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EFFICACY OF FUNGICIDAL AND BIOCIDAL ACTIVITIES OF SOME MEDICINAL PLANTS SOLVENTS EXTRACTS IN MANAGEMENT OF DAMPING OFF DISEASE OF TOMATO

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ABSTRACT

The present study has been carried out for assessment of antifungal activities of acetone, ethanol, methanol and chloroform extracts of three different plants leaves *viz*., *A. marmelos, S. cumini* and *P. pinnata* for management of soil borne fungi *P. debaryanum*, causing damping off disease in tomato, chilli, etc. The maximum inhibition of fungal growth was found in methanolic extract of *A. marmelos* leaves and fruits against, *P. debaryanum*. At 1000 μl concentration (C4), complete inhibition of mycelial growth of *P. debaryanum* was recorded in S3P1 (100%), followed by S3P3 (97.63%), S2P1 (95.21%). Lowest inhibition inhibition was recorded in S4P2 interaction (75.40%). Pot culture study revealed that tomato seed treatment with *P. fluorescens* (10 g/kg) + *T. viride* (4 g/kg) + methanolic extract of *A. marmelos* 4% was effective to control pre and post emergence damping off caused by *P. debaryanum*. The methanolic extract revealed strongest antifungal activity against *P. debaryanum*, followed by ethanolic and lowest antifungal activity found in chloroform extract.

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INTRODUCTION

Traditional fungicides have been practiced for many centuries by a substantial proportion of the population of many countries. The medicinal plants as a source of secondary metabollites have increased worldwide. Medicinal plant extracts represent a continuous effort to find new active compounds with the potential to act against multi resistant bacteria (Mothana and Lindequist, 2005). The potential of higher plants as a source of new drugs isstill largely unexplored (Dubey *et al*., 2004). Hence, last decade as witnessed a source of new bio molecules for plant disease management. This is also true in India, among estimated medicinal plant diversity only a small percentage of plants of this regionhave been investigated for antimicrobial activity against plant pathogenic microorganisms.

A. marmelos phytochemical analysis ascertained the presence of some potential phytochemical groups *i.e*. alkaloids, saponins, tannins, flavonoids and furanocoumarins (Mary Shobha Rani *et al*., 2013). *S. cumini* analysis indicated presence of gallic acid and quercetin in the methanolic extract (Kothari *et al*., 2011). *S. cumini* seeds phytochemical tests of methanol and ethanol extracts revealed the presence of phenols, alkaloids and flavonoids methanol extract of *S. cumini* seeds also contain alkaloids and flavonoids (Yadav *et al*., 2010). They additionally reported presence of saponins, tannins and triterpenoids too. *Pongamia pinnata* methanol extract contented alkaloids, steroids, flavonoids, glycosides, saponins and tannins (Dahikar *et al*., 2008). Soil borne diseases are notoriously difficult to control. Crop rotation, breeding for resistant plant varieties and the application of pesticides are insufficient to control such diseases. The present investigation was undertaken to screen the crude extracts of plants for their antifungal potency in laboratory conditions against *P. debaryanum* causing damping off in tomato.

MATERIALS AND METHODS

P. debaryanum was tested on tomato by soil inoculation technique (Mythukumar *et al*., 2009). The Patho genicity of the fungus was tested on the basis of per cent pre and post-damping off in artificial sick soil. The field soil was sterilized for three successive days at 120ºC. Soil sickness was developed by adding 100 g mass inoculum of *P. debaryanum* in 1 kg of soil. The plastic pots of 15 cm diameter were filled with the above mixture. The pots were watered and incubated for 6 days to multiply the pathogen in soil. The fresh leaves of *A. marmelos, S. cumini* and *P. pinnata* were collected from various places, 5 km^2 area around the headquarter. The identification and authentication of the plants was carried out at Department of Botany Dr. P. D. K. V., Akola (M.S.), India. Collected fresh plant leaves were thoroughly washed under tap water to remove dust and other impurities and once with distilled water and then shade dried separately under shade with occasional shifting for about 3 to 4 weeks. The dried leaves were coarsely powdered with sample grinder and stored in airtight container until further use (Thenmozhi *et al*., 2011).

Acetone, ethanol, methanol and chloroform were used as solvent for preparation of leaves extracts. Forty gram powder of each leaves was separately soaked in 200 ml of acetone, ethanol, methanol and chloroform in 500 ml conical flask and then plugged tightly with cotton and wrapped with paper. All conical flasks were kept on rotary shaker for four days and then allowed to stand for 5 hr to settle the leaves material. Supernatant from each flask was filtered separately through What man No. 1 filter paper and evaporated at room temperature. Residual portion of leaves was repeatedly extracted three times to harvest maximum metabolites from leaves. Air dried extracts were weighed separately and transferred into small vials and kept in refrigerator at 5ºC until further use. The percentage of extraction yield was calculated (Khan *et al*., 2010).

