

PESTALOTIA PALMICOLA ON DATE PALM LEAVES IN EGYPT

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Abstract

A new leaf spot disease symptom was observed on leaves of date palm plants (*Phoenix dactylifera* L.) twice. The first one in beginning year 2001, Barhee cv. resulted from tissue culturing from Bahteem Farm, Qalubya governorate, Egypt and the second one during April, 2004 on plants grown in greenhouse of Central Lab. Res. Development of date palm, A.R.C. Giza governorate, Egypt. Symptoms observed as brown colored spots with yellowish margins. Several spots coalesced to each other and cover large portions on the leaf, finally complete discoloration and dead. The causal pathogen was isolated and identified based on cultural characteristics and light microscope examination as *Pestalotia palmicola* and confirmed its pathogenicity to cause the disease. No significant differences between Bahteem isolate and Central Lab. isolate in disease severity. Host range tests revealed that, most tested ornamental palms Latan palm (*Latania* sp.), Canary island date palm (*Phoenix canariensis*), Mexican fan palm (*Washingtonia robusta*), Coconut palm (*Cocos nucifera*), Royal palm (*Roystonea regia*), Cabbag palmetto (*Sabal palmetto*) and Seaforthia palm (*Seaforthia elegans*) were also attacked by *Pestalotia palmicola*. However, disease severity and disease symptom varied according to plant species.

This is the first report about possibility *Pestalotia palmicola* to attack host range of ornamental palms under Egyptian condition. Royal palm was high susceptible comparison in other tested palms while California fan palm was least susceptible. The relationship between the infection and leaf age showed that, the older leaves (pinnae or leaflet) in adult palm and leaves of tissue culture plants were more susceptible infection than the new leaves of adult palm. Topsin M 70 showed the best effect against the causal pathogen

followed by copper oxychloride, kocide 101 , antracol and kanz while kocide 2000 revealed the lowest effect. Three commercial biofungicides namely AQ10 (*Ampelomyces quisqualis*), Bio-Zaid (*Trichoderma album*), Bio-Arck (*Bacillus megaterium*) and two bioagent isolates namely, *Trichoderma harzianum* and *Bacillus subtilis* were found to be efficient in suppressing Pestolotia leaf spot of date palm caused by *Pestolotia palmicola*. Also they are tested *in vitro* to determine their efficacy in lysing the cell wall of the pathogen. A remarkable relationship between the disease reduction of bioagents and their level of production of mycolytic enzymes viz. Chitinase and β -1, 3-glucanase.

Key words: *Ampelomyces quisqualis*, *Bacillus megaterium*, *Bacillus subtilis* chitinase and β -1, 3-glucanase, Date palm, Leaf spot, ornamental palms, *Pestolotia palmicola*, *Trichoderma album* and *Trichoderma harzianum*.

INTRODUCTION

Leaf spot disease does not kill plants immediately, but crop losses increase gradually with the age of planting. The decrease in functional leaf area caused by the disease results in a reduction in the quality and quantity of yield. *Pestolotia* leaf spot disease caused by *Pestolotia palmicola*, one of the foliage diseases infect wild range of fruit and woody trees as well as ornamental plants (Uchida, 2004). Date palm (*Phoenix dactylifera* L.) is one of important fruit crops planted in Egypt and Arabian lands. Tissue culture technique is important to production of the important varieties of date palm. *Pestolotia* leaf blight caused by *Pestolotia palmicola* attack date palm, coconut palm and washingtonia palm in Florida (El-Sawah, 1965). *Pestolotia palmicola* was recorded for the first time on leaves of date palm trees in Egypt by Rashed, 2001. Obazee and Ikozun (1985). brown leaf spots were surrounded by a chlorotic halo. With age the centre of the spots turned grey and fell out, resulting in shot holes. Noriega, *et al.* (1991) [*Pestalotiopsis*] *palmarum*), very common in all the orchards, and associated with the damage caused by the insect (*Homaledra* sp.). The worst damage was observed in palms not yet in production. Pitta (1994) found that [*Pestalotiopsis*] *palmarum* was isolated and its pathogenicity confirmed by inoculation in the glasshouse. *Pestalotia palmarum* [*Pestalotiopsis palmarum*], one of the most common palm diseases in Brazil Uchida (2004) mentioned that *Pestolotia* leaf spot disease is common on date palm and many of ornamental palm in the world. He also confirmed that fungus *Pestalotiopsis* causes leaf spots, petiole/rachis blights and sometimes a bud

rot of palms. In other words, unlike the other leaf spot and petiole blight pathogens, which attack either the leaf blade or the leaf petiole, *Pestalotiopsis* attacks all parts of the leaf from base to tip. It is also one of the more ubiquitous fungi in the palm canopy, and is easily isolated from healthy palm tissue. Zuart-Macias, *et al.* (1999) In Chiapas, Mexico, the palm *A. mexicana* is used for its sap (beverage), flowers (ornamental and edible), fruits and buds (both edible). They added that, a new disease, which first appeared in 1986, has killed many of the adult trees. A yellowish color appears in the apex of the old leaves and progresses towards the apical bud. The incidence and etiology of the disease were studied. Two species of *Pestalotia*, one isolated from leaf tissue and another from the internal tissues of the rachis were always found to be associated with diseased plants. The new leaves were more susceptible to infection by *Thielaviopsis paradoxa* than the old leaves (Rashed,1998) while the old leaves were more susceptible to infection by *graphiola phoenicis* than the new leaves

