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In vitro efficacy of *Trichoderma* spp. and plant extracts on *Alternaria lini* cause blight disease in Linseed (*Linum usitatissimum* L.)

Narender Kumar and Uma Kant Tripathi

Abstract
Investigations were made on the effect of 3 *Trichoderma* spp. and 8 plants extracts against *Alternaria lini* cause blight disease in linseed. Antifungal activity of *Trichoderma* spp. and selected plant extracts were studied against *Alternaria lini.* The significantly inhibitions of mycelial growth of *A. lini* are recorded in all treatments. Maximum growth of *A. lini* inhibited by *T. harzianum* (72.22%) followed by *T. viride* (67.03%), *T. virens* (63.70%), respectively. When among plant extracts, *Azadirachita indica* showed maximum inhibition (88.52%) followed by *Ocimum tenuiflorum* (76.67%), *Mentha piperita* (74.08%), *Mentha arvensis* (73.70%),*Ginger officinalis* (71.48%), *Parthenium hysterophorus* (59.26%), *Allium sativum* (51.86%) and *Allium cepa* (17.78%), respectively.

Keywords: *Trichoderma, Alternaria lini,* blight disease, linseed

Introduction
Flaxseed or Linseed (*Linum* is *usitatissimum* L.) is known as founding crop [1] which is being evaluated as a crop platform for the production of bio-industrial and nutraceutical products [2]. It is the sixth largest oilseed crop in the world and is one of the oldest cultivated plants [3]. Flaxseed is grown as either oil crop or a fiber crop [4]. Linseed is a *Rabi* crop in India which is a member of family Linaceae. Linseed is an annual dicotyledonous plant [5]. Canada is the world's largest producer of flax (38% of total production) [5]. India contributes about 14.88% and 6.57% to world area and production, respectively. Productivity of Rajasthan state (1351 kg/ha) of India is surpassing the productivity of Asia (728kg/ha) as well as of world (986 kg/ha) [6]. The plant is native to west Asia and the Mediterranean.

Linseed is one of the richest sources of α- linolenic acid (ω-3 fatty acid) and soluble mucilage. An analysis of brown Canadian flax showed about 41% fat, 20% protein, 28% total dietary fibre, 7.7% moisture and 3.4% ash, which is the mineral-rich residue left after samples are burned.[7] Seed contain 20% protein[8] but Indian cultivar Khategaon had a protein content of 21.9% [9] Flax is glutenfree. Linseed oil have ω-3 (57%), ω-6 (16%), monosaturated fatty acid (18%) and saturated fatty acid (9%) in its composition [10] The components present in flaxseed attract the food technologists and nutritionists to explore its activities in health sector [11].

Linolenic (omega-3) fatty acids are reduce the risk of cardiovascular disease.[7] Flaxseed protein was effective in lowering plasma cholesterol and triglycerides (TAG) compared to soy protein and casein protein.[12] The antioxidant activity of the flaxseed has been shown to reduce total cholesterol[14] as well as platelet aggregation [15].

Flaxseed can be incorporated into diet through oil, milled or ground flaxseeds or through eggs, meat produced by animals fed flax meal [16]. The seeds are now widely used as bakery [17] We can also sprinkle ground flax over a salad, cooked vegetables or cold breakfast cereals. Flaxseed is used for the preparation of flaxseed chutney powder [18] and linseed tea [19] Total fibers (cellulose, lignine and hemicellulose) content varies between 22-26 per cent [20].

Linseed is adversely affected by different diseases. Out of 15 fungal diseases of linseed, most important pathogens as *Alternaria linicola* (blight), *Fusarium* spp. (wilt), *Botrytis cinerea* (gray mould) and *Oidium lini* (powdery mildew) [21] *Ascochyta linicola* (foot rot), *Melampsora lini* (Rust), *Rhizoctona solani* (Rhizoctonia seedling blight), *Pythium megalacanthum* (scorch), *Septoria linicola* (pasmo), *Polyspora lini* (browning or stem break) and *Colletotrichum linicolum* (anthracnose) which are affected by seed-treatment and certification, breeding for resistance.

