JEB®

Journal of Environmental Biology

p-ISSN: 0254-8704 • e-ISSN: 2394-0379 • CODEN: JEBIDP

Journal website : www.jeb.co.in ★ **E-mail :** editor@jeb.co.in

Triveni Enterprises (Educational Services Set-up)

Original Research

DOI : http://doi.org/10.22438/jeb/44/3(SI)/JEB-08

Composition of plant growth promoting rhizobacteria in forest and systematic plantations of tasar silkworm food plants and their relationship with soil and leaf nutrients

Manjappa[1]*, T. Pandiaraj[2], R.S. Ekka[3], I.G. Prabhu[3], M.M. Baig[3], A. Sahay[3] and K. Sathyanarayana[3]

[1]Central Sericultural Research and Training Institute, Mysuru-570 008, India

[2]College of Agriculture, Acharya Narendra Deva University of Agriculture Science and Technology, Ayodhya-224 229, India

[3]Central Tasar Research and Training Institute, Ranchi-835 303, India

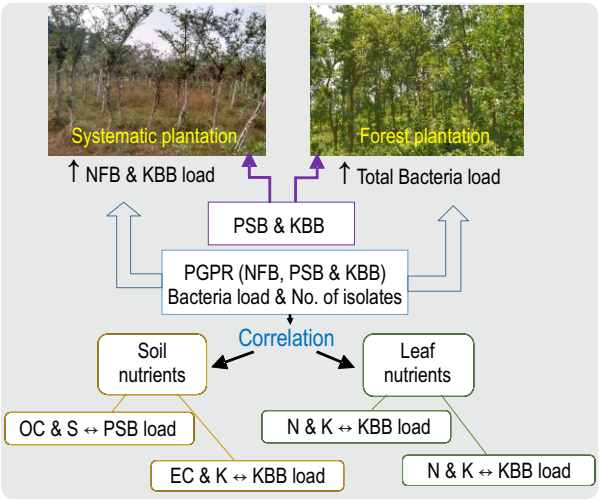
***Corresponding Author Email :** gmanju4132@gmail.com ***ORCiD:** https://orcid.org/0000-0003-2205-2680

Received: 07.12.2022 *Revised*: 09.03.2023 *Accepted*: 28.03.2023

Abstract

Aim: To investigate the status of bacterial load and number of isolates of plant growth promoting rhizobacteria in forest and systematic plantation of *Terminalia tomentosa* and *T. arjuna*, and their relationship with soil and leaf nutrient levels.

Methodology: Soil samples were collected from the rhizosphere of food plants in four tasar silkworm rearing regions of India. Bacterial load of total bacteria, Nitrogen fixing bacteria (NFB), Phosphate Solubilizing Bacteria (PSB) and bacteria grown on King's B media (KBB) was enumerated on specific media by employing serial dilution technique. Based on the colony morphology and cell type, distinct colonies on specific media were counted as isolate. Nutrient levels in soil and leaf samples collected from different plantations were analyzed. Multivariate correlation analysis was performed between PGPR microbial load and isolates with soil physio-chemical parameters and leaf nutrients.



Results: The rhizosphere of forest plants possessed the highest total bacterial load, the systematic plantation possessed the highest NFB load, whereas PSB and KBB load was equal in both the plantations. Forest plantations of Keonjhar and Sundergarh regions had the highest number of NFB isolates per rhizosphere soil sample and systematic plantations possessed the highest number of KBB (Keonjhar and Sundergarh regions) and PSB isolates (West Singhbhum region). Positive correlation was observed between PSB load and the number of isolates with soil organic carbon, KBB load with soil electrical conductivity and available potassium, PSB load and KBB isolates with soil available sulphur. NFB and KBB load and their isolates showed positive correlation with leaf N and K content.

Interpretation: Results suggest the possibility of improving leaf nutrient quality of tasar silkworm food plants by enriching PGPR microbial load through biofertilizer application.

Key words: Rhizobacteria composition, Soil nutrients, Silkworm, *Terminalia tomentosa, Terminalia arjuna*

How to cite : Manjappa, T. Pandiaraj, R.S. Ekka, I.G. Prabhu, M.M. Baig, A. Sahay and K. Sathyanarayana : Composition of plant growth promoting rhizobacteria in forest and systematic plantations of tasar silkworm food plants and their relationship with soil and leaf nutrients. *J. Environ. Biol.*, **44**, 432-438 (2023).

