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In vitro bioefficacy of bioagents against pathogenic mycoflora of linseed seeds

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Abstract
Linseed (*Linum usitatissimum* L.), the most grown oilseed crop is suffered by several diseases causing pathogenic organism, most of which are seed borne, causing significant quantitative and qualitative losses. Though, majority of seed borne fungi are being managed with fungicidal seed treatments, but due to their harmful effects, their use needs to be reduced. Therefore, present research work entitled "*in vitro* bioefficacy of bioagents against pathogenic mycoflora of linseed seeds" was conducted to asses bioefficacy of bioagents against three major pathogenic seedborne fungi *viz*., *Fusarium oxysporum* f. sp. *lini*, *Alternaria lini*, and *Curvularia lunata*, by applying Dual culture technique. The results revealed that all of the seven test bioagents significantly inhibited mycelial growth of *Fusarium oxysporum* f. sp. *lini*, *Alternaria lini*, and *Curvularia lunata* over untreated control. However, in case of *Fusarium oxysporum* f. sp. *lini*, the most effective bioagent was *T. harzianum* (86.85%) followed by *T. hamatum* (85.48%), *A. niger* (83.15%), *T. koningii* (81.60%) while *P. fluorescens* (52.04%) was least effective. Whereas, for *Alternaria lini*, the most effective bioagent was *T. hamatum* (91.60%) followed by *A. niger* (85.77%), *T. harzianum* (82.44%), *T. asperellum* (80.52%) which caused highest mycelial growth inhibition while least inhibition was caused by *P. fluorescens* (57.60%). Whereas, for *Curvularia lunata* bioagent *T. harzianum* (88.15%) followed by *T. hamatum* (85.07%), *T. lignorum* (82.60%) caused highest mycelial growth inhibition while least inhibition was with *P. fluorescens* (54.26%).

Keywords: Mycoflora of linseed, bioagent, *in vitro*

Introduction
Linseed (*Linum usitatissimum* L.) (2n=30) is the sixth largest oilseed crop of the world and is cultivated in more than 50 countries with a production of 27.94 lakh tonnes. In India linseed is known as 'Alsi' in Hindi and 'Javas' in Marathi. Linseed is an important industrial and edible oil and fiber producing crop. It is also used as medicinal plant as it is rich in oil and protein which makes it useful as a dietary supplement (Jhala and Linda, 2010) [6]. Every part of the linseed plant is utilized commercially, either directly or after processing. On a very small scale, the seed is directly used for edible purposes. About 20% of the total oil produced is used at farmers level and the rest 80% oil goes to industries for the manufacturing of paints, oil cloth, varnish, pad-ink, printed ink, linoleum etc. The oil-cake is a good feed for milch cattle and poultries (Singh *et al*., 2018) [10]. In linseed a variety of oil, proteins and carbohydrates are present in the seed, which makes the seed liable to attack by a range of seed-borne pathogens. The predominant fungi associated with linseed seeds causing seed and seedling rot are *Alternaria alternata*, *A. linicola*, *Aspergillus flavus*, *A. niger*, *Colletotrichum linicola*, *Curvularia lunata*, *Fusarium moniliforme*, *F. oxysporum* f. sp. *lini*, *Fusarium pallidoroseum*, *Rhizoctonia bataticola*, *R. solani*, *Phoma exiguea*, var. *linicola* are predominant (Kumar *et al*., 1997) [7]. They prove hazardous for seeds as they cause pre and post emergence mortality and diseases at various stages of crop growth, finally resulting in the reduction of yield and deterioration of seed and fiber quality (Singh *et al*., 2017) [9]. Therefore, present study on *in vitro* bioefficacy of bioagents against pathogenic mycoflora of linseed seeds was planned and conducted at the Department of Plant pathology, College of Agriculture, Latur.

Material and Methods
Isolation, identification and pathogenicity of seed borne fungi
Linseed seeds of varieties NL-97, NL-260, LSL-93 and RLC-4 were collected from Oilseeds Research Station, Latur. These seeds were plated aseptically onto autoclaved and cooled Potato Dextrose Agar medium in separate sterile glass petri plates and incubated at room temperature. After a week of incubation, various fungal colonies developed on PDA plates were observed under stereomicroscope, distinguished on the basis of colony colour and growth habit, further re-isolated on fresh PDA plates and incubated at room temperature. Based on morpho-cultural characteristics and microscopic observations, the most predominant fungi

identified were *Fusarium oxysporum* f. sp. *lini*, *Alternaria lini* and *Curvularia lunata*. The pathogenicity of these three fungi was proved by seed inoculation and standard blotter paper techniques, by using the surface sterilized (2% Sodium hypochlorite solution) seeds of linseed varieties NL-97, NL-260, LSL-93 and RLC-4.

