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Biological, Chemical and Electrolyzed Water Methods for Controlling some Date Palm Diseases in Egypt

Moustafa Sayed Mansour[1], Magdy Mohamed Saber[1], Reda Ali Moussa [2*], Khamis Youssef [3]

[1]*Plant Pathology Department, Faculty of Agriculture, Cairo University, Egypt*

[2]*MSc Student of Plant Pathology, Faculty of Agriculture, Cairo University, Egypt*

[3]*Plant Pathology Research Institute, Agricultural Research Center, Giza, 12619 Egypt*

Abstract

A survey of some fungal diseases on date palm trees recorded the highest disease incidence (DI %) at Giza, New Valley, Aswan, El Beheira and El Sharkia governorates. *Alternaria alternata* recorded the most frequency (67.38%) followed by *Nigrospora oryzae* (9.93%), *Lasiodiplodia theobromae* (9.22%) and *Fusarium solani* (7.80%) respectively; meanwhile, the lowest percentage of frequency was recorded for *Fusarium oxysporum* (5.67%). *L. theobromae* was the main pathogenic fungus (100%) colonizing the treated detached leaves. *Trichoderma harzianum* I1 and I2 effectively inhibited the hyphal growth of the aforementioned tested fungi. The highest percentage (76.85%) of inhibition zone was recorded for *T. harzianum* I1 on *F. oxysporum* followed by *T. harzianum* I2 (70.56 %) on *A. alternata*. On the other hand, *Bacillus* spp. showed an inhibitory effect on all tested fungi. The highest inhibition of *Bacillus* spp. (42.2%) was recorded on *A. alternata* while the lowest inhibition (16.7%) was recorded on *F. solani*. For electrolyzed water tests, acidic EW gave the highest effect on all tested fungi compared with alkaline EW and NaCl without EW, which showed the highest effect on *A. alternata* with (12.67mm) followed by *F. oxysporum*, *F. solani* and *N. oryzae* with (14.67, 25.33, and 26 mm) respectively. Chemical control Tebuzyn 25% EW, Sevron 50% WDG and Sim-mildew 72% WP reduced the growth of the tested fungi. The findings contribute to a better understanding of effective control measures for some date palm diseases.

Keywords: Date palm trees; fungal diseases; Pathogenicity; Management

1. Introduction

Date palm (*Phoenix dactylifera* L.) is one of the most important crops in many regions around the world. Cultivars of date palm grown in Egypt differ in distribution and frequency in all growing regions. Each date palm cultivar is specific and characterized by a region [1]. The Egyptian dates production during 2019 is estimated to be about 1603762 tons and harvested area is nearly 48031 ha with a yield 333901 Kg/ha, where production of the dates in Egypt represents about 17.67% of the total world date production [2]. There are many diseases affecting the production of date palms. Fungi and phytoplasma diseases are the most common diseases affecting date palm trees [3]. In producing countries, several fungal diseases were reported to infect date palm trees *i.e.* Bayoud disease caused by *Fusarium oxysporum* f.sp. *albedinis*; Black scorch disease caused by *Ceratocystis paradoxa* (Hohn); Brown leaf spot caused by *Mycosphaerella tassiana* (De Not) Johns; Diplodia disease caused by *Diplodia phoenicum* (Sacc); Graphiola leaf spot caused by *Graphiola phoenicis* (Moug) Poit; Khamedj disease or inflorescence rot caused by *Mauginiella scaettae* Cav. [4]. Many researchers *i.e.* reported that bio-agents *Bacillus megaterium* and *B. subtilis* gave the highest inhibition percentage of *F. oxysporum* (68.49% and 55.26%), *F. solani* (77.36 and 70.14%) and *F. moniliforme* (52.08%) growth on different date palm offshoots (cv. Saidy).In general, Bacillus spp. more than *T. harzianum* inhibition of redial growth of all the tested fungi and the most significant

*Corresponding author e-mail: dr_redaali@yahoo.com.; (REDA ALI MOUSSA).

