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Variability in Phyllosphere Mycoflora of Tea (*Camellia sinensis L. O. Kuntze*) From Cachar District of Assam.

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ABSTRACT

Phyllosphere is a unique ecosystem for microorganisms, comprising both biotic and abiotic factors. It may aquire the ability to prevent plant pathogens and become resistant to adverse conditions. The present study has been conducted to observe the seasonal variation and species diversity at Silcoorie Tea Estate of Cachar District of Assam. The sampling was carried out for one year (May-2012 – Apr-2013) seasonally after an interval of one month from different sites and different clonal varieties. A total 18 fungal species has been isolated representing 9 saprophytic and 7 parasitic including three unidentified ones. The species composition was seasonal, clonal, and leaf age dependent with considerable environmental fluctuations. The meteorological parameters seem to play an important role in species variability of the phyllosphere mycoflora.

KEYWORDS : Tea, Phyllosphere, Mycoflora.

Introduction:

The habitat adjacent to the leaf surface is known as the phyllosphere and the surface of the leaf is called Phylloplane, (Prece and Dickinson, 1971; and Blackman, 1981). The current inventory of the world's biodiversity is incomplete and we have only a small idea of our true microbial diversity (Stanley, 2002). The leaf surface is a major habitat for microorganisms and study of microbial diversity is an important aspect of functional ecology. The microorganisms on the leaf surface depend on the season and age of the leaf. With diverse ecological functions, leaf surface microflora is considered to be one of the important groups of microorganisms concerned (Ruinen, 1965). Microorganisms on leaf surface play an important role in the plant ecology and can be manipulated to enhance plant growth and to reduce plant diseases. Evuch (2008) used phylloplane fungi as biocontrol agent against *Colletotrichum* leaf disease. The present work aims for the isolation, cultural and morphological characterization of phyllosphere fungi to observe seasonal variation and species diversity of the selected tea garden of Cachar District of Assam.

Study site:

For the present study Silcoorie Tea Estate, Cachar District of Assam was selected. The tea estate is one of the largest gardens which situated in the valley of the river Barak in between 24° 8′ to 24° 5′ North Latitude and 94.28° East longitudes as an elevation varying from 40m above m. s. l.

Materials and Methods:

Healthy leaves of three different growth stages from the tea clone TV21 (Quality category), TV7 and TV9 (Standard category), TV19 and TV23 (yield category) were collected aseptically in a sterile polythene bag, leveled properly and taken in to the laboratory.

Isolation of phyllosphere fungi:

The isolation of fungi was done by leaf impression method and serial dilution method (Aneja, 2003). The cultures were plated in both Czepex- dox- Agar and Potato Dextrose Agar medium and were incubated at 30°C in the BOD incubator for 5days. The colonies developed were examined regularly and counted. Fungi were identified by (Gregory, 1961; Gilman, 1975; and Ellis and Ellis, 1988).

Results and Discussion:

In the entire sampling period a total 18 fungal species were observed. Among this 9 isolates were identified as saprophytic, 7 as parasitic in nature and three were unidentified sterile mycelia forms. The most dominant fungal species were *Aspergillus niger* (23.28%), *A. flavus* (22.80%) *and Penicillium sp* (25.00%). The most prevalent species were *Alternaria alternata* (21.90%), *Curvularia lunata (*16.96%), *Trichoderma sp* (12.62%), *A. fumigatus* (06.12%) *and Fusarium sp* (04.83%) *which were observed in rainy season*. The less frequent species were *Colletotrichum sp* (03.10%), *Geotrichum sp* (02.87%), Cladosporium *cladosporoids* (02.63%), *Pestalotiopsis sp* (02.60%) and *Epicoccum sp* (1.10%) and *Streptomyces sp* (0.95%).

The population of fungi was different in different seasons. The highest population was observed during the month of July to October and sudden decrease started from November up to May. Breeze and Dix (1981) noticed that in summer, the biomass of yeasts on leaves of Acerplatanoids was 50 times greater than of hyphal fungi. Seasonal studies on other tea growing areas also reported the low population of microbes on leaf surface during April and May which might be attributed to increased precipitation which removed appreciable quantities of microflora from the leaf surface (Tanti et al, 2012). The fungal population also showed a significant diversity isolated from different tea varieties. The fungal population recorded from TV23 and TV19 (yield category) was highest than TV7 and TV9 (standard category) where as TV21 exhibited lowest population. Ingold (1971) revealed that the spores deposited on leaves are influenced by the interaction of factors such as spore size, wind speed and the moisture conditions of both the spore and the leaf. The leaf age dependent variation showed gradual increase of the fungal population with the increase in the leaf age the possibility might be due to decrease in biochemical content of the leaves with increase in age of leaves. Osono (2008) examined and compared the seasonal and leaf age-dependent variations in the endophytic and phyllosphere fungal assemblages of *Camellia japonica* and found that frequency of the epiphyte *Cladosporium cladosporoids* varied with both season and leaf age.

