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# VARIATION IN SENSITIVITY TO FUNUGICIDES AMONG ISOLATES OF *Macrophomina phaseolina* FROM COTTON ROOTS

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## ABSTRACT

Twenty isolates of *Macrophomina phaseolina* from cotton roots were evaluated *in vivo* for sensitivity to the seed-dressing fungicides Monceren, Monceren T, Rizolex T, Vitavax 200, and Maxim AP. Monceren and Rizolex T were the best performing fungicides in suppressing *M. phaseolina* under greenhouse conditions. This superiority was attributed to the following reasons: first, they were effective in increasing the percentage of surviving seedlings by 203.09 and 170.10%, respectively, regardless of the tested isolate. Second, they were also effective in increasing the height of surviving seedlings by 93.21 and 62.57%, respectively. Third, Monceren and Rizolex T were effective in increasing the dry weight of surviving seedlings in 50 and 40% of the tested isolates, respectively. A high significant positive correlation was observed between pathogenicity of isolates and their sensitivity to fungicides.

## INTRODUCTION

*Macrophomina phaseolina* (Tassi) Goid., the causal agent of charcoal rot of cotton, is of widespread distribution in the Egyptian soil, and it is easily and frequently isolated form cotton roots particularly during the late period of the growing seasons (Aly *et al.,* 2000).

*M. phaseolina* is a non-specialized fungus, attacking more than 500 host species (Sinclair, 1982); therefore, rotation of cotton with other crops is a questionable practice for controlling this pathogen.

Resistance to *M. phaseolina* has not been found in cotton (Holliday and Punithalingam, 1970). Sources of resistance were considered completely lacking in Pakistan (Akhtar. 1977). Aly *et al.* (1998) reported variation in susceptibility of Egyptian cottons to *M. phaseolina;* however, none of the tested cultivars showed satisfactory level of resistance.

Thus, fungicides have become indispensable for controlling *M. phaseolina* on cotton. For instance, Dwivedi and Ghaube (1985) studied effect of fungicides on the emergence and infection of cotton seedlings by *M. phaseolina* in pot experiments. They found that emergence was maximized after treatment with a TMTD soil drench and the least seedling infection occurred in soils treated with benlate, TMTD, and PCNB (Quintozene).

Chauhan (1986a) reported that control of seedling disease of cotton due to *M. phaseolina* was best and germination maximized when seeds were treated with carbendazim, followed in effectiveness by quintozene.

Chauhan (1986b) evaluated seed treatments of 7 fungicides and selected pair-wise combinations. The percentage of root rot of cotton at harvest ranged from 14 to 22% in treated plots compared with 28% in the control. Benzimidazole-derived fungicides differed in their effects on isolates of *M. phaseolina.* Benlate and carbendazim were most inhibitory but a sesame isolate was less sensitive to all fungicides.

Chauhan (1988) obtained good control of *M. phaseolina* on cotton by seed treatment with carbendazim followed by quintozene, while TMTD and catafol were less effective. A pre-sowing soil drench with quintozene and carbendazim gave very effective control.

Aly *et al.* (2001) evaluated the efficiency of Monceren, Monceren T, Vitavax 200, Rizolex T, and Maxim AP for controlling *M. phaseolina* on cotton, under greenhouse conditions. Monceren, Monceren T, and Rizolex T were equally effective in increasing the percentage of surviving seedlings and the plant height; however, Monceren was the only fungicide, which significantly increased dry weight of seedlings.

The objective of this investigation was to evaluate variation in sensitivity to five seed-dressing fungicides, commonly used for controlling cotton seedling damping-off, among 20 isolates of *M. phaseolina* isolated from cotton roots.

## MATERIALS AND METHODS

### Fungal isolates

Isolates of *M. phaeolina* used in the current study (Table 1) were obtained from the fungal collection of Cotton Disease Research Section, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt. The isolates were originally isolated from cotton roots.

