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SOME FACTORS AFFECTING THE SUSCEPTIBLITY OF COTTONSEED TO *Macrophomina phaseolina*

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ABSTRACT

Colonization of cottonseed by *M. phaseolina* during the early stages of infection was affected by inoculum density, temperature, incubation period (time of seed exposure to inoculum), and pathogenicity of isolates. The higher the inoculum density, the earlier the isolation of *M. phaseolina* from seeds and the higher its isolation frequency. The three main effects of ANOVA (temperature, incubation period, and isolate) were all very highly significant sources of variation in isolation frequency of *M. phaseolina* from seeds regardless of the tested cultivar (Giza 75, Giza 80, or Giza 85). Temperature x incubation period interaction was a very highly significant source of variation in isolation frequency from Giza 75 ($P = 0.0000$) and Giza 80 ($P = 0.0001$), while it was a nonsignificant source of variation ($P = 0.2925$) in isolation frequency from Giza 85. All the other source of variation were nonsignificant ($P > 0.05$) regardless of the tested cultivar.

INTRODUCTION

Macrophomina phaseolina (Tassi) Goid., the causal agent of charcoal rot on cotton, is a seedborne and soilborne pathogen with a wide distribution and a wide host range (Dhingra and Sinclair, 1978). When *M. phaseolina* invades roots or stems of cotton, colonization of internal tissues proceeds rapidly and the plant dies. Examination of affected parts reveals a dry rot, with many tiny black sclerotia distributed throughout the wood and softer tissues (Watkins, 1981).

We believe that the importance of *M. phaseolina*, as a cotton pathogen, is underestimated in Egypt. This view has come from the observation that during the last 50 years, *M. phaseolina* on cotton was almost completely absent from the literature of cotton diseases in Egypt. Thus, a handful of research papers, most of them not dealing with *M. phaseolina per se*, were found in this literature (Mostafa *et al.*, 1957; Mostafa *et al.*, 1959; Mohamed, 1962; Sabet and Khan, 1969; and Omar, 1999). This lack of concern is not justifiable because this fungus is of widespread distribution in the Egyption soil and it is easily and frequently isolated from cotton roots particularly during the late period of the growing season. Thus, when Aly *et al.* (1996) conducted a survey encompassed 88 samples of infected cotton roots from 12 Egyptian governorates. *M. phaseolina* was isolated from 37.5% of the samples examined.

Susceptibility of cotton, as well as other crops, to *M. phaseolina* is markedly affected by the prevailing environmental conditions and such effects are well documented in the literature.

Inoculum density

Rana and Tripathi (1984) found that increased density/unit area of *M. phaseolina* inoculum (sand-oat meal or homogenized mycelia suspension) led to increased collar rot in Indian mustard (*Brassica juncea*). Younger inoculum was more virulent than older mycelium. When Pineda *et al.* (1985) placed sesame seeds in plastic containers filled with autoclaved soil and covered them with soil containing sclerotia of *M. phaseolina*, they found that at 1000 sclerotia/g soil, germination was reduced in 2 of 3 cultivars. Plant mortality, especially of 50 and 60-day-old seedlings, was higher at 333 and 500 sclerotia/g soil than at 200/g. Severe damage thus occurred at the densities tested and might be expected at lower densities in naturally infested soils. Hooda and Crover (1988) found that increase in inoculum quantity of *M. phaseolina* led to greater disease incidence on mungbean (*Vigna radiate*) and accordingly required higher concentrations of fungicides for control. Gupta and Cheema (1990) mentioned that microsclerotia of *M. phaseolina* present on sesame seeds were positively correlated with plant infection and negatively correlated with seed germination, dry matter production, and root and shoot length of seedlings. Umamaheswari *et al.*, (2000) investigated the effect of inoculum levels, 500 and 1000 sclerotia/kg soil, on the incidence of dry root rot (*M. phaseolina*) of groundnut, grown in sterilized and unsterilized soil. They observed a significant increase in disease incidence (48.7 and 98.5%) with increasing inoculum level from 500 to 1000 sclerotia/kg of unsterilized and sterilized soil, respectively. Rettinasababady *et al.* (2000) treated seeds of blackgram (*Vigna mungo*) cv. ADT with different inoculum levels of *M. phaseolina* (0, 2, 5, and 10%). The number of plants showing progressive root rot was counted 15, 30, 45, and 60 days after sowing. The lowest seed germination percentage was at 10% inoculum level. There was a significant increase in root rot incidence that was directly proportional to the increase in plant age. Forty five and 60-day-old plants were severely affected by the pathogen.