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reduction occurred in *F. solani* followed by *F. oxysporum*, and *F. moniliforme* was less affected *in vitro* conditions. On the other hand, *B. megaterium* and *T. viride* gave the highest reduction against *Fusarium* spp, while *B. cereus* and *T. harzianum* presented the lowest reduction against pathogens under field conditions in nurseries and new orchards in New Valley Governorate at Egypt [5].[6] mentioned that *T. harzianum* inhibited the growth of *A. radicina* inciting leaf black spot disease on date palm. [7] reported that Bavistin showed the highest inhibition zone to *A. radicina* compared with Tachigazole 100 mg/L concentration gave the high reduction for fungal radial growth, while 5 mg/L showed the lowest reduction to all isolates. [8] mentioned that in vitro studies, mycelial growths of *F. oxysporum, F. proliferatum,* and *F. solani* were increased for all isolates at 250 mg/L Baiclean®. Recently, several studies have been performed to control plant disease using electrolyzed water. [9] mentioned that using neutral electrolyzed water via overhead irrigation gave the most suppression to *Colletotrichum fructicola* either alone or combined with fungicides.

The main objectives of the present study were (i) Occurrence of date palm off-shoot decline, leaf spots and basal leaf rot at different Giza, New Valley, Aswan, El Behera and El Sharkia governorates, (ii) Isolation of the causal organisms and their pathogenicity tests under laboratory conditions (iii) Control methods including biological, electrolyzed water and chemical fungicides against the pathogenic fungi.

2. Material and Methods

2.1. Survey of date palm tree diseases at five governorates in Egypt

Samples of date palm leaves showing natural infection during seasons (2018 -2020) were collected at five Governorates *i.e.* El Giza, El Behera, El Sharkia, Aswan and New Valley. The percentage of disease incidence (D.I. %) was calculated at each Governorate according to [10].

Disease incidence (%) = Number of diseased plant / Total number of plant tested X100

2.2. Isolation and identification of the causal fungi

Isolation was carried out from infected leaves and leaflets. Leaf-cutting pieces (1cm long) were washed using tap water to remove adhering soil residues and any other particles, surface sterilized in 2% sodium hypochlorite for two minutes, rinsed three times in sterilized distilled water, put on folds of sterilized filter papers, transferred to Petri dishes containing potato dextrose agar (PDA) medium (4 pieces per Petri dish). PDA plates (9-cm-diam.) were then incubated at 25±2ºC for 5-7 days. A hyphal tip for each isolate was moved into Petri dishes containing potato dextrose agar (PDA) medium. All purified fungi were kept in potato dextrose agar slant in a refrigerator at 5ºC until further use according to [11]. The total number of fungal colonies and the frequency percentage of each fungus were separately counted according to [12]. Identification of the purified fungal pathogens was carried out depending on their morphological and cultural characteristics according to [13], and then the isolates were kindly confirmed at Plant Pathology Research Institute, Agricultural Research Center at Giza.

2.3. Pathogenicity tests

Pathogenicity tests were carried out *in vitro* on healthy detached leaves of date palm trees (Zaghlol Variety). Leaflets of palm were removed from the middle part of the date palm trees and cut into pieces (4 cm long); surface-sterilized in sodium hypochlorite 70% for 2 minutes then rinsed in sterilized distilled water for 2 minutes; dried between two sterilized filter papers and moved to Petri dishes (9 cm in diam.) containing 4 leaflets / Petri dish. Fungal discs (5 mm in diam.) of a 7-days-old culture of 5 fungi *i.e. A. alternata, N. oryzae, L. theobromae, F. oxysporum* and *F. solani* isolated from date palm leaves and rachis taken from the edges of colonies (7-days-old) and placed upon the terminal of each leaflet (one disc a leaflet). Petri dishes (3 replicates/treatment) were warped by stretch and incubated at 25 ± 2 °C for four weeks. Fungi free-plates were used as control. After four weeks, the fungal colonizing capability was established as follows: A = 0% no colonization, B = 25% colonization, C = 50% colonization, D = 75% colonization, E = 100% colonization according to [14]. The average colonization capability for each treatment was calculated by the percentages of colonization of four leaflet cuttings.

