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In vitro and in vivo evaluation of selected fungicides against *Alternaria* blight of rapeseed

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Abstract
Under *in vitro* conditions, six fungicide combinations were selected with three different doses analyzed using the poison food technique. In which, Mancozeb 75 WP, Mancozeb 63% + Carbendazim 12% WP and Mancozeb 66.7% + Azoxystrobin 8.3% WP were found best in restricting the mycelium growth completely in all three doses. Under *in vivo* conditions, all the six selected fungicide combinations were significantly effective in controlling the intensity of *Alternaria* blight over control. The minimum% disease intensity was recorded for Carbendazim 50%WP @ 0.2%. While the highest 1000 seed weight and grain yield were recorded for Mancozeb 66.7% + Azoxystrobin 8.3% WP @ 0.05% with 3.88 g and 13.50 kg/ha respectively which is significant over all other treatments.

Keywords: *Alternaria brassicae*, leaf blight, *brassica campestris*, rapeseed, fungicide

Introduction
Rapeseed crops belong to the family cruciferae and genus *Brassica*. Rapeseed (*Brassica campestris*) commonly known as "*Toria*" is herbaceous annual plant shorter than mustard (*Rai*) between 45-150 cm. In India, rapeseed is produced during winter around and needs temperatures between 25 and 30 degrees celsius, low humidity, and essentially no rain, particularly during flowering. Next to China and then Canada, India is the third world's largest producer of rapeseed. Rapeseed is grown on 77.62 lakh acres of land in India, accounting for 19.8% of world production and 9.8% of area. Assam, Bihar, Orissa, and West Bengal are the primary rapeseed-producing states in the nation. In Chhattisgarh, total area under rapeseed cultivation is 0.170 lakh ha while production is 26999 metric tonnes. In Bastar district, rapeseed crops was grown in 0.99 ha among total oilseeds area and production was 0.61 metric tons in Bastar plateau. Bastar district has 13th position in area and 9th position in production of rapeseed in the state. In Chhattisgarh, the major growing districts of rapeseed are Bastar, Narayanpur, Kondagaon, Dantewada, Bijapur, Bemetara, Rajnandgaon, Surajpur, Raigarh and Ambikapur. (Anonymous, 2019. Directorate of Agriculture Chhattisgarh portal) [1]. Rapeseed is susceptible to foliar diseases. There are 42 fungal infections that cause disease in rapeseed species and *Alternaria* blight is one of the most widespread and damaging diseases of cruciferous crops in India. *Alternaria* blight induced by *Alternaria brassicae* (Berk) Sacc. survives in infected debris, most likely as mycelium, and is seed borne as conidia and mycelium on the exterior of the seed coat.

Likewise, *in vitro* assessment can be used when comparing the effectiveness of fungicides. It may further be more useful to know the actual concentration of the newly developed fungicides recently available on the market with regard to improved fungicidal efficiency against the *Alternaria brassicae* isolates of the rapeseed. The *in vitro* evaluation of *Alternaria brassicae* isolates is a method of laboratory bioassay for determining the efficiency of fungitoxic chemicals and their combinations as chemicals successful in suppressing the *Alternaria* blight (Meah *et al*., 1988 and Howlidar *et al*., 1985) [12, 6]. *In vitro* and *in vivo*, Mancozeb + Carbendazim (0.2%) was shown to be most effective in inhibiting mycelial growth and decreasing disease severity. All fungicides suppressed *Alternaria brassicae* mycelial growth *in vitro* compared to control. At 100 and 150 ppm, Mancozeb + Carbendazim have been shown to be significantly superior to Captan + Hexaconazole in terms of fungal growth suppression (Meena *et al*., 2022) [14]. Mancozeb and carbendazim induced 100% suppression of *A. brassicae* fungal isolates. They are known as the best disease control, followed by Captan + Hexaconazole, all of which found that mancozeb alone proved to be superior in lowering disease intensity according to Wagh *et al*. (2017) [20], Chadar *et al*. (2016) [3], Kumar and Rathi (2018) [10], and Choudhary *et al*. (2018) [4].

