J. Agric. Sci. Mansoura Univ., 31 (1): 225 - 239, 2006

EFFECT OF SOME ENVIRONMENTAL FACTORS ON THE INCIDENCE OF CHARCOAL ROT OF COTTON CAUSED BY *Macrophomina phaseolina*

Aly, A.A.[1]; R.M. Youssef[2]; A.M.M. El-Shazley[2]; and M.R. Omar[1]
1- Plant Pathology Res. Institute, Agricultural, Res. Center, Giza, Egypt.
2- Dept. of Agric. Botany, Faculty of Agric., Al-Azhar Univ., Cairo, Egypt.

ABSTRACT

A greenhouse study was carried out to evaluate the effects of temperature, soil moisture, soil texture, fertilization, and intercropping on susceptibility of the Egyptian cotton cultivar Giza 75 to *Macrophomina phaseolina*. The obtained results revealed that the optimum temperature of pathogenicity was somewhere between 24.5±1 .5^{0}C to 38.0±.2.0^{0}C. The results were inconclusive in determining the relationship between soil moisture content and susceptibility of cotton to *M. phaseolina*. The discrepancy in the results may be attributed to the fact that the effect of soil moisture was obscured by other environmental factors interacting with soil moisture. The results suggest that cotton is more susceptible to *M. phaseolina* in clay soil than in sandy clay soil. Nineteen fertilizer treatments were evaluated as to their effects on susceptibility of cotton to *M. phaseolina*. Of these treatments, six were effective in reducing the development of *M. phaseolina* lesions on tap root. However, of the six treatments, the combination of calcium nitrate, potassium sulphate, and calcium superphosphate was the best for two reasons. First, this combination reduced the development of *M. phaseolina* lesions on tap root by 71.86%. Second, this combination was nutritiously balanced because it provided cotton with NPK. Although, other treatments were as effective as this combination in reducing the diseases, they were nutritiously unbalanced. Intercropping onion (Giza 6) and Chinese garlic with cotton significantly suppressed the advancement of *M. phaseolina* lesions on cotton stem by 80.59% and 83.01 %, respectively.

INTRODUCTION

Macrophomina phaseolina (Tassi) Goid., the causal agent of charcoal rot on cotton, is a seed-borne and soilborne pathogen with a wide distribution and 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 in Egypt, is underestimated. 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, 1959; Mohamed, 1962, and Sabet and Khan, 1969). This lack of concern is not justifiable because this fungus is of widespread distribution in the Egyptian 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. For example, Ghaffar and Erwin (1969) found that when cotton roots were inoculated with *M. phaseolina* in a greenhouse at 40°C, severity was not as great as seen at similar temperature in the field. Holliday and Punithalingam (1970) reported that charcoal rot on cotton was more severe at high temperature, between 35 and 39°C. Dhingra and Sinclair (1978) mentioned that *M. phaseolina* was considered as a high temperature pathogen. The severity of the disease it caused increased with increasing temperature with optimum being between 28 to 35°C. Mostafa (1982) found that *M. phaseolina* isolates were more virulent on soybean at 30 than at 20°C.

Radha (1960) reported that infection of cotton with *M. phaseolina* increased and seed germination decreased with increasing soil moisture. Ghaffar and Erwin (1969) showed that cotton plants subjected to water stress developed severe charcoal rot whereas plants growing under adequate moisture conditions were less affected at soil temperatures ranging from 20 to 40°C. Dhingra and Sinclair (1978) mentioned that infection of plants by *M. phaseolina* could occur under high soil moisture conditions but the development of the disease was slow and many plants could withstand the damage through the establishment of new adventitious roots. When Sheikh and Ghaffar (1979) plating out segments of cotton roots on PDA, they found that percentage infection with *M. phaseolina* varied inversely with soil moisture. Blanco-López and Jimenez-Diaz (1983) found that severity and incidence of charcoal rot of sunflower were higher in unirrigated plots compared to irrigated ones. Irrigation at flowering or at flowering and ripening stages decreased disease incidence. Thakar (1984) reported that growing the susceptible cotton cultivar Digvijay under regimes of 25 or 50% available soil moisture reduced the incidence of MP without significantly affecting the yield of seedcotton. Yield was reduced at 75% moisture. Zazzerini *et al.* (1985) studied the resistance of ten sunflower cultivars to *M. phaseolina* under conditions of natural infection in the field, under various irrigation treatments. The cultivar x irrigation treatment was significant. Disease incidence increased with increasing rainfall and irrigation. Bruton *et al.* (1987) found that drought stress was not a requirement for early infection of cantaloupe roots with *M. phaseolina*. The early incidence of infection was favored by soil moisture contents that did not correspond with drought conditions. Taya *et al.* (1988) found that low soil moisture was conducive to chickpea dry root-rot caused by *M. phaseolina*. Pande *et al.* (1989 and 1990) reported that increasing moisture stress increased charcoal rot in sorghum as measured by plant lodging. Walker (1994) mentioned that excessively wet soil by drip irrigation favored infection of muskmelon by *M. phaseolina*. Diourte *et al.* (1995) found that sorghum plants subjected to post-flowering water stress and inoculated with *M. phaseolina* had greater development of charcoal rot symptoms than did inoculated plants not subjected to water stress.

