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Research Article

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Study of fungal diversity with seasonal variation in the Som (*Persea bombycina* Kost.) plantation area of Goalpara district of Assam, India

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ABSTRACT

Persea bombycina Kost. Commonly known as Som, is the primary host plant of golden silk producing Muga silkworm (Antheraea assamensis Helfer.). Muga is very sensitive to the odour of toxic chemicals, temperature & humidity. Muga worms has been dying prematurely for years due to air pollution. Besides pesticides, insecticides & herbicides seasonal change is effecting muga production with food plant. Sands & sediments carried by flood waters covered som bushes up to three to four feet. After the desertification & being affected by stagnant flood water som plant dies within few months. This has been happening for years and most of muga rearing areas are now almost free of this cultivation. Moreover due to climate change outbreaks of various disease causing microbes may occur to the host plants along with other microbes. A study was conducted to study the climatic factor and types of mycoflora present on the som growing areas of Goalpara district during February,2014 to July, 2014. A total of 7 fungal species from air, 12 fungal species from phylloplane, 16 fungal species from rhizosphere and 11 fungal species isolated from non-rhizosphere soil of som plantation area of the study area which show cyclic pattern of occurrence. The major mycoflora which dominates the air, phylloplane, rhizosphere & nonrhizosphere soil of som cultivation area were Rhizopus spp. & Aspergillus spp. The study also reveals that climatic factors such as temperature, humidity & rainfall are also responsible for occurrence of certain mycoflora which effects the Som & ultimately the muga silkworm. Hence recent urbanization, deforestation, pollution & climate changes have all pushed muga silkworm to the danger of declined production & to the very sustenance.

Key words: *Persea bombycina, Climatic factors, Seasonal variation, mycoflora, Goalpara.*

INTRODUCTION

Muga silkworm (Antheraea assamensis Helfer.) is endemic to Northeast India which are polyphagous, multivoltine & semidomesticated in nature. These golden silk producing silkworm feed primarily on two host plants. Persea bombycina Kost. Commonly known as “Som” & Listea polyantha Juss. Commonly known as ” Sualu”. In Assam muga silk culture is practiced in the districts of upper assam & certain parts of lower assam. In lower assam Goalpara and Kamrup distric produces some quantities of muga cocoon.

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Sericulture in Goalpara district existed almost as a practice among the people since a long time. The district is situated at a distance of 146 km from Guwahati, the capital city of assam. The district covers an area of 1,824 sq. km and is bounded by West and East Garo hill districts of the state of Meghalaya on the south Kamrup district on the east, Dhubri district on the west and the Brahmaputra all along the north. It is located between latitudes 25.53 degree and 26.30 degree North and longitudes 90.07 degree and 91.05 degree east. Goalpara district has been given the geographical identification mark because its climate is suitable for silkworm rearing[7].

Muga is very sensitive to the odour of toxic chemicals, temperature & humidity. Muga worms has been dying prematurely for years due to air pollution. Besides pesticides, insecticides & herbicides climate change is effecting muga production with food plant. Sands & sediments carried by flood waters covered som bushes up to three to four feet. After the desertification & being affected by stagnant flood water som plant dies within few months. This has been happening for years and most of muga rearing areas are now almost free of this cultivation. Moreover due to climate change outbreaks of various disease causing microbes may occur to the host plants along with other microbes.

Fungal spores are widely distributed over the world which constitute the major component of the air borne microflora. They are affected by various environmental factors such as temperature, humidity, moisture, wind and geographical location. Seasonal variation affect the distribution of fungi of particular area. Occurence and types of fungal species change with season and geographical locations. Variations in altitude and climatic conditions such as temperature, relative humidity, rainfall etc. prevailing in Northeastern region are responsible for development of different diseases and insect pest as well. The diversity microorganisms in air, phylloplane and soil have been studied by different workers.

It is gradually become evident that a good numbers of fungi do not exist in nature individually, but a number of microorganisms (viz. fungi, bacteria and algae) are present in the air, rhizospere , phylloplane and in other habitats in the host or in close proximity of that host. So the presence of a pathogen doesnot always signify the possibility of initiation of a disease. Sometimes different organisms occurring together may be individually involved in disease syndrome , while in some cases some may not be non-pathogenic.

A study was conducted on climatic factors and its effect on air, phylloplane, rhizosphere & non-rhizosphere mycoflora of Som growing areas of Goalpara district of Assam during February, 2014 to July, 2014.

Climate change with global worming effect has been reported and analysed both at international & national forums and visible effects of global warming on biodiversity in Northeastern zone has been well documented. Climate change over decades has contributed to global warming as endangered fauna & flora in the one of the hotspots of the world i.e Northeastern India with highest biodiversity species congregated. Rapid changes in climate happening, the climate models predict 2.0 to 3.5 C increase in temperature and 250-500 mm increase in precipitation in the Northeastern region with more threats of crop failures thus on survivability itself of the species. Analysis of climatic changes in key locations testifies the changing environment not conducive for success of muga silkworm lifecycle[10].