Materials and methods

In vitro evaluation of selected fungicides against *Alternaria brassicae*

The experiment was conducted at Plant Pathology laboratory of Shaheed Gundadhoor College of Agriculture and Research Station, Jagdalpur (C. G) using poisoned food technique (Schimitz, 1930). Six chemical fungicides *viz*., Mancozeb 75 WP, Carbendazim 50%WP, Chlorothalonil 75% WP, Propineb 70% WP, Mancozeb 63% + Carbendazim 12% WP and Mancozeb 66.7% + Azoxystrobin 8.3% WG were evaluated at three different concentrations, each named by Dose I, Dose II, and Dose III (Table 1) against *Alternaria brassicae* causing blight disease in rapeseed crop. Mycelial growth was examined at 3 and 6 DAI (days after inoculation). Each treatment was repeated five times.

The amount of chemicals required for three doses was measured and aseptically added into a 150 ml flask containing 100 ml sterilized PDA medium. The flask containing PDA medium and chemicals was shaken well to ensure proper mixed solution of poisoned medium. Then, each 20 ml dose was discretely poured into five sterilized petri plates serving as five replications under a laminar flow chamber and allowed to solidify. Five petri plates without fungicide aided as control were also maintained. A 7 mm mycelial disc from a 7-9 days old culture of *Alternaria brassicae* using sterilized cork borer was inoculated in the centre of petri plates with solidified poisoned medium and also in control petri plates. After inoculating all petri plates were incubated at 27±1 °C and the observations of radial growth of mycelia in millimeters (mm) of each treatment at different doses were recorded at 3 DAI and 6 DAI. Collected data were analyzed with Completely Randomized Design (CRD) for significant differences.

In vivo evaluation of selected fungicides against *Alternaria* leaf blight disease

The efficacy of all six chemical fungicides *viz.*, Mancozeb 75%WP, Carbendazim 50%WP, Chlorothalonil 75% WP, Propineb 70% WP, Mancozeb + Carbendazim 63%WP and Mancozeb + Azoxystrobin 66% WP (Table 1) were evaluated against *Alternaria* leaf blight disease of rapeseed (variety Indira Toria-1) at dose II under *in vivo* conditions after evaluation under *in vitro* conditions at different doses. Each treatment was repeated four times in Randomized block design (RBD). Two foliar sprays of fungicides were done at 15 days intervals. The observations on% disease severity were recorded after 10 days of the first and second spray of fungicides. The weight of 1000 seeds (g) and the grain yield (kg/ha) of each plot of different treatments were also taken into account. Collected data were analyzed with Randomized Block Design (RBD) for significant differences.

Results and discussion

In vitro evaluation of selected fungicides against *Alternaria brassicae*

In the present investigation, six fungicides were evaluated *in vitro* against *Alternaria brassicae* by food poison technique each fungicide with low (Dose I), optimum (Dose II) and high (Dose III) doses (Table 1) than recommended dose of fungicides and the results were recorded at 3 and 6 days after inoculations (DAI).

Dose I

Data obtained is shown in Table 2 Fig. 1 and Plate 1 that the mycelial growth of *A. brassicae* on potato dextrose agar (PDA) media poisoned with Mancozeb (Dhanuka M- 45) @ 0.15%, Mancozeb + Carbendazim (Turf) @ 0.15%, Mancozeb + Azoxystrobin (Delma) @ 0.025% was significant completely checked (0.00 mm) at 3 and 6 DAI. Whereas, Chlorothalonil (Kavach) @ 0.2%, Propineb (Antracol) @ 0.2% and Carbendazim (Bavistin) @ 0.15% fungicides were also significantly reduced the mycelial growth recorded as 23.80 mm, 28.40 mm, 31.00 mm radial growth at 3 DAI and 25.40 mm, 52.00 mm, 63.80 mm radial growth at 6 DAI, respectively over the control (without fungicides) recorded as 54.40 mm and 86.40 mm mycelial growth respectively at 3 and 6 DAI.

