

Effect of Physical and Chemical Edaphic Factors on Susceptibility of Cotton Seedlings to *Macrophomina phaseolina*

Aly A. Aly¹ • Mohamed A. Abdel-Sattar² • Kamel A. Abd-Elsalam^{1,3*} • Moawad R. Omar¹

¹ Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt

² Department of Agricultural Botany Faculty of Agriculture, Suez Canal University, Ismailia, Egypt

³ King Saud University, Faculty of Science, Botany and Microbiology Department, P.O. Box: 2455, Riyadh 1145, Saudi Arabia

Corresponding author: * abdel salamka@gmail.com

ABSTRACT

Nineteen soil samples were obtained from different cotton-producing areas in Egypt. The geographic distribution of these samples was as follows: 1 (5.26%) from Minufiya; 4 (21.05%) from Kafr El-Sheikh; 2 (10.53%) from Minya; 3 (15.79%) from Gharbiya; 7 (36.84%) from Sharqiya; and 2 (10.53%) from Faiyum. Clay soil (84.21%) was the predominant type in the samples. Twenty-two physical and chemical parameters were measured in the 19 samples. Data for seedling disease variables (postmergence damping-off and dry weight) and edaphic factors were entered into a computerized stepwise multiple regression analysis. Using the predictors supplied by stepwise regression, two models were constructed. These models showed that edaphic factors accounted for 71.03 and 93.13% of the total variation in postmergence damping-off and dry weight, respectively, in models. From stepwise regression analysis, it was concluded that high soil saturation percent, high Ca⁺⁺ content, low SO₄⁼ content, and high Mg⁺⁺ content were associated with high postmergence damping-off. The present study also showed that high saturation percent, low potassium content, low calcium carbonate content, low Mg⁺⁺ content, high coarse sand content, and high phosphorus content were associated with an increase in the dry weight of seedlings.

Keywords: nitrogen, pH, potassium, sand

INTRODUCTION

Macrophomina phaseolina (Tassi) Goid., the causal agent of charcoal rot (ashy stem) on cotton, is a seed- and soil-borne pathogen with a wide distribution and a wide host range (Dhingra and Sinclair 1978).

M. phaseolina is also a plurivorous fungus attacking more than 500 host species (Sinclair 1982). 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). A negative correlation ($r = -0.85$, $p < 0.01$) was found between disease incidence and yield (Turini *et al.* 2000).

M. phaseolina is of a widespread distribution in Egyptian soil and it is easily and frequently isolated from cotton roots particularly during the late period of the growing season (Aly *et al.* 1996). Resistance to *M. phaseolina* is completely lacking in commercial Egyptian cottons (*Gossypium barbadense* L.) (Aly *et al.* 2006).

Some reports indicate that soil conditions may strongly affect pathogenicity of *M. phaseolina* and its survival in soil. Heavier-textured soils require more nutrients for germination of *M. phaseolina* sclerotia than sandy soils (Filonow *et al.* 1981). The sclerotia of *M. phaseolina* were abundant in very acidic and alkaline soils but were fewer at pH 6.1 (Mukherjee *et al.* 1983). Clay soils yielded more *M. phaseolina* sclerotia than loam or sand (Wyllie and Mekelvey 1983). Bruton and Reuveni (1985) found that soil texture ranging from loamy sand to heavy clay, had no apparent effect on the vertical distribution of sclerotia. Incidence of *M. phaseolina* on chickpea was higher on sandy than clay soils (Taya *et al.* 1988). Red argil soil suppressed the incidence of charcoal rot on the soybean cultivars 'Clark' and 'Cutler 71', while green argil soil enhanced the incidence of

the disease on both cultivars, followed by clay, sandy loam, and sandy soils (Salam 1997). The results of Omar (1999) suggested that cotton is more susceptible to *M. phaseolina* in clay than in sandy clay soil. To our knowledge, no attempts have been made to study the effects of edaphic factors on the incidence of charcoal rot on cotton under Egyptian conditions. Therefore, the main objective of this study was to determine the relationships of physical and chemical edaphic factors to incidence of charcoal rot of cotton. Such information could improve soil management practices for predicting and reducing the incidence of charcoal rot.

