Journal Homepage: -www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI:10.21474/IJAR01/8402
DOI URL: http://dx.doi.org/10.21474/IJAR01/8402
ISSN NO. 2320-5407

RESEARCH ARTICLE

DIVERSITY OF PHYLLOPLANE MYCOFLORA OF SOME MEDICINAL PLANTS OF MAHE, U. T. OF PUDUCHERRY.

Mari Bhat M., Rashna K. and Subramaniam S.
P. G. Department of Botany, Mahatma Gandhi Govt. Arts College, Mahe, U.T. of Puducherry, India.

Manuscript Info

Manuscript History
Received: 12 November 2018
Final Accepted: 14 December 2018
Published: January 2019

Key words:-
Incidence, phylloplane, mycoflora, medicinal plants.

Abstract

Leaves constitute the major part of exposed plant surface. They are open to infestation or sporotropic colonization by air dispersed or splash dispersed mycoflora. The leaves provide unique environment to their surface occupants. The topography of the leaf surface, the micro-climate around the leaves and the typical leaf exudate influence the growth and development of varieties of leaf surface micro-organisms and in turn determine the vitality of the plant concerned. In this context survey on the occurrence of phylloplane fungi on leaf surfaces of five important medicinal plants viz; Azadirachta indica, Centella asiatica, Justicia adhatoda, Ocimum tenuiflorum and Plectranthus amboinicus in Mahe, U. T. of Puducherry was carried out. The survey showed altogether 18 fungal species under 13 genera of which Aspergillus had the highest mean incidence followed by Cercospora, Cladosporium, Curvularia and Diplococcium.

Introduction:-

Plants have been used for medicinal purposes since prehistoric period. They have played a critical role in maintaining human health and civilizing the quality of human life for thousands of years. Treatment with medicinal plant is considered to be very safe and there is less or no side effects because these remedies are linked with nature and they contain components of therapeutic value. Numerous phytochemicals are extracted from medicinal plants. Various parts of these plants are used in treatment of various diseases and disorders. Sometimes the whole plant may be used in the treatment of certain diseases. The medicinal plants might be used either directly or after processing. Economic importance of medicinal plants are higher in countries like India and China. These countries provide two third of plants in traditional and modern system of medicine. In India large number of plants are used in primary health care and they have got various traditional uses. They include Azadirachta indica, Curcuma longa, Leucas aspera, Ocimum tenuiflorum, Phyllanthus amarus, Tinospora cordifolia etc. These medicinal plants are destructed by various microorganisms, which infects roots, stems, leaves, flowers and fruits. Large number of microorganisms inhabit the phyllosphere of plants. While a few microbial species can be isolated from within the plant tissues and many microbes are recovered from the surface of healthy plants.

Phyllosphere is the immediate vicinity of leaf surface, where microbial communities are constantly in dynamic state due to exo and endogenous sources of nutrients. The environment of phyllosphere includes physical, chemical and biological components occupying the surrounding space. Leaf is the main photosynthetic and exposed plant part. It is easily colonized by a variety of microorganisms. The leaf surface is usually known as phylloplane. It is a complex, terrestrial habitat characterized by variety of microorganisms including bacteria, filamentous fungi, yeasts,

Corresponding Author:- Dr. Mari Bhat M.
Address:-P. G. Department of Botany, Mahatma Gandhi Govt. Arts College, Mahe, U.T. of Puducherry, India.

algae and less frequently protozoa and nematodes. Filamentous fungi are considered transient inhabitants of leaf surface. The phylloplane microorganisms may play a vital role with regard to the host plant. They influence the growth of their host plants either negatively as pathogens or positively by increasing stress tolerance and disease resistance. The leaf surface is the suitable environment for microbial growth, because there is a thin film of nutrients deposited on the leaf. The growth and development of leaf surface microorganisms depends on the external and internal factors such as topography of the leaf surface, the micro-climate around the leaf, leaf age, temperature, humidity, moisture content, aeration, light, presence of inhibitors, nutrient availability and typical leaf exudate.