Chemical control using fungicides is still until now the main method for controlling leaf spots disease of date palm in different countries. Chemical treatments to protect fruit trees from infection by certain diseases are routinely applied each year. Many phytopathologists used chemical fungicides to control the disease. Ramaswamy, *et al.* (1989) resulted that, in field trials, the best control of *Pestalotia psidii* on guava was given by Difolatan [captafol], followed by Dithane M-45 [mancozeb]. (Djerbi, 1999) revealed that Methyl thiophanate and new copper-based fungicides were used to control date palm diseases as black scorch, diplodia diseases and *Graphiola* leaf spot. They added that, Topsin M70 at 10 ppm and kocide 101 at 100 ppm recorded complete inhibition on mycelia growth of *Pestalotia palmicola*.

Many bacterial genera as well as *Traichoderma* spp. had a good bioagent activity against wide range of oomycetes and others Elad *et al.* (1982), Buchenauer (1998) and Rajan *et al.* (2002). Kamhawy(2006) found the commercial biofungicides efficient in suppressing banana leaf spot disease caused by *Phyllosticta* sp. He also added that all tested biofungicide prevent disease incidence when applied biofungicide and pathogen at the same time or pathogen a day later.

The aim of this work was to determine possible inoculum sources of the disease especial on date and ornamental palm, so as to be able to plan to method to measures control.

MATERIALS AND METHODS

Source of *Pestalotia palmicola* culture

Two isolates of *Pestalotia palmicola* were used in these studies. The first one (Bahteem isolate) was obtained by Rashed (2001) from infected date palm off- shoots

leaves (*Phoenix dactylifera* L.) Barhee cv. and the second isolate (Central Lab. Isolate) was obtained season 2004 by isolation from infected samples of cv. Barhee date palm off-shoots after acclimation stages from greenhouse of Central Lab. Res. Development of date palm,, Agriculture Research Center, Egypt. Infected samples of cv. Barhee date palm off-shoots were collected. Samples for isolation were taken from the primary leaves of date palm off-shoots. Diseased samples were cut to small portions and were surface sterilized in 0.5% NaOCl solution for 2min. washed in sterilized water and transferred onto potato dextrose agar (PDA) medium in Petri dishes. Plates were incubated at 25-27 °C for 7 days. The emerged fungus was sub cultured, then single spore was picked up and transferred to PDA for the identification that was made according to taxonomic system of Barnett and Hunter(1972) and confirmed at the Mycol. and Dis. Survey Res. Dept., Agric Res. Center, Giza .

Pathogenicity tests

Preparation of pathogen inoculum *Pestalotia palmicola* inoculum was prepared as previously described by KerChung and Hoch, 1996 in brief, with some modification, spores of each isolate grown in plate contained P.D.A.medium (10 days ago at 25-27 °C) . were harvested in 10 ml sterile distilled water amended with 0.3% Tween 80 by gently rubbing the colonies with a glass rod, the plates then left on rotary for one hour. The resulted spore suspension was strained through two layers of cheesecloth to remove mycelial clumps. Spores concentration was determined and adjusted with a hemacytometer to reach 10^6 spore / ml. approximately.

Test plants

Tow years old date palm plants. Originated from seeds of local date palm cv. Siwi, grown in plastic pots contained mixture of sand and clay soil (1:1 v/v) were used. Healthy leaves of date palm plants were surface sterilized with 1.5%mercuric chloride and then rinsed with sterile distilled water before treatment. Inoculation was performed by dusting the leaves with 600 mesh carborandum powder Then sprayed using an atomizer to the point of suspension run off. Inoculated plants were covered with large plastic bags to maintain high humidity for five days. Control plants were similarly treated but with only sterile distilled water. Five plants were used for each treatment. Inoculated seedlings were kept under greenhouse condition at 25 °C. and symptoms were recorded 7,15,21 days after inoculation. Disease severity rate (DSR) were determined using the formula suggested by Chastanger and Ogawa (1979) as follows: $DSR = \frac{\sum (n \times v)}{N} \times 100$. where N= total number of infected leaves, n= number of leaves per category and v= category number witch expresses as follows:
0 = No symptoms

- 1 = Number of spots ranged 1 -25% of leaf
- 2 = Number of spots ranged 25 -50 % of leaf
- 3 = The spots were collected and covered from 50 –75% of leaf
- 4 = Over 75% of leaf was already infected.

