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Short Communication

Effect of biopriming of radish (*Raphanus sativus*) seed with some antagonistic isolates of *Trichoderma*

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Application of biocontrol agent can be done in three ways (i) seed treatment, (ii) soil treatment and (iii) foliar spray. Seed treatment has been considered the most convenient of the three as it is required in less amounts with higher efficacy. Seed being an essential component of agriculture acts as a passive carrier of many diseases. Seed treatment with biocontrol agents may serve as an important tool for managing many soil-borne and seed borne diseases. This process is known as 'biopriming'. Goals for biological seed treatment include control of soil borne pathogens, colonization and protection of subterranean plant parts, and increased plant growth. Effective biocontrol agents have been developed for control of severe seed and seedling pathogens. Several researchers have reported the biological seed treatments for the protection of seed and control of pathogens causing seedling diseases (1,15,3). Similarly several scientists have got positive results of disease control and increased plant growth and yield *in vivo* and under field condition (6,4,2,11). The efficacy of biological seed treatment is influenced by soil pH and iron concentration (14), moisture, temperature and inoculums density of the pathogen (9). *Trichoderma* spp. has been to be dominant in both the rhizosphere and rhizoplane and associated with the roots throughout the life of plant (16). The number of active fungal propagules in the rhizosphere was highest in the seedling stage and decreased until about 50 days after which there was a gradual rise culminating in another peak at nearly 100 days (10). Two species of *Trichoderma viz*. *T. viride* and *T. harzianum* were isolated from collected soil samples of alluvial zone of Nadia district, West Bengal, India. Radish (*Raphanus sativus*) seeds were surface sterilized with 0.1% sodium hypochlorite solution for 3 minutes and then washed thoroughly with sterile distilled water. The seeds were treated with macerated mycelia mat of *Trichoderma* isolates grown in PDB @ 4g/kg of seed. The seeds of radish with culture filtrate and homogenised mycelia mat were shaken gently and separately in a wrist action shaker for 5 minutes for uniform mixing and seed coating. A pinch of carboxy methyl cellulose (CMC) sodium salt was added with the cultural filtrate and mycelia mat as adhesive during treatment so that both mat and cultural filtrate form a coating over the seed surface. After the completion of treatments the seeds were air dried under a laminar air flow hood with cover for 48 hours. The treated seeds were subsequently

used for germination in moist sand bed and under field conditions.

It is revealed that the germination of seed in all bioprimed treatments (TvO, ThC and ThR) were significantly increased over that of non-bioprimed seed (CD~ 1.18). Highest germination percentage (84.00%) was obtained when seeds were treated with ThC and TvO. Root length in all treatment 8.51 cm (TvO), 8.30 cm (ThC) and 8.09 cm (ThR) were significantly higher over that of non-bioprimed seed (CD~1.18). Highest shoot length and germination percentage was obtained with seed treated with TvO. Accordingly highest fresh and dry weight of root and shoot of 50 seedlings was obtained with isolate TvO. It is clear from the Table-2 that germination percentage of seed were significantly higher in case of isolate TvO (80.67%) and ThC (77.00%) in case of bioprimed radish seeds irrespective of *Trichoderma* isolates. The highest root length was obtained when seeds were bioprimed with ThC (5.55 cm) and the highest shoot length was obtained when seeds were bioprimed with ThR (11.96 cm). Vigour index of seedling in case of all bioprimed seeds irrespective of isolates were much higher than the control (non bioprimed, 776.01) seeds. The highest fresh weight of root (4.28 g) was obtained in radish seed bioprimed with isolate ThC and highest shoot weight 65.60 g was obtained in case of ThR. Highest root and shoot dry weight was obtained in case of radish seed bioprimed with ThR followed by seeds bioprimed with ThC. Table-3 clearly envisaged that there was no further increase in germination percentage of radish seeds. The root length in case of ThR was significantly higher (CD~ 1.43) than that of root length over control. Maximum shoot length (27.28 cm) was obtained when seeds were presowing treatment with ThR. The highest vigour index (3017.87) was obtained in case of radish seed bioprimed with *Trichoderma* isolates TvO followed by ThC and ThR, respectively. While considering the

Table 1.

Performance of bioprimed radish seed 14 days after sowing in moist sand bed*

Isolate no.	Germination Percentage (%)	Root Length (cm)	Shoot Length (cm)	Seedling vigour index	Fresh weight (g)		Dry weight (g)	
					Root	Shoot	Root	Shoot
TvO[#]	84.00	8.51	7.50	1345.20	0.510	3.405	0.104	0.436
ThC[##]	84.00	8.30	6.01	1202.00	0.440	2.630	0.160	0.278
ThR[###]	80.00	8.09	5.21	1064.00	0.261	2.008	0.103	0.179
Control	76.00	6.27	6.38	961.60	0.224	2.916	0.083	0.236
SEm±	0.394	0.312	0.330	-	0.019	0.168	0.018	0.023
CD (P=0.05)	1.180	0.961	1.017	-	0.059	0.506	0.054	0.069
CV	21.737	8.007	10.532	-	12.402	13.789	35.922	18.215

* Based on the observation of 50 seeds in each replication; [#] TvO- *Trichoderma viride* isolated from soil of okra; [##]ThC- *Trichoderma harzianum* isolated from soil of cauliflower; [###]ThR- *Trichoderma harzianum* isolated from soil of rice

fresh and dry weight of root and shoot for both bioprimed and non bioprimed seeds the highest were obtained in case of ThR. The leaf area was recorded using a Leaf Area Meter was maximum in case of isolate TvO.

