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EFFECT OF SEED COATING MATERIAL IN THE EFFICACY OF MICROBIAL ANTAGONISTS FOR THE CONTROL OF ROOT ROT FUNGI ON OKRA AND SUNFLOWER

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Abstract

The biological potential of different microbial antagonists viz., *Bacillus thuringiensis, Rhizobium meliloti, Aspergillus niger* and *Trichoderma harzianum* was examined by coating the seeds with gum arabic, glucose, sugar and mollases in the suppression of root rot fungi viz., *Macrophomina phaseolina, Rhizoctonia solani* and *Fusarium* spp., on okra and sunflower plants. All biocontrol agents enhanced the germination and growth of plant as compared to control. Shoot length, shoot weight, root length, root weight were significantly increased in both okra and sunflower. Maximum plant height was observed where seeds of okra and sunflower were coated with *T. harzianum* using 2% of glucose followed by gum arabic, mollases and sugar solution. Gum arabic was found more effective in reducing infection by root rot fungi viz., *M. phaseolina, R. solani* and *Fusarium* spp. Of the different microbial antagonists used, *T. harzianum* was found more effective followed by *B. thuringiensis, R. meliloti* and *A. niger* in the control of root rot fungi.

Introduction

Plants make up the majority of the earth surface. Directly or indirectly, plants also make up all the foods on which all the animals depend. Crop plants have great importance in political, social and agricultural economy of a country. Diseases of crop plants adversely affect the agricultural economy of countries depending upon the severity of diseases. Of the disease causing organism, the soil borne pathogens viz., *Macrophomina phaseolina* (Tassi) Goid, *Rhizoctonia solani* Kühn and *Fusarium* spp., attack roots, limiting nutritional uptake and produce root rot disease complex resulting in the death of plants. The genus *Fusarium* contains a number of species, which have been recognized for a long time as being important plant pathogens (Booth, 1971; Nelson *et al*., 1983). An average yield loss of 2.2 ha in pea was observed in Ontario due to root rot diseases caused by *F. solani* and *F. oxysporum* with complete loss in many cases (Tu, 1987). Similarly, *M. phasoelina* is reported to produce charcoal rot of over 500 species of plants (Sinclair, 1982), where at least 72 hosts have been reported from Pakistan (Mirza & Qureshi, 1978; Shahzad *et al*., 1988). *R. solani* exists as active mycelium in soil and attacks more than 2000 species of plant (Parmeter, 1970), of which at least 63 hosts have been reported from Pakistan (Mirza & Qureshi, 1978).

Seed treatment promote seedling establishment, help ensure yield and reduce the quality losses due to many diseases and insects. The ability of seed treatment to control many fungal diseases has made them one of the biggest success stories of plant disease prevention. Seed treatment control the fungi residing on the surface of seed or inside the seed and are affective against pathogen that reside in the soil and cause seed rot, damping off and root rots. There are two main categories of seed treatment; protectant (contact on the seed surface) and systemic (within plants). Protectants help control pathogens that

reside on the seed surface whereas systemic seed treatments control seed borne fungi that reside within the seed or infect the seed surface (Martha *et al.*, 2003). Due to increase in cost of chemical pesticides and environmental hazards involved with their application emphasis is now given on the biological control agent against plant pathogen (Agrios, 2004). In previous studies several microbial antagonists and biocontrol agents have shown promising results in the control of soil-borne pathogens (Ghaffar, 1978, 1988,1992). *Trichoderma* has gained considerable success (Denis & Webster, 1971). *T. harzianum* protects the root system against *F. solani*, *R. solani* and *M. phasoelina* infection on a number of crops (Malik & Dawar, 2003). *Aspergillus versicolor* (Vuill.) Tirab., a native heat tolerant strain, is highly antagonistic to *Fusarium oxysporum* f.sp., *cumini* was isolated from arid soil and is even multiplied even at 65˚C (Israel & Lodha, 2005). *B. thuringiensis* is a plant growth promoting bacterium which produces bacteriocin compounds (Gray *et al*., 2006). Seed treatment with *Bacillus subtilis* have since been shown to control various diseases in a variety of crops, including diseases caused by *Rhizoctonia solani* Khün in wheat, brown spot of rice and damping off in tomato and sugarbeet (Merriman *et al.*, 1974). The objective of the present studies was to study the effect of seed coating with biocontrol agents in the control of root rot fungi.

