Pathological and Physiological Studies on Macrophomina Phaseolina The Causal Organism of Bean Charcoal Rot Disease

By

I.N.M. ALI*, M.I. GHONIM**; HEBA M. ABDEL-NABI* AND M.I. AHMED**

From

*Faculty of Agric., Suez Canal Univ., Ismailia, Egypt.

** Plant Path. Res. Institute, Kassassien Hort. Res. Station, Agric. Res. Center, Giza, Egypt

Abstract

Pathogenicity tests of Macrophomina *phaseolina* (Tassi) Goid. (pycnidial stage of Sclerotium bataticola Taub.) isolates proved that, all they were pathogenic to Xera bean cultivar. The isolate, which isolated from sweet melon roots was the most pathogenic one, followed by that isolated from bean roots and then that isolated from sesame stem. *In vitro* studies revealed that the most suitable degrees of temperature for the linear growth of the tested isolates ranged between 25-35°C. On the other hand, at 15°C, the linear growth was low and differed with the different isolates. Three infested barley seeds /bean seed as inoculum was enough to incidence the disease. Sesame was the most susceptible host followed by wheat and maize, which were moderate, however cantaloupe was the lowest susceptible host. Fungal culture filtrates at different concentrations reduced the percentage of seed germination and had harmful effect on rootlet length and dry weight. Enzymatic activity of the fungus (pectin methyl esterase, polygalacturonase and cellulase) was assessed in the culture filtrate and/or extract of infected bean plants.

Keywords: Beans; Phaseolus vulgaris; pectin methyl esterase; PME, polygalacturonase; PG; cellulase; Macrophomina *phaseolina*; charcoal rot.

Introduction

Bean (*Phaseolus vulgaris* L.) is one of the most important and widely distributed vegetable crops in the world, as well as, in Egypt due to its high nutritional value. Charcoal rot disease of bean caused by *Macrophomina phaseolina* (Tassi) Goid. is considered one of the most destructive and serious diseases of bean in the greenhouses and open fields.

Salem (1997) showed that, there was a slight growth at the low temperature degree (18°C) within the first 24 hrs. After 24 hrs, the growth continued to increase until covered the Petri dishes after 120 or 192 hrs depending on the fungal isolates. At 28°C, all *M. phaseolina* isolates covered the Petri dishes within 3 days with growth rate of 1.19 mm/hr except two isolates. At 38°C, isolates varied in their growth rates. Miklas *et al.*, (1998) indicated that, charcoal rot or ashy stem blight incited by *M. phaseolina* of a great serious disease for common bean under drought and high temperature conditions in some region.

M. phaseolina has a wide host range and geographic distribution and is a major pathogen of many crops including sorghum, cotton, soybean, chickpea, sunflower, corn, cowpea and peanut (Mihail 1992). Ibrahim (1996) isolated *M. phaseolina* from various hosts such as sesame, cotton, sunflower, syngonium, lentil, bean, roselle and soybean. Sarhan (2000) evaluated the cultural filtrate of *M. phaseolina* at different concentrations, *i.e.* 100, 50 and 25% on the percentage of seed germination of two bean cultivars (Giza 3 and Giza 6).

The maximum polygalacturonase (PG) activity occurred in culture filtrate of *M. phaseolina* after 3-6 days of incubation depending on the isolate. *In vitro*, as well as, *In vivo* PG production was correlated with virulence. Cellulase (Cx) activity increased with increasing culture age. *In vitro*, Cx production was not closely correlated with pathogenicity of the moderately pathogenic isolates, while it correlated with virulence of the virulent and avirulent ones. However, Cx activity was much higher in case of diseased soybean plants than healthy ones, and the activity was increased with increasing seedling age (Abdelal *et al.*, 1983).

Ibrahim (1996) showed that, in vitro all tested isolates of M.*phaseolina* exhibited variable different levels of PME, PG and Cx activity indicating a remarkable increase as the age of culture increased. In vivo PME, PG and Cx activity

were correlated with the virulent isolate.

The present investigation was conducted to study the pathogensis and temperature requirements for the linear growth of three isolates of *M. phaseolina* and their ability to produce pectolytic and cellulotyic enzymes. The effect of fungal culture filtrates on the germination and length of radical roots of three bean cultivars was determined. Also, the inoculum levels and host range as factors affecting bean infection by *M. phaseolina* were investigated.

Materials and Methods

1- Pathogenicity test:

Three isolates of *M. phaseolina* previously isolated by (Ahmed, 2002) from sweet melon roots (collected from Eltal-El kabeer), bean roots (collected from Ain Ghosin) and sesame stems (collected from El Wasfia) were chosen and individually tested for their pathogenicity on Xera bean cultivar under the greenhouse conditions. Pots (25 cm in diameter) were sterilized by immersing them in 5% formalin solution for 15 mins and left to dry for 7 days. Clay-sandy soil used in this study was also sterilized with 5% formalin solution, then left to dry for 3 weeks to allow formaldehyde evaporation.