Host Range

Different hosts of ornamental palms were tested for susceptibility to *Pestotia palmicola* i.e. date palm tissue culture plants , Royal palm (*Roystonea regia*), Latan palm (*Latania* sp.), Canary island date palm (*Phoenix canariensis*), Cabbag palmetto (*Sabal palmetto*),Seaforthia palm (*Seaforthia elegans*), Coconut palm

(*Cocus nucifera*) and Mexican fan palm (*Wachingtonia robusta*). The Common and Latin names according to Chase and Broschate eds(1991) Each four leaves from three plants (2 years old) of ornamental palm were inoculated with the fungus as shown previously . Disease reading were recorded by the measured the infected area after 15days from inoculation On the other hand, severity of artificial infection on individual leaves was determined as the length of the lesions in mm.

Effect of Leaf age

Four seedlings 2years old were inoculated with *Pestotia palmicola* as mentioned previously at four external and four internal leaves. Seedling were covered with plastic pages and disease readings were assessed after 15 days of inoculation. Four primary leaves of tissue culture plants of date palm were Inoculated in the same time to compare with he old (external) and new (internal) leaves of date palm seedlings.

Disease control

Chemical control

Disease control experiments were carried out at A.R.C.,Giza under greenhouse at Berhee var., 2years old . Five fungicides i.e Antracol, Kocde 2000 (Copper Hydroxid D.F.),Unicopper (Copper Oxychloride), Kocide101 (Copper Hydroxid W.P.), Topsin M 70(Thiophanat methyl) and organic as Kanz (Gogoba oil).

Each 10 plants (natural infection)were sprayed with one material of fungicides. Data were recorded before and after spraying according to the scale of disease severity rate. Disease incidence and decrease in disease incidence(Pv)were calculated 15 day after spraying according to the following equation suggested by Munkovold and Marios (1993). $Pv = \frac{Ie - Iv}{Ie} \times 100$. Whereas:

Pv =Decrease(%) in disease incidence.

Ie = Proportion of disease incidence in each treatment.

Iv = Proportion of disease incidence in each treatment.

Proportion of disease incidence was calculated as differences between before and after spraying in each treatment.

Biological control

Biological control is considered one of the most prospective methods to control various diseases that affect fruit production. The possibility to use this method to control leaf spot diseases as the safe and might be cheap method of control were investigated.

Evaluation of commercial biofungicides and two isolates of bioagent on the control of *Pestalotia palmicola* leaf spot:

The efficiency of three tested biofungicides prepared by the commercial companies, namely, AQ10 (*Ampelomyces quisqualis*), Bio-Zaid (*Trichoderma album*) and Bio-Arc (*Bacillus megaterium*) as well as *Trichoderma harzianum* isolate and *Bacillus subtilis* isolate obtained kindly from Department of Botany, Faculty of Agric., Zagazig Univ. for controlling date palm leaf spot disease caused by *Pestalotia palmicola* under greenhouse condition. The bio fungicides were prepared at rate of recommended doses of producing company, *Trichoderma harzianum* used at the same rate of *Trichoderma album* and *Bacillus subtilis* used at the same rate of *Bacillus megaterium*. Date palm plants Berhee var., 2years old ago, were used in this study. The experiment was achieved according to the following regimen: spray the biofungicide one day before inoculation, plants were inoculated with the causal pathogen as mentioned before and sprayed with the tested bio fungicides at the same time of inoculation as well as 15 days after inoculation. All plants were covered with sterile cellophane bags for one week to maintain high relative humidity for spore germination. The bags were then removed when control plants exhibited disease symptoms to expose the plants to ambient environmental conditions for 30 days. Three replicates were used for each treatment. Each replicate contained three plants

Decrease in disease severity (Ps) = (Ic – Iv / Ic) 100 where: Ps=disease reduction %, Ic= disease severity before application (control treatment) and Iv = disease severity after application

Determine Mycolytic enzymes produced by Bio fungicides against *Pestalotia palmicola*,

Three isolates microorganisms of biofungicides namely *Ampelomyces quisqualis*, *Bacillus megaterium* and *Trichoderma album*., were obtained by cultured spor suspension of the commercial biofungicides used previously, *Trichoderma harzianum* isolate and *Bacillus subtilis* isolate obtained kindly from Department of Botany, Faculty of Agric., Zagazig Univ. to determine Mycolytic enzymes viz. β -1,3-glucanases and chitinase produced by bioagents against *Pestalotia palmicola*, the leaf spot pathogen of date palm

