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Original Research Article

Antagonistic Effect of Fungicide, Botanicals and Bio-agents against *Fusarium* wilt of Lentil caused by *Fusarium oxysporum* f. sp. *lentis*

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A B S T R A C T

Lentil wilt caused by *Fusarium oxysporum* f. sp. *lentis* is an important disease and it is a limiting factor to production of lentil. The present study to evaluate the antifungal activity of fungicide, botanicals through food poison technique and bio-agents through dual culture. Results revealed that minimum radial growth was obtained at 500ppm and 1000ppm in metalaxyl 8% + mancozeb 64% (00.00) followed by Neem leaf extract @ 10% (10.90 mm) having per cent inhibition (75.78%), Garlic @ 10% (11.00mm, 75.56%), Onion @ 10% (11.60mm; 74.22%), Neem leaf extract @ 5% (12.4mm; 72.45%). Among bio-agents, *P. fluorescence* showed maximum per cent inhibition (78.92%) followed by *T. viride* (66.82%), *T. harzianum* (60.76%), *B. subtilis* (58.94%), *T. virens* (55.62%).

Keywords

Antagonist, bio-agents, botanicals, in vitro, lentil

Introduction

Lentil *(Lens culinaris* Medik) is a member of Leguminaceae family. It has a high nutritional value and major source of dietary proteins (25%) after soybeans (Zia *et al.*, 2011). In India, most of the population is primarily vegetarian. Pulses have a special place in the daily diet of people due to its high protein contents and several uses (Ali and Mishra, 2000). Major lentil growing states in India are Madhya Pradesh (42.50%), Uttar Pradesh (31.25%), West Bengal (9.38%) and Bihar (8.75%). In India, lentil was grown in 1.49 mha with production of 1.61 mt with an average production of 1006 kg/ha (Anonymous, 2018).

Lentil crop is affected by many fungal diseases. Among them *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *lentis* is one of the major soil borne disease causes an economic loss all over the world (Bayaa *et al.*, 1998 and Taylor, 2007). *Fusarium oxysporum* f. sp. *lentis* infection range from 25-95 per cent depending on the cultivars

tested. It was first reported from Hungary (Fleischmann, 1937) and later from many countries including India (Padwick, 1941), USA (Wilson and Brandsberg, 1965), USSR (Kotava *et al.,* 1965), Syria (Bayya *et al.,* 1986) and Turkey (Bayya *et al.,* 1998). Widespread use of synthetic chemicals has harmful effect on the environment and beneficial microbes present in the soil. The use of biocontrol agents and botanicals for controlling the disease is regarded as an interesting alternative to synthetic fungicides due to their eco-friendly nature as well as economic feasible. The aim of the present study to test the antagonistic effect of botanicals and bio-agents against wilt of lentil.

Materials and Methods

Antagonistic Effect of fungicide and botanical against *F. oxysporum* f. sp. *lentis* using poison food technique

One fungicide i.e. Metalaxyl 8% + Mancozeb 64% @ 500ppm and 1000ppm and botanicals namely neem, garlic, ginger, onion and parthenium @ 5% and 10% against the test pathogen under laboratory condition to find out their relative efficacy for inhibiting the mycelial growth of the pathogen by poison food technique (Nene and Thapliyal, 2000).

Plant extract were prepared as suggested by Madhavi and Singh, 2005. 500ppm and 1000ppm concentration of fungicides and 5% and 10% of botanicals extract were incorporated in 100 ml PDA and mixed thoroughly by sacking, prior to pouring into Petri plates. After the pouring of PDA in Petri plates, the medium was allowed to solidify and these plates were centrally inoculated with the 5 mm diameter disc of pathogen (cut by sterilized cork borer), taken from the margin of actively growing 7 days old culture. Control was used without adding fungicide in the medium. Three replications of each were kept and incubated at 28±2^{o}C. The efficacy of fungicides was observed by measuring the radial growth of the fungal colony at 7 days after incubation.

Per cent growth inhibition was calculated by using formula

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

Where,

I = Per cent inhibition of fungal growth

C = Radial growth of control

T = Radial growth of treated Petri plates.

