

### Original Research Article

## ***In Vitro* Potency of Chemical Fungicides over *Alternaria lini* (Dey) Growth Inhibition Causing Black Bud Disease of Linseed**

### **Abstract**

Linseed, the only economically significant species of family Linaceae is a dense plant with sound branches that gives rise to more production of seed per plant. It is a significant oilseed and fibre crop that grows in diverse range of climate from temperate to tropical regions of India. So, it is crucial to preserve the excellence and yield of such an oilseed crop due to its commercial significance. Linseed cultivation is impacted by a variety of fungal and bacterial diseases. *Alternaria* blight or often called as Black Bud Disease is one of the most important fungal diseases of this oilseed crop which causes yield loss ranging from 28-60%. The present study emphasized upon two objectives. First one is the survey of different cultivars that was conducted in various parts of Uttar Pradesh during the month of March-April, 2021-22 to assess the disease prevalence and severity of Black Bud Disease which revealed that cultivar Neelumhad the highest disease intensity of 45%, while the variety Rashmi had the lowest disease intensity of 10%. Second one involves the study regarding *in vitro* potency of chemical fungicides that revealed the potency of three fungicides i.e. Tilt, Vitavax and Matco over 11 fungicides examined at 0.2 % concentration in fully inhibiting the growth of corresponding fungal pathogen, *Alternaria lini*. Similar result was obtained by Holi, S. K. and Meena, S. (2015). Rovral, Thiram, Folpet, Captan, Ridomil, Companion, Mancozeb, Zineb, Sulfex, Topsin-M, and Bavistin were the remaining fungicides in order of their inhibitory activity against the pathogen.

**Keywords:** *Alternaria lini*, Black Bud Disease, Disease Severity, Fungicides, Growth inhibition

### **Introduction**

Linseed, the only economically significant species of family Linaceae having chromosome no. ( $2n = 30$ ) is a dense plant with an approximate height of 80 cm and having sound branches that gives rise to more production of seed per plant. It is a significant oilseed and fibre crop that grows in diverse range of climate from temperate to tropical regions of India. In India the crop is grown both as a seed crop and fibre crop to manufacture Linen Fibre. It is believed that the origin of small seeded type (oil purpose) Linseed crop has been located in the South-Western Asia. Bold seeded type (fibre purpose) Linseed crop has been originated from Mediterranean coastal lands such as Asia, Egypt, Spain, Italy, and Greece. National production is reported to be 1.207 lakh tonnes from an area of 1.799 lakh ha having average productivity of 671 kg /ha (Directorate of Oilseeds Development Ministry of Agriculture and Farmers Welfare GOI -2020). India is having fifth position in area in the global map and secured sixth position in production after Russia, Canada, Kazakhstan, China and USA (FAO, 2016). On Global scenario, India is having contribution of 14.88% to world area and is having 6.57% contribution to world production level. Linseed production in U.P is about 0.10 lakh tonnes

(2013-14) which accounts as 9.18 percent of total production of the country. Linseed productivity in U.P is about 385 kg/ha (Annual Report on Linseed, 2017-18). It is known to be susceptible to a variety of fungal, viral, nematode, and bacterial infections. Disease, particularly that caused by fungi, is regarded as the most serious. Fungi produce various diseases in the Linseed crop, including damping off, root rot, wilt, *Alternaria* leaf spot, powdery mildew, and rust. The pathogen develops dark brown spot near the bottom of the calyx which grows in size, darkens in colour and spreads throughout the bud, eventually reaching the pedicel which prevents the opening of buds and dark coloured ring appears on pedicel followed by collapse of the entire flower bud in most severe cases. Hence often called as Black Bud Disease of Linseed. However, very little research has been done on the nature of disease and how to control it. Thus considering the above facts and past research activities, the present study involves a survey that was conducted in different parts of Uttar Pradesh during the month of March-April, 2022 to assess the disease prevalence and severity of Black Bud Disease in Linseed and *in vitro* efficacy of chemical fungicides (bioassay test) upon growth inhibition of corresponding fungal pathogen *Alternaria lini*.