MATERIALS AND METHODS

The study was conducted at Goalpara district of Assam, represents a rural & semi urban area during February to July, 2014. A total of 8 sites were selected depending upon the direction namely Budlung pahar & Matia on the north, Lengopara & Baida on the south, Dorapara Agia & Buraburi on the east and Bhalukdubi & Kalyanpur on the west respectively for collecting the samples during the 3 muga crop seasons. Air samples were collected using the culture (gravitatational setting) method with petridishes containg Potato Dextrose Agar (PDA), Martins Rose Bengal Agar (MRBA) and Czapek's Dox Agar medium supplemented with Chloramphenicol (250mg/ml) to prevent bacterial growth. After exposing the plate for 10-15 minutes at 2-3 meter height above the ground level they were transferred to the laboratory and kept for incubation at 25±1°C for a period of 5-7 days and then the the plates are examined for the development of fungal colonoies.

To study Phylloplane mycoflora, different age leaves depending upon the size and shape viz. tender, semi mature and mature were randomly collected during rearing (outdoor) season from February to July, 2014 in sterile polybags and taken back to the laboratory from 8 different places mentioned above during the 3

muga crop seasons .Serial washing technique as described by *Garg et al.* (1971) and leaf sectioning and plating method described by Preece & Dickinson[5] were employed. Leaf discs were cut for each leaf categories with the help of sterilized borer. Pieces from leaf categories were placed separately in 20 ml of sterile distilled water in 250 ml of Erlenmeyer flask shaken for 20 minutes at 120 rpm. The extract of the detachable fungal propagules from the leaf surface was determined by plating 1 ml solution from washing to the petriplates containing PDA media. The cut out leaf discs dorsal and ventral surface were impringed on the surface of PDA media containing petridishes. The petridishes were inoculated at 25 ± 1° C for 5 days and then the plates are examined for the development of fungal colonies. The isolated fungi were identified .

Similarly for soil mycoflora studies soil samples of the muga food plantation area were collected following standard sampling method from the above mention study sites during the 3 muga crop seasons. Soils were collected from the surface layer (0-30cm depth) from each of the locations. For collection of rhizosphere soil sample each Som plantlet was carefully uprooted and the soil adhering to the roots was gently shaken into a strerile polythene bag; the bag was tied and labelled. The non rhizosphere soil samples were collected by digging a few centimeters deep into the field with a sterile hand trowel; the soil collected was then tied and labelled. Isolation and evaluation of microfungi from soil and rhizosphere will be done by serial dilution agar plating method[2]. The petridishes were inoculated at 25±1°C for 5 days and then the plates are examined for the development of fungal colonies. The isolated fungi were identified. The mycelia and spore characters of fungi were studied under microscope (Labomed, Germany) using Lactophenol cotton blue staining and with the help of "A manual of soil fungi by Gilman[6] and illustrated genera of imperfect fungi by H.L. Baranatt[3].

Table 1. Climatic factors: from February to July, 2014

Climatic factors	**Feb**		**Mar**		**Apr**		**May**		**June**		**July**	
Temp	Max 32°C	Min 8°C	Max 36°C	Min 12°C	Max 36°C	Min 19°C	Max 38°C	Min 20°C	Max 30°C	Min 22°C	Max 38°C	Min 23°C
RH	Max 89%	Min 41%	Max 83%	Min 31%	Max 84%	Min 32%	Max 92%	Min 55%	Max 92%	Min 58%	Max 92%	Min 63%
Rainfall	220ml		155ml		220ml		2720ml		3710ml		2510ml	
Total rainy days	2 days		3 days		5 days		14 days		17 days		15 days	

Fig.1: Images of collection of air sample from Som plantation area and their growth after 7 days of incubation period

Table.2. Percentage of occurrence of aeromycoflora from Som growing areas during February to July, 2014 at Goalpara district, Assam during various Muga crop seasons

Fungal isolates	Feb-Mar (Chatua)	Apr-May (Jethua)	Jun-Jul (Aherua)
Aspergillus niger	25.0	18.5	27.5
Aspergillus flavus	0.0	22.5	35.5
Alternaria alternata	22.0	20.0	0.0
Cladosporium cladosporioides	10.5	0.0	0.0
Curvularia lunata	9.5	9.0	8.0
Fusarium oxysporum	10.5	9.5	0.0
Rhizopus stolonifer	22.5	25.5	29.0

