JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Dec. 2016. Vol. 10(4), p. 2849-2856

In vitro and *In vivo* Management of Root Rot/Wilt of Fenugreek through Biological and Chemical Methods

N. Rani and Yashoda R. Hegde

Department of Plant Pathology, University of Agricultural Sciences,
Dharwad - 580 005, Karnataka, India.

http://dx.doi.org/10.22207/JPAM.10.4.46

(Received: 17 April 2016; accepted: 20 June 2016)

Fenugreek (*Trigonella foenum graecum* L.) is an important seed spice, belonging to the family Fabaceae. Now a days, fenugreek suffers from many fungal diseases out of which wilt caused by *Fusarium oxysporum* is becoming severe. *In vitro* evaluations revealed that, among bioagents tested highest per cent inhibition (78.71%) was observed in *T. viride.* Among botanicals, garlic extract recorded the mean maximum inhibition (46.87%). Jeevamrutha was found effective and recorded the maximum inhibition of mycelial growth (55.48%). *In vivo* study revealed that Carbendazim and combi product carbendazim 25% + mancozeb 50% were very effective in managing the disease completely up to 60 DAS.

Keywords: Fenugreek, *Trichoderma viride,* Jeevamrutha, Garlic extract, Carbendazim, Management, Root Rot/Wilt.

Fenugreek (*Trigonella foenum graecum* L.) is an important seed spice, originated in South-Eastern Europe belonging to the family Fabaceae. It is native of India and leading fenugreek producing country in the world. It is the third largest seed spice in India after coriander and cumin. In India, it is grown in about 66,000 ha with an annual production of about 90,000 tonnes (Anon., 2014)[1]. Rajasthan is the fenugreek bowl of country, contributing 90 per cent to the country's production. It has some pharmacological properties such as antitumor, antiviral, antimicrobial, anti-inflammatory, hypotensive and antioxidant activity (Kor and Moradi, 2013)[2].

Fenugreek is mainly grown as leafy vegetable throughout Karnataka and there is ample scope for its cultivation as seed spice. But fenugreek suffers from many fungal diseases *viz.,* Cercospora leaf spot caused by *Cercospora traversiana,* root rot (*Rhizoctonia solani),* leaf spot (*Ascochyta sp.),* powdery mildew (*Erysiphe polygoni),* downy mildew (*Peronospora trigonellae)* and Fusarium wilt (*Fusarium oxysporum*) (Prasad *et al.,* 2014)[3]. Fenugreek wilt complex caused by the fungi like *Fusarium oxysporum, Rhizoctonia solani* and *Sclerotium rolfsii* for the first time in India, Shivpuri and Bansal (1987)[4] reported the *Fusarium oxysporum* Schlecht as the causal agent of wilt of fenugreek from Jaipur district of Rajasthan. Although many diseases are reported in fenugreek, wilt is becoming more severe in recent years. However no much study has been conducted on this disease, So present study was carried out in order to know the efficacy of different bioagents, biorationals and botanicals against *Fusarium oxysporum* causing wilt of fenugreek both under laboratory conditions including all the available management practices and further their efficacy was tested under glasshouse conditions.

* To whom all correspondence should be addressed.
E-mail: ranipgs13agr6199@gmail.com

MATERIALS AND METHODS

In vitro evaluation of bioagents against *Fusarium oxysporum*

The efficacy of six bioagents was tested against *Fusarium oxysporum* for radial growth inhibition using dual culture technique under *in vitro* condition. The cultures of antagonistic microorganisms used in the present study were obtained from Department of Plant Pathology, Institute of Organic Farming, UAS, Dharwad, Karnataka.

List of bioagents used against *Fusarium oxysporum*

1. *Bacillus subtilis*
2. *Pseudomonas fluorescens*
3. *Trichoderma harzianum*
4. *T. koningii*
5. *T. viride*
6. *T. virens*

For maintenance of *P. fluorescens* culture, King's B medium was used whose composition is as follows. For maintenance of *Bacillus subtilis* culture, nutrient agar medium was used.

