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Chemical and Biological Management of Alternaria Leaf Spot of *Aloe Vera*

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ABSTRACTS

Aloe is one know therapeutic nature plant used to keep individuals out of diseases and make them healthy but this medicinal plant gets affected by some health retarding elements and used to lose its medical reputation in therapeutic world. One of these health retarding elements is *Alternaria alternata* found causing leaf spot and reducing qualitative and quantitative grades of *Aloe vera*, the miracle plant, that is why to save medical grades of *Aloe vera*, in current study, seven fungicides belonging to different groups and nine different group fungi as bio-control agents were tested in vitro and in vivo for their efficacy to inhibit the growth of the pathogen to a maximum extent. In vitro poisoned food and dual culture methods were used for fungicides and antagonists respectively. 0.05, 0.1 and 0.2% concentration of each fungicide was added separately in sterilized 2% Malt extract agar medium. Three fungicides named Myclobutanil, Hexaconazole and Tebuconazole proved most effective causing highest growth reduction of mycelium i.e., 100% and got significantly superior then others while carbendazim with 70.20% reduction recorded last. To the best concentrations, Tebuconazole, Myclobutanil and Hexaconazole at each concentration checked 100% growth were significantly superior to all other treatment combinations. Amongst antagonists, maximum reduction in diameter of colonial progress of pathogenic entity (48.80%) has made by *Trichoderma koningii* followed by other species of *Trichoderma* found best, controlling pathogen's development. Field observations to calculate percent disease index (PDI) were taken following 0-9 point scale and recorded that maximum PDI in fungicidal experiments was noticed in carbendazim (47.41%) and minimum in Tebuconazole (42.22%) while in case antagonistic foliar applications maximum PDI was noticed

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in *Aspergillus niger* (48.89%) and least PDI observed in *T. koningii* (40.74%) was followed by *T. harzianum* (43.70%).

Key words: Systemic fungicides; Biocontrol agents; Percent disease index; Percent disease control; Dual culture; Poisoned food technique.

INTRODUCTION

One of the medically known plants and used for its medicinal and healing power worldwide so called *Aloe vera*[1]. The first written record using miracle plant found six thousand years back in Mesopotamia over old clay tablets. *Aloe vera* belonging to Liliaceae family is one perennial, succulent and drought resistant plant of origin of South Africa from where it is distributed to other tropical countries like South Arab, India, East Asia and South East Asia[2]. In India it is grown in Maharashtra, Tamil Nadu, Andhra Pradesh, Gujarat and Rajasthan states. On the account of varieties these some are *Aloe saponaria*, *Aloe forex*, *Aloe barbadensis*, *Aloe variegata*, *Aloe chinensis*, *Aloe curacao* and *Aloe lalifolia* those are predominantly grown[3]. *Aloe barbadensis* Mill is most popular species and possess more therapeutic values so named and called 'True Aloe'[1,4].

People used to call *Aloe vera* a 'Miracle Plant' since it helps in curing heart burns, diabetes and cancer being an antiseptic, anti- inflammatory agent[5,6]. It is also used as beauty aid and health accelerator what revealed from *Aloe vera* gel's photochemistry that ensures about 200 active compounds like minerals, enzymes, vitamins, sugars, phenols, amino acids, saponins, sterols, salicylic acid and lignin7,8.

Leaf spot of *Aloe vera* has been a serious and major concern through the globe and has been reported from various commercially *Aloe vera* spp. harvesting places worldwide[9,10,11]. First Kamalakannan *et. al*., 2008 reported leaf spot disease of *Aloe vera* from India[12] and they confirmed the causal agent as *Alternaria alternata* (Fr.) Keissler and this disease was called one of the most serious fungal diseases in commercially grown *Aloe vera* from North India[13].

Being a major medically used plant, *Aloe vera* adds its great contribution in therapeutic world since used in making medicines, cosmetics, processed foods & drinks and thereof, the disease

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Alternaria leaf spot set limitations to quantitative and qualitative production. Therefore it is very urgent to restrict loss by pathogen, hence, present study focused on isolation of pathogen in order to confirm pathogenic strain and use this information in pathological, physiological and ecofriendly management of leaf spot pathogen using fungicides and bio-agents.

New generation chemicals used in present study were found very effective in controlling disease and these were not used earlier to manage disease on *Aloe vera*. Use of these chemicals alone and in combination with BCAs those were shown good results *in vitro* and *in vivo* proposes an integrated management of disease considering the importance of the disease and the raising concerns for fungicidal resistance development by pathogen due to current trend of unjustified usage of non-systemic chemicals and adjacent approaches.

MATERIALS AND METHODS

In vitro experiment was carried out in the Plant Pathology departmental laboratory and pot experiments were carried out at HRC of Sardar Vallabhbhai Patel University of Agri. & Tech., Meerut (Uttar Pradesh).

