RESEARCH ARTICLE

SEASONAL VARIATION IN MICROBIAL BIOMASS ON PHYLLOSPHERE OF DIFFERENT FRUIT TREE SPECIES

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Abstract: The aim of the present study was to analyze seasonal variations in phyllospheric microbial biomass of different fruit tree species. Leaf samples were collected from seven fruit tree orchards viz. mango, guava, *aonla*, *ber*, bael, jamun, sweet orange during summer, rainy and winter season for estimation of microbial population. In phyllosphere of different fruit tree orchards maximum TBC was observed in mango during summer, jamun during rainy, *aonla* and sweet orange during winter season. Highest diazotrophs were observed in *aonla* during summer and in jamunphyllosphere during rainy and winter season. Microbial populations and fungal count decreased by 13.06 % to 42.02 % from summer to rainy season, whereas increased by 10.80 % to 32.39 % from rainy to winter season in respect to phyllosphere of all the fruit tree species.

Keywords: Fruit tree species, Phyllosphere, Microbial Population

INTRODUCTION

India is blessed with diverse agro-climatic zones having second largest arable land in the world. Different fruit crops are grown in these agro-climatic zones of India. Being the second largest producer of fruits in the world, after China, India is producing 97055 thousand metric tonnes of fruits from area of 6514 thousand hectares (NHB-2018). The major fruits grown in India are mango, citrus, banana, apple and guava. Apart from these fruits, ber, papaya, pineapple, litchi, sapota and grapes are major ones in tropical and sub-tropical conditions. Pear, peach, plum, almond, apricot, walnut and strawberry are grown successfully in the temperature region. Fruits are the nature's gift to mankind. These are not only delicious and refreshing but are also the main source of vitamins, minerals and proteins. Fruits also have medicinal value that makes the imperative use of fruit in our daily diet. It may not be an exaggeration to say that 80-90% of problems arising from under nutrition or malnutrition can be well managed with indigenous medicines and fruits. There is a complexity in environmental factors which affect and alter assemblage structure and functions of microbes. Therefore, it is well known that environmental and seasonal variables such as temperature, moisture or humidity, soil pH and nutrient availability influence the distribution and activity of soil micro-organisms (Oleveira and Oleveira 2005). Similarly, different seasons and plant species are also known to have significant impact on phyllosphere population its quality, composition and abundance.

Micro-organisms are associated with various parts of plants. Some are commensals, some pathogens and some are symbionts. Phyllosphere micro-organisms are found on the aerial surfaces of plants (leaves, stems, buds, flowers etc.) Phyllosphere, referred to all aboveground surfaces of any plant, acts as a landing stage where spores or other propagules can develop and multiply and has been reported as probably the largest ecosystem on earth colonized by microorganisms, mainly bacteria and fungi. Microbial phyllosphere communities are complex and composed by many uncultured microorganisms which are adapted to the harsh environmental conditions. In particular, microbial epiphytes of the phyllosphere are exposed to the atmosphere and must deal with direct UV radiation, wide fluctuations in temperature, low water availability and limited access to nutrients. Therefore, the compositions of phyllosphere communities are affected by environmental factors such as UV radiation, air pollution and nitrogen fertilization as well as by biotic factors, such as plant species and invading microorganisms. Moreover, the phyllosphere is an open system and microbes can also invade plant leaves by migration from the atmosphere, soil, other plants, insects, and animals. It has great commercial importance to the agricultural industry in understanding the survival of plant disease causing bacteria and fungi, for developing new ways to control their spread. The phyllosphere has been less intensively studied than the rhizosphere and is receiving considerable attention in recent years.

In the present study, microbial communities in the phyllosphere of seven fruit species that are common in Northern India were studied during different seasons. It will be helpful for the better understanding of community's structure and multitrophic interactions in the phyllosphere, as a key to develop new strategies for plant protection and better health management in fruit orchards through nutrient cycling.

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MATERIALS AND METHODS

Experimental Site

The leaf and soil samples were collected from the experimental orchards of the Department of Horticulture, CCS Haryana Agricultural University, Hisar, situated at 215.2 m above mean sea level with coordinates of 29º10′ N latitude and 75º46′ E longitudes.

