EFFECT OF MICROBIAL ANTGONISTS AND CHEMICAL FERTILIZERS IN THE CONTROL OF *MACROPHOMINA PHASEOLINA* (TASSI) GOID. ON SUNFLOWER

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Abstract

An experiment was carried out in the green house of Botany Department to investigate the effect of microbial antagonists and chemical fertilizers for the control of *Macrophomina phaseolina on* sunflower (*Helianthus annuus* L.). Plant length was significantly ($p<0.01$) increased in all treatments where soil was amended with chemical fertilizers and seeds were coated with microbial antagonists. Larger plant length and weight was obtained when soil was treated with Ammonium nitrate alone and Potassium sulphate in combination with *Trichoderma viride* or *reesei* followed by DAP in combination with *Rhizobium meliloti.*

The colonization of *M. phaseolina* was completely inhibited when soil was treated with urea, DAP, potassium sulphate, ammonium nitrate and urea in combination with *T. viride* and ammonium nitrate in combination with *R. meliloti*. Significant ($p<0.001$) reduction in colonization of *M. phaseolina* was observed when soil was amended with urea and DAP and seeds were treated with *T. reesei* followed by urea in combination with *R. meliloti* and DAP with *R. meliloti.*

Introduction

Macrophomina phaseolina (Tassi) Goid., is an important root pathogen and causes dry root rot,stem canker, stalk rot or charcoal rot of over 500 plant species including sunflower (Sinclair, 1982, Shahzad *et al*., 1988, Ghaffar, 1992). Various disease management methods have been implemented to combat and eradicate pathogenic fungi. These include cultural, regulatory, physical, chemical and biological methods.

All these methods are effective only when employed well in advance as precautionary measure (Sharma, 1996, Katan, 2000). Once a disease has appeared these methods become impractical / ineffective. Due to increase in cost of chemical pesticides and environmental hazards involved with their application, emphasis is now placed upon the biological control agents against plant pathogens (Agrios, 2004). The objective of the present study was to investigate the effect of seed coating with biocontrol agents and chemical fertilizers in the control of sunflower root rot disease caused by *M. phaseolina*.

Materials and Methods

Soil was collected from experimental plots of Department of Botany, University of Karachi. The soil was sandy loam (Sand, Silt, Clay, 70, 19, 11), pH ranged from 7.5 – 8.1 with moisture holding capacity (MHC) of 40% (Keen & Raczkowski, 1921), total nitrogen 0.077 – 0.099% (Mackenzie & Wallace, 1954), total organic matter 4.17 – 4.59%. The soil had a natural infestation of sclerotia of Macrophomina phaseolina (4 – 6 sclerotia / g soil).Sheikh & Ghaffar 1975 Two *Trichoderma* species; *T. viride* (KUMCC-65) and T. *reesei* (KUMCC-28) were obtained from Karachi University Mycological Culture Collection (KUMCC) and *Rhizobium meliloti* isolated from roots of *Melilotus* sp. were maintained on Potato Dextrose Agar (PDA). Chemical fertilizers viz., urea, DAP, potassium sulphate and ammonium nitrate obtained from local market Soil was amended with chemical fertilizers viz., urea, DAP, potassium sulphate and ammonium nitrate @ 0.5% w/w. After 7 days of soil amendment seeds of sunflower were sown in each pot. Soil without organic substrate and non-treated seeds served as control.

In another set seeds of sunflower were coated with 5 days old culture of *T. reesei, T. viride* and *R. meliloti* using 2% gum arabic as a sticker. Five seeds/pot were sown in 8 cm diameter, plastic pots, each containing 300 g soil There were three replicates of each treatment and pots were kept randomized on screen house bench. After 30 days of growth, plants were uprooted and growth parameters were estimated in term of shoot and root length, shoot and root weight The vigour index (VI) was calculated by multiplying germination percentage with the means plant length and divided by 100:

$$VI = \frac{Plant\ length\ \times Germination\ \%}{100}$$

Roots of sunflower were washed in running tap water, surface sterilized in 1% Ca(OCl)2 and then five 1 cm long root pieces were transferred on PDA plates containing penicillin @ 100,000/ litre and streptomycin @ 200mg/l. Petri plates were incubated for 5 days, at room temperature to confirm infection in roots by root-rot fungi *M. phaseolina*. Data were analyzed for one way analysis of variance (ANOVA by SPSS Ver. 10) The treatment means were compared as Least significance difference (LSD) (Sokal & Rohlf, 1995).