© **Triveni Enterprises**, Lucknow (India)

Introduction

Tasar culture is a forest based agro-industrial avocation forms livelihood for more than 3.5 lakhs rural aboriginal families of the country (Satyanarayana, 2022). India is the only producer of tropical tasar silk with the production of 2689 MT (CSB, 2020-21). Tasar sericulture is an age old culture that has been practiced by the tribals of India by collecting naturally formed cocoons from the forest (Jolly *et al.,* 1979). However, in recent decades it has taken commercial facet, wherein tasar silkworm are being reared on Arjun and Asan food plants grown in forest and systematic plantations to obtain high cocoon yield. In forest plantation, food plants are grown naturally interspersed with other vegetation at random spacing whereas under systematic plantations food plants are raised with definite spacing (6'×4', 10'×6' or any other recommended spacing). Higher income from low input (Jolly *et al.,* 1979) has made tasar sericulture as an attractive occupation for the villagers residing in the edges of forest area (Vishaka *et al.,* 2020).Considering the potential livelihood creation of tasar sericulture the area under systematic plantation has been expanded by the establishment of plantations in the forest, government waste land and marginal lands through various government schemes (Gargi *et al.,* 2015).

But, of late, the tasar cocoon quality has been deteriorating in systematic plantations due to decreasing leaf nutrient quality. This is intern due to decreasing soil nutrient level in systematic plantations (Pandiaraj *et al.,* 2018), which could be attributing to the continuous silkworm rearing with rare application of manures. Tribal tasar farmers cannot afford sufficient quantity of manures due to their poor economic status. It has been widely reported that, beneficial soil microorganisms known as plant growth promoting rhizobacteria (PGPR) have pivotal role in plant nutrition, phyto-stimulation and biological control in agri-horticulture crops and forest species (Doaa, 2012). Significance of PGPR has been also demonstrated in silkworm food plants *viz.,* mulberry (Baqual and Das, 2012; Rajaram *et al.,* 2013; Ou *et al.,* 2022) and *Machilus bombycina* (Som) Muga silkworm food plant (Unni *et al.,* 2008; Rabha *et al.,* 2014). PGPR species have diverse beneficiary properties, in which certain group of beneficial microbes are involved in plant nutrition through various mechanisms.

A group of bacterial species can solubilize/ mineralize inorganic/organic form of phosphate by secreting organic acids, phytase and phosphatase enzymes (Mitra *et al.,* 2022) known as Phosphate Solubilizing Bacteria (PSB) (Kumar *et al.,* 2022). Certain bacterial species which are able to fix atmospheric nitrogen in soil are known as Nitrogen Fixing Bacteria (Kumar *et al.,* 2022). Some bacterial species have biocontrol properties by secreting some antimicrobial compounds besides possessing plant growth promoting properties (Mitra *et al.,* 2022; Kumar *et al.,* 2022); generally these bacteria are isolated using King's B agar and are designated as King's B Bacteria (KBB). In some cases, same PGPR can show multi-functional properties like nutrient mobilization, solubilization, nitrogen fixation, phyto-hormone synthesis, suppression of phyto-pathogens by secreting siderophores, ammonia, hydrogen cyanide, and chitinase (Mitra *et al.,* 2022). As the rhizosphere is very dynamic environment harbors variety of microflora with different types of metabolic and adoptive responses (Vijai and Indu, 1998). Flores-Nunez *et al.* (2018) reported that in a same region pine forest soil had a greater richness of bacterial genera than the agricultural soil. Soils of pine forest and agriculture soil of same region possess different physio-chemical properties on the other hand agricultural soil has greater proportion of IAA-producing and siderophore producing bacteria than in pine forest soil. Differential silkworm rearing intensity and plant nutrient management practices can alter the composition of PGPR under systematic plantation and forest plantations. Hence, present study was undertaken to understand the differences in bacterial load and diversity in three group of rhizosphere bacteria *viz.,* NFB, PSB and biocontrol bacteria under systematic plantation and forest plantation. Further, study aimed to understand the relationship between PGPR composition with nutrient content of soil and leaf of tasar host plants by analyzing rhizosphere soil samples of tasar rearing regions.

Materials and Methods

Laboratory experiments were carried out at Soil Science and Chemistry Laboratory, Central Tasar Research and Training Institute, Ranchi, Jharkhand, India. A total of 62 rhizosphere soil samples were collected from Arjun (34) and Asan (27) food plants grown under systematic plantation (44) and forest plantation (18) in four tasar silkworm rearing regions viz.,West Singhbhum in Jharkhand state and Mayurbhanj, Keonjhar and Sundergarh in Odisha state.

Sampling of rhizosphere soil: Rhizosphere soil adhering to roots was collected at one feet depth, one meter apart from the tree trunk. At each sampling location, three rhizosphere soil samples were collected and mixed to form representative sample of the respective location. Soil samples were collected in polythene covers, immediately preserved in a cooling box, brought to laboratory and stored at 8ºC in a refrigerator until further use.

