~~Screening of Alternariablightof Linseed (*Linum usitatissimum*)caused by (*Alternarialini* L.) in *In vitro* condition~~

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Screening of Linseedgermplasm against Alternaria blight caused by *Alternaria lini*and Control the disease *in-vitro* condition

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Abstract

Linseed (*Linumusitatissimum* L.) is an important oilseed and fiber crop grown both for its seed as well as fibre.It is widely cultivated in Northern India during Rabi season. It suffers from a variety of diseases among which Alternaria blight caused by *Alternarialini* is one of the most yield limiting factors in India. The efficacy of botanicals (neem, ginger, garlic, bougainvillaand eucalyptus extract),bio-agents (*T.harzianum*and *T.viride)* and chemical fungicides (Mancozeb (0.2%), Carbendazim (0.2%)~~,~~ etc. were tested against *Alternarialini*in *in-vitro* conditions.The result of *in vitro* studies revealed that Neem extract @ 150 ppm significantly reduced the mycelial growth of *Alternarialini*as 68.6%~~percent~~ inhibition followed by Garlic bulb extract @ 150 ppm and Ginger rhizome extract@ 150 ppm as 65.45~~%~~ and 58.65% inhibition, respectively.Among fungicide, Carbendazim at 500 ppm proved to be most effective in inhibiting mycelial radial growth of pathogen with 100% inhibition over control followed by Mancozeb that showed 93.94% inhibition over control under *in-vitro* conditions.

Introduction

Linseed or flax (*Linumusitatissimum*L.) (2n=30) belong to the family "Linaceae" and the genus "*Linum"* is widely cultivated in Northern India during Rabi season and is the only species in the family, which is of economic importance. Some cultivated species are *Linumusitatissimum*and *L. angustifolium.* Linseed is an important oilseed and fiber-bearing crop of temperate and subtropical India and grown both for its seed as well as fibre which is used for the manufacture of linen**(Small, 2015**). It has nutritional, medicinal and agricultural use, ~~Thus~~thus linseed is a multipurpose crop and very effort should be taken to boost up production of this economic crop. Linseed crop is a major source of both oilseed and fiber in the country. Linseed is an important oilseed and fiber crop grown for its seed as well as fiber which is used for the manufacture of linen. The seed contains a good percentage of oil varying from 33 to 47 percent in different varieties. The oil is edible and also due to its quick drying property is used for the preparation of paints, varnishes, printing ink, oilcloth, soap, patent leather, and water proof fabrics. About 20% of the total oil is used in domestic purpose and 80% goes to industries for the manufacturing of paints, varnish, oil cloth, pad ink etc.(Kerkhi*et al.*,1999)**.**Linseed is nutritionally very rich, consisting moisture (6.5%), protein (20.3%), fat (37.1%), mineral (2.4%), fiber (4.8%), other carbohydrates (28.8%), energy 530 calories, calcium (170 mg/100g), phosphorus (370 mg/100g), iron (2.7 mg/100g), vitamin A (50 IU), thiamine (0.23 mg/100g), riboflavin (0.07 mg/100g), nicotinic acid (0.05 mg/100g) (Aykroyed*et al.*, 1963)**.**Round the globe linseed crop occupies an area of 2.62 million ha yielding out 2.65 million tones having an average productivity of 1011 kg/ha, National production of 1.41 lakh tones is realized from an area 2.84 million ha,with average productivity of 496 kg/ha,**(**Anonymous, 2017-18**).** India contributes about 14.88% and

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6.57% to world area and production, respectively.The major linseed growing lies in M.P, Chhattisgarh, U.P, Maharashtra, Bihar, Orissa, Jharkhand, Karnataka and Assam which together account for more than 97% of the total area. Among several factors responsible for hampering the yield, disease play a vital role in reduction of yield.It is known to suffer from a number of fungal, viral,nematode and bacterial diseases.Disease, especially those caused by fungi, is considered to be most important one. In linseed crop many diseases such as damping off,root rot, wilt, *Alternaria*leaf spot, ~~powdry~~powderymildew and rust are caused by fungi.

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Material and Methods

A periodical and active survey of Linseed was made during the month of Feb - March 2016 at Oilseed Research farm, Kalyanpur and CRS, Nawabganj, Kanpur to observe the Alternaria blight of Linseed in field. The data were collected randomly to insure uniform data collection. The disease intensity was taken by counting the number of healthy and unhealthy plants in 27 plots, each of size 3x4 m^2. Five plants were selected in each plot and the number of healthy and unhealthy leaves and buds were counted thoroughly.

Screening of various germplasm of linseed against pathogen.

Screening of available germplasms of linseed against Alternaria blight caused by *Alternarialini*was carried out under natural conditions in field at Oilseed Research farm of Chandra Shekhar Azad University of Agriculture and Technology, Kanpur during crop during the period of 2019-2020. Fourteen germplasms were sown along with one resistant and one susceptible check. The categorization was based on leaf and bud infection.

In vitro efficacy of chemical fungicides.