Temperature

Patel and Patel (1990) studied meteorological correlation of charcoal-rot of sesame. They found that optimum temperature for growth and sclerotia formation by *M. phaseolina* was 35°C, both declined at temperature below 15° and above 40°C. Under field conditions, disease intensity increased with a progressive rise in temperature. Mukhopadhyay *et al.* (1991) mentioned that low temperature (25°C) favoured damping-off of mungbean seedlings in pots of soil with maize meal-sand inoculum of *M. phaseolina*. Manici *et al.* (1995) investigated temperature responses of 64 isolates of *M. phaseolina* from different climatic regions of sunflower production in Italy. The isolates were subjected to growth rate (GR) tests at 15, 25, 30, 35, and 40°C. The optimum temperature for growth was 30°C for 62 isolates and 35°C for 2 isolates. Isolate GR varied considerably at all temperatures ($P < 0.01$) but the maximum variability between isolates occurred at 15 and 40°C. Isolates from the north (colder area) grew better at lower temperature than other isolates and also showed a good adaptability to 40°C. Isolates from the midwest (Mediterranean climate) had the faster GR at 40°C but the worst GR at the lowest temperature tested. Isolates from the mideast and south, with

Mediterranean climate, grew better at the optimum temperature (30 and 35°C) and showed the poorest adaptability to the limit temperature (15 and 40°C). Viana and De-Souza (1997) conducted a study to investigate the effects of temperature on sclerotial germination of *M. phaseolina* under laboratory conditions. The tested temperatures were 30, 40 and 50°C. The results indicated that the temperature significantly affected germination of microsclerotia and that the greatest percentage germination was obtained at 30°C. The role of temperature on ashy gray blight of cowpea was studied by Ratnoo *et al.* (1997a) in pot experiment. Seeded pots were subjected to 4 temperature regimes, 10-25, 15-30, 20-35, and 25-40°C. The results clearly indicated that the disease development was most favoured by higher temperatures. All the plants developed the disease in temperature regime of 25-40 and 20-35°C with respective disease indices of 100 and 94.5%. Omar (1999) found that the optimal temperature for pathogenicity of *M. phaseolina* on cotton was somewhere between 24.5±1.5 to 38.0±2.0°C. Viana and de-Souza (2002) evaluated the effect of five temperatures (24, 27, 30, 33 and 39°C) on the germination of microsclerotia of *M. phaseolina* in sand substratum under laboratory conditions. The results showed ($P < 0.05$) that the greatest percentage of germination occurred at 30 and 33°C.

Inoculum quality (Variability in pathogenicity among isolates of *M. phaseolina*)

Lee *et al.* (1986) tested isolates of *M. phaseolina* from bean, cotton and groundnut for pathogenicity on cotton. There were some variations in resistance but all isolates infected cotton. Pearson and Schwerk (1986) found that the isolates of *M. phaseolina* from maize grew on a medium with potassium chlorate, but the isolates from soybean did not. They postulated that these differences might reflect metabolic activities associated with host specialization. Diourte (1987) inoculated sorghum, groundnut, bean and cotton with isolates from each of these hosts. There was a general trend to host preference for the same-host isolate. Vilela *et al.* (1987) tested 18 isolates of *M. phaseolina* causing charcoal rot in cotton. Significant differences in many characters were found but all isolates were highly pathogenic to cotton in Northern Peru. Diourte *et al.* (1995) found that an isolate of *M. phaseolina* originally isolated from sorghum genotype with resistance to charcoal rot caused greater symptoms development than did two other isolates originally obtained from sorghum genotype susceptible to charcoal rot. Ratnoo *et al.* (1997b) established two isolate types (M1 and M2) of *M. phaseolina* isolated from cowpea. The isolate M2 was more virulent with a disease index of 86.11% compared with M1 (77.7%). M2 produced symptoms in 7-10 days and blighted the plants in 10-15 days, whereas M1 produced symptoms in 9-14 days and blighting in 25-35 days. Mayek-Perez *et al.* (1997) determined variation in *in vitro* cultural characteristics and virulence to common beans (*Phaseolus vulgaris*) of 15 *M. phaseolina* isolates obtained from different hosts and geographical areas in Mexico. Results showed that quantitative characteristics such as *in vitro* relative growth rate of the colony, are an appropriate tool to characterize the pathogen' development. *M. phaseolina* isolates obtained from other hosts such as sesame, sorghum, maize and soybean infected bean, indicated the non-specificity of the pathogen. It was concluded that severity of disease and percentage of dead plants preemergence could be useful criteria

