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Original Research Paper

Phyllosphere Mycoflora of *Celosia Argentea* L.

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ABSTRACT

A total number of eight fungal species *viz*., *Fusarium oxysporium*, *Fusarium equisiti, Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Rhizopus arrhizus, Alternaria alternata* and *Penicillium notatum* were isolated by both dilution plate method and leaf impression method from phyllosphere of young to mature to senescent but healthy leaves of *Celosia argentea* L. The total population of these microorganisms showed a considerable increase from young to mature to senescent leaves. A possible role of these microorganisms as bio-control agents of the weed has been discussed.

INTRODUCTION

The term phyllosphere was introduced by Last (1955) to denote the leaf surface environment. Last & Deighton (1965) suggested that the term phylloplane should be used while referring to leaf surface habitat. The terms phyllosphere and phylloplane are interchangeably used in literature. Phylloplane is a natural habitat on leaf surface which supports heterogeneous population comprising both pathogen and non-pathogens (Chandra 1998, Leben 1965). The fungal propagules get deposited on leaf surface in several ways such as by wind-rain splash or through biological activities. These propagules face interference created by the nature of the epidermis and climatic factors. Only after this interaction some of the propagules are able to establish fungal population on the leaf surface. Phyllosphere has been found to be one of the most important factors in the development of a disease (Last 1955). The organisms inhabiting phyllosphere of a plant have a great influence on the course of events in the infection of the host and are ultimately related to the formulation of methods of disease control. The activity and the establishment of a pathogen is an ecological niche depends on an interplay between the host, pathogen and environmental factors i.e., other living microorganisms which affect the course of disease development either by their direct influence on the pathogen or indirectly through influence on the host or an environment. This provides the basis of the concept of bio-control. The mycoflora of phyllosphere of *Celosia argentea* was studied to find out the potential bio-control agents of the weed.

MATERIALS AND METHODS

The phyllosphere mycoflora was isolated by 2 methods i.e., dilution plate method and leaf impression method. The medium used for isolating the fungal flora was potato-dextrose agar with Rose Bengal and streptomycin (Martin 1950).

Dilution plate method: Leaves of different ages (young, mature and senescent but healthy) were taken. About 25 disks of 7mm diameter were cut from each group of leaves with the help of a sterilized cork borer. The discs of each age group were then put in 250 mL conical flasks containing 100mL of distilled and sterilized water. The contents were vigorously shaken for 30 minutes using automatic magnetic shaker to obtain spore suspension of the microbial population of the phyllosphere. Successive dilutions of the spore suspension were made by transferring 10mL of the original spore suspension with the help of a sterilized pipette to another flask containing 90mL of distilled and sterilized water and so on. 1.0 mL of spore suspension from different dilutions was added into each sterilized Petri plate by taking five replicates. The medium was over poured into the plates in warm and melted form (35-40°C). Immediately after the addition of the medium, Petri plates were slightly rotated to mix the spore suspension with the medium uniformly.

Leaf impression method: Leaf discs/segments from each age group of the leaves were pressed against the surface of already cooled and solidified medium. Each segment was pressed from dorsal as well as ventral surface separately. In both the cases the Petri plates were incubated at 25 ± 2°C. The isolation of phyllosphere microorganisms made by the two methods described above was then subjected to purification by hyphal tip method. These purified cultures were then identified up to specific level. PDA medium was used for screening purposes.

Plate 1

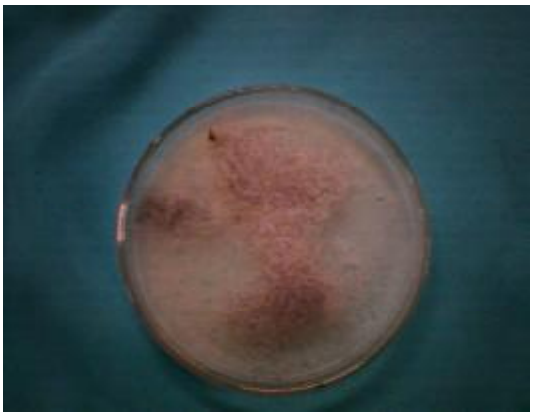

Plate 1.1

Plate 2

Plate 2.1

Plate 3

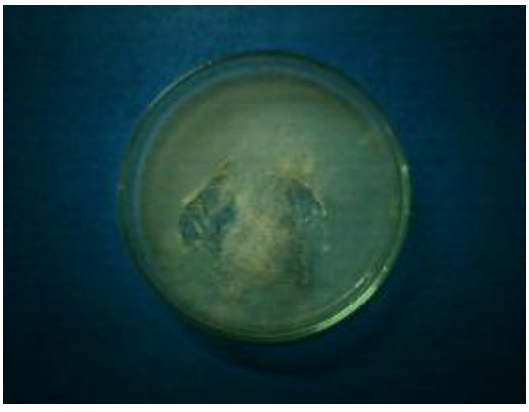

Plate 3.1

Fig. 1: Plate 1: Senescent leaf of *C. argentea*; Plate 1.1: Senescent leaf of *C. argentea* leaf impression on PDA plate showing the growth of fungi. Plate 2: Mature leaf of *C. argentea;* Plate 2.1: Mature leaf of *C. argentea* leaf impression on PDA plate showing the growth of fungi. Plate 3: Young leaf of *C. argentea;* Plate 3.1: Young leaf of *C. argentea* leaf impression on PDA plate showing the growth of fungi.