The leaf areas obtained in all bioprimed seeds had no significant differences among themselves but differ significantly with that of non bioprimed seeds. Chlorophyll content of leaves was measured using Spectrophotometer showed that highest chlorophyll content was obtained in case of isolate TvO. The chlorophyll content in case of all bioprimed seeds irrespective of *Trichoderma* isolates was significantly much higher than that of control (non bioprimed seeds). It was seen from the Table-3 that chlorophyll content of bioprimed varied insignificantly among themselves.

Final data on performance of biopriming was recorded at harvest (57 DAS). Table-4 showed that the highest root length was obtained in seeds bioprimed with TvO and the root length of treated seeds varied significantly (C.D.= 2.77 at P≤ 0.05) with that of control. Highest root circumference was obtained in case of TvO followed by control and had no significant differences.

Table 2.

Field performance of bioprimed radish seeds 15 days after sowing*

Isolate no.	Germination Percentage (%)	Root Length (cm)	Shoot Length (cm)	Seedling vigour index	Fresh weight (g)		Dry weight (g)	
					Root	Shoot	Root	Shoot
TvO	80.67	4.67	10.564	1178.2	2.04	38.20	0.46	3.98
ThC	77.00	5.55	11.75	1332.56	4.28	55.70	0.74	5.86
ThR	68.67	4.89	11.96	1118.12	4.26	65.60	0.76	6.08
Control	59.33	3.87	9.206	776.01	2.77	43.50	0.72	4.74
SEm±	3.604	0.140	0.300	-	0.25	2.16	0.03	0.26
CD (P=0.05)	12.472	0.432	0.925	-	0.78	6.64	0.08	0.79
CV	-	6.657	6.172	-	16.95	9.49	8.43	11.06

* Based on the observation of 50 seeds

Table 3.

Field performance of bioprimed radish seeds 28 days after sowing*

Isolate no.	Germination Percent (%)	Root Length (cm)	Shoot Length (cm)	Root circumference	Seedling vigour index	Leaf area (sq cm)	Fresh weight (g)		Dry weight (g)		Chlorophyll content#
							Root	Shoot	Root	Shoot	
TvO	80.67	12.11	25.29	4.80	3017.87	101.38	9.8	12.2	0.5	0.84	6.52
ThC	77.00	12.59	25.46	4.14	2929.70	100.51	7.38	10.1	0.28	0.61	5.72
ThR	68.67	14.64	27.28	5.13	2878.37	100.23	14	12.8	0.92	0.87	6.21
Control	59.33	11.30	22.25	5.29	1990.64	58.95	6.4	8.8	0.43	0.82	3.06
SEm±	3.60	0.47	0.39	0.37	-	2.67	0.29	0.69	0.05	0.05	0.27
CD (P=0.05)	12.47	1.43	1.23	1.15	-	8.22	1.34	2.37	0.17	0.19	0.95
CV	-	8.22	3.55	17.37	-	6.61	4.62	10.88	15.42	11.71	9.32

* Based on the observation of 50 seeds; #chlorophyll content (mg/g of fresh weight)

Field performance of bioprimed radish seeds 57 days after sowing

Isolate no.	Root Length (cm)	Root circum-ference	Fresh weight of root (g)	Dry weight of root (g)
TvO	20.28	10.45	77.1	5.76
ThC	20.02	8.71	76.3	5.44
ThR	19.91	8.83	75.2	5.38
Control	17.15	10.28	70.4	4.8
SEm±	0.89	0.35	0.59	0.11
CD (P=0.05)	2.77	1.09	2.05	0.39
CV	10.40	8.25	1.37	3.62

*Based on the observation of 50 seeds

When fresh weight and dry weight of root of radish were considered maximum average fresh and dry weight of roots was obtained in case of isolate TvO. Enhancement of plant growth in association with microbial antagonist was in accordance with Harman *et al.* (6). They observed that biocontrol agents are effective as seed treatment organisms since they colonize roots, increase root mass, vigour and frequently provide increase in yield. Kleifeld and Chet (8) noted that *T. harzianum* applied to pathogen free soil induced an increase in seedling emergence, plant height, leaf area and dry weight. Several species of *Trichoderma* upon infection and colonization of the host rhizosphere may trigger the production of phenyl propanoic biosynthetic pathway leading to the production of some phenolic compounds with a significant range of biological functions including production of phytoalexin, a low molecular weight antimicrobial secondary metabolite (17). These organisms are known to produce growth promoting metabolites under a condition of very good rhizosphere competence and endophytism. Gravel *et al.* (5) have also claimed that isolates of *Trichoderma atroviride* produces Indole Acetic Acid which was responsible for growth promotion and yield increases in radish. Harman *et al.* (6) concluded that *T. harzianum* is capable of increasing the uptake of nutrients by secreting enzymes that solubilises the insoluble nutrients. β-1,3 glucanase and peroxidise being pathogenesis related protein have important roles in defence mechanism of host plant against many plant pathogens. Cowpea and pea seeds when primed with antagonist *Trichoderma* isolates and allowed to germinate on two different substrates, moist blotter and moist sand bed, the highest vigour index (3045) of seedling was obtained with isolate TvMar-1 using mycelial mat and the lowest (732) was with TvMar-2 using cultural filtrate for cowpea. The highest biomass (3206 mg) of the seedling shoot on dry weight basis was obtained with TvMar-2 while the highest biomass of root (528 mg) was obtained using ThLc-3 (12). Rawat *et al.* (13) revealed the data that rice seeds treated with *Trichoderma* alleviated the stress condition and significantly increased length and fresh weight of shoot and root, number of leaves, leaf area, photosynthetic rate, chlorophyll fluorescence, chlorophyll content and soil plant analysis development (SPAD) value in comparison to control at all stress levels.

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