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Management of Damping off of nursery seedlings of Tomato with integration of Microbial Bio-agents and Botanicals

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A greenhouse experiment was conducted to evaluate the efficacy of five bio agents (three fungi, *Trichoderma viride, T. harzianum, Aspergillus niger* and two bacteria, *Pseudomonas fluorescens, Bacillus subtilis)* in combination with five plant products, Citronella *(Cymbopogon winterianus)* oil, Palmarosa (*C. martini v*ar. *motia)* oil, Lemon grass (*C. citratus*) oil, Babul (A*cacia nilotica*) leaf extract and Tamarind (*Tamarindus* indica) leaf extract against *Pythium aphanidermatum* (Edson) Fitz., causing damping off disease of nursery seedlings of Tomato. ED_{50} value was calculated for each extract against the pathogen as well as the bio-agents following poisoned food technique. Seed coating with five bio-agents followed by soil application of five botanicals resulted in increased germination of tomato seeds as well as reduction of seedling diseases in comparison to untreated control. The best result was observed in seed treatment with *T. viride* @ 10 ml g^{-1}($4x10^6$ spores ml^{-1}) + soil drenching with 0.020% citronella oil or 0.020% lemon grass oil or 0.025% palmarosa oil closely followed by seed treatment with *P. fluorescen*s @10 ml g^{-1} ($3x\ 10^8$cfu ml^{-1}) or *T. harzianum* @ 10 ml g^{-1} (4×10^6 spores ml^{-1}) + soil drenching with 0.025% palmarosa oil or 0.020% citronella oil in reducing the damping off of tomato seedlings.

Keywords : Bio-antagonists, Botanicals, Damping off, integrated management, seed coating

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill) is one of the most popular vegetable crops, grown all over the country with an estimated global production of 120 million metric-tons. The crop is attacked by several diseases, caused by fungi, bacteria and viruses leading to severe crop losses. Among the fungal diseases, the damping off disease incited by *Pythium aphanidermatum* (Edson) Fitz. is a major constrain in the production of tomato seedlings caused 5 to 80% death of the seedlings and thereby inducing heavy economic consequences for farmers (Lamichhane *et al*. 2017).

Fungicides are mostly used in soil to manage the disease in nurseries but frequent and indiscriminate use of fungicides often leads to soil pollution and development of fungicide resistance in pathogens which necessitates an alternative method for its management and sustainable crop production. 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 (Sreenivasa *et al*. 2011) as well as against *Pythium*-damping off (Ambikapathy *et al*. 2011, Hooda *et al*. 2011). Plant-derived natural substances are non-phytotoxic compounds and potentially effective against plant pathogenic fungi. Similarly, various fungal and bacterial agents have been used experimentally for control of damping off of tomato seedling (Jeyaseelan *et al*. 2012). Some workers have also tried to manage the disease by integrated approaches (Ismael and Mahmood, 2016; Sharma *et al*. 2022).

The objective of the present investigation was aimed to find out the possibility of controlling the

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plant diseases by integration of bio agents along with botanicals as seed coating & soil drenching respectively. This method has a great potential in sustainable crop production in the framework of organic farming system. Integration with microbial bio agents as seed treatments in combination with soil application of botanicals may enhance the establishment of desired bio-agents 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* from Indian Type culture collection, Division of Plant Pathology, IARI. Pathogenicity test was conducted following Koch's postulates.

Collection of botanicals

The oils were extracted from the aromatic grasses, Citronella and Palmarosa, Lemon grass and from Neem seed through hydro distillation process and the leaf extracts were obtained through methanolic extraction of leaves of Tamarind, Neem and Babul in the Dept. of agricultural chemicals, BCKV. 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-agents

The fungal bio-agents *Trichoderma harzianum, Trichoderma viride* and *Aspergillus niger* were collected from Indian Type Culture Collection, Division of Plant Pathology, IARI, New Delhi. Among the two bacterial agents*, Pseudomonas fluorescens* was collected from Centre of Advanced Training in Plant Pathology, Gobind Ballabh Pant University of Agriculture and Technology, Uttarakhand and *Bacillus subtilis* from Department of Plant Pathology, BCKV, Mohanpur, Nadia, West Bengal. Efficacies of the bio-agents were tested using Dual culture plate method (Dennis and Webster, 1971).