6-1.Determination of Chitinases

Isolates of tested biofungicides were cultured in 250 ml conical flasks containing 50 ml of chitin-peptone medium for bacterial isolates (glucose 0.5% peptone 0.2%, colloidal chitin 0.2%, K₂HPO₄ 0.1%, MgSO₄ · 7H₂O 0.05% and NaCl 0.05%, pH 6.8) (Lim *et al.*, 1991) or in liquid Czapek-Dox containing 0.2 % sucrose for fungal isolates at 28°C for 96 h in a rotary shaker incubator. After the incubation period the cultures were centrifuged at 12,000 r.p.m. for 20 min at 4°C and the supernatant was used as enzyme source. Colloidal chitin was prepared from crab shell chitin (Sigma) according to Berger and Reynolds (1958). The reaction mixture contained 0.25 ml of enzyme solution, 0.3 ml of 1M sodium acetate buffer (pH 5.3) and 0.5 ml of colloidal chitin (0.1%) and incubated in a water bath at 50°C for 4 hours. Chitinase activity was determined by measuring the release of reducing sugars by the method of Nelson (1944). One unit of chitinase was determined as 1 nmol of reducing sugar released per hour per ml

6-2. Determination of β -1,3-glucanase

Bacterial isolates were grown in 250 ml conical flasks containing 50 ml of peptone medium and fungal isolates were grown in liquid Czapek-Dox medium. Both media contained laminarin (0.2%) (from *Laminaria digitata*, Sigma) (Lim *et al.*, 1991), incubated at 28°C for 3 days for bacterial isolates and one week for the fungal isolates on a rotary shaker incubator. Then the cultures were centrifuged as mentioned previously and the resulted supernatant filtered through 0.22 μ m Millipore filters and preserved to be use as enzyme source. The reaction mixture contained 0.25 ml of enzyme solution, 0.3 ml of 0.1M phosphate buffer (pH5.5) and 0.5 ml of laminarin (0.2%) (Lim *et al.*, 1991), then incubated at 40°C for 2 h in a water bath. One unit of B-1,3- glucanase activity was determined as 1 nmol of glucose released per hour per ml.

RESULTS AND DISCUSSION

In 2001, Date palm (tissue culture plants) showing leaf spots symptoms were observed on 10% of plants grown under greenhouse in Bahteem Farm (Agric. Res. Center). The fungus was recorded in the second international conference on date palm (Al-Ain, United Arab Emarates, March 25 – 27. 2001. In 2004, the same symptoms were observed on 16% of plants grown in A.R.C. Under greenhouse of Central Lab. Res. Development of date palm. The symptoms were started as small round and fusiform or elliptical lesions brown in outline area on both leaf surface. on the leaves as shown in These lesions were increased in size and numbers during spring and summer. Several

spots coalesced to each other and cover large portions on the leaf, which gives a blighted appearance as shown in Fig.1, A&B and C. These results were in harmony with Obazee and Ikozun (1985)

Pathogenicity tests

Pestalotia palmicola isolate were used to investigate the pathogenic capabilities on leaves of date palm seedling one year ago . Data in(Table,1) revealed that, both *Pestalotia palmicola* isolate was able to induce symptoms similar to those observed under natural condition after (Fig., 2A&B).Moreover, the same fungus was re isolation from the different inoculated leaves and found to be identical with that used in artificial inoculation. The most studies on this disease were described the fungus and symptoms only. These results were agreement with Uchida (2004) He reported and demonstrated that the fungus usually requires wounds for the plant penetration (infection) necessary for disease development. It is not uncommon to isolate *Pestalotiopsis* and another pathogen

