ISSN: 0974 - 0376

The Ecoscan : Special issue, Vol. IX: 645-651: 2016
AN INTERNATIONAL QUARTERLY JOURNAL OF ENVIRONMENTAL SCIENCES
www.theecoscan.com

EVALUATION OF INHIBITORY EFFECT OF MEDICINAL PLANTS SOLVENT EXTRACTS AGAINST *FUSARIUM OXYSPORUM* F. SP. *CICERI* CAUSING WILT OF CHICKPEA

More Yogeshwar *et al.,*

KEYWORDS

Extracts
Antifungal activity
A. marmelos
P. pinnata
F. oxysporum f. sp. *ciceri*

Proceedings of National Conference on Harmony with Nature in Context of Resource Conservation and Climate Change
(HARMONY - 2016)
October 22 - 24, 2016, Hazaribag,
organized by
Department of Zoology, Botany, Biotechnology & Geology
Vinoba Bhave University,
Hazaribag (Jharkhand) 825301
in association with
National Environmentalists Association, India
www.neaindia.org

The Ecoscan
AN INTERNATIONAL QUARTERLY JOURNAL OF ENVIRONMENTAL SCIENCES

MORE YOGESHWAR, R. M. GADE, A. V. SHITOLE*, S. ASWATHI AND S. KAPALI
Department of Plant Pathology,
Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola - 444 104 (M.S.) INDIA
e-mail: amolshitole1690@gmail.com

ABSTRACT

The present experiment was carried out by using different solvent extracts of plants (Acetone, ethanol, methanol and chloroform extract) and bioagents against *F. oxysporum* f. sp. *ciceri*. Effect of plant extracts and bioagents alone and in combination on wilt incidence of chickpea was assessed under pot culture experiment. Methanol extracts of *Aegle marmelos*, was found most sensitive (88.64%) followed by methanol extract of *Pongamia pinnata* (85.30%). Different solvents *viz.*, acetone, ethanol, methanol and chloroform were tested to compare the maximum extraction yield from four different plants. Methanol extracts of *A. marmelos* at all concentration (250, 500, 750 and 1000 μl) found compatible with *Trichoderma viride* and *Pseudomonas fluorescens*. Methanol was found useful for getting highest per cent extraction yield. Seed treatment with *P. fluorescens* 10 g/kg seed + *T. viride* 4 g/kg seed + methanolic extract of *Aegle marmelos* 4% was proved effective to reduce incidence of chickpea wilt disease caused by *F. oxysporum* f. sp. *ciceri* (69.31%).

***Corresponding author**

INTRODUCTION

The indiscriminate use of synthetic chemicals for the control of pests and diseases of crop plants has posed serious threat to human health and environment leading to disturbed biodiversity, outbreaks of secondary pests, and resurgence development of resistance in the pathogens and contamination of food chain in the ecosystem. Fungal diseases of crop plants have always been one of the major constraints in successful crop production, which causes severe yield loss every year. However, the researchers are optimistic in developing alternatives to chemical fungicides. Eco-friendly systems involving plant products and biological agents, which act directly on the pathogens or indirectly by inducing resistance in plants, have gained considerable importance as an alternative to synthetic fungicides (Mishra and Raja, 1999). Plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environment impact and danger to consumers in contrast to the synthetic pesticides (Varma and Dubey, 1999). Plant extracts have unique antimicrobial properties, which act in holistic mode. Various workers have reported that plant extracts and their secondary metabolites; alkaloids, terpenoid, glycosides and phenolic acids have a number of medicinal properties and affect biological functions at very low concentrations; some of them also possess antimicrobial activity (Singh *et al*., 1999). These products are known by their active substances, like *A. marmelos* phytochemical analysis ascertained the presence of some potential phytochemical groups *i.e.* alkaloids, saponins, tannins, flavonoids and furanocoumarins (Phulan *et al.,* 2013). Because of these associated problems, use of aqueous extracts of many allelopathic plants and utilization of microbial antagonists as the environmentally safe alternative methods for disease management seems to be the need of the hour in efficient integrated disease management strategies.

The wilt of Chickpea incited by *F. oxysporum* f. sp. *ciceri* is one of the serious diseases (Gupta *et al.,* 1986). This pathogen is soil borne and seed borne cause profound losses (20 to 100%) depending upon phase of illness and wilting (Haware and Nene, 1980). A systematic investigation was undertaken to screen the antifungal activity and an attempt to explore the possibilities to utilize such huge biological waste for management of wilt of chickpea and their antifungal potency in laboratory conditions against *F. oxysporum* f. sp. *ciceri*.