Screening of botanicals

Five botanicals, Citronella (Cy*mbopogon* w*interianu*s) 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 (Dhingra and Sinclair, 1995) against *Pythium aphanidermatum,* the causal organism of damping off of Tomato ((S*olanum esculentum* cv. *pusa rub*y) as well as against the non-target beneficial fungal bio-agents and ED_{50} values for all botanicals for each of the test organisms were calculated. Both of Neem (*Azadirachta indica)* seed oil and leaf extract failed to show any growth inhibition of the test pathogen even @ 15% and 10% respectively, so they were excluded from further experiment. For bacterial bio-agents, 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 following paper-disc plate method (Thornberry, 1950). For an integrated approach of disease management, seed coating with bio-agents in combination with soil application of botanicals were applied.

Seed coating

Seed coating was done by dipping the surface sterilized Tomato seeds in spore suspension of bio-antagonistic fungi @ 10 ml g^{-1} containing ($4x10^6$ spores ml^{-1}) and bacteria @ 10 ml g^{-1} containing (3×10^8 cfu ml^{-1}) and 0.1% carboxy methyl cellulose for 1 hour and then shade-dried. CMC was used as sticking agent. Seeds, coated with CMC (0.1%) without any bio-agents, served as control.

Green House Experiments

The experiment was conducted for consecutive two years (2021 and 2022) in Department of Plant Pathology, BCKV, Mohanpur, Nadia, West Bengal. Seed coating with five microbial bio-agents followed by soil drenching of five botanicals were used one day after showing. Polythene 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 of Tomato seeds, coated with bio-agents, were sown individually. Simultaneously, the soil was drenched with different botanicals *(@* 0.025% palmarosa oil, 0.020% lemon grass oil, 0.020% citronella oil, 4.0% *A. nilotica* leaf extract and 1.5% *T. indica* leaf extract) separately, 24 hours after sowing of the seeds, so that each individual bio-agents coated seeds could interact with five botanicals separately. 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 (0.1%) coated seeds in artificially inoculated soil. Data was recorded on germination, pre- and post- emergence damping off at 14 and 28 days after sowing.

RESULTS AND DISCUSSION

Different concentrations of each botanical were tested against the test pathogen *P. aphanidermatum* as well as the non-target fungal bio-agents and the ED_{50} values were calculated. Calculations revealed that Table 1, the ED_{50} values of every plant extract were lower for *P. aphanidermatum* (0.021%, 0.017%, 0.017%, 3.381%, 1.253% for Palmarosa oil, Lemon grass oil, Citronella oil, *A. nilotica* leaf extract and *T. indica* leaf extract respectively) than the other test fungal bio-agents. This clearly indicated that the fungal bio-agents could grow well in that concentrations of the respective extracts required in minimizing the target pathogen by 50%.

For bacterial bio-agents, the tolerance level was tested by measuring the inhibition zone at the concentrations of the ED_{50} value of 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 *B. subtilis* 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 *P. fluorescens* (Table 2). Those were taken into account for further study to observe their efficacy in combination with the botanicals in field condition.

Germination percentage

Pooled mean of two years' data on germination of tomato seeds showed that soil application with all the botanicals increased the germination percentage significantly as compared to pathogen check (60.0%). Highest germination was observed in citronella (90.92%, 51.53% increase over control) followed by lemon grass oil (88.55%), statistically at par with palmarosa oil (87.42%) and minimum was observed in case of *T. indica* leaf extract *(*75.51%, 25.85% increase over control) at 28 DAS irrespective of seed coating with different bio-agents (Fig. 1).

Seed coating with different bio-agents also increased germination percentage significantly in comparison to untreated control (60.0%) irrespective of soil application of different botanicals. Maximum germination was obtained in *A. niger* (80.91%, 34.85% increase over control), statistically at par with *T. harzianum* (80.89%)*, T. viride* (80.88%) and *P. fluorescens* (80.42%) and minimum was observed in case of *B. subtilis (*71.78%, 19.63% increase over control) (Fig. 1).

