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PATHOLOGICAL STUDIES ON ALTERNARIA LEAF SPOT DISEASE OF DATE-PALM IN EAST DELTA

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ABSTRACT: Date-palm leaf spot disease was observed on July 2004 in some inspected localities belong to certain governorates. The isolation procedures yielded different *Alternaria* spp. isolates which were capable of inducing the same disease through the pathogenicity test. *In vitro* control studies confirmed that *Trichoderma* spp., *Bacillus* spp., garlic and camphor extracts as well as Kema-Z and Cure-Plus were able to control the pathogen's mycelial growth and suppress its spore germination. Cure-Plus, Kema-Z and *Trichoderma* spp. decreased the severity of the leaf spot disease when investigated in the field trials.

Key words: Alternaria leaf spot, date-palm, *Trichoderma* spp., *Bacillus* spp., pathogenicity test.

INTRODUCTION

Alternaria leaf spot disease is a moderately prevalent disease dispersing on the old leaflets of date-palm (*Phoenix dactylifera* L.) (El-Deeb *et al.*, 2007). The initiated spots reduced the green surface of the total plant leaves resulting a decrease in the photosynthesis process. Pathogenicity tests confirmed that the *Alternaria* spp. isolates were capable of inducing spots on the date-palm leaflets (Marei, Thanaa and Zak, 2007).

It was documented that *Alternaria* spp. could be controlled by fungal and bacterial bio-agents such as *Trichoderma* spp. (Stankeviciene and Snieskiene, 2003) and *Bacillus* spp. Several plant extracts such as artemisia and camphor (Sokovic *et al.*, 2002), garlic (Satya *et al.*, 2005) and sweet marjoram (Leeja and Thoppil, 2007) have antifungal effect against the plant pathogenic fungi.

Otherwise, fungicides are still the fast aid in alarming diseases. Carbendazim, copper derivatives and metalaxyl were reported as effective groups of chemical fungicides in controlling different

plant diseases. Some chemical formulation derivatives were manufactured to be used as fungicides; such as Kocide 2000, Kema-Z and Cure-Plus. The antifungal activity of these fungicides was evaluated on the different date-palm diseases and reported by different investigators (Ruchi, Sood and Sharma, 2002 and Shahid *et al.*, 2003). These control methods (bio-agents, plant extracts and fungicides) not only affect on the pathogen's mycelial growth but also its spores germination, thus it could prevent the disease initiation and development.

The present work was designed to survey, isolate and confirm the pathogenic capability of the isolated causal organism of Alternaria leaf spot disease. It was also aimed to find the best control way (biologically, plant extracts and chemically) that can suppress the fungal growth and inconsequence suppress the leaf spot disease.

MATERIALS AND METHODS

Survey of Date-Palm Alternaria Leaf Spot

This study was performed in several localities belong to El-Sharkia, El-Ismaelia and El-Dakahlia governorates. The disease was assessed on 50 trees per each locality by calculating percentages of the disease incidence (DI) and the disease severity (DS) by the formulas described by Cooke *et al.* (2006).

$$\text{Disease incidence (DI)} = \frac{\text{Number of infected trees}}{\text{Total number of inspected trees}} \times 100$$

$$\text{Disease severity (DS)} = \frac{E\,(n \cdot v)}{X \cdot N} \times 100$$

N = total number of all inspected trees, **n** = number of trees at the rate v, **v** = the rate of the disease on the previously mentioned scale starting at zero till **4** which represent the highest disease severity rate (X).

The rate of Alternaria leaf spot was conducted using the scale described by (Fayud and Mania, 2006) rating the infected leaflets into the following categories:

0 = no symptoms (healthy leaflets), 1 = small spots appeared at the lower leaflets, 2 = the spots elongated and covered most of the lower leaflets, 3 = the spots started to appear at the uppermost leaflets and 4 = the spots covered the whole old leaflets extending to the top one.

The observed disease symptoms were described and figured as

possible. Diseased looking samples were collected and immediately transferred to the laboratory of Plant Pathology, Agric. Bot. and. Pl. Pathol. Dept., Fac. of Agric. Zagazig Univ., using cooled plastic boxes, for the isolation procedures.

Pathological Studies

Isolation, purification and identification of the causative organisms

The diseased samples were carefully washed, cut into small pieces 1 cm length and surface sterilized with 1 % Sodium hypochlorite solution for 3 minutes (Burr *et al.*, 1978). The specimens were then washed several times using sterilized distilled water. The pieces were then dried between sterilized filter papers. The sterilized specimens were plated onto water agar (WA) isolation medium under sterilized conditions then incubated at 28± °C for 7-14 days. After seven days, the developed colonies were purified using single spore isolation technique (Hansen, 1926) and identified according to the fungal morphological characteristics as described by Moubasher (1993) and Barnett and Hunter (1998).

Pathogenicity tests

The pathogenic capability of the isolated *Alternaria* spp. isolates as well as their culture filtrates were tested on detached and attached date-palm leaflets (cultivar Hayani). The inoculum of *Alternaria* spp. isolates numbers 1, 2, 3 and 4 were prepared as spore suspension and culture filtrates by the methods described by Dhingra and Sinclair (1995).