Antagonistic Effect of bio-agents on *Fusarium* wilt of lentil through dual culture technique

The antagonistic potential of *T. virde, T. harzianum, T. virens, P. fluorescens* and *B. subtilis* against *F. oxysporum* f. sp. *lentis* was assessed in dual culture technique. The mycelia disc of 3 mm diameter from the margin of 7 day old culture of bio-agents and test pathogen were placed on solid PDA in paired combination at distance of 2.5 cm from each other in three replication. Control set was made by inoculating test pathogen singly on the medium. Dual Petri dishes were incubated at 28^{0}C in BOD incubator and the extent of interaction was observed by measuring area covered by in dual culture and in the control at 7 days of incubation. The per cent inhibition of the interacting fungi was calculated as follows:

$$I = \frac{A1 - A2}{A1} \times 100$$

Where,

A_1 = Area covered by *Fusarium oxysporum* f. sp. *lentis* in control.

A_2 = Area covered by the *Fusarium oxysporum* f. sp. *lentis* in dual culture.

Results and Discussion

Antagonistic Effect of fungicide and botanicals against *F. oxysporum* f. sp. *lentis* on radial growth

It is clear from the data that minimum radial growth was obtained at 500ppm in metalaxyl 8%+ mancozeb 64% (00.00) followed by 5% concentration in, Neem (12.4 mm), Garlic (13.1 mm), Onion (15.1 mm), Ginger (17.3 mm) and Parthenium (20.1mm) while maximum radial growth was observed in check (45.0 mm). There was no significant differences in radial growth among, Garlic and Onion were at par to each other and rest treatment were significantly superior at 5% concentration.

It is clear from the data that the minimum radial growth was found in Metalaxyl 8 % + Mancozeb 64 % (00.00) at 1000 ppm which was followed by 10 % concentration, Neem (10.90 mm), Garlic (11.0 mm), Onion (11.60 mm), Ginger (15.30 mm) and Parthenium (18.20 mm) while maximum radial growth was observed in check (45.0 mm). There was no significant difference in radial growth among Neem, Garlic and Onion were at par to each other and rest treatment were significantly superior at 10 % concentration (Fig 4.1).

Thus results clearly indicated that plant extracts reduced the radial growth of *F. oxysporum* f. sp. *lentis* and the effectiveness of extracts increased with the increase of their concentration.

Antagonistic Effect of different concentration of fungicide and plant extracts against *F. oxysporum* f. sp. *lentis* on per cent inhibition

Results indicated the maximum per cent inhibition was recorded in Metalaxyl 8 % + Mancozeb 64 % @ 500 ppm (100%) followed by, Neem (72.45 %), Garlic (70.89 %), Onion (66.45 %), Ginger (61.56 %), and Parthenium (55.33 %) @ 5% concentration. The per cent inhibition between Neem and Garlic were at par with each other.

The maximum per cent inhibition was recorded in Metalaxyl 8 % + Mancozeb 64 % @ 500 ppm (100%) followed by Neem (75.78 %), Garlic (75.56 %), Onion (74.22 %), Ginger (66.00 %), and Parthenium (59.56 %) @ 10 % concentration. The per cent inhibition among Neem, Garlic and Onion were at par to each other. However, per cent inhibition in rest of the treatment differed significantly to each other.

The per cent inhibition in radial growth was higher at 10 per cent concentration as compared to 5 per cent concentration at 7 days of incubation. Thus, it is very clear that the efficacy of plant extracts increased with an increased concentration and time of incubation.

Several plants are known to presses antifungal properties against *Fusarium oxysporum* f. sp. *lentis. Azadirachta indica*, *Allium sativum*. *Allium cepa*, and *Zingiber officinale*, have been found effective against wilt of lentil caused by *Fusarium oxysporum* f. sp. *lentis*. Sinha and Sinha (2004), Mandhore and Suryawansi (2008), Garkoti *et al.,* (2013), Sunderrao *et al.,* (2017), Ghante *et al.,* (2019) and Dubey (2020).

Table.1 List of plants with common name, English name, botanical name and the part used.

