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Management of Alternaria blight disease (*Alternaria brassicae*) of mustard through plant extracts and fungicides

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Abstract
Brassica juncea (L.) Czern. & Coss. is commonly known as Indian mustard and globally used as oilseed, vegetable and cosndiments. *Alternaria brassicae* is the most destructive pathogen of oilseeds. In this present investigation five plant extracts and five fungicides were used under *in vitro* through the use of poison food technique (PFT) and Standard Blotter Test (SBT) and under *in vivo* conditions through the use of seed application, foliar application and seed + foliar application. Present data revealed that, among fungicides used as seed treatment *in vitro*, mancozeb + carbrendazim, proved to be the most effective against *Alternaria brassicae* followed by captan + hexaconazole in improving seed germination and vigour index by reducing pre and post-emergence mortality. Similarly, among botanicals tested *in vitro*, garlic extract proved to be effective in inhibition of mycelial growth and improving seed germination and vigour index by reducing pre and post-emergence mortality followed by NSKE. Seed treatment and then foliar application (50 DAS) of mancozeb + carbendazim (0.2%) and garlic extract (10%) were proved most effective in reducing disease intensity. Mancozeb + carbedazim and garlic were found most effective in inhibition of mycelial growth in *in vitro* and in reducing disease intensity as well in *in vivo*.

Keywords: Oilseeds, SBT, emergence, mortality, vigour index

1. Introduction

India is one of the largest economies of the world. The contribution of oilseeds to the agriculture economy of India ranks second only to food grains (Rathore *et al*., 2018) [24]. *Brassica juncea* (L.) Czern. & Coss. belongs to family Brassicaceae (Crucifereae) is commonly known as Indian mustard and globally used as oilseed, vegetable and condiments (Saleem *et al*., 2017) [22]. Oilseed Brassica also referred to as rapeseed-mustard, an important group of oilseed crops in the world, comprises eight cultivated crops of tribe Brassicas within the family Cruciferae (Brassicaceae). The word 'rape' and 'mustard' have been derived from the word "rapum" meaning turnip and European practice of mixing the sweet 'must' of old wine with crushed seeds of black mustard [*Brassica nigra* (L.) Koch] to prepare a hot paste, respectively (Hemingway, 1976) [12].

India is one of the leading oilseeds producing country in the world accounting for 11.2 per cent of the world's rapeseed-mustard production and ranks third in the world next to China and Canada. It is the second most important oilseed crop of India after groundnut in terms of area and production. In India, the crop was grown over 6.412 million hectares of land producing 6.33 million tonnes (Anonymous, 2017-18) [3]. It is mostly grown in Rajasthan, Madhya Pradesh, Haryana, Uttar Pradesh, Gujarat, Bihar and Punjab (Shekhawat *et al*., 2012) [24]. In Rajasthan, rapeseed-mustard occupies a prime place amongst all the oilseed crops grown. Rajasthan ranks first both in area and production of rapeseed-mustard in the country. It is grown in the districts namely Ajmer, Alwar, Bharatpur, Dausa, Jaipur, Sawai-Madhopur, Bikaner, Kota, Baran, Tonk. Rapeseed-mustard comprising an area under cultivation was about 2.379 million hectares in the year 2018-19 and the production was 3.588 million tonnes (Anonymous, 2018-19) [4]. Indian mustard is the premier oilseed Brassica which covers about 85-90 per cent of the total area under cultivation of all these oilseed crops (Rao *et al*., 2017) [20]. In India, mustard crop grown in Rabi season from September-October to February-March. Sandy loam to clay loam soils but thrive best on light loam soils. Soil having neutral pH are ideal for their proper growth and development. Rapeseed-mustard oil is considered the best quality oil for human consumption as compared to other edible oils because of the lowest amount of harmful saturated fatty acids and adequate amount of two essential fatty acids i.e. linoleic acid and linolenic acid (Porter and Crompton, 2008).

Mustard seed contains about 38 to 43 per cent oil which is yellowish in colour (Patel *et al*., 2012) [16].

In agriculture, ninety per cent of food crops are propagated by seed. Most widely grown oilseed crops in world agriculture (groundnut, sesame, mustard etc.) are all affected by seed borne diseases. Depending upon the presence of fungi either on seed coat or in the seed, it is further called as external seed-borne fungi and internal seed-borne fungi, respectively. The literature survey reveals that seeds of mustard (*Brassica campestris* L.) are known to carry several fungal pathogens which alter physio-chemical properties of the seeds during storage, losses of the seed weight, germination potential, medicinal properties and discoloration, causing the losses to the extent of 24 per cent (Ashraf and Choudhary, 2008) [6].

In India, various researchers have studied the incidence of seed borne fungi of several species of Brassica under storage environment from various geographical locations (Ghotekar and Hedawoo, 2010; Ghugal and Thakre, 2014) [8, 9]. Siddiqui (2013) [25] concluded that the full potential of this crop is far from being exploited due to several abiotic and biotic stresses. The infected seeds may fail to germinate, transmit disease from seed to seedling and from seedling to growing plants.

2 Material and Methods

2.1 Efficacy of plant extracts against *Alternaria brassicae* (*in vitro*)

2.1.1 Poisoned Food Technique (PFT)

In present era, many botanicals are being used as fungitoxicants for the cure of various plant diseases. Plant extracts tested are as follows (Table 1).

Table 1: List of plant extracts against *Alternaria brassicae in vitro*

Common name of plant	Botanical name	Plant part used	Concentration (%) for PFT	Concentration (%) for SBT
Garlic	*Allium sativum*	Clove	5, 10	10
Ginger	*Zingiber officinale*	Rhizome	5, 10	10
Neem	*Azadirachta indica*	Seed kernel	5, 10	10
Neem	*Azadirachta indica*	Leaves	5, 10	10
Alstonia	*Alstonia scholaris*	Leaves	5, 10	10
Control	-	-	-	-

The effect of each plant extract was tested at two different concentrations (5 and 10%) following the method suggested by Singh and Majumdar (2001) [26] with slight modifications. To get these, the required plant part was thoroughly washed with sterilized water and grind separately in electric grinder using equal amount of sterilized distilled water (1:1 w/v). The mixture was squeezed with double layered sterilized muslin cloth. The extracts thus obtained were considered as of 100 per cent concentration.