The attached and detached date-palm leaflets were divided into eight treatments according to the number of the tested *Alternaria* spp. isolates and the types of the inoculum used. Each treated detached leaflets were placed in a plastic box padded using wetted and sterilized filter papers. One ml of the spore suspension (10^5 spores/ml) and/or culture filtrate was placed on the detached leaflets. The boxes were covered with transparent plastic sheets then incubated under laboratory conditions (Hatzipapas *et al.*, 2002) until symptoms appearance. On the other hand, the attached leaflets were densely sprayed with ten ml of the previously prepared inoculum (spore suspension and/or culture filtrate) using a sterilized atomizer, 100 ml in capacity, because it wasn't easy to place the inoculum on the surface of the leaflets as done before. The inoculated leaflets were bagged in

a transparent plastic bags (1.5 m length) and wetted with sterilized water. Alternaria leaf spot symptoms were described and its severity was recorded after three weeks of inoculation according to Babu *et al.*, (2004) using the previously mentioned scale.

Isolation, Purification and Identification of the Phyllospheric and Rhizospheric Microorganisms

Date-palm phyllospheric and rhizospheric samples were collected from each inspected locality and used for isolation procedures as described by Aneja (2003). The specific isolation medium (Peptone Dextrose Agar plus Rose Bengal and Streptomycin (Johnson *et al.*, 1960) for fungi, Soil extract agar medium (Lochhead, 1940) for bacteria and Jensen's agar medium (Jensen, 1930) for actinomycetes) were used to isolate these microorganisms.

The inoculated plates were incubated until microbial colonies developed. The developed colonies were purified as mentioned before. The isolated fungi were identified according to descriptions mentioned by Moubasher (1993) and Barnett and Hunter (1998). While, The isolated bacteria were identified according to the classification of Buchanon *et al.*, (1974).

In vitro Control Studies

Through the lab. experiments, the antifungal effect of some bio-agents, plant extracts and fungicides was evaluated on the mycelial growth and the spore germination of the most pathogenic *Alternaria* spp. isolates (Nos.3 and 4) which isolated from El-Quorain and El-Salhia. Poison food technique (Finholt, 1951) and depressed slide technique (Anonymous, 1943) were used to evaluate the antifungal effect of the different control methods on *Alternaria* spp. isolates mycelial growth and spore germination, respectively.

Poison food technique was done by mixing a distinct amount or volume of the tested material with potato dextrose agar (PDA) medium prior to pour in order to obtain the desired concentration. The poisoned medium was inoculated with a disk of the tested *Alternaria* spp. isolates. Petri dishes that inoculated only with *Alternaria* spp. were served as control treatment. Three replicates were used for each

treatment. All previous treatments were incubated at 28 ± 2 °C until completion of *Alternaria* spp. colonies in the control treatment. After incubation period, the mean diameter of the developed colonies were measured and converted into a reduction percentage by the following formula:

$$G.R.\% = \frac{A - B}{A} \times 100$$

Whereas:

A = Diameter of the pathogenic fungal colony in the control treatment.

B= Diameter of pathogenic fungal colony in a treatment.

Depressed slide technique was done by placing 0.5 ml of *Alternaria* spp. spore suspension (10^5 spores/ml) plus 0.5 ml of the tested material on the cavity of the depressed slide which laid on a moistened Petri dish. Two days were enough for the spore germination. After incubation period, the average of the germinated spores were calculated comparing with the control treatment and converted into reduction percentage (R %) using the following formula:

$$R\% = \frac{A - B}{A} \times 100$$

Whereas:

A = Average number of germinated spores in the control.

B = Average number of germinated spores of each treatment.

Biological Control

For the Mycelial Growth of *Alternaria* spp.

By *Trichoderma* spp.

Four *Trichoderma* spp. were tested in this experiment using two disks technique described by Henis and Inbar (1968) on Gliotoxin fermentation medium (GFM) (Brain and Hemming, 1945). These species were known as *T. hamatum, T. viride* and *T. album* were obtained from Agric. Bot. and. Pl. Pathol. Dept., Fac. of Agric. Zagazig Univ. and the fourth *Trichoderma* sp. was isolated from the date-palm rhizosphere zone. After incubation period, the data were calculated as mentioned before.

By *Bacillus* spp.

The antagonistic effect of four

Bacillus spp. isolates isolated from date-palm phyllospheric and rhizospheric regions was evaluated against *Alternaria* spp. isolates Nos.3 and 4 using different methods (streaking plate method, pouring plates method and streaking plate method 2 days after pathogen inoculation) as described by Henis and Inbar (1968). The data were recorded as mentioned before.

By culture filtrate of *Trichoderma* spp. and *Bacillus* spp.

The bio-agent's culture filtrates were obtained from culture of the bio-agents grown on gliotoxin fermentation medium (GFM), filtered and sterilized as described by Abd El-Moity and Shatla (1981). The different concentrations (10, 20 and 30%) were prepared from the stalk filtrates by mixing with PDA medium prior to pour. The medium was poured, inoculated, incubated and the results were recorded as mentioned before.

For the spore germination of *Alternaria* spp.

The previously prepared culture filtrates were evaluated as control agents against the spore germination of *Alternaria* spp. isolates by the depressed slide technique which previously explained.

Plant extracts

Garlic cloves as well as camphor, marjoram, and artemisia leaves were used in separate treatments for this experiment. The sap of the garlic cloves was extracted using several ways being: Cool Water extraction method under 25 °C as described by Aly *et al.* (2003), Hot Water extraction method under 100 °C as described by Zedan (1993) and Organic Solvents extraction method under 5 °C as described by Kshirsagar and Mehta (1972). Ten grams dry weight/100 ml of each solvent was used to prepare the extract. The resultant extracts were filtered, sterilized and kept in a refrigerator at 5 °C for further experimental purposes.