The inoculum of the three isolates was separately prepared by growing them on moistened sterilized barley medium for 20 days at 25°C. Soil infestation was carried out using each isolate of *M. phaseolina* at the rat of 3 inoculated barley seeds around each used bean seed according to the methods of Pastor-Corrales and Abawi (1988). The inoculum was added at the planting time. Control pots were filled with the same amount of sterilized barley medium without inoculum. A set of three pots with five-bean seeds / pot was employed for each tested isolate. The percentages of pre and post-emergence damping-off were assessed 15 and 30 days after sowing, respectively. Meanwhile, charcoal rot and survived plants were recorded 60 days after sowing.

2- Effect of different degrees of temperature on the linear growth of M. phaseolina isolates:

The effect of different degrees of temperature, *i.e.* 15,25 and 35°C on the linear growth of the three *M. phaseolina* isolates was tested. Disks of 4 mm in diameters of each isolate were taken from 5 days old cultures, then transferred on to the center of 9-cm Petri dishes containing PDA medium. Linear growth of the tested isolates was determined after 28,48,72,96,120,144 and 168 hrs. Three replicates were used for each degree of temperature.

3- Inoculum level:

Minimum inoculum level means the minimum amount of fungal inoculum required to cause approximately 50% diseased plants. Inoculum types and its levels were carried out according to Abawi and Pastor-Corrales (1989) as follows: A- Number of barley seeds from barley medium inoculated by the tested isolate per one bean seed, at the rate of 1,2,3,4 and 5 barley seeds per bean seed. B- Weight of barley medium inoculated by the isolate to the weight of the soil, at the rate of 1,2,3,4 and 5%. C- Weight of dried sclerotia of tested isolate to the weight of sterilized soil at the rate of 1,2,3,4 and 5 g dry sclerotia/ Kg soil.

Inoculum preparation, soil infestation and sowing were individually carried out as mentioned before. The healthy seeds of Xera bean cultivar were planted at the rate of 5 seed / pot. Three replicates were used for each treatment. Other 3 pots containing non-infested soil were served as control.

The percentages of pre and post emergence damping-off, survived plants and charcoal rot were assessed as mention before.

4- Host range:

The response of differe.. hosts to infect on *M. phaseolina* was studied under the greenhouse conditions. Twenty different species of different families namely, broad bean, maize, cowpea, okra, peas, cotton, cantaloupe, cucumber, peanut, tomato, watermelon, sorghum, pepper, soybean, castorbean, sesame, wheat, sunflower, lupines and chickpea were tested in this experiment.

Five surface sterilized seeds of any of the tested hosts were sown in each pot containing soil inoculated by the pathogen. Three replicate pots were sown for each host. Also, five surface sterilized seeds were sown in each pot containing uninfected soil and served as control. The percentage of pre and post emergence damping-off, survived plants and charcoal rot was calculated as mentioned before.

5- Effect of fungal cultural filtrate on the germination, rootlet length and weight of bean cultivars:

M. phaseolina isolates were grown in 250-ml conical flasks, containing 100 ml of Czapek's liquid medium. Inoculation was carried out using one disc (5 mm in diameter) taken from 7 days old cultures. Each fungal isolate was inoculated in 4 flasks, in addition to 4 flasks were not inoculated and used as control. All inoculated and noninoculated flasks were incubated at 25°C for 21 days. The fungal filtrate was obtained by filtration the content of each flask through Watman's No1 filter paper and then sterilized by filtration through bacterial filter (G5).

The effect of sterilized filtrate on the seed germination, length and dry weight of radical roots was studied. Three concentrations of the filtrate, *i.e.* 100, 50 and 25% were prepared by addition of proper amounts of sterilized distilled water or sterilized Czapek's medium. Also, 100 % medium and sterilized distilled water were used as control. Ten ml from any of the different filtrate concentrations were purred on sterilized filter papers in sterilized Petri dishes. Five surfaces sterilized healthy seeds of any of the three bean cultivars; namely Nebraska, Morgan and Xera were put in each dish (5 dishes/treatment). After 15 days of incubation at 25°C, the percentage of germinated seeds, length and dry weight of radical roots was calculated.

6- Enzymological studies:

The ability of each isolate of *M. phaseolina* to produce pectinolytic and cellulolytic enzymes was evaluated in vitro and i*n vivo*.

1-*In vitro*: The activity of Pectin methyl esterase (PME), Polygalacturonase (PG) and Cellulase (Cx) produced individually by three isolates of *M. phaseolina* was determined. Each isolate of *M. phaseolina* was grown in conical flasks (250 ml) each containing 100 ml of a medium consists of 0.05% $MgSO_4$-$7H_2O$ + 0.05 % KCl + 0.001% $FeSO_4$-$7H_2O$ + 0.3% L-arginine + 1.8% KH_2PO_4 + 1.2% K_2HPO_4 + 2.0% glucose, with 2.0% pectin for the determination of PG and PME and with 2.0% Carboxymethyl cellulose (CMC) for the determination of Cellulase enzyme (Cx) instead of the glucose (Talboys and Buch 1970).

