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Influence of *Glomus* Species and Soil Phosphorous on *Verticillium* Wilt in Bt Cotton

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Abstract: Amendment of single superphosphate to the soil was done at 20mg /kg and 300mg /kg. After that soil was treated with different treatments like a) *Verticillium dahliae*, b) *Glomus* species, c) both *Verticillium* and *Glomus* species d) None of *Glomus* species and *Verticillium*. The *Verticillium* wilt was more severe in plants infected with *Glomus* species than non-mycorrhizal plants fertigated at 20mg P / kg of soil. However, in plants fertigated with 300mg P / kg of soil, *Verticillium* wilt was equally severe in mycorrhizal and non-mycorrhizal plants. More propagules of *V. dahliae* were found in the plants fertigated with 20mg P / kg of soil, in petioles of mycorrhizal plants than that of non-mycorrhizal plants.

The plants fertigated with 300mg P / kg of soil reported maximum number of propagules of *V. dahliae* were not significantly different in mycorrhizal and non-mycorrhizal plants. It was found that infection of *V. dahliae* in Bt cotton by *Glomus* species was not affected in plants fertigated with 20mg P / kg of soil. The infection was inhibited by phosphorous and further by *V. dahliae* in plants fertigated with 300mg P / kg of soil. The concentrations of phosphorous in the leaves of the treated plants were found similar to the treated mycorrhizal and non-mycorrhizal plants fertigated with 20mg P / kg of soil. The plants infected with *V. dahliae* alone were found lower than the plants infected with both *V. dahliae* and *Glomus* species.

Keywords: Single superphosphate, Mycorrhiza, *Verticillium*, *Glomus* species, Bt cotton, wilt.

1. Introduction

Worldwide it has been accepted that AMF enhances plant, mineral nutrition, especially phosphorus (Mosse, 1973; Hayman, 1986). The majority of cultivated plants like Bt cotton (*Gossypium hirsutum* L.), is normally infected with the beneficial arbuscular mycorrhizal fungi. Moreover, mycorrhizal infection results in growth increase in cotton (Hurlimann, 1974; Rich and Bird, 1974). It is found that in the soil of low fertility where the symbiont increases efficiency of nutrient absorption by the roots. Arbuscular mycorrhizae can explore greater amounts of soil and absorb more phosphorous and certain other minerals than non-mycorrhizal roots (Hattingh *et al.*, 1974; Ross and Harper, 1970). Nehl *et al.*, (1998) have shown that the mycorrhizal colonization in root browning and soil properties associated with a growth disorder in Australian cotton. The environmental factors support the mycorrhizal colonization of roots in the soil (Ross and Harper, 1970; Schonbeck and Dehne, 1977). Zak *et al.*, (1998) studied Arbuscular mycorrhizal colonization *Glomus mosseae* dynamics of cotton (*Gossypium hirsutum* L.) growing under several production systems on the Southern High Plains of Texas. McGee *et al.*, (1999) have reviewed the relationship between density of *Glomus mosseae* propagules and the initiation and spread of arbuscular mycorrhiza in cotton roots. Several studies indicate that mycorrhizal fungal root infections increase in mineral absorption, which influences diseases caused by soil borne fungi. Mycorrhizal roots of a cultivar of soybean susceptible to *Phytophthora* were more susceptible than non-mycorrhizal roots to *P. megasperma* (Ross, 1972).

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In contrast, VAM fungi caused to decrease in production and germination of chlamydospores of *Thielaviopsis basicola* (Baltruschat and Schonbeck, 1972) and increased resistance of tobacco to disease caused by *T. basicola* (Baltruschat and Schonbeck, 1975). Non-mycorrhizal cotton roots were more severely damaged than mycorrhizal roots by *T. basicola* (Schonbeck and Dehne, 1977). The only one report on the influence of VAM on a vascular wilt of tomato caused by *Fusarium oxysporum* f.sp. *lycopersici* was reduced when plants were pre-infected with the mycorrhizal fungus *Glomus mosseae* (Dehne and Schonbeck, 1975).

