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*In vitro*Evaluation of Fungicides, Bio-control Agents and Botanicals against Major Seed Borne Fungus, *Alternariasesami*

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ABSTRACT

Eleven fungicides including three combination products, six isolates of biocontrol agents and six botanical extracts were tested against *Alternaria sesami*, major seed borne pathogen of sesame under *in vitro* conditions using poisoned food and dual culture techniques. Among the fungicides evaluated, combination product of carbendazim 12% + mancozeb 63% @ 0.2% was most effective with minimum mycelial growth (5.27 mm) and highest inhibition of mycelial growth (94.14%) which was significantly superior to all other fungicides tested. Azoxystrobin @ 0.1% was the least effective with 38.46 mm growth and 57.27% inhibition of mycelial growth. Among biocontrol agents, minimum radial growth (12.01 mm) and maximum inhibition of mycelial growth of *A. sesami* over control (86.66%) was obtained with *Trichoderma viride* (isolate-2) followed by *Pseudomonas fluorescens* (isolate-1) with 12.50 mm radial growth and 86.11% mycelial inhibition. Garlic clove extract 10% was significantly superior to other botanical extracts with 20.59 mm radial growth and 72.55% mycelial inhibition over control.

Keywords: *Alternaria sesami*, *Biocontrol agent, Botanicals and Fungicides.*

Seed-borne diseases cause deterioration of seed in storage,reduce the seed viability, vigour and weaken the initial growth of seedlings. They cause considerable damage during germination, at early and late crop growth stages. Several fungi including *Alternaria Curvularia, Fusarium, Helminthosporium,Memnoniella,Penicillum* and *Rhizophus* sp. have been found associated with sesame. Among these, *Alternaria*is the most destructive pathogen of sesame which causes yield loss to the extent of 28.9 per cent (Prasad *et al.,* 1997). In the recent years, *Alternaria* leaf blight has become a serious problem. The incidence of the disease has been found to be on increase year after year in local as well as improved varieties of sesame in Andhra Pradesh and Telangana.

Sesame varieties presently under cultivation do not possess proven field resistance or tolerance and majority of them are more or less prone to the leaf spot/blight caused by *A. sesami*. Under such circumstances fungicides provide the most reliable means of controlling diseases. *In vitro* techniques measuring toxicity of fungicides to fungal hyphae are often useful, especially with fungi that fail to sporulate or sporulate poorly in the laboratory (Vyas, 1984). Recently, biological control using bio-agents and phytoextracts has received much attention in both conventional and organic farming to suppress plant diseases and to overcome some extent the public concerns regarding chemical fungicides (Samuels, 2006). Bioprotectants like *Trichodermaviride, T. harzianum, Bacillus* sp. and *Pseudomonas fluorescens* applied to seed may not only protect seed but also may colonize and protect roots and increase the plant growth (Taylor and Harman, 1990). Several plant extracts have been demonstrated to possess excellent antifungal properties and their exploitation as bio-fungicides has been emphasized within the broad strategy of environmental protection (Savitha*et al*., 2011). Hence, the present investigation was carried out to evaluate the efficacy of fungicides, bio-control agents and botanicals against *A. sesami* under *in vitro* conditions .

MATERIAL AND METHODS

The present investigation was carried out in the laboratory of Plant Pathology, Regional Agricultural Research Station (RARS), Lam and the Department of Seed Science and Technology, Advanced Post Graduate Centre, Lam, Guntur, Andhra Pradesh during 2017-2018. The efficacy of eleven fungicides including three combinations (Table 1), bio-control agents (Table 2) and botanicals (Table 3) in controlling the growth of *A. sesami* was studied under *in vitro* conditions.

*Alternaria sesami*was isolated from infected seed of sesame. Sesame seed, prior to plating, were surface sterilized with 0.1% mercuric chloride for 30-45 sec. followed by four washings in sterile distilled water. The surface sterilized seed was kept on potato dextrose agar medium aseptically in laminar air flow chamber and incubated at 25±2°C for seven days. The infected seed were surrounded by fast growing fungal colonies. The fungal culture of *Alternaria sesami* was

purified by single spore isolation and maintained by sub-culturing at 8-10 days interval for further studies.