Required quantity of each plant extract (*i.e.* stock solution) was mixed thoroughly in melted PDA, to get desired concentration, just before pouring in sterilized 9 cm diameter glass Petri plates and was allowed to solidify for an hour. Each plate was inoculated with 5 mm disc of mycelial bit taken with the help of sterilized cork borer from the periphery of 7 days old culture of *Alternaria brassicae* growing on PDA. The inoculated Petri plates were incubated at 25±1 ℃. Three Petri plates were used for each treatment serving as three replications. A control was also maintained where medium was not supplemented with any plant extract. The experiment was conducted in Completely Randomized Design (CRD). Colony diameter (two diagonals) was measured at 7th day of incubation.

2.1.2 Standard Blotter Test (SBT)

Hundred seeds per sample, surface sterilized with 0.1 per cent mercuric chloride and artificially inoculated with fungus than seeds dipped in concentration of plant extracts which is above in table, were plated on moistened blotters in Petri plates and were incubated at 25±1 ℃ under 12h of alternating cycles of day light and darkness for 7 days. Data were recorded as per 3.

2.2 Efficacy of fungicides against *Alternaria brassicae* (*in vitro*)

2.2.1 Poisoned Food Technique (PFT)

An efficacy of five systemic and non-systemic fungicides (Table 2.) was tested against mycelial growth of *Alternaria brassicae* by Poisoned Food Technique (Schimitz, 1930) [23]. Required quantity of each fungicide added aseptically to 100 ml of sterilized PDA medium in 150 ml flask separately so as to get concentration of 50, 100 and 150 ppm and autoclaved. The flasks were shaken several times to ensure proper and uniform distribution of the fungicide and poured 20 ml PDA separately in sterilized Petri plates and allowed to solidify. Medium without fungicides served as check. Each plate was inoculated with 5 mm diameter mycelial bit of fungus. Inoculated plates were incubated at 25±1℃ for 7 days. The linear growth of test fungus was recorded on 7th day of inoculation and per cent growth inhibition was calculated by using Vincent's (1947) formula:

$$\text{Per cent inhibition} = \frac{C - T}{C} \times 100$$

Where,

C = Radial growth of *Alternaria brassicae* in control (average of both diagonals)

T = Radial growth of *Alternaria brassicae* in treatment (average of both diagonals)

Following fungicides were used during experimentation:

2.2.2 Standard Blotter Test (SBT)

Hundred seeds per sample, surface sterilized with 0.1 per cent mercuric chloride and artificially inoculated with fungus than treated with fungicide (Table 2.) separately, were plated on moistened blotters in Petri plates and were incubated at 25±1 ℃ under 12h of alternating cycles of day light and darkness for 7 days. Data on seed germination and mortality were recorded up to 7 days.

Table 2: List of fungicides against *Alternaria brassicae in vitro*

Common name	Trade name	Concentration (ppm)
Mancozeb (50% WP) + Carbendazim (25% WP)	Sprint	50, 100 and 150
Captan (70% WP) + Hexaconazole (5% SC)	Steam	50, 100 and 150
Fluopicolide (4.44%WG) + Foestyl aluminium (66.6%WG)	Profiler	50, 100 and 150
Difenoconazole (25% EC)	Score	50, 100 and 150
Thiophanate Methyl (75% WP)	Topsin-M	50, 100 and 150
Control	-	-

Table 3: List of fungicides against *Alternaria brassicae in vitro*

Fungicides	Trade Name	Dose (g/kg seed)
Captan(70% WP) + Hexaconazole(5% SC)	Steam	0.1
Mancozeb(50% WP) + Carbendazim(25% WP)	Sprint	0.2
Difenoconazole (25% EC)	Score	0.1
Fluopicolide(4.44% WG) + Foestyl aluminium (66.6% WG)	Profiler	0.1
Thiophanate methyl (75% WP)	Topsin –M	0.2
Control	-	-

2.3 Efficacy of effective plant extracts and fungicides (*in vivo*)

Plant extracts and fungicides which proved efficacious *in vitro* were also evaluated by seed application, foliar application and seed-cum-foliar application (*in vivo*) in cemented pots (30 cm diameter) as per following detail. Prior to sowing, these pots were sterilized with copper sulphate solution and filled with sterilized soil + FYM (soil: FYM = 3:1), sterilized at 15 lb pressure for one hour for three consecutive days. Experiments were laid out in the pots in Completely Randomized Design (CRD) with four replications. These treated seeds were sown in pots @ 5 seeds/ pot × 2 with four replications. The pots were watered as and when required. All the pots were maintained under identical conditions. Inoculation of fungus and foliar application of fungicides and plant extracts were carried on 45 DAS and 50 DAS, respectively. Per cent disease intensity was recorded on 90 DAS. The overall disease scoring was done at 0-6 rating scale (Conn *et al*., 1990) [7] on the basis of disease assessment (Table 4) key for Alternaria blight in rapeseed-mustard.

Table 4: Disease rating scale for Alternaria leaf blight

Rating	Leaf and Pod	Reaction
0	No infection	I
1	Upto 5% area covered	HR
2	>5-10% area covered	R
3	>10-20% area covered	MR
4	>20-30% area covered	MS
5	>30-50% area covered	S
6	>50% area covered	HS

Where, I = Immune, HR = highly resistant, R = Resistant, MR = moderately resistant, MS = moderately susceptible, S = Susceptible and HS = highly susceptible

2.3.1 Through seed application

The seeds of susceptible variety of mustard (Varuna) were sown in cemented pots in cage house. Before sowing, seeds were treated with following fungicides and plant extracts (Table 5).

Table 5: List of fungicides and plant extracts against *Alternaria brassicae in vivo*

Treatment	Dose
Mancozeb (50%WP) + carbendazim (25%WP)	0.2%
Captan (70% WP) + Hexaconazole (5% SC)	0.1%
Garlic	10%
NSKE	10%
Control	-

2.3.2 Through foliar application

The method is same as described at 2.3.1 except that instead of seed treatment, treatments were given foliar application at 50 DAS.

