Prevalence of Fungal Diseases in Medicinally Important Cassia Alata L. under Tropical Conditions on the Coromandel Coast, India

Savitha Veeraragavan[1] Ramamoorthy Duraisamy[1] and Sudhakaran Mani[2]
[1]*Department of Ecology and Environmental Sciences & Pondicherry University, India*
[2]*Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India*

Abstract

Present investigation is focused on fungal infection and consequent disease prevalence in a commercially important plant Cassia alata L., profusely growing in and around Pondicherry region covering four seasons in 2015. Observation and collection of infected leaves, flower buds and pods are done monthly infected leaves and flower buds are subject to culture adopting suitable methods. The common fungal diseases recorded in leaves of C. alata are leaf spot disease and Anthracnose and fungal species namely slimy Aureobasidium, two species of Aspergillus, Rhizopus sp. Alternaria sp. are recorded from these infected plant parts. One of the important results obtained from the present study is that cut ends of the leaves made by folivory insects are found to be infected by the fungi whereas the cut ends of the leaves left by caterpillars are not infected. Relevant photographs in support of fungal disease are also given. The report includes information on the progress of leaf spot and Anthracnose in leaves- stage with photographs which is considered as an important useful input for commercial cultivators in disease management. The uniqueness/ highlight of this work includes one interesting observation that the cut ends of the leaves left by the caterpillars are not infected with fungal species even under ambient climate. This opens up a new avenue in the field of bioactive compounds from caterpillars particularly of the migrant butterfly, Catopsilia pyranthe. Taxonomic authentication of the host plant Cassia alata is also done by the molecular method and obtained NCBI Accession number after submitting sequences.

Keywords— Cassia alata L., Anthracnose, caterpillar, mouth secretion, bio-prospecting

I. INTRODUCTION

As the seeds and leaves are of much commercial importance by virtue of its phytochemical constituents with medicinal and ornamental properties and ZAUBA,2015), presently an attempt is made to study the fungal infection- induced diseases viz. Leaf spot and Anthracnose in *C. alata* growing in Pondicherry region under tropical climate. Primarily, the taxonomic position of the study speceis has been authenticated by molecular sequencing. Successful amplification is achieved using a single set of primer for the enough length of readable *matK* barcode sequences. The BLAST searches by sample sequence in Gen Bank revealed the closest matches with the same species and nearest neighbour (NN) of same or different genus. *matK* sequence of sample AUMP16 showed 100% identity with *Senna alata Cassia alata* Fig. 1.b (NCBI: sequence Accession Number-KX034078. Like other plants, medicinal plants are also succumb to a variety of diseases and also bear the devastating effects of folivory insect pests, facilitating pathogenic fungal growth and affect its medicinal value. The literature review revealed that a variety of pathogenic fungi have been isolated and reported throughout the world in many species of genus *Cassia* but least in *Cassia alata*. Pathogenic fungus *Alternaria cassia* causing foliar blight of several *Cassia* species in India and USA and heavy seedling blight in *C. obtusifolia* (sickle pod) and *C. occidentalis* (coffee *Senna*) in the USA (Boyette, 1988), and *C. fistula* in the USA (Farr et al., 1989). have been reported . Fourteen different species of *Cercospora* have been described on *Cassia* species (Brown & Morgan-Jones, 1977 and Cock and Evan 1984). *Endophyllum cassia*, short cycled rust, has been recorded on *C. obtusifolia* in Ghana, Nigeria and Tanzania and on *C. tora* in India, Malaysia and the phillipines (Cock, and Evans, 1984; Ebbels, and Allen, 1979. Rangaswami et al., 1970, Singh, 1973). *Revelenia barkeleyii* causes severe blight of leaves, stems and pod of *C. absus* in India, Tanzania and Zambia and *C. fistula* and *C. tora* in India (Cock and Evans, 1984; Ebbels and Allen, 1979; Rangaswami et al., 1970; Singh, 1973). Five *Colletotrichum* species have been recorded on *Cassia* species including *C. rotundifolia. Colletotrichum capsici* (Syd.) has been recorded on *C. occidentalis* in Malaysia (Singh, 1973) and *C. tora* in India, Malaysia and Venezueia (Howard and Albregts 1973), *C. fragariae* (Rhind, and Seth 1945) *Colleotrotrichum gloeosporioides* (Penz) has been recorded on *C. alata* in Tanzania (Ebbels, & Allen, 1979). and Venezuela CWMI, *C. occidentalis* in India CWMI, and *C. tora* in Burma (Rhind & Seth, 1945), India CWMI and Malaysia (Kheng, 1976), *C. lindemuthianum* has been found on *C. fistula, C. occidentalis* and *C. tora* in India CWMI, *C. truncatum* (Schw.) has been recorded on *C.*

occidentalis in India CWMI and the USA (Gudauskas, et al.1977).

