Plant Archives Volume 20 No. 1, 2020 pp. 731-737 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

STUDY ON DIVERSITY OF PHYLLOPLANE FUNGI ASSOCIATED WITH THE DRIED-DECAYING LEAVES OF *SOLANUM NIGRUM* L. AND INHIBITION OF CONIDIAL GERMINATION OF *ALTERNARIA ALTERNARA* BY THE PHYLLOPLANE FUNGI

Jyoti Chauhan[1]* and D.K. Jain[2]

[1*]Department of Botany, Kurukshetra University, Kurukshetra (Haryana), India.
[2] Department of Botany, Meerut College Meerut (U.P.), India.

Abstract

Leaf surfaces which termed as phylloplane are the main photosynthetic part of the plant and also constitute a highly diverse and complex habitat of Algae, bacteria filamentous fungi, nematodes, and yeast. Bacteria are the primary colonizer of young leaves while fungal species present during the later stages. These microorganisms may be beneficial or harmful to their host plants. The phylloplane topography, microclimatic conditions around the phylloplane and exudates of leaf have their impact on growth and development of microbial communities of phylloplane. Phylloplane microfungi of dried-decaying leaves of *Solanum nigrum* L. was investigated in this work. Deuteromycotina were the dominant fungal component. Biocontrol agents widely utilized for the control of pathogens. Maximum inhibition was exhibited by the *Chaetomium globosum*.

Key words : Phylloplane, microbial communities, *Solanum nigrum*, Deuteromycotina, *Chaetomium globosum*

Introduction

Phylloplane is that natural leaf surface habitat which provides shelter to heterogeneous microbial population of both pathogenic and non-pathogenic microorganisms. The leaf surface resident fungal species are referred as Phylloplane fungi. Resident and casuals are the two categories of phylloplane fungi (Leveau, 2009, Prabakaran *et al*., 2011; Saha *et al*., 2013). The resident fungal species can multiply on the healthy leave surfaces without influencing the host but the phylloplane casual species colonize the leaf surface but cannot multiply themselves (Saha *et al*., 2013). Algae, protozoa and nematodes also colonize leaf surfaces (Kim *et al*., 2013; Bhuyan, *et al*., 2013; Manjit *et al*., 2014;) and the phylloplane microbial population interacted in various manner (Vorholt, 2012; Bulgarelli *et al*., 2013; Brader *et al*., 2017) with the host plant. Different types of chemical subtances produced by the phylloplane fungi and provide protection to their host against the deleterious effect of pathogens (Innerebner *et al*., 2011; Ritpitakphong *et al*., 2016).

***Author for correspondence :** E-mail: drjyotichauhan2013@gmail.com

The phylloplane microflora influenced the plant populations and ecosystem functioning. Competition among the native microbial communities of plants and fungal pathogen exhibited (Brandl *et al*., 2013). The fluctuating environmental, geographical, seasonal conditions largely affected the phylloplane microorganisms (Knief *et al*., 2010; Wellner *et al*., 2011; Rastogi *et al*., 2012; Copeland *et al*., 2015; Ding and Melchner, 2016). Various scientist have worked on the phylloplane fungi (Prabakaran and Pannerselvam, 2011; Grbic *et al*., 2015; and Waill *et al*.,2016).

The decomposition of leaf litter or dried-decaying leaves is a significant nutrient cycling process and plays a major role in balancing the various components of ecosystem by providing the energy and nutrients. The decomposition of dried-decaying leaves provides the nutrients which are essential for the growth of plants and productivity of ecosystem. Fungi are the important and primary decomposers of nature that decompose dead remains of plants and animals. Dead leaves of plants form the litter layer of the surface of soil that decomposes

by fungal activity. Plant litter served as a large source of organic carbon content of soils. The process of decomposition is highly complex that includes mineralization and transformation of organic matter (Voriskova and Badrian, 2013).

