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SCREENING OF CARRIER MATERIALS TO FORMULATE *TRICHODERMA HARZIANUM* BASED BIO-FUNGICIDE AGAINST FOOT AND ROOT ROT DISEASE OF TOMATO *(Lycopersicon esculentum* L.)

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Abstract

Eight different organic matters were tested for their suitability as carrier materials to prepare *Trichoderma harzianum* based bio-fungicides for controlling foot and root rot disease of tomato caused by *Sclerotium rolfsii.* Four independent experiments were conducted and found that the carrier materials used singly or in combinations were suitable to prepare the bio-fungicides. Mixed use of carrier materials gave better results as compared to single ones. When wheat bran + rice bran, wheat bran + MOC+ rice bran, grasspea bran + rice bran, and grasspea bran +MOC+ rice bran were used as carrier materials. *T. harzianum* based bio- fungicides reduced seedling mortality of tomato by 20.33, 19.33, 24.33, and 19.34%, respectively. Treatment of soil with those bio-fungicides previously infested with S. *rolfsii* caused considerable increased in shoot and root growth of tomato. Based on the findings of investigation, the above mentioned carrier materials might be used to prepare *T. harzianum* based bio-fungicides.

Keyword: *Trichoderma harzianum, Sclerotium rolfsii*, tomato seedling, bio-fungicide.

Introduction

Tomato *(Lycopersicon esculentum* L.) is one of the most important vegetable crops of Bangladesh in terms of area (24,800 ha) and production (232,459 mt) (Anon., 2012). It contributes about 20% of the total vegetable grown in the country and plays a vital role in human nutrition containing 15% vitamin, which is 100% edible (Gowda and Kaul, 1982). The average yield of tomato in Bangladesh is 56 t/ha, which is far below as compared to other countries of the world. Diseases are the major constraints for such low yield of the crop incurring about 30-40% yield loss annually (Anon., 1992). Among the diseases, seedling mortality due to the soil borne fungal pathogen, *Sclerotium rolfsii* is the prevalent one throughout the tomato growing areas of Bangladesh (Anon., 1992). It is very difficult to control the soil borne diseases of tomato caused by *S. rolfsii* through

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conventional method, such as application of fungicides, cultural methods, etc. On the other hand, application of chemical fungicides is expensive and may cause health hazards (Brown and Hendrix, 1980; Punja *et al.*, 1982). The beneficial microbes, such as *T. harzianum* have been identified as effective bio-control agents to formulate bio-fungicides against common soil borne plant pathogens like *S. rolfsii* (Elad *et al.*, 1983; Roy *et al.*, 1989). The bio-control agents usually present in soil is in low population. So, augmentation of their population density to higher level through artificial inoculation is necessary to achieve successful control of soil borne pathogens. For mass culture of bio-control fungi like *T. harzianum*, suitable carrier materials is yet to find out (Harman *et al.*, 1991). Research reports reveal that rice bran, wheat bran, maize bran, sawdust (Das *et al.*, 1997); rice straw, chickpea bran, grasspea bran, rice course powder, black gram bran (Shamsuzzaman *et al.*, 2003) and cow dung, poultry manure, groundnut shell, black ash (Rettinassababady and Ramadoss, 2000) are good carrier materials for multiplication of *T. harzianum.*

Considering the above facts, an investigation was conducted to screen eight organic materials available in Bangladesh as carrier materials for preparation of *T. harzianum* based bio-fungicides effective against *S. rolfsii* causing seedling mortality of tomato.