Dhingra and Sinclair (1978) mentioned that *M. phaseolina* diseases of crop plants were more severe on sandy soils than on heavy soils. Filonow *et al.* (1981) demonstrated that heavier-textured soils required more nutrients for germination of *M. phaseolina* sclerotia than sandy soils. Wyllie and Mekelwey (1983) reported that clay soils yielded greater numbers of *M. phaseolina* sclerotia than loam or sand. Bruton and Reuveni (1985) found that sclerotia of *M. phaseolina* were concentrated in the top 3 cm of soil regardless of host crop. In general, sclerotia were almost absent in the 40-100 cm soil depth. Soil texture, ranging from loamy sand to heavy clay, had no apparent effect on the vertical distribution of sclerotia. Taya *et al.* (1988) found that incidence of *M. phaseolina* on chickpea was higher on sandy than clay soils. Salem (1997) found that the red argil soil suppressed the incidence of charcoal rot on the soybean cultivars Clark and Cultivar 71, while the green argil soil enhanced the incidence of the disease on the two cultivars, followed by clay, sandy loam, and sandy soils.

Ghaffar *et al.* (1971) reported that deficiency or excess of N and K had no effect on susceptibility of cotton to *M. phaseolina*. Prasad (1983) found that the incidence of root and stem rot of jute, caused by *M. phaseolina*, was inversely proportional to K levels. Maximum disease was observed at the 0 kg potash/ha and minimum at 70 kg. Chun and Lockwood (1985) amended sandy loam soil, infested with *M. phaseolina*, with urea at 0.10, 0.25, 0.50, and 1.0% (w/w) in field microplots, ammonia concentration increased with the amount of urea applied and population density of *M. phaseolina* was significantly and often markedly decreased by urea at 1.00, 0.50, and 0.25%; 0.10% urea effectively reduced soil populations after 31 days at high temperature but was ineffective even after 34 days at low soil temperature. Rao *et al.* (1991) applied nitrogen at 0, 60, 120 and 180 kg/ha to 7 maize genotypes. Charcoal rot increased with N levels for all the genotypes and was the maximum at 180 kg/ha. At 60 kg/ha, disease incidence was low. In a field experiment, Khune *et al.* (1993) applied nitrogen at the rates of 0, 20, 40, 60, and 120 kg/ha and the incidence of charcoal rot of sorghum was observed, maximum disease incidence occurred where nitrogen was applied at 120 kg/ha showing 26.72% lodging of plants compared with 0.18% in 0 kg N/ha. Cloud and Rupe (1994) found that lesions of *M. phaseolina* on sorghum stalks tended to increase with increased amounts of nitrogen fertilization, while root infection was not affected by N treatment.

Rajpurghit (1983) found that intercropping was effective in controlling charcoal rot of cotton by using *Phaseolus aconitifolius,* which it was claimed, acted by reducing soil temperature. The control obtained was equal to that obtained by a range of different chemical treatments. Lodha and Mahander (1985) studied the relation of *M. phaseolina* sclerotia to death and yield of legumes *(Cyamopsis tetragonoloba,* cowpea and *Vigna aconitifolius)* in grass *(Cenchrus ciliaris)* intercropping. Most death occurred in cowpea (no yield) with or without grass, but the pathogen population declined sharply in cowpea plots at harvest, in contrast with the increased population in other crops. The results suggested that during drought, legumes intercropped with *C. ciliaris* were highly susceptible to *M. phaseolina*. Cowpea proved the most susceptible and *V. aconitifolius* the least. Singh *et al.* (1989) reported that extracts of soil amended with crop residues of mature and immature wheat, *Cicer arietinum*, pea, and lentil inhibited growth of *M. phaseolina*. Singh *et al.* (1990) recorded high counts of *M. phaseolina* sclerotia in the intercropping systems of sorghum or cowpea with pigeon pea than in single-cropping systems. The highest counts of sclerotia were recorded in plots where sorghum was intercropped continuously with pigeon pea in both the rainy and postrainy seasons.

However, due to the lack of studies, the effects of the previously mentioned factors on susceptibility of the Egyptian cotton *(Gossypium barbedense* L.) to *M. phaseolina* are unclear. Therefore, the objective of this study was to evaluate the effects of temperature, soil moisture, soil texture, fertilization and intercropping on susceptibility of the Egyptian cotton to *M. phaseolina*.

MATERIALS AND METHODS

Effect of temperature on susceptibility of cotton to *M. phaseolina*

M. phaseolina was grown on autoclaved sorghum for three weeks. Fungus-sorghum mixture was used to infest clay loam soil at a rate of 50g/kg soil. Artificially infested soil was dispensed in 15-cm-diameter clay pots, and these were planted with 10 seeds per pot (cultivar Giza 75). Pots of regimes 1, 2, and 4 (Table 1) were placed in air conditioned greenhouses, whereas pots of regime 3 were placed outdoors. In the control (noninfested) treatments, autoclaved sorghum grains were mixed with soil at the rate of 50 g/kg soil. There were five replications (pots) for each treatment. Pots were randomly distributed under each temperature regime. Preemergence damping-off, postemergence damping-off, survival, plant height (cm), and dry weight (mg/plant) were recorded 45 days after planting.

Effect of soil moisture on susceptibility of cotton to *M. phaseolina*

Artificially infested field soil (50 g/kg soil) were dispensed in 25-cm-diameter clay pots, and these were planted with 20 seeds per pot (cultivar Giza 75). When the seedlings were 45 days old, they were thinned to 5 seedlings per pot. After thinning, the plants were watered periodically by adding a liter of water/pot. The irrigation intervals used were 1, 2, 3, and 4 days. In the control (noninfested) treatments, autoclaved sorghum grains were mixed with soil at the rate of 50 g/kg soil. There were five replications (pots) for each treatment. Pots were randomly distributed outdoors. The recommended production practices for cotton were followed during the growing season. At the end of the growing season, seedcotton yield of each treatment was picked, and length of lesions on tap root was measured. The advancement of the lesions on stem was also measured after peeling the bark off the stem. The tissues within root and stem lesions were plated on PDA to verify the presence of *M. phaseolina.*