Table- 1: Percent occurrence of phyllosphere fungi on tea leaf surface in different study sites.

Microbial isolates	Study Site			
	Dharamkhal Division	Dargakona Division	Main division of Silcoorie T. E.	Average %
Aspergillus niger	21.65	23.00	20.28	21.64
Aspergillus flavus	14.43	17.12	19.80	16.91
Penicillium sp	13.33	13.48	22.00	16.27
Alternaria alternata	07.75	05.32	18.09	10.65
Curvularia lunata	11.07	09.00	13.98	11.56
Trichoderma sp	05.34	04.21	09.62	6.39

Aspergillus fumigatus	04.38	05.78	03.12	4.42
Fusarium sp	02.98	06.38	05.13	04.83
Colletotrichum sp	00.00	03.20	06.30	03.16
Geotrichum sp	00.00	05.87	02.75	02.87
Cladosporium sp	03.15	04.76	00.00	02.63
Pestalotiopsis sp	02.00	00.00	05.80	02.60
Epicoccum sp	02.31	01.00	00.00	1.10
Streptomyces sp	00.00	00.00	02.87	0.95

Table- 2 Seasonal Variation of phyllosphere fungi on tea leaf surface:

Microbial isolates	Jan-Feb	Mar-Apr	May-Jun	Jul-Aug	Sep-Oct	Nov-Dec
Aspergillus niger	++	++	+	+++	+++	++
A. flavus	+	++	+	++	+++	++
A. fumigatus	+	-	++	-	-	+
Penicillium sp	+	+	++	++	+++	++
Alternaria alternata	-	+	++	++	+	+
Curvularia lunata	-	-	-	++	++	+
Trichoderma sp	-	-	+	+++	++	+
Fusarium sp	-	-	-	+	+	+
Colletotrichum sp	-	-	+	+	+	-
Geotrichum sp	-	-	-	-	+	+
Cladosporium sp	-	-	+	+	-	-
Pestalotiopsis sp	-	-	+	+	+	-
Epicoccum sp	-	-	+	-	-	-
Streptomyces sp	-	-	-	+	-	-

+++ (Prominent), ++ (Moderate), + (Less), - (No growth).

Fig 1: Fungal population in phyllosphere of tea.

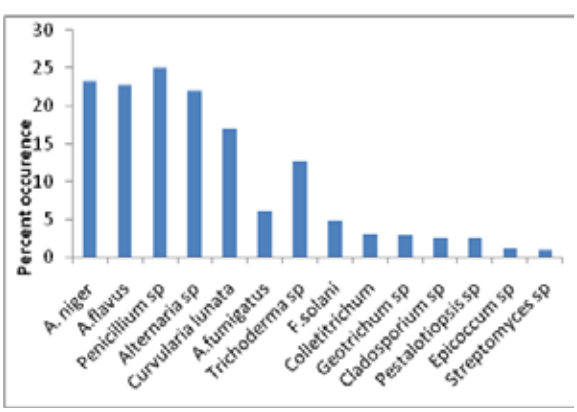


Fig2: Seasonal variation in fungal population in phyllosphere of tea.

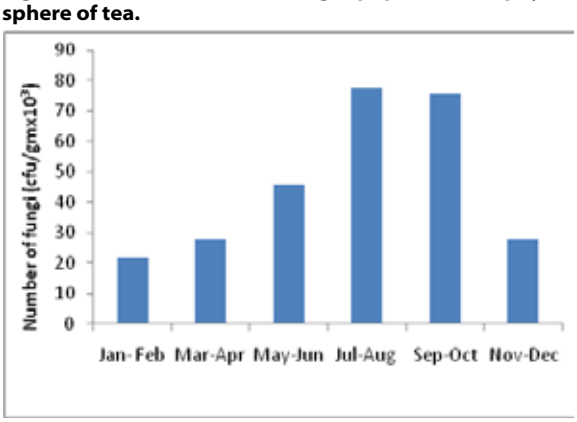


Fig3: Maximum and minimum temperature during sampling period.

