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EFFICACY OF *BACILLUS THURINGIENSIS* AND *RHIZOBIUM MELILOTI* WITH NURSERY FERTILIZERS IN THE CONTROL OF ROOT INFECTING FUNGI ON MUNG BEAN AND OKRA PLANTS

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Abstract

Bacillus thuringiensis (Bt10) and *Rhizobium meliloti* (R5) with and without locally available nursery fertilizers viz., flourish, frutan, NPK, urea and fish meal were used to study their effect on the growth and suppression of soil borne root infecting fungi viz., *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium* spp., on mung bean and okra plants. Nursery fertilizers were applied in the soil @ 0.01% w/w whereas fish meal @ 0.1% v/w. *B. thuringiensis* and *R. meliloti* applied as seed dressing and soil drenching after 1 week of amendment showed a significant increase in seed germination, shoot length, shoot weight, root length, root weight and root nodulation providing better plant growth with a significant decrease in infection by root infecting fungi. Use of NPK was found to increase the efficacy of *B. thuringiensis* and *R. meliloti* in the control of root infecting fungi and growth of mung bean and okra plants.

Introduction

The soil borne pathogens, the major cause of the development of root rot disease of crop plants are difficult to eliminate since they produce resting structures like sclerotia and chlamydospores which are well adapted to survive for longer periods under adverse environmental conditions. These pathogens infect roots of the plants, limiting nutrient uptake by plants and produce root rot and root knot diseases complex resulting in death of the plant. Losses to the crop plants through such diseases are underestimated and generally go unnoticed (Baker & Cook, 1974). Of the soil borne root infecting fungi *Macrophomina phaseolina* (Tassi) Goid is reported to produce charcoal rot, seedling blight, root rot, stem rot, pod rot on more than 500 species of plants (Dhingra & Sinclair, 1978) where at least 72 hosts have been reported from Pakistan (Mirza & Qureshi, 1978; Shahzad *et al*., 1988). *Rhizoctonia solani* exist as active mycelium in soil which is known to produce seed rot, damping off of seedling, wilt and root rot on over 2000 species of plants (Parmeter, 1970), of which at least 63 hosts have been reported from Pakistan (Mirza & Qureshi, 1978; Ghaffar, 1988). *Fusarium solani* and *F. oxysporum*, which are very common in agriculture fields of Pakistan, are known to cause root rot, stem rot and wilt disease on a wide range of plants (Booth, 1971; Ghaffar, 1992).

Use of fungicides, fertilizers and biocontrol agents in the control of soil borne root rot of crop plants is a common practice. Different fertilizers are used for better plant growth. Nitrogen present in the fertilizer is absorbed by the plant which is utilized in a protein synthesis and seed production whereas potassium is involved in many cellular functions including photosynthesis, phosphorylation, water maintenance, reduction of nitrates and reproduction. Potassium is also known to reduce *F. oxysporum* infection on tomato (Ellet, 1973) and *Rhizoctonia solani* infection on hemp (Pal & Choudhary, 1980). Urea also inhibits soil borne root-infecting fungi (Dawar & Ghaffar, 2003). Many plant

growth promoting rhizobacteria eg., *Rhizobium* spp., have a beneficial effect on plants including biological control of soil borne pathogens, induce systematic resistance to plant pathogen, improvement of nutrient and water uptake of plant (Seuk Bae *et al*., 2000). Similarly *Bacillus thuringiensis* is also a plant growth promoting bacterium which produces bacteriocin thuricin compounds (Gray *et al*., 2006). *B. thuringiensis* (commonly known as 'Bt') is an insecticidal bacterium, marketed worldwide for control of many important plant pests, mainly caterpillars of the Lepidoptera, mosquito larvae and black flies etc. Bt products represent about 1% of the total 'agrochemical' market (fungicides, herbicides and insecticides) across the world (Knowles, 1994). Application of bacteria either as seed dressing or as soil drenching has shown a significant suppression of root infecting pathogens on leguminous and non-leguminous plants (Zaki & Ghaffar, 1987; Ehtesham-ul-Haque *et al*., 1990).The aim of the present investigation was to determine the efficacy of bacteria viz., *Bacillus thuringiensis* and *Rhizobium meliloti* with and without fertilizers in the control of root rot disease on mung bean (*Vigna radiata* L.) and okra (*Abelmoschus esculentus* L.) used as test plants.