**Table 1. Geographic origins of *M. phaseolina* isolates used in this study.**

| Isolate no. | Geographic origin |
|---|---|
| 1 | Manzala, Daqagliya |
| 2 | Sakha, Kafr El-Sheikh |
| 3 | Minouf,Minufiya |
| 4 | Santa, Gharbiya |
| 5 | Damanhoor, Beheira |
| 6 | El-Riyad, Kafr El-Sheikh |
| 7 | Giza, Giza |
| 8 | Faiyoum |
| 9 | Sohag, Sohag |
| 10 | Manfaloot, Assiute |
| 11 | Manfaloot, Assiute |
| 12 | Abou-Korkas, Minya |
| 13 | El-Minya, Minya |
| 14 | Sohag, Sohag |
| 15 | Sohag, Sohag |
| 16 | Shandaweel, Sohag |
| 17 | Meet Ghamr, Daqahilya |
| 18 | Abou-Kibeer, Sharqiya |
| 19 | Sirs El-Lian, Minufia |
| 20 | Damanhoor, Beheira |

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

Substrate for growth of isolates was prepared in 500-ml glass bottles, each bottle contained 50 g of sorghum grains and 40 ml of tap water. Contents of each bottle were autoclaved for 30 minutes. Isolated inoculum, taken from one-week-old culture on PDA, was aseptically introduced into the bottle and allowed to colonize sorghum for three weeks.

**_In vivo_ interaction between isolates of _M. phaseolina_ and seed-dressing fungicides**

Twenty batches of autoclaved soil were placed on greenhouse benches and individually infested with each *M. phaseolina* isolate (Table 1) at a rate of 40 g/kg soil. After thoroughly mixing, infested soil was dispensed into 15-cm-diameter clay pots. Seeds of cotton cultivar Giza 89 were treated with fungicides (Table 2). Flowable fungicides were added to dry seeds, while powdered ones were added to slightly moist seeds. The seeds were shaken thoroughly in plastic bags for five minutes and allowed to dry before being planted in the infested pots (10 seeds/pot). In the control treatment, no fungicide was added to seeds. The pots were randomly distributed on a greenhouse bench under temperature regime ranged from 22.5±3.5°C to 39±7°C. Preemergence damping-off was recorded 15 days after planting. Postemergence damping-off, survivals, plant height (cm), and dry weight (mg/plant) were recorded 45 days after planting.

**Table 2. Fungicides used in the study and their active ingredients.**

| Fungicide [a] | Rate (per kg seed) | Active ingredient [b] | Formulation |
|---|---|---|---|
| Monceren | 3 g | 25 % Pencycuron | WP |
| Monceren T | 3 g | 15% Pencycuron + 32% Thiram | WP |
| Rizolex T | 3 g | 20% Tolclofos-methyl + 30% Thiram | WP |
| Vitavax 200 | 4 g | 37% Carboxin + 37.5 Thiram | WP |
| Maxim AP | 2 ml | 3.5% [c] (Metalaxyl M + Fludioxanil) | FS |

**[a] Trade name.**
**[b] Common name.**
**[c] Total active ingredient of the two compounds.**
**[d] WP = Wettable powder and FS = Flowable formulation for seed treatment.**

### Statistical analysis of the data

The experimental design of the present study was a randomized complete block with 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 between means of fungicides within *M. phaseolina* isolates. Percentage data were transformed into arc sine angles before carrying out the ANOVA to produce approximately constant variance. Correlation analysis was performed with a computerized program.

## RESULTS AND DISCUSSION

ANOVA (Table 3) showed that isolate was a nonsignificant source of variation (P = 0.10) in preemergence damping-off, while it was a significant source of variation in postemergence damping-off (P = 0.01), survival (P = 0.0000), plant height (P = 0.02), and dry weight (P = 0.0000). Fungicide was a very highly significant source of variation (P = 0.0000) in all the tested parameters except preemergence damping-off. Isolate x fungicide interaction was a significant source of variation only in preemergence damping-off (P = 0.003) and dry weight (0.0000). Isolate x fungicide interaction accounted for

78.38 and 61.08% of the explained (model) variation in preemergence damping-off and dry weight, respectively (Table 4). Fungicide was more important than isolate as a source of variation in postemergence damping-off, survival, and plant height, while isolate was slightly more important than fungicide as a source of variation in dry weight.