Dual culture test

Bioagents were evaluated for their efficacy through dual culture technique. Both biocontrol agents and test pathogen were cultured on potato dextrose agar in order to get fresh and active growth of fungus. Twenty ml of sterilised and cooled potato dextrose agar was poured into sterile Petriplate and allowed to solidify. For evaluation of fungal bio control agents, mycelial disc of test fungus was inoculated at one end of the Petriplate and antagonistic fungus was placed opposite to it on the other end. In case of evaluation of bacterial antagonist the bacterium was streaked at the middle of the Pertiplates and mycelial disc of the test fungus was placed on either side at the centre of each half of the plate. The plates were incubated at 27±1°C and zone of inhibition was recorded by measuring the clear distance between the margin of the test fungus and antagonistic organism. The colony diameter of the pathogen in control plate was also recorded. The per cent inhibition of the growth of the pathogen was calculated by using the formula given by Vincent (1947)[5].

$$I = \frac{C - T}{C} \times 100$$

I= Per cent inhibition

C= Radial growth in control

T= Radial growth in treatment

In vitro evaluation of botanicals against *Fusarium oxysporum*

Plant based pesticides which are relatively economical, safe and non hazardous can be used successfully against the plant pathogenic fungi. In the present study following plant extracts were selected.

Preparation of cold aqueous extract

Fresh plant materials were collected and washed first in tap water and then in distilled water. Hundred grams of fresh sample was chopped and then crushed in a surface sterilized pestle and mortar by adding 100 ml sterile water (1:1 w/v). The extract was filtered through two layers of muslin cloth. Finally thus obtained extract was used as stock solution. To study the antifungal mechanism of plant extracts, the poison food technique was used (Nene and Thapliyal, 1973)[6]. Five and ten ml of stock solution was mixed with 95 and 90 ml of PDA medium respectively and sterilized, so as to get 5 and 10 per cent concentration. The medium was thoroughly shaken for uniform mixing of extract. Twenty ml of medium was poured into sterile Petriplates, mycelial discs of five mm size from periphery of actively growing culture were cut out by sterile cork borer and one such disc was placed at the centre of each plate. Control was also maintained by growing the pathogen on PDA plates. Then such plates were incubated at 27^0C±1°C temperature and radial growth was taken when maximum growth was observed in control plate. The efficacy of plant products or botanicals was expressed as per cent inhibition of radial growth over the control which was calculated by using the Vincent (1947) formula.

In vitro evaluation of biorationals against *Fusarium oxysporum*

The efficacy of seven organic products was tested against *Fusarium oxysporum* for radial growth inhibition on the potato dextrose agar medium using poison food technique under *in vitro* condition. The biorationals used in this study are biodigester slurry, cow urine, vermivash, raw neem oil, jeevamrutha, beejamrutha and neem seed kernel extract at 10 and 20 per cent concentrations which were obtained from Institute of Organic Farming, UAS, Dharwad, Karnataka.

Ten and 20 ml of individual organic

products was added separately into 90 and 80 ml of potato dextrose agar so as to get the desired concentrations of 10 and 20 per cent and sterilized. Later, 20 ml of the poisoned medium was poured into sterilised Petriplate. Mycelial disc of five mm size from actively growing zone of seven days old culture was cut by a sterile cork borer and one such disc was placed at the centre of each agar plate. Control treatment was maintained without adding any organic products to the medium. Three replications were maintained for each treatment. Then such plates were incubated at room temperature and radial growth was measured when fungus attained maximum growth in control plates. Per cent inhibition of mycelial growth over control was calculated by using the formula given by Vincent (1947)[5].

***In vivo* evaluation of bioagents, biorationals and fungicides**

A pot experiment was conducted in the glasshouse of Department of Plant Pathology, University of Agricultural Sciences, Dharwad to find out best treatment for control of wilt of fenugreek. Five fenugreek seeds were sown per pot. The effective bio agents, biorationals and fungicides evaluated under *in vitro* studies were further evaluated in pot culture. Each treatment was replicated thrice. The giant culture was inoculated to each pot at the rate of 8 per cent. The bioagents, botanicals and organic products listed below were applied individually as seed treatment as well as drenching at 15 and 30 days after sowing. Seed treatment with plant extracts and biorationals were done by soaking in the solution for 30 min. Pots without any treatment served as control.

