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AYANA CHAKRABORTY



J. Mycopathol. Res. 60(3) : 345-352, 2022;
ISSN 0971-3719

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Department of Botany,
University of Calcutta,
Kolkata 700 019, India

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Integrated application of fungicides and biological antagonists in management of pre- and post-emergence Damping off of nursery seedlings of tomato caused by *Pythium aphanidermatum*

AYANA CHAKRABORTY

Department of Botany, Basirhat College, Basirhat, North 24 Parganas- 743412, West Bengal

Received : 10.04.2022

Accepted : 01.07.2022

Published : 26.09.2022

Green house experiment was conducted to evaluate the combined effect of five biological antagonists (three fungi, *Trichoderma viride*, *T. harzianum*, *Aspergillus niger* (AN-27) and two bacteria, *Pseudomonas fluorescens*, *Bacillus subtilis* st. 12) and five chemical fungicides, Mancozeb 75% WP, Blitox-50 (COC 50% WP), Captan 50% WP, Metalaxyl 35% WP and Ridomil MZ 72% WP (metalaxyl 8% + mancozeb 64%) against *Pythium aphanidermatum* (Edson) Fitz., causing pre- and post- emergence Damping off disease of nursery seedlings of tomato. ED₅₀ value was calculated for each fungicide against the pathogen and the compatibility of the fungal bioantagonists was also tested at the same requisite ED₅₀ value following poisoned food technique. Seed coating with five fungicides were used as five main treatments and soil application of bio-antagonists used as sub-treatments under each main treatment. The results revealed that, all the treatment combinations as integration of fungicides as seed treatment and soil application of bio antagonists reduced the seedling disease as well as increased the germination percentage significantly in comparison to untreated control. The best result was obtained in seed coating with Metalaxyl 35% and Ridomil MZ 72% in combination with soil application of *Trichoderma harzianum*, *Pseudomonas fluorescens* and *Trichoderma viride*.

Key words: Compatibility, management, microbial BCAs, *Pythium* damping-off, seed treatment, soil borne disease

INTRODUCTION

Now-a-days, hybrid varieties of all the cash crops are being extensively used for maximum return. Introduction of high yielding genotypes, 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. Tomato (*Lycopersicon esculentum* Mill.) has been regarded as one of the most important cash crop grown under a wide range of agro-climatic conditions throughout the year in India.

Major constraints for cultivation of this crop are pre- and post- emergence damping off caused by *Pythium aphanidermatum* (Edson) Fitz. Raising of seedlings in seed-bed often faces maximum threat and sometimes total loss of costly seeds by the notorious soil-borne pathogens. Management of the disease by application of chemicals is a

common practice but excessive use of chemical pesticides at high concentrations affects the soil health as well as environmental pollution causing ecological imbalance. Some workers have tried to manage this disease by using bio-antagonists through different methods of application on other crops (Kanimozhi *et al.* 2019; Nelson, 2004). Keeping in mind the problems of excessive use of chemicals, focus has been shifted to develop an alternative approach for a better efficacy by integrating fungicide with bio-antagonists (Dubey *et al.*, 2015; Mohiddin *et al.* 2013; Someya *et al.* 2007). Further, integration of biological treatments with fungicidal seed protectants, that eliminate competitors, may enhance establishment of desired bio-antagonists and provide better control of seeds and seedlings diseases, than when either of them are used separately (Ons *et al.* 2020; Wojtkowiak-Gêbarowska *et al.* 2006). With this objective, the present investigation was carried out with effective combinations of five bio-antagonists (*Trichoderma harzianum*, *Trichoderma viride*,

*Correspondence : ayana98361@gmail.com

Aspergillus niger, *Pseudomonas fluorescens* and *Bacillus subtilis* strain 12) with compatible level of fungicides for the optimum control of pre- and post-emergence damping off of tomato seedlings.