Effect of soil texture on susceptibility of cotton to *M. phaseolina*

Bulk samples of soil were collected in the growing season from six fields in different cotton growing areas. Each bulk sample consisted of five subsamples randomly collected from the same field. Soil subsamples were obtained from the upper 10-15 cm of soil with a hand spade. Composite soil sample from each field was artificially infested with *M. phaseolina* (40 g/kg soil), and dispensed in 10-cm-diameter clay pots. The pots were planted with seeds of cultivar Giza 75 (10 seeds/pot). The pots were distributed on a greenhouse bench under temperature regime ranging from 24 ± 20°C to 39±30°C. Postemergence damping-off, was recorded 45 days after planting. Roots of infected plants were plated on PDA to verify the presence of *M. phaseolina.*

Table 1. The temperature regimes (°C) used in study the effect of temperature on susceptibility of cotton to *M. phaseolina.*

Test No.	Temperature (°C)	
	Minimum	Maximum
1	28.0±1.5	43.0±2.0
2	24.0±1.5	38.0±2.0
3	23.0±3.5	34.0±3.0
4	15.0±3.0	24.0±4.0

Effect of fertilization on susceptibility of cotton to *M. phaseolina*

Field soil was artificially infested with *M. phaseolina* (40 g/kg soil), and dispensed in 30-cm-diameter clay pots. Each pot was planted with 50 seeds of cultivar Giza 75. When the seedlings were 45 days old, they were thinned to five seedlings per pot. The plants were fertilized with different combinations of urea, ammonium nitrate, ammonium sulphate, calcium superphosphatte, and potassium sulphate. Calcium superphosphate was the only fertilizer which was applied at the time of sowing, while the other fertilizers were applied after thinning. The amount of fertilizer was calculated according to the following formula: (Amount of fertilizer (g/pot) = [(Soil weight in a pot x quantity of fertilizer per feddan)/1000000 (weight of soil in the upper 15 cm for feddan area)] x1000). Fertilizers used in study and their application rates are shown in Table 2. There were five pots for each treatments. Pots were randomly distributed outdoors. Data were recorded at the end of the growing season as previously mentioned in the soil moisture experiment.

Table 2. Concentrations and application rates of fertilizers used in study

Fertilizer	Concentration	Application rate	
	(%)	Kg/feddan	g/pot
Urea	46.0 N_2	163	1.96
Ammonium sulphate	20.6 N_2	364	4.37
Calcium nitrate	15.5 N_2	484	5.00
Ammonium nitrate	33.5 N_2	224	2.60
Calcium superphosphate	15.0 P_2O_5	150	1.80
Potassium sulphate	52.0 K_2O	100	1.20

Effect of intercropping on susceptibility of cotton to *M. phaseolina*

Field soil was artificially infested with *M. phaseolina* (50 g/kg soil), and dispensed in 25-cm-diameter clay pots. On the middle of January, pots were planted with onion (Giza 6), and garlic (Balady and Chinese). Five onion bulbs or garlic cloves were planted in each pot. In the control treatment, pots were left without planting. There were five pots for each treatment. Pots were randomly distributed outdoors. On the middle of April, all pots were planted with cotton cultivar Giza 75 (10 seeds per pot). Preemergence dampingoff was recorded 20 days after planting, while postemergence damping-off was recorded 45 days after planting. On the first week of June, onion and garlic plants were uprooted, and cotton seedlings were thinned to five plants/pot. At the end of the growing season, data were recorded as previously mentioned in the soil moisture experiment.

RESULTS

Effect of temperature on susceptibility of cotton to *M. phaseolina*

In this study, temperature was maintained at four regimes (Table 3). It was obvious that *M. phaseolina* was unable to induce significant increases in seedling mortality in the preemergence stage at all the temperature regimes. On the contrary, *M. phaseolina* was pathogenic in the postemergence stage at all the temperature regimes. It was evident that raising the temperature within certain limit led to an increase in the postemergence damping-off. The reduction in the surviving seedlings was nonsignificant at the coolest temperature regime, whereas it was significant at all the other regimes. However, the magnitude of reduction in the surviving seedlings (relative susceptibility) varied from one regime to another. Thus, the relative susceptibility was 38.46% when the temperature was ranging from 23.5±3.5 to 34.0±3.0°C, while it reached its maximum level (65.57%) when the temperature was ranging from 24.5±1.5 to 38.0±2.0°C. On the other hand, when the temperature was ranging from 28.5±1.5°C to 43.0±2.0°C, the relative susceptibility decreased from 65.57% to 60.32%. Plant height was significantly reduced by *M. phaseolina* only at the coolest temperature regime. Dry weight was not affected by *M. phaseolina* at all the tested regimes.

Effect of soil moisture on susceptibility of cotton to *M. phaseolina*

In the present study, soil moisture was manipulated by changing watering intervals (Table 4). *M. phaseolina* lesions on tap root reached their maximum length when the plants were watered every day or every two days. A considerable reduction in the length of lesions occurred when the plants were watered every three days. The length of lesions was intermediate when the plants were watered every four days. It was obvious that watering regime had no effect on the length of lesions on stem. The percentage of dead plants reached its maximum level when the plants were watered every day, whereas it reached its minimum level when the plants were watered every four days. Watering every two or three days caused intermediate level of plant death. *M. phaseolina* caused highly significant reduction in seedcotton yield (g/plant) at all the irrigation regimes (Table 5); however, the maximum reduction was observed when the plants were watered every two or three days.

Table 3. Effect of temperature regimes on pathogenicity of *Macrophmina phaseolina* on cotton cultivar Giza 75 under greenhouse conditions.