The treatments are as follows:

T_1 - *Trichoderma harzianum* (0.6%)
T_2 - *Trichoderma viride* (0.6%)
T_3 - *Duranta repens* @ 20%
T_4 - Cow urine @ 20%
T_5 - Captan @ 0.3%
T_6 - Carbendazim (Bavistin 50%WP) @ 0.1%
T_7 - Carbendazim 25% + Mancozeb 50% WP (Sprint 75 % WP) @ 0.1%
T_8 - Carboxin 37.5% + Thiram 37.5% (Vitavax power 75% WS) @ 0.1%
T_9 - Control

Since leaves are the economic parts of the fenugreek, the observations were recorded on plant height and number of leaves at 15, 30, 45 and 60 days after sowing. Per cent disease incidence (PDI) was calculated at 30 and 60 days after sowing by using following formula.

$$PDI = \frac{\text{Number of plants infected} \times 100}{\text{Total number of plants observed}}$$

RESULTS AND DISCUSSION

In the present study the experiments were carried out both under laboratory conditions including all the available management practices and further their efficacy was tested under glasshouse conditions.

***In vitro* evaluation of biocontrol agents**

The results on the efficacy of biocontrol agents against *Fusarium oxysporum* are presented in the Table 1 and Plate 1. The data revealed that the efficacy of bio control agents was significant. Among the bioagents tested during investigation, *T. viride* (78.71%) was found to be best in inhibiting the mycelial growth of *F. oxysporum* followed by *T. koningii* (78.55%) and least inhibition was recorded in *Bacillus subtilis* (57.60%). Present investigation recorded significant mycoparasitism of species of *Trichoderma viz., T. viride, T. koningii, T. harzianum* and *T. virens,* which showed maximum inhibition of mycelial growth of pathogen compared to bacterial antagonists. It may be due to production of antibiotic substance (viridin). Similar results were reported by Chaudhary, (2010)[7] and Rani *et al.,* (2014)[8] against *Fusarium oxysporum* causing wilt of fenugreek.

Among all the bioagents tested fungal bio-agents were found to be effective than bacterial bioagents in inhibiting the mycelial growth of *Fusarium oxysporum.* The use of biocontrol agents for the management of soil borne diseases involves the application of these agents to soil and planting material.

***In vitro* evaluation of botanicals**

Botanicals next to bioagents are safe, ecofriendly and cost effective means of managing the crop diseases effectively. Most of the studies on use of natural plant extracts for control of many soil borne pathogens are still at laboratory level and few of these studies lead to development of commercial product for field use.

In the present investigation, eight plant extracts were evaluated under *in vitro*

condition against *Fusarium oxysporum* to know the fungitoxic nature. Though complete inhibition of the pathogen was not observed in any of the plant extracts tested, but considerable amount of inhibition was noticed in some of them.

The data (table 2) revealed that the efficacy of eight botanicals against *Fusarium oxysporum* was found statistically significant. Among the eight botanicals tested, garlic extract recorded the mean maximum inhibition (46.87%) this was followed by neem (44.00%) was recorded. Maximum inhibition of mycelial growth was recorded in garlic at 10% (55.92%) which was statistically on par with duranta (55.33%) at ten per cent concentration. Garlic and duranta were statistically superior to all other treatments. Least inhibition of mycelial growth was recorded in eucalyptus (20.89%), lantana (29.89%) and tulasi (29.89%) at five per cent concentration. Among the different concentrations tested 10 per cent concentration was found effective in inhibiting the mycelial growth than at five per cent concentration (Table 2 and Plate 2). Similar, results were reported by Shivapuri and Bansal (1987)[4], Shukla and Dwivedi (2012)[9] and Rani *et al.,* (2014)[8]. The antimicrobial properties of onion and garlic were attributed to the presence of sulphur as an active principle (Mangamma and Sreeramulu, 1991)[10].

