The Bioscan 10(4): 1877-1881, 2015 (Supplement on Plant Pathology)
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

IN VITRO SCREENING FOR ANTAGONISTIC POTENTIAL OF *TRICHODERMA* SPECIES AND FLUORESCENT *PSEUDOMONAS* AGAINST *FUSARIUM SOLANI* FOR MANAGEMENT OF SOYBEAN WILT

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KEYWORDS
Fusarium solani
Trichoderma
Pseudomonas
Fusarium wilt
Soybean

Received on :
17.09.2015

Accepted on :
20.11.2015

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ABSTRACT
Soybean wilt disease caused by *Fusarium solani* f. sp. *glycine* is one of the most important diseases of soybean. *In vitro* evaluation among three isolates of *Trichoderma* and twenty four isolates of fluorescent *Pseudomonas* against ten and one aggressive isolates of *F. solani* respectively. The maximum mycelial growth inhibition of test pathogen was observed 13.66 mm (84.40%) with *T. harzianum* 2 in SF2 followed by 15.33 mm (82.37%) with *T. harzianum* 1 in SF8 isolate. All the isolates were effective in inhibiting the mycelial growth (65.15 - 84.40 %) over control. Among all the isolates of fluorescent *Pseudomonas* significantly superior in reducing the mycelial growth over control except P141 and P179, whereas highest mycelial growth inhibition 35.66 mm (59.13%) was observed in P85 followed by 45 mm (48.46%) in P167 isolate. These antagonist isolates inhibited 6.85 to 59.13 per cent mycelial growth of test fungus.

INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) is a legume of the family Fabaceae, subfamily Faboideae. Soybean has become a miracle crop of the twentieth century. It is a triple beneficiary crop, a unique food, a valuable feed and an industrial raw material with considerable potential (Chavan *et al.,* 2014) which made it as a "wonder crop". It's seed contains 40% protein, 20% oil, 30% carbohydrates, excellent amounts of dietary fibre, vitamins, minerals and high level of amino acids such as lysine, leucine, lecithin and large amount of phosphorus. Soybean plants build up the soil fertility by fixing large amounts of atmospheric nitrogen through root nodules. In India it's occupies an area (*kharif*) of 108.83 lakh ha. with a production of 104.36 lakh mt and productivity of 959 kg/ha (Anonymous, 2014). Sustainable soybean production is continuously challenged by diseases that cause quantitative and qualitative losses in yield. It's suffered from a number of diseases such as many fungal, bacterial and viral diseases which are responsible for low producing. Among the fungal pathogens, Fusarium wilt is very common and important disease of soybean. These pathogens cause significant loss in yield and primarily responsible for wide gap in the yield levels in farmers field (Zape *et al.,* 2014). In India root rot of soybean caused by *F. solani* was first reported by Agarwal and Sarbhoy (1975). Foliar symptoms of SDS appear before flowering of late maturing varieties or after flowering in cultivars of early maturing group (Verma *et al*., 2009).

It's difficult to manage the disease by the application of chemicals only. Further, their applications cause several problems such as hazards to human, plant health and building of resistance to these chemicals. In addition to target organism, pesticides also kill various beneficial organisms. Their toxic forms persist in soil and contaminate the whole environment. In order to control the plant diseases, biological control is gaining greater attention due to low cost and ecofriendly application (Dewangan *et al*., 2014). Bacterial antagonists have the twin advantage of faster multiplication and higher rhizosphere competence hence, *P. fluorescens* have been successfully used for biological control of several plant pathogens (Ramamoorthy *et al*., 2002) and biological control using PGPR strains especially from the genus *Pseudomonas* is an effective substitute for chemical pesticides to suppress plant diseases (Compant *et al*., 2005). Several fungal (*Trichoderma* sp.) and bacterial (*Pseudomonas* sp. and *Bacillus* sp.) antagonists, have been successfully used as biocontrol agents in the control of seed and soil borne pathogens like *Sclerotium rolfsii*, *Rhizoctonia solani*, *Fusarium oxysporum* and *Sclerotinia sclerotiorum* in the various crops. (Sharma *et al*., 1999; Mukhopadhyay *et al*., 1992; Raguchander *et al*., 1997; Sankar and Jeyarajan, 1996; Abrahm Mathew and

Gupta, 1998; Kehri and Chandra, 1991).

Looking to the immense importance of this disease appropriate strategies of management like exploitation of biocontrol need to be formulated. Therefore, present research proposal has been taken up.

