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RELATIONSHIP BETWEEN SOIL MICROBES AND PLANT PATHOGENS OF *BACCAUREA RAMIFLORA* IN NOKREK BIOSPHERE RESERVE OF MEGHALYA

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Abstract

Burma grapes (*Baccaurea ramiflora*), locally known as 'gasampe', is a plant growing naturally in Nokrek Biosphere Reserve of Garo Hills, Meghalaya. The fruits are succulent, sweet and sour to taste, andrich in iron. Ripe fruits are consumed fresh and also used for making wine, pickles and jam. A brown red dye is obtained from the root, bark and wood. In traditional medicine, fresh bark is chewed or juice is consumed to treat indigestion and constipation. The objective of the study was to identify disease causing plant pathogens and soil microbes prevalent in the rhizosphere of *Baccaurea ramiflora* in the three zones of Nokrek Biosphere Reserve, and to assess the relationship between soil microbes and plant pathogens. Five diseases caused by fungal pathogens were observed. Change in zone, season and year did not have any significant influence on occurrence of pathogens in *Baccaurea ramiflora* plants. Occurrence of soil microbes in the rhizosphere was significantly influenced by change in zone, soil depth and season. Soil microbes were found to be more in top soil than subsoil. Soil microbe *Aureobasidia pullulans* was isolated only from the subsoil (15-30 cm soil depth) from core zone and transition zone. Among the identified soil microbes, *Aspergillus flavus, Penicillium digitatum* and *Rhizopus stolonifer* were observed in all the three zones of the Biosphere Reserve. Soil microbes *Fusarium oxysporum*, *Aspergillus versicolor*, *Aspergillus niger, Rhizoctonia solani* and *Trichoderma viride* inhabiting the rhizosphere of *Baccaurea ramiflora* at 0-15 cm depth showed positive association with plant pathogens *Perenospora parasitica, Colletotrichum gloeosporoides and Alternaria alternata* while exerting an antagonistic effect on pathogens *Alternaria* sp. and *Phyllostica sulata*. Presence of soil microbes *Cephalosporium roseum, Aureobasidia pullulans and Mucor racemosus* at 15-30 cm soil depth exerted significant positive influence on occurrence of plant pathogens *Alternaria* sp. and *Alternaria alternate* on the plant, whereas an antagonistic effect was observed on pathogens like *Phyllostica sulata*, *Colletotrichum gloeosporoide s*and *Perenospora parasitica*.

Key words*:* Nokrek Biosphere Reserve, *Baccaurea ramiflora*, plant pathogens, rhizosphere, soil microbes.

Introduction

Burma grapes (*Baccaurea ramiflora*) locally known as 'gasampe' is a small evergreen tree with narrowly elliptic or obovate leaves spirally clustered at intervals along the twigs. Flowers are small and borne on branches and the trunk. Male and female flowers are borne separately ondifferent trees. Male flowers are smaller and are 10 cm long mostly borne at the end of the branches. Female flowers are slightly bigger (30 cm long) borne on old branches and main trunks. The fruits of Burma grapes are succulent with sweet and sour taste. Fruits are a rich source of iron containing 5.34 mg /100 g of fruit (Haque *et al.,* 2009). Ripe fruits are consumed fresh and also used for making wine, pickles and jam. Young tender leaves and flowers are used as vegetable and for flavouring curries and minced meat in Bangladesh (Howlader *et al.,* 2012). A brown red dye is obtained from the root, bark and wood. In traditional medicine, fresh bark is chewed or juice is consumed to treat indigestion and constipation. In Chinese medicine, the

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whole plant is utilized as antiphlogistic and anodyne against rheumatoid arthritis, cellulitis and abscesses and to treat injuries (Howlader *et al.*, 2012). The objective of the study was to identify the disease causing pathogens and soil microbes prevalent in the rhizosphere of *Baccaurea ramiflora,* and to assess the relationship between soil microbes and plant pathogens.

