DOI: http://dx.doi.org/10.18782/2320-7051.6734

ISSN: 2320 – 7051 *Int. J. Pure App. Biosci.* **6** (3): 587-596 (2018)







Seasonal Fluctuations in the Microbial Composition of Mulberry Rhizosphere in Various Regions under Temperate Climatic Conditions of Kashmir

Saima Khursheed^{*} and M. R. Mir

Temperate Sericulture Research Institute, Mirgund, SKUAST-K, Post Box 674, GPO-Srinagar Kashmir J&K. *Corresponding Author E-mail: saimaiftikhar0485@gmail.com Received: 18.05.2018 | Revised: 22.06.2018 | Accepted: 28.06.2018

ABSTRACT

Mulberry (Morus sps.) a perennial heterozygous plant can be grown in diverse climatic conditions and mulberry leaf has been found to contribute 38% towards the success of silkworm rearing, the maximum among all factors. Mulberry plant should get better inputs because there is higher production of biomass as against other tree species. The only source of nutrients for mulberry tree seems to be from microbial decomposition of organic matter. Soil microflora plays a pivotal role in evaluation of soil conditions and in stimulating plant growth, development and foliage production. The present study reveals the influence of different seasons on the microflora of mulberry soils under temperate climatic conditions in different regions of Kashmir valleyduring 2014-2015 and the results showed that the variations in the population of microflora were found to be maximum during Spring season which showed a decline towards the Winter season. Amongst the regions, northern region registered the maximum population oftotal bacteria, fungi and Azotobacter whereas, it was central region in case of Azospirillum, phosphorus solubilizing bacteria and Actinomycetes and the lowest values were registered in central region in case of totalbacteria and southern region in case of fungi, Azotobacter, Azospirillum, phosphorus solubilizing bacteria and Actinomycetes. The subsequent decrease in microbial population from spring to winter and the variations among the regions may be because of fluctuations in organic matter, temperature, moisture content, pH and available nitrogen in soil which suppress microbial activity during these seasons and in the particular regions.

Key words: Kashmir, Mulberry, Microflora, Rhizosphere, Regions, Season, Temperate.

INTRODUCTION

Mulberry (*Morus* sps.) is the only food to silkworm (*Bombyx mori*, L.), a monophagus lepidopteron insect, belonging to family *Bombycidae*. It is grown to produce leaf which has been found to contribute 38% towards the

success of silkworm rearing amongst a host of factors¹⁴. Mulberry is a perennial deep rooted plant; the soil should be capable of supplying sufficient air, water and nutrients even at the deeper layers upto which the root system penetrates.

Cite this article: Khursheed, S. and Mir, M.R., Seasonal Fluctuations in the Microbial Composition of Mulberry Rhizosphere in Various regions under Temperate Climatic Conditions of Kashmir., *Int. J. Pure App. Biosci.* **6(3):** 587-596 (2018). doi: http://dx.doi.org/10.18782/2320-7051.6734

Being a high biomass producing plant and the main factor for cocoon production, mulberry plant does not receive due attention as far as frequent management is concerned, owing to its continous utilization of nutrients. However, the leaf productivity and quality are greatly influenced by nutrient availability which in turn is affected by environmental conditions and microbial population. Mulberry plant, therefore, depends on the soil microflora and going transformations the on in the rhizosphere of soil due to these microbes for its growth, development and foliage production. Rhizosphere microflora is generally responsible for the conversion of complex organic substances present in the soil into stable product "humus" which increases the fertility of the soil. Thus, the soil supplies all the nutrients to this plant and it appears that the compensatory source of these nutrients to the soil could be organic matter (litter) decomposition in the soil. Thus, the type and relative population of microorganisms in the soil are very important for decomposition, as they change the plant nutrients to readily available forms and stabilize desirable soil structure for luxuriant plant growth. It is therefore, necessary that the site, where litter decomposition is the main source of nutrients, inhabited with must be suitable microorganisms having reasonably good density and an active stage of growth which finally lead to enhanced microbial interaction for sustainable plant growth. As such in farming, the exploitation mulberry of microbial complex present in the rhizosphere of soil is of paramount importance not only because of their eco-friendly and beneficial nature but also for overall sustainability of sericulture. However, beneficial effects of some of the microbial inoculants procured from other sources have been established both under field as well as nursery conditions^{3, 10} but, no information is available about the native species inhabiting the mulberrv rhizosphere in various seasons and regions.A more specific description of the microbial diversity and distribution in a particular environment would improve our understanding of microbial functionality and interactions

found in that ecosystem. Therefore, the present study entitled, "Seasonal Fluctuations in the Microbial Composition of Mulberry Rhizosphere in Various Regions under Temperate Climatic Conditions of Kashmir" was undertaken to know the population of rhizosphere microflora in different regions and the influence of seasons on their population, so that they could be put to use in the management of soil fertility for sustainable growth of mulberry.