The efficacy of acetone, ethanol, methanol and chloroform extracts of *A. marmelos, S. cumini* and *P. pinnata* at 250, 500, 750 and 1000 μlconcentration were tested against *P. debaryanum* under *in vitro* condition following poisoned food technique on (Al-Rahmah *et al*., 2013). One gram crude extract of all the plant leaves extracted with acetone, ethanol, methanol and chloroform were diluted in 10 ml dimethyl sulphoxide (DMSO) separately and from this 250, 500, 750 and 1000 μl suspension were poured separately in conical flasks which containing 60 mL sterilized melted PDA medium for 3 plates. The conical flask wasshacked well for uniform mixing of plant extract with media and poured in plates then allowed for solidification. In control set, only 250, 500, 750 and 1000 μl DMSO were used. For each treatment, 3 replicates (plates) were used. All the plates were inoculated individually with 5 mm diameter discs of the test fungal cultures and then incubated at 28 ± 2ºC, until the control plates reached full growth. To know the effect of different plant extracts. The per cent growth inhibition (I) of test fungus was calculated (Vincent, 1947).

Antagonistic activity of *P. fluorescens* and *T. viride* on growth of *P. debaryanum* was studied by dual culture technique on PDA plates. The inoculated plates were incubated at 22 ± 2ºC for 3 days. Observations regarding antagonistic effect of all these bioagents against test pathogens were recorded on 3 days after inoculation. The growth inhibition of test pathogen was calculated (Vincent, 1947).

Pot culture experiments were carried out for studying antagonistic activity of *P. fluorescence*, *T. viride* and *A. marmelos* methanol extract alone or in combination as seed treatment against *P. debaryanum* causing damping off of tomato. Three replications were maintained for each treatment in a Factorial completely randomized design (FCRD) in a glasshouse. Incidence of damping off of tomato was recorded at 7, 14 and 21 days after sowing.

Treatments were S1P1 (acetone extract of *A. marmelos*), S1P2 (acetone extract of *S. cumini*), S1P3 (acetone extract of *P. pinnata*), S2P1 (ethanol extract of *A. marmelos*), S2P2 (ethanol extract of *S. cumini*), S2P3 (ethanol extract of *P. pinnata*), S3P1 (methanol extract of *A. marmelos*), S3P2 (methanol extract of *S. cumini*), S3P3 (methanol extract of *P. pinnata*), S4P1 (chloroform extract of *A. marmelos*), S4P2 (chloroform extract of *S. cumini*) and S4P3 (chloroform extract of *P. pinnata*).

In vitro effect of medicinal plants solvent extract on test pathogen was done by using Factorial completely randomized design (FCRD) with three factors having four levels in each factor. Pot culture studies were carried out by using completely randomized design and each treatment had three replications. The statistical analysis of the data was done by statistical method as suggested by Gomez and Gomez, 1984.

RESULTS AND DISCUSSION

The results revealed that all of the tested medicinal plant extracts at each concentration inhibited the growth of *P. debaryanum* (Table 3). The rate of growth inhibition was corroborated with its concentrations in case of all the tested plant extracts.

P. debaryanum was isolated on Vaartaza's medium from infected tomato plants. The extraction yield was affected significantly by the solvent used for extraction. This is principally related to the polarity and capability to extract

Table 1: Effect of different solvents on per cent extraction yield from dry weight of leaves

Sample and local name	Extraction yield (%) in solvents			
	Acetone	Ethanol	Methanol	Chloroform
A. marmelos (Beal)	1.82	2.01	7.44	1.81
S. cumini (Jamun)	3.42	3.22	8.52	2.01
P. pinnata (Karanj)	3.03	4.21	7.21	1.85

Table 2: Efficacy of bioagents on mycelial growth of *P. debaryanum*

Tr. No.	Treatment	Radial mycelial growth (mm) *P. debaryanum*	Per cent inhibition *P. debaryanum*
1	*T. viride*	44.00	51.11 (45.64)*
2	*P. fluorescens*	46.71	48.10 (43.90)
3	Control	90.00	0.00 (0.00)
	F test	Sig	Sig
	S.E (M) ±	0.90	0.59
	C.D. at (p = 0.01)	3.53	2.30

