Egypt. J. Phytopathol., Vol. 34, No. 2, pp. 1-15 (2006)

Host Range and Control of *Phyllosticta* sp. the Cause of Banana Leaf Spot and Blight

M.A.M. Kamhawy
Plant Pathol. Res. Inst., Agric. Res. Centre, Giza, Egypt.

Pathogenicity test proved the ability of *Phyllosticta* sp. to cause the disease symptoms on banana leaves and fruits. In addition, all the tested banana cultivars were susceptible to infection by the pathogen but varied in their response. Host range test revealed that, apple, date palm, grapevine, lychee, mango, plum, prickly pear, fig and black mulberry were also attacked by *Phyllosticta* sp. However, disease severity and disease symptoms varied according to plant species. This is the first report about *Phyllosticta* sp. to attack some fruit trees from different families.

Copral 50% WP. (Copper oxychloride) and Cabriotop 38% WG. (Pyraclostrobin + Metiram) were the most effective fungicides against *Phyllosticta* sp. followed by Nimrod 25%EC. (Bupirirmate) and Bellis 60%WG. (Pyraclostrobin+Boscalid), as mean of growth reduction respectively. On the other hand, Kema-z 50%WP (Carbendazim), and Mancoper 69.5%WP. (Mancozeb+Copper oxychloride) were the lowest effective. Under greenhouse conditions the same fungicides were used as spray treatment and gave the same trend of laboratory screening.

Three commercial biofungicides, *i.e.* AQ10 (*Ampelomyces quisqualis*), Bio-Zaid (*Trichoderma album*) and Bio-Arck (*Bacillus megaterium*) were found to be efficient in suppressing Phyllosticta leaf spot of banana caused by *Phyllosticta* sp. The antagonists produced mycolytic enzymes viz. chitinase and β-1, 3-glucanase. Variations among the biofungicide were found in their ability to produce these enzymes.

Key words: *Ampelomyces quisqualis*, *Bacillus megaterium*, banana, chitinase, leaf spot, *Phyllosticta* sp. and *Trichoderma album*

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. Phyllosticta leaf spot disease (PLS) one of the foliage diseases infect wild range of fruit and woody trees as well as ornamental plants (Singh, 1978; Adhikari and Asha Tiwari, 1991; Madriz *et al.*, 1991and Ali and Saikia, 1997). The disease was recorded first time in Egypt on Banana orchards (*Musa* spp.) cv. Williams during March, 2005 in Bader Centre, Menuofya governorate by Kamhawy *et al.* (2005). Symptoms observed as fusiform or elliptical lesions in outline area on both leaf surfaces. The spots are dark brown in the centre surrounding with water soaked faint brown tissue which associated with bright yellow at the spot border. Several spots coalesced to each other and cover large portions on the leaf, which gives a blighted appearance.

Amusa *et al.* (2001) recorded leaf blight symptoms associated with infection by *Phyllosticta hibiscini* on roselle (*Hibiscus sabdariffa* var. *sabdariffa*). Madriz, *et al.* (1991) isolated *Phyllosticta musae* [*P. musarum*] from lesions on leaves and inflorescences of *Heliconia* spp. grown in parks, gardens and indoors in Venezuela. Herrera Isla (1993) reported that, *Phyllosticta* sp. is the causal agent of leaf spot and blight in *Sterculia apetala*. Muthumary (1993) cleared the presence of a mucilaginous membrane in SEM studies of a *Phyllosticta* sp. on *Codiaeum variegatum*. The remnants of a sheath were seen on some of the conidia after partial gelatinization. McMillan (1995) recorded *Phlyllosticta* sp. as a causal pathogen of some diseases in Brazil. Saata *et al.* (1994) described 23 rare species of Sphaeropsidales, including *Ascochyta, Phoma, Phyllosticta, Septoria* and *Stagonospora*, occurring in Poland. Khan *et al.* (1995) recorded pathogenic *Phyllosticta* spp. on forest tree and bamboo in India. Luo LuYi and Zhang XiaoYan (2000) reported brown spot disease (*Phyllosticta actinidia*) seriously attack kiwifruit leaves.

Chemical control using fungicides is still the main method for controlling leaf spots disease of banana in different countries. Chemical treatments to protect fruit trees from infection by certain diseases are routinely applied each year. Many phytopathologiests used chemical fungicides to control the disease. Sujan Singh *et al.* (1991) revealed that in laboratory tests, of 5 fungicides tested (Captaf, Dithane M-45 [mancozeb], Captafol, Blitox [copper fungicides] and Bavistin [carbendazim]), Bavistin at 0.05%and DithaneM-45 at 0.25% were effective against *P. adjuncta*. Kamla Uniyal *et al.* (2001) revealed that, Diathan M-45 and Radomil were effective in controlling leaf blight disease of poplar caused by *Phyllosticta adjuncta*. Luo LuYi and Zhang XiaoYan (2000) reported that, spraying 5-6 degree lime sulfur before and after flowering decreased the infection. Spraying brown spot disease (*Phyllosticta actinidia*) during July-August with mancozeb, Thiophanate-methyl or carbendazim gave good disease reduction. The aim of this work was to determine possible inoculum sources of the disease, so as to be able to plan to method of measures control.

Materials and Methods

Source of Phyllosticta sp.:

A pathogenic isolate of *Phyllosticta* sp. obtained by Kamhawy *et al.* (2005) from infected banana leaves (*Musa* spp.) cv. William was used in presented study.

