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Efficacy of Different Plant Oils/Extract and Bio-Agent against Wilt of Lentil Caused by *Fusarium oxysporum* f. sp. *lentis*)

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A B S T R A C T

Lentil is one of the major *rabi* pulse crop grown in India. Lentil is a rich source of protein, minerals and vitamins for human nutrition and straw is also valued animal feed. For eco-friendly and sustainable management of the disease by using plant oils/extract. The plant oils/extracts inhibits the fungal growth range between 16.11 to 72.78%. Among then incorporation of Neem oil was found significantly superior over all the treatments resulting maximum mycelia growths inhibits (72.78%) followed by Garlic oil, Mustard oil, Ginger, and Black piper while Onion was least effective to inhibits mycelia growth. The used of bio-agents to inhibits the fungal growth ranged between 21.53 to 73.84%. Evaluation of bio-agent, the maximum inhibition of radial growth (73.84%) of *Fusarium oxysporum* f. sp. *lentis* was in *T. harzianum*, followed by *T.hamatum*, *B.subtils* and *T.viride*. Among the bio-agents tried *Aspergillus niger* was the least effective bio-agent and showed minimum inhibition in 21.53%. The fungal growth range varied from 17.00 to 65.50 mm and these were showed all the bio-agents suppressed the colony growth of the pathogen.

Introduction

Lentil (*Lens culinaris* Medik.) is one of the major *rabi* pulse crop grown in India. It's can be provides several dietary nutrients, these includes protein, essential minerals and vitamins for beneficial to the promotion of good health of humans and straw is also valued animal feed (Grusak, 2009; Iqbal *et al.*, 2006 and Erskine *et al.*, 1990). It provides the major protein requirement of millions of people of the world. It is usually grown after the rainy season on conserved soil moisture. Lentil plants are affected by a wide range of pathogens with fungal diseases being the most important. These decrease productivity through infection and damage to leaves, stems, roots and pods, and reduces marketability by discoloring seed quality (Taylor *et al.*, 2007). Vascular wilt (*Fusarium oxysporum f. sp. lentis*) of lentil is an important soil borne disease and causing significance yield losses under dry and warm

condition (Bayaa *et al.*, 1997 and Booth, 1971). In Madhya Pradesh wilt incidence as 50 to 78 per cent has been reported in some fields (Khare, 1981; Agrawal *et al.*, 1991 and Vasudeva and Srinivasan, 1952). As a result of environmental awakening and the side effects, most of the common fungicides cause on the human body the scientists are now in search of some alternative methods for controlling the plant diseases (Shashikant *et al.*, 1989). Higher plants contain a wide spectrum of secondary substances *Viz*. phenols, flavonoids, quinones, tannins, essential oils alkaloids, saponins and steroids. These plant chemicals may be fruitfully exploited for their different biological properties (Wain, 1977; Kubo and Nakanishi, 1979 and Mahadevan, 1982). In a search for better alternatives to pesticides and insecticides and other chemical control agents, natural products are considered to be environmentally safe for control of pest and diseases. In the present study experiment has been given on the effect of different bio-agent and different plant oils/extract check the radial growth of *Fusarium oxysporum* f. sp. *lentis* causing wilt disease of lentil.

Materials and Methods

Collection of the samples and isolation of causal pathogen

Diseased plants were collected from the field of different location of Bundelkhand region. Samples of wilt affected root tissues were collected in paper bags for the isolation of *Fusarium oxysporum* f. sp. *lentis*. Tissue isolation technique was followed after through surface sterilization of root pieces (2-3 mm size) with 0.1 per cent mercuric chloride solution for a minute. After this, the cut pieces thoroughly washed thrice in sterile distilled water to remove mercuric chloride from the treated pieces. These pieces were transferred in petri plates containing solidified PDA medium. The inoculated plates were then incubated at 25±2 ° temperature. These plates were regularly observed for the fungal growth. The fungal growth appearing on the root pieces were examined and purified by following single hyphal tip cut methods (Rangaswami, 1958). *Fusarium oxysporum* f. sp. *lentis* was identified based on the spores and conidiophores morphology. The pathogenicity of the isolates was proves by Koch's postulates.