In the recent years, researchers have participated in the study of phylloplane mycoflora of various plants. Lot of investigations have been carried out on the phylloplane flora of leaf surfaces of plants, growing in garden or cultivated in many parts of the world by several researchers. Shamim Shamsi and Razia Sulthana (2014) isolated Alternaria alternata, Arthrinium saccharicola, Aspergillus flavus, Curvularia brachyspora and Monilia species from the herbal medicinal plants. Puja Gawas Sakhalkar and Bhat (2010) examined the mycoflora associated with the medicinal plant, Aegle marmelos of the forests of Western Ghats and reported species in the genera such as Aspergillus, Cladosporium and Penicillium. Prabakharan et al., (2011) reported the occurrence of phylloplane fungi on the leaf surface of three important medicinal plants such as Ocimum sanctum, Phyllanthus amarus and Azadirachta indica. Aspergillus flavus, Penicillium expansum, Fusarium semitectum, Fusarium oxysporum were isolated from Ocimum sanctum. Scrophulariopsis species was isolated from phylloplane of Phyllanthus amarus. Penicillium janthinellum, Aspergillus fumiculosis, Aspergillus species, Curvularia lunta and Fusarium moniliforme were isolated from the phylloplane of Azadirachta indica. Studies of Jalander and Gachande (2012) on phylloplane mycoflora of medicinal plants like Ocimum, Withania, Catharanthus and Azadirachta shows dominance of fungal species belonging to the genera Aspergillus and Penicillium. Aspergillus, Curvularia and Fusarium are most common fungal genera on phylloplane flora of some medicinal plants of Ficaceae family. Phylloplane is highly sensitive to environmental factors (Dalal, 2014). Studies of leaf surface mycoflora of some plants like Hibiscus rosa-sinensis, Annona squamosa, Bryophyllum pinnata and Croton species showed the dominance of Fusarium sp., Curvularia sp. and Aspergillus niger. The phylloplane fungal taxa associated with Tinospora cordifolia include Alternaria alternata, Aspergillus niger, Cladosporium herbarum and Penicillium chrysogenum (Nayak, 2015). Yadav (2015) isolated four fungal genera from Ocimum santum. Among them Aspergillus, Mucor and Rhizopus were isolated from the phylloplane; Aspergillus and Fusarium were isolated from phyllosphere. Angela and Usha Shri (2016) reported 10 fungal taxa from four different medicinal plants and isolate were Alternaria alternata, Yeast, Cunninyhamella species and Mucor from Hibiscus rosa-sinensis; Aspergillus flavus and Syncephalastrum racemosum from Rauwolfia serpentina; Trichoderma species and Penicillium species from Datura alba; Cladosporium species and Curvularia lunata from Curcuma longa. Studies of Shikha Thakur (2017) shows Aspergillus niger, Trichoderma harzianum, Penicillium frequentans were common fungal taxa in Rauwolfia serpentina.

Variety of phylloplane and endophytic fungi is used to imitate as one of the rich source of novel compounds of biological accomplishments and have a high level of organizational diversity on the leaf surfaces. Bioactive composites produced by these phylloplane and endophytes have shown inspiring potentiality towards therapeutic medicine. The biodiversity of phylloplane and endophytic fungi from different aged leaves of medicinal mangrove plant Avicennia marina from Puducherry was studied by Nayak and Anandhu (2017). Evueh (2008) reported phylloplane fungi as biocontrol agent against Colletotricum leaf disease. Phytophthora capsici was isolated from vegetable crops belonging to the families Cucurbitaceae, Solanaceae and Fabaceae (Hausbeck and Enzenbacher 2008). FatemaYeasmin and Shamim Shamsi (2013) examined the phylloplane mycoflora of Gerbera species and their pathogenic potentiality. Trichoderma, Phoma, Fusarium, Aspergillus niger and Curvularia are isolated from Mussaenda frondosa, Ixora coccinea and Bougainvillea spectabillis by Dalal (2014). Nayak (2015) isolated altogether 17 phylloplane and endophytic fungal species from the ornamental plant, Mangifera indica and he identified the dominance of Aspergillus niger and Trichoderma species. Mari Bhat and Anusree (2015) isolated 15 fungal species from some vegetable crops of Mahe, U. T. of Puducherry.The newly expanded leaves are infested by bacteria while yeast and filamentous fungi dominate later in growing season, Taxa belonging to filamentous Ascomycetes, Zygomycetes, Basidiomycetes and Deuteromycetes have been recorded on the phylloplane of different plants (Dickinson, 1976). Phylloplane is usually open to infestation or sporotropic colonization by mycoflora. The phylloplane fungi are mycota growing on leaf surface. There are two groups of fungi – residents and casuals. Residents can multiply on the surface of healthy leaves without noticeably affecting the host. Whereas casuals land on the leaf surface but cannot grow (Prabakaran et al., 2011). The fungi are the group of organisms having a great biodiversity and they are the largest group of microbes of typical ecosystems throughout the world (Nayak, 2015). The fungal spoilage of medicinal plant is responsible for the significant loss in their medicinal

