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Research Article

Biointensive management of wilt disease of lentil through biocontrol agents and organic amendments

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ABSTRACT: Lentil (*Lens culinaris* M.) is an important dietary source of protein and other essential nutrients in South and West Asia, North and East Africa. Lentil crops are susceptible to a number of diseases caused by fungi and other parasites. Among them, the most significant and serious soil-borne disease is Fusarium wilt (*Fusarium oxysporum* f.sp. *lentis*: *Fol*). Systematic studies in laboratory, cage house and field trials were conducted (2016-17 to 2017-18). Laboratory experiments were carried out in completely randomized design with four replications and field trials were carried out consecutively during 2016-17 and 2017-2018 crop season in Randomized Block Design (RBD) with four replications using L9-12, a susceptible cultivar. Under *in vitro*, bio efficacy of selected bio agents, maximum 81.33 per cent mycelial growth inhibition of pathogen was recorded in *Trichoderma harzianum* (local isolate) and minimum mycelial growth inhibition 55.00 per cent was recorded in *Bacillus subtilis*. Under i*n vivo* condition, effect of selected bio agents and organic amendments on disease incidence, percent disease control, pathogen spore in per gram soil and yield ha^{-1} of lentil was recorded. Application of *Trichoderma harzianum* (Local isolate) @ 6g/Kg seed was found most effective with maximum seed germination 91.95 and 90.62 per cent, lowest disease incidence of 24.22 and 25.39 per cent and higher pooled mean grain yield 774.31 kg/ha over check during 2016-17 and 2017-18, respectively. Among organic amendments, maximum disease control (42.02 per cent), minimum average disease incidence (36.33 per cent) and minimum pathogen spores ($1.3x10^4$ per gram soil) were observed in neem cake amendment followed by poultry manure and vermicompost amendments. This indicates that these treatments can be effectively used for controlling *Fusarium* wilt disease under organic mode of lentil cultivation in Rajasthan.

KEY WORDS: Biocontrol agents, *Fusarium oxysporum* f.sp. *lentis*, lentil wilt, organic amendments

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INTRODUCTION

The role of food legumes in improving the health and nutrition of human (Tharanathan and Mahadevamma, 2003), maintaining good stock of cattle (Siddique *et al*., 2012), ameliorating the condition of soil (Biederbeck *et al*., 2005), and in alleviating greenhouse gases (Lemke *et al*., 2007). In the global lentil scenario, India ranked first in the area and second in the production with 39% and 22% of world area and production respectively (Tiwari *et al*., 2018). Among the several biotic stresses limiting lentil yield, wilt of lentil is one of serious diseases caused by *Fusarium oxysporum* f.sp. *lentis* and plays a major role in reducing lentil yield (Choudhary and Mohanka, 2012). Management with cultural practices and fungicides is not potential because of it have mainly soil born nature. Environmental concerns of pesticides use and development of fungicidal resistance have necessitated use of eco-friendly tools for disease management. Biological control is known to be the best and effective method, against soil-borne pathogens. This method has many advantages such as environment friendly, cost effective and extended plant protection. Many fungal and bacterial species like *Pseudomonas*, *Trichoderma, Bacillus* and *Streptomyces* have antagonistic effect on Fusarium wilt of lentil (Tiwari *et al*., 2018). Use of bio-agents and organic amendments and formulation of bio agent with organic amendments against lentil wilt is the best alternative to chemical control measures (Garkoti *et al*., 2014). Laboratory, cage house and field studies were conducted (2016-17 to 2017-18) to evolve the individual use and best combination for controlling lentil wilt.

MATERIALS AND METHODS

Isolation/collection and maintenance of cultures of pathogen/bio-agents

Wilted plants of lentil were used to prepare pure culture and maintained on Potato Dextrose Agar (PDA) medium at 25 + 1˚C. The identification of pathogen was confirmed on the basis of morphology and cultural characters. Mass multiplication of the pathogen was carried out on sorghum grains. Five bio-agents were used during entire study i.e., *Trichoderma. harzianum* (Local isolate), *T. harzianum* (Commercial), *Trichoderma viride, Pseudomonas fluorescens, Bacillus subtilis.* These isolates were collected from Rajasthan Agriculture Research Institute, Durgapura, Jaipur, Rajasthan.

