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Investigation of phylloplane mycoflora of some vegetable crops

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ABSTRACT

India is one of the largest vegetable producing country in the world. Vegetables such as Brinjal, Ladies finger, Tapioca, Amaranthus, Murraya, etc are extensively used in Mahe for the food purpose. Pathogens adversely affect the production and quality of vegetables. The leaf borne fungi are one of the major causes of serious diseases in crops because of poor health and quality of vegetables. To realize this aspect the study has been undertaken to find out the phylloplane mycoflora employing BPT and PDA method. The phylloplane mycoflora ofvegetables is enumerated in the present communication. Leaves samples of different vegetables showed varied level of fungal incidence in both culture methods. A totals of 15 fungal types were observed in PDA culture method viz; Cladosporium cladosporioides(98.8%), Penicillium funiculosum(74.4%), Aspergillus flavus(57.6%), P. notatum(46.8%), A. niger(40.4%), Fusarium oxysporum(35.2%), Penicillium sp(34.4%), A. fumigatus(28.4%), P. frequentans(20%), Cercosporasp(15.6%), Rhizopusstolonifer(2.4%), Helminthosporiumsp (1.6%), Alternaria alternata(1.6%), Curvularia lunata(0.8%) and Rhizopus sp(0.4%). On the other hand on BPT method a total of 11 fungal species were reported in varied level of incidence viz; Cladosporium cladosporioides (26.4%) Fusarium oxysporum (24.4%), Cercosporasp (23.6%), Alternaria alternate (9.2%), Curvularia lunata (5.6%), Aspergillus niger (4.8%), Rhizopus sp(2.8%), Helmintho sporium sp(4.8%), Penicillium sp (1.2%), R. stolonifer (0.4%) and P. funiculosum(0.4%).

Key words: Incidence, phylloplane, mycoflora, vegetables

INTRODUCTION

Vegetable crops are grown worldwide as a source of nutrients and fibre in the human diet. Leafy vegetables are excellent sources of minerals, vitamins, dietary fibres and aromatic substances. Vegetables are used as leaf, stem, fruits, flower and tuber. These crops might be consumed fresh or after processing and are produced either on forms with conventional or organic agricultural production methods.

India is one of the largest vegetable producing countries in the world. In India different vegetables such as Brinjal, Ladies finger, Tapioca, Amaranthus, Murraya etc. are cultivated in Kerala, Karnataka, Tamil Nadu, Andra Pradesh, Gujrat, Kashmir, Madhy Pradesh, etc. These vegetable crops are not spared from destruction by fungal pathogens which infect roots, stems, leaves, flowers and fruits. It is now well established that a large number of microorganisms inhabit the phyllosphere of crop plants. While a few microbial species can be isolated from within pant tissues, many more are recovered from the surfaces of healthy plants. The phylloplane, the surface of plant leaves is a complex terrestrial habitat that is characterised by a varity of microorganisms including bacteria, filamentous fungi and yeasts. Phylloplane fungi are the mycota growing on the surface of the leaves. There are two groups of phylloplane fungi – residents and casuals. Residents can multiply on the surface of healthy leaves without noticeable affecting the host. Whereas, casuals land on leaf surface but cannot grow. The microbial diversity of phylloplane communities is influenced by plant age, species, micro and macro habitat, changes to environmental regimes and position of leaf on plant. The fungal spoilage of vegetables is responsible for the significant loss in the world food supply. Fungal infection during pre-harvest condition and subsequent colonization during storage bring about vegetable deterioration. Fungal growth on vegetables has two consequences such as deterioration in quality and production of secondary metabolites known as mycotoxins, which are carcinogenic, mutagenic and teratogenic

and dermatitic to man and animals and to cause hepatic carcinoma in man. Therefore enumeration and correct identification of phylloplane fungi is very important in alleviating the loss due to biodeterioration and also to reduce the exposure of human and animal life to toxic substances.

The microbiology of aerial plant surface has received increased attention. It is now well established that a large number of microorganisms inhabit the phylloplane of crop plants. The term "phylloplane" has been introduced independently by Last [11] and subsequently "phyllosphere" by Dickinson. Since then the study of phyllospheremicroflora has received attention of different workers.