***In vitro* evaluation of biorationals**

As biorationals are cost effective and environment friendly means of management, Besides chemical control, biological method of control is an effective, eco-friendly and cost effective means of management. An effort was made to know the efficacy of different biorationals against *Fusarium oxysporum.*

The results revealed that, the effect of biorationals on fungal growth was significant. Among biorationals evaluated cow urine was found effective in inhibiting mean maximum mycelial growth (45.30%), this may be due to acidic pH. This was significantly superior to all other treatments. Jeevamrutha was found effective and recorded the maximum inhibition of mycelial growth (55.48%) which was statistically on par with cow urine (55.00%) and was followed by raw neem oil (34.96%) at 20 per cent concentration. Least inhibition of mycelial growth was observed in vermiwash and bejamrutha at both the concentrations. Biodigester slurry was least effective in inhibition of mycelial growth at both the concentrations tested. Twenty per cent concentration was found effective than at 10 per cent (Taate 3) Similar results were reported by Raja *et al.,* (2006)[11] and Sapre and Verma (2006)[12].

Sl.No.	Botanical name	Common Name	Parts used
1	*Azadirachta indica* Juss.	Neem	Leaf
2	*Duranta repens* L.	Duranta	Leaf
3	*Allium cepa* L.	Onion	Bulb
4	*Lantana camara* L.	Lantana	Leaf
5	*Ocimum sanctum* L.	Tulsi	Leaf
6	*Parthenium hysterophorus* L.	Congress weed	Leaf
7	*Eucalyptus globes* L.	Nilgiri	Leaf
8	*Allium sativum* L.	Garlic	Bulb

Table 1. *In vitro* evaluation of bioagents against *Fusarium oxysporum*

Bioagent	Inhibition of mycelial growth (%)
Trichoderma harzianum	77.91 (62.62)*
Trichoderma viride	78.71 (61.62)
Trichoderma virens	68.44 (55.87)
Trichoderma koningii	78.55 (61.39)
Bacillus subtilis	57.60 (50.94)
Pseudomonas fluorescens	58.52 (48.49)
S.Em.±	1.14
CD at 1%	4.65
CV %	4.02

*Arcsine transformed values

In vivo* evaluation of bioagents, biorationals and fungicides against *Fusarium oxysporum

The bioagents, botanicals and biorationals and fungicides which were found effective under *in vitro* conditions were tested in pot culture experiment as explained in Material and Methods.

Observations were recorded on plant height, number of leaves and per cent disease incidence at 15, 30, 45 and 60 days after sowing (DAS) (Table 4).

Wilt incidence

Wilt incidence was less at 30 Days after sowing compared to 60 DAS. Carbendazim and combi product carbendazim 25% + mancozeb 50% were very effective in managing the disease completely up to 60 DAS which was statistically on par and significantly superior to all other treatments. This was followed by Vitavax power, duranta and *T. viride* which were on par with each other. Cow urine was less effective at 30 and 60 DAS with 73.33 and cent per cent wilt incidence disease respectively.

Table 2. *In vitro* evaluation of botanicals against *Fusarium oxysporum*

Botanicals	Inhibition of mycelial growth (%) Concentrations		Mean
	5%	10%	
Allium cepa L.	31.63 (34.21)*	42.25 (40.53)	36.94 (37.41)
Allium sativum L.	37.81 (37.93)	55.92 (48.38)	46.87 (43.19)
Azadirachta indica Juss.	38.44 (38.30)	49.55 (44.73)	44.00 (41.54)
Duranta repens L.	32.63 (34.82)	55.33 (48.04)	43.98 (41.52)
Eucalyptus globules L.	20.89 (27.18)	34.85 (36.16)	27.87 (31.85)
Lantana camara L.	29.89 (33.13)	43.74 (41.39)	36.81 (37.34)
Ocimum sanctum L.	29.89 (33.13)	40.29 (39.39)	35.09 (36.31)
Parthenium hysterophorus L.	40.77 (39.67)	43.25 (41.11)	42.01 (40.39)
Mean	32.74 (34.66)	45.65 (42.44)	39.20 (38.55)
	S.Em.±	CD at 1%	
Botanicals (B)	0.90	3.47	
Concentrations (C)	0.45	1.74	
BxC	1.27	4.91	
CV %	0.15		

