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In vitro evaluation of new fungicide combinations against *Alternaria brassicae* causes leaf blight of rapeseed

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Abstract
Rapeseed crops belong to the family Cruciferae and genus *Brassica*. *Alternaria* blight of rapeseed is caused by *Alternaria brassicae* (Berk.) Sacc. is a well-known destructive disease worldwide. An experiment was conducted *in vitro* to evaluate the effectiveness of fungicide combinations at each with three different doses against *Alternaria brassicae*.
Among six new fungicides combinations, Mancozeb (@ 0.15%, 0.20% and 0.25%), Mancozeb + Carbendazim (@ 0.15%, 0.20% and 0.25%), and Mancozeb + Azoxystrobin (@ 0.25%, 0.05% and 0.1%) fungicides were significantly superior to completely check (0.00 mm) the mycelial growth of *Alternaria brassicae* followed by Chlorothalonil (@ 0.2%, 0.25% and 0.3%) Propineb (@ 0.2%, 0.25% and 0.3%) and Carbendazim (@ 0.15%, 0.20% and 0.25%) fungicides were also significantly reduced the mycelial growth of *Alternaria brassicae* at all three different doses of fungicides.

Keywords: *Alternaria brassicae*, leaf blight, rapeseed, *Brassica juncea*, fungicides

Introduction
Rapeseed crops belong to the family Cruciferae and genus *Brassica*. Rapeseed (*Brassica juncea* L.) commonly known as "Toria" is herbaceous annual plant shorter than mustard (Rai) between 45-150 cm. Rapeseed seed oil meal may contain anti-nutritional factor such as goitrogen (thioglycoside or glucosinolate), tannic acid, erucic acid, sinapine (cholinesterase), pectin and oligosaccharide. Rapeseed seeds have high energy content, having 42-45% oil with relatively high protein content (42-45%). Rapeseed seed oil has less than 2% erucic acid which is responsible for causing heart lesions and solid components of glucosinolates less than 30 micromoles/g. Rapeseed oil also known as canola oil, is a rich source of omega-3 fatty acids. (NIN, 2021)
India is the largest rapeseed producing country in the world next to China followed by Canada. The total area under rapeseed in India is 77.62 lakh ha with globally 19.8% and 9.8% area and production respectively. The major rapeseed growing states in the country are Rajasthan, Haryana, Madhya Pradesh, Gujarat, Uttar Pradesh and Chhattisgarh.
In Chhattisgarh, total area under rapeseed cultivation is 0.170 lakh ha while production is 26999 metric tonnes. In Bastar district, rapeseed crops were 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 (Anonymous, Directorate of Agriculture, 2019). In Chhattisgarh, the major growing districts of rapeseed are Bastar, Narayanpur, Kondagaon, Dantewada, Bijapur, Bemetara, Rajnandgaon, Surajpur, Raigarh and Ambikapur.
Huge demand for edible oil is still imported to fulfill the need of ever-growing household needs, which can be easily met by reducing the gap between crop's prospective and probable yield at farmer's field. A major responsible factor to this gap is unattended exposure of numerous biotic, mesobiotic and abiotic stresses. Major biotic stress of rapeseed is powdery mildew, downy mildew and *Alternaria* blight, in which *Alternaria* blight causes widespread and destructive disease. *Alternaria* blight is also called black spot (Louvet, 1958) [10]. This disease attacks all aerial parts of plants and leads to huge yield losses.
Alternaria blight of rapeseed is caused by *Alternaria brassicae* (Berk.) Sacc. is a well-known destructive disease worldwide. Characteristic symptoms of *Alternaria* blight are the formation of concentric ring targeted board like spots usually of brown color and are formed on leaves, stems and siliqua. Their characteristic symptoms may vary with differences with the host and

environmental factors. Initially the symptoms develop on the lower leaves as small spots, later enlarged to form prominent concentric spots. As the disease progresses, it causes lower leaf defoliation and disease appears on upper plant parts. Besides destruction of leaves and stem, it invades siliqua and causes severe infection which in result leads to shriveling of seeds, reduction in oil quantity and chemical composition of seed (Kaushik *et al*., 1984) [6].

