Research Paper :

Antifungal activity of *Trichoderma* spp. against *Alternaria lini* responsible for bud blight of linseed

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SUMMARY

Linseed blight caused by *Alternaria lini* is an economically important and major disease of linseed. Isolates of *A. lini* were collected from different linseed growing areas of Vidarbha and were tested by using culture filtrates of four species of *Trichoderma* on the basis of mycelial growth, spore germination and sporulation behaviour. The ten isolates showed less variation in per cent inhibition of mycelial growth, spore germination and sporulation behaviour. The maximum inhibition in mycelial growth was observed with 10% concentration of culture filtrate. Among four species of *Trichoderma, T. hamatum* inhibited 38.46% radial mycelial growth whereas *T. viride* showed 78.38% and 82.20% inhibition sporulation intensity and spore germination, respectively.

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Alternaria blight caused by *Alternaria lini* Dey is a serious disease of linseed causing losses to the extent of 28-60 per cent. The disease appears from seedling stage to seed setting stage (Chaudhary and Srivastava, 1975) and the losses are found with the increased disease intensity where it appears on bud forming stage as a bud blight. In Maharashtra, the disease appears almost every year on the linseed crop grown, where 10 to 25 per cent incidence of linseed blight was recorded (Anonymous, 2007). Being a devastating disease of linseed, Alternaria blight causes heavy losses and oil per cent is also reduced which is having commercial value (Kolte and Fitt, 1997). Due to the conventional and continuous use of fungicides, the resistance and residue problem were developing in the pathogen. Looking at this problem, it is necessary to find out new areas for strengthening the management of this pathogen like use of bioagents. Therefore, the present study was taken for eco-friendly management of the disease.

MATERIALS AND METHODS

The present investigation was carried out *in vitro* condition of Department of Plant Pathology, PGI, Dr. PDKV, Akola during 2007-2008. The experiment was done by using culture filtrates of 4 bioagents *viz.*, *Trichoderma hamatum, T. harzianum, T. virens and T. viride* against 10 isolates of *A. lini*.

Preparation of culture filtrates:

Bioagents were grown in 150 ml Potato dextrose broth in 250ml conical flask for 20 days. The broth of bioagents culture containing mycelium and spores were filtered through Whatman filter paper No.4, were centrifuged at 5000 rpm for 10 min. to collect cell free supernatant. Considering the supernatant as 100% concentration was used in poisoned food technique at 10% concentration. (Mane and Pal, 2008).

Effect of radial mycelial growth:

Ten isolates of the *A. lini* were used to study the antifungal activity of bioagents by poisoned food technique. The PDA added with 10% of culture filtrates separately poured in Petri plates and after solidification the plates

were inoculated with 6mm disc of individual *A. lini* isolates at the centre maintained on PDA medium and incubated at 28 ± 2^{0}C for 7 days. Observations on diameter of fungal growth were measured. The per cent inhibition was calculated with the help of mean colony diameter by using the following formula given by Vincent, 1927.

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

where,
I = Per cent growth inhibition
C = Growth of fungus in control
T = Growth of fungus in treatment

Effect of culture filtrates on sporulation behaviour of *A. lini*:

To study the effect of culture filtrates of bioagents, on sporulation intensity of the pathogen, three discs of 6 mm size one from centre, one from middle and one from periphery were removed from the same plates used for per cent growth inhibition. The individual disc was mixed in 10 ml of sterile distilled water and spore load was counted by heamocytometer and per cent inhibition of sporulation was calculated by using the following formula:

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

where,
I = Per cent spore inhibition
C = Sporulation in control
T = Sporulation in treatments

Effect of culture filtrates on spore germination of *A. lini:*

The spores of *A. lini*, grown on PDA for 10 days were removed with the help of a sterilized needle and brush in sterilized water. One drop each of spore suspension and double strength solutions (20%) of different non-pathogenic isolates were put separately into cavity slides under aseptic conditions. Three replications of each treatment were maintained. The slides were placed in Petri plates, lined with moist blotter paper to serve as moist chambers. For check, the spores were added to sterilized water. Per cent germination was recorded after 36 hrs. of incubation at 28 ± 2^{0}C. The per cent inhibition of spore germination was calculated by using the following formula (Bhatiya and Awasthi, 2007).

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

where,
I = Per cent inhibition of spore germination
C = Spore germination in control
T = Spore germination in treatments

Statistical analysis:

Statistical analysis was done by applying Completely Randomized Design (CRD) for *in vitro* studies (Gomez and Gomez, 1976).