Materials and Methods

The study was conducted in Nokrek Biosphere Reserve situated in between 25°25'- 25°30'N latitudes and 90°15'- 90°35'E longitudes at an elevation of about 4650 feet above sea level and stretched over an area of 820 sq. Km, located in the Garo Hills of Meghalaya, India. The Biosphere Reserve is divided into three zones: the core zone which is known as Nokrek National Park with an area of 47.48 sq. Km, the buffer zone covering an area of 227.92 sq. Km followed by transition zone with an area of 544.60 sq. Km. The hottest months of the year are March to May and the coolest months are December to February. Both southwest and north-eastern monsoons bring rain to the area. The temperature varied from 8^0C-38^0C in the year 2014 and 10^0C-36^0C in 2015. The total rainfall in 2014 was 2778.9 mm and in 2015 was 3809.9 mm.

Five plants of *Baccaurea ramiflora* were selected randomly from each zone of the Biosphere Reserve. Diseased plant parts were collected during pre monsoon and post monsoon period of years 2014 and 2015 and cultured in the laboratory in different media under aseptic conditions. For isolation of fungal pathogen from diseased plant samples, single spore technique and single hyphal tip technique of culturing were used. The isolated fungal pathogen were then purified by streaking and single spore isolation method. Pathogenicity tests were conducted thereafter by pin prick method (Tomkins and Trout, 1931). Pathogenicity was confirmed by Koch's postulates.

Soil samples were collected from two different depths of the rhizosphere *viz.* 0-15 cm and 15-30 cm, from four directions of the plant with the help of soil auger. The soil samples collected from four directions of each depth were combined to form a composite sample. A representative sample was drawn from the composite sample for culturing and identification of microbes.Soil microorganisms were identified by the Direct method and Plate method. Isolation from soil samples were carried out by dilution plate method (Warcup, 1960) using appropriate media. The colonies were identified bystudying the macro and micro morphological characteristics. Microphotographs of the slides from the colony were taken and compared with standard literature for proper identification of microbes.

Redundancy Analysis (RDA) was followed to study the occurrence of plant pathogens and soil microbes. Canonical Correspondence Analysis (CCA) was used to find out the relationship between soil microbes and occurrence of plant pathogens (TerBraak, 1995).

Results and Discussion

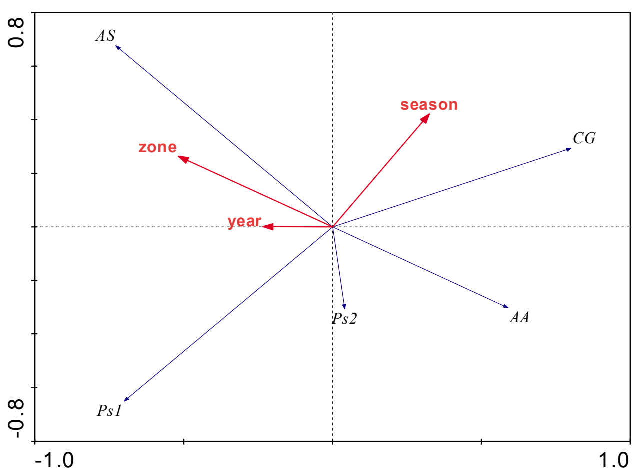
Five diseases caused by fungal pathogens were recorded in *Baccaurea ramiflora* during the observation period. During pre-monsoon period, pathogenic infestation was more intransition zone while in post monsoon period it was more in buffer zone. The independent factors likezone, season and year did notexhibitany significant influence on occurrence of plant pathogens in *Baccaurea ramiflora*. However, change in zone from core to buffer and transition, and advancement of year showed an increase in occurrence of plant pathogens like *Phyllostica sulata* and *Alternaria spp.* while a negative trend was observed in occurrence of pathogens *Colletotrichum gloeosporioides, Alternaria alternate* and *Perenospora parasitica*. Advancement of season had positive effect on occurrence of *Colletotrichum gloeosporioides* and *Alternaria* sp. while it had a negative effect on *Alternaria alternata, Phyllostica sulata* and *Perenospora parasitica* (Fig. 1).