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 fungicides

Bio-assay of eight fungicides viz., Mancozeb 75% WP, Blitox-50 (COC 50% WP), Captan 50% WP, Captafol 80% WP, Kitazin 48% EC, Propiconazole 25% EC, Metalaxyl 35% WP and Ridomil MZ 72% WP (metalaxyl 8% + mancozeb 64%) against the test pathogen was carried out to evaluate the tolerance limit by poisoned food technique (Dhingra and Sinclair, 1995) at different dosages and ED₅₀ and ED₉₀ values were calculated.

Collection of Bio-antagonists

The fungal bio-antagonists *Trichoderma harzianum* (Acc. No. 6017.05), *Trichoderma viride* (Acc. No. 6018.05) and *Aspergillus 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 of Advanced Faculty Training in Plant Pathology, Gobind Ballabh Pant University of Agriculture and Technology, Uttarakhand and *Bacillus subtilis* st.12 (CMI No. 349545) from Department of Plant Pathology, BCKV, Mohanpur, Nadia, West Bengal. Efficacy of the bio-antagonists were tested using Dual culture plate method (Dennis and Webster, 1971)

Compatibility study of bio-antagonists with fungicides

In vitro study was carried out to evaluate the compatibility of the bio-antagonists with different dosages of fungicides by poisoned food technique.

Green-house Experiment

Green-house experiment was carried out in Department of Plant Pathology, BCKV, Mohanpur, Nadia, West Bengal, for two consecutive years 2018- 2019. Host plant chosen was Tomato

(*Lycopersicon esculentum* cv. Pusa ruby). Five biological antagonists (three fungi, *T. viride*, *T. harzianum*, *A. niger* and two bacteria, *Ps. fluorescens*, *B. subtilis* st.12) and five chemical fungicides, Mancozeb, Blitox-50 (COC), Captan, Metalaxyl 35% and Ridomil MZ (metalaxyl 8% + mancozeb 64%), selected from laboratory experiment through poisoned food technique against the test pathogen were used to carry out the experiment. For an integrated approach of disease management, seed coating with fungicides (@ 100 ppm, 102 ppm, 100 ppm, 200 ppm and 450 ppm for Mancozeb, Blitox-50, Captan, Metalaxyl and Ridomil MZ respectively) combined with soil application of bio-antagonists (@ 10 g/ kg soil [4×10^6 spores/ ml], multiplied in Sand-maize meal medium) were applied. Seed coating with five fungicides were done and applied as five main treatments and soil application of five bio-antagonists were used as sub-treatments under each main treatment.

Seed coating

Seed coating with selected fungicides was done by dipping the surface sterilized seeds in requisite dosages of fungicidal suspension, screened through ED₅₀ values (@ 100 ppm, 102 ppm, 100 ppm, 200 ppm and 450 ppm for Mancozeb, Blitox-50, Captan, Metalaxyl and Ridomil MZ respectively) and 0.1% carboxy methyl cellulose for 1 hour and then shade-dried. CMC was used as sticking agent.

Three fungicides, namely, Captafol, Kitazin and Propiconazole were discarded due to incompatibility with the bio-antagonists.

Multiplication of bio-antagonists

Fungal bio-antagonists were multiplied in Sand-maize meal medium and the bacterial bio-antagonists were multiplied in Potato Dextrose-broth culture.

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. aphanidermatum* (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 fungicide coated seeds of tomato were sown individually. Simultaneously, the soil application with different bio-antagonists @ 10 g/ kg soil (4×10^6 spores/ ml) and 10 ml/ kg soil (3×10^8 cfu/ ml) for

fungal and bacterial respectively were done separately at the time of sowing of the seeds. Trays were kept in complete randomized block design (CRBD) with three replications for each of the test treatments. Control treatment was maintained by sowing only CMC coated seeds in artificially inoculated soil. Data were recorded regarding germination, pre- and post- emergence damping off after 14 and 28 days of sowing.