2.4. Biological control

Two bio agents *i.e. Bacillus* spp. and *Trichoderma harzianum* against five fungi *i.e. L. theobromae, F. oxysporum, F. solani, N. oryzae* and *A. alternata*,

isolated from leaves of the date palm trees were tested. Discs (5 mm in diam.) were taken from the edge of the cultures 7 days old of the aforementioned fungi and inoculated separately on one side of plates. *Bacillus* spp. (48-hr-old) was streaked 2 – 3 cm away from the fungal disc and incubated at 25ºC. Bacillus–free plates were used as a control. Two isolates of *Trichoderma harzianum* I_1 and I_2 were studied *in vitro*. Discs (5 mm in diam.) of the aforementioned fungi (7 days old) were individually placed on one side of PDA plate and a disc (5 mm in diam.) of *T. harzianum* was placed on the other side of the same dish. Plates inoculated only with the pathogenic fungi were used as a control. Three plates were used for each treatment. All Petri dishes were incubated at 25±2 ºC until plates of one treatment were full of fungal growth. The percentage of inhibition zone was calculated as formula dual cultured by [15].

2.5. Chemical Control

In this trial, these fungicides *i.e.* Tebuzyn 25% EW, Sevron 50% WDG and Sim-mildew 72% WP Table (1) were tested *in vitro* for their efficacy against five fungi *i.e. L. theobromae, F. oxysporum, F. solani, N. oryzae* and *A. alternata*. Tested fungicides were prepared in distilled sterilizing water and then added to the melted sterilizing PDA culture medium at 45 ºC to obtain the dishes concentrations *i.e.* 0, 10,100, 500, and 1000 mg/L. Then inoculation with aforementioned fungi. Fungicide-free medium was used as a control treatment. Three replicates were used for each treatment. Colony growth inhibition (%) was calculated according to [16] as the following formula: $P = (C - T) / C \times 100$

Where P = mycelia growth inhibition.

C = colony diam. in control.

T = tested colony diam. in treatment

2.6. Electrolyzed water:

2.6.1. Preparation of alEW and acEW

Sodium chloride (NaCl, Adwic) was used in this study as a stock solution (10%) to prepare electrolyzed water (EW). Two kinds of EW alkaline electrolyzed water (alEW) and acidic electrolyzed water (acEW) were produced by ROX-10 WBE (Hoshizaki Electric Co., LTD.), using water flow rate (high=6 L min^{-1}) and current intensity (13A). Water supply pressure was maintained within 0.1–0.75 Mpa by using the pressure regulation valve. The alEW and acEW were collected from their respective outlets in separate flasks just before the experiments. The properties of generated alEW and acEW were measured, such as; pH, oxidation/reduction potential, total dissolved solids, and electrical conductivity using a pH meter (Adwa- AD8000); free chlorine using a photometer (Lovibond photometer- MD100).

2.6.2. In vitro testing of EW against fungal species

Agar plugs (5 mm in diam.) containing mycelia from the growing edge of 1-week-old culture were placed in the center of PDA plates. Plates were amended with 250 mg L^{-1} of ampicillin and streptomycin to avoid contamination. For each compound, three Petri dishes were utilized as replicates. For each PDA plate, three holes (5 mm in diam.) at the corner of the plates were inoculated with 50 µL of alkaline electrolyzed water (alEW) or acidic electrolyzed water (acEW). Non-amended PDA plates were used as a control. The plates were incubated at 24± 2°C for one week. Colony diameter (mm) for each fungus was recorded as the average of the longest and the shortest diameter.

2.7. Statistical analysis

The data obtained were statistically analyzed using Completely Randomized Design suggested by [17]. Data were subjected to ANOVA. Averages were compared at 0.05 level of probability using the least significant difference (L.S.D.) as mentioned by [18].