Phylloplane microorganisms interact with each other by means of antibiosis, competition and parasitism and due to this interaction they protect the plants against the pathogens (Stromberg *et al.,* 2000). Plant pathogens cause severe damage to the agricultural crops. Every year farmers faces the severe economic loss due to the damage created by fungal plant pathogens. For the control of these plant pathogens various types of fungicides are largely used by the farmers in the agricultural crops due to which environment become polluted and the biotic component suffers so many severe diseases. Therefore biocontrol agents are used for the control of plant pathogens (Chisholm *et al.,* 2006; Heydari, 2007). Mycoparasitism and hyperparasitism are the two main characteristics by which these biocontrol agents protect the plants against the pathogens (Ghorbanpour *et al.,* 2018). For the biocontrol of plant pathogens, phylloplane antagonistic microorganism are used worldwide as an alternative to the chemical bactericides and fungicides and these are also ecofriendly (Prakasam and Sharma, 2012). *Alternaria alternata* is common fungal pathogen of the foliar plant diseases.

Therefore, the present study based on the two main objectives:

1. Isolation of phylloplane fungi of dried-decaying leaves *Solanum nigrum,* and
2. To study the antagonistic effect of phylloplane fungi on the conidial germination of *Alternaria alternata*

Materials and Methods

Solanum nigrum (makoi) plant belongs to family Solanaceae that has various medicinal properties, used as an experimental plant. The seeds were collected from mature berries and were sown by maintaining the proper distance at the were collected and then brought to the laboratory. For the isolation of fungal mycoflora following methods was used.

Dilution plate method

Twenty-five dried-decaying leaves of the plant were collected. 100 discs of 6mm diameter were cut by sterilized corkborer. These were placed in a sterilized conical flask (Borosil) of 250 ml capacity containing 100 ml of sterilized distilled water. The flasks were hand-shaken for 20 min. to get a homogenous suspension of the fungal propagules. From this suspension with 90 ml of sterilized distilled water. From this dilution, 1ml aliquot of the suspension per Petri dish was added into each of ten sterilized Petri dishes of 9 cm diameter. Approximately, 15 ml of molten, cool, sterilized Czapek's Dox yeast extract agar (CDYA) medium was added to the inoculated Petri dishes. The Petri dishes were incubated for 7 days at 25± 1^0C after which colonies of fungi were identified, counted and recorded. Results were expressed as average number of colonies cm^{-2} leaf surface of each fungus. Quantitative estimation of total fungal population was made by the following formula:

Total fungal population cm^{-2} leaf surface =

$$\frac{\text{Average no. of colonies} \times \text{dilution}}{\text{The total area of leaf discs}}$$

This method was used to isolate detachable propagules present on the leaves.

Media Used

Initially, a few media were tried during the present investigation such as PDA (Potato Dextrose agar) and CDYA (Czapek's Dox yeast agar). The CDYA medium was selected to get the maximum number of fungi. Composition of CDYA medium: Sucrose, 30g; $NaNO_3$, 3g; $K_2HPO_4.7H_2O$, 1g; $MgSO_4.7H_2O$, 0.5 g; FeSO4.7H2O, 0.01 g; agar-agar, 20 g; yeast extract, 1g; distilled water, 1 liter, supplemented with rose Bengal (33 mg/liter) and streptomycin(30 µg/ml).

Antagonistic effect of phylloplane fungi studied as

Effect of supernatants of conidial suspension of phylloplane fungi on conidial germination of *Alternaria alternata* : The following leaf surface fungi were maintained in pure culture on sterilized PDA slants at 25± 1^0C for the study: *Aspergillus flavus, A. nidulans, A. niger, Chaetomium globosum, Cladosporium cladosporioides, C. herbarum, Curvularia lunata, Drechslera australiensis, Fusarium oxysporum, Myrothecium indicum, Nigrospora sphaerica, Phoma humicola* and *Trichoderma lignorum.* Conidia from 10-day old cultures were scraped and one loop full of conidia of each fungus was stored in 5 ml of sterilized distilled water for 97 hr. The suspension were centrifuged at 10000 r.p.m. for 15-20 min. at room temperature. The supernatants were collected in sterilized test tubes. Hanging drop method was used to study the germination of the conidia and germ tube growth. Three replicates were taken for each fungus. Sterilized distilled water was used as control.