Fig. 2: Occurrence of Aeromycoflora from Som growing areas during Feb-Jul, 2014

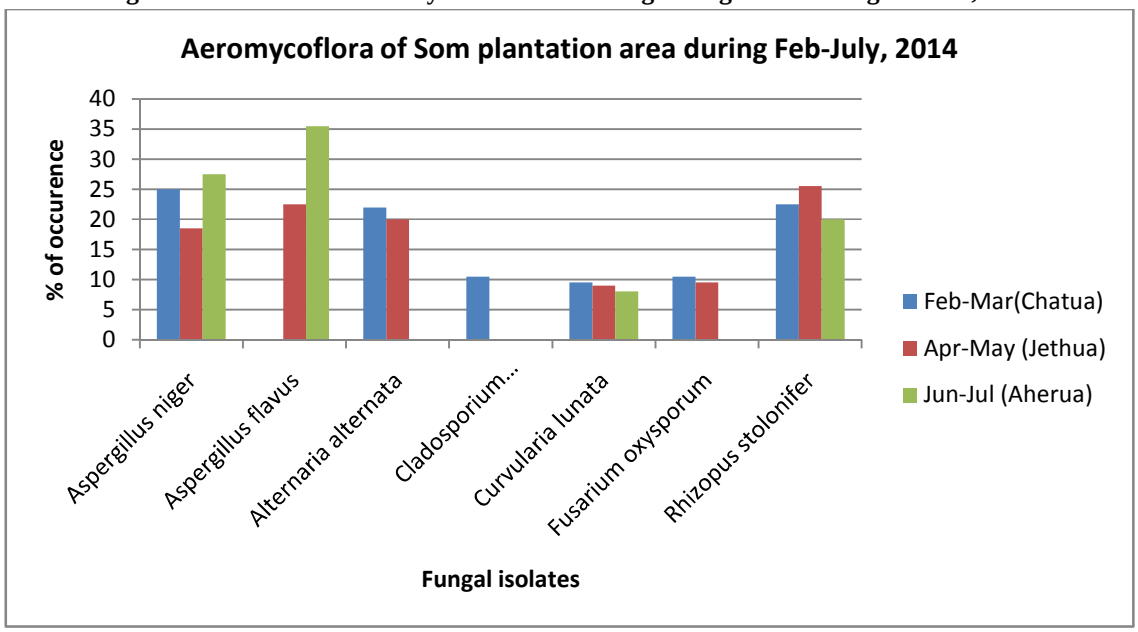


Table.3. Fungal isolates from phylloplane of Som during Chatua generation of *A. assamensis* (Feb-March, 2014)

Climatic factors	Status of leaves	Types of surface	No. of leaves	Fungi isolated	% of Occurence
February Max Min Temp 32ºC 8 ºC RH 89% 41% Rainfall - 220 ml. Total rainy days- 2 March Max Min Temp 36ºC 12 ºC RH 83% 31% Rainfall – 155 ml. Total rainy days- 3	Tender	Dorsal	10	*Rhizopus stolonifer* *Alternaria alternata* *Curvularia lunata* *Mucor hiemalis* *Penicilliumchrysogenum* *Fusarium oxysporum*	27.50% 23.50% 17.50% 12.50% 10.50% 8.50%
		Ventral	10	*Rhizopus stolonifer* *Alternaria alternata* *Curvularia lunata* *Mucor hiemalis* *Penicillium chrysogenum*	38.50% 25.50% 22.50% 10.00% 3.50%
	Semi - mature	Dorsal	10	*Rhizopus stolonifer* *Curvularia lunata* *Alternaria alternata* *Fusarium oxysporum* *Geotrichum sp.*	40.50% 26.50% 20.00% 10.50% 2.50%
		Ventral	10	*Rhizopus stolonifer* *Alternaria alternata* *Aspergillus niger*	55.50% 30.50% 14.00%
	Mature	Dorsal	10	*Rhizopus stolonifer*	40.00%

				Aspergillus niger	25.00%
				Mucor hiemalis	23.50%
				Penicillium chrysogenum	11.50%
		Ventral	10	*Rhizopus stolonifer*	45.50%
				Aspergillus niger	40.00%
				Penicillium chrysogenum	14.50%

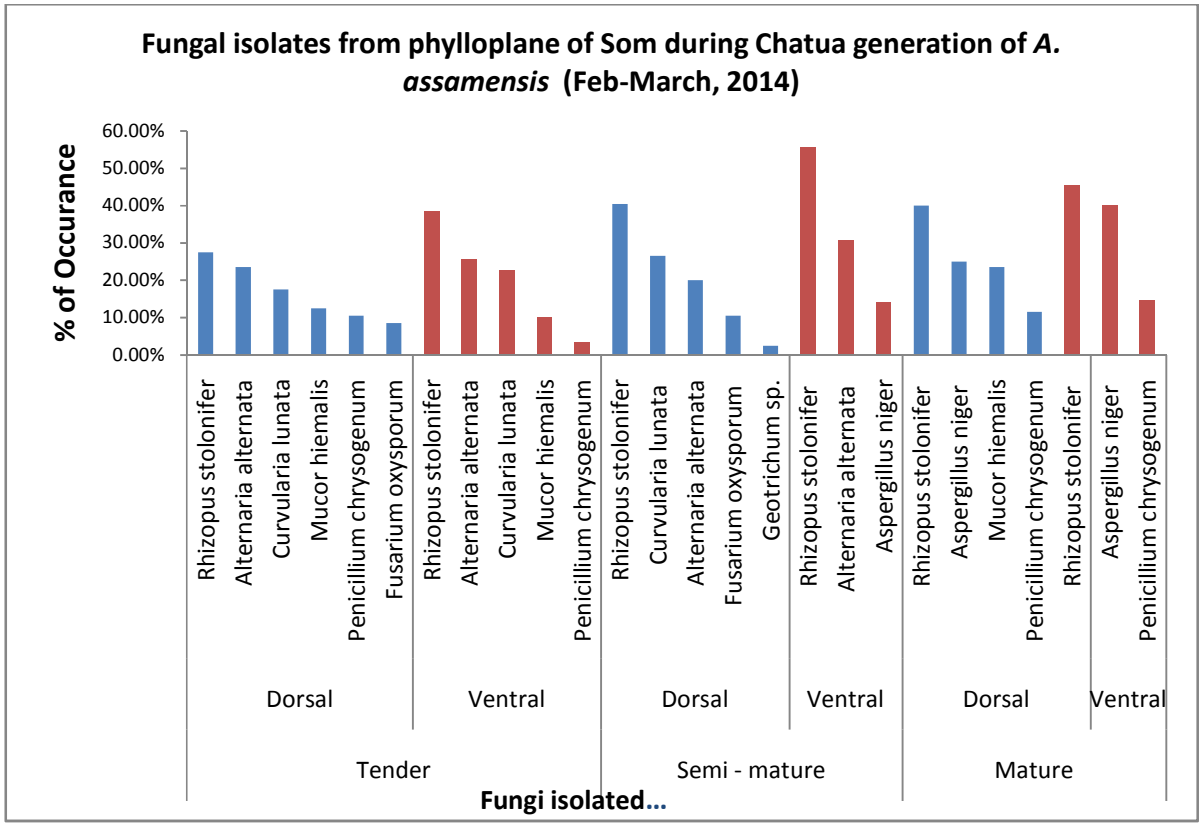
Table.4. Fungal isolates from phylloplane of Som during Jethua generation of *A. assamensis* (April-May, 2014)

