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RESEARCH ARTICLE

IN VITRO EVALUATION OF PLANT EXTRACTS, BIO AGENTS AND FUNGICIDES AGAINST *ALTERNARIA ALTERNATA* INCITANT OF BROWN LEAF SPOT OF POTATO

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Abstract: Brown leaf spot disease of potato caused by *Alternaria alternata* has been known as one of the destructive and common diseases of potato and occurred worldwide. *In vitro* evaluation of plant extracts, *Euphorbia tithymaloides* (Nagdon) was found most effective followed by *Jasminum* spp (Jasmine) and *Eclipta prostrate* (Bhringraj). Highest mycelial growth inhibition (71.86%) was recorded at 10% concentration of *Trichoderma viride* culture filtrate followed by *Trichoderma viride* (60.74% reduction) and *Pseudomonas fluorescens* (34.81% growth reduction) over to control. Among the different fungicides, Hexaconazole 4% + Zineb 68% WP at 0.3% concentration exhibited nearly 100% reduction in mycelial growth over to control treatment (90.00 mm radial growth) at par with Hexaconazole 5% EC at 0.2%, Captan 70% + Hexaconazole 5% WP at 0.3% followed by Fluopyram 17.7% + Tebuconazole 17.7% SC at 0.2% with 92.59% mycelial growth reduction and Tebuconazole 50% + Trifloxystrobin 25% SC at 0.2% with 91.48% reduction over to control at 7 DAI.

Keywords: Potato, *Alternaria alternata*, Plant extract, Bio agents, Fungicide, *In vitro*

INTRODUCTION

Potato (*Solanum tuberosum* L.) also known as "The king of vegetables" is an annual plant in the Solanaceae family, grown for its starchy edible tubers and is mainly grown in tropics as well as sub-tropics during the cool as well as dry seasons. Potato is the most potential food crop in the world since being nutritious and remunerative crop considered a friend of poor men. Potato contained carbohydrates (22%), proteins (2%), fats (0.1%), water (74%) along with minerals and trace elements viz. potassium, sodium, iodine and magnesium, folic acid, pyridoxine, vitamin C, ascorbic acid and Iron (Sahar *et al*., 2017). Potato is fourth most important staple food crop in the world after maize, wheat, and rice, globally cultivated in more than 20 million hectares with a total global production of 359 million tons in 2020. India is largest producer of potato after China almost one third of total potato is harvested by both countries (FAO, 2020). India produced 53.03 million tones of potato from 2.16 million hectare area with an average yield of 24.55 t/ha during 2018-19, cultivated in almost all states, among them Uttar Pradesh, West Bengal, Bihar, Punjab and Gujrat are the leading state, while in Chhattisgarh, grown over an area of 556.83 thousand hectares with a production of 659.66 thousand tones.

The diseases caused by fungal, bacterial, nematodes and virus are the major biotic stresses for potato. Among the fungal diseases blight and brown leaf spot, that is caused by two species of genus *Alternaria* (*Alternaria solani* and *Alternaria alternata*), occurs worldwide on potato crops, particularly in the regions with high temperature and alternating periods of dry weather and high humidity and/or irrigated potato soils, light-textured, sandy, low in organic matter (Gudmestad and Pasche, 2007). Foliar lesions of brown leaf spot appear as small, irregular to circular, dark brown spots on lower leaves and range in size from pinpoint to 1/8 inch, leads to drying out leaflets (Van der Waals *et al*., 2011), often confused with those caused by *Alternaria solani* (Giha, 1973) except for the absence of concentric rings within the necrotic spots.

