Int.J.Curr.Microbiol.App.Sci (2020) Special Issue-11: 1367-1371

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Special Issue-11 pp. 1367-1371**
Journal homepage: http://www.ijcmas.com

Original Research Article

Isolation, Identification and Purification of Leaf Spot Disease of *Aloe vera*

Akash Pandey*, S. K. Pande, Rajesh Saini and Krishna Kumar

Department of Plant Pathology, N.D.U.A &T, Faizabad, Uttar Pradesh, India

**Corresponding author*

A B S T R A C T

Aloe vera (*Aloe barbadensis* Miller) is a marvelous medicinal plant well known for its excellent medicinal properties. Leaf spot and root rot diseases were found on Aloe vera in various areas of Gwalior, Madhya Pradesh, India, in winters of 2011-2012. The typical disease symptoms were observed on both abaxial and adaxial surface of leaves as well as on root. Leaf spot, necrosis, root rot, yellowing of plants and even death was recorded as diseases symptoms. On the basis of morphological and microscopic characteristics of the fungus, three species of Fusarium *i.e. F. fusaroides, F. moniliforme* and *F. solani*, were found to be associated with the leaf spot and root rot diseases. Koch's postulate was applied to confirm the causal organisms of the diseases. As per literature till date, this seems to be the first report of leaf spot disease on Aloe vera caused by *F. fusaroides* and *F. moniliforme* while second report of root rot by *F. solani* from India.

Keywords

Aloe vera, Identification, Leaf spot

Introduction

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). The flowers are yellow to red in colour and are borne in dense racemes terminating in the cylindrical spike. The various regional names for Aloe vera are Kuwaargandal (Punjabi), Gheekanwaar (Hindi), Ghrita Kumari, Kumaari, Ghrit Kumaarika (Sanskrit), Indian Aloe (English), Kumari (Malyalam, Oriya), Katarazhai, Kilimukan, Chirukuttali (Tamil), Lolisara (Kannada), Kumarpathu (Gujrati), Chinna Kalabanda (Telgu), Ghrit Kumaari (Bengali), Korepharh (Marathi). The folk name of this plant is "Kanniedood", which means "can-not die".

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 $Hgcl_2$ (mercuric chloride) solution and finally washed thoroughly with tap water .The metallic blade, scissor, forceps, inoculating needle, etc. were sterilized by dipping in the sprit and heating on flame to red hot. Laminar flow was sterilized with ultra violet lamp before use. The sprit was used as disinfectant. The dry glassware's were sterilized at 160^0C 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 (121^0C) 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 ($C_6H_{12}O_6$) - 30.00 g
Sodium nitrate($NaNO_3$) - 20.00 g
Potassium di hydrogen phosphate (KH_2PO_4) - 01.00 g
Magnesium sulphate ($MgSO_47H_2O$) - 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 121^0C 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^{o}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^{0}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^{0}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.

Results and Discussions

Isolation, identification and purification

Isolation

The diseased leaves of the plant were carried to the laboratory in air tight sterilized bio-degradable polythene bags. The collected leaf samples were washed in sterile distilled water and were cut into small pieces of 3-5 mm in size from the diseased portion and passed through 0.1% of $Hgcl_2$ solution one minute for surface sterilization and washed in three changes of sterile distilled water. These leaf cuttings were blotted between sterile filter paper and aseptically plated on Potato dextrose agar (PDA) and Czapeck's dox agar (CZA) and incubated at BOD (28± 2° C) for 7 days, after appearance of mycelia growth it was transferred on to fresh PDA slant.

Purification

The purification of the isolated fungi was undertaken following single spore isolation technique. A dilute spore suspension was poured on petri dish containing medium 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 per cent PDA, after proper growth of the fungi 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 12^0C for further studies. The purified isolate of the fungal pathogen was labelled. The entire procedure of isolation was done under laminar air flow.

Characterization & identification of pathogen

The symptoms appeared on the leaves in form of small dark brown necrotic spots which gradually enlarge to cover up an area of 2-8 cm in diameter. The infected area transforms from dark brown to black.