Preparation of pathogen inoculum:

Phyllosticta sp. inoculum was prepared as previously described by KerChung and Hoch (1996). Spore suspension was strained though two layers of cheesecloth to remove mycelial clamps. Pycnidiospore concentration was determined and adjusted with a haemocytometer to reach 10^5 spore / ml approximately.

Pathogenicity tests and cultivar reactions:

Three cultivars of banana plants, six months age, namely; Grand Nain, Maghrabi and Williams were used to study their response to infection by *Phyllosticta* sp. Healthy leaves of banana were surface sterilized with 1.5% mercuric chloride and

then rinsed with sterile distilled water before treatment. Then, leaves sprayed using an atomizer to the point of suspension run off. Inoculated plants were covered with large plastic bags to maintain high humidity for two days. Control plants were similarly treated with only sterile distilled water. Five replicate plants were used for each cultivar.

Disease assessment:

Disease reading was determined for each leaf according to the disease severity rating which was made to include the size and frequency of the lesion / leaf. The following numerical rates were suggested for disease severity:

0= No symptoms.
1= Few scattered lesions covering about 1-25% of the leaf.
2= Spots covering about 25-50% of the leaf.
3= Spots coalescing and covering about 50-75% of the leaf.
4= Severe infection with coalescing and covering more than 75% of the leaf.

Disease severity was calculated according to the equation suggested by Townsend and Heuberger (1943) as follows:

$$\text{Disease severity (\%)} = \text{sum } (n \times r) \times 100 / 4N.$$

Whereas: (n) is the number of leaves in each numerical grade(r) and (N) is the total number of inoculated leaves multiplied by the maximum numerical grade.

Banana fruits inoculation:

Unwounded immature fruits (cv. Williams) were used to study their response to the infection by *Phyllosticta* sp. Spore suspension reach 10^5 spore/ml approximately was atomized onto banana fruits from all directions until run off. After inoculation, banana fruits packed into transparent plastic bag to serve as a moist chamber 48 h at room temperature(28±2°C) to ensure high humidity during the infection process. After 48 h. the inoculated banana unpacked and left incubated at room temperature with a daily check for symptoms development.

Host range:

Fifteen plant species belonging to thirteen genera, *i.e.* apple (*Malus domestica* Borth.); apricot (*Prunus armeniaca* L.); banana (*Musa* spp.); black mulberry (*Moruse nigra* L.); date-palm (*Phoenix dactylifera* L.); guava (*Psidium guajava* L.); lychee (*Litchi chinensis*); prickly pear (*Opuntia ficus-indica* Mill); fig (*Ficus carica* L.); mango (*Mangifera indica* L.); grape (*Vitis vinifera* L.); pomegranate (*Punica granatum* L.); peach (*Prunus persica* L.); sweet orange (*Citrus sinensis* Osbeck) and plum (*Prunus domestica* L.), were used to test their reaction to infection by *Phyllosticta* sp. The plants were fertilized twice per month 10: 10: 10 (NPK) soluble fertilizer, 2g/l water. Plants chosen for inoculation and disease development were actively growing and had suitable number of leaves except Prickly pear used cladodes. The plants were inoculated by spraying spore suspension and kept under observation for 14 days. Disease severity was determined 14 days after leaf inoculation. Three replicate plants were used for each tested plant species. Inoculation methods as well as disease severity assessment were followed as mentioned before.

Disease control:

Chemical control:

Nine different fungicides namely; Nimrod 25%EC (Bupirirmate), Kocide 101 77% WP. (Cupric hydroxide), Anrtracol 70% (Propineb), Kema-z 50%WP (Carbendazim), Mancoper 69.5 %WP (Mancozeb + Copper oxychloride), Copral 50% WP. (Copper oxychloride), Ridomil plus 50% (Mancozeb + Metalaxyl),Bellis 60%WG (Pyraclostrobin+Boscalid), Cabriotop 38% WG (Pyraclostrobin+Metiram) were used *in vitro* to evaluate their inhibitory effect on linear growth of *Phyllosticta* sp. Nine different concentrations, *i.e.* 0, 10, 50, 100, 200, 300, 400, 500 and 600ppm based of the active ingredient of each fungicides were prepared in previously calculated volume of autoclaved PDA medium before pouring into Petri dishes. Plates were inoculated with one disk (5-mm-diameter) of 14-day-old culture of the desired fungus and incubated at 25°C for 14 days. Three replicates were used for each treatment and the growth (mm) was recorded when the full growth of tested fungus was observed in the check treatment. Percentage of reduction in fungal growth was calculated relative to the check treatment.

Greenhouse experiments:

Greenhouse experiments were achieved in greenhouse of Fruit and Woody Dis. Res. Dept., Plant Pathol. Inst., ARC. Giza, Egypt. Banana plants (cv. William), previous inoculated and exhibited varied degrees of disease symptoms, were used as test plants. Disease severity due to artificial infection was recorded as mentioned before. Banana plants were sprayed twice at 15 day intervals with the recommended dose of the desired fungicide with enough fungicidal solution. Three replicates were used for the evaluation of each applied treatment. Disease severity was recorded 30 days after fungicidal application as mentioned before. Decrease in disease severity was calculated according to the following equation (Munkvold and Morios, 1993):

$$(Ps)= (Ic-Iv / Ic) \times 100$$

Whereas: Ps=disease reduction (%), Ic= proportion of increase in disease severity in control treatment and Iv= proportion of increase in disease severity in each treatment.

