PATHOLOGICAL AND PHYSIOLOGICAL STUDIES ON SOME ISOLATES OF *MACROPHOMINA PHASEOLINA*

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ABSTRACT

Macrophomina phaseolina was isolated more frequently (65.2%) followed by *Fusarium* spp. (25.0%), *Rhizoctonia solani* (7.6%) and *Sclerotium rolfsii* (2.2%) from different samples showing charcoal-rot and root rot symptoms. The highest frequencies of *M. phaseolina* (94.0%), *Fusarium* spp. (52.3%), *R. solani* (37.5%), and *S. rolfsii* (18.5%) were recorded in Menia, Domiat, Menofiya, and Qalubya,. Eleven isolates of *M. phaseolina* obtained from different hosts and locations were studied in this work. The obtained isolates differed significantly in linear growth and sclerotial production. Color of fungal colonies was varied from gray to black also and seems to be correlated with density of sclerotial formation.

In general, culture filtrates sterilized by autoclave, were more toxic and caused significant reduction in seed germination, shoot and root lengths of sesame seedlings than those sterilized through Sintered glass filter. Culture filtrates of sesame isolates No.1 and 5 showed the highest and lowest phytotoxocity, respectively.

Pathogenicity tests carried out in infested soil and piercing sesame stems with toothpicks proved that, *M. phaseolina* isolated from sunflower and sesame (isolate No.7) were less virulent, meanwhile sesame-isolate No.3 was more virulent on sesame plants.

Studying protein patterns proved that, the highest degree of similarity was detected between *M. phaseolina* isolates from host plants grown in the warmer soils in Middle and Upper Egypt.

The interaction between 5 isolates of *M. phaseolina* and 5 host plants was studied. Sunflower was the most susceptible host to infection, followed by sesame, soybean, cotton and peanut, respectively. However, isolates of *M. phaseolina* from sesame, soybean, and sunflower were more aggressive than those isolated from peanut and cotton. Host-plant/pathogen-isolate interactions were studied, the highest disease incidence was associated with peanut/peanut isolate, sesame/sesame isolate, soybean/sunflower isolate, sunflower/soybean isolate, and cotton/sesame isolate combinations. Isolate of *M. phaseolina* isolated from peanut was more aggressive on sunflower than the other tested host plants.

Key Words: Sesame, charcoal-rot, *Macrophomina phaseolina*, culture filtrates, soil infestation, toothpick inoculation, electrophoresis, patterns protein.

INTRODUCTION

Sesame (*Sesamum indeicum* L.) is one of the most important summer oil crops in the tropical and subtropical regions, allover the world. It is grown in many locations of Egypt (i.e., Sharkia, Ismaiellia, Giza, Assiut, El-Minia, Sohag and Qena Governorates). Sesame plants are attacked during the growing season with many diseases causing considerable quantitative and qualitative losses in seed yield and oil content **(Abd El-Kader, 1977; El-Deeb *et al.*, 1987 and El-Barougy, 1990)**.

Charcoal and/or root-rot and wilt diseases caused by many soil-borne pathogens including, *Macrophomina phaseolina* Tassi (Goid.) are the main destructive diseases of sesame crop especially in upper Egypt **(Al-Ani *et al*., 1970; El-Barougy, 1990** and **Khalifa, 1997)**. Isolates of *M. phaseolina* from different host plants and different localities in Egypt greatly varied in inducing damping-off, root-rot and charcoal-rot on sesame plants resulting in considerable decrease in healthy plants **(Khalifa, 1997)**. This fungal pathogen attacks a wide range of plants like sunflower **(Ahmed *et al.*, 1994a)**, soybean **(Hassanein, 1985)**, cotton **(Mostafa *et al*., 1957)**, peanut **(Zayed *et al.*, 1986)** and many others. **Abd El-Ghany (1998)** obtained 8 isolates of *M. phaseolina* from different host plants. She observed considerable variations between isolates in linear growth as well as their pathogenicity on potato tubers and maize stem portions *in vitro.* Pathogenicity test carried out under greenhouse conditions gave contradictory results regarding specificity of isolates on different hosts. *M. phaseolina* could survive in soil, sesame seeds **(Dinakaran and Dharmalingam, 1996),** as well as in residue of sesame plants (**Al-Ahmed and Saidawi, 1988; Azab *et al.*, 1994**)

The present work was conducted to study the severity of *M. phaseolina* isolated from different hosts grown in different localities in Egypt on sesame plants. Toothpick piercing technique was used also for studying the effect of plant age on incidence of charcoal rot incited by different isolates of *M. phaseolina*. Isolation frequency, effect of cultural filtrates sterilized by autoclave or through Sintered glass filter on germination of sesame seeds as well as on length of shoots and radical roots, SDS protein patterns, and host/pathogen interaction were also studied.

MATERIALS AND METHODS

Isolation and identification of the causal organisms:

Sesame, peanut, soybean, sunflower and cotton plants showing charcoal-rot and root rot symptoms were used in this study. Sesame plants were collected from 7 locations i.e. El-Saff "Giza", Etsa "Fayoum" Samllot "Menia" Shandaweel "Sohag", Abo-Hammad "Sharkia", El-Tahrir "Behera" and New Domiat "Domiat", while diseaed peanut, soybean, sunflower, and cotton plants were collected from Somosta "Beni-Suef", Assiut «Assiut", Qaha « Qalubya" , Menof « Menofiya", respectively.

