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ORIGINAL ARTICLE

OBSERVATION OR IDENTIFICATION OF MAJOR FUNGAL FOLIAR DISEASE AND ISOLATION OF PATHOGEN IN THREE MEDICINAL PLANT GARDENS IN THREE DIFFERENT DISTRICTS OF WEST BENGAL

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Abstract: *Plants are very good source of different nutraceuticals. Different parts of the plants are either used directly as medicine or as source of active principles obtained from them. Any microbial attack on them leads to crop loss or interfere with their chemical constituents. In West Bengal medicinal plants are widely used in folk culture, due to humidity and other environmental factors plants become very prone to fungal attack. Foliar diseases are mostly visible through naked eyes, making it easier to study the symptoms. Colletotrichum sp and Alternaria sp were predominant fungus isolated from infected leaves in those months of survey (August, 2020 to March, 2021).*
Keywords: *Colletrotrichum*, conidia. *Rauwolfia*, leaf spot.

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1. INTRODUCTION

In the ancient period, Atharva Veda (Around 1200 BC), the oldest literary monument of Indian medicine, is believed to be the origin of Ayurveda, the Indian science of medicine summarized traditional medical science based on medicinal plants focusing on 600 herbs. Currently, more than 80,000 plants are used for therapeutic purposes. The plant biodiversity of India is one of the largest biodiversity systems in the world. Different plants are grown in different habitats with different therapeutic activities. In India, folk communities are mostly dependent upon and have a rich knowledge on medicinal plants for their therapeutic properties. Plants provide various kinds of therapeutic agents that are used for the treatment of a wide range of diseases such as cancer, diabetes, malaria, inflammation, obesity, cardiovascular [1], and microbial infections [2]. In traditional system of medicine to modern medicine, neutraceuticals, food supplements, pharmaceutical intermediates and chemical entities for synthetic drugs plants serves as the richest resource [3]. They are the source of essential oils, alkaloids, flavonoids, tannin, saponins and antimicrobial agents. Fungal pathogens are very potent to invade them and interfere with their active principles by producing mycotoxins [4]. WHO (World Health Organization) in 1979 reported mycotoxins are hazardous to human and animal health [5]. We surveyed three different districts which are situated on the both banks of river Ganges. They have almost same altitude, soil type and rainfall pattern. In this study we attempted to document the fungal foliar diseases of some medicinally important plants, their symptoms and causal organisms.

2. MATERIALS AND METHOD

Site of sample collection and symptom study

An extensive survey was carried out during post monsoon season (i.e. August, 2020 to March, 2021) around three different districts (Hooghly, Howrah and Nadia) of West Bengal. The survey was conducted in Serampore college medicinal plant garden in Hooghly, State pharmacopeia laboratory and pharmacy for medicine and medicinal garden in Nadia and Charak Udyan and its surrounding of Acharya Jagadish Chandra Bose Indian Botanic Garden in Howrah. The survey was conducted to collect data on occurrence of fungal disease on different plants having medicinal properties and causal organisms. Infected leaves are collected in a sterilized zipper bag and carried to the laboratory to study symptoms under the simple microscope.

Table1: Surveyed location with elevation, annual rainfall and soil type

Area	Latitude and Longitude	Altitude	Annual Rainfall	Soil Type
Serampore College medicinal garden, Serampore, West Bengal	$22^0$75'19" N 88° 35' 18" E	17 m	1359.7 mm	New alluvial plain
State Pharmacopoeial Laboratory and Pharmacy, Kalyani, West Bengal	$22^0$97'47" N $88^0$43'37" E	17 m	1259.4 mm	New alluvial plain
Acharya Jagadish Chandra Bose Indian Botanic Garden, Howrah, West Bengal	22^0 55' 6'' N 88^0 28' 6'' E	16 m	1466.2 mm	New alluvial plain

Isolation of pathogen

Collected leaf samples are thoroughly washed under running tap water to remove excess dirt from the surface. From the collected leaf samples diseased parts are incised into small pieces and dipped into sterile distilled water. Then dipped in 4% Na-hypochlorite (NaOCl) solution for one minute for surface sterilization and again washed with sterile distilled water for three times. The incised leaves were then blotted between two sterile blotting papers and transferred it to potato dextrose agar (PDA) containing Petri plates aseptically with the help of an incinerated inoculating needle. Each plate was incubated in BOD at (25±2° C) for 7 days. After 7 days of mycelial growth the isolation and purification was done by transferring it to a fresh PDA slant by single hyphal tip method. The whole process was done aseptically in a Laminar Air Flow (LAF).

