (2-4). Date palm production in Iraq was reported to be around 250 thousand tons, with an average of 1666.67 kg/ha in 2017 (Iraqi ministry of agriculture).

The date palm is being affected by several pathogenic fungi amongst them are *Alternaria* sp., *Fusarium* sp., *Phytophthora* sp., *Diplodia* sp., *Mauginiella* sp., *Mycosphaerella* sp., *Thielaviopsis* sp., *Glomerella* sp., *Phoma* sp., *Graphiola* sp., *Nigrospora* sp., *Chalara* sp., *Chaetosphaeria* sp., *Phomopsis* sp. etc. These pathogens caused severe damages to different date palm parts such as stem, leaves, fruit and root leading to severe losses and reduction in total yield (5). Worldwide annual economic yield lose due to these pathogens have been estimated more at 50% in 2002 (6, 7).

Among these diseases are the bayoud, the most dangerous of which affects the date palm, especially in Morocco, Algeria and Mauritania and is caused by *Fusarium oxysporum* f. sp. *albedinis*, inflorescence rot caused by *Thielaviopsis paradoxa* or *Mauginiella scaetae*, leaf spot caused by *Mycosphaerella tassiana* and *Alternaria* spp., Khamedj or date palm inflorescences rot caused by *Fusarium moniliforme* or *T. paradoxa*, black scorch caused by *T. paradoxa*, leaf

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blight caused by *Glomerella cingulata*, off-shoot decline caused by *Chalara paradoxa*, belaat disease caused by *Phytophthora* sp., apical drying of palm leaves caused by *Alternaria* sp. and *Phoma* sp. and leaf spot and yellowing diseases (8).

Leaf spot and yellowing diseases in date palms received less attention from researchers. There are several fungi capable of causing this disease, including *Graphiola phoenicis* (9), *Nigrospora sphaerica* (10), *Alternaria* spp. (11), *Fusarium solani* (12). However, most researchers agree that the causes of leaf spots and yellowing diseases in date palms are the genus *Alternaria* (7, 13-17).

Alternaria spp. is a genus of ascomycete fungi, belongs of *Eumycotera* subkingdom, form *Dematiaceae* family, Moniliales order, and Hypomycetes class. Some of the species *Alternaria* are the asexual anamorph of *Pleospora* ascomycete while others are thought to be anamorphs of *Leptosphaeria* (18).

One of the best alternatives to control plant pathogens is by using genetically resistant varieties and chemical treatments (17).

Score and Pentanol fungicides are widely used in agriculture in different parts of the world. Score is a systemic Triazole fungicide for long-lasting preventive and strong curative action against powdery mildew, many date palm diseases and rust disease in fruit trees (19). Score presented high efficacy against date palm diseases when tested under *in vitro* and *in vivo* conditions (6-7, 17). Pentanol is an active organic compound produced by plants and is a component of insect sex pheromones emitted (20). In *Arabidopsis*, it was reported that 3-Pentanol can trigger plant systemic resistance against *Pseudomonas syringae* pv. *tomato* (6), making possible that Pentanol has an effect on many date palm disease.

Recently, a wide range of symptoms were remarked on date palm in Iraq which are light yellow lesions on peduncles and gradually developing to longitudinal pale brown stripes on the whole peduncle. Date fruits wilt usually from the bottom of the strand up and then the pedicel, peduncle and whole bunch wilt dry. The objectives of this investigation were to: (i) isolate the pathogens associated with these symptoms, (i) evaluate the pathogenicity of the isolated phytopathogens and (iii) determine the efficacy of two fungicides widely used by Iraqi farmers (Score and Pentanol) under *in vitro* and *in vivo* conditions.

Materials and Methods

Phytopathogens isolation from date palm

The sampling was done to collect diseased leaves of date palm (cv. Barhi) showing typical symptoms of spotting and yellowing from palm grove located in Basra, Iraq. Infected leaves were cut in small pieces (0.5 - 1 cm) and sterilized by soaking into 3% sodium hypochlorite (NaOCl) for 2 min and washed with sterilized distilled water 3 times. The samples were dried and inserted on the surface of Petri dishes (9 cm in diameter) containing Malt Extract Agar (MEA) medium amended with streptomycin (60 µg/ml). In each Petri dish, seven fragments were placed (with total of 30 Petri dishes). The plates were incubated in the dark at 25±2 °C for 5-7 days and then examined for fungal growth (21, 22). The fungal genus identification is carried by observing the macroscopic (growth, color, colony) and microscopic examination (mycelium, conidiophore, conidia, resistance structures, sexual form) (23).