Materials and Methods

Microbial antagonists viz., *Bacillus thuringiensis* (Bt 10), *Rhizobium meliloti* (R5), *Aspergillus niger* (An 20) and *Trichoderma harzianum* (KUCC 65) obtained from Karachi university culture collection (KUCC) were used.

Seeds of okra (*Abelmoschus esculentus* L.) and sunflower (*Helianthus annus* L.) were surface sterilized with 1% $Ca(OCl)_2$ for three minutes, rinsed thoroughly in running tap water and dried aseptically. The seeds were treated with microbial antagonists viz, *B. thuringiensis, R. meliloti, A. niger, T. harzianum* separately by using 1% and 2% sugar, mollases, glucose and gum arabic solution as a sticker. Ten seeds after treatment with suspension of microbial antagonists were transferred in test tube containing 9ml sterilized distilled water. The test tube was shaken and dilution series was made. One ml suspension was poured on PDA and cells/seed of bacteria and number of conidia/seed of fungi was calculated by using the formula: No. of cells or conidia seed X dilution factor.

Soil used for the experiments was obtained from the experimental plots of Department of Botany, University of Karachi and passed 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 24%. Soil had natural infestation of 1-3 sclerotia of *M. phaseolina* per g of soil as found by wet sieving dilution technique (Sheikh & Ghaffar, 1975), 5-10% colonization of *R. solani* on sorghum seeds used as baits (Wilhelm, 1955) and 3000 cfu of *Fusarium* spp., as assessed by soil dilution technique (Nash & Synder, 1962).

Seed coating with microbial antagonists protects the seeds from seed borne and soil borne pathogens, which enables the seed to germinate and become established as a healthy seedling (Chang & Kommeldahl, 1968). Seeds of sunflower (*Helianthus annus* L.) and okra (*Abelmoschus esculentus* L.) after treatment with 48 hrs. old cultures of *B. thuringiensis, R. meliloti* and 7 days old culture of *A. niger, T. harzianum* used for coating the seeds in 1 and 2% sugar, mollases, glucose and gum arabic solution as a sticker and 5 seeds/pot were sown in 8 cm diam., plastic pots, each pot containing 300g soil. There were three replicates of each treatment and pots without antagonists and

without seed coating material served as control. Pots were kept randomized in a screen house at the Department of Botany, University of Karachi, where soil was kept @ 40% M.H.C. (Keen & Raczkowski, 1922). Plants were uprooted after 30 days. Plant growth parameters in terms of root length, shoot length and fresh weights of root, shoot and incidence of root infecting fungi were recorded. After 30 days, roots of okra and 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 @ 20mg/l. Petri plates were incubated for 5 days, at room temperature to confirm infection of roots by root infecting fungi. Data were analyzed and were subjected to analysis of Variance (ANOVA) following the procedure as given by Gomez & Gomez (1984).

Results and Discussion

Population of bacteria and fungi after seed treatment was counted by serial dilution technique (Table 1). Seed dressing with microbial antagonists viz., *B. thuringiensis, R. meliloti, A. niger* and *T. harzianum* showed efficiency in the control of root rot fungi on crop plants. No significant increase in germination was observed when seeds of okra and sunflower were treated with microbial antagonists using 1 and 2% of sugar, mollases, glucose and gum arabic as stickers. Results showed that growth parameters in terms of shoot length, shoot weight, root length and root weight were significantly increased in sunflower and okra plants when seeds were treated with microbial antagonists viz, *B. thuringiensis, R. meliloti, A. niger* and *T. harzianum* ($p<0.001$) (Table 2). Among the different coating materials used as stickers gum arabic was found effective for growth parameters and in the control of root rot fungi ($p<0.001$) whereas mollases also showed some efficiency in reducing the root infection caused by root rot fungi ($p<0.01$) on sunflower and okra. The 2% gum Arabic solution showed more promising results in increasing shoot length, shoot weight ($p<0.001$), root length, root weight ($p<0.01$) and in reducing infection of root rot fungi viz., *M. phaseolina, R. solani* and *Fusarium* spp., on okra. Of the different microbial antagonists used *T. harzianum* showed most effective results in increasing germination of sunflower and okra seeds. Increase in shoot length, shoot weight, root length and root weights ($p<0.001$) was also observed. There was significant increase in growth parameters ($p<0.01$) and significant reduction in infection of *R. solani, M. phaseolina* and *Fusarium* spp., where seeds of okra and sunflower were treated with *T. harzianum* and *B. thuringiensis* (Table 3).