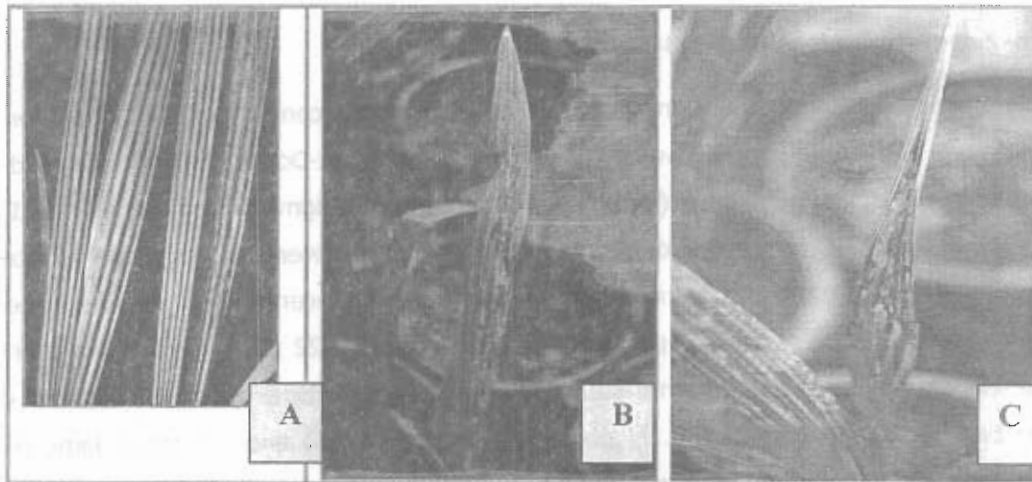


Fig. 1. IA&B and C. Typical symptoms of Natural infection with *Pestalotia* leaf spot disease of date palm leaves caused by *Pestalotia palmicola* (a) Notice small round and fusiform or elliptical lesions brown in outline area on both leaf surface (b) increase in size and numbers during spring and summer. Spots coalesced to each other and cover large portions on the leaf, which gives a blighted appearance (c) from the same diseased tissue. In most cases, it is likely the other pathogen was the primary pathogen (the fungus that invaded the healthy plant tissue first), and *Pestalotiopsis* invaded via the wound created by the primary pathogen

Table 1. Virulence of tow *Pestalotia palmicola* isolates on date palm off-shoot

Codes of <i>Pestalotia</i> <i>palmicola</i> Isolates	% Disease severity , after (days)			Means
	7	15	21	
Bahteem isolate	17.4	26.3	34.0	25.9
Central Lab. isolate	15.7	27.0	32.8	25.17
Control (water treatment)	0.0	0.0	0.0	0.0
L.S.D at 0.05: Days(D)=0.32 Isolates (I)=0.3 Lesio(I)= 0.3 D x I =1.11 D x L =3.31 I x L =1.92 D x I x L =1.22				

Host range

Table (2) show that most the tested hosts from ornamental plants were susceptible to the pathogen. Symptoms on the leaves of plants appeared as brown spots and necrosis of tissue. Royal palm was high susceptible comparison in other tested palms while California fan palm was least susceptible. Ramaswamy, *et al.* (1989) reported that, Isolates of the pathogen from guava infected coconut palm, mango Eucalyptus, *Achras sapota* [*Manilkara zapota*], *Artocarpus*

Table 2. Effect of *Pestalotia palmicola* on some ornamental palms under greenhouse condition

Scientific name	Common name	Area of infection (mm)*
<i>Latania sp</i>	Latan palm	196.6
<i>Phoenix canariensis</i>	Canary island date palm	143.3
<i>Wachingtonia robusta</i>	Mexican fan palm	9.3
<i>Cocus nucifera</i>	Coconut palm	57.3
<i>Roystonea regia</i>	Royal palm	233.3
<i>Sabal blacbournian</i>	Cabbag palmetto	102.6
<i>Seaforthia elegans</i>	Seaforthia palm	58.3

* Area of infection (mm) : length x width / 2 after 10 days (3 replicates)

heterophyllus and *Litchi chinensis* in inoculation tests. Such a non- host specific of the pathogen provides a variety of sources for inoculum in nature Noriega, *et al.* (1991) and Uchida (2004)

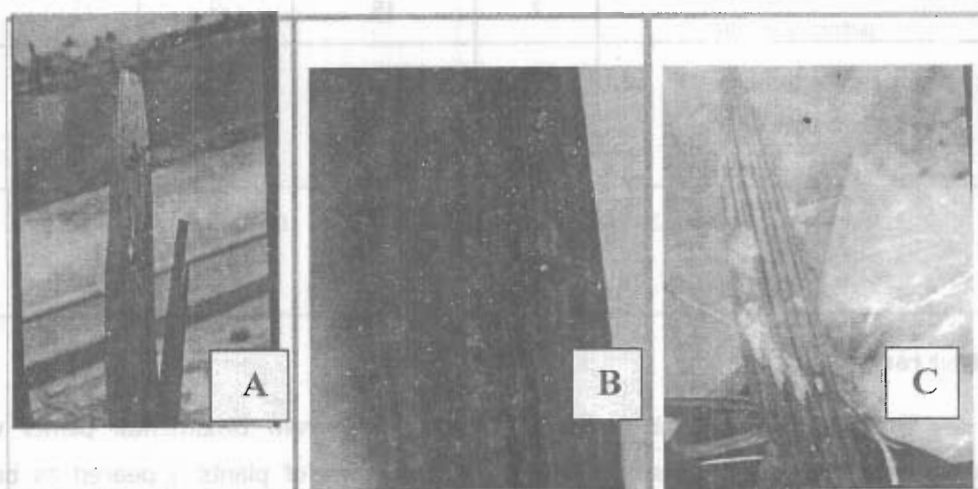


Fig. 2: 2A, 2B and 2C. Typical symptoms of *Pestalotia* leaf spot disease of date palm leaves resulting from artificial inoculation with *Pestalotia palmicola*. After 7days (A) and more than 21 days (C). B, Close-up of typical symptoms of *Pestalotia* leaf spot disease of date palm on leaf.

