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STUDIES ON THE OCCURRENCE AND PATHOGENIC POTENTIAL OF *PHYTOPTHORA* SPP. ON *ALOE VERA* L. IN KOTA DISTRICT, RAJASTHAN

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Aloe vera L. being an excellent source of various antiseptic agents and ingredients of several cosmetic products, commonly grows wild in nature as well as cultivated plant in the state of Rajasthan. In the present investigation, efforts were made to determine the occurrence and pathogenic potential of the fungus *Phytopthora* spp. on *Aloe vera* plants causing leaf spot and leaf blight disease at different *Aloe* growing localities of Kota district, Rajasthan. Various pathological aspects *viz.* symptoms of the disease, etiology, isolation, and culture of the pathogen were considered for the identification of the disease. Besides these pathogenicity test conducted, showed successful development of the disease. A severe attack by the pathogen resulted in a great loss of plant growth and yield of the commercial product *i.e.* gel and latex content. A significant reduction in chlorophyll content was evident due to extensive foliar damage. Thus, there is an urgent need to control this fungal pathogen for quality production of *Aloe* gel and latex.

Keywords: *Aloe vera L;* Chlorophyll content; Gel and Latex; Leaf blight; Leaf spot; *Phytopthora* spp.

Introduction

Aloe vera L. (Family-Liliaceae) is a succulent perennial herb. It has attracted much attention worldwide for its medicinal and cosmetic uses in a rapidly growing market. The plant grows wild in nature as well as cultivated in commercial fields in the state of Rajasthan. Its most common species like *A. barbadensis, A. perryi, A. ferox* etc., are well known for their commercial product Barbadose (a dried leaf exudates *i.e.* gel+latex). The gel is a mucilaginous juice obtained from the inner parenchymatous portion of the leaves. It contains carbohydrate polymers like gluco-mannans plus various organic and inorganic compounds, such as glycoproteins, vitamins, enzymes, aloetic, salicyclic and cinnamonic acids, sulphur and phenols as antiseptic agents responsible for its medicinal and cosmetic value[1]. *Aloe* latex is bitter yellow coloured liquid exudates from the pericyclic tubules just beneath the epidermal layer of the leaves. It is a mixture of Anthroquinone glycosides like Aloin (a-b), Barbolin which are used as laxatives[2].

The use of gel as a cosmetic ingredient and latex as a laxative is approved by U.S. Food and Drug Administration. The latex is recommended to the patients suffering from the ailments like anal fissures, hemorrhoids etc. The use of gel for wound healing, psoriasis and dermabrasion was suggested by Davis *et al.*[3]. In India its fresh gel as well as whole leaves exudates in solid form (Barbados) are used as cathartic, stomachic and antihelminthic.

Despite its antiseptic qualities and immune stimulating actions, the plant suffers from bacterial and fungal infections. Bacterial soft rot caused by *Erwinia chrysanthmii* (biovar 3) was observed by De laat *et al.*[4] at Carribean island of Aruba, biochemical and pathological characteristics of the strain, including results of successful inoculation experiments were also presented by them. Later on Mandal[5] also reported soft rot disease of *Aloe* caused by *Pictobacterium chrysanthemii.*

But little information is available on fungal pathogens of *Aloe vera* plant. In the present investigation an extensive survey of Kota region carried out during the years 2007-2008, *Aloe* was found to be severely infected by some fungal pathogen. Stunted plant growth, leaf tip drying and withering, brownish black lesions on leaf blades and leaf collapse were the prominent symptoms. So, there was an urgent need to diagnose the disease and identify the pathogen infesting such an economically important plant. With this objective pathogenicity test, symptoms and etiological studies were performed. Efforts were made on isolation, culture and identification of the pathogen. The pathogenic effect of the fungus on plant growth and production of gel and latex was estimated in terms of

reduction in chlorophyll content and the amount of leaf exudates in diseased plants as compared to their healthy counterparts.