Soil depth, season and zone showed significant influence on occurrence of soil microbes in the rhizosphere of *Baccaurea ramiflora*. Observations revealed that soil microbes in the rhizosphere of *Baccaurea ramiflora* were significantly higher in topsoil (0-15 cm depth) than subsoil (15-30 cm depth) in all three zones of the Biosphere reserve. Increase in soil depth increased the probability of occurrence of soil microbes like *Penicillium digitatum, Aureobasidia pullulans, Aspergillus flavus, Cephalosporium roseum, Mucor racemosus, Chaetomium globosum, Rhizoctonia solani and Drechslera oryzae;* while occurrence of soil microbes like *Curvularia lunata, Penicillium terrestre, Rhizopus stolonifer, Colletotrichum falcatum, Aspergillus versicolor, Trichoderma viride, Trichophyton vulgare* and *Fusarium oxysporum* were more in top soil. Change of season from pre monsoon to post monsoon as well as change in zone from core to buffer and transition zone increased the probability of occurrence of *Curvularia lunata, Penicillium terrestre, Aspergillus niger, Penicillium digitatum, Chaetomium globosum, Mucor racemosus, Aureobasidia pullulans, Aspergillus flavus* and *Cephalosporium roseum* significantly (Fig. 2). Soil microbe *Aureobasidia pullulans* was isolated only from 15-30 cm soil depth (subsoil) from core zone and transition zone in both years of observation.

Soil microbes like *Aspergillus niger, Fusarium oxysporum, Rhizoctonia solani, Trichoderma viride* and *Aspergillus versicolor* at 0-15 cm depth of rhizosphere showed positive association with plant pathogens *Alternaria alternata, Perenospora parasitica,* and *Colletotrichum gloeosporioides* while they exhibited an antagonistic effect on the pathogens *Alternaria* sp. and *Phyllostica sulata* (Fig. 3). Soil microbes *Cephalosporium roseum, Mucor racemosus* and *Aureobasidia pullulans* at 15-30 cm soil depth exhibited significant positive influence on the occurrence of plant pathogens like *Alternaria* sp. And *Alternaria alternata* while they antagonistically affected pathogens like *Phyllostica sulata, Colletotrichum gloeosporioides* and *Perenospora parasitica* (Fig. 4).

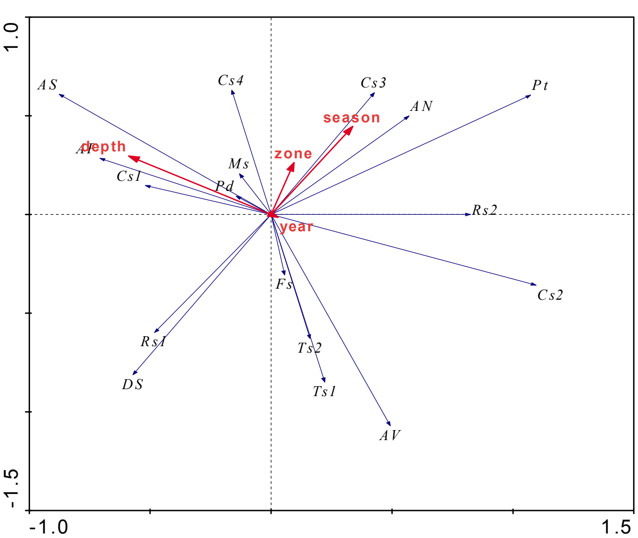
In the present study five diseases caused by fungal pathogens only were observed in plants of *Baccaurea ramiflora* in Nokrek Biosphere Reserve. Ploetz (2007) stated that fungi are the most prevalent and important plant pathogen. Dominance of fungi species from seed mycoflora collected from forest trees was reported by Mehrotra and Mehrotra (2000.) Marak *et al*., (2018) also reported dominance of fungal pathogens in *Emblica officinalis* in Nokrek Biosphere Reserve of Meghalaya. Incidence of diseases in Baccaurea ramiflora were less in core zone compared to transition zone and buffer zone of the Biosphere Reserve. Kutcher *et al.,* (1999) observed that severity of plant diseases vary over a landscape in relation to topography.

Soil microbes in the rhizosphere of *Baccaurea ramiflora* were found to be significantly more in the topsoil (0-15 cm depth) compared to subsoil (15-30 cm depth) in all three zones of the Biosphere Reserve in pre monsoon and post monsoon period during both years of observation. Das *et al.,* (2013) also reported higher microbial population in top soil in Dibru Shaikhowa Biosphere Reserve. Bundt *et al.,* (2001) reported that microbes exist throughout the soil profile but they are most abundant in surface soils. Bhattacharya and Jha (2011) reported that fungal population was higher in surface soil, which might be due to high amounts of organic carbon, higher aeration and favourable moisture. Occurrence of soil microbes in the rhizosphere of *Baccaurea ramiflora* in Nokrek Biosphere Reserve was significantly influenced by change of season. Zhang *et al*., (2014) reported that rhizosphere microbial abundance of different plant species varied greatly across season. Marak *et al.,* (2018) also reported that occurrence of soil microbes in the rhizosphere of *Emblica officinalis* in Nokrek Biosphere Reserve was greatly influenced by season. Seasons cause profound changes in factors such as temperature, humidity, vegetation and nutrient concentrations, which are crucial for microbial survival. Soil microbial community structure and function are known to be sensitive to