Materials and Methods

Different nursery fertilizers viz., flourish, frutan, NPK, urea and fishmeal which contain several essential micro and macronutrients in their composition were purchased from the local market and used for the control of root infecting fungi on okra and mung bean plants. Soil used for the experiment was obtained from the experimental plots of the Department of Botany, University of Karachi and sieved through 2mm sieve to discard particles. The soil used was sandy loam (Sand, Silt, Clay; 70, 19, 11%), pH range from 7.5-8.1 with moisture holding capacity (MHC) of 24.04% (Keen & Raczkowski, 1922), total nitrogen 1.5% (Mackenzie & Wallace, 1954), total organic matter 2.4 %. Soil had natural infestation of 1-3 sclerotia of *Macrophomina phaseolina* as found by wet sieving dilution technique (Sheikh & Ghaffar, 1975), 5-10% colonization of *Rhizoctonia solani* on sorghum seeds used as baits (Wilhelm, 1955) and 3000cfu of *Fusarium* spp., as assessed by soil dilution technique (Nash & Snyder, 1962). The bacterial strains of *Bacillus thuringiensis* (Bt10) was obtained from the Microbiology Department, University of Karachi and *Rhizobium meliloti* (R5) was isolated from *Melilotus indica*. Actively growing culture of *Bacillus thuringiensis* (Bt10) and *Rhizobium meliloti (*R5) were used as seed dressing and soil drenching. Seeds of mung bean and okra after treatment with 48 hrs old cultures of *B. thuringiensis* (Bt10) and *R. meliloti* (R5) using 2% gum arabic as a sticker were sown in 8 cm diam., plastic pots, containing 300gm soil/pot. There were three replicates of each treatment and the pots without bacteria and without fertilizer served as control. Pots were kept randomized in a screen house of the Department of Botany, University of Karachi, where soil was kept at 40% M.H.C. (Keen & Raczkowski, 1922). Soil without bacterial suspension and fertilizers served as control. To determine the infection of pathogenic fungi on roots, the method used by Short *et al*., (1980) was modified where plants were uprooted after 30 days. Data on germination, plant height shoot weight, root weight and root nodulation was recorded. Roots were washed under running tap water and surface sterilized in 1% Ca $(OCl)_2$ for 3 min., and then five 1 cm long root pieces was transferred on PDA plate containing Penicillin @ 100,000/l and Streptomycin @ 20mg/l. Petri dishes were incubated for 5 days at room temperature to confirm infection of roots caused by root rot fungi.

Data were analyzed and subjected to analysis of variance (ANOVA) following the procedure as suggested by Gomez & Gomez (1984).

Results and Discussion

Present study describes the efficacy of nursery fertilizers and bacteria for the control of root infecting fungi viz., *Fusarium* spp., *Macrophomina phaseolina* and *Rhizoctonia solani* on mung bean and okra plants. All nursery fertilizers with bacteria showed a significant increase in germination and plant growth parameters in both mung bean and okra plants as compared to control (Table 1). NPK and fishmeal with bacteria showed 100 % germination in mung bean and okra plants. Nursery fertilizers used in combination with *Bacillus thuringiensis* (Bt10) and *Rhizobium meliloti* (R5) showed significant increase in numbers of nodules/plant in mung bean ($p<0.001$) where frutan and NPK were used with *R. meliloti* and fish meal and NPK was used with *B. thuringiensis.* Bacterization of mung bean and okra seeds with *B. thuringiensis* (Bt10) and *R. meliloti* (R5) improved plant growth and decreased infection caused by root rot fungi. Seeds of mung bean and okra plants coated with *B. thuringiensis* ($11.33x10^8$ cells/seed) and *R. meliloti* ($5.7x10^8$ cells/seed) in mung bean whereas in okra *B. thuringiensis* ($6.1x10^8$cells/seed) and *R. meliloti* ($8.5x10^8$cells/seed), a significant suppression of *Fusarium* spp., was observed when fishmeal was used with *R. meliloti* (Table 2). When coated seeds were sown in fishmeal amended soil, the infection of *M. phaseolina* was completely suppressed by NPK and urea when seeds were coated with both bacteria ($p<0.01$) (Table 2). Infection of *R solani* was inhibited more than 70% by all the fertilizers with bacteria ($p<0.001$). In soil drenching method, infection of *Fusarium* spp., was significantly reduced ($p<0.01$) where soil was amended with NPK @ 0.01% and soil was drenched with both bacteria (Table 2). The infection of *M. phaseolina* was significantly suppressed by all fertilizers in combination with bacteria but completely inhibited where fishmeal was used with both bacteria ($p<0.01$). Infection of *R. solani* was significantly inhibited by all the nursery fertilizers in combination with bacteria ($p<0.001$) (Table 2). Of the two methods used soil drenching with bacteria was better method for the control of root rot fungi as compared to seed coating (Table 2).