The brown leaf spot also appear as necrotic lesion on tuber especially under storage as black pit (Nolte, 2008) and reported post harvest losses as high as 10%. Wharton and Kirk (2008) have been reported around 20% yield loss, however there have been cases of 70–80% yield losses, where the disease has been left uncontrolled. These losses can be increased when the disease is combined with other diseases like early blight, black-leg and *Verticillium* wilt (Jansky *et al*., 2008). Yield losses due to brown leaf spot disease were estimated around 30% in South Africa (Van der Waals *et al*., 2011) can be reached up to 80% in North America if the disease left uncontrolled (Soleimani and Kirk, 2012)

The most common method for controlling *Alternaria* diseases is the cultural practices (Wharton and Kirk, 2012) and use of fungicides (Tsedaley *et al*., 2014). However, the fungicides cannot be considered as a long-term solution, due to concerns of expense, exposure risks and the hazards of its residues. Optimization of fungicide used for the control of

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Alternaria diseases is still a considerable challenge due to the capacity of pathogen to produce huge amounts of inoculum. Now a day, various botanical and bio-control agents available which can reduce populations of foliar pathogens but there effect is comparatively low. Plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides (Verma and Dubey, 1999). In the present study, investigation was carried out for in vitro evaluation of plant extracts, bio agents and fungicides against *Alternaria alternata* incitant of brown leaf spot of potato.

MATERIAL AND METHODS

Present investigations were carried out at Department of Plant Pathology, IGKV, Raipur (Chhattisgarh) during 2021-22. The plant extracts, culture filtrate of *Trichoderma viride* and fungicides were evaluated at different concentrations against the fungal pathogen *Alternaria alternata* through poison food technique. *Trichoderma viride* and *Pseudomonas fluorescens* were evaluated against *Alternaria alternata* through dual culture technique. All the tests along with control treatment were carried out on PDA medium with three replications. The mycelial growth of test fungus was recorded at 7 DAI and per cent inhibition of mycelial growth of the fungus was calculated by using the formula given by Vincent (1947) as

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

Where,
I = Per cent inhibition
C = Mycelial growth in control
T = Mycelial growth in treatment

Isolation

The standard tissue isolation procedure was followed to isolate the pathogen. The infected leaves showed typical symptoms were washed thoroughly in running tap water repeatedly, to remove the soil and dust adhered on surface. The infected leaves were cut into bits of about five mm size and surface sterilized in 0.1 per cent Mercuric Chloride solution for 30 second, rinsed thrice with sterile distilled water. Surface sterilized bits were then placed aseptically on the centre of Petri dish contained sterilized solidified Potato Dextrose Agar (PDA) medium and gently pressed to confer contact with medium. The Petri dishes then incubated in B.O.D. incubator under alternate 12 h light and 12 h dark at 28±2°C for 72 hours. Pure culture of the *A. alternata* was obtained by hyphal tip culture and single spore culture technique. The pathogen was identified as *Alternaria alternata* based on morpho-cultural characteristics.

Preparation of aqueous leaf extract

The apparently healthy leaves of these plants were collected from field, washed thoroughly 2-3 times with running tap water and once with sterile distilled water then air dried. The small pieces of 100 gm leaves were crushed in 100 ml of sterile water by using mortar with pestle. The crushed product was filtered by using muslin cloth then centrifuged at 10000× *g* for 15 min at 4°C and the supernatant solutions were collected and filtered through Whatman No. 1 filter paper, sterilized at 15 lb/inch^2 pressure for 15 minutes. The obtained extracts served as the stock solution (100% concentration).

***In vitro* evaluation of plant extracts**

Twelve different plant species, known for their medicinal value in traditional medicine were evaluated at different concentration viz 1, 3, 5, 7 and 10% in the laboratory for their efficacy against *A.alternata* through poison food technique. The required quantity of stock solution (100%) were added separately with melted potato dextrose agar medium to make required concentration viz., 1, 3, 5, 7 and 10 % and mix thoroughly. The 20 ml melted media mixed with plant extract were then poured in to Petri dishes. After solidification, a mycelial disc (5 mm) of 10 days old culture of pathogen (*Alternaria alternata*) was transferred on centre of the poured Petri dishes. Control was maintained by growing the cultures on PDA without any plant extract. All the plates were incubated at 28±2°C in B.O.D. incubator for 7 days.