Literature on phylloplane mycoflora of vegetable crops was enumerated by different investigators. In this review, we discuss the fungi on vegetable crops and highlight recent developments in this area. Emergence of fungi as potential disease management agents. The plant pathogenic fungi attacking vegetable crops that have been studied from the perspective of using fungi for control, range from biotrophs to necrotrophs. Biotrophic fungi, such as powdery mildew fungi, only grow and reproduce on the living host plant (obligate parasites), whereas necrotrophs, such as *Botrytis cinerea*, which causes gray mold disease, are opportunistic fungi that grow and reproduce on plant debris or organic matter but can rapidly invade wounded or senescing plant tissues. In addition, fungi can infect the roots and colonize the vascular tissues of the plant, causing root rots and wilt diseases (*Pythium* sp. and *Fusarium* sp., respectively). Most plant pathogens affecting vegetable crop species have been reasonably well-studied and information on their biology is available [1]. *Fusarium* sp was isolated from cucumber[13]and *Fusarium proliferatum* reported on onion [14]. The phylloplane fungi such as *Fusarium proliferatum* and *Fusarium fumonisins* on garlic plant was reported from Germany[6]. *Pythium ultimum was reported* from chinese cabbage, cucumber and sugarbeet[9]. Severe attacks by *Phytophthora* sp., *Pythium* sp., *Botrytis cinerea*(De Bary) Whet., *Didimella lycopersici* Kleb., *Alternaria* sp. and *Cladosporium fulvum* Cooke occurred in spring, especially in vegetable crops belongs to solonaceae and cucurbitaceae was repoted[8]. *Phytophthora* sp was reported on colocasia, bottle gourd, egg plant,common bean, sponge gourd and tomato[2]. Various workers have reported different fungal pathogens found on *Brassica* vegetables, which include the *Alternaria* sps. Causing leaf blight or dark spot, the *Leptoshaeria maculans* causing black leg or Phoma stem canker, the clubroot disease caused by *Plasmodio phorabrassicae*, and various others, thus culminating into heavy loss to the *Brassica* crop yield[20]. Fungal diseases of fruits and vegetables of 17 crops were studied[17] and in all 19 fungal pathogens were observed. Among these *Alternaria solani, Aspergillus niger, Aspergillus fumigatus, Fusarium* sp., *Mucor*sp., *Penicillum* sp. and *Rhizopus sp.*, were found to be major disease causing organisms. *Aspergillus flavus, A. niger, Penicillium sp, P. notatum, Fusarium oxysporum*, etc on some medicinal plants such as *Azadiracta*, *Phyllanthus* and *Ocimum was reported[16]*. *Cercospora sp* was reported from bhendi[18]. *Aspergillus sp, Curvularia sp, Penicillium sp* etc were isolated on some green leafy vegetables[10].

MATERIALS AND METHODS

Collection of samples :
The different age leaves viz. tender, semi mature and mature of Brinjal /egg plant(*Solanum melongena* L.) , Ladies finger/bhendi(*Abelomos chusesculentus* (L.) Moench.), Amaranthus/ cheera (*Amaranthus tricolor* L.), Tapioca/ cassava (*Manihotes culentacrantz*) and Murraya/curry leaf *(Murraya koenigii* (L.) Sprenge), were collected from five different locations in Mahe and placed in sterile plastic bags aparetly and immediately brought to the laboratory. A composite sample of each variety was prepared by mixing the individual samples together and the phylloplane mycoflora was isolated by using standard moist blotter paper method (BPT) and potato dextrose agar plate method (PDA).

Blotter paper technique (BPT) method :
In this method petriplates of size 90 mm were wrapped in brown paper, simultaneously Whatman's filter paper no 1 were also wrapped in brown paper for sterilization. Petriplates and blotter paper were sterilized in autoclave at 15 lbs pressure for 20 min. After sterilization in front of laminar flow sterile blotter papers were kept in the petriplates and moistened with sterile distilled water. Each leaf sample were taken in separate petriplates. The leaf fragment of 1 cm. were cut out using sterile scissor. 10 pieces of leaf samples of each above variety were places in the different petriplates and were incubated at 25 ± 2 oC . After 5 days different colonies were developed on leaves that were observed with help of microscope .