Effect of different plant oils/extracts against the pathogen of *Fusarium oxysporum* f. sp. *lentis*

To test the different plant oils/extracts against the test pathogen. The three essential oils such as Neem, Garlic and Mustard oil were tested for their fungitoxicity by the using poisoned food technique against *Fusarium oxysporum* f. sp. *lentis*. Essential oil was separately dissolved in acetone (0.1 ml oil in 1.0 ml of acetone). The mixed essential oil and acetone was used as stock solution. The PDA medium containing10% concentration of each essential oil was prepared.

In order to find out the efficacy of various plant extracts against the Fusarium wilt of lentil, three plant extracts *Viz.*, Ginger rhizome, black piper seed and Onion bulb were used. Fresh plant material were collected and washed thoroughly in distilled water. Hundred gm of each washed plant material was grinded in pestle mortar by adding equal amount (100ml)of sterilized distilled water (1:1w/v) and heated at 40 to 50 ° for 10 minutes in hot water both to avoid contamination. The material was filtering passing through double layered muslin cloth and centrifuged at 1000rpm for 10 minutes.

The supernatant was collected and finally filtered through whatman no.1 filter paper and treated as standard plant extract (100%).The functioning of three essential oils and plant extracts is tested against the test pathogen. Ten ml of stock solution of oils/extracts was incorporated in 90 ml medium to make 10 per cent concentration of the oils/extract. 15 ml melted PDA was poured in sterilized petri plates. After solidification all the plates were inoculated individually with 5 mm diameter culture disc of *Fusarium oxysporum* f. sp. *lentis.* PDA plates without plant oils/extracts but inoculated with *Fusarium oxysporum* f. sp. *lentis* served as control.

Three times were maintained for all the treatments and plates were incubated at 25±2 °. Toxicity of each oils/ extracts against the test fungi was calculated in terms percent inhibition of mycelia growth using the inhibition zone was formed and expressed as per cent inhibition using the formula given by Vincent (1927) and data was analyzed by using OPSTAT statistical program Sheoran (2006).

$$\text{Percent inhibition} = \frac{\text{Colony diameter in Control (mm)} - \text{Colony diameter in treatment (mm)}}{\text{Colony diameter in control (mm)}} \times 100$$

..eqn(1)

Effect of different bio-agents against the pathogen of *Fusarium oxysporum* f. sp. *lentis*

Six bio-agents *Viz. Trichoderma harzianum, Trichoderma hamatum, Bacillus subtilis, Trichodema viride, Trichoderma virens* and *Aspergillus niger* were assessed for their efficacy against *Fusarium oxysporum* f. sp. *lentis* by using dual culture techniques (Nene and Thapliyal, 1993) the culture of test fungus and antagonists was multiplied on PDA medium. 5mm disc of test fungus and the bio-agents cut from the edge of seven days old culture plates were placed on PDA medium in petri plates. The test fungus and bio-agent were placed opposite to each other at a distance of 5mm form the circumstance of petri plates. The same disc of the test fungus was placed alone only one side on PDA medium as control. Each treatment was replicated three times and incubated at 25 ±2 °. The data were recorded after seven days of bio-agent placement, when percent inhibition formula was given in equation 1.