MATERIALS AND METHODS

A virulent isolate of *F. oxysporum* f. sp. *ciceri* isolated from wilt infested chickpea plants was used in the present studies. Chickpea variety JG-62 was used as a host crop. Three antagonists' *viz*., *T. harzianum* and *P. fluorescens* isolated from soil sample. The experiment was conducted *in vitro* and greenhouse conditions during 2015. The sand sorghum medium (SSM) was used for the mass multiplication of *F. oxysporum* f. sp. *ciceri* in the laboratory. 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 Whatman 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 5^0C 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 μl concentration were tested against. *F. oxysporum* f. sp. *ciceri* under *in vitro* condition following poisoned food technique on PDA (Potato Dextrose Agar) (Al-Rahmah *et al*., 2013). One gram crude extract of all 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 was shacked 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 ± 2°C, 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).

Compatibility was determined for *T. viride*, *P. fluorescens* and methanolic plant leaves extract by Poisoned food technique. Spectrophotometric method was followed to study the compatibility of *A. marmelos* plant leaves methanolic extract with potential bacterial bioagents.

Pot culture experiment werecarried out for studying antagonistic activity of *Pseudomonas fluorescence*, *Trichoderma viride* and *Aegle marmelos* methanolic extract alone or in combination as seed treatment against *F. oxysporum* f. sp. *ciceri.* Chickpea JG-62 seeds were surface disinfected in 2% sodium hypochlorite for 30 seconds, rinsed in sterile distilled water and dried overnight. Ten seeds were planted 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 were soaked in 2, 3 and 4% solutions separately for 3 hr and air dried overnight before sowing and inoculated pots with the pathogen alone served as control. Three replications were maintained for each treatment in a Factorial completely randomized design (FCRD) in a glasshouse. Incidence of wilt in chickpea was recorded at 30 and 60 days after sowing.

Treatment details 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*), S3P4 (methanol extract of *P. pinnata*), S4P1 (chloroform extract of *A. marmelos*), S4P2 (chloroform extract of *S. cumini*) and S4P3 (chloroform extract of *P. pinnata*).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 *F. oxysporum* f. sp. *ciceri*. The extraction yield was affected significantly by the solvent used for extraction. This is principally related to the polarity and capability to extract 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* - 7.04%, *Syzygium cumini* – 8.12% and *Pongamia pinnata* – 6.81%) (Table 1).

It was observed from the data presented in Table 2, that, *F. oxysporum* f. sp. *ciceri* was sensitive to all the tested bioagents but *P. fluorescens* recorded maximum (81.59%) mycelial inhibition (Swati Rose Toppo and Preeti Tiwari, 2015; Asha *et al*., 2011). Our results match with Lily Trivedi and Rathi (2016) and Mohmedand El-Hadidy (2016) who reported that the *Trichoderma* species evaluated against the wilt pathogen exhibited great potential in managing chickpea wilt under

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)	1.42	1.61	7.04	1.42
Syzygium cumini (Jamun)	3.01	2.82	8.12	1.61
Pongamia pinnata (Karanj)	2.63	3.81	6.81	1.45

Table 2: Efficacy of bioagents on mycelial growth of *Fusarium oxysporum* f. sp. *ciceri*

Tr. No.	Treatment	Radial mycelial growth (mm)	Per cent inhibition
		F. o. ciceri	*F. o. ciceri*
1	*T. viride*	20.43	77.30 (61.58)*
2	*P. fluorescens*	16.57	81.59 (64.68)
3	Control	90.00	0.00 (0.00)
	S.E (M)±	0.72	0.57
	C.D. at (p=0.01)	2.83	2.21

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

Table 3: Efficacy of bioagents on mycelial growth of *Fusarium oxysporum* f. sp. *ciceri*