With such caustic disease many scientists and research workers have come across chemicals being successful in suppressing *Alternaria* blight disease (Meah *et al*., 1988 and Howlidar *et al*., 1985) [11, 4]. The *in vitro* evaluation of *Alternaria brassicae* isolates is a method of laboratory bioassay in determining efficiency of fungitoxic chemicals and their combinations. One important aspect of *in vitro* assessment is to compare between fungicides effectiveness. Deliberating the above facts, present study was undertaken to evaluate the effect of selected new fungicides and its combinations in controlling growth of *Alternaria brassicae in vitro.*

Review of Literature

Karthikeyan *et al*. (2020) [5] conducted an experiment on *in vitro* examination of fungicides against leaf blight of mustard at five different concentrations *viz*., 50 ppm, 100 ppm, 250 ppm, 500 ppm and 1000 ppm in which concluded that Tebuconazole (100%) found to be most significant in reducing mycelia growth of *Alternaria brassicae followed* by Mancozeb (96.05 mm at 1000 ppm) and the least was Chlorothalonil (53.94 mm at 50 ppm).

Haider *et al*. (2013) [14] conducted a laboratory bioassay of four fungicides against *Alternaria brassicae* and observed minimum mycelia growth at 6 DAI, 9 DAI. 12 DAI and 15 DAI. Mancozeb (Dithane M- 45) @ 0.45% and Carbendazim (Bavistin DF) @ 0.1% were found to restrict the growth 2.2 cm () and 3.0 cm (15 DAI) respectively. Rovral 50 WP was the most effective of all.

Meena *et al*. (2021) conducted an experiment *in vitro* against *Alternaria brassicae* through use of five fungicides at three different concentrations *viz*., 50 ppm, 100 ppm and 150 ppm. Data revealed that Mancozeb + Carbendazim was most effective to inhibiting the growth of *Alternaria brassicae* at 50 ppm (94.32%), 100 ppm (100%) and 150 ppm (100%).

Rajvanshi *et al*. (2020) reported that out of six fungicides at three concentrations of each @ 0.05, 0.1 and 0.2 per cent against *Alternaria brassicae*. Tebuconazole 250 EC + Trifloxystrobin WG 75 and Propiconazole 25 EC were most effective at all concentrations whereas Propineb 70WP inhibited radial growth @ 0.05% (52.56 mm), 0.1% (43.00 mm) and 0.2% (30.84 mm).

Kumar *et al*. (2019) studied on four fungicides at 10 µg/ml, 25µg/ml, 50µg/ml, 75µg/ml and 100µg/ml concentration *in vitro* conditions. Result showed that Mancozeb (Indofil M-45) and Carbendazim (Colt) were moderately effective at each concentration stated as Mancozeb 10µg/ml (24.33 mm), 25µg/ml (16.33 mm), 50µg/ml (13.00 mm), 75µg/ml (12.00 mm) and 100µg/ml (8.66 mm) wherein Carbendazim 10µg/ml (25.33 mm), 25µg/ml (21.00 mm), 50µg/ml (18.00 mm), 75µg/ml (14.00 mm) and 100µg/ml (9.33 mm).

Saha *et al*. (1989) [17] stated that Dithane M- 45 inhibited the *Alternaria brassicae most* effectively *in vitro* conditions over five fungicides included in the study. Hossain *et al*. (2006) [3] conducted an experiment in which Rovral 50 WP significantly and Dithane M- 45 moderately inhibited *Alternaria* blight.

Kumar *et al*. (2004) [8] studied management consisting of six fungitoxic and one antagonist in which Dithane M- 45 revealed best results in restricting spore germination of *Alternaria brassicae*.

Khan *et al.* (2007) [9] and Singh *et al.* (2008) [19] concluded the efficiency of several fungicides namely Carbendazim 50%, Ridomil MZ 72 WP and Apron 35 SD and bioagent *viz*., *Trichoderma harzianum* and *Pseudomonas fluorescens*, as a single entity and also in combination against *Alternaria brassicae* which showed that Carbendazim 50% was efficient in inhibiting growth.