RESULTS AND DISCUSSION

The effect of culture filtrate of *Trichoderma viride, T. harzianum, T. hamatum and T. virens* on radial mycelial growth and sporulation intensity of 10 pathogenic *Alternaria lini* isolates were evaluated through poisoned food techniques and their effect on spore germination was tested with 10 isolates individually. In this all the four species of *Trichoderma* have shown fungistatic as well as antisporulant properties against *Alternaria lini*.

Data presented in Table 1, effect of bioagents on the radial mycelial growth of 10 isolates of *A. lini* ALP-1 to ALP-10 revealed that *T. hamatum* was found effective against ALP-1 showing 49.83mm radial mycelial growth with 34.29 per cent inhibition followed by *T. harzianum* 32.97 per cent growth inhibition and *T. virens* 25.93 per cent inhibition. Similarly, *T. hamatum* recorded 26.13 per cent and 36.67 per cent inhibition against ALP-2 and ALP-5, respectively followed by *T. harzianum* with 24.55 per cent and this treatment was found to be at par with other treatments except control.

While studying ALP-4, ALP-6 and ALP-9 it was found that *T. harzianum* was best in controlling the pathogens with 40.67, 43.00 and 39.17mm radial mycelial growth and 45.78, 43.79 and 45.02 per cent inhibition, respectively. This treatment was found to be at par with *T. virens*, with 43.56 per cent inhibition against ALP-4 while against ALP-9 *T. harzianum* (45.02 per cent inhibition) was followed by *T. hamatum* (44.08 per cent inhibition) in reducing the mycelial growth. The radial mycelial growth was found to be less in treatment *T. virens* against ALP-7 and ALP-8 isolate up to 43.17 and 47.17 mm, respectively with 35.76 and 36.63 per cent inhibition, respectively. In case of ALP-10, *T. hamatum* and *T. virens* was found most superior in reducing the radial mycelial growth with 30.11 per cent.

The data presented in Table 2, revealed that all treatments were significantly superior over control in inhibition of spore intensity. Among the treatments, *T. viride* 10 per cent was found effective giving 1.21x10^5 spores / cm^2 with 78.31 per cent inhibition against ALP-1, which was at par with all the bioagents. Similar trend

Table 1 : Effect of plant extract on mycelial growth of isolates of *Alternaria lini* (*in vitro*)

Treatments	Isolates																			
	ALP 1		ALP 2		ALP 3		ALP 4		ALP 5		ALP 6		ALP 7		ALP 8		ALP 9		ALP 10	
	Av. (mm)	PI	Av. (mm)	PI	Av. (mm)	PI	Av. (mm)	PI	Av. (mm)	PI	Av. (mm)	PI	Av. (mm)	PI	Av. (mm)	PI	Av. (mm)	PI	Av. (mm)	PI
Allium sativum	23	63.3	25.5	63.17	23.17	57.8	24.5	57.44	16.17	70.26	24.17	59.65	15.17	75.99	20.5	59.19	17.5	64.55	17.17	72.95
Annona squamosa	27.83	55.59	29.5	57.39	26.17	52.34	25.33	55.99	20.83	61.68	26.17	56.32	20.83	67.02	28.17	43.93	26.33	46.66	26.67	57.98
Azadirachta indica	22.17	64.63	31	55.22	29.67	45.96	25.5	55.7	31.3 3	42.37	22.83	61.88	21.17	66.49	26.83	46.58	30.17	38.89	32.17	49.32
Datura metal	24.5	60.9	24.83	64.13	24.5	55.37	25.17	56.28	15.5	71.49	21.17	64.66	21.67	65.7	15.5	69.14	14.83	69.95	18.5	70.85
Eucalyptus spp.	38.17	39.1	40.67	41.26	30.67	44.14	30.5	47.02	31.17	42.67	15.17	74.68	30.17	52.24	19.67	60.85	40.5	17.96	40.83	35.66
Ipomea cornea	34.5	44.95	42.83	38.13	25.17	54.16	34.17	40.65	20.17	62.91	16.83	71.9	20.83	67.02	37.83	24.68	31.83	35.52	35.5	44.07
Lantana camara	41.5	33.78	39	43.67	34.33	37.46	34.67	39.78	31.83	41.45	30.83	48.53	32	49.34	32.83	34.64	44.83	9.18	39.17	38.29
Parthenium hysterophorus	17.5	72.07	40.5	41.5	31.17	43.23	32.17	44.12	29.83	45.13	13.83	76.91	24.83	60.69	22.5	55.21	25.5	48.35	53.83	15.18
Pongamia pinnata	26.83	57.18	41	40.78	25.5	53.55	33.5	41.81	24.5	54.94	24.17	59.65	26.33	58.31	25.17	49.9	26.17	47	37.5	40.91
Tridex procumbans	13.67	78.19	38.5	44.39	16.17	70.55	15.83	72.5	12.17	77.62	16.83	71.9	15.17	75.99	12.83	74.45	18.5	62.53	19.33	69.54
Control	62.67		69.23		54.9		57.57		54.37		59.9		63.17		50.23		49.37		63.47	
F test	Sig.		Sig.		Sig.		Sig.		Sig.		Sig.		Sig.		Sig.		Sig.		Sig.	
SE (±)	0.4		0.42		1.87		4.44		0.44		0.43		0.76		0.71		0.46		0.47	
CD (P = 0.01)	2.04		2.15		9.39		2.22		0.21		2.19		3.85		3.56		2.31		2.39	