MATERIAL AND METHODS

The study entitled, "Seasonal Fluctuations in the Microbial Composition of Mulberry Rhizosphere in Various Regions under Temperate Climatic Conditions of Kashmir" was conducted at Biofertilizer Research Laboratory, FoA, Wadura, SKUAST-K as per the following experimental details:-

Experimental details

1. Regions: 03- North (Baramulla and Bandipora), Central (Srinagar and Pulwama north) and South (Anantnag and Kulgam districts)

2. Number of locations : 03 from each region 3. Location names : North (TSRI-Mirgund, P₄ BSF-Manasbal and Sericulture Development Department (SDD)- Bandipora Central (SDD-Poohu, Central Silk Board-Galander and SDD-Srinagar): South (SDD-Y.K. Pora, SDD-Krungsoo and SDD- Bijbehara)

4. Samples per location : 05 (Composite)

5. Year : 2014-2015

6.Seasons: 04 (Spring, Summer, Autumn, and Winter) Spring → 1st fortnight of April Summer →1st fortnight of July Autumn →1st fortnight of October Winter →1st fortnight ofJanuary
6. Design of survey: Purposive sampling.

Soil sampling

Soil samples were collected during spring (1stfortnight of April), summer (1st fortnight of July), autumn (1st ofortnightof October) and winter (1st fortnight of January) seasons from 9 different mulberry farms of Kashmir valley from the soil adhering to the roots of mulberry in sterilized polythene bags with proper labels to prevent moisture loss and as soon as possible were refrigerated at 4°C to avoid

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ISSN: 2320 - 7051

microbial fluctuations. From each location, during each season, five samples were collected to minimize the effect of inherent site variability. Thus a total of 180 samples were collected in all the four seasons and brought to the laboratory for isolation and enumeration (cfu's) of various microorganisms as under:

- Total viable bacteria (cfu/g soil) were isolated by using nutrient agar as growing medium by Serial Dilution Pour Plate Technique¹.
- Total viable fungi (cfu/g soil) were isolated by using Rose Bengal agar medium using Serial Dilution Pour Plate Technique¹.
- ✤ Actinomycetes (cfu/g soil) were isolated using Actinomycetes agar medium by Serial Dilution Pour Plate Technique¹.
- Phosphorous Solubilizing Bacteria (cfu/g soil) were isolated by using Pikovskaya's agar medium using Pour Plate Serial Dilution Technique¹⁵.
- Nitrogen fixers like Azotobacter and Azospirillum species (cfu/g soil) were isolated by using Ashby's and nitrogen free bromothymol media respectively by using Pour Plate Serial Dilution Technique¹.

Isolation of soil microflora

Rhizosphere soil samples were analyzedfor enumeration of soil microflora such as total bacteria, fungi, *Azotobacter*, *Azospirillum*, phosphorus solubilizing bacteria and *Actinomycetes* by isolation and enumeration method following standard Serial Dilution Plate technique²⁶. Specific media for different microorganisms such as Nutrient agar

Bengal medium, Rose agar medium, Azotobacter agar medium, Azospirillum medium. Pikovskava medium and Actinomycetes agar medium were used. For isolation,1gram of rhizosphere soil was taken and mixed well with 9 ml of sterile water to get 10⁻¹ dilution. After thorough mixing, one ml of sample from this test tube was pipetted out and transferred to another test tube containing 9 milliliters of sterile water and mixed thoroughly to get 10^{-2} dilution. The procedure was repeated again and again upto 10^{-7} dilution. From the respective dilution, 0.5 ml of microbial suspension was drawn aseptically with the help of 1ml sterilized pipette and spread uniformly on sterile petri dishes containing 15 ml of the respective media. Dilution of 10^{-3} , 10^{-4} and 10^{-5} were respectively used to isolate fungi (Plate-1), Actinomycetes (Plate-2) and Azotobacter (Plate-3) where as 10^6 , 10^4 and 10^5 for bacteria (Plate-4), Azospirillum (Plate-5) & phosphorus solubilizing bacteria (Plate-6) respectively. The preparation of serial dilutions and inoculation of the respective media were carried out under aseptic conditions in a laminar air flow chamber. The inoculated plates were then inverted upside down and were incubated at $28 \pm 2^{\circ}$ C in dark. The plates were observed for 1-6 days for microbial growth and then enumerations were made by counting the colonies developed on the surface of respective agar media after incubation with the help of digital colony counter. Similarly colonies showing phosphate larger solubilizing zone with respect to other colonies around them PSM's. were considered as