*Arcsine transformed values

Table 3. Effect of different biorationals on mycelial growth of *Fusarium oxysporum*

Biorationals	Inhibition of mycelial growth (%) Concentrations		Mean
	10%	20%	
Biodigester slurry	0.00 (0.00)*	0.00 (0.00)	0.00 (0.00)
Cow urine	35.59 (36.61)	55.00 (47.85)	45.30 (42.28)
Beejamrutha	2.41 (8.92)	4.85 (12.72)	3.63 (10.98)
Jeevamrutha	13.63 (21.66)	55.48 (48.13)	34.56 (35.99)
Neem seed kernel extract	25.44 (30.28)	2.30 (8.71)	13.87 (21.86)
Raw neem oil	39.04 (38.65)	34.96 (36.23)	37.00 (37.45)
Vermiwash	2.11 (8.35)	2.07 (8.28)	2.09 (8.31)
Mean	16.88 (24.25)	22.10 (28.03)	19.49 (26.19)
	S.Em.±	CD at 1%	
Biorationals (B)	0.36	1.42	
Concentration(C)	0.19	0.76	
B×C	0.51	2.01	
CV %	4.05		

*Arcsine transformed values

Table 4. *In vivo* evaluation of bioagents, biorationals and fungicides against *Fusarium oxysporum*

Treatments	Dosage	Number of leaves				Plant height (cm)				Per cent wilt incidence	
		15DAS	30 DAS	45DAS	60 DAS	15DAS	30 DAS	45DAS	60 DAS	30 DAS	60 DAS
Control	-	7.00	*	*	*	10.67	*	*	*	100.00 (10.02)**	100.00 (10.02)**
Trichoderma harzianum	6 g/kg	9.00	16.93	18.53	24.00	16.77	21.20	24.00	30.00	0.00 (0.71)	53.33 (7.31)
T. viride	6 g/kg	11.67	19.17	22.60	25.93	17.81	22.33	28.93	34.87	0.00 (0.71)	20.00 (4.53)
Duranta repens	20%	9.00	10.00	13.80	17.60	15.00	18.43	21.57	26.87	20.00 (4.53)	20.00 (4.53)
Cow urine	20%	6.00	9.27	11.87	15.93	5.00	8.43	17.37	19.73	73.33 (8.57)	100.00 (10.02)
Captan	0.3%	7.00	10.13	15.20	17.93	9.29	13.10	17.70	23.40	0.00 (0.71)	26.67 (5.14)
Carbendazim	0.1%	13.33	20.87	25.53	30.20	16.83	25.70	32.17	38.53	0.00 (0.71)	0.00 (0.71)
Carbendazim 25%+ Mancozeb 50%	0.1%	12.00	18.07	23.60	28.40	15.67	22.10	30.57	35.00	0.00 (0.71)	0.00 (0.71)
Carboxin 37.5% + Thiram 37.5%	0.1%	10.33	17.73	21.27	25.67	14.77	18.95	25.07	31.53	0.00 (0.71)	20.00 (4.53)
S.Em.±		0.27	0.19	0.27	0.23	0.16	0.16	0.11	0.21	0.13	0.26
CD at 5%		1.11	0.78	1.10	0.93	0.66	0.65	0.43	0.85	0.51	1.05
CV %		4.97	2.46	2.77	1.92	2.03	1.62	0.83	1.36	7.56	8.45

**= ÖX+0.5 transformed values

*= Death of plants

Number of leaves

All treatments have increased the number of leaves significantly at 30, 45 and 60 DAS compared to untreated control. Maximum number of leaves was recorded in carbendazim (20.87, 25.53 and 30.20 at three stages respectively) which is significantly superior to all other treatments, this was followed by Sprint (18.07, 23.60 and 28.40), *Trichoderma viride* (19.17, 22.60 and 25.93) and next best was Vitavax power (17.73, 21.27 and 25.67). The minimum number of leaves was recorded in cow urine (15.93) at 60 DAS. Death of plant was noticed at 28 days after sowing in control.