Table (1): Trade name, active ingredient and manufacture of the used chemical component

Trade Name	Active Ingredient	Manufacture
Tebuzyn 25% EW	25%Tebuconazole	CairoChem for agricultural services
Sevron 50% WDG	50% Kresoxim-methyl	Al-Munir for Agriculture and Livestock
Sim-mildew 72% WP	8% Cymoxanil 64% Mancozeb	CairoChem for agricultural services

3. Results and Discussion

3.1. Survey of date palm tree diseases and symptoms

Many symptoms appeared on date palm leaves *i.e.* leaf spots, off-shoot decline and leaf base rot. The main symptoms noticed during the survey were leaf spots at all Governorates followed by off-shoot decline and leaf base rot. The highest disease incidence (75%) appeared at Giza Governorate followed by New Valley; Aswan; El Behera and El Sharkia Governorates (72.86 % - 70 % - 64 % and 57.14 % respectively). These results were in agreement with those reported by [19] who surveyed upper Egypt were limited spread of fungal diseases because the high temperature and dry conditions compared with Giza governorate showed the most elevated humidity conditions and low temperatures.[20]found that *B. theobromae* leaf spot was developed under drought conditions.

3.2. Isolation and identification of the causal organisms

Data in Table (2) show that the highest occurrence of infection was by *Alternaria alternata* (73.17%) at EL Behera Governorate followed by EL Sharkia Governorate (72.73%) while occurrence of *A. alternata* was isolated In three Governorates (Giza 58.93%, New Valley 55.56% and 44.83% at Aswan), On the other hand *Nigrospora oryzae* was isolated from New Valley and Giza Governorates by 25% and 17.86% respectively. *Lasiodiplodia theobromae* was isolated from Aswan and El Behera by 20.69% and 17.07% respectively. *Fusarium oxysporum* was isolated from Aswan and Giza Governorates by 13.79% and 12.50% respectively, while *F. solani* was isolated from El Sharkia by 20.45%. These results indicated that the leaf spot disease was the main problem in date palm leaves caused by *A. alternata*. [19] reported that *A. alternata* was isolated from date palm leaves and basal rot showed a frequency of 43.1% and 33.3% respectively. [21] Isolated *A. alternata, F. oxysporum, F. solani* from date palm roots. These results were in harmony with many investigators *i.e.*[22] and [23] who isolated *A. alternata, L. theobromae, F. solani, F. oxysporum* recording frequency percentages of (6.25%, 10%, 7.50% and 8.75% respectively) from date palms tree. [24] isolated *Nigrospora oryzae* from the infected date palm leaves.

Table (2): Occurrence and frequency (%) of fungi isolated from diseased leaves of date palm collected from five governorates of Egypt

	Percentage of isolation and frequency of causal organisms at Egyptian Governorates											
	Giza		**El Behera**		**El Sharkia**		**New Valley**		**Aswan**		**Total No. of colonies**	**Total frequency%**
Isolated fungi	Occ.	Freq.%	occ.	Freq.%	occ.	Freq.%	occ.	Freq.%	occ.	Freq.%		
A. alternata	33	58.93	30	73.17	32	72.73	20	55.56	13	44.83	95	67.38
N. oryzae	10	17.86	4	9.76	0	0.00	9	25.00	4	13.79	14	9.93
L. theobromae	4	7.14	7	17.07	2	4.55	4	11.11	6	20.69	13	9.22
F. oxysporum	7	12.50	0	0	1	2.27	0	0	4	13.79	8	5.67
F. solani	2	3.57	0	0	9	20.45	3	8.33	2	6.90	11	7.80
Total	56	100	41	100	44	100	36	100	29	100	141	100

Occ. = Occurrence for each fungus, Freq. % = Percentage of frequency for fungi colonies for each fungus

3.3. Pathogenicity test

The highest infection (100%) was recorded on palm leaves artificially infected with *Lasiodiplodia theobromae* isolates followed by *Alternaria alternata, F. oxysporum* and *F. solani*. *Nigrospora oryzae* showed the lowest colonization (25%) on date palm leaflets. The control treatment did not show any colonization (Fig. 1 and Table 3). These results were in agreement with those reported by [25] and [24]. [14] mentioned that *F. solani* recorded a percentage of colonization (6 – 56% colonization) followed by *F. oxysporum*. [26] recorded that *Botryodiplodia theobromae* was the highest pathogenic fungi on date palm trees.