Alternaria blight of linseed was first reported by Dey [22] from Kanpur, Uttar Pradesh in 1933. Later Siddiqui [23] in 1963 reported the occurrence of Alternaria blight on linseed cultures at IARI, New Delhi and other parts of the country.

The fungus was named as *Alternaria lini* after the first report of this disease in 1933 [22]. Alternaria blight is a major disease in which all areal parts of linseed are effected. In severe condition seeds also contaminate. This disease causes heavy loss in terms of quality and quantity of fiber and seed of linseed.

Synthetic chemicals used in disease management create several problems like environmental pollution, residual effect in grain and killing of non-target organism(s). Development of resistant strains of plant pests are serious problem of pests management, increase due to the application of only pesticide strategies for pests management [24]. To escape above problems, *Trichoderma* spp. and plant extracts are best alternate of chemicals. *Trichoderma* is a best biological weapon for crop protection and also act as plant growth promoter [25]. Therefore, the study was undertaken in the present investigation as "*In vitro* efficacy of *Trichoderma* spp. and plant extracts on *Alternaria lini* cause blight disease in Linseed (*Linum usitatissimum* L.)".

Materials and Methods

Collection of Infected Plant Symptoms of Linseed

The culture of plant pathogen used in present investigation was isolated from the diseased plants of linseed (cultivar Shekhar) which were collected from the Oilseed Farm, Kalyanpur of the C. S. Azad University of Agriculture and Technology, Kanpur (U.P) India. Effected leaves show concentric rings. In severe condition concentric ring spots are collapse and form blight symptoms. In severe condition bud and stem also effected.

Isolation and Identification of Plnat Pathogen

For the isolation of causal organism, small pieces of diseased leaf sowing typical symptoms along with healthy tissues were cut with the help of sterilized blade. These pieces washed thoroughly with the tap water and placed into 0.1 per cent mercuric chloride solution followed by washing thrice with sterilized water thoroughly. Excess water was removed by placing on the folds of sterilized blotting paper. Dried pieces were asceptically transferred into Petri-dish containing potato dextrose agar medium with the help of a sterilized forceps. Petri dishes were properly marked with glass marker and incubated at 25±2ºC in B.O.D. incubator.

On 2nd day whitish mycelial colony observed in petri plates and this colony gradually changed into blackish in colour. Some part of colony was taken and slide was prepared by using the method of Aneja [26] and observed under the microcrope. Conidiophores of *Alternaria lini* were branched, septate, dark in colour and produced muriform conidia.

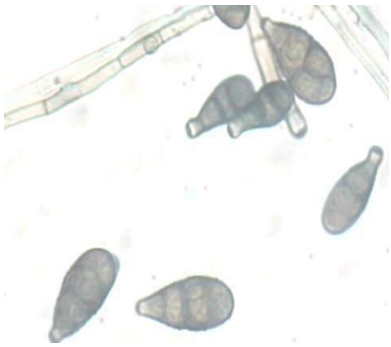
Purification of *A. lini*

Alternaria lini purified by single spore culture technique.[27] After sub culturing the isolate was maintained on PDA medium at 4°C for further use.

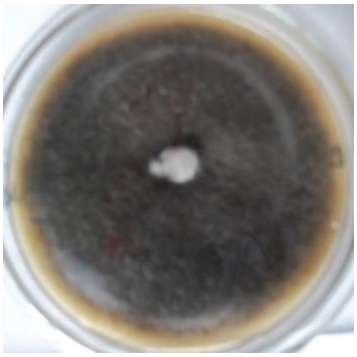
Pathogenecity Test

The Pathogenecity of the isolated fungus was tested following Koch postulates in a plot experiment on linseed which were found most susceptible to Alternaria blight under field condition [26].

Disease symptoms on leaf (a)



Conidia and hyphae of pathogen (b)



Pure culture of *A. lini* (c)

Blight symptoms on leaf, Conidia and hyphae of *A. lini* and its pure culture

Isolation of *Trichoderma* spp.