The medium which containing pectin or CMC was autoclaved, then inoculated with any of the tested isolates, by placing a 5 mm in diameter of agar disc taken from 4 days old cultures grown on PDA medium in Petri dishes. Five flasks were used for each isolate, and the flasks were incubated at 30°C. After 7,15and 21 days of incubation, the cultures were filtrated and centrifuged at 3000 rpm for 20 mins. The clear supernatant was utilized as crud enzyme for determining the enzyme activity (Kertez 1951).

a- Pectin methyl esterase (PME): Pectin methyl esterase (PME) was determined based on the hydrolytic removal of methyl groups from pectin molecule by the action of the enzymes.

b- Polygalacturonase (PG) and Cellulase (Cx) activities: The activities of Polygalacturonase (PG) and Cellulase (Cx) were determined using the viscometric method.

2- *In vivo*: Artificially inoculated Xera bean seedlings by toothpick inoculation (Chan and Sackston 1972), was carried out under the greenhouse conditions for studying cell wall degrading enzymes associated with diseased tissues. Seedlings (15 days old) were inoculated individually with the three isolates of *M. phaseolina* near soil surface, non-inoculated seedlings were used as a check.

After 10,20 and 30 days of inoculation, individual extracts from diseased and healthy tissues of Xera bean seedling were obtained by blending an equal amount of both tissues and distilled water in warring blender. The extract was. employed to estimate the pectolytic and celluloltic activities in both diseased and healthy tissues as described before.

7- Statistical analysis:

The data were statistically analyzed using the analysis of variance procedure for completely randomized design. Treatment means were compared using the protected Least Significant Difference (L.S.D.) analysis according to Snedecor and Cochran (1967).

Results

1- Pathogenicity test:

The pathogenicity test was carried out using three isolates of the fungus *M. phaseolina* . Data shown in Table (1) show that, all the tested isolates were pathogenic to Xera bean cultivar.

Sweet melon isolate had a superior effect on Xera cultivar and was the most pathogenic isolate since it caused 53.33, 13.33, 80.33 and 33.33% of pre and post emergence damping-off, charcoal rot and survived plants, respectively, followed by bean isolate, being 33.33, 0.0, 50.33 and 66.67%, respectively. Sesame isolate seemed to be the less pathogenic isolate which exhibited 20.0, 0.0, 51.33 and 80.0 % of pre and post emergence damping-off, charcoal rot and survived plants, respectively. Control treatment didn't score any infection and gave 100% survived plants.

2- Effect of different degrees of temperature on the linear growth of M. phaseolina isolates:

Data presented in Table (2) reveal that, the diameter of *M. phaseolina* growth was varied according to the used degree of temperature. There was a slight growth at the low temperature degree (15°C) within the first 24 hrs after inoculation for the three tested isolates.

After 24 hrs, the growth continued to increase, but all tested isolates did not covered the Petri dishes after 168 hrs. At 25°C, all *M. phaseolina* isolates covered the Petri dishes within 72 hrs., while at 35°C, isolates of *M. phaseolina* varied in their linear growth. Sweet melon isolate covered the Petri dishes within 72 hrs, while bean isolate covered the Petri dishes after 96 hrs, whereas sesame isolate covered the Petri dishes within 120 hrs.

3-Inoculum potential:

Results shown in Table (3) indicate that there was a proportional relationship between the amount of inoculum added to the soil and the percentage of pre and post emergence damping-off, charcoal rot and survived plants.

Increasing the amount of the inoculum resulted in increasing the percentage of disease incidence. Data, also indicate that methods of using inoculum (3 inoculated barely seeds/bean seed, 3% of inoculum/ soil weight and 3g dried sclerotia/kg soil) were enough to cause pre and post emergence damping-off and charcoal rot.

Three inoculated barely seeds/ bean seed gave the highest percentage of disease incidence, being 53.33,13.33 and 11.1 % for pre and post emergence damping-off and charcoal rot, respectively. The inoculum levels (3% inoculum/soil weight and 3g dried sclerotia/ kg soil) were less effective since exhibited 40.0, 13.33 and 8.14 %, as well as, 26.66, 6.66 and 5.92 %, respectively. So that, 3 infected barely seeds/bean seed was chosen for the further studies.

4- Host range:

The different tested plants were evaluated to their susceptibility to the infection by *M. phaseolina*. Data presented in Table (4) show that, all the used plants were found to be infected but with different degrees. Sesame and sunflower were more susceptible, being 66.66, 4.16, 41.93, 29.16 and 66.66, 4.16, 41.94, 29.16 % for pre and post emergence damping-off, charcoal rot and survived plants, respectively, followed by castor bean, broad bean, cowpea, okra, peas, peanut and soybean. Meantime, wheat and maize were moderate susceptible (33.33, 0.0, 8.14, 66.66 %, as well as, 33.33, 0.0, 15.46, 66.66 %), respectively. Cantaloupe was less susceptible host, which recorded 12.50, 0.0, 11.76 and 87.50 %, respectively, followed by cucumber and tomato.