Table 1: Evaluation of new combinations of fungicides at different doses against *Alternaria brassicae* under *in vitro* and *in vivo*

Treatments		Dose/l. of PDA media (%) under *in vitro*			Dose/l. water (%) under *in vivo*
		Dose I	Dose II	Dose III	
T_1	Mancozeb 75% WP	0.15	0.20	0.25	0.20
T_2	Carbendazim 50% WP	0.15	0.20	0.25	0.20
T_3	Chlorothalonil 75% WP	0.2	0.25	0.3	0.25
T_4	Propineb 70% WP	0.2	0.25	0.3	0.25
T_5	Mancozeb 63% + Carbendazim 12% WP	0.15	0.20	0.25	0.20
T_6	Mancozeb 66.7% + Azoxystrobin 8.3% WG	0.025	0.05	0.1	0.05
T_7	Control (Without fungicide)				

Dose II

Data obtained is shown in Table 2, Fig. 1 and Plate 1 that the mycelial growth of *A. brassicae* on potato dextrose agar (PDA) media poisoned with Mancozeb (Dhanuka M- 45) @ 0.20%, Mancozeb + Carbendazim (Turf) @ 0.20%, Mancozeb + Azoxystrobin (Delma) @ 0.05% was significant completely checked (0.00 mm) at 3 and 6 DAI. Whereas,Chlorothalonil (Kavach) @ 0.25%, Propineb (Antracol) @ 0.25% and Carbendazim (Bavistin) @ 0.20% fungicides were also significantly reduced the mycelial growth recorded as 11.80 mm, 20.60 mm, 22.60 mm radial growth at 3 DAI and 22.40 mm, 32.80 mm, 56.00 mm radial growth at 6 DAI, respectively over the control (without fungicides) recorded as 53.80 mm and 87.80 mm mycelial growth respectively at 3 and 6 DAI.

Table 2: *In vitro* evaluation of new fungicide combinations at different doses against *Alternaria brassicae*

Treatments	Mycelial growth in mm					
	Dose I		Dose II		Dose III	
	3 DAI	6 DAI	3 DAI	6 DAI	3 DAI	6 DAI
T_1: Mancozeb 75% WP	00.00	00.00	00.00	00.00	00.00	00.00
T_2: Carbendazim 50% WP	31.00	63.80	22.60	56.00	8.20	24.20
T_3: Chlorothalonil 75% WP	23.80	25.40	11.80	22.40	6.80	18.00
T_4: Propineb 70% WP	28.40	52.00	20.60	32.80	10.80	26.00
T_5: Mancozeb 63% + Carbendazim 12% WP	00.00	00.00	00.00	00.00	00.00	00.00
T_6: Mancozeb 66.7% + Azoxystrobin 8.3% WG	00.00	00.00	00.00	00.00	00.00	00.00
T_7: Control (Without fungicide)	54.40	86.40	53.80	87.80	54.80	88.40
SEm±	0.59	0.85	0.83	0.89	0.50	1.04
CD(p=0.05)	2.35	2.48	1.71	2.59	1.46	3.03
C.V. (%)	9.05	5.85	8.42	6.98	9.74	10.45

Dose III

Data obtained is shown in Table 2, Fig. 1 and Plate 1 that the mycelial growth of *A. brassicae*on potato dextrose agar (PDA) media poisoned with Mancozeb (Dhanuka M- 45) @ 0.25%, Mancozeb + Carbendazim (Turf) @ 0.25%, Mancozeb + Azoxystrobin (Delma) @ 0.1%was significant completely checked (0.00 mm) at 3 and 6 DAI. Whereas,Chlorothalonil (Kavach) @ 0.3%, Propineb (Antracol) @ 0.3% and Carbendazim (Bavistin) @ 0.25% fungicides were also significantly reduced the mycelial growth recorded as 6.80 mm, 10.80 mm, 8.20 mm radial growth at 3 DAI and 18.00 mm, 26.00 mm, 24.20 mm radial growth at 6 DAI, respectively over the control (without fungicides) recorded as 54.80 mm and 88.40 mm mycelial growth respectively at 3 and 6 DAI.