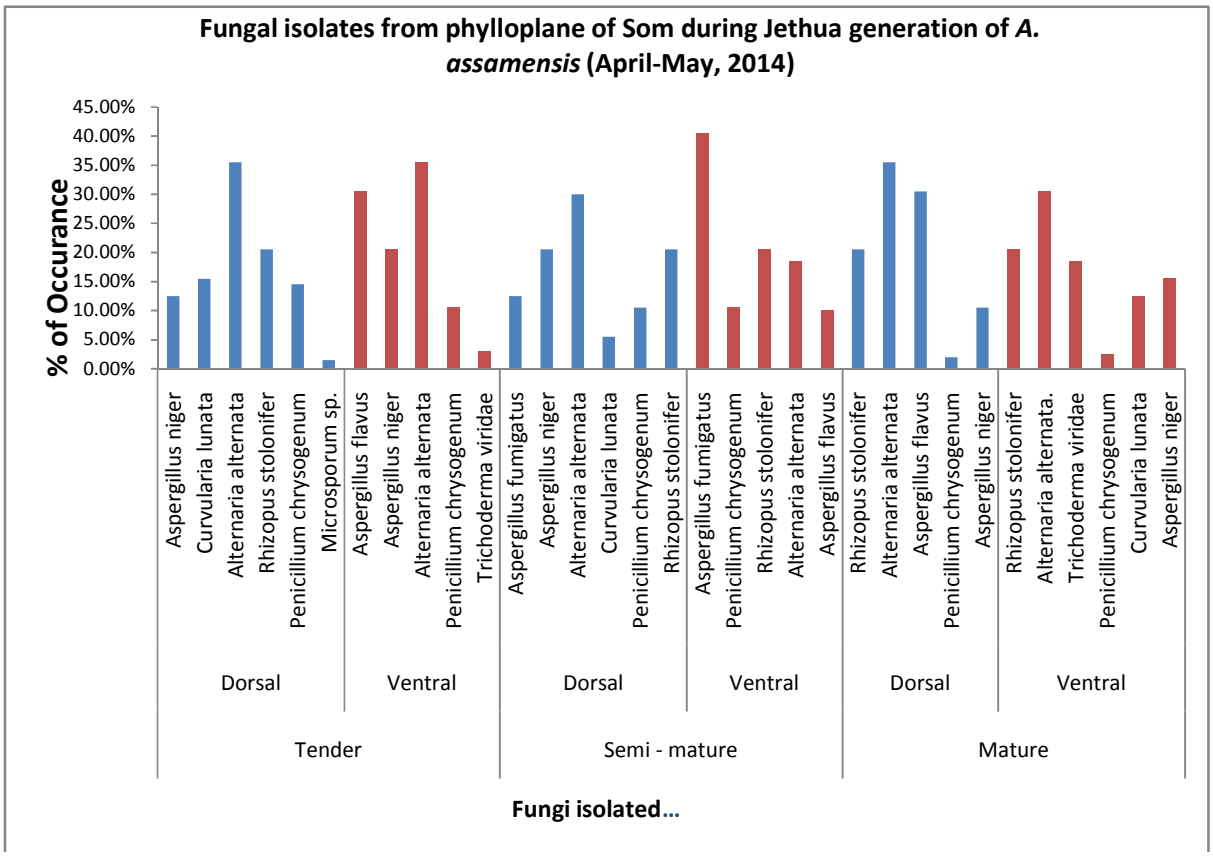
Climatic factors	Status of leaves	Types of surface	No. of leaves	Fungi isolated	% of Occurence
April	Tender	Dorsal	10	*Aspergillus niger*	12.5%
				Curvularia lunata	15.5%
				Alternaria alternata	35.5%
Max Min				*Rhizopus stolonifer*	20.5%
				Penicillium chrysogenum	14.5%
Temp 36°C 19°C				*Microsporum sp.*	1.5%
RH 84% 32%		Ventral	10	*Aspergillus flavus*	30.5%
				Aspergillus niger	20.5%
Rainfall - 220 ml.				*Alternaria alternata*	35.5%
				Penicillium chrysogenum	10.5%
Total rainy days- 5				*Trichoderma viridae*	3.0%
	Semi - mature	Dorsal	10	*Aspergillus fumigatus*	12.5%
				Aspergillus niger	20.5%
				Alternaria alternata	30.0%
May				*Curvularia lunata*	5.5%
				Penicillium chrysogenum	10.5%
Max Min				*Rhizopus stolonifer*	20.5%
		Ventral	10	*Aspergillus fumigatus*	40.5%
Temp 38°C 20° C				*Penicillium chrysogenum*	10.5%
				Rhizopus stolonifer	20.5%
RH 92% 55%				*Alternaria alternata*	18.5%
				Aspergillus flavus	10.0%
	Mature	Dorsal	10	*Rhizopus stolonifer*	20.5%
				Alternaria alternata	35.5%
				Aspergillus flavus	30.5%
Rainfall-2720 ml.				*Penicillium chrysogenum*	2.0%
Total rainy days- 14				*Aspergillus niger*	10.5%
		Ventral	10	*Rhizopus stolonifer*	20.5%
				Alternaria alternata.	30.5%
				Trichoderma viridae	18.5%
				Penicillium chrysogenum	2.5%
				Curvularia lunata	12.5%
				Aspergillus niger	15.5%

