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EVALUATION OF FLORAL EXTRACTS, BIOCONTROL AGENTS AND FUNGICIDES FOR MANAGEMENT OF DAMPING OFF OF TOMATO CAUSED PYTHIUM DEBARYANUM

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ABSTRACT

Present study was undertaken to study the bioefficacy of the four flower (Marigold sp., *Gaillardia* sp., *Chrysanthemum* sp. and *Calotropis* sp.) extracted with four solvents (methanol, acetone, dichloromethane and distilled water), three biocontrol agents (*Pseudomonas fluorescense*, *Trichoderma harzianum* and *Bacillus subtilis*) and three fungicides (Carbendazim, Triram and Metalaxyl) under *in vitro* and pot experiment against tomato damping off caused by *Pythium debaryanum* in Plant Pathology Dept., Dr. PDKV, Akola (M.S.) during 2011-2014. *In vitro* study reveals that of the different floral solvent extracts, marigold distilled water extract was found effective for reducing dry mycelial weight of the test pathogen at all tested concentrations (250, 500, 750 and 1000 μ l). From the bioagents, *T. harzianum* was found most effective and recorded significantly the highest mycelial growth inhibition (51.11%). In case of fungicides, Metalaxyl (0.2%) was found most effective and recorded complete inhibition of the test pathogen. Pot experiment showed that seed treatment with Metalaxyl (0.2%), Thiram (0.2%) and *P. fluorescens* (10 g/kg) + *T. harzianum* (4 g/kg) + Marigold water extract 4% was most effective for reducing damping off incidence in tomato (74.11, 68.37 and 68.16%, respectively).

INTRODUCTION

Chemical fungicides have been the main weapons in controlling soil-borne plant pathogens and in increasing the yields in modern systems of crop production. However, the massive and sometimes inappropriate use of the synthetic fungicides in agricultural practices resulted in severe negative effects on multiple levels. On the one hand, the plant pathogens have developed resistance to the fungicidal treatments in use (Rossall, 2012) and on the other hand, the contamination of the environment soil, water and air and of the final products due to fungicide treatment was reported. Therefore, new control formulations for plant diseases represent a real need in the nowadays context of sustainable development in agriculture and ecology area. Plants produce multiple secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids (Butu *et al.*, 2014a) being in the same time important sources of biologically active molecules possessing antibacterial, antifungal (Negi, 2012) and antioxidant properties. In recent years, plants extracts obtained by various methods have raised scientific research interest for their antimicrobial potential. Moreover, an increasing number of studies are developed for investigation of the antimicrobial effects of medicinal plants for plant disease control (Manasi *et al.*, 2014).

Biological control using antagonistic microbes alone, or as supplements to minimize the use of chemical pesticides in a system of integrated plant disease management, has become more important in recent years. Commonly, the activity and efficacy of biocontrol agents are often profoundly affected by several factors such as sensitivity to environmental conditions in the soil and rhizosphere (Dunne *et al.*, 1998), type and amount of inoculum applied, method and timing of application and age of the inoculum (Lewis and Papavizas, 1987a,b), colonization ability of biocontrol agents in fields rich in infective propagules of pathogen (Deacon and Berry, 1993) and narrow spectrum range of activity against one species pathogens (Whipps, 2001).

An integrated approach consisting of combinations of biological and botanical tools could provide a solution to the problems of individually applying plant extracts and biocontrol agents. In addition to that, there is an opportunity for additive or synergistic control by a combination of botanical and biological control strategies. This work was focused on the use of an integrated disease management strategy that combined biological and botanical tools for compatibility and efficacy in disease control in order to minimize environmental risks while maximizing plant health in a long-term protection programme for this disease. The aim of the investigation was thus to establish compatible interactions between flower extract/s and biocontrol agent/s in order to improve the efficacy of disease control.