Pathogenicity assay

The experiment was carried out in the greenhouse on date palm cv. Barhi by artificial inoculation. The seedlings were placed in a pot containing a mixture of peat moss and vermiculite (1:1). Both compounds were autoclaved twice at 120 °C.

For *Alternaria* sp., the assay was carried out by spraying the leaves of each seedling (4 months old) by 5 ml of conidial suspension (10^5 conidia per ml) for each palm trees leaf of *Alternaria* sp. (4 days old).

The pathogenicity of *Fusarium* sp. was established according to the standard method (24). Roots of each palm trees seedling (4 months old) were inoculated with 10 ml of spore suspension of *Fusarium* sp. (10^6 spores/ml).

The isolated phytopathogens were inoculated separately and in combination. The plants were covered with a transparent plastic to ensure high humidity of 70-90% during 3 days after inoculation to ensure the infection occurred.

Only one control was performed; by inoculating the date palm seedlings with sterilized distilled water (negative control). The pots were then placed in a greenhouse for two weeks with nine date palms seedlings per treatment and per replicate. The entire experiment was repeated three times (21).

The evaluation parameters were measured within four week following inoculation. Area of observed symptoms were scored for disease index using a scale from 0 to 4; 0 = no spots; 1 = number of spots covering leaf about 1-3 spots, with yellowing of 1-25% of the leaf area; 2 = number of spots covering leaf about 4-6 spots, with yellowing of 26-50% of the leaf area; 3 = number of spots covering leaf about 7-9 spots, with yellowing of 51-75% of the leaf area; 4 = spots covering the totality of leaf, with yellowing of 75-100% of the leaf area. The severity data were processed by McKinney's formula, which generates a numeric disease severity index (DSI): DSI (%) = $(\Sigma vn)/(NV)\times100$, where v represents the numeric value of the disease index scale, n is the number of plants assigned to the disease index scale, N is the total number of the plants and V is the numeric value of the highest disease index scale. DSI was calculated from five leaves of each date palm seedlings (21).

In vitro evaluation of fungicides against the isolated phytopathogens

Only the most virulent phytopathogen (*Alternaria* sp.) was used for the *in vitro* and *in vivo* evaluation of fungicides.

Relative efficacy of fungicides on mycelial growth inhibition of the isolated phytopathogens was studied *in vitro*, using poisoned food technique (25). In this experiment, two fungicides (Score (1 ml/l) and Pentanol (3 ml/l)) were used for their efficacy (26, 27). The fungicide suspension was made by adding required quantity of fungicides to the molten potato dextrose agar (PDA) medium to obtain the desired concentration (three concentrations; 100% of commercial recommended dose, 200% of commercial recommended dose, and 300% of commercial recommended dose) on the basis of active ingredient present in the chemical (25). Fourteen milliliter of amended medium was poured into each sterilized petriplates and suitable checks were maintained. One mycelial disc plug (0.5 cm diameter) of pathogen (4 days-old culture) was placed in the center of the plate. A plug of pathogen was used as control treatment (without treatment). Three replicates for each individual treatment were conducted and the plates were incubated at 25±2 °C receiving fluorescent light with 12 hr cycling for 7 days. The percent of pathogen radial mycelial growth inhibition (I) was evaluated according to this formula: I (%) = (1- Cn/C0) x 100; where: Cn is the radial growth diameter of the pathogen in the presence of the treatment. C0 is the growth diameter of the pathogen in the control treatment (21).

Greenhouse evaluation of fungicides against the isolated phytopathogens

Date palm seedlings (cv. Barhi) were placed in a pot containing a mixture of peat and vermiculite (1:1); at the rate of one seedling in each pot (180 pots for each treatment and each replicate (Three replicates)). The entire experiment was repeated twice. Treatment application and inoculation were occurred after four months of the date palm growing. This experiment was concerned only with preventive treatments. Each fungicide (only at the recommended dose) was applied separately with 5 date palm seedlings/treatment (4 treatments: T1: Score; T2: Pentanol; T3: Positive control and T4: Negative control). The treatment application was carried out by spraying the date palm leaves 3 days before the inoculation of the conidial suspension of the pathogen. All seedlings were inoculated at the same time with a spore suspension of 10^6 CFU\ml of the isolated phytopathogens obtained from 7-day-old cultures grown on PDA medium. Fungicides were applied to date palm using a hand-held sprayer at a volume of 40 ml per seedling. Immediately after inoculation plants were enclosed in plastic bags for 24 hr to optimize conditions suitable for infection. The pots were then placed in a greenhouse for two weeks. Assessments were conducted 15 days after inoculation by measuring disease severity index. DSI was calculated from five leaves of each date palm seedlings (21, 27).