Plate- 1 Fungal colonies Copyright © May-June, 2018; IJPAB

Plate- 2 Actinomycetous colonies



Plate- 3 Azotobacter colonies

Plate- 4 Bacterial colonies



Plate- 5 Azospirillum colonies

Experimental Findings

Observations recorded on various parameters under the study entitled, "Seasonal Fluctuations in the Microbial Composition of Mulberry Rhizosphere in Various Regions under Temperate Climatic Conditions of Kashmir" are described under the following headings:

Total bacteria (cfu/g soil $\times 10^6$)

Total bacterial population was maximum (57.75) during spring season which was statistically significant over the other three seasons ranging from 39.33 in winter to 51.71 during autumn. Among the regions, northern region registered the maximum value (50.33) for total bacteria being statistically higher than the values recorded in southern (48.21) and central region (47.93). However the values for southern and central region were at par with each other Table 1).

Total viable fungi (cfu/g soil \times 10⁵)

The value for total viable fungi too was maximum (8.71) during spring season being

Plate- 6 PSB colonies

significant over the other three seasons of the year ranging from 3.82 in winter to 6.93 in autumn. Among the regions, northern region had the maximum value (7.30) for total viable fungi being statistically significant over the other regions with the least value (5.00) recorded in southern region (Table 2).

Azotobacter (cfu/g soil×10⁴)

The population of *Azotobacter* was maximum (22.15) during the spring season being statistically significant than the other seasons wherein it ranged from 12.46 in winter to 18.57 in autumn. Among the regions, northern region registered the maximum value (17.86) and was significant over the other two regions with the *Azotobacter* population of 16.76 and 17.03 for southern and central region respectively (Table 3).

Azospirillum (cfu/g soil \times 10⁵)

The population of *Azospirillum* was maximum (18.97) during spring season and significant over the other three seasons ranging from 11.60 in winter to 17.22 in autumn season.

Int. J. Pure App. Biosci. 6 (3): 587-596 (2018)

ISSN: 2320 - 7051

Among the regions, central region registered maximum (16.11) value and was significant over the other two regions where as the least

value (14.88) for Azospirillum population was registered in southern region (Table 4).

Table 1: Seasonal variation in total viable bacterial count (cfu/g soil × 10⁶) of mulberry soils of Kashmir

Region		Nor	th		Central					Overall			
Season	Mirgund	Manasbal	Bandipora	Sub Mean	Poohu	Galander	Srinagar	Sub Mean	Y.K.Pora	Krungsoo	Bijbehara	Sub Mean	Mean
Spring	58.20	57.20	57.60	57.66	58.00	54.80	59.80	57.33	59.20	57.20	57.80	58.06	57.75
Summer	50.00	48.80	47.80	48.86	43.60	43.60	49.20	45.46	46.40	43.40	45.80	45.20	46.51
Autumn	53.40	52.40	51.40	52.40	52.80	48.80	54.00	51.86	51.60	49.80	51.20	50.86	51.71
Winter	43.40	41.80	42.00	42.40	33.00	38.40	39.20	36.86	39.20	38.20	38.80	39.73	39.33
Mean	51.25	50.05	49.70	50.33	46.85	46.40	50.55	47.93	49.10	47.15	48.40	48.21	
	n<0.05)												

Seasons

Regions

0.94

0.81

0.39 0.34

Table 2: Seasonal variation in total viable fungi (cfu/g soil $\times 10^5$) of mulberry soils of Kashmir