MATERIALS AND METHODS

Isolation and purification of *Fusarium solani*

Fresh diseased roots of soybean plant showing wilt symptoms were collected and washed properly with tap water. Infected roots and stems were cut in small pieces with the help of sterilized blade in such a way that each of them contained healthy as well as diseased tissues. These pieces were surface sterilized with 0.1% mercuric chloride (HgCl2) solution for one minute followed by three subsequent washing in sterilized water to remove traces of mercuric chloride (HgCl2). The pieces were transferred aseptically to Petridishes containing PDA. Inoculated Petridishes were kept in incubator at 25 ± 2°C and were examined at frequent intervals to see the growth of fungus developing from different pieces. Isolation was made and the isolated culture was purified by single spore isolation (Kotasthane and Agrawal, 2014). Morphological characteristics of purified isolates were compared with standard description (Seifert, 1996).

Isolation and maintain of *Trichoderma* and *Pseudomonas*

The different isolates of *Trichoderma* sp. and fluorescent *Pseudomonas* were isolated from rhizosphere soil and maintained *Trichoderma* in PDA slant and *Pseudomonas* were in King's B agar slants.

Evaluation of fungal and bacterial antagonistic

The experiment was conducted to see the antagonistic activity of different sp. of *Trichoderma* (*T. harzianum* and *T. viride*) were used and all of these three isolates were screened for their antagonistic activity against ten isolates of *F. solani* by dual culture on Potato Dextrose Agar contain Petridishes (Morton and Stroube, 1955). Twenty ml of Potato Dextrose Agar medium was poured in sterilized Petridishes of 90 mm diameter. On solidification of medium, dual inoculation technique was adopted, each plate using 6 mm disc of aggressive test pathogen and that isolate placing them on the plates at the opposite points from each other. Three Petridishes were kept for each combination. After inoculation these were incubated at 25 ± 2°C, all the isolates of *Trichoderma* sp. were tested for their effectiveness against the pathogen. Observations of mycelial growth were recorded after 9 days of incubation with the help of a scale.

In laboratory, funnel is commonly used to separate solids from liquids, liquids from liquids and occasionally for pouring something into a container. Our present investigation suggests a simple technique where funnel can be used to inoculate bio-agent (liquid / sporulting bioagent) for confrontation assays (Kotasthane *et al.,* personal communication). Funnels (Borosil make, Diameter 75 mm, Plain, 60° Angle Stem) of different diameters are available and can be used as per the requirement and size of the Petridish. Edges of the glass funnel were sterilized by dipping in alcohol and flaming. Broth containing young growing cell of fluorescent *Pseudomonas* were dispensed in a sterile Petri dish (For each isolate of fluorescent *Pseudomonas* a sterilized container is required). Cool sterilized edge of the funnel was then dipped in the broth culture containing young growing cells of fluorescent *Pseudomonas*. Care was taken to remove the excess inoculums by gently shaking the dipped funnel. Plates were inoculated in the centre with agar plugs (5 mm dia.) containing young growing mycelium of aggressive pathogenic fungi *F. solani* was further used for confrontation assays. Precaution was taken to keep the plugs (containing growth of pathogenic fungus) in the centre. Petriplates pre-inoculated with plant pathogenic fungi were then inoculated by the fluorescent *Pseudomonas* isolate by touching / stamping the edge of the funnel on the surface of the solid media (sterilized potato dextrose agar and King's B solid medium). Keeping the narrow stem of the funnel vertically positioned on the agar plug helped us to stamp the inoculum (present on the edge of the funnel) uniformly surrounding the plugs (containing growth of pathogenic fungus). Narrow stem of the funnel also eased the handling of funnel in all the inoculation steps. Touching / stamping with the edge of funnel (containing bacterial inoculum) uniformly transfer the bacterial inoculum surrounding the pre-inoculated agar plugs (containing mycelial growth of pathogenic fungus). Each isolates were taken three replication and the plates were incubated at 25ºC. The mycelial growth of the pathogen and inhibition zone was measured after 8 days of incubation. The per cent inhibition in mycelia growth of the pathogen over control was calculated using by given formula given by Vincent (1947).

$$\text{Per cent inhibition in mycelial growth} = \frac{C - T}{C} \times 100$$

Where,

C = Mycelial growth in control

T = Mycelial growth in treatment

RESULTS AND DISCUSSION

Observations on the growth and colonization of the test pathogen in dual culture, screening by the antagonistic isolates proved that different isolates of *Trichoderma* differed in their ability to suppress the growth of the different isolates of test pathogen. The fast growing isolates caused more inhibition of the pathogen due to mycoparasitism and competition for space and nutrients. *T. harzianum* 1 significantly inhibited mycelial growth of all isolates of *F. solani* as compared to control presented in Table 1. Among the isolates, significant maximum inhibition of mycelial growth 15.33 mm (82.37%) was observed in SF8 followed by SF6 17.33 mm and SF3 22.66 mm with inhibition percentage 80.07 and 73.94 respectively. The least non significant mycelial growth was found among the isolates was SF9 35 mm (59.77%) followed by SF5 34 mm (60.92%) and SF3 22.66 mm (73.94%).