2.3.3 Through seed-cum-foliar application

The treatments were given through seed-cum-foliar application as per point 2.3.1 and 2.3.2. Inoculum was sprayed at 45 DAS and disease intensity was recorded 90 DAS. The overall per cent disease intensity and per cent disease control were calculated as follows:

$$\text{Per cent disease intensity} = \frac{\text{Total Sum of all disease ratings}}{\text{Total number of leaves observed} \times \text{Maximum rating}} \times 100$$

The per cent disease control (PDC) was also calculated by using the following formula:

$$\text{Per cent disease control} = \frac{\text{Disease in control} - \text{Disease in treatment}}{\text{Disease in control}} \times 100$$

3 Results

3.1 Plant extracts

3.1.1 Efficacy of plant extracts against *Alternaria brassicae* by Poisoned Food Technique: The efficacy of five plant extracts (Table 6 Fig 1 and Plate 1) were tested *in vitro* at two concentrations *viz*., 5 and 10 per cent against *Alternaria brassicae* on PDA by Poisoned Food Technique. Among five plant exracts, the garlic extract was found significantly superior in inhibiting mycelial growth (89.40% and 91.68%) of *Alternaria brassicae* followed by NSKE (85.60% and

89.10%) at 5 and 10 per cent concentrations, respectively. Whereas neem (83.45% and 87.50%), ginger (80.30% and 84.68%) and Alstonia (59.30% and 62.30%) inhibited mycelial growth of the fungus at 5 and 10 per cent concentrations, respectively. Both the concentrations (5% and 10%) of all the tested plant extracts were found significant over control.

Table 6: Efficacy of plant extracts against *Alternaria brassicae* by Poisoned Food Technique on 7th day of incubation at 25 + 1^{0}C

Name of plant	Plant part used	Per cent inhibition of mycelial growth at various concentration* (%)		
		5	10	Mean
Garlic	Clove	89.40	91.68	90.54
		(71.00)	(73.24)	
Neem (NSKE)	Seed Kernel	85.60(67.70)	89.10 (70.72)	87.35
Neem (Leaf)	Leaf	83.45	87.50	85.48
		(65.99)	(69.30)	
Ginger	Rhizome	80.30	84.68	82.49
		(63.65)	(66.96)	
Alstonia	Leaf	59.30	62.30	60.80
		(50.36)	(52.12)	
Control	-	0.00	0.00	0.00
		0.00	0.00	
			SEm+	CD (p=0.05)
		P	0.84	2.47
		C	0.49	1.43
		PxC	1.19	3.49

*Average of four replications

*Figures given in parentheses are angular transformed values

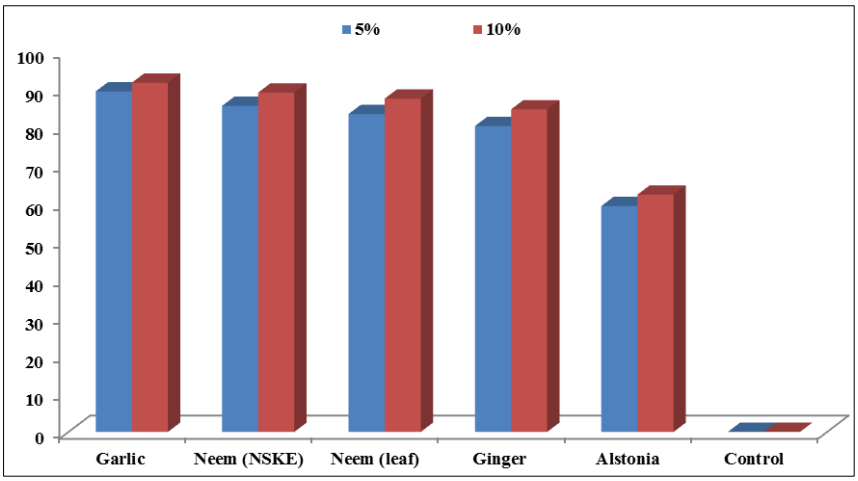

Fig 1: Efficacy of plant extracts against *Alternaria brassicae* by Poisoned Food Technique on 7th day of incubation at 25 + 1^{0}C

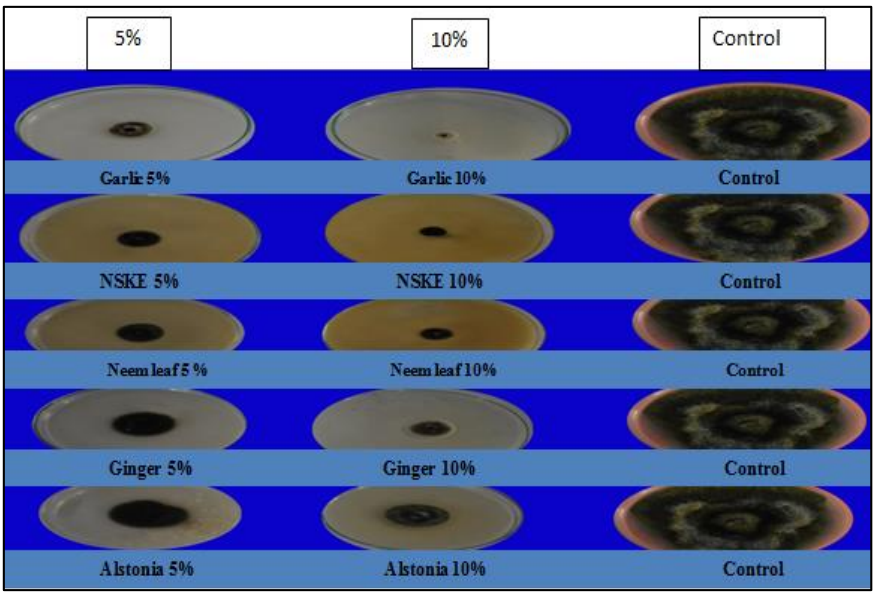

Plate 1: Efficacy of plant extracts against *Alternaria brassicae* causing Alternaria leaf blight of mustard (*in vitro*)

3.1.2 Efficacy of plant extracts against *Alternaria brassicae* by Standard Blotter Technique (*in vitro*)

The results (Table 7 Fig 2 and Plate 2) of plant extracts against *Alternaria brassicae* in indicated that seeds treated with plant extracts gave significantly higher per cent of seed germination, ranged from 68.00 to 85.00 per cent in comparison to control (56.50%). Maximum per cent seed germination was observed in seeds treated with extract of garlic (85.00%) followed by NSKE (80.00%), neem (78.00%), ginger (77.00%) and Alstonia (68.00%). It is evident from the data that plant extract of garlic proved most effective in giving lowr pre- (4.50%) and post- emergence (5.00%) mortality followed by NSKE (5.50% and 4.50%). Neem and ginger extracts also proved effective as they showed less pre- emergence (6.75% / 5.50%) mortality while, it was found at par in post- (5.00% / 5.00%) emergence mortality in comparison to control, respectively.