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

Table 3: Effect of interaction mean of solvents x plants x concentrations (S x P x C)

S x P x C (Solvent x plant x Conc.)	% inhibition over control C1 (250 µl)	C2 (500 µl)	C3 (750 µl)	C4 (1000 µl)
S 1 P 1	13.6(21.68)*	43.04(41.00)	79.69(63.21)	86.57(68.30)
S 1 P 2	10.96(19.33)	29.98(33.19)	50.98(45.56)	81.05(64.19)
S 1 P 3	14.45(22.34)	41.68(40.21)	68.78(56.03)	88.86(70.50)
S 2 P 1	15.86(23.47)	57.63(49.39)	86.41(68.37)	95.21(77.36)
S 2 P 2	12.16(20.41)	33.33(35.26)	57.76(49.46)	85.43(67.56)
S 2 P 3	16.64(24.07)	45.50(42.42)	74.45(59.63)	91.04(72.59)
S 3 P 1	16.99(24.34)	86.35(68.31)	90.91(72.45)	100.00(89.76)
S 3 P 2	14.40(22.30)	37.67(37.86)	63.22(52.66)	88.73(70.39)
S 3 P 3	18.89(25.76)	48.84(44.33)	80.01(63.44)	97.63(81.15)
S 4 P 1	10.96(33.11)	29.85(33.11)	74.11(59.41)	77.64 (61.78)
S 4 P 2	8.83(28.88)	23.33(28.88)	43.01(40.98)	75.40(60.26)
S 4 P 3	13.31(38.57)	38.88(38.57)	62.18(52.02)	77.73(61.84)
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00(0.00)
Source	S.E (M) ±		C.D. at (p = 0.01)	
Solvent (S)	0.04		0.18	
Plants (P)	0.04		0.18	
Concentrations (C)	0.04		0.18	
Solvent x Plants (S x P)	0.08		0.31	
Solvent x Concentrations (S x C)	0.09		0.36	
Plants x Concentrations (P x C)	0.08		0.31	
Solvent x Plants x Concentrations (S x P x C)	0.14		0.62	

Solvents (S)	Plant leaves (P)	Concentrations (C)
S1- Acetone	P1- *Aegle marmelos* leaf extract	C1- 250 µl
S2- Ethanol	P2- *Synzygium cumini* leaf extract	C2- 500 µl
S3- Methanol	P3- *Pongamia pinnata* leaf extract	C3- 750 µl
S4- Chloroform		C4- 1000 µl

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

Table 4: Effect of *Pseudomonas fluorescens, Trichoderma viride* and methanolic extract of *A. marmelos* alone and in combination on damping off of tomato caused by *P. debaryanum.*

Tr. No.	Treatments	Seed emergence (%)	Damping off (%) Pre	Post	Total reduction over control (%)
T1	*P. fluorescens* alone (10 g/kg)	71.33 (57.63)*	14.63 (22.49)*	32.67 (33.54)*	49.27
T2	*T. viride* alone (4 g/kg)	74.89 (59.93)	10.37 (18.79)	33.33 (33.01)	55.01
T3	Methanolic extract of *A. marmelos* alone @ 2%	61.56 (51.68)	26.33 (30.87)	38.33 (40.11)	23.79
T4	Methanolic extract of *A. marmelos* alone @ 3%	65.11 (53.80)	22.07 (28.02)	37.00 (37.99)	32.63
T5	Methanolic extract of *A. marmelos* alone @ 4%	70.00 (56.79)	16.22 (23.75)	34.67 (35.07)	44.69
T6	*P. fluorescens* (10 g/kg) + *T. viride* (4 g/kg)	77.11 (61.42)	7.71 (16.12)	30.33 (30.81)	61.87
T7	*P. fluorescens* (10 g/kg) + Methanolic extract of *A. marmelos* @ 4%	74.67 (59.78)	10.64 (19.03)	29.33 (30.79)	58.62
T8	*T. harzianum* (4 g/kg) + Methanolic extract of *A. marmelos* @ 4%	76.67 (61.12)	8.25 (16.69)	26.33 (28.59)	65.01
T9	*P. fluorescens* (10 g/kg) + *T. viride* (4 g/kg) + Methanolic extract of *A. marmelos* @ 4%	79.11 (62.80)	5.32 (13.33)	27.33 (28.68)	68.16
T10	Control (Pathogen inoculated)	53.56 (47.04)	35.90 (36.81)	42.67 (46.78)	0.00
T11	Soil control	85.12 (52.91)	-	-	-
F test		Sig	Sig	Sig	-
S.E (M) ±		1.16	0.27	0.59	-
C.D. at (p = 0.01)		4.301	1.02	2.20	-

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

substances that can be dissolved in the used solvent. The methanol was found extensively useful for extraction yield and was the most capable to extract more substances or the plants used contain more substances that preferably dissolve in methanol (*A. marmelos* - 7.44%, *S. cumini* - 8.52% and *P. pinnata* - 7.21%) (Table 1).