1.	Neem	Neem	*Azadirachta indica*	Meliaceae	Leaves
2.	Lahsun	Garlic	*Allium sativum*	Liliaceae	Bulb
3.	Pyaz	Onion	*Allium cepa*	Liliaceae	Bulb
4.	Adarakh	Ginger	*Zingiber officinale*	Zingiberaceae	Rhizome
5.	Parthenium	Parthenium	*Parthenium hysteroporus*	Asteraceae	Leaves Stem

Table.2 Antagonistic Effect of fungicide and botanicals against *F. oxysporum* f. sp. *Lentis*

Plant extracts/Fungicide	**Dose**	**Mycelial growth (mm)**	**Per cent inhibition**	**Dose**	**Mycelial growth (mm)**	**Per cent inhibition**
Metalaxyl 8 % + Mancozeb 64 %	500 ppm	00.00	100	1000 ppm	00.00	100
Neem Leaf extract (*Azadirachta indica*)	5%	12.4	72.45	10%	10.90	75.78
Garlic (*Allium sativum*)	5%	13.1	70.89	10%	11.00	75.56
Onion (*Allium cepa*)	5%	15.1	66.45	10%	11.60	74.22
Ginger (*Zingiber officinale*)	5%	17.3	61.56	10%	15.30	66.00
Parthenium *Parthenium hysterophorus*)	5%	20.1	55.33	10%	18.20	59.56
Control		45.00	00.00		45.00	00.00
SEm±		**0.96**	**0.62**		**2.08**	**1.72**
CD at 5 %		**1.91**	**1.87**		**1.45**	**2.21**

Table.3 Antagonistic Effect of bio-agents against *F. oxysporum* f. sp. *lentis* on radial growth and growth Inhibition

Fungal antagonist	**Radial growth (mm)**	**Growth inhibition (%)**
Trichoderma harzianum	19.23	60.76
Trichoderma viride	16.26	66.82
Bacillus subtilis	20.12	58.94
Trichoderma virence	21.75	55.62
Pseudomonas fluorescence	10.33	78.92
Control	49.00	00.00
SEm±	0.84	1.33
CD at 5 %	**2.61**	**3.31**

Fig.1 Antagonistic Effect of fungicide and botanicals against *F. oxysporum* f. sp. Lentis