Evaluation of bioagents

In vitro evaluation of bio-agents: Bio-efficacy of all antagonists was tested against *Fusarium oxysporum* f. sp. *lentis through* dual culture technique developed by (Morton and Stroube, 1955). Twenty ml sterilized melted PDA medium was poured into sterilized Petri-plates, allowed to solidify, then 6mm discs of the fungus and the antagonistic cut with the help of sterilized cork borer were placed on PDA approximately 4 cm apart each other and incubated in BOD incubator at 25 ± 1ºC for 96 hrs. Four replications were maintained in completely randomized design (CRD). The per cent growth inhibition was worked out by using the formula given by (Arora and Dwivedi, 1980).

Inhibition of growth of pathogen = (Colony diameter of pathogen in check - Colony diameter of pathogen in dual culture/ Colony diameter of pathogen in check) x100.

In vivo evaluation of bio-agents: The bioagents used *in vitro* study were taken *in vivo* evaluation during *Rabi* 2016-17 and *Rabi* 2017-18 in sick plot of lentil wilt at Research Farm, Rajasthan Agricultural Research Institute, Durgapura (Jaipur) (Table 1). Four replications were maintained in RBD. Plot size was 3.0 m x 4.0 m, [1]spacing 30 x 10 cm, Seed rate 40 kg/ha variety L9-12 was used during entire study. Data on per cent germination after 10 and 20 days after of sowing, Disease incidence at 10, 30 and 60 days after sowing and grain yield (kg/ha).

In vivo evaluation of soil amendments: Soil was amended with 10 different organic materials including various crop straws, three oil cakes, vermin-compost, goat manure, poultry manure and Farm Yard Manure (FYM) (Table 2). Sterilized

Table 1. Bio-agents evaluated against *Fusarium oxysporum* f.sp. *lentis* as seed dresser in *in vivo*

S.No.	Antagonists	Dose g/kg seed
1	*Trichoderma harzianum* (Local isolate)	6
2	*Trichoderma harzianum* (commercial)	6
3	*Trichoderma viride*	6
4	*Bacillus subtilis*	8
5	*Pseudomonas fluorescens*	8
6	Control	-

Table 2. Following soil amendments tested against *Fusarium oxysporum* f.sp. *lentis* in cage house condition

S. No.	Organic material	Dose g kg-1 soil
	Poultry mannure	20
	Goat manure	20
	Moong bean straw	20
	Vermi-compost	20
	Mustard cake	05
	Groundnut cake	05
	Farm Yard Manure (FYM)	20
	Chickpea straw	20
	Neem cake	05
	Cluster bean straw	20
	Check	-

(4% formalin) earthen pots were filled with autoclaved soil containing inoculum of the pathogen multiplied on sorghum grains @ 20 g/pot. Straws and cakes each individually were thoroughly ground and mixed in the form of powder. Soil was amended 15 days earlier to sowing and then inoculated with the pathogen as done earlier. Seeds of lentil (L9-12) coated with mycelial culture of the bioagents by rolling on 7 days old culture plates were air dried and subsequently sown in pot. One pot of control was maintained by sowing untreated seeds in pots having healthy soil. Each treatment replicated thrice in completely randomized design. As and when required, pots were irrigated with equally measured amount of water and plants affected with wilt were recorded till above 70 per cent plants showed disease incidence in control pots. Observation on disease incidence was recorded starting after 7 days of sowing at continue up to 60 days. Pathogen population was estimated in terms of spores (CFU) g^{-1} of soil from the samples drawn from each treatment.

RESULTS

Laboratory studies

All bio-agents were found to suppress the growth of the pathogen (Table 3 and Fig. 1). Maximum 81.33 per cent mycelial growth inhibition of pathogen was recorded in *T. harzianum* (local isolate) followed by 76.97 per cent in *Trichoderma harzianum* (commercial) and minimum mycelial growth inhibition was recorded 55 per cent in *Bacillus subtilis*.