AA=Alternaria alternata
As= Alternaria spp, CG= Colletotrichum gloeosporioides,
Ps1=Phyllostica sulata,
Ps2=Perenospora parasitica

Fig. 1 : Effect of zone, season and year on occurrence of pathogenic microbes in *Baccaurea ramiflora.*



AF=Aspergillus flavus,
AN=Aspergillus niger,
AS=Aureobasidia pullulans,
AV=Aspergillus versicolor,
Cs1=Cephalosporium roreum,
Cs2=Colletotrichum falcatum,
Cs3=Curvularia lunata,
Cs4=Chaetomium globosum,
DS=Drechslera oryzae,
Fs=Fusarium oxysporum,
Ms=Mucor racemosus,
Pd=Penicillium digitatum,
Pt=Penicillium terrestre,
Rs1=Rhizoctonia solani,
Rs2=Rhizopus stolonifer,
Ts1=Trichoderma viride,
Ts2=Trichophyton vulgare.

Fig. 2 : Effect of zone, soil depth, season and year on occurrence of soil microbes in the rhizosphere of *Baccaurea ramiflora*.

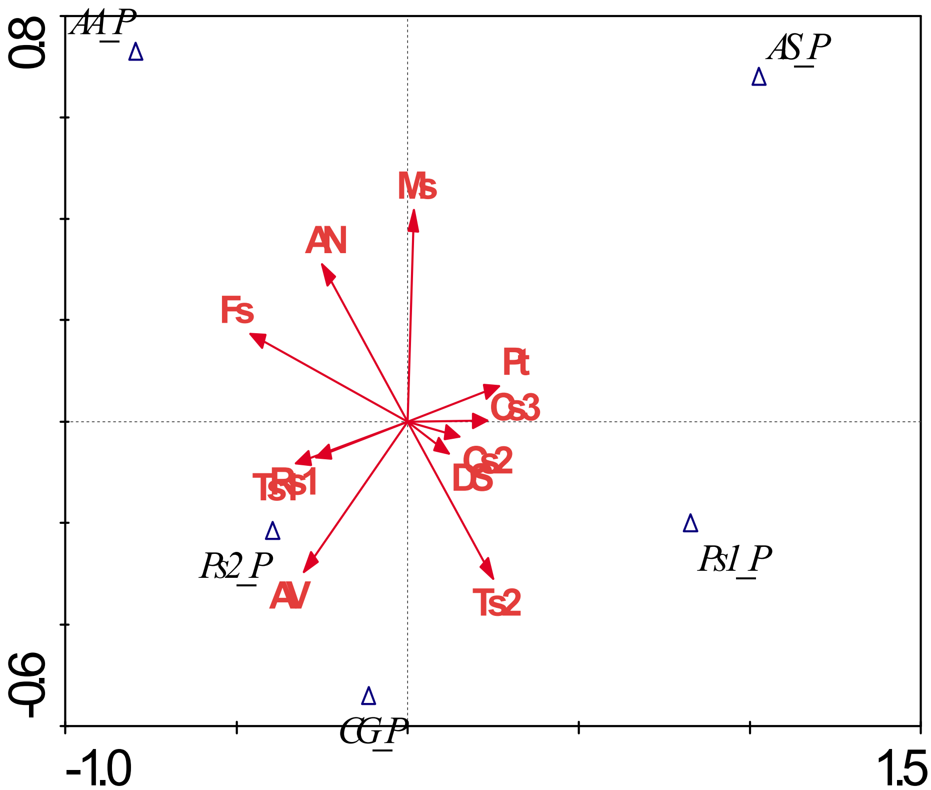


Fig. 3 : Association of soil microbes at 0-15 cm depth of rhizosphere with plant pathogens of *Baccaurea ramiflora*.