In vitro evaluation of bioagents

Fungal and bacterial biocontrol agents were evaluated *in vitro* against *F. oxysporum* f. sp. *lini*, *A. lini* and *C. lunata* the fungi pathogenic to linseed, applying dual culture technique (Dennis and Webster, 1971). Seven days old cultures of the test bioagents and the pathogens were used for the study. Discs of 5 mm diameter of culture growth of the *F. oxysporum* f. sp. *lini*, *A. lini* and *C. lunata*, the test bioagents were cut out with sterilized cork borer. Then two culture discs, one each of the test fungus and test bioagent were placed at equidistance and exactly opposite to each other on autoclaved and cooled PDA medium in petri plates and incubated at 26±2 ^{0}C. Test pathogens were assessed separately. PDA plates inoculated separately with culture disc of *F. oxysporum* f. sp. *lini*, *A. lini* and *C. lunata* alone were maintained as untreated control.

Experimental details

Design : CRD (Completely Randomized Design)
Replications : Three
Treatments : Eight

Tr. No.	Treatments	Tr. No.	Treatments
T_1	*Trichoderma asperllum*	T_5	*T. lignorum*
T_2	*T. harzianum*	T_6	*Aspergillus niger*
T_3	*T. hamatum*	T_7	*Pseudomonas fluorescens*
T_4	*T. koningii*	T_8	Control (Untreated)

Colony diameter of fungal pathogen on medium was recorded and per cent inhibition of the test pathogen with the test bioagents, over untreated control was calculated by applying following formula (Arora and Upadhyay, 1978) [1].

$$\text{Per cent growth inhibition} = \frac{\text{Colony growth in control plate} - \text{Colony growth in intersecting plate}}{\text{Colony growth in control plate}} \times 100$$

Results and Discussion

A total seven bioagents (six fungal and one bacterial bioagent) were evaluated *in vitro* against three major seed-borne fungi *viz*., *F. oxysporum* f. sp. *lini*, *Alternaria lini* and *Curvularia lunata* of linseed by dual culture technique and obtained results on colony diameter (mm) and per cent inhibition of mycelial growth of these three test fungi are presented in Table 1 and Plate I A, B and C.

For *F. oxysporum* f. sp. *lini* (Table 1, Plate I A and Fig. 1 A) significantly highest mycelial growth inhibition was with *T. harzianum* (86.85%) followed by *T. hamatum* (85.48%), *A. niger* (83.15%), *T. koningii* (81.60%), *T. lignorum* (75.44%), *T. asperellum* (71.85%) and *P. fluorescens* (52.04%), respectively.

For *A. lini* (Table 1, Plate I B and Fig. 1 B) significantly highest mycelial growth inhibition was with *T. hamatum* (91.60%) followed by *A. niger* (85.77%), *T. harzianum* (82.44%), *T. asperellum* (80.52%), *T. koningii* (77.04%), *T. lignorum* (74.48%) and *P. fluorescens* (57.60%), respectively.

For *C. lunata* (Table 1, Plate I C and Fig. 1 C) significantly highest mycelial growth inhibition was with *T. harzianum* (88.15%) followed by *T. hamatum* (85.07%), *T. lignorum* (82.60%), *A. niger* (79.30%), *T. asperellum* (75.33%), *T. koningii* (71.48%) and *P. fluorescens* (54.26%), respectively.

Plate: A **Plate: B** **Plate: C**

Plate I: A, B and C: *In vitro* efficacy of several bioagents against *F. oxysporum* f. sp. *lini*, *A. lini*, *C. lunata* of linseed seeds

Table 1: *In vitro* efficacy of several bioagents against *F. oxysporum* f. sp. *lini*, *Alternaria lini* and *Curvularia lunata* of linseed seeds

