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Evaluation of botanical extracts and biocontrol agents for the management of leaf spot disease in *Aloe vera* caused by *Alternaria alternata*

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Abstract
Aloe vera, also known as Gheegwar or Ghritkumari, and scientifically known as *Aloe barbadensis*, belongs to the Aloeaceae family. During the period of 2013-2024, *Aloe vera* leaf spot disease was observed in "Crop cafeteria" of ITM University, Gwalior. The present study conducted an investigation on eco-friendly management of leaf spot disease of *Aloe vera* caused by *Alternaria alternata*. Five botanical extracts namely Neem, Moringa, Garlic, Akanda and Lantana were tested at three different concentrations viz. 5%, 10%, 15% with three replications each using poison food technique. The results revealed that out of all the five botanical extracts, Neem@15% exhibited the highest inhibition percentage of mycelial growth of *A. alternata* (49.59%) followed by Garlic@15% (47.43%). Five biocontrol agents namely *Trichoderma* spp., *Trichoderma viride*, *Trichoderma harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis* were used to study the antagonistic activity against *A. alternata* using dual culture technique. Among the five antagonists, *Trichoderma viride* (75.94%) exhibited the maximum inhibition percentage of the radial growth of *A. alternata* followed by *Trichoderma* sp. (75.47%) and *T. harzianum* (70.34%).

Keywords: *Aloe vera*, *Alternaria alternata*, leaf spot, eco-friendly management, *in vitro*

Introduction
Aloe barbadensis Miller, also known as *Aloe vera* (L.) Burm.f. is a perennial succulent medicinal herb that is drought-resistant and a member of the Aloeaceae family (Barcroft and Myskja, 2003) [2]. It is often called a "miracle plant" and has a extensive record of economic and medicinal applications dating back thousands of years (Daodu, 2000) [3]. Since *Aloe vera* content 96% of water which is a rich source of minerals, amino acids, enzymes, and phytochemicals, it is widely utilized in ayurveda medicine, cosmetics, and as a dietary supplement (Rajeswari *et al*., 2012) [17]. For natural pain relief from burns, rashes, insect bites, and other skin irritations, *Aloe vera* gel has been utilized (Olusegun, 2000) [13]. *Aloe vera* is mostly native to warm regions of Africa, particularly the highlands of tropical Africa and the Café Province of South Africa (Yebpella *et al*., 2011) [25]. In India, *Aloe vera* is grown in Andhra Pradesh, Rajasthan, Gujarat, Madhya Pradesh, Maharashtra, Chhattisgarh, Karnataka, Tamil Nadu, Bihar, West Bengal, and Jharkhand. Thailand is the world's largest grower of *Aloe vera*, contributing around one-third of the plant's entire output. Other leading producers include Mexico, Dominican Republic, United States and Costa Rica. The demand for Aloe Vera is robust globally, with significant growth expected in emerging markets like India, China, and the Middle East. Numerous bacterial and fungal diseases attacked *Aloe vera*, affecting both its therapeutic and antimicrobial potential (IMARC Group, 2021) [7]. Major diseases of *Aloe vera* includes leaf spot disease caused by *Alternaria alternata* (Kamalakannan *et al*., 2008) [9], leaf rot disease caused by *Fusarium oxysporum* (Kawuri *et al*., 2012) [10], anthracnose disease caused by *Colletotrichum gloeosporiodes* (Avasthi *et al*., 2012) [1], soft rot disease caused by *Pectobacterium chrysanthemi* (Pervez *et al*., 2016) [15], rust disease caused by *Uromyces aloes* (Devin *et al*., 2021) [4]. Cultivation of *A. vera* is severely threatened by diseases, which cause enormous losses of between 25 and 75 percent (Syamala and Ciba, 2017) [23]. Bacterial soft rot causes yield losses up to 80 percent (Syamala and Ciba, 2017) [23]. In India, aloe grown in Coimbatore, Erode and Madurai

districts of Tamil Nadu, India, suffered heavy losses due to a leaf spot disease (Kamalakannan *et al*., 2008) [9]. Considering these viewpoints, the objectives of the present study is to identify the suitable eco-friendly management of leaf spot disease of *Aloe vera* caused by *Alternaria alternata*.