Soil microbes:
AF=*Aspergillus flavus,*
AN=Aspergillus niger,
AS=Aureobasidia pullulans,
AV=Aspergillus versicolor,
Cs1=Cephalosporium roreum,
Cs2=Colletotrichum falcatum,
Cs3=Curvularia lunata,
Cs4=Chaetomium globosum,
DS=Drechslera oryzae,
Fs=Fusarium oxysporum,
Ms=Mucor racemosus,
Pd=Penicillium digitatum,
Pt=Penicillium terrestre,
Rs1=Rhizoctonia solani,
Rs2=Rhizopus stolonifer,
Ts1=Trichoderma viride,
Ts2=Trichophyton vulgare.

Plant microbes:
AA_P=Alternaria alternata,
As_P= Alternaria spp,
CG_P= Colletotrichum gloeosporioides,
Ps1_P=Phyllostica sulata,
Ps2_P=Perenospora parasitica

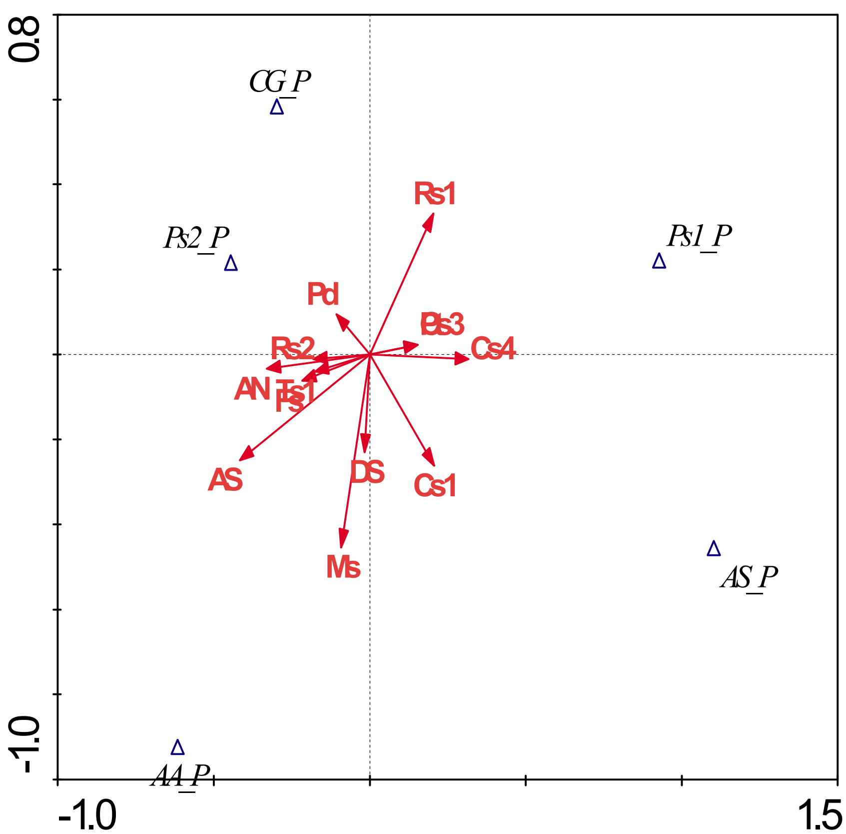


Fig. 4 : Association of soil microbes at 15-30 cm depth of rhizosphere with plant pathogens of *Baccaurea ramiflora*.

Soil microbes:
AF=Aspergillus flavus,
AN=Aspergillus niger,
AS=Aureobasidia pullulans,
AV=Aspergillus versicolor,
Cs1=Cephalosporium roreum,
Cs2=Colletotrichum falcatum,
Cs3=Curvularia lunata,
Cs4=Chaetomium globosum,
DS=Drechslera oryzae,
Fs=Fusarium oxysporum,
Ms=Mucor racemosus,
Pd=Penicillium digitatum,
Pt=Penicillium terrestre,
Rs1=Rhizoctonia solani,
Rs2=Rhizopus stolonifer,
Ts1=Trichoderma viride,
Ts2=Trichophyton vulgare.

Plant microbes:
AA_P=Alternaria alternata,
As_P= Alternaria spp,
CG_P= Colletotrichum gloeosporioides,
Ps1_P=Phyllostica sulata,
Ps2_P=Perenospora parasitica

changes in temperature and water availability (Hartel, 2005). Balser *et al.,* (2010) also stated that the alteration of surface soil temperature and moisture regime is likely to have direct effects on soil microbes.