The infected roots were cleaned from adherent soil, surface sterilized by dipping in 2.0% sodium hypochlorite for 5 minutes, rinsed several times in sterilized distilled water then dried between sterilized filter papers. Small pieces of sterilized roots were plated onto plain-agar and incubated at 26°C for 5-7 days. Hyphal tips taken from the peripheral ends of the growing colonies were transferred to platted potato dextrose agar (PDA). The isolated fungi were furthur purified using both hyphal tip or single spore techniques (**Hildebrand**, **1938**) and identified according to **Dhingra** and **Sinclair** (**1973**). Frequency/percentage of different isolated fungi associated with roots of infected plants collected from different locations was calculated.

Comparative studies on isolates of *Macrophomina phaseolina*:

1- Linear growth, color and nature of fungal growth and sclerotial formation:

Agar discs (5 mm) were cut from the peripheral active mycelial growth of 3-day-old cultures for each of the obtained isolates of ***M. phaseolina*** and transferred to ready PDA plates and incubated at 27°C. Four replicates were used for each isolate. Observations about color and nature of mycelial growth in addition to diameters of fungal colonies "in mm" were recorded when plates were filled with fungal growth of any isolate. Ability of different isolates to produce sclerotia was determined after 10 days by counting the average number of sclerotia per microscopic field (X100).

2- Bioassay of culture filtrates:

Conical 250 ml flasks containing Czapek's liquid medium (100 ml per each flask) were inoculated each with a disc of fungal growth of a given isolate of *M. phaseolina* and incubated for 10 days at 27 °C. Culture filtrates was collected, filtered through Whatman No. 1 filter paper, and centrifuged for 15 minutes at 3000 rpm to separate the fungal propagules. The supernatant culture filtrates were sterilized either by autoclaving at 121°C for 15 min or by filtration through Sintered glass G5 (non-heated filtrates). Ten ml from given filtrates were placed onto sterilized filter paper placed inside 250 ml sterilized flasks and ten sterilized surface sterilized sesame seeds were placed in each flask. . Three replicated flasks were used for each treatment. Flasks were incubated for 10 days under room temperature (26°C). Percentages of germinated sesame (Giza 32 cv.) seeds as well as lengths of radical roots and shoots as affected by toxicity of heated and non-heated culture filtrates for different isolates were investigated.

3- Pathogenicity test:

Two different techniques were used for testing pathogenicity of different isolates of *M.* ***phaseolina*** as follows:

A- Soil infestation technique:

In this technique, pots (ϕ 25 cm) formalin sterilized were then filled with soil previously autoclaved for two hours at 121ºC. Inoculum for a given isolate of *M. phaseolina* was prepared by growing a particular isolate at 26º C for 2 weeks in autoclaved sand-sorghum. Potted soil was infested separately by fungal inocula at the rate of 3.0% (wt./wt.), then watered and left for one week to insure even distribution of the inoculum. Pathogen-free sterilized sand-sorghum medium was used at the same rate for infestation of potted soil in control treatment. Pots were planted with healthy surface sterilized sesame seeds of Giza 32 cultivar at rate of 10 seeds per pot. Three pots were used for each particular treatment.

Disease assessment:

Disease incidence expressed as % pre- and post-emergence damping-off was determined 15 and 45 days after sowing, respectively, and % healthy survived seedlings were also determined. After 90 days from sowing, percentages of sesame plants infected with charcoal-rot as well as healthy standing plants were estimated. The following formulae were used for determining these disease criteria.

% Pre-emergence = No. of non-germinated seeds / No. of sown seeds X 100

% Post-emergence = No. of dead seedlings / No. of sown seeds X 100

% Survived seedlings = No. of seedlings / No. of sown seeds X 100

% Charcoal rot = No. of plants with charcoal-rot / No. of survived seedlings X 100

% Standing healthy plants = No. of healthy plants / No. of survived seedlings X 100

B- Toothpick-piercing technique:

In this technique stems of sesame plants were inoculated by toothpick prepared according **Thirumalacher** *et al*. (**1977**). One toothpick charged with fungal growth was gently pierced into plant stem near soil surface. Toothpicks without fungal growth were used for piercing stems in control plants. Five replicates were used for each particular treatment. After stem piercing, disease symptoms began to appear around piercing site then extend upward and downward. The pathogenicity of a given isolate was evaluated by measuring the length (in cm) of the diseased parts of the stem after 30, 60, and 90 days from sowing in pots.

4- Elctrophoretic patterns protein:

Protein patterns obtained by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to study the relationship between the obtained 11 isolates of *M. phaseolina*. Fungal protein extracts was prepared according to **Rataj-Guranowska *et al.*,** (**1984**). The protein content in the supernatant extracts was estimated according to **Bradford** (**1976**) by using bovine serum albumen as a standard protein. The person product moment correlation coefficient (r) between any pair densitometric tracings of protein patterns was computed by a computerized program and the resulting matrix of correlation coefficient was used for evaluating the level of similarity between any pair of isolates (**Gomez** and **Gomez**, **1984**). Electrophoretic protein patterns obtained by SDS-PAGE from the obtained isolates of ***M. phaseolina*** were clustered (**Joseph** *et al.*, **1992**) by the average linked technique (un-weighted pair-group method). The results were expressed as phenograms. Cluster analysis was performed with a computerized program.