Conc. of *Aegle marmelos* extract (mg)	Mycelial growth (mm) Days after inoculation			Per cent compatibility Days after inoculation		
	2	4	5	2	4	5
25	26.50	71.00	80.50	72.60 (58.50)	78.89 (62.73)	89.44 (71.04)
50	24.50	55.00	67.80	67.12 (55.04)	61.11 (50.83)	75.33 (60.22)
75	16.50	45.00	55.00	45.21 (42.25)	50.00 (45.00)	61.11 (51.42)
100	13.50	44.50	49.00	36.99 (37.46)	49.44 (44.68)	54.44 (47.55)
control	36.50	90.00	90.00	-	-	-
S.E (M)±	0.57	0.89	0.92	0.74	0.90	0.45
C.D. at (p=0.01)	2.32	3.63	3.75	3.02	3.68	1.85

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

Table 4: Compatibility of *Pseudomonas fluorescens* with *Aegle marmelos* methanolic extract by spectrophotometry

Conc. of extract (mg)	Optical density at 610 nm Time (hrs.) after inoculation				Per cent compatibility Time (hrs.) after inoculation			
	12	24	36	48	12	24	36	48
25	1.64	2.24	2.41	2.13	83.25 (9.12)*	97.82 (9.89)	95.26 (9.76)	88.02 (9.38)
50	1.57	2.15	2.17	2.07	79.70 (8.92)	93.89 (9.69)	85.77 (9.26)	85.54 (9.25)
75	1.47	2.10	2.04	2.05	74.62 (8.64)	91.70 (9.58)	80.63 (8.98)	84.71 (9.20)
100	1.45	2.07	1.95	2.01	73.60 (8.58)	90.39 (9.51)	77.08 (8.78)	83.06 (9.11)
Control	0.08	0.08	0.08	0.07	-	-	-	-
Bacterial control	1.97	2.29	2.53	2.42	-	-	-	-
S.E (M)±	0.05	0.11	0.12	0.13	0.09	0.04	0.03	0.04
C.D. at(p=0.01)	0.19	0.43	0.51	0.52	0.38	0.16	0.12	0.16

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

glasshouse and field conditions. Selected isolates of *Pseudomonas fluorescens* were found to be effective in reducing the wilt incidence and increasing the plant growth as well as grain yield of chickpea (Kala *et al.*, 2016; Kumbhar *et al.*, 2016). *P. fluorescens* has revolutionised the field of biological control of soil- borne plant pathogenic fungi because it produces phenazin, pyroluterin, phloroglucinol and siderophores, which might be involved in the supersession of the wilt pathogen (Fridlender *et al.,* 1993). Muneeb *et al*. (2010) observed 84.79% mycelial inhibition of the *F. oxysporum* f.sp. *ciceri* by *T. viride* under *in vitro* condition. Similar types of results also found by Choudhary *et al*. (2013) and Mina Koche *et al*. (2013).

The concentration of 25 and 50 mg/ 20 ml showed 89.44 and 75.33% compatibility and was found to be non-inhibitory at 5 days after inoculation as compared to control. A significant difference in mycelial growth of *T. viride* was observed in concentration from 75 and 100 mg/ 20 ml media (Tapwal *et al.*, 2012) (Table 3). The results of study correlates with the findings of Bagwan (2010) who reported that seed treatment or furrow application of *Trichoderma* would be compatible with neem oil, neem leaves extract, wild sorghum leaves extract, neem cake, castor cake and mustard cake extracts for the integrated management of soil borne diseases of groundnut.

Observations on growth of bacteria (*P. fluorescens* in *Aegle marmelos* ethanolic extract amended broth at various concentrations were assessed by turbidomertic method and the results are presented in Table 4 for *P. fluorescens*. The bacterial growth was not affected by *Aegle marmelos* methanolic extract even at the highest concentration of 100 mg. In *P. fluorescens* inoculated broth the turbidity increased with increase in incubation time (1.45 to 2.10) in *Aegle marmelos* methanolic extract at the concentration of 100 mg, while in control the turbidity did not increase (0.08). When the incubation period was increased beyond 42 hours the turbidity was found to be decreased at all the concentrations (Table 4) (Ahila and Prakasam, 2013).