Materials and Methods

The experiment was conducted in the Plant Pathology Laboratory of Shaheed Gundadhur College of Agriculture and Research Station, Kumhrawand, Jagdalpur (C.G.) in Completely Randomized Design using poisoned food technique. Six chemical fungicides i.e., Mancozeb (Dhanuka M- 45), Carbendazim (Bavistin), Chlorothalonil (Kavach), Propineb (Antracol), Mancozeb + Carbendazim (Turf) and Mancozeb + Azoxystrobin (Delma) were evaluated at three different concentrations each named by Dose 1, Dose 2 and Dose 3 which were examined at 3 and 6 DAI (Days After Inoculation). Each of the treatments was replicated five times. Details of the fungicides used are mentioned in Table 1.

Table 1: *In vitro* evaluation of new fungicide combinations at different doses against *Alternaria brassicae*.

Treatments	Dose/l. of water (%)		
	Dose 1	Dose 2	Dose 3
T1: Mancozeb (Dhanuka M- 45)	0.15	0.20	0.25
T2: Carbendazim (Bavistin)	0.15	0.20	0.25
T3: Chlorothalonil (Kavach)	0.2	0.25	0.3
T4: Propineb (Antracol)	0.2	0.25	0.3
T5: Mancozeb + Carbendazim (Turf)	0.15	0.20	0.25
T6: Mancozeb + Azoxystrobin (Delma)	0.025	0.05	0.1
T7: Control (Without fungicide)			

Isolation and maintenance of pure culture

Plant material infected with *Alternaria brassicae* was visually examined and collected randomly from *Brassica juncea* cultivated field in Shaheed Gundadhur College of Agriculture and Research Station. Infected leaves samples were cut into pieces (8-10 mm) and surface sterilized with Mercuric chloride 0.1% for 30 seconds and washed thrice with distilled water and blot dried using sterilized filter paper. Thereafter the bits were placed in 90 mm sterilized Petri plates containing potato dextrose agar (PDA) medium under laminar flow chamber and incubated in BOD at 27±1 °C for seven days. Based on morphological characteristics the pathogen was identified by preparing slides under microscope as *Alternaria brassicae.* Then the culture was sub cultured by

transferring small discs (6 mm) of medium containing pathogen using sterilized cork borer to another Petri plate containing PDA medium and incubated at 27±1 °C for seven days. The pure culture obtained was preserved.

***In vitro* evaluation of chemical fungicides**

Six fungicides i.e., Mancozeb, Carbendazim, Chlorothalonil, Propineb, Mancozeb + Carbendazim and Mancozeb + Azoxystrobin were used at three different doses and evaluated under *in vitro* by Poisoned food technique (Schimitz, 1930) against *Alternaria brassicae*. The quantity of required chemicals for three doses were measured and added aseptically in 150 ml conical flask containing 100 ml of sterilized PDA medium. The flask with PDA medium and chemicals were shaken properly to ensure proper mixed solution of poisoned medium. Then each dose was poured 20 ml discreetly in five sterilized Petri plates serving as five replications under the laminar flow chamber and allowed to solidify. Five Petri plates without fungicide aided as control were also maintained. A mycelial disc of 7 mm of 7-9 days old culture of *Alternaria brassica* using sterilized cork borer was inoculated in the center of solidified poisoned medium Petri plates and without fungicide incorporated Petri plates as well. After inoculating all the Petri plates, they were incubated at 27±1 °C and the observations on radial growth of mycelia in millimeters (mm) of each treatment at different doses were recorded at 3 DAI and 6 DAI.

Results and Discussion

In the present investigation, six fungicides were evaluated against *Alternaria brassicae* by food poison technique each fungicide with three different doses (Table1) under *in vitro* and the results were recorded at 3 and 6 days after inoculations (DAI).