Present work was initiated to determine the influence of arbuscular mycorrhizae on *Verticillium* wilt of Bt cotton caused by *Verticillium dahliae* Kleb. The mycorrhizal infection increases phosphorus absorption by the roots. Hence two soil phosphorus levels were employed to study the influence of the mycorrhizal fungus on root disease and the effects of phosphorus fertigation on *Verticillium* wilt as well as *Verticillium* mycorrhiza interactions.

2. Materials and Methods

The soil for pot culturing of Bt cotton was collected from the fields of Yeola Taluka, District Nashik, Maharashtra, India (20° 02' 0" N / 74° 30' 0" E). The soil was sterilized twice for an hour at 121^0C and the amendment was done with single superphosphate [$Ca(H_2PO_4)_2.H_2O$] at 20mg /kg or 300mg / kg of soil. The soil contained 7µg of phosphorus per gram of soil before the addition of phosphorus. After that, different treatments of fungi were mixed with the soil before plantation. The first treatment included *Glomus* species. The second treatment was included *Verticillium dahliae* (MTCC no. 1351). The third treatment was given in combination of *Glomus* species and *V. dahliae* whereas fourth treatment treated as a control (Table 1).

Bt cotton rhizosphere fresh soil samples were brought to the laboratory from various fields like Saigaon, Patoda, Golhewadi, Kotamgaon, Kusur, Gawandgaon, Mukhed and Bharam from Yeola Taluka of Nashik district, Maharashtra, India. The soil was air dried in the shade. About 100g of air dried soil was placed into a beaker with 1000ml of tap water. The soil mixture was vigorously mixed with a glass rod for 30 seconds. After settling the soil particles and organic debris, the remaining soil- root- hyphae- spore suspension was slowly poured through a set of 240, 170, 150, 100 and 72µm sieves. The extracts were washed away from sieves to Whatman filter paper. Using a Trinocular research microscope, spores, aggregates and sporocarps were picked up by means of needle (Gerdemann and Nicolson, 1963).

In Petri dish seeds of Jowar were placed on moist filter paper and seeds were allowed to germinate. When the roots were about 2 – 3cm, the picked up *Glomus* spores were surface sterilized by streptomycin. Roots of Jowar were also sterilized by means of placing an alcohol drop. Later with the help of needle, the sterilized spore was contaminated with the sterilized root of Jowar. Single mycorrhizal spore was inoculated on individual root of Jowar seedling. After 1-2 days of inoculation, the seedlings of Jowar were transferred in a pot containing sterilized soil in the greenhouse. Pots were watered regularly, as required. After 45 – 50 days, the roots were analyzed for mycorrhizal colonization by Phillips and Hayman's method (1970). After finding AMF colonization water supply interrupted and shoots cut off at soil level and kept outside. Pots with roots were allowed to dry. Such roots were then cut with the help of the chopper. Roots along with rhizosphere soil were again used for the multiplication of individual species.

Inoculum of *Glomus* species consists of different species like *Glomus albidum, G. botryoides, G. constrictum, G. fasciculatum, G. fecundisporum, G. fistulosum, G. formosanum, G. pansihalos, G. tenebrosum, G. trimurales* etc. Mycorrhizal treatment in the form of *Glomus* species consists of 10g of soil, roots and spores (20-30 spores per gram).

Microsclerotia of *V. dahliae* was mixed into the soil for a final concentration of 100 microsclerotia per gram of soil as well as 300 microsclerotia per gram of soil. In addition, part of the soil was fertigated with 20mg P / kg of soil and 300mg P /kg of soil.

The microsclerotia isolated and used for the experiment by the method of Huisman and Ashworth (1974). One ml of conidial suspension of *V. dahliae* was pipetted onto sterilized cellophane disks that covered the surface of potato dextrose agar in 90mm diameter Petri dishes. The cultures were incubated at 23^0C. After 3 weeks, microsclerotia were scraped off the cellophane and blended at full speed for 20 seconds in 100ml of sterile distilled water in a Mixer cup. The suspension was passed through sieves and only microsclerotia between 100 and 200µg in diameter were used.

