Management of Pre- and Post-emergence Damping off of nursery seedlings of Brinjal with the application of seed coating with Bio-antagonists vis-a-vis soil application with plant products: an integrated approach

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J. Mycopathol. Res. 60(2) : *237-244, 2022;*
ISSN 0971-3719

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J. Mycopathol. Res. **60(2)** : 237-244, 2022; (ISSN 0971-3719)

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Received : 15.01.2022 Accepted : 21.04.2022 Published : 27.06.2022

Green house experiment was conducted to evaluate the combined efficacy of five biological antagonists (three fungal, namely,*Trichoderma viride*, *T. harzianum*, *Aspergillus niger* and two bacterial, namely, *Pseudomonas fluorescens*, *Bacillus subtilis* st. 12) and five plant extracts (essential oils and plant extracts) namely, Citronella (*Cymbopogon winterianus*) oil, Palmarosa (*C. martini* var. *motia*) oil, Lemon grass (*C. citratus*) oil, Babul (*Acacia nilotica*) leaf extract and Tamarind (*Tamarindus indica*) leaf extract against *Pythium aphanidermatum* (Edson) Fitz., causing Pre- and Post- emergence damping off disease of nursery seedlings of Brinjal. ED_{50} value was calculated for each extract against the bio-antagonists and also against the pathogen were tested following poisoned food technique. Seed coating with five bio-antagonists were used as five main treatments and soil application of five plant extracts used as sub-treatments under each main treatment.The results revealed that, integration of bio-antagonists as seed treatment and soil application of plant extracts reduced the seedling diseases as well as increased the germination percentage significantly as compared to untreated control. The best result was observed in seed treatment with *T. viride* (4 x 10^6 spores / ml) + soil drenching with Palmarosa oil (0.025%) or Citronella oil (0.020%) or Lemon grass oil (0.020%) closely followed by seed treatment with *T. harzianum* (4 x 10^6 spores / ml) + soil drenching with Palmarosa oil (0.025%) or Citronella oil (0.020%).

Key words: Botanical pesticides, integrated management, methanolic extract, microbial antagonists, sick soil, soil borne disease

INTRODUCTION

The major vegetables grown throughout the year in the new alluvial zones of West Bengal include tomato, cabbage and cauliflower in the winter and brinjal and chilli in both winter and rainy seasons. Now-a-days, hybrid varieties are being extensively used for maximum return. Introduction of high yielding genotypes are mainly susceptible to different diseases under changing cropping system as well as continuous mono-cropping have resulted in a spurt of diseases practically in all crops including vegetables.

In addition, there has been a dramatic change in disease problems during the last few decades because of excessive use of chemicals with no concern to ecology and non- target effects. Brinjal (*Solanum melongena*) has been regarded as one of the most important cash crop grown respectively under a wide range of agro climatic conditions throughout the year in India as well as in West Bengal. Major constrains for cultivation of this crop are pre- and post- emergence damping off caused by *Pythium aphanidermatum* (Edson) Fitz. Raising of seedlings in seedbed often faces maximum threat and sometimes total loss of costly seeds owing this notorious soil-borne pathogen. Over the last two decades, a lot of focus has been shifted towards developing ecofriendly management within the Integrated Disease Management system without affecting our precious ecosystem. Prospects of use of plant products or botanicals for plant disease control have been explored by different workers in soil-borne, foliar and post harvest diseases (Patil *et. al.* 2002, Tripathi *et. al*. 2003, Singh, *et. al*. 2003, Kulkarni, 2009

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Sreenivasa *et. al*. 2011) and various fungal and bacterial antagonists have been used experimentally for control of certain plant diseases (Gawande, *et.al*., 2009 and Singh *et. al.* 2010). The objective of the present investigation was to evaluate the efficacy of controlling the plant diseases by integration of biological antagonists as seed-coating with common bio-friendly plant extracts as soil drenching. This method has a great potential in sustainable agriculture management in the framework of organic farming system. Integration of seed treatment with microbial bio-agents in combination with soil application of plant extracts may enhance the establishment of desired bio-antagonists and provide better control of seed and seedling diseases than used separately.