Table.5. Observation of fungal isolates from phylloplane of Som during Aherua generation of *A. assamensis* (June-July, 2014)

Climatic factors	Status of leaves	Types of surface	No. of leaves	Fungi isolated	% of Occurance
June	Tender	Dorsal	10	*Rhizopus stolonifer*	45.0 %
				Aspergillus niger	30.0 %
Max Min				*Aspergllus flavus*	5.5 %
Temp 30°C 22°C				*Curvularia lunata*	4.5 %
RH 92% 58%				*Aspergillus fumigatus*	15.0 %
Rainfall – 3710ml		Ventral	10	*Rhizopus stolonifer.*	50.0 %
Total rainy days- 17				*Aspergillus niger*	15.0 %
				Aspergillus flavus	10.0 %
				Aspergillus fumigatus	25.0 %

July Max Min Temp 38°C 28°C RH 92% 58% Rainfall - 2510 ml Total rainy days- 15	Semi - mature	Dorsal	10	*Rhizopus stolonifer* *Aspergillus fumigatus* *Aspergillus niger* *Aspergillus flavus*	39.5 % 20.0 % 10.0 % 25.0 %
		Ventral	10	*Rhizopus stolonifer* *Aspergillus niger* *Curvularia lunata* *Aspergillus flavus* *Aspergillus fumigatus*	55.0 % 25.0 % 10.0 % 5.5 % 4.5 %
	Mature	Dorsal	10	*Rhizopus stolonifer* *Aspergillus niger* *Aspergillus flavus* *Aspergillus fumigatus*	50.0 % 35.0 % 5.0 % 10.0%
		Ventral	10	*Rhizopus stolonifer* *Aspergillus niger* *Curvularia lunata* *Penicillium Chrysogenum* *Aspergillus fumigatus*	45.0 % 25.0 % 15.0 % 10.0 % 5.0 %




Fungal isolates from phylloplane of Som during Jethua generation of A. assamensis (April-May, 2014)
% of Occurance
45.00%
40.00%
35.00%
30.00%
25.00%
20.00%
15.00%
10.00%
5.00%
0.00%
Aspergillus niger
Curvularia lunata
Alternaria alternata
Rhizopus stolonifer
Penicillium chrysogenum
Microsporum sp.
Aspergillus flavus
Aspergillus niger
Alternaria alternata
Penicillium chrysogenum
Trichoderma viridae
Aspergillus fumigatus
Aspergillus niger
Alternaria alternata
Curvularia lunata
Penicillium chrysogenum
Rhizopus stolonifer
Aspergillus fumigatus
Penicillium chrysogenum
Rhizopus stolonifer
Alternaria alternata
Aspergillus flavus
Rhizopus stolonifer
Alternaria alternata
Aspergillus flavus
Penicillium chrysogenum
Aspergillus niger
Rhizopus stolonifer
Alternaria alternata.
Trichoderma viridae
Penicillium chrysogenum
Curvularia lunata
Aspergillus niger
Dorsal
Ventral
Dorsal
Ventral
Dorsal
Ventral
Tender
Semi - mature
Mature
Fungi isolated…