Fungicides were tested by employing poisoned food technique (Nene and Thapliyal, 1993). 10 ml stock solution of 1,00,000 ppm concentration of each fungicide was prepared using sterilized distilled water. To obtain the desired concentration of fungicide (0.1% or 0.2% or 0.3%) in the medium, amount of stock solution to be added was calculated by using the formula:

$C_1V_I = C_2V_2$; where, C_1 = Concentration of the stock solution (ppm); V_1 = Volume of the stock solution to be added (ml); C_2 = Desired concentration (ppm); V_2 = Volume of PDA in which fungicide is to be amended (ml).

Thereafter 20 ml of the poisoned medium was poured into sterilized Petri plate (90 mm diameter) under aseptic conditions in laminar air flow inoculation chamber and allowed to solidify. Each plate was inoculated in the centre with five mm diameter disc cut from the periphery of actively growing seven days old *A. sesami*culture under aseptic conditions and incubated at 25±1°C in a BOD incubator(Model no: RBI- 38/68/108). Four replications were maintained for each treatment. PDA plates with non-poisoned medium inoculated with *A. sesami* served as control.

In order to find out the antagonistic effect of different microorganisms (two isolates of *Pseudomonas fluorescens*collected from Biocontrol Lab, ARS, Amaravathi and three isolates of *Trichodermaviride* and one isolate of *Bacillus subtilis* collected from Agricultural College, Bapatla)against radial growth of *A. sesami,* dual culture experiment was conducted as per the procedure given by Dhingra and Sinclair (1985). Sterilized potato dextrose agar medium, melted and cooled at 45^0C, was poured aseptically into sterilized Petri plates. Mycelial discs of 5 mm diameter from the edge of actively growing culture of *A. sesami*and isolates of fungal bio-control agent were separately cut with the help of a sterilized cork borer and two discs were simultaneously placed on the periphery about one cm from the edge of the Petri plates (90 mm diameter) on opposite sides. In case of bacterial isolates, same procedure was followed but for streaking the bacteria using sterilized bent glass rod instead of placing the disc. Four replications were maintained for each treatment. The Petri plates containing potato dextrose agar medium inoculated with *A. sesami* alone served as control. All the Petri plates were incubated at 25±1°C in BOD incubator.

Fresh plant material of different species *viz*., leaves of neem, Lantana, CalotropisandTulasi; bulbs of onion and garlicclovesswas thoroughly cleaned, surface sterilized with ethanol (70%) and washed well with sterile water. The plant material was ground with sterile water at the rate of 1 ml g^{-1} of plant tissue using sterile pestle and mortar and the macerate was filtered through a muslin cloth to get the crude extract. Poisoned food technique using botanical extract @ 10 % was employed against *A. sesami*to test their efficacy. Four replications were maintained for each treatment. The Petri plates containing PDA alone and inoculated with *A. sesami*served as control.

Radial growth of the *A. sesami*was recorded daily till the full growth of fungus was observed in control. The growth of fungal colony was measured by excluding five mm fungal inoculum disc from the centre of the Petri plate with the help of scale. Per cent inhibition of growth over control was calculated using the formula given by Vincent (1927).

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Per cent inhibition of growth over control;

C = Radial growth of *A. sesami*in non-poisoned food medium (control) (mm);

T = Radial growth of *A. sesami* in poisoned food medium (treatment)(mm).

The data recorded were subjected to angular (arc sin) transformation and analyzed statistically by adopting Completely Randomized Design (CRD) as described by Panse and Sukhatma (1985). The means were compared by Duncan multiple-ranges test (DMRT) at P d" 0.05.

RESULTS AND DISCUSSION

The results clearly revealed that all the fungicides, biocontrol agents and botanical extracts tested *in vitro* against *Alternariasesami*significantly inhibited the mycelial growth. The fungicides were found to be more effective than biocontrol agents and botanical extracts.

In vitro evaluation of fungicides against *A. sesami*

All the test fungicides were significantly effective in reducing the mycelial growth of *A. sesami* (Table 1) with the growth inhibition over the control ranging from 57.27% to 94.14% (Plate 1). Combination product of carbendazim 12% + mancozeb 63% @ 0.2% was found most effective *in vitro* with minimum mycelial growth (5.27 mm) and highest mycelial growth inhibition (94.14%) and was significantly superior to all other fungicides. It was followed by hexaconazole @ 0.2% with 7.26 mm radial growth and 91.93% inhibition. Azoxystrobin @ 0.1% was the least effective with 38.46 mm growth and 57.27% inhibition of mycelial growth.