Seed treatment is an attractive method for introducing biocontrol agents into a soil root environment since it protects the seed from seed-borne and soil-borne pathogens and enable the seed to germinate and become established as a healthy seedling (Chang & Kommedahl, 1968). Antagonists applied to the seeds may have the opportunity to be the first colonizer of the roots (Chao *et al*., 1986). Similarly control of soil-borne pathogens by the addition of antagonistic microorganisms to the soil is also a potential non-chemical means for the plant disease control. *T. harzianum* is an effective biocontrol agent against several soil-borne and seed-borne fungal plant pathogen and has been extensively studied (Lewis & Papavizas, 1991; Elad *et al.*, 1982). Proposed mechanisms of this biocontrol are antibiosis (Ghisalberti *et al*., 1990), mycoparasitism (Singh & Faull, 1990) and competition or fungicidal action because of the ability of *Trichoderma* to produce antibiotics or hydrolytic enzymes (Lorito *et al*., 1994). Present results showed that seed dressing with *T. harzianum* and *A. niger* showed significant increase in plant height, weight and reduce the infection of root rot fungi in okra and sunflower. In the present study shoot length, shoot weight, root length and root weight were significantly increased

Table 1. Population of bacteria and fungi on seeds of okra and sunflower after seed treatment.

Treatments	CFU/ Seed			
	Okra		Sunflower	
	1%	2%	1%	2%
	A. niger			
Sugar	$16x10^5$	$13 x10^5$	$30 x10^5$	$24 x10^5$
Mollases	$14 x10^5$	$14 x10^5$	$16 x10^5$	$21 x10^5$
Glucose	$19 x10^5$	$12 x10^5$	$31 x10^5$	$34 x10^5$
Gum arabic	$13 x10^5$	$15 x10^5$	$15 x10^5$	$15 x10^5$
	T. harzianum			
Sugar	$11 x10^5$	$10 x10^5$	$15 x10^5$	$13 x10^5$
Mollases	$18 x10^5$	$17 x10^5$	$17 x10^5$	$14 x10^5$
Glucose	$12 x10^5$	$8 x10^5$	$22 x10^5$	$20 x10^5$
Gum arabic	$10 x10^5$	$5 x10^5$	$10 x10^5$	$7 x10^5$
	B. thuringiensis			
Sugar	$10 x10^5$	$8 x10^5$	$9 x10^5$	$16 x10^5$
Mollases	$38 x10^5$	$7 x10^5$	$9 x10^5$	$12 x10^5$
Glucose	$18 x10^5$	$10 x10^5$	$10 x10^5$	$12 x10^5$
Gum arabic	$14 x10^5$	$5 x10^5$	$12 x10^5$	$6 x10^5$
	R. meliloti			
Sugar	$53 x10^5$	$76 x10^5$	$60 x10^5$	$30 x10^5$
Mollases	$83 x10^5$	$40 x10^5$	$72 x10^5$	$48 x10^5$
Glucose	$35 x10^5$	$41 x10^5$	$35 x10^5$	$29 x10^5$
Gum arabic	$40 x10^5$	$70 x10^5$	$45 x10^5$	$32 x10^5$

in sunflower and okra when seeds were coated with *R. meliloti* and *B. thuringiensis*. Similar report was made by Siddiqui *et al*., (2000) on okra when *Rhizobia* was used as seed dressing and soil drenching significantly increased growth parameters and number of nodules. In the present investigation, *Rhizobium* used either as seed dressing significantly improved plant growth and reduced disease intensity of plants due to initial colonizers of the rhizosphere of test plants. *Rhizobium meliloti* significantly inhibited the infection of *R. solani* on okra plant when *R. meliloti* was multiplied on leaves powder of *Rhizophora mucronata* plant (Tariq *et al*., 2007). The growth promoting effect appears to be direct or indirect. Direct mechanism of growth promotion include the fixation of atmospheric nitrogen in leguminous plants only, production of growth regulators such as auxins, cytokinins and gibberillins like substances (Sheng, 1993) which act directly on plant itself and affect growth. Indirect mechanism may involve the production of toxic metabolites (Chakraborty & Purkayastha, 1984) which have their inhibitory effect on soil borne plant pathogens, thereby increase in plant growth. *Rhizobia* which are good rhizosphere organism for leguminous or non-leguminous plants presumably prevent the contact of pathogenic fungi on roots by covering the hyphal tips of the fungus and parasitizing it (Tu, 1978). It was also previously observed that *B. thuringiensis* applied as seed dressing showed a significant increase in seed germination, shoot length, shoot weight, root length and root weight (Sheikh *et al*., 2006). The results of the present study indicates the potentialities of seed treatment with different fungal and bacterial antagonists viz., *B. thurigiensis, R. meliloti*, *A. niger*, *T. harzianum* in the suppression of root infecting fungi on okra and sunflower. There is therefore need to characterize fungicidal compound produced by biological antagonists resulting in control of root infecting fungi instead of use of pesticides which are costly and hazardous.