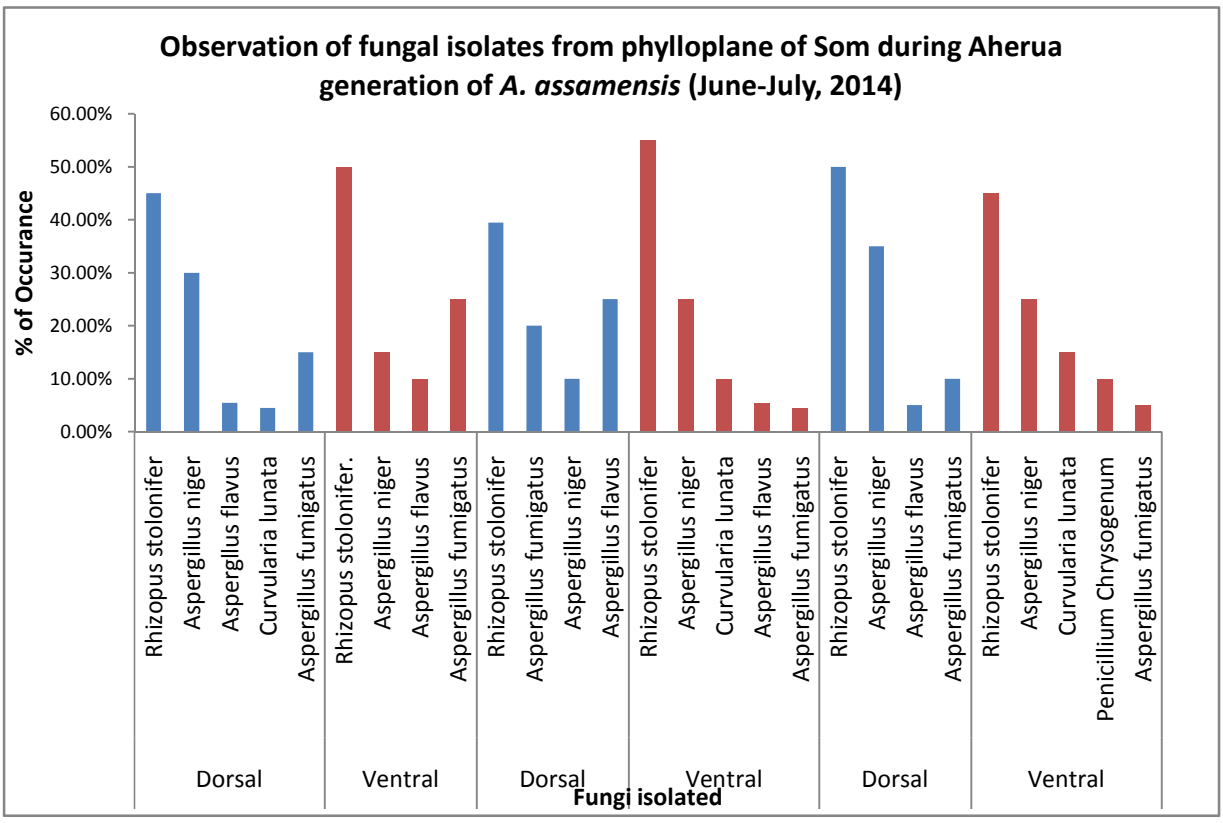
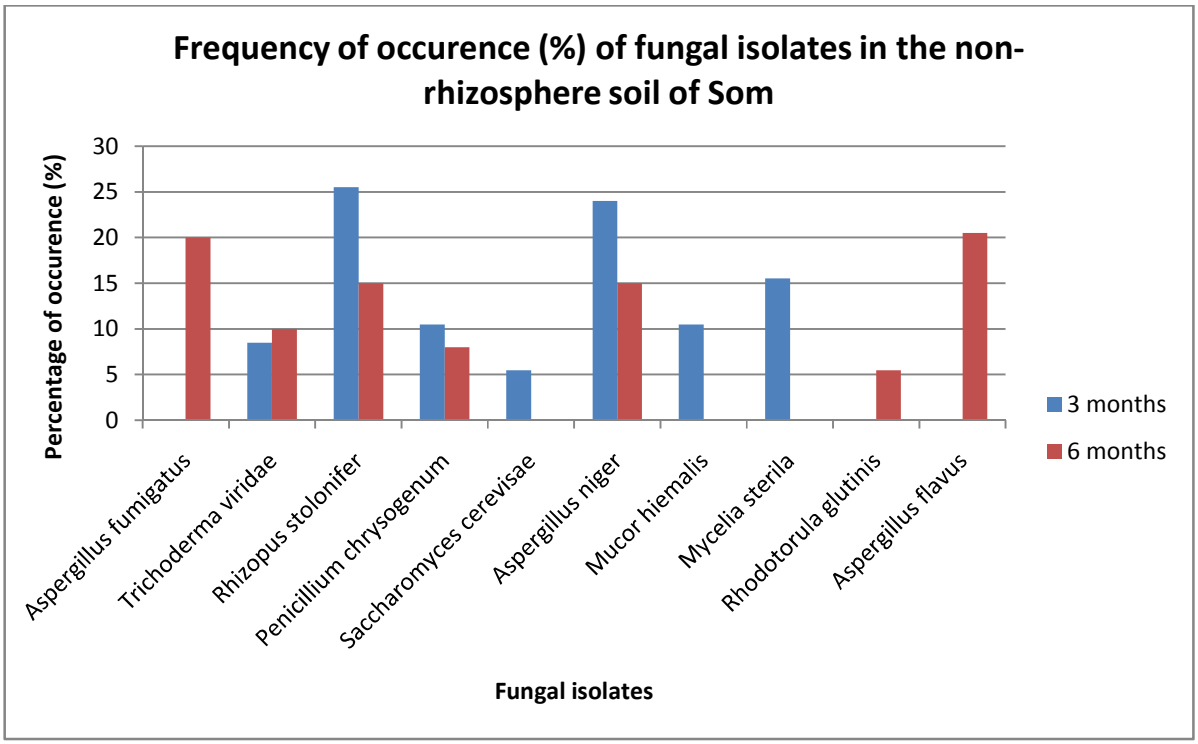

