Comparative efficacy of different biological agents, botanical extracts and chemical in management of linseed wilt incited by *Fusarium oxysporum* f. sp. *Lini.* (Bolley) synder and Hansen under sick field condition

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Abstract

Cultivated Flax is commonly known as “Ulsee” or “Tisee” (*Linum usitatissimum* L.). Linseed wilt caused by *Fusarium oxysporum* f.sp. *Lini.* Pathogen screened out against this fungus. The experiment was conducted in pots under glass house condition, during 2017-18 by using susceptible cultivar Chambal. All the treatments were found significantly superior over check (untreated control) in controlling the disease severity by checking the wilting of plants. Out of 13 treatments, the minimum wilting of 21.11 per cent were recorded with treatment T5 [Seed (5g/kg seed) and soil treatment (10g/kg soil) with *Trichoderma harzianum*] followed by T3 ([Seed (5g/kg seed) and soil treatment (10g/kg soil) with *Trichoderma viride*], (21.57 per cent) and T1 ([Seed + soil treatment with carbendazim (0.2 Per cent)]) (25.00 per cent), respectively. Wilting of the plant started in control pots just 15 to 20 days after sowing while in treated pots, wilting started after 35 to 40 days after sowing. Seed treatments with either *Trichoderma* spp. or *Pseudomonas* spp. or with extracts were less effective as compared to treatments of seed and soil both.

Keywords: Biological agents, botanical extracts, *Fusarium oxysporum* f. sp. *Lini.*

Introduction

Flaxseed or Linseed (*Linum usitatissimum* L.) (2n = 30) is known as founding crop (Genser and Morris, 2003) [7] which is being evaluated as a crop platform for the production of bioindustrial and nutraceutical products (Jhala et al., 2003). The origin of Indian type of linseed is traced to be in Ethiopia, through polyphyletic origin of the same is indicated. It is the sixth largest oilseed crop in the world and is one of the oldest cultivated plants (Bhatt and Rowland, 1990) [6]. Flaxseed is grown as either oil crop or a fiber crop (Diederichsen and Richards, 2003) [6]. Linseed is a Rabi crop in India which is a member of family Linaceae. Linseed is an annual dicotyledonous plant. Globally linseed is an important crop and its production is 21.23 lac tonnes from 21.12 lac/ha with an average yield of 1006 kg/ha. While our national production is 1.54 lac tonnes from an area of 3.42 lac ha with poor productivity of 449 kg/ha. India ranks second in area after Canada in the world, but is at fourth place in term of production after Canada, China and U.S.A. In term of productivity India (449 kg/ha) is far below to Canada (1492 kg/ha), U.S.A (1484 kg/ha), Egypt (1465 kg/ha), Russia (1292 kg/ha) and China (944 kg/ha). In our country, Madhya Pradesh leads in both (Yield 0.328 lakh tonnes and acreage 1.044 lakh ha) followed by Uttar Pradesh (yield 0.271 lakh tonnes and acreage 1.080 lakh ha respectively. In Uttar Pradesh the total area under this crop is about 1.080 lakh hectares and annual production of 0.271 lac tonnes with productivity of 251 kg/ha.