RESULTS AND DISCUSSION

Different concentrations of each fungicide were tested against the test pathogen *P. aphanidermatum* as well as against the non-target bio-antagonists and their ED₅₀ and ED₉₀ (MIC) values were calculated. Calculations revealed that (Table 1), all the eight fungicides tested against the target pathogen, inhibited the mycelial growth up to 50% within 700 ppm and up to 90% within 1000 ppm. The ED₅₀ values of *P. aphanidermatum* found were 98.04, 101.703, 88.462, 645.868, 3.889, 1.575, 191.401, 428.289 ppm and ED₉₀ values found were of 232.425, 279.187, 214.649, 888.203, 5.734, 3.616, 256.893 and 482.335 ppm for Mancozeb, Blitox-50, Captan, Captafol, Kitazin, Propiconazole, Metalaxyl and Ridomil MZ respectively.

Captan, Metalaxyl and Ridomil MZ which inhibited the growth of the test pathogen at low concentration but need higher concentrations for inhibiting the mycelial growth of fungal bio-antagonists, were selected as compatible for inclusion in the integrated management approaches.

Fungicides have been evaluated against *P. aphanidermatum* and several other soil-borne plant pathogens by several workers, but the use of friendly fungicides on bio-antagonists was in limited practice. Compatibility study of *Trichoderma* isolates with fungicides against damping off pathogen has been studied by Sharma *et. al.* (2003). Pesticidal tolerance of fungal bio-control agents has been studied by Singh and Singh (2007), Dubey *et. al.* (2015).

Effect of seed coating with fungicides and soil application of bio-antagonists on germination of tomato

The data for germination was recorded 14 and 28 days after sowing (DAS). Statistical analysis of two years' (2018-19) pooled mean data clearly revealed that (Table 2), every sub-treatment (soil application with bio-antagonists) significantly increased the germination percentage in comparison to untreated control irrespective of fungicide used. Maximum germination was observed in *T.*

Table 1: ED₅₀ and ED₉₀ (MIC) values of fungicides for inhibition of radial growth of *P. aphanidermatum*, *T. harzianum*, *T. viride* and *A. niger*

Microorganism		Fungicide conc. (ppm.)							
		Mancozeb	Blitox-50 (COC)	Captan	Captafol	Kitazin	Propiconazole	Metalaxyl	Ridomil MZ
<i>P. aphanidermatum</i>	ED ₅₀	98.04	101.703	88.462	645.868	3.889	1.575	191.401	428.289
	ED ₉₀	232.425	279.187	214.649	888.203	5.734	3.616	256.893	482.335
<i>T. harzianum</i>	ED ₅₀	358.854	832.984	215.695	335.557	1.082	1.0	Upto 500 ppm no inhibition	735.121
	ED ₉₀	735.803	6349.404	569.391	576.515	4.507	1.0	Upto 500 ppm –no inhibition	945.00
<i>T. viride</i>	ED ₅₀	193.594	2357.045	215.482	296.793	3.152	1.0	Upto 500 ppm –no inhibition	3025.11
	ED ₉₀	471.120	17974.555	740.667	828.642	7.319	1.0	Upto 500 ppm –no inhibition	9821.25
<i>A. niger</i>	ED ₅₀	120.148	796.255	117.515	166.47	8.207	1.0	346.044	730.25
	ED ₉₀	501.737	2366.022	300.256	376.749	15.013	1.0	1124.165	6125.00

Among these, three fungicides, namely, Captafol, Kitazin and Propiconazole were discarded as their ED₅₀ and ED₉₀ values for the beneficial bio-antagonists (*T. harzianum*, *T. viride* and *A. niger*) were found lower than *P. aphanidermatum*. The rest five fungicides, viz., Mancozeb, Blitox-50,

harzianum (82.73% and 86.74%) followed by *Ps. fluorescens* (80.07% and 83.80%) at 14 DAS and 28 DAS respectively. Minimum germination was obtained in *A. niger* (69.53% and 73.33%) followed by *B. subtilis* st. 12 (72.07% and 75.73%), whereas intermediate result was exhibited by *T. viride*