Complete inhibition of the pathogen was reported with mancozeb and propineb at 0.3% concentration

Table 1. Effect of fungicides on mycelial growth of *Alternaria sesami in vitro*

S. No.	Name of the fungicide	Trade name (manufacturing company)	Active ingredient	Recommended dose	Radial growth (mm)*		Inhibition over control
1	Captan	Captaf (Rallis)	50% WP	0.30%	32.89	$(34.98)^{h}$	63.46
2	Mancozeb	Dithane M- 45 (Indofil)	75% WP	0.30%	16.34	$(23.83)^{e}$	81.85
3	Chlorothalonil	Kavach (Syngenta)	75% WP	0.20%	21.22	$(27.42)^{g}$	76.42
4	Carbendazim	Zoom (UPL)	50% WP	0.10%	18.77	$(25.66)^{f}$	79.15
5	Propiconazole	Tilt (Syngenta)	25% EC	0.10%	12.54	$(20.73)^{d}$	86.07
6	Hexaconazole	Contaf (TATA)	5% EC	0.20%	7.26	$(15.63)^{b}$	91.93
7	Tebuconazole	Folicur (Bayer)	25% EC	0.20%	17.76	$(24.92)^{f}$	80.26
8	Azoxystrobin	Amistar (Syngenta)	25% SC	0.10%	38.46	$(38.30)^{i}$	57.27
9	Carboxin 37.5% + Thiram 37.5%	Vitavax Power (Bayer)	75% WP	0.20%	11.32	$(19.65)^{c}$	87.42
10	Carbendazim 12% + Mancozeb 63%	Saaf (UPL)	75% WP	0.20%	5.27	$(13.27)^{a}$	94.14
11	Hexaconazole 5% + Captan 70%	Taqat (Rallis)	80% WP	0.10%	17.54	$(24.74)^{f}$	80.52
12	Control				90	$(71.54)^{j}$	0
	Mean				28.39		
	SEm ±				0.3		
	CD (P ≤ 0.05)				0.87		
	CV (%)				1.83		

Table 2. Effect of bio-control agents on mycelial growth of *Alternaria sesami in vitro*

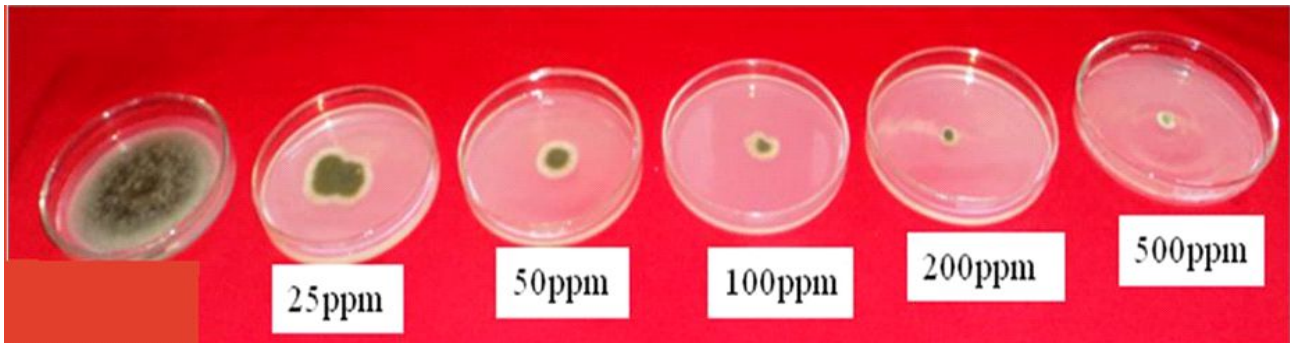
S. No.	Treatment	Radial growth (mm)*	Inhibition over control (%)
B_1	*Pseudomonas fluorescens* (isolate-1)	12.5 $(20.69)^{a}$	86.11
B_2	*Pseudomonas fluorescens* (isolate-2)	18.9 $(25.76)^{c}$	79.00
B_3	*Trichodermaviride* (isolate-1)	21.37 $(27.52)^{d}$	76.26
B_4	*Trichodermaviride* (isolate-2)	12.01 $(20.27)^{a}$	86.66
B_5	*Trichodermaviride* (isolate-3)	13.17 $(21.27)^{b}$	85.37
B_6	*Bacillus subtilis*	24.13 $(29.41)^{e}$	73.19
B_7	Control	90 $(71.54)^{f}$	
	Mean	30.92	
	SEm ±	0.18	
	CD (P ≤ 0.05)	0.54	
	CV (%)	1.00	