Results and Discussion

Table 1 showed that the maximum number of fungi

obtained in the collection made when the dried-decaying leaves were of 135 days and further decline in the number of phylloplane mycoflora was noticed at 165 days old leaves. The dominant fungi were *Fusidium viride, Cladosporium cladosporioides, Fusarium oxysporum,Curvularia lunata, Alternaria alternata, Aspergillus flavus, A. niger, Rhizopus arrhizus,* and *Trichoderma lignorum.*The other fungi viz. *Drechslera australiensis, Phoma humicola,* and *Trichoderma lignorum* were present consistently throughout the investigation period. *Alternaria humicola, Aspergillus luchensis, A. sulphureus, A. sydowi, Drechslera hawaiiensis, Pithomyces chartarum* and sterile hyphae (black) appeared on 105 days old leaves and showed their presence up to last stage of sampling. *Aspergillus candidus, A. fumigatus, Alternaria humicola,* and *Choanephora cucurbitarum* were isolated from the last two samplings.

This method provides valuable data regarding quantitative and qualitative features of mycoflora. The dried decaying leaves of the plant harbors largest mycoflora because the leaves at this stage of partial decomposition have high fungal contamination from the soil. *Aspergillus flavus, A. niger,* and some other Aspergilli appeared as soil contaminants on dried decaying leaves of 105-135- days old. Some members of Deuteromycotina were found to colonize 105-135- day old leaves and they were *Alternaria humicola, A. tenuissima, Fusarium moniliforme, Humicola brevis, Myrothecium indicum, Phoma hiberica, Papulospora sp., Spicaria silvatica, Stemphylium botryosum, Robillarda* sp. and *Sphaeronaema spinella.*

Actinomucor repens, Choanephora cucurbitarum, Circinella simplex, and *Cunninghamella echinulata* were obtained by dilution plate method dried decaying leaves. Similar other reports suggest that a critical study of the factor governing the appearance of the Zygomycotina during advanced stages of decomposition would be of great interest (Sumithra *et al.*, 2016; Voriskova and Baldrian, 2013).

It is evident from table 2 that supernatants of spore suspensions of almost all the test phylloplane fungi inhibited germination of conidia to varying extent. Maximum inhibition was in case of *Chaetomium globosum*, followed by *Aspergillus niger, Fusarium oxysporum, Myrothecium indicum* and *Aspergilhas flavus*. Germ tube growth was inhibited by all test fungi. Maximum inhibition was caused by *Chaetomium globosum*. Some fungi, e.g. *Nigraspora sphaerica, Trichoderma lignorum* and *Cladosporium herbarum* which could not inhibit the conidial germination, but inhibited germ tube growth.

Gveroska and Ziberoski (2012) reported that *Alternaria alternata* inhibited by *T. harzianum.* Gorawar and Hedge (2006), Rajkonda *et al*., (2011) also reported the inhibition of *Alternaria alternata* by Trichoderma sp. *Trichoderma harzianum* and *Bacillus subtilis, B.*

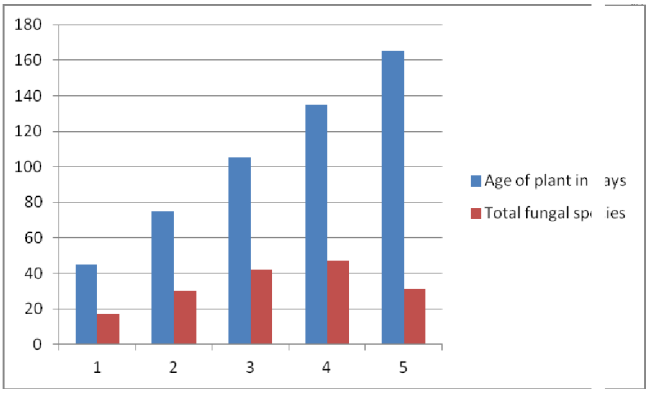

Fig.1: Total fungal species isolated from dried-decaying leaves of different ages.