Plant height

Most of the treatments have increased plant height significantly at 30, 45 and 60 DAS compared to untreated control. Maximum plant height was recorded in carbendazim (25.70, 32.17 and 38.53 cm) which was significantly superior to all other treatments and this was followed by Sprint (22.10, 30.57 and 35.00 cm), *Trichoderma viride* (22.33, 28.93 and 34.87 cm) and next best was Vitavax power (18.95, 25.07 and 31.53 cm). The least plant height was recorded in cow urine (8.43, 17.37 and 19.73cm) at 30, 45 and 60 DAS respectively. In control, there was no further growth of plant at 28 days after sowing because death of plant was noticed.

Among different bioagents, biorationals and fungicides tested, fungicides have given good results. In the present study among the different chemicals tested carbendazim was effective and it completely inhibited the disease up to 60 DAS with maximum plant height (38.53 cm) and number of leaves (30.20), which was significantly on par with carbendazim 25% + mancozeb 50% with 28.40 number of leaves and 35cm height of the plant at 60 DAS. Lowest wilt incidence was recorded at 30 DAS than at 60 DAS. Carbendazim and combi product carbendazim 25% + mancozeb 50% were very effective in managing the disease completely up to 60 DAS. These results are comparable

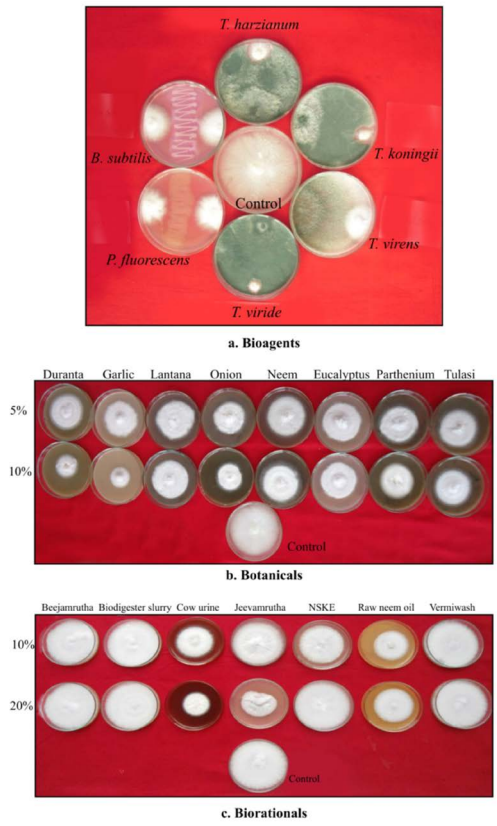

Plate 1. Biological management of *Fusarium oxysporum*

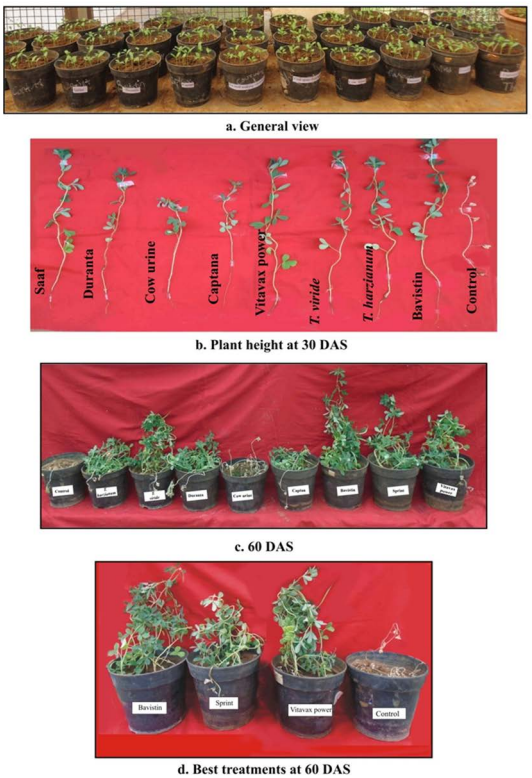

Plate 2. *In vivo* evaluation of bioagents, biorationals and fungicides against *Fusarium oxysporum*