Table (3): Colonization capability of 5 fungi isolated from trees of date palm

Percentages of colonization of 5 fungi on date palm tree leaves *in vitro*		
Pathogenic fungi	**(mm)**	**%**
A.alternata	18.8	50%
N. oryzae	10.6	25%
F. solani	17.8	50%
F. oxysporum	18.9	50%
L. theobromae	37.9	100%
L.S.D: P at 0.05 = 0.6		

3.4. Biological control

Data in Table (4) show that all the tested bio-agents reduced fungal colonies the percentage of liner growth of five fungi *i.e. Lasiodiplodia theobromae, Alternaria alternata, F. oxysporum, F. solani* and *Nigrospora oryzae* isolated from palm trees compared to the control treatment. Data presented in Table (4) show that the highest percentage (76.85%) of inhibition zone was recorded for *T. harzianum* I_1 on *F. oxysporum* followed by *T. harzianum* I_2 70.56% against *A. alternata* while the lowest percentage 30.74% and 23.33% of inhibition zone were recorded for *T. harzianum* I_2 and I_1. Data in the Table show that *T. harzianum* I_1 and I_2 show the heights efficacy compared to Bacillus spp. These results are in harmony with [27] who mentioned that colonies of *T. harzianum* develop faster than isolates of the tested pathogenic fungi *i.e.* The fast growth of Trichoderma spp. gives their chance to compete for the space and nutrients with plant pathogenic fungi, beside they mycotoxin. *Trichoderma* spp. produce number of antibiotics against plant pathogenic fungi like trichodermol, harzianum, trichodermin, harzianolide and trichotoxin [28]. [29] mentioned that the direct fungal parasitic activity of *Trichoderma* spp. suggested as one of the mechanisms involved in their antagonistic activity against phytopathogenic fungi; using Trichoderma coil, hook or appressorium-like structures. On the other hand, the highest antagonism of Bacillus spp. showed on *A. alternata* 42.2%, followed by *N. oryzae*, *F. oxysporum*, *L. theobromae* and *F. solani* (27.4%, 24.3%, 21.1% and 16.7% respectively). Moreover, *L. theobromae* and *F. solani* showed the lowest inhibition zone (21.1% and 16.7% respectively). The antagonistic activity of *B. Subtilis* due to its ability to produce a variety of antifungal lipopeptides [30]. Bacillus strains isolated from the date palm rhizosphere have the potential to control causal organisms of plant diseases in extreme environments [30]. Moreover, *B. Subtilis* produces several kinds of antibiotics *i.e.* bacillomycin, iturin, mycosubtilin, bacilysin, fengymycin and mycobacillin. These results are in harmony with many investigators [31] and [32]. Finally, *Bacillus* spp. produce three natural substances namely lipopeptidesfengycin (F), surfactin (S), and mycosubtilin (M). All these lipopeptides caused morphological damage to the pathogenic fungi *i.e.* vesicle-like swollen structures [33].