Weather and Climate

Hisar has a typical semi-arid climate with hot and dry summers and extremely cold winters. The mean monthly maximum and minimum temperature show a wide range of variations both during summer and winter months. A maximum temperature of around 45ºC during summer months of May to June and temperature as low as freezing point accompanied by occasional frost in winter months of December and January. The total rainfall as well as its distribution in the region is subjected to large variations. About 80 per cent of the annual rainfall (about 450 mm) is received during July to September. A few showers also occur from December to February due to the western disturbances. The rainfall is highly erratic with 20-30 per cent annual and 30-50 per cent seasonal variations.

Study Plants

The following seven orchards of fruit tree species (Table 1) were used during the course of this investigation to assess microbial populations in phyllosphere.

Table 1. List of fruit tree species orchards studied during investigation

S.No.	Fruit Tree Species	Scientific name	Rootstock	Spacing
1	Mango	*Mangiferaindica*	Deshi mango seedling	10 m x 10 m
2	Guava	*Psidiumguajava*	L-49	6 m x 6 m
3	Sweet orange	*Citrus sinensis*	Rough lemon seedling	6 m x 6 m
4	Jamun	*Syzygiumcuminii*	Deshijamun seedling	10 m x 10 m
5	Aonla	*Emblicaofficinalis*	Deshiaonla seedling	10 m x 10 m
6	Bael	*Aeglemarmelos*	Deshibael seedling	10 m x 10 m
7	Ber	*Ziziphusmauritiana*	*Ziziphusrotundifolia*	10 m x 10 m

Plants were kept under uniform orchard management practices during the study, where all the cultural practices were carried out as per package of practices for fruit crops, CCS HAU, Hisar.

Collection of samples

Five representative plants/ replications were selected from each orchard. Fresh and mature leaf samples were collected, during different season's viz. summer, rainy, and winter from seven fruit tree species mango, guava, citrus, jamun, aonla, bael and ber. From all parts of the tree canopy i.e. top, bottom and interior as well as from all the directions east, west, north, south during day time (morning) thoroughly mixed to form a composite sample. Samples were placed in sterile well marked plastic bags and kept at 4^oC, which were then analyzed same day.

Microbiology of phyllosphere

Total microbial count (cfu) using Nutrient Agar, Nitrogen fixers (cfu) using Jensen's N_2 free media, Total fungal count (cfu) using CzapekDoxs' media in the phyllosphere was done by taking 10 g of fresh leaves from the composite sample and adding into 90 ml sterilized distilled water. Then, the samples were placed on rotary shaker for half an hour. Serial dilutions (up to 10^{-5}) of samples were made in 9.0 ml sterilized water blanks and 0.1 ml of appropriate dilution was spread on freshly prepared nutrient agar, media plates. The plates were incubated at 28 ± 2^0C in a BOD incubator for 3-4 days. Based on the morphotypes, different bacterial colonies were counted for total microbial count. The counts were calculated on per g leaf basis using formula:

Total Count:

$$\frac{\text{No. of cfu (colony forming units) x dilution factor}}{\text{Volume taken (ml)}}$$

Statistical Analysis

Data recorded was compiled and subjected to statistical analysis (Panse and Sukhatme, 1987) as per the design of the experiment (Threefactorial RBD) and tested for variances at 5% level of significance for Microbial population data of phyllosphere samples was analyzed through descriptive statistics.

RESULTS AND DISCUSSION

The microbial communities of leaves are diverse and include many genera of bacteria, filamentous fungi, yeasts, algae and less frequently protozoa and nematodes which are important for plant health and growth (Whipps*et al.*, 2008). In the present study, total bacterial count was found highest among other microbial population in phyllosphere of fruit orchards.Lindow and Brandl. (2003) reported that bacteria are by far the most abundant inhabitants of the phyllosphere and factors such as plant genotypes, phenotypes and environmental factors affect bacterial communities in the phyllosphere. Total bacterial count in different seasons was observed highest in summer season. As, dust storms are common in the area of study during summer months, which may have lead to accumulation of bacterial spores on the leaves along with the dust particles. Penny cook and Newhook

(1981) studied population densities of mould, yeast and bacteria increased during summer season and were higher on expanding rosette leaves of plants and mould populations.