Soil and leaf sampling for nutrient estimation: To study the effect of native PGPR microbial effect over soil and food plant leaf nutrients level, respective soil and host plant leaf samples were collected from Keonjhar and Sundergarh regions. Soil samples were drawn from the same pit from where rhizosphere soil sample were collected. Soil samples were collected by V shaped pit method, wherein soil was scraped from the surface to 30 cm depth, mixed thoroughly, three random samples were mixed together and drawn representative sample of 1 kg from each location. For leaf nutritional analysis, five leaves were collected from the middle portion of the branches of sampled host plant. Leaf samples from three host plants from each location were collected as a representative sample.

Estimation of soil and leaf nutrients: All the representative soil samples were air-dried, ground and passed through 2 mm sieve for nutrient analysis. Soil physical properties such as pH and

electrical conductivity (EC) were determined by potentiometer and conductivity meter (Labman) using 1: 2.5 soil water suspensions (Jackson, 1973) and organic carbon was estimated by Walkely and Black (1934) method. Soil samples were analysed for available macronutrients such as nitrogen (Subbiah and Asija, 1956), phosphorus (Bray and Kurtz, 1945), potassium (1N ammonium acetate extractable) and sulphur (turbidimetric method) by following the methods described by Page *et al.* (1982). Leaf samples were processed by shade drying followed by oven dry at 60ºC till constant weight was obtained and grinded to powder. Leaf sample (0.5 g and 1.0 g) was used for the estimation of total nitrogen and remaining elements (P and K). Total leaf nitrogen was estimated by micro-Kjeldahl digestion and distillation as per the procedure suggested by AOAC (1995); total phosphorous by Diacid mixture digestion followed by recording colorimetric reading (Dynamica UV spectrophotometer) of yellow color complex through ammonium molybdate ammonium vanadate solution (Koenig and Johnson, 1942); total potassium by tri-acid digestion, followed by recording diluted extract in flame photometer.

Enumeration of total bacteria from tasar silkworm food plant rhizosphere: Total bacteria was enumerated in the rhizosphere soil samples of six locations covering both forest and systematic plantations equally in West Singhbhum region (Jharkhand). Initially, 1g of rhizosphere soil adhering roots was dissolved in 10 ml of sterilized distilled water in a conical flask and the contents were shaken for 30 min. Thereafter, individual serial decimal dilutions were prepared up to 10^{-9} dilution of original sample and 0.1 ml of inoculum from different dilutions (10^{-7}, 10^{-8} and 10^{-9}) were plated in triplicates on nutrient agar (Peptone 10 g, Meat extract 10 g, Sodium chloride 5 g and Agar 12 g per liter, pH 7.3) by following spread plate technique (Thatcher and Clark, 1968). Plates were incubated overnight at 37°C. Plates having colony-forming units (CFU) ranging from 30-300 were counted by avoiding plates with <30 colony number and with innumerable to count. Colony numbers recorded from triplicates were taken average.

Enumeration of NFB, PSB and KBB from tasar silkworm host plant rhizosphere: Enumeration of NFB and PSB bacterial load was carried out in the rhizosphere soil samples collected from all the 63 sampling locations and KBB was isolated from Keonjhar and Sundergarh regions. For the enumeration of NFB, PSB and KBB group of bacteria different dilutions *viz.*, 10^{-4},10^{-5}, 10^{-5} and 10^{-6}, were prepared by considering their abundance in the soil. To isolate specific group of bacteria, specific growth media were used. NFB, PSB and KBB were isolated on Azotobacter (Sucrose) agar, Pikovskaya's agar and King's B agar, respectively. Inoculum (0.1 ml) from different dilutions were spread on selective media agar plates and incubated at 30°C for 24 hr for NFB and KBB and 28°C for 48 hr for PSB to obtain an optimum growth of respective group of bacteria. Bacterial load was determined as described in previous section. Based on colony morphological features viz., shape, edge, elevation, color and texture unique colonies were considered as isolate (Flores-Núñez *et al.,* 2018). Isolates were confirmed by re-streaking over specific media by following the zig-zag method. Number of isolates per rhizosphere soil sample (per location) were recorded.

Statistical analyses: Differences for total microbial load, PGPR microbial load and number of isolates between systematic plantation and forest plantation was tested using t-test in MS Excel 2013. Correlation between PGPR microbial load and number of isolate per sample with their corresponding soil nutrients and food plant leaf nutrients was tested by using 45 samples in SPSS 16.0v.