Effect of Leaf age

Data shown in table (3) indicate that the old leaves of date palm were more susceptible to infection with the pathogen than the new leaves of adult palms and leaves of tissue culture plants at the same level for susceptible of infection approximately

Table 3. Effect of leaf age on infection with *Pestalotia palmicola*

Type of leaf	Area of infected lesion(mm)
New leaves	147.3
Old leaves	633.3
Leaves of tissue culture	166.6

Biological control

The biofungicides found efficient in suppressing date palm leaf spot disease caused by *Pestalotia palmicola*. all tested biofungicide prevent disease incidence when applied biofungicide and pathogen at the same time or pathogen a day later. Data in (Table, 4) reveal that Bio-Zaid (*Trichoderma album*) were the best biofungicides for controlling leaf spot which recorded the highest percent decrease in disease severity being 83.76 % followed by *Bacillus subtilis* and *Trichoderma harzianum* being

recorded 81.65 and 80.37 % respectively, while *Ampelomyces quisqualis* (AQ10) at the least. Data in table, 4 also indicated that the differences between Bio-Zaid (*Trichoderma album*), *Bacillus subtilis* and *Trichoderma harzianum* were non significant

Table 4. Effect of different biofungicides with the recommended dose in controlling Pestalotia Leaf Spot (PLS) on date palm.

Tested biofungicides	Pathogen first, biofungicide a 15 day later			
	*D.S. before treatment (%)	D.S. after treatment (%)	increscent I in D.S. (%)	Reduction in D.S. (%)
<i>Bacillus megaterium</i> (Bio-Arck)	23	30.16	6.84	78.76
<i>Trichoderma album</i> (Bio-Zaid)	21.44	26.66	5.22	83.79
<i>Ampelomyces quisqualis</i> (AQ10)	27	34.59	7.59	76.43
<i>Trichoderma harzianum</i>	22	28.32	6.32	80.37
<i>Bacillus subtilis</i>	21	26.91	5.91	81.65
Control (Water)	26.38	58.59	32.21	0

L.S.D. at 5% level for: bioagent (Ba)= 3.00 & Disease severity (DS)= 4.11 (Ba x Ds) = 4.9

*D.S.=Disease severity

Estimation of β -1,3-glucanases and Chitinase activity

The antagonists found efficient in suppressing date palm leaf spot disease caused by *Pestalotia palmicola*. were tested *in vitro* for their efficacy in lysing the cell wall of the pathogen. The antagonists produced mycolytic enzymes viz. β -1,3-glucanases, and chitinase. Variations among the isolates were found for the production of these enzymes. The findings demonstrate mechanism of antagonism by the tested biofungicides through production of pathogen cell wall lysing enzymes, chitinase and β -1,3-glucanases. Data in Table (5) indicate clearly that, all tested biofungicides released Chitinase and β -1,3-glucanases enzymes varied according to bioagent when grown in mixed culture with *Pestalotia palmicola*. In this respect *Bacillus megaterium* recorded the highest chitinase activity being 0.374 followed by *Trichoderma harzianum* and *Ampelomyces quisqualis* being recorded. 0.346 and 0.314 unit respectively and, while *Trichoderma album* at the least

Table 5. Chitinase , β -1,3-glucanases activity produce by bioagents after 3days incubation in peptone medium

Tested Bio fungicide	Chitinase activity unit/ml/h.	β -1,3-glucanases unit/ml/h.
<i>Bacillus megaterium</i>	0.374	0.178
<i>Trichoderma album</i>	0.201	0.396
<i>Ampelomyces quisqualis</i>	0.314	0.160
<i>Trichoderma harzianum</i>	0.346	0.210
<i>Bacillus subtilus</i>	0.270	0.198

On the other hand *Trichoderma album* recorded the highest β -1,3-glucanases activity being 0.396 unit followed by *Trichoderma harzianum* and *Bacillus subtilus* being recorded 0.210 and 0.198 unit respectively. Chitin is an unbranched polysaccharide composed primary of beta 1,4 linked N- Acetyl glucose amine residues. It can be regarded as a cellulose analog, in which the hydroxyl groups have been replaced by N-Acetyl glucoseamino groups. The degradation of chitin is catalyzed by chitinases, which hydrolyze chitin to chitodextrins. Sundheim (1992). El-Tarabily *et al.* (2000). Many bacterial genera as well as *Trichoderma* spp. had a good bioagent activity against wide range of oomycetes and others Elad *et al.* (1982), Buchenauer (1998) and Rajan *et al.* (2002)

Data presented in Table (6) reveal that topsin was the most effective fungicides against Pestalotia Leaf Spot (PLS) being recorded 91.66 followed by Copper oxychlorid being recorded 75% as reduction in disease incidence, while kanz at the least being 33.33. The differences between topsin and other tested fungicides were highly significant. These differences between the fungicides tested in their fungicidal effect on the pathogenic fungus tested might be due to mode or degree of the antagonistic action of the fungal cell to specific fungicides (Watkins *et al.*, 1977) and chemical composition of the fungicides (Carnegie *et al.*, 1990).