Material and Methods

Isolation, purification and pathogenicity test of the pathogen

The infected leaves of *Aloe vera* were collected from the Crop cafeteria of ITM University, SoAG, Gwalior. For further investigation, the leaves samples were brought to Plant Pathology PG and the entire work of isolation and purification was done successfully under aseptic condition. Pathogenicity test was also performed successfully inside the greenhouse, confirming Koch's postulates.

Collection and preparation of plant extracts

Five botanicals (Table 1) namely Neem *(Azadirachta indica)*, Moringa (*Moringa oleifera*), Lantana (*Lantana camara*), Garlic (*Allium sativum*) and Akanda (*Calotropis gigantea*) were collected inside the campus of ITM University, Gwalior.

The plant extracts were prepared according to the method described by Shabana *et al*. (2017) [20] and Draz *et al*. (2019) [5]. Freshly collected healthy leaves and bulb were well cleansed with tap water before air dried on newspaper for 3-4 days by keeping away from sunlight. Dry leaves weighing 70g were thoroughly crushed and placed in 1000ml beaker overnight with distilled water that is four times the quantity of botanical extracts to make it upto 25% concentration. The pulp was filtered using double layered muslin cloth followed by Whattman's filter paper No. 1.

Table 1: List of the plant extracts used for management of Leaf spot of *Aloe vera*, common name, scientific name, part used, concentration and reference for extraction method.

Plant name	Scientific name	Part used	Concentration	Reference
Neem	*Azadirachta indica*	Leaf	5%, 10%, 15%	Rasheed *et al*. (2019) [18]
Moringa	*Moringa oleifera*	Leaf	5%, 10%, 15%	Regmi *et al*. (2014) [19]
Lantana	*Lantana camara*	Leaf	5%. 10%, 15%	Nayak *et al*. (2023) [11]
Akanda	*Calotropis gigantea*	Leaf	5%, 10%, 15%	Regmi *et al*. (2014) [19]
Garlic	*Allium sativum*	Bulb	5%, 10%, 15%	Singh *et al*. (2014) [22]

In vitro efficacy of botanical extracts against *Alternaria alternata*

The five botanical extracts were evaluated for the study of *in vitro* efficacy against *A. alternata* to checked the mycelial growth using poison food technique (Nene and Thapliyal, 1982) [12]. The experiment was conducted at three different concentrations (5%, 10% and 15%) with three replication each and one chemical fungicide namely Carbendazim 50% was used for comparison. A PDA medium was aseptically filled with different concentrations of 5%, 10%, and 15% to create a volume of 100 ml, and the medium was then autoclaved. A pinch of streptomycin sulfate was added on PDA and mixed thoroughly to prevent bacterial contamination and then poured in 9cm petri plates. After the solidification of media, 8.8 mm discs of the pathogens were taken from 5-7 days old culture and placed in the centre of the plates. The inoculated petri plates were incubated at 25°C for 9 days. For every treatment, three replications were kept in addition to the control. After the completion of 9 days, the percent inhibition of mycelial growth of the pathogen was calculated using the formula described by Vincent (1947) [24]:

Inhibition % = [C–T/C] × 100

Where, C = Diameter of fungus colony (mm) in control plate
T = Diameter of fungus colony (mm) in treated plate

Statistical analysis

Two statistical programs, SPSS and MS-Excel were used to evaluate the data. Using the Least Significant Differences following Duncan test (DMRT) post hoc test in SPSS, the treatment means were divided.