Results and Discussion

Tasar silkworms are reared on the food plants grown naturally in the forest as well as systematically raised plantations. Generally, the intensity of silkworm rearing is high under systematic plantations compared to forest plantation. It is generally observed that cocoon quality derived from systematic plantations is inferior than cocoon derived from forest plantations which could be due to poor leaf nutrient quality in systematic plantations, and in turn due to poor soil nutrient levels (Subbaswamy *et al.,* 2004) in systematic plantations. Most of the systematic plantations of major tasar rearing regions are deficient in available nitrogen and phosphorous (Sinha *et al.,* 2017; Pandiaraj *et al.,* 2018).

PGPR have significant role in plant nutrition and growth promotion and hence, PGPR has been used as inoculants in many regions around the globe with the aim to reduce the application of potentially polluting chemical fertilizers and pesticides (Montañez *et al.,* 2012). Therefore, an attempt was made to study the composition of three group of PGPR species, *i.e.,* atmospheric nitrogen fixing bacteria (NFB), phosphate solubilizing bacteria (PSB) and bacteria having bio-control properties (KBB) in the tasar silkworm food plants rhizosphere grown under forest and systematic plantations. Present study is the first report to understand the composition of PGPR species in forest and systematic plantation of tropical tasar silkworm food plants, and also to study their relationship with soil and food plant nutrient contents. Enumeration of total bacterial count on nutrient agar plates revealed that the highest total bacterial load was observed in rhizosphere soil samples of forest plantation (1.30×10^{9} $CFUg^{-1}$) as compared to systematic plantation (7.9×10^{8} $CFUg^{-1}$). Similar observation was also observed by Flores-Núñez *et al.* (2018), where pine forest had the highest microbial load compared to agriculture soil with respect to food plant species. As a rhizosphere had the highest bacterial load (1.35×10^{9} $CFUg^{-1}$) as compared to Arjun rhizosphere (8.9×10^{8} $CFUg^{-1}$).

The NFB, PSB and KBB isolates isolated in this study have already been tested for their nitrogen fixing ability, phosphate solubilization and biocontrol property, respectively (Annual Report, 2020-21). In the West Singhbhum region of Jharkhand and Mayurbhanj region of Odisha, the bacterial load of NFB was highest in the rhizosphere of systematic plantations (79×10^{5} $CFUg^{-1}$ and 72×10^{5} $CFUg^{-1}$) as compared to forest

Table 1: Bacterial load (CFU g^{-1}) and number of isolates of nitrogen fixing bacteria and phosphate solubilizing bacteria under forest and systematic plantation of West Singhbhum region of Jharkhand and Mayurbhanj, Keonjhar and Sundergarh regions of Odisha

Region/State	Plantation type	NFB (x 10^5)	No. of NFB isolates	PSB (x 10^6)	No. of PSB isolates
West	Systematic plantation	79 ± 27.7	4 ± 0.8	59 ± 21	4.8± 0.94
Singhbhum	Forest plantation	15 ± 4.4	3.6 ± 0.6	62 ± 8	3.5± 0.66
(Jharkhand)	p-value	0.05	0.42	0.65	0.0005
Mayurbhanj	Systematic plantation	72 ± 15	2.83 ± 0.83	59 ± 23	3.8 ± 0.7
(Odisha)	Forest plantation	54 ± 5	2.67 ± 0.58	66 ± 28	5.3 ± 1.5
	p-value	0.02	0.7	0.57	0.21
Keonjhar and	Systematic plantation	53 ± 13	3.5 ± 1.0	49 ± 20.6	4.8 ± 1.5
Sundergarh	Forest plantation	30 ± 25	6 ± 1.4	59 ± 24.7	5.1 ± 1.0
(Odisha)	p-value	0.18	0.03	0.45	0.66

Values are mean ± S.D.

Table 2: Bacterial load (CFU g^{-1}) and number of isolates of king's B bacteria under forest and systematic plantation of Keonjhar and Sundergarh regions of Odisha

Plantation type	KBB (x 10^6)	No. of KBB isolates
Systematic plantation	89 ± 103	4.2 ± 1.47
Forest plantation	68 ± 58	2.4 ± 1.48
p-value	0.28	0.008

Values are mean ± S.D.

Table 3: Bacterial load and number of isolates of NFB and PSB in the rhizosphere of Arjun and Asan food plants of West Singhbhum region of Jharkhand and Mayurbhanj, Keonjhar and Sundergarh regions of Odisha.