When necessary, the results were statistically analyzed using factorial experimental design suggested by Snedcor and Cochran (1982).

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 safe and might be cheep method of control was investigated.

Evaluation of commercial biofungicides on the control of Phyllosticta leaf spot:

The efficiency of three tested commercial biofungicides, *i.e.* AQ10 (***Ampelomyces quisqualis***), Bio-Zaid (***Trichoderma album***) and Bio-Arc (***Bacillus megaterium***) for controlling banana leaf spot disease under greenhouse conditions were evaluated. The biofungicides were prepared at recommended doses of

producing company. Banana plants (cv. William), six months age, 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 biofungicides at the same time of inoculation as well as 15 days after inoculation when plants exhibited disease symptoms. All plants were covered with sterile cellophane bags for one week to maintain high relative humidity for spore germination. The bags were then removed 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 was calculated according to Munkvold and Morios (1993) as mentioned before.

Determination the activity of mycolytic enzymes produced by the microorganisms of bio fungicides against Phyllosticta sp.:

Three microorganisms: *i.e. Ampelomyces quisqualis; Bacillus megaterium* and *Trichoderma album*, were obtained by culturing spore suspension of the commercial biofungicides previously used in order to determine mycolytic enzymes viz. β-1,3-glucanases and chitinase produced by commercial biofungicides against *Phyllosticta* sp., the leaf spot pathogen of banana.

Determination of Chitinases:

Isolates of tested biofungicides were cultured in 250 ml conical flasks containing 50ml 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 medium containing 0.2% sucrose for fungal isolates. All tested micro organisms were incubated at 28°C for 96 h. in a rotary shaker incubator. After the incubation period the cultures were centrifuged at 12,000 rpm. 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 0.25ml of enzyme solution, 0.3ml of 1M sodium acetate buffer (pH 5.3) and 0.5ml of colloidal chitin (0.1%) and incubated in a water bath at 50°C for 4 h. 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/ml.

Determination of β-1,3-glucanase:

Bacterial isolates were grown in 250 ml conical flasks containing 50ml of peptone medium and fungal isolates were grown in liquid Czapek-Dox medium. Inoculated both media contained laminarin 0.2% (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 previously mentioned and the resulted supernatant filtered though 0.22μm Millipore filters and preserved to be use as enzyme source. The reaction mixture contained 0.25ml of enzyme solution, 0.3ml of 0.1M phosphate buffer (pH5.5) and 0.5ml 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/ml.

Results and Discussion

Pathogenicity test and varietal reaction:

Three banana cultivars namely; Grand Nain, Maghrabi and Williams were inoculated by *Phyllosticta* sp. Data in Table (1) and Fig. 1 (A, B and C) showed that Percentages of the disease index revealed significant in the interaction between the tested cultivars and pathogen. The highest disease index was always recorded on cv. Williams being 32.86% followed by cvs. Maghrabi and Grand Nain which recorded 20.51 and 16.43%, respectively.

Table 1. Pathogenic ability of *Phyllosticta* sp. on some banana cultivar

Tested banana cvs.	Disease severity (%) after days			
	7	15	21	Mean
Grand Nain	04.17	18.75	26.38	16.43
Maghrabi	08.33	20.83	32.38	20.51
Williams	14.29	35.42	48.7	32.86
L.S.D. at 5% for: Days (D)= 0.8. Cultivar (C)= 1.1 and D x C=1.6				

Also, pathogenicity test indicated the ability of the pathogen to attack banana fingers. Bunches sprayed with spores of *Phyllosticta* sp. showed brown spots characteristic of the fungus as shown in Fig. 1 (G). It appeared first as small brown spots, which gradually enlarged. Although circular at first, the spots later became irregular formed like cavity in shape. These results are in harmony with those of Meredith (1968) and McMillan (1995).

Host range:

Fifteen plant species representing eleven families (thirteen genera) were tested for their reaction to Phyllosticta leaf spot disease caused by *Phyllosticta* sp. isolated from banana (*Musa* spp.). Obtained results (Table 2 and Fig. 2) show that *Phyllosticta* sp. is wide spread pathogen, the pathogen was able to attack banana, apple, prickly pear, date-palm, plum, lytchee, black mulberry, mango, grape and Fig. However, disease severity and disease symptoms varied according to plant species. Banana was severely attacked (39.16% as disease severity), followed by Apple, Prickly pear (27.40 and 26.24%, respectively). Meanwhile, date-palm, plum, lychee, mango and black mulberry recorded 21.54, 18, 16.24, 14.11 and 13.81%, respectively. Fig and grape showed slight infection, while other tested plant species remained healthy. Such a non-host specific of the pathogen provides a variety of sources for inoculum in nature. Symptoms on apple leaves can be seen as reported by Ikase and Dumbravs (2004). They reported that *Phyllosticta* sp. was the causal of apple leaf blotch. Disease symptoms on prickly pear consisted of tiny circular, light grey colured spots with pycnidia on them and a rusty halo. Spots became bigger, tended to coalesce and darken (Fig.2 N) finally resulting in stem death. These results are in agreement with those reported by Wright *et al.* (2004) who stated that *Phyllosticta* sp. is the cause of prickly pear stems blight. They also added that