Table 2: Effect of seed coating with Fungicides and soil application of Bio-antagonists on germination percentages of Tomato (2 years' pooled mean)

Fungicide Treatment (seed coating)	Germination percentage														
	Soil application of bio-antagonists												Mean (Main treatment)		
	<i>B. subtilis</i> st. 12		<i>Ps. fluorescens</i>		<i>T. harzianum</i>		<i>T. viride</i>		<i>A. niger</i>		Control		14	28	
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	14 DAS	28 DAS
Mancozeb	68.33 (55.79)	71.0 (57.47)	78.67 (62.56)	81.67 (64.75)	79.67 (63.49)	82.67 (65.46)	75.33 (60.43)	77.67 (62.04)	70.33 (57.02)	72.67 (58.50)	58.33 (49.82)	62.0 (51.96)	71.77 (58.19)	74.61 (60.09)	
Blitox 50	70.0 (56.90)	73.0 (58.78)	76.0 (60.75)	80.33 (63.76)	78.33 (62.29)	82.67 (65.46)	74.33 (59.57)	77.67 (61.84)	63.0 (52.56)	66.0 (54.97)	58.33 (49.82)	62.0 (51.96)	70.00 (56.98)	73.61 (59.36)	
Captan	70.0 (56.82)	74.33 (59.59)	75.67 (60.47)	79.33 (63.0)	79.33 (63.04)	82.67 (65.46)	73.33 (68.93)	77.67 (61.84)	68.33 (55.77)	72.67 (58.50)	58.33 (49.82)	62.0 (51.96)	70.83 (57.47)	74.78 (60.06)	
Metalaxyl	78.33 (62.42)	82.67 (65.58)	83.67 (66.21)	88.33 (70.12)	89.33 (71.06)	94.67 (77.09)	84.67 (67.03)	89.33 (71.06)	75.0 (60.04)	79.33 (63.0)	58.33 (49.82)	62.0 (51.96)	78.22 (62.76)	82.72 (66.47)	
Ridomil MZ	73.67 (59.15)	77.67 (61.84)	86.33 (68.37)	89.33 (71.06)	87.0 (69.01)	91.0 (72.97)	83.67 (66.21)	89.33 (71.06)	71.0 (57.47)	76.0 (60.75)	58.33 (49.82)	62.0 (51.96)	76.67 (61.67)	80.89 (64.94)	
Mean (Sub-treatment)	72.07 (58.22)	75.73 (60.65)	80.07 (63.67)	83.80 (66.54)	82.73 (65.78)	86.74 (69.29)	78.27 (64.43)	82.33 (65.57)	69.53 (56.57)	73.33 (59.14)	58.33 (49.82)	62.0 (51.96)	-	-	
	SEm ±												CD at 5%		
Treatment (seed coating)	0.53												1.49		
Bio-antagonists	0.49												1.36		
Days after sowing ((DAS)	0.31												0.86		
Treatment × Bio-antagonists	1.19												3.33		
Treatment × DAS	0.57												NS		
Bio-antagonists × DAS	0.69												NS		
Treatment × Bio-antagonists × DAS	1.68												NS		

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

(78.27% and 82.33%) at 14 DAS and 28 DAS respectively.

While considering the main treatment, seed coating with fungicides on germination of seeds, most striking result was obtained in seed treatment with metalaxyl (78.22% and 82.72%) followed by Ridomil MZ (76.67% and 80.89%) at 14 DAS and 28 DAS respectively in increasing the germination percentage in comparison to untreated control (58.33% and 62%) under artificially inoculated soil condition. Application of the other fungicides gave intermediate results and statistically significant though their differences were statistically at par with each other.