Statistical analysis

The data were analyzed by ANOVA using SPSS version 20.0 statistical software (SPSS, SAS Institute, USA), to evaluate parameter values differences. Differences between treatments were determined by least significant difference (LSD) test at 5% of significance level.

Results and Discussion

Phytopathogens isolation from date palm

We successfully isolated two phytopathogens species (*Alternaria* sp. and *Fusarium* sp.) based on their visual morphological characterizations from the diseased leaves of date palm cultivated in Basra, Iraq (Fig. 1). The isolation frequencies of these phytopathogens species were showed that the higher was registered for *Alternaria* sp. (89%), followed by *Fusarium* sp. (9%), *Trichoderma* spp. (1.7%), *Aspergillus* sp. (0.2%) and *Penicillium* sp. (0.1%). Several research works from different date palm growing areas of the world reported various fungal pathogens (*Fusarium* sp. and *Alternaria* sp.) associated with the symptoms we detected (14, 16, 28-30).

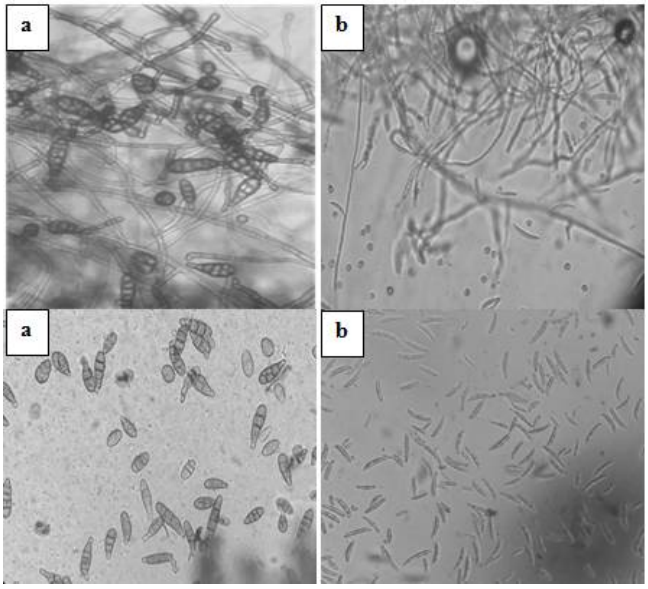

Fig. 1. The isolated phytopathogens on date palm. a) *Alternaria* sp.; b) *Fusarium* sp.

Pathogenicity assay

The results of pathogenicity test of *Alternaria* sp. and *Fusarium* sp. exhibited high degree of pathogenicity according to the measured DSI. Statistical analysis revealed a significant difference of DSI ($P<0.01$). Variations in pathogenicity level were indicated between the isolated phytopathogens. *Alternaria* sp. inoculated separately or in combination with *Fusarium* sp. was highly virulent to date palm leaf with DSI values 67.33 and 65.99%, respectively. It was pathogenic 14 days after inoculation (Table 1). Symptoms initially appeared on the leaves in the form of small yellow spots in the form of concentric circles then turned brown surrounded by halo furs and the progress of the injury takes spots broad (Fig. 2). Present results are in analogy with (31-34) which reported the pathogenicity of *Alternaria* sp. (DSI = 70%) and *Fusarium* sp. (DSI = 50%) on date palm. The isolated species of *Fusarium* sp., *Alternaria* sp., *Mycosphaerella* sp., *Diplodia* sp., *Phomopsis* sp.,

Chaetosphaeropsis sp., *Botryodiplodia* sp., *Omphalia* sp. and *Phoma* sp. were recorded as fungal pathogens of date palm trees on the basis of pathogenicity examinations (15, 34). The varieties used in the discussion part are moderately resistant to *Fusarium* sp. and *Alternaria* sp.