The previously prepared extracts were separately used to prepare the aforementioned concentrations (10, 20 and 30%) which then evaluated against *Alternaria* spp. isolates mycelial growth by poison food technique method and the spore germination by depressed slide technique method as mentioned before.

Chemical control

Different concentrations (0, 1,

5, 10, 50, 100, 250, 500 and 1000 ppm of active ingredient) for each of Kema-Z, Cure-Plus and Kocide 2000 were prepared to evaluate their inhibitory effect on *Alternaria* spp. isolates mycelial growth by poison food technique and the spore germination by depressed slide technique as mentioned before.

Management of Alternaria Leaf Spot Disease, *In vivo*

The most effective control methods through the *in vitro* studies (Kema-Z, Cure-Plus, garlic extract, camphor extract, *Trichoderma album*, *T. harzianum*, *Bacillus* sp. isolate No. 4 and 9) were selected to control the Alternaria leaf spot disease, *in vivo*.

The inoculation was performed as previously explained in the " Pathogenicity test ". The following notes were taken under concern: 1) The concentration of each formulation was prepared according to its commercial recommended dose (fungicides, 10% for plant extracts and 4 g/l for the bio-agents). 2) The control agent formulations were treated as a spraying solution. 3) The control test was performed on detached and attached rachis.

The whole experiment was divided into three treatments named T1, T2 and T3 according to the treating time of the control agent and the fungal pathogens. In T1 treatment, the control agent was applied one week before fungal inoculation. The control agent and the fungal inoculum were applied at the same time in the second treatment, T2. In T3 treatment, the control agent was applied one week after the fungal inoculation. Additional treatment was included and served as a control treatment whereas water was used instead of the control agents and/or fungicides.

Data were recorded after complete death occurred in the control treatment. The recorded data were calculated as disease severity which was previously explained at the end of the "Pathogenicity test". Re-isolation was performed as previously mentioned. The isolated fungi were compared with the original cultures to confirm Koch's postulates.

Statistical Analysis

The results of the previous experiments were statistically analyzed according to the procedures reported by Snedecor and Cochran (1980). The means of all treatments were compared by

the least significant difference value "L.S.D." at 5% level of probability.

RESULTS AND DISCUSSIONS

Data presented in Table 1 and illustrated by Fig. 1 show that, the highest infection percentage of Alternaria leaf spot disease in El-Sharkia Governorate was observed in El-Zagazig (35%) followed by El-Salhia (25%) and El-Khattarah (24.6%). The lowest infection percentage (5%) was detected in El-Quorain. Alternaria leaf spot disease was not detected in Abo-Kabeer locality. Percentages of disease severity were 5, 20, 10 and 5% in El-Zagazig, El-Salhia, El-Khatarah and El-Quorain, respectively.

Alternaria leaf spot infection percentage in El-Ismaelia governorate as well as its severity were 7 and 15% in El-Quassasin as well as 5 and 15% in Abo-Sweer. In El-Dakahlia governorate where Koom El-Noor district (one of the most famous date palm producing areas) the Alternaria leaf spot infection percentage was 12% and its severity reveal 5% per each infected tree. The typical symptoms of Alternaria leaf spot were appeared as scattered elongated brown lesions with black margins on the old leaflets (Fig. 1).

These results were agreed with those obtained by Hilal *et al.*, (2002) as well as Fayud and Mania (2006). The explanation of such results might be due to the variation in the environmental conditions over the inspected diseases (El-Deeb *et al.*, 2007). It also might be due to one or more of the following factors: i) pathogen frequency differs through the localities either qualitatively or quantitatively. ii) climatic conditions vary considerably between regions. iii) varietal sensitivity. iv) dissemination factors available in the region. v) it also might be affected by the cultural practices (Turner, 1981).

Data presented in Table 2 show that *Alternaria* spp. isolates were the most frequently isolated from the shoot of the date-palm trees located in each inspected district. This result was in line with Fayud and Mania (2006) who reported that the date-palm leaf spots often yielded by different air-borne fungi including *Alternaria* spp. This might be due to that the phyllospheric zones always exposed to infection with a wide range of the pathogenic microorganisms.

Alternaria spp. was able to cause spots on the inoculated date-

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Table 1. Survey of the date-palm Alternaria leaf spot disease in three Egyptian governorates

Governorate & Locality	Cultivars	Infection percentage	Disease severity
1. El-Sharkia			
El- Zagazig	Hayani	35	5
El- Salhia	Hayani	25	20
El- Khattarah	Zaghlol	24.6	10
El-Quorain	Hayani	5	5
Abo-Kabeer	Hayani	0	0
2. El-Ismailia			
El- Quassasin	Hayani	7	15
Abo-Sweer	Hayani	5	15
3. El-Dakahlia			
Koom-El-Noor	Zaghlol	12	5