In vitro efficacy of biocontrol agents against *Alternaria alternata*

Five biocontrol agents viz. *Trichoderma* spp., *T. harzianum*, *T. viride*, *Pseudomonas fluorescens* and *Bacillus subtilis* were obtained from the Plant Pathology PG Lab, SoAG, ITM University, Gwalior. The experiment for biological management was performed using dual culture technique on five treatments of biocontrol agents with three replications each to evaluate their antagonistic activities against *Alternaria alternata* (Fokkema, 1978) [6]. Autoclaved melted Potato Dextrose Agar and Nutrient Agar Medium were used for fungi and bacterial bio-agents respectively, and were poured in 9cm petri plates which allowed to solidified in aseptic condition. A pinch of streptomycin sulfate was added on PDA and mixed thoroughly to prevent bacterial contamination. Using a cork borer, culture discs of 5-7 days old pathogen and biocontrol agents measuring 8.8mm were cut and transferred to petri plates after solidification of media and then petri plates were incubated at BOD incubator at 25°C for 9 days. These discs were put on the opposite side of the periphery of petri plates. The pathogen grown in same condition on PDA without bio-agents served as control. After nine days of incubation, the mycelial inhibition was calculated using the formula described by Vincent (1947) [24]:

Inhibition % = [C–T/C] × 100

Where, C = Diameter of fungus colony (mm) in control plate
T = Diameter of fungus colony (mm) in treated plate

Results and Discussion

In vitro efficacy of botanical extracts against *Alternaria alternata*

The five botanical extracts were tested at 5%, 10% and 15% concentrations to evaluate *in vitro* efficacy on radial growth of mycelium against *Alternaria alternata* using poison food technique. The results of the recorded data represented in Table 2 and Figure 1, 2 & 3 showed the effect of botanical extracts on mycelial growth of *Alternaria alternata*. Among the five botanical extracts, Neem@15% was recorded the highest inhibition percentage (49.59%) of mycelium growth

of *A. alternata* followed by Garlic@15% (47. 43%) and Moringa@15% (46. 56%). The lowest percentage and least effective in inhibition of mycelial growth of *A. alternata* was observed in Akanda @5% (8.94%). The toxicity of fungus against botanical extracts might be due to antifungal metabolites present in different plant species (Rani et al., 2018) [16].

In almost similar study, Singh et al. (2014) [22] also observed the effectiveness of Neem and Garlic extracts against *A. alternata*. Likewise, Regmi *et al*. (2014) [19] also reported that Neem (*Azadirachta indica)* manifested the highest inhibition of mycelial growth of *A. alternata causing Aloe vera* leaf spot disease when evaluated with six plant extracts namely *Jatropa curcas, Datura strumarium, Morus alba Moringa oleifera, Calotropis gigantea, Azadirachta indica* and *Morus alba* using poison food technique.

Table 2: Radial growth and inhibition percentage of *Alternaria alternata* against botanical extracts.

