

Management of Leaf Spot Disease of Aloe vera

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Abstract: *Aloe barbadensis* (L.) is a perennial, drought-resisting, xerophytic plant belonging to the family 'Liliaceae'. The name, Aloe, is derived from the Arabic "alloeh" or Hebrew "halal" meaning bitter shiny substance. The leaves are 40-60 cm long, erect, broad, thick and fleshy succulent, glaucous-green in colour, narrow-lanceolate in shape with long acuminate tip with small thorns on both edges. The central bulk of the leaf contains colourless mucilaginous pulp (Aloe vera gel), made up of large, thin walled mesophyll cells. The plant contains 95 – 96% water and over 75 other constituents which include vitamins, minerals, enzymes, sugars, phenolic compounds, saponins and amino acids (Boudreau and Beland, 2006). Aloe vera is also used as a food substance, possibly for its gelling properties. It has been reported that leaf spot of Aloe vera was incited by *Alternaria alternata* that can cause economic losses in the cultivation of the Aloe vera. (Rukhsana *et al.*, 2010) A total of 15 fungi, including *Alternaria alternata*, *Fusarium roseum*, *Fusarium oxysporum*, *Alternaria dianthi*, *Aspergillus niger*, *Aspergillus fumigatus*, *Drechslera australiensis*, *Curvularia senegalensis*, *Colletotrichum dematium*, *Nigrospora oryza*, and *Trichoderma viride* were isolated from the leaf samples of Aloe vera collected from different areas (Singh *et al.*, 2014). Surveys have shown that the common disease on the Aloe vera plants was leaf spots which causes harmful effects on the medical value of the plant parts, and other fungi *Fusarium solani*, *Aspergillus niger*, *Penicillium spp.* were recorded (Chavan and Korekar, 2011)

MATERIALS AND METHODS

Collection of infected leaf spot sample

Leaves showing characteristic leaf spot symptom were collected from field of main experiment station, Department of Medicinal & Aromatic Plant, Narendra Deva University of Agriculture & Technology, Kumarganj, Faizabad (Uttar Pradesh). The infected leaves were kept in rough dry envelop and marked clearly mentioning characters and date of collection etc. and brought to the laboratory for isolation of the pathogen.

Cleaning and sterilization of glassware's and metals

The glassware's used were cleaned with washing powder and after cleaned using 0.1

percent HgCl₂ (mercuric chloride) solution and finally washed thoroughly with tap water. The metallic blade, scissor, forcep, inoculating needle, etc. were sterilized by dipping in the spirit and heating on flame to red hot. Laminar flow was sterilized with ultra violet lamp before use. The spirit was used as disinfectant. The dry glassware's were sterilized at 160°C for 1 hr's in hot air oven.

Preparation of culture media

Potato dextrose agar (PDA)

Composition

Peeled potato	- 200g
Dextrose	- 20g
Agar-agar	- 20g
Distilled water	- 1000 ml.

METHOD

Potato were peeled and chopped in to small pieces and boiled in 500 ml distilled water. The dextrose was dissolved in prepared potato decoction, in another beaker Agar-agar was put in to 500 ml boiling water. After both potato decoction and the molten agar were mixed together. The final volume was made up 1000 ml by adding distilled water then the prepared medium was sterilized in autoclave at 15 psi pressure at temperature (121°C) for 30 minutes.

Preparation of PDA slants

The culture tubes containing sterilized medium were taken out from the autoclave and kept at 45 degree angle on a support to prepare slant and stored in the refrigerator for further use.

Czapeck's (Dox) agar (CZA)

Composition

Sucrose (C ₆ H ₁₂ O ₆)	-30.00 g
Sodium nitrate(NaNO ₃)	- 20.00 g
Potassium di hydrogen phosphate (KH ₂ PO ₄)	-01.00 g
Magnesium sulphate (MgSO ₄ 7H ₂ O)	- 00.50 g
Potassium chloride (KCl)	- 00.50 g
Ferric chloride	- 00.01 g
Agar-agar	- 20.00 g
Distilled water	- 1000 ml
	(volume to make up)

METHOD

All the chemical ingredients excluding agar were dissolved in 300 ml water and Agar-agar was melted separately in 500 ml distilled water. Two solutions were mixed thoroughly and the volume was made up to 1000 ml by using distilled water and sterilized at 15 psi pressure and 121°C temperature for 30 minutes.

Method of isolation

The diseased samples, showing distinct characteristics of specific disease, were selected

for isolation of the pathogen. The selected plant parts were washed with fresh sterilized water to remove the dust particles and surface contaminates. The washed diseased plant parts were cut into small bits, with some healthy portions, with the help of sterilized scalpel . The cut pieces were surface sterilized with 0.1 percent mercuric chloride solution under aseptic condition inside the Laminar flow and washed thoroughly 3 to 4 times with sterilized water to remove the traces of mercuric chloride, the excess moisture was removed by placing them in the fold of sterilized blotting papers.