5- Effect of fungal culture filtrate:

Data in Table (5) show the effect of different concentrations, *i.e.* 25, 50 and 100 % of *M. phaseolina* cultural filtrate on the percentage of seed germination, rootlet length and dry weight of three bean cultivars namely Nebraska, Morgan and Xera. The data indicate that, fungal cultural filtrate decreased seed germination %, rootlet length and dry weight of the three tested cultivars.

Xera bean cultivar, was highly affected by all tested concentrations of the pathogen. The cultural filtrate caused the most harmful effect on seed germination %, rootlet length and dry weight.

Concerning with Nebraska bean cultivar, the obtained data are in contrast than that recorded in case of cv. Xera. The cultural filtrate caused the least effect on the percentage of seed germination, rootlet length and rootlet weight. With respect to Morgan cv., the effect was moderately under all the tested concentrations of culture filtrate.

6- Enzymatic activity:

A-*In vitro*: Pectin methyl esterase (PME), polygalacturonase (PG) and cellulase (CX) were determined in the culture filtrate of the three tested isolates of *M. phaseolina*. The tested isolates were grown on pectin and carboxy methyl cellulose (CMC) media for 7,15 and 21days.

Data presented in Table (6) show that, the activity of (PME) was detected in the culture filtrate of the three tested isolates with different degrees at all incubation periods. The highest enzymatic activity of pectin methyl esterase (PME) was observed with the virulent sweet melon isolate, while the least activity was noted for the least virulent one (Sesame isolate), whereas bean isolate was intermediate in this respect.

Concerning with polygalacturonase (PG) enzyme activity, the least activity of (PG) was noted for the least virulent sesame isolate. Meanwhile, the highest activity was recorded for the virulent sweet melon isolate, but isolate obtained from bean was intermediate activity. On the other hand, cellulase (CX) enzyme activity was increased by increasing culture age and was not correlated with the virulence of the tested isolates. In all cases, cellulase activity was higher after 21 days of incubation for the three tested isolates.

B-In vivo: Pectinolytic and cellulolytic enzyme activities were determined in bean seedlings infected by any of the three isolates of *M. phaseolina*. Extracts of healthy and infected bean seedlings were used for this purpose after 10, 20 and 30 days of inoculation.

Data shown in Table (7) indicate that, the activity of (PME) was increased in the infected seedlings, compared with the uninfected ones. The virulent sweet melon isolate produced the highest activity of (PME), while the least virulent sesame isolate produced the lowest activity of (PME). Polygalacturonase enzyme activity was also low in the uninfected seedlings.

The highest activity of (PG) was recorded in the seedlings that infected by the virulent isolate, while the lowest activity was found in the seedlings infected by the least virulent one. Concerning with (CX) enzyme activity, data show that, it increased in the seedlings infected by the tested isolates, compared with the healthy seedlings. Activity of (CX) was higher in the seedlings infected by the virulent sweet melon isolate, while the lowest activity was found in the seedlings infected by the least virulent one. In general, (PME, PG and CX) enzyme activities were increased in the seedlings infected by the virulent isolate and /or with increasing of periods after inoculation.

Table (1): Pathogenicity test of three isolates of *M. phaseolina* on Xera bean cultivar, 15, 30 and 60 days after sowing under greenhouse conditions.

Isolates	% Damping-off.		%Charcoal rot	%Survived plants
	Pre emergence	Post emergence		
Sweet melon isolate	53.33	13.33	80.33	33.33
Bean isolate	33.33	0.0	50.33	66.67
Sesame isolate	20.00	0.0	51.33	80.00
Control	00.00	0.0	0.0	100.00
L.S.D. at 0.05	20.8	N.S	18.4	14.91

Table (2): Effect of three different temperatures for 7 days on linear growth (mm) of three isolate of *M. phaseolina* grown on PDA medium.

Isolates (I)	Temper-ature (T)	Linear growth (mm) after (hrs) of incubation period (H)							Mean
		24	48	72	96	120	144	168	
Sweet melon isolate	15 °C	17.3	32.3	44.7	53.0	61.3	73.3	77.0	51.27
	25 °C	29.0	55.3	90.0	90.0	90.0	90.0	90.0	76.32
	35 °C	48.7	65.3	90.0	90.0	90.0	90.0	90.0	80.57
	Mean	31.67	50.97	74.90	77.67	80.43	84.43	85.67	69.39
Bean isolate	15 °C	15.7	26.3	36.7	41.0	52.7	65.3	73.7	44.48
	25 °C	26.3	44.3	90.0	90.0	90.0	90.0	90.0	74.37
	35 °C	47.7	57.0	81.3	90.0	90.0	90.0	90.0	78.00
	Mean	29.90	42.53	69.33	73.67	77.57	81.77	84.57	65.62
Sesame isolate	15 °C	11.3	26.7	33.7	41.3	55.3	62.0	72.0	43.18
	25 °C	22.0	46.3	90.0	90.0	90.0	90.0	90.0	74.04
	35 °C	40.3	50.3	76.7	85.7	90.0	90.0	90.0	74.71
	Mean	24.53	41.10	66.80	72.33	78:43	80.67	84.00	63.98
	Mean	28.70	44.87	70.34	74.56	78.81	82.29	84.75	