MATERIALS AND METHODS

Production of *M. phaseolina* inoculum used in soil infestation

Isolate of *M. phaseolina* used in the present study for soil infestation was obtained from the fungal collection of the Cotton Disease Research Section, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt. This isolate was originally isolated from cotton roots. Substrate for growth of isolates was prepared in 500-ml glass bottles, each of which 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 at 28°C.

Soil sampling and assessment of susceptibility of cotton seedlings to *M. phaseolina* in different soils

Soil samples obtained from 19 cotton-producing areas in Egypt varied considerably in their physical and chemical make-up (Table 1). Each bulk sample consisted of five sub-samples arbitrarily collected from the same field. Soil sub-samples were obtained from the upper 10-15 cm of soil with a hand spade. A composite soil

sample from each field was infested with *M. phaseolina* inoculum described above at a rate of 30 g/kg of soil. The infested soil was dispensed in 10-cm-diameter clay pots and these were planted with 20 seeds/pot (cultivar 'Giza 89'). Pots (five/soil sample) were randomly distributed on a greenhouse bench under a temperature regime ranging from 24 ± 2 to $39 \pm 3^\circ\text{C}$. Percentage of *M. phaseolina*-infected seedlings and dry weight (mg/plant) of surviving plants were recorded 45 days after planting.

Soil analysis

Particle size analysis was made by the pipette method according to Piper (1950). Soil paste extract was analyzed according to Jackson (1967) for determining soil salinity, cations, anions, and pH. Nitrogen content was determined according to Markus *et al.* (1985). Calcium carbonate was determined by Collin's calcimeter, and calculated as CaCO_3 percent (Wright 1939). Phosphorus, potassium, and micronutrients were extracted by ammonium bicarbonate-DTPA and determined by inductively coupled plasma (ICP 400) according to Soltanpour (1985).

Statistical analysis

Pots were distributed on a greenhouse bench in a completely randomized block design with five replications. Simple correlation coefficients were calculated to evaluate the degree of association between each of postemergence damping-off and dry weight and the edaphic factors. Stepwise regression technique with the greatest increase in R^2 as the decision criterion was used to describe the relationship between each of postemergence damping-off and dry weight and the edaphic factors. Statistical analyses were performed using SPSS for Windows (Rel. 11.0.1. 2001. SPSS Inc., Chicago, IL).

RESULTS

Nineteen soil samples were obtained from cotton-producing areas in Egypt (Table 1). The geographic distribution of the samples was as follows: 4 (21%) from Middle Delta (Minufiya and Gharbiya); 4 (21%) from North Delta (Kafr El-Sheikh); 7 (37%) from East Delta (Sharqiya) and 4 (21%) from Middle Egypt (El-Faiyum and El-Minya).

Clay soil was the predominant type representing 84.21% of the samples (Table 2). Measurements of *M. phaseolina* pathogenicity parameters and 22 physical and chemical edaphic factors in the 19 soil samples are shown in Tables 3 and 4. Saturation percent was the only factor positively correlated with each of postemergence damping-off and dry weight (Table 5).

Data for pathogenicity variables and edaphic factors were entered into a computerized stepwise multiple regression analysis. Based on the predictors supplied by stepwise regression, two models were constructed (Tables 6, 7). These models showed that the edaphic factors accounted for 71.03 and 93.13% of the total variation in postemergence damping-off and dry weight, respectively. Simple correlation (Table 5) and stepwise regression models (Tables 6, 7) showed that saturation percent was the most important edaphic factor contributing to the incidence of postemergence damping-off and dry weight. It accounted for 23.99% of the total variation in postemergence damping-off and 31.26% of the total variation in dry weight. Mg^{++} (meq/100 g soil) was included in the two regression models, where it accounted for 5.73% of the total variation in postemergence damping-off and 18.14% of the variation in dry weight.

DISCUSSION

A positive regression coefficient (slope) in a regression model indicates that the particular factor under consideration would increase intensity of the disease if it is in excess. In this study, slope of saturation percent (X9) and coarse sand (X18) were positive in the regression models of post-emergence damping-off and dry weight, respectively. Taken together, these results implied that soils, which had high

Table 1 Geographic origins of soil samples used in study.

Governorate	Soil sample	
	No	%
Minufiya	1	21.05
Kafr El-Sheikh	4	5.26
El-Minya	2	10.53
Gharbiya	3	15.79
Sharqiya	7	36.84
El-Faiyum	2	10.53
Total	19	100.00

Table 2 Texture of soil samples used in study.