5- Host-pathogen interaction:

In this experiment, susceptibility of five host plants namely peanut, sesame, soybean, sunflower and cotton to infection with five isolates of *M. phaseolina* isolated from peanut (isolate No.8), sesame (isolate No.9), soybean (isolate No.13), sunflower (isolate No.17), and cotton (isolate No.27) plants was investigated. Inoculum of each of these isolates was prepared and added to sterilized-potted soil as above mentioned. Individual pots infested with a given isolate were planted separately with a surface sterilized seeds of peanut, sesame, soybean, sunflower or cotton at the rate of 10 seeds per pot. Three pots were used for each host plant in each isolate of *M. phaseolina*. Disease assessment was determined as early described in pathogenicity test.

Results and Discussion

1. Isolated fungi and their frequency's:

Data in Table (1) indicated that, *Macrophomina phaseolina* was isolated more frequently followed by *Fusarium* spp., *Rhizoctonia solani* and *Sclerotium rolfsii* from roots of sesame and other crops. Out of 793 fungal isolates, 464 belong to *M. phaseolina* (59.0%), 206 to *Fusarium* spp. (26.2%), 101 to *R. solani* (12.4%), and only 22 were *S. rolfsii* (2.5%). Isolation frequency's ranged in different locations between 28.8-94.0%, 6.0-52.3%, 0.0-37.5%, and 0.0-18.5% for these fungi, respectively.

Diseased sesame plants collected from Menia "Samallot" yielded the highest frequency of *M. phaseolina* (94%), followed by those collected from Behera "El-Tahrir" (92.3%), Giza "El-Saff" (72.3%), Sharkia "Abo-Hammad" (58.3%), Fayoum "Etsa" (55.6%), and Sohag "Shandaweel" (42.5%). However, *Fusarium* spp. shows the highest frequency on sesame plants collected from Domiat "New Domiat" (52.3%) followed by *M. phaseolina* (36.4%) and *R. solani* (11.3%). These results are in agreement with **Acimovic** (**1962**) who reported that all sunflower infection with *M. phaseolina* was frequently associated with *Fusarium* spp. and **El-Zarka** (**1976**) who isolated *M. phaseolina*, *F. oxysporum, F. solani*, and *R. solani* from charcoal rotted sunflower plants. Also, **Shaarawy** (**1980**) recorded that, *M. phaseolina* and *F. oxysporum* were more frequently isolated from sunflower diseased plants collected from different localities followed by *R. solani* and *S. rolfsii*. Filtrates of the tested fungi decreased percentages of sunflower-seed germination

Table (1): Frequency of fungi isolated from roots of sesame plants and some other oil **crops (a, b, c and d) grown at different locations.

Location	Total number of isolated fungi	Number and frequency of isolated fungi							
		M. phaseolina		*Fusarium spp.*		*Rhizoctonia solani*		*Sclerotium rolfsii*	
		No.	%	**No.**	%	**No.**	%	**No.**	%
El-Saff	94	68	72.3	19	20.2	3	3.2	4	4.3
Etesa	72	40	55.6	28	38.9	4	5.6	-	-
Samalot	50	47	94.0	3	6.0	-	-	-	-
Shandaweel	80	34	42.5	31	38.8	15	18.8	-	-
Abo-Hamad	84	49	58.3	26	31.0	9	10.7	-	-
El-Tahrir	65	60	92.3	5	7.7	-	--	-	-
Somosta (a)	83	65	78.3	10	12.0	8	9.6	-	-
Assiut (b)	60	31	51.7	12	20.0	14	23.3	3	5
Qaha (e)	81	31	38.3	22	27.2	13	16.0	15	18.5
Menouf (d)	80	23	28.8	27	33.8	30	37.5	-	-
Total number	793	464	59.0	206	26.2	101	12.4	22	2.5

** a, b, c, and d means that the fungi were isolated from Peanut, Soybean, Sunflower and Cotton, respectively.

As for the other tested host plants, peanut plants collected from Beni-Suef "Somosta" shows the highest frequency of *M. phaseolina* (78.3%) followed by soybean collected from Assiut "Assiut" (51.7%), and sunflower collected from Qalubya "Qaha" (38.3%). In cotton plants collected from Menofiya, *R. solani* was more frequently isolated (37.5%) followed by *Fusarium* spp. (33.8%) and *M. phaseolina* (28.8%). These results could be explained in light of environmental conditions especially temperature and water stress rather than host plant. The high temperature prevailing in Beni-Suef and Assiut seems to be the most important factors affecting occurrence of *M. phaseolina*. In this regard, **Agarwal *et al.*** (**1973**) tested 15 soybean varieties against *M. phaseolina* and found that the maximum infection occurred at 35-40 °C. Meanwhile, **Ghaffar and Erwin** (1969) reported that water stress of plants appeared to be more important predisposing factor than high temperature. **Sackston (1978)** reported also that, the occurrence and severity of charcoal rot of sunflower, *M. phaseolina*, varied from year to year with climatic conditions and host cultivars in Hungary.

Macrophomina phaseolina and *Fusarium* spp. were isolated from all locations *Rhizoctonia solani* was not isolated from plant materials collected from Menia and Behera governorates. While *Sclerotium rolfsii* was isolated only from three locations i.e. Giza, Assiut, and Qalubya. These results indicate the wide spread of ***M. phaseolina*** and holds fairly good with **Khalifa** (**1997**) who recorded that, *Macrophomina phaseolina* was most frequently isolated from sesame plants collected from 7 localities followed by *R. solani* and *F. ventricosum* (three locations) and *F. oxysporum*, *F. solani* and *S. rolfsii* (two locations).