Material and Method

Survey: To assess disease incidence an intensive survey was conducted during 2007-2008 from July to Feb. at different localities in Kota region. A total of 180 plant samples (30 from each locality) were randomly selected. Plants were keenly observed with the help of hand lenses and dissecting microscope. On the basis of the presence of lesions and blight on the leaves and stunted growth, diseased plants were counted out of the samples brought from each locality and percent disease incidence was calculated.

Diagnosis and Identification of the Pathogen: For this efforts were made on following lines-

1- Isolation, culture and identification of the pathogen: Culture technique was adopted for this purpose. The pathogen was isolated and its culture was tried both on pure PDA medium and PDA medium supplemented with healthy fresh *Aloe* leaf extract. For isolation the lesions having diseased tissue were cut with a sterile scalpel and surface sterilized with 0.1% mercuric chloride solution, washed and blot dried. Then transferred (in aseptic conditions) into the culture tubes containing PDA slants, plus 0.5 ml of fresh *Aloe* leaf extract. Cultures were maintained at 20±2°C temperature. After 2 days onwards cultures were examined for the growth of fungus. For identification purpose stained preparations were made from the cultures and slides were observed under microscope. On the basis of the structure of mycelium, reproductive bodies and etiological measurements, the fungus was identified. Taxonomic determinations were made with the help of the literature and expertise available. For confirmation of the disease and to study disease development and affect of the *Phytopthora* spp. on various plant growth parameters of *Aloe vera* pathogenicity test was carried out.

2- Pathogenicity: For pathogenicity test, surface sterilized healthy root sucker of *Aloe* at 4 leaf stages were transplanted singly in 15 cm earthen pots filled with autoclaved sand and soil mixture (1:3) in replicates of 3. A separate set of healthy suckers was kept uninoculated which served as control. 15 days after transplantation suckers were inoculated for disease development. For inoculation highly spotted leaves and infected plant debries collected from the surveyed area were taken. With the help of the sterile scalpel, the spots with mycelium and spores were cut and spread on moist leaves. In addition to this spotted leaf tissue and plant debries was also mixed in the pot soil. The pots were kept in moist chamber at 20±2°C temperature and covered with polythene for spore germination. After 3 days pots were transferred in green house and watered thrice a week. Observations regarding disease development were noted. After 120 days plants were uprooted and observed for the symptoms of the disease and pathogenic effects of the fungus on various growth attributes of the plants as compared to healthy counterparts. Chlorophyll content was estimated by the method of Arnon[6]. The amount of gel plus latex content per plant was measured by making cuts near the base of leaves and the leaves were placed in tilted position along the sides of V shaped wooden through to drain out all gel and latex. Then it was collected and measured with measuring cylinder.

3. Symptoms and etiology- For disease diagnosis leaves were observed both externally as well as anatomically. Leaf anatomy of both healthy and infected plant was studied. On the basis of symptoms disease was confirmed. For etiological observations the mycelium from the pure cultures and infected leaf was observed under microscope and with the help of micrometer size of the chlamydopores, sporangia and oospore was measured.

Results and Discussion

Survey : Survey study conducted at six different *Aloe* growing localities of Kota region indicated that leaf spot and leaf blight disease was more prevalent in the area. High rainfall, warm and humid climate favoured the growth of the pathogen. The pathogen identified as *Phytopthora* spp. was consistently affecting the plant. The percent disease incidence varied at different localities (Table 1). It was found to be maximum (100%) at Graveyards of Kota and minimum (60%) in the Gardens where proper care of the plant being taken. It is important to note that previously these localities were never surveyed for the presence of the pathogen on *Aloe vera* plant. Though, *Phytopthora* fungus is well distributed and well established constraint to the cultivation of various crops in the state of Rajasthan.