Potato dextrose Agar media (PDA) :
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 min. The supernatant were decanted and filtered by muslin cloth and the filtrate was collected. Dextrose (20g) and agar (15g) were transferred into the extract and shanked to dissolve the

ingredients. The medium was made up to 1 litre by addition of distilled water and added chloramphenicol (150 mg/l) to control the bacterial growth. The pH of the medium was adjusted to 5.6. Finally, the medium was cotton plugged and autoclaved at 121ºC for 15 minutes

Isolation of fungi

Leaf samples of Brinjal, Ladies finger, Tapioca, Amaranthus and Murraya were collected and placed in sterile plastic bags, and immediately brought to the laboratory. From the basal part of the leaves a fragment of 1cm of leaf blade was cut out (10 pieces) using sterile scissor and shaken in flasks filled with 100ml of distilled water. From the suspension of microorganisms prepared in this way 1ml was transferred into petridishes containing potato dextrose agar medium. The inoculum was spreaded uniformly and kept undisturbed in dust free chamber at room temperature for a period of 3-5 days. The fungal colonies were observed and pure cultures were maintained.

Identification :

The identification of phylloplane mycoflora was done referring standard manuals. Morphology and Taxonomy of Fungi [4],An Introduction to Fungi[5],The illustrated kingdom of fungi[15], Dematiaceous hypomycetes[7] and Illustrated genera of imperfect fungi[3]. The total number of fungal species isolated from each leaf sample and the percentage of fungal occurrence was calculated as follows -

$$\text{Fungal occurrence (\%)} = \frac{\text{Number of leaves with fungal growth} \times 100}{\text{Total number of leaves examined}}$$

RESULTS

Qualitative and quantitative incidence of leaf mycoflora as evaluated by BPT method:

Total count: Leaf samples of five different vegetables showed a varied level of incidence of mycoflora. A total of 11 different fungal species were isolated from BPT method on tested leaves of vegetables viz: *Alternaria alternata, Aspergillus niger, Cercospora sp, Cladosporium cladosporioides, Curvularia lunata, Fusarium oxysporum, Helmintho sporium sp, Peniciliumfuniculosum, Penicilliumsp, Rhizopus stolonifer,* and *Rhizopus sp*.

On the individual basis 8 fungal species were reported on Brinjal and Ladies finger, 7 on Tapioca and 5 on , Amaranthus and Murraya leaf.

Alternaria alternata were found common in Brinjal and Ladies finger, Tapioca and Murraya leaf whereas *Cercospora sp, Cladosporium cladosporioides and Fusarium oxysporum* were found common in Brinjal, Ladies finger, Tapioca, Amaranthus and Murraya leaf. *Aspergillus niger* and *Curvularia lunata* were found common in Brinjal, Ladies finger and Tapioca. The species of *Helmintho sporium* was found common in Ladies finger, Tapioca and Murrayaleaf. *Penicillium* species were found in Ladies finger and Amaranthus. *Rhizopus stolonifer* were found only in Amaranthus and *Rhizopus* species were found in Brinjal (Table-1). 50 pieces of leaves from each vegetable samples were tested for phylloplane mycoflora.

Relative abundance: Among the vegetable leaves screened Ladies finger a highest mean incidence (11.8%) was reported. Followed by on Tapioca (11.2%), on Brinjal (10.1%), on Murraya leaf (7.6%) and on Amaranthus (5.2%) respectively (Table-1). It was observed that *Cladosporium cladosporioides* showed the highest percentage of mean incidence in all the five different vegetables (26.4%) and it is followed by *Fusarium oxysporum* (24.4%), *Cercosporasp* (23.6%), *Alternaria alternata* (9.2%) and others (Table-1).

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

Leaf samples of five different tested vegetables showed a varied level of incidence of mycoflora. 15 different fungal species were isolated from PDA method on tested vegetables viz: *Alternaria alternaria, Aspergillus flavus, A. fumigatus, A. niger, Cercosporasp, Cladosporium cladosporioides, Curvularia lunata, Fusarium oxysporum, Helminthos porium sp, Penicillium frequentans, P. funiculosum, P. notatum, Rhizopus stolonifer, Rhizopus sp* respectively. On the individual basis 12 different fungal species were observed on Ladies finger, 11 on Brinjal, 10 on Tapioca, 9 on Amaranthus and 8 on Murraya leaf. *Aspergillus flavus, Cladosporium cladosporioides, Fusarium oxysporum, Penicillium funiculosum, Penicilium notatum, Penicilium sp.* were found common in all vegetable leaves samples. *Alternaria alternata* were found common in Brinjal and Tapioca. *A. fumigatus* were found common in Brinjal, Ladies finger, Tapioca and Murraya leaf. *A. niger* were found common in Ladies finger, Brinjal, Amaranthus and Tapioca. The species of *Helmintho sporium* were found common inBrinjal and Ladies finger. *Rhizopus stolonifer* were found in Ladies finger and Tapioca whereas *Curvularia lunata* was observed only in