Region/State	Food plant	NFB (x 10^5)	No. of NFB isolates	PSB (x 10^6)	No. of PSB isolates
West	Arjun	24.5 ± 19	4 ± 1.1	66.3 ± 16.8	5.3 ± 1.2
Singhbhum	Asan	92 ± 22	3.6 ± 1.5	50.4 ± 17.0	4.1 ± 2.2
(Jharkhand)	p-value	0.47	0.42	0.07	0.25
Mayurbhanj	Arjun	64.8 ± 26.4	3.0 ± 0.89	55.5 ± 23.2	3.5 ± 0.8
(Odisha)	Asan	61.5 ± 15.3	2.8 ± 0.75	60.2 ± 25.2	4.3 ± 1.2
	p-value	0.7	0.7	0.7	0.1
Keonjhar and	Arjun	53 ± 13	3.5 ± 1.0	48.5 ± 23	4.8 ± 1.2
Sundergarh	Asan	30 ± 25	6 ± 1.4	58.7 ± 21	5.1 ± 1.5
(Odisha)	p-value	0.18	0.03	0.45	0.66

Values are mean ± S.D.

plantations (15 x 10^5 $CFUg^{-1}$ and 54 x 10^5 $CFUg^{-1}$) (Table 1). Soil with nitrogen deficiency have higher load of NFB species in order to fix more atmospheric nitrogen in soil. Soil nutrient analyses of systematic plantations revealed that majority of tasar rearing regions soils are deficient in available nitrogen (Sinha *et al.,* 2017; Pandiaraj *et al.,* 2018). Low nitrogen content in soil could be due to continuous intensive tasar silkworm rearing in systematic plantations with low/no application of manures/fertilizers. Similar observation was also made by Naher *et al.* (2013) in rice, where soil without application of nitrogen fertilizer had higher load of nitrogen fixing bacteria. The results of correlation analysis in the present study corroborates with the findings of Flores-Núñez *et al.* (2018) who also observed higher load of nitrogen fixing bacteria in cultivated soil than in pine forest soil, where in negative correlation (r= -0.18, but weak correlation) was observed between NFB population and available nitrogen content in the soil samples collected from forest and systematic plantations. This indicates that soil with low nitrogen content will have higher microbial load of atmospheric nitrogen fixers. However, in case of bacterial load of Phosphate solubilizing bacteria (PSB) and King's B bacteria (KBB) no significant difference was observed between forest and systematic plantations in the study areas (Table 1, 2). Thus, intensive silkworm rearing in systematic plantations did not affect the microbial load of PSB and KBB in the study areas. The diversity of NFB, PSB and KBB was measured based on the number of isolates isolated per rhizosphere soil sample.

Online Copy

Table 4: Bacterial load (CFU g^{-1}) and diversity of NFB, PSB and KBB in the rhizosphere of Arjun and Asan food plants of Keonjhar of Odisha.

Plantation type	KBB (x 10^6)	No. of KBB isolates
Arjun	92.8 ± 100	4.0 ± 1.5
Asan	59.8 ± 55	2.4 ± 1.4
p-value	0.34	0.03

Values are mean ± S.D.

Table 5: Correlation between soil physical and nutrient parameters with PGPR (NFB, PSB and KBB) bacterial load and number of isolates in the rhizosphere soil samples collected from forest and systematic plantation.

Parameters	PGPR bacteria	Soil physical and nutrient parameters						
		OC	EC	pH	N	P	K	S
Bacterial load	PSB	0.29*	-0.13	0.06	0.03	-0.21	-0.20	0.53**
	NFB	-0.13	0.06	-0.10	-0.18	-0.17	0.16	-0.18
	KBB	0.07	**0.51****	-0.13	0.07	-0.05	**0.36***	0.00
No. of isolates	PSB	**0.25***	-0.01	0.21	-0.17	-0.14	0.27	0.09
	NFB	0.11	-0.18	0.06	-0.15	-0.14	0.23	-0.04
	KBB	0.14	-0.10	0.09	0.15	0.02	0.02	**0.39***

The highest diversity of NFB was found in the rhizosphere of forest plantations (6 isolates) as compared to systematic plantation (3.5 isolates) in Keonjhar and Sundergarh regions (Table 1). Systematic plantations had shown the highest diversity of PSB in West Singhbhum (4.8 isolates) and KBB in Keonjhar and Sundergarh regions (4.2 isolates) as compared to the forest plantations (3.5 and 2.4 isolates) (Table 1, 2). These results indicate that the comparison between forest and systematic plantations for bacterial load and diversity varies from regions to regions. These differences between plantations could be due to various factors such as soil physio-chemical properties (Flores-Núñez *et al.,* 2018), intensity of silkworm rearing, nutrient management, cultural practices, vegetation type, climate, etc. Diversity and distribution of rhizospheric soil bacteria are reported as niche-specific and shared between two or more host plants (Yadav *et al.,* 2018). Therefore, a study was undertaken at three tasar rearing regions to understand whether bacterial load and diversity of NFB, PSB and KBB are common or different in the rhizosphere of Arjun and Asan host plants.