Interaction of main treatment and sub-treatments showed no significant difference in respect to

germination. Maximum germination was observed in seed treatment with Metalaxyl + soil application of *T. harzianum* (89.33% and 94.67%) closely followed by seed treatment with Ridomil MZ + soil application of *T. harzianum* (87.0% and 91.0%) at 14DAS and 28 DAS respectively. Soil application with *T. viride* and *Ps. fluorescens* in combination with the seed treatment of Metalaxyl (89.33%) and Ridomil MZ (89.33%) showed statistically at par results in increasing the germination percentage of tomato seeds. Soil application of *T. harzianum* in combination with Mancozeb and Blitox 50 gave intermediate results (82.67%) and significantly higher than untreated control (62.0%).

Interaction in between treatments × methods, treatments × DAS and treatments × DAS × methods of applications were statistically insignificant.

Table.3: Effect of seed coating with Fungicides and soil application of Bio-antagonists on pre-emergence damping off of Tomato (2 years' pooled mean)

Fungicide Treatments (seed coating)	Percentage of pre-emergence damping off						
	Soil application of bio-antagonists						Mean (Main treatment)
	<i>B. subtilis</i> st. 12	<i>Ps. fluorescens</i>	<i>T. harzianum</i>	<i>T. viride</i>	<i>A. niger</i>	Control	
28 DAS	28 DAS	28 DAS	28 DAS	28 DAS	28 DAS	28 DAS	
Mancozeb	22.92 (28.60)	21.50 (27.62)	20.67 (27.03)	22.83 (28.54)	23.50 (28.97)	30.33 (33.40)	23.62 (29.03)
Blitox 50	20.00 (26.56)	18.00 (25.10)	17.33 (24.59)	18.67 (25.59)	20.83 (27.15)	30.33 (33.40)	20.86 (27.07)
Captan	19.50 (26.19)	17.00 (24.34)	15.50 (23.17)	16.50 (23.96)	19.67 (26.32)	30.33 (33.40)	19.75 (26.23)
Metalaxyl	11.67 (19.93)	8.67 (17.10)	5.33 (12.90)	8.33 (16.76)	11.17 (19.49)	30.33 (33.40)	12.58 (19.93)
Ridomil MZ	13.67 (21.69)	10.33 (18.68)	10.83 (18.89)	10.42 (18.74)	13.17 (21.26)	30.33 (33.40)	14.79 (22.11)
Mean (Sub-treatment)	17.55 (24.59)	15.10 (22.57)	13.93 (21.32)	15.35 (22.72)	17.67 (24.64)	30.33 (33.40)	-
			SEm ±			CD at 5%	
Treatment (seed coating)			0.31			0.89	
Bio-antagonists			0.29			0.81	
Days after sowing ((DAS)			-			-	
Treatment × Bio-antagonists			0.70			1.99	
Treatment × DAS			-			-	
Bio-antagonists × DAS			-			-	
Treatment × Bio-antagonists × DAS			-			-	

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

Effect of seed coating with Fungicides and soil application of Bio-antagonists on pre-emergence damping off

The data were recorded for pre-emergence mortality on 28 days after sowing (DAS). Statistical analysis of two years' (2018-19) pooled mean data clearly indicated that (Table 3), every sub-treatment (soil application with bio-antagonists) significantly reduced the pre-emergence mortality in comparison to untreated control (30.33%) irrespective of fungicides used. The best result was obtained in soil application of *T. harzianum* (13.93%) followed by *Ps. fluorescens* (15.10%) and *T. viride* (15.35%) at 28 DAS whereas, intermediate results obtained in case of *B. subtilis* st. 12 (17.55%) closely followed by *A. niger* (17.67%).

In case of seed coating with fungicides on pre-emergence mortality of seeds, the best result was obtained in seed treatment with Metalaxyl (12.58%) followed by Ridomil MZ (14.79%) at 28 DAS in comparison to untreated control (30.33%) under artificially inoculated soil condition.