The efficacy of three systemic and six non-systemic fungicides were evaluated against *A. lini*at three concentrations on potato dextrose agar medium using poisoned food technique. Non-systemic fungicides were tested in laboratory at concentration of 500 ppm, 750 ppm and 1000 ppm and systemic fungicides at 100 ppm, 250 ppm and 500 ppm with three replications in each treatment. The radial growth of the fungus on poisoned food medium was recorded at 12 days after inoculation. The different fungicides tested are mentioned below.

Table 1. List of fungicides used in the experiment

Sl. No	**Common Name**	**Trade name**
Non-systemic fungicides		
1	Copper oxy chloride	Blitox 50WP
2	Carbendazim 12% + Mancozeb 63%	Saaf 75 WP
3	Mancozeb	Indofil M-45 75WP

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4	Carbendazim 25%+ Iprodione 25%	Quintal 50 WP
5	Metalaxyl 4% + Mancozeb 64%	Ridomil 68WP
6	Hexaconazole 4% + Zineb 68%	Avtar 72 WP
Systemic fungicides		
1	Tricyclazole	Beam 75WP
2	Hexaconazole	Contaf 5EC
3	Propiconazole	Tilt 25EC

Results and ~~discussion~~Discussion

Screening of various germplasm of linseed against pathogen~~:~~

The use of resistant variety is cheapest and the best means for controlling any plant disease. Therefore in present studied, the screening for source of resistance against Alternaria blight of linseed caused by *Alternaria lini*was also taken.Fourteen germplasms were screened against Alternaria blight of linseed under natural condition and graded in different categories according to their reaction on leaves and buds as mentioned in ~~table~~ Table 1 of materials and methods (**Table 2**).

Comment [DZ3]: Write results of germplasm here.

Table 2: Evaluation of linseed germplasm/cultivar against *Alternaria lini* infection under natural conditions

S.No.	Germplasm	Grade	Reaction
1.	SJKO-17	2	MR
2.	SJKO-18	1	R
3.	SJKO-19	1	R
4.	SJKO-20	1	R
5.	SJKO-21	1	R
6.	T-397	1	R
7.	KL-214	0	I
8.	KL-215	4	S
9.	KL-216	3	MS
10.	Neelam	2	MR
11.	SJKO-22	0	I
12.	SJKO-23	1	R
13.	SJKO-24	2	MR

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14.	SJKO-25	5	MR

In vitro* evaluation of botanicals against *Alternaria lini

Six plant extracts were evaluated at three concentrations (50ppm, 100ppm, 150ppm) in the laboratory for their efficacy against *A. lini*through poisoned food technique as detailed in Materials and Methods(Table -3).The plant extracts inhibited the growth of the pathogen in the medium. The resultsof*in-vitro* studies revealed that Garlic bulb extract @ 150ppm significantly reduced the mycelial growth of *Alternarialini*as 68.6%~~percent~~ inhibition followed byNeem leaf extract @ 150 ppm and Ginger rhizome extract@ 150 ppm as 64.3% and 57.2% inhibition respectively~~.~~(Shovan *et~~.~~ al*., 2008).

Table-3In vitro evaluation of botanicals against *A. lini*causing Alternaria blight disease on linseed

S.No.	Fungicides/ concentration	Percent inhibition			
		50 ppm	100 ppm	150 ppm	Mean
1	*AzadirachtaIndica*	44.45	59.49	68.6	57.51
2	*Allium sativum*L.	44.35	59.90	65.45	56.57
3	*Zingiberofficinale*	33.40	49.65	58.65	47.23
4	*Allium cepa*	28.45	45.25	56.75	43.48
5	*Bougainvillea sp. L.*	23.70	34.85	58.25	38.93
6	*Eucalyptus globules Labill*	17.80	30.55	39.35	29.23
7	Control	0.0	0.0	0.0	0.0
	SEm ±	**0.2726**	**0.3817**	**0.4363**	**0.4956**
	CD @ 5%	**0.8273**	**1.1580**	**1.3235**	**1.5032**

CD=?????

In vitro* evaluation of bio-agents against *Alternarialini

Two bio-agents obtained from different sources were used for these experiments. The experiments were conducted to know their antagonistic potential against *A. lini*through dual culture technique.

The data pertaining to the effect of bio-agents on the fungus growth were taken at 4 and 9 days after inoculation(Table ~~-~~4). The percent inhibition caused by the bio-agents was higher at 9 days than 4 days after inoculation. The fungal antagonists (*T. harzianum*and *T. ~~Viride~~viride)* were found to completely inhibit the fungal growth. The results of *in vitro* studies revealed that *T.harzianum*significantly reduced the mycelial growth of *Alternarialini*as 37.95%~~percent~~

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inhibition followed by *T. viride* as 34.05%~~percent~~ inhibition, respectively (Perveen~~&~~and Bokhari, 2012;~~),(~~Yassin~~,~~*et al*., 2021).