*Mean of four replications; Values in the parenthesis indicate angular transformed values;
Values followed by same alphabet in the same column do not differ significantly at P ≤ 0.05 according to DMRT

Table 3. Effect of botanical extracts on mycelial growth of *Alternaria sesami in vitro*

S. No.	Treatment	Radial growth (mm)*	Inhibition over control (%)
P_1	Neemleaf extract 10%	22.77 (28.49)^{b}	69.64
P_2	Lantana leaf extract 10%	37.71 (37.87)^{d}	49.72
P_3	Calotropis leaf extract 10%	44.51 (41.83)^{f}	40.66
P_4	Tulasileaf extract 10%	41.47 (40.07)^{e}	44.70
P_5	Onionbulb extract 10%	24.19 (29.45)^{c}	67.74
P_6	Garlicclove extract 10%	20.59 (26.97)^{a}	72.55
P_7	Control	75.00 (59.98)^{g}	
	Mean	39.46	
	SEm ±	0.06	
	CD ($P \leq 0.05$)	0.18	
	CV (%)	0.25	

*Mean of four replications; Values in the parenthesis indicate angular transformed values;
Values followed by same alphabet in the same column do not differ significantly at $P \leq 0.05$ according to DMRT



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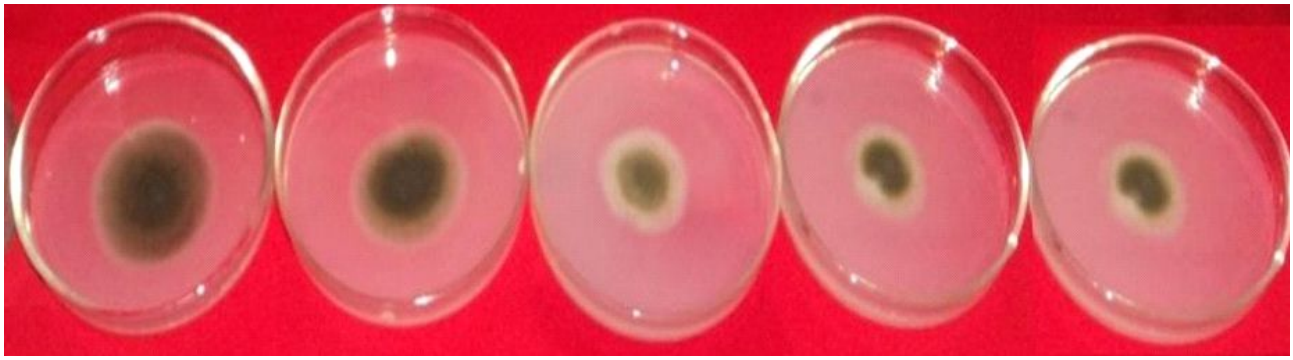
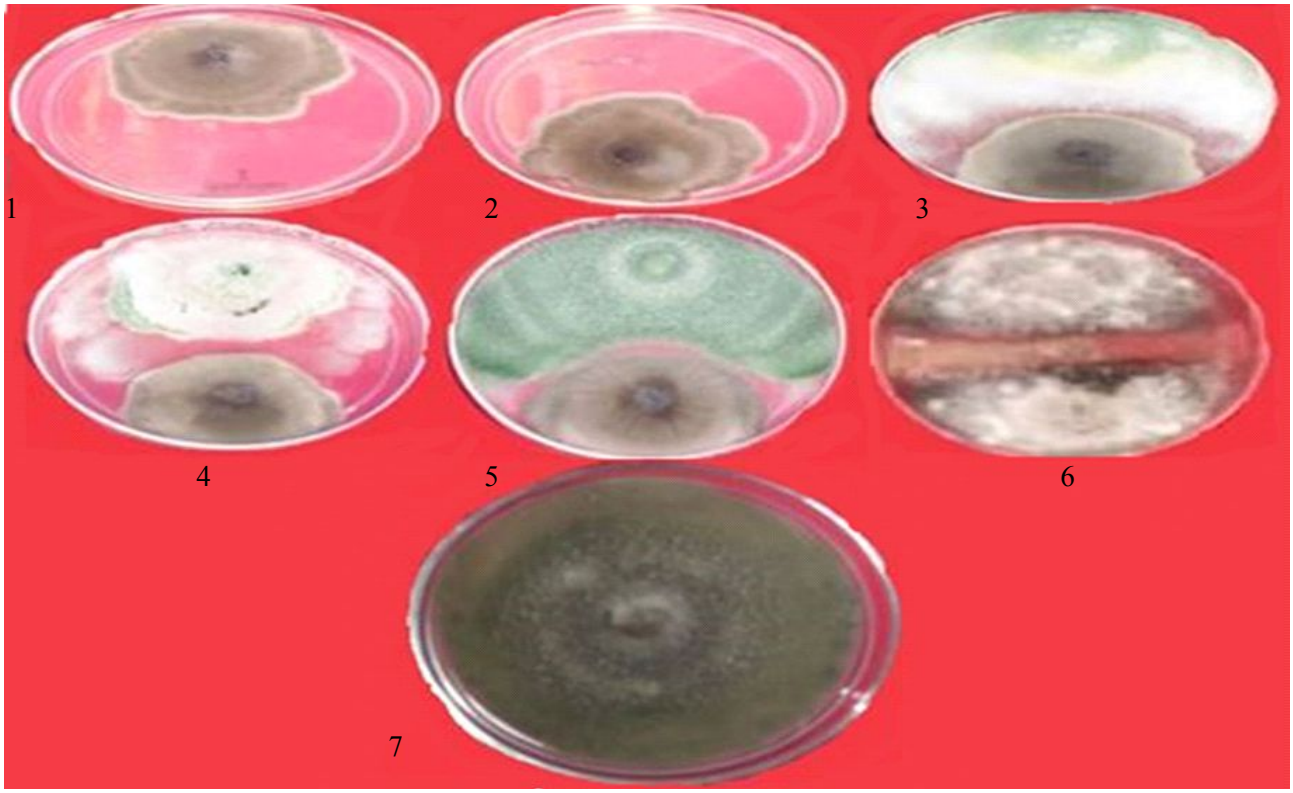