Collection, isolation and pure culturing of pathogen

For the detection of natural occurrence of pathogen from *Aloe vera* a visit was made to HRC where crop was found moderately to severely infected and critical symptoms showing infected leaves of *Aloe vera* were collected from the centre. The collected samples were first washed in tap water to remove dust particles then pieces of 3 mm were cut and surface sterilized deeping in 0.1% mercuric chloride ($HgCl_2$) for 30 seconds. Traces of $HgCl_2$ were removed by transferring bits through parallel kept three Petri dishes contains sterilized water. Excess moisture from the cutting pieces was removed by placing them in the fold of sterilized blotting paper and finally transferred to Petri dishes contains malt extract agar (MEA) medium that were put to be incubated and temperature of biological oxygen demand (BOD) incubator was set to 25 ± 2^0C. As the mycelial growth is achieved and seen around the pieces, tips of advancing mycelium were cut and placed into MEA poured culture slants and pure culture of fungus was obtained by adopting single hyphal tip culture technique[15,16].

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Identification of Fungus

The pathogen was grown on Malt extract agar medium to get best growth and for identification based on their cultural and morphological characters[17,18].

Symptomatology and pathogenicity test

The symptoms of the leaf spot disease were carefully observed and recorded from initiation to mortality of the plant parts. Isolated fungus was introduced on healthy leaves in order to confer pathogenicity following Koch's Postulates[19,20]. A pot experiment was conducted determining pathogenic capabilities of *A. alternata* isolated from infected *Aloe vera* plants. Plants were sown in sterilized soil filled in earthen pots of 30 cm diameter @ 3.5 kg sterilized soil each pot. Leaves were then inoculated by spraying with conidial suspension @ 2×10^4 conidia per ml concentration of the inoculum that was prepared from 10 days old growing culture of *A. alternata* on malt agar medium. After inoculation plants were covered with polythene bags for 48 hours to provide humidity and to prevent secondary infection. A set of un-inoculated plants was also maintained as control that received only sterilized water spray. Plants were given water on regular intervals to maintain the requisite moisture for proper growth and development of the pathogen and production of disease symptoms. The development of the disease symptoms was regularly observed and recorded. Re-isolation of pathogen from affected plant parts was made in order to confirm the presence of inoculated fungus and proving of Koch's postulates.

Evaluation of BCAs:

In vitro efficacy of antagonists against *A. alternata* had performed following well known technique for dual culture proposed by Dennis and Webster[21,22,23]. Mechanism of interaction was observed and the data was expressed as percent inhibition in mycelial growth of pathogen by following formula[24].

$$\text{Percent Inhibition} = \frac{\text{Growth in control} - \text{Growth in treated plates}}{\text{Growth in control}} \times 100$$

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In vivo experiments with three replications were performed in pots following Randomized Block Design (RBD) and finally data was calculated as PDI. The intensity of the disease was recorded after 15 days of spraying by following formula.

$$\text{Percent Disease Control (PDC)} = \frac{\text{PDI in control} - \text{PDI in treatments}}{\text{PDI in control}} \times 100$$

Evaluation of Fungicides

Seven different group fungicides enlisted in Table 1, were tested in vitro for their efficacy to inhibit the growth of the pathogen to a maximum extent and performed by Poisoned Food Technique[25].

Table 1. Active ingredients and doses of fungicides used in experiment

1	Mancozeb	DithaneM-45 75% WP	Manganese + zinc ethylene	0.05	0.1	0.2
3	Myclobutanil	Systhane 10%WP	α–butanil-α-(4- chlorophenyl)-1h-	0.05	0.1	0.2
5	Metalaxyl +	Sanchar 72% WP	N-(2-Dimethylphenyl)-N-	0.05	0.1	0.2
7	Hexaconazole	Contaaf 5 % EC	(RS)-2-(2,4-dichlorophenyl)-1- (1h-	0.05	0.1	0.2

The requisite quantity of each fungicide was added separately in sterilized 2% Malt extract agar medium, poured into sterilized Petri plates and inoculated with 5mm disc of 14 days old culture of the pathogen. Discs were placed in reverse position so that culture growing side could come in direct contact of medium. Three replications for each treatment were kept and Petri dishes were incubated at 25 ± 2^0C. Three replicates of control in which the medium was not mixed with

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any fungicides but simply inoculated with pathogen were kept side by side. The colony diameter was recorded after 14 days till the fungal colony fully covered the Petri plates in control. The percent inhibition over control was calculated by Bliss's formula[24]. *In vivo* experiments in replicates of three were set and RBD was performed to collect data into percent disease index (PDI) to evaluate the relative efficacy of different fungicides whereas control was left without treatment only plain water was sprayed. The intensity of the disease was recorded after 15 days of spraying. The observations on leaf spot were recorded before spray. Further, these observations were taken by following 0-9 disease scale (Table 2) and converted into percent disease index (PDI).