Data obtained is shown in Table 2, Figure 1 and Plate 1 that the mycelial growth of *A. brassicae* was significant completely checked (0.00 mm) in potato dextrose agar (PDA) media poisoned with Mancozeb (Dhanuka M- 45) @ 0.15%, Mancozeb + Carbendazim (Turf) @ 0.15%, Mancozeb + Azoxystrobin (Delma) @ 0.025% fungicides followed by 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 but not at par with Mancozeb (Dhanuka M- 45) @ 0.15%, Mancozeb + Carbendazim (Turf) @ 0.15%, Mancozeb + Azoxystrobin (Delma) @ 0.025% fungicides.

Data obtained is shown in Table 3, Figure 2 and Plate 2 that the mycelial growth of *A. brassicae* was significant completely checked (0.00 mm) in potato dextrose agar (PDA) media poisoned with Mancozeb (Dhanuka M- 45) @ 0.20%, Mancozeb + Carbendazim (Turf) @ 0.20%, Mancozeb + Azoxystrobin (Delma) @ 0.05% fungicides followed by 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 and 87.80 mm mycelial growth respectively but not at par with Mancozeb (Dhanuka M- 45) @ 0.20%, Mancozeb + Carbendazim (Turf) @ 0.20%, Mancozeb + Azoxystrobin (Delma) @ 0.05% fungicides.

Data obtained is shown in Table 4, Figure 3 and Plate 3 that the mycelial growth of *A. brassicae* was significant completely checked (0.00 mm) in potato dextrose agar (PDA) media poisoned with Mancozeb (Dhanuka M- 45) @ 0.25%, Mancozeb + Carbendazim (Turf) @ 0.25%, Mancozeb + Azoxystrobin (Delma) @ 0.1% fungicides followed by Chlorothalonil (Kavach) @ 0.3%, Carbendazim (Bavistin) @ 0.25% and Propineb (Antracol) @ 0.3% fungicides were also significantly reduced the mycelial growth recorded as 6.80 mm, 8.20 mm, 10.80 mm radial growth at 3 DAI and 18.00 mm, 24.20 mm, 26.00 mm radial growth at 6 DAI, respectively over the control (without fungicides) recorded as 54.40 mm and 86.40 mm mycelial growth respectively but not at par with Mancozeb (Dhanuka M- 45) @ 0.25%, Mancozeb + Carbendazim (Turf) @ 0.25%, Mancozeb + Azoxystrobin (Delma) @ 0.1% fungicides. Meena *et al*. (2004) and Wagh *et al*. (2017) [20] who concluded that mancozeb and carbendazim was effective in 100 per cent inhibition in mycelia growth of *A. brassicae*. Kumar *et al*. (2004) [8] founded that among 6 fungicides and 1 plant extract, Dithane M-45 (Mancozeb) was superior and the best result against inhibiting *Alternaria brassicae*.

Table 2: *In vitro* evaluation of new fungicide combinations at different doses against *Alternaria brassicae*. (Dose 1)

Treatments	Dose/l. of media (%)	Mycelial growth in mm	
		3 DAI	6 DAI
T1: Mancozeb	0.15	00.00	00.00
T2: Carbendazim	0.15	31.00	63.80
T3: Chlorothalonil	0.2	23.80	25.40
T4: Propineb	0.2	28.40	52.00
T5: Mancozeb + Carbendazim	0.15	00.00	00.00
T6: Mancozeb + Azoxystrobin	0.025	00.00	00.00
T7: Control (Without fungicide)		54.40	86.40
S.Em±		0.59	0.85
CD(p=0.05)		2.35	2.48
C.V. (%)		9.05	5.85