Interaction with seed coating with bio-agents and soil application of botanicals showed no significant difference in respect to germination of Tomato seed. Maximum germination was observed in seed treatment with *T. harzianum*+ soil application of 0.020% citronella oil (94.17%, 56.95% increase over control) closely followed by *B. subtilis*+ soil application of 0.020% lemon grass oil (93.84%, 56.40% increase over control). Soil application of citronella oil proved to be mostly promising in increasing germination percentage with combination of each and every seed treatment and they were statistically at par with each other. The other treatment combinations gave intermediate results at 28 days after sowing (DAS). Minimum germination was observed in seed treatment with *B. subtilis*+ soil application of 1.5% *T. indica* leaf extract (72.05%, 20.08% increase over control).

Table.1: ED_{50} values of botanicals for inhibition of radial growth of *P. aphanidermatum, T. viride, T. harzianum* and *A. niger* in *in vitro* condition

Plant extracts (%)		Microorganisms			
		P. aphanidermatum	*T. viride*	*T. harzianum*	*A. niger*
Palmarosa Oil	ED_{50}	0.21	0.041	0.036	0.029
Lemon grass oil		0.17	0.019	0.036	0.021
Citronella oil		0.17	0.043	0.042	0.032
A. nilotica leaf extract		3.381	5.880	8.131	4.136
T. indica leaf extract		1.253	1.605	1.374	1.651
Neem oil		Up to 15% no inhibition			
Neem leaf extract		Up to 10% no inhibition			

Table. 2: Effect of botanicals on inhibition of *P. fluorescens* and *B. subtilis* in *in vitro* condition

Plant extracts	Concentration (%)	Inhibition zone (mm) of Microorganisms (6 DAI)	
		P. fluorescens	*B. subtilis*
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

Pre-emergence damping off

Two years' pooled mean data (Fig. 2) showed that all the botanicals decreased the pre-emergence damping off as compared to pathogen check (34.0%) and their differences were statistically significant irrespective bio agents used. Minimum mortality was obtained in 0.020% citronella oil (5.94%, 82.53% disease reduction) followed by 0.020% lemon grass oil (8.46%) which was statistically at par with 0.020% palmarosa oil (8.86%) irrespective of bio-agent used. Among the bio-agents, the lowest mortality was observed in *T. viride (*13.63%, 59.91% disease reduction), statistically at par with *T. harzianum* (13.70%), and *A. niger* (13.73%) while used as seed treatment.

Soil drenching with citronella oil (0.020%) in combination with almost all the seed coating with bio agents reduced pre-emergence mortality. Best result was obtained in combination of *T. harzianum*+ citronella oil (3.67%, 89.21% disease reduction) followed by *B. subtilis*+ palmarosa oil (4.81%, 85.85% disease reduction) and *T. viride*+ palmarosa oil (5.89%, 82.68% disease reduction) combination. The rest combination also gave fair results in disease reduction in comparison to untreated control (34.0%).

Post-emergence damping off

Post-emergence mortality was reduced by most of the botanicals as compared to pathogen check irrespective of the bio agents used as seed coating. Minimum mortality was obtained in 0.020% lemon grass oil (32.82%) statistically at par with 0.020% citronella oil (33.41%) and 0.025% palmarosa oil (33.59%), whereas to

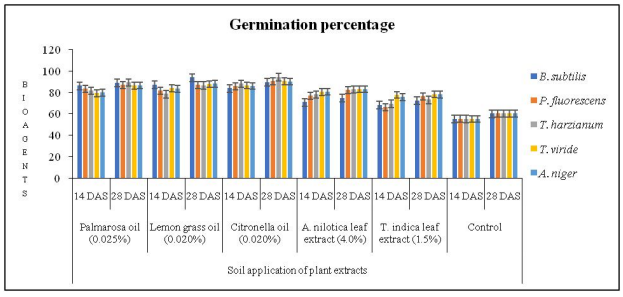

Fig.1: Effect of seed coating with Bio-antagonists and soil drenching with Plant extracts on germination percentages of Tomato (2 years' pooled mean)