Present results showed that bacteria in combination with NPK and fishmeal increased the germination of the seeds. Seed coating with microbial antagonists protect the seeds from seed borne and soil borne pathogens, which enables the seeds to germinate and become established as a healthy seedling (Chang & Kommedahl, 1968).Main purpose of the control of plant pathogens is to improve growth quality and yield. This can be achieved by the reduction of plant parasitic pathogens to a low and safe level to reduce economic losses. Control of root infecting fungi with the use of mineral fertilizers could presumably be due to the increase in tolerance with the development of thicker cuticle and cell wall or more schlerenchyma tissue with different nutrient regimes which has been correlated with the difficulty in penetration of pathogen (Huber, 1980). Siddiqui *et al.,* (1999) also reported that root rot diseases in mung bean caused by root infecting fungi viz., *Fusarium* spp., *M. phaseolina* and *R. solani* also reduced by the addition of urea and potash. Toxicity of ammonia ion released during degradation of urea exerted an adverse effect on soil borne pathogen (Oteifa, 1995). Pal & Choudhary (1980) also found that root rot disease caused by *Fusarium oxysporum* and *R. solani* reduced by the addition of mineral fertilizers. Similarly Siddiqui *et al.,* (2000) reported that *Rhizobium* and *Trichoderma harzianum* showed better biocontrol and growth promoting effect when used with urea. Bai *et al*., (2002a) reported that *B. thuringiensis* enhance the root nodulation and plant growth in soybean when applied as a co inoculation with

Table 1. Effect of nursery fertilizers on efficacy of bacteria in the growth of mung bean and okra plants.

Mung bean						
Treatments	**Germination %**	**Shoot length (cm)**	**Shoot weight (gm)**	**Root length (cm)**	**Root weight (gm)**	**Nodulation**
	Seed dressing					
Control	66.67	14.35	1.74	6.33	0.15	1.33
Control+*B. thuringiensis*	86.60	14.26	2.01	9.30	0.24	2.433
Control+*R. meliloti*	73.33	10.72	1.08	7.71	0.25	1.42
Flourish	60.0	9.83	0.50	7.22	0.16	0
Flourish+*B. thuringiensis*	100.0	8.75	1.04	3.42	0.18	0.776
Flourish+*R. meliloti*	86.70	8.75	1.03	4.72	0.19	1.66
Frutan	66.67	6.66	0.54	3.78	0.15	0.66
Frutan+*B. thuringiensis*	86.67	11.83	1.18	4.44	0.23	1.106
Frutan+*R. meliloti*	86.67	9.55	1.03	4.22	0.16	3.553
NPK	80.0	7.18	0.91	2.93	0.13	0.776
NPK+*B. thuringiensis*	100.0	7.22	1.13	3.13	0.14	2.11
NPK+*R. meliloti*	100.0	11.32	1.72	5.93	0.17	7
Urea	80.0	8.86	0.96	3.32	0.09	0
Urea +*B. thuringiensis*	93.33	7.11	0.61	2.13	0.10	0
Urea+*R. meliloti*	86.67	7.78	0.82	2.42	0.17	1
Fish meal	80.0	11.30	0.71	2.08	0.13	3.776
Fishmeal+*B. thuringiensis*	93.33	14.17	1.59	7.77	0.35	6.696
Fishmeal+*R. meliloti*	100.0	10.42	1.41	4.77	0.24	6.28
LSD0.05=	**25.850**	**6.874**	**0.868**	**4.412**	**0.167**	**3.985**
	Soil drenching					
Control	60.0	14.35	1.74	6.33	0.15	0.443
Control+*B. thuringiensis*	86.67	13.66	2.01	9.0	0.24	0.553
Control+*R. meliloti*	66.67	10.33	1.08	8.33	0.25	1.33
Flourish	73.33	9.76	1.04	3.05	0.14	0.22
Flourish+*B. thuringiensis*	80.0	6.66	0.83	3.08	0.12	0.0
Flourish+*R. meliloti*	86.67	11.97	1.62	4.01	0.20	2.66
Frutan	80.0	9.44	0.74	1.94	0.10	1.33
Frutan+*B. thuringiensis*	100.0	11.77	1.50	4.88	0.27	1.106
Frutan+*R. meliloti*	86.67	11.10	1.12	4.83	0.16	0.553
NPK	73.37	13.12	1.78	6.10	0.15	0.553
NPK+*B. thuringiensis*	86.67	7.86	1.38	2.77	0.19	0.0
NPK+*R. meliloti*	100.0	3.66	0.66	1.33	0.07	0.22
Urea	60.0	9.66	0.85	2.60	0.07	0.553
Urea +*B. thuringiensis*	93.33	4.66	0.55	1.05	0.07	0.0
Urea+*R. meliloti*	80.0	6.33	0.41	2.27	0.09	1.33
Fish meal	80.0	10.53	0.60	2.88	0.08	0.33
Fishmeal+*B. thuringiensis*	100.0	5.93	1.07	2.33	0.10	0.66
Fishmeal+*R. meliloti*	93.33	3.76	0.53	2.21	0.18	0.0
LSD0.05=	**27.546**	**6.640**	**23.12**	**0.929**	**0.143**	**2.704**