In case of *T. harzianum* 2, all the isolates exhibited significantly less growth as compared to control. Significantly maximum mycelial growth inhibition was observed 13.66 mm (84.40%) in SF2 followed by SF10 16.33 mm (81.43%) and SF3 17.33 mm (80.30%) as compared with other isolates. Among the isolates, whereas the isolate SF7 43 mm (51.33%), SF9 37.66

Table 1: Evaluation of antagonistic *Trichoderma* isolates against aggressive isolates of *F. solani* under *in vitro* condition

Isolates	Mycelial growth (mm)			Per cent inhibition mycelial growth*		
	Th 1	Th 2	Tv 3	Th 1	Th 2	Tv 3
SF1	30.66	33.00	33.66	64.75 (53.56)	62.50 (52.21)	61.74 (51.77)
SF2	33.33	13.66	30.66	61.68 (51.73)	84.40 (66.77)	65.15 (53.80)
SF3	22.66	17.33	32.33	73.94 (59.28)	80.30 (63.62)	63.25 (52.66)
SF4	32.00	31.66	31.66	63.22 (52.64)	64.01 (53.11)	64.01 (53.11)
SF5	34.00	32.00	35.00	60.92 (51.28)	63.63 (52.89)	60.23 (50.88)
SF6	17.33	31.33	33.66	80.07 (63.46)	64.39 (53.34)	61.74 (51.77)
SF7	34.33	43.00	31.33	60.53 (51.06)	51.13 (45.61)	64.39 (53.34)
SF8	15.33	35.33	33.33	82.37 (65.15)	59.85 (50.66)	62.12 (51.99)
SF9	35.00	37.66	39.66	59.77 (50.61)	57.19 (49.11)	54.92 (47.80)
SF10	38.33	16.33	38.33	55.93 (48.39)	81.43 (64.46)	56.44 (48.68)
Control	87.00	88.00	88.00			
CD(5%)	1.73	5.56	1.54	2.10 (1.26)	6.67 (3.89)	1.84 (1.09)
SEm	0.85	1.88	0.52	0.70 (0.42)	2.24 (1.31)	0.62 (0.36)

* Mean of three replication, Th - *T. harzianum*, Tv – *T. viride* Arc sine transformed values are in paranthesis

Table 2: Evaluation of antagonistic fluorescent *Pseudomonas* isolates against aggressive isolate of *F. solani under in vitro* condition

Isolates	Mycelial Growth(mm)	Per cent mycelial growth inhibition *
P5	75.66	13.34 (21.22)
P6	62.33	28.63 (32.33)
P11	75.66	13.32 (21.28)
P67	73.00	16.40 (23.86)
P72	65.33	25.02 (26.26)
P76	65.33	25.17 (30.09)
P85	35.66	59.13 (50.24)
P99	75.66	13.34 (21.39)
P124	59.33	32.04 (34.45)
P126	76.00	12.95 (20.97)
P129	74.00	15.25 (22.94)
P141	81.33	6.85 (15.04)
P143	53.66	38.54 (38.36)
P151	54.33	37.77 (37.90)
P161	78.33	10.30 (18.64)
P167	45.00	48.46 (44.10)
P176	52.00	40.46 (39.48)
P179	80.00	8.38 (16.79)
P201	74.33	14.88 (22.68)
P205	71.33	18.28 (25.22)
P216	51.33	41.20 (39.91)
P233	56.66	35.11 (36.32)
P247	77.33	11.43 (19.70)
P248	75.66	13.34 (21.39)
Control	87.33	-
C.D. at 5 %	10.33	12.40 (8.63)
SE(m)	3.62	4.35 (3.02)

mm (57.19%), SF8 35.33 mm (59.85%), SF1 33 mm (62.50%), SF5 32 mm (63.63%), SF4 31.66 mm (64.01%) and SF6 31.33 mm (64.39%) exhibited non significantly higher mycelial growth among them.