Table 2 : Effect of plant extract on sporulation intensity of isolates of *Alternaria lini* (*in vitro*)

Treatments	Isolates																			
	ALP 1		ALP 2		ALP 3		ALP 4		ALP 5		ALP 6		ALP 7		ALP 8		ALP 9		ALP 10	
	Av. (x105/ cm^2)	PI	Av. (x105/c m^2)	PI	Av. (x105/c m^2)	PI	Av. (x105 /cm^2)	PI	Av. (x105 /cm^2)	PI	Av. (x 105/c m^2)	PI	Av. (x105 /cm^2)	PI	Av. (x105/ cm^2)	PI	Av. (x105 /cm^2)	PI	Av. (x105 /cm^2)	PI
Allium sativum	2.21	62.31	1.06	82.78	1.15	80.79	1.12	81	1.15	78.45	1.3	74.86	1.27	74.1	1.24	79.1	1.18	73.33	1.18	80
Annona squamosa	2.24	61.81	1.24	79.9	1.24	79.31	1.15	80.5	0.97	81.77	0.94	81.71	1.24	74.7	1.33	77.61	1.21	72.67	1.33	77.5
Azadirachta indica	2.24	61.81	1.21	80.38	1.21	79.8	1.18	80	1.09	79.56	1.12	78.29	1.27	74.1	1.24	79.1	1.15	74	1.09	81.5
Datura metal	2.18	62.81	1.24	79.9	1.03	82.76	1.15	80.5	1.3	75.69		78.29	1.24	74.7	1.33	77.61	1.12	74.67	1.24	79
Eucalyptus spp.	1.97	66.33	1.3	78.95	1.41	76.35	1.18	80	1.09	79.56	1.33	74.29	1.03	78.92	1.15	80.6	1	77.33	1.09	81.5
Ipomea cornea	1.33	77.39	1.21	80.38	1.06	82.27	1.15	80.5	1.06	80.11	1.15	77.71	1.21	75.3	1.06	82.09	1.18	73.33	1.18	80
Lantana camara	1.77	69.85	1.24	79.9	1.21	79.8	1.21	79.5	1.27	76.24	1.27	75.43	1.33	72.89	1.36	77.11	1.21	72.67	1.27	78.5
Parthenium hysterophorus	2	65.83	1.24	79.9	1.3	78.33	1.3	78	1.27	76.24	1.27	75.43	1.27	74.1	1.27	78.61	1.24	72	1.33	77.5
Pongamia pinnata	1.97	66.33	1.18	80.86	1.12	81.28	1.15	80.5	1.21	77.35	1.18	77.14	1.15	76.51	1.21	79.6	1.21	72.67	1.03	82.5
Tridex procumbans	2.09	64.32	1.27	79.43	1.24	79.31	1.27	78.5	1.3	75.69	1.24	76	1.12	77.11	1.15	80.6	1.24	72	0.97	83.5
Control	5.86		6.16		5.98		5.89		5.33		5.16		4.89		5.92		4.42		5.89	
F test	Sig.		Sig.		Sig.		Sig.		Sig.		Sig.		Sig.		Sig.		Sig.		Sig.	
S.E. (±)	0.08		0.07		0.08		0.07		0.08		0.07		0.09		0.09		0.07		0.11	
C.D. (P = 0.01)	0.42		0.37		0.4		0.38		0.44		0.35		0.46		0.48		0.37		0.58	

was also observed in the ALP-2, ALP-4, ALP-8, ALP-5 and ALP-10 isolates against *T. viride* 10 % superior with 1.03, 1.03, 1.18, 1.00 and 1.00 x10^5 spores / cm^2 with 79.77, 81.87, 80.77, 82.01 and 84.40 per cent inhibition, respectively over the respective isolates and control.