with findings of Haque and Ghaffar (1992)[13], Maheshwari *et al.* (2008)[14] and Sundaramoorthy and Balabaskar (2013)[15].

REFERENCES

1. Anonymous, 2014, http://www.indiastat.com/agriculture/2/spices/262/fenugreek/20663.
2. Kor, N. M. and Moradi, K. Physiological and pharmaceutical effects of fenugreek (*Trigonella foenum-graecum*) as a multipurpose and valuable medicinal plant. *Glob. J. Med. Pl. Res*., 2013; **1**: 199-206.
3. Prasad, R., Acharya, S., Erickson, S, and Thomas, J. Identification of cercospora leaf spot resistance among fenugreek accessions and characterization of the pathogen. *Australian J. Crop. Sci.,* 2014; **8**(6): 822-830.
4. Shivpuri, A. and Bansal, P. K. Fusarium wilt of *Trigonella foenum-graecum* L. *Indian J. Mycolo. Pl. Pathol.*, 1987; 26: 749-751.
5. Vincent, J. M. Distortion of fungal hyphae in presence of certain inhibitors, *Nature,* 1947; **159**: 239-241.
6. Nene, Y. L. and Thapliyal, P. N.: Fungicide in plant diseases control 2nd edn. Oxford and IBH publishing Co. Pvt. Ltd., New Delhi, 1973; p. 325.
7. Chaudhary, H. J., Patel, D. S. and Patel, R. L. Efficacy of bioagent and phyto extracts against *Fusarium oxysporum* causing wilt of fenugreek. *J. Mycol. Pl. Pathol.,* 2010; **41**(1): 148.
8. Rani, N., Hegde, Y. R., Nargund, V. B., Veena and Hegde, R. V., 2014, Efficacy of bioagents against *Fusarium oxysporum* causing wilt of fenugreek. *Nation. Symp. Plant diseases: New perspectives and innovative management strategies*. 1. Biological and other organic practices, 11-12, December, 2014, UAS, Dharwad, pp. 29.
9. Shukla, A. and Dwivedi, S. K. Bioeffecacy of plant extracts against *Fusarium species* causing wilt in pulses. *IOSR J. Eng.,* 2012; **2**(1): 136-144.
10. Mangamma, P. and Sreeramulu. Garlic extract inhibitory to the growth of *Xanthomonas campestris* pv. *vesicatoria*. *Indian Phytopath.,* 1991; **44**: 372-374.
11. Raja, J., Suganthi, C. and Kurucheve, V. Use of animal urine for the management of sheath blight of rice. *Phytopathol*., 2006; **96**.
12. Sapre, J. K. and Verma, R. K. *In vitro* evaluation of cow urine and buttermilk against three major soil borne pathogens of soybean. *Soybean Res*., 2006; **4**: 33-39.
13. Haque, S. E. and Ghaffar, A. Efficacy of *Trichoderma* spp. and *Rhizobium meliloti* in control of root rot of fenugreek. *Pakistan J. Bot.,* 1992; **24**: 217-221.
14. Maheshwari, S. K., Nazir, A. Bhat, Masoodi, S. D. and Beig, M. A. Chemical control of lentil wilt caused by *Fusarium oxysporum* f. sp. *lentis. Ann. Pl. Protec. Sci.,* 2008; **16**(2): 419-421
15. Sundaramoorthy, S. and Balabaskar, P. Biocontrol efficacy of *Trichoderma* spp. against wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici. J. App. Biol. Biotechnol.,* 2013; **1**(03): 036-040.