Characterization and Identification

Phenotypical characterization and identification of the isolated pathogen was done with the help of Nagamani *et al.* (2006) [6].

Table 2: Surveyed area along with plants having fungal foliar disease symptoms during post monsoon season (August, 2020 – March, 2021)

Area	Plant sample	Total no. of selected plants (A)	No. of affected plants (B)
Serampore	*Cymbopogon citratus* (DC.) Staph.	40	35
Serampore	*Cinnamomum tamala* (Buch.-Ham.) Th. G.G. Nees.	1	1
Serampore	*Holarrhena antidysenterica* (L.) Wall. ex A. DC.	2	2
Serampore	*Andrographis paniculata* (Burm.f.) Wall. ex Nees.	15	8
Serampore	*Rouwolfia serpentina* (L.) Benth. ex Kurz.	8	4
Serampore	*Ocimum sanctum* L.	11	7
Kalyani	*Ocimum sanctum* L.	32	9
Kalyani	*Mentha arvensis* L.	23	23
Kalyani	*Rouwolfia serpentina* (L.) Benth. ex Kurz.	20	18
Howrah	*Andrographis paniculata* (Burm.f.) Wall. ex Nees	15	4
Howrah	*Ocimum sanctum* L.	4	4

Study of the symptoms

In *Cymbopogon citratus* (DC.) Staph: Purple spot appeared on the leaf sheath and lamina. Gradually two spots colease with each other and form big spots. Tip of the leaves became dry and turns grey to black in colour. Leaf starts to dry from the apex and finally falls off (Figure 1A and 1B).

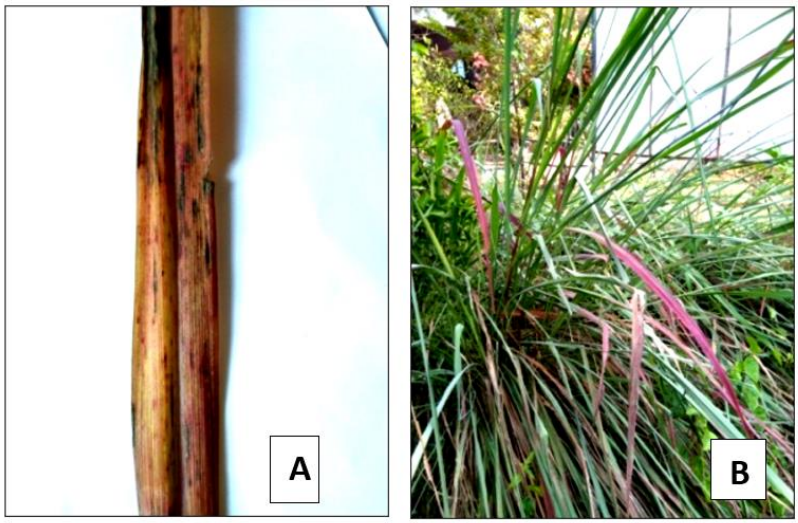

Figure 1: (A) A single leaf lamina and (B.) whole plant of *Cymbopogon citratus* showing disease symptom.

In *Cinnamomum tamala* (Buch.-Ham.) Th. G.G. Nees.: Dark brown spots appeared at the apex of the leaf at first and gradually towards the middle of the leaf. Infected zone appears as dry and ultimately become brittle. Lesions coalesce to form irregular shaped brown patches. Lesion drops out of leaves leaving a hole. Usually, the healthy leaves are dark green in colour but become light green with yellow zone when brown spots appear (Figure 2A and 2B).