2. Cultural studies on isolates of *Macrophomina phaseolina*:

I- Characters of mycelial growth and sclerotial formation:

Data in Table (2) show that 10 isolates of *M. phaseolina* produced extensive and profuse aerial hyphae. Linear growth was significantly higher in sesame isolates No. 2, 3, 4, 7, and in peanut and soybean isolates (88.3-90.0 mm) followed by sesame isolates No.5, 1, cotton and sunflower isolates (84.3-85.5 mm). However, sesame isolate No.6 produces the lowest linear growth (81.8 mm) with scanty aerial mycelium.

Table (2): Growth nature, mycelial color, linear growth and sclerotial production of the different *M. phaseolina* isolates (*in vitro*).

Isolate .and No.	Location	Growth nature and Mycelial color	Linear growth (mm.)	Sclerotial formation*
Sesame. 1	El-Saff	Gray $^{-}$	85.0	86.3
Sesame. 2	Etesa	Gray $^{+}$	90.0	72.0
Sesame. 3	Samalot	Light gray $^{+}$	90.0	50.5
Sesame. 4	Shandaweel	White $^{+}$	89.3	2.3
Sesame. 5	Abo-Hamad	Gray $^{+}$	84.3	81.0
Sesame. 6	El-Tahrir	Light gray $^{+}$	81.8	46.3
Sesame. 7	New Domiate	Black gray $^{+}$	90.0	113.5
Peanut	Somosta	Black gray $^{+}$	88.3	115.3
Soybean	Assiut	Black $^{+}$	90.0	127.0
Sunflower	Qaha	Light gray $^{+}$	85.5	54.5
Cotton	Menouf	Black gray $^{+}$	80.0	87.8
L.S.D. at 0.05			1.82	5.55

* Number of sclerotia was counted under field microscopic X100

\- = Scanty aerial growth, + = Profusely aerial growth

Number of sclerotia produced by different isolates ranged between 2.3-127.0 sclerotia/microscopic field. The highest number of sclerotia was produced by isolates of soybean, peanut and sesame.No.7 without significant differences between the latter two isolates. However, the lowest number of sclerotia was produced by sesame isolate No. 4. Mycelial color of a given isolate of *M. phaseolina* seems to be correlated with density of sclerotial. The color of mycelial growth of soybean isolate, which produced the highest number of sclerotia, was black or greyish black loss of black pigment in the white isolate No. 4 produced the lowest number of sclerotia. Similar findings were reported by **Ahmed** and **Ahmed** (**1969**). **Dhingra** and **Sinclair** (**1973**) who observed variation among the *M. phaseolina* isolates obtained from the different parts of a single soybean plant. Also, **Hassanein (1985)** reported that, his 13 isolates of *M. phaseolina* isolated from soybean grown in different localities differed in the rate of growth, nature of growth, color of the mycelium and sclerotial formation.

II. Bioassay toxicity of cultural filtrates:

Data in **Table** (**3**) proved that, germination of sesame seeds, length of shoots and roots of sesame seedlings were deteriously affected by culture filtrates of the different tested isolates of *M. phaseolina*. In general, culture filtrates sterilized by autoclaving were more toxic than those sterilized by filtration through Sintered glass filter. Filtrates sterilized through Sintered glass filter of sesame isolate No.5 and filtrates of isolate No.4 sterilized by autoclaving showed the highest toxicity against seed germination, shoot and root lengths. These results could be explained in light of toxin/growth regulator balance. Presence of growth regulators in fungal filtrates may be suppressed by the harmful effects of fungal toxins. In such cases, growth regulatores might be collapsed in autoclaved filtrates, while the thermostable toxin is still effective. Production of heat-stable toxins by *S. bataticola* (*M. phaseolina*) was reported by several investigators (**Raj** and **Singh**, **1973** and **Dhingra** and **Sinclair, 1974**). These results could be supported also by **Hodges** (**1962**) who reported that, *S. bataticola* produces indol acetic acid (IAA) *in vitro*. IAA might be responsible for the proliferation of seedling-roots affected by black root-rot. **Chan** and **Sackston** (**1973**) reported also that, *S. bataticola* could produce a non-specific toxin which induced necrotic spots in sunflower leaves. The virulence of four isolates of *S. bataticola* was not correlated with their toxin production in culture. The present results indicated clearly that, the root length was more favourable for assaying toxicity of both types of cultral filtrates than shoot length, while seed germination test was less sensitive test. Seed germination was not significantly affected while both shoot and root lengths were significantly decreased by culture filtrates of sesame isolates No.1 and 3 and cotton isolate sterilized by filtration. Similar findings were recorded by **Shaarawy (1980)** who found that, sunflower-seedlings were more sensitive to fungal filtrates of *M. phaseolina*, than seed germination.

Table (3): Effect of the non-autoclaved and autoclaved culture filtrate of different *M. phaseolina* isolates on % of sesame seed germination(*in vitro*).