Table 1. Pathogenicity of the isolated phytopathogens

Treatments	Disease severity index (%)
Negative control	0±0
Alternaria sp.	67.33±0.77
Fusarium sp.	55.89±0.52
Alternaria sp. + *Fusarium* sp.	65.99±0.96
$LSD_{0.05}$	<0.01

Data are the average of 3 date palm seedlings per treatment and per replicate (with 3 replicates).
Disease severity index was calculated from five leaves of each date palm seedlings.

Statistically, significant increase in mycelial growth inhibition was observed with an increase in concentration of commercial recommended dose. The highest inhibition percent was registered at 300% of commercial recommended dose and obtained data ranging from 82.91% (Pentanol) to 88.76% (Score). However, the lowest inhibition was recorded at 100% of commercial recommended dose. It can be concluded that Score seemed to be the most effective treatment with inhibition rate above 77.79% under *in vitro* conditions (Table 2). These findings are in concordance with those of (13) showed that Score, Cuprosate, Mizeb and Carbendazim provided highly significant mycelial growth inhibition with 90%. In another study, there are reports stating that score is capable of decreasing the mycelial growth and colonization of *Alternaria* sp. and inhibited their

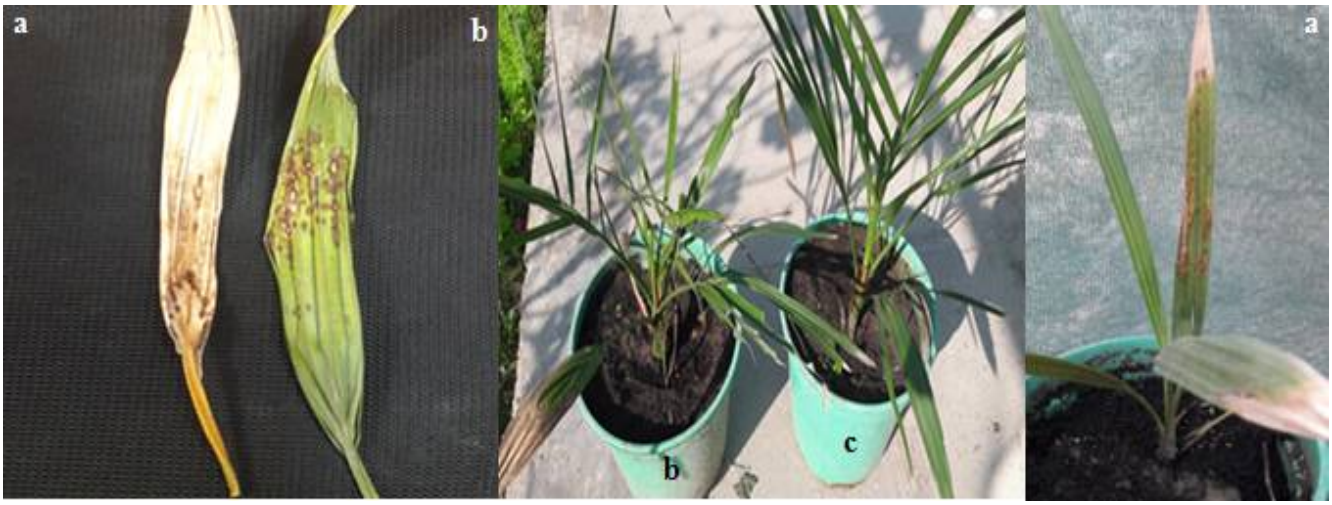

Fig. 2. Symptoms of artificial inoculation of *Alternaria* sp. applied separately **(a)** or in combination with *Fusarium* sp. **(b)** comparing with the negative control **(c)** on date palm.

Table 2. Effect of two fungicides on mycelial growth inhibition of *Alternaria* sp. after 7 days of incubation at 25±2 °C

Treatments	Concentrations		
	100% of commercial recommended dose	200% of commercial recommended dose	300% of commercial recommended dose
Score	77.79±0.37	79.81±0.28	88.76±0.75
Pentanol	68.96±0.85	71.03±0.90	82.91±0.66
$LSD_{0.05}$	For concentrations = 2.13		For pesticides = 1.74
	Interaction = 1.01		

Data are the average of 3 Petri dishes per treatment per replicate (with 3 replicates).

Alternaria spp. proved to be pathogenic on date palm leaves. This agrees with previous studies that indicated the prevalence of this genus and its pathogenicity on date palm leaves (16, 30).