Materials and Method

Screening of carrier materials for *T. harzianum* bio-fungicides in plastic tray

The experiment was conducted in plastic tray to screen out the suitable substrates to formulate the *T. harzianum* based bio-fungicide against the tomato seedling mortality caused by *S. rolfsii*. The experiment was conducted in the net-house of Plant Pathology Division, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur in the year of 2009-10. A pure culture of bio-control agent *T. harzianum* (TM7) was used in the experiment. The fungus *T. harzianum* (TM7) was grown in potato dextrose agar (PDA) medium, which was used as inoculum of bio-fungicide. The substrates used in this study were rice husk, rice bran, wheat bran, maize bran, grasspea bran, soybean bran, and saw dust. Six hundred g of each substrate materials was mixed thoroughly in the plastic tray and then transferred to four 1000 ml Erlenmeyer flask. The flask with substrate materials were sterilized in an autoclave at 121^0C for 15 minutes and cooled down to make it ready for inoculation. The sterilized substrate was inoculated with 5 mm diameter mycelial disc of 5-day old culture of *T. harzianum* grown on PDA and then incubated at room temperature (25±2 ^{0}C) for 15 days. After incubation, the colonized substrates were removed from the flasks and air dried and finally preserved in refrigerator at 10 ^{0}C for future use. Besides, the pathogenic fungi *S. rolfsii* was multiplied on sterilized barley grains in 1000 ml Erlenmeyer flask at temperature of 25±2 ^{0}C for 15 days. The plastic trays were

filled with sterilized soil @ 4 kg/tray. In this experiment, both pathogenic fungus *S. rolfsii* and *T. harzianum* based bio-fungicide were used @ 20 g/kg soil each and seeds of BARI Tomato-2 (Raton) were sown in the tray soil @100 seeds per tray. The tray experiment was laid out in completely randomized design (CRD) with six replications. Data were collected on percent emergence and mortality of tomato seedlings.

Efficacy of carrier material based *T. harzianum* bio-fungicides in seed bed

The experiment was conducted to evaluate the different carrier materials to formulate the *T. harzianum* based bio-fungicides against foot and root rot disease of tomato caused by *S. rolfsii* in seed bed under net house condition. In this seed bed experiment, the colonized barley grains with *S. rolfsii* were incorporated in the seed bed soils @100 g/m^2 soil. Inoculated seed bed soil (one meter square) was allowed to multiply the pathogen *S. rolfsii* for 10 days with proper soil moisture. From plastic tray experiment, the effective four substrates viz., rice bran, wheat bran, maize bran, and grasspea bran were selected to formulate *T. harzianum* based bio-fungicide. The chemical fungicide Provax was used as seed treatment for comparison. The inoculums of *T. harzianum*, colonized on these substrates, were incorporated in the seed bed soils @100 g/m^2 soil and kept for 7 days with proper soil moisture for establishment of *T. harzianum* in the soils. The seeds of BARI Tomato-2 (Raton) were sown in the seed bed @ 200 seeds per seed bed. The experiment was laid out in completely randomized design (CRD) with four replications. Data were collected on percent seedling emergence, seedling mortality, shoot height, shoot weight, root length, and root weight of tomato seedlings. The percent data were converted into arcsine transformation values before statistical analysis. Data were analyzed statistically by using the MSTATC program. The treatment effects were compared by applying the least significant different (LSD) test.

Efficacy of mixed carrier material of *T. harzianum* based bio-fungicides

The experiment was conducted to evaluate the individual and combined effect of *T. harzianum* bio-fungicides in seed bed under net house condition. In this seed bed experiments, the colonized barley grains were incorporated in the seed bed soils @100 g/m^2 soil. Inoculated seed bed soil was allowed to multiply the pathogen *S. rolfsii* for 10 days with proper soil moisture. Three substrates viz., rice bran, wheat bran, grasspea bran and their combination (with equal proportion) with mustard oilcake were used. The inoculum of *T. harzianum*, colonized on different substrates were incorporated in the seed bed soils @ 100 g/m^2 soil and kept for 7 days with proper soil moisture for establishment of *T. harzianum* in the soils. The seeds of BARI Tomato-2 (Raton) were sown in the

seed bed @ 200 seeds per seed bed. The experiment was laid out in a completely randomized design (CRD) with four replications.

Data were collected on percent seedling emergence, seedling mortality, shoot height, shoot weight, root length, and root weight of tomato seedlings. The percent data were converted into arcsine transformation values before statistical analysis. Data were analyzed statistically by using the MSTATC program. The treatment effects were compared by applying the least significant different (LSD) test.