Table 6. Effect of some fungicides with the recommended dose in controlling Pestalotia Leaf Spot (PLS) on date palm under natural infection.

Tested fungicides	Pathogen first, biofungicide a 15 day later			
	*D.S. before treatment (%)	D.S. after treatment (%)	I increment	Reduction in D.S. (%)
Kenz	15	23	8	33.33
Kocid 101	15	20	5	58.33
Topsin M70%	15	16	1	91.66
Antracole	16	22	6	50
Kocid 2000	16	25	9	25
Copper oxychlorid	15	18	3	75
control	16	28	12	100

L.S.D. at 5% level for: Fungicides (cid) = 3.00 & Disease severity (DS) = 1.31 (cid x DS) = 6.9
*D.S=Disease severity

REFERENCES

1. Barnett, H. L. and B. B. Hunter. 1972. Illustrated genera of Imperfect Fungi. Third Ed. Burgess publ. co. Minneapolis, Minnesota pp241.
2. Berger, L. R. and D. M. Reynolds. 1958. The chitinase system of an isolate of *Streptomyces griseus*. *Biochem. Biophysic. Acta*, 29: 522-534.
3. Buchenauer, H. 1998 . Biological control of soil-borne diseases by rhizobacteria. *J. Plant Dis. And Protec.*, 105: 329 – 348
4. Carnegie, S. F., A. Ruthven, D. A. Lindsay and T. D. Hall. 1990. Effects of fungicides applied to seed potato tubers at harvest or after grading on fungal storage diseases and plant development. *Ann. Appl. Biol.*, 116: 61-72.
5. Chase, A. R. and T. K. Broschat. 1991. Diseases and disorders of Ornamental palms. APS Press, The American Phytopathol. Soc. St. Paut, Minn, USA. 56p.
6. Chatanger, G. A., J. M. Ogawa. 1979. Fungicides treatment to suppress *Botrytis cinerea* and protect fresh market tomatoes. *Phytopathology*, 69: 59-63
7. Djerbi, M. 1981. Diseases of date palm, tech. Bull. No.2, FAO, Baghdad.
8. Elad Y., I. Chet and Y. Henis. 1982. Degradation of plant pathogenic fungi by *Trichoderma harzianum*. *Can. J. Microbiol.* 28: 719 - 725.
9. El-Swah, M. W. 1965. Diseases of fruits trees and its control (In Arabic). Dar- El-Maaref pub. Pp. 510
10. El Tarabily K. A., M. L. Sykes, I. D. Kurtbohe, G. E. Hardy, A. M. Barbosa and R. F. H. Dekke. 2000. Synergistic effects of a cellulase producing *Micromonospora carbonacea* and an antibiotic producing *Streptomyces violascens* on the suppression of *Phytophthora cinnamomi* root rot of *Banksia grandis*. *Can. J. Bot.*, 74: 618-624
11. Kamhawy, M. A. M. 2006. Host range and control of *Phyllosticta* sp. The cause of banana leaf spot and blight. *Egypt J. Phytopathol.*, 34, 2: 1-15
12. Ker Chung, Kuo and H. C. Hoch. 1996. The parasitic relationship between *Phyllosticta ampelica* and *Vitis vinifera*. *Mycologia*. 88: 626-634
13. Lim, H., Y. Kim and S. Kim. 1991. *Pseudomonas stutzeri* YLP-1 genetic transformation and antifungal mechanism against *Fusarium solani*, an agent of plant root rot. *Appl. Environ. Microbiol.*, 57: 510-516.
14. Munkvold, G. P. and J. J. Morios. 1993. Efficacy of natural epiphytes and colonizers of grapevine wounds for biological control of *Eutypa dieback*. *Phytopathology*, 83: 624-629.
15. Nelson, N. 1944. Photometric adaptation of Somogi method for determination of glucose. *J. Biol. Chem.*, 152: 135-175.