Observations on interaction effect of solvents, leaves and concentrations on dry mycelial weight of *F. oxysporum* f. sp. *ciceri* were recorded and per cent inhibition were determined and presented in Table 5. Statistically analyzed results clearly indicated the higher fungitoxicity of methanolic extract of *A.*

Table 5: 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	58.63(49.97)*	73.22(58.84)	76.60(61.07)	78.78(62.58)
S 1 P 2	56.42(48.69)	67.67(55.35)	73.10(58.76)	74.34(59.57)
S 1 P 3	57.69(49.42)	70.97(57.40)	74.44(59.63)	78.76(62.56)
S 2 P 1	58.80(50.07)	75.43(60.29)	79.78(63.28)	82.14(65.00)
S 2 P 2	57.52(49.32)	71.91(57.99)	76.49(61.00)	79.75(63.26)
S 2 P 3	57.45(49.28)	70.78(57.28)	74.13(59.43)	78.79(62.58)
S 3 P 1	59.86(50.69)	79.81(63.31)	83.56(66.09)	88.64(70.30)
S 3 P 2	58.53(49.9)	75.38(60.25)	79.80(63.30)	82.04(64.92)
S 3 P 3	58.89(50.12)	76.42(60.95)	82.11(64.98)	85.30(67.45)
S 4 P 1	57.68(49.42)	67.35(55.15)	69.72(56.61)	72.15(58.15)
S 4 P 2	55.40(48.10)	64.32(53.32)	68.51(55.87)	68.83(56.06)
S 4 P 3	57.41(49.26)	66.34(54.54)	68.68(55.97)	70.98(57.40)
Control	0.00	0.00	0.00	0.00
Source			S.E (M) $\pm$	C.D. at (p = 0.01)
Solvent (S)			0.04	0.17
Plants (P)			0.03	0.13
Concentrations (C)			0.08	0.33
Solvent x Plants (S x P)			0.07	0.29
Solvent x Concentrations (S x C)			0.08	0.33
Plants x Concentrations (P x C)			0.07	0.29
Solvent x Plants x Concentrations (S x P x C)			0.14	0.54

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 6: Effect of *Pseudomonas fluorescens*, *Trichoderma viride* and methanolic extract of *Aegle marmelos* alone and in combination on chickpea wilt caused by *F. oxysporum* f. sp. *Ciceri*

Tr. No.	Treatment	Germination (%) 30 DAS	Wilt (%) 60 DAS	% disease control
T1	*P. fluorescens* alone (10 g/kg)	83.33 (65.91)*	24.00 (33.42)*	40.00 (43.66)*	56.70
T2	*T. viride* alone (4 g/kg)	86.67 (68.58)	30.33 (29.33)	47.67 (39.23)	48.38
T3	Methanolic extract of *Aegle marmelos* alone @ 2%	73.67 (59.13)	41.33 (40.01)	58.00 (49.60)	37.18
T4	Methanolic extract of *Aegle marmelos* alone @ 3%	76.33 (60.89)	39.33 (38.84)	56.33 (48.64)	38.99
T5	Methanolic extract of *Aegle marmelos* alone @ 4%	81.00 (64.16)	35.67 (36.67)	53.00 (46.72)	42.60
T6	*P. fluorescens* (10 g/kg) + *T. viride* (4 g/kg)	90.67 (72.21)	22.00 (27.97)	32.00 (34.45)	65.33
T7	*P. fluorescens* (10 g/kg) + Methanolic extract of *Aegle marmelos* @ 4%	89.67 (71.25)	22.00 (31.09)	32.33 (38.25)	64.98
T8	*T. viride* (4 g/kg) + Methanolic extract of *Aegle marmelos* @ 4%	92.33 (73.93)	26.67 (27.97)	38.33 (34.65)	58.48
T9	*P. fluorescens* (10 g/kg) + *T. viride* (4 g/kg) + Methanolic extract of *Aegle marmelos* @ 4%	94.33 (76.23)	14.33 (22.25)	28.33 (32.16)	69.31
T10	Control (pathogen inoculated)	68.67 (55.96)	59.67 (50.57)	92.33 (73.93)	0.00
S.E (M) $\pm$		1.41	0.72	1.06	-
C.D. at (p = 0.01)		5.22	2.65	3.90	-

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

marmelos plant to control mycelial growth of *F. oxysporum* f. sp. *ciceri*.

At highest concentration (1000 μl), 88.64% inhibition of mycelial growth of test fungus was recorded in S3P1 and 85.30% inhibition observed in interaction S3P3. Lowest inhibition of test fungus 68.83% and 70.98% was recorded in interaction S4P2 and S4P3, respectively. The trend was observed similar in lower concentrations *viz.*, 250, 500 and 750 μl.