Region	North					Central				South				
Season	Mirgund	Manasbal	Bandipora	Sub Mean	Poohu	Galander	Srinagar	Sub Mean	Y.K.Pora	Krungsoo	Bijbehara	Sub Mean	Mean	
Spring	10.00	9.60	9.60	9.73	7.60	9.40	9.00	8.66	9.40	6.40	7.40	7.73	8.71	
Summer	6.40	6.20	6.00	6.20	5.00	6.20	5.40	5.53	6.00	3.20	3.60	4.26	5.33	
Autumn	8.60	9.20	8.40	8.73	5.60	7.20	7.20	6.66	7.80	4.00	4.40	5.40	6.93	
Winter	5.00	4.40	4.20	4.53	3.80	5.20	4.00	4.33	3.20	2.40	2.20	2.60	3.82	
Mean	7.50	7.35	7.05	7.30	5.50	7.00	6.40	6.30	6.60	4.00	4.40	5.00		

C. D (p≤0.05) Seasons

Regions

Table 3: Seasonal variation in azotobacter population (cfu/g soil × 10⁴) of mulberry soils of Kashmir

Region		Nor		Central				South					
Season	Mirgund	Manasbal	Bandipora	Sub Mean	Poohu	Galander	Srinagar	Sub Mean	Y.K.Pora	Krungsoo	Bijbehara	Sub Mean	Overall Mean
Spring	22.80	21.40	23.20	22.46	19.80	22.40	21.60	21.26	21.60	24.80	21.80	22.73	22.15
Summer	17.60	16.40	17.20	17.06	15.20	15.80	14.20	15.06	16.40	15.20	13.20	14.93	15.68
Autumn	19.40	17.80	18.60	18.60	18.80	19.40	17.40	18.53	17.80	21.40	16.60	18.60	18.57
Winter	13.80	13.20	13.00	13.33	11.80	13.00	11.80	12.20	11.80	12.00	11.80	11.86	12.46
Mean	18.40	17.20	18.00	17.86	16.40	17.65	16.25	16.76	16.90	18.35	15.85	17.03	
C.	C. D (p≤0.05) Seasons : 0.57 Regions : 0.49												

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T	able 4: Seasonal v	variation in azosp	oirillum (cf	fu/g soil × 10°)) population	of mulberry	soils of Kashmir

\backslash		Nor	Central										
Region Season	Mirgund	Manasbal	Bandipora	Sub Mean	Poohu	Galander	Srinagar	Sub Mean	Y.K.pora	Krungsoo	Bijbehara	Sub Mean	Overall Mean
Spring	20.40	20.20	18.80	19.80	19.20	19.40	19.40	19.33	17.20	17.00	19.20	17.80	18.97
Summer	16.80	14.40	13.80	15.00	15.20	16.40	15.40	15.66	13.80	13.80	15.00	14.20	14.95
Autumn	19.00	18.00	16.20	17.73	17.20	19.00	16.80	17.66	15.40	15.60	17.80	16.26	17.22
Winter	13.20	11.40	10.60	11.73	11.40	12.20	11.80	11.80	11.20	11.00	11.60	11.26	11.60
Mean	17.35	16.00	14.85	16.06	15.75	16.75	15.85	16.11	14.40	14.35	15.90	14.88	

C. D (p≤0.05)

Seasons : 0.64 Regions :0.55

Phosphorus solubilizing bacteria (cfu/g soil $\times 10^5$)

The population of phosphorus solubilizing bacteria was maximum (18.60) during spring season being significantly more than the rest of the seasons ranging from 9.66 in winter to 14.08 in autumn season. Among the regions, central region registered the maximum value (14.11) for phosphorus solubilizing bacteria and was statistically at par with the population (13.91) recorded in northern region but significantly higher than the population

(12.65) recorded in southern region (Table 5). Actinomycetes (cfu/g soil 10⁶)

The population of *Actinomycetes* was maximum (14.22) during spring season being statistically significant over the other seasons ranging from 7.00 in winter to 11.48 in autumn. Among the regions, central region registered the maximum value (10.80) which was, however, at par with the value (10.73) recorded in northern region but different from the value (9.90) registered by the southern region (Table 6).