Oidim sp. has been recorded on *C. fistula* in India CWMI, *C. floribunda* in Australia (Simmonds 1966), India CWMI and Pakistan (Khan and Kamal 1968), *C. obtusifolia* in Tanzania (Ebbels and Allen 1979); in *C. surattensis* in Pakistan (Wiehe 1953) and on *C. tora* in Cuba, Gambia, Malaysia, Nigeria (CWMI) and in India (Rangaswami et al., 1970). However, it is to be noted from the above literature on fungal disease in species of genus *Cassia* that reports related to this aspect has been dates back to more than 20 years; (Shamsi et al. 2014), but now, the scenario on the use of members of genus *Cassia* is reported to be most important in terms of their medicinal and export potential. It is also true that except for the reports of (Ebbels, and Allen, 1979) and (Wiehe 1953) there is no report on the prevalence of fungal disease in *Cassia alata* (Fig 1a) and hence present study has been undertaken to examine pathogenic fungal species and disease caused by them in *C. alata* growing in Pondicherry region under tropical climate.

II. MATERIALS AND METHODS

Pondicherry is located along the Coromandel Coast of peninsular India with the geographical coordinates $11^0$52'N, $79^0$45'E and $11^0$59'N and $79^0$52' E. The study was undertaken during January-December, 2015 covering four seasons viz. post-monsoon, summer (south west monsoon- less intense) pre-monsoon and Monsoon (north east monsoon),. The mean annual rainfall of the study area is about 1311-1172mm.The mean number of annual rainy a day is 55; the mean monthly temperature ranges between 21^0 C and 30^0 C in the study area. This region gets more rainfall during north east monsoon. Humidity is also high in this region as the study area is located near the coast. *Cassia alata*, one of the members of the family Caesalpiniaceae is an invasive plant found growing profusely in the Pondicherry urban region. (Fig.1b).

For fungal studies, two types of infected plant parts are sampled viz. 1. Infected leaves and flower buds with the fungal attack; 2. Leaves that are browsed by caterpillars left out cut ends without any visible infection; besides infected buds and pods are collected aseptically and brought to the laboratory. All samples are examined for fungal species. The culture was conducted in two phases. Phase 1: all the four types of samples are screened for fungal species; phase 2: only the leaves with cut ends left by caterpillars are cultured for the second time to confirm the result obtained in Phase 1. The infected plant parts collected are grown on potato dextrose agar (PDA) medium. For this PDA medium comprising of potato 20 %, dextrose 2 % was prepared and pH adjusted to 7.0. This medium was complemented with agar 1.5 % and autoclaved at 15 psi for 15 min. Autoclaved medium was poured in sterile Petri plates (25 ml/plate) under laminar flow (Horizontal laminar air flow - Matrimake model Mc 7000) and allowed to solidify and finally, the cut bits of samples are placed on the solidified agar aseptically. All inoculated Petri plates are incubated for two days at 23±2ºC and light – darkness cycle of 12/12 hour. The fungi grow on the plates were identified based on their colony forming traits and reproductive materials viz. mycelia and spores under light microscope and genus level identification are done by biochemical methods described by (Sangeetha and Thangadurai, 2013). Photographs are also taken both in the field and cultured fungi under microscopes (Phase contrast Microscope ZEISS-Primo star and Trinocular stereoscopic -CETI). Instruments used in the present study are available with the department of Ecology & Environmental Sciences, Pondicherry University.