Interaction of main treatment and sub-treatments showed significant differences in respect to pre-emergence mortality. Minimum mortality was observed in seed treatment with Metalaxyl + soil application of *T. harzianum* (5.33%) followed by soil application of *T. viride* (8.33%) and *Ps. fluorescens* (8.67%) under the same fungicide treatment, which was followed by seed treatment with Ridomil MZ + soil application of *Ps. fluorescens* (10.33%) followed by *T. viride* (10.42%)

Table.4: Effect of seed coating with Fungicides and soil application of Bio-antagonists on post-emergence damping off of Tomato (2 years' pooled mean)

Fungicide Treatments (seed coating)	Percentage of post-emergence damping off														Mean (Main treatment)	
	Soil application of bio-antagonists															
	<i>B. subtilis</i> st. 12		<i>Ps. fluorescens</i>		<i>T. harzianum</i>		<i>T. viride</i>		<i>A. niger</i>		Control		14	28		
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	14 DAS	28 DAS	
Mancozeb	21.92 (27.91)	27.92 (31.89)	18.83 (25.69)	24.83 (29.88)	17.17 (24.44)	23.17 (28.75)	18.50 (25.47)	24.50 (29.67)	21.83 (27.84)	27.83 (31.83)	52.0 (46.15)	59.17 (50.29)	21.21 (22.10)	21.21 (22.10)		
Blitox 50	24.17 (29.45)	29.58 (32.95)	22.50 (28.30)	28.0 (31.94)	20.17 (26.67)	26.17 (30.76)	22.17 (28.07)	28.17 (32.04)	26.17 (30.76)	32.17 (34.55)	52.0 (46.15)	59.17 (50.29)	27.86 (31.57)	33.87 (35.42)		
Captan	20.17 (26.67)	26.17 (30.76)	17.50 (24.72)	23.33 (28.88)	16.17 (23.65)	22.17 (28.06)	18.0 (25.10)	23.83 (29.22)	21.17 (27.39)	26.67 (31.09)	52.0 (46.15)	59.17 (50.29)	24.17 (28.95)	30.22 (33.05)		
Metalaxyl	10.17 (18.57)	16.0 (23.56)	5.83 (13.85)	11.98 (20.21)	3.33 (10.49)	8.63 (17.05)	6.50 (14.64)	11.0 (18.53)	12.17 (20.38)	17.83 (24.97)	52.0 (46.15)	59.17 (50.29)	15.0 (20.68)	20.77 (25.77)		
Ridomil MZ	11.83 (20.09)	17.83 (24.96)	7.30 (15.63)	13.17 (21.23)	4.50 (12.18)	10.50 (18.89)	7.50 (15.86)	13.50 (21.54)	13.50 (21.54)	19.50 (26.19)	52.0 (46.15)	59.17 (50.29)	16.11 (21.91)	22.28 (27.19)		
Mean (Sub-treatment)	17.65 (24.54)	23.50 (28.82)	14.39 (21.64)	20.26 (26.43)	12.29 (19.49)	18.19 (24.70)	14.53 (21.83)	20.20 (26.20)	18.97 (25.58)	24.80 (29.73)	52.0 (46.15)	59.17 (50.29)	-	-		
	SEm ±														CD at 5%	
Treatment (seed coating)	0.24														0.68	
Bio-antagonists	0.22														0.62	
Days after sowing ((DAS)	0.14														0.39	
Treatment × Bio-antagonists	0.55														1.53	
Treatment × DAS	0.35														NS	
Bio-antagonists × DAS	0.32														NS	
Treatment × Bio-antagonists × DAS	0.77														NS	

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

and *T. harzianum* (10.83%) under the same fungicide treatment at 28 DAS and they were statistically at par with each other. Maximum mortality was obtained in mancozeb seed treatment along with application of all the bio-antagonists though significantly lower than untreated control (30.33%).

Interaction in between treatments × methods, treatments × DAS and treatments × DAS × methods of application were statistically insignificant.