Table 5: Seasonal variation in phosphorus solubilizing bacterial (cfu/g soil $\times 10^5$) population of mulberry
soils of Kashmir

Region		Nor	th		Central					Overall			
Season	Mirgund	Manasbal	Bandipora	Sub Mean	Poohu	Galander	Srinagar	Sub Mean	Y.K.pora	Krungsoo	Bijbehara	Sub Mean	Mean
Spring	19.40	18.40	18.00	18.60	19.00	19.40	18.80	19.06	18.40	18.20	17.80	18.13	18.60
Summer	13.20	12.40	11.80	12.46	12.40	13.60	12.00	12.66	11.00	10.40	10.20	10.53	11.88
Autumn	15.00	14.60	13.20	14.26	14.40	13.80	15.80	14.66	12.80	13.40	13.80	13.33	14.08
Winter	11.20	10.00	9.80	10.33	9.80	10.80	9.60	10.06	9.20	8.20	8.40	8.60	9.66
Mean	14.70	13.85	13.20	13.91	13.90	14.40	14.05	14.11	12.85	12.55	12.55	12.65	

C. D (p≤0.05) Seasons : Regions :

0.46

0.40

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iursneed and wirr	ini. J. Pure App. Diosci. 0 (3): 387-390 (2018) ISSN: $2520 - 7051$									
Table 6: Seasonal variation in actinomycetes (cfu/g soil 10 ⁶) population of mulberry soils of Kashmir											
N		a a									

Region		Nor	th			Central				South				
Season	Mirgund	Manasbal	Bandipora	Sub Mean	Poohu	Galander	Srinagar	Sub Mean	Y.K.pora	Krungsoo	Bijbehara	Sub Mean	Mean	
Spring	17.00	14.40	14.80	15.40	14.00	15.20	13.80	14.33	12.80	13.40	12.60	12.93	14.22	
Summer	10.00	9.40	8.60	9.33	10.00	10.20	8.40	9.53	8.60	8.60	9.00	8.73	9.20	
Autumn	12.40	11.40	10.60	11.46	12.20	12.60	11.00	11.93	11.00	11.00	11.20	11.06	11.48	
Winter	7.40	6.60	6.20	6.73	7.20	8.20	6.80	7.40	6.40	6.80	7.40	6.86	7.00	
Mean	11.70	10.45	10.05	10.73	10.85	11.55	10.00	10.80	9.70	9.95	10.05	9.90		
	C. D (p≤0.05)		•			•	•	•	•	•	•	•	•	

Seasons

Regions

: 0.46

DISCUSSION

0.54

The results obtained in the study, "Seasonal Fluctuations in the Microbial Composition of Mulberry Rhizosphere in Various Regions under Temperate Climatic Conditions of Kashmir" are discussed below:-

Rhizosphere microflora of mulberry growing soils

Total bacteria: -Total bacterial count was the highest during spring season being statistically significant over other seasons with the least during winter. Among the regions, northern region registered the maximum value; whereas, central region registered the least. The increase in the size of microbial community was proportional to the increased organic matter content of the soil. In addition to organic matter, some other factors like soil moisture also influence microbial populations in soil. Tate and Terry²⁵ found positive correlation between bacterial population and soil moisture and concluded that moisture was generally a limiting factor to the microbial activity. These results suggest that microbial activity in soil was perhaps influenced by the inputs added to it. Further increased bacterial count and their enhanced activity may probably be due to soil pH because most of the microbes prefer neutral to slightly alkaline pH^7 the favorable temperature, soil besides moisture and organic carbon in the soil. During spring, rains are common in the valley and the soil temperature and moisture improve after the winter chill. Least bacterial count during winter may be attributed to snow cover

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and cold soil conditions retarding bacterial growth. Sharma²² has also reported adverse effect of cold soil conditions and snow cover on the bacterial population. Tiwari²³ also reported higher bacterial population in Pineapple orchard soils of different ages in North-Eastern India during wet rainy seasons (May) and also observed decreased bacterial population during winter.

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Total fungi: - Total fungi were maximum during spring season being statistically significant over the rest of the seasons. The least value was recorded during winter. It has been reported that the density of fungal population increased during the rainy season when the soil moisture was significantly higher. Among the regions, northern region registered the maximum value; whereas, southern region registered the least. The increased population of fungi during spring among the seasons and in northern region among the regions could be attributed to soil pH, organic carbon, moisture, nitrogen and phosphorus content of the soil which are the main factors affecting the fungal population and diversity²⁷. These findings are in conformity with the results of Deka⁶ and Bissett⁵ who reported that environmental factors such as pH, moisture, temperature, organic carbon and soil nitrogen play an important role in the distribution of mycoflora. Gupta *et al.*⁸ also reported that organic carbon content of soils had highly significant and positive correlation with the fungal population. The soil organic carbon, nitrogen, phosphorus,

ISSN: 2320 - 7051

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potassium are important for fungi. In the absence of any of these the growth and sporulation of moulds as well as other microorganisms are hampered a lot^{20} .