**Table 3. Analysis of variance of the effects of isolate of *M. phaseolina*, seed-dressing fungicide and their interaction on cotton seedling disease variables (cultivar Giza 89) under greenhouse conditions.**

| Parameter and source of variation [a] | D.F. | M.S. | F. value | P > F |
|---|---|---|---|---|
| **Preemergence damping-off** | | | | |
| Replication | 4 | 135.580 | 1.670 | 0.1558 |
| Isolate (S) | 19 | 117.708 | 1.449 | 0.0990 |
| Fungicide (F) | 5 | 86.762 | 1.068 | 0.3771 |
| S x F | 95 | 122.574 | 1.509 | 0.0031 |
| Error | 476 | 81.209 | | |
| **Postemergence damping-off** | | | | |
| Replication | 4 | 3197.978 | 16.888 | 0.0000 |
| Isolate (S) | 19 | 365.696 | 1.931 | 0.0106 |
| Fungicide (F) | 5 | 2297.188 | 12.131 | 0.0000 |
| S x F | 95 | 206.372 | 1.090 | 0.2805 |
| Error | 476 | 189.360 | | |
| **Survival** | | | | |
| Replication | 4 | 4942.379 | 19.2649 | 0.0000 |
| Isolate (S) | 19 | 610.877 | 2.3811 | 0.0009 |
| Fungicide (F) | 5 | 3084.311 | 12.0223 | 0.0000 |
| S x F | 95 | 240.607 | 0.9379 | |
| Error | 476 | 256.549 | | |
| **Plant height** | | | | |
| Replication | 4 | 3817.911 | 17.928 | 0.0000 |
| Isolate (S) | 19 | 393.898 | 1.850 | 0.0160 |
| Fungicide (F) | 5 | 19888.751 | 9.339 | 0.0000 |
| S x F | 95 | 184.757 | 0.868 | |
| Error | 476 | 212.960 | | |
| **Dry weight** | | | | |
| Replication | 4 | 174001.936 | 10.0003 | 0.0000 |
| Isolate (S) | 19 | 72035.026 | 4.1400 | 0.0000 |
| Fungicide (F) | 5 | 236960.896 | 13.6187 | 0.0000 |
| S x F | 95 | 53672.566 | 3.0847 | |
| Error | 476 | 17399.650 | | |

**[a] Replication is random, while each of fungicide and *M. phaseolina* isolate is fixed.**

**Table 4. Relative contribution of isolate of *M. phaseolina*, seed-dressing fungicide and their interaction to variation in cotton seedling disease variables (cultivar Giza 89) under greenhouse conditions.**

| Source of variation | Relative contribution[a] to variation in | | | | |
|---|---|---|---|---|---|
| | Preemergnece damping-off | Postemergnece damping-off | Survival | Plant height | Dry weight |
| Isolate (S) | 15.05 | 13.67 | 16.66 | 14.89 | 16.39 |
| Fungicide (F) | 2.92 | 22.60 | 22.14 | 19.79 | 14.19 |
| S x F | 78.38 | 38.57 | 32.82 | 34.93 | 61.08 |

**[a] Calculated as percentage of sum squares of the explained (model) variation.**

Due to the high significance of isolate x fungicide interaction as a source of variation in preemergence damping-off, a LSD was calculated to compare between fungicide means within each isolate (Table 5). These comparisons showed that the differences in preemergence damping-off between fungicides and the control was not the same for each isolate- that is, *M. phaseolina* isolates responded differently to the application of fungicides. For example, Monceren significantly increased preemergence damping-off caused by isolate 6, while it significantly reduced preemergence damping-off caused by isolate 15. On the other hand, Monceren did not show any effect on the other isolates. Pathogenicity of isolate 6 was also increased by Maxim AP;however, this fungicide significantly suppressed pathogenicity of isolate 8.