Results and Discussion

Screening of carrier materials for *T. harzianum* bio-fungicides in plastic tray

Results of the first experiment conducted to determine the efficacy of *T harzianum* based bio-fungicides having different carrier materials against S. *rolfsii* causing seedling disease of tomato, where the soil was artificially inoculated with the pathogen and treated with the bio-fungicides are presented in Table 1. Seedling emergence of tomato was 55% under control (untreated soil). Pre-sowing soil treatment with the *T. harzianum* based bio-fungicides multiplied on different carrier materials increased the seedling emergence to 61.00 -78.00%. The lowest increase of seedling emergence was recorded from saw dust and the highest was recorded from wheat bran carrier.

Maximum pre-emergence and post-emergence mortality of 45.00% and 43.00%, respectively, were recorded from the untreated control tray. Pre-sowing treatment of *S. rolfsii* inoculated soil with the bio-fungicides reduced pre-emergence seedling mortality to 22.00-39.00% and post-emergence mortality to 0.00-17.95%. Total seedling mortality was reduced by 31.05 - 66.00% over untreated control. The results showed that *T. harzianum* treated tray soil gave higher percentage (43.05 - 78.00%) of healthy seedlings, while untreated control tray soil produced only 12% healthy seedling in *S. rolfsii* inoculated soil. Due to pre-sowing soil treatment with *Trichoderma* based bio-fungicides, the highest percentage of healthy seedlings was achieved with the carrier material, wheat bran followed by maize bran, rice bran, and soybean bran. The lowest percentage of healthy seedlings was recorded from the carrier material sawdust followed by grasspea bran and rice husk. The overall performance of the bio-fungicides with respect to reduction of tomato seedling disease and increase of healthy seedlings indicated that the best carrier material of *T. harzianum* based bio-fungicides was wheat bran followed by maize bran, rice bran, and soybean bran.

Efficacy of carrier material based *T. harzianum* bio-fungicides in seed bed

Pre-sowing treatment of *S. rolfsii* inoculated soil with *T. harzianum* based bio-fungicides having grasspea bran, wheat bran, and maize bran as carrier materials

gave significant increase in seedling emergence of tomato as compared to control. The efficacy of three carrier materials of the bio-fungicides to enhance seedling emergence was not significantly different. The lowest seedling emergence of 40.75% was found in treatment Provax-200, which was statistically similar to untreated control and rice bran carrier material (Table 2).

Table 1. Effect of different carrier based *T. harzianum* bio-fungicides on the emergence and mortality of tomato seedling in *Sclerotium rolfsii* inoculated soils in plastic tray.

Name of carrier materials for *T. harzianum*	Seedling emergence (%)	Pre-emergence mortality (%)	Post-emergence mortality (%)	Total mortality (%)	Mortality reduction (%) over control	Total healthy seedling (%)
Rice husk	68.00 b (55.54)	32.00 bc (34.45)	3.05 c (10.06)	35.05 bc (36.30)	52.95	64.95 ab (53.69)
Rice bran	75.00 a (59.99)	25.00 c (30.00)	0.00 c (1.62)	25.00 c (30.00)	63.00	75.00 a (59.99)
Wheat bran	78.00 a (62.02)	22.00 c (27.97)	0.00 c (1.62)	22.00 c (27.97)	66.00	78.00 a (62.02)
Maize bran	77.00 a (61.34)	23.00 c (28.66)	0.00 c (1.62)	23.00 c (28.86)	65.00	77.00 a (61.34)
Grasspea bran	68.00 b (55.54)	32.00 bc (34.45)	5.76 bc (13.89)	37.76 bc (37.91)	50.24	62.24 ab (52.08)
Soybean bran	76.00 a (60.66)	24.00 c (29.33)	2.38 c (8.87)	26.38 c (30.90)	61.62	73.62 a (59.09)
Saw dust	61.00 bc (51.35)	39.00 ab (38.64)	17.95 b (25.06)	56.95 b (48.99)	31.05	43.05 a (41.00)
Untreated Control	55.00 c (47.86)	45.00 a (42.13)	43.00 a (40.97)	88.00 a (69.73)	--	12.00 c (20.27)
LSD (p=0.05)	5.15	5.16	13.85	14.28	--	14.29

Values in a column having same letter did not differ significantly (p=0.05) by LSD and the values within the parentheses were the Arcsine Transformed values.