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 (Hurteau, 2004) [9]. Seeds contain 20% protein (Halligudi, 2012) [8] but Indian cultivar Khategaon has a protein content of 21.9%. Linseed oil has ω-3 (57%), ω-6 (16%), monosaturated fatty acid (18%) and saturated fatty acid (9%) in its composition. (Mishra and Verma, 2013) [14]. Linolenic (omega-3) fatty acids reduce the risk of cardiovascular disease. Flaxseed protein is effective in lowering plasma cholesterol and triglycerides (TAG) compared to soy protein and casein protein (Bhathena et al., 2002) [2].
The antioxidant activity of the Flaxseed has been shown to reduce total cholesterol as well as platelet aggregation (Allman et al., 1995) [1]. Despite considerable increase in productivity and production a wide gap exists between potential yield and the yield realized at farmer’s field, which is largely because of a number of biotic and abiotic diseases, to which linseed crop is exposed. Linseed crop is adversely affected by different biotic diseases. Out of 15 fungal diseases of linseed, most important pathogens are Alternaria liniocola (blight), Fusarium spp. (wilt), Botrytis cinerea (gray mould) and Oidium lini (powdery mildew), Ascochyta liniocola (foot rot), Melampsora lini (Rust), Rhizoctonia solani (Rhizoctonia seedling blight), Pythium megalecanthum (scorch), Septoria liniocola (pemsio), Polyspora lini (browning or stem break) and Colletotrichum lincolum (anthracnose) (Mercer et al., 1991) [13]. Among these diseases, wilt caused by Fusarium oxysporum f. sp. lini is the most important disease and known to inflict 80% of theoretical yield losses under conditions favourable for wilt development in linseed (Sattar and Hafiz, 1952) [17]. The plants are attacked at all the stages of growth. The edges of infected cotyledons of very young seedlings roll inwards and further growth of the plant ceases and seedlings die (Thind, 2005) [21]. Therefore, management of the disease is very difficult and single method is not sufficient for management of the disease. Mukhopadhyay (1987) [15] found an integrated approach of using cultural measures, biological control, chemical control for management of the disease. An integrated approach using Carbendazim, T. viride along with Neem seed kernel extract resulted in reduction of wilt incidence caused by Fusarium oxysporum against cumin (Bhatnagar et al., 2013) [3]. Considering the above point’s in view current research was done to develop integrated disease management strategies against Fusarium oxysporum f.sp. Lini in Linseed.

Materials and Methods
Collection of diseased material
Naturally affected plants of linseed showing symptoms of wilt disease were collected during Rabi 2017-18 from crop research farm Nawabganj, C.S. Azad University and Technology, Kanpur, (U.P.). Such affected plants were brought to the laboratory and critically examined for the presence of causal organism. The freshly collected diseased materials were used for isolation of the pathogen.

Isolations of pathogen
The causal organisms were isolated from affected roots of linseed plants. The affected roots were first washed in tap water to remove dust particles and then thoroughly washed with sterilized water in order to remove the surface contaminants. Instruments to be used were sterilized by using 95 per cent methylated alcohol. Small pieces of diseased portion along with healthy parts were cut into pieces with a sterilized blade. The cut pieces were surface sterilized with 0.1 per cent mercuric chloride solution under aseptic conditions inside the laminar flow and washed thoroughly 3-4 times with sterilized water to remove the traces of mercuric chloride. Excess moisture was removed by placing them in the fold of sterilized blotting papers. These pieces were transferred to 2 per cent Potato Dextrose Agar (PDA) medium in 90 mm Petri dishes, previously autoclaved at 15 p.s.i. for 20 minutes with the help of sterilized needles. The Petri dishes were then transferred at 28±2°C temperature for 7 days in B.O.D. incubator. These incubated plates were observed for mycelial growth of the causal fungus after 24 hours of inoculation daily once till the growth of the fungus was noted. As soon as the mycelial growth was visible around these pieces, the hyphal tips from the advancing mycelium were cut and transferred into the culture tubes containing Potato-Dextrose Agar medium for further purification, identification and maintenance of culture.

Purification of pathogen
The purification of fungal isolates was taken following single spore isolation technique (Choi, 1999) [5]. A dilute spore suspension was poured on plain agar Petri dishes to form a very thin layer on it and spores were allowed to settle down on the agar surface. Settled spores were separated out from each other, selected under the microscope and encircled with the help of dummy cutter in Petri dishes. They were lifted along with agar blocks and transferred to Petri dishes containing sterilized 2 per cent PDA medium. After proper growth of fungus obtained by single spore culture regular sub-culturing was done to check contamination, till pure cultures were obtained. These cultures were sub cultured at monthly intervals and maintained on Potato-Dextrose-Agar slants under refrigeration at 6 to 8°C for further studies.

Preparation of culture media
Modified Czapek-Dox-Agar medium was used for isolation of Fusarium wilt pathogen using method of Singh and Chaube (1970) [19], Potato-Dextrose-Agar medium prepared by using method described by Johnston and Booth (1983) [11], was used for present study used for maintaining of pure culture of the wilt pathogen.

Collection of Bio – agents
Bio - agents viz., Trichoderma harzianum, Trichoderma viride and Pseudomonas fluorescens were collected from Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture & Technology, Kanpur to conduct the present investigation.