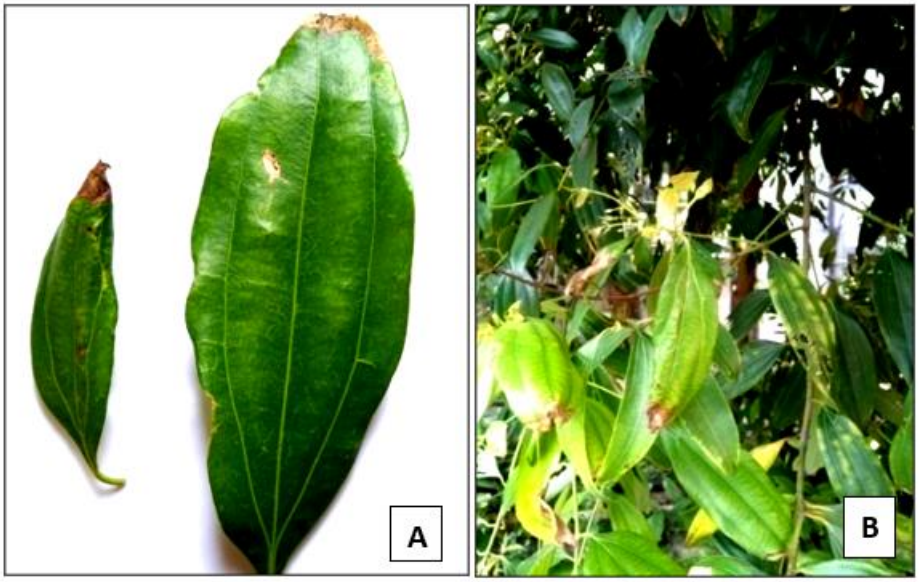

Figure 2: A branch bearing diseased leaf (A.) and solitary leaf (B.) of *Cinnamomum tamala* showing symptom

In *Holarrhena antidysenterica* (L.) Wall. ex A. DC.: Brownish and reddish-brown spots are scattered all over the lamina. Spots are roughly 1mm in diameter or even small. Leaves became slightly dry. Spots are abundant towards apex. Highly infected leaves became very dry and finally fall off (Figure 3A, 3B).

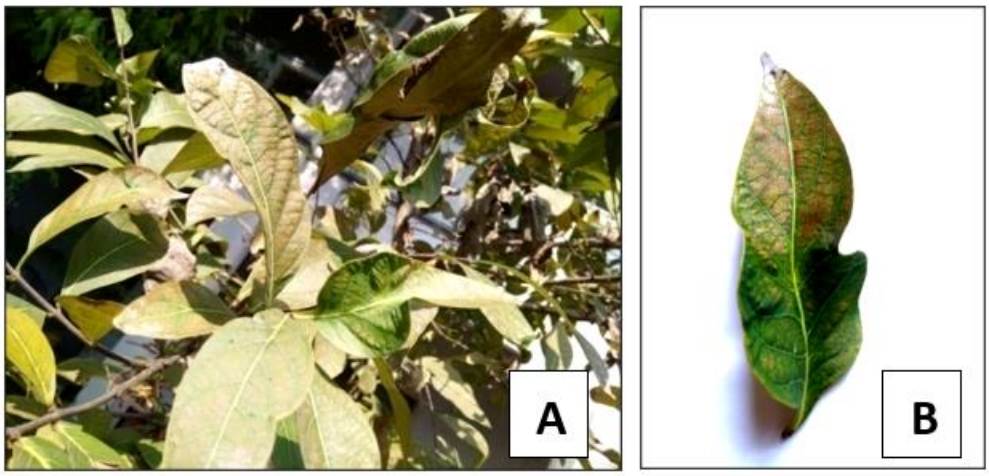

Figure 3: A bunch of leaves (A) and a single leaf (B) of *Holarrhena antidysenterica* showing symptom.

In *Andrographis paniculata* (Burm.f.) Wall. ex Nees.: Leaves contain dark black lesions without definite border. Spots appear along with the margin and the apex. As symptoms progresses whole leaf turned black and finally falls off. Ventral side of the leaf shows dotted brown spots in addition with totally dark necrotic area (Figure 4A, 4B).

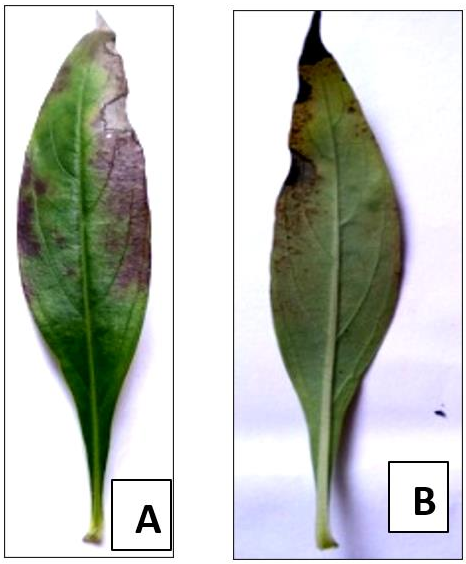

Figure 4: Dorsal (A) and ventral (B) side of leaf of *Andrographis paniculata* showing symptom.