Soil samples collected from various rhizosphere soils of wilt diseased fields of linseed of Nwabganj Farm, C. S. Azad University of Agriculture and Technology. Trichoderma selective medium (TSM) was used for isolation of the isolates of *Trichoderma* [28]. *Trichoderma* spp. was isolated from soil sample by using the serial dilution technique.

Identification of *Trichoderma* spp.

Different *Trichoderma* spp. identified on the basis of their colour, septation and branching pattern of mycelium which were recorded microscopically. The colour shape and size of sclerotia were observed at 5-6 days of incubation.

Efficacy of *Trichoderma* spp. against *Alternaria lini*

The efficacy of *T.harzianum*, *T. viride* and *T. virense* were tested for mycelial growth and per cent inhibition of *A. lini* by using dual culture in laboratory, Department of Plant Pathology of this university. Efficacy assessed by using dual culture technique to measuring the radial growth of *A. lini* as well as that of *Trichoderma* spp.

Observations were recorded on colony growth of *Trichoderma* spp. and *A. lini* at 72 hours in dual culture as well as control. The per cent growth inhibition of *A. lini* was calculated as follows:

$$\text{Per cent growth inhibition} = \frac{A1 - A2}{A1} \times 100$$

Where,

A_1 = Area covered by the *A. lini* in control.

A_2 = Area covered by the *A. lini* in dual culture.

Preparation of plant extract

Hundred gram of each washed plant material was grinded in Pestle and Mortar by adding equal amount (100 ml) of sterilized water (1: 1 w/v) and heated at 80 ^{0}C for 10 minutes in hot water both. The materials was filtered through double

layered muslin cloth followed by filtering through sterilized Whatman No. 1 filter paper and treated as standard plant extract (100%). The 5.0 per cent concentration was made by adding in requisite amount of sterilized PDA medium. Ten ml plant extract of stock solution were added to the 90.0 ml of sterilized cooled PDA medium. All the plant extracts were tested at 5.0 per cent concentration under *in vitro* condition by using poison food technique to study the inhibitory effect of these botanicals on mycelial growth of *A. lini*. The observation was recorded on radial growth at 72 hours of incubation in plant extracts amended Petri dishes as well as in control. Per cent growth inhibition was calculated by using formula [29].

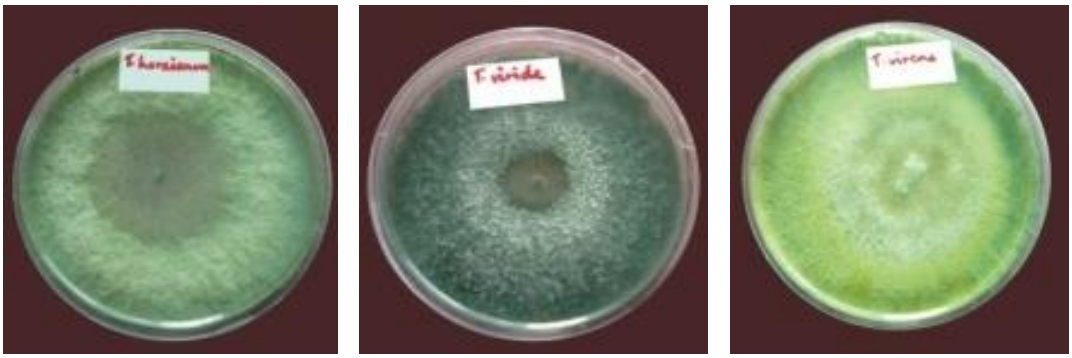
$$I = \frac{C - T}{C} \times 100$$

Where,

I = Per cent inhibition of fungal growth
C = Radial growth of control
T = Radial growth of treated Petri dishes

Result and Discussion

Results clearly indicated that minimum radial growth and maximum inhibition per cent was noticed in *T. harzianum* (25.00mm, 72.22%) followed by *T. viride* (29.67mm, 67.03%) and *T. virinse* (32.67mm, 63.70%) as compared to control (90.00mm). The radial growth significantly differed to each other in all the treatments.



