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POPULATION DENSITY BACTERIAL EPIPHYTES AND ENDOPHYTES OF DIFFERENT MEDICINAL PLANTS IN SAME ECOLOGICAL ENVIRONMENT

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ABSTRACT

Population density of bacterial epiphytes on dorsal as well as on ventral side of the leaves of different medicinal plants were calculated per square centimeter leaf area. On some medicinal plants, the minimum population of bacteria was on the dorsal side, while the maximum on ventral side, whereas in other medicinal plant, it was a *vice versa*. The minimum population of bacterial epiphytes was found on the leaves of *Cymbopogon winterianus*, *Ocimum canum*, *Tecomella undulate* and *Glycyrrhiza glabra*, while the maximum population was observed on the leaves of *Ocimum kilimand, Holarrenna antidysenterica*, *Madhuca indica*, *Piper longum* and *Tinospora cardifolia*. The endophytic bacterial population was enumerated per leaf. The results indicated that in some medicinal plants there was no presence of endophytic bacteria, whereas in other medicinal plants the entophytic bacterial population ranged from 1 to 4 per leaf. Interestingly, three plants *viz.*, *Lawsonia inemis*, *Piper longum* Linn. and *Tinospora cardifolia* (wild) *miers*, had uncountable bacterial endophytic population. These results indicated that the population density of bacterial leaf epiphytes and leaf endophytes in the same ecological environment was dependent/ govern by the concern plant species.

KEYWORDS: bacterial leaf epiphytes, bacterial leaf endophytes, medicinal plant.

INTRODUCTION

Bacteria are common inhabitants of both the surfaces and the internal tissues of most plants and may have diverse effects on host plant development. Leaves constitute a very large microbial habitat. It is estimated that the terrestrial leaf surface area that might be colonized by microbes is about 6.4 X 10^8 km^2 (Morris and Kinkel, 2002). Plant associated bacteria isolated from rhizoplane and phylloplane surfaces are known as epiphytes (Andrews and Harris, 2000) whereas those isolated from the interior of tissues, which they inhabit without causing harm to the host, are called endophytes (Petrini *et al.* 1989; Azevedo *et al.* 2000), with some bacterial populations fluctuating between endophytic and epiphytic colonization (Hallmann *et al.*, 1997). Reflective of marked differences in the physicochemical environments of above-ground versus subterranean plant surfaces, the leaf bacterial flora differs substantially from that of roots. For example, pigmented bacteria, which are rarely found in the rhizosphere, dominate leaf surfaces (Fokkema and Schippers, 1986; Stout, 1960a,b), presumably because solar radiation influences the ecology of the phyllosphere (Jacobs and Sundin, 2001; Sundin and Jacobs, 1999). The differential composition of leaf and root bacterial communities is further evidenced by the failure of common root colonizers such as *Rhizobium* (O’Brien and Lindow, 1989) and *Azospirillum* (Jurkevitch and Shapira, 2000) to become established on leaves.

Endophytic and epiphytic bacteria can contribute to the health, growth and development of plants. Plant growth promotion by endophytic and epiphytic bacteria may result either from indirect effects such as the bio control of soilborne diseases through competition for nutrients, siderophore-mediated competition for iron, antibiosis or the induction of systemic resistance in the plant host, or from direct effects such as the production of phytohormones or by providing the host plant with fixed nitrogen or the solubilization of soil phosphorus and iron (Glick, 1995; Shishido *et al*., 1999; Kinkel *et al*., 2000 and Sturz *et al*., 2000). The utilization of endophytic and epiphytic bacteria in agricultural production depends on our knowledge of the bacteria–plant interaction and our ability to maintain, manipulate and modify beneficial bacterial populations under field conditions (Hallmann *et al*., 1997). The study of plant-associated bacteria is important not only for understanding the ecological role of such bacteria in their interaction with plants.

MATERIAL AND METHODS

Leaves of 56 medicinal plants grown in the same ecological environments were collected from All India Medicinal and Aromatic Plants Project, Mahatma Phule

Krishi Vidyapeeth, Rahuri to assess population density of bacterial leaf epiphytes and endophytes.

Numeration of bacterial leaf epiphytes on leaves of medicinal plants

Leaf imprint method was used to numerate the bacterial epiphytes on the leaves of the medicinal and aromatic plants. For this, the leaves of medicinal plant were imprinted (Fig. 1) on the nutrient agar (NA) plates (both dorsal and ventral side of leaves were imprinted on the respective plates). The leaf imprinted plates were incubated for 48 hrs in BOD incubator at 28 ± 1°C temperature and the growth of bacterial colonies were noted with their population density.