Effect of seed coating with fungicides and soil application of Bio-antagonists on post-emergence damping off

The data were recorded for post-emergence damping off on 14 DAS and 28 days after sowing

(DAS). Statistical analysis of two years' (2018-19) pooled mean data clearly revealed that, every sub-treatment (soil application with bio-antagonists) significantly reduced the post-emergence mortality (18.19% to 24.80%) as compared to untreated control (52.0% at 14 DAS and 59.17% at 28 DAS) irrespective of fungicide treatments (Table 4). Minimum post-emergence mortality was observed in *T. harzianum* (12.29% and 18.19%) closely followed by *T. viride* (14.53% and 20.20%) and *Ps. fluorescens* (14.39% and 20.26%) at 14 DAS and 28 DAS respectively, whereas intermediate result was exhibited by *B. subtilis* st. 12 (17.65% and 23.50%) and *A. niger* (18.97% and 24.80%) at 14 DAS and 28 DAS respectively irrespective of fungicides used as compared to untreated control (52.0% at 14 DAS and 59.17% at 28 DAS).

While considering the main treatment, all the fungicides (seed coating) significantly reduced the post-emergence mortality, but the best result was obtained in seed treatment with metalaxyl (15.0% and 20.77%) followed by Ridomil MZ (16.11% and 22.28%) at 14 DAS and 28 DAS in comparison to untreated control (52.0% and 59.17%) under artificially inoculated soil condition. Application of the other fungicides gave intermediate results and were statistically significant in comparison to untreated control though their differences were statistically at par.

Interaction of main treatment and sub-treatments showed significant differences in respect to post-emergence mortality. Minimum mortality was observed in seed treatment with Metalaxyl + soil application of *T. harzianum* (3.33% and 8.63%) followed by soil application of *Ps. fluorescens* (5.83% and 11.98%) and *T. viride* (6.50% and 11.0%) under the same fungicide treatment. The next best result was obtained in seed treatment with Ridomil MZ + soil application of *T. harzianum* (4.50% and 10.50%) followed by soil application of *Ps. fluorescens* (7.30% and 13.17%) and *T. viride* (7.50% and 13.50%) under the same fungicide treatment at 14DAS and 28 DAS. Maximum mortality was observed in soil application of *A. niger* in combination with seed treatment of Blitox -50 (32.17%), though significantly lower than untreated control (59.17%).

Interaction in between treatments× methods, treatments× DAS and treatments× DAS× method of application were statistically insignificant.

The results therefore indicated that seed treatment with Metalaxyl 35% WP along with soil application of *T. harzianum* or *Ps. fluorescens* or *T. viride* not only increased the germination percentage but also reduced the pre- and post-emergence damping off tomato to a high extent. It was also evident that almost similar result was obtained in seed treatment with Ridomil MZ 72% WP in combination with soil application of *T. harzianum* or *T. viride* or *Ps. fluorescens* in reducing the seedling mortality. In this study, the integration of chemical and biological method proved to be beneficial in disease management for their synergistic effect. Sultana *et. al.* (2013) reported that, seed rot of bottle gourd and cucumber can be reduced by combined application of fungicides and microbial antagonists. *Pythium* -damping off can be

minimized by integration of fungicides and *Trichoderma* isolates has also been reported by Sharma *et. al.* (2003). Someya *et. al.* (2007) had reported that combined application of *Pseudomonas fluorescens* with low dosage of Benomyl can effectively control *Fusarium oxysporum*. Compatibility of *Bacillus subtilis* (BS 16) with fungicides on solanaceous crops like chilli has been studied by Rajkumar *et. al.* (2018).

CONCLUSION

In conclusion it can be mentioned that, bio-control agents in combination with compatible fungicides have great potential in reducing the seedling diseases like *Pythium* -damping off of tomato. These treatments may have the potential to develop new antifungal strategies for integrated pest management since the chances of resistance development are lower and the fungicide dose might be reduced in comparison to traditional management by using higher dose of single fungicides. The present study therefore suggested that, Metalaxyl 35% WP and Ridomil MZ 72% WP are effective in reducing pre- and post-emergence damping off of nursery seedlings when applied in combination with microbial bio-antagonists, *T. harzianum* or *T. viride* or *Ps. fluorescens*.

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