***In vitro* evaluation of bio-agents**

The antagonistic efficiency of bio control agents, *Trichoderma viride* and *Pseudomonas fluorescens* were evaluated against *Alternaria alternata* through dual culture technique. 20 ml PDA medium poured on Petri dish and after solidification five mm mycelial disc of *Alternaria alternata* taken from ten days old culture was placed at one end of Petri dish and 5 mm mycelial disc of *Trichoderma viride* was inoculated on the opposite in same Petri dish, both discs were in equal distance from periphery of Petri dish. In case of *Pseudomonas fluorescens*, the pathogen *A. alternata* was placed at one end of the Petri dish and bacterial culture was streaked on opposite end on same Petri dish. The culture filtrate of *Trichoderma viride* at different concentrations viz., 1, 3, 5, 7 and 10% were evaluated through poison food technique. Appropriate volumes of the filtrates were added to the molten PDA medium to obtain final concentrations of 1, 3, 5, 7 and 10% and poured into sterilized petri dishes. After solidification of the medium placed in the Petri dish was inoculated on centre with 5 mm mycelial disc of the test pathogen taken from 10 days old culture. The plates were then incubated at 28±2^{0}C.

***In vitro* evaluation of fungicides**

Fungicides Carbendazim 12% + Mancozeb 63% WP, Hexaconazole 4% + Zineb 68% WP, Captan 70% + Hexaconazole 5% WP, Chlorothalonil 75% WP and

Mancozeb 75% WP were evaluated at concentration of 0.1, 0.15, 0.2, 0.25 and 0.3%, while six fungicides viz., Myclobutanil 10% WP, Tebuconazole 50% + Trifloxystrobin 25% WG, difenoconazole 25% EC, Fluopyram 17.7% + Tebuconazole 17.7% SC, Azoxystrobin 11% + Tebuconazole 18.3% SC and Hexaconazole 5% EC were evaluated at concentration of 0.05, 0.1, 0.15,0.2 and 0.25% against *Alternaria alternata* in laboratory through poisoned food technique on PDA medium. Fungicide suspension was prepared in PDA by adding required quantity of fungicide to obtain the desired concentration of the fungicide. Twenty ml poisoned PDA medium were poured in to Petri dishes and after solidification, a 5 mm mycelial disc of pathogen from ten days old culture were placed aseptically in the centre. A control treatment was maintained without adding fungicides. All the treatments were incubated at 28±2^{0}C in B.O.D. incubator

RESULTS AND DISCUSSION

***In vitro* evaluation of plant extracts:** Result presented in table 1 showed that all the plant extracts tested against *Alternaria alternata* were found reduced mycelial growth in comparison to control at all the concentrations at 7 DAI. Among the different concentrations viz., 1, 3, 5, 7 and 10%, the most effective concentration was 10% at par with 7%, at which all the plant extracts exhibited minimum mycelial growth of test pathogen, while maximum fungal growth was recorded at 1% concentration. Irrespective of different concentrations, *Euphorbia tithymaloides* (Nagdon) was the most effective plant extract among the plant extracts tested, which inhibited the mycelial growth 30% followed by *Jasminum* spp (Jasmine) with 28.52% inhibition and *Eclipta prostrate* (Bhringraj) with 25.19% at concentration of 10%. However the least effective plant extract in inhibiting the mycelial growth was *Alkana tinctoria* (Ratanjot) with 2.97% growth inhibition followed by *Lantana camera* with 5.56% and *Aegle marmelos* (Bel) with 7.41% mycelial growth reduction of test fungus over to control. The results also showed the gradual reduction of mycelium growth with gradual increase in concentration of plant extract.