Maximum seedling vigour was observed in seeds treated with garlic (1045.50) followed by NSKE (900.00) while, it was minimum in control (350.30).

Table 7: Efficacy of plant extracts against *Alternaria brassicae* by Standard Blotter Technique (*In vitro*)

S. No.	Name of plant	Plant part used	Conc. (%)	Per cent germination	Per cent mortality		Seedling length*		Vigour index
					Pre-emergence	Post-emergence	Radicle length (cm)	Plumule length (cm)	
1.	Garlic	Clove	10	85.00	4.50	5.00	6.00	6.30	1045.50
				(67.21)	(12.25)	(12.92)			
2.	Neem (NSKE)	Seed kernel	10	80.00 (63.43)	5.50 (13.56)	4.50 12.25)	5.50	5.75	900.00
3.	Neem (Leaf)	Leaf	10	78.00	6.75	5.00	5.00	5.50	819.00
				(62.03)	(15.06)	(12.92)			
4.	Ginger	Rhizome	10	77.00	5.50	5.00	4.75	5.10	758.45
				(61.34)	(13.56)	(12.92)			
5.	Alstonia	Leaf	10	68.00	11.00	6.50	4.20	5.10	632.40
				(55.55)	(19.37)	(14.77)			
6.	Control	-	-	56.50	42.00	15.75	3.00	3.20	350.30
				(48.73)	(40.40)	923.38)			
	S.Em+			1.01	0.25	0.26			18.21
	CD (p=0.05)			3.05	0.75	0.78			54.89

*Average of four replications

*Figures given in parentheses are angular transformed values

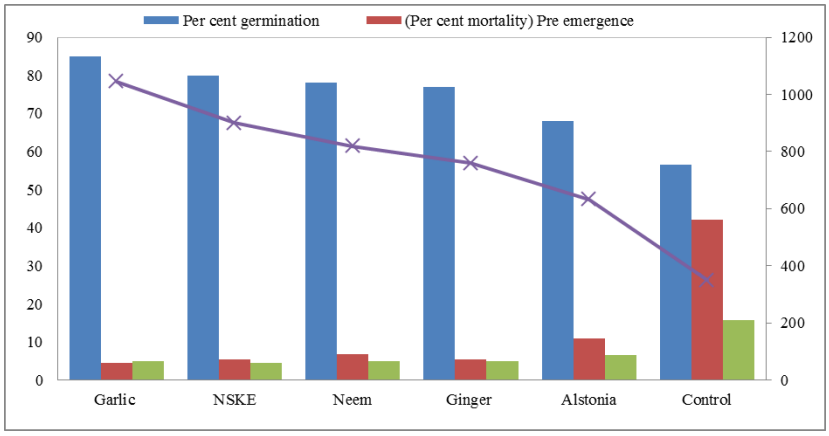

Fig 2: Efficacy of plant extracts against *Alternaria brassicae* by Standard Blotter Technique (*in vitro*)

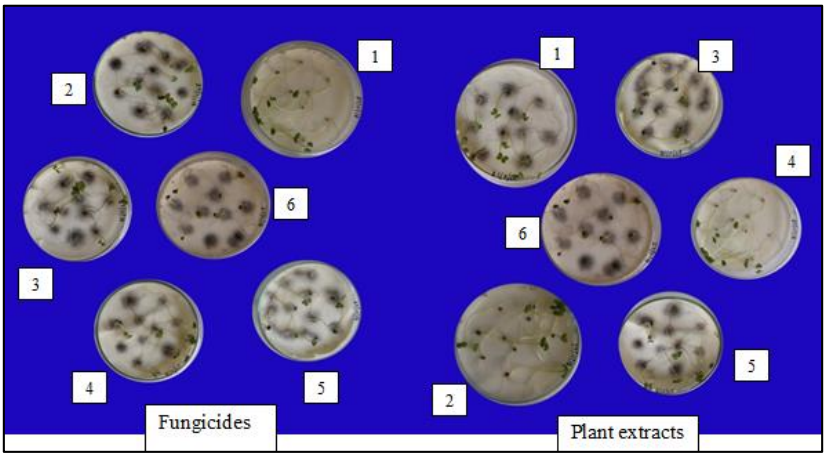

Plate 2: Efficacy of plant extracts and fungicides against *Alternaria brassicae* by Standard Blotter Technique (*in vitro*)

3.2 Fungicide

3.2.1 Efficacy of fungicides against *Alternaria brassicae* by Poisoned Food Technique

Efficacy of five systemic and non-systemic fungicides *viz.*, captan + hexaconazole, mancozeb + carbendazim, difenoconazole, thiophanate methyl and fluopicolide + fosetyl aluminium were tested at 50, 100 and 150 ppm concentration for inhibition of mycelial growth of *Alternaria brassicae*. The data (Table 8 Fig 3 and Plate 3) revealed that all the fungicides significantly inhibited the mycelial growth of *Alternaria brassicae* as compared to check. Mancozeb + carbendazim was found significantly superior at 100 and 150 ppm with cent per cent inhibition of mycelial growth followed by captan + hexaconazole (81.25%, 88.79% and 89.13%) at 50, 100 and 150 ppm, respectively. Inhibition of mycelial growth was at par in difenoconazole (60.83%, 69.40% and 75.60%) and fluopicolide + fosetyl aluminium (64.85%, 68.14% and 74.65%) which were also higher over control. Thiophanate methyl (59.40%, 64.15% and 72.00%) was found least effective in inhibiting mycelial growth. The inhibition of mycelial growth was increased and maximum at higher concentration that was 150 ppm concentration. Fungicides and concentration interaction was also significant.