The identification of fungi was made by preparing slides of the fungi and observing them under microscope. The fungi mounted on slides, stained with lacto phenol-cotton blue, on the basis of morphological and cultural characteristics using manuals. The fungal colony was olivaceous black with dark olive-green margins, and abundant branched septate, golden brown mycelium. The conidiophores were branched, straight, golden-brown and smooth walled. The conidia were obpyriform produced in long branched chains, with a short pale beak.

Pathogenicity test

Pathogenicity test was conducted on *Aloe barbadensis* leaf was placed in 30 petri plates, inoculated with spore suspension of *Alternaria alternata* using moist sterilized filter paper, plates were incubated at 25 ± 2°C and were regularly observed for development of symptoms. The re-isolation was done to confirm the test pathogen from infected portion of the leaves taken for pathogenicity test were cultured in petri plate and incubated at 25 ± 2°C. Olivaceous black colonies with dark olive-green margins, and abundant branched septate, golden brown mycelium growth similar to that of test fungus appeared after 7 days of incubation in petri plate; slides were prepared and examined under microscope. The cultural and morphological behaviour of the pathogen i.e., isolate from naturally infected was similar to the standard pure culture proves its pathogenicity.

Maintenance of culture

To maintain the cultures of *Alternaria alternata* were grown on sterilized PDA. The plates were inoculated with a bit of 10 days old culture grown on potato dextrose agar (PDA) at 25 ±2^0C temperature, one set of each pathogen was used for sub-culturing on PDA at regular intervals.

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 development of disease was recorded at 10 days interval starting from the first initiation of disease symptoms up to leaf cutting stage. The meteorological data on temperature, relative humidity and rainfall was recorded. The Aloe vera crop suffers from many diseases among which the leaf spot holds the lions share. The Percent disease intensity (PDI) was recorded at ten days interval starting from the start of first disease symptom up to harvesting. The percent disease intensity (PDI) was recorded during April to August 2016.

The pathogen was isolated on PDA from infected *Aloe barbadensis* 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, Infantion *et al*., 2009 and Bassimba and Mira, 2012).

Acknowledgment

The authors wish to thank HOD, Department of Plant Pathology, N.D.U.A& T, Faizabad- 224229 (U.P.) for providing need based facilities for conducting this research work.

References

Aneja, K. R. and Singh, K. (1989). *Alternaria alternata* (Fr.) Keissler, a pathogen of water hyacinth with bio control potential. *Trop. Pest Manag.* 35:354-358.

Bassimba, D. D. M and Mira, J.L. (2012). First report *Alternaria petroselini* causing leaf blight of Fennel in Spain, *Plant Disease* 96(6): 907-908.

Bello, G. M., Dal and Ello, A. E. (1988). Severe blight of *Borrage officinalis L.* a new disease caused by *Alternaria alternata* (Fr.) Keissler. *Investigation Agraria, Production Y Protection vegetables* 3: 347-354.

Bhatt, J. C., Gahlain, A. and Pant, S.K. (2000). Record of *Alternaria alternata* on tomato, capsicum and spinach in Kuman Hills. *Indian Phytopath* 53:495-496.

Boudreau, M. D., Beland, F.A. (2006). An evaluation of the biological and toxicological properties of *Aloe barbadensis* (Miller). *J. of Environmental Sci. and Health*, Part C Environmental Carcinogenesis and Ecotoxicology Reviews 24: 103–154.

Gunasekhar ,V., Govindlah and Datta, R.K.(1994). Occurrence of Alternaria leaf blight of mulberry and a key for disease assessment. *International .J. Trop. Dis*., 12: 52-57.

Infantino, A., Giambattista. G. Di., Pucci, N., Pallottini, L., Poletti, F. and Boccongelli, C. (2009). First report of *Alternaria petroselini* on fennel in Italy. *New Dis. Repts* ., 19:26.

Singh, N. (2005). Studies on Alternaria blight of Adusa (*Adhatoda vasicanees*) with emphasis of epidemiology of the disease. *Ph.D Thesis Rajasthan Agricultural University , Bikaner*, pp-48.