Collection of botanicals
Seed kernel of Azadirachta indica from university campus, Chandra Shekhar Azad University of Agriculture & Technology, Kanpur and Allium sativum from local market were collected to conduct the experiment.

Collection of fungicide
Chemical fungicide i.e., carbendazim was collected from local market.

Effect of different treatments against disease under sick field condition
Linseed cultivar Chambal was raised in earthen pots of (25x20 cm) size containing approximately 40 kg of potting mixture consisting of sterilized farm yard manure, sand and soil (3:2:1). The mass culture of the antagonists was prepared on Bajra grains following the method of Singh at el. (1996) [20]. The sterilized soil was well mixed separately with 1 per cent (w/v) pure inoculums of the pathogen. The seed (5g/kg seed) and soil treatment (10g/kg soil), with T. viride and T. harzianum, seed (10g/kg seed) and soil treatment with Pseudomonas fluorescens (20g/kg soil), and botanicals (seed kernel extract) with Azadirachta indica (10 per cent w/v) and extract of Allium sativum (10 per cent w/v) were mixed
separately with the pathogen infected soil. The pots containing the soil pathogen inoculums without the antagonist served as control. These treatments were compared with the treatment of seed and soil treatments with chemical fungicide carbendazim (0.2 per cent). The seeds treated with different bio-agents, plant products and fungicide were sown in pot soil at the rate of 15 seeds per pot. The soil moisture was maintained by adding water when required. Appearance of disease development was noted in each treatment reported. The final incidence of wilted plants was recorded. The percent disease incidence was calculated by using date of sowing of experiment. The percent disease control was calculated as per formula given below:

\[
\text{Per cent disease control} = \frac{U-T-T}{T} \times 100
\]

Where,

UT- Untreated  
T- Treated

The details of the treatments were given as follows

\begin{align*}
T_1: & \text{ Seed treatment with } Trichoderma viride (5g/kg seed) \\
T_2: & \text{ Seed treatment + soil treatment with } Trichoderma viride (10g/kg soil) \\
T_3: & \text{ Seed treatment with } Trichoderma harzianum (5g/kg seed) \\
T_4: & \text{ Seed treatment + soil treatment with } Trichoderma harzianum (10g/kg soil) \\
T_5: & \text{ Seed treatment with } Pseudomonas fluorescens (10g/kg seed) \\
T_6: & \text{ Seed treatment + soil treatment with } Pseudomonas fluorescens (20g/kg soil) \\
T_7: & \text{ Seed treatment with seed kernel extract of Azadirachta indica (10 per cent w/v)} \\
T_8: & \text{ Seed treatment + soil treatment with seed kernel extract of Azadirachta indica (10 per cent w/v)} \\
T_9: & \text{ Seed treatment with extract of Allium sativum (10 per cent w/v)} \\
T_{10}: & \text{ Seed treatment + soil treatment with extract of Allium sativum 10 percent w/v)} \\
T_{11}: & \text{ Seed treatment with carbendazim (0.2 per cent)} \\
T_{12}: & \text{ Seed treatment + soil treatment with carbendazim (0.2 per cent)} \\
T_{13}: & \text{ Control}
\end{align*}

Results and Discussion

Evaluation of different treatments against disease under sick field condition

In this study the efforts have been made to evaluate the active bio-agents/plant products as seed dressers and as a soil application in comparison to fungicide (Carbendazim) against Fusarium wilt of linseed under sick pot culture. The experiment was conducted in pots under glass house condition, during 2017-18 by using susceptible cultivar Chambal.