MATERIALS AND METHODS

Isolation of pathogen

The pathogen was isolated from the infected seedlings of tomato by tissue segment method (Rangaswami, 1968) and identified as *Pythium aphanidermatum* (Acc. No. 5614.03, 5613.03) from Indian Type culture collection, Division of Plant Pathology, IARI. Pathogenicity test was conducted following Koch's postulates.

Screening of botanicals

Bio-assay of seven botanicals viz. Citronella (*Cymbopogon winterianus*) oil, Palmarosa (*C. martini* var. *motia*) oil, Lemon grass (*C. citratus*) oil, Neem (*Azadirachta indica*) seed oil, Babul (*Acacia nilotica*) leaf extract, Tamarind (*Tamarindus indica*) leaf extract and Neem (*Azadirachta indica*) leaf extract against the test pathogen was carried out to evaluate the tolerance limit by poisoned food technique at different dosages. The oils were extracted from the aromatic grasses, Citronella and Palmarosa, Lemon grass and Neem seed through hydrodistillation and the leaf extracts were obtained through methanolic extraction of leaves of Tamarind, Neem and Babul in Centre for Aromatic and Medicinal plant, Central Regional Research Farm, Gayeshpur, Nadia, West Bengal. Tween- 80 (0.1%) was used as emulsifier for preparing the aqueous solution of the essential oils and preserved as 100% stock solution.

Collection of Bio-antagonists

The fungal bio-antagonists *Trichoderma harzianum* (Acc. No. 6017.05), *Trichoderma viride* (Acc. No. 6018.05) and *A. niger* (AN-27, Kalisena) were collected from Indian Type culture collection, Division of Plant Pathology, IARI, New Delhi. Among the two bacterial antagonists, *Pseudomonas fluorescens* was collected from Centre in advanced Studies in Plant Pathology, Gobinda Ballav Pant University of Agriculture and Technology, Uttarakhand and *B. subtilis* st.12 (CMI No. 349545) from Department of Plant Pathology, BCKV.

Laboratory Experiment

The experiment was carried out under Laboratory condition in Departmentof Plant Pathology, BCKV, Mohanpur, Nadia, West Bengal. Five biological antagonists (three fungal namely, *T. viride*, *T. harzianum*, *A. niger* and two bacterial namely *Ps. fluorescens*, *B. subtilis* st.12) and five plant extracts, namely,Citronella (*Cymbopogon winterianus*) oil, Palmarosa (*C. martini* var. *motia*) oil, Lemon grass (*C. citratus*) oil, Babul (*Acacia nilotica*) leaf extract, Tamarind (*Tamarindus indica*) were selected from laboratory experiment through poisoned food technique against *Pythium aphanidermatum*, the causal organism of pre- and post emergence damping off of Brinjal (*Solanum melongena* cv. *pusaswani*) and ED_{50} values for all plant extracts were calculated (Table 1). As both Neem (*Azadirachta indica*) seed oil and leaf extract failed to show any growth inhibition of the test pathogen even @ 15% and 10% respectively, they were excluded from further experiment to continue.The efficacy of all the fungal bio-antagonists to withstand the previously selected ED_{50} values of the plant extracts were tested following poisoned food technique (Table 2).For bacterial bio-antagonists, the tolerance level was tested by measuring the inhibition zone at the concentrations just higher than the ED_{50} value of each extract found for the pathogen (Table 3) following paper-disc plate method. For an integrated approach of disease management, seed coating with bio-antagonists combined with soil application of plant extracts were applied. Seed coating with five microbial bio-antagonists were done and applied as five main treatments and soil application of five plant extracts were used as sub-treatments under each main treatment.