Table 4. ~~:~~Percent inhibition of *A. lini*over control in presence of bio-control agents~~-~~

No.	Treatment	Radial growth of pathogen (mm)	Percent inhibition
1	*A.lini + T. viride*	4.38	**34.05**
2	*A.lini + T.harzianum-*	4.68	**37.95**
3	**Control**	7.15	**-**
SEm ±		**0.1265**	
CD (5%)		**0.4372**	

CD=????

In vitro* evaluation of chemical fungicides against *Alternarialini

Considering the economic importance and serious nature of the disease and in the absence of suitable resistance genotype of the crop, chemical method of control is a dependable method to control disease(Table 6 and 7). The efficacy of three systemic and six non-systemic fungicides were evaluated against *A. lini*at three concentrations as described in "Material and Methods". The results indicated that there was difference among non systemic and systemic fungicides in inhibiting the growth of *A.lini.* Among the six non systemic fungicides evaluated at 1000 ppm, Mancozeb completely inhibited of the fungus growth (100%) and significantly superior over other treatments, followed by Quintal 50WP (88.89%), Ridomil 68 WP (86.30%), Saaf 75WP (82.96%). Least growth inhibition was observed in Blitox50WP followed by Avtar 72 WP (77.78%). Higher concentrations of the fungicides were found more effective against *A. lini*compare to lower concentrations. Mancozebwas found completely in inhibiting the mycelial growth of *A. lini*at 1000 ppm and found significantly superior over the other lower concentrations.Among the different systemic fungicides, percent inhibition of Carbendazim at 500 ppm (100%) gave complete growth inhibition of the fungus, followed by Contaf 5EC (94.60%). Least inhibition of mycelial growth was observed in Beam 75WP (64.75%) at 500 ppm and it was not effective in reduction of growth of the fungus at 100 ppm (17.65%). Carbendazim was found less effective with 86.20%~~per cent~~ reduction in growth of *A. lini*at 100 ppm than at 250 ppm (91.40%) and 500 ppm (100%) (Yadav *et al*., 2021;~~),(~~Brahmankar*et al*., 2021).

Table ~~-~~5.~~:~~Treatment details~~:-~~

T1.	Copper oxy chloride	T6.	Hexaconazole 4% + Zineb 68%
T2.	Carbendazim 12% + Mancozeb 63%	T7.	Tricyclazole
T3.	Mancozeb	T8.	Hexaconazole
T4.	Carbendazim 25%+ Iprodione 25%	T9.	Propiconazole

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T5.	Metalaxyl 4% + Mancozeb 64%	CK	Control

Table 6.÷*In vitro* evaluation of non-systemic fungicides against *A. lini*

S.No.	Trade name	Common name/ concentration	Per cent inhibition			
			500 ppm	**750 ppm**	**1000 ppm**	**mean**
1.	Blitox 50WP	Copper oxy chloride 50WP	58.15	69.63	77.04	68.27
2.	Saaf 75 WP	Carbendazim 12% + Mancozeb 63%	49.26	61.85	82.96	64.69
3.	Avtar 72 WP	Hexaconazole 4% + Zineb 68%	54.81	58.89	77.78	63.83
4.	Quintal 50 WP	Carbendazim 25%+ Iprodione 25%	55.93	67.04	88.89	70.62
5.	Ridomil 68WP	Metalaxyl 4% + Mancozeb 64%	55.56	71.85	86.30	71.23
6.	Indofil M-45 WP	Mancozeb	84.07	87.78	100.00	90.62
7.	**Control**		0.00	0.00	0.00	0.00
SEm ±			**0.66**	**0.43**	**1.15**	-
			2.53	**1.66**	**4.38**	-

Table 7.÷*In vitro* evaluation of systemic fungicides against *A. lini*

No.	Trade name	Common name/ concentration	Percent inhibition			
			100 ppm	**250 ppm**	**500 ppm**	**mean**
1	Beam 75WP	Tricyclazole	17.65	44.10	64.75	42.17
2	Contaf 5EC	Hexaconazole	85.45	88.85	94.60	89.63
3	Bavistin	Carbendazim	86.20	91.40	100.00	92.53
4	Control		0.00	0.00	0.00	0.00
SEm ±			**0.47**	**0.51**	**0.72**	**0.62**
CD (1%)			**1.55**	**1.62**	**2.35**	**2.04**

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Conclusion

Plant extracts inhibited the growth of *A. linia* in the medium, with Garlic bulb extract at 150ppm having the highest inhibition rate. Bio-agents (*T. harzianum* and *T. viride*) significantly inhibited the mycelial growth of *Alternaria linia*, with *T. harzianum* having 37.95% inhibition and *T. viride* having 34.05% inhibition respectively and the efficacy of three systemic and six non-systemic fungicides were evaluated against *A. lini* at three concentrations. Mancozeb was found to be the most effective, followed by Quintal 50WP, Ridomil 68 WP, Saaf 75WP, Blitox 50WP, and Avtar 72 WP. Carbendazim was found less effective with 86.20%~~per cent~~ reduction in growth of *A. linia*100 ppm than at 250 ppm (91.40%) and 500 ppm (100%). These are the treatment to inhibit the growth of *Alternaria linia***.**

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