Observation of fungal isolates from phylloplane of Som during Aherua generation of A. assamensis (June-July, 2014)
% of Occurance
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
Rhizopus stolonifer
Aspergillus niger
Aspergillus flavus
Curvularia lunata
Aspergillus fumigatus
Rhizopus stolonifer.
Aspergillus niger
Aspergillus flavus
Aspergillus fumigatus
Rhizopus stolonifer
Aspergillus fumigatus
Aspergillus niger
Aspergillus flavus
Rhizopus stolonifer
Aspergillus niger
Curvularia lunata
Aspergillus flavus
Aspergillus fumigatus
Rhizopus stolonifer
Aspergillus niger
Aspergillus flavus
Aspergillus fumigatus
Rhizopus stolonifer
Aspergillus niger
Curvularia lunata
Penicillium Chrysogenum
Aspergillus fumigatus
Dorsal
Ventral
Dorsal
Ventral
Dorsal
Ventral
Fungi isolated

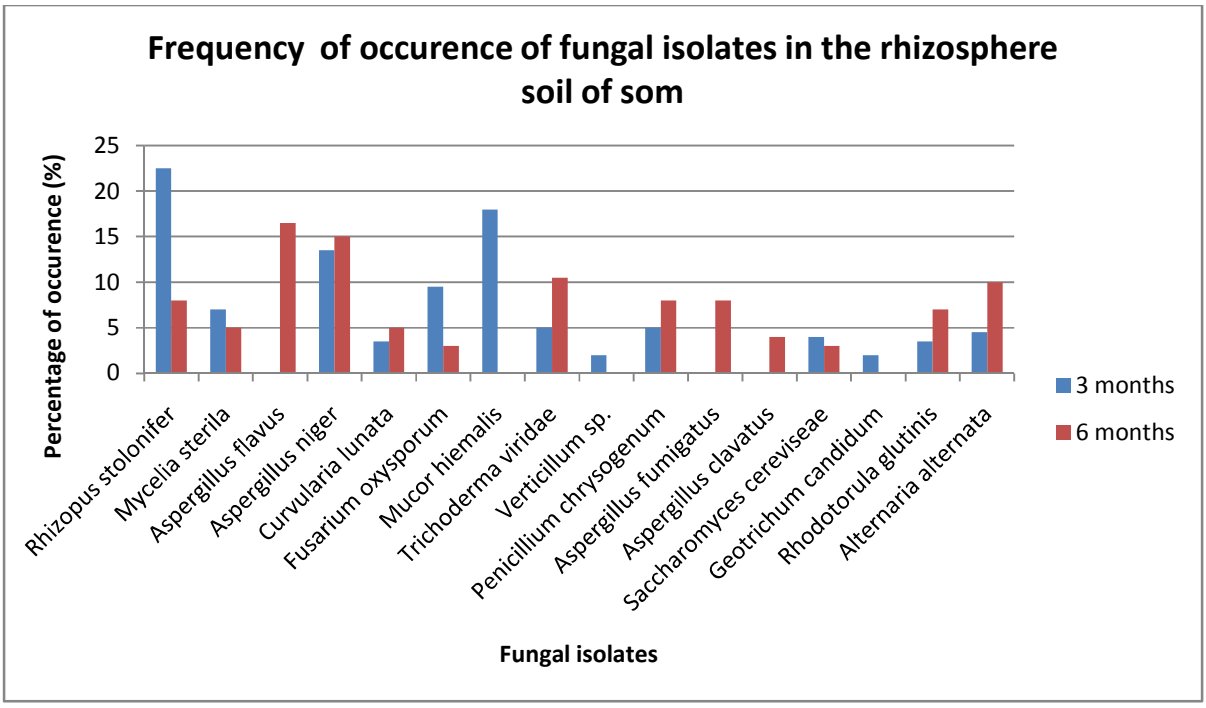
Table .6. Frequency of Occurence (%) of fungal isolates in the Non-rhizosphere soil of Som

S. No.	Fungal isolates	Age of Plants (months)	
		3	**6**
1	*Aspergillus fumigatus*	-	20.0
2	*Trichoderma viridae*	8.50	10.0
3	*Rhizopus stolonifer*	25.50	15.0
4	*Penicillium chrysogenum*	10.50	8.0
5	*Saccharomyces cereviseae*	5.50	-
6	*Aspergillus clavatus*	-	5.50
7	*Aspergillus niger*	24.0	15.0
8	*Mucor hiemalis*	10.50	-
9	*Mycelia sterile (white)*	15.50	-
10	*Rhodotorula glutinis*	-	5.50
11	*Aspergillus flavus*	-	20.50