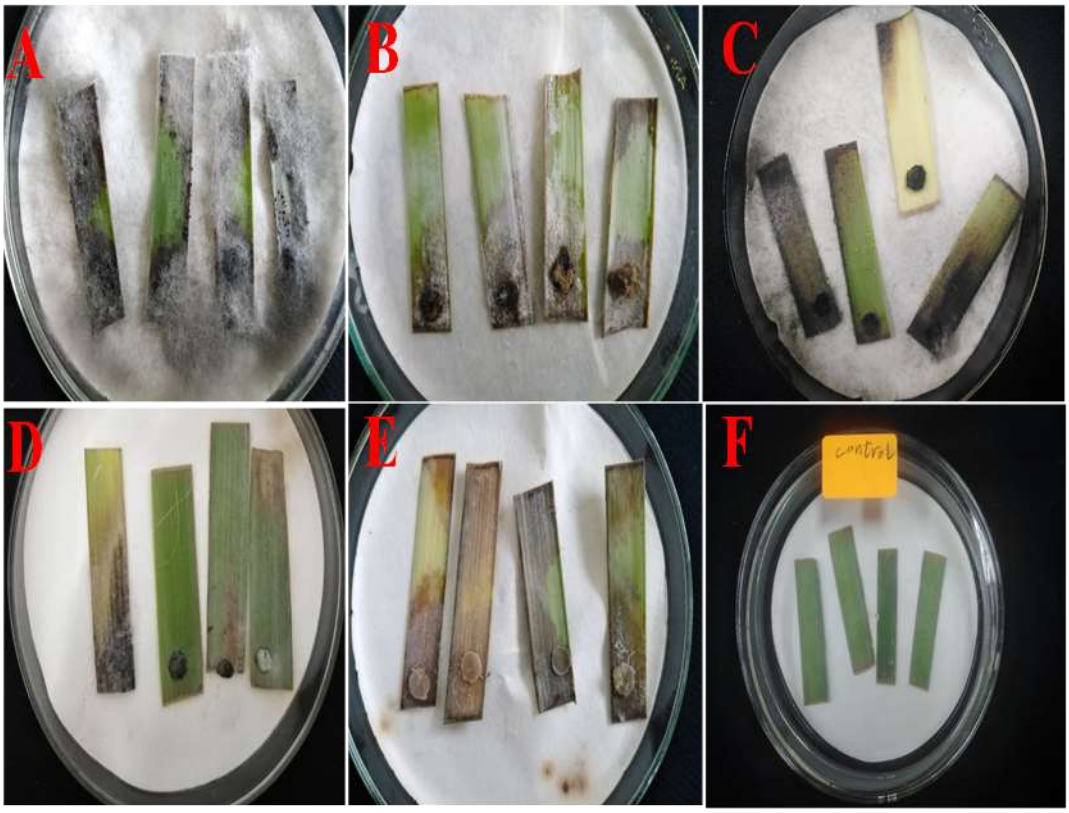

Figure (1): Fungal colonizing capability on detached date palm leaves infected by 5 fungi isolated from date palm leaves. (A) *L. theobromae*, (B) *A. alternata*, (C) *F. oxysporum*, (D) *F. solani*, (E) *N. oryzae*, (F) Control

Table (4): Antagonistic effect of two isolates of *Trichoderma harzianum* and *Bacillus* spp. on the mycelial growth (mm) of five fungi isolated from leaves of date palm trees on PDA medium at 25 ºC *in vitro*

Bio agents	Percentages of growth inhibition (PGI %) of 5 fungi									
	A. alternata		***N. oryzae***		***F. solani***		***F. oxysporum***		***L. theobromae***	
	Mean (mm)	*%*	Mean (mm)	*%*	Mean (mm)	*%*	Mean (mm)	*%*	Mean (mm)	*%*
Trichoderma harzianum I_1	47.6	47	33.1	63.15	47.3	47.59	27.5	76.85	69	23.33
Trichoderma harzianum I_2	26.5	70.56	42.6	52.78	54.4	39.63	28.6	68.33	62.3	30.74
Bacillus spp.	52	42.2	65.3	27.4	75	16.7	68.1	24.3	71	21.1
Control	90	0	90	0	90	0	90	0	90	0
L.S.Dat 0.05	Bio agent= 0.317, Fungi = 0.409, Bio agent X Fungi= 1.41									

% = Percentage of inhibition zone was calculated as formula dual cultured by [15]

