

Screening of *Alternaria* blight of Linseed (*Linum usitatissimum*) caused by (*Alternaria* L.) in *In-vitro* condition.

Abstract

Linseed (*Linum usitatissimum* L.) is an important oilseed and fiber crop grown both for its seed as well as fiber. It is widely cultivated in Northern India during the Rabi season. It suffers from a variety of diseases among which *Alternaria* blight caused by *Alternaria* is one of the most yield limiting factors in India. The efficacy of botanicals (neem, ginger, garlic, bougainvillea and eucalyptus extract), bio-agents (*T. harzianum* and *T. viride*) and chemical fungicides (Mancozeb (0.2%), Carbendazim (0.2%), etc. were tested against *Alternaria* in *in-vitro* conditions. The result of *in vitro* studies revealed that Neem extract @ 150 ppm significantly reduced the mycelial growth of *Alternaria* 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 (*Linum usitatissimum* 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 *Linum usitatissimum* and *L. angustifolium*. Linseed is an important oilseed and fiber-bearing crop of temperate and subtropical India and is grown both for its seed as well as fiber which is used for the manufacture of linen (Small, 2015). It has nutritional, medicinal, and agricultural use. Thus linseed is a multipurpose crop and every 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 waterproof fabrics. About 20% of the total oil is used for domestic purposes and 80% goes to industries for the manufacturing of paints, varnish, oilcloth, pad ink, etc. (Kerkhiet *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 tons having average productivity of 1011 kg/ha, National production of 1.41 lakh tones is realized from an area of 2.84 million ha, with average productivity of 496 kg/ha, (Anonymous, 2017-18). India contributes about 14.88% and 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 plays a vital

Comment [AO1]: Suggestions : Linseed (*Linum usitatissimum* L.) is an important oilseed and fiber crop grown for its seed and fiber. It is widely cultivated in Northern India during the Rabi season. It suffers from various diseases among which *Alternaria* blight caused by *Alternaria* is one of India's most yield-limiting factors. The efficacy of botanicals (neem, ginger, garlic, bougainvillea, and eucalyptus extract), bio-agents (*T. harzianum* and *T. viride*), and chemical fungicides (Mancozeb (0.2%), Carbendazim (0.2%), etc. were tested against *Alternaria* in *in-vitro* conditions. The result of *in vitro* studies revealed that Neem extract @ 150 ppm significantly reduced the mycelial growth of *Alternaria* with 68.6 percent inhibition followed by Garlic bulb extract @ 150 ppm and Ginger rhizome extract @ 150 ppm with 65.45% and 58.65% inhibition respectively. Among fungicides, Carbendazim at 500 ppm proved to be most effective in inhibiting mycelial radial growth of pathogens with 100 % inhibition over control followed by Mancozeb which showed 93.94% inhibition over control under *in-vitro* conditions

role in the 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 the most important one. In linseed crops many diseases such as damping off, root rot, wilt, *Alternaria* leaf spot, ~~powdery~~ powdery mildew and rust are caused by fungi.

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 the field. The data were collected randomly to ensure uniform data collection. The disease intensity was taken by counting the number of healthy and unhealthy plants in 27 plots, each of size 3x4 m². 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 *Alternaria lini* 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~~ were evaluated against *A. lini* at three concentrations on potato dextrose agar medium using the poisoned food technique. Non-systemic fungicides were tested in the laboratory at concentrations 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 the 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
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

Result and discussion

Screening of various germplasm of linseed against pathogen:

The use of resistant variety is the cheapest and best means for controlling any plant disease. Therefore in the present study, the screening for the 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 conditions and graded in different categories according to their reaction on leaves and buds as mentioned in table 1 of materials and methods (Table 2).

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
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 the poisoned food technique as detailed in Materials and Methods (Table -3). The plant extracts inhibited the growth of the pathogen in the medium. The results of *in-vitro* studies revealed that Garlic bulb extract @ 150ppm significantly reduced the mycelial growth of *Alternaria linis* 68.6 percent inhibition followed by Neem leaf extract @ 150 ppm and Ginger rhizome extract @ 150 ppm at 64.3% and 57.2% inhibition respectively. (Shovan *et. al.*, 2008).

Table-3 *In 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	<i>Azadirachta indica</i>	44.45	59.49	68.6	57.51
2	<i>Allium sativum</i> L.	44.35	59.90	65.45	56.57
3	<i>Zingiber officinale</i>	33.40	49.65	58.65	47.23
4	<i>Allium cepa</i>	28.45	45.25	56.75	43.48
5	<i>Bougainvillea sp. L.</i>	23.70	34.85	58.25	38.93
6	<i>Eucalyptus globules</i> <i>Labill</i>	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

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***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*) were found to completely inhibit the fungal growth. The results of *in vitro* studies revealed that *T. harzianum* significantly reduced the mycelial growth of *Alternarialinias* 37.95 percent inhibition followed by *T. viride* as 34.05 percent inhibition respectively (Perveen & 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	<i>A.lini</i> + <i>T. viride</i>	4.38	34.05
2	<i>A.lini</i> + <i>T.harzianum</i> -	4.68	37.95
3	Control	7.15	-
SEm ±		0.1265	
CD (5%)		0.4372	

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* compared to lower concentrations. Mancozeb was 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
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
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			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

Conclusion

Plant extracts inhibited the growth of *A. lini* 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 lini*, 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

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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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