Seed coating

Seed coating with fungal spores was done by dipping the surface sterilized seeds in spore suspension of bio-antagonistic fungi @ 10 ml/ 10g

Table 1: ED_{50} and ED_{90} (MIC) values of plant extracts for inhibition of radial growth of *P. aphanidermatum*, *T. viride*, *T. harzianum* and *A. niger* in *in vitro* condition

Microorganism		Plant extracts (%)						
		Palmarosa Oil	Lemon grass oil	Citronella oil	*A. nilotica* leaf extract	*T. indica* leaf extract	Neem oil	Neem leaf extract
Pythium aphanidermatum	ED_{50}	0.021	0.017	0.017	3.381	1.253	Upto 15% no inhibition	Upto 10% no inhibition
	ED_{90}	0.077	0.023	0.055	4.370	2.343		
T. viride	ED_{50}	0.041	0.019	0.043	5.880	1.605		
	ED_{90}	0.082	0.039	0.066	26.585	2.313		
T. harzianum	ED_{50}	0.036	0.036	0.042	8.131	1.374		
	ED_{90}	0.088	0.047	0.065	20.353	2.414		
A. niger	ED_{50}	0.029	0.021	0.032	4.136	1.651		
	ED_{90}	0.065	0.042	0.115	4.370	2.384		

Table 2: Effect of plant extracts on inhibition of *Ps. fluorescens* and *B. subtilis* st. 12 in *in vitro* condition

Plant extracts	Concentration (%)	Inhibition zone (mm) of Microorganisms (6 DAI)	
		Ps. fluorescens	*B. subtilis* st. 12
Palmarosa oil	0.010	2.0	3.0
	0.25	4.0	5.5
Lemon grass oil	0.010	0.0	0.0
	0.020	2.0	1.0
Citronella oil	0.010	0.0	0.0
	0.020	3.0	2.0
A. nilotica leaf extract	3.0	10.0	9.0
	4.0	30.0	25.0
T. indica leaf extract	1.0	12.0	14.0
	1.5	20.0	22.0
Control	-	0.0	0.0

containing (4×10^6 spores/ ml) and 0.1% carboxy methyl cellulose for 1 hour and then shade-dried. CMC was used as sticking agent.

Bacterial seed coating was done by dipping the surface sterilized seeds in bio-antagonistic bacterial suspension containing (3×10^8cfu/ ml) and 0.1% carboxy methyl cellulose for 1 hour and then shade-dried. 10 ml suspension was used for 10g of seeds.Seed coated with CMC without any bio-antagonists served as control.

Green House Experiments

The green house experiments were conducted for consecutive two years (2018-2019).Seed coating with five microbial bio-antagonists were applied as five main treatments and soil drenching of five plant extracts were used as sub-treatments under each main treatment.

Soil application

Wooden trays of 30 cm× 20 cm× 6 cm dimension were filled with sterilized potting compost (65 kg loamy garden soil: 20 kg compost: 150 g wood ash) and were inoculated with *P. aphanide-rmatum* (multiplied in sand maize meal medium) at a ratio of 1: 20 w/w ratio of pathogen and soil and kept under polythene cover for 2 days allowing pathogenic growth. After 2 days of inoculation, 75 bio-antagonists coated seeds of Brinjal were sown individually. Simultaneously, the soil was drenched with different plant extracts (@ 0.025% palmarosa oil, 0.020% lemon grass oil, 0.020% citronella oil, 4.0% *A. nilotica* leaf

Table 3: Effect of seed coating with Bio-antagonists and soil drenching with Plant extracts on germination percentages of Brinjal (2 years' pooled mean)