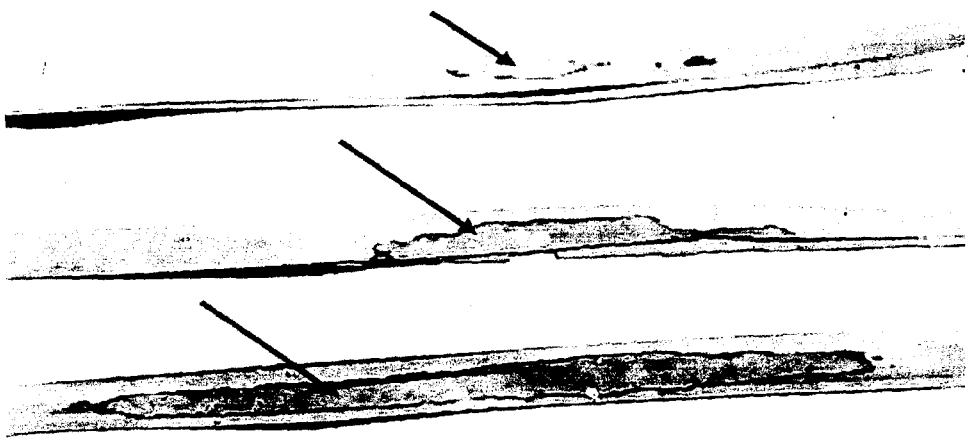

Fig. 1. Showing the typical date-palm Alternaria leaf spot symptoms on leaflets

-palm leaflets. The symptoms appeared as blights on the leaflets inoculated with spraying but they were appeared as spots on the leaflets inoculated with placing a drop of the liquid inoculum on the leaf surface. These symptoms were observed on the mature leaflets after three weeks of inoculation as scattered small brownish spots with blackish margins (Fig. 2). The developed spots were enlarged and coalesced to cover most of the inoculated leaf surface.

The obtained data in Table 3 and Fig. 2 reveal that the detached leaflets were more sensitive than the attached ones exhibiting the typical symptoms of Alternaria leaf spot disease. The disease severity percentages on the detached leaflets were ranged between 2.2 and 44.98%, while they ranged between 1.6 and 13.5% on the attached ones.

The culture filtrates of *Alternaria* spp. isolates significantly caused Alternaria leaf spot symptoms in high severity percentages in comparison with the spore suspension of the same fungus. *Alternaria* spp. isolates No. 3 and 4 were found to be the most virulent recording 14.85 and 44.98% severity percentages, respectively, on the detached leaflets and 7.65 % and 13.5 % on the attached ones.

The tested isolates of *Alternaria* spp. appeared to be date-palm pathogens causing leaf spot disease, as reported by Babu *et al.* (2004) as well as Fayud and Mania (2006). The results also showed that the leaf spot symptoms always appear after 20 days of inoculation, as also recorded by Babu *et al.* (2004). The obtained data showed that the detached leaflets were more susceptible to the fungal infection than the attached one. These results were similar to those obtained by Liu *et al.* (2007) who reported that *Colletotrichium linicola* A1 could cause a typical infection only on detached, but not attached Arabidopsis leaves. That was also agreed with Brooks (2008) who revealed that attached leaves had smaller lesion diameters than detached ones incubated in closed containers.

The immature leaflets were also immune to the infection in contrast to the mature leaflets. This finding might be due to the high levels of silica, wax and tannin contents in the young immature leaflets which make it immune against the fungal infections (Agrios, 2005 and Fayud and Mania, 2006).

Table 2. Frequency percentage (F%) of the isolated microorganisms from Alternaria leaf spot date-palm trees

Governorate		El-Sharkia					El-Ismaelia		El-Dakahlia
Locality / Isolated microorganisms		El- Zagazig	El- Quorain	El-Salhia	El-Khattarah	Abo-Kabeer	El-Quassasin	Abo-sweer	Koom El-Noor
Alternaria spp.	No	18	1	8	12	0	4	1	0
	F%	90	16.7	88.9	100	0	33.3	14.3	0
Nigrospora sp.	No	0	5	0	0	0	3	3	0
	F%	0	83.3	0	0	0	25	42.9	0
Stymphillium sp.	No	2	0	1	0	0	0	0	0
	F%	10	0	11.1	0	0	0	0	0
Thielaviopsis sp.	No	0	0	0	0	0	5	3	0
	F%	0	0	0	0	0	41.7	42.9	0

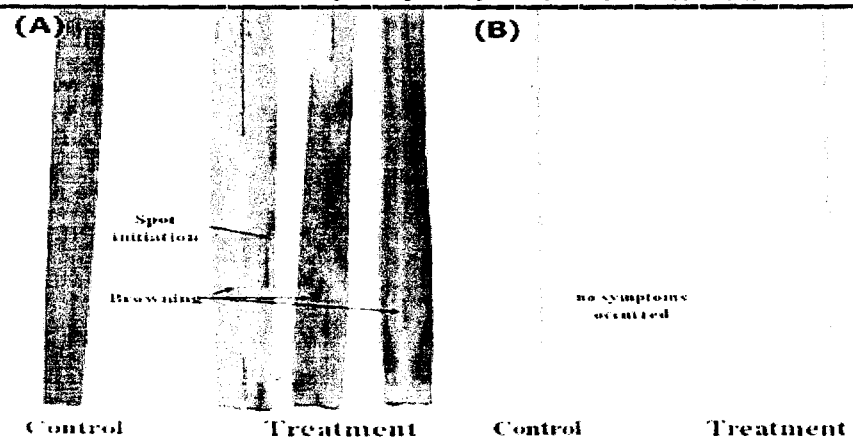

Fig.2. Symptoms resulted from the pathogenicity test of *Alternaria* spp. isolates
(A) Mature leaflets (B) Immature leaflets of cultivar Hayani

Table 3. Pathogenicity tests of *Alternaria* spp. isolates on date-palm leaflets (cultivar Hayani).