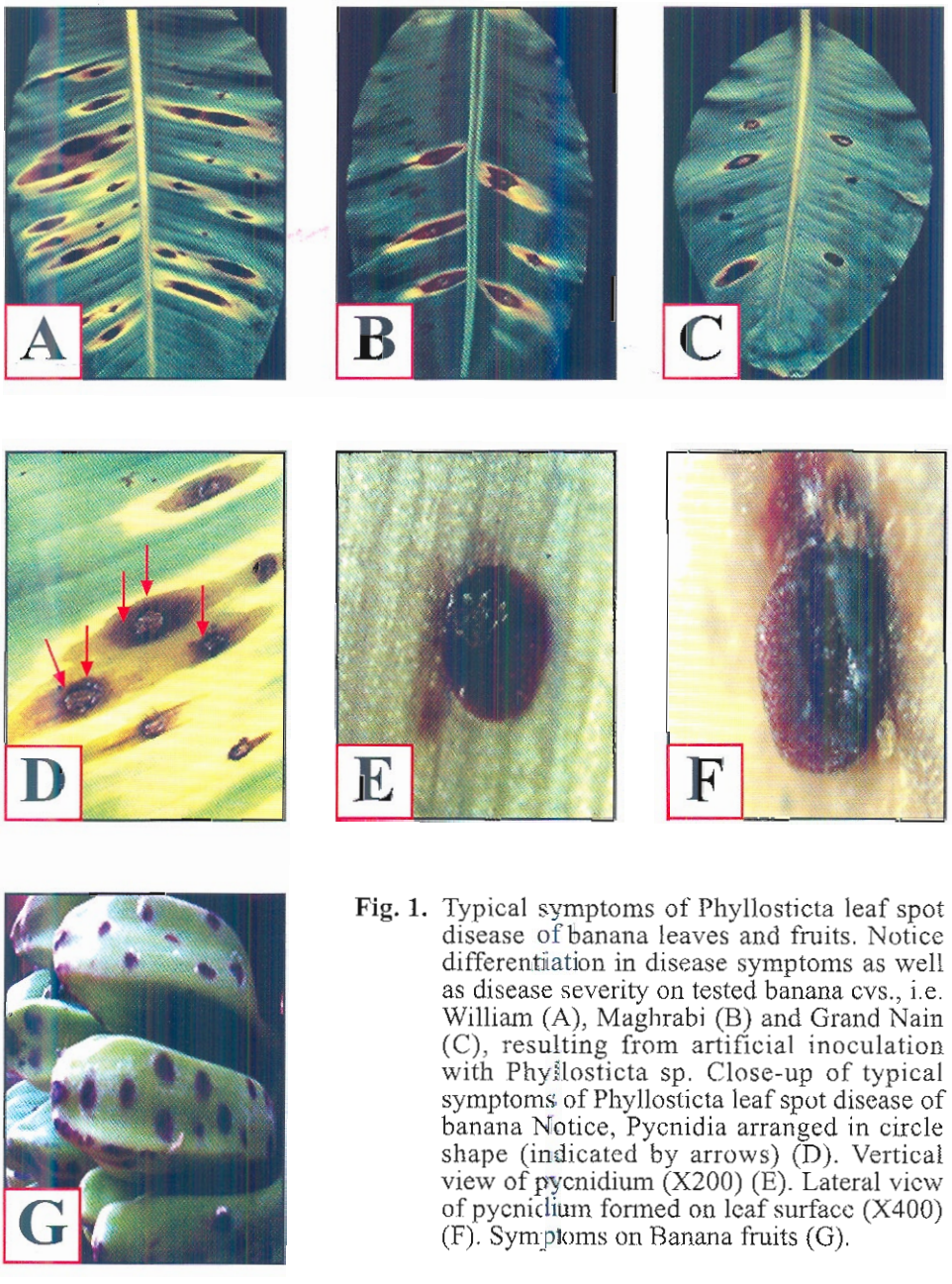

Fig. 1. Typical symptoms of Phyllosticta leaf spot disease of banana leaves and fruits. Notice differentiation in disease symptoms as well as disease severity on tested banana cvs., i.e. William (A), Maghrabi (B) and Grand Nain (C), resulting from artificial inoculation with Phyllosticta sp. Close-up of typical symptoms of Phyllosticta leaf spot disease of banana Notice, Pycnidia arranged in circle shape (indicated by arrows) (D). Vertical view of pycnidium (X200) (E). Lateral view of pycnidium formed on leaf surface (X400) (F). Symptoms on Banana fruits (G).

Table 2. Reaction of plant species from different families to inoculation with *Phyllosticta* sp. under greenhouse conditions

Plant species	Family	Disease severity (%)
Apple (*Malus domestica*, Borth)	Rosaceae	27.40
Apricot(*Prunus armeniaca* L.)		****
Plum (*Prunus domestica* L.)		18.00
Peach (*Prunus persica* Batsch.)		****
Black mulberry (*Moruse nigra* L.)	Moraceae	13.81
Fig (*Ficus carica* L.)		12.33
Banana (*Musa* spp.)	Musaceae	39.16
Date-palm (*Phoenix dactylifera* L.)	Palmaceae	21.54
Guava (*Psidium guajava* L.)	Myrlaceae	****
Grapevine (*Vitis vinifera* L.)	Vitaceae	12.77
Mango(*Mangifera indica* L.)	Anacardiaceae	14.11
Pomegranate (*Punica granatum* L.)	Punicaceae	****
Sweet orange(Citrus *sinensi* Osbeck)	Rutaceae	****
Prickly pear (*Opuntia ficus-indica* Mill)	Cactaceae	26.92
Lychee (*Litchi chinensis* Sonn)	Sapindaceae	16.24