III. RESULTS

Two types of fungal diseases are known to be prevalent viz. leaf spot and Anthracnose both in leaves and flower- buds. Leaf spot is noticed during post-monsoon and summer (Fig.2a&d) whereas Anthracnose is noticed in almost all types of plant parts sampled viz. leaves, dorsal and ventral sides of leaf lamina, mid-ribs, flower buds as well in matured flowers during summer and pre monsoon. It is also noticed in the field that Anthracnose is more prevalent in leaves and also in flowers where the initial infection is by fungi on the cut ends made by bud borer *Hendecasis duplifascialis* spotting of the foliage caused by the fungus, Alternaria is the most conspicuous symptom of blight next to Anthracnose. Primarily leaf-spot appears flowed by Anthracnose after a spell of rains i.e. post-monsoon and post summer seasons. Individual spots are at first seen as circular in outline but became irregular. The spots varied from 2 to 10 millimeters. As the spots increase in size and coalesce. (Fig.2e-f). Different stages of Anthracnose developed from leaf spot disease are illustrated in Fig.3. Similarly, development of Anthracnose in flower bud is depicted in Fig. 4. and Fig. 5 show the different dimension of anthracnose attack in pods. The noteworthy observation made in the study is the cut ends of leaves made by folivory insects by nibbling, promotes more fungal infection whereas the cut ends in leaves left after browsing by caterpillars showed no fungal growth. Unlike the cut ends of leaves caused by the insects, these leaves have not shown any infection. The second attempt is also made and confirmed that observation. From fungal culture studies, the swamps from all infected samples revealed 5 fungi viz. *Aspergillus niger*, *A. fumigates*, *Rhizopus sp.* *Aureobasidium* and *Alternaria sp*; among those *A. niger* seems to be common in all plants parts including pods the slimy colonies of *Aureobasidium sp*. are also isolated along with the overgrown *Rhizopus* from infected parts of

the plant *Aspergillus niger* the black colony forming fungi and green varieties with fuming colony growth are recorded (Fig. 6a-f).