In *Rouwolfia serpentina* (L.) Benth. Ex Kurz.: Dark brown irregular spots appear on the leaf lamina. At first spots are small, circular but eventually it merges with neighbouring spots. Symptoms appear as necrotic. Spots are 1-2 mm in diameter. Leaves turn yellow with time and finally fall off (Figure 5).

Figure 5: A single leaf of *Rouwolfia serpentina* showing symptom

In *Mentha arvensis L.:* Infection appears in the form of irregular dark brown spots often surrounded by a yellow margin on the dorsal side of leaves. Affected leaves gradually become yellowish, dry and finally fall off. Leaves from the bottom side of the plant affected first gradually it progresses further upward ultimately leading to heavy defoliation (Figure 6).

Figure 6: A single leaf of *Mentha arvensis* showing symptom

In *Ocimum sanctum* L.: Small dark brown spots on the upper surface of the leaves. These enlarge to prominent dark brown circular lesions. Diseased leaves finally turn brown and falls off (Figure 7).

Figure 7: *Ocimum sanctum* leaves showing symptom

3. RESULTS

Isolated pathogens and their morphological and microscopic characteristic

Among the isolated pathogens from leaves 3 out of 5 pathogens are morphologically and microscopically similar and appear to be same. Culture plates of isolated fungal pathogens from infected leaves of *Cinnamomum tamala*, *Holarrhena antidysenterica, Andrographis paniculata* and *Rouwolfia serpentina* shows relatively low growth rate. PDA plates show growth rate of almost 2 mm per day. Colony appears to be dense white in colour gradually turns into dark grey to black (Figure 8A). Hyphae are hyaline and compact or loose under light microscope. Conidia present (Figure 8C.). Conidia were single celled and ovoid to oblong with rounded apices. They vary in size, 10-15µm in length and 5-7 µm in breadth. Acervuli present. On the basis of phenotypical characters, the pathogen was identified as *Colletotrichum sp*.

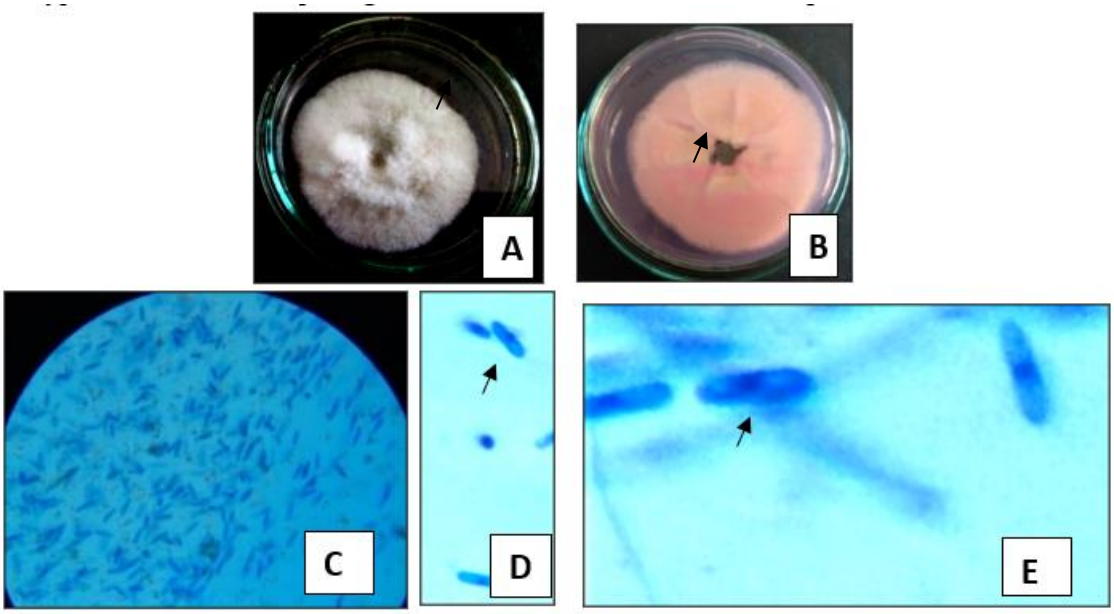

Figure 8: (A) Single colony. (B) Reverse plate. (C, D, E) Conidia of *Colletotrichum sp*.