**** No disease symptoms appeared.

Cucurbita maxima and Cucumis sativus seedlings and fruits that were artificially inoculated showed disease symptoms. On the other hand, symptoms on date palm firstly appear as shown in Fig. 2 (I), irregular red spot 2mm in diameter surrounding with bright at the spot border. Several spots may coalesce to form large spots (Pitta, 1994 and Ramos Mariano *et al.* 1998). Grapevine, fig and plum (Fig. 2 K, L and P) were similar in symptoms against *Phyllosticta* sp. these symptoms can seen as dark brown spots with darkness margin, the spots often detached to produce shot-hole symptoms. Ferreira *et al.* (1989) reported that *Phyllosticta* sp. associated with dieback and pruning wounds of grapevines in South Africa. Also, Prikhod'ko (1975) reported that *Phyllosticta prunicola* the cause of shot hole spot of stone fruit trees. Togawa (1998) revealed that brown leaf spot disease of cherry caused by *Phyllosticta* sp. On the other hand, symptoms on lychee (Fig.2 M) appear as circular red spot (McMillan 1995). Mango leaves exhibited disease symptoms as circular necrotic lesions with tiny colour are shown in Fig. 2 (O). Vala *et al.* (1989), Madriz *et al.* (1991) and Muthumary *et al.* (1993) isolated *Phyllosticta musae* [*P. musarum*] from lesions on leaves and inflorescences of Heliconia spp. grown in parks, gardens and indoors in Venezuela. According to the available literature, this is the first report about possibility of *Phyllosticta* sp. to attack several hosts in Egypt. Addition to host rang of *Phyllosticta* sp., the obtained results may be illustrating the source of inoculum.

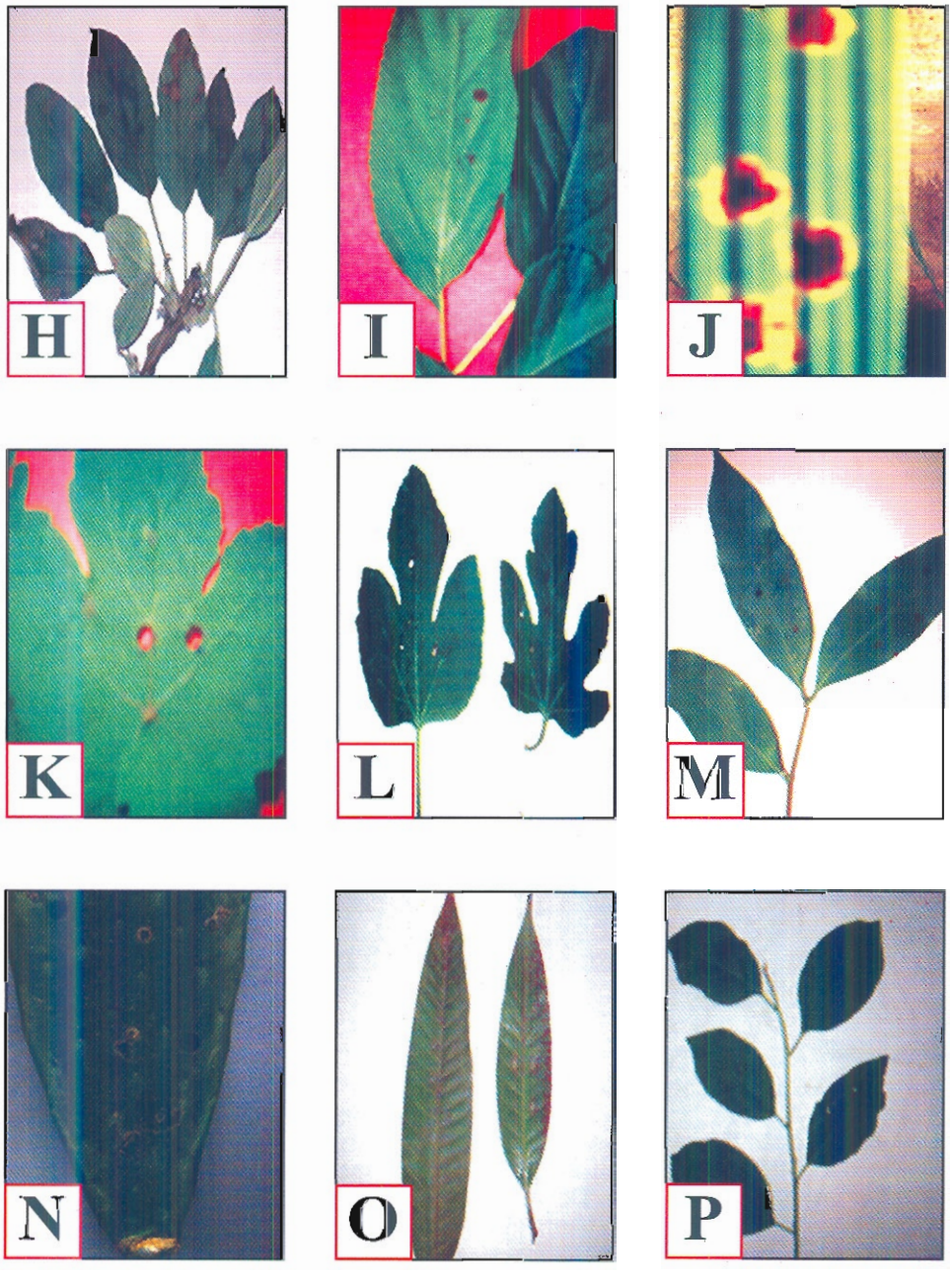

Fig. 2. Disease symptoms resulted from artificial inoculation by Phyllosticta sp. on different hosts, i.e. apple (H), black mulberry (I), date-palm (J), Grape (K), Fig (L), lytchee (M), prickly pear (N), mango (O) and plum (P).