Field studies

All the bio-agents were found significantly superior in minimizing the disease over control. Application of *Trichoderma harzianum* (Local isolate) @ 6g/Kg seed was found most effective with maximum seed germination 91.95

Table 3. Bio-efficacy of different bio-agents on growth of *F. oxysporum* f.sp. *lentis* under *in vitro* by dual culture method

S.No.	Treatments (bio-agents)	Colony diameter of antagonist (mm)*	Colony diameter *Fusarium oxysporum* f.sp. *lentis**	Inhibition of growth of *Fusarium oxysporum* f.sp. *lentis**
1.	*T. harzianum* (Local isolate)	73.15 (58.79)	16.80 (24.20)	81.33 (64.22)
2.	*T. harzianum* (Commercial)	69.23 (56.31)	20.70 (27.06)	76.97 (61.32)
3.	*T. viride*	64.25 (53.28)	25.70 (30.46)	71.44 (57.70)
4.	*P. fluorescens*	57.35 (49.23)	32.60 (34.82)	63.78 (52.99)
5.	*Bacillus subtilis*	49.50 (44.71)	40.50 (39.52)	55.00 (47.87)
6.	Control	00.00	90.00 (71.57)	00.00
	S.Em.±	00.18	00.09	00.28
	C.D. at 5%	00.58	00.26	00.86
	C.V. %	00.56	00.46	00.80

*Mean of four replications
Figures in parentheses are angular transformed values

and 90.62 per cent and lowest disease incidence of 24.22 and 25.39 per cent as against 57.02 and 58.59 per cent in check during 2016-17 and 2017-18, respectively followed by *T. harzianum* (commercial) @ 6g/Kg seed attaining wilt disease incidence 27.58 and 28.75 per cent in both the year of testing. Over all maximum seed germination, minimum wilt disease incidence, maximum grain yield in lentil was observed with *T. harzianum* (local isolate) and *T. harzianum* (commercial). Higher pooled mean grain yield 774.31 kg/ha and minimum mean wilt incidence 24.28 per cent and maximum disease control 57.09 per cent was recorded when the seeds were treated with *T. harzianum* (local isolate) and *T. harzianum* (commercial) followed by *T. viride and Pseudomonas fluorescens.* Maximum mean disease incidence 40.97 per cent, minimum germination 81.34 per cent, minimum mean disease control 29.12 per cent and minimum pooled yield (670.50 kg/h) was found in bio-agent *Bacillus subtilis* treated plots (Table 4).

Cage house conditions

Coating of seeds with mycelial preparation of different bioagents significantly improved seed germination, reduced disease incidence, increased per cent disease control and minimum pathogen population in soil. It is evident from the data presented in Table 5 and Fig. 2 that all the amendments provided significant reduction in disease incidence over control. Soil amendment with neem cake was found most effective in reducing the disease during both the years of testing. The maximum disease control 42.02 per cent, minimum average disease incidence 36.33 per cent and minimum pathogen spores ($1.3x10^4$ per gram soil) were observed in neem cake amendment Followed by poultry manure and vermicompost amendments. Minimum disease control 20.27 per cent, maximum average disease incidence 49.96 per cent and maximum pathogen spores ($2.1x10^4$ per gram soil) was recorded in cluster bean straw followed by chickpea and mung bean straw. Results also indicates that when soil was amended with pulses straw like mung bean and chickpea an increase in wilt incidence and pathogen population per gram soil was recorded in both the year of testing.

The results of present study clearly indicate that bio-control agents and organic amendments play important role in minimizing the lentil wilt incidence in both in vitro and in vivo conditions. Among all bio agents and organic amendments, seed treatment with *Trichoderma harzianum* (native & commercial) @ 6 gm/kg and soil amend with neem cake 5 gm/kg soil were found best control for wilt disease management.