Total Bacterial Count (TBC)

Total bacterial count varied among different (Table 2, Fig 1) fruit tree species and was found highest in mango phyllosphere (5.25 x 10^4cfu/g) during summer. Jager*et al*. (2001) reported that bacterial diversity in mango phylloplane was lowest in winter and increased gradually through spring to summer. But, aonla and sweet orange orchards had comparable results (5.57 x10^4cfu/g) and were found highest among other fruit tree species during the winter season. Lindow and Anderson. (1996) found the population sizes of bacteria of different types on navel orange leaves, with low bacterial populations during the summer and increased in size during the winter and spring months. Percent variation over the seasons revealed that total bacterial count decreased by 4.70% to 6.68% in most of the fruit tree species and increased by 5.09 % to 6.01 % in ber and jamun orchards from summer to rainy season. However, with seasonal shift from rainy to winter season TBC declined in bael (2.08%) and jamun (3.74 %) orchards but increased in other fruit tree species, TBC increased from 0.62 % to 14 %. Similarly,Jackson *et al*. (2006) found that when the plants are exposed to rainfall after a long period of desiccation, the environmental factors like temperature, rain fall and water availability affect the bacterial community on plant leaves and the complex phyllosphere community undergoes change in overall structure and activity.

Table 2. Effect of seasonal variation on total bacterial count (cfu/g) in phyllosphere of different fruit tree species

Fruit tree species	**Total bacterial count***			**Seasonal variation (%)**	
	Summer	Rainy	Winter	Summer to Rainy	Rainy to Winter
Mango	5.25	4.95	5.04	-6.06**	1.79
Guava	5.07	4.81	4.84	-5.41**	0.62
Ber	4.66	4.91	5.54	5.09	11.37
Jamun	4.69	4.99	4.81	6.01	-3.74**
Sweet orange	4.90	4.68	5.57	-4.70**	15.98
Aonla	5.11	4.79	5.57	-6.68**	14.00
Bael	5.14	4.90	4.80	-4.90**	-2.08**

*Values indicated in table are log values of total bacterial count (x 10^4) ** (-) indicates decrease