Numeration of bacterial leaf endophytes in leaves of medicinal and aromatic plants

For numeration of bacterial endophytes in the leaves of different medicinal plants the leaves of respective plants were washed with tap water to remove dirt. Then these were blotter paper dried and surface sterilized in Mercury Chloride (0.1%) for 2 minutes and then washed thrice with distilled sterilized water. The sterilized leaves were macerated in the sterile mortal and pestle in 10 ml of water and allowed to stand for 10 minutes. Then a loop of the suspension was streak on the NA plate with the help of sterilize inoculating needle. The inoculated plates were incubated at 28 ± 1°C temperature and observed for the development of bacterial colonies upto 3 days and the growth of different colonies were noted with their population density.

RESULT AND DISCUSSION

A total of 56 medicinal plants were tested for the presence and numeration of bacterial leaf epiphytes and endophytes on/ in the leaves. The population density of bacterial epiphytes on dorsal as well as ventral side of the leaves was calculated per square centimeter leaf area (Fig. 2). The results (Table 1) indicated that the medicinal plant varied in harboring the epiphytic bacterial population on the dorsal and ventral side of the leaves. The minimum bacterial population of epiphytes per square centimeter on the leaf surface was 1, whereas the maximum population of epiphytes was uncountable and these population varied with the medicinal plants. On the same medicinal plants, the minimum population of bacterial epiphytes was on the dorsal side, while the maximum was on the ventral side, whereas in other medicinal plant it was *vice versa*. The minimum population of bacterial epiphytes were found on the leaves of *Cymbopogon winterianus jowitt*, *Ocimum canum*, *Tecomella undulatew* and *Glycyrrhiza glabra*, while the maximum was observed on the leaves of *Ocimum kilimand*, *Holarrenna antidysenterica*, *Madhuca indica*, *Piper longum* and *Tinospora cardifolia*.

Goodman (1967) and Blackman and Bordle (1976) reported that saprophytic bacteria residing on the plant surface to protect plants against bacterial diseases. Several bacterial species were known to occur on leaf surfaces but their relation with phytopathogenic bacteria occurred only in few cases (Verma *et al.*, 1980, 1983). Sinha *et al.*, (1983) showed that phylloplane bacteria associated with cotton include *Aeromonas*, *Corynebacterium*, *Flavobacterium*, *Micrococcus* and *Pseudomonas* and protected the leaves from *Xanthomonas campestris* pv. *malvacearum* infection. Maintenance of adequate population of these bacteria might be significant in integrated management of disease. On some medicinal plants, the minimum population of bacteria was on the dorsal side, while the maximum on ventral side, whereas in other medicinal plant, it was a *vice versa*. The minimum population of bacterial epiphytes was found on the leaves of *Cymbopogon winterianus*, *Ocimum canum*, *Tecomella undulate* and *Glycyrrhiza glabra*, while the maximum population was observed on the leaves of *Ocimum kilimand*, *Holarrenna antidysenterica*, *Madhuca indica*, *Piper longum* and *Tinospora cardifolia*. Similarly, in some medicinal plants there was no presence of endophytic bacteria, whereas in other medicinal plants the entophytic bacterial population ranged from 1 to 4 per leaf. Several different types of bacterial colonies were seen in a single plant. Generally, endophytic bacterial populations are larger in roots and decrease in the stems and leaves (Lamb *et al.* 1996). The bacterial endophytes are used to control fusarium wilt of cotton (Chen *et al.*, 1995).

Kinkel *et al.* (2000) and O'Brien and Lindow (1989) reported that plant species appear to influence the microbial carrying capacity of the leaf, since the total number of culturable bacteria recovered from broad-leaf plants such as cucumber and beans was significantly greater than that recovered from grasses or waxy broad-leaf plants.

Morris and Kinkel (2002) reported that the large number of bacteria on leaves in temperate regions of the world and that populations in tropical regions are probably even larger, the planetary phyllosphere bacterial population may be as large as 1026 cells.

Steven and Maria (2003) reviewed on miclobial ecology and reported that bacteria are sufficiently numerous to contribute in many processes of importance to global processes, as well as to the behavior of the individual plants on which they live.

The population density of endophytes in these leaves of medicinal plants indicated that in some medicinal plants, there was no presence of endophytic bacteria, whereas in others the endophytic bacterial population ranges from 1 to 4 per leaf. Interestingly three plants *viz.*, *Lawsonia inemis*, *Piper longum* and *Tinospora cardifolia*, had uncountable bacterial endophytic population.