The petri-dish used in the experiment were sterilized at 160°C for 1 hr's in hot air oven and poured with 2 percent potato dextrose agar (PDA) medium and after one or two pieces of infected leaf parts were transferred in each petri-dish with the help of sterilized needle.

The petri-dishes were properly marked with glass marking pencil indicating date of isolation and isolate number etc. The petri-dishes were then transferred at 28±2°C in an incubator.

Cultural and Morphological characters of the fungus

Culture characteristics of the fungus were studied on PDA , 20 ml of sterilized medium was poured into each petri plate and allow to solidify. The petri plate was inoculated with actively growing mycelia disc(5mm) and incubated at (28±2°C).

Pure-culture of the pathogen

The purification of the fungal isolation was undertaken following single spore isolation technique. A dilute spore suspension was poured in petri-dish to form a very thin layer on it and spores allowed to settle down on the agar surface, settled spores were separated out from each other, selected under the microscope and encircled with the help of dummy cutter in petri-dish and were lifted along with agar blocks and transferred to petri-dish containing

sterilized 2 percent PDA, then proper growth of the fungus obtained by single spore culture, regular sub-culturing were done, to check contamination, till pure-culture was not obtained. These cultures were sub-cultured at 15 days interval and maintained on PDA slants under refrigeration at 10 to 12°C for further studies.

Identification of the fungus

An isolated and raised pure culture of *Alternaria* fungus was identified on the basis of morphological and cultural characters with the help of available manuals, (Subramanian, 1971;) and also get it confirmed from ITCC (Indian Type Culture Collection), IARI Division of Plant Pathology, New Delhi.

Efficacy of fungicides against *Alternaria alternata* in vitro

The experiment was conducted in completely randomized block design (RBD) in three replication with six treatments viz. fungicides and botanicals namely Carbendazim -50% WP @ (0.25%) Mancozeb -75% WP @ (0.25%) Propiconazole -25 EC @ (0.25%) and Neem (Water leaf extract) @ (5.0%) Garlic (Water bulb extract) @ (5.0%) Tulsi (Water leaf extract) @ (5.0%).

Meteorological data

The experimental site falls under sub-tropical climatic of eastern part of India. The District Faizabad comes under eastern region of Uttar Pradesh, distributed in three seasons viz., **rainy, winter** and **summer**. The rainy season occurs from mid June to mid of September. The winter months prevails from November to March with mild to severe cool temperature. The severe cold was recorded in the month of December and occasionally winter rains and frost was also noticed. The summer months occur from April to June. The dry and hot wind waves were also noticed in the months of mid May and June.

Weekly meteorological, data was recorded at the Meteorological observatory of Narendra Deva University of Agriculture and Technology, Kumarganj, Faizabad and presented in Table -(3&4)

Efficacy of different fungicides and botanicals under natural field condition against leaf spot disease of *Aloe vera*

The experiment having following details, was conducted in view to work out an effective fungicidal and botanical management schedule for the disease.

The experiment was conducted as follows

Treatment	:	- 7
Plot size	:	- 7.0×2.0 m ²
Row to row distance	:	- 50 cm
Design	:	- RBD
Plant to plant	:	- 40 cm
Replication	:	- 3

Treatments details

T₁ = Three foliar sprays with Carbendazim 50 WP @ 0.25%

T₂ = Three foliar sprays with Mancozeb 75 WP @ 0.25%

T₃ = Three foliar sprays with Propiconazole 25 EC @ 0.25%

T₄ = Three foliar sprays with Neem leaf extract @ 5%

T₅ = Three foliar sprays with Garlic bulb extract @ 5%

T₆ = Three foliar sprays with Tulsi leaf extract @ 5%

T₇ = Control (Untreated)

Preparation of botanicals extract

The leaf extract of Neem, Tulsi and bulb extract of Garlic were prepared by cold water extraction method. The samples were washed separately in tap water. They were crushed in mortar and pestle by adding distilled water @ 2

Table 1: Plants used as botanicals with their common name, english name, family and their part used

S. No	Common Name	English Name	Botanical Name	Family	Part used	Active compound	Activity	Class
1	Neem	Neem	<i>Azadirachta indica</i> (A.Juss.)	Meliaceae	Crude extracts	Azadirachtin	Fungi	Terpenoides
2	Lahsun	Garlic	<i>Allium sativum</i> . Linn	Liliaceae	Bulbs	Allicin	Fungi & bacteria	Sulfoxide
3	Tulsi	Ocimum	<i>Ocimum grattissimum</i>	Lamiaceae	Crude extracts	Thymol & Eugenol	Fungi & bacteria	Allylbenzene

ml/g fresh weight. The extracts were clarified by passing through two layers of cheese cloth and finally through Whatman no. 1 filter paper. The filtered extracts were quoted in the study as 100 % extract.

Preparation of botanical concentration

The appropriate volume of plant extract was mixed in sterilized distilled water to make the desired concentration (w/v) for experiments. For bioassay, double strength concentrations

Table 2: List of fungicides, trade name, chemical name and supply source.

