RESEARCH PAPER

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Evaluation of culture filtrates of non-pathogenic isolates on mycelial growth and sporulation on pathogenic isolates of *Alternaria lini*

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Blight of linseed caused by *Alternaria lini* is most important disease in linseed grown areas of Vidarbha. From 22 different isolates of *A. lini* collected from the AICRP (Linseed) Nagpur, 10 were found pathogenic and 12 were non pathogenic. The effects of these 12 non pathogenic isolates were evaluated by poisoned food technique against the 10 pathogenic isolates on the basis of radial mycelial growth, sporulation inhibition and spore germination by hanging drop method. From the study it was observed that the non pathogenic isolate ANP-4 had shown maximum fungistatic potential with an average of 77.74 per cent mycelial growth inhibition. However, antisporulant properties of all the treatments showed significant results over control but among that the treatment ANP-6 had given maximum reduction in sporulation (77.64 per cent) whereas maximum spore reduction (79.74 per cent) was observed in ANP-4 treatment.

Key words : linseed , Alternaria blight, Biological management, *Alternaria lini*

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INTRODUCTION

Alternaria blight of linseed caused by *Alternaria lini* is a serious disease causing losses to the extent of 28-60 per cent. The disease appear from seedling stage to seed setting stage (Chaudhary and Srivastava,1975) and losses appears on bud forming stage as a bud blight. Fifteen to thirty per cent incidence of linseed blight was also recorded in the experimental fields of Agriculture College, Nagpur and on farmers fields (Annonymous, 2007). In Maharashtra, the disease appear almost every year on the linseed crop grown in the tune of 10 to 25 per cent (Anonymous, 2007). 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 non pathogenic isolates of *Alternaria* against predominant pathogenic isolates of *Alternaria*. Therefore, the present study was undertaken for ecofriendly management of the disease.

RESEARCH METHODOLOGY

The present investigation was carried out in the laboratory of Dept. of Plant Pathology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola during 2007-2008. The experiment was done by using 12 non pathogenic isolates of *Alternaria* spp. culture filtrates. *viz.,* ANP-1, ANP-2, ANP-3, ANP-4, ANP-5, ANP-6, ANP-7, ANP-8, ANP-9, ANP-10, ANP-11 and ANP-12 against 10 pathogenic isolates of *Alternaria lini.*

Preparation of culture filtrates :

To prepare the culture filtrates of 12 non pathogenic isolates of *Alternaria* spp. ANP-1 to ANP-12, the isolates were grown in 150 ml of Potato dextrose broth (PDB) in 250 ml conical flask for 20 days. The broth containing mycelium and spores were filtered through Whatman filter paper No. 4 and were centrifuged at 5000 rpm for 10 min. to collect cell free supernatant and was considered 100 per cent concentration for poisoned food technique as 10 per cent concentrations (Mane and Pal, 2008).

Effect on radial mycelial growth :

Ten pathogenic isolates of *Alternaria lini* were used to study the antifungal activity against non pathogenic isolates of *Alternaria*. The 10 per cent nonpathogenic culture filtrates were added to the medium and the plates were inoculated individually 6mm disc pathogenic isolates of *A. lini* at the centre and incubated at 28 ± 20C for 7 days. Observations on mean colony diameter were measured and the per cent inhibition was calculated by using following formula given by Vincent (1927)

$$I = \frac{C - T}{C} X100$$

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

Effect on sporulation intensity :

To study the effect of culture filtrates of non pathogenic isolates on sporulation intensity, 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 1 ml of sterile distilled water and spore load was counted by haemocytometer and per cent inhibition of sporulation was calculated by using following formula.

$$I = \frac{C - T}{C} X100$$

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

Effect on spore germination :

The spores of *A. lini*, removed with the help of a sterilized needle and brush in sterilized water and from which one drop each of spore suspension and double strength solutions *i.e*. 20 per cent was 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 chamber. For check, the spores were added to sterilized water. Per cent germination was recorded after 36 hrs. of incubation at 28± 2^0C. The per cent inhibition of spore germination was calculated by using following formula (Bhatiya and Awasthi, 2007).

$$I = \frac{C - T}{C} X100$$

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 ANALYSIS

The effect of culture filtrate of 12 non pathogenic isolate of *Alternaria i.e.* ANP-1 to ALP-12 on radial mycelial growth and sporulation intensity of ten *A. lini* isolate were evaluated through poisoned food technique and their effect on spore germination was tested by hanging drop method.

