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Phyllospheric Microbial Population in Relation to Atmospheric Pollution of Ranchi

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Abstract

A comparative study was undertaken surrounding Ranchi, capital of Jharkhand to study the effect of pollution caused either due to vehicle emission or industrial discharge on phyllospheric microbial communities of *Oryza sativa, Triticum aestivum. Pisum sativum, Cajanus cajan, Eucalyptus viminalis* and *Lantana camara.* Leaf samples of the different plant species were collected from three major road sides and three industrial sites and also one —two km away from road and industries. The homogenized leaf samples were plated on suitable media for each microbial group and total counts were performed by serial dilution technique. It was observed that pollution had significant influence on quantum of living microorganisms on leaf surface of different plant species. The effect of pollution caused by vehicle emission showed more pronounced negative effect on microorganisms on all four crops than the wild species. However, industrial discharge more significantly reduced the phyllospheric microbial population of the wild species as compared to crops. The maximum per cent decrease in bacterial count was 41.6, 56.0, 51.9 and 51.0%, fungal population was 60, 7, 56.5, 43.5 and 55.5 and population of actinomycetes was 60.3, 46.4, 72.2 and 58.2% for *Oryza sativa, Triticum aestivum, Pisum sativum* and *Cajanus cajan*, respectively. In case of wild species i.e. *Eucalyptus viminalis* and *Lantana camara,* the maximum reduction in bacterial count and its reduction magnitude was (62.0 and 53.0% respectively), fungal population to the tune of (65.6 and 34.5% respectively) and actinomycetes count was observed (57.8 and 46.7% respectively). The data showed non-significant effect of location and pollution interactions.
Key words : Phyllosphere, Pollution, Microorganisms, Population.

The external surface of the leaf as an environment for microorganisms has been termed the "phyllosphere" in analogy with the rhizosphere of roots (1). Microorganisms in the phyllosphere are widespread and are encountered in a variety of host plants, such as forest trees, herbs, graminoids, cultivated plants and mosses. Many microorganisms present on the phyllosphere support plant growth and development through biological control of disease, nitrogen fixation, acceleration of senescence, production of growth regulators and controling plant parasites either by stimulating plants to synthesize phytoalexins or by producing antibacterial (2). The microbial layers of the phyllosphere show marked differences in species composition and surface spread, which are seemingly characteristics for particular plant species. Not only do different plant species show striking differences in their phyllospheric populations but different leaves of same plant show variations depending on the position and age of the leaves and it is evident that during the development of a leaf microorganisms from the leaf surface for only a short time will be absent and vigorous development of the microorganisms occurs during the maturity of the leaf when it is photosynthesing and transpiring.

Deposition of toxic particulate substances containing heavy metals and soluble harmful gases in the form of acid rains on the leaf surface through rapid industrialization and increasing qualitative and quantitative impact on phyllospheric micro flora. Air pollutants influence biological systems in different ways at a global level. At regional or local levels these effects are more significantly detectable, specially in urban and industrial areas or when the pollution is associated with roadways (3). Studies concerning air pollution impacts are difficult to undertake because of the different nature of pollutants, meteriology and orography of the considered area. The microbial ecosystems established on plant surfaces are strongly influenced by pollutants (4) and

Table 1. Bacterial population (× 10^7) on phyllosphere of different plants at different location. UPS—Unpolluted site ; PS—Polluted site.

Location	*Oryza sativa*		*Triticum aestivum*		*Pisum sativum*	
	UPS	PS	UPS	PS	UPS	PS
L_1	12.2	9.1	16.0	8.8	19.9	10
L_2	8.9	5.2	20.9	9.2	14.4	9
L_3	11.6	8.2	18.6	14.0	16.8	9
L_4	13.0	7.9	19.0	10.0	11.9	8
L_5	11.1	8.4	18.9	13.7	14.3	9
L_6	11.5	8.7	20.7	11.1	16.3	11
	SE ±	CD 5%	SE ±	CD 5%	SE ±	CD 5%
Location	0.37	0.75	0.53	1.10	0.52	1.07
Pollution	0.12	0.25	0.18	0.37	0.17	0.36
L × P	1.26	NS	1.26	NS	1.26	NS
CV%	5.35		5.04		5.95	

Table 1. Continued.

Location	*Cajanus cajan*		*Eucalyptus viminalis*		*Lantana camara*	
	UPS	PS	UPS	PS	UPS	PS
L_1	19.4	9.5	14.2	6.9	8.8	5.8
L_2	15.3	11.6	11.3	4.9	11.0	5.8
L_3	13.4	7.3	12.2	7.1	10.4	6.3
L_4	12.7	6.5	13.8	5.2	12.8	6.0
L_5	17.6	11.0	11.1	8.9	8.0	7.8
L_6	18.6	9.6	11.7	5.1	7.6	3.7
	SE ±	CD 5%	SE ±	CD 5%	SE ±	CD 5%
Location	0.47	0.98	0.61	NS	0.47	NS
Pollution	0.16	0.33	0.20	0.42	0.16	0.33
L × P	1.26	NS	1.26	NS	1.26	NS
CV %	5.34		9.21		8.71	

the capacity of these microbial populations to withstand environmental changes can influence their activity and consequently the whole host plant condition.