Texture	Soil sample	
	No	%
Clay	16	84.21
Sandy Clay	3	15.79
Total	19	100.00

Table 3 Pathogenicity parameters of *M. phaseolina* (Y1 and Y2) and edaphic factors (X1 and X22) used in study.

Variable	No.
Postemergence damping-off (%)	Y1
Dry weight (mg/plant)	Y2
Nitrogen (ppm)	X1
Phosphorus (ppm)	X2
Potassium (ppm)	X3
Iron (ppm)	X4
Zinc (ppm)	X5
Manganese (ppm)	X6
Copper (ppm)	X7
pH	X8
Saturation percent	X9
Electric conductivity (ds/m)	X10
HCO_3^-	X11
Cl^- (meq/1000g soil)	X12
SO_4^{--} (meq/100 g soil)	X13
Ca^{++} (meq/100 g soil)	X14
Mg^{++} (meq/100 g soil)	X15
Na^{++} (meq/100 g soil)	X16
K^+ (meq/100 g soil)	X17
Coarse sand (%)	X18
Fine sand (%)	X19
Silt (%)	X20
Clay (%)	X21
Calcium carbonate (%)	X22

saturation percent were often excessively wet, which delayed seed germination, reduced root development of seedlings, and may have increased root infection by *M. phaseolina*. On the contrary, coarse-textured soils, which contained a high level of coarse sand remained drier, and may have reduced infection by limiting mycelial growth and formation of microsclerotia by *M. phaseolina* – that is, coarse sand decreased infection; consequently, it increased dry weight when it was in excess. This interpretation is in agreement with Hillocks (1992) that excessive soil moisture often predispose cotton seedlings to seedling disease by reducing their rate of growth. It is also coincides with some early reports, which showed a strong association between wet soil and incidence of charcoal rot. For example, infection of cotton with *M. phaseolina* increased and seed germination decreased with increasing soil moisture (Radha 1960). Philip *et al.* (1969) showed that seedling blight and ashy stem blight of common beans caused by *M. phaseolina* was dependent upon high soil moisture. Growing the susceptible cotton cultivar 'Digvijay' under regimes of 25 or 50% available soil moisture reduced the incidence of *M. phaseolina* without significantly affecting the yield of seed cotton. Yield was reduced at 75% moisture (Thakar 1984). Zizzerini *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.

Table 4 Measurements of pathogenicity parameters and edaphic factors^a in 19 soil samples.