T. viride 3 significantly reduced the growth of all isolates of test pathogen as compared to control. Among all isolates, SF2 30.66 mm (65.15%), exhibited highest significant reduced mycelial growth followed by SF3 32.33 mm (63.25%) and SF8 33.33 mm (62.12%), while non significant reduced mycelial growth was observed with the isolates SF7 31.33 mm (64.39%) followed by SF4 31.66 mm (64.01%). The least

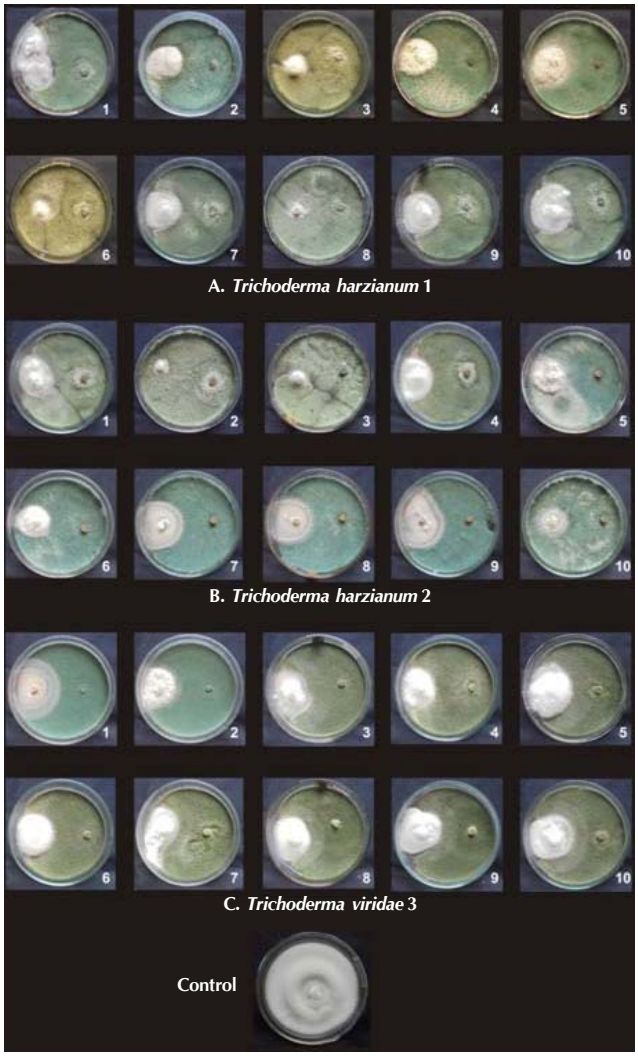

Figure 1: Antagonistic interaction between different isolates of *Trichoderma* and different isolates of *F. solani*

inhibition mycelial growth was observed in SF9 39.66 mm (54.92 %) followed by SF10 38.33 mm (56.44 %). The similar results were observed by Bohra and Mathur (2004); Begum *et*

Figure 2: Antagonistic interaction between *F. solani* (SF7) and different isolates of fluorescent *Pseudomoas* by funnel technique

al., (2007) and Zape *et al*., (2014) in *Fusarium* spp. causing wilt of soybean. Similar work done by (Gupta *et al*., 2003; Sangle and Bambawale, 2004; Mehta *et al*., 2010; Sundaramoorthy and Balabaskar, 2013; Tetarwal *et al*., 2013 and Kumari *et al* 2014).

Twenty four isolates of fluorescent *Pseudomonas* screened against virulent isolates of *F. solani* (SF7). All the isolates of *Pseudomonas* significantly superior in reducing the mycelial growth over control except P141 and P179 presented in Table 2, isolate P85 was significantly superior in reducing the mycelial growth of test pathogen 35.66 mm over other isolates of *Pseudomonas* followed by P167 45 mm and inhibited the mycelial growth by 59.13 and 48.46 per cent respectively. The least inhibition mycelial growth was recorded in isolate P141 81.33 mm (6.85 %) followed by P179 80 mm (8.38 %). Mycelial growth in control was 87.33 mm. The similar results were observed that *P. fluorescens* was an important antagonist inhibiting the growth of *Fusarium* and reported by various workers Vidhyasekaran *et al*., (1995); Saikia *et al*., (2003); Rini *et al*., (2007); Naik, (2010); Kumar *et al*., (2010); Asha *et al*., (2011); Rajeswari and Kannabiran, (2011); Kapoor *et al*., (2012); Adhikari *et al*., (2013) and Dewangan *et al*., (2014).