Temperature regime		Pre-emergence damping-off (%)[a]		Post-emergence damping-off (%)		Survival %		Plant height (cm)		Dry weight (mg/plant)	
Minimum	**Maximum**	Non-infested soil	Infested soil	Non-infested soil	Infested soil	Non-infested soil	Infested soil	Non-infested soil	Infested soil	Non-infested soil	Infested soil
28.5±1.5°C	43±2°C	37.00^{A}	38.00^{A}	0.00^{B}	37.00^{A}	63.00^{A}	25.00^{B}	30.26^{A}	32.07^{A}	652.60^{A}	611.60^{A}
24.5±1.5°C	38±2°C	39.00^{A}	36.00^{A}	0.00^{B}	43.00^{A}	61.00^{A}	21.00^{B}	25.89^{A}	23.92^{A}	337.80^{A}	261.00^{A}
23.5±3.5°C	34±3°C	34.00^{A}	31.00^{A}	1.00^{B}	29.00^{A}	65.00^{A}	40.00^{B}	19.37^{A}	18.34^{A}	631.60^{A}	715.40^{A}
15.0±3.0°C	24±4°C	28.00^{A}	38.00^{A}	0.00^{B}	16.00^{A}	72.00^{A}	46.00^{A}	10.42^{A}	8.24^{B}	108.40^{A}	88.40^{A}

[a] Percentage data were transformed into arc sine angles before carrying out the analysis of variance to produce approximately constant variance. For each variable, values in a row followed by the same letter(s) are not significantly different ($P < 0.05$) according to Duncan's multiple range test.

Table 4. Effect of soil moisture on pathogenicity of *Macrophomina phaseolina* on cotton cultivar Giza 75 in an outdoor pot experiment.

Watering regime	Length of lesion on tap root (%) [a]	Length of lesion on stem (%) [b]	Dead plants (%)
Watering every day	26.94$^{c\,A}$	4.24$^{d\,A}$	68$^{c\,A}$
Watering every two days	30.06^{A}	8.62^{A}	28^{B}
Watering every three days	6.90^{B}	6.30^{A}	36^{AB}
Watering every four days	17.30^{AB}	12.02^{A}	20^{B}

[a]
$$\frac{\text{Length of lesion on tap root (cm)}}{\text{Length of tap root (cm)}} \text{ X } 100$$

[b]
$$\frac{\text{Length of lesion on stem (cm)}}{\text{Length of stem (cm)}} \text{ X } 100$$

[c] Percentage data were transformed into arc sine angles before carrying out the analysis of variance. Values in a column followed by the same letter(s) are not significantly different ($P < 0.5$) according to Duncan's multiple range test.

[d] Percentage data were transformed into $\sqrt{X} + 0.05$ before carrying out the analysis of variance. Values in a column followed by the same letter(s) are not significantly different ($P < 0.05$) according to Duncan multiple range test.

Table 5. Effect of *Macrophomina phaseolina* on seedcotton yield of cotton cultivar Giza 75 under the effect of four watering regimes in an outdoor experiment in natural soil.

Watering regime	Yield (g/plant)		Reduction	Reduction[a] (%)
	Non infested soil	Infested soil		
Watering every day	2.008	1.030	0.978**	48.71
Watering every two days	2.026	0.786	1.240**	61.20
Watering every three days	2.106	0.894	1.212**	57.55
Watering every four days	2.086	1.218	0.868**	41.61

** Reduction in yield was significant at $P < 0.01$ according to LSD (0.298).

[a] Reduction was expressed as percentage of the noninfested control.

Effect of soil texture on susceptibility of cotton to *M. phaseolina*

Three clay soils and three sandy clay soils from different governorates (Table 6) were artificially infested with a pathogenic isolate of *M. phaseolina*. *M. phaseolina* was highly pathogenic in the clay soil no. 3, while it was weakly pathogenic in the clay soil no. 2 and the sandy soils no$_s$. 5 and 6. In the clay soil no. 1 and the sandy soil no. 4, the fungus was moderately pathogenic. Isolation frequency of *M. phaseolina* of the sandy clay soil no. 6 was significantly lower than that of any of the other samples. However, no significant differences in the isolation frequency were observed among these samples (samples from no. 1 to no. 5).

Table 6. Effect of soil texture on pathogenicity of *M. phaseolina* on cotton cultivar Giza 75 under greenhouse conditions.

	No. and texture of soil sample	Geographic origin	Dead plants (%) [a]	Root tissues colonized by *M. phaseolina* (%) [b]
1.	Clay	Kafr El-Sheikh – Sakha	59.27 ^{AB}	95.00 ^{A}
2.	Clay	Minufiya – Sirs El-Lian	38.90 ^{B}	95.00 ^{A}
3.	Clay	Sharqiya – Zagazig	65.09 ^{A}	100.00 ^{A}
4.	Sandy clay	Minya – Mallawy	46.87 ^{AB}	85.00 ^{A}
5.	Sandy clay	Faiyum – Faiyum	37.61 ^{B}	90.00 ^{A}
6.	Sandy clay	Faiyum – Sunnoris	38.41 ^{B}	65.00 ^{B}

[a] Means followed by the same letter(s) are not significantly different ($P < 0.05$) according to Duncan's multiple range test. Each value is the mean of five replications.

[b] Percentage data were transformed into $\sqrt{X}$ before carrying out the analysis of variance where X is the percentage value. Each value is the percentage of root segments (0.5 cm long) that yielded *M. phaseolina*.