The nonsignificance of isolate x fungicide interaction as a source of variation in postemergence damping-off indicated that isolate and fungicides under consideration acted independently of each other-that is, fungicidal efficiency was not affected by the tested isolate. Therefore, LSD was calculated to compare between the general means of fungicides (Table 6). These comparisons showed that all the tested fungicides were effective in reducing postemergence damping-off regardless of the tested isolate; however, they showed different levels of efficiency. Thus, Monceren and Monceren T were the best performing fungicides in reducing infection regardless of *M. phaseolina* isolate. They reduced infection by 38.17 and 27.42%, respectively. Rizolex T and Maxim AP were almost equally effective because they reduced infection by 23.89 and 22.94%, respectively. Vitavax 200 was the least effective fungicide because it reduced infection only by 17.38%. Monceren, Monceren T, Rizolex T, Vitavax 200, and Maxim AP were all effective in increasing the percentage of surviving seedlings by 203.09, 145.36, 170.10, 115.46, and 130.93%, respectively (Table 7). The fungicides were also effective in increasing the height of surviving seedlings by 93.21, 60.80, 62.57, 54.96, and 57.27%, respectively (Table 8). The comparisons between fungicides and the control within isolates revealed that the efficiency of the tested fungicides in increasing the dry weight of surviving seedlings varied from one isolate to another. Thus, Monceren, Monceren T, Rizolex T, Vitavax 200, and Maxim AP were effective against 10, 6, 8, 4, and 5 isolates, respectively (Table 8).

The results of the present study demonstrated that Monceren and Rizolex T were the best performing fungicides in controlling *M. phaseolina* on cotton. This superiority was attributed to the following reasons: first, they were effective in increasing the percentage of surviving seedlings by 203.09 and 170.10%, respectively, regardless of the tested isolate. Second, they were also effective in increasing the height of surviving seedlings by 93.21 and 62.57%, respectively. Third, Monceren and Rizolex T were effective in increasing the dry weight of surviving seedlings in 50 and 40% of the tested isolates, respectively. In general, the results of the present study agree with those of some other workers. For example, El-Barougy (1990) found that Rizolex inhibited the linear growth of *M. phaseolina*. In seed-dressing trails, Anaso (1995) found that Vitavax 200 and Metalaxyl were ineffective against charcoal rot of sorghum. Aly *et al.* (2001) reported that Monceren and Rizolex T were effective against *M. phaseolina* on cotton.

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A highly significant positive correlation was observed between pathogenicity of isolates and their *in vivo* sensitivity to fungicides (Table 9). This correlation indicates that the highly pathogenic isolates had the highest sensitivity to fungicides, while the weakly pathogenic ones had the lowest sensitivity.

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**التباين فى الحساسية للمبيدات الفطرية بين عزلات فطر ماكروفومينا فاسيولينا المعزولة من جذور القطن**

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أختبرت عشرون عزلة من فطر ماكروفومينا فاسيولينا – المعزولة من جذور القطن – تحت ظروف الصوبة لتقييم التباين فى حساسيتها للمبيدات الفطرية الكاسية للبذرة مونسرين ومونسرين تى وريزولكس تى وفيتافاكس 200 وماكسيم إيه بى. كان المبيدان مونسرين وريزولكس تى هما الأكثر فاعلية فى مقاومة الإصابة بالفطر، ويعزى تفوق هذين المبيدين إلى الأسباب التالية: أولاً ، أظهرا فاعلية فى زيادة النسبة المئوية للبادرات الباقية على قيد الحياة بمقدار 203.9 و 170.10% ، على الترتيب ، وذلك بغض النظر عن العزلة المختبرة. ثانياً ، أظهرا فاعلية فى زيادة أطوال البادرات الباقية على قيد الحياة بمقدار 93.21 و 62.57% ، على الترتيب. ثالثاً ، أظهرا فاعلية فى زيادة الوزن الجاف للبادرات الباقية على قيد الحياة فى 50 و 40% من العزلات المختبرة ، على الترتيب. لوحظ إرتباط موجب عالى المعنوية بين القدرة المرضية للعزلات وحسساسيتها للمبيدات.