Comparison was made between two bioagents for their ability to control mycelial growth of *P. debaryanum* by dual culture technique. All the bioagents were effective in reducing fungal growth. Among two bioagents tested, maximum inhibition of mycelial growth was noticed in *Trichoderma viride* (51.11%) and was found to be significantly superior over other treatments (Table 2). Similar results were observed by Muthu kumar *et al*. (2011) in which he reported 53.8 to 71.5% and

57.8 to 76.7% inhibition with *Trichoderma* and *Pseudomonas* isolates, respectively. *Pseudomonads* were more efficient in inhibiting various *Pythium* isolates than bacilli (Georgakopoulos *et al*., 2002; Shitole *et al*., 2013; Archana Zalte *et al*., 2013).

Interaction effect of solvents, plants and concentrations were evaluated for their effectiveness against test fungus. There was varied degree of fungi toxicity among all the interactions. Results of Table 3, represent that, at 1000 μl concentration (C4), complete inhibition of mycelial growth of test fungus was recorded in S3P1, followed by S3P3, S2P1. Lowest inhibition (75.40%) inhibition was recorded in S4P2. Similar results were seen in lower concentrations (Chakrabarty *et al*., 2013; Choudhary *et al*., 2013). Earlier study of 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.

Methanolic extract of *A. marmelos*, *P. fluorescens* and *T. viride* alone and in combination in pot culture experiment were screened against *P. debaryanum* causing damping off in tomato (Table 4). Among all the treatments, maximum 79.11% germination was observed in treatment T9 (*P. fluorescens* 10 g/kg seed + *T. viride* 4 g/kg seed + methanolic extract of *A. marmelos* 4%) followed by 76.67% in T8 (*T. viride* 4 g/kg seed + methanolic extract of *A. marmelos* 4%) and both these treatments were at par with each other. Lowest germination was reported in control T10 (53.56%) (Maurya *et al*., 2014) (Table 4).

It was observed from the results of current study, that maximum efficacy was recorded in combined application of both the bioagents and methanolic extract of *A. marmelos*. It was due to the synergetic activity of their components. These results are in agreement with Muthu kumar *et al*. (2010) who reported that *T. viride, P. fluorescens* and Zimmu leaf extract significantly reduced damping off incidence. It has been reported that application of *P. fluorescens* triggers / activates plants' latent defense mechanisms in response to infection by pathogen (Shitole *et al*., 2013; Archana Zalte *et al*., 2013).

Minimum post emergence damping off (26.33) was recorded in T8 (*T. viride* 4 g/kg seed + methanolic extract of *A. marmelos* 4%) and 27.33 in T9 (*P. fluorescens* 10 g/kg seed + *T. viride* 4 g/kg seed + methanolic extract of *A. marmelos* 4%), which are at par with each other (Table 4). 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). Similar results were also observed by Mina koche *et al*. (2013) and Muthu kumar *et al*. (2010).

Eleven treatments were screened to see the comparative effect of methanolic extract of *A. marmelos* and bioagents for reducing damping off in tomato. All the treatments were effective to reduce damping off. There were significant differences among all the treatments for reducing damping off. Maximum reduction in tomato damping off (68.16%) was observed under pot experiment in Treatment T9 (*P. fluorescens* 10 g/kg seed + *T. viride* 4 g/kg seed + methanolic extract of *A. marmelos* 4%), followed by 65.01% in treatment T8 (*T. viride* 4 g/kg seed + methanolic extract of *A. marmelos* 4%) and both these treatments were found to be at par with each other (Table 4).

Results indicated a gradual decrease in damping off with corresponding increase in concentration of methanolic extract of *A. marmelos* when integrated with bioagents. Earlier it was reported by Muthukumar *et al*. (2010) that *T. viride, P. fluorescens* and zimmu leaf extract significantly reduced damping off incidence which corroborated the present findings.

In the present study combined application of bacterial and fungal bioagents with methanolic extract of *A. marmelos* resulted in maximum activity against *P. debaryanum* than individual application. It may be due to synergetic effect of combined treatment. As above stated bioagents have promising effect due to presence of different antifungal constituents. This may go long way in providing better alternative to the over dependency on synthetic fungicides.

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