Mean of Temperatures (T) 15°C = 46.31 25°C = 74.91 35°C = 77.67

L.S.D. at 0.05 for :

Isolates (I) = 0.29 (IXT) = 0.82 (IXTX H) = 1.36

Temperatures (T) = 0.47 (IX H) = 0.78

Hours (H) = 0.45 (TXH) = 0.78

Table (3): Effect of different inoculum levels and inoculum methods of *M. phaseolina* on percentages of pre and post emergence damping-off, charcoal rot and survived plants of Xera bean cultivar.

Inoculum levels (R)	%Damping off								%Charcoal rot				%Survived plants			
	Pre emergence				Post emergence											
	* A	B	C	Mean	A	B	C	Mean	A	B	C	Mean	A	B	C	Mean
1	6.66	13.33	20.0	13.33	6.66	0.0	0.0	2.22	4.44	4.44	2.82	3.90	86.66	86.66	80.0	84.44
2	20.0	40.0	26.66	28.89	6.66	6.66	0.0	4.44	7.03	5.92	5.04	5.99	73.33	53.33	73.33	66.66
3	53.33	40.0	26.66	39.99	13.33	13.33	6.66	11.11	11.10	8.14	5.92	8.39	33.33	46.66	66.66	48.88
4	60.0	40.0	33.33	44.44	13.33	13.33	6.66	11.11	14.80	8.51	23.31	15.54	46.66	46.66	60.0	51.11
5	73.33	53.33	33.33	53.33	13.33	13.33	13.33	13.33	37.0	25.90	38.86	33.92	13.33	33.33	53.33	33.33
Mean.	42.66	37.33	28.0		10.66	9.33	5.33		14.87	10.58	15.19		50.66	53.33	66.66	

* Inoculum methods: A = Seeds treatment (No. of inoculated barley seeds/bean seed).
B = Soil treatment (%Weight of barley/ Weight of soil).
C = Soil treatment (Weight of sclerotia (g)/kg soil).

L.S.D. at 0.05 for:

Inoculum methods (I)	13.71	3.71	3.39	13.48
Inoculum levels (R)	20.69	3.18	9.98	25.12
Interaction (I x R)	15.39	8.51	11.39	25.51

Table (4): Susceptibility of different plant hosts to infection by *M. phaseolina*, the causal agent of charcoal rot of bean.

The tested plants	% Damping-off		% Charcoal rot	% Survived plants
	Pre emergence	Post emergence		
Broad bean	41.66	4.16	33.30	54.16
Cowpea	41.66	0.00	37.00	58.33
Peas	50.00	0.00	33.30	50.00
Soybean	58.33	4.16	37.00	37.50
Lupins	41.66	0.00	9.62	58.33
Chickpea	41.66	4.16	6.66	54.16
Cantaloupe	12.50	0.00	11.76	87.50
Cucumber	16.66	0.00	11.10	83.34
Watermelon	29.16	0.00	9.62	70.80
Castor bean	54.16	4.16	29.60	41.66
Peanut	41.66	0.00	33.30	58.33
Sesame	66.66	4.16	41.93	29.16
Sunflower	66.66	4.16	41.94	29.16
Maize	33.33	0.00	15.46	66.66
Sorghum	41.66	0.00	18.50	58.33
Wheat	33.33	0.00	8.14	66.66
Cotton	37.50	0.00	29.60	62.50
Okra	41.66	4.16	37.00	54.16
Paper	37.50	0.00	8.14	62.50
Tomato	16.66	0.00	14.80	83.33
L.S.D. at 0.05	9.87	7.9	10.43	29.47

Table (5): Percentage of seeds germination, length (mm) and dry weight (g) of rootlet of three bean cultivars, at different concentrations of fungal culture filtrates 15 days after incubation at 25°C.

Conc. of culture filtrate (F)	% Germination				Rootlet length (mm)				Rootlet dry weight (g)			
	Nebraska cv.	Morgan cv.	Xera cv.	Mean	Nebraska cv.	Morgan cv.	Xera cv.	Mean	Nebraska cv.	Morgan cv.	Xera cv.	Mean
25% filtrate	93.33	93.33	86.0	90.88	55.40	60.50	51.30	55.73	0.66	0.40	0.26	0.44
50% filtrate	85.66	80.0	73.33	79.66	52.90	59.40	49.10	53.80	0.53	0.36	0.30	0.39
100% filtrate	73.33	66.66	46.66	62.21	50.40	58.70	47.80	52.30	0.46	0.30	0.20	0.32
100% medium	100.0	100.0	100.0	100.0	50.30	58.0	50.90	53.06	0.73	0.40	0.33	0.48
Distilled water	100.0	100.0	100.0	100.0	55.50	60.50	52.40	56.13	0.80	0.50	0.36	0.55
Mean	90.46	87.99	81.19		52.90	59.42	50.30		0.66	0.39	0.29	

L.S.D. at 0.05 for

Filtrate (F)	8.19	1.49	0.08
Cultivar (cv.)	8.66	1.76	0.10
(F×cv.)	12.71	9.19	3.06

Table (6): Pectin methyl estrase (PME), Polygalacturonase (PG) and cellulase (CX) enzyme activity in fungal culture filtrates of three *M. phaseolina* isolates after 7,15 and 21 days of incubation at 30°C *in vitro*.