Soil Sample No.	Pathogenicity Parameters													Edaphic factors										
	Y1	Y2	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14	X15	X16	X17	X18	X19	X20	X21	X22
1	33.34	246.4	11.80	4.07	69.30	1.17	0.31	1.86	0.44	7.83	56.7	1.15	3.03	2.91	6.62	4.63	3.72	3.12	1.09	1.86	32.66	22.40	22.40	3.50
2	59.27	216.3	25.60	5.47	741.10	14.82	1.08	13.54	5.12	7.92	79.0	0.89	3.03	2.91	3.93	3.61	3.25	2.51	0.50	1.65	20.60	17.60	57.94	3.30
3	50.51	246.2	24.80	3.23	400.10	51.03	1.86	9.08	8.99	7.93	56.3	0.89	4.04	2.91	2.99	3.61	3.25	2.51	0.57	4.24	40.19	17.11	38.63	1.90
4	26.69	170.4	26.00	7.06	370.50	63.17	4.53	10.50	12.63	9.15	500	1.28	6.06	3.88	3.13	2.58	1.51	8.45	0.53	1.88	30.7	24.00	43.45	2.60
5	40.49	273.6	69.00	5.28	458.60	17.57	2.87	13.08	6.46	8.10	61.7	0.98	2.02	4.85	4.63	4.12	2.74	4.25	0.39	1.64	15.96	21.15	57.53	1.80
6	38.38	196.3	46.20	2.76	370.50	23.07	1.89	5.27	6.22	8.05	57.3	0.89	3.03	2.91	3.33	3.09	2.79	3.12	0.27	1.94	19.80	19.08	55.25	2.60
7	33.46	292.0	35.00	4.67	361.10	18.18	1.89	4.99	4.48	8.37	73.3	1.62	2.02	3.88	10.40	2.06	2.84	10.83	0.57	1.61	16.00	20.98	55.22	3.60
8	65.09	400.3	32.30	2.20	497.60	15.55	2.39	7.30	6.50	8.18	77.7	1.15	3.03	4.85	4.14	3.09	1.81	6.35	0.77	0.92	21.06	20.50	55.24	2.00
9	39.33	177.4	25.50	3.81	634.10	19.12	1.30	7.25	5.14	8.15	66.7	0.81	3.03	2.91	3.17	3.09	1.81	3.89	0.32	2.43	16.98	22.30	57.15	3.50
10	45.36	224.5	21.40	1.78	292.50	17.34	1.63	4.30	5.62	8.29	63.3	1.19	3.03	4.85	4.62	3.09	1.81	7.28	0.32	1.46	9.65	16.78	66.70	4.10
11	54.72	177.3	18.97	2.42	292.50	18.20	0.94	2.55	4.15	8.29	55.7	1.79	3.03	8.73	8.00	7.21	3.57	8.45	0.53	2.09	20.46	24.00	55.71	3.00
12	26.35	222.8	27.20	3.60	331.50	43.98	1.37	2.85	5.60	8.55	53.3	0.70	2.02	2.91	3.47	3.09	1.81	3.12	0.38	1.92	17.93	19.99	60.58	2.60
13	38.65	229.5	13.98	3.17	341.60	19.27	0.88	2.96	4.52	8.51	56.7	0.81	2.02	3.88	3.32	3.09	1.81	3.89	0.43	1.36	21.87	23.42	54.21	2.40
14	38.48	190.2	30.50	0.60	263.60	23.32	1.56	2.44	5.94	8.44	55.0	1.28	3.03	4.85	5.29	3.09	2.79	6.72	0.57	2.16	22.39	18.26	57.58	2.70
15	35.98	255.8	14.40	1.99	370.50	35.55	1.59	4.41	6.82	8.80	71.7	1.40	3.03	4.85	7.05	3.09	1.81	9.38	0.65	14.02	22.35	5.89	46.13	6.70
16	32.78	187.7	23.70	2.95	604.50	14.62	1.96	4.16	5.31	8.29	60.0	1.62	3.03	2.91	10.98	6.18	2.64	7.12	0.98	15.49	26.78	10.46	45.81	4.20
17	14.55	208.0	41.70	1.99	370.50	30.54	1.38	4.43	5.75	8.25	57.7	1.28	3.03	5.82	4.97	3.09	1.81	8.45	0.47	0.80	17.02	20.66	57.72	1.80
18	36.01	198.0	23.00	1.56	341.60	16.73	1.64	6.16	5.43	8.35	66.7	2.17	2.53	8.73	13.65	4.12	7.24	13.12	0.43	0.55	17.48	25.04	60.15	2.10
19	58.00	282.0	48.10	3.81	331.50	38.02	1.60	4.61	5.08	8.10	63.3	2.21	3.03	12.61	8.54	7.21	2.59	13.85	0.53	3.29	17.69	17.35	60.67	3.00

^a Identification of pathogenicity parameters and edaphic factors are shown in Table 3.**Table 5** Correlation between pathogenicity parameters of *M. phaseolina* and edaphic factors.

Edaphic factor	Pathogenicity parameter	
	Post-emergence Damping-off (%)	Dry weight (mg/plant)
Nitrogen (ppm)	0.024	0.250
Phosphorus (ppm)	-0.036	-0.009
Potassium (ppm)	0.276	-0.006
Iron (ppm)	-0.205	-0.151
Zinc (ppm)	-0.114	0.061
Manganese (ppm)	0.280	0.098
Copper (ppm)	-0.077	-0.106
pH	-0.432	-0.231
Saturation percent	0.490*	0.559*
Electric conductivity (ds/m)	0.104	0.017
HCO ₃ ⁻	-0.031	-0.278
Cl ⁻ (meq/100 g soil)	0.302	0.109
SO ₄ ⁼ (meq/100 g soil)	-0.063	-0.008
Ca ⁺⁺ (meq/100 g soil)	0.365	-0.102
Mg ⁺⁺ (meq/100 g soil)	0.103	-0.156
Na ⁺⁺ (meq/100 g soil)	-0.025	0.109
K ⁺ (meq/100 g soil)	0.030	0.269
Coarse sand (%)	-0.107	-0.087
Fine sand (%)	-0.001	-0.069
Silt (%)	-0.050	-0.105
Clay (%)	0.146	0.031
Calcium carbonate (%)	-0.038	-0.070

* Pearson correlation coefficient (r) is significant at P ≤ 0.05 (*).