Results and Discussion

Effect of different plant oils/extracts against the pathogen of *Fusarium oxysporum* f. sp. *lentis*

Use of fungicides is one of the major components in the disease management but regular use of fungicides caused adverse effect on environment. It also encourages development of resistance among pathogen. Therefore, six pant oils/extracts contents of Neem oil, Garlic oils, Mustard oil, Ginger, Black piper and Onion tested against radial growth of *Fusarium oxysporum* f. sp. *lentis*. The result obtained from (Table 1) and its corresponding histogram (Fig.1) reveled the maximum inhibits the radial growth (72.78%) *Fusarium oxysporum* f. sp. *lentis* was in Neem oil followed by Garlic oil, and Mustard oil. Among the tested plant extracts Ginger was most effective which showed 44.45% inhibition zone followed by Black paper and Onion which showed 20.00mand 16.11% inhibition zone respectively. All the treatments were significant superior than control. In the similar reported that several higher plants are known to posses fungitoxicity against spore germination or mycelia growth (Dixit *et al.,* 1982; Dubey *et al.,* 1983).

Amongst several metabolites of higher plants, the essential oil has been reported to be highly efficacious against different plant pathogens (Gaur and Raychaudhury, 1970; Pandey and Dubey, 1992).

The *Allium sativum* and *Azadirachta indica* has been reported highly toxic to Fusarium spp. was inhibits the mycelia growth and spore germination (Shivpuri *et al.,* 1997; Abdulrahman, 2005 and Sahayaraj *et al.,* 2006). In conclusion present study demonstrated that different bio-agent and different plant oils/extract can be used for protecting of huge losses with wilt disease of lentil caused by *Fusarium oxysporum* f. sp. *lentis.*

Effect of different bio-agents against the pathogen of *Fusarium oxysporum* f. sp. *lentis*

The use of chemicals is being discouraged now a-days for the reason that the fungicides are not eco-friendly for being hazardous to mammalian group and responsible for creating the environmental pollution in air, soil and water. Therefore, eco-friendly management of the disease by six bio-agents *Viz. Trichoderma harzianum,Trichoderma hamatum, Bacillus subtilis, Trichodema viride, Trichoderma virens* and *Aspergillus niger* were evaluated against *Fusarium oxysporum* f. sp. *lentis In vitro*.

Table.1 Effect of different plant oils/extracts against the pathogen *in vitro.*

S.No.	Treatments	Dose (%)	Av. diameter of fungal growth (mm)	Inhibition over control (%)
1	Neem oil	10	16.33	72.78
2	Garlic oil	10	19.00	68.33
3	Mustard oil	10	30.00	50.00
4	Ginger	10	33.33	44.45
5	Black piper	10	48.00	20.00
6	Onion	10	50.33	16.11
7	Control	-	60	-
CD at 5%			4.40	
SE(m)			1.41	

Fig.1 Effect of different plant oils/extracts against the pathogen.