MATERIALS AND METHODS

An experiment was conducted to check the efficacy of plant extracts, biocontrol agents and fungicides against *P. debaryanum* under *in vitro* and *in vivo* conditions. Pathogen was isolated from infected tomato seedlings. Required biocontrol agent's viz., *P. fluorescens*, *B. subtilis* and *T. harzianum* were collected from Department

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Efficacy of flower extracts

Marigold sp., *Chrysanthemum* sp. and *Gaillardia* sp. *Calotropis* sp. flowers used in this study were collected from various temples from Akola city of Maharashtra. These were thoroughly washed under tap water to remove dust and other impurities and dried separately under shade with occasional shifting for about 3 to 4 weeks. The dried flowers were powdered with grinder and stored in airtight container until further use (Thenmozhi *et al.*, 2011). Forty gram powder of each flower was separately soaked in 200 ml of Methanol, Acetone, Dichloromethane and Sterilized distilled for four days at 150 rpm. Resultant extract was filtered separately through Whatman No. 1 filter paper and evaporated at room temperature. Air dried extracts were kept in refrigerator at 5°C until further use.

One gram residue of four plant extracts from all the solvents were diluted in 10 ml DMSO separately and from this 250, 500, 750 and 1000 μ l suspensions were poured separately into 250 ml conical flasks containing 50 ml sterilized potato dextrose broth (PDB). In control set only 250, 500, 750 and 1000 μ l DMSO were used. For each treatment 3 replicates (flasks) were used. All conical flasks were inoculated individually with 5 mm diameter discs of the *Pythium debaryanum* cultures and then incubated at $28 \pm 2^\circ\text{C}$ for 3 days and then each flask was filtered off using pre-weighted Whatman filter paper. Mycelial mat along with filter paper from each treatment were dried up to a constant dry weight at 70°C for 6 hr. Then the dry weights of each fungus along with filter paper were noted and actual weight of each filter paper was subtracted and resultant value was used to determine the growth inhibition. The per cent growth inhibition (I) was calculated (Vincent, 1947).

Efficacy of bioagents

Three bioagents *viz.*, *T. harzianum*, *B. subtilis* and *P. fluorescens* were evaluated *in vitro* against *P. debaryanum* by Dual culture technique (Stack *et al.*, 1986) on PDA medium. The experiment was conducted by completely randomized design with four treatments and three replications. Observations on radial mycelial growth of the fungal pathogen was measured and per cent inhibition of the test fungus (*P. debaryanum*) was calculated (Vincent, 1947).

Efficacy of fungicides

The efficacy of three fungicides namely, Carbendazim (0.1%), Metalaxyl (0.2%) and Thiram (0.2%) against *P. debaryanum* was evaluated *in vitro* by applying liquid bioassay technique and using PDB as basal medium. The experiment was conducted by Completely Randomized Design with three replications. Required quantity of fungicidal formulations were added in 250 ml conical flasks containing 50 ml sterilized potato dextrose broth (PDB) separately to make a desired concentration of each fungicide. In control set only 50 ml PDB was used. All conical flasks were inoculated individually with 5 mm diameter discs of the test fungal cultures and then incubated at $28 \pm 2^\circ\text{C}$ for 3 days. The remaining procedure was as discussed under bioefficacy of floral extracts.

Evaluation of floral extracts, fungicides and bioagents by paper towel method

This method was used to know the effect of seed treatments with Marigold water extract, *P. fluorescens*, *T. harzianum* and fungicides *viz.*, Carbendazim, Thiram and Metalaxyl on growth promoting activity in tomato. Randomly selected 100 seeds treated with Marigold flower extract, bioagents and fungicides were placed on two layers of moist germination paper and rolled carefully to avoid any excess pressure on seeds. These towels were incubated in seed germinator at $20 \pm 2^\circ\text{C}$ for 10 days. Similarly, control set was maintained by using non treated seeds of tomato. Three replications were maintained for each treatment separately. The first count was taken on 10th day. All morphologically normal seedlings were counted and germination was expressed in percentage. To find out the seedling vigour, ten normal seedlings were taken from the germination test at random and the root length was measured from the collar region to the tip of the primary root and the mean root length was expressed in cm. The same seedlings were used for the measurement of shoot length. The shoot length was measured from the collar region to the point of junction of cotyledons. The mean shoot length was expressed in cm. Vigour index was calculated by the following formula, given by Abdul Baki and Anderson (1973).