Treatment	Detached mature leaflets				Attached mature leaflets			
Pathogen	Filtrate	Spores	Index	Mean	Filtrate	Spores	Index	Mean
Alternaria sp. No. 1	15.71	6.66	9.05	**11.13**	5.63	3.8	1.83	**4.5**
Alternaria sp. No. 2	3.3	1.1	2.2	**2.2**	2.23	0.93	1.3	**1.6**
Alternaria sp. No. 3	27.7	2	25.7	**14.85**	14.3	0.9	13.4	**7.65**
Alternaria sp. No. 4	50.66	39.3	11.36	**44.98**	21.5	5.5	16	**13.5**
Mean	**24.34**	**12.26**	**12.1**	**18.29**	**10.92**	**2.78**	**8.13**	**6.8**
LSD at 5%	**9.34**	**1.34**			**4.09**	**3.07**		

Whereas: Filtrate= Culture filtrate. Spores= Spore suspension. 3= Increase in disease severity (Disease index).
*Note: Immature leaflets showed no infection

Phyllospheric and rhizospheric isolation yielded several kinds of microorganisms including two isolates of *Trichoderma* spp., two isolates of *Trichothecium* spp., three isolates of *Penicillium* spp. and nine isolates *Bacillus* spp., while there wasn't actinomycetes colonies. Such antagonists were also isolated from the rhizosphere and/or phyllosphere of the date-palm by Sariah *et al.* (2005).

Data presented in Table 4 show that the linear growth of *Alternaria* sp. isolate No. 3 was significantly suppressed by *Bacillus* sp. No. 4 and 9 at the rate of 92.2 and 91.83% using the pouring plate method. *Alternaria* sp. isolate No. 4 was significantly suppressed by *Bacillus* sp. No. 4 and 9 at the rates 92.93 and 91.1%, respectively, using the same method.

Data in Table 5 state that the mycelial growth of *Alternaria* sp. isolate No. 3 was significantly reduced by *Trichoderma* sp. and *T. viridi* exhibiting 94.4% reduction. On the other hand, isolate No. 4 was significantly suppressed by *T. album* at a rate being 91.93 % reduction. In general, the obtained data revealed that there was significant difference between

Table 4. Effect of different bacterial isolates on the mycelial growth of *Alternaria* spp. using different methods, *in vitro*.

Pathogen / Bio-agent	Streaking Plate Method		Pouring Plate Method		Streaking 2 days after pathogen inoculation	
	Alternaria spp. Isolates No. 3 and 4					
	No. 3	No. 4	No. 3	No. 4	No. 3	No. 4
Bacillus sp. No. 4	71.11	78.51	92.2	92.93	87.37	87.53
Bacillus sp. No. 7	65.11	76.66	88.8	88.83	42.57	57.33
Bacillus sp. No. 8	68.88	81.48	71.43	88.07	80	88.83
Bacillus sp. No. 9	77.03	72.22	91.83	91.1	89.2	66.23
Mean	**70.53**	**77.22**	**86.07**	**90.09**	**74.79**	**74.98**
Control	0	0	0	0	0	0
LSD at 5%	3.73	5.24	5.07	1.95	4.35	1.99

the tested bio-agents as well as their filtrates especially those of *Trichoderma album* in suppressing *Alternaria* spp. isolates mycelial growth even at their low concentrations.

The culture filtrate of *Bacillus* sp. isolate No. 9 (diluted up to 30%) significantly suppressed the mycelial growth of both isolates (3 and 4) at the rates being 80.4 and 85.9% reduction percentages, respectively (Table, 6).

Data in Table (7 reveal that, the spore germination of isolate No. 3 of *Alternaria* sp. was completely suppressed by *T. album*. The effective action of *Trichoderma* spp. against the fungal pathogens thought to be due to the mycoparasitism, antifungal substances and lytic enzymes like chitenase (Harman, 2006; Howell, 2006; Reino *et al.*, 2008 and Vinale *et al.*, 2008).

Spore germination percentage of the of isolate No. 4 was also reduced by *Bacillus* sp. No. 7 to be 90%. These results were in line with those of Stankeviciene and Snieskiene (2003). The antagonistic action of *Bacillus* spp. might be due to the antibiotic secretion (Farahat, 1998), enzymes and metabolites which reflect permeability changes in the protoplasmic membrane (Austin *et al.*, 1977), competition for

Table 5. Mycelial growth reduction percentages of *Alternaria* spp. isolates by different *Trichoderma* spp., *in vitro*

Bio-agent \ Pathogen	*Alternaria* spp. isolates		Mean
	No. 3	No. 4	
Trichoderma sp.	94.4	87.97	91.1
T. viridi	94.4	87.23	90.8
T. harzianum	91.66	86.63	89.2
T. album	93.87	91.93	92.9
Mean	93.58	88.44	91
Control	0	0	0
LSD at 5%	3.08	3.43	

Table 6. Mycelial growth reduction percentages of *Alternaria* spp. isolates using bio-agents culture filtrates at various concentrations, *in vitro*