for selecting resistant bean germplasm to *M. phaseolina* in early stages of growth.

However, due to the lack of studies, the effects of the previously mentioned factors on susceptibility of cottonseed to *M. phaseolina* are unclear. Therefore, the objective of this study was to evaluate the effects of inoculum density, temperature, incubation period, and isolate on susceptibility of seed from some of the Egyptian cottons (*Gossypium barbadense* L.) to *M. phaseolina*.

MATERIALS AND METHODS

Isolates of *M. phaseolina*

Isolates of *M. phaseolina* used in the following studies were obtained from the fungal collection of Cotton Disease Research Section, Plant Pathology Research Institute, Agric. Res. Center. These isolates were originally isolated from cotton roots as well as from roots of other hosts of *M. phaseolina*.

Effect of inoculum density on susceptibility of cottonseed to *M. phaseolina*

Substrate for growth of a highly pathogenic isolate of *M. phaseolina* was prepared in 500-ml glass bottles, each bottle contained 100 g of sorghum grains and 80 ml of tap water. Contents of each bottle were autoclaved for 30 minutes. Isolate inoculum, taken from one-week-old culture on PDA, was aseptically introduced into the bottle and allowed to colonize sorghum for three weeks. The present study was carried out by using autoclaved clay loam soil. Soil was infested with *M. phaseolina* inoculum at a rate of 10 and 50 g/kg of soil. In the control treatment, autoclaved sorghum grains were mixed thoroughly with soil at a rate of 10 or 50 g/kg of soil. Infested and non-infested soils were dispensed in 10-cm-diameter clay pots and these were planted with 10 seed per pot (cultivar Giza 75). The pots were randomly distributed on a greenhouse bench. The prevailing temperatures during the experiment were 22.5±0.5 (minimum) and 30.5±0.5 (maximum). Twenty five seeds were randomly removed from soil each six hrs. and used for isolation on PDA. There were five pots (replicates) for each sampling interaval.

Effects of temperature, incubation period, isolate, and their interactions on susceptibility of cottonseed to *M. phaseolina*

Three isolates, differing in their pathogenicity, were used in this study. The isolates were weakly pathogenic, moderately pathogenic, and highly pathogenic. Batches of autoclaved clay loam soil were separately infested with inoculum of each isolate at a rate of 50 g/kg of soil. Infested soil was dispensed in 15-cm-diameter clay pots and these were planted with 15 seeds per pot for each of the tested cultivars (Giza 75, Giza 80, and Giza 85). Pots were randomly distributed on greenhouse benches under four temperature regimes (Table 1). There were four pots (replicates) for each treatment. Seeds or radicles of germinating seeds were randomly removed from soil after 24, 48, 72, and 96 hr. from planting and used for isolation on PDA.

Table 1: Temperature regimes (°C) and incubation periods (hr) used in studying the effects of temperature, incubation period, isolate and their interactions on susceptibility of cotton seeds to *M. phaseolina*.

Treatment No.	Temperature regime		Incubation period (hr)
	Min.	Max.	
T1	27.5±3.5	38.5±0.5	24, 48, 72, 96
T2	25±1	35±1	24, 48, 72, 96
T3	22.5±0.5	30.5±0.5	24, 48, 72, 96
T4	18±0.0	25.5±0.5	24, 48, 72, 96

Statistical analysis of the data

The experimental design of all studies was a randomized complete block with four or five replicates. Analysis of variance (ANOVA) of the data was performed with the MSTAT-C Statistical Package. Least significant difference (LSD) was used to compare treatment means. Regression analysis was performed with a computerized program.