Evaluation of fungicides, botanicals and bioagents in pot culture

Tomato seeds were surface disinfected in 2% sodium hypochlorite for 30 seconds, rinsed in sterile distilled water and dried overnight. Tomato seeds were planted at 50 seeds per pot filled with sterilized potting soil (1.5 kg) (Latha *et al.*, 2009). The inoculum of fungal pathogens multiplied on sand: sorghum medium was incorporated in to the separate pots at 1:20 (w/w) ratio of pathogen and soil. In every treatment, the talc-based formulation of *T. harzianum* and *P. fluorescens* was applied as a seed treatment at 4 and 10 g/kg of seed, respectively. In Marigold water extract treatment, seeds of tomato were soaked in 2, 3 and 4% solutions for 3 hr and air dried overnight before sowing. The fungicides Metalaxyl (0.2%), Carbendazim (0.1%) and Thiram (0.2%) were also used for comparison and inoculated and non-inoculated pots with the pathogen alone served as control. Three replications were maintained for each treatment in a completely randomized design in a glasshouse. Incidence of damping-off in tomato was recorded at 7, 14 and 21 days after sowing.

Treatment details

S1F1 (Methanol extract of Marigold flower), S1F2 (Methanol extract of *Gaillardia* sp. flower), S1F3 (Methanol extract of *Chrysanthemum* sp. flower), S1F4 (Methanol extract of *Calotropis* sp. flower), S2F1 (Acetone extract of Marigold flower), S2F2 (Acetone extract of *Gaillardia* sp. flower), S2F3 (Acetone extract of *Chrysanthemum* sp. flower), S2F4 (Acetone extract of *Calotropis* sp. flower), S3F1 (Dichloromethane extract of Marigold flower), S3F2 (Dichloromethane extract of *Gaillardia* sp. flower), S3F3 (Dichloromethane extract of *Chrysanthemum* sp. flower), S3F4 (Dichloromethane extract of *Calotropis* sp. flower), S4F1 (Distilled water extract of Marigold flower), S4F2 (Distilled water extract of *Gaillardia* sp. flower), S4F3 (Distilled water extract of *Chrysanthemum* sp. flower) and S4F4 (Distilled water extract of *Calotropis* sp. flower).

RESULTS AND DISCUSSION

The results revealed that all of the tested floral extracts at each concentration inhibited the growth of *P. debaryanum* (Table 1). The rate of growth inhibition was corroborated with its concentrations in case of all the tested plant extracts.

Effect of floral extracts on reduction of dry mycelia weight of *P. debaryanum*

Interaction effects of all three factors (flower, solvent and concentration) were studied to find out effective interaction against test fungus. At highest concentration *i.e.* 1000 μ l, complete inhibition of dry mycelial weight of test fungus was recorded in interactions S4F1, S4F3, S4F4, S1F1, S1F3, S1F4 and S3F1. Lowest inhibition 50.51% was reported in interaction S2F2 (Chakrabarty *et al.*, 2013) (Table 1). From the above results it is observed that floral water extracts were effective in reducing biomass production. Al-Rahmah *et al.* (2013) reported that *T. vulgaris* extract was most effective in suppressing growth of *P. aphanidermatum* due to presence of phenolic compounds as thymol and carvacrol which played vital role in growth inhibition of phytopathogenic fungi.

Effect of bioagents on mycelia growth of *P. debaryanum*:

Result reveals that all the bioagents evaluated exhibited antagonistic activity against *P. debaryanum*. *T. harzianum* was found most effective and recorded significantly highest mycelial

inhibition (51.11%) of the test pathogen over control. The second best antagonists found was *P. flueroscens* which recorded 48.10 % inhibition. *B. subtilis* was found relatively less effective with 34.13% inhibition of the test pathogen (Table 2). Results of the present study on inhibitory effects of the test antagonists: *T. harzianum*, *B. subtilis*, and *P. flueroscens* are in conformity with those reported earlier by several workers (Swati Rose Toppo and Preeti Tiwari, 2015).