Culture study : Culture study revealed that the fungus isolated from the diseased plants of *Aloe vera* was an obligate parasite, as it failed to grow on pure PDA medium (Fig. 4). It required host leaf extract as a supplement in culture medium for its growth at the optimum temperature of 20±2°C. Proper growth of the mycelium was observed after 8 days of inoculation. Microscopic examination of the culture revealed mycelium to be hyaline, aseptate coenocytic and branched. 12 days old mycelium was bearing sporangiophores with sporangia. Development of antheridia, oogonia and oospores was evident on the 18

Fig. 1

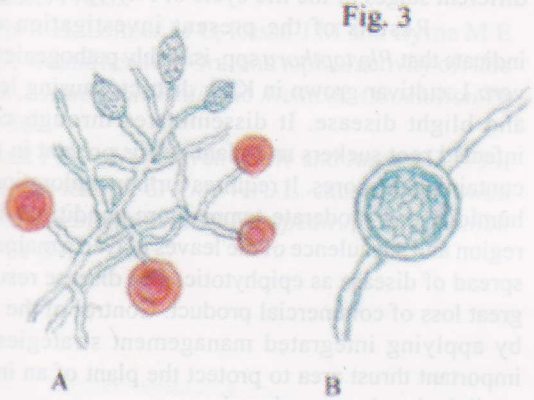
Fig. 2

A

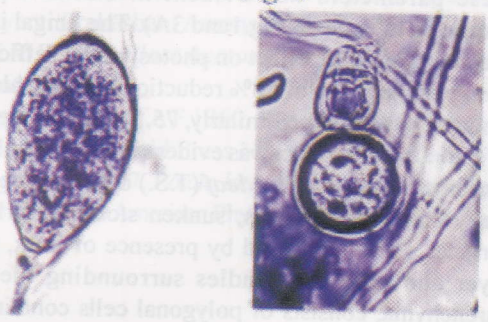
B

Fig. 3

Fig. 4



A B



C D

Fig.5.

Fig.1. Healthy plant 1. Infected plant (3A) Healthy leaves (3B) Infected leaves with shot-hole symptom 4. *Phytopthora culture* (5A) Mycelium with sporangia and oospores (5B) Chlamydospores (5C) Sporangium (5D) Oogonium and oospore.

days old mycelium (Fig.5A-D). Oospores development was also seen by Clinton[7] in artificial cultures of *P. infestans.* Thus, on the basis of nutritional requirement, structure of the mycelium and reproductive bodies the fungus identified was *Phytopthora* spp.parasitic on *Aloe vera* plant.

Pathogenicity: Pathogenicity test conducted showed successful development of the disease under pot trial. Penetration of the fungus was both from the soil and surface of the leaves through stomata after spore germination. High humidity, moderate temperature (20±2°C), water logging condition of the soil and succulence of the leaves favoured disease development. The fungal mycelium developed after spore germination invaded leaf tissue intercellularly by producing small haustoria. Further growth of the mycelium resulted in appearance of the symptoms like leaf tip drying and withering and development of brownish black lesions on both the surfaces and edges of the leaves (Fig.2 and3B). After 15 days of inoculation, lesions turned into white fuzz, containing mycelium with sporangiophores and sporangia. After 25 days of inoculation mycelium had developed oogonia, antheridia and oospores. Under dry conditions chlamydospores were developed. It was also evident that the sporangia and chlamydospores so produced, served as the source of inoculum for reinfection on healthy foliage through rain water and wind. The fungus perennated in the form of oospores in the infected leaves, root suckers and plant debris in the soil that also observed as a source of inoculum for reinfection. The present findings were also confirmed by Tilak[8] who also reported *Phytopthora* de bary infection during humid conditions in rainy season through spore germination.