Fig 1a. Casssia Alata L Syn Senna Alata

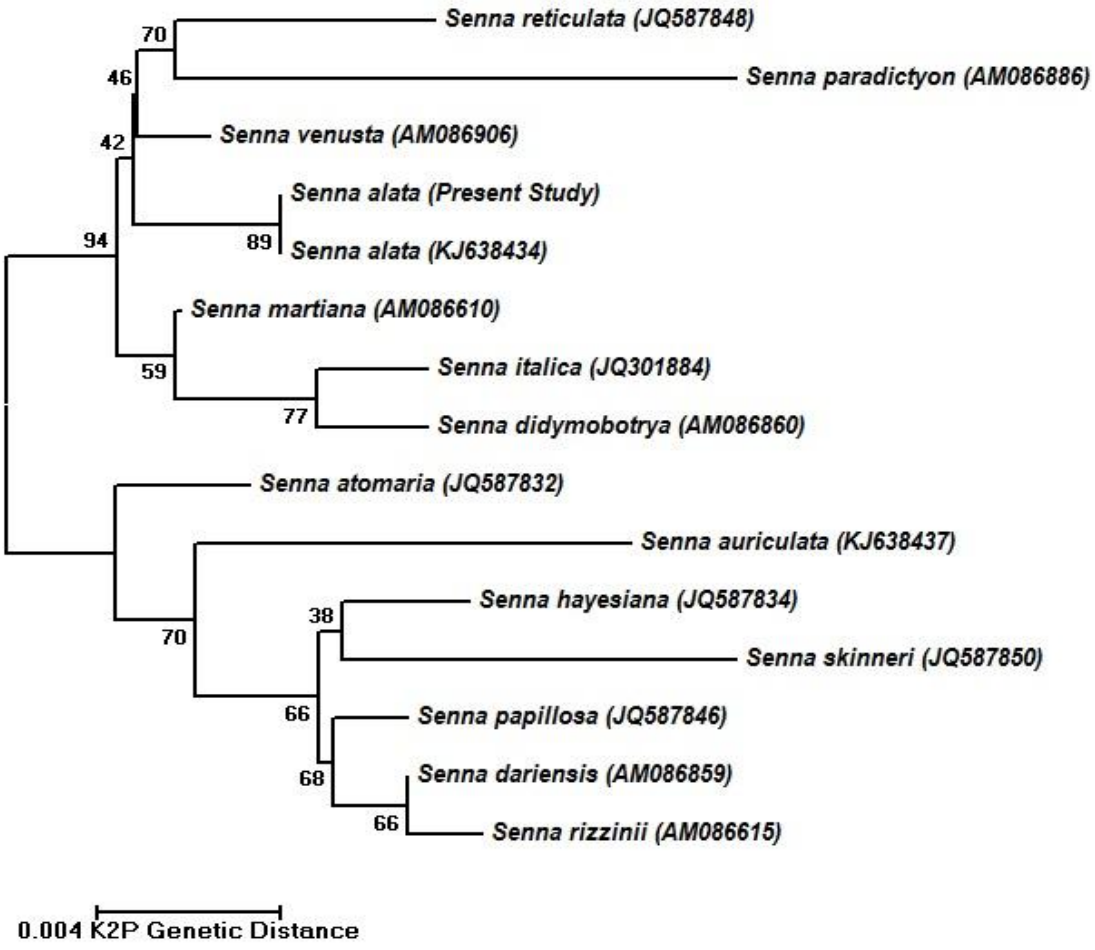

Fig 1.B Phylogenetic Tree

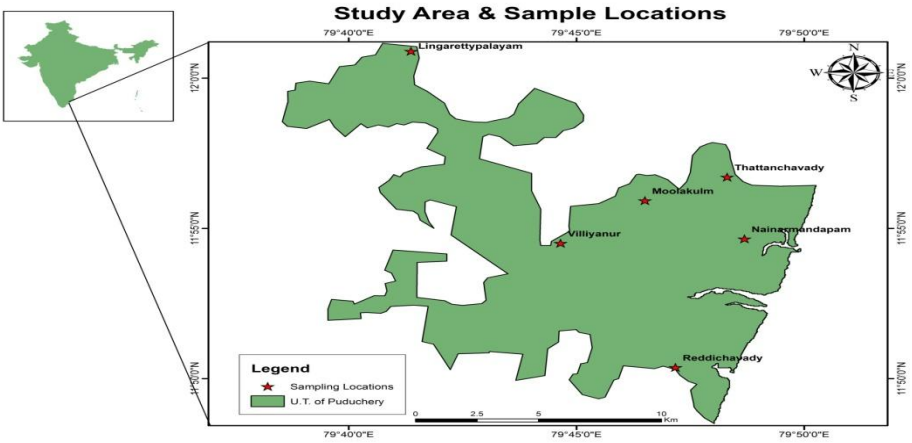

Fig 1c. Study Site- Pondicherry Region

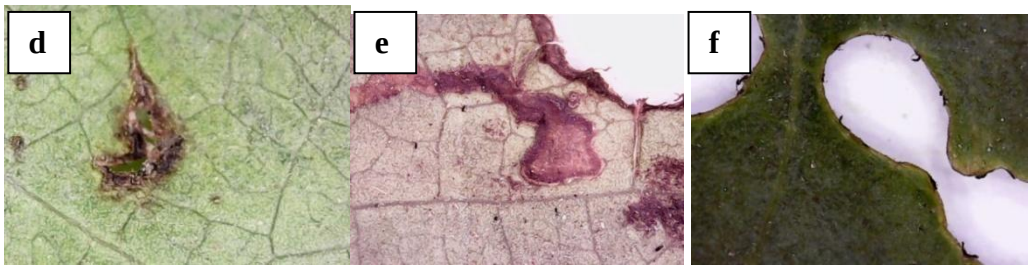

Fig.2. Leaf Spot- Different Stages In Leaf Spot Disease. A. Spot on Leaf; B. Both Spot of Second Stage of Tanning.; C. Distinct Tanning on Leaves D. Close Up View Affected Part of the Leaf; E. Infected Part Ready to Coalase; F. Coalased After Leaving a Hole on the leaf.