Effect of fungicides on reduction of dry mycelia weight of *P. debaryanum*

Complete mycelial inhibition was recorded with Metalaxyl (0.2%). This was followed by the Thiram (84.91%) whereas; Carbendazim (0.1%) was not effective and did not show inhibition of *P. debaryanum* (Table 3). Earlier report of Al-Rahmah *et al.* (2013) suggests that Carbendazim at 4 ppm concentration completely inhibited growth of *P. aphanidermatum*. The present results are in contrast with the findings of Al-Rahmah *et al.* (2013). Results of Metalaxyl in present study are in support of Locke *et al.* (1983) who reported that Metalaxyl at 0.2% concentration effectively suppressed mycelial growth of *Pythium ultimum*.

Effect of water extract of Marigold flower, bioagents and fungicides on tomato seeds by paper towel methods

Three seed dressing fungicides were also tested for comparison (Table 4). Germination per cent, mean root length, mean shoot

Table 1: Efficacy of different flower extracts on dry mycelial weight of *P. debaryanum*

S x F x C(SolventxFlowerx Conc.)	% inhibition over control			
	C1	C2	C3	C4
S 1 F 1	22.01 (27.16)*	62.34 (52.15)	84.63 (67.63)	100.00 (90.00)
S 1 F 2	5.19 (13.14)	33.67 (35.12)	54.66 (47.68)	65.73 (54.21)
S 1 F 3	3.21 (10.25)	49.91 (44.95)	70.05 (57.01)	100.00 (90.00)
S 1 F 4	12.83 (20.59)	48.86 (44.36)	63.15 (52.79)	100.00 (90.00)
S 2 F 1	0.00 (0.00)	34.17 (35.77)	66.47 (54.63)	81.51 (64.57)
S 2 F 2	0.00 (0.00)	25.43 (30.23)	40.63 (39.53)	50.51 (45.30)
S 2 F 3	6.89 (14.77)	31.42 (33.94)	51.09 (45.63)	61.96 (51.55)
S 2 F 4	8.90 (16.17)	36.66 (37.26)	56.32 (48.63)	82.60 (65.35)
S 3 F 1	16.54 (23.99)	46.14 (42.78)	64.01 (53.14)	100.00 (90.00)
S 3 F 2	0.00 (0.00)	25.94 (30.61)	49.99 (45.00)	58.72 (50.09)
S 3 F 3	19.99 (26.53)	40.40 (39.45)	60.70 (51.21)	80.43 (63.76)
S 3 F 4	5.66 (11.23)	44.39 (41.78)	64.28 (53.32)	77.72 (61.84)
S 4 F 1	42.21 (40.51)	76.06 (60.81)	100.00 (90.00)	100.00 (90.00)
S 4 F 2	1.24 (3.71)	39.90 (39.16)	61.53 (51.67)	82.06 (64.94)
S 4 F 3	20.00 (26.55)	66.58 (54.69)	96.43 (79.12)	100.00 (90.00)
S 4 F 4	30.37 (32.42)	67.58 (55.30)	100.00 (90.00)	100.00 (90.00)
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Source	S.E (M) \pm	C.D. at (p=0.01)		
Solvent (S)	0.49	1.81		
Flowers (F)	0.49	1.81		
Concentrations (C)	0.49	1.81		
Solvent x Flowers (S x F)	0.98	3.62		
Solvent x Concentrations (S x C)	0.98	3.62		
Flowers x Concentrations (F x C)	0.98	3.62		
Solvent x Flowers x Concentrations (S x F x C)	1.96	7.24		