Table 2. Effect of seed dressing with fungal and bacterial antagonists on growth parameters of okra and sunflower.

Treatments	Okra				Sunflower			
	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)
Fungal antagonists								
Control	8.3	0.7	12	0.5	12	1.1	8	0.23
A. niger 1 % sugar	7.7	0.4	5	0.1	20	1.3	10	0.24
A. niger 2 % sugar	12	0.6	9	0.3	24	1.5	7.7	0.14
A. niger 1 % mollases	11	0.4	5.7	0.4	32	1.7	10	0.16
A. niger 2 % mollases	9.7	1.2	11	0.4	14	1.8	6.3	0.15
A. niger 1 % glucose	10	1.2	8	0.2	22	3.2	9.3	0.8
A. niger 2 % glucose	16	1.3	16	0.1	13	3.3	15	0.53
A. niger 1 % gum arabic	11	0.9	11	0.1	9.7	1.5	10	0.09
A. niger 2 % gum arabic	19	0.3	19	0.2	27	1.5	5.7	0.06
LSD0.05=	3.2	0.1	4	0.2	96	0.4	3.7	0.13
Control	8.3	0.7	12	0.5	12	1.1	8	0.23
T. harzianum 1 % sugar	17	1.7	18	0.8	17	1.5	9.7	0.06
T. harzianum 2 % sugar	9	0.4	8.3	0.1	13	1.4	5.3	0.07
T. harzianum 1 % mollases	15	0.7	11	0.2	11	1.4	5	0.21
T. harzianum 2 % mollases	12	0.8	8.6	0.2	17	1.5	6.3	0.09
T. harzianum 1 % glucose	11	1.6	13	0.2	10	2.9	7	0.16
T. harzianum 2 % glucose	15	0.6	6.3	0.3	13	2.7	8	0.38
T. harzianum 1 % gum arabic	12	1.9	18	0.4	16	1.7	5.3	0.07
T. harzianum 2 % gum arabic	12	1.7	10	1.5	10	1.6	4.7	0.04
LSD0.05=	1.4	25	2	0.2	3	0.4	2	0.08

Table 2. (Cont'd.).

Treatments	Okra				Sunflower			
	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)
	Bacterial antagonists							
Control	8.33	0.73	12	0.5	12	1.1	8	0.23
B. thuringiensis 1 % sugar	12.3	0.24	15	0.4	29	2.6	17	1.07
B. thuringiensis 2 % sugar	10.8	0.48	18	0.1	32	4.1	20	1.36
B. thuringiensis 1 % mollases	9.83	0.18	11	0.1	26	2.4	14	15
B. thuringiensis 2 % mollases	13.7	0.36	9	0.1	16	2.1	13	0.37
B. thuringiensis 1 % glucose	9	0.37	11	0.1	22	3	10	0.51
B. thuringiensis 2 % glucose	12	0.64	6.7	0.1	15	2.5	9	0.83
B. thuringiensis 1 % gum arabic	13.3	0.58	17	0.1	39	2.6	20	0.41
B. thuringiensis 2 % gum arabic	10.3	0.18	5	0.1	17	1.3	9.7	0.61
LSD0.05=	3.19	0.16	49	0.2	3.8	0.3	4.7	0.17
Control	8.33	0.73	12	0.5	12	1.1	8	0.23
R. meliloti 1 % sugar	11.7	0.26	9.7	0.4	22	1.9	6	0.21
R. meliloti 2 % sugar	8.66	1.02	7.3	0.6	34	1.8	18	0.56
R. meliloti 1 % mollases	15.7	2.3	7	0.3	18	1.9	13	0.35
R. meliloti 2 % mollases	12.7	1.53	5	0.1	13	2.5	8.7	0.39
R. meliloti 1 % glucose	11	1.9	7.7	0.2	11	1.6	10	0.55
R. meliloti 2 % glucose	10	0.59	7	0.3	14	1.4	8.7	0.45
R. meliloti 1 % gum arabic	10	0.85	6.7	0.1	12	1.3	8.7	0.11
R. meliloti 2 % gum arabic	9.66	0.75	6	0.1	22	1.8	7	0.15
LSD0.05=	2.23	0.22	1.9	0.2	4.8	0.2	2.7	0.13

Table 3. Effect of seed dressing with fungal and bacterial antagonists in the control of root infecting fungi on okra and sunflower.