Sr. No	Treatments	*F. oxysporum* f. sp. *lini*		*Alternaria lini*		*Curvularia lunata*	
		Colony diameter (mm)	Mycelial growth Inhibition (%)	Colony diameter (mm)	Mycelial growth Inhibition (%)	Colony diameter (mm)	Mycelial growth Inhibition (%)
T_1	*T. asperellum*	25.33	71.85 (57.95)	17.53	80.52 (63.80)	22.20	75.33 (60.21)
T_2	*T. harzianum*	11.83	86.85 (68.73)	15.80	82.44 (65.22)	10.66	88.15 (69.86)
T_3	*T. hamatum*	13.06	85.48 (67.60)	7.56	91.60 (73.15)	13.43	85.07 (67.26)
T_4	*T. koningii*	16.56	81.60 (64.59)	20.66	77.04 (61.36)	25.66	71.48 (57.72)
T_5	*T. lignorum*	22.10	75.44 (60.29)	22.96	74.48 (59.65)	15.66	82.60 (65.34)
T_6	*Aspergillus niger*	15.16	83.15 (65.76)	12.80	85.77 (67.83)	18.63	79.30 (62.93)
T_7	*Pseudomonas fluorescens*	43.16	52.04 (46.16)	38.16	57.60 (49.37)	41.16	54.26 (47.44)
T_8	Control (Untreated)	90.00	0.00 (00.00)	90.00	0.00 (00.00)	90.00	0.00 (00.00)
SE±		0.47	0.52	0.53	0.57	0.63	0.70
CD (P=s0.01%)		1.38	1.53	1.56	1.68	1.85	2.05

Figures in parentheses are Arcsine transformed values

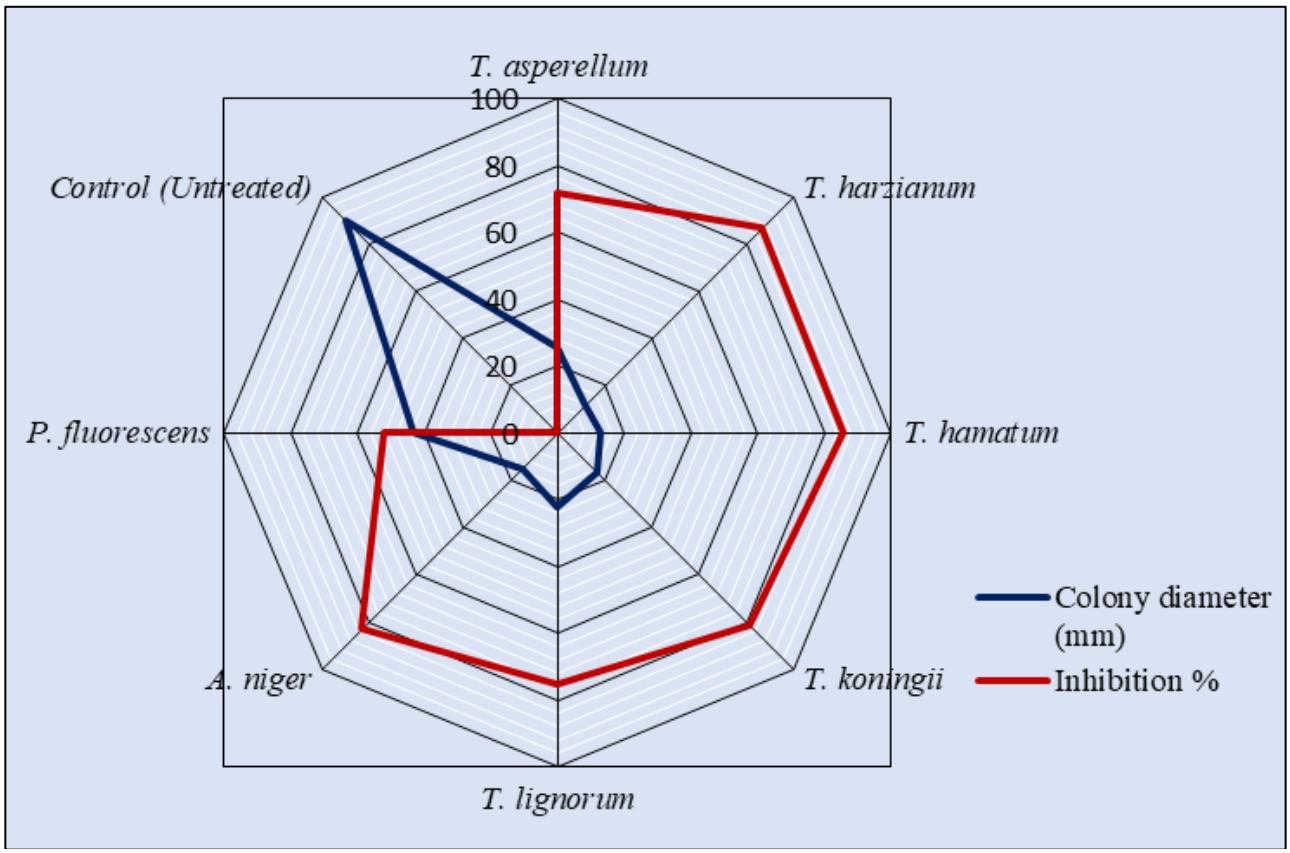

Fig 1A: *In vitro* efficacy of several bioagents against *F. oxysporum* f. sp. *lini* of linseed seeds