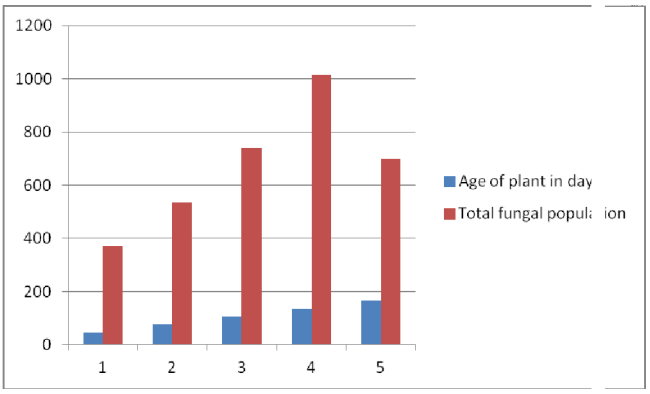

Fig. 2: Total fungal population isolated from dried-decaying leaves of different ages.

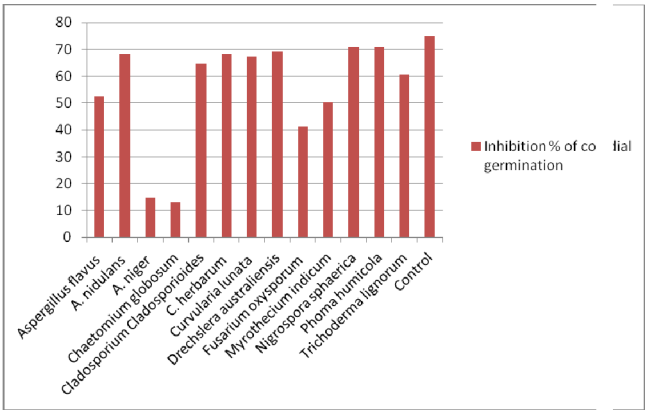

Fig. 3: Effect of supernatants of some phylloplane fungi on condial germination percentage of *Alternaria alternata.*

Table 1: Mycoflora isolated from dried-decaying leaves of *Solanum nigrum* by Dilution plate method (the number against a species represents the average number of colonies cm^{-2} leaf surface).

Name of Fungi	Age of plant in days				
	45	75	105	135	165
1. Zygomycotina					
Actinomucor repens Schostakowitsch	7	_	_	_	6
Choanophora cucurbitarum (Berkeley and Ravenel) Thaxter	_	_	_	14	16
Circinella simplex Van Tighem	_	13	14	_	_
Cunninghamella echinulata Thaxter	_	8	9	13	4
Mucor racemosus Fresenius	13	_	14	16	18
Rhizopus arrhizus Fischer	10	16	18	32	30
Syncephalastrum racemosus (Cohn.) Schrocter	_	_	_	10	12
2. Ascomycotina					
Aspergillus nidulans (Eidam) Winter	_	13	13	15	_
Melanospora sp. Corda (Frontispiece)	_	12	14	13	_
3. Deuteromycotina					
Alternaria alternata (Fr.) Keissler	30	34	36	39	36
A. humicola Oudemans	_	_	18	20	23
A. tenuissima (Kunze ex pers.) Wiltshire	_	_	12	_	_
Aspergillus candidus Link	_	_	_	6	10
A. clavatus Desmazieres	_	4	6	_	_
A. flaviceps Bainer and Sartory	_	_	_	3	_
A. flavus Link ex Fries	26	28	30	40	43
A. fumigatus Fresenius	_	_	_	39	36
A. humicola Chaudhuri	_	_	_	14	10
A. luchuensis Inui	_	_	30	35	40
A. niger Van Tieghem	26	29	30	40	40
A. repens (Corda) de bary	_	_	7	_	_
A. sulphureus (Fresenius) Thom. and Church	_	_	8	10	12
A. sydowi (Bainer and Sartory)Thom. and Church	_	_	22	38	20
A. terreus Thom.	11	16	_	18	_
Beltrania rhombica O. Penzig	_	10	_	_	_
Candida albicans Berkhout	_	2	8	10	_
Chaetomella sp. Fuckel	_	11	16	_	_
Cladosporium cladosporioides (Fresen.) de Vries	50	54	60	60	57
C. herbarum (Pers.) Link ex Gray	16	16	_	_	_
Coniothyrium sp. Corda	_	5	7	18	_
Curvularia lunata (Walker) Boedijn	36	35	40	49	38
C. pallescens Boedijn	_	_	6	_	_
Drechslera australiensis (Bugnicourt) Subram. and Jain ex Ellis	16	18	18	24	16
D. halodes (Drechsler) Subram. and Jain	_	7	_	9	_
D. hawaiiensis (Bugincourt) Subram. and Jain	_	_	6	10	12
D. state of *Cochliobolus bicolor* Paul and Parbey	_	_	_	9	7
D. state of *C. spicifer* Nelson	_	3	7	_	_
Epicoccum purpurascens Ehrenb. ex Schlecht.	_	13	12	7	_
Fusarium moniliforme Sheldon	_	_	16	20	_
F. oxysporum Schlechtendahl	38	38	42	50	48