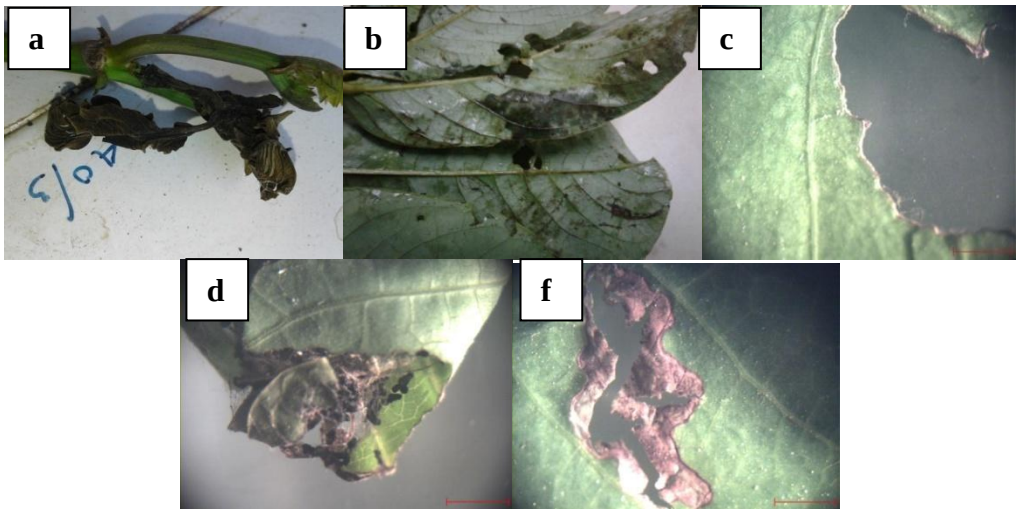

Fig3. Different Stages of Anthrocnose- Different Stages (Leaf And Flower Bud) A. Leaves With Tanning In Vein Lamina on the Ventral Side; C.Cut Ends in Leaves Left By Insects. D. Infection at the Cut Ends- Initiating Anthracnose. E. Anthrocnose Ready To Coalase.

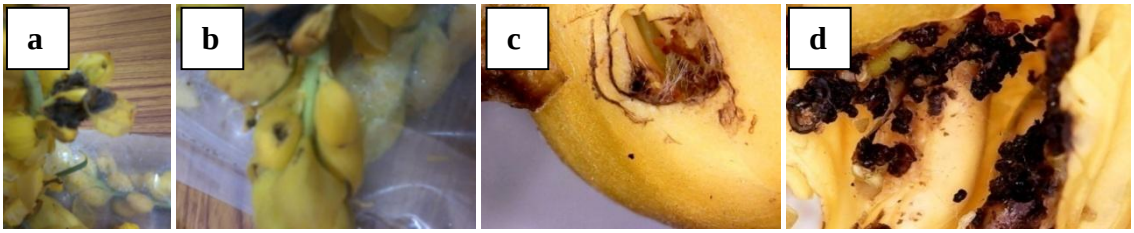

Fig. 4.Infected in Flower Bud A . Initial Infection by Aspergillus Sp. Infection On Hole Made By Bud Borer. C. Prominent Hole Inviting Infection D. Anthronose Destroying the Hole Flower.

Fig.5 Infection in Pods A. C.S of Healthy Pod ; B. C.S Of Infected Pod: C. Infection Starts from Stalk Base; D. Progress of Anthracnose.

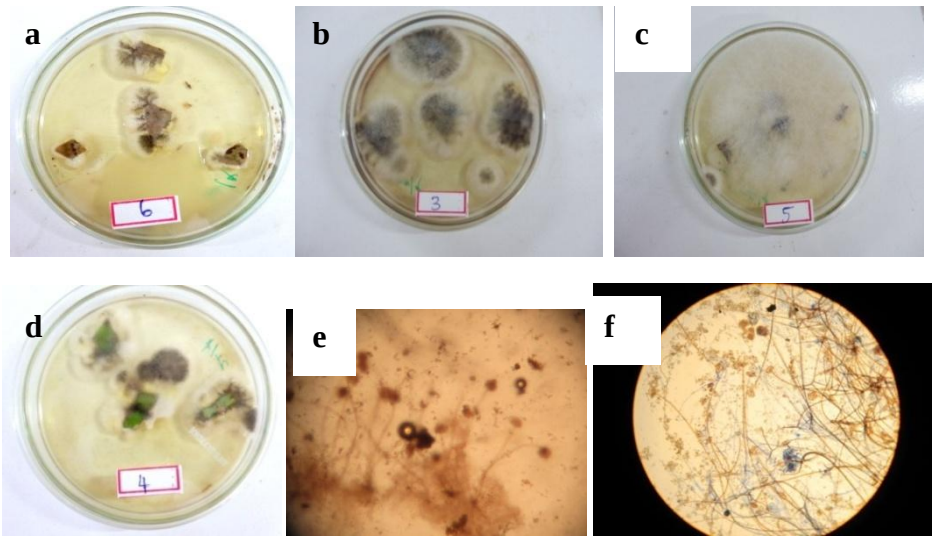

Fig.6.Fungal Pathogenic Fungi Isolated. A. Aureobasidium, B.Aspergillusc, E.Rhizopus D. Aspergillus With Aureobasidium,