Pre-emergence seedling mortality was 40.75 and 41.00% under the carrier materials grass pea bran and wheat bran, respectively, which were statistically similar but significantly lower compared to control and Provax-200 treatment. The highest pre-emergence seedling mortality of 59.25% was recorded from the treatment with Provax-200, which was statistically similar to the treatments with *Trichoderma* based bio-fungicides having the carrier materials maize bran, rice bran and untreated control (Table 2).

Table 2. Effect of selected carrier based *T. harzianum* bio-fungicides on the emergence and mortality of tomato seedling in *Sclerotium rolfsii* inoculated soils in seed bed.

Name of carrier materials for *T. harzianum*	Seedling emergence (%)	Pre-emergence seedling mortality (%)	Post-emergence seedling mortality (%)	Total seedling mortality (%)	Mortality reduction (%) over control
Grasspea bran	59.25 a (50.37)	40.75 b (39.64)	8.50 cd (16.77)	49.25 c (44.57)	38.77
Wheat bran	59.00 a (50.23)	41.00 b (39.77)	6.82 d (14.82)	47.82 c (43.75)	40.20
Maize bran	49.75 ab (44.88)	50.25 ab (45.15)	14.65 b (22.38)	64.90 bc (53.66)	23.12
Rice bran	52.50 abc (46.44)	47.50 ab (43.56)	13.00 bc (21.11)	60.50 c (51.06)	27.52
Provax-200	40.75 c (39.56)	59.25 a (47.45)	18.56 b (25.43)	77.81 ab (61.89)	10.21
Untreated Control	43.50 bc (40.97)	56.50 a (49.02)	31.52 a (34.08)	88.02 a (69.74)	--
LSD (p=0.05)	6.059	5.128	4.606	10.13	--

Values in a column having same letter did not differ significantly (p=0.05) by LSD and the values within the parentheses were the Arcsine Transformed values.

Significant decrease in post-emergence seedling mortality was achieved with all bio-fungicides having different carrier materials and also treatment with Provax-200. The maximum of 40.20% reduction in total seedling mortality was recorded when the carrier material was wheat bran, which was followed by grasspea bran, rice bran, and maize bran. The lowest reduction of only 10.21% was found when the soil was treated with Provax-200 (Table 2).

Shoot length and shoot weight of tomato seedlings increased significantly (P=0.05) over control due to treatment of *S. rolfsii* inoculated seed bed soil with *T. harzianum* based bio-fungicides where the carrier materials were grasspea bran, wheat bran, maize bran, rice bran and with a fungicide Provax-200 (Table 3). The highest increase was achieved with grasspea bran. Its effectiveness was statistically similar to wheat bran and rice bran. The maximum increase in shoot weight was also recorded from grasspea bran followed by wheat bran, maize bran, and rice bran. Their efficacy to increase shoot weight was significantly different. The lowest increase of shoot growth was recorded from Provax-200 treatment. Treatment of seed bed soil with four bio-fungicides gave significant increase in length and weight of roots as compared to untreated control. The maximum increase was achieved with the carrier material grasspea bran, which

was statistically similar to wheat bran and rice bran. Increase in root growth over control was found minimum under Provax-200 treatment (Table 3).

Table 3. Effect of selected carrier based *T. harzianum* bio-fungicides on the vegetative growth of tomato seedling in *Sclerotium rolfsii* inoculated soils in seed bed.

Name of carrier materials for *T. harzianum*	Shoot length (cm)	Shoot wt (g/plant)	Root length (cm)	Root wt (g/plant)
Grasspea bran	27.10 a	4.24 a	13.10 a	0.55 a
Wheat bran	25.88 ab	3.32 b	13.00 a	0.53 a
Maize bran	24.05 b	2.99 c	10.95 b	0.39 b
Rice bran	25.10 ab	2.70 d	11.10 ab	0.52 a
Provax-200	20.33 c	1.77 e	10.23 bc	0.45 ab
Untreated control	16.05 d	1.26 f	8.73 c	0.23 c
LSD (p=0.05)	2.632	0.2235	1.223	0.095

Values in a column having same letter did not differ significantly (p=0.05) by LSD.

