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EFFECTS OF FERTILIZERS ON GROWTH AND SCLEROTIA FORMATION OF *MACROPHOMINA PHASEOLINA* (TASSI) GOID.

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Abstract

Macrophomina phaseolina was exposed to various concentrations of N, P and K fertilizers in PDA medium. Most of the fertilizers did not affect and some slightly increased the mycelial growth. All the fertilizers significantly affected sclerotia of the fungus. Urea and CAN at 10 ppm N reduced sclerotia by 85 to 90%. Similar reduction was noted with ammonium sulfate and NPK-(20 : 20 : 20) at or above 20 ppm N. DAP decreased sclerotia count whereas SSP increased it up to 300%. Under the influence of potassium fertilizers sclerotia count was increased by 200 to 400%, maximum with potassium nitrate followed by MOP and SOP.

Macrophomina phaseolina is a destructive soil borne plant pathogen which causes leaf blight, dry rot, damping off, wilt and charcoal rot in economically important crop plants. The fungus can multiply in and out of the host through mycelium and/or asexual spores. Severity of a disease caused by *M. phaseolina* is thought to be directly related to the viable sclerotia in soils (Kendig *et al*. 2000). Fertilizers reduced inoculum level in soil and controlled the infection of *M. phaseolina* possibly by favoring microbial competition against the pathogen (Irshad *et al*. 2006). However, the direct influence of fertilizers on *M. phaseolina* is not thoroughly investigated. In this study various nitrogen (N), phosphorus (P) and potassium (K) fertilizers were evaluated for their impact on mycelium growth and sclerotia of *M. phaseolina*.

Pure culture of *M. phaseolina* (Tassi) Goid. was obtained from First Fungal Culture Bank of Pakistan, Faculty of Agricultural Sciences, University of the Punjab, Lahore. The study was comprised of three experiments conducted according to two-factors completely randomized design (CRD) with each treatment replicated thrice. In each experiment, the factors were the source and rate of a single mineral nutrient out of N, P or K. Synthetic fertilizers used were urea, calcium ammonium nitrate (CAN), ammonium sulfate (AS) and NPK-(20 : 20 : 20) (NPK) for nitrogen, diammonium phosphate (DAP), single super phosphate (SSP) and NPK for phosphorus, and sulfate of potash (SOP), muriate of potash (MOP) and potassium nitrate for potassium. Through these fertilizers, N at 10, 20, 40, and 80 ppm, P at 5, 10, 20 and 40 ppm and K at 50, 100, 200 and 400 ppm were mixed to PDA medium by using food poison technique (Ogawa *et al*. 2016). PDA medium without any fertilizer was considered as control. The 3 mm agar discs of *M. phaseolina* were implanted into the wells in the PDA plates and incubated at 28 ± 2°C for 7 days in dark. At 4th day of incubation, radial mycelial colonial growth was measured. At 7th day, number of

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sclerotia per 3 mm agar disc was estimated by using haemocytometer. The data of all the three experiments were subjected to analysis of variance (ANOVA) and means were compared through Tukey's HSD test at $p \leq 0.05$ by using Statistix 8.1 computer software.

Colony size of *M. phaseolina* did not respond to most of the N, P and K fertilizers. No fertilizer negatively affected colony size, instead, CAN and SOP significantly increased it at every nutrient concentration. Urea at and above 20 ppm N and DAP at and above 20 ppm P also increased colony size of *M. phaseolina* (Tables 1-3).

Table 1. Effects of nitrogen fertilizers on colony growth and sclerotia formation of *Macrophomina phaseolina.*

Name of fertilizer	Concentration	Colony diameter (cm)	Number of sclerotia
	0 (control)	6.2 c	63.0 a
Urea	10	6.2 c	2.0 e
	20	7.7 a	2.3 e
	40	7.3 b	3.7 e
	80	7.3 b	2.3 e
Calcium ammonium nitrate	10	7.2 b	2.3 e
	20	7.2 b	4.0 e
	40	7.3 b	3.0 e
	80	7.3 b	4.0 e
Ammonium sulfate	10	6.5 c	45.3 b
	20	6.5 c	14.0 de
	40	6.5 c	11.6 de
	80	6.3 c	7.0 e
NPK-20 : 20 : 20	10	6.3 c	37.6 bc
	20	6.3 c	25.0 cd
	40	6.3 c	13.0 de
	80	6.3 c	6.3 e
LSD ($p \leq 0.05$)		0.37	16.02

Means sharing common letter(s) in a column do not differ significantly at $p < 0.05$.