**Table 1: *Alternaria* leafspot of Linseed disease severity ratings**

Rating	Category/Reaction	Disease Percentage	
		Leaf infection (%)	Bud infection (%)
0	Immune(I)	0	0
1	Resistant (R)	<10	<10
2	Moderately resistant(MR)	10.10-25	10.10-25
3	Moderately susceptible(MS)	25.1-50.0	25.1-50.0
4	Susceptible(S)	50.1-75.0	50.1-75.0
5	Highly susceptible(HS)	75.1-100	75.1-100

The following formula was used to determine the percentage of Bud/Leaf infection:-

$$\text{Percent leaf /bud infected or diseased} = \frac{\text{Sum of numerical rating}}{\text{Total number of bud/Leaf observed} \times 5} \times 100$$

## Materials and Methods

### Survey for assessing prevalence and severity of disease:

A regular and constant observation was carried out on Linseed crop at ten places i.e. CSA University Research Farm, Nawabganj; Oilseed Research Farm, Kalyanpur; NARP Research Farm, DillipNagar, Kanpur Dehat; RRS, Bharari, Jhansi; Crop Research Station, Mauranipur, Jhansi; Crop Research Farm, Gursharai, Jhansi; RRS, Bilatal, Hamirpur; KVK Farm, Bohatpurajalaun; KVK Farm, Hamirpur; KVK Farm, Mahoba during the month of March-April, 2022 for leaf spot and black bud disease caused by *Alternaria lini* in the Linseed fields. The data were collected randomly in the selected fields using standard method to keep uniformity in the data collection. The disease incidence was calculated by counting the number of healthy and infected plants in ten plots, each of one square meter size in each field. In other case, disease intensity was calculated by counting percentage of infection, which was randomly observed in fields and calculated with the help of 0-5 artificial rating

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**Comment [NP2]:** Make the standard tables, no vertical lines

scales mentioned in Table 1. In five selected plants, total buds infected were recorded, and infested leaf area was recorded in ten selected leaves.

#### ***In vitro* potency of chemical fungicides (bioassay test)**

The following 14 fungicides belonging to different groups were tested under present study to select the appropriate and effective fungicide which could effectively inhibit the growth of pathogen. This could be accomplished by the technique of Poisoned Food (Schmitz, 1930). Before putting the mixture into sterile Petri Plates, the necessary amount of each fungicide was added to a two percent potato dextrose agar medium and thoroughly mixed by shaking. The medium was then left to attain the solidify state followed by inoculation with 5 mm disc of inoculum from 7 days old culture of the pathogen. The fungal discs were reversed so that the pathogen could come in contact with the medium directly. Three replications were kept for each treatment. Incubation of the Petri Plates were done at the temperature of  $25 \pm 2^{\circ}\text{C}$  along with one set of control in which the medium was not mixed with any fungicide but simply inoculated with the pathogen. The radial growth of fungal colony were measured in mm after 24 hours till the fungal colony fully covered the control Petri plates. According to Bliss, 1934 the percent inhibition over control was calculated by using following formula.

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$$\text{Percent inhibition over control} = \frac{C-T}{C} \times 100$$

Where; C = Growth of fungus in control, T = Growth of fungus indifferent treatments

**Table 2: Active ingredients and doses of fungicides used in experiment**

Comment [NP4]: Standard table please

SI NO.	Name of fungi toxicant	Active ingredient	Doses (%)
1.	Thiram	80% Tetramethyl thiuram disulphide	0.2
2.	Indofil M-45	Manganese ethylene bisdithiocarbamate+ Zinc ion	0.2
3.	Indofil Z-78	75% Zinc ethylene-bis-dithiocarbamate	0.2
4.	Sulfex	Elemental wettable Sulphur	0.2
5.	Captan	50% N Trichloromethyl thio-4 cyclohexene-1,2 dicarboximide	0.2
6.	Folpet	[N(1,1,2,2-tetrachloro ethyl sulfenyl),Cis-4 cyclohexane-1,2-dicarboximide	0.2
7.	Rovral	[3-(3,5 dichlorophenyl)-N-isopropyl 2,4-Dioxiomidazolidine-1 carboxamide	0.2
8.	Bavistin	2-(Methoxy-carbamyl) benzimidazole carbamate	0.2
9.	Vitavax	5,6-dihydro-2-methyl-1,4-oxathin-3-Carboxanilide	0.2
10.	Ridomil	Methyl-DL-N-(2,6-dimethyl phenyl-N)2 methoxyacetyl-alaninate	0.2