Bio-agent (A) \ Pathogen	*Alternaria* sp. No. 3			*Alternaria* sp. No. 4			Mean		
	10%	20%	30%	10%	20%	30%	10%	20%	30%
Trichoderma sp.	0	0	0	0	0	0	0	0	0
T. viridi	0	0	0	0	0	4.43	2.22	0	0
T. harzianum	0	10	37.2	1.1	25.6	49.3	43.25	17.8	0.55
T. album	43.3	57.8	69.3	38.9	57.8	67.9	68.6	57.8	41.1
Bacillus sp. No. 4	24.4	43.3	56.7	45.6	60	85.6	71.15	51.65	35
Bacillus sp. No. 7	15.6	27.8	47.3	8.9	30	45	46.15	28.9	12.25
Bacillus sp. No. 8	0	21.1	45.6	0	14.4	42.8	44.2	17.75	0
Bacillus sp. No. 9	76.7	78.9	80.4	80	81.1	85.9	83.15	80	78.35
Control	0	0	0	0	0	0	0	0	0
Mean	12.3	18.4	25.9	13.4	21.3	31.1			
LSD (A) at 5%	3.41	3.41	3.41	2.51	2.51	2.51			
LSD (B) at 5%	1.34	1.34	1.3	1.21	1.21	1.21			
A x B	**	**	**	**	**	**			

Whereas: B = different concentrations.

Table 7. Effect of bio-agent culture filtrates on the spore germination of the different *Alternaria* spp. isolates, *in vitro*

Pathogen / Bio-agent (A)	*Alternaria* sp. isolates		Mean
	No. 3	No. 4	
Trichoderma sp.	0	0	**0**
T. viridi	20	40	**30**
T. harzianum	0	0	**0**
T. album	100	58	**79**
Control	0	0	**0**
Mean	**24**	**19.6**	
LSD (A) at 5%	1.15	1.75	
LSD (B) at 5%	0.81	1.24	
A x B	**	**	
Bacillus **sp. No. 4**	43.2	70	**56.6**
Bacillus **sp. No. 7**	65	90	**77.5**
Bacillus **sp. No. 8**	7	19	**13**
Bacillus **sp. No. 9**	0	0	**0**
Control	0	0	**0**
Mean	**23.04**	**35.8**	
LSD at 5%	2.81	4.53	

Whereas: B = types of tested media.

nutrients (Droby *et al.*, 1990) and bulb formation and lysis of the fungal hyphae (Korsten and De Jager, 1995).

Data presented in Tables 8 and 9 detect that the mycelial growth of *Alternaria* spp. isolates No. 3 and 4 were significantly affected by the different tested extracts under 10, 20 and 30% concentrations, being 8.86, 12.89 and 13.47% reduction percentages, respectively, for the isolate No. 3 and 9.84, 17.95 and 20.91% for the isolate No.4.

Spore germination of each isolate was completely inhibited when garlic bulbs extract using the cool water method. Significant differences between the tested plant extracts were obtained as well as between their concentrations and the extraction methods reporting that the garlic cloves extracted with the cool water extraction method significantly suppressed *Alternaria* spp. isolates even at its low concentrations.

The active compounds of garlic (sulfur compounds) might destroy the fungal cells resulting in decreasing the oxygen uptake, reducing cellular growth, inhibiting the synthesis of lipids, proteins and nucleic acids, changing the lipid profile of the cell membrane and inhibiting the synthesis of the fungal cell wall (Gupta and Porter, 2001).

Data in Tables 10 and 11 show that, the mycelial growth of *Alternaria* spp. isolates was completely inhibited at 1000 ppm of Cure-Plus. On the other hand, these isolates were moderately affected by the high concentrations of Kocide 2000 and Kema-Z even at 1000 ppm, respectively. However, the high tested concentrations of such fungicides inhibited the fungal spore germination. These findings were similar to those of Moharram *et al.* (2004).

However, the mycelial growth and the obtained data also revealed that there was significant difference between the tested fungicides as well as their concentrations where Cure-Plus resulting significant suppression of *Alternaria* spp. isolates even at its low concentrations. The inhibition effect of Kema-Z might be due to its effect on the development of the germ tubes, the formation of appressoria and the growth of mycelia. On the other hand, Cure-Plus contained metalaxyl which inhibit protein synthesis in fungi by interference with the synthesis

Table 8. Mycelial growth reduction percentages of the different *Alternaria* spp. isolates by plant extracts at various concentrations, *in vitro*

Extract(A)	Pathogen e.m.(B)	*Alternaria* sp. No. 3				*Alternaria* sp. No. 4				Mean
		10	20	30	mean	10	20	30	mean	
Garlic	Cool	100	100	100	**100**	100	100	100	**100**	**100**
	Hot	0.00	10.33	10.73	**7.02**	10.73	31.47	44.43	**28.88**	**17.95**
	organic	5.50	20.33	22.20	**16.01**	11.10	24.80	27.67	**21.19**	**18.6**
Artemisia	Cool	5.67	11.10	11.10	**9.29**	0.00	15.50	21.07	**12.19**	**10.74**
	Hot	0.00	6.60	7.73	**4.78**	5.13	25.50	32.97	**21.20**	**12.99**
	organic	0.00	5.13	7.87	**4.33**	0.00	0.00	3.30	**1.10**	**2.72**
Marjoram	Cool	0.00	4.40	5.50	**3.30**	0.00	3.67	5.13	**2.93**	**3.12**
	Hot	0.00	0.00	0.00	**0.00**	0.00	9.23	11.10	**6.78**	**3.39**
	organic	0.00	4.40	5.13	**3.18**	0.00	6.97	8.83	**5.27**	**4.22**
Camphor	Cool	11.07	15.90	16.23	**14.40**	10.73	20.30	21.83	**17.62**	**16.01**
	Hot	0.00	0.00	0.00	**0.00**	5.50	18.10	20.73	**14.78**	**7.39**
	organic	10.73	15.13	15.50	**13.79**	4.40	13.67	16.60	**11.56**	**12.67**
Control	Cool	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	**0.00**	**0**
	Hot	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	**0.00**	**0**
	organic	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	**0.00**	**0**
Mean		**8.86**	**12.89**	**13.47**	**11.74**	**9.84**	**17.95**	**20.91**	**16.23**	**13.99**
F.test		**	**	**		**	**	**		
AxLxG		**	**	**		**	**	**		
L.S.D at 5% (A)		1.78	1.78	1.78		3.04	3.04	304		
L.S.D at 5% (C)		0.69	0.69	0.69		2.35	2.35	2.35		
L.S.D at 5% (B)					0.69				2.35	