DISCUSSION

Biological control is known to be the best and effective method, against soil-borne pathogens. This method has many advantages such as eco-friendly and cost effective. Many fungal and bacterial species like *Pseudomonas*, *Trichoderma* and *Bacillus* have antagonistic effect on Fusarium wilt of lentil (Tiwari *et al*., 2018). The pathogenic fungus *F. oxysporum* f.sp. *lentis* is supposed to be responsible for severe disease damage under hot and dry weather conditions with the temperature ranges of 22–25°C, suitable for disease development, thus causing huge losses in areas with such conditions (Mohammadi *et al*., 2011). In the present study, five known antagonists were evaluated for their antagonism against *F. oxysporum* f. sp. *lentis in vitro* by dual culture method and observed that all the bio agents *viz*., *T. harzianum* (local isolate), *T. harzianum* (commercial), *T. viride, P. fluorescens* and *Bacillus subtilis* were antagonistic to the growth of

Table 4. Efficacy of different bio control agents against lentil wilt incidence and yield in field condition

S. No	Test organism	Dose g/kg seed	2016-17				2017-18				Mean**			
			Germination* (%)	Disease Incidence* (%)	% Disease control	Yield (kg/ha)*	Germination* (%)	Disease incidence* (%)	% Disease control	Yield (kg/h)*	Germination (%)	Disease incidence (%)	% Disease control	Pooled Yield (kg/h)
1.	*T. harzianum* (local isolate)	6	091.45	024.22	057.52	778.60	090.62	025.39	056.66	770.00	091.04	024.28	057.09	774.31
2.	*T. harzianum* (commercial)	6	088.31	027.58	051.63	756.50	087.51	028.75	050.93	748.50	087.91	028.16	051.28	752.54
3.	*T. viride*	6	085.16	033.59	041.09	714.00	083.98	035.15	039.97	704.90	084.57	034.37	040.53	709.43
4.	*P. fluorescens*	8	082.35	038.83	031.90	680.30	080.79	039.61	032.39	680.00	081.57	039.22	032.15	680.15
5.	*B. subtilis*	8	081.34	040.46	029.04	670.90	079.54	041.48	029.20	670.10	080.44	040.97	029.12	670.50
6.	Control		075.00	057.02	000.00	572.50	074.06	058.59	000.00	566.10	074.53	057.80	00.00	569.30
	S.Em.±		000.93	001.84		016.30	000.68	001.79		016.07				016.22
	C.D. at 5%		002.81	005.55		050.22	002.06	005.40		049.52				049.91
	CV%		002.22	009.97		004.56	001.65	009.40		004.66				004.89

*Mean of four replications, Figures in parentheses are angular transformed value, **Mean of two years (2016-17 and 2017-18)

Table 5. Effect of different soil amendments on lentil wilt development induced by *F. oxysporum* f.sp. *lentis*

S. No.	Organic material	Dose g kg^{-1} soil	Wilt disease incidence* (%)		**Mean wilt incidence	% Disease control	Pathogen Spores g-1 soil
			2016-17	2017-18			
1.	Poultry manure	20	36.67	38.21	37.44	40.25	1.4 x 10^4
2.	Goat manure	20	41.67	43.27	42.47	32.22	1.7 x 10^4
3.	Mung bean straw	20	46.67	48.20	47.44	24.29	1.9 x 10^4
4.	Vermicompost	20	37.83	39.50	38.67	38.29	1.6 x 10^4
5.	Mustard cake	5	46.50	47.13	46.82	25.28	1.3 x 10^4
6.	Groundnut cake	5	44.33	46.37	45.35	27.63	1.8 x 10^4
7.	Farm Yard Manure (FYM)	20	40.00	42.04	41.02	34.54	1.6 x 10^4
8.	Chickpea straw	20	48.25	49.13	48.69	22.29	2.0 x 10^4
9.	Neem cake	5	35.42	37.23	36.33	42.02	1.3 x 10^4
10.	Cluster bean straw	20	49.92	49.99	49.96	20.27	2.1 x 10^4
11.	Check	-	62.17	63.14	62.66	00.00	2.4 x 10^4
	S.Em. ±		00.93	00.68			
	C.D.at 5 %		02.69	01.95			
	C.V. %		04.18	02.96			

*Mean of four replications
Figures in parentheses are angular transformed values
**Mean of two years (2016–17 and 2017–18)