RESULTS AND DISCUSSION

Effect of inoculum density on susceptibility of cottonseed to *M. phaseolina*

Cottonseeds were removed from infested soil every six hours and used for isolation on PDA. Results shown in Table (2) indicated that the higher the inoculum density, the earlier the isolation of MP from seeds and the higher its isolation frequency.

Table 2:Effect of inoculum density on susceptibility of cotton (cultivar Giza 75) to *M. phaseolina*.

Inoculum Density	Time (hr) required for colonization of seeds	Isolation frequency
1%	96	4%
5%	12	12%

Effects of temperature, incubation period, isolate, and their interactions on susceptibility of cottonseed to *M. phaseolina*

ANOVA (Table 3) showed that the three main effects of this study (temperature, incubation period, and isolate) were all very highly significant sources of variation in susceptibility of cotton seed to *M. phaseolina* regardless of the tested cultivar. The two-way interaction for temperature x incubation period was a very highly significant source of variation in the case of cultivars Giza 75 ($P = 0.0000$) and Giza 80 ($P = 0.0001$), while it was a nonsignificant source of variation ($P = 0.2925$) in the case of Giza 85. All the other sources of variation were nonsignificant ($P > 0.05$) regardless of the tested cultivar.

Table 3: Analysis of variance of the effects of temperature regime, incubation period, isolate and their interaction on isolation frequency of *M. phaseolina* from seeds of three cotton cultivars.

Source of variation	D. F	M. S	F- value	P>F
	Giza 75			
Temperature regime (T)	3	1091.519	15.9403	0.0000
Incubation period (P)	3	4157.836	60.7203	0.0000
TxP	9	880.015	12.8516	0.0000
Isolates (S)	2	9164.365	133.8348	0.0000
TxS	6	59.185	0.8643	
PxS	6	25.229	0.3684	
TxPxS	18	75.819	1.1073	
Error	192	68.475		
	Giza 80			
Temperature regime (T)	3	327.678	4.4695	0.0064
Incubation period (P)	3	8411.917	114.7376	0.0000
TxP	9	300.001	4.0920	0.0001
Isolates (S)	2	5098.703	69.5457	0.0000
TxS	6	75.297	1.0270	0.4090
PxS	6	153.828	2.0982	0.0552
TxPxS	18	38.646	0.5271	
Error	192	73.314		
	Giza 85			
Temperature regime (T)	3	1238.073	18.8412	0.0000
Incubation period (P)	3	4460.693	67.8835	0.0000
TxP	9	79.332	1.2073	0.2925
Isolates (S)	2	5406.890	82.2828	0.0000
TxS	6	137.501	2.0925	0.0559
PxS	6	89.900	1.3681	0.2294
TxPxS	18	103.257	1.5714	0.0707
Error	192	65.711		

Relative contribution of each source of variation to variation in isolation frequency of *M. phaseolina* from seeds is shown in Table (4). Incubation period was the first in importance as a source of variation in isolation frequency from Giza 80 and Giza 85. It accounted for 61.27% of the explained (model) variation in isolation frequency from Giza 80 and 42.02% from Giza 85. In the case of Giza 75, isolate was the first in importance as a source of variation in isolation frequency followed by incubation period. Isolate was the second in importance as a source of variation in isolation frequency from Giza 80 and Giza 85. The highest relative contribution of temperature x incubation period interaction to variation in isolation frequency was observed in the case of Giza 75.

Table 4: Relative contribution of temperature regime, incubation period, isolate and their interaction on isolation frequency of *M. phaseolina* from seeds of three cotton cultivars.

Source of variation	Relative contribution [a] to variation in isolation frequency of *M. phaseolina* from seeds of		
	Giza 75	Giza 80	Giza 85
Temperature regime (T)	7.46	2.39	11.66
incubation period (P)	28.43	61.27	42.02
TxP	18.05	6.56	2.24
Isolate (S)	41.78	24.76	33.26
TxS	0.81	1.10	2.59
PxS	0.35	2.24	1.69
TxPxS	3.11	1.69	5.84

[a] calculated as percentage of sum of squares of the explained (model) variation

Table 5: Effect of temperature regime, incubation period, isolate and their interactions on isolation frequency of *M. phaseolina* from seeds of cotton cultivar Giza 75.