Isolate	Seed germination%			Shoot length "mm"			Root length "mm"		
	Non-autoclaved	Autoclaved	Mean	Non-autoclaved	Autoclaved	Mean	Non-autoclaved	Autoclaved	Mean
Sesame. 1	93.3	86.7	90.0	96.2	70.0	83.1	51.0	30.0	40.5
Sesame. 2	86.7	56.7	71.7	86.8	9.8	48.3	55.8	2.0	28.9
Sesame. 3	93.3	56.7	75.0	108.4	70.6	89.5	52.8	21.0	36.9
Sesame. 4	83.3	46.7	65.0	64.0	14.6	39.3	38.4	3.0	20.7
Sesame. 5	3.3	56.7	30.0	2.2	19.0	10.6	0.8	2.4	1.6
Sesame. 6	53.3	66.7	60.0	77.2	63.2	70.2	25.0	27.4	26.2
Sesame. 7	90.0	53.3	71.7	94.4	49.0	71.7	37.4	17.6	27.5
Peanut	86.7	83.3	85.0	70.8	43.4	57.1	47.6	20.2	33.9
Soybean	66.7	96.7	81.7	95.8	66.8	81.3	26.2	27.6	26.9
Sunflower	76.7	86.7	81.7	54.2	86.4	70.3	15.2	7.6	11.4
Cotton	96.7	53.3	75.0	103.6	19.2	61.4	17.0	6.2	11.6
Control	100	100.0	100.0	120.0	120.0	120.0	71.0	71.0	71.0
Mean	77.5	70.29	73.9	81.13	52.26	66.9	36.52	19.67	28.09

LSD at 5% for:			
Isiolates:	6.83	5.93	6.73
Sterilization	2.79	2.42	2.75
Interaction	9.66	8.38	9.51

non-autoclaved: culture filtrated through Sintered glass G5

3. Pathogenicity tests:

A. Using soil infestation technique:

Tested isolates of *M. phaseolina* have significantly varied in pathogenicity to sesame plants (**Table 4-a**). In pre-emergence stage sesame isolate No.3 and peanut isolate proved to the most virulent while sesame isolate No.6 and peanut isolate were the most virulent in post emergence stage. Sesame isolates No.5 and 7 and sunflower isolate showed no significant effect in both pre- and post-emergence stages compared with control. On the other hand, peanut isolate produce the lowest survived seedlings followed by sesame isolates No.3 and 4 without significant differences inbetween. However, survivals were not significantly affected by isolates of sunflower and sesame No.7. For charcoal-rot incidence and healthy standing plants, sesame isolate No.3 was the most virulent as it produced the highest disease incidence and lowest healthy standing plants followed by sesame isolates No.1, 2 and 4. While sesame isolates No.5 and No. 7 and sunflower isolate seemed to be less virulent as they caused the lowest incidence of charcoal rot and produced the highest healthy standing plants. It is worthy to state that disease-free plants were significantly decreased due to infection by all tested isolates of *M. phaseolina*. Significant variations in pathogenicty of *M. phaseolina* isolates were reported by several researchers. **Hassanien** (**1985**) in

Egypt found that, El-Menia-isolate of *M. phaseolina* was more virulent on soybean than Alexandria-isolate. **Khalifa (1997)** working on root-rot of sesame recorded that, ***M. phaseolina*** isolated from El-Nobaria was not significantly effective for inducing pre-emergence damping-off while those isolated from Sharkia and Behera had no significant effect in post-emergence disease stage if compared with control. He added that, an isolate from Beni-Suef was the most virulent causing root-rot.

Because wide variations were detected between isolates of *M. phaseolina* during different stages of disease development, the following procedure named "accumulative virulence" was innovated to facilitate quantitative comparisons between these isolates. The data in **Table (4-a)** were rearranged in ascending order for pre- and post-emergence damping-off and charcoal-rot and in descending order for both survived seedlings and healthy standing plants. The numerical position "rank" of the rearranged data for each isolate in these five disease criteria was recorded. Then, the total number of ranks across all disease criteria was calculated for each isolate. The final total number of ranks was arranged ascendingly for determining quantitative virulence of the different isolates as shown in **Table (4-b)**. According to acummulative virulence illustrated in **Table (4-b)** it could noticed that virulence of an isolate was proportionally increased by increasing its total numbers of ranks. Thus, both isolates of sunflower and sesame No..7 were considered a less virulent isolates because they have the lowest score of (= 6) followed by sesame isolate No..5 (=11), cotton isolate (= 17), soybean isolate (= 25), sesame isolate No.6 (= 27), sesame isolate No.2 (= 29), sesame isolate No.1 (= 30), sesame isolate No.4 (= 31), peanut isolate (= 33) and sesame isolate No.3 (= 38). Reasonably sesame isolate No.3 was considered aggressive and more virulent on sesame plants.

Table (4-a): Effect of different isolates of *M. phaseolina* on damping off and charcoal-rot diseases incidence on Giza 32 sesame cv using soil infestation technique.

Isolate	**Disease incidence**				
	% Pre	% Post	% Survival	% Charcoal rot	% Healthy
Sesame. 1	16.7	23.3	60.0	55.6	26.7
Sesame. 2	16.7	20.0	63.3	57.9	26.7
Sesame. 3	33.3	20.0	46.7	85.7	6.7
Sesame. 4	23.3	23.3	53.3	50.0	26.7
Sesame. 5	3.3	10.0	86.7	15.4	73.3
Sesame. 6	13.3	26.7	60.0	38.9	36.7
Sesame. 7	0.0	6.7	93.3	14.3	80.0
Peanut	30.0	26.7	43.3	38.8	26.7
Soybean	26.7	13.3	60.0	38.9	36.7
Sunflower	3.3	3.3	93.3	14.3	80.0
Cotton	10.0	16.7	73.3	31.8	50.0
Control	0.0	0.0	100.0	0.0	100.0
LSD. at 5%	10.90	10.94	12.26	14.74	15.67

B. Using stem piercing technique:

Charcoal rot disease was successfully induced on stems of sesame plants by piercing them with toothpicks carrying fungal growth of the different isolates of *M. phaseolina.* The length of stem parts showing charcoal rot significantly differed and depended on fungal isolate and plant age. The data in **Table** (**5**) show that, sesame isolate No.3 was the most virulent as it caused the tallest length of diseased stem part (average 40.6 cm), while sesame isolate No.7 and soybean isolate seemed to be less pathogenic as they caused the lowest increase in length of diseased stem portion (average 0.2-1.13 cm) within 10 days after stem piercing. These results are similar to those recorded in soil infestation technique. Thus, the easy and inexpensive stem piercing technique could be used for studying pathogenicity of charcoal rot, even on plants grown under field conditions, instead of the hard and expensive soil infestation technique which might be used only in pot experiments.