Only the most virulent phytopathogen (*Alternaria* sp.) was used for the *in vitro* and *in vivo* evaluation of fungicides.

In vitro evaluation of fungicides against the isolated phytopathogens

Data presented in Table 2 demonstrated clearly that the two fungicides exerted high significant reduction on radial mycelial growth of *Alternaria* sp. after 7 days of incubation using poison food technique. sporulation and spore germination (25, 34). The active ingredient of both fungicides has the potential to limit the growth of a wide range of pathogens (21, 22).

Greenhouse evaluation of fungicides against the isolated phytopathogens

The disease severity index showed that the treatments fungicides differed significantly (P<0.01) (Table 3). Foliar treatment of date palm seedlings with Pentanol in the presence of *Alternaria* sp. reduced significantly the DSI with 17.87%, followed by treatment with Score (22.65%) (Table 3). It was demonstrated that foliar treatment with difenoconazole decreased the disease incidence and severity (25). Similar results were obtained with Swift (Carbendazim 50%) reporting that the disease incidence was ranged from 23.4% to 6.3% (North of Basra) and from 6.8% to 1.9% (South of Basra) after date palm fungicide treatment with Swift. The disease severity was 18% (North of Basra) and 12.7% (South of Basra) and reduced significantly up to 4.7% and 4.3% (12). There are several reports demonstrated that Score, Pentanol, Benlate, Bayleton, Tilt, Ortiva, Naturame, Phyton-27, Revus Top and Dazim have been used to control different fungal pathogens on date palm under *in vivo* conditions (13, 23, 36-39).

Table 3. Disease severity index (DSI) recorded on date palm leaf inoculated with *Alternaria* sp. (10^5 conidia per mL) and treated separately by two fungicides

Treatments	Disease severity index (%)
Negative control	0±0
Positive control	60.83±1.05
Score (1 mL/L)	22.65± 0.86
Pentanol (3 mL/L)	17.87± 0.49
$LSD_{0.05}$	<0.01

Data are the average of 5 date palm seedlings per treatment per replicate (with 3 replicates).

Disease severity index (DSI) was calculated from five leaves of each date palm seedlings.

DSI (%) = (Σvn)/(NV)×100, where v represents the numeric value of the disease index scale, n is the number of plants assigned to the disease index scale, N is the total number of the plants and V is the numeric value of the highest disease index scale. DSI was calculated from five leaves of each date palm seedlings.

There are reports stating that the Difenoconazole and Pentanol are capable of decreasing of the mycelial growth and colonization of *Alternaria solani* and inhibited their sporulation and spore germination (25, 39, 40). The high concentration of fungicides had a good inhibitory effect on the growth of plant pathogen fungi and reduced disease severity. The mycelial growth inhibition and the disease severity reduction were significantly higher with the high concentrations of fungicides (41-43). The appressorium is an important fungal structure during the penetration process; therefore, this fungicides show a spore germination inhibition and appressorium formation blocking (39, 40). Studies also revealed that the active ingredient could be responsible for their higher antifungal activity against fungal plant pathogen (39-44).

Conclusion

Alternaria sp. and *Fusarium* sp. have caused leaf spot and yellowing diseases with isolation frequencies of 89% and 9%, respectively. The results of pathogenicity test proved the ability of *Alternaria* sp. to infect date palm leaves. The chemical fungicide Pentanol at the recommended rate was the most effective in controlling *Alternaria* sp. under *in vivo* conditions which decreased the disease severity on date palm. Based on the current results, it is deduced that tested Pentanol could be employed in foliar treatments to may induce date palm systemic resistance, through a specific signal transduction cascade. The systemic resistance induction of date palm by Pentanol against *Alternaria* sp. is a subject of future research. The fungicides used have wide-ranging effects, and it seems likely that the treatment with fungicides killed both pathogenic fungi as well as beneficial micro organism (mycorrhiza, antagonitic etc). For this reason, we must appeal to integrated pest management strategies on integrating different approaches (chemical control, biological control, prophylactic technique, genetic control etc), while reducing the use of fungicides.

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Authors' contributions

All authors contributed equally to this work.

Conflict of interests

The authors declare that they have no conflict of interest. This article does not contain any studies with human participants or animals performed by any of the authors. Informed consent was obtained from all individual participants included in the study. All authors have approved the manuscript for submission.

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