Incubation period (hr)	Isolate	Temperature regime T1	T2	T3	T4	Mean
24	S1	12.00(15.94)[a]	8.00 (10.64)	12.00 (15.94)	8.00 (10.62)	10.00(13.29)
	S2	28.00 (31.63)	20.00 (26.56)	24.00 (29.09)	12.00 (15.94)	21.00 (25.81)
	S3	44.00 (41.54)	28.00 (31.63)	48.00 (43.85)	24.00 (29.09)	36.00 (36.53)
	Mean	28.00 (29.70)	18.67 (22.24)	28.00 (29.63)	14.67 (18.55)	22.34 (25.21)
48	S1	40.00 (39.00)	20.00 (26.56)	16.00 (21.25)	12.00 (15.94)	22.00 (25.69)
	S2	52.00 (46.15)	36.00 (36.70)	28.00 (31.63)	28.00 (31.63)	36.00 (36.53)
	S3	56.00 (48.46)	52.00 (46.15)	52.00 (46.15)	44.00 (41.54)	51.00 (45.58)
	Mean	49.33 (44.54)	36.00 (36.47)	32.00 (33.01)	28.00 (29.70)	36.33 (35.93)
72	S1	52.00 (46.15)	28.00 (31.63)	28.00 (31.63)	20.00 (26.56)	32.00 (33.99)
	S2	72.00 (58.37)	32.00 (34.16)	52.00 (46.15)	48.00 (43.85)	51.00 (45.63)
	S3	88.00 (74.06)	52.00 (46.15)	56.00 (55.84)	56.00 (48.64)	63.00 (56.13)
	Mean	70.67 (59.53)	37.33 (37.31)	45.33 (44.45)	41.33 (39.62)	48.67 (45.25)
96	S1	24.00 (29.09)	24.00 (29.09)	12.00 (15.94)	44.00 (41.54)	26.00 (28.92)
	S2	36.00 (36.70)	28.00 (31.63)	24.00 (29.09)	60.00 (51.00)	37.00 (37.11)
	S3	48.00 (43.85)	64.00 (53.30)	44.00 (41.54)	72.00 (58.37)	57.00 (49.27)
	Mean	36.00 (36.55)	38.67 (38.01)	26.67 (28.86)	58.67 (50.30)	4.00(38.43)
Overall mean		46.00 (42.58)	38.67 (33.68)	33.00 (34.01)	35.67 (34.54)	36.84 (36.21)
Mean of isolate	S1	22.50(25.47)				
	S2	36.25(36.27)				
	S3	51.75(46.88)				

LSD (transformed data)	P≤0.05	P≤0.01
For Period (P)	2.98	3.90
Temperature(T)	2.98	3.93
P x T	5.96	7.86
Isolate(S)	2.58	3.40
P x S	NS	NS
T x S	NS	NS
P x T x S	NS	NS

[a] Mean of five replicates. Percentage data were transformed into arc sine angles before carrying out the analysis of variance. Transformed means are shown in parentheses.

Due to the significant interaction between temperature regime and incubation periods in the case of Giza 75, a least significant difference (LSD) was used to compare between the incubation periods within temperature regimes. These comparisons showed that the magnitude of the difference between incubation periods was affected by temperature regime (Table 5). For example, the increase of incubation period from 24 hr to 48 hr caused highly

significant increase in the isolation frequency under the first temperature regime; however, the increase in isolation frequency was nonsignificant under the third temperature regime. Similarly, the increase of incubation period from 48 hr to 72 hr caused highly significant increase in isolation frequency under the first temperature regime, however, almost no increase in isolation frequency was observed under the second temperature regime. Under the first temperature regime, the increase of incubation period from 72 hr to 96 hr caused an appreciable decrease in isolation frequency, while it caused an appreciable increase in isolation frequency under the fourth temperature regime.