IV. DISCUSSION

Any environmental factor that favours the growth of fungi is unfavorable for the growth of the plants and that leads to increases in infection affecting the normal function of the plant; in this persuit, present investigation is focused on occurrence, nature and seasonality of fungal diseases and their causative organisms in a medicinally/commercially important, Cassia alata coming up in the urban agglomeration. Totally five fungal pathogens viz. Aureobasidium, Rhizopus, Alternaria sp., Aspergilllus niger and A. fumigatus. have been isolated and identified from infected leaves, flower buds and pods of C. alata in the present investigation. It has been reported that Alternaria cassia causes foliar blight of several Cassia species in India and USA (Boyette, 1988; Wiehe (1953) have reported eight species of fungi belonging to eight genera associated with C. alata in which A. niger is also recorded in the present study. Flower bud infected with A. niger is seriously destroyed on the progress of the infection ending with the total destruction of the flower. It is also more evident from previous reports that phytotoxic effects of A. niger caused loss of yield in wheat (Singh, 1973) groundnut, sunflower and safflower (Sangeetha and Thangadurai, 2013). Further, it is learnt from the field and laboratory screening study, that the fungal species Alternaria cassia, a very common pathogen in Cassia species, causing foliar blight of several Cassia species in India and USA and serious seedling blight

of C. obtusifolia (sickle pod) and *C. occidentalis* (coffee senna). As given in (RPD No. 648/ July 1998), Alternaria causes small, circular to angular often zoned brown spots on the leaves that drops out. According to records of International Mycological Institution as quoted by Lenne, (1990) foliar flight and leaf sports are recorded in C. alata in which fungal species of Cercospora was reported; (Chupp, 1953) recorded Phaesorcropsis stimutae in C. alata from Columbia, USA. Therefore occurrence Alternaria sp. in C. alata and causing foliar destruction is quite obvious from the study. Prevalence of all these fungi during different seasons revealed that leaves of C. alata are encounted with fungal diseases viz. leaf spot leading to Anthracnose caused by the pathogenic fungal species viz. Rhizopus sp., Alternaria sp., and Aspergillus niger. Spots on the leaves of C. alata, are most often brownish and tan or black (Ebbels, and Allen 1979) similar to the present findings. Pataky et al (1998).

It is clearly illustrated in Fig 2a-c that small dot like holes in leaf formed due to Alternaria sp., slowly enlarges in size and succumb to much fungal growth resulting from a tanned appearance. It is also more clear from Fig.2 d-f that such the condition later ends with coalase leaving a hole on the lamina. Present observation is in conformity with reports of (Pataky et al 1998) that species of Alternaria fungus is the most common causative factor for leaf spot diseases in cassia species the same has been recorded from the infected plant part with leaf spots.

The second most prevalent disease noted in Cassia alata is Anthracnose condition in plants particularly in leaves and flower buds means a pathogenic condition of the host caused by more than one fungal species on a single microclimate i.e. in leaves and flower buds. All five species of fungi isolated and identified are considered to be the causative factor one way or other causing the pathogenic condition called anthracnose in leaves and flower buds in the present study. As explained by (RPD no.648) the spots varied in size and colour depending on the plant affected, the specific organism involved, and the stage of development. Infected plants have brown or black water-soaked spots on the foliage. The spots enlarge at a faster rate during wet conditions and the spots have a speckled appearance under dry climate. (Fig 2. a-c). Besides, such a tanned or brownish black markings on the veins on the ventral side are also noticed and weakens the strength of the leaves (Fig.2 c) and the deep and intensive tanning in older leaves might impair the vascular tissues of the leaves as recorded in the present study. It is also to be noted that mostly freshly formed terminal leaves and flower buds (Fig.3a) are heavily infected. Reports from Florida University confirms the present observation that the anthracnose attacked leaves turn into Tan to brown irregular shaped spots or blotches on leaves; often located close to leaf veins. Moreover, most of these diseases are caused due to cool weather, light and frequent rains, fog or heavy dews, high humidity, and crowded or shady plantings (Chupp, 1953). Such an environmental condition prevails in the Pondicherry region both during post monsoon and post summer. As Cassia alata being a bushy plant, and growing denser at its peak period, the shady and crowdedness also enhances the fungal infection.