Disease control:
Evaluation of different fungicides on the linear growth of tested pathogen:

Data presented in Table (3) reveal that Copral and Cabriotop were the most effective fungicides against Phyllosticta sp. being recorded 68.4 and66.9% followed by Nimrod and Bellis being recorded 60.9 and 58.3% as mean of growth reduction, respectively. The differences between Copral and Cabriotop as well as Nimrod and Bellis were not significant and highly significant compared with the other tested fungicides. On the other hand, Kema-z and Mancoper were the lowest effective fungicides being recorded 27 and 17.7% respectively. Data also indicate that the tested fungus varied in its sensitivity against the fungicides used. The growth of Phyllosticta sp. showed more positive response to Copral 50% WP. and Cabriotop 60% where, complete growth inhibition was recorded at 100 and 200ppm followed by Nimrod recorded at 300 ppm and Bellis38% at 400, respectively while, no complete growth reduction for the fungus tested occurred when Kocid 101 was used until 600 ppm. 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 3. Growth reduction (%) of the tested pathogenic fungi resulted from the *in vitro* tested fungicides

Tested fungicide	Growth reduction (%) at fungicide used concentrations (ppm)									
	0	10	50	100	200	300	400	500	600	Mean
Anrtracol 70%	0	0	0	48	55	62	77	100	100	49.1
Copral 50%WP.	0	0	16	100	100	100	100	100	100	68.4
Kemazed 50%	0	0	0	0	0	13	61	69	100	27
Kocid 101	0	0	0	22	33	40	44	67	81	31.9
Mancoper 69.5	0	0	0	0	0	0	11	48	100	17.7
Nimrod 25%EC	0	0	35	52	61	100	100	100	100	60.9
Bellis 60%	0	0	17	50	77	81	100	100	100	58.3
Cabriotop 38%	0	0	32	70	100	100	100	100	100	66.9
Ridomil Plus50%	0	0	50	50	50	61	83	100	100	49.3
L.S.D. at 5% for: Concentration (C)=2, Fungicide (F)= 3.19 and F x C= 4.4										

Greenhouse experiments:

In vitro treatments indicate that Copral 50% WP and Cabriotop 38% WG followed by Nimrod 25%EC and Bellis 60%WG were the most effective fungicides against the tested fungus. Under greenhouse conditions these four fungicides were used as spray treatment to control Phyllosticta leaf spot of banana and gave the same trend of laboratory screening. Data in Table (4) reveal that Copral and Cabriotop were the best fungicides for controlling leaf spot which recorded the highest percentages of decrease in disease incidence, being 86.57 and 84.16 %, respectively, followed by Nimrod and Bellis recorded 71.51 and 68.28%, respectively. Many plant pathologists recorded that several fungicides decreased the percentage of Phyllosticta leaf spot disease severity (Sujan Singh et al., 1991, Luo LuYi and Zhang XiaoYan, 2000 and Kamla Uniyal et al., 2001).

Table 4. Effect of different fungicides at the recommended dose in controlling Phyllosticta leaf spot of banana

Tested fungicide	D.S. before* treatment (%)	D.S. after treatment (%)	Increase in D.S. (%)	Reduction in D.S. (%)
Copral 50% WP.	42.33	46.00	3.67	86.57
Cabriotop 38% WG.	41.87	46.20	4.33	84.16
Nimrod 25%EC.	48.21	56.00	7.79	71.51
Bellis 60%WG.	53.33	62.00	8.67	68.28
Control (water)	48.66	76.00	27.34	00.00
L.S.D. at 5% for: Fungicide (F)= 3.38. Disease severity (D)= 4.11 and F x D= 5.8				

* D.S.= disease severity.

Biological control:

The biofungicides found efficient in suppressing Banana leaf spot disease caused by *Phyllosticta* sp. Data in Table (5) reveal that all tested biofungicides prevent disease incidence when applied at the same time of pathogen inoculation or one day later. Bio-Zaid (*Trichoderma album*) was the best biofungicide for controlling leaf spot which recorded the highest decrease percentage in disease severity being 94 .85 % followed by Bio-Arck (*Bacillus megaterium*) and AQ10 (*Ampelomyces quisqualis*) being recorded 88.09 and 76.43 %, respectively.

Table 5. Effect of different biofungicides with the recommended dose in controlling Phyllosticta leaf spot on banana

Tested biofungicide	Pathogen first, then biofungicide 15 days later			
	D.S. before* treatment (%)	D.S. after treatment (%)	Increase in D.S. (%)	Reduction in D.S. (%)
Bacillus megaterium (Bio-Arck)	29.16	33.00	3.84	88.09
Trichoderma album (Bio-Zaid)	25.00	26.66	1.66	94.85
Ampelomyces quisqualis (AQ10)	27.00	34.59	7.59	76.43
Control (water)	26.38	58.59	32.21	00.00
L.S.D. at 5% for: Fungicide (F)= 3.38. Disease severity (D)= 4.11 and F x D= 5.8				

* D.S.= disease severity.