properties. Therefore enumeration and correct identification of phylloplane fungi on medicinal plants is very important in alleviating the loss due to bio deterioration and also to reduce the exposure of human and animal life to toxic substances. In the present study which is botanical in nature, detailed phylloplane mycoflora of five different important medicinal plants were conducted in Mahe during the year 2018.

Modern plant pathology has made significant contribution to the study of various diseases of plants. Impact of fungi on leaves is to reduce the medicinal property of the plants. Relatively very less information related to fungi associated with medicinal plants is available and therefore, the data procured through the systemic studies of phylloplane mycoflora of medicinal plants help in formulating the preventive measure and check the phylloplane fungal diseases. With this background the objectives of the present study were to: 1) Isolate and identify the phylloplane fungi associated with the different medicinal plants, 2) to conduct a qualitative and quantitative study of the naturally occurring phylloplane mycoflora and 3) to compare the phylloplane fungi grown in two different culture methods.

Materials And Methods:-

Sample collection:-

Fresh leaves of different different developmental stages of Azadirachta indica, Adhatoda vasica, Centella asiatica, Ocimum tenuiflorum and Plectranthus amboinicus were used in this study because they come in abundant source, easily available and they are utilized in traditional medicine. Random samples of different leaves were collected from five different regions of Mahe viz., Chalakkara, Cherukallayi, Manjakkal (Mahe), Palloor and Pandakkal and placed in sterile polythene bags and carefully brought to the laboratory. A composite sample of each variety was prepared and the phylloplane mycoflora was isolated by using Blotter paper technique (BPT) and Potato dextrose agar plate method (PDA). Five different types of medicinal plants which are common were selected for the investigation viz., Azadirachta indica, Centella asiatica, Justicia adhatoda, Ocimum tenuiflorum and Plectranthus amboinicus.

Sterilization of glass wares:-

Standard methods were followed to sterilize the glass wares. All the glasswares were thoroughly washed with detergent, rinsed several times with tap water followed by distilled water, then dried and sterilized in hot air oven at 160^0C for 2 hours. The media used were sterilized by autoclaving at 121^0C for 15 minutes under 15 lbs pressure.

Mycoflora detection on phylloplane:-

The phylloplane mycoflora of medicinal plants were detected by using two methods viz., Blotter paper technique (BPT) and Potato dextrose agar plate method (PDA).

Blotter paper technique (BPT):-

The blotter paper technique was developed by Doyer in 1938. In this method petri plates of size 90 mm were wrapped in brown paper. Simultaneously the Whatman's number 1 filter paper was also wrapped in brown paper for sterilization. Petri plates and blotter paper were sterilized in autoclave at 15 lbs pressure for 20 minutes. After sterilization, sterile blotter papers were kept in the petri plates and moistened with sterile distilled water in front of the laminar air flow. Each leaf sample was taken in separate petri plates. The leaf fragment of 1 cm was cut out using sterile scissor. 10 pieces of leaf samples of each variety were placed in different petri plates and were incubated at 25 ± 2^0C. After 7 days of incubation different colonies were developed on leaves that were observed with the help of microscope.

Potato Dextrose Agar Plate (PDA) method:-

Preparation of Potato dextrose agar medium was prepared by combining peeled potato (200g), dextrose (20g), agar (15g) and distilled water (100ml). The potato tubers were peeled and weighed for about 200g. The tubers were chopped into small pieces with the help of sterile knife. The chopped potatoes were transferred into a conical flask containing about 1000ml of distilled water. The content was boiled for 20 minutes. The supernatant was decanted and filtered with muslin cloth and the filtrate was collected. 20g of dextrose and 15g of agar were transferred into the extract and swirled to dissolve the ingredients. The medium was made up to 1 litre by the addition of distilled water. Antibiotic Chloramphenicol (150mg/L) was added to the medium to control the bacterial growth. The pH of the medium was adjusted to 5.6. Finally the medium was cotton plugged and autoclaved at 121^oC for 15 minutes. After preparation of PDA media, it was poured into sterile petri plates. Then it is allowed to cool at room temperature.