Data presented in Table 2 indicated pathogenic effects of *Phytopthora* spp. infestation on morphological features, chlorophyll content and the amount of leaf exudates of *Aloe vera* plant. A significant reduction in all these parameters was evident in diseased plant as compared to control (Fig.1and 3A). The fungal infection showed an adverse effect on photosynthetic efficiency of *Aloe* plant in terms of 48% reduction in total chlorophyll content over healthy. Similarly, 75.5% loss in the amount of commercial product was evident in diseased plant. The anatomy of healthy *Aloe leaf* (T.S.) depicted presence of waxy cuticle, epidermis, sunken stomata on both the surfaces of leaf followed by presence of latex, palisade layer and vascular bundles surrounding the central parenchyma, consists of polygonal cells containing gel. Same anatomical features were also observed by Tayal[9]. However, the infected leaf (T.S.) showed shrinkage of leaf tissues and formation of grey to tan, dry and dead necrotic brown tissue instead of healthy epidermal palisade and central parenchymatous tissue filled with gel. Thus, death of the tissue resulted in a great loss of chlorophyll, latex and gel contents.

During initial stage of the infection leaf parenchyma showed presence of inter cellular hyphae with haustoria. That was followed by the development of sporangiophores with sporangia. Some diseased cells also showed presence of chlamydospores, oogonia, antheridia and oospores.

Symptoms and etiology : Under pot trial, due to *Phytopthora* spp.infestation on *Aloe vera* plant, the earliest symptoms observed were wilting and withering of leaf tips on the outside of a crown i.e., leaf damage started and dead part turned brown (Fig.2and3B). Similar observations were also made by Chupp and Sherf[10] during White tip disease of *Allium cepa* caused by *Phytopthora porri* Foister. On the surface of the leaves, first indication of the disease was off colouring of the foliage instead of normal green colour. This was followed by appearance of rose tints. After this brownish to blackish more or less circular to oval or irregular, separate or coalesce lesions were observed on the surfaces and edges of the leaves (Fig.2). Under moist, cloudy weather condition the disease was more severe. Lesions were more in number and some became necrotic and their dead part fall away, leaving shot-hole symptom (Fig.3B), whereas others were not delimited in size. At this stage drying, shrivelling and tissue death was evident, that ultimately caused leaf bending or leaf collapse. The lesions especially on the upper surface of the leaf were having whitish fuzz formed by the fungal mycelium bearing sporangiophores and sporangia, which is a useful distinguishing characteristic to help to differentiate late blight infections from other diseases[11]. As said above the anatomical study of infected leaf showed different stages of the life cycle of *Phytopthora* spp.

Results of the present investigation clearly indicate that *Phytopthora* spp. is highly pathogenic to *Aloe vera* L.cultivar grown in Kota district, causing leaf spot and blight disease. It disseminates through contact, infected root suckers and plant debris present in the soil containing oospores. It requires further exploration. High humidity and moderate temperature conditions in Kota region and succulence of the leaves played a major role in spread of disease as epiphytotic. The disease results in a great loss of commercial product. Control of the disease by applying integrated management strategies is the important thrust area to protect the plant of an immense medicinal and ecc nomic value.

Table 1. Distribution and incidence of leaf spot and leaf blight disease of *Aloe vera* L. in Kota district (Raj.)

S. No.	Localities studied	Total No. of Plants studied	No. of plants infected	% Disease Incidence
1.	Saji Dehra	30	27	90.0%
2.	Darah Forest	30	28	93.3%
3.	Jawahar Sagar Dam Forest	30	25	83.3%
4.	Graveyards of Kota	30	30	100.0%
5.	Gardens	30	18	60.0%
6.	Houses	30	21	70.0%
Total	6	180	149	Average% D.I.82.7%

Table 2. Effect of *Phytopthora* spp. infestation on various plant growth parameters of *Aloe vera* plant as compared to healthy plant after 120 days of inoculation (Each value is a mean of 3 replicates)

S. No.	Growth characters	Healthy Plant	Infected Plant	% Decrease over Healthy
1.	Fresh wt.(gms)/plant	320	159	50.3%
2.	No. of leaves/plant	12	8	33.3%
3.	Length of leaf (cms)	35	18	48.6%
4.	Total chlorophyll content (mg/gm)	2.491	1.266	49.2%
5.	Leaf exudates (gel+latex) (ml/plant)	170	80	53.0%

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