Isolates / Culture age (days)		[b] PME	[c] PG		[d] Cx	
			[a] 15 m.	30 m.	15 m.	30 m.
Sweet melon isolate	7	0.27	8.5	18.1	13.5	26.1
	15	1.63	20.4	34.6	18.9	29.3
	21	2.19	33.7	47.3	20.4	40.1
Bean isolate	7	0.22	5.8	12.5	5.4	9.6
	15	0.29	15.5	28.8	8.6	13.1
	21	0.82	24.6	45.4	10.3	26.7
Sesame isolate	7	0.13	5.2	12.2	2.6	7.4
	15	0.18	6.5	15.1	9.5	9.8
	21	0.19	18.6	34.4	10.2	10.7

Table (7): Pectin methyl estrase (PME), Polygalacturonase (PG) and cellulase (CX) enzymes activity in the extracts of (Xera) bean seedlings inoculated with three *M. phaseolina* isolates, after 10, 20 and 30 days of inoculation at 30°C in vivo.

Isolates	Days after inoculation	[b] PME	[c] PG		[d] Cx	
			[a]15min	30 min.	15 min.	30 min.
Sweet melon isolate	10	0.33	10.2	24.9	19.7	33.6
	20	1.37	23.3	38.1	35.4	41.5
	30	1.66	32.5	43.3	46.5	42.4
Bean isolate	10	1.24	9.1	15.6	19.2	20.7
	20	0.75	18.7	22.5	25.1	34.7
	30	1.17	23.4	37.1	27.7	30.3
Sesame isolate	10	0.21	8.2	12.1	3.7	3.9
	20	0.22	7.2	9.7	5.1	10.2
	30	0.26	13.5	22.3	12.8	17.3
Control	10	0.11	2.6	1.8	2.8	3.1
	20	0.14	3.1	4.2	2.9	1.5
	30	0.13	3.5	6.7	4.4	3.2

Disscussion

Pathogenicity test proved that all *M. phaseolina* isolates were pathogenic with different degrees, where sweet melon isolate was the most virulent one, followed by bean and sesame isolates. Saleh 1997 and Salem 1997 reported similar results. Hussien 1997 noted that, isolate MG_2 of *M. phaseolina* (isolate 2 from Giza) recorded the highest figures of pre and post emergence damping-off, followed by isolates MB_3 (isolate 3 from Bani-Sweif), MI_4 (isolate 4 from Ismailia) and MB1 (isolate 1 from Bani-Sweif).

The tested degrees of temperature greatly affected the growth of different isolates of *M. phaseolina*. There was a slight growth at low temperature 15 C for the three tested isolates. At 25°C, all *M. phaseolina* isolates covered the Petri dishes within 24 hrs, while at 35°C, the isolates varied in their linear growth. Salem 1997 previously reported a similar result.

Three different cultivars (Nebraska, Morgan, and Xera) showed obvious difference in their reaction to cultural filtrate of *M.* Phaseolina isolates. This variation in reaction could be utilized in a rabid technique for screening the different bean cultivars for resistance to *M. phaseolina*. Sarhan 2000 proved that cultural filtrate of all the tested fungi (F. oxysporum, A. alternata, R. solani and *M. phaseolina*) decreased seed germination percentage and rootlet length of the three-tested bean cultivars. This effect was very clear especially in case of F. oxysporum cultural filtrate and might be due to some toxic material and/or enzymes.

Disease severity depends on the inoculum potential and/or amount of fungal propagules in the soil. The obtained results indicated that, there was a gradual increase in the disease severity with increasing the inoculum levels and inoculum types. This was agreement with the results obtained by Agha 1977 and Eisa, Nour-Jehan 1998.

Abawi and Pastor-Corrales 1986 evaluated the efficiency of several inoculum preparations and screening procedures for determining the reactions of bean germplasm accessions to infection by *M. phaseolina*. The most effective methodology in the greenhouse tests was to cover bean seeds with 2-3 cm of soil artificially infested with *M. phaseolina* (2 g of dry sclerotia / kg of pasteurized soil). Whole rice seeds colonized with *M. phaseolina* also were highly efficient in causing charcoal rot, and the resulting symptoms permitted the differentiation of susceptible and resistant bean germplasm. Large volumes of the colonized rice seeds can be produced easily and rapidly, and thus, this inoculum is suitable for extensive field evaluation trials.