Sclerotia of the fungus were negatively affected with applied nitrogen (Table 1). Irrespective to N concentration applied, CAN and urea significantly reduced number of sclerotia up to 90%. Sclerotia count in AS and NPK-(20 : 20 : 20) treated media was found to gradually decrease (85%) with increasing rate of N (Table 1). Irrespective to P concentration applied, DAP did not affect and SSP increased sclerotia count to 300% (Table 2).

Sclerotia count was increased with the application of potassium fertilizers. In case of SOP, increase (200%) in sclerotia count was noted at 200 and 400 ppm K concentrations. MOP increased sclerotia at and above 100 ppm K concentration. Among all tested K fertilizers, potassium nitrate conferred maximum favor to sclerotia and resulted in 380% increase in the count even at 50 ppm K concentration (Table 3).

Negative response of *M. phaseolina* sclerotia count to applied nitrogenous fertilizers seems to be due to decrease in C : N ratio of the PDA medium to a less favorable range. Compared to AS and NPK, urea and CAN retarded sclerotia formation at lower nitrogen concentrations. This might

be due to comparatively better absorption of nitrogen as urea and CAN, attributed to organic nature of the former and presence of calcium in the latter.

Table 2. Effects of phosphorus fertilizers on colony growth and sclerotia formation of *Macrophomina phaseolina*.

Name of fertilizer	Concentration	Colony diameter (cm)	Number of sclerotia
	0 (Control)	6.2 cd	10.7 cde
Diammonium phosphate	5	5.7 e	3.3 de
	10	5.9 de	3.7 de
	20	6.7 b	3.0 e
	40	7.4 a	5.3 cde
Single super phosphate	5	6.3 c	43.0 a
	10	6.3 c	39.0 a
	20	6.3 c	38.0 a
	40	6.3 c	38.7 a
NPK-20 : 20 : 20	5	6.3 c	37.7 a
	10	6.3 c	13.0 c
	20	6.3 c	25.0 b
	40	6.3 c	6.3 cde
LSD ($p < 0.05$)		0.33	8.99

Means sharing common letter(s) in a column do not differ significantly at $p < 0.05$.

Table 3. Effects of potassium fertilizers on colony growth and sclerotia formation of *Macrophomina phaseolina*.

Name of fertilizer	Concentration	Colony diameter (cm)	Number of sclerotia
	0 (Control)	6.3 b	55.67 e
Sulfate of potash	50	7.3 a	59.0 e
	100	7.4 a	66.6 e
	200	7.7 a	151.3 d
	400	7.2 a	173.7 d
Muriate of potash	50	6.4 b	83.3 e
	100	6.3 b	312.3 abc
	200	6.4 b	331.0 ab
	400	6.4 b	368.6 a
Potassium nitrate	50	6.5 b	266.6 c
	100	6.4 b	294.3 bc
	200	6.4 b	352.0 ab
	400	6.2 b	355.0 ab
LSD ($p < 0.05$)		0.62	64.0

Means sharing common letter(s) in a column do not differ significantly at $p < 0.05$.

Depressive effect of DAP on sclerotia seems to be owing to that 18% N compounded with P in the fertilizer. Otherwise, P seems to have promontory effect on sclerotia as it did in case of SSP where no N was accompanied with P in the fertilizer. This is supported by the response of fungi to N fertilizers which clearly improved mycelial growth and suppressed sclerotia (Table 1).

Positive impact of K on *M. phaseolina* is supported from literature where K application significantly increased charcoal rot of sugar beet caused by the fungus (Stojsin *et al.* 1999). The fungus produced fewer sclerotia with SOP than that of other K sources. This might be related to the addition of sulfate ion along with potassium in SOP treated PDA.

A comparison of various fertilizers revealed that urea and sulfate of potash are the best N and K sources to comparatively suppress or give less benefit to the fungus, respectively. Compound phosphate fertilizers which also supply nitrogen along with do not increase sclerotia count contrary to P - only fertilizers. The effective fertilizers need to be further investigated in soils against *M. phaseolina*.

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