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

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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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

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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 Neelum had 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*. 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.

1. 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 pedicle 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 Uttarpradesh during the month of March-April, 2022 to assess the disease prevelence 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*. Considering the economic importance, seriousness of the disease and absence of suitable resistance genotypes of the crop, chemical method of control is a dependable method to control this disease.

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

> Percent leaf /bud infected or diseased = Sumof numerical rating / Total number of bud/Leaf observed x 5 *100

2. MATERIALS AND METHODS

2.1 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, Dillip Nagar,Kanpur Dehat; RRS, Bharari, Jhansi; Crop Research Station, Mauranipur, Jhansi; Crop Research Farm, Gursharai, Jhansi; RRS, Bilatal, Hamirpur; KVK Farm, Bohatpura jalaun; KVK Farm, Hamirpur; KVK Farm, Mahoba during the month of March-April, 2022 for leaf spot and black bud disease caused by *A. 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 scales mentioned in Table 1. In five selected plants, total buds infected were recorded, and infested leaf area was recorded in ten selected leaves.

2.2 *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 [1]. For preparation of 0.2 percent fungicide formulation; 500mg of each fungicide was dissolved in small quantity of two percent potato dextrose agar medium and then the volume was made up to 250ml and the formulation was thoroughly mixed by shaking. Two percent potato dextrose agar medium was prepared by boiling 200g peeled potatoes in 1000ml of distilled water followed by mixing of 20 g dextrose and 20 g agar-agar in the filtered potato effluent. Then the medium was sterilized under autoclave at 1.1 kg/cm² pressure for 20 minutes at 121.6°C temperature prior to pouring into the Petri Plates. Before putting the mixture into the Petri Plates, they were sterilized under hot air oven at 160^0C for one hour. 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 concerned pathogen in laminar air flow cabinet under aseptic condition. 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 inoculated Petri Plates were done in BOD (Biological Oxygen Demand) incubator at the temperature of 25 ± 2^0C 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 [2] the percent inhibition over control was calculated by using following formula.

Percent inhibition over control = $\frac{C-T}{C}X100$

Where;

C = Growth of fungus in control,
T = Growth of fungus in different treatments

3. RESULTS

3.1 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 inTable-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 %.

3.2 *In vitro* Potency of Chemical Fungicides over Pathogen Growth Inhibition

In order to find suitable fungicides effective against the pathogen *A. 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 [1]. 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±2ºC, 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 *A. 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.

Table 1. Alternaria leaf spot of linseed disease severity ratings

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

Table 2. Active ingredients and doses of fungicides used in experiment

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

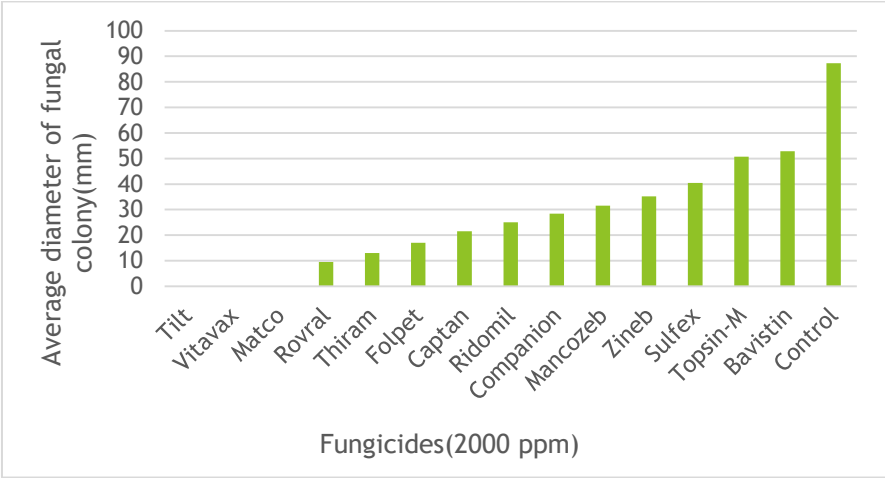

Fig. 1. Effect of different fungicides on average diameter of fungal colony (mm) of *A. lini* *in vitro*

Table 3. Disease intensity of black bud disease in different cultivars at adjoining area of Kanpur, Uttar Pradesh

Sl no.	Location	Variety	Disease Intensity (%)
1.	CSA University Research Farm, Nawabganj	Shekhar	15.0
		T-397	20.0
		Sweta	30
2.	Oilseed Research Farm,Kalyanpur	Neelum	45
		Neela	30-40
		Rashmi	10
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
		Rashmi	10
		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