Table 1 contd....

Table 1 contd....

Name of Fungi	Age of plant in days				
	45	75	105	135	165
Fusidium viride Grove	48	55	60	76	60
Humicola brevis (Gilman and Abbott) Gilman	_	_	16	21	_
Myrothecium indicum Rama Rao	_	_	18	23	_
Nigrospora sphaerica (Sacc.) Mason	_	16	14	23	10
Papulospora sp. Preuss	_	_	10	15	_
Penicillium frequentans Westling	13	13	_	14	16
P. nigricans Bainer	_	10	_	18	12
Phoma hibernica Grimes, O' Conner and Cummins	_	_	6	8	_
P. humicola Gilman and Abbott	6	9	10	20	10
Pithomyces chartarum (Berk. and Curt.) M.B. Ellis	_	_	11	12	8
Sclerotium sp. Tode	_	_	_	_	6
Spicaria silvatica Oudemans	_	_	14	17	_
Stemphylium botryosum Wallroth	_	_	7	12	_
Sterile hyphae (black)	_	_	16	18	26
Sterile hyphae (white)	10	16	_	_	_
Torula herbarum (Pers.) Link ex Gray	_	10	_	16	_
Trichoderma lignorum (Tode) Harz.	16	20	24	25	17
Ulocladium sp. Precuss	_	_	3	6	_
Verticillium albo-atrum Reinke and Berthold	_	_	10	10	_
Total fungal species	17	30	42	47	31
Total fungal population					
(Average number of colonies of fungi cm^{-2} leaf surface)	372	534	738	1015	699

Table 2: Effect of supernatants of some phylloplane fungi on conidial germination percentage and germ tube length (µm) of *Alternaria alternata* (incubated for 12 hr at 25 + 1°C).

Test Fungi	Conidial germination %	Value of t	% inhibition	Mean germ tube length (µm)
Alternaria alternata	69.8±2.65	1.46	4.2	160
Aspergillus flavus	52.4±1.45	14.16**	26.3	86
A. nidulans	68.1±1.62	1.00	18.7	170
A. niger	14.7±1.00	46.82**	80.2	48
Chaetomium globosum	13.1±0.92	49.03**	82.3	42
Cladosporium cladosporioides	64.8±3.15	2.54	9.4	132
C. herbarum	68.0±2.03	2.18	20.2	54
Curvularia lunata	67.4±4.01	0.06	9.8	150
Drechslera australiensis	69.3±2.52	1.38	3.9	182
Fusarium oxysporum	41.3±1.21	23.13**	42.3	48
Myrothecium indicum	50.2±1.20	15.19**	29.5	63
Nigrospora sphaerica	71.0±3.72	0.52	2.0	102
Phoma humicola	70.9±4.72	0.54	2.2	116
Trichoderma lignorum	60.4±3.95	4.79	15.5	98
Control	75.0±1.95	——	0	162

*Significant at 5% level.

**Significant at 1% level.

pumilus and *B. megaterium* were found to be more effective against *A. alternata* (Tozlu *et al.*, 2018).