From infected *Cymbopogon citrates* PDA plate shows another type of fungal colony which differs from previous. In PDA plates the fungal colony grows 2-3mm/day, fast growing. Initially whitish gradually turned little dark to grey (Figure 9A, 9B). Hyphae septate, conidiophores bearing conidia, brownish, unbranched;conidia septate, 3septa present, elongated with tapering end, cylindrical, smooth and slightly curved with rounded end, arise in group, dark brown in colour (Figure 9C). Varied in length and breadth. 26-31µm in length and 11-13µm in breadth. On the basis of phenotypical characters the pathogen was identified as *Curvularia sp.*

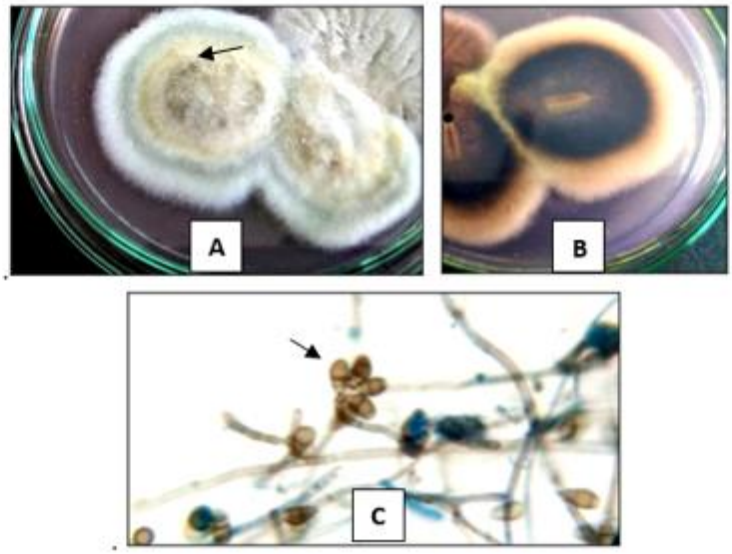

Figure 9: (A) colony on culture plate. (B) reverse plate. (C) Conidiophore bearing conidia of *Curvularia sp.*

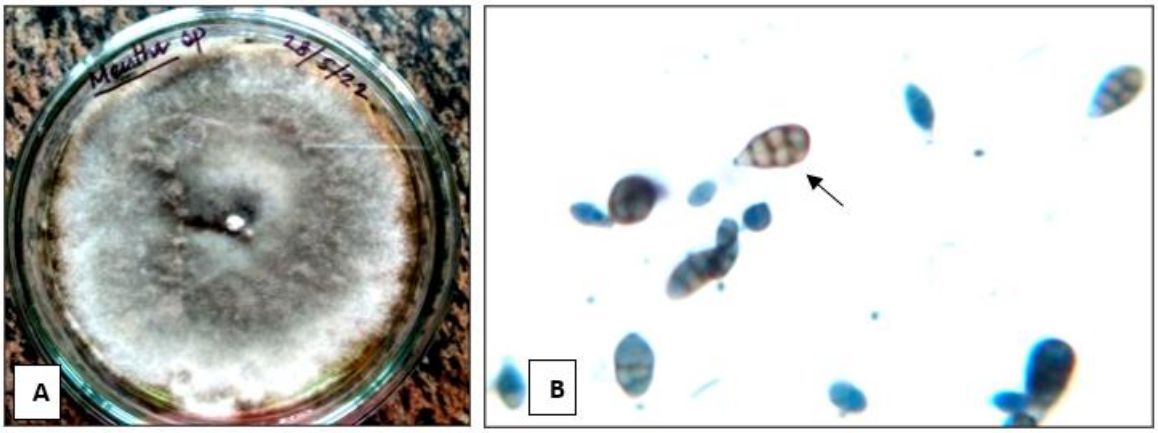

Figure 10. A: plate showing fungal colony. B: Microscopic view of single spore of *Alternaria alternata*

In *Mentha sp* plate, the colony is white at first gradually become dark grey, compact and cotony (Figure 10 A). Reverse PDA plate black, conidia present in chains. Conidia ellipsoid, septa present, 4-5 horizontal and 1-3 longitudinal septa present (Figure 10B). on the basis of phenotypical characters the pathogen was identified as *Alternaria alternata.*