Table 4. Potency of fungicides over growth inhibition of *A. lini in vitro* after 7 days of incubation at 25±2ºC

Sl 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	

4. DISCUSSION AND CONCLUSION

4.1 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

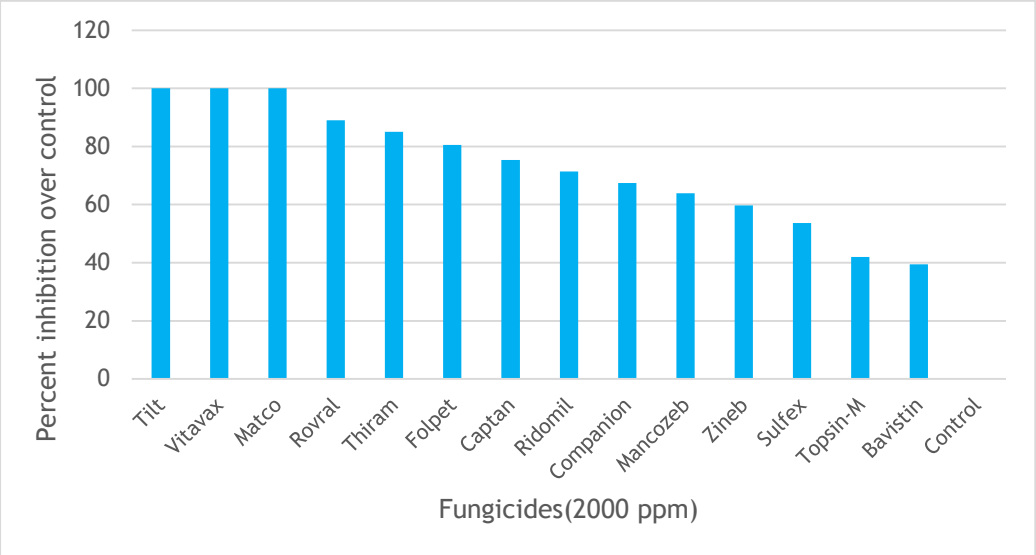

Fig. 2. Effect of different fungicides on percent inhibition over control of *A. lini in vitro*

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.

4.2 *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 *A. 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 [1]. Each of the fungicide was found to be significantly effective in inhibiting *A. 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. The results indicated that these three fungicides are proved to be superior over other 11 treatments. Rovral (89.064), Thiram (85.079), Folpet (80.533), Captan (75.323), Ridomil (71.372), Companion (67.433), Mancozeb (63.872), Zineb (59.727), Sulfex(53.624), Topsin-M (41.978) and Bavistin (39.482) were the remaining fungicides in decreasing order of their inhibitory activity against the pathogen. Among these 11 fungicides; Rovral, Thiram, Folpet, Captan, Ridomil, Companion, Mancozeb, Zineb and Sulfex were proved to be potential in effectively inhibiting the fungal growth to more than 50 percent in laboratory condition. But rest of the fungicides such as Topsin-M and Bavistin were not proved that much potential in inhibiting the pathogen growth as it reduced the pathogen growth to less than 50 percent in laboratory condition and considered as less effective in *in vitro*. Amisha et al. [3] conducted similar study on inhibition of *A. lini* growth and found that Propiconazole 25 EC proved the best at all the concentrations (100, 150 and 200 ppm) as it showed 100% of mycelial growth inhibition over control. Brahmankar et al. [4] found that Captan 75% WP, Carbendazim 50% WP and Thiophanate methyl 70% WP were found effective against *A. lini* growth inhibition and showed 72.37%, 22.15% and 12.26% growth inhibition respectively which is in accordance with the present investigation. Holi and Meena [5] also found the similar result that Propiconazole

(0.1%) completely inhibited the growth of *A. lini* in *in vitro* and was found significantly superior over the rest of fungicides; Rovral, Copper oxychloride, Dithane M-45, Propiconazole, Difenconazole, Carbendazim and Topsin in *in vitro* condition. Yadav et al. [6] tested various plant protection chemicals and found that Rovral@ 0.2% and mancozeb@ 0.2% were found most effective. Propiconazole@ 0.1% and hexaconazole@ 0.1% were found to be effective in reducing severity of Alternaria blight. Iprodione, Propiconazole, Hexaconazole, Carbendazim 0.10%, Capton 0.20% have been proved to manage the disease economically.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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