The different host plants were evaluated to their susceptibility to infect by *M. phaseolina*. All the tested hosts were infected by different degrees. Sesame and sunflower were more susceptible, followed by castor bean, broad bean, cowpea, okra,

peas, peanut and soybean. Wheat and maize were moderate susceptible. Cantaloupe was less susceptible host, followed by cucumber and tomato. Most of the tested hosts gave percentage of disease incidence. This proved that *M. phaseolina* had a wide host range and this might be due to, it is a seed and soil borne pathogen. Abdon *et al.*, 1980 mentioned that *M. phaseolina* was isolated from the roots of cowpea, common bean, hyacinth bean, pigeon pea, green gram and one weed of Crotalaria spp 15 days after inoculation. They added that, in case of common bean, soybean, cowpea and hyacinth bean, the pathogen was found in the lower stem 15 days after inoculation. Symptoms were not apparent on green gram until 60 days after inoculation, this implied a good level of resistance in this crop. The absence of symptoms in case of chickpea and pigeon pea suggests more resistance to the pathogen in these crops and would therefore, be suitable for crop rotation in the infested fields. These crops are drought tolerant and would fit well in farming systems of areas where charcoal rot disease is prevalent. Songa and Hillocks 1996 noted that, the plurivorous nature of *M. phaseolina* enables to survive on many alternatives hosts in the absence of crop hosts.

Cultural filtrate of the virulent sweet melon isolate decreased seed germination percentage and rootlet length and dry weight of the three tested cultivars. This effect was very clear especially in case of concentration 100 % culture filtrate, which caused strong inhibition of seed germination and decreased the rootlet length and dry weight, followed by concentration 50 % and 25 %. The tested culture filtrates may be had inhibitors or toxic substances.

Three isolates of *M. phaseolina*, which previously showed different degrees of virulence were examined for their polygalacturonase (PG), pectin methyl estrase (PME) and cellulase (CX) enzymes activity both in vitro and *in vivo*. *In vitro* studies revealed that the activity of pectolytic and cellulolytic enzymes was increased with increasing the period of incubation for all tested isolates. The highest activity of (PG) and (PME) enzymes was recorded for the most virulent isolate, while the least activity was noted for the least virulent isolate. All tested isolates produced cellulase (CX) in culture filtrates at different incubation periods, and the activity of cellulase enzyme was increased with increasing of incubation periods. These results are in complete agreement with those recorded by Rai and Srivastava 1975, Chan and Sackston 1972.

The obtained results *in vivo* experiments are agreed with those obtained by Rai and Srivastava 1975, Abdelal *et al.*, 1983 and Ibrahim 1996. Chan and Sackston 1972 who recorded that PG and PME are important in penetration and early stages of pathogenesis and virulence of the isolates is correlated with their ability to produce these enzymes in host tissues. They also found that, (CX) activity in the plant extracts was increased with time after inoculation.

Generally, the high activity of both PME and PG enzyme could be taken as an indicator for the virulence of *M. phaseolina* isolates. Meanwhile, for CX enzyme its activity increases by increasing the incubation period of some isolates.

References

1. ABAWI, G. S. and PASTOR CORRALES, *M.* A. 1986: Screening procedure and virulence of isolates of Macrophomina *phaseolina* to beans. Phytopathology. 76 (10):1064.
2. ABAWI, G. S. and PASTOR CORRALES, *M.* A. 1989: Charcoal rot screening procedure and virulence of Macrophomina *phaseolina* isolates on dray edible bean. Turrialba, 39 (2): 200-207.
3. ABDELAL, H. R.; EL-FAHL, A. *M.*; SHATTA, H.A. and MAHROUS, *M. M.* 1983: Production of pectolytic and cellulolytic enzymes by six isolates of Macrophomina *phaseolina*. Agric. Res. Rev., 61:154-165.
4. ABODON, Y. A.; EL-HASSAN, S. A. and ABBAS, H. K. 1980: Seed transmission and pycnidial formation in sesame wilt disease caused by Macrophomina *phaseolina* (Maubl.) Ashby. Agric. Res. Rev., 52:93-69.
5. AGHA, *M.* S. 1977: Root rot disease of soybean and its control. *M.* Sc. Thesis, Fac. Agric., AL- Azhar Univ., Egypt.
6. AHMED, *M.* I. 2002: Studies on charcoal rot disease of bean in Ismailia. *M.* Sc. Thesis. Fac. Agric., Suez Canal Univ., Ismailia, Egypt.
7. CHAN, Y. H. and SACKSTON, W. F. 1972: Production of pectolytic and celluloltic enzymes by virulent and avirulent isolates of Sclerotium bataticola during disease development in sunflowers. Can. J. Bot., 50:2449-2453.
8. EISA, NOUR-JEHAN, *M. M.* E. 1998: Studies on

root rot disease of bean in Egypt. Ph. D. Thesis, Fac. Agric., Suez Canal Univ., Ismailia, Egypt.