16. Noriega, C. D. H., L. N., Becerra, R. F. Hernandez. 1991. Coconut diseases in the Coast of Guerrero, Mexico. Source *Revista Mexicana de Fitopatologia*. 1991. 9, 2: 84- 93.
17. Obazee, E. N. and T. Ikozun. 1985. The occurrence of a leaf spot disease of coconut palm caused by *Pestalotia palmarum* Cooke in Nigeria *Fitopatologia Brasileira*. 10, 1:167-169.
18. Pitta, G. P. B. 1994. Diseases of palms in Brazil. *Acta Horticulture*. 360, 231- 234
19. Rashed, M. F. 1998. Pathological studies on black scorch disease of date palm. Ph.D Thesis, Fac. Agric., Cairo Univ. 110p.
20. Rashed, M. F. 2001. Phytopathological note. *Pestalotia* sp. on date palm leaves in Egypt. The second international conference on date palm. Al- Ain, United Arab Emirates. 1:401
21. Rajan P. P., Y. R. Sarma and M. Anandaraj. 2002. Management of foot rot of black pepper with *Trichoderma* species. *Indian Phytopath.* 55: 17-21
22. Ramaswamy, G. R., H. S. Sohi, H. C. Govindu. 1989. Studies on the host range of *Pestalotia psidii*, the causal agent of guava canker and evaluation of fungicides against the pathogen. Source *Indian Journal of Mycology and Plant Pathology*. 1988, publ. 1989. 18: 2, 180-181. 5 ref.
23. Sundhein, L. 1992. Effect of Chitinase encoding genes in biocontrol *Pseudomonas* spp. *Biological Control of Plant Diseases*, Edited by E. S. Tjamos et al., Plenum Press, New York, 1992
24. Uchida, J. Y. 2004. *Pestalotiopsis* diseases. Pages 27-28 in: *Diseases and Disorders of Ornamental Palms*. M. L. Elliott, T. K. Broschat, J. Y. Uchida, and G. W. Simone, eds. American Phytopathological Society, St. Paul, MN.
25. Watkins, J. E., L. J. Littefield and G. D. Statler. 1977. Effect of the systemic fungicide 4-n-butyl-1, 2,4-triazole on the development of *Puccinia recondite* f.sp. *tritici* in Wheat. *Phytopathology*, 67: 985.
26. Zuart-Macias, J. L., P. Ponce-Diaz, G. Santiago Marroquin and R. Quiroga. 1999. Coyol palm (*Acrocomia mexicana*), a phylogenetic resource from Chiapas, Mexico. *Acta Horticulturae*. 486, 305-310.

بيستالوشيا بالميكولا على أوراق نخيل البلح في مصر

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في اول عام ٢٠٠١ لوحظت أعراض تنقع أوراق على أوراق نباتات نخيل البلح ناتج زراعة الأنسجة صنف بارحى مزرعة بهتيم، محافظة القليوبية، مصر. تظهر على هيئة بقع بنية ذات حواف صفراء قد تلتحم هذه البقع ببعضها البعض وتغطي أجزاء كبيرة على الورقة، تؤدي في النهاية الى اصفرار وموت كامل للورقة تم عزل المسبب المرضي و بناء على الصفات المظهرية والفحص المجهرى عرف المسبب المرضي على أنه فطر بيستالوشيا بالميكولا وأكدت قدرته على احداث الأصابة. نفس الأعراض لوحظت على النباتات المنزرعة فى صوبة الأقلمة فى المعمل المركزى لتطوير النخيل ، مركز البحوث الزراعية، محافظة الجيزة، مصرفي أبريل ، ٢٠٠٤.. ولم توجد أى اختلافات بين العزلة المتحصل عليها من بهتيم والمتحصل عليها من المعمل المركزى إختبار المدى العوائلى أثبت أن كل نخيل الزينة التى تم إختبارها كانت قابلة للإصابة ولكن بدرجات متفاوتة.

هذا هو التقرير الأول حول امكانية فطر بيستالوشيا بالميكولا لمهاجمة مدى عوائلى من نخيل الزينة تحت الظروف المصرية. النخيل الملوكى كان الأكثر قابلية للأصابة بينما النخيل المروحي كان أقل قابلية. كذلك وجد من خلال الدراسة أن هناك علاقة بين عمر الورقة وقابليتها للأصابة ، الأوراق الأقدم فى النخيل البالغ وأوراق نباتات زراعة الأنسجة أكثر تأثراً من الأوراق الجديدة للنخيل البالغ.

مبيد النوسين م ٧٠ أعطى أعلى تأثير ضد المسبب المرضي يليه أوكسى كلوريد النحاس يليه الكوسيد ١٠١ ثم الأنتراكول والكانز بينما كان الكوسيد ٢٠٠٠ أقلهم تأثيراً. اختبرت ثلاث مركبات حيوية تجارية هي بيو - ارك ؛ بيو - زيد و ايه كيو ١٠ وعزلة من فطر ترايكوديرما هيرزيانم وعزلة من بكتريا باسيلس سبتلس من حيث قدرتها على مكافحة المرض تحت ظروف الصوبة و أثبتت إختبارات الصوبة قدرتهم على مكافحة المرض ولقد اختبرت قدرة الميكروبات الحيويه المستخدمه فى إنتاج المبيدات الحيويه وذلك بزراعتها على بيئات صناعيه لمعرفة قدرتها على افراز الانزيمات المحللة للكيتين مثل ٣،١ بيتا جلوكانيز والكيتينيز ولقد وجدت علاقة واضحة بين قدرة هذه المركبات الحيوية فى مكافحة المرض وقدرتها على إنتاج ٣،١ بيتا جلوكانيز والكيتينيز .