The results revealed no significant difference between Arjun and Asan host plants for the bacterial load (Table 3, 4) and diversity of NFB, PSB and KBB in all study areas, except for Keonjar and Sundergarh regions, where Asan food plant rhizosphere had the heighest NFB isolates (6 isolates) than Arjun (3.5 isolates) and Arjun plant had the highest KBB isolates (4 isolates) than Asan (2.4) (Table 4). Overall results suggest that bacterial load of NFB, PSB and KBB varied with the plantation type rather than food plant species. Both food plants (*Terminalia arjuna* and *T. tomentosa*) belong to same genera and are naturally cross compatible (Gargi *et al.,* 2015) and hence, rhizosphere of both host plants could have shared common niche for the most of the studied bacterial group and shown less differences in their composition. Considering the vital properties of PGPR on plant nutrition and growth promotion, association between the bacterial load and diversity of PSB, NFB and KBB with soil and leaf nutrient status was studied by using the rhizosphere soil of tasar rearing regions. Soil microbial composition is determined by several biotic and abiotic factors. Abiotic factors like edaphic and environmental factors have been hypothesized and demonstrated to influence on microbial communities (Calderon *et al.,* 2001). Organic carbon availability as a source of energy for microbes determines the microbial composition (Drenovsky *et al.,* 2004). Positive correlation was observed between soil organic carbon with PSB load and its number of isolates per rhizosphere soil samples (Table 5). This indicates that the soil organic matter content could have enhanced the PSB load and diversity.

This is in accordance with the study of Anwar *et al.* (2021), wherein application of PGPR along with organic supplements has enhanced microbial load and their efficiency in improving plant growth and nutrient uptake in Okra. KBB bacterial load was positively correlated with (r = 0.51) with electrical conductivity (EC) of soil. In a similar study by Flores-Núñez *et al.* (2018) in pine forest rhizosphere, the diversity of PGPR was related to physico-chemical property of the soil and showed positive association of PSB and siderophore producers with EC and CEC of soil. The bacterial load of NFB and PSB and their diversity did not show any correlation with soil EC and pH. Soil sulphur content was positively associated with the bacterial load of PSB (r = 0.53), and number of KBB isolates (r = 0.39) present in the corresponding rhizosphere soil samples (Table 5).

Whereas, bacterial load of KBB was positively associated with the available soil potassium content (r = 0.36). NFB, PSB and KBB load, but diversity had not show any significant association

Table 6: Correlation between leaf nutrient contents of food plants in forest and systematic plantation with their corresponding rhizosphere PGPR (NFB, PSB and KBB) bacterial load and number of isolates

Parameters	PGPR bacteria	Leaf nutrient content (%)			
		N	P	K	S
Bacterial load	PSB	-0.01	-0.26	**-0.41***	0.02
	NFB	**0.46***	0.28	**0.41***	0.11
	KBB	**0.39***	0.06	**0.39***	-0.07
No. of isolates	PSB	0.13	-0.3	0.18	0.07
	NFB	0.27	0.2	**0.44***	-0.32
	KBB	0.39*	0.24	-0.02	0.01

NFB: Nitrogen fixing bacteria; PSB: Phosphate solubilizing bacteria; KBB: King's B Bacteria; N: Total nitrogen, P: Total Phosphorous, K: Total Potassium; S: Total Sulphur.

with the available nitrogen and phosphate content in soil. However, leaf N content was positively associated with the bacterial load of NFB (r = 0.46) and KBB (r = 0.39), and diversity of KBB (r = 0.39) (Table 6). This could be explained as the plants consume majority of available N and K from the soil for their growth and development by leaving soil with low level of N and K. Besides other factors can also have influenced the level of available soil N and K. Similarly, leaf K was also correlated positively with the bacterial load of NFB (r = 0.41) and KBB (r = 0.39), and number of isolates of NFB (r = 0.44). However, leaf K was negatively correlated with the PSB load. Leaf P and S contents did not show any correlation with bacterial load and diversity of PSB, NFB and KBB. In most of the study regions differential PGPR composition was evident between forest and systematic plantations, however, no difference was observed between Arjun and Asan food plant species. Under native tasar food plant habitat, PGPR composition had significant positive associationship over leaf nutrient levels.

The results of this study, highlight the scope for improvement of leaf nutrient quality of tasar food plants through inoculation of PGPR in the form of biofertilizer.

Acknowledgment

The authors are thankful to Dr. P. K. Kar, BSMTC Pali,for the collection of soil samples from Mayurbhanj region of Odisha.