Three to four Bt cotton seeds (MRC- 7347 BG-II) were sown in 10cm diameter clay pots containing soil from the various treatments (Table 1). Four kg soil was weighed into each pot. Seedlings were thinned to one plant per pot and kept in a greenhouse of Ashwamedh Agritech and Farm Solution Systems at 22-23^0C. All plants were watered whenever necessary with 14% Hoagland's solution minus phosphorus (Hoagland and Arnon, 1938). Each treatment was replicated 10 times.

After 13 weeks of seedling germination, the numbers of propagules of *V. dahliae* in infected plant tissue was estimated by a modification of Buchenauer and Erwin's method (1972). The petioles were collected

from third and fourth uppermost leaves from each plant. The surface was disinfected in 1.0% sodium hypochlorite solution for 3 minutes. Each group of petioles was cut into smaller sections by knife and blended with 200ml of sterile distilled water in a mixer at full speed for three times 20-seconds. The suspension was diluted 1:6 with water and 1ml was spread on each of six Petri dishes of sodium polypectate agar (Huisman and Ashworth, 1974). Plates were incubated at 23^0C (± 1^0C) for 6 days. The colonies of *V. dahliae* produce microsclerotia and were easily recognized.

At the end of the experiment, the percentage of roots infected with *Glomus* species was estimated. The root samples were selected randomly from each pot after thorough washing in distilled water. Roots were autoclaved for 15 to 20 minutes in 10% KOH solution. It was cleared in distilled water and neutralized with 2% HCl. The roots were stained with trypan blue (0.05%) in lactophenol. The percentage root infection was measured by Phillips and Hayman's method (1970). Dry weights and mineral content of Bt cotton leaves were determined as described by Labanauskas *et al.,* (1967).

3. Results

Studies on the effect of different treatments given to the Bt cotton plants found that the soil fertilized with 20mg of P per kilogram of soil shows the difference in height, shoot and root weights. The plants infected with *Glomus* species were significantly greater *(P* = 0.05) than those of non-mycorrhizal plants (Table 1). However, the growth response due to the mycorrhizal association was not evident with 300mg of P / kg of soil, because the greater amount of soil phosphorus caused greater heights, shoot and root weights. It was found that the height, shoot and root weight were not affected by the presence or absence of *Glomus* species. Despite the greater growth of mycorrhizal plants fertilized with 20mg of P per kg of soil, *Glomus* species did not significantly improve growth in plants infected with *V. dahliae* at either soil phosphorus concentration (Table 1).

The *Verticillium* wilt of plants grown in soil amended with 20mg of P and 100 microsclerotia of *V. dahliae* per gram of soil was significantly *(P* = 0.05) more severe in plants infected with *Glomus* species (Table 2). The degree of vascular discoloration was almost three times greater in mycorrhizal plants than in non-mycorrhizal plants. Whereas, it was found that about 14 times more propagules of *V. dahliae* was recovered from petioles of mycorrhizal plants than from non-mycorrhizal plants. In soil amended with 20mg of P and 300 microsclerotia per gram of soil were shown vascular discoloration in plants uniformly severe whether or not the plants were mycorrhizal. The greater numbers of propagules of *V. dahliae* was recovered from petioles of mycorrhizal plants than non-mycorrhizal plants. In the plants fertigated with 300mg of P per kg of soil, vascular discoloration and numbers of propagules of *V. dahliae* recovered from petioles of mycorrhizal and non-mycorrhizal plants did not differ significantly. The symptoms of diseased plants were correlated with vascular discoloration and propagules counts. Mycorrhizal plants fertilized with 20mg of P / kg of soil and both mycorrhizal and non-mycorrhizal plants fertilized with 300mg of P / kg of soil had the earliest and most, severe wilt symptoms.