ACKNOWLEDGEMENT

This study was supported by Department of Plant Pathology, College of Agriculture (IGKV), Raipur, Chhattisgarh . We are greatful to Dr. K. P. Verma, Principal Scientist, Dr. A. S. Kotasthane, Professor and Head, Dr. C. S. Shukla, Professor, Dr. R .K. Dantre, Professor and Shri H. K. Singh, Assistant Professor, Department of Plant Pathology for their necessary guidance in this research.

REFERENCES

Abrahm Mathew, K. and Gupta, S. K. 1998. Biological control of root rot of french bean caused by *Rhizoctonia solani*. *J. Mycol. Pl. Pathol.* **28:** 202-205.

Adhikari, A., Dutta, S., Nandi, S., Bhattacharya, I., De Roy, M., Sarkar, G. and Mandal, T. 2013. Antagonistic potentiality of native rhizobacterial isolates against root rot disease of okra, incited by *Rhizoctonia solani*. *Afr. J. Agric. Res.* **8(4):** 405-412.

Agarwal, D. K. and Sarbhoy, A. K. 1975. Nectria haematocoa, aperfect state of *Fusarium solani* (Mart.) Sacc. on soybean from India. *Curr. Sci*. **44:** 602.

Anonymous 2014. Estimates of area, productivity and production of soybean in India during *kharif*. SOPA., pp. 1-4.

Asha, B. B., Nayaka, S. C., Udayshankar, 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.

Begum, M. M., Sariah, M., Zainal Abidin, M. M., Puteh, A. B. and Rahman, M. A. 2007. Histopathological studies on soybean seeds infected by *Fusarium oxysporum* f. sp. *glycines* and screening of potential biocontrol agents. *Res. J. Micro.* **2(12):** 900-909.

Bohra, B. and Mathur, K. 2004. Biocontrol agents and neem formulations for suppression of *Fusarium solani* root rot in soybean. J. Mycol. *Pl. Pathol.* **34(2):** 408-409.

Compant, S., Reiter, B., Sessitsch, A., Nowak, J., Clement, C. and Barka, E. A. 2005. Endophytic colonization of *Vitis vinifera* L. by plant growth-promoting bacterium *Burkholderia* sp. strain PsJN; Appl. Environ. *Microbiol.* **71:** 1685-1693.

Chavan, N. G., Bhujbal, G. B. and Manjare, M. R. 2014. Effect of seed priming on field performance and seed yield of soybean varieties. *The Bioscan.* **9(1):** 111-114.

Dewangan, P. K., Koma, B., Baghel, S., Khare, N. and Singh, H. K. 2014. Characterization of *Pseudomonas fluorescens* in different media and its antagonistic effect on phytopathogenic fungi. The Bioscan, **9(1):** 317-321.

Gupta, S. B., Thakur, M. P., Tedia, K., Singh, A. K., Bachkaiya, K. K. and Thakur, K. 2003. Studies on local isolates of *Trichoderma viride* and their relationship with wilt/root rot causing fungi of chickpea. In: Chickpea Research for the Millennium. International Chickpea Conference, Raipur, Chhattisgarh, India. January 20-22, 2003. pp. 184-191.

Kapoor, S., Jaiswal, A. and Shukla, D. N. 2012. Eco- friendly strategies for management of Fusarium wilt of *Pisum sativum L. Afr. J. Microbiol. Res.* **6(48):** 7397-7400.

Kehri, H. K. and Chandra, S. 1991. Antagonism of *Trichoderma* viride to *Macrophomina phaseolina* in the conrol of dry root rot of mung. *Ind. Phytopathol.* **44:** 60-63.

Kotasthane, A. S. and Agrawal, T. 2014. Simple technique for single basidiospore isolation . Paper presented in National seminar on *Plant Genomic Era* from 26-28 May, 2014 held at Dept. of Plant Pathology, IGKV, Raipur.

Kumar, H., Bajpai, V. K., Dubey, R. C., Maheshwari, D. K. and Kang, S. C. 2010. Wilt disease management and enhancement of growth and yield of *Cajanus cajan* (L.) by bacterial combinations amended with chemical fertilizer. *Crop Protec.* **29:** 591-598.

Kumari, A., Kumar, R. and Kumar, H. 2014. Efficacy of fungicides

and *Trichoderma viride* against *Fusarium oxysporum* f. sp. *cubense in vitro*. *The Bioscan*. **9(3):** 1355-1358.