Due to the significant interaction between temperature regime and incubation period in the case of Giza 80, a LSD was used to compare between the incubation periods within temperature regimes. These comparisons showed that the magnitude of the difference between incubation periods was affected by temperature regime (Table 6). For example, the increase of incubation period from 48 hr to 72 hr caused highly significant increase in the isolation frequency under the first temperature regime; however, the increase in isolation frequency was nonsignificant under the second temperature regime.

Table 6: Effects of temperature regime, incubation period, isolate and their interactions on isolation frequency of *M. phaseolina* from seeds of cotton cultivar Giza 80.

Incubation period (hr)	Isolate	Temperature regime T1	T2	T3	T4	Mean
24	S1	12.00(15.94)[a]	12.00(15.94)	8.00(10.62)	4.00(5.31)	9.00 (11.25)
	S2	20.00(26.56)	16.00(21.25)	(12.00)(15.4)	8.00(10.62)	14.00 (18.9)
	S3	32.00(34.16)	24.00(29.09)	24.00(29.09)	20.00(23.78)	25.00(29.03)
	Mean	21.33(25.55)	17.33(22.09)	14.00(18.55)	10.67(13.24)	15.83(19.86)
48	S1	20.00(26.56)	24.00(29.09)	16.00(21.25)	8.00(10.62)	16.50(21.88)
	S2	32.00(34.16)	28.00(31.63)	24.00(29.09)	16.00(21.25)	25.00(29.03)
	S3	36.00(36.70)	36.00(36.70)	44.00(41.54)	36.00(36.69)	38.00(37.91)
	Mean	29.33(32.47)	29.33(32.47)	28.00(30.63)	20.00(22.85)	26.67(29.61)
72	S1	36.00(36.70)	24.00(29.09)	28.00(31.63)	24.00(29.09)	28.00(31.63)
	S2	52.00(46.15)	32.00(34.16)	48.00(43.85)	36.00(36.69)	42.00(40.21)
	S3	72.00(58.37)	52.00(46.16)	60.00(51.00)	60.00(55.84)	61.00(52.84)
	Mean	53.33(47.07)	36.00(36.47)	45.33(42.16)	40.00(40.54)	43.67(41.55)
96	S1	44.00(41.54)	32.00(34.16)	44.00(41.54)	48.00(43.85)	42.00(40.27)
	S2	52.00(46.15)	48.00(43.85)	56.00(48.46)	64.00(53.53)	55.00(48.00)
	S3	52.00(46.15)	56.00(48.69)	56.00(48.46)	68.00(55.84)	58.00(49.79)
	Mean	49.33(44.61)	45.33(42.23)	52.00(46.15)	60.00(51.07)	51.67(46.02)
Overall mean		38.33(37.43)	32.00(33.32)	34.83(34.37)	32.67(31.93)	34.46(34.26)
Mean of isolate	S1	23.88 (26.43)				
	S2	34.00 (33.96)				
	S3	45.50(42.39				

LSD (transformed data)	P≤0.05	P≤0.01
For Period (P)	3.08	4.07
Temperature(T)	3.08	4.07
P x T	6.17	8.13
Isolate(S)	2.67	3.52
P x S	NS	NS
T x S	NS	NS
P x T x S	NS	NS

[a] **Mean of five replicates. Percentage data were transformed into arc sine angles before carrying out the analysis of variance. Transformed means are shown in parentheses.**

Due to the lack of a significant interaction between incubation period and temperature regime in the case of Giza 85, a LSD was calculated to compare between the general means of incubation periods and temperature regimes (Table 7). The highest isolation frequency of *M. phaseolina* from seeds was found when the seeds were incubated in infested soil for 72 hours. The increase of the incubation period to 96 hr did not significantly increase the isolation frequency of *M. phaseolina* from seeds. Seeds yielded the highest frequency of *M. phaseolina* when they were incubated under the 2nd and the 3rd temperature regimes.

Table 7: Effects of temperature regime, incubation period, isolate and their interactions on isolation frequency of *M. phaseolina* from seeds cotton cultivar Giza 85.