Conclusion

The Phylloplane mycoflora of dried-decaying leaves of *Solanum nigrum* was isolated by Dilution plate method. During this study, it was observed that *Fusidium viride, Cladosporium cladosporioides, Fusarium oxysporum, Alternaria alternata, Aspergillus flavus, Curvularia lunata, Aspergillus niger,* and *Rhizopus arrhizus* were the dominant fungal species present on the dried-decaying leaves of *Solanum nigrum.* Chemical fungicides are harmful for the human being and other components of ecosystem, due to their biomagnification these causes serious threat to biodiversity, recently, the biological control agents are used widely for the control of plant diseases and provide resistance against the plant pathogens.

Acknowledgement

I would like to convey my sincere thanks to Dr. P.N. Singh for their valuable suggestions and constant encouragement.

References

Angela, G. and S.P. Usha (2016). Studies on phylloplane mycoflora and medicinal plants. *Int. J. Adv. Tech. Eng. Sci.*, **4(9):** 230-245.

Bhuyan, P.M., S.P. Sandilya and D.K. Gogoi (2013). Phyllosphere microflora of Muga silkworm host plant *Persea bombycina* Kost (Som) leaves in Jorhat District of Assam, India. *Int.Res.J.Biol.Sci.*, **2(12):** 60-65.

Brandl, M.T., C.E. Cox and M. Teplitski (2013). *Salmonella* interactions with plants and their associated microbiota. *Phytopathology*, **103(4):** 316–325.

Brader, G., S. Compant, K. Vescio, B. Mitter, F. Trognitz, L.J. Ma and A. Sessitsch (2017). Ecology and genomic insights into plant-pathogenic and plant-nonpathogenic endophytes. *Annu. Rev.Phytopathol.*, **55(1):** 61–83.

Bulgarelli, D., K. Schlaeppi, S. Spaepen, E.V.L.V. Themaat and P. Schulze-Lefert (2013). Structure and functions of the bacterial microbiota of plants. *Annu. Rev.Plant. Biol.*, **64(1):** 807–838.

Chisholm, S.T., G. Coaker, B. Day and B.J. Staskawicz (2006). Host-microbe interactions : Shaping the evolution of plant immune response. *Cell.*, **124**: 803-814.

Copeland, J.K., L. Yuan, M. Layeghifard, P.W. Wang and D.S. Guttman (2015). Seasonal community succession of the phyllosphere microbiome. *Mol. Plant. Microbe. Interact.*, **28(3):** 274–285.

Ding, T. and U. Melchner (2016). Influences of plant species, season and location on leaf endophytic bacterial communities of non-cultivated plants. *PLoS.ONE.*, **11(3):** e0150895.

Ghorbanpour, M., M. Omidvari, P. Abbaszadeh-Dahaji, R. Omidvar and K. Kariman (2018). Mechanisms underlying the protective effects of beneficial fungi against plant diseases. *Biol. Control.*, **117:** 147–157.

Gorawar, M.M. and R.Y. Hedge (2006). Role of biocontrol agents in management of foliar diseases of turmeric. *Int. J. Plant. Sci.*, **1(2):** 145-146.

Grbic, M.L., M. Stupar, N. Unkovic, V.J. Jelena, B. Stevanovic and D. Grubisic (2015). Diversity of microfungi associated with phyllosphere of endemic Serbian plant *Nepeta tanjensis* Diklic & Milojevic. *Braz. J. Bot.*, **38(3):** 597–603.

Gveroska, B. and J. Ziberoski (2012). *Trichoderma harzianum* as a biocontrol agent against *Alternaria alternata* on tobacco. *Appl. Techno. Inovations.*, **7(2):** 67-76.

Heydari, A. (2007). Biological control of Turfgrass fungal Diseases. *In : Turfgrass management and physiology, pessarakli,* M.(Ed.). CRC Press, Florida, USA.

Innerebner, G., C. Knief and J.A. Vorholt (2011). Protection of *Arabidopsis thaliana* against leaf-pathogenic *Pseudomonas syringae* by *Sphingomonas* strains in a controlled model system. *Appl. Environ. Microbiol.*, **77(10):** 3202–3210.

Kim, M., D. Singh, A. Lai-Hoe, R. Go, R.A. Rahim, A.N. Ainuddin, J. Chun and J.M. Adams (2012). Distinctive phyllosphere bacterial communities in tropical trees. *Microb. Ecol.*, **63 (3)**: 674-681.