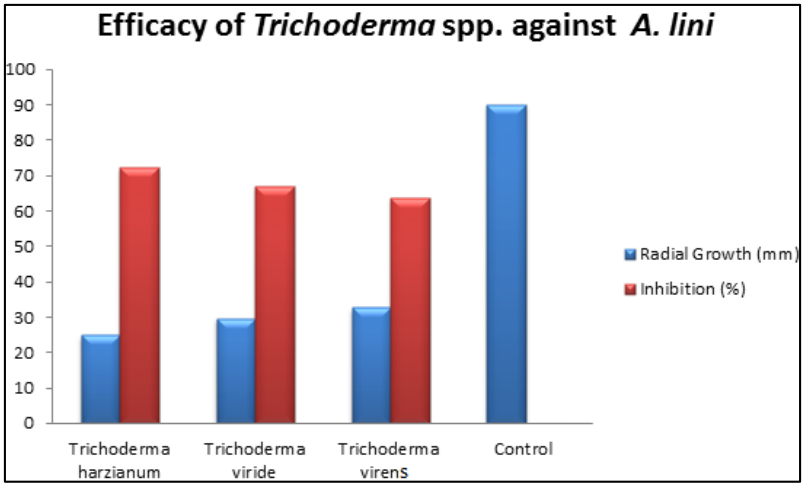
T. harzianum *T. viride* *T. virens*

Pure culture of bioagents (*T. harzianum*, *T. viride* and *T. virens*)

Table 1: Efficacy of *Trichoderma* spp. against the mycelial growth and per cent inhibition of *A. lini* in dual culture technique after 72 hrs of incubation

***Trichoderma* spp.**	**Radial Growth (mm)**	**Inhibition (%)**
Trichoderma harzianum	25.00	72.22
Trichoderma viride	29.67	67.03
Trichoderma virence	32.67	63.70
Control	90.00	00.00
C.D.	1.213	-
SE(m)	0.344	-

Tagaram [30] recorded percentage growth inhibition of *Alternaria alternata* by *T. viride* and *T. harzianum* was 80.1% and 72.2% respectively. Among four species of *Trichoderma*, *T. hamatum* inhibited 38.46% radial mycelial growth whereas *T. viride* showed 78.38%, respectively [31].