With majority of tested isolates, length of diseased stem portion was increased proportionally and significantly by the increase in plant age from 30 to 90 days. This means that, the older plants were more susceptible to these isolates than the younger one. On the other hand, isolates No.1 and 5 were pathogenic to 60 more than 90 days-old plants. In soil infested with charcoal rot pathogen(s), disease symptoms started from roots and extended to different extent upwardly in plant stem. Wounds caused in stem through applying toothpick piercing technique might be necessary for accelerating stem infection with some isolates and might be retarded in some other isolates. This explaination could be supported by **Sackston (1978)** who reported that, the root-rot caused by *M. phaseolina* on young sunflower plants appeared to be of little economic importance, but a disease of mature plants caused by the same pathogen caused serious damage. **Tikhonov *et al.* (1976)** suggested also that, the virulence of charcoal rot on sunflower plants is due to the long period of time from infection to development of symptoms on arial parts of the plant.

Table (5): Effect of different isolates of *M. phaseolina* on charcoal-rot disease incidence on Giza 32 sesame cv using stem piercing technique.

Isolate	Length (cm) of diseased stem after			Mean
	30 days	60 days	90 days	
Sesame. 1	6.7	9.4	2.1	6.1
Sesame. 2	9.9	21.5	24.0	18.5
Sesame. 3	16.7	35.1	70.1	40.6
Sesame. 4	5.5	4.3	32.1	14.0
Sesame. 5	0.0	27.3	4.0	10.4
Sesame. 6	11.7	29.2	31.1	24.0
Sesame. 7	0.0	0.5	0.0	0.2
Peanut	10.7	24.8	38.7	24.7
Soybean	1.6	1.1	0.68	1.13
Sunflower	3.0	6.4	1.2	3.5
Cotton	13.5	25.5	1.6	13.5
Control	0.0	0.0	0.0	0.0
Mean	6.61	15.43	17.13	--

L.S.D. at 5% for: Isolates: 1.51 Plant age: 0.49 Interaction: 2.61

4. Elctrophoretic patterns protein:

Protein bands derived from the gel electrophoretic pattern of soluble proteins extracted from fungal hyphae of 11 isolates of *M. phaseolina* as well as similarity between these isolates are illustrated by **Fig. (1 a&b)**. The position of each band was recorded by densitometry scanning as rate of flow. The obtained results indicated that the 11 tested isolates of *M. phaseolina* belonged to 2 distinct separate clusters. Sesame-isolate from Giza location alone (Lane-1) was located in the first cluster; while the other 10 isolates were located in a distinct. The second cluster consisted three sub-clusters. The first sub-cluster includes the more closest similar isolates (similarity 98.59%) isolated from soybean (Lane-8) and peanut (Lane-9) grown at Assiut and Beni-Suif, respectively. The second sub-cluster includes isolates obtained from cotton plants grown at Menofiya (Lane-11) and sunflower plants grown at Qalubya (Lane-10). Similarity between the later 2 isolates was 76.88%. These results indicate that, similarity was higher between isolates obtained from host plants grown in the warmer soils (as in the first sub-cluster) than those isolated from host plants grown in less warm soils (as in the second sub-cluster). The third sub-cluster contains the other 6 sesame-isolates. Again, degree of similarity between isolates in this sub-cluster (70.07-95.25%) was depended also on soil temperature in which sesame was grown. Similarity reached its maximum between isolates obtained from sesame grown in warmer soils i.e. Sohag and El-Minia and gradually decreased by decrease in soil temperature as in El-Sharkia, El-Fayoum, El-Behera, and Domiat, respectively.

These results indicated clearly that, similarity between isolates of *M. phaseolina*, based on their protein patterns, was controlled by soil temperature in which the pathogen survived more than by the host plants. **Abd-El-Ghany (1998)** studied protein patterns in 8 isolates of *M. phaseolina* isolated from different locations and host plants and found that the fungus isolated from sunflower plants grown at Giza was located in single cluster while those isolated from sesame and cotton plants grown at Qalubya were located in another different “cluster”

5. Effect of host-pathogen combination on charcoal rot disease incidence:

Data presented in **Table (6)** show that, pre-emergence damping-off differed significantly mean % range from (6.7-22.7%) between tested host plants. Also, tested host plants showed different reaction to the isolates, cotton shows the highest incidence (22.7%) followed by soybean (18.7%), sesame and sunflower (12.0%) and peanut (6.7%). However, it was not significantly affected by variation of isolates of *M. phaseolina* or interaction between isolates and host plant. Also, post emergence damping-off showed no significant differences due to variety of the tested host plant, isolate of *M. phaseolina* or interaction between them.