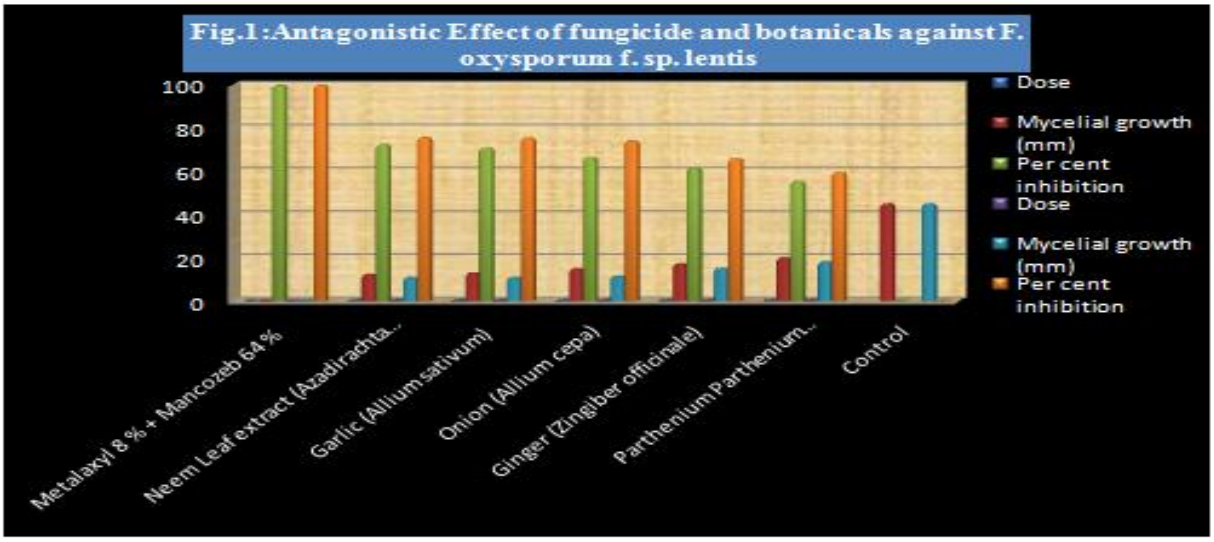
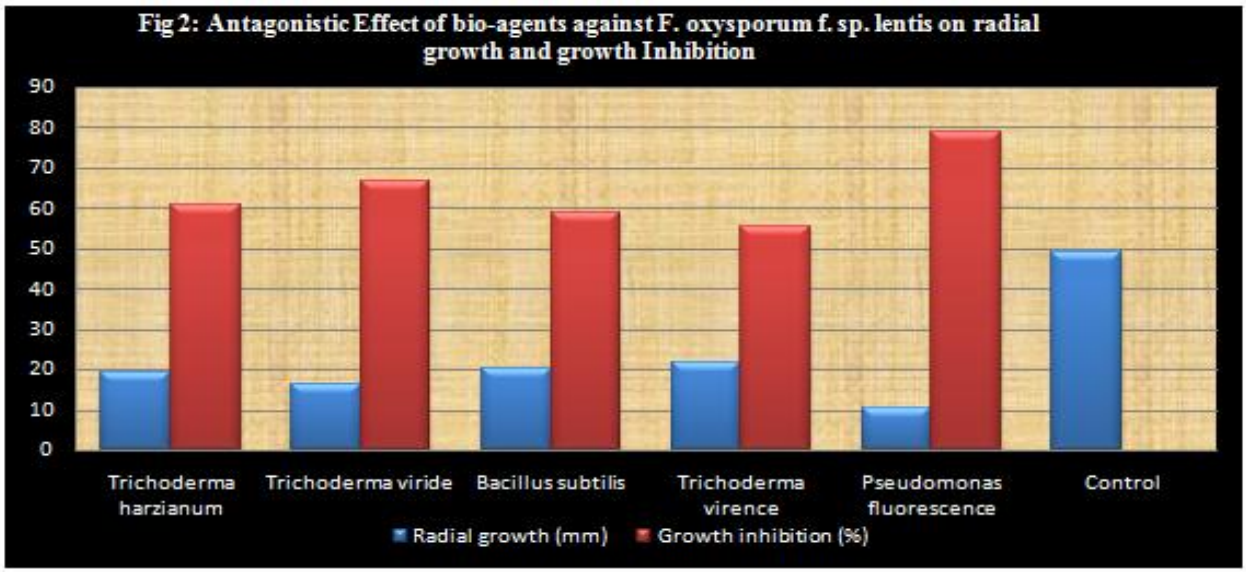


Fig.2 Antagonistic Effect of bio-agents against *F. oxysporum* f. sp. lentis on radial growth and growth Inhibition



Antagonistic Effect of bio-agents against *F. oxysporum* f. sp. *lentis* on radial growth and growth Inhibition using dual culture technique

The efficacy of bio-agents *T. harzianum, P. fluorescence, T. virence, T. viride* and *B. subtilis* were tested for radial growth and per cent inhibition of *F. oxysporum* f. sp. *lentis* by using dual culture technique. The result were obtained at 7 days of incubation, however, the radial growth were *T. harzianum* (19.23mm), *T. viride* (16.26mm), *B. subtilis* (20.12mm), *T. virens* (21.75mm) and *P. fluorescence* (10.33mm). The radial growth in control was (49.0 mm). There were

no significant differences in radial growth among *T. harzianum*, *B. subtilis* and *T. viride* were at par to each other and all treatments were significantly differed with each other after 7 days of incubation.

P. fluorescence showed maximum per cent inhibition (78.92%) followed by *T. viride* (66.82%), *T. harzianum* (60.76%), *B. subtilis* (58.94%), *T. virens* (55.62%) after 7 days of incubation, respectively this significantly differed with each other. The result obtained in the present study is analogous with the reports of many earlier workers. Bana *et al.,* (2017) reported that *T. harzianum* and *T, viride* is most effectively inhibit the growth of *Fusarium oxysporum.* Prasad *et al.,* (2019) and Dubey (2020) also found the same result as stated above.

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