Treatments	Okra			Sunflower		
	Fusarium spp.	*Rhizoctonia solani*	*Macrophomina phaseolina*	*Fusarium* spp.	*Rhizoctonia solani*	*Macrophomina phaseolina*
	Fungal antagonists					
Control	55.55	55.55	100	88.88	55.55	100
A. niger 1 % sugar	0	0.00	0	0.00	0.00	66.66
A. niger 2 % sugar	0	0.00	0	0.00	0.00	44.44
A. niger 1 % mollases	88.88	0.00	88.88	0.00	0.00	100
A. niger 2 % mollases	33.33	0.00	44.44	11.11	0.00	44.44
A. niger 1 % glucose	11.33	0.00	33.33	11.11	44.44	55.55
A. niger 2 % glucose	22.22	0.00	44.44	0.00	33.33	44.44
A. niger 1 % gum arabic	0	0.00	100	0.00	33.33	66.66
A. niger 2 % gum arabic	0	0.00	33.33	0.00	11.11	0.00
LSD0.05=	49.64	11.1	49.35	20.01	52.95	49.5
Control	55.55	55.55	100	88.88	55.55	100
T. harzianum 1 % sugar	88.88	0.00	0.00	0.00	33.33	100
T. harzianum 2 % sugar	0.00	0.00	0.00	0.00	22.2	88.88
T. harzianum 1 % mollases	0.00	0.00	0.00	11.11	0.00	100
T. harzianum 2 % mollases	0.00	0.00	0.00	0.00	0.00	100
T. harzianum 1 % glucose	55.55	22.22	0.00	0.00	0.00	100
T. harzianum 2 % glucose	0.00	0.00	0.00	0.00	0.00	66.66
T. harzianum 1 % gum arabic	0.00	0.00	22.22	0.00	22.22	66.66
T. harzianum 2 % gum arabic	0.00	0.00	11.11	0.00	11.11	55.55
LSD0.05=	40.28	24.19	23.55	15.20	48.55	351

Table 3. (Cont'd.).

Treatments	Okra			Sunflower		
	Fusarium spp.	*Rhizoctonia solani*	*Macrophomina phaseolina*	*Fusarium* spp.	*Rhizoctonia solani*	*Macrophomina phaseolina*
	Bacterial antagonists					
Control	55.55	55.55	100	88.88	55.55	100
B. thuringiensis 1 % sugar	44.44	0.00	100	0.00	44.44	100
B. thuringiensis 2 % sugar	0.00	77.77	77.77	0.00	11.11	66.66
B. thuringiensis 1 % mollases	100	0.00	0.00	22.22	0.00	100
B. thuringiensis 2 % mollases	22.22	0.00	0.00	0.00	0.00	88.88
B. thuringiensis 1 % glucose	0.00	0.00	100	0.00	11.11	44.44
B. thuringiensis 2 % glucose	0.00	0.00	66.66	0.00	0.00	44.44
*B. thuringiensis*1%gum arabic	100	0.00	100	0.00	0.00	88.88
*B. thuringiensis*2%gum arabic	44.44	0.00	100	0.00	0.00	11.11
LSD0.05=	30.64	25.44	30.91	24.19	35.11	40.22
Control	55.55	55.55	100	88.88	55.55	100
R. meliloti 1 % sugar	0.00	0.00	100	0.00	0.00	100
R. meliloti 2 % sugar	33.33	0.00	44.44	0.00	0.00	11.11
R. meliloti 1 % mollases	0.00	11.11	100	0.00	0.00	88.88
R. meliloti 2 % mollases	33.33	0.00	0.00	0.00	0.00	22.22
R. meliloti 1 % glucose	100	55.55	0.00	0.00	0.00	44.44
R. meliloti 2 % glucose	100	33.33	0.00	0.00	0.00	33.33
R. meliloti 1 % gum arabic	0.00	0.00	100	0.00	11.11	88.88
R. meliloti 2 % gum arabic	0.00	0.00	100	0.00	0.00	0.00
LSD0.05=	31.64	38.25	22.2	11.10	32.6	49.34

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