Solvents (S)	Flowers(F)	Concentrations (C)
S1- Methanol	F1- Marigold flower extract	C1- 250 μ l
S2- Acetone	F2- <i>Gaillardia</i> sp. flower extract	C2- 500 μ l
S3- Dichloromethane	F3- <i>Chrysanthemum</i> sp. Flower extract	C3- 750 μ l
S4- Distilled Water	F4- <i>Calotropis</i> sp. Flower extract	C4- 1000 μ l

length and seedling vigour index of tomato showed significant variation. Significantly highest performance of growth characters of tomato seedlings were observed where seeds were sown after treated with crude marigold water extract at 4% in combination with biocontrol agents. Among all treatments *P. fluorescens* 10 g/kg seed + *T. harzianum* 4 g/kg seed + Marigold water extract 4% showed better performance of growth characters as compared to other treatments (Mourya *et al.*, 2014) (Table 4). Present findings were well supported by previous reports of Muthukumar *et al.* (2010) who reported that a combination of *T. viride* + *P. fluorescens* + zimmu leaf extract resulted in increased plant growth and yield of chilli. This result indicates that plant extracts as an excellent alternative of chemicals for seed treatment.

Evaluation of fungicides, botanicals and bioagents in pot

Table 2: Efficacy of bioagents on mycelial growth of *Pythiumde baryanum*

Tr. No.	Treatments	Per cent inhibition
1	<i>T. harzianum</i>	51.11 (45.64)*
2	<i>P. fluorescens</i>	48.10 (43.90)
3	<i>B. subtilis</i>	34.13 (35.70)
4	Control	0.00 (0.00)
	S.E (M) ±	0.59
	C.D. at (p=0.01)	2.30

*Figures in parenthesis are arc sin transformed values; Average of five replications

Table 3: Efficacy of fungicides on mycelial growth of *Pythiumde baryanum*.

Tr. No.	Treatments	Per cent inhibition
1	Carbendazim (0.1%)	0.00 (0.00)*
2	Metalaxyl (0.2%)	100.00 (90.00)
3	Thiram (0.2%)	84.91 (67.39)
4	Control	0.00 (0.00)
	S.E (M) ±	0.62
	C.D. at (p=0.01)	1.19

*Figures in parenthesis are arc sin transformed values; Average of five replications

Table 4: Effect of Marigoldwater extract, *P. fluorescens* and *T. harzianum* alone and in combination on seedling growth parameters of tomato by paper towel method

Tr. No.	Treatments	%Germination	Mean shoot length (cm)	Mean root length (cm)	SVI
T1	<i>P. fluorescens</i> alone (10 g/kg)	75.00	4.50	5.50	750.00
T2	<i>T. harzianum</i> alone (4 g/kg)	79.60	4.50	6.00	835.80
T3	Marigold water extract alone @ 2%	64.33	2.90	3.10	385.98
T4	Marigold water extract alone @3%	66.67	3.80	3.30	473.36
T5	Marigold water extract alone @4%	69.67	4.10	3.50	529.49
T6	<i>P. fluorescens</i> (10 g/kg) + <i>T. harzianum</i> (4 g/kg)	80.33	6.20	7.50	1100.52
T7	<i>P. fluorescens</i> (10 g/kg) + Marigold water extract 2%	77.33	4.50	5.60	781.03
T8	<i>P. fluorescens</i> (10 g/kg) + Marigold water extract 3%	78.33	4.40	5.70	791.13
T9	<i>P. fluorescens</i> (10 g/kg) + Marigold water extract 4%	79.67	4.80	6.20	876.37
T10	<i>T. harzianum</i> (4 g/kg) + Marigold water extract 2%	81.33	4.50	6.20	870.23
T11	<i>T. harzianum</i> (4 g/kg) + Marigold water extract 3%	82.33	4.60	6.20	889.16
T12	<i>T. harzianum</i> (4 g/kg) + Marigold water extract 4%	84.00	4.80	7.20	1008.00
T13	<i>P. fluorescens</i> (10 g/kg) + <i>T. harzianum</i> (4 g/kg) + Marigold water extract 4%	86.33	7.50	8.30	1364.01
T14	Metalaxyl (0.2%)	77.90	6.00	4.80	841.32
T15	Thiram (0.2%)	77.00	4.50	7.20	900.90
T16	Carbendazim (0.1%)	74.00	8.00	4.30	910.20
T17	Control	65.00	2.70	2.30	325.00