Change of zone had significant effect on the distribution of soil microbes in the rhizosphere of *Baccaurea ramiflora.* Boerner (2006) reported that topographic characteristics of an ecosystem influence the below ground microbial community. Tsai *et al.,* (2007) also stated that topography might influence the quantity and diversity of fungal population in soil. Among the soil microbes identified in the three zones of Nokrek Biosphere Reserve, *Aspergillus flavus*, *Penicillium digitatum* and *Rhizopus nigricans* were the dominant ones. Marak *et al.,* (2018) also observed the dominance of these three species in their study with *Emblica officinalis* in Nokrek Biosphere Reserve. Tangjang *et al.,* (2009) and Das *et al.* (2013) also reported dominance of genus *Aspergillus, Penicillium* and *Rhizopus* in their study site.

A close relationship was observed between soil microbes in the rhizosphere of *Baccaurea ramiflora* and pathogens infecting these plants. Some soil microbes exhibited a strong positive influence on the occurrence of certain plant pathogens while exerting an antagonistic effect on others. Cook and Baker (1983) reported that soil biodiversity suppresses pathogens via complex ecological inter-actions or specific, where one or few antagonists act against single pathogen. Pathogen suppressive soils have been known for over 100 years and suppressiveness may be mediated by biotic or abiotic mechanisms (Chandrashekara *et al*., 2012). Diverse soil microbial communities contain taxa with a wide range of effects on plant growth, from strongly positive to strongly negative with the outcome of many interactions dependent on abiotic soil conditions (Huang *et al.,* 2014).

Conclusion

Five diseases caused by fungal pathogens were recorded in *Baccaurea ramiflora* plants in Nokrek Biosphere Reserve of Meghalaya. Change in zone, season and year did not have any significant influence on the occurrence of pathogenic microbes. Change in soil depth, season and zone significantly influenced the occurrence of soil microbes in the rhizosphere of *Baccaurea ramiflora.* Soil microbes were more in topsoil (0-15 cm) compared to subsoil (15-30 cm depth) of the rhizosphere of *Baccaurea ramiflora*. Soil microbe *Aureobasidia pullulans* was isolated only from the subsoil (15-30 cm soil depth) from core zone and transition zone in both years of observation. Among the identified soil microbes *Aspergillus flavus, Penicillium digitatum* and *Rhizopusnigricans* were prevalent in all three zones of the Biosphere reserve. The soil microbes *Fusarium oxysporum*, *Aspergillus versicolor*, *Aspergillus niger, Rhizoctonia solani* and *Trichoderma viride* inhabiting the rhizosphere of *Baccaurea ramiflora* at 0-15 cm depth showed positive association with the plant pathogens *Perenospora parasitica, Colletotrichum gloeosporoides and Alternaria alternate* while exerting antagonistic effect on pathogens *Alternaria* sp. And *Phyllostica sulata.* Presence of soil microbes *Cephalosporium roseum, Aureobasidia pullulans and Mucor racemosus* at 15-30 cm soil depth exerted significant positive influence on occurrence of *Alternaria* sp. and *Alternaria alternata* on the plant, whereas an antagonistic effect was observed on pathogens like *Phyllostica sulata, Colletotrichum gloeosporoides* and *Perenospora parasitica*.

References

Balser, T.C, J.L.M. Gutknecht and C. Liang (2010). How will climatic change impact soil communities? In: Soil Microbiology and Sustainable Crop Production G.R.Dixon and Emma Tilston (eds). *University of Reading UK*, 373-379.

Bhattacharya, P.N. and D.K. Jha (2011). Seasonal and depth wise variation in microfungal population numbers in Nameri forest soils, Assam, Northeast India. *Mycosphere,* **2(4):**297-305.

Boerner, R.E.J (2006). Unraveling the Gordian Knot: Interactions among vegetations, topography and soil properties in the Central and Southern Appalachiam. *J. Torrey Bot. Soc*., **133**: 321-361.

Bundt, M., F. Widmer, M. Pesaro, J. Zever and P. Balser (2001). Preferential flow paths: biological 'hotspots' in soils. *Soil, Biology and Biochemistry,* **33**: 369-379.