Table 8: Efficacy of fungicides against *Alternaria brassicae* by Poisoned Food Technique on 7th day of incubation at 25 + 1 ^{0}C

S. No.	Fungicides	Trade name	Per cent inhibition of mycelial growth at various concentration* (ppm)			
			50	100	150	Mean
1.	Captan + Hexaconazole	Steam	81.25	88.79	89.13	86.39
			(64.34)	(70.44)	(70.75)	
2.	Mancozeb + carbendazim	Sprint	94.32	100	100	98.11
			(76.21)	(90.00)	(90.00)	
3.	Difenoconazole	Score	60.83	69.40	75.60	68.61
			(51.25)	(56.42)	(60.40)	
4.	Fluopicolide + Fosetyl aluminium	Profiler	64.85 (53.64)	68.14 (55.64)	74.65 (59.77)	69.21
5.	Thiophanate methyl	Topsin-M	59.40	64.15	72.00	65.18
			(50.42)	(53.22)	(58.05)	
6.	Control	--	0.00	0.00	0.00	0.00
			0.00	0.00	0.00	
					S.Em+	CD (p=0.05)
				F	0.85	2.44
				C	0.60	1.72
				FxC	1.47	4.22

*Average of four replications
Figures given in parentheses are angular transformed values

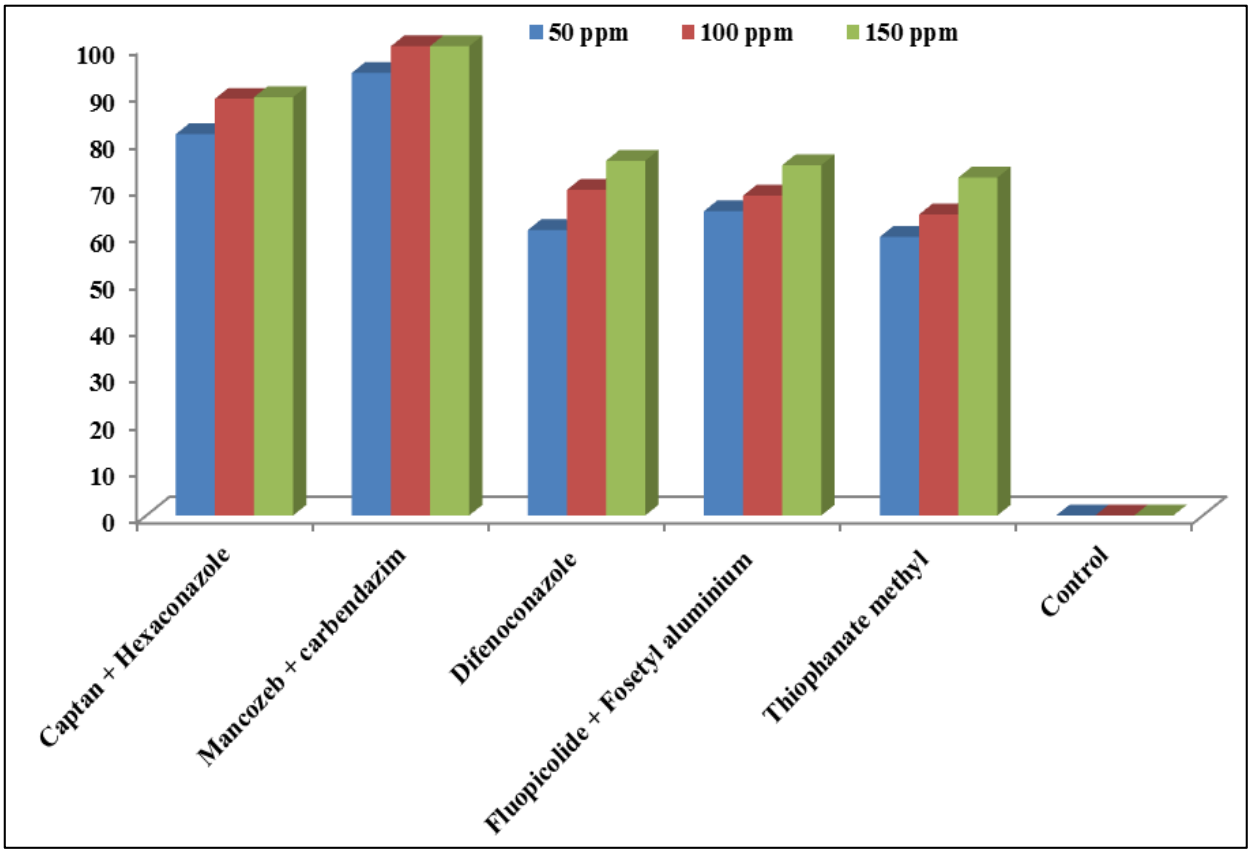

Fig 3: Efficacy of fungicides against *Alternaria brassicae* by Poisoned Food Technique on 7th day of incubation at 25 + 1 ^{0}C