Table 1: Population density of bacterial epiphytes and endophytes on/in the leaves of medicinal plants in same ecological environment

Sr. No	Medicinal plants	Population density of bacterial epiphytes on the leaves. Area (cm^2)		Population density of endophytic in leaf
		Dorsal side	Ventral side	
1.	*Abrus precatorius* Linn.	uc	2	0
2.	*Acacia catechu wild*	7	6	0
3.	*Acorus calamus* Linn.	uc	3	2
4.	*Adhatoda vesica nees*	3	10	3
5.	*Aegle marmelos corr*	5	10	0
6.	*Aleo Vera* Linn.	3	2	1
7.	*Asparagus Racemosus Wild*	2	9	0
8.	*Azadirachta indica* A. Juss	6	33	2
9.	*Bacopa monnieri* (Linn.)	2	7	2
10.	*Bixa orellana* Linn.	15	20	0
11.	*Butea monosperma*	18	6	0
12.	*Calotropis gigantean*	7	7	0
13.	*Careya arborea* Roxb	4	4	1
14.	*Carissa carandas* Linn.	20	23	2
15.	*Cassia fistula* Linn.	26	8	0
16.	*Centella asiatica* (Linn.)	3	10	0
17.	*Commiphora mukul* Hook	15	12	0
18.	*Cymbopogon flexuosus*	3	5	1
19.	*Cymbopogon winterianus jowitt*	1	3	1
20.	*Eugenia jambolana lam*	5	2	0
21.	*Feronia elephantum correa*	2	6	0
22.	*Gemelina arborea* Linn.	4	25	0
23.	*Glycyrrhiza glabra* Linn.	1	1	0
24.	*Hibiscus Rosa Synensis* Linn.	23	24	3
25.	*Holarrenna antidysenterica wall*	5	uc	2
26.	*Ixora coccinee*	4	7	0
27.	*Lawsonia inemis*	3	5	uc
28.	*Madhuca indica* J.F. Gmel	10	uc	0
29.	*Memecylon edule* Roxb.	3	12	1
30.	*Mentha arvensis* Linn.	12	19	3
31.	*Mimosa pudica*	14	-	2
32.	*Myristica fragrans* Houtt.	Uc	20	0
33.	*Nerium indicum* Mill.	4	5	1
34.	*Ocimum* canum sims	1	3	1
35.	*Ocimum kilimand – scharicum* Guerke.	30	14	2
36.	*Oroxylum indicum* Vent.	2	13	0
37.	*Piper longum Linn.*	5	uc	uc
38.	*Plumbago rosea* Linn.	22	13	0
39.	*Populus dettoiys*	17	8	0
40.	*Premna integrifolia* Linn.	4	13	0
41.	*Pterocarpus marsupium* Roxb	11	20	0
42.	*Rauwolfia serpentia* Benth Ex kurz	20	9	4
43	*Ruta graveloens* Linn.	3	5	1
44.	*Salmalia malabarica* Schott & Endl.	3	8	0
45.	*Samecarpus anacardium* Linn.	6	5	2
46.	*Sapindus trifoliatus* Linn.	6	15	0
47.	*Simarouba glauca*	18	21	0
48.	*Solanum khasianum* Clarke.	4	16	0
49.	*Tecomella undulate*	1	5	0
50.	*Terminalia arjune*	23	12	3
51.	*Terminalia bellerica Retz*	17	8	2
52.	*Terminalia chebula*	16	5	0

53.	*Tinospora cardifolia* (wild)	50	uc	uc
54.	*Vicna rosea* Linn.	2	9	0
55.	*Vitex negundo* Linn.	4	3	2
56.	*Withania somnifera dunal*	5	15	10

uc = Uncountable.