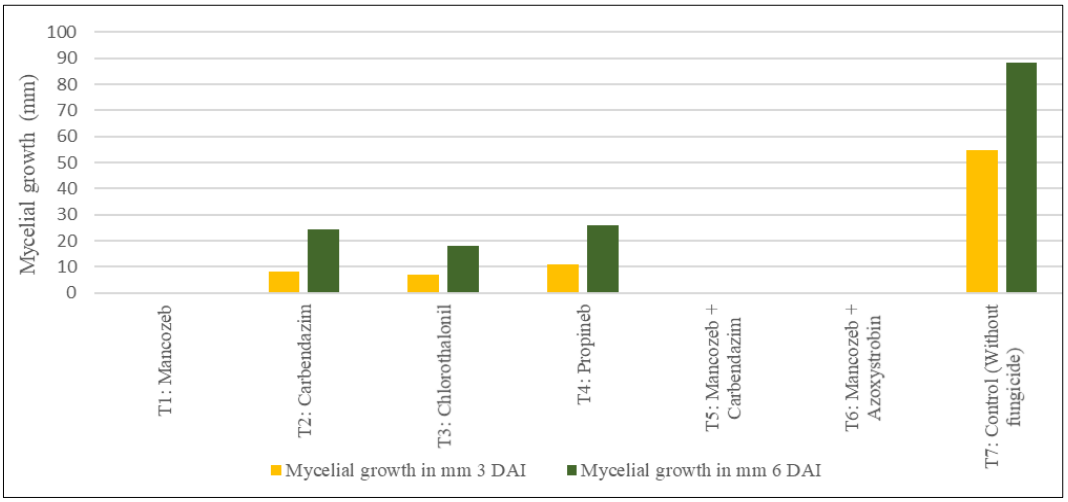


Fig 1: *In vitro* evaluation of new fungicide combinations at different doses against *Alternaria brassicae*. (Dose 1)

Table 3: *In vitro* evaluation of new fungicide combinations at different doses against *Alternaria brassicae*. (Dose 2)

Treatments	Dose/l. of water (%)	Mycelial growth in mm	
		3 DAI	6 DAI
T1: Mancozeb	0.20	00.00	00.00
T2: Carbendazim	0.20	22.60	56.00
T3: Chlorothalonil	0.25	11.80	22.40
T4: Propineb	0.25	20.60	32.80
T5: Mancozeb + Carbendazim	0.20	00.00	00.00
T6: Mancozeb + Azoxystrobin	0.05	00.00	00.00
T7: Control (Without fungicide)		53.80	87.80
S.Em±		0.83	0.89
CD(p=0.05)		1.71	2.59
C.V. (%)		8.42	6.98

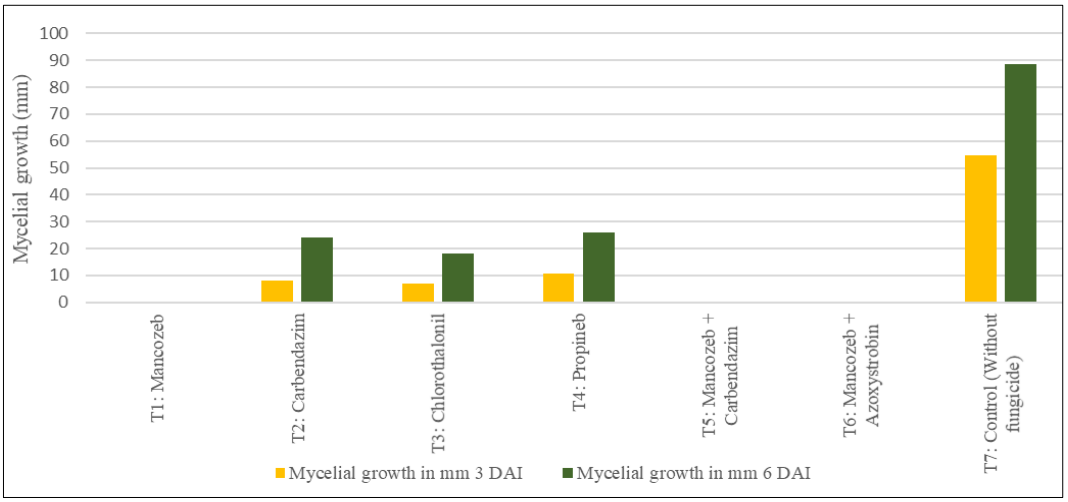


Fig 2: *In vitro* evaluation of new fungicide combinations at different doses against *Alternaria brassicae*. (Dose 2)