Table 2. 0-9 scale to assess per cent disease index

S.No	Numerical rating	Description
1	0	No symptoms on leaf
2	1	Small, irregular brown spots covering 1% or less of the leaf area
3	3	Small, irregular, brown spots with concentric rings covering 1-10% of the leaf area
4	5	Lesions enlarging, irregular, brown with concentric rings covering 11-25% of the leaf area
5	7	Lesions coalesce to form irregular brown patches with concentric rings covering 26-50% of the leaf area. Lesions also on stem and petioles

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6	9	Lesions coalescing to form irregular, dark brown patches with concentric rings covering 51% or more of the leaf area. Lesions seen on the stem and petiole

RESULTS AND DISCUSSION

Symptomatology

External expressions resulting in symptoms appears as olive towards dark brown, small to collapsed, initially circular and takes oval to irregular shapes on maturity and becomes necrotic, sunken, seen abundantly nearby leaf topology that was found achieving 1.0-3.0 mm

diameter. On maturity of sports black sporulation used to come out through the center of the almost necrotic spots. Later on severely affected leaves dried up downward and lost its mucilaginous content (Figure 1).

Figure 1. Symptoms of leaf spot disease of *Aloe vera*

Pathogenicity test

Two random leaves were selected plant wise and these selected leaves were pin pricked and then sprayed with spore suspension @2×10^4 conidia ml^{-1} of *Alternaria alternata* to achieve inoculation. On the other hand sterile distilled water was sprayed where treatment served as control. Experiment was monitored for appearing of typical symptoms from inoculated leaves on daily basis.

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In order to confer Koch's postulates pathogen was re-isolated and compared with the original culture of the pathogen on culture and morphological characterization which was similar in all respects. Hence, the causal agent of the disease was confirmed as *Alternaria alternata*.

Identification of fungus

Morphological characters are the basis of identification of the pathogen[26] harvested on Malt extract agar medium as under.

Colony

Figure 2a, exhibited that colony were brown grey by color, short and suede-like with a white periphery by appearance. From backside of Petri dishes it seems dark brown to black in color.

Conidiophore

Conidiophores are short to long, simple or branched arising singly and measuring 76.2-227.6 µm long and 9.6-22.3 µm wide.

Conidia

Conidia were observed to arise singly and in chains at the tip of each conidiophore measuring between 63.37-197.48 µm long and 17.28-48.53 µm wide. They were muriform, thick walled, dark brown, beaked and non-beaked with 6-7 transverse septa and 0-3 longitudinal septa in long chains of 9 to 15 were seen during microscopy (Figure 2b).

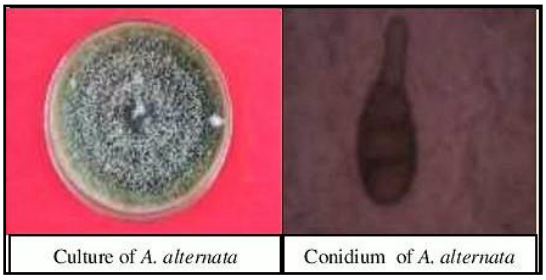

Figure: 2a. Culture of *A. alternata* **2b.** Conidium of *A. alternate*

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Cultural studies of the pathogen in different solid media

Fungi can be grown on a variety of media comprising of known and unknown constituents but, *in vitro* they require some specific medium for their best vegetative as well as reproductive growth. The pathogen was grown on different culture media in Petri dishes replicated four times. The radial growth was measured after 10 days of inoculation at 25±1°C. The data thus obtained are summarized in Table 3.

Table 3. Growth pattern of *Alternaria alternata* on different media

Culture Media	R1	R2	R3	Mean(SE±)
Potato dextrose agar medium	49	53	51	51.00b±1.16
Czapek's medium	40	43	44	42.33c±1.20
Water agar medium	0	0	0	0.00d±0
Malt extract agar medium	82	86	84	84.00a±1.15

Different letter (s) in the column shows means are significantly different at P = 0.05

Table 3 and Figure 3 data expressed best mycelial progress was obtained on Malt extract agar medium (Figure 3c) followed by Potato dextrose agar medium (Figure 3a) and Czapek's medium (Figure 3b). The growth of *A. alternata* on water agar medium (Figure 3d) was found to be nil.