Table.7. Frequency of Occurence (%) of fungal isolates in the Rhizosphere soil of Som

S.No.	Fungal isolates	Age of Plants (months)	
		3	**6**
1	*Rhizopus stolonifer*	22.5	8.0
2	*Mycelia sterile (white)*	7.0	5.0
3	*Aspergillus flavus*	-	16.5
4	*Aspergillus niger*	13.5	15.0
5	*Curvularia lunata*	3.5	5.0
6	*Fusarium oxysporum*	9.5	3.0
7	*Mucor hiemalis*	18.0	-
8	*Trichoderma viridae*	5.0	10.5
9	*Verticillium sp.*	2.0	-
10	*Penicillium chrysogenum*	5.0	8.0
11	*Aspergillus fumigatus*	-	8.0
12	*Aspergillus clavatus*	-	4.0
13	*Saccharomyces cereviseae*	4.0	3.0
14	*Geotrichum candidum*	2.0	-
15	*Rhodotorula glutinis*	3.5	7.0
16	*Alternaria alternata*	4.5	10.0





RESULT AND DISCUSSION

During the study period Maximum temperature reported on the month of May and July and minimum temperature recorded in the month of February with relative humidity maximum in the month of May, June & July and minimum in the month of March. The district received a highest amount of rainfall in the month of June with a total rainy days of 17 days and minimum rainfall in the month of March. During the study period, a total of 7 species of fungi were isolated and identified from air on the basis of colony morphology, mycelia, sporangiophore and spore structure from different groups. Among them

Aspergillus niger, *Rhizopus stolonifer* and *Curvularia lunata* species dominate over the som air flora throughout the study period.The other species includes *A. flavus*, *Alternaria alternata*, *Cladosporium cladosporioides*, *Fusarium oxysporium* and *Penicillium chrysogenum*. It is seen that on the month of Feb-march occurrence of *A.niger* is higher followed by *Rhizopus stolonifer*, *Alternaria alternata*, *Cladosporium cladosporioides*, *Fusarium oxysporum*, *Curvularia lunata*. During April-May occurrence of *Rhizopus stolonifer* is higher followed by *Aspergillus flavus* which were absent in the month of Feb-march. In the month of June-July it was seen that the occurrence of *Aspergillus flavus* were higher. While few species *A. alternata, C. cladosporioides, C. lunata, F. oxysporum* showing a decreasing pattern from Feb to July.

While from the som phylloplane a total of 12 fungal species were isolated from different types of leaves of Som viz. tender, semi mature and mature based on shape and size from both the dorsal and ventral surface of the leaves. During February-March, *Rhizopus stolonifer*, during April-May *Alternaria alternata* and during June-July again *Rhizopus stolonifer* dominates the phylloplane mycoflora.

On the other hand from non-rhizosphere soil a total of 11 fungal species were isolated. Among which *Rhizopus stolonifer*, *Aspergillus niger*, *Penicillium chrysogenum* and *Trichoderma viridae* were the dominant fungal species for all the age group plants. The other genera includes *A. fumigatus, Mycelia sterila(white), A. flavus, S. cereviseae, A. clavatus, Aspergillus sp., Mucor hiemalis*, and *Rhodotorula glutinis.* Similarly from the rhizosphere soil a total of 16 fungal species were isolated among which *Rhizopus stolonifer*, *Penicillium chrysogenum*, *A. niger* , *Curvularia lunata*, *Fusarium oxysporium*, *Alternaria alternata*, *Saccharomyces cereviseae*, *Rhodotorula glutinis*, *Mycelia sterile(white)* and *Trichoderma viridae* dominates all the fungal flora for all the age group plants. The other genera includes *A. flavus*, *A. fumigates*, *A. clavatus*, *Mucor hiemalis*, *Verticillium sp.*, and *Geotrichum sp.* Both in the non-rhizopsphere and rhizosphere of som Rhizopus stolonifer is dominant over other fungi among 3 months age group of plants, while among 6 months of age groups of plants occurrence of the Aspergillus flavus were dominant over other mycoflora.

From the result it is clearly observed that mainly *Rhizopus sp.* and *Aspergillus spp.* completely dominates all the air, phylloplane & soil Mycoflora of Som. It also indicates that seasonal as well as monthly variation of climatic factors such as temperature, humidity & rainfall etc of the Goalpara district, Assam affect the distribution of air, phylloplane and both the rhizosphere & non rhizosphere soil mycoflora of Som. It is also seen that few fungi were available only for a particular season in a particular climatic condition while some other prevail in the air, phylloplane, nonrhizosphere & rhizosphere soil throughout the study period with variation on the occurrence.

CONCLUSION

The initial studies over the study period gives qualitative & quantitative data on air, phylloplane & soil mycoflora over the Som, the host plant of Muga silk worm with the seasonal variation and climate change. The systematic studies will lead to the illustration of identification characters of pathogenic and non-pathogenic fungus occurring in Som ecosystem. The systematic characters will help to develop diagnostic keys supplemented with information on symptoms of diseases, its extent of damage, life cycle, and distribution and management strategies. More works will be carried and communicated due course of time.

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