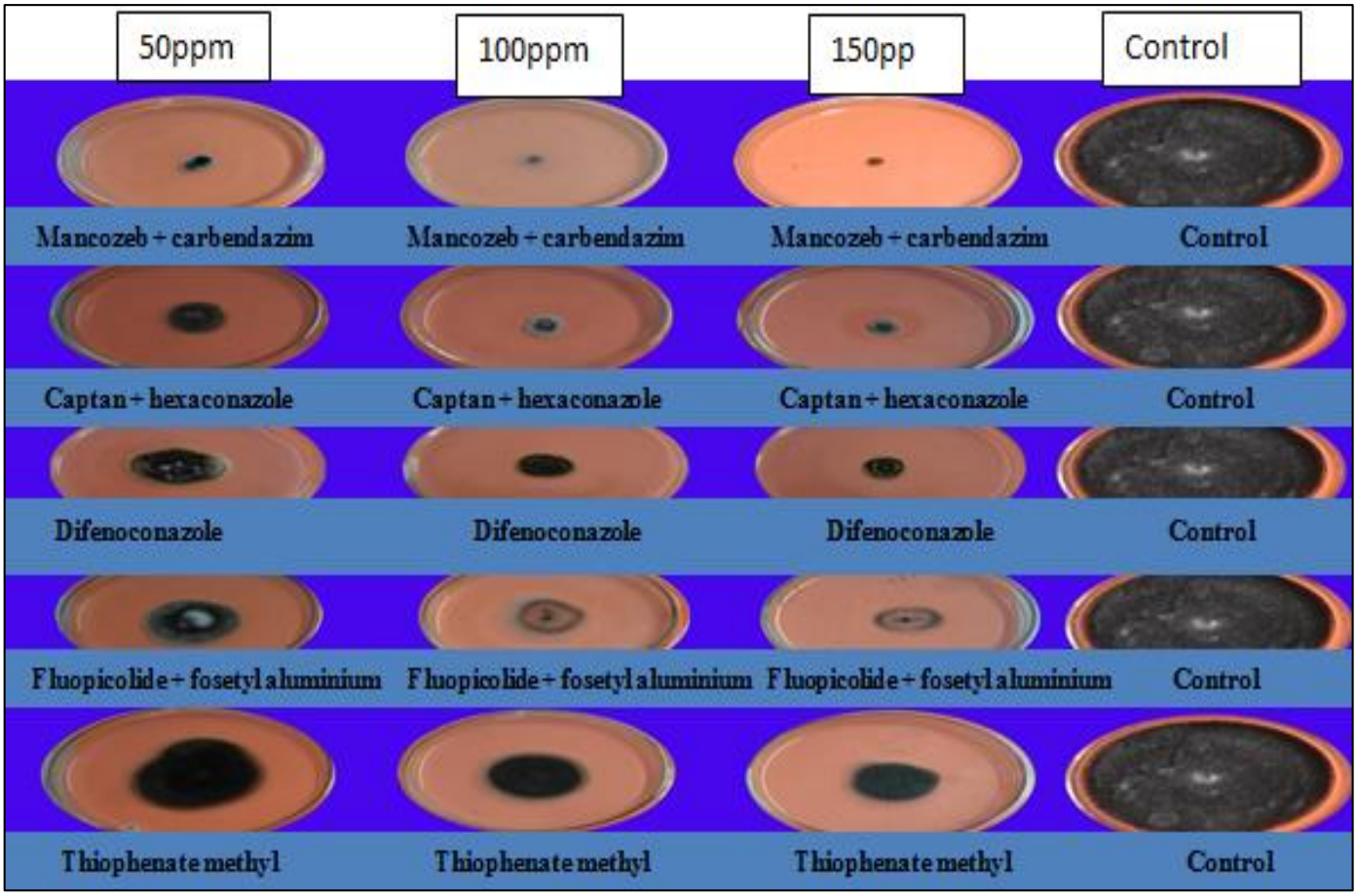

Plate 3: Efficacy of fungicides against *Alternaria brassicae* causing Alternaria leaf blight of mustard (*in vitro*)

3.2.2 Efficacy of fungicides against *Alternaria brassicae* by Standard Blotter Technique (*in vitro*)

Data (Table 9, Fig 4 and Plate 2) on fungicidal treatments of seeds inoculated with *Alternaria brassicae* indicated that seeds treated with mancozeb + carbendazim was found most effective in giving higher seed germination (89.00%) followed by captan +hexaconazole (87.00%) and difenoconazole (76.00%). Whereas fluopicolide + fosetyl aluminium showed (72.00%) seed germination. Thiophanate methyl was found least effective with (69.00%) seed germination. All the fungicides, under study showed significant reduction in pre- and post- emergence mortality against *Alternaria brassicae* over control. Lowest mortality was observed when seeds treated with mancozeb + carbendazim. Pre–emergance mortality was at par with captan + hexaconazole and difenoconazole. All the fungicidal treatments increased the root, shoot length and vigour index over control. Longest root length (5.65 cm and 5.12 cm), shoot length (6.10 and 6.00 cm) and vigour index (1045.75 and 967.44) were recorded in treatment with mancozeb + carbendazim and captan + hexaconazole, respectively.

Table 9: Efficacy of fungicides against *Alternaria brassicae* by Standard Blotter Technique (*In vitro*)

S. No.	Fungicides	Dose (%)	Per cent germination	Per cent mortality		Seedling length*		Vigour index
				Pre emergence	Post emergence	Radicle length (cm)	Plumule length (cm)	
1.	Captan + Hexaconazole	0.1	87.00	5.00	4.50	5.12	6.00	967.44
			(68.87)	(12.92)	(12.25)			
2.	Mancozeb + carbendazim	0.2	89.00	4.50	3.00	5.65	6.10	1045.75
			(70.63)	(12.25)	(9.97)			
3.	Difenoconazole	0.05	76.00	5.00	9.00	4.50	5.75	779.00
			(60.67)	(12.92)	(17.46)			
4.	Fluopicolide + Fosetyl aluminium	0.1	72.00	10.50	5.25	4.50	4.50	648.00
			(58.05)	(18.91)	(13.25)			
5.	Thiophanate methyl	0.2	69.00	7.50	12.00	4.20	5.00	634.80
			(56.17)	(15.89)	(20.27)			
6.	Control	-	52.00	37.50	17.00	2.90	3.50	332.80
			(46.15)	(37.76)	(24.35)			
	S.Em+		0.17	0.31	0.34			13.52
	CD (p=0.05)		0.50	0.94	1.01			40.76

* Average based on 4 replications
Figures given in parentheses are angular transformed values