Brinjal. The species of *Rhizopus* observed only in Murraya leaf. 50 pieces of leaves from each vegetable sample were tested for phylloplane mycoflora

Relative percentage: Among the vegetable leaves samples highest mean incidence was found in Tapioca(43.6%) followedby Brinjal (36.8%), Ladies finger (30.2%), Amaranthus(27.3%) and Murraya leaf (14.8%) respectively(Table-1). It was observed that *Cladosporium cladosporioides* showed the highest percentage of mean incidence in all five tested vegetables (98.8%) followed by *Penicillium funiculosum*(74.4%). *Aspergillus flavus* (57.6%), *P. notatum*(46.8%), *A.niger*(40%), *Fusarium oxysporum*(35.2%), *Penicillium* sp(34.4%), *A. fumigatus (*28.4%) and others (Table-1)

Comparison of the incidence of leaf mycoflora by two different culture methods :
The incidence of leaf borne fungi was determined on five different vegetables by two different culture methods carried out simultaneously employing BPT and PDA culture methods. The data were analysed to know comparative account of phylloplane mycoflora by two different culture methods and the results are presented here.

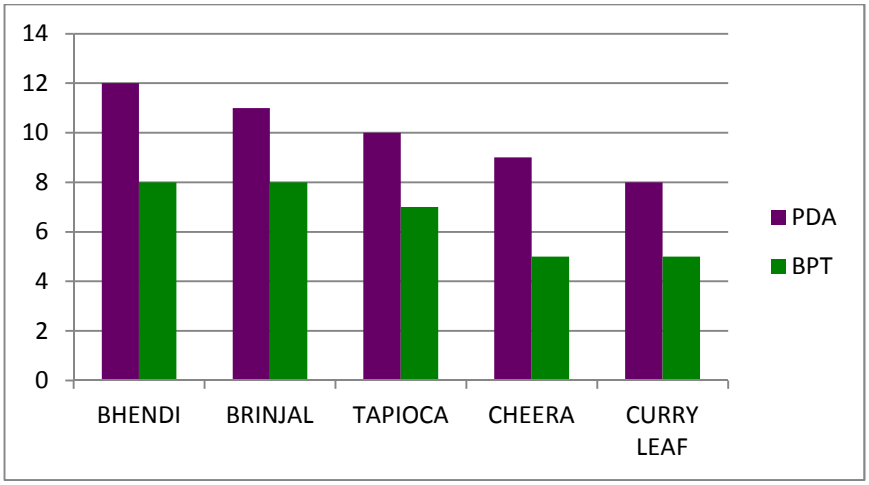
A total of 15 fungal types were observed on both culture methods. Out of these 11 types were reported on BPT culture method whereas 15 fungi observed on PDA culture method. A total of 11 fungal types were common in both culture methods, however the quantity of incidence of fungi varied in both culture methods on different vegetable leaf sample (Table-1).The highest fungal incidence 8 types was observed on Brinjal leaves in BPT and 11 types of fungi on PDA culture method. Similarly 5 fungal types in BPT and 9 in PDA on Amaranthus,5 fungi in BPT and 8 in PDA on Murraya leaf,8 fungi in BPT and 12 fungal types in PDA on Ladies finger,7 fungal types in BPT and 10 fungal types in PDA on Tapioca respectively(Figure-1)

.

Table1Comparative incidents of leave borne fungi on different vegetable leaves in BPT culture (A) and PDA culture (B) method

SL NO	FUNGI	INCIDENCE OF LEAF BORNE FUNGI (%)										MEAN	MEAN
		BRINJAL		LADIES FINGER		AMARANTUS		MURRAYA		TAPIOCA			
		A	B	A	B	A	B	A	B	A	B	A	B
1	*Alternaria alternate*	16	2	4	0	0	0	12	0	14	6	9.2	1.6
2	*Aspergillus flavus*	0	146	0	36	0	30	0	6	0	70	0	57.6
3	*Aspergillus fumigatus*	0	24	0	32	0	0	0	2	0	84	0	28.4
4	*Aspergillus niger*	8	56	8	88	0	22	0	0	8	36	4.8	40.4
5	*Cercosporasp*	20	0	16	12	12	66	28	0	42	0	23.6	15.6
6	*Cladosporium cladosporioidis*	22	54	52	50	20	72	22	44	16	274	26.4	98.8
7	*Curvularia lunata*	6	4	8	0	0	0	0	0	14	0	5.6	0.8
8	*Fusarium oxysporum*	24	52	30	32	20	4	20	26	28	62	24.4	35.2
9	*Helminthos poriumsp*	0	2	10	6	0	0	2	0	2	0	2.8	1.6
10	*Penicillium frequentans*	0	0	0	52	0	48	0	0	0	0	0	20
11	*Penicillium funiculosum*	2	128	0	36	0	92	0	84	0	32	0.4	74.4
12	*Pencillium natatum*	0	78	0	36	0	50	0	48	0	22	6	46.8
13	*Pencillium sp*	0	6	2	64	4	26	0	10	0	66	1.2	34.4
14	*Rhizopus stolonifer*	0	0	0	10	2	0	0	0	0	2	0.4	2.4
15	*Rhizopus sp*	14	0	0	0	0	0	0	2	0	0	2.8	0.4
	MEAN	10.1	36.8	11.8	30.2	5.2	27.3	7.6	14.8	11.2	43.6	9.2	30.5