Results and Discussion

Sunflower seeds germination significantly ($p<0.01$) varied between the treatments (Table 1). Reduced germination was observed in urea treated seeds alone and in combination with fungal biocontrol agents. Maximum germination of seeds was noticed in control and ammonium nitrate with *Trichoderma viride* treatments (Fig. 1). Results showed that plant length (Root+ Shoot Length) significantly ($p<0.01$) increased in all treatments where soil was amended with chemical fertilizers and seed were coated with microbial antagonists.Greater plant length and weight was obtained when soil was treated with Ammonium nitrate alone and Potassium sulphate in combination with *Trichoderma viride* or *Trichoderma reesei* followed by DAP in combination with *Rhizobium meliloti (*Fig. 1). Vigour index increased when seeds treated with Ammonium nitrate in combination with *T. viride* followed by DAP in combination with *T reesei.* Colonization of *M. phaseolina* was completely inhibited when soil was treated with urea, DAP, potassium sulphate, ammonium nitrate and urea in combination with *T. viride* and ammonium nitrate in combination with *R. meliloti (*Fig 2). Significant ($p<0.001$) reduction in colonization of *M. phaseolina* was observed when soil was amended with urea and DAP and seeds were treated with *T. reesei* followed by urea in combination with *R. meliloti* and DAP with *R. meliloti* (Fig 2).

Table 1. One Way ANOVA for the effect of microbial antagonists and chemical fertilizers on plant growth and root colonization by *Macrophomina phaseolina*.

		SS	df	MS	F	Sig.
Germination %	Treatment	21440	19	1128.421	2.989	0.002
	Error	15101.33	40	377.533		
	Total	36541.33	59			
Plant Length (cm)	Treatment	1920.209	19	101.064	6.666	0.001
	Error	606.408	40	15.16		
	Total	2526.617	59			
Plant Weight(g)	Treatment	70.635	19			0.001
	Error	35.067	40			
	Total	105.702	59			
Vigour Index	Treatment	2555.215	19	134.485	3.129	0.001
	Error	1719.383	40	42.985		
	Total	4274.598	59			
Colonization %	Treatment	39416.98	19	2074.578	13.376	0.001
	Error	6204	40	155.1		
	Total	45620.98	59			

Use of fertilizers is a general practice to enhance the growth of plant. Presence of fertilizers in soil affecting populations of microorganisms in soil either directly or indirectly (Curl & Rodrguez Kabana, 1973). Huber (1980) in his study concluded that increase tolerance is due to the development of thicker cuticle and cell wall in nutrient regions make penetration of pathogen difficult. Siddiqui *et al.* (1999) also reported significant reduction of root rot diseases in mungbean caused by the root infecting fungi viz., *M. phaseolina, F. solani* and *R. solani* by the addition of urea and potash. The present investigation showed that plant length significantly ($p<0.01$) increased in those treatments where soil was amended with chemical fertilizers and seeds were coated with microbial antagonists. Irshad *et al*. (2006) reported that plant growth was significantly improved in both okra and mung bean when urea was used @ 0.001 %. Greater plant length and weight was obtained when soil was treated with ammonium nitrate alone and in combination of potassium sulphate with *T. viride* or *T. reesei* followed by DAP in combination with *R. meliloti*. Otsyula & Ajanga (1994) reported that nutrient supply increased plant growth, height, vigor. Marschner (1995) found that nitrogen absorbed by plant is utilized in protein synthesis and seed production where potassium is involved in essentially all cellular function. Presently