Effect of fertilization on susceptibility of cotton to *M. phaseolina*

Cotton plants were fertilized with different combinations of urea, ammonium nitrate (AN), ammonium sulphate (AS), calcium nitrate (CN), calcium superphosphate (CS) and potassium sulphate (PS). Data of Table (7) showed that the development of *M. phaseolina* lesions on tap root was not significantly affected by the application of urea treatments (urea alone and urea in combination with other fertilizers). The combination of AS and PS significantly suppressed the development of *M. phaseolina* lesions by 64.83%, while all the other treatments of AS had no significant effects on the development of the lesions.

Evidently, a synergistic interaction occurred between AS and PS when they were applied in combination. This is because neither of them, when it was applied alone, was able to suppress the lesions development. All CN treatments significantly suppressed the development of lesions, however, no significant differences in the suppressive ability were observed among these treatments. AN was effective in suppressing the development of lesions only when it was applied alone. CS and PS, whether they were applied singly or in combinations, had no significant effects on the development of lesions. Most of the treatments significantly increased plant height and dry weight.

Effect of intercropping on susceptibility of cotton to *M. phaseolina*

Four variables were used to evaluate the effects of three intercropping regimes on susceptibility of cotton to *M. phaseolina*. These variables were the development of lesions on the tap root, the colonization of the root tissues by the fungus, the advancement of lesions on the stem, and the seedcotton yield (Table 8). Onion and Chinese garlic significantly reduced the advancement of lesions on stem by 80.59% and 83.01%, respectively. On the other hand, all the other variables were not significantly affected by any of the intercropping regimes.

Table 7. Effect of different combinations of fertilizers on pathogenicity of *M. phaseolina* on cotton cultivar Giza 75 in outdoor pot experiment.

Treatment	Lesions on tap root[a] (%)	Plant height (cm)	Dry weight (g/plant)
Urea	$3.342^{\text{B-E}}$	78.00^{ABC}	$10.50^{\text{EFG}*}$
Urea+Calcium superphosphate	7.37^{AB}	$75.85^{\text{A-D}*}$	$10.75^{\text{D-G}*}$
Urea+Potassium sulphate	10.268^{A}	$80.30^{\text{AB}*}$	$11.75^{\text{B-E}*}$
Urea+Calcsium superphosphate+Pot. sulphate	$4.885^{\text{A-D}}$	$77.25^{\text{ABC}*}$	$12.85^{\text{B}*}$
Ammonium sulphate	8.260^{AB}	$80.50^{\text{AB}*}$	$11.00^{\text{C-G}*}$
Amm. sulphate+Calcium superphosphate	6.250^{ABC}	$82.35^{\text{A}*}$	$12.17^{\text{BCD}*}$
Amm. sulphate+Potassium sulphate	$1.985^{\text{CDE}*}$	$81.85^{\text{AB}*}$	$11.35^{\text{CDE}*}$
Amm. sulphate +Calcsium superphosphate+Pot. sulphate	$5.117^{\text{B-E}}$	$74.70^{\text{A-E}*}$	$11.85^{\text{B-E}*}$
Calcium nitrate	$0.000^{\text{E}*}$	$73.75^{\text{A-E}*}$	$11.15^{\text{C-F}*}$
Calcium nitrate+Calcium superphosphate	$2.222^{\text{DE}*}$	$71.40^{\text{B-E}*}$	$10.80^{\text{D-G}*}$
Calcium nitrate+Potassium sulphate	$0.000^{\text{E}*}$	$74.18^{\text{A-E}*}$	$12.38^{\text{BC}*}$
Calcium nitrate+Cal. superphosphate+Pot. sulphate	$1.588^{\text{DE}*}$	$78.15^{\text{ABC}*}$	$11.90^{\text{B-E}*}$
Ammonium nitrate	$1.811^{\text{CDE}*}$	$64.20^{\text{EF}*}$	8.50^{HI}
Amm. nitrate+Calcium superphosphate	7.155^{AB}	$65.45^{\text{DEF}*}$	$9.60^{\text{GH}*}$
Amm. nitrate+Potassium sulphate	10.887^{A}	$68.70^{\text{CDE}*}$	$9.75^{\text{FGH}*}$
Amm. nitrate+Cal. superphosphate+Pot. sulphate	9.052^{AB}	$68.15^{\text{CDE}*}$	$12.38^{\text{BC}*}$
Calcium superphosphate	8.838^{AB}	48.85^{G}	6.10^{J}
Potassium sulphate	$3.461^{\text{B-E}}$	56.15^{FG}	8.50^{HI}
Calcium superphosphate+Potassium sulphate	$6.072^{\text{A-D}}$	49.00^{G}	6.85^{J}
Infested control	5.644^{AB}	49.45^{G}	7.25^{IJ}
Noninfsted control	$0.000^{\text{E}*}$	$66.00^{\text{DE}*}$	$14.25^{\text{A}*}$

[a] Percentage data were transformed into VX + 0.5 to produce approximately constant variance. Values in a column followed by the same letter(s) are not significantly different ($P < 0.5$) according to Duncan's multiple range test.
An asterisk (*) denotes a significant difference from the infested control.

Table 8. Effect of intercropping regime on susceptibility of cotton cultivar Giza 75 to *Macrophomina phaseolina* in an outdoor pot experiment.

Intercropping regime	Lesions on tap root (%)	Root tissues colonized by *M. phaseolina*	Lesions on stem (%)	Yield (g/plant)
Onion (Giza 6)	9.398^{aA}	96^{bA}	1.203^{aB}	0.880^{B}
Garlic (Chinese)	9.580^{A}	92^{AB}	1.053^{B}	0.768^{B}
Garlic (Balady)	12.214^{A}	68^{B}	3.308^{A}	0.602^{B}
Infested control	9.932^{A}	88^{AB}	6.197^{A}	0.698^{B}
Noninfested control	0.000^{B}	8^{C}	0.000^{B}	1.766^{A}

[a] Percentage data were transformed into $\sqrt{X} + 0.05$ before carrying out the analysis of variance.