Table 1: Phyllosphere fungal flora of young, mature and senescent leaves of *C. argentea L.* (Leaf impression method).

S.No.	Fungal species	Young leaves replicates					Mature leaves replicates					Senescent leaves replicates				
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1	*Aspergillus flavus*											+		+	+	+
2	*Aspergillus fumigatus*						+	+	+		+	+	+	+	+	+
3	*Aspergillus niger*											+	+			+
4	*Fusarium oxysporium*	+	+	+	+	+										
5	*Rhizopus arrhizus*						+	+	+		+					

Table 2: Phyllosphere fungal flora of young, mature and senescent leaves of *C. argentea* L. (Dilute plate method).

S.No.	Fungal species	Young leaves replicates					Mature leaves replicates					Senescent leaves replicates				
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1	*Alternaria alternata*											+	+	+	+	+
2	*Aspergillus flavus*						+		+	+		+			+	+
3	*Aspergillus fumigatus*						+	+	+	+	+	+		+	+	+
4	*Aspergillus niger*							+		+	+	+	+		+	+
5	*Fusarium equisiti*	+	+	+		+										
6	*Fusarium oxysporium*	+	+		+	+										
7	*Penicillium notatum*											+		+		+
8	*Rhizopus arrhizus*						+	+	+	+	+					
	Total number of species			6					13					19		

Table 3: Comparative account of various fungi on young, mature and senescent leaves of C*. argentea (*isolated by both the methods).

S.No.	Fungal species	Leaves		
		Young	Mature	Senescent
1	*Alternaria alternata*			+
2	*Aspergillus flavus*		+	+
3	*Aspergillus fumigatus*		+	+
4	*Aspergillus niger*		+	+
5	*Fusarium equisiti*	+		
6	*Fusarium oxysporium*	+		
7	*Penicillium notatum*			+
8	*Rhizopus arrhizus*		+	
	Total number of species	2	4	5

RESULTS AND DISCUSSION

Young, mature and senescent leaves along with the growth of fungi by lead impression method are shown in Fig. 1. The isolated fungi from these leaves by different methods are given in Tables 1-3.

Phyllosphere fungal flora of senescent, mature and young leaves of *Celosia argentea*: A total number of 8 fungal species viz., *Aspergillus flavus, A. fumigatus, A. niger, Fusarium equisiti, F. oxysporium, Penicillium notatum, Rhizopus arrhizus* and *Alternaria alternata* were isolated from the phyllosphere of young, mature and senescent but healthy leaves of *C. argentea*. The total population of the microorganisms showed a considerable increase from young to mature to senescent leaves (Table 3). This may be due to increased availability of the nutrients.

Five fungal species were isolated by leaf impression method (Table 1). *Fusarium oxysporium* was isolated from young leaves. Two genera were isolated from mature leaves, *Aspergillus* having 2 species *Aspergillus fumigatus* and *A. niger*, and other genera was *Rhizopus arrhizus*. Two genera were isolated from the senescent but healthy leaves of *C. argentea viz.*, *Alternaria alternata* and *Aspergillus* having three species *Aspergillus fumigatus, A. niger, A. flavus* (Table 2).

Eight fungal species belonging to five genera were isolated from the leaves of the *C. argentea* by dilution plate

method (Table 2). Two fungal species *Fusarium oxysporium* and *F. equisiti* were isolated from the young leaves. Two genera were isolated from the mature leaves. *Aspergillus* having *A. furmigatus, A. flavus, A. niger* and the other genera *Rhizopus arrhizus*. Three genera of fungal species were isolated from the senescent but healthy leaves, showing, *Aspergillus fumigatus, A. flavus, A. niger, Alternaria alternata, Penicillium notatum.*

The total phyllosphere population of myco-organisims showed an increase from young to mature to senescent but healthy leaves of *C. argentea* (Table 3). These findings show that the population of epiphytic myco-organisms increase with advance in age and maturity of leaves. This may be due to the increased availability of nutrients.

The young leaves were found to be infested with only 2 fungal species *viz., Fusarium oxysporium* and *F. equisiti*. The mature leaves were infested with two genera containing four species. These are *Aspergillus fumigatus, A. flavus, A. niger* and *Rhizopus arrhizus*. The senescent leaves were infested with 3 genera of 5 fungal species *viz., Aspergillus fumigatus, A. flavus, A. niger, Alternaria alternata* and *Penicillium notatum* (Table 3).

The two fungal species *Fusarium oxysporium, F. equisiti* were found only on young leaves, and were not present on mature and senescent leaves of *C. argentea*. However, *Aspergillus fumigatus, A. flavus* and *A. niger* were associated with both mature and senescent leaves. But *Rhizopus arrhizus* was found only on mature leaves, and *Alternaria alternata* and *Penicillium notatum* were found only from the senescent leaves.

All these fungal species are weak pathogenic and not host specific and also found from various other hosts. They do not cause any disease to the plant. Only *Alternaria alternata* was found to cause a mild leaf spot disease of weed (Dhawan 1988). They do not complete their life cycle on the living host and they do not damage the host tissue due to less virulence. However, if some strains of any of these fungi are developed which meet the requirements of using them as bio-control agents *viz.*, host specificity strong virulence so they can be considered as bio-herbicides for bio-control of *C. argentea.*

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