Authors' contribution: Manjappa: Conceptualization and designing of the experiments; collection of soil samples from study regions, laboratory experiments; **T. Pandiaraj:** Analysis of physio-chemical properties of soil and leaf nutrients; **R.S. Ekka:** Execution of laboratory work as a JRF; **I.G. Prabhu:** Constant technical support in laboratory work; **M.M. Baig:** Preparation of manuscript; **A. Sahay and K. Sathyanarayana:** Support in execution of work.

Funding: Central Silk Board, Ministry of Textiles, Government of India.

Research content: The research content of manuscript is original and has not been published elsewhere.

Ethical approval: Not applicable.

Conflict of interest: The authors declare that there is no conflict of interest.

Data availability: Not applicable.

Author statement: All authors have read, revised, agreed and approved the final manuscript.

Consent to publish: All authors agree to publish the paper in *Journal of Environmental Biology.*

References

Annual Report: Central Tasar Research and Training Institute, Central Silk Board, Ministry of Textiles, Govt. of India, Piska Nagri, Ranchi, Jharakhand, India, 108 pages (2020-21).

Anwar, H., X. Wang, A. Hussain, M. Rafay, M. Ahmad, M. Latif, M.U. Jamshaid, I. Khalid, A. Dar and A. Mustafa: Comparative effects of bio-wastes in combination with plant growth promoting bacteria on growth and productivity of Okra. *Agronomy,* **11**, 2065 (2021).

AOAC, Official Methods of Analysis. 16th Edn.: Association of Official Analytical Chemists, Washington, DC (1995).

Baqual, M.F. and P.K. Das: Effect of inoculation with *Azotobacter* and phosphate solubilizing microorganisms on mulberry (*Morus* spp.). *Int. J. Sci. Nat.,* **3**, 608-612 (2012).

Bray, R.H. and L.T. Kurtz: Determination of total, organic and available forms of phosphorus in soils. *Soil Sci.,* **59**, 39-45 (1945).

Calderon, F.J., L.E. Jackson, K.M. Scow and D.E. Rolston: Short term dynamics of nitrogen, microbial activity and phospholipid fatty acids after tillage. *Soil Sci. Soc. Am. J.,* **65**, 118–126 (2001).

CSB: Central Silk Board, Bengaluru, Ministry of Textiles, Govt. of India, Functioning of Central Silk Board and Performance of Indian Silk Industry, 19 pages (2020-21).

Doaa, A.R. Mahmoud: Forest life under control of microbial life, new advances and contributions to forestry research. (Eds.: Dr. Andrew and A. Oteng-Amoako), In Tech (2012).

Drenovsky, R., D. Vo, K. Graham and K.M. Scow: Soil water content and organic carbon availability are major determinants of soil microbial community composition. *Microb. Ecol.,* **48**, 424–430 (2004).

Online Copy

Flores-Núñez, V.M., E. Amora-Lazcano, A. Rodriguez-Dorantes, J.A. Cruz-Maya and J. Jan-Roblero: Comparison of plant growth-promoting rhizobacteria in a pine forest soil and an agricultural soil. *Soil Res.,* **56**, 346-355 (2018).

Gargi, R. Kumar, M. Deka, H. Yadav and A. Sahay: Crossability studies in *Terminalia arjuna* and *T. tomemtosa. Int. J. Appl. Bio. Pharma. Technol.,* **6**, 211-216 (2015).

Jackson, M.L.: Soil Chemical Analysis. Prentice Hall of India Pvt. Ltd., New Delhi, 498 pages (1973).

Jolly, M.S., S.K. Sen and T.N. Sonwalker: Non-mulberry silks. FAO Agricultural Services Bulletin, Food and Agriculture Organization of the United Nations, Rome, pp. 1-15 (1979).

Koenig, R.A. and C.R. Johnson: Colorimetric determination of biological materials. *Ind. Eng. Chem. Analyt. Edn.,* **14**, 155-156 (1942).

Kumar, M., Poonam, S. Ahmad and R.P. Singh: Plant growth promoting microbes: Diverse roles for sustainable and ecofriendly agriculture. *Ener. Nexus*, **7**, 1-11 (2022).

Mitra, D., R. Mondal, B. Khoshru, A. Senapati, T.K. Radha, B. Mahakur, N. Uniyal, E.M. Myo, H. Boutaj , B.E. Guerra Sierra, P. Panneerselvam, A.N. Ganeshamurthy, S.A. Elković, T. Vasić, A. Rani, S. Dutta and P.K. Das Mohapatra: Actinobacteria-enhanced plant growth, nutrient acquisition, and crop protection: Advances in soil, plant, and microbial multifactorial interactions. *Pedosphere*, **32**, 149-170 (2022).