Incubation period (hr)	Isolate	Temperature regime T1	T2	T3	T4	Mean
24	S1	8.00 (10.62)[a]	16.00 (21.25)	12.00 (15.94)	12.00 (15.94)	12.00 (15.94)
	S2	12.00 (15.94)	20.00 (26.56)	20.00 (26.56)	16.00 (21.25)	17.00 (22.85)
	S3	24.00 (29.09)	28.00 (31.63)	36.00 (36.70)	24.00 (29.09)	28.00 (31.63)
	Mean	14.67 (18.55)	21.33 (26.48)	22.67 (26.40)	17.33 (22.09)	19.00 (23.38)
48	S1	16.00 (21.25)	24.00 (29.09)	20.00 (26.56)	12.00 (15.94)	18.00 (23.21)
	S2	16.00 (21.25)	36.00 (34.16)	28.00 (31.63)	20.00 (26.56)	25.00 (28.40)
	S3	28.00 (31.63)	40.00 (39.00)	52.00 (46.15)	28.00 (31.63)	37.00 (37.10)
	Mean	20.00 (24.71)	33.33 (34.08)	33.33 (34.78)	20.00 (24.71)	26.67 (29.57)
72	S1	32.00 (34.16)	28.00 (31.63)	28.00 (31.63)	24.00 (29.09)	28.00 (31.83)
	S2	32.00 (34.16)	32.00 (34.10)	52.00 (46.15)	28.00 (31.63)	36.00 (36.51)
	S3	48.00 (43.85)	72.00 (58.37)	64.00 (53.30)	68.00 (55.84)	63.00 (52.84)
	Mean	37.33 (37.39)	44.00 (41.37)	48.00 (43.69)	40.00 (38.85)	42.33 (40.33)
96	S1	28.00 (31.63)	32.00 (34.16)	44.00 (41.54)	28.00 (31.63)	33.00 (34.74)
	S2	32.00 (34.16)	52.00 (46.15)	56.00 (48.46)	28.00 (31.63)	42.00 (40.10)
	S3	32.00 (34.16)	52.00 (46.15)	72.00 (58.37)	68.00 (55.84)	56.00 (48.63)
	Mean	30.67(33.32)	45.33(42.15)	57.33 (49.46)	41.33 (39.70)	43.67 (41.16)
Overall mean		25.67(28.49)	36.00(63.02)	40.33 (38.58)	29.67 (31.34)	32.92 (33.61)
Mean of isolate	S1	22.75(26.43)				
	S2	30.00(31.90)				
	S3	46.00(42.55)				

LSD (transformed data)	P ≤0.05	P ≤0.01
For Period (P)	2.92	3.85
Temperature(T)	2.92	3.85
PxT	NS	NS
Isolate(S)	2.53	3.34
PxS	NS	NS
TxS	NS	NS
PxTxs	NS	NS

[a] **Mean of five replicates. Percentage data were transformed into arc sine angles before carrying out the analysis of variance. Transformed means are shown in parentheses.**

The isolates of *M. phaseolina* maintained their ranking regardless of the tested cultivar. Thus, S1 was the least aggressive isolate in colonization of seeds, while S3 was the most aggressive isolate. S2 was intermediate in aggressiveness (Tables 5, 6, and 7).

A highly positive linear relationship was found between incubation period and isolation frequency ($P < 0.01$). R^2 of the regression model was 0.83 when the inoculum density was 1% and increased to 0.96 when the inoculum density was 5% (Figs. 1 and 2).

The results of the present study demonstrated that colonization of cotton seeds by *M. phaseolina* during the early stages of infection was affected by inoculum density, temperature, incubation period (time of seed exposure to inoculum), and pathogenicity of isolates. The findings of this study highlight the importance of some cultural practices in controlling *M. phaseolina* on cotton. For example, in normal cropping sequence of cotton it would not be desirable to introduce soybean, which would tend to increase the *M. phaseolina* inoculum density (Kenerley and Jeger, 1992). Burying and subsequent decomposition of the residues from previous crops may aid in controlling *M. phaseolina* by reducing population of the pathogen in the residues through antibiosis (Watkins, 1981). Control of *M. phaseolina* may be achieved by preventive measures such as acid delinting and coating seeds with protective fungicides, which prevent infection when the seeds are sown in infested soil. It is also recommended to avoid extreme high temperature through early sowing.