From the data presented in Table 1. it was revealed that all the treatments were superior over control. The treatment ANP-4 @10 per cent was found to be most effective showing 16.17mm radial mycelial growth and 78.58 per cent inhibition against ANP-1 isolate. This treatment was followed by ANP-7 with 71.07 per cent growth inhibition as well as ANP-8 inhibiting mycelial growth up to 68.20 per cent. Similar kind of results were obtained against isolate ALP-5, ALP-6, in which minimum radial mycelial growth *i.e.* 12.17 mm and 16.83mm with 83.02 per cent and 79.5 per cent growth inhibition, respectively, in ANP-4 followed by treatments ANP-7 with 64.42 and 67.63 per cent inhibition, respectively. While, studying the isolate ALP-2, ALP-8, it was found that treatment ANP-4 was best among the treatments showing 17.83mm and 18.17mm mycelial growth with 75.87 per cent and 75.44 per cent growth inhibition. And this treatment was followed by ANP-8 with 65.94 per cent and 64.4 per cent growth inhibition, respectively.

Data presented in Table 2 showed that all the treatments were significantly superior over control giving good effect on the sporulation intensity of pathogenic *A. lini* isolates. While studying ALP-1 isolate, it was found that ANP-9 was best with $0.91x10^5$ spores/cm^2 giving 85.97 per cent inhibition and this treatment was found to be at par with all the treatment except control. Treatment ANP-10 was found to be the best against ALP-2 and ALP-5 giving 1. $12x10^5$ and $1.03x10^5$ spores/cm^2, respectively.

Similar kind of observations were recorded in studying spore germination of ALP-3, ALP-4, ALP-6, ALP-8 and ALP-9 in which treatment ANP-4, ANP-1, ANP-6, ANP-5, ANP-6 and ANP-7, respectively was found best among all the treatments showing $0.97x10^5$ spores/cm^2 (82.90 per cent), $1.06x10^5$ spores/cm^2 (82.94 per cent), $1.00x10^5$ spores/cm^2 (77.03 per cent), $0.97x10^5$ spores/cm^2

Table 2: Effect of culture filtrates of non-pathogenic isolates of *Alternaria lini* on mycelial growth of pathogenic isolates of *Alternaria lini* (in vitro).

Treatments	ANP 1		ANP 2		ANP 3		ANP 4		ANP 5		ANP 6		ANP 7		ANP 8		ANP 9		ANP 10	
	Av. (mm)	%	Av. (mm)	%	Av. (mm)	%	Av. (mm)	%	Av. (mm)	%	Av. (mm)	%	Av. (mm)	%	Av. (mm)	%	Av. (mm)	%	Av. (mm)	%
ANP 1	54.5	21.78	65.83	10.92	24.67	65.9	36.67	23.12	64.67	9.71	59.83	19.81	60.77	16.11	56.11	21.06	60	15.85	61.11	17.11
ANP 2	64	15.19	60.83	11.68	20.33	71.89	65.67	9.91	60.5	13.58	64.83	13.11	62.5	13.9	51.5	30.31	61.83	13.28	50.83	31.61
ANP 3	51.11	18.95	63.67	13.85	32.11	55.53	60.33	18.11	64.5	10	55.5	25.67	60.67	15.11	11.67	39.6	58.33	18.19	60.5	18.5
ANP 4	46.11	28.58	71.83	19.81	19.5	78.01	45.11	19.5	72.11	83.02	16.83	71.16	71.5	75.69	18.11	75.11	11.5	19.65	13.5	79.15
ANP 5	45.5	39.11	55	25.58	25.83	64.29	31.83	48.81	38.11	16.11	36.83	50.61	28.33	60.65	11.11	80.29	35.11	30.68	36.5	50.9
ANP 6	53.5	29.11	63.83	13.52	21.83	61.52	55.67	21.71	71.11	31.19	61.11	18.08	45.83	36.31	52.5	29.02	60.83	11.68	51	31.39
ANP 7	21.83	11.01	26	64.82	21.5	66.13	35	58.11	23.5	61.12	24.11	61.63	11.18	38.64	31.18	51.85	25.83	63.11	25.5	65.11
ANP 8	21	68.2	25.11	65.9	21.11	66.59	23.83	61.19	31.67	51.63	25.11	66.29	16.11	35.88	26.33	64.1	31.83	46.9	51.11	23.09
ANP 9	61.83	18.01	56.83	23.09	25.33	64.98	45.67	38.29	56.11	21.63	53	29.02	54.83	23.84	50.83	31.28	48.11	32.45	65.33	12.11
ANP 10	51.83	31.32	60.83	11.68	12.11	81.11	60.67	18.02	60.5	15.58	62.11	16.11	52.83	25.62	50.5	31.73	54.67	23.05	61.5	11.26
ANP 11	64.5	11.51	62.11	13.88	10.67	83.78	53.11	28.15	54.67	23.12	61.83	17.19	55.83	21.95	54.11	26.71	56.83	20.29	54.67	25.45
ANP 12	44.67	10.82	48	35.05	21.11	66.59	46.33	31.39	39.83	44.12	36.67	50.89	42.67	40.71	42	43.22	36.5	48.8	49.5	33.11
Control	78.11		78.9		72.33		71		77.67		71.67		72		78.91		77.3		71.33	
S.Em.			S.Em.		S.Em.		S.Em.		S.Em.		S.Em.		S.Em.		S.Em.		S.Em.		S.Em.	