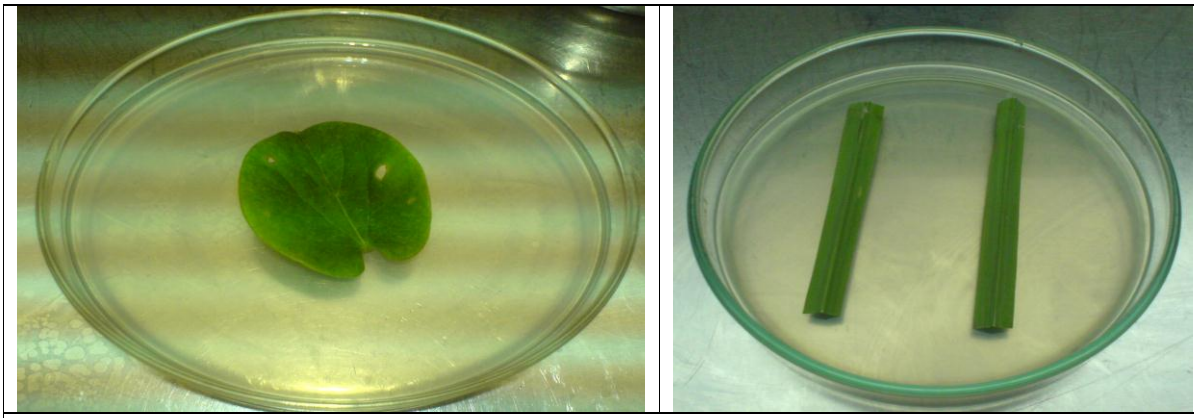
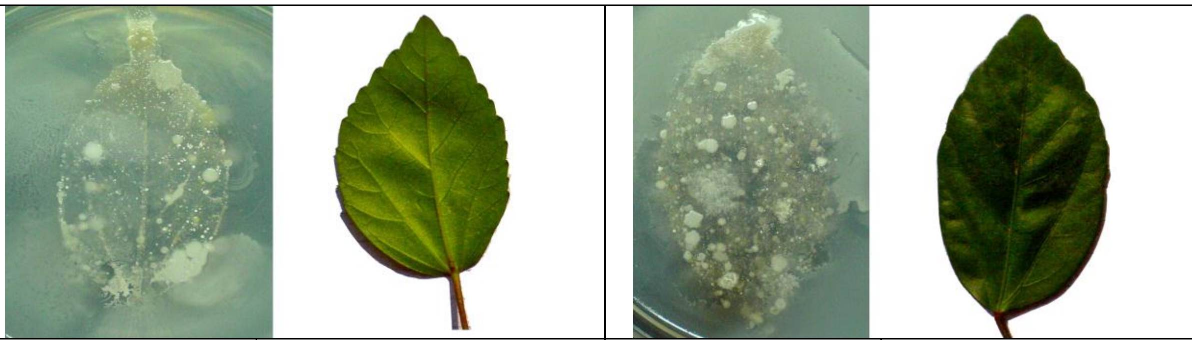
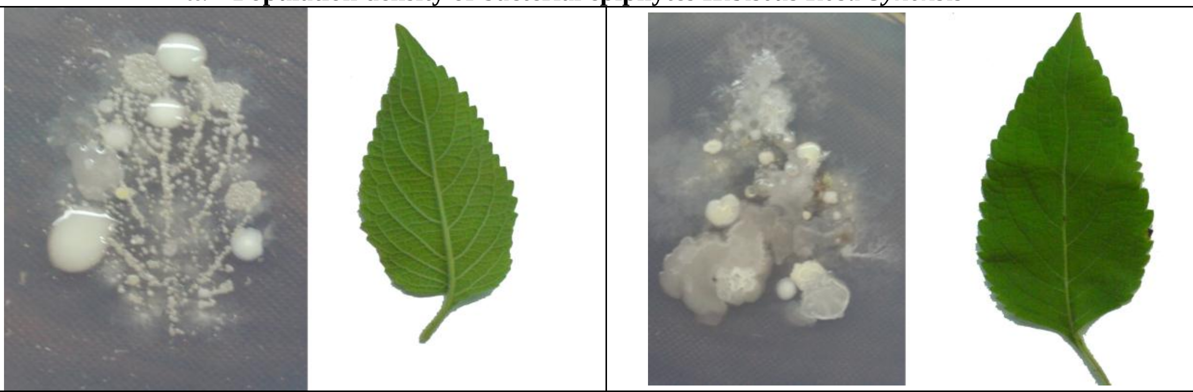


Fig 1: Method for numeration of bacterial epiphytes on the leaves of medicinal plants.



In the culture plate	Dorsal side of the leaf	In the culture plate	Ventral side of the leaf

a. Population density of bacterial epiphytes *Hibiscus Rosa Synensis*



In the culture plate	Dorsal side of the leaf	In the culture plate	Ventral side of the leaf

b. Population density of bacterial epiphytes *Ocimum kilimand*

Fig 2: Numeration of bacterial epiphytes on the leaves of medicinal plants.

CONCLUSION

The medicinal plant varied in harbouring the epiphytic bacterial population on their dorsal and ventral side of the leaves in the same ecological environments; and the population density of bacterial leaf epiphytes and leaf endophytes in the same ecological environment was dependent/ govern by the concern plant species.

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