Treatment details	Radial growth of pathogen (cm)				Inhibition % (Final day) *
	2 DAI	5 DAI	7 DAI	9 DAI	
Neem (5%)	1.5±O.06^{d}	3.2±0.10^{ef}	4.4±0.10^{ef}	4.93±0.06^{fg}	36.75(37.32) ^{def}
Neem (10%)	1.27±0.06^{abc}	2.73±0.15^{c}	3.87±0.15^{d}	4.57±0.15^{de}	41.44(40.06) ^{fg}
Neem (15%)	1.13±0.06^{a}	2.13±0.15^{a}	2.73±0.21^{a}	3.93±0.31^{b}	49.59(44.76) ^{i}
Garlic (5%)	1.4±0.06^{cd}	3±0.10^{de}	3.83±0.06^{d}	4.77±0.06^{ef}	38.89(38.58) ^{defg}
Garlic (10%)	1.37±0.12^{bcd}	2.77±0.06^{cd}	3.63±0.12^{bcd}	4.40±0.10^{cd}	41.43(40.06) ^{gh}
Garlic (15%)	1.23±0.06^{ab}	2.27±0.15^{ab}	3.43±0.06^{b}	4.10±0.10^{b}	47.43(43.53) ^{hi}
Moringa (5%)	1.5±0.10^{d}	3.17±0.15^{ef}	4.6±0.26^{f}	5.03±0.15^{fg}	35.45(36.53) ^{de}
Moringa (10%)	1.4±0.10^{bcd}	2.87±0.23^{cd}	4.40±0.17^{ef}	4.77±0.15^{ef}	38.87(37.09) ^{defg}
Moringa (15%)	1.27±0.06^{abc}	2.40±0.10^{b}	3.73±0.12^{cd}	4.17±0.15^{bc}	46.56(43.03) ^{hi}
Lantana (5%)	0.19±0.12^{h}	3.33±0.06^{f}	4.9±0.10^{g}	5.50±0.10^{h}	29.48(32.89) ^{c}
Lantana (10%)	1.87±0.12^{gh}	3.17±0.15^{ef}	4.63±0.12^{f}	5.10±0.10^{g}	34.60(36.06) ^{d}
Lantana (15%)	1.77±0.15^{fg}	2.90±0.10^{cd}	4.27±0.15^{e}	4.90±0.17^{fg}	37.17(37.56) ^{def}
Akanda (5%)	2.2±0.06^{i}	4.63±0.15^{i}	6.27±0.15^{j}	7.10±0.26^{j}	8.94(17.06) ^{a}
Akanda (10%)	1.87±0.15^{gh}	4.17±0.15^{h}	5.90±0.17^{i}	6.97±0.15^{j}	10.69(19.05) ^{a}
Akanda (15%)	1.53±0.06^{de}	3.87±0.15^{g}	5.23±0.25^{h}	6.63±0.06^{i}	14.95(22.75) ^{b}
Carbendazim	1.67±0.06^{ef}	3.23±0.06^{ef}	3.53±0.06^{bc}	3.6±0.10^{a}	40(39.23) ^{efg}
Control	2.33±0.06^{i}	5.10±0.10^{j}	6.63±0.15^{k}	7.80±0.10^{k}	-
CD @5%	0.155	0.268	0.482	0.776	2.699
SE(m)	0.054	0.093	0.167	0.269	0.933
SE(d)	0.076	0.131	0.236	0.38	1.319
CV	5.777	5	6.513	9.075	4.548

The represented data are Mean ± Standard deviation, and mean in the column followed by the same letter are not significantly differently according to DMRT(P<0.05).

* Figure in parenthesis are arcsine/angular value. *DAI=days after inoculation

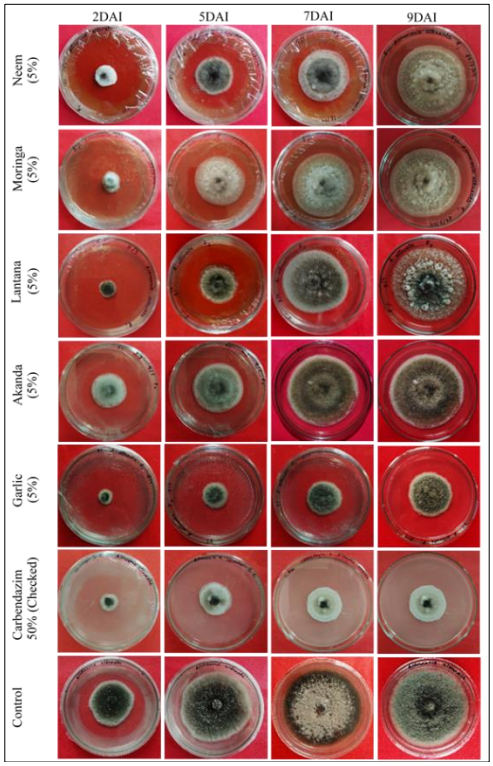

Fig 1: *In vitro* efficacy of botanical extracts against *Alternaria alternata* at 5% concentration using poison food technique for 2, 5, 7 and 9 days on PDA incubated at 25 ℃