Azotobacter: - Azotobacter was maximum during spring and significant over other seasons with least value recorded in winter. Among the regions, maximum value was reported from northern region followed by southern region whereas central region registered the least. Highest count of Azotobacter during spring season could be due to the highest content of available N, O.C in the season. This is in conformity with the findings of Milosevic et al.¹³ who reported a positive correlation between Azotobacter with O.C and N in the soil. The highest count of Azotobacter in the northern region could, however, be correlated with the highest content of available nitrogen in the region amongst all the regions. Shilpkar²¹ reported that the count of all microbial communities decreased in post-monsoon compared to monsoon and pre-monsoon.

Azospirillum: *Azospirillum*were maximum during spring season being statistically significant over the rest of the seasons. The least value was recorded in winter. This may be because of highest content of organic carbon prevailing during spring season. Rinkee et al.¹⁸ observed that the population of Azospirillum showed an increasing trend with the increasing organic carbon content in the soil. The results are corroborative with the findings of Rafi and Charvulu¹⁶ who also reported seasonal variation in the population of Azospirillum being more during Kharif season as compared to Rabi season. Among the regions, central region registered the maximum value being statistically at par with northern region and significant over southern region which registered the least value. Highest population of Azospirillum in northern and central region may be attributed to the increased pH and increased N content in the regions.

Phosphorussolubilizingbacteria:Phosphorus solubilizing bacteria were highestduring spring season, being significant over

other seasons and the lowest in winter. Among the regions, highest values were recorded in northern region and the lowest values in southern region. Highest population of PSB during spring could be due to the presence of nitrogen in larger quantities which they utilize as nitrite, nitrate or in amino form and in turn greatly influence phosphorus solubilization activity⁹. The decreased number of phosphorus solubilizing bacteria during summer and winter may be due to less carbon and unfavorable temperature during these two seasons as compared to the other two seasons. Bajpai and Rao² stated that efficient phosphate solubilizers always prefer soils having good carbon content for their survival. However, the population of phosphorus solubilizing bacteria being more in northern and central region than the southern region could be explained due to the highest population of AM fungal population¹¹ and also due to alkaline pH in the regions as also reported by Ravikumar et al.¹⁷, in Mangrove ecosystem.

Actinomycetes: -Actinomyceteswere highest during spring season and statistically significant over the other seasons with least values recorded in winter. The low availability of air and free oxygen during the rainy/spring season could have created a favourable condition for the growth of facultative hypothesis behind anaerobes. The the maximum population of Actinomycetes in the spring is attributed to the nutrient rich conditions, favourable temperature and moisture, thus depicting a significant effect on the number of Actinomycetes. Ritu et al.¹⁹ have also reported variations in the population of Actinomycetes in bitter gourd soil during different seasons with significantly maximum values during the spring season. The population of Actinomycetes was higher in central and northern region than southern region which could be due to slightly alkaline/higher pH nature of the regions. Basilio et al.⁴ have also reported that Actinomycetes prefer to grow in neutral and slightly alkaline conditions.

CONCLUSION

The findings of the presentstudy led to the following conclusion:-

- Microbial population of all the mulberry gardens showed highest values during spring and the lowest during winter.
- Spring represented the favourable season for the proliferation and activity of microbes, thereby, enhancing the fertility in soils.
- Mulberry soils depict great variations among the seasons in the soilmicroflora,which differ in their relative density as well as their activities too.
- Soil microorganisms, in general thrived well during spring and autumn as compared to summer and winter owing to favorable climatic conditions especially moisture, temperature, etc which also had a pronounced influence on enzyme activities.
- Nutrient availability in the form of organic matter in the soil seems to have pronounced influence on the microbial population in mulberry growing soils.
- The growth stage of the plant, soil type and the cultural operations followed; too have a modifying effect on soil microflora.
- In general, soil microorganisms could be inoculated to the soil during the spring seasonbecause at this stage, plant has a high demand for mineral nutrients.

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