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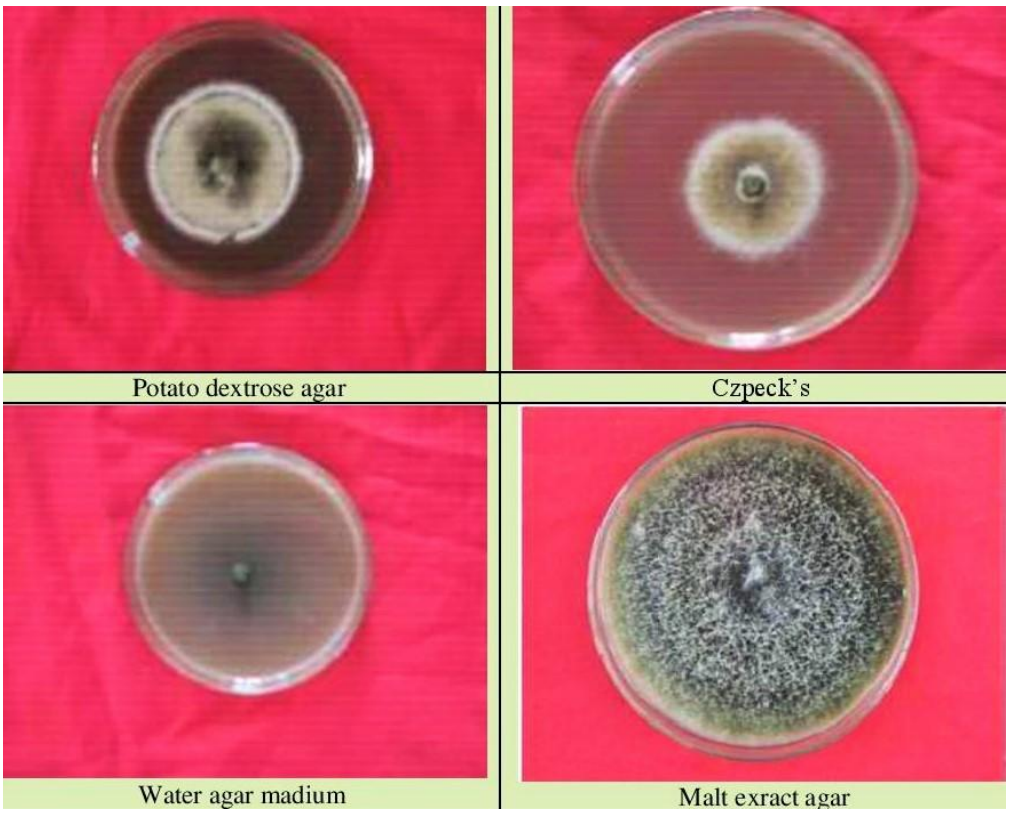

Figure 3(a-d) Clockwise: *Alternaria alternata* grown on 3a. Potato dextrose agar medium 3b. Czpeck's medium 3c. Malt extract agar medium 3d. Water agar medium

In vitro evaluation of Fungicides

In vitro studies contributed seven fungicides used to evaluate by poisoned food technique to be find out best for their efficacy to *A. alternata*. It is clear that all the fungicides tested at 3 different concentrations enlisted in Table 4 were significantly effective in reducing the mycelial growth of *A. alternata* and percent inhibition to mycelial growth of *A. alternata* in comparison with control showed an inhibition ranging 63% to 100.00% at 0.05% concentration (conc.) from all seven fungicides enlisted in Table. 3. Tebuconazole, Myclobutanil and Hexaconazole at 0.05, 0.1 and 0.2 percent were significantly superior to all other treatment combinations controlling 100% mycelia growth of the pathogenic fungi. Carbendazim at 0.05 and 0.2% and a combo of Metalaxyl + Mancozeb at 0.1% were recorded least performer controlling mycelial growth.

At 0.05% conc. all these chemicals were found significantly different with each other while Mancozeb and Carbendazim were statistically at par with each other at 0.1% conc. and both

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were found at par with a combination of Metalaxyl + Mancozeb at 0.2% conc. Table 4.

The effect of concentrations on *A. alternata*, irrespective of chemicals was found significant and most effective at 0.2 percent conc. except Tebuconazole, Myclobutanil and Hexaconazole where all given conc. i.e., 0.05, 0.1 and 0.2 percent used to control 100% mycelial growth of *A. alternata*.

Table 4. *In vitro* evaluation of fungicides against *Alternaria alternata*

Fungicides	0.05%			Mean	Percent Inhibition over control	0.10%			Mean	Percent Inhibition over control			0.20 %	Mean	Percent Inhibition over control
Mancozeb	20	23	23	22d±1	73.80c±1.17	21	21	21.5	21.17c±0.17	74.80c±0.61	22.5	22	19	21.17b±1.09	74.80c±1.91
Tebuconazole	0	0	0	0f±0	100a±0	0	0	0	0e±0	100a±0	0	0	0	0d±0	100a±0
Mycl	0	0	0	0f±0	100a	0	0	0	0e±0	100	0	0	0	0d±0	100

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obutanil					±0					a±0					a±0
Tricyclazole	12.5	14	12	12.83e±0.6	84.70b±1.71	6	7	6	6.33d±0.33	92.50b±1.26	0	6	7	4.33c±2.19	92.0b±1.0
Metalaxyl + Mancozeb	24	24	26	24.67c±0.67	70.57d±1.29	25	22	25	24.0b±1.0	71.43d±0.79	22	20	21	21.0b±0.58	75.0c±1.53
Carbendazim	28	33	32	31b±1.53	63.0e±1.53	21	23	22	22.0c±0.58	73.80c±0.62	20	22	24	22.0b±1.15	73.80c±0.99
Hexaconazole	0	0	0	0f±0	100a±0	0	0	0	0e±0	100a±0	0	0	0	0d±0	100a±0
Control	84	84	84	84a±0	0.00f±0	84	84	84	84.0a±0	.000e±0	84	84	84	84.0a±0	.000d±0

Different letter (s) in the column shows means are significantly different at P = 0.05

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In vitro evaluation of bio-agents

Nine antagonistic spp. viz., *Trichoderma harzianum*, *T. koningii*, *T. atraviride*, *T. viride*, *T. lonyizruciatum*, *Penicillium notatum*, *Aspergillus niger*, *Chaetomium globosum*, and *Metrahizium anisoplae* on growth of *A. alternata* was studied in vitro by dual culture method[36,39]. Results in Table 5 represented maximum inhibition of pathogen's colony was exhibited by *T. koningii* where colony diameter achieved by *A. alternata* was 24.5 centimeter (cm) with inhibition percent of 48.80. This value was followed by *T. harzianum* and recorded significantly superior then other BCAs were being tested. *T. viride* and *T. atraviride* showed no statistical difference but found statistically superior then other treatments in row i.e., *T. Lonyizruciatum, Metrahizium anisoplae, Penicillium notatum. Chaetomium globosum* has secured second last position since *Aspergillus niger* proved to be last.