The progress of anthracnose in infected leaves as well as in buds is quite evident from Fig.3 a-d. (leaves) and 4 a-d (bud). The infection is initiated by the Aspergillus sp. on the surface of the buds. The bored surface of the bud is succumbed to secondary infection by more than one fungus and ends with anthracnose. It is reasonably presumed that excreta of bud borer worm present in the bored bud might have facilitated the heavy fungal growth/infection as these excreta are rich in moisturized organic substances. It is also relevant to state that fungal infection is initiated by the folivore insects-the leaf-nibbling insects such as Helicoverpa sp. which are already reported among species of genus Cassia and other chewing type or sucking type of insects make a cut in the lamina or petal of the flower which facilitates the availability of nutrients oozing out of the cut ends and the humid climate favors the growth of fungal spores on that spot; further on progress of the fungal growth on the leaves and buds ends with diseases like leaf spot and anthracnose. But on closer examination on the fungal cultures, it is learnt that the bits of leaves sampled from the leaves browsed by caterpillar has not supported any fungus. Unlike the cut ends of leaves caused by the insects, these leaves have not shown any infection. It also is pointed out that these findings are also checked in the second trial and even in the second trial, no fungus has grown on the leaves left by caterpillars. This could be reasonably explained that mouth secretions of the caterpillar might have prevented the spore development on such leaves. To be precise, mouth secretion of the caterpillar might possess some bioactive property that would have inhibited fungal growth at the cut ends.

The pods of C. alata are also found to be infected by fungi. Fig 5a-d clearly show fungal attack even in the young pods. The infection starts from the base of the stalk and slowly permeate into the developing seeds and destroy the growing pods. It is also to be mentioned that even the bore formed by the pod borers also speed up the pathogenicity. The infection starts from the base of the stalk and slowly permeate into the developing seeds and destroy the growing pods. It is also to be mentioned that even the bore formed by the pod borers also speed up the pathogenicity.

Therefore, from the present study, it is reported that leaf spot and anthracnose are the common fungal diseases found in the leaves of Cassia alata caused by pathogenic fungi viz. Aureobasidium, Rhizopus, Alternaria sp.,

Aspergilllus niger and A. fumigatus. It is also to be noted that studies on these pathogenic fungal species and their infection in Cassia alata under tropical climate, is not done so far and hence presently this study contributes additional scientific information in fungal infection in Cassia alata and its seasonality. The data on the prevalence fungal diseases and their seasonality in addition to already existing data (Ebbels and Allen 1979 & Shamsi et al., (2014). are useful for further studies in disease management. The outcome finding from the study is more useful in understanding the type and gravity of fungal attack and its seasonality and would help to collect quality leaves of *Cassia alata* from the study area during summer and pre monsoon so as to get better quality leaves. Secondly, it is emphasized that the observation made with regard to fungal infection in cut ends of insects and caterpillar, is the point to ponder in terms of the bioactive property of mouth secretions of caterpillar feeding on leaves of *Cassia alata*; this interesting finding opens up new vistas in the field of bio-activity and bio-control.

V. CONCLUSIONS

The present study highlights existing scenario on pathogenic fungal species and adds field information pertaining to medicinally/commercially important leaves of *Cassia alata* and seasonality**,** helps to collect quality leaves to fetch the better price for the leaves. The study has recorded in detail the progress of leaf spot and Anthracnose in leaves -stage by stage with photographs which is considered as an important input for commercial cultivators in disease management. First time reported in the *Cassia alata* Anthracnose leaf spot disease in the tropical condition in the coromandel Coast of India. The uniqueness/ highlight of this work includes one interesting observation that the cut ends of the leaves left by the caterpillars are not infected with fungal species even under ambient climate. This opens up the new avenue in the field of
bioactive compounds from caterpillars particularly of the migrant butterfly, *Catopsilia pyranthe.* The outcome of the study highlights the fungal species and their seasonality which would help commercial leaf-collector to get quality leaves and better prize**.**

ACKNOWLEDGMENT

We thank Dr. A. Yogamoorthy, Dr. S M. Sundarapandian, Department of Ecology and Environmental Sciences, Pondicherry University for their timely help. We thank the Pondicherry University and University Grants Commission for providing fellowship during the study period**.**

REFERENCES

[1] Boyette, C. D. Biocontrol of three leguminous weed species with Alternaria cassiae. Weed Technology, 414-417. 1988.