11.	Tilt	1-[2-(2,4-dichlorophenyl)-4-propyl-1,3 dioxalan-2-methyl]-H-1,2,4-triazole	0.2
12.	Matco	Metalaxyl 8% + Mancozeb 64% WP	0.2
13.	Companion	Carbendazim 12%+ Mancozeb 63% WP	0.2
14.	Topsin-M	1,2- bis(methoxy carbonyl-2 thiouredo) benzene	0.2

## Results

### Survey for assessing prevalence and severity of disease

During the crop season of 2021-22, a survey was done in important agricultural areas of Uttar Pradesh to determine the prevalence and severity of disease caused by *Alternaria lini* Dey and findings are mentioned in Table-3. The data recorded in Table-3 illustrated that the maximum disease intensity was observed with cultivar Neelum having disease intensity of 45% and minimum with cultivar Rashmi having disease intensity of 10%. Rest of the varieties have a disease intensity ranging from 15-30 %.

**Table-3: Disease intensity of Black Bud Disease in different cultivars at adjoining area of Kanpur, Uttar Pradesh** Comment [NP5]: Standard table please

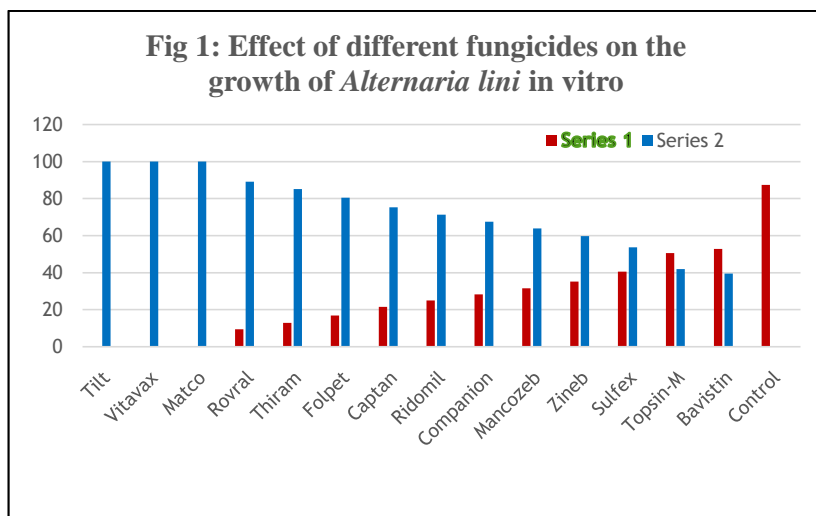
SI NO.	Location	Variety	Disease Intensity (%)
1.	CSA University Research Farm, Nawabganj	Shekhar	15.0
		T-397	20.0
		Sweta	30
2.		<b>Neelum</b>	<b>45</b>
		Neela	30-40
		<b>Rashmi</b>	<b>10</b>
3.	NARP Research Farm, Dillip Nagar, Kanpur Dehat	Shekhar	10-15
		T-397	15-20
4.	RRS, Bharari, Jhansi	JL-66	20-25
		Shekhar	15
5.	Crop Research Station, Mauranipur, Jhansi	Shekhar	15-20
		<b>Rashmi</b>	<b>10</b>
		Sweta	30
6.	Crop Research Farm, Gursharai, Jhansi	Shekhar	15
		Rashmi	10
7.	RRS, Bilatal, Hamirpur	Parvati	10-15
		Sharda	15-20

8.	KVK Farm, Bohatpurajalaun	JL-66	15-20
9.	KVK Farm, Hamirpur	Mau Alsi-1	20
		Mau Alsi-2	15-20
10.	KVK Farm, Mahoba	Mau Alsi-1	20
		Mau Alsi-2	15-20