Table 5. Evaluation of BCAs *in vitro* conditions against *Alternaria alternata*

Bio agents	Colony diameter of pathogen			Mean (±SE)	Percent inhibition over control			Mean(±SE)
	R1	R2	R3		R1	R2	R3	
Trichoderma harzianum	25	27	24	25.33g±0.88	48	48	45	47.00a±1.00
T. koningii	22	25.5	26	24.5g±1.26	47	48.4	51	48.8a±1.17
T. atraviride	27	25	27.5	26.5fg±0.76	43	44	46.8	44.6b±1.14
T. viride	28	27.5	29.4	28.3ef±0.57	42	40.4	40	40.8c±0.61

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T. lonyizruciatum	27	31	32	30e±1.53	37	39	36	37.33d±0.88
Penicillium notatum	39	39	41	39.67bc±0.67	17.5	16.5	17	17.00f±0.29
Aspergillus niger	44	45	46.5	45.17b±0.73	5.4	6.2	5.3	5.63g±0.28
Chaetomium globosum	41	40.3	39.66	40.32c±0.39	15	15.4	16.7	15.7f±0.51
Metrahizium anisoplae	39	36	36.9	37.3d±0.89	22.6	22.4	21	22.00e±0.50
Control	48.2	46.3	49	47.83a±0.80	0	0	0	0h±0.00

Different letter (s) in the column shows means are significantly different at P = 0.05

In vivo evaluation of fungicides

It was observed from Table 6 during field studies after 15 days of sowing that all the treatments differ significantly over unprotected check (control) in which maximum PDI was measured. The treatment seemed closer to control was Carbendazim. Least PDI was observed in Tebuconazole (42.23) followed by Hexaconazole (42.9) and Myclobutanil (42.97). Plant disease control (PDC) is directly correlated with PDI; less the PDI more the PDC, therefore, from the results maximum PDC was reported wherein minimum PDI was recorded i.e., Tebuconazole (19.57) followed by Hexaconazole, Myclobutanil and Trycyclazole were significantly superior over other treatments.

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Table 6. Field efficacy of fungicides in the management of leaf spot of *Aloe vera*

Treatments	R1	R2	R3	Per cent Disease Index (PDI)	R1	R2	R3	Per cent Disease Control (PDC)
Mancozeb	42	48.2	43	44.40b±1.92	25	3.21	17.15	15.12ab±6.37
Tebuconazole	43.7	41	42	42.23b±0.79	21.96	17.67	19.08	19.57a±1.26
Myclobutanil	39.5	44.8	44.6	42.97b±1.73	29.46	10.04	14.07	17.86a±5.92
Tricyclazole	39.8	46	45.3	43.70b±1.96	28.93	7.63	12.72	16.43a±6.42
Metalaxyl + Mancozeb	46.6	42.7	46.3	45.20b±1.25	16.79	14.26	10.79	13.95ab±1.64
Carbendazim	44.9	49.4	47.9	47.40b±1.32	19.82	0.8	7.71	9.44ab±5.56
Hexaconazole	43.9	46.3	38.5	42.90b±2.31	21.61	7.03	25.82	18.15a±5.69
Control	56	49.8	51.9	52.57a±1.82	0	0	0	0b±0

Different letter (s) in the column shows means are significantly different at P = 0.05

In vivo evaluation of biocontrol agents

Data presented in Table 7 in respect to evaluation of BCAs shows maximum PDI, was noticed in control that was followed by *Aspergillus niger*. Least PDI and maximum PDC were

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observed in *T. koningii* which was followed by *T. harzianum* that means less the PDI shown in a treatment more the PDC is achieved.

Aloe vera crop is subjected to attack by a number of diseases, of which leaf spot caused by *Alternaria alternata* is serious and also a major limiting factor in cultivation of *Aloe vera*, thus, seven fungicides and nine BCAs were evaluated to check their efficacy in controlling growth of fungus *in vitro* and under natural occurring conditions. Biological control through the use of antagonistic microorganisms is a potential, non-chemical means of controlling plant diseases by reducing inoculum levels of pathogens. Such a management would help in preventing the pollution and also health hazards.

In the present investigation, the antagonistic effect of different bio-agents was assessed against *A. alternata* by dual culture technique. In laboratory all the fungicides and BCAs showed inhibition in mycelial growth in the form of reduction in radial diameter. On field all the tested fungicides and BCAs had shown decrease in PDI when compared with control (check).