Percentage of survived seedlings was affected significantly by host plant, and isolate of *M. phaseolina*. As for host plant, peanut exhibited the highest percentage of survival (85.32%). However, sesame (70.68%), soybean (69.36%), sunflower (74.68) and cotton (62.68) came next without significant differences in between. As for isolates, peanut-isolate induced the lowest damping off and highest % survival (82.68%) followed by cotton-isolate (77.34%), sunflower-isolate (70.68%), soybean-isolate (68.02%) and sesame-isolate and (64.0%).

Regarding charcoal rot, the obtained results indicated that sunflower plants showed the highest disease incidence (57.34%) followed by sesame plants (35.84%), soybean plants (29.96%), cotton plants (20.54%) and peanut plants (6.66%). Thus peanut seems to be the most resistant host while sunflower was the most susceptible one to infection with charcoal rot pathogen. These results agree with those reported by several onvestigators. **Boewe (1942)** found that, *Sclerotium bataticola* attacked germinating seeds of corn and soybeans. **Sackston (1978)** reported also that, *M. phaseolina* causes serious damage on mature sunflower plants. As for virulence of isolates, the present results indicated that, *M. phaseolina* isolated from sesame induced the highest charcoal rot disease incidence (38.16%) followed by isolates of soybean (37.88%) and sunflower (36.88%) without significant differences in between. Isolates of *M. phaseolina* isolated from peanut and cotton were significantly equal and exhibited the lowest disease incidence i.e. 21.16% and 16.26%, respectively. As for interaction, the obtained results showed different responses between host plant and isolate of *M. phaseolina*. Isolates of *M. phaseolina* isolated from sesame, soybean and sunflower were significantly equal in inducing charcoal rot on sesame, soybean and sunflower plants but they were ranked differently for each host. The highest disease incidence on sesame, soybean, and sunflower plants was induced by isolates of *M. phaseolina* from sesame, sunflower and soybean, respectively. On cotton plants the highest disease incidence was induced by sesame isolate followed by isolates from sunflower, cotton and soybean, respectively without significant variation in between. Among tested host plants, sunflower only exhibited severe infection with peanut isolate. Percentage of diseased plants of sunflower due to peanut-isolate recorded 50.0% compared with 8.3-16.7% on the other tested host plants. These results hold fairly good with **Abd El-Ghany (1998)** who compared 8 isolates of *M. phaseolina* isolated from different host plants and found considerable variations in pathogenicity of these isolates on potato tubers and maize stem portions *in vitro.* She added that, pathogenicity test of carried out under greenhouse conditions gave contradictory results regarding specificity of isolates on different hosts.

Table (6): Effect of different *M. phaseolina* isolates isolated from some host plants on damping-off and charcoal-rot diseases incidence on some oil crops.

Disease measurement	Tested host	Tested isolate					Mean
		M8	M9	M13	M17	M27	
Pre-emergence %	Peanut	6.7	6.7	13.3	6.7	0.0	6.7
	Sesame	6.7	20.0	6.7	13.3	13.3	12.0
	Soybean	6.7	20.0	13.3	26.7	26.7	18.7
	Sunflower	6.7	13.3	20.0	13.3	6.7	12.0
	Cotton	13.3	33.3	26.7	20.0	20.0	22.7
Mean		8.0	18.7	16.0	16.0	13.3	-
Post-emergence %	Peanut	13.3	13.3	6.7	0.0	6.7	8.0
	Sesame	6.7	20.0	26.7	20.0	13.3	17.3
	Soybean	6.7	13.3	20.0	13.3	6.7	12.0
	Sunflower	13.3	13.3	13.3	20.0	6.7	13.3
	Cotton	6.7	26.7	13.3	13.3	13.3	14.7
Mean		9.3	17.3	16.0	13.3	9.3	-
Survivals %	Peanut	80.0	80.0	80.0	93.3	93.3	85.3
	Sesame	86.7	60.0	66.7	66.7	73.3	70.7
	Soybean	86.7	66.7	66.7	60.0	66.7	69.4
	Sunflower	80.0	73.3	66.7	66.7	86.7	74.7
	Cotton	80.0	40.0	60.0	66.7	66.7	62.7
Mean		82.7	64.0	68.0	70.7	77.3	-
Charcoal rot %	Peanut	13.3	6.7	6.7	0.0	0.0	5.3
	Sesame	13.3	33.3	33.3	26.7	13.3	24.0
	Soybean	13.3	20.0	26.7	26.7	13.3	20.0
	Sunflower	40.0	46.7	53.3	46.7	20.0	41.3
	Cotton	6.7	13.3	6.7	20.0	13.3	12.0
Mean		17.3	24.0	25.3	24.0	12.0	
Healthy plants %	Peanut	66.7	73.3	73.3	93.3	93.3	80.0
	Sesame	73.3	26.7	33.3	40.0	60.0	46.7
	Soybean	73.3	46.7	40.0	33.3	53.3	49.3
	Sunflower	40.0	26.7	13.3	20.0	66.7	33.3
	Cotton	73.3	26.7	53.3	46.7	53.3	50.7
Mean		65.3	40.0	42.6	46.7	65.3	

L.S.D. 5%	Pre-	Post-	Survival	Charcoal-rot	Healthy plants
Host	7.51	n.s.	10.7	7.52	9.76
Isolates	n.s.	n.s.	10.7	7.52	9.76
Interaction	n.s.	n.s.	n.s.	n.s.	21.82