9. HUSSIEN, S. H. *M.* H. 1997: Studies on acquired resistance to fungicides in some fungi-causing root rot diseases of bean. *M.* Sc. Thesis, Fac. Agric., Cairo Univ., Egypt. 95 pp.

10. IBRAHIM, S. A. I. A. 1996: Phytopathological studies on the variability in virulence of some isolates of Macrophomina *phaseolina* from different hosts. Ph. D. Thesis, Fac. Agric., Cairo Univ., Egypt.

11. KERTEZ, Z. I. 1951: The pectic substances. Interscience publishers, New York, 268 pp (c.f. Talboys and Bush 1970).

12. MIHAIL, J.D.1992: Macrophomina. Pages 134-136 in: Methods for research on soil borne phytopathogenic fungi. (c.f. Olaya *et al.*, 1996).

13. MIKLAS, P. N.; VALERIS SYONE; CARLOS, A. U.; JOHNSON, E. and BEAVER, J. S. 1998: Inheritance and QTL analyses of field resistance to ashy stem blight in common bean. Crop Science. 38:916-921.

14. OLAYA, G.; ABAWI, G.S. and WEEDEN, N.F. 1996: Inheritance of the resistance to Macrophomina *phaseolina* and identification of RAPD markers linked the resistance genes in bean. Phytopathology. 86, 6 : 674-679.

15. PASTOR-CORRALES, *M.* A. and ABAWI, G. S. 1988: Reactions of selected bean accessions to infection by Macrophomina *phaseolina*. Plant disease 72: 39-41.

16. RAI, J. N. and SRIVASTAVA, S. K. 1975: Production of endo-polygalacturonase and cellulase by isolates of Macrophomina *phaseolina* causing Barassica junicea. Indian J. Myco. Plant Pathol., 5: 169-173.

17. SALEH, O. I. 1997: Wilt, root rot and seed diseases of groundnut in El-Minia Governorate. Egypt. J. Phytopathol., 25 (1-2): 1-18.

18. SALEM, M.F.*M.* 1997: Suppression of Macrophomina *phaseolina*, the causal organism of charcoal rot of soybean. *M.* Sc. Thesis, Fac. Agric., Minufiya Univ., Shibin El-Kom, Egypt.

19. SARHAN, E.A. D. 2000: Studies on some seed borne diseases of bean and their control. *M.* Sc. Thesis, Fac. Agric., Zagazig Univ. 153 pp.

20. SNEDECOR, G.W. and COCHRAN, W.G. 1967: Statistical methods. 6th ed. Iowa State university press, Iowa, U.S.A.

21. SONGA, W. and HILLOCKS. R. J. 1996: Legume hosts of Macrophomina *phaseolina* in Kenya and effect of crop species on soil inoculum levels. J. Phytopathology 144: 387-391.

22. TALBOYS, P. W. and BUSH, L. V. 1970: Pectic enzymes produced by Verticillium spp. Trans Br. Mycol. Soc., 55: 367-381.

دراسات مرضية وفسيولوجية على فطر ماكروفومينا فاصولينا المسبب لمرض العفن الفحمي في الفاصوليا

إبراهيم ناجى محمد على[1] - مجدي إبراهيم غنيم[2]- هبه محمد عبدالنبى[1] _ محمد إسماعيل محمد أحمد [2]

١- كلية الزراعة, جامعة قناة السويس - الإسماعيلية, مصر.

٢- معهد بحوث أمراض النباتات, محطة بحوث البساتين بالقصاصين - مركز البحوث الزراعية, الجيزة, مصر.

أوضحت الدراسة أن عزلة الفطر ماكروفومينا فاصولينا المعزولة من جذور الشمام كانت الأكثر قدرة على إحداث المرض يليها العزلة المعزولة من جذور الفاصوليا ثم العزلة المعزولة من سوق السمسم, كما أوضحت دراسة تأثير درجات الحرارة لثلاث عزلات من الفطر ماكروفومينا فاصولينا في المعمل أن درجة الحرارة المثلى للنمو الميسليومى تتراوح بين ٢٥ - ٣٥ م وانخفضت عند ١٥م وذلك باختلاف العزلات كما أدى زيادة تركيز اللقاح إلى زيادة النسبة المئوية لحدوث المرض وكان المستوى ٣ بذور شعير/ بذرة فاصوليا كافي لإحداث المرض. وعند استخدام الراشح المزرعى للفطر بتركيزات مختلفة قلل النسبة المئوية لإنبات بذور الفاصوليا, كما أن له تأثير ضار على طول الجذور ووزنه الجاف. كانت نباتات السمسم من أكثر العوائل النباتية قابلية للإصابة يليها نباتات القمح ثم الذرة أما الكنتالوب فقد كان أقل النباتات المختبرة حساسية للإصابة بالفطر. زاد النشاط الإنزيمي للإنزيمات بكتين ميثيل استريز وبولى جلاكتورونيز وسليلوز في الراشح المزرعى لعزلات الفطر وكذلك في مستخلص بادرات الفاصوليا المصابة بالفطر.