Mehta, A. N., Chauhan, H. L., Makwana, K. V., Gohel, N. M. and Patel, S. J. 2010. Bioefficacy of phytoextract, antagonist and fungicides against *Fusarium udum* of pigeonpea wilt. J. Pl. Dis. Sci., 5(1): 56-60.

Morton, D. T. and Stroube, N. H. 1955. Antagonistic and stimulatory vects of microorganisms upon *Sclerotium rolfsii*. *Phytopathology*. **45:** 419-420.

Mukhopadhyay, A. N., Shrestha, S. M. and Mukherjee, P. K. 1992. Biological seed treatment for control of soil born plant pathogens. *FAO Plant Prot. Bull.* **40:** 221-30.

Naik, B., Nagaraja, R. and Basavaraja, M.K. 2010. Biological control of *Fusarium oxysporum* f. sp. *vanillae*, the casual agent of stem rot of vanilla *in vitro*. I.J.S.N.**1(2):** 259-261.

Raguchander, T., Rajappan, K. and Samiappan, R. 1997. Evaluating methods of application of biocontrol agents in the control of mungbean root rot. *Ind. Phytopathol.* **50:** 229-234.

Rajeswari, P. and Kannabiran, B. 2011. *In vitro* effects of antagonistic microorganisms on *Fusarium oxysporum* infecting *Arachis hypogaea* L. J. Phytol. **3(3):** 83-85.

Ramamoorthy, V., Raguchander, T. and Samiyappan, R. 2002. Enhancing resistance of tomato and hot pepper to pythium diseases by seed treatment with fluorescent *Pseudomonads*. *Eur. J. Plant Pathol*. **108:** 429-441.

Rini, C. R. and Sulochana, K. K. 2007. Usefulness of *Trichoderma* and Pseudomonas against *Rhizoctonia solani* and *Fusarium oxysporum* infecting tomato. *J. Trop. Agric.* **45(1):** 21-28.

Sangle, U. R. and Bambawale, O. M. 2004. New strains of *Trichoderma* spp. strongly antagonistic against *Fusarium oxysporum* f. sp. *sesami*. *J. Mycoogy and Plant Pathology*. **34(1):** 107-109.

Saikia, R., Singh, T., Kumar, R., Srivastava, J., Srivastava, A. K., Singh, K. and Arora, D. K. 2003. Role of salicylic acid in systemic resistance induced by *Pseudomonas fluorescens* against *Fusarium oxysporum* f. sp. *ciceri* in chickpea. *Microbiol. Res.* **158(3):** 203-213.

Sankar, P. and Jeyarajan, R. 1996. Seed treatment for biological control of *Macrophomina phaseolina* in sesamum. *Ind. Phytopathol.* **49:** 148-151.

Seifert, K. 1996. *Fusarium* interaction key pp. 15-55.

Sharma, S. K., Verma, B. R. and Sharma, B. K. 1999. Biocontrol of *Sclerotinia sclerotiorum* causing stem rot of chickpea. *Ind. Phytopathol.,* **52:** 44-46.

Sundaramoorthy, S. and Balabaskar, P. 2013. Biocontrol efficacy of *Trichoderma* spp. against wilt of tomato caused by *Fusarium oxysporum f .sp . lycopersici*, *J. App. Bio. & Biotechnology*. **1(3):** 36-40.

Tetarwal, J. P., Trivedi, A. and Mathur, K. 2013. Sustainable and eco-friendly management of root rot of soybean caused by *Rhizoctonia solani* and *Fusarium solani*. *Indian Phytopath.* **66(2):** 215-219.

Verma, K. P., Dantre, R. K. and Thakur, M. P. 2009. Effect of date of sowing on incidence of Colletotrichum pod blight, rust, fusarial wilt and target leaf spot of soybean. *Ann. Pl. Protec. Sci.* **17(1):** 133-135.

Vidyasekaran, P. and Muthamilan, M. 1995. Development of formulations of *Pseudomonas fluorescens* for control of chickpea wilt. *Plant Dis.* **79:** 782-786.

Vincent, J. M. 1947. Distortion of fungal hyphae in the presence of certain inhibitiors. *Nature.* **159:** 850-850.

Zape, A. S., Gade, R. M., Singh, R. and Deshmukh, V. A. 2014. Efficacy of different antagonist against the *Sclerotium rolfsii*, *Rhizoctonia solani* and *Fusarium solani*. *The Bioscan.* **9(4):** 1431-1434.