3.5. Chemical control

Data in Table (5) show that all the tested fungicides *i.e.* Tebuzyn 25% EW, Sim-mildew 72% WP and Sevron 50% WDG gave reduction for 5 fungi *Alternaria alternata, Nigrospora oryzae, Fusarium solani, Fusarium oxysporum* and *Lasiodiplodia theobromae* isolated from palm leaves. The most effective fungicide was Tebuzyn 25% EW at all concentrations followed by Sim-mildew 72% WP and Sevron 50% WDG. Moreover, Tebuzyn 25% EW showed complete inhibition for the mycelia growth of all pathogenic fungi at 500 and 1000 mg/L, except that 96.67% with *Fusarium oxysporum* at 500 mg/L, in the same case, Tebuzyn 25% EW recorded completely inhibited the growth of *F. solani* (100 %) at all concentration, while at 10 and 100 mg/L concentrations have different inhibition zone percentages with *Lasiodiplodia theobromae, Alternaria alternata, Fusarium oxysporum* and *Nigrospora oryzea*. Sim-mildew 72% WP at a concentration 1000 mg/L recorded completely inhibited the growth of *N. oryzae* (100 %) followed by *F. oxysporum, A. alternata, F. solani* and *L. theobromae* (70.93 %, 69.26 %, 68.89 % and 61.67 % respectively). Sevron 50% WDG showed the lowest growth reduction of all concentrations of 10,100, 500 and 1000 mg/L. sevron 50% WDG was not affected on *Lasiodiplodia theobromae* at all concentrations with 0%, moreover at 1000 mg/L recorded the highest effect on *F. solani* with 53.89% followed by *N. oryzae* with 50.56%, *A. alternata* with 45.74% and *F. oxysporum* with 36.85% respectively. These results were in harmony with those recorded by [34]. Tebuconazole (Tebuzyn 25% EW) inhibited the growth and sterol composition of *Botrytis cinerea, Erysiphe graminis* f.sp. *hordei, Rhynchosporium secalis, Ustilago maydis, Pyrenophora teres* and *Pyricularia oryzae*. [35] mentioned that Tebuconazole and kresoxim-methyl inhibited mycelial growth of *Marssonina coronaria*, but had a low effect on conidial germination in East Asian apples. [36] reported that tebuconazole could inhibit the germination of spores by inhibiting the elongation of the germination tube, which results in malformations, thickening and branching of the germination tube. However, it did not affect the swelling of the spores and failed to invade the spores. Mycelial morphology and cell structure also changed significantly when treated with fungicides, the morphological changes in the mycelium were mainly swelling, increased hyphal branches, and coarsening of the hyphae. Cytological changes mainly include increased and thickened cell septa, irregular thickening of cell walls, increased membranes, thickened or irregular contractions of mitochondria, increased cell nuclei, nuclear dispersal, and strong cell vacuolation.

Table (5): Effect of five concentrations of three fungicides on the linear growth (mm) of five fungi isolated from leaves of date palm trees

Fungicides	Conc. (mg/L)	*L. theobromae*	*A. alternata*	*F. oxysporum*	*N. oryzea*	*F. solani*
Tebuzyn 25% EW	0	90	90	90	90	90
	10	22.5	21	26.8	51.1	0
	100	8.8	12.1	14	25.3	0
	500	0	0	3.0	0	0
	1000	0	0	0	0	0
Sevron 50%WDG	0	90	90	90	90	90
	10	90	90	90	89.1	55.5
	100	90	90	90	85.8	43.3
	500	90	89.1	88.3	80	47.8
	1000	90	48.8	56.8	44.5	41.5
Sim-mildew 72%WP	0	90	90	90	90	90
	10	90	90	90	90	88.3
	100	51.5	85.6	86.7	81.5	86.7
	500	44.5	67.5	48.8	6.7	69.8
	1000	34.5	27.6	26.7	0	28.0
L.S.D: *P* at 0.05	Fungicide (F)	0.17				
	Conc.(C)	0.22				
	Fungi (G)	0.221				
	F X C	1.04				
	G X C	1.23				
	F X G	2.02				
	F X C X G	1.48				

3.6. Electrolyzed water

Results in Table (6) show that two types of EW water *i.e.* acidic EW and alkaline EW and the salt solution of NaCl without EW, in addition, to the use of distilled water as a control. The results showed that acidic EW gave the highest effect on all tested fungi compared with alkaline EW and NaCl without EW, which showed the highest effect on *A. alternata* with (12.67mm) followed by *F. oxysporum, F. solani* and *N. oryzae* with (14.67, 25.33, and 26 mm) respectively, while *L. theobromae* showed the lowest effect with (52.67 mm). In addition, alkaline EW showed the most effect on *A. alternata* with (16.67 mm) followed by *F. oxysporum, F. solani, N. oryzae* and *L. theobromae* (20.67, 30.67, 38.67 and 56 mm) respectively. In the other case, using NaCl salt without EW showed a weak effect compared to Acidic EW and Alkaline EW where fungi colony diameter for all fungi was close to using control treatment. NaCl without EW gave the most effect on *F. solani* with (70.67mm) followed by *F. oxysporum, A. alternata, L. theobromae* and *N. oryzae* with (73.33, 81.33, 84.67 and 85.33 mm) respectively.