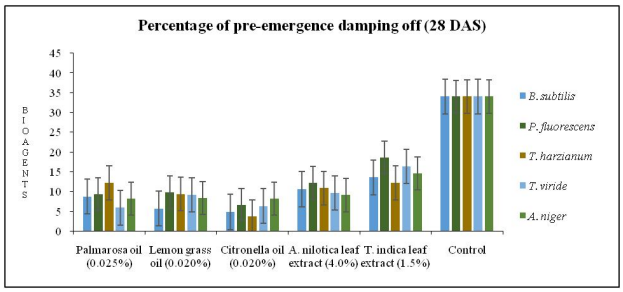

Fig.2 : Effect of seed coating with Bio-agents and soil drenching with Botanicals on pre- emergence damping off of Tomato (2 years' pooled mean)

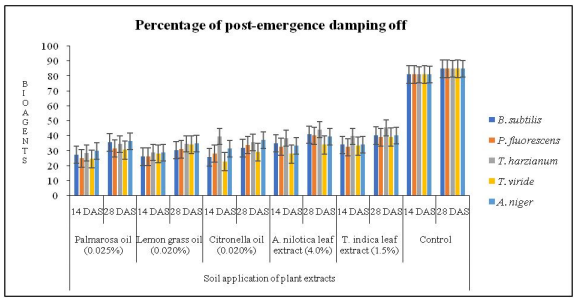

Fig. 3: Effect of seed coating with Bio-agents and soil drenching with Botanicals on post-emergence damping off of Tomato (2 years' pooled mean)

some extent higher rate of mortality was noticed in case of *A. nilotica* (39.50%) and *T. indica* (40.65%) but far lower than the control (84.67%) (Fig. 3).

Seed treatment with different bio-agents also reduced the incidence of post-emergence damping off. Lowest mortality was observed in *T. viride* (41.81%), statistically at par with *P. fluorescens* (43.24%) *T. harzianum* (43.36%). No significant difference in mortality was observed among the effect of the rest bio-agents.

Minimum post-emergence mortality was observed in seed treatment with *T. viride* + soil drenching of citronella oil (28.94% at 28 DAS, 65.82% disease reduction), closely followed by seed treatment with *B. subtilis*+ soil drenching of lemon grass oil (30.26%, 64.26% disease reduction) and seed treatment with *T. viride* + soil drenching of palmarosa oil (30.44%) and seed treatment with *T. viride*+ soil drenching of lemon grass oil (36.33%). Maximum mortality was observed in seed treatment with *T. harzianum*+ soil drenching of *T. indica* leaf extract (45.23%, 46.58% disease reduction) followed by seed treatment with *T. harzianum*+ soil drenching of *A. nilotica* leaf extract (44.0%), still far lower than untreated control (84.67%) (Fig. 3).

The overall result suggested that seed coating with different bio-agents and soil drenching with different botanicals increased the germination percentage and reduced the pre and post-emergence mortality of tomato seedlings individually as well as in combined applications. Seed treatment of *T. viride* @ 4 x 10^6 spores ml^{-1} followed by soil drenching of 0.020% citronella oil or 0.020% lemon grass oil or 0.025% palmarosa oil gave maximum germination and the seedling stand closely followed by seed treatment of *P. fluorescens* @ 3x 10^8cfu ml^{-1} or *T. harzianum* @ 4 x 10^6 spores ml^{-1} in combination with soil drenching of citronella oil or lemon grass oil @ 0.020%.

Botanicals can reduce the damping off of several nursery seedlings due to presence of some alkaloids which is fungitoxic (Allameh, *et al*. 2002, Matan *et al*. 2006). The result of the present study obtained in respect of management of damping off tomato with botanicals and bio-products are in conformity with those reported earlier by several workers in tomato (Hooda *et al*. 2011) and other crops (Arya 2004, Muthukumar *et al*. 2010). Similarly seed coating with different bio-agents also reduced the pre- and post emergence damping off of tomato (Manoranjitham *et al*. 2001), cucumber seedlings (Patricio *et al*. 2001) and of cotton seedlings (Howel, 2002). 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.

CONCLUSION

The present study therefore suggests that, seed coating with *T. viride* or *P. fluorescens* @ 10 ml

g^{-1} seeds along with soil drenching of 0.020% citronella oil or 0.020% lemon grass oil or 0.025% palmarosa oil may be used as a powerful tool for management of *Pythium*-damping off disease of tomato in present day organic farming crop production system as it is safe, economical and eco-friendly.

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DECLARATIONS

Conflict of interest. Authors declare no conflict of interest.

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