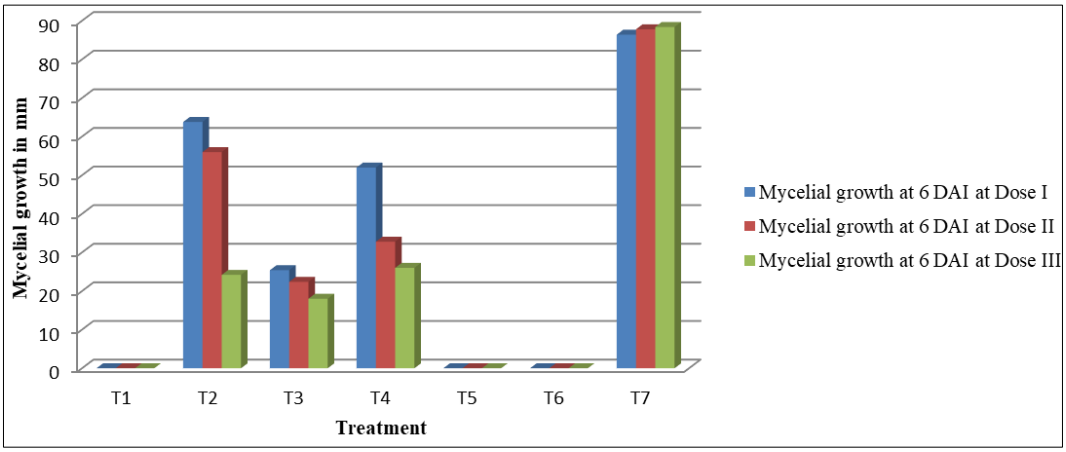

Fig 1: Mycelial growth of *Alternaira brassicae* on PDA media poisoned with different doses of selected fungicide

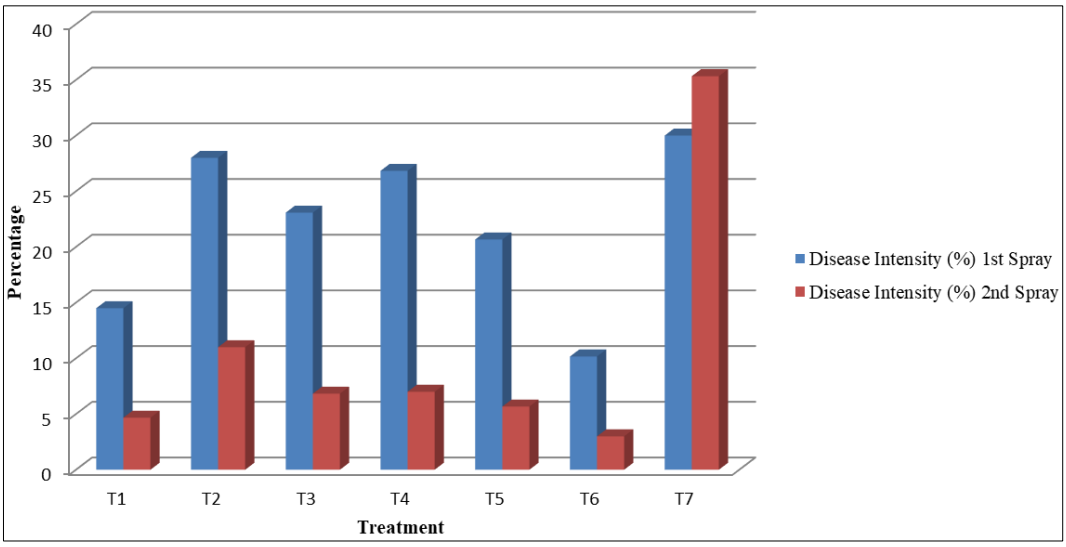

Fig 2: *In vivo* evaluation of selected fungicides against *Alternaria* blight of rapeseed