The present findings are agreeing with the results of Nashwa *et al*. (2012) they reported leaf extracts of *D. stramonium*, *A. indica* and *A. sativum* @ 5% inhibited mycelial growth of *A. solani* (44.4, 43.3 and 42.2%, respectively) highest. Rani *et al*. (2017) reported maximum inhibition by *Lantana camara* (65.07%) which was followed by *Datura stramonium* (63.33%) and *Azadirachta indica* kernel (61.33%) at 20% concentration. Similar effects of various other plant products against *Alternaria* spp were reported by Patni *et al*., 2005 and Roopa *et al*., 2014. The mechanisms of disease suppression of plant products have been suggested the presence of active principles that may either directly act on the pathogen (Amadioha, 2000) or induce systemic resistance in host plants resulting in a reduction of the disease development (Kagale *et al*., 2004).

Table 1. *In vitro* evaluation of plant extracts against *Alternaria alternata*

S.No	Plant species	Plant parts used	Mean mycelial growth (mm)* 7 DAI**					% reduction over control at 10%
			1%	3%	5%	7%	10%	
1	*Lantana camara*	Leaves	88.33	87.67	87.33	87.33	85.00	5.56
2	*Alkana tinctoria*	Leaves	90.00	90.00	90.00	88.33	87.33	2.97 (lowest)
3	*Calotropis gigantean*	Leaves	84.67	87.33	87.33	87.33	82.33	8.52
4	*Tribulus terrestris*	Leaves	88.00	88.00	85.33	83.67	82.00	8.89
5	*Eclipta prostrate*	Leaves	74.33	76.00	70.33	67.67	67.33	**25.19 (III)**
6	*Dhatura stramonium*	Leaves	76.33	78.33	68.67	70.33	70.33	21.86
7	*Hibiscus* spp	Leaves	84.33	84.00	80.00	77.33	75.67	15.92
8	*Ricinus communis*	Leaves	83.00	85.33	78.67	77.00	74.33	17.41
9	*Aegle marmelos*	Leaves	85.00	84.67	82.33	83.33	83.33	7.41
10	*Euphorbia tithymaloides*	Leaves	77.33	77.67	66.33	64.33	63.00	**30.00 (I)**
11	*Jasminum* spp	Leaves	71.00	70.67	66.67	65.33	64.33	**28.52 (II)**
12	*Cascabela thevetia*	Leaves	83.00	83.33	80.00	78.67	79.00	12.22
13	Control	-	90.00	90.00	90.00	90.00	90.00	-
	SE(m)±		0.018	0.021	0.347	0.372	0.348	
	C.D.(p=0.05)		0.052	0.061	1.015	1.087	1.017	

*Mean of three replications, **Days after inoculation

***In vitro* evaluation of bioagents:** Data presented in table 2 revealed that the bioagents significantly inhibited the mycelial growth of test fungus and also *Trichoderma viride* culture filtrate at all concentration inhibited the mycelial growth of the *Alternaria alternata* significantly over untreated control. Among the different concentration of *Trichoderma viride* culture filtrate, the minimum

mycelial growth of test pathogen was recorded at concentration of 10% (25.33 mm) followed by 7% concentration (28 mm), most effective among the different concentration including control (90.00 mm) at 7 DAI, while maximum mycelial growth (61.00 mm) was observed at 1% concentration followed by 3% concentration (52.33 mm). The gradual reduction of mycelial growth was observed as the concentration of culture filtrate increased. *Trichoderma viride* (35.33 mm mycelial growth) and *Pseudomonas fluorescens* (58.67 mm) also found significantly in reducing the mycelial growth of test fungus in comparison to control treatment on dual culture test at 7 DAI. Highest mycelial growth inhibition (71.86%) was recorded at 10% concentration of *Trichoderma viride* culture filtrate followed by *Trichoderma viride* (60.74% reduction) and *Pseudomonas fluorescens* (34.81% growth reduction) over to control. Odebode (2006) who found undiluted culture filtrates of the two *Trichoderma* species completely inhibited germination of conidia/spores of all the rot pathogens and percentage inhibition decreases by diluting the culture filtrates. The enhanced antifungal activity of *Trichoderma* spp and *Pseudomonas fluorescens* against *Alternaria* spp in *in vitro* were also reported by Thaware *et al*. (2010). Chaitali (2014) recorded significant reduction of mycelial growth of *Alternaria* fungus by *Trichoderma viride* (81.4%) and *Pseudomonas fluorescens* (66%). The results of the present study corroborates with the results of Zade *et al*. (2018) who reported *Trichoderma* spp most effective against *Alternaria alternata* causing leaf spot of Soybean, with maximum mycelial per cent inhibition 79.65% and 76.55%, respectively and concluded that the inhibition may have been due to the secretion of extracellular cell degrading enzymes such as chitinase β-1, 3-glucanase, cellulose and lectin, which may have myco-parasitic.