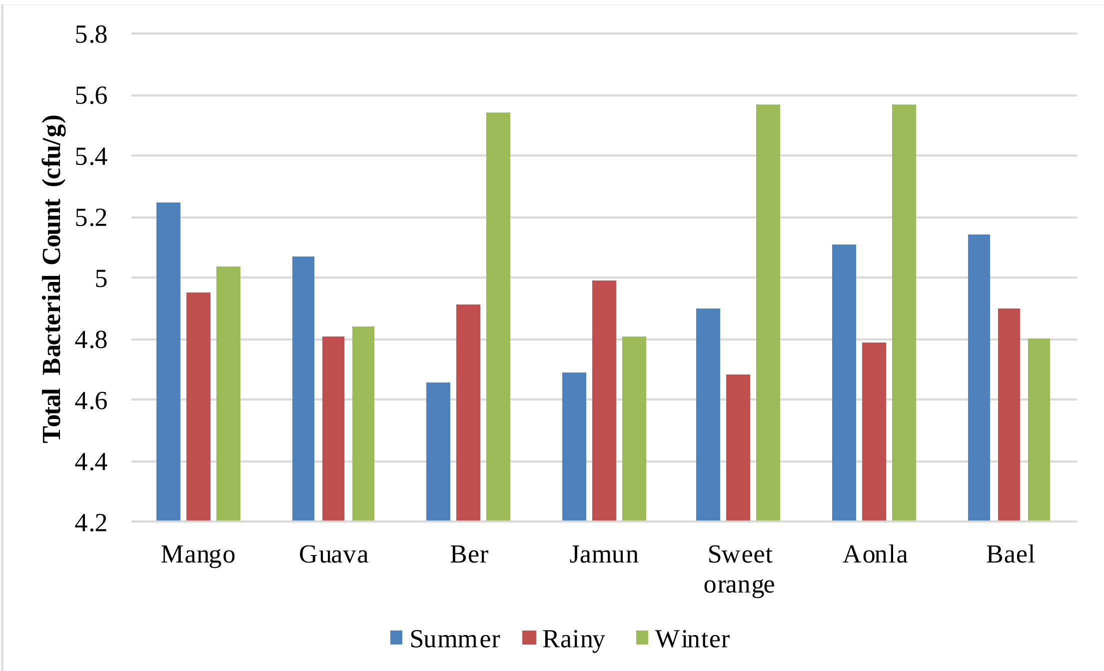

Fig 1. Effect of seasonal variation on total bacterial count (cfu/g) in phyllosphere of different fruit tree species

Fungal Count (cfu/g)

Fungal count was found lesser than (Table 3, Fig 2) total bacterial count during the summer season. It may be due to susceptibility of fungus to UV radiation. Kadivar and Stapleton (2006) and Stapleton and Simmons (2006)also commented that phyllosphere microorganisms are capable of withstanding in high UV radiations and this is an important selection pressure in the phyllosphere habitat for survival and growth.

The highest fungal count was recorded in winter season as compared to summer and rainy season, in all the fruit tree species. Highest fungal count during winter season was observed in aonlaphyllosphere (5.00 x 10^3cfu/g) followed by bael (4.60 x 10^3cfu/g) and jamunphyllosphere (3.87 x 10^3cfu/g) It may be due to lesser solar radiations and low temperature with high humidity in the winter months, which favor growth of fungi on leaf surface. Jager*et al*. (2001) reported that filamentous fungi were found abundant during winter and lowest in spring season. Seasonal trend in diversity of fungi showed an increase from winter to spring, followed by a slight increase in summer and rise during autumn. Moreover, density and diversity increased with leaf age. Gradual increase from the foliole stage, flush and juvenile to the mature leaf stage was found in fungal count, supporting the fact that fungal count was highest in aonla, bael and jamun orchards in winter month.The effect on community composition was also reported by plant factors i.e. cultivars, plant species and leaf age (Raschee*t al*., 2006; Hunter *et al*., 2010; Rastogi*et al*., 2012.)

The highest fungal count was observed in guava (4.38 x 10^3cfu/g) during summer season, in aonla (3.77 x 10^3cfu/g) during rainy and in aonlaphyllosphere (5.00 x 10^3cfu/g) during winter season. While comparing the percent variation over the seasons, it was found that fungal count declined in all the fruit tree species by 13.06 % to 42.02 % from summer to rainy season, whereas count increased from 10.80 % to 32.39 % with seasonal shift from rainy to winter season. The microbial population in phyllosphere of all the fruit orchards decreased during the rainy season because heavy rains washed the microbes and their spores from the leaves surface, further decreasing the microbial counts while maximum increase in fungal count was found in baelphyllosphere (32.39 %)during rainy to winter season.

Table 3. Effect of seasonal variation on fungal count (cfu/g) in phyllosphere of different fruit tree species

Fruit tree species	Fungal count*			Seasonal variation (%)	
	Summer	Rainy	Winter	Summer to Rainy	Rainy to Winter
Mango	4.36	3.07	3.51	-42.02**	14.32
Guava	4.38	3.23	3.69	-35.60**	12.46
Ber	4.10	3.11	3.56	-31.83**	12.64
Jamun	4.20	3.04	3.87	-38.16**	21.44
Sweet orange	3.55	3.14	3.52	-13.06**	10.79
Aonla	4.41	3.77	5.00	-16.98**	24.6
Bael	3.96	3.11	4.60	-27.33**	32.39

*Values indicated in table are log values of fungal count (x 10^3) ** (-) indicates decrease