*Figures in parenthesis are arc sin transformed values. Average of five replications(SVI = Seedling vigour index)

culture

The significantly highest germination percentage and lowest percent of pre emergence damping-off of tomato were recorded in seed treatment with *P. fluorescens* 10 g/kg seed + *T. harzianum* 4 g/kg seed + Marigold water extract 4%. Moreover, *T. harzianum* 4 g/kg seed + Marigold water extract 4% showed better performance in increasing seed germination and suppressing damping-off disease of tomato (Table 5). Minimum post emergence damping off (16.33%) was recorded in Metalaxyl (0.2%) and was significantly superior over control and other treatments. *P. fluorescens* 10 g/kg seed + *T. harzianum* 4 g/kg seed + Marigold water extract 4% recorded 27.33% post emergence damping off. Maximum post emergence damping off (47.33%) was exhibited in Carbendazim (0.1%) and was not effective as compared to control (42.67%). In case of reduction in total damping of, all the treatments were effective to reduce damping off except Carbendazim which showed stimulating effect to the fungus and observed inferior than control. Maximum reduction in tomato damping off (74.11%) was observed in Metalaxyl (0.2%), followed by 68.37% in Thiram(0.2%) and both these treatments were found to be at par with treatment *P. fluorescens* 10 g/kg seed + *T. harzianum* 4 g/kg seed + Marigold water extract 4% which showed 68.16% reduction in total damping off (Shitole *et al.*, 2013) (Table 5).

Results indicated a gradual decrease in damping off with corresponding increase in concentration of crude Marigold water extract when integrated with bioagents. Earlier it was reported by Muthukumar *et al.* (2010) that the combined application of *T. viride*, *P. fluorescens* and zimmu leaf extract significantly reduced damping off incidence which corroborated the present findings. The broad spectrum antifungal activity of plant species was observed to be related to the presence of saponins, tannins and alkaloids (Ndukwe *et al.*, 2005). Many scientists suggested that Metalaxyl is effective fungicide to control diseases caused by *P.*

Table 5: Effect of *P. fluorescens*, *T. harzianum* and Marigold water extract alone and in combination on damping off of tomato caused by *P. debaryanum*.

Tr. No.	Treatments	Seed emergence (%)	Damping off (%)		Total reduction over control (%)
			Pre	Post	
T1	<i>P. fluorescens</i> alone(10 g/kg)	71.33 (57.63)*	14.63 (22.49)*	32.67 (33.54)*	49.27
T2	<i>T. harzianum</i> alone(4 g/kg)	74.89 (59.93)	10.37 (18.79)	33.33 (33.01)	55.01
T3	Marigold water extract alone@ 2%	61.56 (51.68)	26.33 (30.87)	38.33 (40.11)	23.79
T4	Marigold water extract alone @3%	65.11 (53.80)	22.07 (28.02)	37.00 (37.99)	32.63
T5	Marigold water extract alone @4%	70.00 (56.79)	16.22 (23.75)	34.67 (35.07)	44.69
T6	<i>P. fluorescens</i> (10 g/kg) + <i>T. harzianum</i> (4 g/kg)	77.11 (61.42)	7.71 (16.12)	30.33 (30.81)	61.87
T7	<i>P. fluorescens</i> (10 g/kg) + Marigold water extract 2%	71.33 (57.63)	14.62 (22.48)	32.00 (33.15)	49.99
T8	<i>P. fluorescens</i> (10 g/kg) + Marigold water extract 3%	71.56 (57.67)	14.36 (22.27)	31.00 (32.51)	51.42
T9	<i>P. fluorescens</i> (10 g/kg) + Marigold water extract 4%	74.67 (59.78)	10.64 (19.03)	29.33 (30.79)	58.62
T10	<i>T. harzianum</i> (4 g/kg) + Marigold water extract 2%	75.11 (60.07)	10.10 (18.53)	32.33 (32.38)	56.42
T11	<i>T. harzianum</i> (4 g/kg) + Marigold water extract 3%	75.78 (60.52)	9.31 (17.76)	31.67 (31.87)	58.23
T12	<i>T. harzianum</i> (4 g/kg) + Marigold water extract 4%	76.67 (61.12)	8.25 (16.69)	26.33 (28.59)	65.01
T13	<i>P. fluorescens</i> (10 g/kg) + <i>T. harzianum</i> (4 g/kg) + Marigold water extract 4%	79.11 (62.80)	5.32 (13.33)	27.33 (28.68)	68.16
T14	Metalaxyl (0.2%)	76.22 (60.82)	8.78 (17.23)	16.33 (22.20)	74.11
T15	Thiram (0.2%)	75.11 (60.07)	10.11 (18.54)	20.33 (25.14)	68.37
T16	Carbendazim (0.1%)	58.00 (47.68)	30.58 (33.58)	47.33 (47.53)	4.52
T17	Control (pathogen inoculated)	53.56 (47.04)	35.90 (36.81)	42.67 (46.78)	0.00
S.E (M)±		1.165	0.277	0.597	-
C.D. at (p=0.01)		4.301	1.022	2.206	-