Table 2. *In vitro* evaluation of bioagents against *Alternaria alternata*

S.No.	Bio control agents & culture filtrate	Mycelial growth (mm) 7 DAI*					% reduction over control
1	*Trichoderma viride* culture filtrate	**1%**	**3%**	**5%**	**7%**	**10%**	71.86
		61.00	52.33	37.33	28.00	25.33	
	SE(m)±	0.28					
	C.D.(p=0.05)	0.873					
	Bioagents						
2	*Trichoderma viride*	35.33					60.74
3	*Pseudomonas fluorescens*	58.67					34.81
4	Control	90.00					-

* Days after inoculation

***In vitro* evaluation of fungicides:**

The efficacy of eleven fungicides at different concentrations was evaluated against *Alternaria alternata* in laboratory through poisoned food technique on PDA medium. The results presented in table 3 revealed that all the treatments were significantly effective in reducing the mycelial growth of the *Alternaria alternata* at all concentrations, when compared to control treatment at 7 days after inoculation. The results also indicated that least inhibition of mycelial growth observed at lowest concentration and per cent inhibition increased with increased in concentration till the maximum inhibition, at this concentration and subsequent concentration, the maximum inhibition was nearly to similar.

Among the fungicides tested, Hexaconazole 4% + Zineb 68% WP at 0.3% concentration found minimum mycelial growth (5.00 mm) and exhibited nearly 100% reduction in mycelial growth over to control treatment (90.00 mm radial growth) at par with Hexaconazole 5% EC at 0.2% (5.00 mm), Captan 70% + Hexaconazole 5% WP at 0.3% (5.00 mm) followed by Fluopyram 17.7% + Tebuconazole 17.7% SC at 0.2% (6.67 mm) with 92.59% mycelial growth reduction at par with Tebuconazole 50% + Trifloxystrobin 25% SC at 0.2% (7.67 mm) with 91.48% reduction over to control at 7 DAI. The maximum mycelial growth and minimum mycelial growth reduction among the fungicides was observed in Chlorothalonil 75% WP at 0.3% (42.67 mm) with 52.59% mycelial growth reduction followed by Carbendazim 12% + Mancozeb 63% WP at 0.3% (22.67 mm) with 74.81% growth reduction at par with Mancozeb at 0.3% (21.67 mm) with 75.92% mycelial growth reduction over to control treatment. However, other fungicides viz Difenoconazole 25% EC (11.00 mm), Azoxystrobin 11% + Tebuconazole 18.3% SC (12.33 mm) and Myclobutanil 10% WP (17.67 mm) also observed significant at all concentration but most effective at concentration of 0.2% in reduction of mycelial growth of *Alternaria alternata* in comparison to that of control.