While studying the isolates ALP-3, ALP-6 and ALP-9 it was found that treatment *T. harzianum* (10 %) was found to be best showing minimum *i.e.* 1.15x10^5, 1.06x10^5 and 1.06x10^5 spores / cm^2 with 79.26, 79.07 and 67.57 per cent spore inhibition, respectively. This treatment was found at par with all other treatments. In case of isolate ALP-7, *T. virens* (10 %) was found to be best effective in reducing the sporulation intensity showing 1.03x10^5 spores/cm^2 with 77.17 per cent inhibition.

In spore germination study (Table 3), it was found that *T. viride* @10 % was the most effective in reducing spore germination 87.15 per cent showing 12.00 per cent spore germination followed by *T. hamatum* having 15.50 per cent spore germination.

Similarly, *T. viride* was found to be best among all the treatments against isolates ALP-2, ALP-3, ALP-5, ALP-7 ALP-8 and ALP-10 over control showing 13.20%, 11.20%, 15.20%, 17.00%, 14.40% and 14.60% spore germination with 83.70%, 86.16%, 81.23%, 77.72%,

Table 3 : Effect of fungal extracts on spore germination of *Bipolaris sorokiniana* (In vitro)

Treatments	ALP-2		ALP-3		ALP-7		ALP-5		ALP-6		ALP-7		ALP-8		ALP-9		ALP-10	
	Av. (%)	PI	Av. (%)	PI	Av. (%)	PI	Av. (%)	PI	Av. (%)	PI	Av. (%)	PI	Av. (%)	PI	Av. (%)	PI	Av. (%)	PI
Allium sativum	71.0 (57.55)	80.23	19.0 (25.22)	78.75	18.90 (25.71)	78.15	21.90 (25.02)	78.69	23.20 (28.70)	73.35	13.50 (21.56)	84.79	20.30 (26.78)	76.8	17.60 (22.16)	82.68	17.10 (22.06)	82.93
Annona squamosa	73.30 (21.39)	84.89	15.50 (23.03)	82.98	16.20 (23.73)	81.21	15.10 (22.81)	82.02	17.60 (22.76)	83.24	17.10 (22.30)	83.17	15.10 (23.13)	82.7	13.00 (21.13)	84.58	15.80 (23.12)	80.87
Azadirachta indica	20.80 (26.80)	76.36	15.90 (23.91)	82.31	17.00 (25.18)	80.82	20.30 (26.78)	75.83	18.10 (25.10)	78.81	16.00 (23.56)	81.26	7.00 (21.91)	91	15.00 (22.76)	82.2	17.10 (24.13)	79.3
Datura metal	73.10 (21.72)	84.73	17.30 (24.58)	80.76	18.10 (25.10)	78.73	21.20 (21.72)	77.76	20.10 (25.85)	76.58	18.70 (25.52)	78.1	15.50 (23.19)	82.29	20.50 (26.92)	76.58	22.90 (28.59)	72.28
Eucalyptus sp.	15.10 (22.87)	82.84	16.40 (23.89)	81.76	17.10 (22.06)	83.1	15.10 (22.81)	82.02	12.60 (20.15)	85.53	13.00 (21.13)	84.78	13.00 (21.13)	85.1	17.00 (22.30)	80.92	12.80 (20.96)	84.5
Ipomea cornea	71.50 (24.80)	80	17.50 (24.13)	80.53	15.80 (23.73)	81.73	19.10 (25.92)	77.26	16.60 (24.04)	80.94	20.50 (26.92)	76	22.00 (27.97)	71.85	17.50 (24.13)	79.24	20.80 (27.13)	71.82
Lantana camara	21.50 (27.53)	75.51	25.30 (30.20)	71.86	22.00 (27.97)	71.51	18.80 (25.70)	77.52	19.10 (25.33)	83.71	18.30 (25.33)	78.51	16.50 (23.97)	81.7	19.20 (25.99)	77.22	17.20 (24.50)	79.18
Parthenium hysterophorus	21.50 (27.53)	75.51	18.20 (25.25)	79.76	18.50 (25.78)	78.51	15.60 (23.26)	81.73	19.10 (25.18)	77.73	18.10 (25.18)	78.81	16.50 (23.97)	81.7	15.30 (23.03)	81.85	13.70 (21.72)	83.71
Pongamia pinnata	19.10 (25.53)	77.95	18.50 (25.55)	79.2	21.20 (27.12)	75.19	18.10 (25.70)	78.1	21.10 (27.56)	75.73	19.20 (25.99)	77.52	19.10 (26.13)	77.83	21.20 (27.12)	77.85	20.70 (27.05)	77.94
Tridex procumbens	15.30 (23.81)	81.18	22.80 (28.52)	71.64	15.30 (23.03)	82.31	18.30 (25.33)	78.21	15.90 (23.79)	81.75	17.10 (22.55)	82.19	15.20 (22.95)	82.63	16.10 (23.89)	80.55	17.60 (22.76)	82.32
Control	88.00 (69.73)		89.50 (71.71)		85.50 (67.77)		87.00 (68.73)		87.10 (68.81)		85.10 (67.57)		87.50 (69.30)		87.30 (66.66)		82.60 (65.35)	
C.D. (P = 0.01)	2.46	Sig.	2.56	Sig.	2.65	Sig.	2.58	Sig.	2.75	Sig.	3.09	Sig.	2.56	Sig.	2.71	Sig.	3.15	Sig.
S.E.(±)	0.8		0.87		0.58		0.87		0.94		0.67		0.53		0.9		0.63	
C.D. (P = 0.01)	2.46		2.56		2.65		2.58		2.75		3.09		2.56		2.71		3.15	