The leaves had carried oval to circular, olivacious (on initiation) to dark brown (on maturity) sunken and necrotic spots progressed downward and achieved a diameter reached 3.0-5.0 mm or more. Findings of present investigation were supported to those were described by Kamalakannan[12] on the *Aloe vera* leaves in India and across the globe[9,10,11].

Table 7. Field efficacy of BCAs in the management of leaf spot of *Aloe vera*

Bio agents	PDI			Mean (±SE)	PDC			Mean(±SE)
	R1	R2	R3		R1	R2	R3	
Trichoderma harzianum	43	42	46.1	43.70±1.23	21.82	24.54	10.31	18.89±4.36

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T. koningii	37	39.1	46	40.70±2.72	32.73	29.75	10.51	24.33±6.96
T. atraviride	38.6	48	49	45.20±3.31	29.82	13.76	4.67	16.08±7.35
T. viride	45.7	47	45	45.90±0.59	16.91	15.56	12.45	14.97±1.32
T. lonyizruciatum	51	45.9	43.1	46.67±2.31	7.23	17.54	16.15	13.64±3.23
Penicillium notatum	48	45.8	48.5	47.43±0.83	12.73	17.71	5.64	12.03±3.50
Aspergillus niger	45.3	51.9	49.4	48.87±1.92	17.64	6.76	3.89	9.43±4.19
Chaetomium globosum	45	47.6	49.7	47.43±1.36	18.18	14.48	3.31	11.99±4.47
Metrahizium anisoplae	50.8	45	46.5	47.43±1.74	7.64	19.15	9.53	12.11±3.56
Control	55	55.66	51.4	54.02±1.32	0	0.00	0	0.00±0.00

Different letter (s) in the column shows means are significantly different at P = 0.05

Morphological identification

Identification of fungus was made on the basis of important morphological characters such as shape, size, septation of conidiophores and conidia in the culture medium. The characteristics

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of the fungus in this context were compared with all *Alternaria* spp. reported on the host. On the basis of its culture and morphological characters the fungus causing leaf spot disease of *Aloe vera* has been proved *Alternaria alternata*. These findings were supported by findings of Subramanian[19], Carmichael et al.[27] and Goettel et al.[17].

Best culture medium and poisoned food technique

In order to find out the best culture medium, the fungus was grown on different solid media for determining their comparative suitability for vegetative and reproductive growth. Among these solid media, malt extract agar medium proved to be the best medium for growth and sporulation of the fungus, followed by potato dextrose agar medium and czapek's medium. Although, potato dextrose agar has been reported the best medium for growth and sporulation of *Alternaria alternata*[28,29]. In *in vitro* studies on the efficacy of fungicides seven tested fungicides were evaluated by means of poisoned food technique. The results revealed that Tebuconazole, Myclobutanil and Hexaconazole at the entire concentrations used i.e., 0.05%, 0.1% and 0.2% were found highly effective resulting in 100 percent inhibition of mycelial growth. Other fungicides found effective to check the growth of the fungus were Tricyclazole, Mancozeb, Metalaxyl + Mancozeb and Carbendazim. Hexaconazole at 0.1 percent gave maximum inhibition of mycelial growth and spore germination[30,31] of pathogen. Hundred percent inhibition of mycelial growth was recorded in Tebuconazole at 0.50 percent concentration and Carbendazim @ 1.0 percent was next best inhibiting mycelial growth of fungi [32]. Mancozeb caused 100 percent inhibition of mycelial growth[33].

Percent Disease Index (PDI)

Under field experiments, fungicides found effective during *in vitro* studies were used in the management of leaf spot of *Aloe vera*, where, results revealed that Tebuconazole was most effective in minimizing the percent disease index followed by Hexaconazole and Myclobutanil in terms of efficacy. Hexaconazole 5EC followed by Myclobutanil 10 WP and Carbendazim 50 WP proved to be significantly superior by exhibiting average disease incidence[31]. Bavistin @2 percent was found reducing PDI up to an acceptable level (59.77%)[34]. Mancozeb has been reported to be effective fungicide against *A. alternata*[31]. The antagonistic evaluation of different

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bio-agents was also assessed against *A. alternata* by dual culture technique. Greatest reduction in colonial growth of *A. alternata* has been recorded in *T. koningii* and found significantly superior over all the other bioagents tested. *T. harzianum* and *T. atraviride* were found next best to *T. koningii.* Species of *Trichoderma,* viz. *T. koningii, T. harzianum, T. atraviride* and *T. viride* showed more mycelial inhibition of organism compared to other antagonists. *T. harzianum* followed by *T. viride* found best inhibiting mycelial growth of fungus[33]. Findings revealed that antagonist *T. asperellum* was recorded a maximum percentage of inhibition of radial growth in mycelium (70.39%) through non-volatile metabolites followed by *T. viride* (67.22%), *T. harzianum* (65.92%), *T. longibrachiatum* (64.80%) and *Beauveria bassiana* (64.61%) in a dual-culture-method[34].