Table 4: *In vitro* evaluation of new fungicide combinations at different doses against *Alternaria brassicae*. (Dose 3)

Treatments	Dose/l. of water (%)	Mycelial growth in mm	
		3 DAI	6 DAI
T1: Mancozeb	0.25	00.00	00.00
T2: Carbendazim	0.25	8.20	24.20
T3: Chlorothalonil	0.3	6.80	18.00
T4: Propineb	0.3	10.80	26.00
T5: Mancozeb + Carbendazim	0.25	00.00	00.00
T6: Mancozeb + Azoxystrobin	0.1	00.00	00.00
T7: Control (Without fungicide)		54.80	88.40
S.Em±		0.50	1.04
CD(p=0.05)		1.46	3.03
C.V. (%)		9.74	10.45

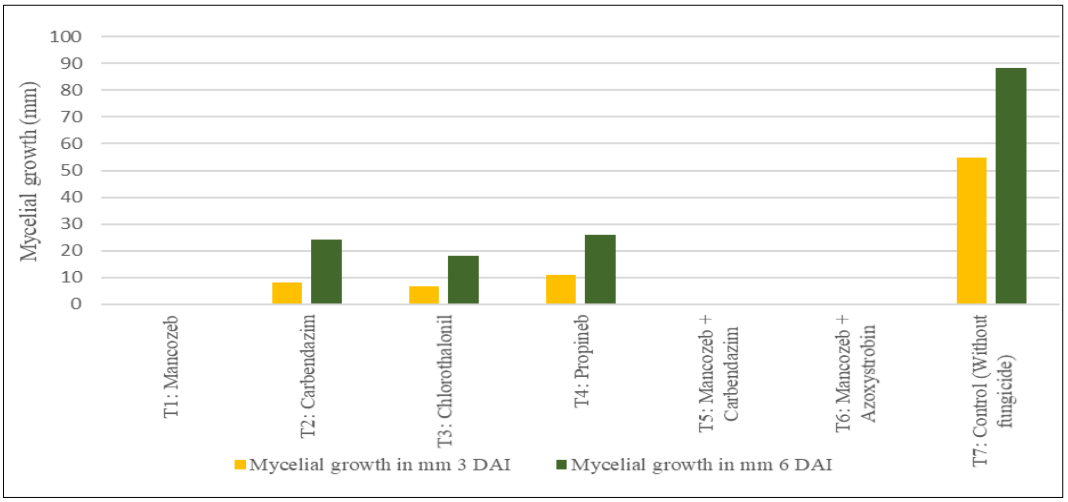
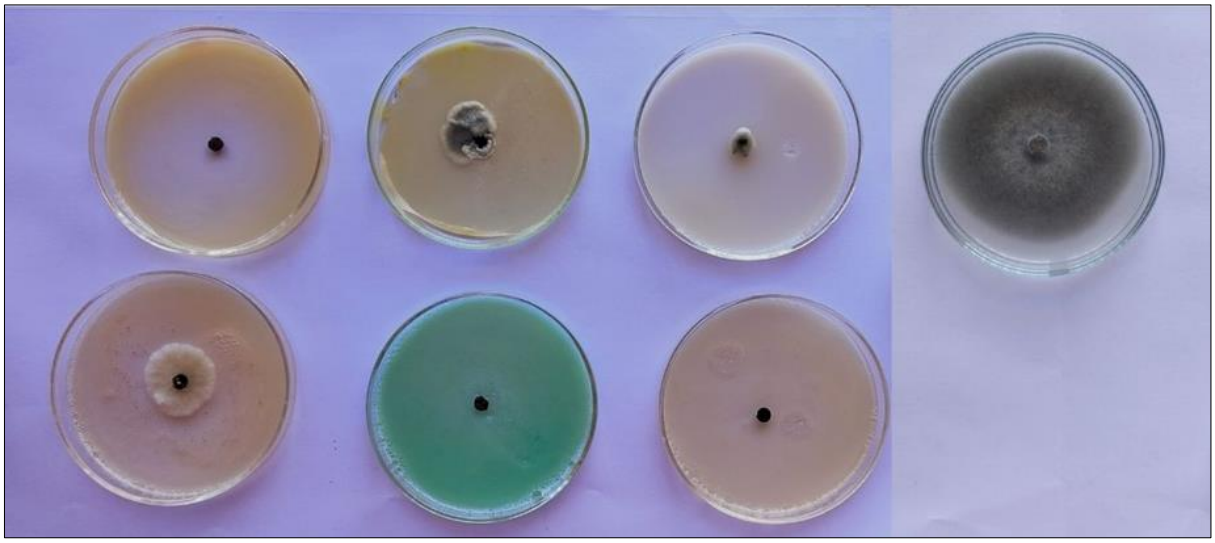