Bio-antagonists (seed coating)	Germination percentage													
	Soil application of plant extracts													
	Palmarosa oil (0.025%)		Lemon grass oil (0.020%)		Citronella oil (0.020%)		*A. nilotica* leaf extract (4.0%)		*T. indica* leaf extract (1.5%)		Control		Mean (Main treatment)	
	14 DAS	28 DAS	14 DAS	28 DAS	14 DAS	28 DAS	14 DAS	28 DAS	14 DAS	28 DAS	14 DAS	28 DAS	14 DAS	28 DAS
B. subtilis st.12	61.36 (51.59)	68.78 (56.07)	57.83 (49.51)	61.95 (51.93)	50.44 (45.25)	58.08 (49.65)	51.50 (45.86)	59.50 (50.48)	52.58 (46.48)	56.83 (48.94)	44.25 (41.67)	50.33 (45.19)	52.99 (46.73)	59.25 (50.37)
Ps. fluorescens	61.73 (51.79)	67.25 (55.11)	60.11 (50.84)	63.0 (52.57)	56.01 (48.46)	62.21 (52.09)	56.67 (48.83)	64.24 (53.28)	55.43 (48.11)	61.07 (51.39)	44.25 (41.67)	50.33 (45.19)	55.69 (48.28)	61.35 (51.61)
T.harzianum	69.75 (56.65)	75.58 (60.39)	62.92 (56.76)	73.42 (59.01)	64.92 (53.70)	71.08 (57.51)	64.45 (53.42)	72.60 (58.52)	61.41 (51.61)	69.58 (56.59)	44.25 (41.67)	50.33 (45.19)	62.45 (52.30)	68.77 (56.20)
T. viride	71.42 (57.69)	79.25 (62.91)	72.08 (58.13)	76.75 (61.22)	66.25 (54.52)	74.50 (59.68)	67.13 (55.05)	76.54 (61.10)	65.77 (54.22)	73.95 (59.38)	44.25 (41.67)	50.33 (45.19)	64.48 (53.54)	71.89 (58.25)
A. niger	62.16 (52.07)	72.72 (58.66)	61.58 (51.75)	68.08 (55.69)	57.33 (49.24)	65.92 (54.33)	58.42 (49.88)	68.08 (55.73)	55.92 (48.43)	64.92 (53.77)	44.25 (41.67)	50.33 (45.19)	56.61 (48.84)	65.01 (53.89)
Mean (Sub-treatment)	65.28 (53.96)	72.72 (58.63)	62.90 (53.40)	68.64 (56.08)	58.99 (50.23)	66.36 (54.65)	59.63 (50.61)	68.19 (55.82)	58.22 (49.77)	65.27 (54.01)	44.25 (41.67)	50.33 (45.19)	58.45 (49.94)	65.25 (54.06)

	SEm ±	CD at 5%
Treatment (soil application)	0.59	1.66
Bio-antagonists	0.54	1.51
Days after sowing ((DAS)	0.34	0.96
Treatment × Bio-antagonists	1.32	NS
Treatment × DAS	0.84	NS
Bio-antagonists × DAS	0.76	NS
Treatment× Bio-antagonists × DAS	1.87	NS

* Figures in the parenthesis are indication of angular transformed value.

extract and 1.5% *T. indica* leaf extract) separately 24 hours after sowing of the seeds, so that each individual bio-antagonist coated seeds used as main treatment could interact with five plant extracts separately, used as sub-treatment. Trays were kept in complete randomized block design (CRBD) with three replications for each of the test botanical. Control treatment was maintained by sowing only CMC coated seeds in artificially inoculated soil. Data recording regarding germination, pre- and post- emergence damping off was done on 14 and 28 days after sowing.

RESULTS AND DISCUSSION

Different concentrations of each plant extracts were tested against the test pathogen *P. aphanidermatum* as well as the non-target fungal bio-antagonists and the ED_{50} and ED_{90} (MIC) values were calculated. Calculations revealed that (Tables 1 & 2), the ED_{50} and ED_{90} values of every plant extract were lower for *P. aphanidermatum* (0.021% and 0.077%, 0.017% and 0.023%, 0.017% and 0.055%, 3.381% and 4.370%, 1.253% and 2.343% for Palmarosa oil, Lemon grass oil, Citronella oil, *A.nilotica* leaf extract and *T.indica* leaf extract respectively)than the other test fungus. This clearly indicated that the fungal bio-antagonists could grow well in that concentrations of the respective extracts required to minimize the target pathogen by 50% and 90%. Minimum ED_{50} against *P. aphanidermatum* was observed in lemon grass oil and citronella oil (0.017%) followed by palmarosa oil (0.021%), *T.indica* leaf extract an *A.nilotica* leaf extract.