The bio-agents those were found effective in the laboratory conditions were used to evaluate under field conditions and results revealed that *T. koningii, T. harzianum, T. atraviride T. viride* and *T. lonyizruciatum* were effective in management of disease.

Similar results were obtained by many workers where a significant decrease in terms of pool data of PDI as a result of the application of fungal biocontrol agents *T. asperellum, T. viride* and *T. harzianum* has been achieved [34,35]. *Trichoderma viride* exhibited 36.33% disease control and proved to be superior in providing protection against Alternaria leaf spot of *Vicia faba*[36,37] under *in vivo* conditions. *Burkholderia cenocepacia* VBC7 and *Pseudomonas poae* two of the biocontrol organisms have shown the potential to decrease the disease severity and to maintain the good health as well as antimicrobial potential of *A. vera* plants[38].

CONCLUSION

In conclusion, results showed in this project a visible and valid correlation between application of systemic fungicides and the reduction in colonial growth in vitro, PDI in vivo and Increment in percent inhibition of mycelial growth in vitro, percent disease control over control in vivo. Similar kind of correlation was recorded though biocontrol agents in vivo and in vitro.

STATEMENT OF SIGNIFICANCE

This study discovers the new generation systemic fungicides and BCAs that can be beneficial for

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the management of Alternaria leaf spot of *Aloe vera* and lessen the pesticidal load (residual load) over foliar parts that are of medicinal value used for various purposes.

This study will help the researcher to uncover the critical areas of minimal dose of chemicals to overcome fear of mammalian toxicity in human consumption and use of soil micro-flora for eco-safe and healthy approach for the management and improvement in qualitative and quantitative yield of the plant and bi-products. A new theory on integrated applications of above used approaches may be arrived at upcoming researches being held simultaneously and in nearby future.

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REFERENCES

1. Shelton, R. M. (1991). Aloe vera: its chemical and therapeutic properties. *International journal of dermatology*, *30*(10), 679-683.
2. Surjushe, A., Vasani, R., & Saple, D. G. (2008). Aloe vera: a short review. *Indian journal of dermatology*, *53*(4), 163.
3. Chavan, S. P., & Korekar, S. L. (2011). A survey of some medicinal plants for fungal diseases from Osmanabad district of Maharashtra state. *Recent Research in Science and Technology*, *3*(5).
4. Verma, S., & Singh, S. P. (2008). Current and future status of herbal medicines. *Veterinary world*, *1*(11), 347.
5. Boudreau, M. D., & Beland, F. A. (2006). An evaluation of the biological and toxicological properties of Aloe barbadensis (miller), Aloe vera. *Journal of Environmental Science and Health Part C*, *24*(1), 103-154.
6. Sahu, P. K., Giri, D. D., Singh, R., Pandey, P., Gupta, S., Shrivastava, A. K., ... & Pandey, K. D. (2013). Therapeutic and medicinal uses of Aloe vera: a review. *Pharmacology & Pharmacy*, *4*(08), 599.
7. Grindlay, D., & Reynolds, T. (1986). The Aloe vera phenomenon: a review of the

ISSN:0971-1260
Vol-22-Issue-16-August-2019

properties and modern uses of the leaf parenchyma gel. *Journal of ethnopharmacology*, *16*(2-3), 117-151.

8. Ji, H. F., Li, X. J., & Zhang, H. Y. (2009). Natural products and drug discovery. *EMBO reports*, *10*(3), 194-200.
9. Bajwa, R., Mukhtar, I., & Mushtaq, S. (2010). New report of Alternaria alternata causing leaf spot of Aloe vera in Pakistan. *Canadian Journal of Plant Pathology*, *32*(4), 490-492.
10. Da Silva, W. L., & Singh, R. (2012). First report of Alternaria alternata causing leaf spot on Aloe vera in Louisiana. *Plant disease*, *96*(9), 1379-1379.
11. Maiti, C. K., Sen, S., Acharya, R., & Acharya, K. (2007). First report of Alternaria alternata causing leaf spot on Stevia rebaudiana. *Plant Pathology*, *56*(4), 723.
12. Kamalakannan, A., Gopalakrishnan, C., Renuka, R., Kalpana, K., Lakshmi, D. L., & Valluvaparidasan, V. (2008). First report ofAlternaria alternata causing leaf spot onAloe barbadensis in India. *Australasian Plant Disease Notes*, *3*(1), 110-111.
13. Shukla, R. S., Abdul-Khaliq, S. H., & Alam, M. (2008). Phytotoxin production by Alternaria alternata and its role in black leaf spot disease of Aloe vera. *4th National Interactive Meet Souvenir (NIM-08)*.
14. Choi, Y. W., Hyde, K. D., & Ho, W. H. (1999). Single spore isolation of fungi. *Fungal diversity*.
15. Goh, T. K. (1999). Single-spore isolation using a hand-made glass needle. *Fungal Diversity*, *2*, 47-63.
16. Ho, W. C., & Ko, W. H. (1997). A simple method for obtaining single-spore isolates of fungi. *Botanical Bulletin of Academia Sinica*, *38*.
17. Goettel, M. S., & Inglis, G. D. (1997). Fungi: Hyphomycetes. In "Manual of Techniques in Insect Pathology"(LA Lacey, Ed.).
18. Subramanian, O.V. (1971). Hyphomycetes. I.A.R.I., New Delhi, India. Pages: 804-819.
19. Geison, G. L. (2014). *The private science of Louis Pasteur* (Vol. 306). Princeton University Press.
20. Brock, T. D. (1988). *Robert Koch: a life in medicine and bacteriology* (pp. 96-104). Madison, WI: Science Tech Publishers.
21. Dennis, C., & Webster, J. (1971). Antagonistic properties of species-groups of