Montañez, M., A.R. Blanco, C. Barlocco, M. Beracochea and M. Sicardi: Characterization of cultivable putative endophytic plant growth promoting bacteria associated with maize cultivars (*Zea mays* L.) and their inoculation effects *in vitro*. *Appl. Soil Ecol.,* **58**, 21-28 (2012).

Naher, U.A., R. Othman and Q.A. Panhwar: Culturable total and beneficial microbial occurrences in long-term nutrient deficit wetland rice soil. *Austr. J. Crop Sci.,* **7** , 1848-1853 (2013).

Ou, T., M. Zhang, Y. Huang, L. Wang, F. Wang, R. Wang, X. Liu, Z. Zhou, J. Xie and Z. Xiang: Role of rhizospheric *Bacillus megaterium* HGS7 in maintaining mulberry growth under extremely abiotic stress in hydro-fluctuation belt of three Gorges reservoir. *Front. Plant Sci.,* **13**, 880125 (2022).

Page, A.L., R.H. Miller and D.R. Keenay: Methods of Soil Analysis. Part - 2. Soil Society of America, Inc. Publishers, Madison, Wisconsin, USA, pp. 228-534 (1982).

Pandiaraj, T., P.P. Srivastava, S. Das and A.K. Sinha: Assessing soil fertility status of tasar host plants growing soils in Purulia district of West Bengal state. *J. pharmacogn. phytochem.,* **7**, 2966-2970 (2018).

Rabha, J., D. Jha, H. Sarma, A. Acharya, U. Chakraborty and B. Chakraborty: Characterization of bacteria from the rhizosphere of *Persea bombycina* with multiple growth promoting traits. *Indian Phytopath.,* **67**, 126-133 (2014).

Rajaram, S., K. Nongrang, S.K. Mandal, M.K. Ghosh and B.B. Bindroo: *Azotobacter chroococcum* mass culture for production of bio-fertilizer, its sustained efficacy on nitrogen fixation and crop productivity in mulberry garden. *Int. J. Comput. Eng. Res.,* **3**, 332-340 (2013).

Sathyanarayana, K.: Research advancements and increasing multi-stakeholder base: A paradigm shift in Indian tasar silk promotion. Souvenir cum book of abstracts. National Symposium on Vanya Sericulture: Opportunities Galore, October 28-29, 2022. ISBN: 978-81-958770-0-3, pp. 1-15 (2022).

Sinha, A.K., P.P. Srivastava, T. Pandiaraj and S. Das: Evaluation of soil fertility status of tasar silk growing fields of Bihar and Jharkhand states for balanced nutrition to tasar host plant, *Terminalia arjuna*. *Res. J. Chem. Env. Sci.,* **5**, 63-67 (2017).

Subbaswamy, M.R., N.R. Singhvi, B.V. Naidu, M.M. Reddy, H. Jayaram, and N. Suryanarayan: Effect of source of nitrogen on phosphorus uptake and arginine content in mulberry. *Indian J. Sericul.*, **40**, 182–184 (2004).

Subhiah, B.V. and G.S. Asija: A rapid procedure for the estimation of available nitrogen in soils. *Curr. Sci.,* **25**, 259-260 (1956).

Thatcher, F.S. and D.S. Clark: Microorganisms in Foods: Their significance and methods of enumeration. University of Toronto Press, Canada, pp. 1-234 (1968)

Unni, B.G., U. Bora, H.R. Singh, B.S. Dileep Kumar, B. Devi, S.B. Wann, A. Bora, B.S. Bhau, K. Neog and R. Chakravorty: High yield and quality silk fibre production by Muga silkworm, *Antheraea assama* through the application of plant growth promoting rhizobacteria. *Curr. Sci.,* **94**, 768-774 (2008).

Vijai, P. and J. Indu: Rhizosphere bacteria for biocontrol of plant diseases. *Indian J. Microbiol.*, **38**, 187- 204 (1998).

Vishaka, G.V., M.S. Rathore, M. Chandrashekharaiah, H.A. Nadaf and R.B. Sinha: Tasar for tribes: A way of life. *J. Ent. Zoo. Stud.,* **8**, 374-377 (2020).

Walkley, A. and T.A. Black: An experimentation of the vegetative method for determining organic matter and proposed modification of the chromic acid titration method. *Soil Sci.,* **37**, 38-39 (1934).

Yadav, A., P. Verma, S. Kumar, V. Kumar, M. Kumar, T.C.K. Sugitha, B.P. Singh, A.K. Saxena and H.S. Dhaliwal: Actinobacteria from rhizosphere: molecular diversity, distributions, and potential biotechnological applications. In: New and Future Developments in Microbial Biotechnology and Bioengineering (Eds.: B.P. Singh, V.K. Gupta and A.K. Passari) Elsevier, pp. 13-41 (2018).

Online Copy