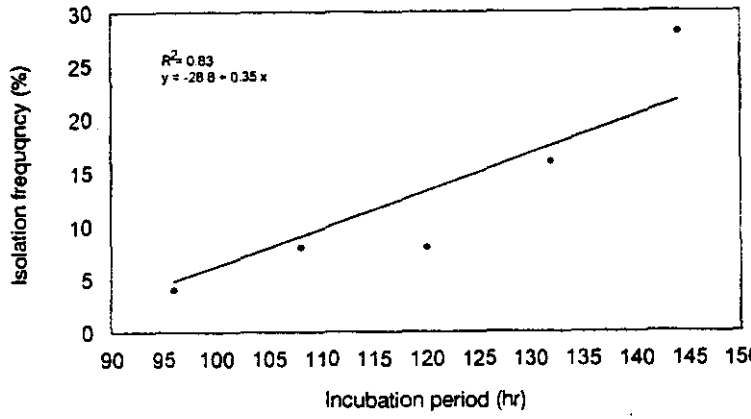

Figure 1: Relationship between incubation period of cotton seeds (cv. Giza 75) in autoclaved soil infested with *M. phaseolina* and frequency of the fungus isolated from these seeds. The autoclaved soil was infested with *M. phaseolina* at the rate of 1% (w/w).

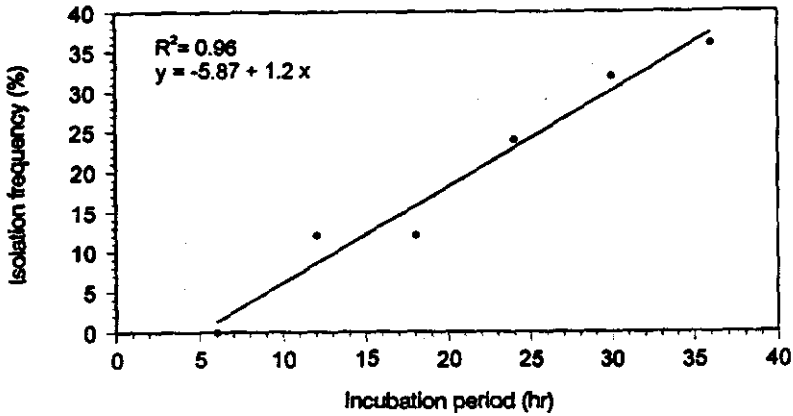

Figure 2: Relationship between incubation period of cotton seeds (cv Giza 75) in autoclaved soil infested with *M. phaseolina* and frequency of the fungus isolated from these seeds. The autoclaved soil was infested with *M. phaseolina* at the rate of 5% (w/w).

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بعض العوامل المؤثرة على قابلية بذرة القطن للإصابة بفطر ماكروفومينا فاسيولينا

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إن استعمار بذرة القطن بفطر ماكروفومينا فاسيولينا خلال المراحل المبكرة من الإصابة يتأثر بالعديد من العوامل هى على النحو التالى: كثافة اللقاح الفطرى ودرجة الحرارة وفترة الحضانة (مدة تعرض البذرة للقاح الفطرى) والقدرة المرضية لعزلات الفطر. بخصوص كثافة اللقاح الفطرى ، يمكن القول أنه كلما إزدادت كثافة اللقاح الفطرى كلما كان توقيت عزل الفطر من البذرة أكثر تبكيراً ، وكلما إزداد تكرار العزل من البذرة. التأثيرات الرئيسية التى تضمنها تحليل التباين (درجة الحرارة وفترة الحضانة والقدرة المرضية للعزلة) كانت كلها مصادر عالية المعنوية للتباين فى تكرار عزل الفطر من البذرة ، بصرف النظر عن صنف القطن المستخدم فى العزل (جيزة ٧٥ أو جيزة ٨٠ أو جيزة ٨٥). تفاعل درجة الحرارة × فترة الحضانة كان مصدراً عالى المعنوية للتباين فى تكرار عزل الفطر من بذرة جيزة ٧٥ وجيزة ٨٠ فى حين كان مصدراً غير معنوياً للتباين فى تكرار العزل من جيزة ٨٥. جميع مصادر التباين الأخرى كانت غير معنوية ، بصرف النظر عن الصنف المستخدم فى العزل.