Finally, because losses in tested host plants occurred during different stages of disease development, % healthy standing plants might be the best criterion to reflect the significant effects of variety of host plant, source of pathogen-isolate and the interaction between them. As for host plant, results of Table (6) showed that, the highest significant difference in healthy standing plants was detected between peanut (80.0%) and sunflower (33.3%). Percentages of healthy standing plants in sesame (46.7%), soybean (49.3%) and cotton (50.7%) were significantly equal. Concerning with source of isolate, the obtained results proved that, isolates of *M. phaseolina* isolated from sesame, soybean, and sunflower were significantly equal and more pathogenic than those isolated from peanut and cotton. Peanut-isolate was more severe on peanut plants followed by sesame- and soybean-isolates. However, sunflower- and cotton-isolates seemed to be the least pathogenic to peanut plants. On sesame plants, sesame-isolate was the more pathogenic, followed by soybean- and sunflower-isolates without significant variations in between. As for soybean plants, the lowest % healthy standing plants was induced by sunflower-isolate followed by isolates of sesame, cotton, and peanut, respectively. On sunflower plants, the soybean-isolate was the most severe followed by sunflower-, sesame-, peanut- and cotton-isolates, respectively. Sesame- and sunflower-isolates were more pathogenic on cotton plants than the cotton-isolate

The present results conclude that, sunflower plants were affected by peanut-isolate more than peanut plants. The harmful effect of sesame-isolate was greater and similar on sesame, sunflower, and cotton plants. Soybean-isolate was more severe on sunflower and sesame plants than soybean plants. Among all tested host plants, peanut only seems to be not significantly affected by cotton-isolate. Effect of cotton-isolate on sesame, soybean, sunflower, and cotton plants was significantly equal. These findings may be very important for planning strategy for integrated control of charcoal rot caused by *M. phaseolina*. Peanut seems to be the safest preceding host in sesame, soybean or cotton but not sunflower rotations. In rotation concerned with oil crops sowing sesame after sesame, sunflower or cotton must be prevented for minimizing disease incidence on sesame plants. Sunflower suffered great loss in healthy plants if it was sown after soybean.

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دراسات باثولوجية وفسيولوجية على بعض عزلات فطر ماكروفومينا فاسيولينا

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كان الفطر ماكروفومينا فاسيولينا الأكثر تكراراً بين الفطريات المعزولة من عينات مختلفة بها أعراض العفن الفحمي وعفن الجذور (65.2%) يليه أنواع من فطر فيوزاريوم (25.0%) ثم فطر ريزوكتونيا سولانى (7.6%) وكان الفطر سكليروشيوم رولفسياى أقلها تكرارا (2.2%). وقد سجلت العينات النباتية المجموعة من مناطق المنيا ، دمياط ، المنوفية ، القليوبية أعلى تكرار لعزل تلك الفطريات الأربعة على التوالى.

تم إختيار عدد 11 عزلة من الفطر ماكروفومينا فاسيولينا معزولة من عوائل نباتية ومناطق مختلفة وقد أثبتت الدراسة وجود اختلاف واضح فى لون النمو الميسيليومى وسرعة النمو وإنتاج الأجسام الحجرية بين تلك العزلات. كما كانت رواشحها المعقمة فى الأوتوكلاف بشكل عام أكثر سمية وأدت إلى تقليل إنبات بذور السمسم وأطوال السيقان والجذور بدرجة أكبر من تلك المعقمة بواسطة الترشيح – ولكن لوحظ إنعكاس هذا الوضع فى بعض العزلات تحت الدراسة كما قد تفاوت معدل النقص بسبب السمية بين العزلات المختلفة.

تم دراسة القدرة المرضية لعزلات فطر ماكروفومينا عن طريق الزراعة فى تربة ملوثة بلقاح تلك العزلات أو عن طريق وخز سيقان السمسم عند أعمار مختلفة بسلاكات الأسنان الحاملة للقاح تلك العزلات. وقد أظهرت كلتا الطريقتين ضعف القدرة المرضية لعزلة عباد الشمس وعزلة السمسم رقم 7 وشدة القدرة المرضية لعزلة السمسم رقم 3 على نباتات السمسم. وقد تم خلال هذه الدراسة إبتكار طريقة حسابية سهلة لمقارنة العزلات ببعضها وترتيبها تبعا لتراكم قراءات قدراتها المرضية.

أظهرت دراسة أنماط البروتين فى العزلات المختلفة لفطر ماكروفومينا فاسيولينا إرتفاع درجة القرابة بين عزلات الفطر المعزولة من العوائل النباتية النامية فى الأراضى الأكثر دفئا وقلة درجة القرابة بين العزلات الناتجة من عوائل نامية فى أراضى متفاوتة فى درجة الدفء.

تم أيضا دراسة التفاعل بين عدد من العزلات الفطرية والعوائل النباتية المختلفة وقد أظهرت النتائج أن نباتات عباد الشمس كانت أكثر العوائل النباتية المختبرة قابلية للإصابة يليها نباتات السمسم ثم فول الصويا والقطن بينا كانت نباتات الفول السودانى أكثرها مقاومة للإصابة بالعزلات المختلفة. اتضح أيضا أن عزلات الفطر المعزولة من السمسم وفول الصويا وعباد الشمس كانت أشد فى قدرتها المرضية عن تلك المعزولة من الفول السودانى أو القطن. وبدراسة التفاعل بين العائل النباتى وعزلة الفطر – لوحظ أن نباتات الفول السودانى والسمسم كانت أكثر قابلية للإصابة بنفس العزلات المعزولة منها بينما كانت نباتات فول الصويا وعبادالشمس والقطن أكثر قابلية لإصابة بعزلات عباد الشمس وفول الصويا والسمسم على التوالى.