The findings from the present investigation agrees with the findings of Meena *et al*. (2004) [13] and Wagh *et al.* (2017) [20] who concluded that mancozeb and carbendazim was effective in 100% inhibition in mycelia growth of *A. brassicae*. Kumar

et al. (2004) found that among 6 fungicides, Dithane M-45 (Mancozeb) was superior and the best result against inhibiting *Alternaria brassicae*. Karthikeyan *et al*. (2021) [8] Tebuconazole also reported that the most effective chemical fungicide, inhibiting fungal growth 100% at all doses tested, followed by Mancozeb at 1000 ppm (96.05) and Metalaxyl + Mancozeb at 1000 ppm (92.10), but 50 ppm Chlorothalonil was the least efficient in suppressing fungal growth (53.94). Similarly, Panwar *et al*. (2013) [15] found that tebuconazole completely inhibited *Alternaria* growth followed by Mancozeb while Carbendazim had the least suppression. Biswas and Ghosh (2018) [2] and Kantwa *et al*. (2014) [7] were also reported that mancozeb had a strong growth inhibitory molecule for *Alternaria* sp. Thaware *et al*. (2010) [19] discovered a similar inhibition in *Alternaria*. Synthetic fungicides suppress pathogens by damaging their cell membranes orpermeability, or by blocking the pathogen's metabolic activities, and hence are effective. Saha (1989) [16] stated that Mancozeb (Dithane M-45) was most effective against *A. brassicae in vitro* under five fungicides study. Hossain *et al*. (2006) [5] performed an experiment in which Iprodione (Rovral 50 WP) and Mancozeb (Dithane M-45) was significant superior to check the mycelia growth of *Alternaria*. Kumar *et al*. (2004) studied six fungicides and one antagonist and found that Mancozeb (Dithane M-45) showed the best results in limiting spore germination of *A. brassicae*.