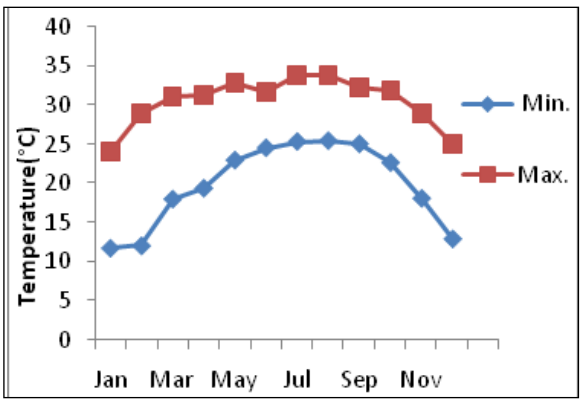


Fig4: Maximum and minimum humidity during sampling period.

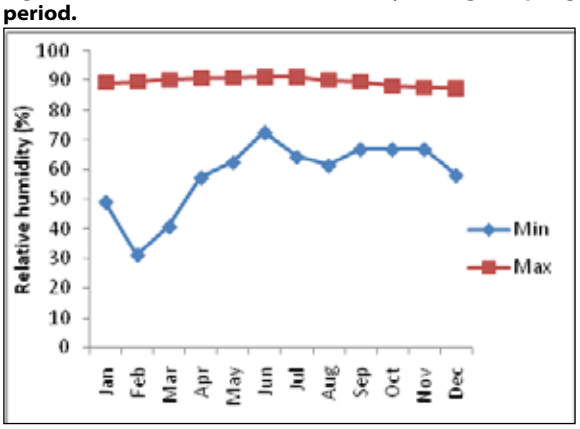


Fig 5: Average rainfall (mm) during sampling period.

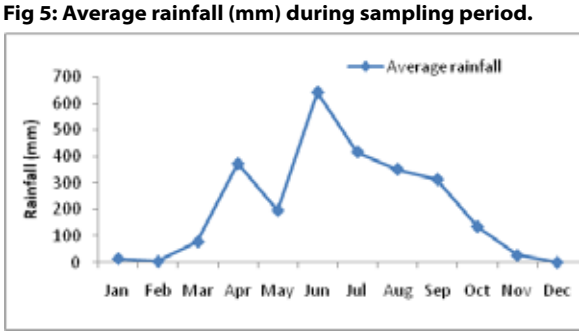


Fig 6: Clonal variation of the fungal population.

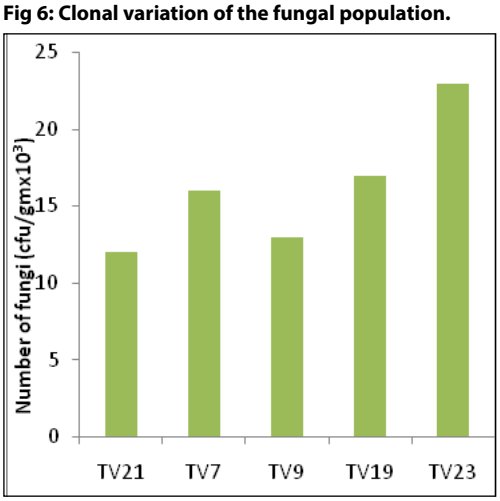
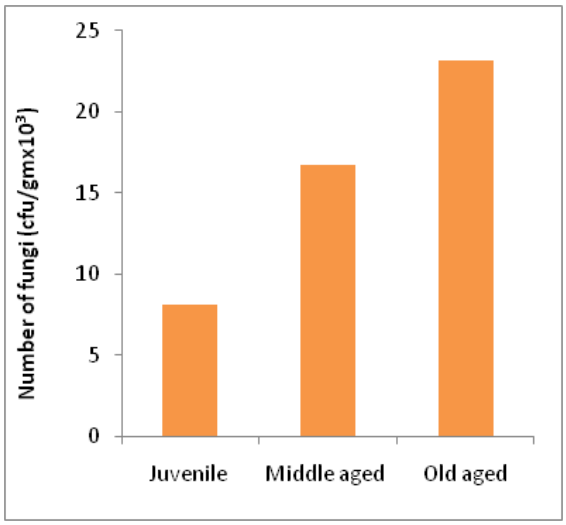


Fig 7: Population of fungi on different stages of leaf development.



The present investigation showed a significant diversity of the phyllosphere fungi from the selected sites of tea gardens. The phyllosphere saprophytes can be manipulated to reduce plant diseases, decomposition of pollutants and dead plant biomass. Blakeman et al (1982) revealed that the phyllosphere saprophytes can have antagonistic effects on foliar pathogens and thus functions as natural biological control organisms. Voriskova and Petr (2012) considered that fungi are the primary decomposers of dead plant biomass in terrestrial ecosystems.

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