S. No.	Common name	Trade name	Chemical name	Source
1.	Carbendazim 50WP	Bavistin	Methyl 1, 2-Benzimidazole -2yl carbamate	BASF, India Ltd.
2.	Mancozeb 75 WP	Indofil M-45,	Manganese ethylene bisdithio-carbamate	Indofil Chemicals, Ltd.
3.	Propiconazole 25 EC	Tilt	1-[2-(2,4dichlorophenyl) -4-propyl-1,3-dioxolan-2ymethyl]-1H-1,2,4 triazole	Hindustan Ciba Geigy Ltd.

of botanicals were prepared by dissolving 5 ml of plant extract in 95 ml of sterilized distilled water, respectively to get the final concentration of 5 percent.

Observations recorded

Five plants from each plot were randomly selected and tagged. observations were recorded on percent disease incidence, percent disease control, leaf yield per hectare. Percent disease incidence and percent disease control was calculated as per formula :

Per cent disease intensity(PDI)

Percent disease intensity and percent disease control were calculated by using the following formula given by (Kushalappa and Ludwig, 1982).

$$\text{Per cent disease intensity (PDI)} = \frac{\text{Sum of all numerical ratings}}{\text{Total number of leaves examined} \times \text{Highest rating}} \times 100$$

Per cent disease control (PDC)

$$\text{Per cent disease control (PDC)} = \frac{\% \text{ disease in control} - \% \text{ disease treatment}}{\% \text{ disease in control}} \times 100$$

Or

$$\text{PDC} = \frac{C-T}{C} \times 100$$

C = Percent disease incidence in untreated control.

T = Percent disease incidence in treated plants.

DISCUSSION

The *Aloe vera* crop suffers from vagaries of fungal, bacterial and viral disease. Among fungal disease, *Alternaria* leaf spot caused by *Alternaria alternata* (Fr.) Keissler, earlier considered to be a minor disease, is now becoming increasingly destructive & widely damaging in recent years. During the course of this study, the disease appeared in the month of April and reached at its peak in the month of July. In view of the economic importance of

the disease and limited information available on status, variability and disease management, studies were carried out on present scenario of the disease, variability, effect of different weather parameters on disease development and effective management of the disease through chemical fungicides and botanicals. The maximum disease severity (86.58) was found in month of July was correlated with maximum temperature (0.818°C), minimum temperature (0.398°C), rainfall (-0.69mm) and relative humidity (-0.024) followed by June with disease severity (82.67) was correlated with maximum temperature (0.727°C), minimum temperature (0.033°C), rainfall (-0.110mm), and relative humidity (-0.545). The minimum disease severity (53.44) was recorded in month of April was correlated with maximum temperature (0.482°C), minimum temperature (-0.818°C), rainfall (00), and relative humidity (-0.843).

The pathogen was isolated on PDA from infected *Aloe vera* leaves showing characteristic symptoms of disease i.e. small light to dark brown lesions on leaves starting from margin or tip and having concentric rings in brown lesions. The fully developed spots were round to oval in shape, sunken on both sides with light brown margins and brown to black centre and were similar to *Alternaria* diseases on other hosts (Bellow *et al.*, 1988, Aneja and Singh, 1989, Gunasekhar *et al.*, 1994, Bhatt *et al.*, 2000, Singh, 2005, Infanton *et al.*, 2009 and Bassimba and Mira, 2012).

Exclusive reliance on fungicides for the control of disease of various crops has resulted in residue and environmental hazards. Therefore, efforts are being diverted to employ higher plants (botanicals) and their derivatives as a tool for integrated disease management because they do not cause bio-accumulation, bio-magnification and environmental pollution. Plant extracts have assumed special significance in the present day strategy of developing ecologically safe

method of plant disease management. Singh and Majumdar (2001). Three plant extracts namely Neem, Garlic and Tulsi and three chemical fungicides Propiconazole, Mancozeb and Carbendazim were tested at different concentration and inhibited mycelial growth of *Alternaria alternata* *in vitro*. The minimum disease intensity (30.76%) and highest yield (1059 q/ha) and highest disease control (62.54) was recorded with Propiconazole (0.25%). Our results are in agreement with the result of Singh and Majumdar (2001) they observed that Garlic clove (5%) concentration effective in reducing disease intensity of Pomegranate fruit. The *Alternaria alternata* causes leaf spot disease in all three seasons on *Aloe barbadensis* in India which limits the cultivation of *Aloe vera* production to reduce the disease the foliar spray of Carbendazim, Mancozeb, Propiconazole, Neem, Garlic, and Tulsi combination with respect to disease intensity, yield q/ha and amount of gel data recorded (**Table-**), indicate that all treatments were found significant over control during 2016-17. The minimum percent disease incidence were recorded in Propiconazole 25 EC @ 0.25% PDI (30.76) and among the botanicals Neem leaf extract @ 5% PDI (68.51) shows encouraging results where as Reddy *et al.* (2009) tested antifungal activity of botanical extracts such as *Azadirachta indica*, *Allium cepa*, *Allium sativum*, against the growth of pathogenic fungi.

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