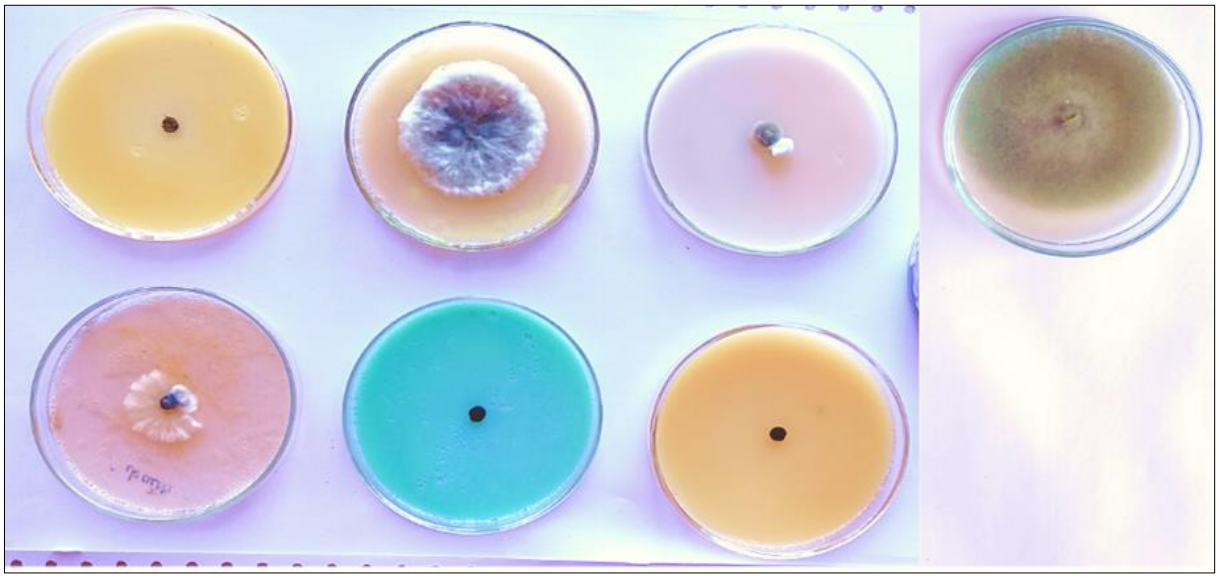
Fig 3. *In vitro* evaluation of new fungicide combinations at different doses against *Alternaria brassicae*. (Dose 3)

Fig 4: Leaf blight of rapeseed crop



T1: Mancozeb
T2: Carbendazim
T3: Chlorothalonil
T4: Propineb
T5: Mancozeb + Carbendazim
T6: Mancozeb + Azoxystrobin
T7: Control (Without fungicide)

Plate 1: Mycelial growth *Alternaria brassicae* on PDA media poisoned with Dose 1 fungicide



T1: Mancozeb
T2: Carbendazim
T3: Chlorothalonil
T4: Propineb
T5: Mancozeb + Carbendazim
T6: Mancozeb + Azoxystrobin
T7: Control (Without fungicide)

Plate 3: Mycelial growth *Alternaria brassicae* on PDA media poisoned with Dose 3 fungicide

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