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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 T_4 [Seed (5g/kg seed) and soil treatment (10g/kg soil) with *Trichoderma harzianum*] followed by T_2 [(Seed (5g/kg seed) and soil treatment (10g/kg soil) with *Trichoderma viride*], (21.57 per cent) and T_{12} [(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 bio-industrial 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 (Bhatty and Rowland, 1990) [4]. 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 lac 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 linicola* (blight), *Fusarium* spp. (wilt), *Botrytis cinerea* (gray mould) and *Oidium lini* (powdery mildew), *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) (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 oxyporium* 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±2ºC 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 8ºC 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 fallowing 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-

Per cent disease control= $\frac{UT-T}{T}$×100

Where,
UT- Untreated
T- Treated

The details of the treatments were given as follows

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

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 T_4 [Seed (5g/kg seed) and soil treatment (10g/kg soil) with *Trichoderma harzianum*] followed by T_2 [(Seed (5g/kg seed) and soil treatment (10g/kg soil) with *Trichoderma viride*], (21.57 Per cent) and T_{12} [(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 T_6 Seed treatment + soil treatment with *Pseudomonas fluorescens* (10g/kg soil) (28.25) and T_8 Seed treatment + soil treatment with seed kernel extract of *Azadirachta indica* (10 Per cent w/v) (39.90 Percent), respectively. Treatments only seed treatments with either *Trichoderma* spp. or *Pseudomonas* spp. or with 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 T_1 [Seed treatment with *Trichoderma viride* (5g/kg seed)], T_5 [Seed treatment with *Pseudomonas fluorescens* (10g/kg seed)], T_7 [Seed treatment with seed kernel extract of *Azadirachta indica* (10 Per cent w/v)] and T_9 [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 (5t/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], Companion, Mancozeb and Ridomil [Metalaxyl] on the growth and development of *F. oxysporum* f.sp. *Lini* 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 oxysporium* f. sp. *lini* causing wilt in linseed in present study was found maximum which a new record is.

Table 1: Effect of treatments on disease severity of *Fusarium oxysporium* f. sp. *lini* during 2017-18.

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

T6	9.33	6.67	28.52	65.11
T7	9.33	3.00	68.33	16.42
T8	10.00	6.00	39.90	51.19
T9	9.67	2.33	75.74	7.36
T10	10.67	5.33	50.00	38.84
T11	10.67	5.00	49.96	38.89
T12	10.00	7.33	25.00	69.42
T13	9.33	1.67	81.76	-
G.M.	9.49	4.87	48.82	-
SEm±	0.66	0.44	4.68	-
CD at 5%	N.S.	1.28	13.66	-

Conclusion

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 T_4 [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 T_2 (5 g /kg seed) + soil treatments with *Trichoderma viride* (10 g/soil)) (21.57 per cent) and T_{12} [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

1. Allman MA, Pena MMm, Pang D. Supplementation with flaxseed oil versus sunflower seed oil in healthy young men consuming a low-fat diet: effects on platelet composition and function. Eur. J Clin. Nutr. 1995; 49:169-178.
2. Bhathena SJ, Ali AA, Mohamed AI, Hansen CT. and Velasquez MT. Differential effects of dietary flaxseed protein and soy protein on plasma triglyceride and uric acid levels in animal models. Journal of Nutritional Biochemistry. 2002; 13(11):684-689.
3. Bhatnagar K, Tak SK, Sharma RS, Majumdar VL, Meena RL. Management of cumin wilt caused by *Fusarium oxysporum* f. sp. *cumini* through chemical and biological agents. Indian Phytopath. 2013; 66(1):101-102.
4. Bhatty RS, Rowland GG. Measurement of alpha linolenic acid in the development of edible oil flax. J Am. Oil Chem. Soc. 1990; 67:364-367.
5. Choi YW, Hyde KD, Ho WH. Single spore isolation of fungi. Fungal Diversity. 1999; 3:29-38.
6. Diederichsen A, Richards K. Cultivated flax and the genus *Linum* L.: taxonomy and germplasm conservation. *In*: Flax, The genus Linum (*Eds, Muir AD, Westcott ND.*), Taylor & Francis Group, 2003, 22-54.
7. Genser MV, Morris DH. Introduction: History of the cultivation and uses of flaxseed. In: *Flax*- The Genus Linum, (Eds. Muir AD, Westcott ND.). Routledge, Taylor & Francis Group, London, 2003, 1-21.
8. Halligudi N. Pharmacological properties of Flax seeds: A Review. Hygeia. J D. Med. 2012; 4(2):70-77.
9. Hurteau MC. Unique new food products contain good omega fats. Journal of Food Science Education. 2004; 3(4):52-53.
10. Jhala AJ, Hall LM, Hall JC. Potential Hybridization of Flax with Weedy and Wild Relatives: An Avenue for Movement of Engineered Genes? Crop Sci. 2008; 48:825-840.
11. Johnston A, Booth C. Plant pathologists Pocket Book. Oxford and IBH Publishing Co, 1983, 439.
12. Kishor Ram, Jyoti Singh, Manjul Pandey. Management of wilt disease caused by *Fusarium oxysporum* f.sp. Lini in linseed through cultural practices. Annals of Plant Protection Science.1991; 16(2):245-247.
13. Mercer PC, Hardwick NV, Fitt BDL, Sweet JB. Status of diseases of linseed in the UK. Oilseeds Research Review. 1991; (OS3):76.
14. Mishra S, Verma P. Flaxseed- Bioactive compounds and health significance. IOSR Journal of Humanities and Social Science. 2013; 17(3):46-50.
15. Mukhopadhyay AN. Biological control of soil borne plant pathogens by *Trichoderma* spp. J Mycol. Pl. Pathol. 1987; 17:1-9.
16. Rekha Patil D, Naik MB, Ravi MK, Bhemanna MV, Amaresh YS. Evaluation of native isolates of *Trichoderma* spp. against *Fusarium oxysporum* f.sp. *ciceri* causing wilt in chick pea. J Pl Disease Sci. 2011; 6(2):163-16.
17. Sattar A, Hafiz A. Researches on plant diseases of Punjab. Sci. Mongr. Pak. Assoc. Adv. Sci. 1952; 1(3):158.
18. Singh N, Palat R, Narain U, Mathuria OP. Efficacy of bio-agent in management of linseed wilt. Annual of Plant Protection Sciences. 2008; 16(1):245-246.
19. Singh RS, Chaube HS. Selective agar media for the isolation of fungi. Indian Journal of Mycol. and PI. Pathology. 1970; 3:67-70.
20. Singh RS, Chaubey HS, Rai B. Selective agar media for the isolation of fungi. Indian Journal of Mycol. and PI. Pathology. 1996; 18:67-70.
21. Thind TS. Diseases of field crops and their management, 2005, 136.