Isolation of Fungi:-
Leaf samples of Azadirachta indica, Centella asiatica, Justicia adhatoda, Ocimum tenuiflorum and Plectranthus amboinicus were used for making inoculum. Fragment of 1cm of leaf blade was cut out from the basal part of the leaf using sterile scissor. 10 pieces of each leaf variety was collected from different locations cut out and shaken in flasks filled with 100ml of distilled water. From this suspension of microorganisms 1ml was transferred into petri plates containing PDA medium. The inoculum was spread well in the media plates.

Incubation for the growth of Fungi:-
All the petri plates with the inoculum were kept undisturbed in dust free chamber at room temperature for 3-5 days of incubation period. The fungal colonies were observed under the microscope.

Identification of leaf borne Fungi:-
After incubation period the phylloplane fungal colonies monitored by blotter paper method and agar plate method were identified. In agar plate method, the fungal colonies were counted for individual species and the total number was enumerated after 3 days of incubation. A small quantity of the material was transferred to a clean microscopic slide and stained with cotton blue or safranin. It was then mounted in glycerin and observed under microscope and phylloplane mycoflora were identified. The fungal colonies were identified on the basis of colony characteristics such as colony appearance, colony colour, shape and other morphological features of the mycelia and spores to the lowest taxonomic rank possible. In addition identifications were confirmed by referring to the standard manuals, of Ellis (1971) and Barnett & Hunter (1998). In BPT method the total number of fungal species isolated from each leaf sample and the percentage of fungal occurrence was calculated as follows:

Fungal occurrence (%) = (Number of leaves with fungal growth /Total number of leaves examined) × 100

Weather Recording:-
Throughout the period of investigation, continuous data on daily temperature, relative humidity and rainfall were recorded (Table 1). The analysis of the weather data over the period of investigation from January to March 2018 in Mahe reveals the prevalence of hot and humid weather in the area. The temperature touches minimum of 22.84^0C and reaches maximum of 35^0C. The relative humidity touches minimum of 72% and records maximum of 93% during the period of investigation. From January to March 2018 a total of 1.90 mm of rainfall was recorded.

Results:-
Studies of phylloplane mycoflora of five important medicinal plants viz; Azadirachta indica, Centella asiatica, Justicia adhatoda, Ocimum tenuiflorum and Plectranthus amboinicus in Mahe, U. T. of Puducherry showed altogether 18 fungal species under 13 genera.

Qualitative and quantitative incidence of leaf mycoflora as evaluated by BPT method:-
Leaf samples of five important medicinal plants such as Centella, Holy Basil, Malabar nut, Neem and Panikoorka showed a varied level of incidence of mycoflora. A total of 7 different fungal species were isolated and identified from different aged leaf samples viz; tender semi-mature and mature of tested plants by BPT method. The isolated fungi include Alternaria alternata, Aspergillus niger, Cercospora sp., Cladosporium cladosporioides, Fusarium oxysporum, Penicilium chrysogenum and Torula graminis. Six fungal species were reported on Panikoorka, 4 on Centella and Ocimum, 3 on Malabar nut and Neem. Cercospora species and Fusarium oxysporum were found common in all the tested medicinal plants. On the other hand Cladosporium cladosporioides was found in Centella, Malabar nut and Panikoorka. Torula graminis was found in Holy basil (Tulsi) and Neem. Alternaria alternata was found common in Centella and Panikoorka. Penicillium chrysogenum was found in both Holy basil and Panikoorka. Aspergillus niger was found only in Panikoorka (Figure 1, Table 1). 50 pieces of leaves from each medicinal plant were tested for phylloplane mycoflora.

Relative abundance:-
Among the medicinal plants, leaves screened for highest mean incidence was found in Holy basil (7.8%), followed by Panikoorka (7.3%), Centella (5%), Malabar nut (5%) and Neem (4.4%) respectively (Table 1).Cercospora species showed the highest percentage of mean incidence in all the five medicinal plants (27.8%) followed by Fusarium oxysporum (9.6%) and Cladosporium cladosporioides (1.2%). Aspergillus niger, Alternaria alternata, Penicillium chrysogenum and Torula graminis showed very less percentage of mean incidence (Figure 2).