***In vitro* potency of chemical fungicides over pathogen growth inhibition:**

In order to find suitable fungicides effective against the pathogen *Alternaria lini*, 14 fungicides (mentioned in materials & methods) from various groups were evaluated in the laboratory against the pathogen. The experiment was conducted in Petri plates of 90 mm diameter using Potato dextrose agar medium of two percent concentration and Food Poisoning technique (Schmitz, 1930). Three times each treatment was carried out. As a control, culture medium without any fungicide was used. The inoculated Petri dishes were then kept to be cultured for 7 days at  $25 \pm 2^\circ\text{C}$ , with radial growth data recorded independently for each treatment. The potency of fungicides were determined by the percentage inhibition of fungal growth compared to the control. Recorded data were analysed statistically and mentioned in the **table-4**. The results in table-4 illustrated that each fungicide examined was significantly more effective in inhibiting *Alternaria lini* growth over control. Three fungicides i.e. **Tilt, Vitavax, and Matco** were shown to have more potential out of 14 fungicides examined in fully inhibiting the growth of pathogen. Rovral, Thiram, Folpet, Captan, Ridomil, Companion, Mancozeb, Zineb, Sulfex, Topsin-M, and Bavistin were the remaining fungicides in order of their inhibitory activity against the pathogen.

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Table-4: Potency of fungicides over growth inhibition of *Alternaria lini* in vitro after 7 days of incubation at 25±2°C

Series 1- Average Diameter of Fungal Colony (mm)

Series 2- Percent inhibition over control

SI NO.	Fungicides tested	Dose(ppm)	Average diameter of fungal colony(mm)	Percent inhibition over control
1.	Tilt	2000	0.00	100
2.	Vitavax	2000	0.00	100
3.	Matco	2000	0.00	100
4.	Rovral	2000	9.55	89.064
5.	Thiram	2000	13.03	85.079
6.	Folpet	2000	17	80.533
7.	Captan	2000	21.55	75.323
8.	Ridomil	2000	25	71.372
9.	Companion	2000	28.4	67.433
10.	Mancozeb	2000	31.55	63.872
11.	Zineb	2000	35.17	59.727
12.	Sulfex	2000	40.5	53.624
13.	Topsin-M	2000	50.67	41.978
14.	Bavistin	2000	52.85	39.482

15.	Control	2000	87.33	
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## Discussion & Conclusion

### Survey for assessing prevalence and severity of disease

In the Rabi season in 2021-2022, the disease was found in a severe form in Kanpur and other parts of Uttar Pradesh. Among various cultivars under study, variety Neelum had the highest disease intensity which is 45%, while the variety Rashmi had the lowest disease intensity which is 10%. Intensity of Black Bud Disease was found to be ranged from 15 to 30% in Kanpur's adjacent Linseed growing area. During the survey, Alternaria blight (leaf spot and black bud disease) was found to be more severe at the pod bearing stage in the months of March and April. All of aerial components of plants get diseased. Patches of small, dark brown, uneven spots developed, eventually covering the stem. The leaves eventually dried out and curled up. The most prevalent symptoms of disease appeared on buds and flowers. The disease can be characterised by the formation of dark brown patches near the bottom of the calyx that enlarge and darken in colour and spread throughout the bud, eventually reaching the pedicel. The petals and other floral elements entirely shrivelled and on the pedicel, a dark-coloured fungus mycelial growth ring appeared. With an outward jolt, the pedicel usually get separated from this point in the mature plant.

### *In vitro* potency of chemical fungicides over pathogen growth inhibition:

In order to find the suitable fungicide effective towards inhibiting the growth of the pathogen *Alternaria lini*, 14 fungicides i.e. Tilt, Vitavax, Matco Rovral, Thiram, Folpet, Captan, Ridomil, Companion, Mancozeb, Zineb, Sulfex, Topsin-M and Bavistin from various groups were evaluated in the laboratory against the pathogen. Each of the fungicide were examined at the concentration of 0.2% by mixing in two percent potato dextrose agar medium by using the technique of Food Poisoning (Schmitz, 1930). Each of the fungicide was found to be significantly effective in inhibiting *Alternaria lini* growth over control. Three fungicides i.e. Tilt, Vitavax, and Matco were found to have more potency out of 14 fungicides examined in fully inhibiting the growth of fungal pathogen i.e. Percent inhibition over control was found to be 100% in case of these three fungicides with average diameter of fungal colony growth zero mm. Rovral, Thiram, Folpet, Captan, Ridomil, Companion, Mancozeb, Zineb, Sulfex, Topsin-M and Bavistin were the remaining fungicides in order of their inhibitory activity against the pathogen. Similar result was obtained by Holi, S. K. and Meena, S. (2015).

**Comment [NP11]:** A. lini

**Comment [NP12]:** Conclusion have not been conveyed explicitly addressing the objectives

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