For bacterial bio-antagonists, the tolerance level was tested by measuring the inhibition zone at the concentrations just higher than the ED_{50} value of

Table 4: Effect of seed coating with Bio-antagonists and soil drenching with Plant extracts on pre-emergence damping off of Brinjal (2 years' pooled mean)

	Percentage of pre-emergence damping off						
	Soil application of plant extracts						
Bio-antagonists (seed coating)	Palmarosa oil (0.025%)	Lemon grass oil (0.020%)	Citronella oil (0.020%)	*A. nilotica* leaf extract (4.0%)	*T. indica* leaf extract (1.5%)	Control	Mean (Main treatment)
	28 DAS	28 DAS	28 DAS	28 DAS	28 DAS	28 DAS	28 DAS
B. subtilis st.12	13.13 (21.24)	13.67 (21.67)	13.83 (21.83)	14.70 (22.54)	15.33 (23.05)	35.58 (36.61)	17.71 (24.49)
Ps. fluorescens	13.68 (21.68)	14.44 (22.29)	14.33 (22.22)	15.68 (23.29)	16.08 (23.63)	35.58 (36.61)	18.29 (24.96)
T. harzianum	11.33 (19.67)	12.67 (20.84)	12.63 (20.82)	13.25 (21.34)	14.33 (22.24)	35.58 (36.61)	16.63 (23.59)
T. viride	11.67 (19.97)	12.33 (20.53)	13.0 (21.13)	16.0 (23.57)	14.67 (22.51)	35.58 (36.61)	17.21 (24.05)
A. niger	13.67 (21.69)	13.83 (21.83)	14.83 (22.65)	15.83 (23.45)	16.17 (23.71)	35.58 (36.61)	18.32 (24.99)
Mean (Sub-treatment)	12.70 (20.85)	13.39 (21.43)	13.72 (21.73)	15.09 (22.84)	15.32 (23.03)	35.58 (36.61)	17.63 (24.42)

	SEm ±	CD at 5%
Treatment (soil application)	0.30	0.86
Bio-antagonists	0.28	0.78
Days after sowing ((DAS)	-	-
Treatment × Bio-antagonists	0.68	NS
Treatment × DAS	-	-
Bio-antagonists × DAS	-	-
Treatment× Bio-antagonists × DAS	-	-

* Figures in the parenthesis are indication of angular transformed value.

each extract found for the test pathogen. The inhibition zones were found to be 5.5mm, 1.0 mm, 2.0 mm, 15 mm, and 14 mm for palmarosa oil, lemon grass oil, citronella oil, *A.nilotica* leaf extract, *T. indica* leaf extract respectively in case of *B.subtilis* st. 12 and 4.0 mm, 2.0 mm, 3.0 mm, 20 mm and 12 mm for palmarosa oil, lemon grass oil, citronella oil, *A.nilotica* leaf extract, *T.indica* leaf extract respectively in case of *Ps. fluorescens*. Still those were taken into account for further study to observe their efficacy in combination with the plant extracts in field condition.

Germination percentage

Pooled mean of germination of brinjal seeds showed that all the sub- treatments (soil application with plant extracts) increased the germination percentage significantly as compared to pathogen check. Highest germination was observed in palmarosa oil (72.72%) followed by lemon grass oil (68.64%), statistically at par with *A. nilotica* leaf extract (68.19%) and minimum was observed in case of *T. indica* leaf extract (65.27%) at 28 DAS irrespective of seed coating with different bio-antagonists (Table 3).

Seed coating with different bio-antagonists also increased germination percentage significantly in comparison to untreated control irrespective of soil application of different plant extracts. Maximum germination was obtained in *T. viride* (71.89%) followed by *T. harzianum* (68.77%) and minimum was observed in case of *B. subtilis* st. 12 (59.25%) (Table 3).