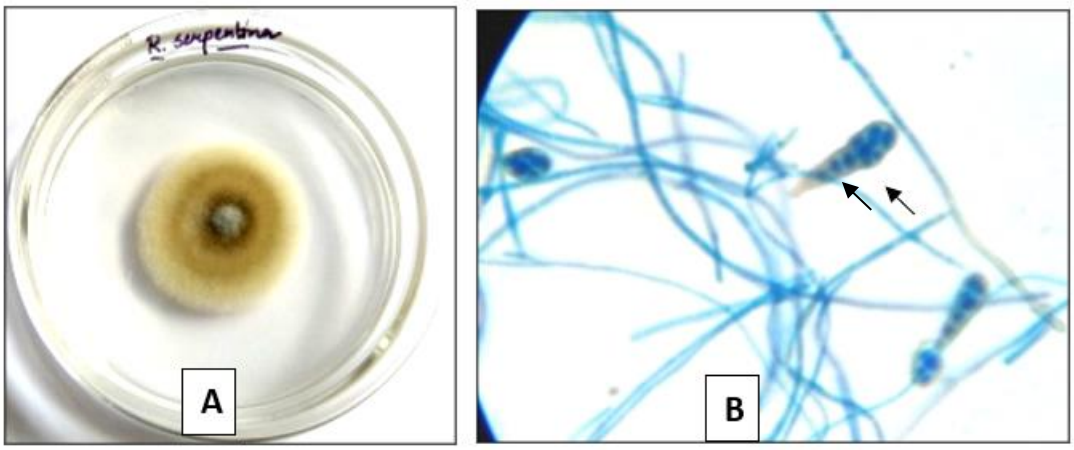

Figure 11. A: Plate showing fungal colony. B: Microscopic view of single spore of *Alternaria* sp

Another *Rouwolfia serpentina* plates shows relatively low growth rate which is different from other plate of the same. In this plate the colony is very compact. At first stage of growth the colony was white but gradually it turns brownish to black having concentric rings (Figure 11A). Conidia elongated having a stalk like structure at the base and head is swollen, colour dark brown, horizontal septa present (Figure 11B). On the basis of phenotypical characters the pathogen was identified as *Alternaria* sp.

4. DISCUSSION

The symptoms and pathogens we found are more or less same on the four plants. There are previous reports on these disease. The symptom which we found on *Rouwolfia serpentina* was similar to the work of Varadarajan [7] who first reported anthacnose disease, Ghosh and Chakraborty [8] and Ghosh and Banerjee [9]. Another report suggested that *Cercospora rauwolfiae* cause leaf spot disease in *R. serpentina* and *Alternaria alternata* is a minor seedborne pathogen in Bhadra Wildlife Sanctuary during 2006–2009 [10]. Leaf blight disease caused by

Macrophomina phaseolina in *R. serpentina* was previously reported [11] . Thakur *et al.* (1974) [12] reported *Curvularia* leaf spot in *R. serpentina* in Jammu and Kashmir. Although in West Bengal Ghosh and Chakraborty (2012) [8] identified anthracnose disease by *Colletotrichum gloeosporioides* and tried to control the disease by biological agents. Koike and Molinar [13] reported a rust disease in *Cymbopogon citratus* by *Puccinia nakanishikii*. Bhat *et al.* (1988) [14] reported a partial drying of *Cinnamomum zeylanica* seedling by *Colletotrichum gloeosporides*. Prakasham [15] observed elongated spots at the margin and finally defoliation caused by *Phytophthora capsici*. In later stage this caused shot hole symptoms in India. *Cinnamomum verum* leaves showed black spots caused by *Colletotrichum gloeosporoides* in Taiwan [16]. In *Andrographis paniculata* different diseases were repoted previously. In 2016, it was reported that wart diseses caused by *Synchytrium lepidagathidis* in tendar aerial parts of the plant and inflorescense, leaf web blight by *Rhizoctonia solani*, leaf spot by *Cercospora sp* [17]. A thorough study was done on medicinal plants of Vellore district in Tamil Nadu, India and found different fungal disease such as foot rot disease by *Fusarium moniliforme*, blight disease by *Alternaria alternata*, leaf spot by *Colletotrichum gloeosporoides, Curvularia lunata, Curvularia penneseti, Cercospora adhatodae* [18].

5. CONCLUSION

From the results we can conclude that during our survey we found *Colletotrichum sp* was the predominant fungus. And at that time (August – March) leaf spot disease was dominant disease. *Colletotrichum* affects wide range of medicinal plants as well as fruit bearing plants. The weather condition such as temperature, humidity, rainfall of tropical region is very perfect for growth of the fungus and to attack any plant to cause foliar disease.

6. ACKNOWLEDGEMENT

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