Ahanjan *et al*. (2009) reported 40.00 and 42.38% inhibition of mycelial growth of *F. oxysporum* by aqueous and methanolic extract of *Parrotia persica,* respectively at 500 ppm concentration. Abdel-Monaim *et al*. (2011) tested extracts including *Calotropis procera* suppressed growth of *F. oxysporum* f. sp. *lupine* with different percentages. Effects of methanolic extract of *A. marmelos* and bioagents alone and in

combination on chickpea wilt caused by *F. oxysporum* f. sp. *ciceri* under pot experiment was studied in the present investigation. Observations on per cent seed germination and wilt incidence at 30 and 60 DAS was noted and results are presented in Table 6.

Results in Table 6 revealed that, T9 (*P. fluorescens* 10 g/kg seed + *T. viride* 4 g/kg seed + methanolic extract of *A. marmelos*) gave maximum seed germination (94.33%) and was significantly superior over all the other treatments. Lowest per cent germination (68.67%) was reported in T10 (Control). Minimum wilt incidence (14.33%) at 30 DAS was exhibited in T9 (*P. fluorescens* 10 g/kg seed + *T. viride* 4 g/kg seed + methanolic extract of *Aegle marmelos*), followed by 22.00% in T6 (*P. fluorescens* 10 g/kg seed + *T. viride* 4 g/kg seed) and T7 (*P. fluorescens* 10 g/kg seed + *T. viride* 4 g/kg seed + methanolic extract of *Aegle marmelos*). Maximum wilt incidence (59.67%) at 30 DAS was observed in control treatment, followed by 41.33% in T3 (methanolic extract of *A. marmelos* 2%) (Table 6). Similar trends were recorded at 60 DAS. Per cent disease reduction was calculated and was found higher in T9 (69.31%).

Abdel-Monain *et al*.(2011) reported water extracts of *E. jambonala* leaves, *C. colocynthis* fruits and *N. oleander* leaves resulted in highest reduction in disease severity caused by *F. oxysporum* f. sp. *lupini*. Present study showed that all the tested plant extracts suppressed growth of *F. oxysporum* f.sp. *ciceri* with different degrees. Also, methanolic extracts inhibited growth of the pathogen more than the other solvents. The *A. marmelos* plant extracts showed the highest effect in reducing radial growth of the pathogen compared with other extracts. Several higher plants have been found to possess outstanding fungitoxicity against mycelial growth of different phytopathogenic fungi (Mohamad and El-Hadidy, 2008).

The present study revealed that methanol extract of *A. marmelos* in poisoned food technique inhibited highest growth of test pathogen at the rate of 1000 μl. Methanol was the best solvent for extraction of antifungal constituents from tested medicinal plant leaves. Methanolic extract of *A. marmelos* was proved for promising activity against *F. oxysporum* f. sp. *ciceri*. *T. viride* and *P. fluorescens* were found effective against *F. oxysporum* f.sp. *ciceri*. *T. viride* and *P. fluorescens* were compatible with each other and also with methanolic extract of *Aegle marmelos*. In pot culture study combined application of *T. viride* and *P. fluorescens* and methanolic extract of *A. marmelos* as seed treatment was found effective. Thus, chickpea wilt could be managed by the integration of various practices like, seed treatment with bioagents and plant extract. Further field experiments are suggested to investigate *in vivo* effects of these medicinal plants extracts in comparison with some chemical fungicides for the management of wilt of chickpea.

REFERENCES

Abdel-Monaim, M. F., Abo-Elyousr, K. A. M. and Morsy, K. M. 2011. Effectiveness of plant extracts on suppression of damping-off and wilt diseases of lupine (*Lupinus termis* Forsik). *Crop Prot.* **30:** 185-191.

Ahanjan, M., Raghavendra, M. P. and Raveesha, K. A. 2009. A novel antifungal phenolic compound from *Parrotia persica*. *African J. Biochem. Res.* **3(5):** 174-180.

Ahila Devi and Prakasam, V. 2013. Compatibility nature of Azoxystrobin 25 SC with *Pseudomonas fluorescens* and *Bacillus subtilis* on chilli Plants. *World J. Agril. Sci.* **1(8):** 258-264.

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.