Among the 15 fungi highest fungal incidence was observed by *Cladosporium cladosporioides* in all the vegetables in both culture methods. The incidence of *Penicillium funiculosum* was observed in all the vegetable leaves samples in PDA culture method whereas *P. funiculosum* was reported only on Brinjal in BPT method. The incidence of *Aspergillus flavus* was observed in all the vegetable leaves samples in PDA method. However it was not observed in BPT culture method (Table-1).in all the vegetable leaves samples in PDA method. However it was not observed in BPT culture method (Table-1).



X-axis: types of species **Y- axis: Number of Fungi(%)**

Figure-1.Incidence of fungi on five different vegetable leaf samples in PDA and BPT methods

DISCUSSION

Leaf samples of different vegetables showed a varied level of incidence of mycoflora. It was observed that the incidence of phylloplane fungi in both quantitatively and qualitatively greater in PDA culture method than BPT culture method. Similar reports regarding the incidence of phylloplane fungi have been reported in spices[12].. It was observed that *Cladosporium cladosporioides* showed the highest percentage of incidence (98.8%) on PDA culture method whereas its incidence was (26.4%) on BPT culture method. *Penicillium funiculosum* contributed maximum fungal incidence on PDA (74.4%) whereas (0.4%) only in BPT method. *Aspergillus flavus* (57.6%) and *P. notatum*(46.8%) reported only in PDA culture method. *A.niger*(40.4% and 4.8%), *Fusarium oxysporum*(35.2% and24.4%), and *Penicillium sp* (34.2%and1.2%) contributed to the total fungal incidence on both PDA and BPT culture methods. Similar reports were observed on the phylloplane mycoflora of some medicinal plants [16]. *A. fumigatus* (28.4%) and *P. Frequentans*(20%) reported only in PDA culture method. *Cercospora sp* contributed to the total fungal incidence on both PDA (15.6%) and BPT(23.6%). Similar reports were observed in Ladies fingerleaves[18]. *Alternaria alternata* contributed maximum fungal incidence on PDA (1.6%) and (9.2%) on BPT culture method. Similar reports were observed [19] on *Spilanthe soleracea* and in some green leafy vegetables[10]. Maximum fungal incidence of *Rhizopus stolonifer* (2.4%) on PDA and (0.4%) on BPT culture method. *Helmintho sporium* species (1.6% and 2.8%) and *Curvularia lunata* (0.8% and 5.6%) table and figure reported on different vegetable in PDA and BPT culture methods. Maximum appearance of saprophytic fungi such as *Cladosporium* sp and *Rhizopus sp* etc in unsterilized vegetable leaves reduce the appearance of some deep seated fungi such as *Alternariasp, Aspergillus sp, Fusarium sp* etc have been reported as most dominant fungi on all the tested vegetable leaves due to the raise in temperature level(33.9^{o}C)

Greater number of fungi was encountered on the leaves of Ladies finger is 12, 11 on Brinjal, 10 on Tapioca, 9 on Amaranthus and 8 on Murraya leaf in PDA culture method whereas the highest number of fungi reported on Brinjal and Ladies finger 8 each and 7 on Tapioca followed by 5 on Murraya leaf and Amaranthus.

CONCLUSION

The phylloplane mycoflora of five different vegetables in Mahe is rich in quantity and quality. During the period of investigation the leaf borne fungi of one or other types have been reported. The overall results reveal that the PDA culture method is more supporting media than BPT culture method for the isolation of leaf borne fungi.

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