Methods

Selection of Site

Six polluted (three road side and three industrial sites)sites dominated by cultivated plants *(Oryza sativa, Triticum aestivum, Pisum sativum and Cajanus cajan)* and wild type (*Eucalyptus viminalis and Lantana camara*) plants, located a busy road intersection and heavy industrial discharge of Ranchi (altitude 625 a.s. l., latitude $23^0 17'$ N, longitude $85^0 19'$ E) taken as the polluted site while the other was approximately 1—2 km away from either roadside or industries and considered as the unpolluted site. Care was taken to ensure similarity in topography and micro-environmental conditions. 15—20 leaves/plant from three plant replicates each belonging to six plant spp. were taken randomly from the sites of six location. Matured whole leaf samples were removed with the help of sterilized scissors and were kept separately in sterilized poly bags. The leaf samples were stored at low temperature (4 C) till completion of the experiment.

Enumeration of Microbial Population

Dilution plate technique was employed (5) for assessing of phyllospheric micro floral population. Discs of 1 cm diameter were excised from the leaves with the help of a sterilized cork borer under laminar flow chamber to avoid contamination. Fifty such discs per sample were placed in a 250 ml conical flask containing 100 ml of sterilized distilled water. The flask was shaken vigorously for 15—20 minutes to detach the surface propagules. A suspension of 10^{-2} was obtained. 10 ml of this suspension was transferred asep-

Table 2. Fungi population (× 10^5) on phyllosphere of different plants at different location. UPS—Unpolluted site ; PS—Polluted site.

Location	*Oryza sativa*		*Triticum aestivum*		*Pisum sativum*		*Cajanus cajan*		*Eucalyptus viminalis*		*Lantana camara*	
	UPS	PS	UPS	PS	UPS	PS	UPS	PS	UPS	PS	UPS	PS
L_1	7.2	3.2	6.2	3.1	6.9	3.9	6.2	2.8	6.6	4.0	4.7	4.2
L_2	6.9	2.7	7.4	3.2	5.0	3.4	5.2	4.1	9.5	5.0	4.1	3.0
L_3	7.7	5.9	7.1	6.4	7.2	4.1	5.4	3.6	6.9	3.2	4.5	4.1
L_4	8.9	5.4	5.9	4.1	6.9	4.5	4.1	2.6	7.8	2.7	4.2	2.8
L_5	6.5	5.3	8.0	5.6	7.3	4.9	5.1	4.0	6.0	5.1	5.4	4.5
L_6	5.4	3.3	4.5	2.7	5.2	5.1	4.5	3.8	4.9	3.4	4.5	4.2
	SE ±	CD 5%	SE ±	CD 5%	SE ±	CD 5%	SE ±	CD 5%	SE ±	CD 5%	SE ±	CD 5%
Location	0.29	0.60	0.25	0.53	0.24	NS	0.18	NS	0.37	NS	0.25	NS
Pollution	0.09	0.20	0.08	0.18	0.08	0.17	0.05	0.12	0.12	0.25	0.08	0.17
L × P	1.02	NS	0.86	NS	0.86	NS	0.86	NS	0.86	NS	0.86	NS
CV %	7.33		6.88		6.56		5.97		9.68		8.54	

tically to a 250 ml conical flask containing 90 ml of sterilized distilled water to get a dilution of 10^{-3}. The process was repeated to get a suspension of 10^{-7}dilution. For the counting of bacteria dilution of 10^{-7}, for fungi dilution of 10^{-5} and for actinomycetes dilution of 10^{-3} suspensions were used. One ml required dilution was transferred aseptically into triplicate sterilized petriplates. Thereafter 15—20 ml of sterilized, liquefied and cooled appropriate media for specific micro flora was poured and mixed to and fro. After solidification the petriplates were incubated for one week at 28 ± 2 C for bacteria and actinomycetes and at 30 ± 2 C for fungi in an incubator. After the incubation period, the colonies which had developed on the plates were counted. Three replicates were maintained in each case and the mean was taken for comparison.

Results and Discussion

Effects of pollutants on bacterial population on phyllosphere of different plant species under present study are given in Table 1. The data show that population was significantly reduced due to different pollution sources. Bacterial population was maximum reduced at location L_2 (41.6 and 56.0%) was noted in cereals crop (*Oryza sativa* and *Triticum aestivum*), however, location L_1 recorded maximum reduction of bacterial population of pulses (*Pisum sativum* and *Cajanus cajan*) phyllosphere i.e., 51.9 and 51.0% while in case of wild type (*Eucalyptus viminalis* and *Lantana camara*) plants bacterial reduction was recorded at location L_4 to the tune of 62.0, 53.0% respectively. Range of decrease in the population of bacteria at all locations of Oryza sativa, Triticum aestivum, Pisum sativum, Cajanus cajan Eucalyptus viminalis and Lantana camara plants were to the tune of 24.8 to 41.6, 24.6 to 56.0, 30.7 to 51.9, 24.1 to 51.0, 19.5 to 62.0 and 2.9 to 53.0% respectively. The interaction effect of location and pollution on bacterial count of different plant phyllosphere was non-significant.