[b] Percentage data were transformed into arc sine angles before carrying out the analysis of variance to produce approximately constant variance. Values in a column followed by the same letter(s) are not significantly different ($P < 0.05$) according to Duncan's multiple range test. Each value is the percentage of root segments (0.5 cm long) that yielded *M. phaseolina*.

DISCUSSION

In this study, it was evident that raising the temperature within certain limit led to an increase in the pathogenicity of *M. phaseolina*. On the other hand, the fungus was not pathogenic at the coolest temperature regime. Although, it was not possible to identify the exact optimum temperature for pathogenicity, it can be concluded that it was somewhere between 24.5±1.5°C to 38.0±2.0°C. These results are in concert with numerous reports, which indicate that *M. phaseolina* is a high temperature pathogen (Dhingra and Sinclair, 1978).

Conflicting views have been suggested regarding the effect of soil moisture on susceptibility to *M. phaseolina*. On the one hand, some workers found that susceptibility to *M. phaseolina* was favored by high soil moisture content (Radha, 1960; Philip *et al.*, 1969; Zazzeriri *et al.*, 1985; Bruton *et al.*, 1987; Walker, 1994), but on the other hand, other workers reported that susceptibility to *M. phaseolina* was favored by low soil moisture content or even drought conditions (Hodges, 1962; Ghaffar and Erwin, 1969; Sheikh and Ghaffar, 1979; Blanco-Lόpez and Jimenez-Diaz, 1983; Taya *et al.*, 1988). The results of this investigation were inconclusive in determining the relationship between soil moisture content and susceptibility of cotton to *M. phaseolina* . the discrepancy in the results may be attributed to the fact that the effect of soil moisture was obscured by other environmental factors interacting with soil moisture such as soil temperature.

Effect of soil texture on susceptibility of cotton to *M. phaseolina* was studied in three clay soils and three sandy clay soils artificially infested with a pathogenic isolate of *M. phaseolina*. In general, the mean of the dead plants in the sandy clay soil was 40.96%, while that of the clay soil was 54.42%. Isolation frequency of *M. phaseolina*, which was 80.00% in the sandy clay soils increased to 96.67% in the clay soil. Taken together, these results suggest that cotton is more susceptible to *M. phaseolina* in clay soil than in sandy clay soil. The conduciveness of clay soil to susceptibility of cotton to *M. phaseolina* could attributed to the fact that clay soil yields greater number of *M. phaseolina* sclerotia than sand (Wyllie and Mekelvey, 1983). However, it is worthy of mention that the results of the present study do not agree with the previous investigations, which reported that *M. phaseolina* disease on crop plants were more severe on sandy soils than on heavy soils (Dhingra and Sinclair, 1978; Taya *et al.*, 1988).

Fertilizers can influence plant disease by altering plant resistance, by directly affecting the pathogens, or by affected the soil microbiota, which may in turn influence the pathogen-host interaction (Huber and Watson, 1974). From pathological point of view, a good fertilizer should meet two requirements. First, it should reduce the development of the disease. Second, it should be nutritiously balanced. In this investigation, 19 different fertilizer treatments were evaluated as to their effect on susceptibility of cotton to *M. phaseolina*. Of these treatments, six were effective on reducing the development of *M. phaseolina* lesion on tap root. However, of the six treatments, the combination of CN, PS, and CS was the only combination which met the two requirements. First, this combination reduced the development of *M. phaseolina* lesions on tap root by 71 .86%. Second, this combination was nutritiously balanced because it provided cotton with NPK. Although other treatments were as effective as this combination in reducing the disease, they were nutritiously unbalanced. For example, the combination of As and PS lacked phosphorus. Another example was CN, which lacked potassium and phosphorus. The combination of CN and CS was effective in reducing the disease; however it lacked potassium. Therefore, it seems reasonable to conclude that the combination of CN, PS, and CS should be used for the fertilization of cotton in *M. phaseolina* -infested soil.

Members of the Lilaceae produce antimcirobial compounds. Garlic oil inhibited growth and sclerotium production in *R. solani* (Sing and Sing, 1930) and growth and spore production in 10 other fungi (Murthy and Amonkar, 1974). Agrawal (1978) reported the *in vitro* inhibitory effect of onion root and bulb extracts on the growth of several rhizosphere fungi. Parkinson and Clarke (1964) showed that the microflora levels in rhizosphere of onion and garlic were significantly lower than those of other plants. Onion bulb extract or root exudates inhibited both sclerotial germination and mycelial growth of *Sclerotium rolfsii* (Zeidan *et al.*, 1986). In the present work, intercropping onion (Giza 6) and Chinese garlic with cotton significantly suppressed the advancement of *M. phaseolina* lesions on cotton stem. These results indicate that intercropping onion (Giza 6) or Chinese garlic with cotton in *M. phaseolina* -infested fields may be useful and inexpensive means of reducing susceptibility of cotton to *M. phaseolina.*

REFERENCES

Agrawal, P. 1978. Effect of root and bulb extracts of *Allium* spp. on fungal growth. Trans. Br. Mycol. Soc., 70: 439-441.

Aly, A.A., E.M. Hussein, M.A. Mostafa and A.I. Ismail. 1996. Distribution, identification and pathogenicity of *Fusarium* spp. Isolated from some Egyptian cottons. Menofiya J. Agric. Res. 21: 819-836.

Blanco-Lopez, M.A. and R.M. Jiménez-Diaz. 1983. Effect of irrigation on susceptibility of sunflower to *Macrophomina phaseolina.* Plant Disease 67: 1214-1217.