The cultivar x irrigation treatment was significant. Disease incidence increased with increasing rainfall and irrigation. Walker (1994) mentioned that excessively wet soil by drip irrigation favoured infection of muskmelon by *M. phaseolina*. Since saturation percent was accompanied by less surviving seedlings, it is expected that less competition occurred among these seedlings leading to an increase in dry weight, hence the positive slope of saturation percent in the regression model of dry weight.

Table 7 Identification of predictors included in stepwise regression models in Table 6 and their relative contribution.

Predictor	Number	Relative contribution (%)
Postemergence damping-off (%)		
Saturation percent	X9	23.98992
Ca ⁺⁺ (meq/100 g soil)	X14	19.72808
SO ₄ ⁼ (meq/100 g soil)	X13	21.58219
Mg ⁺⁺ (meq/100 g soil)	X15	5.730319
Dry weight (mg/plant)		
Saturation percent	X9	31.26000
Potassium (ppm)	X3	12.54617
Calcium carbonate (%)	X22	10.08217
Mg ⁺⁺ (meq/100 g soil)	X15	18.14451
Coarse sand (%)	X18	18.21478
Phosphorus (ppm)	X2	2.799428

Soil nutrients play an important role in disease susceptibility of cotton. Nutrients may influence disease susceptibility in a number of ways. They may directly promote or inhibit the disease agents or antagonists of the disease agent. They may also affect the function of tissues within the cotton plant, which resist or encourage pathogens. Even though genetic make-up in large measure controls the resistance to disease, resistance is expressed through complex physiological and biochemical processes that are linked to the nutritional status of the plant or of the pathogen (Hodges 1992). In the present study, of the chemical edaphic factors studied, postemergence damping-off was affected by Ca⁺⁺ (meq/100 g soil), SO₄⁼ (meq/100 g soil) and Mg⁺⁺ (meq/100 g soil), while dry weight of the surviving seedlings was affected by potassium (ppm), calcium carbonate (%), Mg⁺⁺ (meq/100 g soil), and phosphorus (ppm). The results of the present study imply that under Egyptian conditions, certain physical and chemical edaphic factors favour infection of cotton with *M. phaseolina*, and that control of the pathogen may be possible by modifying the nutritional status of the plant.

The improving and controlling soil habitat factors for survival of *V. dahlia* in cotton, it was possible to effectively

Table 6 Stepwise regression models that describe the effects of edaphic factors on pathogenicity of *M. phaseolina*.

Dependent variable (Y)	Stepwise linear regression model ^a	Coefficient of determination (R ²)	F-value ^b
Postemergence damping-off (%)	Y = -43.61022 + 1.12267X9 + 6.273973X14 - 3.12311X13 + 3.111516X15	71.03%	8.58**
Dry weight (mg/plant)	Y = -45.53856 + 9.7767X9 - 0.3990798X3 - 55.73462X22 - 23.34064X15 + 10.90636X18 + 6.538946X2	93.13%	27.10 **

^a Identification of the predictors and their relative contribution to R² are shown in Table (7).^b F-value is significant at P ≤ 0.01 (**).

minimize the numbers of microsclerotia survived in the soil and to prevent disease occurrence and development (Yang *et al.* 2004).

In conclusion, the regression model that described the effects of edaphic factors on pathogenicity of *M. phaseolina* was constructed based on seedling mortality in the post-emergence stage. Seedling mortality at the preemergence stage was excluded from the analysis because the involvement of the indigenous fungi of soil samples in preemergence damping-off may interfere with *M. phaseolina* effects increasing the experimental error associated with the model. On the contrary, the use of seedling mortality at the post-emergence stage minimized the experimental error because *M. phaseolina*-infected seedlings were easily distinguished in this stage by external visible signs in particular microsclerotia, which were present in the tap root and the lower portion of stem.

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