Efficacy of mixed carrier material of *T. harzianum* based bio-fungicides

Seedling emergence of tomato sown in *S. rolfsii* inoculated soil in seed bed without pre-sowing treatment (control) was only 50%. Treatment of the inoculated soil with *T. harzianum* based bio-fungicides having mixed carrier materials and with Provax-200 increased the seedling emergence to 60.00-66.33%. Maximum seedling emergence was observed in case of Provax-200 treatment followed by the bio-fungicides having mixed carrier materials grasspea bran + rice bran and grasspea bran alone. Pre-emergence and post-emergence seedling mortality was 50% and 17%, respectively, under control. Due to treatment of soil before sowing reduced pre-emergence mortality to 33.67-40.00% and post-emergence mortality to 6.00-8.00. Application of Provax-200 gave maximum of 25% reduction in total seedling mortality (25.33%) over control followed by combined use of grasspea bran + rice bran based bio-fungicide and the minimum seedling mortality was observed in case of wheat bran based bio-fungicide. The findings indicated that combined use of grasspea bran + rice bran based bio-fungicides was equally effective as Provax-200 in controlling seedling mortality of tomato (Table 4).

Length of shoot and weight of shoot and root of tomato seedlings was increased significantly due to treatment of *S. rolfsii* inoculated seed bed soil with *T. harzianum* based bio-fungicides having single and mixed carrier materials and seed treatment with Provax-200. The longest root (29.77 cm) of tomato was recorded from the treatment with bio-fungicides having mixed carrier materials

grasspea bran + MOC + rice bran based *T. harzianum* bio-fungicide, while the shortest root length (23.43 cm) was obtained from the control bed. Shoot weight of tomato seedlings also followed the similar trend of results due to the bio-fungicides and Provax-200.

Table 4. Effect of carrier based *Trichoderma harzianum* bio-fungicides and Provax-200 on suppression of tomato seedling disease caused by *Sclerotium rolfsii* in seed bed.

Name of carrier materials for *T. harzianum*	Seedling emergence (%)	Pre-emergence seedling mortality (%)	Post-emergence seedling mortality (%)	Total seedling mortality (%)	Seedling mortality reduction (%) over control
Wheat bran	60.00 b (50.77)	40.00 b (39.23)	8.67 b (17.12)	48.67 b (44.23)	18.33
Grasspea bran	63.33 ab (52.73)	36.67 bc (37.27)	8.00 b (16.43)	44.67 b (41.94)	22.33
Rice bran	60.00 b (50.77)	40.00 b (39.23)	8.33 b (16.78)	48.33 b (44.04)	18.67
Wheat bran + Rice bran	60.00 b (50.77)	40.00 b (39.23)	6.67 b (14.97)	46.67 b (43.09)	20.33
Wheat bran + MOC+ Rice bran	60.00 b (50.77)	40.00 b (39.23)	7.67 b (16.08)	47.67 b (43.66)	19.33
Grasspea bran + Rice bran	64.33 a (53.33)	35.67 c (36.67)	7.00 b (15.34)	42.67 c (40.78)	24.33
Grasspea bran +MOC + Rice bran	60.67 b (51.16)	39.33 b (38.84)	8.33 b (16.78)	47.66 b (43.65)	19.34
Rice bran + MOC	60.00 b (50.77)	40.00 b (39.23)	7.33 b (15.71)	47.33 b (43.47)	19.67
Wheat bran + Grasspea bran + MOC	60.33 b (50.96)	39.67 b (39.03)	7.67 b (16.08)	47.34 b (43.47)	19.66
Wheat bran + Grasspea bran+ Rice bran + MOC	62.00 ab (51.94)	38.00 bc (38.05)	6.00 b (14.18)	44.00 b (41.65)	23.00
Seed treatment with Provax-200	66.33 a (54.53)	33.67 c (25.47)	8.00 b (16.43)	41.67 c (40.20)	25.33
Untreated Control	50.00 c (45.00)	50.00 a (45.00)	17.00 a (24.35)	67.00 a (54.93)	--
LSD (p=0.05)	3.52	2.28	3.25	3.76	--

Values in a column having same letter did not differ significantly (p=0.05) by LSD and the values within the parentheses were the Arcsine Transformed values.