Interaction with seed coating with bio-agents and soil application of plant extracts showed no significant difference in respect to germination of brinjal seed. Maximum germination was observed in seed treatment with *T. viride* + soil application of 0.025% palmarosa oil (79.25%) closely followed by 0.020% lemon grass oil (76.75%),followed by seed treatment with *T. harzianum*(4 x 10^6 spores / ml) + soil application of 0.025% palmarosa oil (75.58%) at 28 days after sowing (DAS). Minimum

Table 5: Effect of seed coating with Bio-antagonists and soil drenching with Plant extracts on post-emergence damping off of Brinjal (2 years' pooled mean)

Bio-antagonists (seed coating)	Percentage of post-emergence damping off													
	Soil application of plant extracts													
	Palmarosa oil (0.025%)		Lemon grass oil (0.020%)		Citronella oil (0.020%)		*A. nilotica* leaf extract (4.0%)		*T. indica* leaf extract (1.5%)		Control		Mean (Main treatment)	
	14 DAS	28 DAS	14 DAS	28 DAS	14 DAS	28 DAS	14 DAS	28 DAS	14 DAS	28 DAS	14 DAS	28 DAS	14 DAS	28 DAS
B.subtilis st.12	38.98 (38.63)	42.0 (40.36)	32.83 (34.94)	40.67 (39.61)	35.91 (36.81)	41.50 (40.10)	41.67 (40.20)	47.50 (43.56)	40.76 (39.68)	45.52 (42.43)	74.17 (59.61)	78.33 (62.44)	44.05 (41.64)	49.25 (44.75)
Ps. fluorescens	36.17 (36.96)	43.33 (41.17)	34.83 (36.15)	42.33 (40.59)	37.33 (37.66)	42.50 (40.69)	41.58 (40.15)	47.02 (43.29)	41.50 (40.10)	45.11 (42.19)	74.17 (59.61)	78.33 (62.44)	44.26 (41.77)	49.77 (45.06)
T. harzianum	32.50 (34.75)	36.83 (37.36)	32.92 (35.01)	37.17 (37.56)	31.92 (34.39)	35.92 (36.81)	37.37 (37.68)	41.50 (40.11)	38.88 (38.58)	43.31 (41.16)	74.17 (59.61)	78.33 (62.44)	41.29 (40.0)	45.51 (42.57)
T. viride	31.92 (34.40)	35.25 (36.42)	31.83 (34.33)	36.33 (37.06)	30.67 (33.62)	34.67 (36.07)	38.0 (38.05)	42.52 (40.70)	37.83 (37.95)	41.67 (40.19)	74.17 (59.61)	78.33 (62.44)	40.74 (39.66)	44.79 (42.15)
A. niger	34.67 (36.07)	38.83 (38.54)	34.50 (35.97)	39.17 (38.74)	35.17 (36.36)	39.17 (38.74)	42.33 (40.58)	46.42 (42.94)	41.92 (40.34)	46.0 (42.70)	74.17 (59.61)	78.33 (62.44)	43.79 (41.49)	47.99 (44.02)
Mean (Sub-treatment)	34.85 (36.16)	39.25 (38.77)	33.38 (35.28)	39.13 (38.71)	34.20 (35.77)	38.75 (38.48)	40.19 (39.33)	44.99 (42.12)	40.19 (39.34)	44.64 (41.95)	74.17 (59.61)	78.33 (62.44)	42.83 (40.91)	47.46 (43.71)

	SEm ±	CD at 5%
Treatment (soil application)	0.45	1.25
Bio-antagonists	0.41	1.14
Days after sowing ((DAS)	0.26	0.72
Treatment × Bio-antagonists	1.0	NS
Treatment × DAS	0.63	NS
Bio-antagonists × DAS	0.58	NS
Treatment× Bio-antagonists × DAS	1.42	NS

* Figures in the parenthesis are indication of angular transformed value

germination was observed in seed treatment with *B. subtilis* st. 12 and soil application of 1.5% *T. indica* leaf extract (56.83%).

The interactions in between soil drenching with plant extracts × days after sowing (DAS), seed treatment × DASand seed coating × soil application DAS were statistically significant in respect to germination of brinjal seedlings.