ISSN:0971-1260
Vol-22-Issue-16-August-2019

Trichoderma: I. Production of non-volatile antibiotics. *Transactions of the British Mycological Society*, *57*(1), 25-IN3.

22. Dennis, C., & Webster, J. (1971). Antagonistic properties of species-groups of Trichoderma: II. Production of volatile antibiotics. *Transactions of the British Mycological Society*, *57*(1), 41-IN4..
23. Dennis, C., & Webster, J. (1971). Antagonistic properties of species-groups of Trichoderma: III. Hyphal interaction. *Transactions of the British Mycological Society*, *57*(3), 363-IN2.
24. Bliss, C. I. (1934). The method of probits—a correction. *Science*, *79*(2053), 409-410.
25. Shukla, R., Kumar, A., Prasad, C. S., Srivastava, B., & Dubey, N. K. (2008). Antimycotic and antiaflatoxigenic potency of Adenocalymma alliaceum Miers. on fungi causing biodeterioration of food commodities and raw herbal drugs. *International Biodeterioration & Biodegradation*, *62*(4), 348-351.
26. Frankland, J. C., Dighton, J., & Boddy, L. (1990). 11 Methods for Studying Fungi in Soil and Forest Litter. In *Methods in microbiology* (Vol. 22, pp. 343-404). Academic Press.
27. Carmichael, J. W., Kendrick, W. B., Conners, I. L., & Sigler, L. (1980). *Genera of Hyphomycetes*. Univ. Alberta Press.
28. Pandey, B. N., Srivastava, S. P., & Srivastava, R. K. (2006). Studies on effect of various culture media on growth sporulation and morphological variations of Alternaria alternata (Fr.) Keissler. *Flora and Fauna*, *12*(2), 247-248.
29. Saeed, M. A., Ahmad, M., & Khan, M. A. (1995). Effect of different media, temperatures, pH levels, nitrogen and carbon sources on the growth of Alternaria alternata. *Pakistan Journal of Phytopathology (Pakistan)*.
30. Roopa, R. S., Yadahalli, K. B., & Kavyashree, M. C. (2014). Evaluation of natural plant extracts, antagonists and fungicides against early blight caused by A. solani in vitro. *The Bioscan*, *9*(3), 1309-1312.
31. Badri, Z. A. (2013). Evaluation of fungitoxicants against Alternaria alternata causing umbel blight disease of kala zeera (Bunium persicum). *Indian Phytopathology*, *66*(2), 195-198.
32. Jakatimath, S. P., Mesta, R. K., Biradar, I. B., Mushrif, S. K., & Ajjappalavar, P. S.

ISSN:0971-1260
Vol-22-Issue-16-August-2019

(2017). In vitro evaluation of fungicides, botanicals and bio-agents against Alternaria alternata causal agent of fruit rot of brinjal. *Intl. J. Curr. Microbiol. Appl. Sci*, *6*(5), 495-504.

33. Chethana, B. S., Ganeshan, G., Rao, A. S., & Bellishree, K. (2013). In vitro evaluation of plant extracts, bioagents and fungicides against Alternaria porri (Ellis) Cif., causing purple blotch disease of onion. *Pest Management in Horticultural Ecosystems*, *18*(2), 194-198.
34. Ghosh, S. K., Banerjee, S., Pal, S., & Chakraborty, N. (2018). Encountering epidemic effects of leaf spot disease (Alternaria brassicae) on Aloe vera by fungal biocontrol agents in agrifields—An ecofriendly approach. *PloS one*, *13*(3), e0193720.
35. Gveroska, B., & Ziberoski, J. (2012). Trichoderma harzianum as a biocontrol agent against Alternaria alternata on tobacco. *ATI-Applied Technologies & Innovations*, *7*(2), 67-76.
36. Sanjeet, K., Upadhyay, J. P., & Sanjeev, K. (2006). Biocontrol of Alternaria leaf spot of Vicia fiba using antagonistic fungi. *Journal of Biological Control*, *20*(2), 247-250.
37. Meena, P. D., Meena, R. L., Chattopadhyay, C., & Kumar, A. (2004). Identification of critical stage for disease development and biocontrol of Alternaria blight of Indian mustard (Brassica juncea). *Journal of phytopathology*, *152*(4), 204-209.
38. Ghosh, R., Barman, S., Khatun, J., & Mandal, N. C. (2016). Biological control of Alternaria alternata causing leaf spot disease of Aloe vera using two strains of rhizobacteria. *Biological control*, *97*, 102-108.