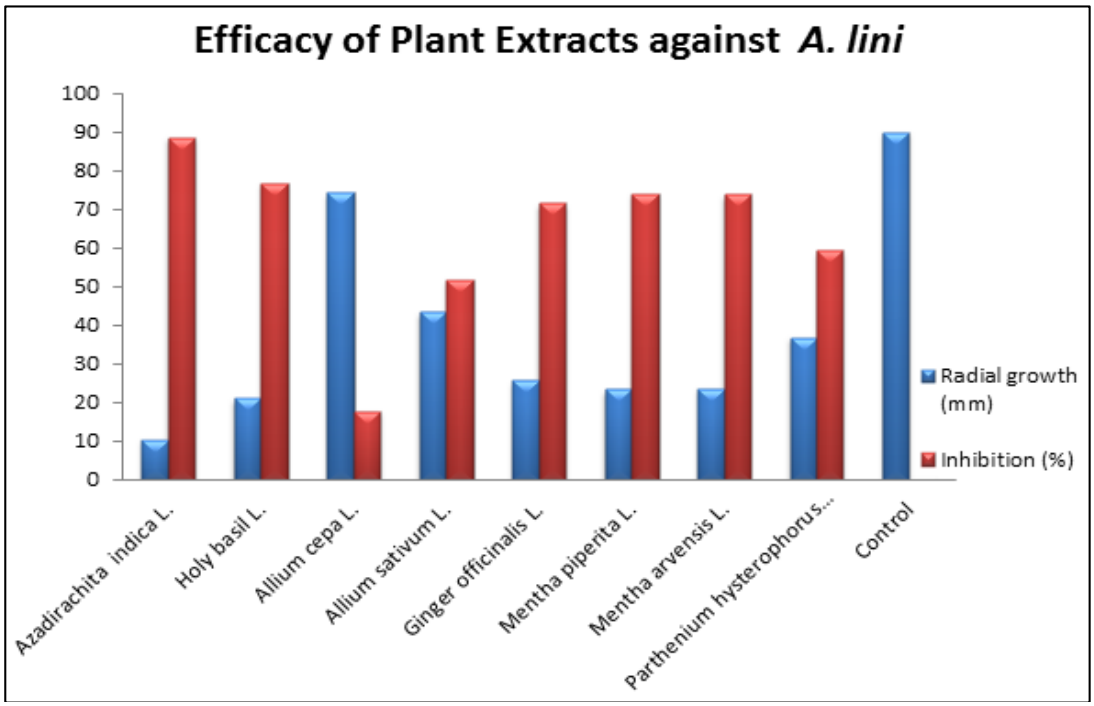


Efficacy of Plant extracts against *Alternaria lini*

The minimum radial growth and maximum inhibition per cent was obtained in *Azadirachita indica* (10.33mm, 88.52%) followed by *Ocimum tenuiflorum* (21.00mm, 76.67%), *Mentha piperita* (***23.33***mm, 74.08), *Mentha arvensis* (***23.67***mm, 73.70%), *Ginger officinalis* (***25.67***mm, 71.48%), *Parthenium hysterophorus* (***36.67***mm, 59.26%), *Allium sativum* (***43.33***mm, 51.86%) and *Allium cepa* (***74.33***, 17.78%) respectively as compared to control.

Table 2: Inhibitory effect of plant extracts against test fungus

Test plants	Radial growth (mm)	Inhibition (%)
Azadirachita indica L.	***10.33***	88.52
Ocimum tenuiflorum L.	***21.00***	76.67
Allium cepa L.	***74.33***	17.78
Allium sativum L.	***43.33***	51.86
Ginger officinalis L.	***25.67***	71.48
Mentha piperita L.	***23.33***	74.08
Mentha arvensis L.	***23.67***	73.70
Parthenium hysterophorus L.	***36.67***	59.26
Control	90.00	00.00
C.D.	2.30	-
SE(m)	0.77	-



Effectivity of the extract of neem leaf (*Azadirachita indica* L.) in inhibitory the mycelia growth of fungus have been reported by Singh *et al* (2007) [32]. Yadav [33] found out of 8 plant extracts, Garlic (*Allium sativum* L.) extract gave maximum inhibition in mycelial growth followed by Ginger (*Zingiber officinale*), Neem, Onion (*Allium cepa* L.), Dhatura, Tulsi (Holy basil=*Ocimum tenuiflorum* L.) against *R. solani* causing web blight of french bean. Shinde and Patel [34] found that bulb extract of Garlic gave hundred per cent inhibition of mycelial growth of *R. solani* causing black scurf of potato followed by Ginger (*Zingiber officinale*), Tulsi, *Eucalyptus* and Neem.

Conclusion

Linum usitatissimum L. occupies an important position in world market because of its multiple trade use. It is a valuable crop and every part of the plant has specific economic importance. Disease is one of the major constraints that limit productivity of linseed. Now-a-days, *Trichoderma* and plant extracts are ecofriendly weapons used in organic farming. Antifungal activity was confirmed by all of the selected *Trichoderma* and plant species. The results revealed that different *Trichoderma* spp. and plant extracts varied in their efficacy for inhibiting the mycelial growth of *A. lini*. Although the selected species of *Trichoderma* and concentration of tested plant species were unable to completely inhibit the pathogens but they could be used in combination with the fungicides as IDM (Integrated Disease Management) strategy to minimize the use of fungicides. The finding of the present investigation could be an important step towards the possibilities of using *Trichoderma* spp. and natural plant products as bio-pesticides in the plant disease management.

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