Root infection by *Glomus* species in Bt cotton fertilized with 20mg of P / kg of soil was not reduced by *V. dahliae* (Table 3). However, the amount of infection was reduced in plants fertilized with 300mg of P per kg of soil. It was further reduced in plants fertilized with 300mg of P per kg of soil and infected with *V. dahliae.*

Although concentrations of phosphorus in leaves of non-mycorrhizal and mycorrhizal plants were similar, concentrations of phosphorus were significantly greater in leaves of plants infected with both *Glomus* species and *V. dahliae* than the plants infected with either fungus alone (Table 4). The plants fertilized with 20mg of P per kg of soil and not infected with *V. dahlia* were shows higher zinc concentrations in mycorrhizal plants than that of non-mycorrhizal plants. Whereas, it was found that potassium concentrations were less in mycorrhizal plants than in non-mycorrhizal plants.

Table 1. Effect of *Glomus* species, *V. dahliae* and two levels of soil phosphorus on dry weight (g) of shoot and root of Bt cotton plant.

Treatment	20mg P / kg of soil			300mg P / kg of soil		
	Height (cm)	Shoot dry weight (g)	Root dry weight (g)	Height (cm)	Shoot dry weight (g)	Root dry weight (g)
C	44.77	15.01	4.50	53.90	18.00	6.00
G	50.09 ± 0.68[a]	17.50 ± 0.39[a]	5.50 ± 0.29[a]	57.13± 0.72	19.03 ± 0.54[a]	6.32 ± 0.51
V100	35.60	11.00	4.00	35.11	10.70	3.50
V100+G	36.3 ± 0.44[a]	12.05 ± 0.62	4.10 ± 0.28[a]	40.55 ± 0.59	13 ± 0.40[a]	4.20 ± 0.27[a]
V300	30.38	10.50	3.20	0.00	0.00	0.00
V300+G	32 ± 0.74[a]	10.18 ± 0.59[a]	3.1 ± 0.26[a]	0.00	0.00	0.00

Values are mean of three replicates; [a] = Values are significant at P ≤ 0.05 level over corresponding control plants; Value ± SE; ± Standard error; C = Control; G = *Glomus* species; V100 = *V. dahliae* (100 MS /g of soil); V300 = *V. dahliae* (300 MS /g of soil); V100+G = *V. dahliae* (100 MS /g of soil) + *Glomus* species; V300+G = *V. dahliae* (300 MS /g of soil) + *Glomus* species; MS = Microsclerotia.

Table 2. Effect of *Glomus* species, *Verticillium dahliae* and two levels of soil phosphorus on vascular discolouration index and propagules per gram of petiole tissue of Bt cotton plant.

Treatment	20mg P / kg of soil		300mg P / kg of soil	
	VDI	Propagules/g	VDI	Propagules/g
V100	3.07	795.00	6.51	23599.00
V100+G	5.86 ± 4.24	110269	7.32 ± 6.09a	21581[a]
V300	5.22	17202.00	0.00	0.00
V300+G	716.67	26682[a]	0.00	0.00

Values are mean of three replicates; [a] = Values are significant at $P \leq 0.05$ level over corresponding control plants; Value ± SE; ± Standard error; V100 = *V. dahliae* (100 MS /g of soil); V300 = *V. dahliae* (300 MS /g of soil); V100+G = *V. dahliae* (100 MS /g of soil) + *Glomus* species; V300+G = *V. dahliae* (300 MS /g of soil) + *Glomus* species; MS = Microsclerotia.

Table 3. Effect of *Verticillium dahliae* and soil phosphorus on percentage of root tissue infected with *Glomus* species in Bt cotton.

Treatment	20mg P / kg of soil	300mg P / kg of soil
G	70.83	37.37
V100+G	71.79 ± 3.3[a]	20.00 ± 3.3 [a]
V300+G	74.01 ± 2.4 [a]	0.00

Values are mean of three replicates; [a] = Values are significant at $P \leq 0.05$ level over corresponding control plants; Value ± SE; ± Standard error; G = *Glomus* species; V100+G = *V. dahliae* (100 MS /g of soil) + *Glomus* species; V300+G = *V. dahliae* (300 MS /g of soil) + *Glomus* species; MS = Microsclerotia.

Table 4. Effect of *Glomus* species, *Verticillium dahliae* and two levels of soil phosphorus on concentration of P (%), K (%) and Zn (ppm) in leaves of Bt cotton plants.