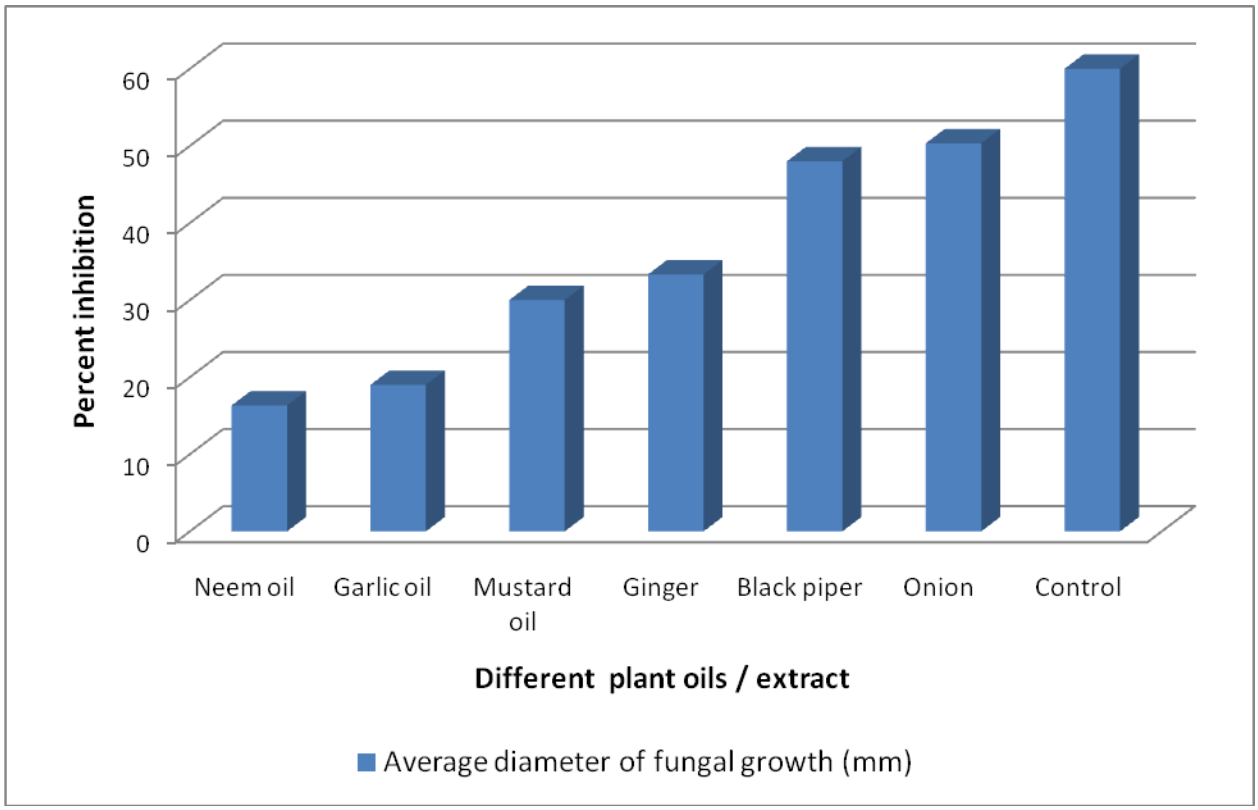
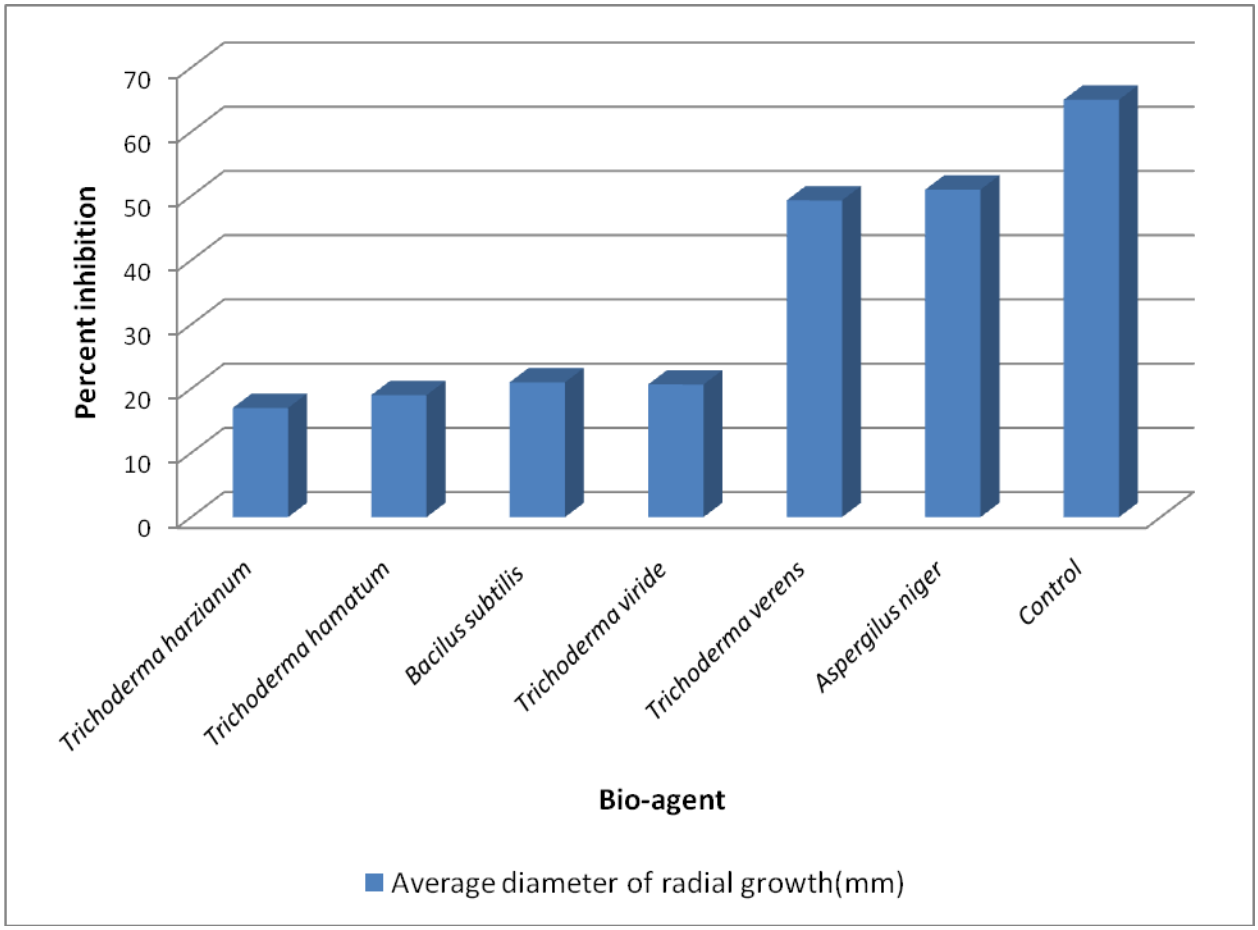