Bruton, B.D. and R. Reuveni. 1985. Vertical distribution of microsclerotia of *Macrophomina phaseolina* under various soil types and host crops. Agric. ecosystems Environ. 12: 165-169.

Bruton, B.D., M.J. Jeger, and R. Reuveni. 1987. *Macrophomina phaseolina* infection and vine decline in cantaloupe in relation to planting date, soil environment, and plant maturation. Plant Disease, 71: 259-263.

Chun, D. and J.J. Lockwood. 1985. Reduction of *Pythium ultimum, Thielaviopsis basicola,* and *Macrophomina phaseolina* in soil associated with ammonia generated from urea. Plant Disease, 69:154-158.

Cloud, G.E. and J.C. Rupe. 1994. Influence of nitrogen, plant growth stage, and environment on charcoal rot of grain sorghum caused by *Macrophomina phaseolina* (Tassi) Goid. Plant and Soil 158: 203-210.

Dhingra, O.D. and J.B. Sinclair. 1978. "Biology and pathology of *Macrophomina phaseolina*". Imprensia Universidade Federal de Viscosa, Brazil. 166 p.

Diourte, M., J.L. Starr, M.J. Jeger, J.P. Stack, and D.T. Rosenow. 1995. Charcoal rot *(Macrophomina phaseolina)* resistance and the effects of water stress on disease development in sorghum. Plant Pathology 44:196-202.

Filonow, A.B., D. Chun, and J.E. Lockwood. 1981. Enhanced loss of endogenous nutrients from fungal propagules treated with ammonia. Phytopathology 71: 1116 (Abstr.).

Ghaffar, A. and D.C. Erwin. 1969. Effect of soil water stress on root rot of cotton caused by *Macrophomina phaseoli.* Phytopathology 59: 795-797.

Ghaffar, A., M.A.B. Mallik, and T.H. Khan. 1971. Some factors affecting infection of cotton seedlings by *Macrophomina phaseoli.* Pakistan Journal of Botany 3: 83-87.

Hodges, C.S. 1962. Black root rot of pine seedlings. Phytopathology 52: 210-219.

Holliday, P. and E. Punithalingam. 1970. *Macrophomina phaseolina.* CMI Description of Pathogenic Fungi and Bacteria, no. 275. Commonwealth Agricultural Bureaux, Farnham Royal, Bucks, UK.

Huber, D.M. and R.D. Watson. 1974. Nitrogen form and plant disease. Annu. Rev. Phytopathol. 12:139-165.

Khune, N.N., K.D. Thakur, R.N. Shrawankar and J.D. Chrade. 1993. Influence of nitrogen fertilization on incidence of charcoal rot of sorghum. PKV Research Journal 17: 81-83.

Lodha, S. and S. Mahander. 1985. Quantitative determination of *Macrophomina phaseolina* (Tassi) Goid. in grass-legume intercropping systems. Annals of Arid Zone 23: 259-261.

Mohamed, H.A. 1962. Effect of date of planting on fungi and other microorganisms isolated from cotton seedlings. Plant Dis. Reptr. 46: 801-803.

Mostafa, M.A. 1959. Review of fungal diseases of cotton in Egypt. Egypt Rev. Sci. 3:1-55.

Mostafa, M.A., M.S. Naim, and S.K. Moawad. 1957. Studies in the interaction between *Fusarium oxysporum* (Schlecht.) and *Macrophomina phaseoli* (Maubl.) in parasitizing :Karnak" and "Giza 30" cotton varieties and in culture. I. Comparative cultural studies of *Fusarium* and *Macrophomina.* The third Arab Sci. Cong. 57-67.

Mostafa, M.M.M. 1982. Studies on some rots of soybean plants. Ph.D. Thesis, Al-Azhar Univ., Cairo, 196p.

Murthy, N.B.K., and S.V. Amonkar. 1974. Effect of natural insecticide from garlic *(Allium sativum)* and its synthetic form (diallyldisulphide) on plant pathogenic fungi. Indian J. Exp. Biol. 12: 208-209.

Pande, S., L.K. Mughogho, and R.I. Karunakar. 1990. Effect of moisture stress, plant population density, and pathogen inoculation on charcoal stalk rot of sorghum. Annals of Applied Biology 116: 221-232.

Pande, S., L.K. Mughogho, N. Seettharama, and R.I. Karunkar. 1989. Effects of nitrogen, plant density, moisture stress, and artificial inoculation with *Macrophomina phaseolina* on charcoal rot incidence in grain sorghum. Journal of Phytopathology 126: 343-352.

Parkinson, D. and J.H. Clarke. 1964. Studies on the fungi in the root region. III. Root surface fungi of three species of *Allium* Plant Soil,20:166-174.

Phillip, C.T., K.K. kartha, R.K. Joshi, and K.G. Nema. 1969. A *Rhizoctonia* diseases of "Mung" *(Phaseolus aureus* Roxb.) in Madhya Pradesh. JNKVV Res. J. 3: 40-43.

Prasad, Y. 1983. Effect of different doses of potash on the incidence of root and stem rot of jute. Journal of Research, Assam Agricultural Univ. 4: 163-164.

Radha, K. 1960. The genus *Rhizoctonia* in relation to soil moisture. II. *Rhizoctonia bataticola* cotton root rot organism. Indian Coconut J. 13: 137-144.

Rajpurghit. T.S. 1983. Control of root rot of cotton caused by *Rhizoctonia bataticola* (Taub) Butler. Madras Agricultural Journal 70: 751-754.

Rao, G.K., R.B. Raj, S. Mohammad, and H. Begum. 1991. Influence of nitrogen fertilization on the incidence of charcoal rot in different maize cultivars. Indian Phytopathology 44:118-119.