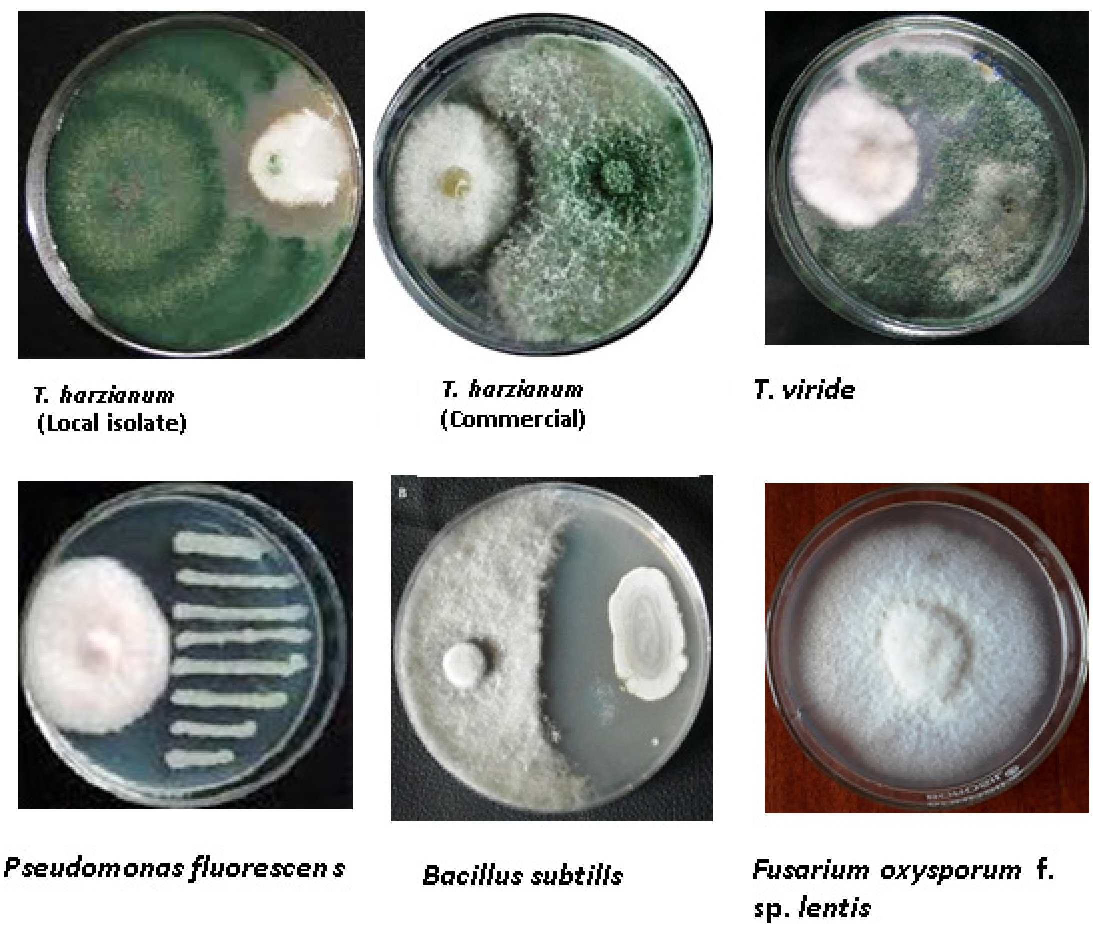

Fig.1. Growth of different antagonistic in dual culture

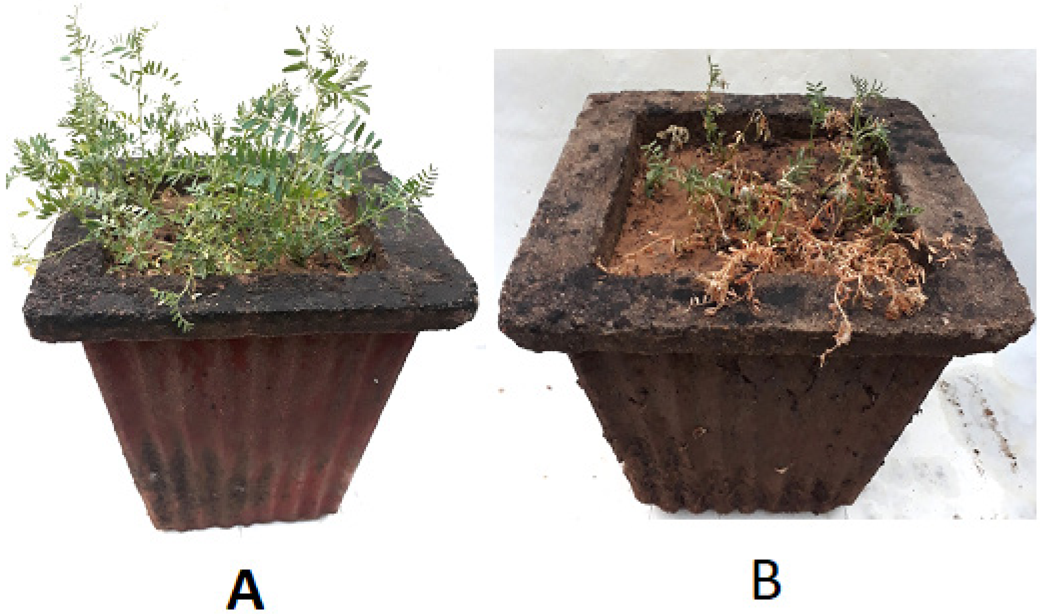

Fig 2. Effect of neem cake soil amendment on lentil wilt development induced by *F. oxysporum* f. sp. *lentis.*, A: Neem cake amend pot B: Control

Fusarium oxysporum f.sp. *lentis*. Maximum mycelial growth inhibition of pathogen was recorded in *T. harzianum* (local isolate) followed by *T. harzianum* (commercial) and minimum mycelial growth inhibition was recorded in *Bacillus subtilis*. The results of the treatments suggested that *T. viride* was found most effective 72.85 per cent inhibition on mycelia growth of test pathogen *Fusarium oxysporum* f.sp. *lentis* in among four bioagents *viz. T. viride, T. harzianum, P. fluorescens* and G. *virens* against (Singh *et al*., 2014). In field condition, also all the bio-agents were found significantly superior in minimizing the disease over control. *T. harzianum* (Local isolate) @ 6g/Kg seed was found most effective with maximum seed germination (91.45 and 90.62 %), higher pooled grain yield 774.31 kg/ha and highest per cent disease control (24.22 and 25.39) against 57.02 and 58.59 per cent disease incidence and minimum pooled yield 569.30 kg/ha in check during 2016-17 and 2017-18, respectively. (Kumar *et al*., 2013) recorded that seed treatment with *T. harizanum* + *P. fluorescens* gave minimum disease incidence and maximum grain yield. Among tested bio-agents, *T. viride* was found significant giving maximum seed germination with 85.0 per cent and minimizing disease incidence by 1.12 per cent which was followed by *T. harzianum* in paper towel method (Singh *et al*., 2014).

In the effect of soil amendments trial neem cake amendment was found most effective in reducing the disease during both the years of testing. The maximum disease control 42.02 per cent, minimum average disease incidence 36.33 per cent and minimum pathogen population per gram soil ($1.3x10^4$) was observed in neem cake amendment followed by poultry manure and vermicompost amendments and minimum disease control 20.27 per cent, maximum average disease incidence 49.96 per cent and maximum pathogen population per gram soil was recorded in cluster bean. (Manthan and Balabaskar, 2002) recorded that neem leaf extract 60 per cent, poultry litter 40 per cent and buffalo urine 20 per cent were capable to inhibit the mycelial growth of *Fusarium moniliforme.* (Rajiv and Dubey, 2003) recorded the outcome of soil amendments alone or in combination with fertilizers on *Fusarium oxysporum* f.sp. *lentis* at seedling, flowering and pod formation stages. Similar observations have been recorded by (Garkoti *et al*., 2013).

CONCLUSION

The results of the present study suggest that greater emphasis on the T. *harzianum* (Local isolate) was shows maximum inhibition of the growth of *F. oxysporum* f.sp. *lentis in vitro* and field condition also. In the soil amendments, Neem cake was shows maximum disease control and minimum pathogen population as compared to other amendment.

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