Asha, B. B., Nayaka, S. C., Udaya Shankar, A. C., Srinivas, C. and Niranjana, S. R. 2011. Selection of effective bio-antagonistic bacteria for biological control of tomato wilt caused by *Fusarium oxysporum* f. sp. *lycopersici*. *The Bioscan*. **6(2):** 239-244.

Bagwan, N. B. 2010. Evaluation of *Trichoderma* compatibility with fungicides, pesticides, organic cakes and botanicals for integrated management of soil borne diseases of soybean [*Glycine max* (L.) Merril]. *Int. J. Plant Prot.* **3(2):** 206-209.

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.

Fridlender, M., Inbar, J. and Chet, I. 1993. Biological control of soil borne pathogens by a â-13 glucanase producing *Pseudomonas cepacia*. *Soil Biol. Biochem*. **25:** 1211-1221.

Gomez, K. A. and Gomez, A. A. 1984. Statistical procedures for Agriculture research, IRRI, Monila, Phillippines.

Gupta, O., Katasthane, S. R. and Khare, M. N. 1986. *Fusarium* wilt of chickpea (*Cicer arietinum* L.). *Agric. Rev*. **7:** 87-97.

Haware, M. P. and Nene, Y. L. 1982. Symptomless carriers of the chickpea wilt *Fusarium*. *Plant Dis*. **66:** 250-251.

Kala, C., Gangopadhyay, S. and Godara, S. L. 2016. Eco-friendly management of wilt caused by *Fusarium oxysporum* f. sp. *ciceri* in chickpea. *Legume Res*. **39(1):** 129-134.

Khan, Z. S., Shinde, V. N., Bhosale, N. P. and Sahera Nasreen, 2010. Chemical composition and antimicrobial activity of angiospermic plants. *Middle-East J. Scientific Res.* **6(1):** 56-61.

Kumbhar, A. S., Gade, R. M., Shitole, A. V. and Bandgar, M. S. 2016. Utilization of spent mushroom substrate with bio control agents for management of wilt of chickpea. *J. Pl. Dis. Sci*. **11(1):** 113-115.

Latha, P., Anand, T., Ragupathi, V., Prakasam, R. and Samiyappan 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.

Lily Trivedi and Rathi, Y. P. S. 2016. Integrated management of seed borne *Fusarium oxysporum* f. sp. *ciceri* in chickpea wilt complex. *World J. Pharm. and Pharmaceu. Sci*. **5(6):** 2392-2402.

Mina, D. Koche, Gade, R. M. and Deshmukh, A. G. 2013. Antifungal activity of secondary metabolites produced by *Pseudomonas fluorescens*. *The Bioscan*. **8(2):** 723-726.

Mishra, S. K. and Raja, R. 1999. Systemic acquired resistance: review. *Ann. Agric. Res.* **20:** 249-259.

Mohamed, E. H., Nayla, E., Haroun and AbdAlla, M. A. E. 2016. Control of chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceri* with botanical extracts and fungicides. *Int. J. Curr. Microbiol. and Appli. Sci*. **5(4):** 360-370.

Mohamed, N. H. and El-Hadidy, A. M. 2008. Studies on biologically active constituents of *Verbas cumeremobium* Murb. and its inducing resistance against some diseases of cucumber. *Egypt. J. Phytopathol*. **36:** 133-150.

Muneeb, A., Vaid, A. and Razdan, V. K. 2010. Evaluation of different measures to control wilt causing pathogens in chickpea. *J. Plant Prot. Res*. **51(1):** 55-59.

Phulan, R., Vasisht, K. and Khullar, N. 2013. Bio-autography an

efficient method to check the *in vitro* antimicrobial activity of *Aegle marmelos* against enteric pathogens. *IIOABJ*. **4:** 4-9.

Singh, S. K., Sarma, B. K., Srivastava, J. S., Singh, U. P. and Ray, A. B. 1999. Antifungal activity of ¢3-Alstovenine, a plant alkaloid isolated from *Alstonia venenata*. *Folia. Microbiol*. **44:** 510-512.

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.

Tapwal Ashwani, R. Kumar, Nisha, Nandini Gautam and Shailesh Pandey 2012. Compatibility of *Trichoderma viride* for selected fungicides and botanicals. *Int. J. Plant Pathol*. **3(2):** 89-94.

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.

Varma, J. and Dubey, N. K. 1999. Prospectives of botanical and microbial products as pesticides of tomorrow. *Curr. Sci*. **76(2):** 172-179.

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