Table 3: Effect of culture filtrates of non-pathogenic isolates of *Alternaria lini* on sporulation intensity of pathogenic isolates of *Alternaria lini* (in vitro)

Treatments	ANP 1		ANP 2		ANP 3		ANP 4		ANP 5		ANP 6		ANP 7		ANP 8		ANP 9		ANP 10	
	Av. (X 10 (n=2))	%	Av. (X 05 (n=2))	%	Av. (X 05 (n=2))	%	Av. (X 05 (n=2))	%	Av. (X 05 (n=2))	%	Av. (X 05 (n=2))	%	Av. (X 05 (n=2))	%	Av. (X 05 (n=2))	%	Av. (X 05 (n=2))	%	Av. (X 05 (n=2))	%
ANP 1	1.15	82.35	1.21	78.13	1.11	71.67	1.05	80.97	1.21	71.1	1.09	75	1.15	70.11	1.33	75.11	1.21	61.11	1.3	75.97
ANP 2	1.21	80.57	1.3	77.08	1.11	71.09	1.38	71.73	1.38	67.69	1.11	66.89	1.11	65	1.5	67.82	1.11	62.02	1.3	75.97
ANP 3	1.18	81.9	1.33	75.55	1.21	71.12	1.21	80.09	1.33	72.89	1.33	69.59	1.15	72.11	1.21	75.21	1.36	61.31	1.21	80.31
ANP 4	1.21	81	1.21	78.13	0.91	82.9	1.15	81.52	1.18	75.9	1.15	73.65	1.53	62.86	1.3	75.69	1.21	65.61	1.21	80.82
ANP 5	1.55	76.02	1.21	78.65	1.15	79.79	1.12	81.99	1.12	77.11	1.21	70.93	1.55	62.11	0.91	81.71	1.09	71.32	1.59	75.37
ANP 6	1.03	81.16	1.33	75.55	1.12	80.31	1.12	81.99	1.12	77.11	-	71.03	0.91	71.86	1.68	68.51	1.06	72.09	1.21	80.82
ANP 7	1.15	82.35	1.18	79.11	1.09	80.83	1.11	75.78	1.18	75.9	-	71.03	1.21	70	1.11	73.18	1.06	72.09	1.18	81.11
ANP 8	1.09	83.26	1.3	77.08	1.3	71.2	1.15	81.52	1.21	69.88	1.21	72.3	1.35	61.11	1.33	75.11	1.06	72.09	1.15	82.19
ANP 9	0.91	85.97	1.38	75.52	1.11	71.67	1.09	82.46	1.15	76.51	1.03	76.35	1.33	61.86	1.18	71.9	1.21	66.51	1.18	81.11
ANP 10	1.03	81.16	1.12	80.21	1.21	71.12	1.33	78.51	1.03	78.92	1.21	71.62	1.53	62.86	1.15	78.15	1.18	68.99	1.33	75.15
ANP 11	0.91	85.97	1.11	71.87	1.36	75.11	1.21	80.51	1.86	62.05	1.11	61.51	1.21	59.29	0.91	81.71	1.33	65.12	0.91	84.93
ANP 12	1.21	80.57	1.18	79.11	1.33	76.68	1.21	80.09	1.15	76.51	1.21	71.62	1.11	65.11	1.18	71.9	1.09	71.32	1.12	82.65
Control	6.51		5.65		5.69		6.22		7.89		7.36		7.13		5.33		3.8		6.15	
S.Em.	S.Em.		S.Em.		S.Em.		S.Em.		S.Em.		S.Em.		S.Em.		S.Em.		S.Em.		S.Em.	