Qualitative and Quantitative incidence of leaf mycoflora as evaluated by PDA method:-

Leaf samples of five important medicinal plants such as Centella, Holy basil, Malabar nut, Neem and Panikoorka showed a varied level of incidence of mycoflora. Altogether 16 different fungal species under 11 genera were isolated and identified from different aged leaf samples of the tested medicinal plant leaves by the PDA method. Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus terreus, Cercospora sp., Cladosporium cladosporioides, Curvularia lunata, Diplococcium spicatum, Fusarium oxysporum, Mucor sp., Nigrospora sp., Penicillium chrysogenum (P. notatum), Penicillium citrinum, Penicillium funiculosum, Phoma sp., and Rhizopus stolonifer were identified as the phylloplane fungi from the five medicinal plants. On the individual basis 10 different fungal species under 7 genera were observed on Centella, 9 fungal species of 6 genera on Holy basil (Tulsi) and Panikoorka, 8 on Malabar nut and 9 on Neem. Aspergillus flavus, Aspergillus niger, Cladosporium cladosporioides, Fusarium oxysporum, Phoma sp. were found common in all the medicinal plant leaf samples. Nigrospora species was found only in Panikoorka. Aspergillus flavus, A. niger, Cladosporium cladosporioides, Fusarium oxysporum, Mucor sp., Penicillium chrysogenum, P. citrinum, P. funiculosum, Phoma sp. and Rhizopus stolonifer were isolated from phylloplane of Centella. Aspergillus flavus, A. fumigatus, A. niger, A. terreus, Cladosporium cladosporioides, Fusarium oxysporum, Mucor sp., Phoma sp. and Rhizopus stolonifer were isolated from Holy basil. Aspergillus flavus, A. niger, Cercospora sp., Cladosporium cladosporioides, Curvularia lunata, Fusarium oxysporium, Penicillium chrysogenum and Phoma sp. were isolated from phylloplane of Malabar nut. Aspergillus flavus, A. niger, Cladosporium cladosporoioides, Diplococcium spicatum, Fusarium oxysporum, Mucor sp., Penicillium chrysogenum, Phoma species and Rhizopus stolonifer were identified from the leaf surface of Neem. Aspergillus flavus, A. fumigatus, A. niger, Cladosporium cladosporioides, Fusarium oxysporum, Nigrospora sp., Penicillium chrysogenum, P. citrinum and Phoma species were isolated from the phylloplane of Panikoorka plant.

Comparison of the incidence of Phylloplanemycoflora by two different culture methods:-

The incidence of leaf borne fungi was determined on five important medicinal plants by two different culture methods such as BPT and PDA culture methods. The data were analyzed to know comparative account of phylloplane mycoflora by two different culture methods and the results are presented here (Table 1). During the present study altogether 18 fungal species under 13 genera were isolated and identified from the healthy leaves of the tested plants by employing both BPT and PDA culture methods. Out of these 7 fungal taxa were observed by BPT culture method whereas 16 fungal species of 11 genera were observed by PDA culture method. A total of 5 fungal types were common in both culture methods, however the quantity of incidence of fungi varied in both culture methods on different medicinal plant leaf samples. The fungal incidence on the leaves of medicinal plants varies in number. 5 types of fungi were observed on the leaves of Centella in BPT and 10 in PDA culture method. Similarly 3 fungi in BPT and 9 in PDA on Holy basil and Neem leaves, 3 fungal types in BPT and 8 in PDA on the leaves of Malabar nut, 6 fungi in BPT and 9 in Panikoorka respectively. Among the 18 fungi, highest fungal incidence was observed by Aspergillus niger in all the medicinal plants in PDA method. Cercospora species was observed in highest number in BPT method in all tested plants. Aspergillus niger was identified in all medicinal leaf samples in PDA culture method whereas it was observed on Centella and Panikoorka in BPT method. The incidence of Aspergillus flavus was observed in all the leaf samples of tested plants in PDA method. However it was not observed in BPT culture method. Nigrospora species was identified only in Panikoorka leaves by PDA method. Mean percentage of fungi isolated by both BPT and PDA method differ from each other. In BPT method Holy basil leaves showed highest percentage of fungi. It is followed by Panikoorka, Malabar nut, Centella and Neem. But PDA method showed varied mean percentage of fungi. In PDA method highest mean percentage of fungi isolated from Centella and it is followed by Malabar nut, Neem, Holy basil and Panikoorka respectively (Figure 1, Table 1). Mean percentage of leaf borne fungi on Holy basil and Panikoorka is high in BPT method, whereas in PDA method it shows less percentage.