Whereas: Cool= Cool water extraction, Hot= Hot water extraction, Organic= Organic solvent extraction (ethyl alcohol 95%), e.m. = extraction method and C = the different concentrations (10, 20 and 30%).

Table 9. Effect of plant extracts on the spore germination of *Alternaria* spp. isolates, *in vitro*

Pathogen / Bio-agent (A)	*Alternaria* sp. No. 3			*Alternaria* sp. No. 4			Mean
	Cool	Hot	Organic	Cool	Hot	Organic	
Garlic	100	12	32	100	52	44	**56.67**
Artemisia	24	0	0	40	8	0	**12**
Marjoram	0	0	0	0	40	0	**6.67**
Camphor	12	0	8	28	12	16	**12.67**
Control	0	0	0	0	0	0	**0**
Mean	**27.2**	**2.4**	**8**	**33.6**	**22.4**	**12**	
LSD at 5% (A)	2.55	2.55	2.55	4.15	4.15	4.15	
LSD at 5% (B)	1.98	1.98	1.98	3.22	3.22	3.22	
A x B	**	**	**	**	**	**	
F test	**	**	**	**	**	**	

Whereas: Cold= Cold water extraction, Hot= Hot water extraction, Organic= Organic solvent extraction (Ethyl alcohol 95%) and B = different extraction methods.

Table 10. Effect of different fungicides on the mycelial growth of the different *Alternaria* spp. isolates, *in vitro*

Fungicide(A)	Pathogen Con(B)	*Alternaria* spp. isolates		Mean
		No. 3	No. 4	
Kema-Z	50	6.26	0	**3.13**
	100	9.63	0	**4.815**
	250	14.06	27.77	**20.915**
	500	28.14	44.44	**36.29**
	1000	29	44.44	**36.72**
	Control	0	0	**0**
Cure-Plus	50	32.22	27.77	**29.995**
	100	55.18	64.44	**59.81**
	250	72.59	79.62	**76.105**
	500	86.66	86.66	**86.66**
	1000	100	100	**100**
	Control	0	0	**0**
Kocide 2000	50	15.92	10.37	**13.145**
	100	18.51	16.66	**17.585**
	250	34.44	43.33	**38.885**
	500	51.11	71.48	**61.295**
	1000	65.55	77.03	**71.29**
	Control	0	0	**0**
Mean		**34.4**	**38.56**	
LSD at 5% (A)		2.53	1.44	
LSD at 5% (B)		2.82	1.61	
A x B		**	**	

Whereas: Con. = concentration by ppm.

Table 11. Effect of different fungicides on the spore germination of the different *Alternaria* spp. isolates, *in vitro*

Pathogen	*Alternaria* spp. isolates								
	No. 3				No. 4				Mean
Fungicide (A)	100	250	500	1000	100	250	500	1000	
Kema-Z	30	50.3	100	100	83	95	100	100	**82.29**
Cure-Plus	37	90	100	100	90	100	100	100	**89.63**
Kocide 2000	10	37.5	90	100	30.3	54	100	100	**65.23**
Control	0	0	0	0	0	0	0	0	**0**
Mean	**19.3**	**44.5**	**72.5**	**75**	**50.8**	**62.3**	**75**	**75**	
LSD at 5%(A)	1.95	1.95	1.95	1.95	3.35	3.35	3.35	3.35	
LSD at 5%(B)	1.95	1.95	1.95	1.95	3.35	3.35	3.35	3.35	
A x B	**	**	**	**	**	**	**	**	
F test	**	**	**	**	**	**	**	**	

Whereas: B = the tested concentrations.

of ribosomal RNA and copper oxychloride having a copper-II ion (Cu^{++}) which could be taken up by the spores during the germination and accumulates until a sufficiently high concentration is achieved to destroy the spore cell as well as its germ tube. Kocide-2000 which contains copper hydroxide can inhibit respiration of the fungal cells. Its copper ions also do the previously mentioned action against the fungal spores (Anonymous, 2002).

Alternaria spp. isolate Nos. 3 and 4 were found to be able to cause leaf spot disease in the presence of the different tested fungicides. Data in Table 12 show that leaf spot disease caused by the isolate No. 3 was completely managed by Kema-Z, Cure-Plus and *Trichoderma album* when applied at the T1 and T2 times when detached leaflets were tested. The same disease was moderately controlled by *T. harzianum* when applied at the T1 and T2 times, while it was not affected with the other control methods (garlic and camphor extracts as well as *Bacillus* sp. No. 4 and 9).