Treatment		C	G	V100	V100+G	V300	V300+G
20mg P / kg of soil	**P (%)**	**0.12**	**0.11± 0.03[a]**	**0.09**	**0.16 ± 0.03 [a]**	**0.15**	**0.21 ± 0.03 [a]**
	K (%)	**1.08**	**0.87 ± 0.03[a]**	**0.85**	**0.87 ± 0.03[a]**	**1.06**	**0.86 ± 0.20**
	Zn (ppm)	**10.35**	**13.15 ± 0.28[a]**	**9.15**	**17.95 ± 0.28[a]**	**10.15**	**12.95 ± 0.29[a]**
300mg P / kg of soil	**P (%)**	**0.41**	**0.42± 0.03[a]**	**1.05**	**1.12 ± 0.03[a]**	**0.00**	**0.00**
	K (%)	**0.85**	**0.77± 0.03[a]**	**1.52**	**2.23 ± 0.03[a]**	**0.00**	**0.00**
	Zn (ppm)	**15.55**	**16.75± 0.32[a]**	**20**	**19.55 ± 0.31[a]**	**0.00**	**0.00**

Values are mean of three replicates; [a] = Values are significant at $P \leq 0.05$ level over corresponding control plants; Value ± SE; ± Standard error; C = Control; G = *Glomus* species; V100 = *V. dahliae* (100 MS /g of soil); V300 = *V. dahliae* (300 MS /g of soil); V100+G = *V. dahliae* (100 MS /g of soil) + *Glomus* species; V300+G = *V. dahliae* (300 MS /g of soil) + *Glomus* species; MS = Microsclerotia.

4. Discussion

The results obtained from the present experiment shows adequate phosphorus nutrition to Bt cotton, whether due to phosphorus fertilizer in the soil or due to increased phosphorus uptake by mycorrhizal fungi. The more severe wilt was observed due to inadequate P nutrition in non-mycorrhizal plants as compared to mycorrhizal plants. The plants fertigated with a low level of soil P, (20mg of P / kg of soil), in this case, the *Verticillium* wilt was more severe in mycorrhizal than in non-mycorrhizal Bt cotton plants. This is apparent from measurements of vascular discoloration and propagules counts and from the reduction of growth in plants infected with *V. dahliae*.

Possible reasons for the increase in disease severity in mycorrhizal plants include:

a) An increase in the number of avenues for penetration by *V. dahliae*, since *Glomus* species may produce chlamydospores in cortical tissue in such abundance that the cortex ruptures.
b) Dilution of the concentration of potassium in mycorrhizal plants;
c) A larger population of *V. dahliae* in mycorrhizal plants due to their improved nutrient status; and
d) More movement of microconidia in the mycorrhizal plants where the greatest amount of tissue resulted in greater transpiration.

These experiments support the latter three hypotheses since the severity of disease increased as the phosphorus nutrition of the plants improved. From the results, it is found that both mycorrhiza and heavy phosphorus fertigation improve vigor and growth of Bt cotton. This situation favors sever *Verticillium* Wilt. Adequate amounts of all nutrients except phosphorus were supplied to all the plants, found that the growth was inferior in non-mycorrhizal plants fertigated with 20mg of P /kg of soil and poor plant growth observed in less severe *Verticillium* Wilt. The concentration of potassium was generally reduced in mycorrhizal plants. This could be a factor in the increased severity of disease in mycorrhizal plants; hence Bt cotton plants with high levels of potassium are more resistant to *Verticillium* (Hafey *et al.*, 1975).

V. dahliae did not affect mycorrhizal development in soil with 20mg of P / kg of soil since infection by

Glomus species was not reduced at either low or high inoculum densities of *V. dahliae*. Furthermore, concentrations of phosphorus and zinc were not reduced in mycorrhizal plants infected with *V. dahliae.* With 300mg of P / kg of soil, infection by *Glomus* species was reduced in plants infected with *V. dahliae* because very high levels of phosphorus accumulated in diseased tissue. It is well known that high phosphorus levels inhibit mycorrhizae formation (Daft and Nicolson, 1969).

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