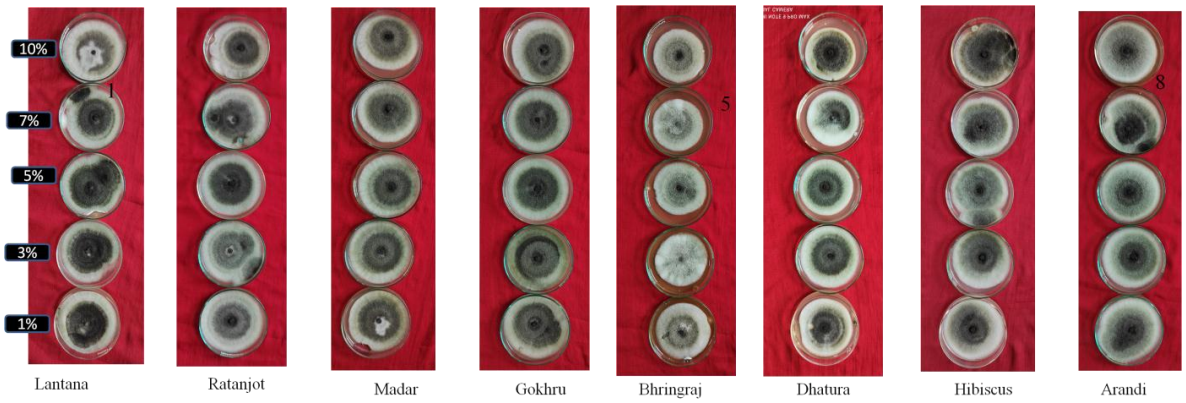
The above results were in conformity with the findings of Singh and Singh (2006) who evaluated the Azoxystrobin, Chlorothalonil, Propineb, Mancozeb, Copper oxychloride and Copper hydroxide at 250, 500, 1000, 2000 and 2500 ppm and Hexaconazole at 50, 100, 200, 500 and 1000 ppm against *Alternaria alternata* causing early blight of tomato and observed that Hexaconazole 5% EC was very effective as it caused 100% growth inhibition.

The present results were also supported by the results of Sukrutha *et al*. (2014) who recorded Zineb 68% + Hexaconazole 4% WP and Captan 68% + Hexaconazole 5% WP at 0.1, 0.2 and 0.25% concentration equally effective and on par with Difenoconazole (100%) at 0.1 and 0.15% concentration, Mancozeb (99.33%) at 0.25% concentration, Propineb (99.11%) at 0.25 %, least effective chemical was Chlorothalonil at all the concentration tested against *A. solani*. Sharma *et al*. (2022) found Hexaconazole most effective in inhibition of mycelial growth (94.44%, 100% and 100%) of *Alternaria alternata* at all the tested concentrations i.e. at 100 ppm, 300 ppm and 500 ppm concentrations, respectively followed by Tebuconazole + Trifloxystrobin with inhibition of 88.86, 94.40 and 100% at 100, 300 and 500 ppm, respectively.

Table 3. *In vitro* evaluation of fungicides against *Alternaria alternata*

S.No.	Treatments	Mycelial growth (mm) 7 DAI*					% reduction over control
		0.1%	**0.15%**	**0.2%**	**0.25%**	**0.3%**	
1	Carbendazim 12% + Mancozeb 63% WP	36.33	29.33	25.67	25.00	22.67	74.81
2	Hexaconazole 4% + Zineb 68% WP	6.00	6.00	5.33	0.00	0.00	100.00
3	Captan 70% + Hexaconazole 5% WP	18.33	13.33	7.67	0.00	0.00	100.00
4	Chlorothalonil 75% WP	51.67	46.67	44.33	42.00	42.67	52.59
5	Mancozeb 75% WP	47.67	30.00	26.33	23.00	21.67	75.92
	SE(m)±					0.134	
	C.D.(p=0.05)					0.401	
		0.05%	**0.1%**	**0.15%**	**0.2%**	**0.25%**	
6	Myclobutanil 10% WP	38.33	23.33	22.67	17.67	17.33	80.37
7	Tebuconazole 50% + Trifloxystrobin 25% SC	18.67	11.00	8.00	7.67	7.33	91.48
8	Difenoconazole 25% EC	19.33	19.33	18.67	18.33	18.00	79.63
9	Fluopyram 17.7% +Tebuconazole 17.7% SC	9.33	8.33	7.33	6.67	6.00	92.59
10	Azoxystrobin 11% + Tebuconazole 18.3% SC	16.67	15.00	12.67	12.33	12.00	85.92
11	Hexaconazole 5% EC	8.33	7.33	6.33	0.00	0.00	100.00
12	Control	90.00	90.00	90.00	90.00	90.00	-
	SE(m)±	0.43	0.333	0.284	0.3	0.185	
	C.D.(p=0.05)	1.308	0.979	0.838	0.88	0.542	