*Figures in parenthesis are arc sin transformed values. Average of three replications

debaryanum. As per the scientific reports, *P. fluorescens* (Ramamoorthy *et al.*, 2002), *T. harzianum* (Warin *et al.*, 2008) and plant extracts (Mitali *et al.*, 2012) were effective in controlling damping off caused by *P. debaryanum* in various plants.

In the present study combined application of bacterial and fungal bioagents with Marigold water extract resulted in maximum activity against *Pythium debaryanum* than individual application. It may be due to synergetic effect of combined treatment. The flowers used in the present study are readily available with no or low cost and with easy method of extraction it can be exploited in the control of damping off of tomato. Further field experiments are suggested to investigate the *in vivo* effects of these extracts as compared to some chemical fungicides for the management of damping off of tomato.

REFERENCES

- Abdul baki, A. A. and Anderson, J. P. 1973. Vigour determination in soybean seeds by multiple criteria. *Crop Sci.* **1**: 630-633.
- Al-Rahmah, A. N., Mostafa, A. A., Abdel-Megeed, A., Yakout, S. M. and Hussein, S. A. 2013. Fungicidal activities of certain methanolic plant extracts against tomato phytopathogenic fungi. *African J. Microbiol. Res.* **7**(6): 517-524.
- Butu, M., Rodino, S., Butu, A. and Butnariu, M. 2014. Screening of bioflavonoid and antioxidant activity of *Lens culinaris* medikus. *Digest J. Nanomaterials and Biostructures.* **9**(2): 519-529.
- Choudhary, C. S., Jain, S. C., Kumar, R. and Choudhary, J. S. 2013. Efficacy of different fungicides, biocides and botanical extract seed treatment for controlling seed borne *Colletotrichum* sp. in chilli (*Capsicum annuum* L.). *The Bioscan.* **8**(1): 123-126.
- Deacon, J. W. and Berry, L. A. 1993. Biocontrol of soil-borne plant pathogens: concepts and their application. *Pestic. Sci.* **37**: 417-426.
- Dunne, C., Moe`nne-Loccoz, Y., McCarthy, J., Higgins, P., Powell, J., Dowling, D. N and O`Gara, F. 1998. Combining proteolytic and phloroglucinol-producing bacteria for improved biocontrol of *Pythium*-mediated damping-off of sugar beet. *Plant Pathol.* **47**: 299-307.
- Latha, P., Anand, T., Ragupathi, V., Prakasam, R. and Samiyappan, R. 2009. Antimicrobial activity of plant extracts and induction of systemic resistance in tomato plant by mixture of PGPR strains and Zimmu leaf extract against *Alternaria solani*. *Biol. Control.* **50**: 85-93.
- Lewis, J. A. and Papavizas, G. C. 1987a. Reduction of inoculums *Rhizoctonia solani* in soil by germlings of *Trichoderma hamatum*. *Soil Biol. Biochem.* **19**: 195-201.
- Lewis, J. A. and Papavizas, G. C. 1987b. Application of *Trichoderma* and *Gliocladium* in alginate pellets for control of *Rhizoctonia* damping-off. *Plant Pathol.* **36**: 438-446.
- Locke, J. C., Papavizas, G. C., Lewis, J. A., Lumsden, R. D. and Kantzes, J. G. 1983. Control of *Pythium* blight of snap beans by seed treatment with systemic fungicides. *Plant Dis.* **67**: 974-977.
- Manasi, K. Bhagwat and Datar, A. G. 2014. Antifungal activity of herbal extracts against plant pathogenic fungi. *Archives of Phytopath and Pl. Protec.* **47**(8): 959-965.
- Maurya, M. K., Singh, R. and Tomer, A. 2014. Determination of population dynamics of *Pseudomonas fluorescens* in the soil planted with tomato grown on de-oiled cakes of mahua and karanja. *The Bioscan (Supplement on Plant Pathology).* **9**(3): 1337-1340.
- Mitali Madhusmita Pattnaik, Kar, M. and Sahu, R. K. 2012. Bioefficacy of some plant extracts on growth parameters and control of diseases in *Lycopersicon esculentum*. *Asian J. Plant Sci. Res.* **2**(2): 129-142.
- Muthukumar, A., Eswaran, A., Nakkeeran, S. and Sangeetha, G. 2010. Efficacy of plant extracts and biocontrol agents against *Pythium aphanidermatum* inciting chilli damping-off. *Crop Protec.* **29**: 1483-1488.
- Ndukwe, K. C., Okeke, I. N., Lamikanra, A., Adesina, S. K. and Aboderin, O. 2005. Antibacterial activity of aqueous extracts of selected chewing sticks. *J. Contemp. Dental Pract.* **6**(3): 86-94.
- Negi, P. 2012. Plant extracts for the control of bacterial growth: Efficacy, stability and safety issues for food application, *Int. J. Food Microbiol.* **156**(1): 7-17.
- Ramamoorthy, V., Raguchander, T. and Samiyappan, R. 2002. Enhancing resistance of tomato and hot pepper to *Pythium* diseases