Sabet, K.A. and L.D. Khan. 1969. Competitive saprophytic ability and inoculum potential of cotton root-infecting fungi in five soils. Cott. Grow. Rev. 46: 119-113.

Salem, M.F.M. 1997. Suppression of *Macro phomina phaseolina*. The causal organism of charcoal rot of soybean. M.Sc. Thesis, Minufiya Univ., Shibin El-Kom, I7Ip.

Sheikh, A.H. and A. Ghaffar. 1979. Relation of sclerotial inoculum density and soil moisture to infection of field crops by *Macrophomina phaseolina*. Pakistan Journal of Botany 11: 185-189.

Sing, H. and U.P. Sing. 1980. Inhibition of growth and sclerotium formation in *Rhizoctonia solani* by garlic oil. Mycologia 72:1022-1025.

Singh, D., K.G. Nema, and S.C. Vyas. 1989. Toxicity of some of the crop residues to soilborne pathogens of chickpea *in vitro.* International Chickpea Newsletter 21:19-20.

Singh, S.K., Y.E. Nene and M.V. Reddy. 1990. Influence of cropping systems on *Macrophomina phaseolina* populations in soil. Plant Disease 74: 812-814.

Taya, R.S., N.N. Tripathi, and M.S. Panwar. 1988. Influence of soil type, soil moisture, and fertilizers on the severity of chickpea dry root-rot caused by *Rhizoctonia bataticola* (Taub.) Butler. Indian Journal of Mycology and Plant Pathology 18: 133-1 36.

Thakar, N.A. 1984. Influence of moisture on root-rot of cotton. Madras Agricultural Journal 71: 629-630.

Walker, G.E. 1994. First report of *Macrophomina phaseolina* associated with vine decline of muskmelon in South Australia. Plant Disease 78: 640.

Watkins, G.M. ed. 1981. Compendium of cotton diseases. The American Phytopathological Society. St. Paul, Minnesota. 87 p.

Wyllie, T.D. and S.B. Mekelvey. 1983. Factors affecting populations of *Macrophomina phaseolina* in Missouri soils. Phytopathology 73: 814 (Abstr.).

Zazzenini, A., M. Monotti, R. Buonaurio,and V. Pirani. 1985 Effects of some environmental and agronomic factors of charcoal rot of sunflower. Helia 8: 45-49.

Zeidan, 0., Y. Elad, Y. Hadar, and I. Chet. 1986. Integrating onion in crop rotation to control *Sclerotium rolfsii.* Plant Disease 70: 426-428.

تأثير بعض العوامل البيئية على حدوث مرض العفن الفحمى فى القطن الناجم عن الإصابة بفطر ماكروفومينا فاسيولينا

على عبد الهادى على[1] ، رشاد محمد يوسف[2] ، أحمد محمد محجوب الشاذلى[1] و معوض رجب عمر[1]
١ – معهد بحوث امراض النباتات – مركز البحوث الزراعية – الجيزة – مصر
٢ – قسم النبات الزراعى – كلية الزراعة – جامعة الأزهر – القاهرة – مصر

اجريت دراسة تحت ظروف الصوبة لتقييم تأثيرات كل من الحرارة ورطوبة التربة والتسميد والتحميل على قابلية صنف القطن المصرى جيزة ٧٥ للإصابة بفطر ماكروفومينا فاسيولينا. اظهرت النتائج المتحصل عليها أن درجة الحرارة المثلى لحدوث الإصابة تقع ضمن نطاق حرارى يتراوح ما بين ٢٤,٥ ± ١,٥ °م إلى ٣٨ ± ٢,٠ °م. لم تتوصل الدراسة إلى نتائج قاطعة بخصوص تأثير رطوبة التربة على حدوث الإصابة ، والتضارب فى النتائج المتحصل عليها قد يرجع إلى ان العوامل البيئية الأخرى مثل حرارة التربة تتفاعل مع الرطوبة بطريقة تؤدى إلى حجب تأثيرها . تدل نتائج الدراسة على أن التربة الطينية أكثر ملاءمة لإصابة القطن بفطر ماكروفومينا فاسيولينا من التربة الطينية الرملية. قيمت ١٩ معاملة سمادية من حيث تأثيرها على قابلية القطن للإصابة بالفطر ، ٦ من هذه المعاملات أدت إلى نقص معنوى فى طول القرح المتكونة على الجذر الوتدى للنبات. من بين هذه المعاملات السمادية الست ، كان الخليط المتكون من نترات الكالسيوم وكربونات البوتاسيوم وسوبرفوسفات الكالسيوم هو المعاملة السمادية الأفضل ، هذه الأفضلية تعزى الى سببين هما: (١) أدت هذه المعاملة إلى نقص معنوى فى طول القرح المتكونة على الجذر الوتدى للنبات بحوالى ٧١,٨٦%. (٢) هذه المعاملة هى الوحيدة المتوازنة غذائيا نظرا لاحتوائها على كل من النيتروجين والفسفور والبوتاسيوم . وبالرغم من أن العديد من المعاملات الأخرى لم تختلف معنويا عن هذه المعاملة من حيث القدرة على الحد من نمو القرح على الجذر الوتدى الا ان هذه المعاملات كانت غير متوازنة غذائيا. تحميل البصل (صنف جيزة ٦) والثوم الصينى مع القطن أحدث نقصا معنويا فى قدرة الفطر على استعمار أنسجة الساق ، مما أدى إلى نقص فى طول قرح الفطر المتكونة على الساق بحوالى ٨٠,٥٩% فى حالة البصل و ٨٣,١% فى حالة الثوم.