Monthly Variation of phylloplane fungi on Medicinal plants by both BPT and PDA Methods:-

Population of phylloplane fungi is different in different months. Highest population was observed during month of March (222) and less in January (158). Environmental factors such as temperature, moisture, humidity, pH and rainfall during the sampling period also affect the growth and population of the phylloplane fungi. The fungal growth increases with increasing relative humidity and rainfall. Particular temperature also affects the growth of fungi on the leaves.

Figure 1. Histogram showing incidence of phylloplane mycoflora

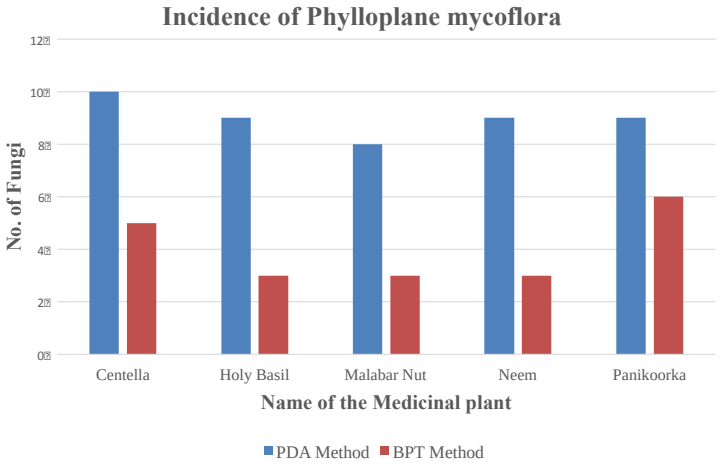

Figure 2. Relative abundance of some dominant phylloplane fungal types and their percentage

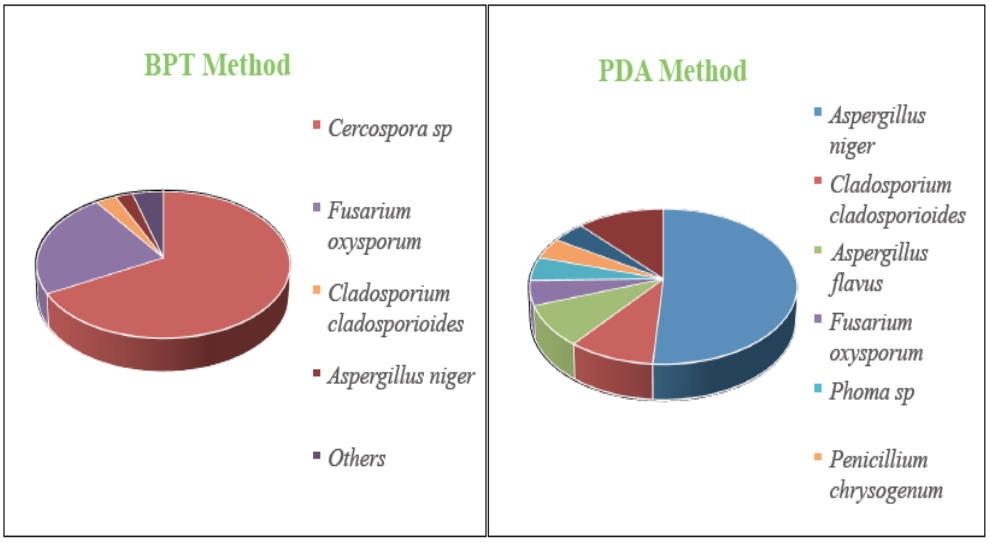

Table 1:-Comparative incidents of phylloplane fungi on different vegetable leaves in BPT culture (A) and PDA culture (B) methods

S. N.	Name of the phylloplane fungi	Incidence of phylloplane fungi										Mean (A)	Mean (B)
		Centella		Holy Basil		Malabar Nu		Neem		Panikoorka			
		BPT	PDA	BPT	PDA	BPT	PDA	BPT	PDA	BPT	PDA		
1	Alternaria alternata	3	0	0	0	0	0	0	0	1	0	0.8	0
2	Aspergillus flavus	0	10	0	2	0	11	0	3	0	8	0	6.8
3	Aspergillus fumigatu	0	0	0	2	0	0	0	0	0	4	0	1.2
4	Aspergillus niger	3	37	0	38	0	42	0	47	2	34	1	39.6
5	Aspergillus terreus	0	0	0	2	0	0	0	0	0	0	0	0.4
6	Cercospora sp.	15	0	47	0	25	3	21	0	31	0	27.8	0.6