Maximum of 8.03 cm root length was obtained from rice bran + MOC based bio-fungicide followed by seed treatment with Provax-200 (7.90 cm) and the minimum root length was found in untreated control. Maximum root weight of tomato seedlings were recorded from the wheat bran + rice bran based *T. harzianum* bio-fungicides and minimum weight from the control seed bed. The shoot and root growth of tomato seedlings indicate that the combined use of carrier materials for *T. harzianum* based bio-fungicides gave better results compared to single carrier material and seed treatment with Provax-200 (Table 5).

Table 5. Effect of carrier based *Trichoderma harzianum* bio-fungicides and Provax-200 on the vegetative growth of tomato seedling in *Sclerotium rolfsii* inoculated seed bed.

Name of carrier materials for *T. harzianum*	Shoot length (cm)	Shoot wt (g/plant)	Root length (cm)	Root wt (g/plant)
Wheat bran	27.87 b	4.21 bcd	7.27 abcd	0.42b
Grasspea bran	27.20 b	4.21 bcd	6.67 cde	0.41c
Rice bran	27.67 b	4.18 cd	7.20 abcd	0.42 b
Wheat bran + Rice bran	27.87 b	4.11 d	7.50 abc	0.43 a
Wheat bran + MOC+ Rice bran	27.73 b	4.07 d	7.00 bcd	0.40 d
Grasspea bran + Rice bran	27.93 b	4.29 abc	6.67 cde	0.42 b
Grasspea bran +MOC+ Rice bran	29.77 a	4.38 abc	7.60 abc	0.42 b
Rice bran + MOC	28.33 ab	4.24 bcd	8.03 a	0.41 c
Wheat bran + Grasspea bran + MOC	28.27 ab	4.50 a	7.27 abcd	0.40 d
Wheat bran + Grasspea bran+ Rice bran + MOC	28.80 ab	4.43 ab	6.47 de	0.40 d
Seed treatment with Provax-200	28.20 ab	4.27 bcd	7.90 ab	0.42 b
Untreated Control	23.43 c	3.24 e	5.73 e	0.32 e
LSD (p=0.05)	1.428	0.1515	0.897	0.05

Values in a column having same letter did not differ significantly (P=0.05) by LSD.

Emergence of tomato seedlings in *S. rolfsii* inoculated seed bed soil was significantly (p=0.05) higher in soil treated with *T. harzianum* based bio-fungicide having different carrier materials. Maximum seedling emergence of 89.33% was recorded from the seed bed treated with wheat bran + MOC + Rice bran based bio-fungicide followed by rice bran (80.67%). The minimum of 58.33% seedling emergence was recorded from untreated control; post-emergence mortality of tomato seedling was the highest (41.67%) under control. All the treatments with

T. harzianum based bio-fungicides and seed treatment with Provax-200 gave significant decrease of post-emergence seedling mortality. The efficacy of all the treatments to reduce seedling mortality was statistically similar.

Table 6. Effect of carrier based *Trichoderma harzianum* bio-fungicides on reduction of seedling disease (*Sclerotium rolfsii*) and vegetative growth of tomato in seed bed.