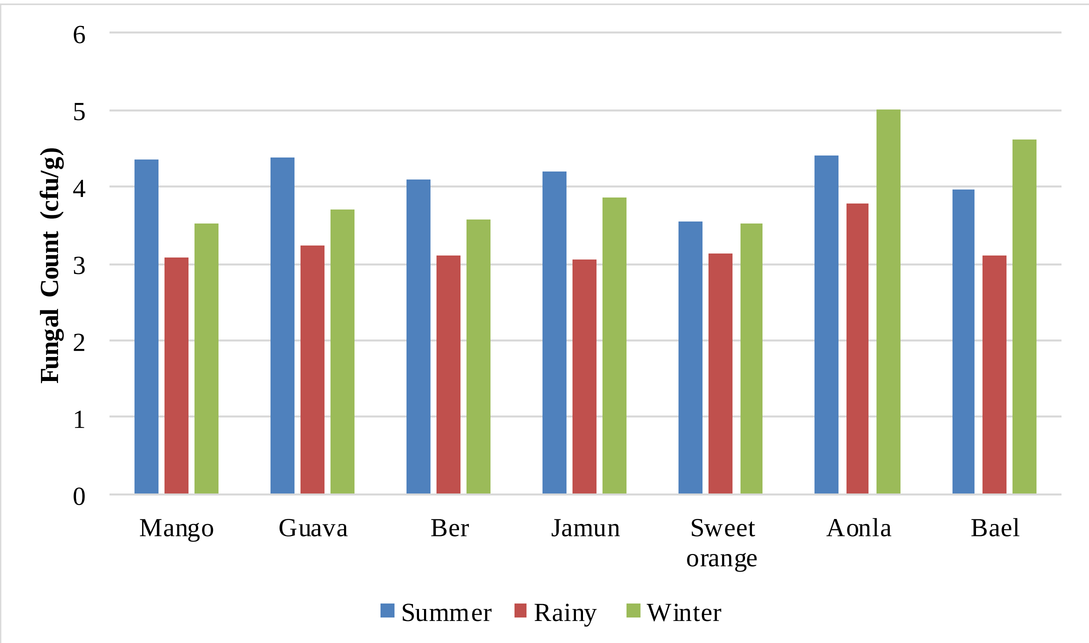

Fig 2. Effect of seasonal variation on fungal count (cfu/g) in phyllosphere of different fruit tree species

Nitrogen Fixers or Diazotrophs (cfu/g)

Nitrogen fixers or diazotrophs (Table 4, Fig 3) were recorded highest in summer season. Ercolani (1991) found distinct patterns of microbial composition at different times of the year, while bacterial community diversity being lowest during the warmest and driest months and highest in cooler and rainy months. In different fruit tree species, aonlaphyllosphere had highest diazotrophs during summer (5.05 x 10^4cfu/g) and winter season while in jamun during rainy season.

Maximum increase in diazotrophs was found in jamunphyllosphere (10.84 %) during seasonal change from summer to rainy season and in aonla phyllosphere (13.27%) during rainy to winter season. This may be due to blooming season of aonla in spring and maturity and harvesting during winter season. Exudates from surface of leaves, flowers and fruits may have supported the growth and survival of diazotrophs during summer and winter season. Miyamoto *et al*. (2004)reported the presence of N_2 fixing bacteria in the phyllosphere of many crop plants as free living N_2 fixing bacteria, which fix atmospheric nitrogen. Use of N_2 fixers, phosphate and potassium solubilizers contribute in enhancing uptake of plant nutrients **(**Afifi*et al*., 2014).

While comparing the percent variation over the seasons, it was found that diazotrophs decreased from 0.89 % to 18.82 % during seasonal change from summer to rainy season in different fruit tree species, whereas diazotrophs decreased by 2.29 % to 10.79 % and increased by 4.27 % (sweet orange) to 13.27 % (aonla) in fruit tree species with seasonal shift from rainy to winter. Batool*et al*. (2016) reported that leaves are dominant part of the tree and bacteria are the supreme inhabitant of the leaves. It was also supported by Beattie & Lindow.1995; Jacques & Morris.1995;Andrews & Harris.2000. Ercolani (1991)and suggested that epiphytic bacterial communities are dynamic and greatly differ in size, within and among plant of same species and over the growing season.