7	Cladosporium cladosporioides	1	7	0	10	2	6	0	6	3	8	1.2	7.4
8	Curvularia lunata	0	0	0	0	0	4	0	0	0	0	0	0.8
9	Diplococcium spicatum	0	0	0	0	0	0	0	3	0	0	0	0.6
10	Fusarium oxysporum	13	10	6	2	8	5	8	3	13	3	9.6	4.6
11	Mucor sp.	0	2	0	6	0	0	0	2	0	0	0	2
12	Nigrospora sp.	0	0	0	0	0	0	0	0	0	1	0	0.2
13	Penicillium chrysogenum	0	10	2	0	0	3	0	0	1	0	0.6	2.6
14	Penicillium citrinum	0	2	0	0	0	0	0	2	0	2	0	1.2
15	Penicillium funiculosum	0	2	0	0	0	0	0	0	0	2	0	0.8
16	Phoma sp.	0	2	0	9	0	3	0	2	0	3	0	3.8
17	Rhizopus stolonifer	0	1	0	1	0	0	0	7	0	0	0	1.8
18	Torula graminis	0	0	0	0	0	0	2	0	0	0	0.4	0
	Mean	1.94	4.61	3.05	4	1.94	15.4	6.2	15	10.2	13		

Discussion:-

Leaf samples of different medicinal plants showed a varied level of incidence of mycoflora. It was observed that the incidence of phylloplane fungi is quantitatively and qualitatively greater in PDA culture method than BPT culture method. Similar reports regarding the incidence of phylloplane fungi have been reported in some vegetable crop leaves by Mari Bhat and Anusree in 2015. During the investigation altogether 18 fungal species were isolated and identified. Among this 16 fungal species were isolated by PDA and 7 by BPT culture method respectively. It was observed that Aspergillus niger showed the highest percentage of incidence (34.6%) on PDA culture method whereas Cercospora species (27.8%) shows highest percentage on BPT method. Aspergillus flavus, A. fumigatus, A. terreus, Curvularia lunata, Diplococcium spicatum, Mucor sp., Nigrospora sp., Penicillium citrinum, P. funiculosum, Phoma species reported only in PDA culture method. Alternaria alternata and Torola graminis were found only in BPT method. The overall result showed greater number of fungi on the leaves of Holy basil (126) and lesser on Neem (104). Similarly 119 fungi on phylloplane of Panikoorka, 113 on Centella and 112 on Malabar nut. In BPT culture method Holy basil showed highest percentage of incidence (7.8%) and lowest percentage in Neem. In PDA culture method percentage of fungal incidence is high in Centella and less in Panikoorka. The overall results reveal that the PDA culture method is more supporting media than BPT culture method for the isolation of leaf borne fungi. The frequency of fungal species differed significantly in different months. The number of fungi and frequency of colonization were greater in the month of March and less in January. This may be because the environmental condition. Rainfall in March promotes the dispersal of fungal spores.

Conclusion:-

The present investigation showed a significant diversity of the phylloplane fungi from the selected sites of Mahe, U. T. of Puducherry. The study of phylloplane mycoflora of important medicinal plants showed varied level of fungal incidence in both the culture method employed. Altogether 18 fungal species under 13 genera were identified. The isolated fungi belonged to the class Deuteromycetes and Hyphomycetes and Zygomycetes. Among this 16 species, 11 genera were isolated by PDA method. 7 types of fungi were observed by BPT method. A total of 5 fungal types were common in both culture methods, however the quantity of incidence of fungi varied in both culture methods on different medicinal plant leaf samples. Cladosporium cladosporioides and Fusarium oxysporum were identified on all the tested leaf samples by both culture methods. In BPT method Cercospora species showed highest percentage of incidence (27.8%). Alternaria alternata and Torula graminis were found only by BPT culture method. Aspergillus niger shows highest percentage of incidence in PDA method (39.6%). It is followed by Cladosporium cladosporioides (7.4%), Aspergillus flavus (6.8%), Fusarium oxysporum (4%), Phoma species (3.8%) and Penicillium chrysogenum (3.4%). Other fungi are found in less amounts. A total of 371 fungi were isolated and identified by PDA method and 207 by BPT method. The study on Phylloplane mycoflora of medicinal plants is of great importance, because nowadays plants are easily infested by fungi. Leaf samples of different medicinal plants showed varied level of incidence of mycoflora. It indicates the ability of fungi developing an association with broad