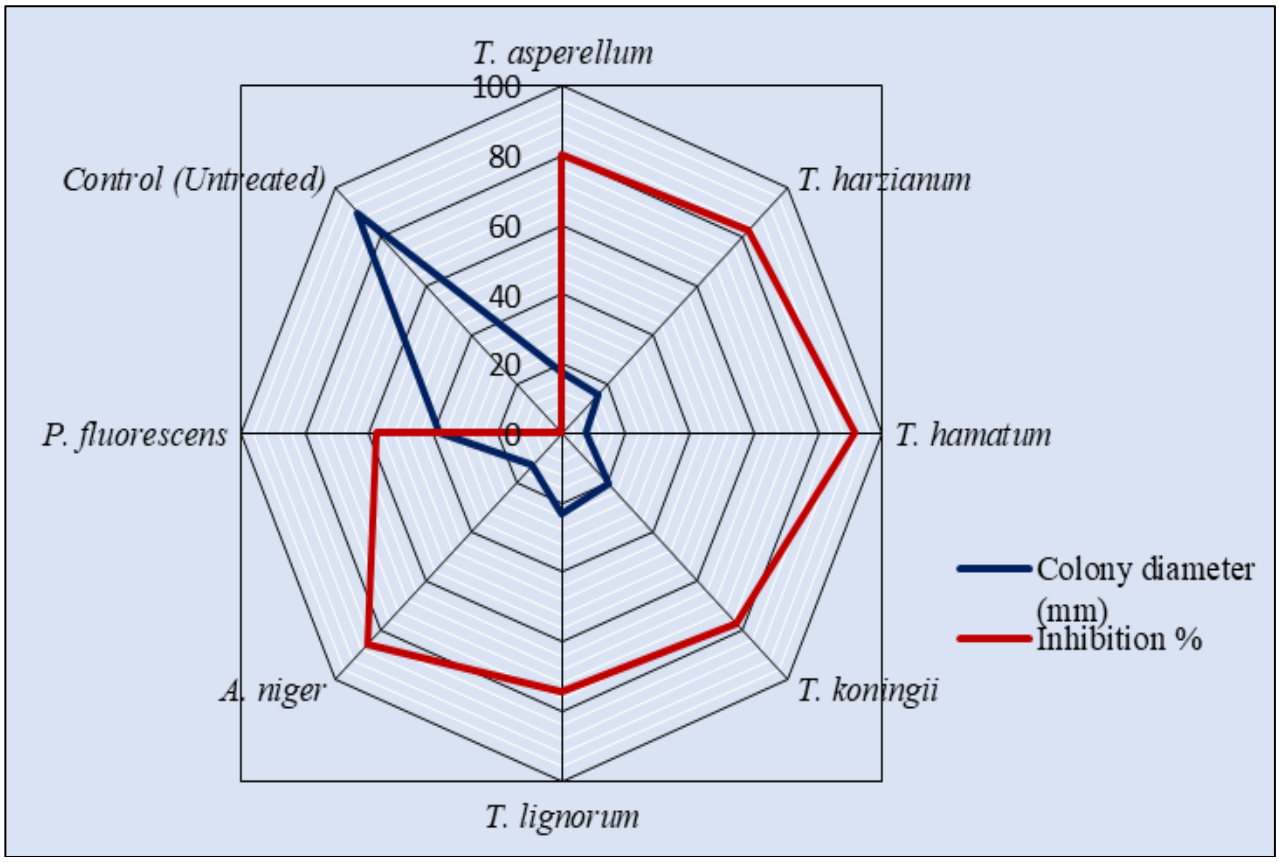

Fig 1B: *In vitro* efficacy of several bioagents against *A. lini* of linseed seeds