7 8 9 10 11

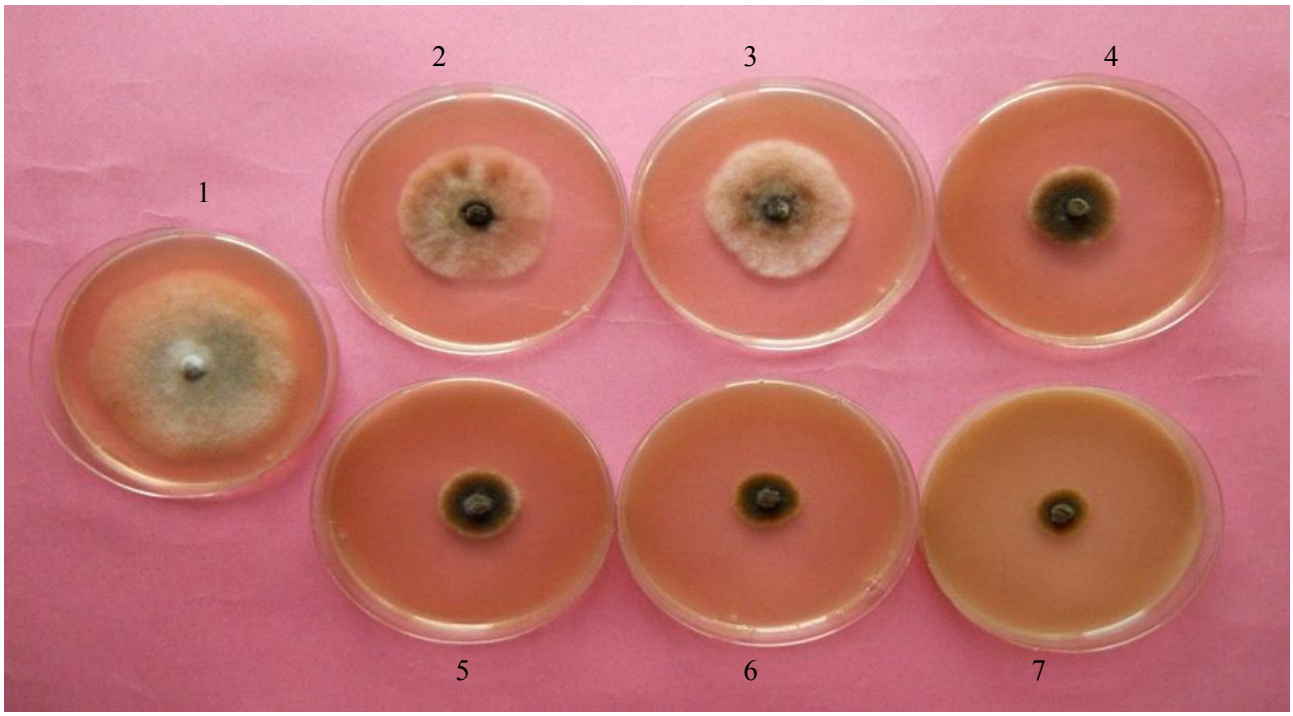
1. Control; 2. Propiconazole @ 0.1 %; 3. Tebuconalole @ 0.2 %; 4. Combination product of Carboxin 37.5% + Thiram 37.5 % @ 0.2 %; 5. Hexaconazole @ 0.2 %; 6. Combination product of Carbendazim 12% + Mancozeb 63% @ 0.2 %; 7. Azoxystrobin @ 0.1 %; 8. Captan @ 0.3 %; 9. Chlorothalonil @ 0.2 %
10. Carbendazim @ 0.1 %; 11. Mancozeb @ 0.3 %

Plate 1. *In vitro* evaluation of fungicides against *Alternaria sesami*



1. Pseudomonas fluorescens (isolate-1); 2. Pseudomonas fluorescens (isolate-2)
3. Trichoderma viride (isolate-1); 4. Trichoderma viride (isolate-3)
5. Trichoderma viride (isolate-2); 6. Bacillus subtilis; 7. Control

Plate 2. *In vitro* evaluation of bio-control agents against *Alternaria sesami*



1. Control; 2. Tulasileaf extract 10%; 3. Calotropis leaf extract 10% ; 4. Lantanaleaf extract 10%
5. Onionbulb extract 10%; 6. Neemleaf extract 10%; 7. Garlicclove 10%

Plate 3. *In vitro* evaluation of botanicals against *Alternaria sesami*

whereas copper oxy chloride was reported with 72.27 per cent inhibition (Chethana*et al*., 2012). Maheshwari and Krishna (2013) observed that hexaconazole (400 and 300 ppm) completely inhibited the growth of *A. alternata*. Wanggikar*et al*. (2014)observed cent per cent (100.00 %) inhibition of *A. porrii* with Hexaconazole followed by Difenoconazole (83.91%) and Mancozeb (63.58%) and minimum inhibition with chlorothalonil (31.40%). Maximum growth inhibition (100.00%) was reported with mancozeb at 1000 ppm followed by copperoxychloride at 1000 ppm (95.20%) and mancozeb 500 ppm (90.50%) (Kantwa*et al*., 2014).Carbendazim (94.44%) was found with significantly high mycelial inhibition of *A. carthami* followed by mancozeb (85.43%) and thiram (83.33%) (Taware, *et al*., 2014). Dithane M-45 (Mancozeb) was found to be the most effective fungicide against *A. helianthi (*Athira,2017*)*. Mancozeb, carbendazim, hexaconazole, propiconazole and carbendazim + mancozeb completely inhibited the growth of *A. macrospora* (Prasad *et al*., 2018).Hexaconazole, difenconazole, propiconazole, Zineb 68% + Hexaconazole 4% 72 WP @ 0.1 to 0.25% and Carbendazim 25% + Iprodione 25% 50 WP at 0.25%were effective with 100 per cent inhibition of *A. carthamii* (Madhu*et al.*, 2018).