Wilting of the plant started in control pots just 15 to 20 days after sowing while in treated pots, wilting started after 35 to 40 days of sowing. All the treatments were found significantly superior over check (untreated control) in controlling the disease severity by checking the wilting of plants. Minimum wilting of 21.11 Per cent were recorded with treatment T1 [Seed (5g/kg seed) and soil treatment (10g/kg soil) with Trichoderma harzianum], followed by T2 [Seed (5g/kg seed) and soil treatment (10g/kg soil) with Trichoderma viride], (21.57 Per cent) and T12 [(Seed + soil treatment with carbendazim (0.2 Per cent)] (25.00 Per cent), respectively. All these treatments were found at par in controlling the disease severity. These treatments were also found statistically at par with treatments T1, Seed treatment + soil treatment with Pseudomonas fluorescens (10g/kg soil) (28.25) and T8, Seed treatment + soil treatment with seed kernel extract of Azadirachta indica (10 Per cent w/v) (39.90 Per cent), respectively. Treatments only seed treatments with either Trichoderma spp. or Pseudomonas spp. or leaf extracts were less effective as compared to treatments of seed and soil both. Only seed treatments have no long-term effect on controlling the disease incidence. Maximum percent wilting of 81.76 Per cent were recorded in untreated pot. The treatments T1 [Seed treatment with Trichoderma viride (5g/kg seed)], T3 [Seed treatment with Pseudomonas fluorescens (10g/kg seed)], T7 [Seed treatment with seed kernel extract of Azadirachta indica (10 Per cent w/v)] and T9 [Seed treatment with extract of Allium sativum (10 Per cent w/v)] were found less effective in controlling the disease in comparison to others (Table-1). Singh et al. (2008) tested the efficacy of Trichoderma harzianum, Trichoderma viride, Thiram and Farm yard manure (50/ha) alone and in combination against wilt of linseed at Kanpur and reported all the treatment significantly increased the plant density and seed yield and reduced the disease incidence over untreated control. Kishor and Singh (2008) evaluated the effects of Bavistin [carbendazim], Benlate [Benomyl], Thiram + Bavistin, Roko (thiophanate-methyl), Thiram, Agrosan G.N. [phenylmercury acetate], Captan, Vitavax [carboxin], Companio, Mancozeb and Ridomil [Metalaxyl] on the growth and development of F. oxysporum f.sp. Linii in linseed (cv. Chambal) and found systemic fungicides Bavistin, Benlate and Roko were the most effective (reduced wilt incidence by 82.4, 69.0 and 53.5%, respectively), followed by Thiram, Agrosan G.N., Captan and Vitavax. The fungicides increased the yield by 59-97%. Rekha et al. (2011) also evaluated 10 promising isolates of Trichoderma spp., from Raichur, Karnataka under sick soil condition and reported maximum germination wilt minimum incidence with soil application T. viride as compared to check. Effectivity of Trichoderma viride and T. Harzianum in present study supports the above findings. Effectivity seed and soil treatment against Fusarium oxysporum f. sp. lini causing wilt in linseed in present study was found maximum which a new record is.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Initial plant population average of 3 pots</th>
<th>Final plant population/pot</th>
<th>% plant wilted</th>
<th>% Disease control over check</th>
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<tbody>
<tr>
<td>T1</td>
<td>9.67</td>
<td>4.00</td>
<td>58.15</td>
<td>28.87</td>
</tr>
<tr>
<td>T2</td>
<td>9.00</td>
<td>7.00</td>
<td>21.57</td>
<td>73.61</td>
</tr>
<tr>
<td>T3</td>
<td>9.33</td>
<td>4.33</td>
<td>53.70</td>
<td>34.31</td>
</tr>
<tr>
<td>T4</td>
<td>9.33</td>
<td>7.33</td>
<td>21.11</td>
<td>74.18</td>
</tr>
<tr>
<td>T5</td>
<td>8.67</td>
<td>3.33</td>
<td>60.90</td>
<td>25.51</td>
</tr>
</tbody>
</table>

Table 1: Effect of treatments on disease severity of Fusarium oxysporum f. sp. lini during 2017-18.
The investigation was carried out to find out the effectiveness of bio-agents/plant products as seed dressers and as soil application in pot in comparison to fungicides (Carbendazim) against *Fusarium oxysporium* f.sp. *Lini* causing wilt in linseed. Treatment T₄ [seed treatment (5g/kg seed) + soil treatment with *Trichoderma harzianum* (10g/kg soil)] showed minimum per cent wilting with maximum disease control followed by treatment T₉ (5 g/kg seed) + soil treatments with *Trichoderma viride* (10 g/soil) (21.57 per cent) and T₁₂ [seed and soil treatment with carbendazim (0.2 per cent)] (25.0 per cent), respectively under pot culture condition. From this investigation, it is concluded that *Trichoderma harzianum*, when applied to both seed and soil enhanced disease control against *Fusarium oxysporium* f.sp. *Lini*.

Fig 1: Effect of different treatments application on disease severity
References