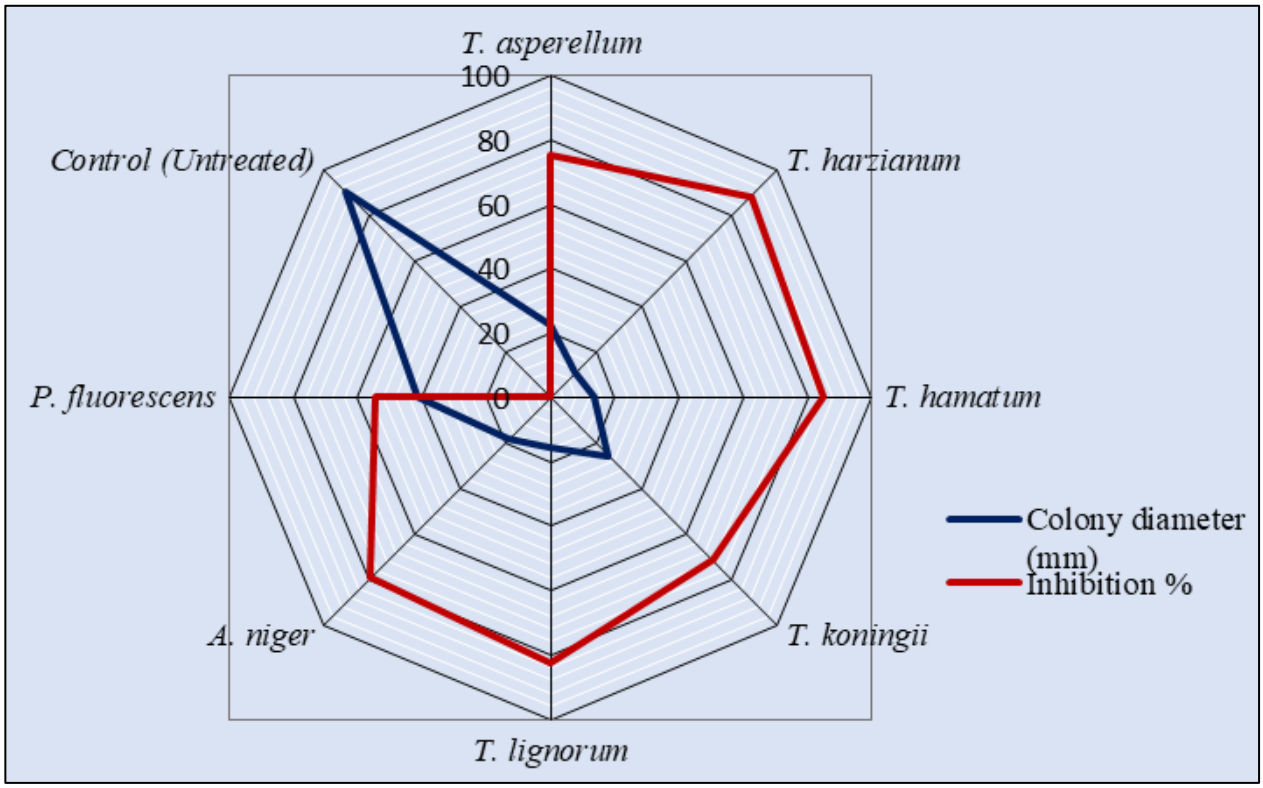

Fig 1C: *In vitro* efficacy of several bioagents against *C. lunata* of linseed seeds

Results of the present study are in agreement with previous findings of the earlier workers (Ghosh *et al*. 2002; Meena, 2005; Bhoye *et al*. 2011; Savitha *et al*. 2012; Toua *et al*. 2013; Ashwini *et al*. 2014 and Dhawan *et al*. 2019) [5, 8, 2, 11, 2, 4]. Ghosh *et al*. (2002) [5] reported that, *T. viride* and *T. harzianum* effectively inhibited the growth of *Alternaria alternata in vitro*. Meena (2005) [8] reported that, local isolates of *T. viride* and *T. harzianum* was highly effective in reducing total seed mycoflora of linseed with enhanced seed germination. Bhoye *et al*. (2011) [3] recorded maximum inhibition in mycelial growth with 10% concentration of culture filtrate. Among four species of *Trichoderma, T. harzianum* found the most effective against *A. lini*.

Conclusion

Biological control is an effective, ecofriendly and alternative approach for disease control. The results revealed that among six fungal (five *Trichoderma* spp. and *Aspergillus niger*) and one bacterial (*P. fluorescens*) bioagent evaluated *in vitro* against *viz*., *Fusarium oxysporum* f. sp. *lini*, *Alternaria lini* and *Curvularia lunata* of linseed seeds, *Trichoderma harzianum* and *Trichoderma hamatum* were most effective against tested pathogens, while *Pseudomonas fluorescens* was least effective.

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