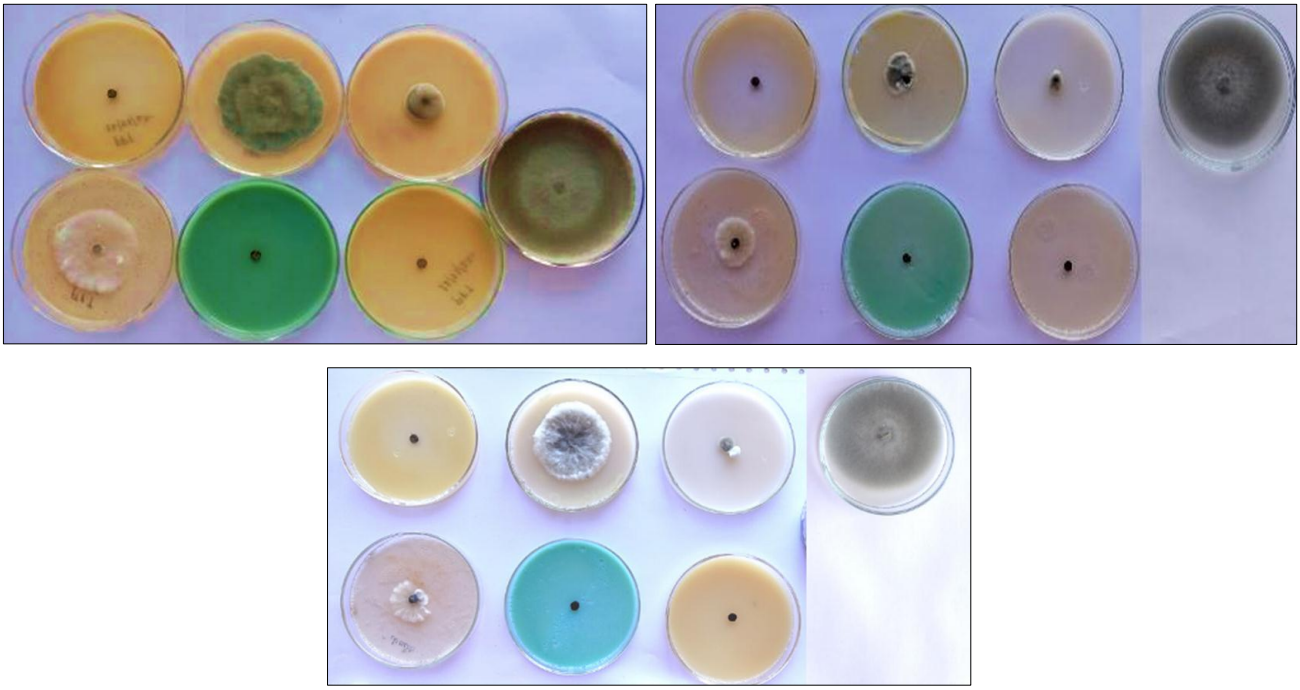

Plate 1: Mycelial growth of *Alternaria brassicae* on PDA media poisoned with different doses of selected fungicides

In vivo evaluation of selected fungicides against *Alternaria* leaf blight disease

In Rabi 2021-22, efficacy of selected fungicides was studied against *Alternaria blight* of rapeseed crop under *in vivo* conditions. The results in Table 3, Plate 2 and fig 2 revealed that all tested six fungicides were significantly effective in reducing the intensity of *Alternaria* blight after 1st and 2nd foliar spray of selected fungicides and also increased grain yield over the treatment of control (without any spray).

Plate 2: *In vivo* evaluation of selected fungicides against *Alternaria* blight of rapeseed

Table 3: *In vivo* evaluation of selected fungicides against *Alternaria* blight of rapeseed

Treatments	Dose/l. of water (%)	Disease Intensity (%)		1000 Seed weight (g)	Grain yield (q/ha)
		1st Spray	2nd Spray		
T_1: Mancozeb 75% WP	0.20	14.50	4.67	3.48	11.95
T_2: Carbendazim 50% WP	0.20	28.00	11.00	3.77	8.85
T_3: Chlorothalonil 75% WP	0.25	23.08	6.83	3.84	6.65
T_4: Propineb 70% WP (Antracol)	0.25	26.83	7.00	3.83	7.75
T_5: Mancozeb 63% + Carbendazim 12% WP	0.20	20.67	5.67	3.45	11.15
T_6: Mancozeb 66.7% + Azoxystrobin 8.3% WG	0.05	10.17	3.00	3.88	13.50
T_7: Control (Without fungicide)		30.00	35.33	3.28	5.62
SEm±		0.91	0.37	0.26	0.08
CD(p=0.05)		2.82	1.14	NS	0.25
C.V. (%)		7.16	6.03	12.28	1.78