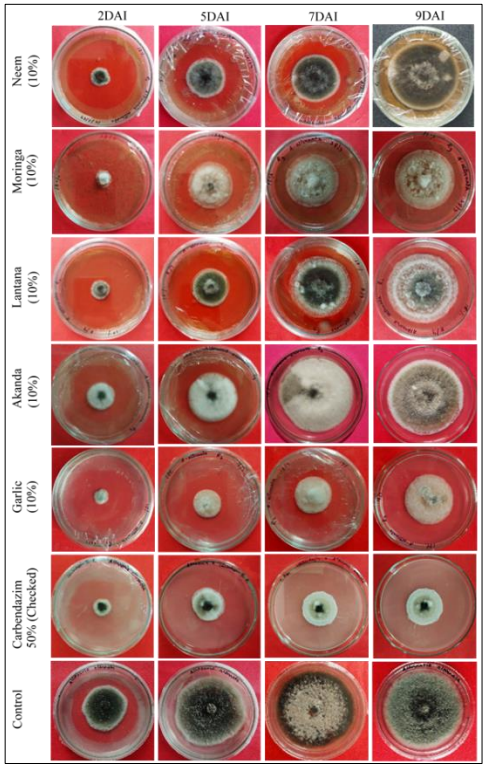

Fig 2: *In vitro* efficacy of botanical extracts against *Alternaria alternata* at 10% concentration using poison food technique for 2, 5, 7 and 9 days on PDA incubated at 25 ℃