Name of carrier materials for *T. harzianum*	Seedling emergence (%)	Post-emergence seedling mortality (%)	Shoot length (cm)	Shoot wt (g/plant)	Root length (cm)	Root wt (g/plant)
Wheat bran	79.00 b (63.26)	12.00 b (20.26)	5.83 c	2.32 c	6.20 ab	0.49
Grasspea bran	72.33 b (61.59)	12.33 b (20.54)	6.07 c	2.37 c	6.10 ab	00.53
Rice bran	80.67 b (58.31)	12.00 b (20.26)	6.10 c	2.41 c	5.93 ab	0.48
Wheat bran + Rice bran	79.67 b (63.30)	11.33 b (19.60)	7.10 b	2.90 b	6.20 ab	0.49
Wheat bran + MOC+ Rice bran	89.33 a (71.06)	12.33 b (20.49)	6.73 bc	2.95 b	6.53 a	0.48
Grasspea bran + Rice bran	79.67 b (63.35)	11.67 b (19.96)	8.23 a	3.82 a	6.80 a	0.57
Grasspea bran +MOC+ Rice bran	80.00 b (63.46)	11.33 b (19.67)	8.87 a	3.86 a	6.73 a	0.51
Rice bran + MOC	79.00 b (62.90)	12.67 b (19.67)	8.50 a	3.84 a	6.40 a	0.56
Wheat bran + Grasspea bran + MOC	77.33 (61.70 b)	13.00 b (19.96)	8.42 a	3.84 a	6.80 a	0.52
Wheat bran + Grasspea bran+ Rice bran + MOC	73.33 b (62.15)	12.00 b (20.26)	8.73 a	3.89 a	6.53 a	0.58
Seed treatment with Provax-200	77.67 b (59.12)	13.00 b (21.09)	6.00 c	2.25 c	5.87 ab	0.46
Untreated Control	58.33 c (49.83)	41.67 a (34.43)	5.10 d	1.90 c	5.40 b	0.45
LSD (p=0.05)	6.373	2.156	0.86	0.475	0.881	NS

Values in a column having same letter did not differ significantly (P=0.05) by LSD and the values within the parentheses were the Arcsine Transformed values.

Under untreated control, shoot length, root length, shoot weight, and root of tomato were 5.10 cm, 1.90 g, 5.40 cm, and 0.45 g, respectively. The parameters increased to 5.83-8.73 cm, 2.32-3.89 g, 6.10-6.80 cm, and 0.48-0.58 g, respectively, due to soil treatment with bio-fungicides having single or mixed carrier materials. The increase in length of shoot and root, and length of root was significant compared to control. Mixed use of carrier materials to prepare *T. harzianum* based bio-fungicides gave better root growth of tomato seedlings compared to single carrier material and seed treatment with Provax-200 (Table 6). *T. harzianum* based bio-fungicides were seemed to be superior for tomato seedling disease (*S. rolfsii)* management in seed bed condition.

Soil amendment with formulated *Trichoderma* proved to be effective in controlling *S. rolfsii,* the causal fungal pathogen of seedling disease of many crops. Many researchers reported antagonistic activity of *Trichoderma* isolates against plant pathogens, especially against fungal pathogens, such as *Rhizoctonia solani, Fusarium oxysporum,* and *Sclerotium rolfsii* (Lo *et al.,* 1996; Tran, 1998; Bari *et al., 2000;* Shamsuzzaman *et al.,* 2003; Ngo *et al.,* 2006; Shalini *et aI.,* 2006). The formulated *T. harzianum* grown on peat soil based black bran was found to be effective in controlling nursery diseases like damping off, tip over, and seedling blight of egg plant and also promoted seed germination (Meah *et al.,* 2004). Rice bran gave 35% higher seed germination in eggplant, which is supported by the report of Sangeetha *et al.* (1993) who found wheat and rice bran as the best substrate for the formulation of *T. harzianum* bio-fungicides. The disease incidence of tomato, water melon, and cotton was reported to be reduced considerably by the application of *T. harzianum* (Sivan and Chet, 1986). Ghaffar (1987) reported that the *In-vitro* growth of *S. oryzae* and formation of sclerotia was drastically inhibited by the presence of *T. harzianum.* Similarly, Jee and Kim (1987) reported that *T. harzianum* was the best *In-vitro* antagonist soil borne pathogens. Begum *et al.* (1999) reported that *T. harzianum* treated seeds of black gram gave 86.70/00 to 100%) reduction of foot and root rot disease caused by *S. rolfsii* over the control. Tehroni and Nazari (2004) recognized *T. harzianum* as an effective remedy against *Phytophthora* damping-off of cucumber. Shoresh *et al.* (2005) stated that *Trichoderma* spp. are effective bio-control agents for a number of soil borne plant pathogens and induced a potentate state in the plant enabling it to be more resistant to subsequent pathogen infection.

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