spectrum of leaves irrespective of their types. The actual diversity may depend on the methods used for gathering and handling of leaf samples, size of the leaf fragment and culture. From the present study, it may be concluded that phylloplane flora is highly sensitive to environmental factors. Study of phylloplane mycoflora is highly interdisciplinary in nature and has tremendous scope to find the significant applications in the field of medicine, epidemiology, disease forecasting and plant pathology.

Acknowledgements:-

The authors are thankful to Principal, MGGA Collage, Mahe, U.T. of Puducherry for providing facilities.

References:-

1. Shamsi S and Sultana R. Report on phylloplane mycoflora associated with Catharanthus roseus (L.) G. Don – A herbal medicinal Plant in Bangladesh. Bangladesh J. Sci. Res. 2014: 27(2): 201-207.
2. Sakhalkar PG and Bhat DJ. Mycoflora associated with Aegle marmelos, a medicinal plant of the forests of Western Ghats, India. Kavaka 2010: 37-46.
3. Prabakaran M, Merinal S and Panneerselvam A. Investigation of phylloplane mycoflora from some medicinal plants. European Journal of Experimental Biology. 2011: 1(2): 219-225.
4. Jalander V and Gachande BD. PhylloplaneMycoflora of Some Medicinal Plants. Asian Journal of Biology and Biotechnology. 2012: 1(1): e112.
5. Dalal LP. Phylloplane Flora of Some Novel Medicinal Plants of Family Ficaceae. International Journal of Scientific & Engineering Research. 2014: 5(3): 1117-1123.
6. Nayak BK. Biodiversity of phylloplane and endophytic fungi studied on the medicinal plant, Tinosporacordifolia. International Journal of Chemical Concepts. 2015: 1(3): 109-113.
7. Yadav AM. Investigation of mycoflora from medicinal plant Ocimum sanctum L.IJRBAT, Special Issue. 2015: (6): 24-26.
8. Angela G and Usha Shri P. Studies on phylloplanemycoflora and medicinal plants. International Journal of Advanced technology in Engineering and Science. 2016: 4(9): 230-245.
9. Thakur S. Study of phylloplane mycoflora of some selected medicinal plants. International Journal of Applied Biology and Pharmaceutical technology.2017: 8(2).
10. Nayak BK and Anandhu R. Biodiversity of Phylloplane and Endophytic Fungi from Different Aged Leaves of Medicinal Mangrove Plant Species Avicennia marina. J. Pharm. Sci. & Res. 2017: 9(1): 6-9.
11. Evueh AG. Use of phylloplane fungi as biocontrol agent against Colletotrichum leaf disease of rubber (Hevea brasiliensis Muell. Arg.). African Journal of Biotechnology.2010: 7.
12. Hausbeck MK and Enzenbacher TB. An evaluation of susceptibility of cucurbitaceous, solanaceous and fabaceous vegetables. Plant disease report. 2008: 10: 1404-1414.
13. Yeasmin F and Shamsi S. Phylloplane mycoflora of Gerbera spp. and their pathogenic potentiality. Journal of Bangladesh Academy of Sciences. 2013: 37 (2): 211-217.
14. Nayak BK. Isolation and identification of phylloplane and endophytic fungi from one ornamental plant, Mangifera indica, International Journal of Techno Chem Research. 2015: 1(3): 188-192.
15. Mari Bhat M and Anusree ME. Investigation of phylloplane mycoflora of some vegetable crops, Asian Journal of Plant Science and Research. 2015: 5(6): 63-68.
16. Dickinson CH and Wallace B. Effects of late applications of foliar fungicides on activity of micro-organisms on winter wheat flag leaves. Transactions of the British mycological Society.1976: 67(1): 103-112.
17. Doyer LC. Manual for wageningen, Netherland: International Seed Testing Association, 1938: Pp 59.
18. Ellis MB. Dematiaceous Hypomycetes. Commonwealth Mycological Institution, Kew, England, 1971: 608 pp.
19. Barnett and Hunter B B. Illustrated genera of imperfect fungi, Fourth Edition. APS Press, Minnesota, 1998: 218 pp.