[2] Brown, L. G., and Morgan-Jones, G. Notes on Hyphomycetes. XX. "Cercospora–complex" fungi of Cassia and Psoralea. Mycotaxon, 6; (2), 261-276. 1977.

[3] Chupp, C. Monographs of the genus *Cercospora*. (Ithaca Press; New York. 1953

[4] Cock, M. J. W., and Evans, H. C. Possibilities for biological control of *Cassia tora* and *C. obtusifolia*. International Journal of Pest Management, 30;(4), 339-350. 1984.

[5] Commonwealth Mycological Institute, Kew Surrey, England. pp. 696.

[6] Deshpande, K. S., and Kulkarni, G. M. Metabolites of seed-borne fungi in relation to the viability of some oil seeds. Indian Botanical Reporter, 9; 1, 20-21. 1990.

[7] Ebbels, D. L., and Allen, D. J. A supplementary and annotated list of plant diseases, pathogens and associated fungi in Tanzania, Commonwealth Mycological Institute. 1979.

[8] Farr, D. F., Bills, G. F., Chamuris, G. P., and Rossman, A. Y. Fungi on plants and plant products in the United States. APS press. 1989.

[9] Gudauskas, R. T., Teem, D. H., and Morgan-Jones, G. Anthracnose of *Cassia occidentalis* caused by *Colletotrichum dematium* f. truncata. Plant Disease Reporter, 61 (6), 468-470. 1977.

[10] Howard, C. M., and Albregts, E. E.. *Cassia obtusifolia*, a possible reservoir for inoculum of *Colletotrichum fragariae*. Phytopathology, *63*(4), 533-534. 1973

[11] Khan, S. A., and Kamal, M.. The fungi of South West Pakistan. Part 1. Pak. J. Sci. & Ind. Res, 11, 61-80. 1968

[12] Kheng, K. T. New plant disease records for Sarawak for 1973 and 1974. Sawawak Museum Journal Malaysia. 24; 45, 217-225. 1976.

[13] Leather, R. I. A catalogue of some plant diseases and fungi in Jamaica. Bulletin, Ministry of Agriculture and Lands, Jamaica, 61. 1967.

[14] Lenne, J. M. Diseases of Cassia species—a review. Tropical Grasslands, *24*;(4), 311-324. 1990.

[15] Pataky, N. R. Fungal leaf spot diseases of shade and ornamental trees in the midwest. *Plant Disease*, *648*, 1-8.

[16] Rangaswami, G., Seshadri, V. S., and Channamma, K. L. Fungi of south India. 1970.

[17] Report on *plant disease* RPD no. 648/july Department of Crop Sciences, University of Illinois USA. 1998.

[18] Rhind, D., and Seth, L. N. The fungi of Burma. The Indian Journal of Agricultural Science, 15, 142-155. 1945.

[19] Sangeetha, J., and Thangadurai, D. Identification Key for the Major Growth Forms of Lichenized Fungi. In Laboratory Protocols in Fungal Biology (pp. 91-112). Springer New York. 2013.

[20] Shamsi, S., Chowdhury, P., and Naher, N. Mycoflora associated with the leaves of *Senna alata* (L.) Roxb. Journal of Bangladesh Academy of Sciences, 37, (2), 249-252. 2014.

[21] Simmonds, J. H. Host index of plant diseases in Queensland. Host index of plant diseases in Queensland. 1966.

[22] Singh, K. G. A Check-list of host and diseases in Peninsular Malaysia. Ministry of Agriculture and Fisheries Malaysia. Bulletin, 132. 1973.

[23] Wiehe, P. O. The plant diseases and fungi recorded from Mauritius. Common wealth Mycological Institute. 1948.