***Alternaria* leaf blight disease intensity**

At 15 DAS**,** Table 3, Plate 2 and fig 2 showed that during the 1st foliar spray of selected fungicides, the disease intensity of *Alternaria* leaf blight in rapeseed crop was significantly lowest recorded in the foliar spray ofMancozeb + Azoxystrobin (Delma) @ 0.05% with 10.17% followed by Mancozeb (Dhanuka M- 45) @ 0.20%with 14.50%, Mancozeb + Carbendazim (Turf) @ 0.20% with 20.67%,Chlorothalonil(Kavach)@ 0.25% with 23.08%, Propineb (Antracol) @ 0.25%with26.83%, Carbendazim (Bavistin) @ 0.20% with 28.00% over the highest disease intensity was recorded in control (Without fungicide)as 30.00%.Data at 2nd foliar spray of fungicides and its combinations revealed that among selected fungicides, lowest disease intensity or maximum inhibition of *Alternaria* leaf blight was recorded in the foliar spray of Mancozeb + Azoxystrobin (Delma)@ 0.05% with 3.00% followed by Mancozeb (Dhanuka M- 45) @ 0.20% with 4.67%, Mancozeb + Carbendazim (Turf) @ 0.20% with 5.67%, Chlorothalonil(Kavach)@ 0.25% with 6.83%, Propineb (Antracol) @ 0.25% with 7.00%, Carbendazim(Bavistin)@ 0.20% with 11.00% over the highest disease intensity was recorded in control (Without fungicide)as 35.33%. In the present study Mancozeb + Azoxystrobin(Delma) @ 0.05% after 1st and 2nd spray was found to be most effective under field conditions. Similarly, Kumar*et al*., (2019) [9] recorded Azoxystrobin to be highly proficient in controlling foliar pathogen like *Alternaria* as compared to conventional pesticides available in the market. Consequently, the later best fungicide Mancozeb (Dhanuka M-45) @ 0.2% was found statistically at par with Mancozeb + Azoxystrobin(Delma) @ 0.05% in regard to reduction in disease intensity%.

1000 Seed weight

The data on 1000 seed weight were obtained from all treatment plots of rapeseed crop Table 3 and found highest seed weight from the plots treated with Mancozeb + Azoxystrobin (Delma) fungicide @ 0.05% was 3.88 g followed by Chlorothalonil (Kavach) @ 0.25% with 3.84 g per, Propineb (Antracol) @ 0.25% with 3.83 g per, Carbendazim (Bavistin) @ 0.20% with 3.77 g per, Mancozeb (Dhanuka M- 45) @ 0.20% with 3.48 g per, Mancozeb + Carbendazim (Turf) @ 0.20% with 3.45 g per whereas lowest seed weight of 3.28 g per 1000 seed weight was recorded in plots of without fungicides spray (control). All the treatments independently gave significantly higher weight of 1000 seed over the control treatment.

4.4.3 Grain yield

Data of grain yield from all treatments was summarized in Table 3 and data revealed that Mancozeb + Azoxystrobin (Delma) fungicide @ 0.05% spray was proved to give highest yield (13.50 q/ha) as well as less outbreak of leaf blight and other diseases followed by Mancozeb (Dhanuka M- 45) @ 0.20% (11.95 q/ha), Mancozeb + Carbendazim (Turf) @ 0.20% (11.15 q/ha), Carbendazim (Bavistin) @ 0.20% (8.85 q/ha), Propineb (Antracol) @ 0.25% (7.75 q/ha) and Chlorothalonil (Kavach) @ 0.25% (6.65) over control (5.62 q/ha).

The present outcome corroborated the findings of Singh (2011) who found that the mycelial growth of *Alternaria* was significantly reduced in Amistar (Azoxystrobin) fungicide incorporated media under *in vitro*. It was also superior in the field condition to reduce the *Alternaria* disease pressure with the sprays of Amistar (Azoxystrobin) @ 0.1% at 3 times with 2.33% disease severity compared to 43.33% in the control plot.

Amistar (Azoxystrobin) was also the most successful in lowering severity of disease under natural conditions, according to Sidlauskiene *et al*. (2001) [17]. Kumar *et al*. (2019) [9] shows that 1000 grains from Amistar (Azoxystrobin) (5.83 g) had the highest grain weight of 1000 grains, followed by Tilt (Propiconazole) (5.83 g). The weight of 1000 grains of Indofil M-45 (Mancozeb) and Colt (Mancozeb + Carbendazim) was 4.96 g and 4.90 g, respectively, which was similar to Control (4.76 g). Average yield per hectare, the yield potential for each treatment was computed in terms of hectares. The largest yield was reported in Amistar at 2536.21 kg/ha, followed by Indofil M-45, Tilt, and Colt at 2079.17, 1959.13, and 1671.49 kg/ha, respectively, all of which were greater than the Control (1375.11 kg/ha). Singh *et al*. (2018) had comparable effects with Mancozeb on rapeseed.

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