The leaf spot disease which caused by *Alternaria* sp. isolate No. 4 was not significantly affected by the tested fungicides. In general, Kema -Z and Cure-Plus were found to be the most effective fungicides in controlling Alternaria leaf spot disease on the attached leaflets, which caused by *Alternaria* sp. isolate No. 3, while Cure-Plus and the bio-agent *T. album* were the most effective ones in controlling the same disease on the detached leaflets which caused by the same isolate. On the other hand, Cure-Plus and *T. harzianum* were found to be the most effective agents in controlling the disease on the attached leaflets, which caused by isolate No. 4. Kema-Z and Cure-Plus were the most effective in controlling the disease on the detached leaflets, which caused by the same isolate.

The treating time of the tested control methods is important in reducing Alternaria leaf spot severity. These results were agreed with El-Morsi (2004) who said that treating with the control methods one hour before pathogen inoculation caused more reduction in the disease severity than treating three days after inoculation. Such results agreed with those reported by Molan *et al.* (2003). The explanation of the obtained results might be due to the killing effect of the control methods which keep the plant surface free of the infectious spores.

Table 12. Effect of different control methods on the severity of the date-palm leaf spot disease, caused by different *Alternaria* spp. isolates

Pathogen	*Alternaria* sp. Isolate No. 3								*Alternaria* sp. Isolate No. 4							
	Detached leaflets				Attached leaflets				Detached leaflets				Attached leaflets			
Treatment	T1	T2	T3	Mean	T1	T2	T3	Mean	T1	T2	T3	Mean	T1	T2	T3	Mean
Kema-Z	17.67	10.1	6.17	11.31	100	79	76.9	85.3	8.9	1.4	0	3.43	10.33	6.95	0	5.76
Curoplus	39.4	28.9	14.5	27.6	100	100	93.01	97.67	25	26.9	0	17.3	19.3	31.97	23.7	24.99
Garlic	0	8.9	0	2.97	29.07	69.7	3.6	34.12	0	0	0	0	4.43	18.83	13	12.09
Camphor	0	0	0	0	0	3.1	0	1.03	0	0	0	0	0	0	0	0
Trichoderma album	0	29.4	12.4	13.93	100	100	25.9	75.30	2.5	6.4	0	2.97	16.63	23.3	0.4	13.4
T. harzianum	0	1.1	0	0.37	86.5	70	25	60.5	0	0.4	0	0.13	27.33	22	0.33	16.56
Bacillus sp. 4	0	0	0	0	0	0	0	0	0	0	0	0	9.4	16.67	0	8.69
Bacillus sp. 9	0	0	0	0	0	0	0	0	0	0	0	0	7	11.03	0	6.01
Mean	7.13	9.8	4.13	7.02	51.95	52.73	28.05	44.24	4.55	4.39	0	2.98	11.8	16.34	4.68	10.98
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LSD at 5%	5.53	5.68	4.16		2.25	2.07	5.33		1.79	2	0		5.04	8.15	4.48	

Whereas: the T1, T2 and T3: the different application times of the control agents.
T1= the control agent was applied one week before fungal inoculation.
T2 = the control agent and the fungal inoculum were applied at the same time.
T3= the control agent treating time was delayed one week after fungal inoculation.

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دراسات باثولوجية علي مرض تبقع الاوراق الالترناري في نخيل البلح في شرق الدلتا

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يعتبر نخيل البلح واحد من أهم أشجار الفاكهة في مصر والوطن العربي نظرا لأهميته الاقتصادية المستمدة من محصوله وكذا من الصناعات القائمه علي المنتجات الثانويه من تلك الاشجار كالسعف والجذع وخلافه. تصاب تلك الاشجار بالعديد من الامراض والافات الضارة التي تؤدي الي حدوث خسائر اقتصاديه متفاوته علي النخل والتي قد تصل إلى تدمير النخلة باكملها. ولعل من أهم تلك الأمراض امرض تبقعات الأوراق التي تقلل المسطح الأخضر المسئول عن التمثيل الضوئي ومن ثم محصول البلح. لذا قامت تلك الدراسه لحصر مرض تبقع الأوراق الالترناري المنتشر في بعض محافظات شرق الدلتا بمصر وكذلك عزل المسبب المرضي وايجاد طرق مقاومه المرض حيويا او كيميائيا تحت ظروف المعمل والحقل.

لوحظ خلال دراسات الحصر التي تم اجراؤها خلال شهر يوليو لعام 2004 انتشار مرض تبقع الاوراق الالترناري علي اشجار نخيل البلح المنزرعه في المراكز المختلفه التابعه لمحافظات الشرقية والإسماعيلية والدقهليه. ومن خلال العزل من العينات المصابه تم الحصول علي عدة عزلات من الفطر الترناريا. وباختبار قدرتها المرضيه تبين أن لها القدرة علي إحداث مرض تبقع الاوراق. اثبتت الدراسه ايضا امكانيه مكافحه الفطر الممرض معمليا باستخدام العديد من عوامل المكافحه الحيويه مثل بعض انواع الفطر تريكوديرما والبكتريا باسيللس بالاضافه الي مستخلصات نباتات الثوم والكافور وكذلك مبيدات الكيما-زد والكيوربلس والتي قامت اما بتثبيط نموه الميسيليومي أو وقف إنبات جراثيمه. أما علي المستوي الحقلي فكان لمبيد الكيوربلس والكيما-زد وكذلك انواع الجنس تريكوديرما القدرة علي خفض شدة الاصابه بمرض تبقع الاوراق الالترناري بشكل ملحوظ.