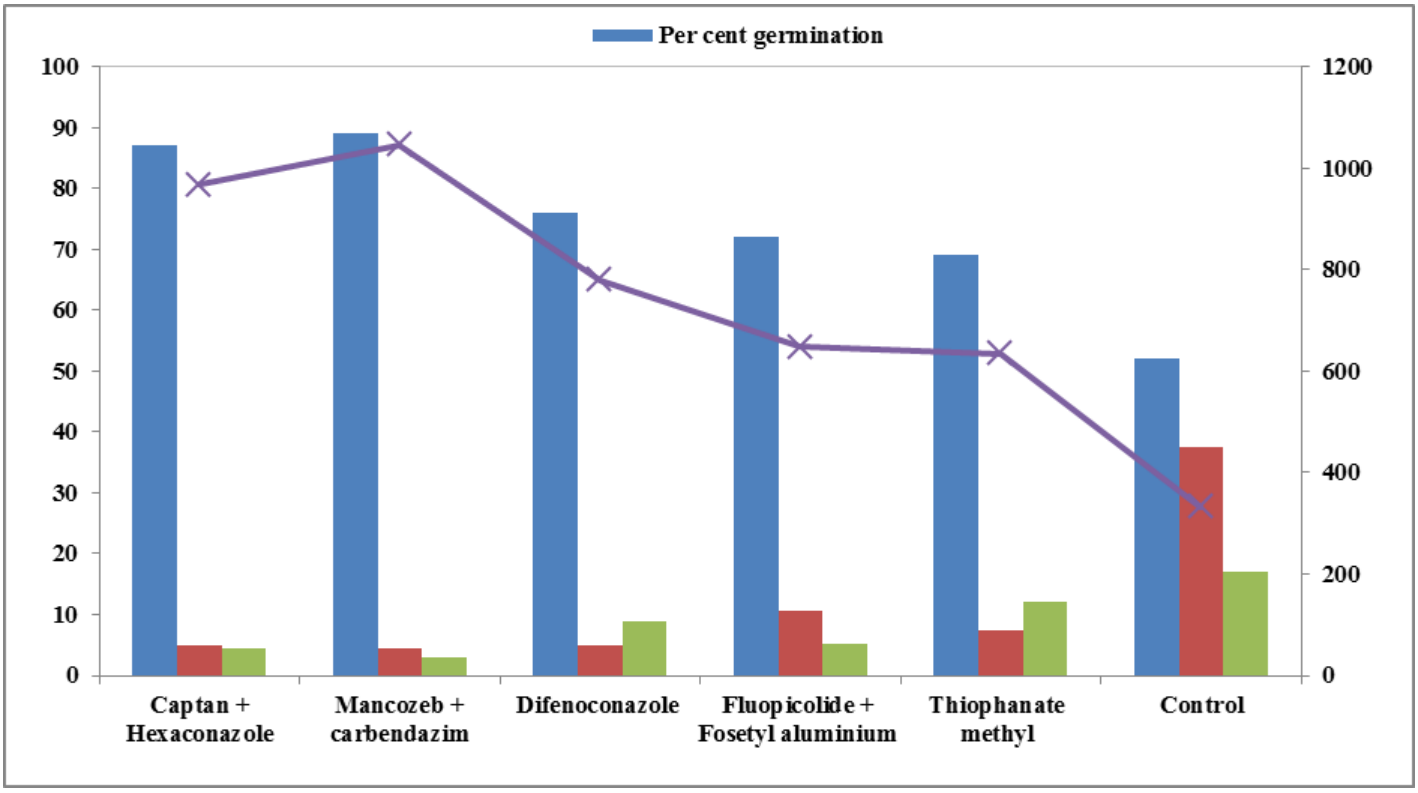

Fig 4: Efficacy of fungicides against *Alternaria brassicae* by Standard Blotter Technique (*in vitro*)

3.3 Efficacy of plant extracts and fungicides against *Alternaria brassicae* through seed and foliar application (*in vivo*): Plant extracts and fungicides which were found most effective in *in vitro* were also tested in pots through seed, foliar and seed-cum-foliar application against *Alternaria brassicae* and these were garlic and NSKE as plant extracts whereas mancozeb + carbendazim and captan + hexaconazole as fungicides.

Table 10: Efficacy of plant extracts and fungicides against *Alternaria brassicae* through seed and foliar applications (*In vivo*)

Treatment	Conc. (%)	Seed application		Foliar application		Seed-cum-foliar application	
		Per cent disease intensity*	Per cent disease control	Per cent disease intensity*	Per cent disease control	Per cent disease intensity*	Per cent disease control
Mancozeb (50% WP) + carbendazim (25% WP)	0.2	31.65 (34.23)	47.22	16.66 (24.09)	73.68	11.66 (19.97)	81.08
Captan (70% WP) + hexaconazole (5% SC)	0.1	34.98 (36.26)	41.67	18.33 (25.35)	71.04	14.99 (22.78)	75.68
Garlic	10	41.65	30.54	29.98	52.63	24.99	59.45
		(40.19)		(33.20)		(29.99)	
NSKE	10	44.98	24.99	33.32	47.37	28.32	54.05
		(42.12)		(35.26)		(32.15)	
Control		59.97	-	63.30	-	61.64	-
		(50.75)		(52.71)		(51.73)	
S.Em+		0.37		0.51		0.58	
CD (P=0.05)		1.06		1.45		1.67	

*Average of four replications
Figures given in parentheses are angular transformed values

The results (Table 10 and Fig 5) revealed that all plant extracts and fungicides were found significantly superior over control in reducing per cent disease intensity. Through seed application, minimum disease intensity (31.65%) was recorded with mancozeb + carbendazim followed by captan + hexaconazole (34.98%), garlic (41.65%) and NSKE (44.98%) while, through foliar application it was reduced at 16.66 per cent disease intensity with mancozeb + carbendazim followed by captan + hexaconazole (18.33%), garlic (29.98%) and NSKE (33.32%) over control. Whereas, seed cum foliar application of mancozeb + carbendazim was found significantly superior in all three application methods that it showed lowest disease intensity (11.66%) followed by captan + hexaconazole (14.99%), garlic (24.99%) and NSKE (28.32%) over control (61.64%).