(81.77 per cent) and $1.06x10^5$ spores/cm^2 (72.09 per cent) inhibition of sporulation, respectively against the respective isolates.

Data summarized in Table 3 indicates that all the 12 non pathogenic isolate had shown antisporulant properties against pathogenic isolates of *A. lini.* Treatment ANP-3 10 per cent was found to be the best against ALP-1 isolate giving 14.33 per cent spore germination with 84.65 per cent growth inhibition which was at par with ANP-11 (83.44 per cent), ANP-1 (83.30 per cent) and ANP-7 (83.80 per cent). Similarly in studying spore germination of ALP-4 and ALP-9 isolates, ANP-3 treatment was found best showing 12.57 per cent and 13.63 per cent spore germination with 83.66 per cent and 83.63 per cent inhibition, respectively. Treatment ANP-10 was also found best in reducing 14.67 per cent spore germination of ALP-2 isolate and 81.89 per cent growth inhibition followed by ANP-8 showing 80.78 per cent inhibition.

More or less in the same way, it is found that the treatments ANP-6 (12.27 per cent), ANP-4(15.60 per cent), ANP-11(14.80 per cent), ANP-8 (16.47 per cent), ANP-8 (13.47 per cent) and ANP-4 (12.87 per cent) were best among in reducing the spore germination of ALP-3, ALP-5, ALP-6, ALP-7, ALP-8 and ALP-10 isolates of *Alternaria lini*.

The data summarized in Table 4 revealed that ANP-4 had the maximum fungistatic potential with an average 77.74 per cent inhibition of mycelial growth. Whereas, regarding antisporulant properties though all the treatments show significant results over control and ANP-6 had given maximum (77.64 per cent) reduction of sporulation and ANP-4 had given maximum (79.74 per cent) reduction in spore germination, distinctly superior over other treatments. On the basis of variation present in their fungistatic potentials (Table 1-3) treatment ANP-4 @10 per cent was the most effective.

Table 4 : Comparison of culture filtrates of non pathogenic *Alternaria* isolates on radial mycelial growth, sporulation intensity and spore germination (Average of ten isolates)

Treatments	% inhibition of radial mycelial growth	% inhibition of sporulation intensity	% inhibition of spore germination
ANP-1	23.17	76.18	76.85
ANP-2	23.18	72.68	72.50
ANP-3	23.46	75.18	78.20
ANP-4	77.74	75.91	79.74
ANP-5	47.83	75.51	76.92
ANP-6	29.27	77.64	75.60
ANP-7	61.81	76.94	78.32
ANP-8	55.67	75.78	78.99
ANP-9	29.47	76.56	72.64
ANP-10	23.97	76.11	78.01
ANP-11	23.78	74.74	75.86
ANP-12	44.13	76.22	77.77
Control	00.00	00.00	00.00

Previously, antagonistic potential of non pathogenic isolates against its pathogenic isolates of *Fusarium oxysporum* infecting celery roots have been reported by Schneider (1984), *F. oxysporum* f.sp. *cucumerinum* infecting cucumber (Paulitz *et al*., 1987; Mandeel and Baker, 1991), *F. lini* infecting linseed (Philippe and Alabouvette, 1991), *F. oxysporum* f.sp. *cieris* infecting chickpea (Hervas *et al*., 1995), *F. oxysporum, F. moniliformae* infecting cyclamen (Minuto *et al*., 1995), Fusarium wilt of tomato (Robert *et al.,* 1998) and Fusarium wilt of banana (Nel *et al.,* 2006). All these reports justify the biocontrol potential of non pathogenic isolates of similar microorganisms against the mycelial growth of isolates in soil borne diseases. The efficacy of these spore germination against *Fusarium oxysporum* f.sp. *vasinfectum* was also found by Garett and Robinson (1969). Similar results of sporulation intensity were also obtained by using on filtrate of *Bacillus subtilis* against *Colletotrichum goeosporiodes* (Kelemu an Badal, 1994) and used culture filtrates of *Calearisporium arbuscula* against *Sphaerotheca fuliginea.* (Hijwegen, 1989). In the current study non pathogenic isolates of *Alternaria* spp. have antagonistic potential against the pathogenic isolates of same fungus responsible for blight of linseed, that indicates apart from soil borne diseases this phenomenon is existing in foliar diseases as well.

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