Alkaline EW reduced the growth of *L. theobromae, F. oxysporum, N. oryzae, A. alternata* and *F. solani* by 34.12, 74.80, 53.23, 80 and 63.49%, respectively. Also, acidic EW reduced the growth of *L. theobromae, F. oxysporum, N. oryzae, A. alternata* and *F. solani* by 38.04, 82.11, 68.55, 84.80 and 69.84, respectively. Acidic electrolyzed water plays a good role in controlling the damage of grape downy mildew, *Botrytis cinerea*, anthracnose, grape Alternaria leaf spot, and anthracnose of grape, with foliar control effect of 93.5, 96.2, 93.20, 100 and 87.9%, respectively [37].

Cl-free anions produced by the electrical dissociation of the Cl salt, a fortified NaCl salt for ROX electrolysis machine, are powerful oxidants that intervene in several biological structures and functions [38], [39], [40]. Also, the treatments by alkaline (alEW) and acidic (acEW) electrolyzed water caused irregular mycelia growth, irregular branching of hyphae in the apical part and loss of linearity in the tested fungal structures, the results showed by scanning electron microscopy (SEM). The effect of alkaline and acidic on the physical and chemical properties of 'Valencia' sweet orange quality, including mass loss, total soluble solids (TSS), citric acid, pH, ascorbic acid and fruit color index was examined [41].

Table (6): Effect of alkaline EW, acidic EW and sodium chloride on colony diameter (mm) of five pathogenic fungi isolated from date palm trees

Treatments	Fungi colony diameter (mm)				
	L. theobromae	***A. alternata***	***F. oxysporum***	***N. oryzea***	***F. solani***
Control	85	82	82.67	83.33	84
Alkaline EW	56	20.67	38.67	16.67	30.67
Acidic EW	52.67	14.67	26	12.67	25.33
NaCl without EW	84.67	73.33	85.33	81.33	70.67
LSD: *P* at 0.05 =	Treatments (T) = 1.10,	Fungi (F) =1.23,	T X F = 4.28		

4. Conclusions

The current study aimed to follow up on the occurrence of date palm diseases *i.e.* leaf spots and basal leaf rot at five Egyptian governorates. Survey results recorded the highest infection was at Giza governorate followed by Aswan governorate, while El Sharkia governorate showed the lowest infection rate. Several methods were validated including biological, electrolyzed water and fungicides to control the isolated fungi under laboratory conditions. Further experiments are needed to confirm the efficacy of promising control methods under field conditions. The heights isolation *Alternaria alternata* were isolated from date palm trees, while *F. solani* showed the lowest isolation. Pathogenicity test showed that *Lasiodiplodia theobromae* was the most pathogenic fungus. Biological control shows that *Bacillus* spp. recorded the highest inhibition effect on *Alternaria alternata* and the lowest inhibition on F. *solani. Trichderma harzianum* (I_1) isolate showed strong inhibition of *F. oxysporum* and (I_2) isolate showed the strongest inhibition on the fungus *Alternaria alternata*. The highest inhibitory effect was the fungicide Tebuzyn 25% EW, which inhibited the growth of all tested fungi. Electrolyzed water on Acidic EW showed the highest effect on *Alternaria alternata*, while the lowest effect on the isolated fungi *Lasiodiplodia theobromae*. Alkaline showed the highest effect on *Alternaria alternata*, while the lowest effect on the isolated fungi *Lasiodiplodia theobromae.*

5. Conflicts of interest

The authors declare that there is no conflict of interest to declare.

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