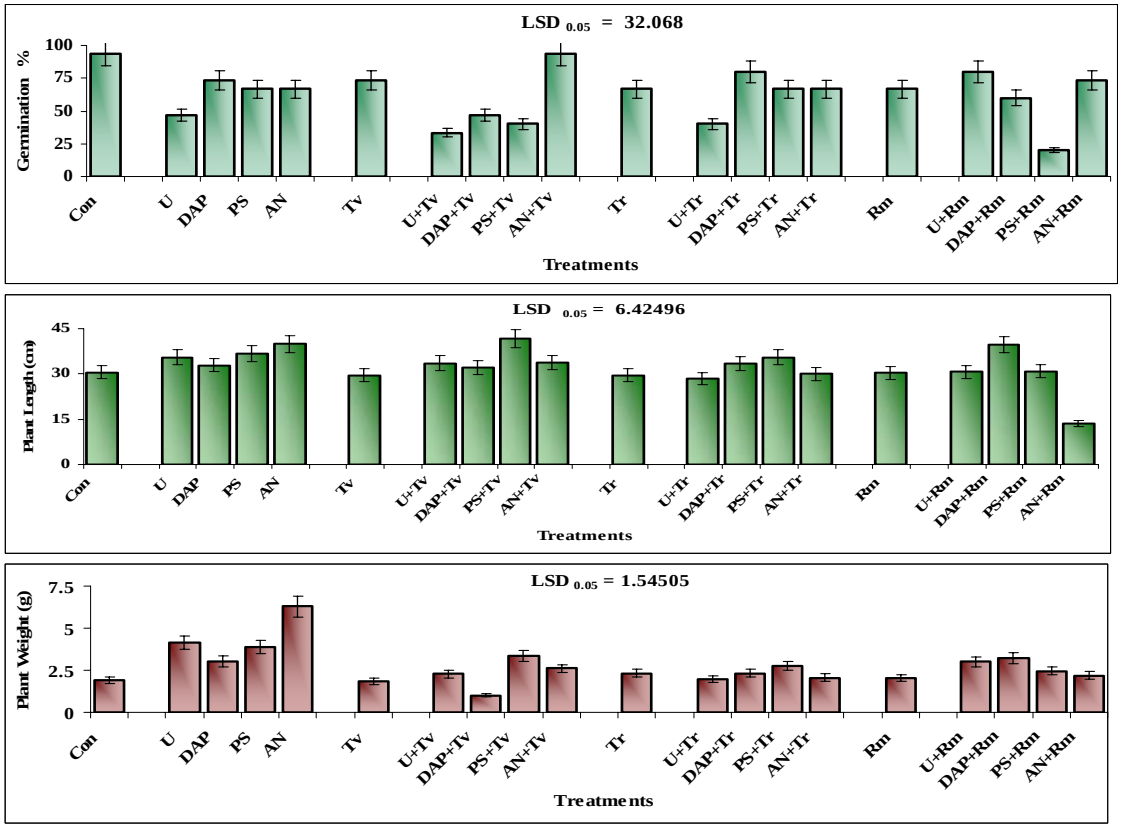

Fig.1. Effect of microbial antagonists and chemical fertilizers on seed germination, plant length, plant weight. Bars show standard error (SE±).

Con = Control Tv= *Trichoderma viride*, Tr = *T. reesei*, Rm = *Rhizobium meliloti.* U = Urea, DAP = Di-ammonium phosphate, PS= Potassium sulphate, AN= Ammonium nitrate.

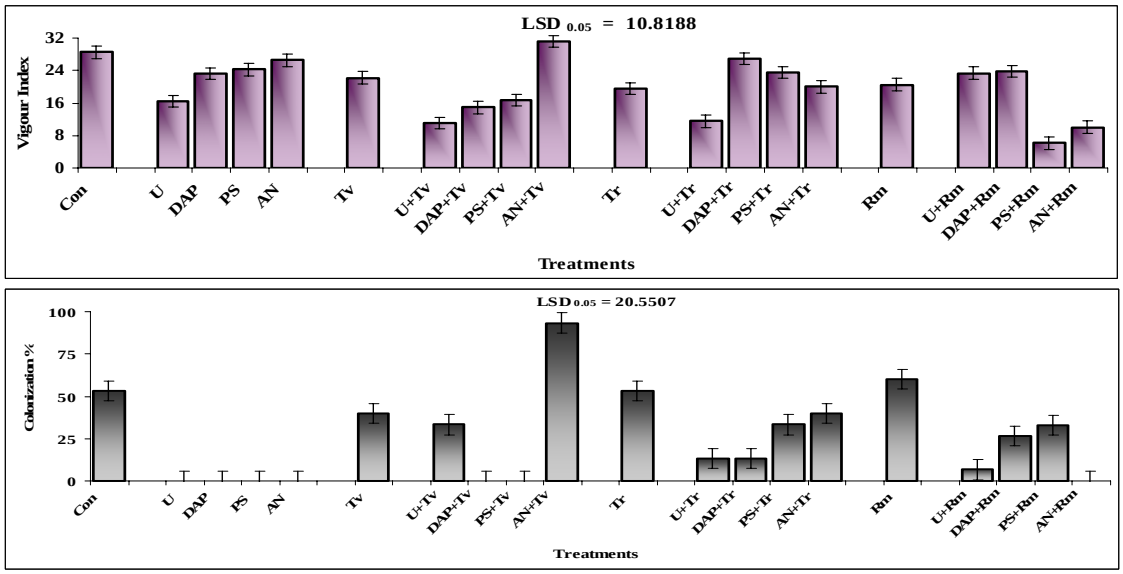

Fig.2. Effect of microbial antagonists and chemical fertilizers on vigour index and root colonization by *M. phaseolina*. Bars show standard error (SE±).

Con = Control Tv= *Trichoderma viride*, Tr = *T. reesei*, Rm = *Rhizobium meliloti.* U = Urea, DAP = Di-ammonium phosphate, PS= Potassium sulphate, AN= Ammonium nitrate

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colonization of *M. phaseolina* was completely inhibited when soil was treated with urea, DAP, potassium sulphate, ammonium nitrate and urea in combination with *T. viride* and ammonium nitrate in combination with *R. meliloti*. Parveen *et al.* (2008) recorded that urea and potash separately and in combination significantly reduced the M. phaseolina infection. Marschner (1995) observed that high nitrogen level have been reported to increase the root exudation which stimulates higher population of *Pseudomonas aerogunisa* and *Rhizobium* around root. Significant reduction in colonization of *M. phaseolina* was observed when soil was amended with urea and DAP and seeds were treated with *T. reesei* followed by urea in combination with *R. meliloti* and DAP with R. meliloti. The results of the present study are similar to the report of Ellet, (1973), Pal & Chaudhary (1980) where diseases caused by root infecting fungi reduced by the addition of mineral fertilizers. The adverse effect of urea on nematode population might be due to toxicity of ammonia ions released during degradation (Oteifa, 1955). Potassium and nitrogen suppressed the root infecting fungi where potassium deficiency increases root exudates (Huber, 1980; 1991; Huber & Arny, 1985).

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