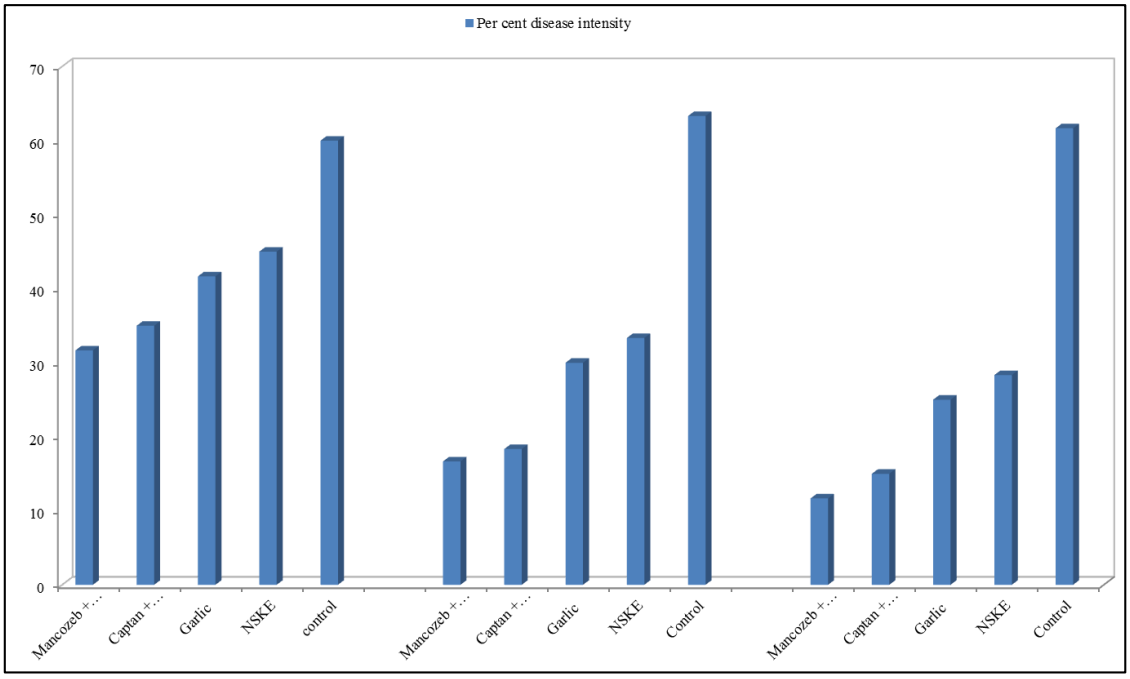

Fig 5: Efficacy of plant extracts and fungicides against *Alternaria brassicae* through seed and foliar application (*in vivo*)

4. Discussions

Seed treatment is the cheapest and easiest method of plant disease control. Seed treatment with plant extracts and fungicides is well known for the control of seed borne fungi (Abou-Heilan, 1984).

4.1 Efficacy of plant extracts (*in vitro*)

Five plant extracts of namely garlic, neem, ginger, NSKE and Alstonia were tested at 5 and 10 per cent concentrations and significantly inhibited mycelial growth of *Alternaria brassicae in vitro*. Among these, garlic gave maximum inhibition of mycelial growth of the fungus followed by NSKE. This finding is supported by the work of Meena *et al*. (2004) [15] who reported that the extract of *Allium sativum* showed maximum reduction in mycelial growth of *Alternaria brassicae* causing Alternaria blight of Indian mustard.

Seed treatment with plant extracts have reported to be the safest in comparison to fungicides. Now days, besides chemical control attention has been paid towards of higher plant products as novel chemotherapeutants in plant protection (Gurjar *et al*., 2012) [10]. In this investigation, Standard Blotter Method was employed to know the efficacy of plant extracts against *Alternaria brassicae*. It was observed that seed treatment with different plant extracts increased seed germination and vigour index with reduced pre and post-emergence mortality. Among all the plant extracts tested, garlic gave maximum seed germination and minimum pre and post- emergence mortality with higher vigour index followed by NSKE. This finding is in line with the result of Singh and Majumdar (2001) [26], Ferdous *et al*. (2002) [11], Yadav (2009) [29], who reported that *Allium sativum* (garlic) performed better against mustard diseases, than the other botanicals over untreated control.

4.2 Efficacy of fungicides (*in vitro*)

The importance of chemicals cannot be denied in disease management. In laboratory, out of five fungicides tested by Poisoned Food Technique, mancozeb + carbendazim was found highly effective followed by captan + hexaconazole in inhibiting mycelial growth of *Alternaria brassicae*. This work draws support from the findings of Meena *et al*. (2004) [15] and Wagh *et al*. (2017) [28] who reported that mancozeb and carbendazim caused 100 per cent inhibition in mycelial growth of *A. brassicae*.

In the present investigation, Standard Blotter Method was also employed to know the efficacy of fungicides against *Alternaria brassicae*. It was observed that seed treatment with different fungicides enhanced seed germination and vigour index with reduced pre- and post- emergence mortality. Among all the seed dressing fungicides tested, mancozeb + carbendazim gave maximum per cent seed germination and minimum pre- and post- emergence mortality with higher vigour index followed by captan + hexaconazole. Our results are supported by the findings of Pathak and Godika (2002) [18], Chadar *et al*. (2016) [5], Ahmed *et al*. (2018) [1] who reported that seed dressing with fungicides performed better than the untreated control.

4.3 Efficacy of fungicides and plant extracts (*in vivo*)

Two botanicals and two fungicides were evaluated in *in vivo*, which were found highly effective in in vitro, by applying as seed and foliar treatment, gave promising results in reducing per cent disease intensity of Alterneria blight disease of mustard. Among fungicides, maximum per cent disease control was recorded with mancozeb + carbendazim followed by captan + hexaconazole while in plant extracts garlic gave minimum per cent disease intensity followed by NSKE. Our results are supported with the outcome of Chadar *et al*. (2016) [5], Rakesh *et al*. (2018) [19] and Chaudhary *et al*. (2018) [4] who found mancozeb as a superior in reducing disease intensity, whereas Singh and Majumdar (2001) [26], Yadav (2009) [29], Mahapatra and Das (2013) [13], Meena and Sharma (2012) [14] concluded that extract of Allium sativum showed minimum disease severity.

5. Summery and Conclusion

Among fungicides used as seed treatment *in vitro*, mancozeb + carbrendazim, proved to be the most effective against

Alternaria brassicae followed by captan + hexaconazole in improving seed germination and vigour index by reducing pre and post-emergence mortality. Similarly, among botanicals tested *in vitro*, garlic extract proved to be effective in inhibition of mycelial growth and improving seed germination and vigour index by reducing pre and post-emergence mortality followed by NSKE. Seed treatment and then foliar application (50 DAS) of mancozeb + carbendazim (0.2%) and garlic extract (10%) were proved most effective in reducing disease intensity.

Mancozeb + carbedazim and garlic were found most effective in inhibition of mycelial growth in *in vitro* and in reducing disease intensity as well in *in vivo*.

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