Knief, C., A. Ramette, L. Frances, C. Alonso-Blanco and J.A. Vorholt (2010). Site and plant species are important determinants of the *Methylobacterium* community composition in the plant phyllosphere. *ISME. J.*, **4(6):** 719–728.

Leveau, J. (2009). Life on leaves. *Nature,* 461- 741.

Prabakaran, M., S. Merinal and Panneerselvam (2011). Investigation of phylloplane mycoflora from some medicinal plants. *Eur. J. Exp. Bio.*, **1(2):** 219-225.

Prakasam, V. and P. Sharma (2012). *Trichoderma harzianum* (Th-3) a potential strain to manage the purple blotch of onion (*Allium cepa* L.) caused by *Alternaria porri*. *J. Agric. Sci.*, **4(10) :** 266-272.

Rajkonda, J.N., V.S. Sawant, M.G. Ambuse and U.N. Bhale (2011). Inimical potential of *Trichoderma* species against pathogenic fungi. *Plant. Sci. Feed.*, **1(1):** 10-13.

Rastogi, G., A. Sbodio, J.J. Tech, T.V. Suslow, G.L. Coaker and J.H. Leveau (2012). Leaf microbiota in an agroecosystem: spatio temporal variation in bacterial community composition on field-grown lettuce. *ISME. J.*, **6(10):** 1812–18.

Ray, M.K., P.K. Mishra, P.K. Burah and D. Choudhary (2014). Isolation and a comparative study of Phylloplane mycoflora of Muga host plants Som and Sualu from Goalpara district of Assam. *Int. J. Pure App. Biosci.*, **2(6):** 78-83.

Ritpitakphong, U., L. Falquet, A. Vimoltust, A. Berger, J.P. Métraux and F. L'Haridon (2016). The microbiome of the leaf surface of *Arabidopsis* protects against a fungal pathogen. *New.Phytol.*, **210(3):** 1033–1043.

Saha, A., A. Ray and P. Das (2013). Fungal colonization of the phylloplane of Psidium Guineense Sw. growing in Suryamaninagar, Tripura, northeast India. *Int. J. Basic Appl. Chem. Sci.*, **3(1):** 62-67.

Stromberg, K.D., L.L. Kinkel and K.J. Leonard (2000). Interactions between *Xanthomonas translucens* pv. *translucens*, the causal agent of bacterial leaf streak of wheat, and bacterial epiphytes in the wheat phyllosphere. *Biolo. Control*., **17:** 61-72.

Sumithra, N., M. Dorai and K.S. Rajesh (2016). Fungal diversity on the leaf litter of *Syzygium calophyllifolium* Walp. *Env. Sci. Ind. J*., **12(12):** 129.

Tozlu, E., N. Tekiner, R. Kotan and S. Ortucu (2018). Investigation on the biological control of *Alternaria alternata*. *Indian J. Agric. Sci.*, **88 (8):** 1241–7.

Vorholt, J.A. (2012). Microbial life in the phyllosphere. *Nat. Rev.Microbiol.*, **10(12):** 828–840.

Voriskova, J. and P. Baldrian (2013). Fungal community on decomposing leaf litter undergoes rapid successional changes. *ISME Journal*, **7:** 477–486.

Waill, A., A. Elkhateeb, Abdel-Nasser, B. Zohri, Mohamed, Mazen, H. Mohamed and M.D. Ghoson (2016). Investigation of diversity of endophytic, phylloplane and phyllosphere mycobiota isolated from different cultivated plants in new reclaimed soil, Upper Egypt with potential biological applications. *Int. J. Medi. Pharm. Res.*, **2(1):** 23-31.

Wellner, S., N. Lodders and P. Kampfer (2011). Diversity and biogeography of selected phyllosphere bacteria with special emphasis on *Methylobacterium* spp. *Syst. Appl. Microbiol.*, **34(8):** 621–630.

Whipps, J.M., P. Hand, D. Pink and G.D. Bending (2008). Phyllosphere microbiology with special reference to diversity and plant genotype. *J. Appl. Microbiol.*, **105(6):** 1744–1755.