Pre-emergence damping off

Two years' pooled mean data showed that all the plant extract decreased the Pre-emergence damping off as compared to pathogen check and their differences were statistically significant irrespective bio agents used. Minimum mortality was obtained in 0.025% palmarosa oil (12.70%) followed by 0.020% lemon grass oil(13.39%) which was statistically at par with 0.020% citronella oil (13.72%) irrespective of bio-agent used (Table 4).

Seed treatment with different bio-antagonists showed differential reactions in management of pre-emergence damping off and their differences were statistically significant. Lowest mortality was observed in *T. harzianum*@ 4 × 10^6 spores / ml (16.63%), closely followed by *T. viride*@ 4 × 10^6 spores / ml (17.71%) and they were at par (Table 4).

The interactions in between seed coating and soil drenching with plant extracts showed no significant differences in reducing pre-emergence mortality. Whereas, individual soil application of plant extracts and seed coating with bio agents reduced the pre emergence mortality of brinjal seeds significantly in comparison to untreated control.

Post-emergence damping off

Two years' pooled mean data on degree of severity in post-emergence mortality was reduced by plant extracts as compared to pathogen check irrespective of the bio agents used as seed coating. The minimum mortality was obtained in citronella oil @ 0.020% (38.75%), followed by lemon grass oil @ 0.020% (39.13%) and palmarosa oil @ 0.025% (39.25%), whereas maximum mortality was noticed in case of *A. nilotica* (44.99%) and *T. indica* (44.64%)but far lower than the control (Table 5).

Seed treatment with different bio-antagonists also reduced the post-emergence damping off and their differences were statistically significant. Lowest mortality was observed in *T. viride* (44.79%), statistically at par with *T. harzianum* (45.51%). No significant difference in mortality was observed among the effect of the two bacterial bio-antagonists.

Minimum post-emergence mortality was observed in seed treatment with *T. viride* + soil application of citronella oil (34.67%) closely followed by palmarosa oil (35.25%) under the same main treatment, seed treatment with *T. harzianum* + soil application of citronella oil (35.92%)and seed treatment with *T. viride* + soil application of lemon grass oil (36.33%), followed by seed treatment with *T. harzianum* + soil application of palmarosa oil (36.83%) at 28 DAS. Maximum mortality was observed in seed treatment with *B. subtilis* st. 12 + soil application of *A. nilotica* leaf extract (47.50%) followed by seed treatment with *Ps. fluorescens* + soil application of *A. nilotica* leaf extract (47.02%), still far lower than untreated control (78.33%) (Table 5).

The interactions between soil application of plant extracts × days after sowing (DAS), seed coating × DAS and treatment with plant extracts× bio-antagonists × DAS were statistically insignificant in respect to post-emergence mortality of brinjal seedlings.

The overall result suggested that seed coating with different bio-antagonists and soil drenching with different plant extracts increased the germination and reduced the pre- and post-emergence mortality of brinjal seedlings individually as well as in combined applications. Seed treatment of *T. viride* @ 4×10^6 spores / ml in combination with soil drenching of citronella oil @ 0.020% or palmarosa oil @ 0.025% or lemon grass oil @ 0.020% gave maximum germination and the seedling stand closely followed by seed treatment of *T. harzianum* in combination with soil drenching of citronella oil or palmarosa oil. Plant extracts reduced the damping off of several nursery seedlings due to presence of some alkaloids which is fuungitoxic (Allameh, *et. al*. 2002; Matan *et. al*. 2006). Similarly seed coating with different bio-antagonists also reduced the Pre- and Post-emergence Damping-off of forest nursery seedlings reported by several workers such as Sanjay *et. al*. (2001) in forest nurseries, Patricio *et. al*. (2001) in cucumber seedlings, and Howel (2002) in cotton seedlings Several workers attempted to manage the other diseases by using botanicals and bio agents as reported by Marak (2015) in green gram and Padder*et al*. (2010) in common bean against *Colletotrichum lindemuthianum.* Here, an attempt has been made to minimize the important disease like Damping-off of seedlings by combined application of both the common plant products and microbial bio-pesticides. The combinations may be used as a powerful tool for plant disease management in present day organic farming crop production system as it is safe, economical and eco-friendly.

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