Chandrasekhara, C., J.C. Bhatt, R. Kumar and K.N. Chandrasekhara (2012). Suppressive soils in plant disease management. In: *Eco-Friendly Innovative Approaches in Plant Disease Management.* V.K. Singh, Y. Singh and Singh, A. (eds). *International Book Distributors, Dehradun*, 241-256.

Cook, R.J. and K.F. Baker (1983). The Nature and Practice of Biological Control of Plant Pathogens. The American Phytopathology Society, Minnesota.

Das, K., Nath, R. and P. Azad (2013). Soil Microbial diversity of Dibru-Saikhowa Biosphere Reserve forest of Assam, India. *Global Journal of Science Frontier Research (C)*, **13 (3)**: 7-13.

Haque, M.N., B.K. Saha, M.R. Karim and M.N.H. Bhuiyan (2009). Evaluation of Nutritional and Physiochemical Properties of Several Selected Fruits of Bangladesh. *Bangladesh J. Sci. Ind. Res.,* **44(3):**353-358.

Hartel, P.G. (2005). Soil abiotic environmental factors. In:

Principles and Applications of Soil Microbiology. Second Edition. D.M. Sylvia, J.J. Fuhrmann, P.G. Hartel and D.A. Zuberer(eds). *Upper Saddle River, New Jersey*, 41-51.

Howlader, M.A., A.S. Apu, R.K. Saha, F. Rizwan, N. Nasrin, and M. Asaduzzaaman (2012). Cytotoxic activity of N-Hexane, Chloroform and Carbon Tetrachloride fractions of the Ethanolic extracts of leaves and stems of *Baccaurea ramiflora* (Lour). *International Journal of Pharmaceutical Sciences and Research,* **3(3):** 822-825.

Huang, X.F., J.M. Chopparo, K.F. Reardon, R. Zhang, Q. Shen and J.M. Vivanco (2014). Rhizosphere interactions: root exudates, microbes and microbial communities. *Botany*, **92**: 267-275.

Kutcher, H.R., S.S. Malhi, A.M. Johnston and G. Hnatowich (1999). Impart of topography and management on diseases of canola and wheat. *In: Precision Agriculture, P. C. Robert, R.H. Rust and W.E. Larson (eds) ASA, CSSA, SSSA, Madison, W.I.* 599-562.

Marak, L.C., L.S. Pereira, R. Chakraborty and D. Mazumdar (2018). Diversity of plant pathogens and soil microbes associated with *Emblica officinalis* in Nokrek Biosphere Reserve of Meghalaya. *Environment and Ecology,* **36 (1):**1-6.

Mehrotra, M.D. and A. Mehrotra (2000). Seed-borne fungi of forest trees and their management-An overview. *Indian J. For***, 23**: 78-97.

Ploetz, R.C. (2007). Diseases of Tropical Perennial Crops.Challenging Problems in Diverse Environments. *Plant Disease,* **91**:644-663.

Tangjang, S., K. Arunachalam, A. Arunachalam and A. Shukla (2009). Microbial Population Dynamics of Soil under Traditional Agroforestry Systems in northeast India. *Research Journal of Soil Biology,* **1**: 1-7.

TerBraak, C.J.F. (1995). Ordination. Chapter 5 In: Data Analysis in Community and Landscape Ecology (Jongman, R.H.G., TerBraak, C.F.G and Van Tongeren, O.F.R.,Eds) Cambridge *University Press, Cambridge, UK*, 91-173.

Tomkin,s R.G. and S.A.Trout (1931). The use of ammonium salts for the prevention of green moulds in Citrus. *Jour. Pomo. Hort. Sci*., **9**: 257-264.

Tsai, S.H., A. Selvam and S.S. Yang (2007). Microbial diversity of topographical gradient profiles in Fushan Forest Soils of Taiwan. *Ecological Research,* **22**: 814-824.

Warcup, J.H. (1960). Methods for isolation and estimation of activity of soil. In: The Ecology of Soil Fungi Parkenson D and Ward J S (eds.) Liverpool University Press, Liverpool, 321.

Zhang, L.H., L.P. Song, G. Xu, P. Chen, J.N. Sun and H.B. Shao (2014). Seasonal dynamics of rhizosphere soil microbial abundances and enzyme activities under different vegetation types in the coastal zone, Shandong, China. *Clean Soil, Air and Water,* **42(8):** 1115-1120.