The fungal counts were significantly reduced due to pollution at all locations. (Table 2). The maximum reduction in population was observed 60.7 and 56.5% at location L_2 for cereals (*Oryza sativa* and *Triticum aestivum*), 43.5 and 55.5% at location L_1 of pulses (*Pisum sativum* and *Cajanus cajan*) and 65.6 and 34.5% for wild type plants (*Eucalyptus viminalis* and *Lantana camara*) at location L_4. However, the reduction range was 18.4 to 60.7, 9.4 to 56.5, 1.4 to 43.5, 16.1 to 55.5, 65.5 to 14.5 and 5.8 to 34.5% for *Oryza sativa, Triticum aestivum, Pisum sativum, Cajanus cajan Eucalyptus viminalis* and *Lantana camara,* respectively. Non significant interaction among locations and pollution was noted in all cases of fungal count. Table 3 predicts that the significant reduction in phyllospheric actinomycetes count of all plants except *Pisum sativum* phyllosphere due to variation in nature of the pollutants at the specific locations.The location L_2 showed maximum reduced population of actinomycetes of cereals (*Oryza sativa* and *Triticum aestivum*) phyllosphere and its magnitude was 60.3 and 46.4% respectively, however, L_1 location was observed for maximum reduction for actinomycetes of pulses (Pisum sativum and Cajanus cajan) phyllosphere to the tune of 72.4 and 58.2%, respectively. While, wild type plants phyllospheric

Table 3. Actinomycetes population (× 10^3) on phyllosphere of different plants at different location.

Location	*Oryza sativa*		*Triticum aestivum*		*Pisum sativum*		*Cajanus cajan*		*Eucalyptus viminalis*		*Lantana amara*	
	UPS	PS	UPS	PS	UPS	PS	UPS	PS	UPS	PS	UPS	PS
L_1	2.2	1.4	2.4	1.5	5.4	1.5	2.6	1.1	2.8	1.2	2.1	1.4
L_2	3.6	1.4	2.8	1.5	2.0	1.6	2.7	1.3	3.1	1.9	2.7	2.1
L_3	2.6	1.4	2.7	1.5	2.6	2.1	2.3	1.2	2.5	1.4	2.3	1.7
L_4	2.9	2.7	2.0	1.4	2.1	2.1	2.6	1.4	1.9	1.5	3.0	1.6
L_5	2.0	1.7	2.1	1.5	1.9	1.23	2.1	1.4	2.8	1.6	2.2	1.8
L_6	2.0	1.4	2.4	1.8	1.8	2.2	2.2	1.7	2.4	1.7	1.9	1.6
	SE ±	CD 5%	SE ±	CD 5%	SE ±	CD 5%	SE ±	CD 5%	SE ±	CD 5%	SE ±	CD 5%
Location	0.19	NS	0.07	NS	0.10	NS	0.08	NS	0.12	NS	0.17	NS
Pollution	0.06	0.13	0.02	0.05	0.03	0.07	0.02	0.06	0.03	0.07	0.05	0.12
L × P	0.86	NS	0.86	NS	0.86	1.77	0.86		0.86	NS	0.86	NS
CV %	13.13		5.73		7.35		6.57		7.23		11.87	

actinomycetes was observed at L_1 for Eucalyptus (57.8%) and Lantana camara phyllosphere at L_4 (46.7%). Range of reduction percentage was observed for the different plants actinomycetes was observed to in this order 6.9 to 60.3, 23.8 to 46.4, -23.9 to 72.4, 24.1 to 58.2, 21.1 to 57.8 and 17.4 to 46.7%, respectively.

Number of phyllospheric bacteria, fungi and actinomycetes was significantly reduced in polluted sites as compared to the unpolluted sile ; analysis of data indicated the detrimental effect of pollutants on the leaf surface microbial community. The findings were supported by industrially derived metal pollution studies (6), automobiles discharge a number of gases and trace metal contaminants get settled on leaf surfaces at roadsides and enter in contact with phyllospheric microorganisms (7). Density and diversity of epiphytic microbial population may varied depending on the host plant species, their physiological stages, crop-growing season and variation in morphological and physio-chemical properties of leaf surface such findings have been reported earlier (8). Therefore, different variety of a particular crop species could show substantial variation in the density and diversity of their epiphytic microbial population. Since the plants were exposed to same environment and air micro flora, above differences may be attributed to the differences in microhabitat offered by different plant species (9). The data revealed that the phyllosphere microflora of plants living in a sub-tropical road side, industries side and remote environment changed in terms of numerical composition of bacteria, actinomycetes and fungi with respect to plants living in unpolluted environment, probably it might be due to change in crop types, varieties and its physiological stages (9).

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