Table.2 Effect of bioagents against the pathogen *in vitro.*

S.No.	Treatments	Av. Diameter of radial growth(mm)	% inhibition
1	*Trichoderma harzianum*	17.00	73.84
2	*Trichoderma hamatum*	19.00	70.76
3	*Bacilus subtilis*	21.00	67.69
4	*Trichderma viride*	20.66	68.21
5	*Trichoderma verens*	49.33	24.10
6	*Aspergilus niger*	51.00	21.53
7	Control	65.00	-
CD at 5%		4.17	
SE(m)		1.34	

Fig.2 Effect of bio-agents against the pathogen.



The result presented in (Table 2) and corresponding histogram (Fig.2), revealed that maximum per cent inhibition (73.84%) of *Fusarium oxysporum* f. sp. *lentis* was in *T. harzianum*, followed by *T.hamatum*, *B.subtils* and *T.viride*. Among the bio-agents *Aspergillus niger* was the least effective bio-agent. The inhibition of the fungal growth range from 21.53% to 73.84% that showed all the bio-agents

suppressed the colony growth of the pathogen. All the treatments were significantly superior to control. *T.virens* proved better only than *Aspergillus niger* inhibiting the growth of the pathogen. The radial growth of the pathogenic fungus was statistically similar in case of *T.hrzianum, T.hamatum, Bacilus subtils* and *T.viride* as there was no significant difference among these treatments. In Similar observations on mycelial growth inhibition of soil borne fungal pathogens by *T. harzianum. T. viride* and *T. virens* were reported by Mukherjee and Tripathi (2000), Upadhyay and Mukhopadhyay (1986). Our finding is close agreement with the finding of the Choudhary and Mohankain (2012) that reported *T.harzianum* is the most effective and reduce radial growth of *Fusarium oxysporum* sp. The *T.harzianum* and *T.viride* were found maximum mycelia growth inhibits of the pathogen *Fusarium oxysporum* f. sp. *ciceri* caused by wilt of chickpea (Dubey *et al.*, 2007).

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