* Days after inoculation



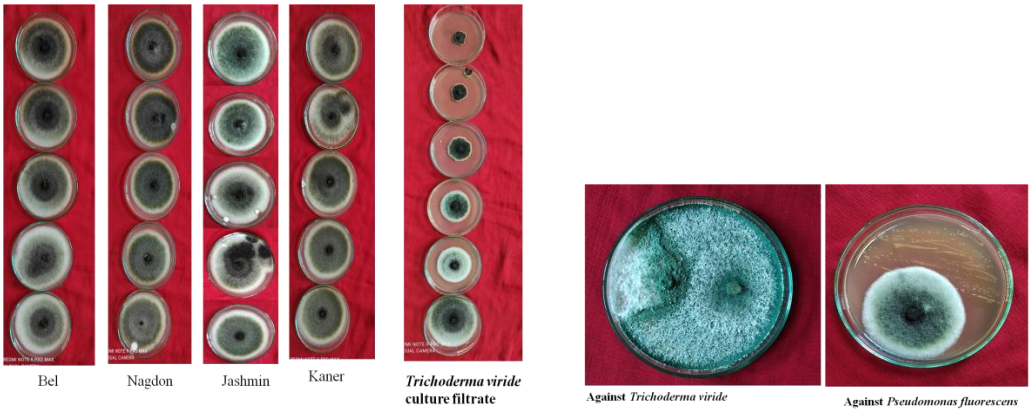


Plate 1: *In vitro* evaluation of plant extracts and bio agents against *Alternaria alternata*

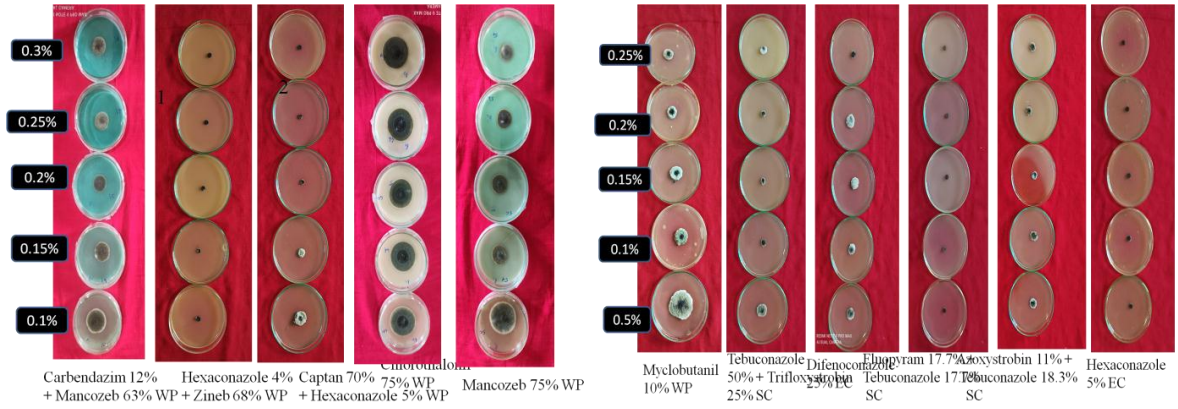


Plate 2: *In vitro* evaluation of fungicides against *Alternaria alternata*

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