In vitro* evaluation of bio-control agents against *A. sesami

All the bio-control agents significantly reduced the mycelial growth of *A. sesami* over control (Table 2).Minimum radial growth (12.01 mm) and maximum inhibition of mycelial growth of *A. sesami* over control (86.66%) was obtained with *Trichoderma viride* (isolate-2) followed by *Pseudomonas fluorescens* (isolate-1) with 12.50 mm radial growth and 86.11% mycelial inhibition. *Bacillus subtilis* was the least effective with 24.13 mm radial growth and 73.19% mycelial inhibition (Plate 2). The results revealed that the antagonists significantly reduced the growth of *A. sesami* either by competition (over growing) or by antibiosis (exhibiting inhibition zones).The inhibition of mycelial growth of *A. sesami* by *Trichoderma* isolates could be obviously attributed to several possibilities of existence of microbial interactions such as higher competitive ability, stimulation and antibiosis by these isolates over the test pathogen.The antibiotics/volatile and non-volatile metabolites produced by the bio-control agents might have inhibited the mycelial growth (Karimi*et al*., 2012).

Dalpati *et al*.(2010) reported superiority of *T. harzianum* with 76.66% inhibition of *A. macrospora* followed by *Bacillus subtilis* (73.66%). Chethana*et al*. (2012) also found *T. harzianum*as most effective against *A. porri*causing 79.35 per cent inhibition of mycelial growth whereas Wanggikar *et al*. (2014) recorded 58.94 % and 54.45%inhibition of *A. porrii* by *P. fluorescens* and *T.viride*, respectively. *Pseudomonas*was reported with better growth inhibition (77%) of *A. helianthi* than *Trichoderma* strains (71%) (Athira, 2017). Prasad *et al.* (2017) found *B. subtilis* strain to cause 51.68% growth inhibition of *A. macrospora*followed by P.*fluorescens* strain 1 (41.89%) and P.*fluorescens* strain 3 (39.94%).

In vitro* evaluation of botanicals against *A. sesami

Significant differences in the efficacy of botanicals were observed in reducing the radial growth of *A. sesami*(Table 3).Garlicclove extract 10% was significantly superior with 20.59 mm radial growth and 72.55% mycelial growth inhibition over control in comparison to other botanicals tested (Plate 3). Least inhibition (40.66%) of mycelia growth over control was noticed in case of *Calotropis* leaf extract 10%.

Allium sativum, *Azardirachtaindica*, *Daturastramonium* L. and *Ocimum sanctum*were found to be most effective against *A. alternata* in pomegranate crop (Singh and Majumdar, 2001). Aqueous neem leaf extract (1-5%) was found to be most effective against *A. alternata* causing leaf blight of groundnut (Nandagopal and Ghewande, 2004). The highest growth inhibition of *A.alternata*and *A. helianthi* by *A. indica* was also observed by Kumar *et al*. (2005) and Mesta*et al*. (2009).Lantana (44.59%) and Datura (30.88%) were found effective by restricting mycelial growth of *A. macrospora* (Dalpati*et al*., 2010).Garlic clove extract was found most effective in inhibiting the mycelial growth (46.60 %) of *A. alternata* followed by neem (43.30%) and Daturaleaf extract (40.30%) (Singh and Verma, 2010 andKantwa*et al.*, 2014). Neem leaf extract was reported superior with 68.52% inhibition of *A. macrospora* followed by garlic clove (68.40%) (Prasad *et al*., 2017).

CONCLUSION

The present results clearly indicated the combination product of carbendazim 12% and mancozeb 63% to be highly effective in controlling the mycelia growth of *A. sesami*.*T. viride* (isolates-2 and 3) and *P. fluorescens* (isolate-1) inhibited *A. sesami* similar to fungicides *viz*., propiconazole and combination product of carboxin + thiram under *in vitro* conditions. The extracts of garlic clove, neem leaf and onion bulb showed comparable efficacy with biocontrol agent, *B. subtilis* and fungicide, chlorothalonil against *A. sesami*. Hence, both biocontrol agents and botanicals need to be further tested for their efficacy in controlling this major seed borne fungus under *in vivo* conditions.

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