82.84% and 80.27 per cent spore inhibition, respectively. However, *T. virens* was found most effective in reducing spore germination of isolate ALP-6 *i.e.* 12.60 per cent with 84.84 per cent inhibition followed by *T. viride* with 16.40 per cent spore germination. In case of isolate ALP-9, *T. harzianum* was found superior to reduce the spore intensity up to 15.50 per cent with 81.39 per cent inhibition.

To select the most effective treatment to be taken forward for pot culture study, the treatments were compared for their average performance. The data presented in Table 4 revealed that *T. hamatum* had the maximum fungistatic properties with an average 38.46 % inhibition of mycelial growth, where as, *T. viride* had maximum antisporulant activity with 78.38% inhibition of sporulation intensity and 82.20% inhibition of spore germination. Although, there was not much variation observed among the bioagents for antisporulant properties, enough variation present in fungistatic potential was observed due to *T. harzianum* @ 10%, was the most superior among all the bioagents (Table 1 to 3). Therefore, *T. harzianum* @ 10 per cent was selected for evaluation under pot culture condition.

Table 4 : Performance of bioagents on radial mycelial growth, sporulation intensity and spore germination of *Alternaria lini* (average of ten isolates)

Treatments	% inhibition of radial mycelial growth	% inhibition of sporulation intensity	% inhibition of spore germination
Trichoderma viride	22.96	78.38	82.20
T. harzianum	37.15	75.84	76.60
T. hamatum	38.46	74.42	79.01
T. virens	33.87	75.24	79.13
Control	00.00	00.00	00.00

In the current study four species of *Trichoderma i.e. T. harzianum, T. viride, T. hamatum* and *T. virens* had shown antifungal properties against *A. lini.* This finding indicate that *Trichoderma* spp. possess broad spectrum antifungal property against *Alternaria*. Among these *T. harzianum* was found the most effective against *A. lini*. The species of Trichoderma were varying their in performance to inhibite the myceilium growth, of *A. lini* Similar result were reported by Gulhane *et al.* (2005) against *Pythium,* Zade *et al.* (2005) has also reported the similar results against *Puccinia arachidis*. In the spore germination study the culture filtrates of bioagents had antagonistic properties against *A. lini*. Similar results were obtained by Mane and Pal (2008) for *T. hamatum* and *T. harizianum* along with few more bioagents against *Fusarium oxysporum* f.sp. *ciceri.* In the sporulation study the spore inhibition of A. lini due to culture filtrates of bioagents was observed. Similar results were obtained by Kelemu and Badel (1994) while studying pathogenic culture filtrates of *Bacillus subtilis* against some genera of pathogenic fungi such as *Colletotrichum gloeospoiriodes* Hijwegen. (1989) had obtained *Sphaerotheca fuliginea* due to the effect of culture filtrates of seventeen different antagonistic fungal species like *Calcarisoprium arbuscula* on sporulation.

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