Estimation of β-1, 3-glucanases and Chitinase activities:

The antagonists found to be efficient in suppressing Banana leaf spot disease caused by *Phyllosticta* sp. were tested *in vivo* for their efficacy for lysing the cell wall of the pathogen. The antagonists produced mycolytic enzymes viz. β-1,3-glucanases and chitinase. Variations among the isolates could be referred for the

production of these enzymes. The findings demonstrate mechanism of antagonism by the tested biofungicides though production of pathogen cell wall lysing enzymes, chitinase and β-1, 3-glucanases. Data in Table (6) indicate clearly that, all tested biofungicides released Chitinase and β-1, 3-glucanases enzymes varied according to biofungicide when grown in mixed culture with *Phyllosticta* sp. In this respect *Bacillus megaterium* recorded the highest chitinase activity being 0.35 followed by *Ampelomyces quisqualis* and *Trichoderma album* being recorded 0.31 and 0.13 unit, respectively. On the other hand, *Trichoderma album* recorded the highest β-1,3-glucanases activity being 0.372 unit followed by *Bacillus megaterium* and *Ampelomyces quisqualis* being 0.172 and 0.101unit, 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 glucose amino groups. The degradation of chitin is catalyzed by chitinase, which hydrolyze chitin to chitodextrins (Sundhein, 1992 and 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).

Table 6. Estimation of β-1, 3-glucanases and Chitinase activities as Glucose unit released per hour /ml

Tested biofungicide	Chitinase activity unit/ml/h.	β-1,3-glucanases unit/ml/h.
Bacillus megaterium	0.35	0.172
Trichoderma album	0.130	0.372
Ampelomyces quisqualis	0.31	0.101

References

Adhikari, R.S. and Asha Tiwari 1991. Some new host records of fungi from India. *Indian Phytopathol.*, **44**: 147-151

Ali, M.S. and Saikia, U.N. 1997. Coelomycetes of Assam. *Indian Phytopathol.*, **50**: 200-205

Amusa, N.A.; Kogbe, J.O.S. and Ajibade, S.R. 2001. Stem and Foliar blight in roselle (*Hibiscus sabdariffa* L. var. *sabdariffa*) in the tropical forest region of south- western Nigeria. *J. Hort. Sci. & Biotechnol.*, **76**: 681-684.

Berger, L.R. and. Reynolds, D.M. 1958. The chitinase system of an isolate of *Streptomyces griseus. Biochem. Biophysic. Acta*, **29**: 522-534.

Buchenauer, H. 1998. Biological control of soil-borne diseases by Rhizobacteria. *J. Plant Dis. & Protec.*, **105**: 329-348.

Carnegie, S.F.; Ruthven, A.; Lindsay, D.A. and Hall, T.D. 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.

Elad Y.; Chet I. and Henis Y. 1982. Degradation of plant pathogenic fungi by *Trichoderma harzianum. Can. J. Microbiol.*, **28**: 719-725.

El-Tarabily, K.A.; Sykes, M.L.; Kurtbohe, I.D.; Hardy, G.E.; Barbosa, A.M. and Dekker, R.F.H. 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.

Ferreira, J.H.S.; Matthee, F.N. and Thomas, A.C. 1989. Fungi associated with dieback and pruning wounds of grapevines in South Africa. *South African J. for Enology and Viticulture*, **10**: 62-66.

Herrera Isla. 1993. *Phyllosticta* sp., causal agent of leaf spot and Blight in *Sterculia apetala* (Jacq.) Karst. [Spanish]. *Centro Agricola.*, **20**: 82-83.

Ikase, L. and Dumbravs, R. 2004. Apple breeding for disease resistance in Latvia. *Acta Hort.*, **663**: 713-716

Kamhawy, M.A.M.; Tohamy, M.R.A. and Hassan, E.A.M. 2005. First record of Phyllosticta leaf spot disease of banana in Egypt. *Egypt. J. Phytopathol.*, **33**: 93-94. (Note).

Kamla Uniyal; Ajab Singh and Rajiv Pandey, 2001. Leaf blight of poplar and its control. *Indian Forester*, **127**: 1394-1396.

KerChung, Kuo and Hoch, H.C. 1996. The parasitic relationship between *Phyllosticta ampelicda* and *Vitis vinifera. Mycologia*, **88**: 626-634.

Khan, S.N.; Misha, B.M. and Tivari, R.K. 1995. New host records of pathogenic fungi from India. *Indian J. of Forestry*, **18**: 89-92.

Lim, H.; Kim, Y. and Kim, S. 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.

Luo LuYi and Zhang XiaoYan. 2000. The control of two kinds of leaf spot diseases for kiwifruit. *South China Fruits*, **29**: 40.

Madriz, R.; Smits B.G. and Noguera, R. 1991. Main pathogenic fungi affecting some ornamental species of the genus Heliconia. *Agronomia Tropical*, **41**: 265-274.

McMillan, R.T.Jr. 1995. Diseases of *Litchi chinensis* in south Florida. Proceedings of the Florida State. *Horticultural Society*, **107**: 360-362.

Meredith, D.S. 1968. Freckle disease of banana in Hawaii caused by *Phyllosticta musarum* (Cke) Petr. *Ann. Appl. Biol.*, **62**: 328-340.