Table 1. (Cont'd.)

Okra					
Treatments	**Germination %**	**Shoot length (cm)**	**Shoot weight (gm)**	**Root length (cm)**	**Root weight (gm)**
	Seed dressing				
Control	43.33	12.91	7.31	5.15	0.37
Control+*B. thuringiensis*	53.33	11.86	1.93	5.15	0.38
Control+*R. meliloti*	60.0	12.48	1.74	5.47	0.27
Flourish	60.0	11.73	1.04	3.68	0.16
Flourish+*B. thuringiensis*	86.67	8.50	1.37	4.52	0.14
Flourish+*R. meliloti*	73.37	8.26	0.75	3.63	0.08
Frutan	66.67	11.32	1.14	5.05	0.17
Frutan+*B. thuringiensis*	80.0	9.77	1.67	4.76	0.31
Frutan+*R. meliloti*	86.67	10.24	2.0	3.78	0.21
NPK	66.67	15.32	2.47	7.83	0.36
NPK+*B. thuringiensis*	100.0	11.75	15.0	4.80	0.21
NPK+*R. meliloti*	86.67	12.29	2.0	4.82	0.28
Urea	60.0	10.55	1.79	4.81	0.34
Urea +*B. thuringiensis*	73.33	11.75	1.96	5.81	0.35
Urea+*R. meliloti*	70.0	11.78	1.84	4.33	0.22
Fish meal	80.0	16.77	2.61	5.39	0.44
Fishmeal+*B. thuringiensis*	100.0	13.59	1.36	5.55	0.25
Fishmeal+*R. meliloti*	100.0	13.38	2.09	4.44	0.27
LSD0.05=	**23.976**	**5.43**	**3.649**	**2.569**	**0.210**
	Soil drenching				
Control	53.33	12.91	7.31	5.15	0.37
Control+*B. thuringiensis*	66.67	11.86	1.93	5.15	0.38
Control+*R. meliloti*	66.67	12.49	1.74	5.47	0.27
Flourish	56.67	10.5	2.2	3.78	0.24
Flourish+*B. thuringiensis*	66.67	13.0	2.34	4.56	0.27
Flourish+*R. meliloti*	73.33	13.08	2.60	4.6	0.25
Frutan	60.0	11.73	0.60	4.4	0.08
Frutan+*B. thuringiensis*	86.67	15.6	0.71	5.92	0.10
Frutan+*R. meliloti*	66.67	13.75	2.61	5.05	0.48
NPK	60.0	13.61	1.72	4.86	0.39
NPK+*B. thuringiensis*	80.0	12.55	2.55	4.19	0.40
NPK+*R. meliloti*	100.0	7.86	3.55	4.31	0.21
Urea	56.67	9.33	1.55	3.53	0.23
Urea +*B. thuringiensis*	66.67	11.70	1.79	4.47	0.22
Urea+*R. meliloti*	66.67	13.75	1.75	4.35	0.34
Fish meal	60.0	16.07	2.0	5.44	0.26
Fishmeal+*B. thuringiensis*	80.0	17.34	2.26	5.67	0.31
Fishmeal+*R. meliloti*	80.0	15.89	1.57	5.76	0.18
LSD0.05=	**23.03**	**8.733**	**3.755**	**3.051**	**2.412**

Table 2. Effect of nursery fertilizers on efficacy of bacteria in the control of root infecting fungi of mung bean and okra plants.