Table 4. Effect of seasonal variation on nitrogen fixers count (cfu/g) in phyllosphere of different fruit tree species

Fruit tree species	Nitrogen fixers count*			Seasonal variation (%)	
	Summer	Rainy	Winter	Summer to Rainy	Rainy to Winter
Mango	4.77	4.62	4.17	-3.25	-10.79
Guava	4.79	4.57	4.41	-4.81	-3.63
Ber	4.30	4.74	4.62	9.28	-2.60
Jamun	4.36	4.89	4.54	10.84	-7.71
Sweet orange	4.46	4.11	4.30	-8.52	4.42
Aonla	5.05	4.25	4.90	-18.82	13.27
Bael	4.51	4.47	4.37	-0.89	-2.29

*Values indicated in table are log values of nitrogen fixers count (x 10^4) ** (-) indicates decrease

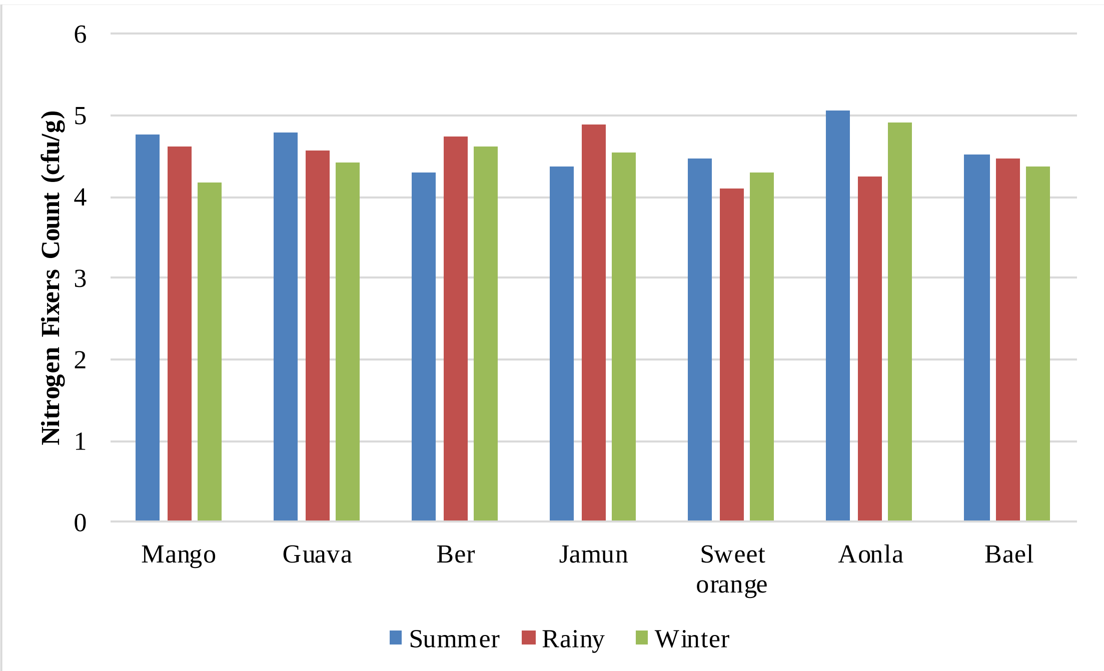

Fig 3. Effect of seasonal variation on nitrogen fixers count (cfu/g) in phyllosphere of different fruit tree species

CONCLUSION

Total bacterial count in phyllosphere decreased by 4.70 % to 6.68 % (sweet orange, aonla, bael, guava and mango) and increased in few fruit orchards by 5.09 % to 6.01 % (ber and jamun) with seasonal change from summer to rainy season. Fungal count in phyllosphere decreased by 13.06 % to 42.02 % from summer to winter season in all the fruit tree species, whereas from rainy to winter season fungal count again increased from 10.80 % to 32.39 % in all fruit tree species. Nitrogen fixer's count in phyllosphere decreased from 0.89 % to 18.82 % (sweet. orange, aonla, bael, mango and guava) or increased in few orchards from 9.28 % to 10.84 % (jamun and ber) during seasonal change from summer to winter season. Similarly, count decreased by 2.29 % to 10.79 % (mango, guava, jamun, ber and bael) or increased in few orchards by 4.27 % to 13.27 % (sweet. orange, aonla) with seasonal shift from rainy to winter season.

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