by seed treatment with *Fluorescent pseudomonads*. *European J. Plant Pathol.* **108**: 429-441.

Rossall, S. 2012. Fungicide Resistance in Crop Protection: Risk and Management. *Plant Pathology.* **61**: 820.

Shitole, A. V., Gade, R. M., Archana Zalte and Bandgar, M. S. 2013. Utilization of spent mushroom substrate for management of tomato damping off. *J. Pl. Dis. Sci.* **8(2)**: 196-199.

Stack, J. P., Kenerley, C. M. and Pettit, R. E. 1986. Application of biological control agents. In: *Biological Control of Plant Diseases*. Butterworth, London. p. 71.

Swati Rose Toppo and Preeti Tiwari 2015. Biocontrol potentialities of native *Pseudomonas* isolates against plant pathogenic fungi *Rhizoctonia* spp., *Fusarium* spp. and *Colletotricum* spp. in tomato rhizosphere under greenhouse condition. *The Bioscan (Supplement on Agronomy)*. **10(1)**: 373-377.

Thenmozhi, M., Bhavya, P. K. and Rajeshwari Sivaraj 2011.

Compounds identification using HPLC and FTIR in *Eclipta alba* and *Emilia sonchifolia*. *Int. J. Engin. Sci. and Technol.* **3(1)**: 292-298.

Vincent, J. H. 1947. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature.* **15**: 850.

Warin Intana, Prakong Yenjit, Taksin Suwanno, Supalak Sattasakuchai, Manoon Suwanno and Chiradej Chamswarn 2008. Efficacy of antifungal metabolites of *Bacillus* spp. for controlling tomato damping-off caused by *Pythium aphanidermatum*. *Walailak J. Sci. Tech.* **5(1)**: 29-38.

Whipps, J. M. 2001. Microbial interactions and biocontrol in the rhizosphere. *J. Exp. Bot.* **52**: 487-511.

Zagade, S. N., Deshpande, G. D., Gawade, D. B., Atnoorkar, A.A. and Pawar, S. V. 2012. Biocontrol agents and fungicides for management of damping off in chilli. *World J. Agri. Sci.* **8(6)**: 590-597.