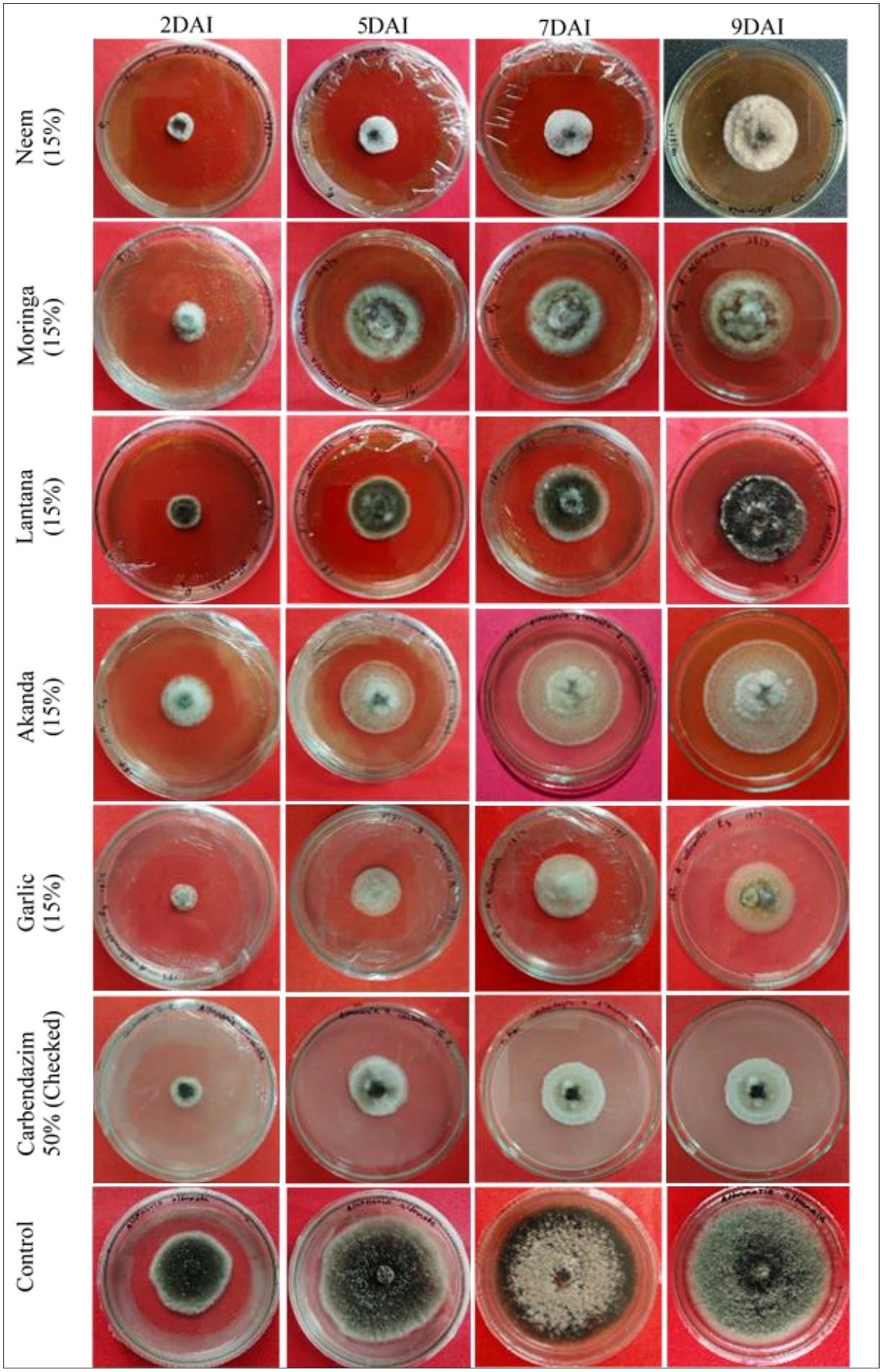

Fig 3: *In vitro* efficacy of botanical extracts against *Alternaria alternata* at 15% concentration using poison food technique for 2, 5, 7 and 9 days on PDA incubated at 25 ℃

In vitro efficacy of biocontrol agents against *Alternaria alternata*

Using a dual culture technique, five bio-control agents viz. *Trichoderma* spp., *T. harzianum*, *T. viride*, *Pseudomonas flourescens*, and *Bacillus subtilis* were investigated to study the antagonistic activity against *A. alternata*. The findings represented in Table 3 and Figure 4 indicated that all five of the biocontrol agents were significantly effective when compare with the control. Antagonist Trichoderma viride (75.94%) inhibits the maximum percentage of radial mycelial growth of *A. alternata* followed by *Trichoderma* spp. (75.47%) and *Trichoderma harzianum* (72.34%). The lowest percentage of inhibition was exhibited in *Pseudomonas fluorescens* (75.94%). After the completion of 9 days of incubation, the mycelium growth of *A. alternata* was outgrown by all three of the *Trichoderma* species. This overgrowth may be due to its fast-growing nature, rapid sporulation or secretion of cell wall lytic enzymes in dual culture (Sharma and Trivedi, 2010) [21].

The current results are aligned with those of Jakatimath *et al*. (2017) [8], which showed that *Trichoderma* spp. exhibited the highest efficacy against *Alternaria alternata* as compared *Pseudomonas fluorescens* and *Bacillus subtilis*. Moreover, Pareek *et al*. (2012) [14] also reported that *T. viride* and *Trichoderma harzianum* shown significant antagonistic relationships against *Alternaria alternata* that caused leaf spot disease in cucumber.

Table 3: Radial growth and inhibition percentage of *Alternaria alterna* against biocontrol agents.