Treatments	Infection%					
	Seed dressing			Soil drenching		
	Fusarium spp.	*Macrophomina phaseolina*	*Rhizoctonia solani*	*Fusarium* spp.	*Macrophomina phaseolina*	*Rhizoctonia solani*
	Mung bean					
Control	100.0	77.76	100.0	100.0	77.76	100.0
Control+*B. thuringiensis*	88.86	11.10	33.30	88.86	11.10	33.30
Control+*R. meliloti*	88.86	22.2	33.30	88.86	22.20	33.30
Flourish	77.76	11.10	0.0	33.30	33.30	0.0
Flourish+*B .thuringiensis*	44.43	0.0	0.0	55.50	11.10	0.0
Flourish+*R. meliloti*	22.20	11.10	11.10	55.50	11.10	0.0
Frutan	33.30	11.10	0.0	44.43	11.10	11.10
Frutan+*B. thuringiensis*	33.30	11.10	0.0	66.63	0.0	0.0
Frutan+*R. meliloti*	33.30	0.0	0.0	66.63	0.0	0.0
NPK	55.56	0.0	0.0	33.30	11.10	0.0
NPK+*B. thuringiensis*	44.40	0.0	0.0	11.10	0.0	0.0
NPK+*R. meliloti*	44.40	0.0	0.0	0.0	0.0	0.0
Urea	33.30	0.0	0.0	0.0	0.0	0.0
Urea+*B. thuringiensis*	44.40	0.0	0.0	44.40	11.10	0.0
Urea+*R. meliloti*	55.50	11.10	0.0	33.30	33.30	11.10
Fish meal	22.20	11.10	0.0	66.60	0.0	0.0
Fishmeal+*B. thuringiensis*	11.10	22.20	0.0	11.10	0.0	0.0
Fishmeal+*R. meliloti*	33.30	11.10	0.0	11.10	0.0	0.0
LSD0.05=	**52.409**	**34.572**	**23.588**	**37.756**	**50.851**	**24.149**

Table 2. (Cont'd.)

Treatments	Infection%					
	Seed dressing			Soil drenching		
	Fusarium spp.	*Macrophomina phaseolina*	*Rhizoctonia solani*	*Fusarium* spp.	*Macrophomina phaseolina*	*Rhizoctonia solani*
	Okra					
Control	100.0	88.60	100	100.0	88.60	100.0
Control+*B. thuringiensis*	88.66	88.60	66.6	88.66	88.60	66.60
Control+*R. meliloti*	66.60	77.60	44.4	66.60	77.60	44.40
Flourish	44.30	22.20	11.1	44.40	44.40	0.0
Flourish+*B .thuringiensis*	66.60	11.10	0.0	44.40	22.20	55.60
Flourish+*R. meliloti*	22.20	0.0	0.0	33.30	22.20	33.30
Frutan	44.30	0.0	0.0	55.50	33.30	0.0
Frutan+*B. thuringiensis*	11.10	0.0	0.0	22.20	0.0	0.0
Frutan+*R. meliloti*	55.50	0.0	0.0	22.20	0.0	11.10
NPK	33.30	0.0	0.0	66.60	11.10	0.0
NPK+*B. thuringiensis*	33.30	0.0	0.0	33.30	33.30	0.0
NPK+*R. meliloti*	44.40	11.10	0.0	33.30	0.0	0.0
Urea	22.20	0.0	0.0	33.30	0.0	0.0
Urea+*B. thuringiensis*	33.30	0.0	0.0	0.0	0.0	0.0
Urea+*R. meliloti*	11.10	11.10	0.0	11.10	0.0	11.10
Fish meal	22.20	22.20	0.0	44.30	0.0	0.0
Fishmeal+*B. thuringiensis*	11.10	0.0	0.0	33.30	0.0	0.0
Fishmeal+*R. meliloti*	11.10	0.0	0.0	0.0	0.0	0.0
LSD0.05=	**64.596**	**44.866**	**4.966**	**23.623**	**27.306**	**37.425**

Bradyrhizobium japonicum. Present results showed that soil amendment with nursery fertilizers and bacteria for the control of root rot fungi showed that infection of *Fusarium* spp., *M. phaseolina* and *R. solani* were significantly reduced in okra and mung bean. Dawar & Ghaffar (2003) reported that urea showed significant reduction in *M. phaseolina* infection on mung bean. The shoot length, shoot weight, root length and root weight were also significantly increased in mung bean and okra plants.

The results of the present study showed that NPK increased the efficacy of *B. thuringiensis* and *R. meliloti* in the control of root rot of mung bean and okra plants. Similarly Ghaffar, (1992), Dawar & Ghaffar, (2003) reported that Urea and NPK increased the efficacy of *Paecilomyces lilacinus* against root rot fungi. The plants with proper nutrients are able to produce new roots to replace the older roots, which are destroyed by soil borne pathogens. The best root and shoot growth requires a balanced level of the major nutrients. The newly developed roots have the capacity to become more resistant against root infecting fungi. Use of *B. thuringiensis* and *R. meliloti* could therefore be applied in the control of soil borne root infecting fungi and increased the crop production.

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