Muthumary, J. 1993. The gelatinous covering of a *Phyllosticta conidium* - SEM study. *Mycologist*, **7**: 1-16.

Munkvold, G.P. and Morios, J.J. 1993. Efficacy of natural epiphytes and colonizers of grapevine wounds for biological control of Eutypa dieback. *Phytopathology*, **83**: 624-629.

Nelson, N. 1944. Photometric adaptation of Somogi method for determination of glucose. *J. Biol. Chem.*, **152**: 135-175.

Pitta, G.P.B. 1994. Diseases of palms in Brazil. *Acta Hort.*, **360**: 231-234.

Prikhod'ko, V.P. 1975. Shot hole spot of stone fruit trees. *Zashchita Rastenii*, **9**: 47.

Rajan, P.P.; Sarma, Y.R. and Anandaraj, M. 2002. Management of foot rot of black pepper with *Trichoderma* species. *Indian Phytopathol.*, **55**: 17-21.

Ramos Mariano, R.L.; Fernandesde, Lira R.V.; Silveira, E.B. and Menezes, M. 1998. Survey of endophytic and epiphytic fungi from coconut leaves in the northeast of Brazil. II. Effect of the locality on the fungal population. *Agrotropica*, **10**: 1-8.

Saata, B.; Muenko, W. and Woczanska, A. 1994. New and rare species of Sphaeropsidales in Polish flora. *Acta Mycologica*, **29**: 81-93.

Singh, S.M. 1978. Some Sphaeropsidales from Balaghat. *Indian Phytopathol.*, **31**: 176-179.

Snedcor, G.W. and Cochran, W.G. 1982. *Statistical Methods.* 7th Ed., The Iowa State Univ. Press, Ames, Iowa, USA.

Sujan Singh; Pandey, P.C.; Ajab Singh and Ram Bahadur. 1991. Studies on foliage diseases of poplars. *Indian Forester*, **117**: 135-142.

Sundhein, L. 1992. Effect of Chitinase encoding genes in biocontrol *Pseudomons* spp. In: *Biological Control of Plant Diseases.* E.S. Tjamos *et al.* (Ed.). Plenum Press, New York.

Togawa, M. 1998. Occurrence of brown leaf spot disease of cherry caused by *Phyllosticta* sp. Proc. of the Kanto-Tosan Plant Protect. Soc., **45**:151-153.

Townsend, G.R. and Heuberger, J.W. 1943. Method for estimating losses caused by diseases in fungicide experiments. *Plant Dis. Reptr.*, **27**: 340.

Watkins, J.E. Littefield, L.J. and Statler, G.D. 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.

Wright, E.R.; Peralta, S. and Carballo, S. 2004. Determination of inoculum sources of *Phyllosticta* sp., causal agent of *Opuntia ficus-indica* stem blight. *ALF, Lima, Peru.*, **39**: 91-95. (C.f. CAB Abst., 1973-2005, Access. No. 20053037034).

Vala, D.G.; Solanky, K.U.; Desai, V.D. and Joshi, H.U. 1989. Diseases of mango occurring in Gujarat State. *Acta Hort.*, **231**: 544-550.

(Received 10/07/2006;
in revised form 12/09/2006)

المدى العوائلى لفطر الفلوستيكتا المسبب لمرض تبقع ولفحة اوراق الموز وطرق مقاومته

محمود أحمد محمود فحاوى
معهد بحوث أمراض النباتات- مركز البحوث الزراعية- جيزة.

اظهرت اختبارات العدوى وحساسية أصناف الموز قدرة الفطر فلوستيكتا على احداث أعراض المرض على أوراق و ثمار الموزبدرجات متفاوته على حسب الصنف. واتقد كان الصنف ويليام هو اكثر الاصناف قابلية للاصابة يليه الصنف مغربى فى حين كان الصنف جراند نين أقلهم اصابة.ظهر من خلال البحث أن فطر فلوستيكتا له مدى عوائلى واسع حيث اظهرت اختبارات المدى العوائلى قدرة الفطر على احداث الاصابه على التفاح – نخيل البلح – اينشى – تين شوكى – مانجو – برقوق – عنب – تين – توت ولقد اختلفت أعراض وشدة المرض باختلاف النبات المختبر.كانت أكثر المبيدات فعالية سواء فى الصوبة أو الحقل هم مبيدى الكوبرال والكوبريوتوب يليهما النمرود والبايز فى حين كان أقلهم كيمازد والمانكوبر. اختبرت ثلاث مركبات حيوية تجارية هى بيو- ارك ؛ بيو- زيد و ايه كيو ١٠ فى قدرتها على مكافحة المرض تحت ظروف الصوبة و كان أكثرهم فعاليه هو المبيد الحيوى بيو- زيد يليه بيو- ارك ثم ايه كيو ١٠. ولقد اختبرت قدرة الميكروبات الحيويه المستخدمه فى انتاج المبيدات الحيويه وذلك بزراعتها على بيئات صناعيه لمعرفة قدرتها على افراز الانزيمات المحللة للكيتين مثل ١،٣بيتا جلوكنيز والكيتينيز ولقداظهر المبيد الحيوى بيو-زيد اعلى نشاط بالنسبة لأنزيم ١ و ٣ بيتا جلوكانيز يليه بيو أرك ثم ايه كيو ١٠ أما بالنسبة لأنزيم الكيتينيز فقد كان المبيد الحيوى بيو أرك هو الأعلى نشاطا يليه ايه كيو ١٠ ثم بيو زيد.