Treatment details	Radial growth of pathogen (cm)				Inhibition % (Final day) *
	2 DAI	5 DAI	7 DAI	9 DAI	
Trichoderma spp.	1.43±0.12^{a}	1.77±0.64^{a}	1.77±0.64^{a}	1.77±0.64^{a}	75.47(60.54) ^{b}
Trichoderma harzianum	1.50±0^{ab}	1.87±0.21^{ab}	2.13±0.23^{a}	2.13±0.23^{a}	70.34(57.03) ^{b}
Trichoderma viride	1.57±0.06^{a}	1.73±0.15^{a}	1.73±0.15^{a}	1.73±0.15^{a}	75.94(60.64) ^{b}
Pseudomonas flourescens	2.03±0.06^{bc}	2.37±0.06^{bc}	3.37±0.15^{b}	3.50±0.10^{b}	51.37(45.79) ^{a}
Bacillus subtilis	1.53±0.06^{c}	2.63±0.15^{c}	3.03±0.35^{b}	3.30±0.20^{b}	54.15(47.38) ^{a}
Control	2.23±0.06^{d}	4.90±0.10^{c}	6.47±0.12^{c}	7.2±0.10^{c}	-
CD @5%	0.12	0.528	0.592	0.545	5.51
SE(m)	0.038	0.169	0.19	0.175	1.73
SE(d)	0.054	0.24	0.269	0.247	2.44
CV	3.884	11.533	10.675	9.253	5.51

The represented data are Mean ± Standard deviation, and mean in the column followed by the same letter are not significantly differently according to DMRT(P<0.05).

* Figure in parenthesis are arcsine/angular value. *DAI=days after inoculation

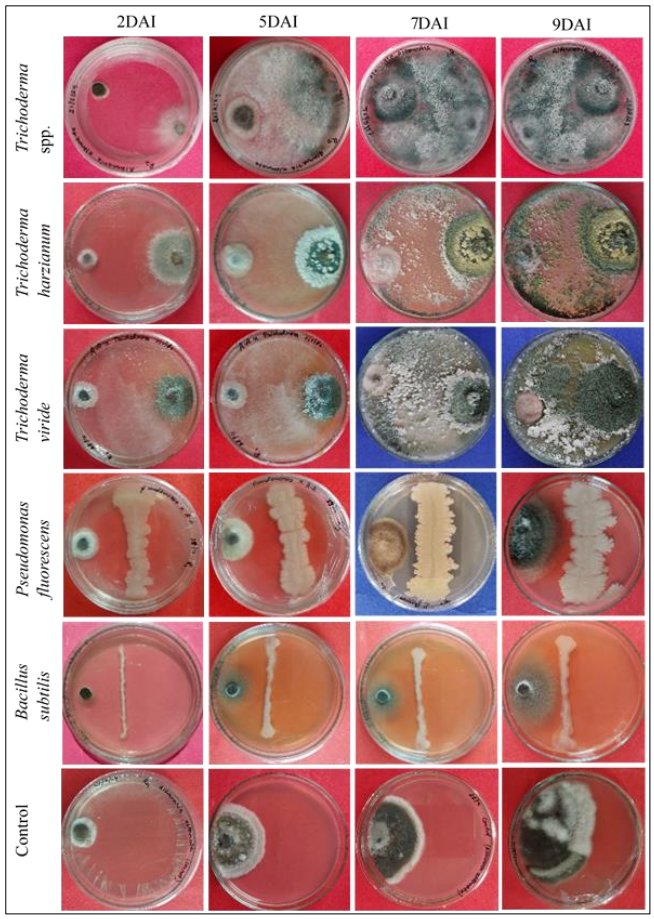

Fig 4: Dual culture study for antagonistic effect of biocontrol agents against *Alternaria alternata* for 2, 5, 7 and 9 days incubated at 25 °C

Conclusion

Aloe vera is seriously threatened by the prevalence of leaf spot disease caused by *Alternaria alterna*. Based on the current study, it can be concluded that among the five tested botanical extracts, the most effective was found in Neem leaf extract @15% followed by Garlic bulb extract @15% in inhibiting the pathogen growth of leaf spot disease of *Aloe vera*. Out of the five biocontrol agents, *Trichoderma viride* recorded the maximum inhibition of mycelial growth of *A. alternata*. This study showed that, despite the effectiveness of chemical fungicides, eco-friendly management has a promising effect in lowering the occurrence and severity of disease while maintaining the medicinal and aesthetic value of the *Aloe vera* plants.

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