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Microbial community on leaf surface of *Lantana camera* as influenced by industrial and road side pollution

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Abstract
Present investigation was carried out to isolate, characterize and identify the microbial communities on leaf surface of Lantana camera collected from different polluted (near road or industrial sites) and unpolluted (one km away) sites of Ranchi district of Jharkhand. The population of Bacterial, Fungi and Actinomycetes decreased significantly in polluted site as compared to unpolluted sites. The bacterial isolate found on leaf surface of *Lantana camera* from different locations have been placed in five groups on the basis of morphological, physiological and biochemical characterizations. These groups of bacterial isolates were identified as *Methylovorous* spp., *Aminobacter* spp., *Beijerinckia* spp., *Azotobacter* spp. *Pseudomonas* spp. Less no. of bacterial isolates (9) were noted in polluted site as compare to unpolluted site (12). Dominant genus reported on polluted site was *Aminobacter* and *Pseudomonas* however, *Beijerinckia* spp. showed their dominancy in polluted sites. 17 fungal isolates found on leaf surface of *Lantana camera* from different location have been placed in 8 different groups based on cultural characterization. The prominent genera at polluted site were *Aspergillus*, while *Rhizopus* was found only at unpolluted sites. Twelve isolates of actinomycetes, corresponding to five genus were noted from *Lantana camara* leaf surface at polluted and unpolluted sites. The genera *Herbidospora* was found at both site but, dominant at unpolluted site.

Keywords: Microbial community, leaf surface, *Lantana camera,* industrial, road side pollution

Introduction
Lantana camara is a semi woody ever green shrub belongs to family Verbenaceae. It has become one of the most widely spread weeds in wastelands and roadsides. The foliar exudates which are washed down in the crevices of the bark provide nutrients sufficient to maintain a complex microbial community. Dust, moisture and debris deposited between such leaf epiphytes and these in turn would provide an environment for micro-organisms. The microbial layers of leaf surface show marked differences in species composition and surface spread, which are seemingly characteristics for particular plant species.
The kind of micro-organisms occurring in the population and the degree to which the surface is covered vary for different plant species under different environmental conditions capping these ideas in mind, present investigation was carried out to isolates, characterizes and identify the microbial communities on leaf surface of *Lantana camera*.

Material and methods
Selection of location & sample collection
Lantana camara leaves were collected from polluted (near road, industrial sites) and unpolluted (one km away) sites & samples were prepared from six different locations of Ranchi district.

Table 1: Leaf sample collected from different Location

Location	Area name	Geographical situations	Site name	
			Polluted site (PS)	Unpolluted site (UPS)
L1	Ranchi – Hazaribagh	23^0 22′ N - $85^0$21′ E	Road side (RSL1)	Remote side (RRL1)
L2	Ranchi – Jamshedpur	23^0 22′ N - $85^0$21′ E	Road side (RSL2)	Remote side (RRL2)
L3	Ranchi – Gumla	23^0 22′ N - $85^0$19′ E	Road side (RSL3)	Remote side (RRL3)
L4	HEC, Sec- II, Ranchi	23^0 19′ N - $85^0$17′ E	Industrial site (ISL4)	Remote side (IRL4)
L5	HEC, Sec-III, Ranchi	23^0 18′ N - $85^0$17′ E	Industrial site (ISL5)	Remote side (IRL5)
L6	Usha Martin, Ranchi	23^0 22′ N - $85^0$25′ E	Industrial site (ISL6)	Remote side (IRL6)

Source: J.S.P.C.B. (2010) [7].

15 – 20 leaves/plant from three plant replicates were taken randomly from the sites of all location. Matured whole leaf samples were removed with the help of sterilized scissors between 9.00 AM to 3.00 PM during bright sunshine and were kept separately in sterilized poly bags. The leaf samples were stored at low temperature (4 ^{0}C) till completion of the experiment.

Isolation and Identification of bacteria isolates

Isolation

Bacterial counts were determined by nutrient agar medium by serial dilution plate technique (Aneja, 2007) [1]. 1 cm diameter discs leaves were excised with the help of a sterilized cork borer. Per sample fifty such discs were placed in a 250 ml conical flask containing 100 ml of sterilized distilled water. Flask was shaken vigorously for 15 – 20 minutes to detach the surface propagules. For the counting of bacteria 10^{-7} dilution were taken. Pure cultures of bacterial isolates were obtained through repeated streaking of well differentiable colonies on solidified media for microbial groups. Pure cultures thus obtained were maintained by frequent sub culturing on slants throughout the experiment.

Characterization

During total bacterial count, plate showing maximum number of colonies was taken for further investigation in all cases. Assignment of appropriate genera to the bacterial isolates were done based on morphological (Shape and arrangement of cells, size, gram stain, and colony size, colour & shape on agar), physiological (O_2 relation & motility) and biochemical (Ammonia production, Urease test, Catalase test, Starch hydrolysis, Casein hydrolysis, H_2S production, NO_3 reduction, Growth on N free media & Growth on PSB media) characters as described in Bergey's Manual of Determinative Bacteriology (Holt, *et al.*, 1994) [5].

Morphological characterization

For examination of size and shape, smears on slides fixed by gentle heat were stained with carbol fuchsin. Gram stained bacterial cells on clean micro slides were used for measurement of bacterial size (length and width) using ocular micrometer. 24 hours old cultures of bacteria were stained and examined under microscope for shape and arrangement of cells. Isolates were examined for Gram staining characters. Shape, size and colour of isolates colony under study were streaked and plate were incubated as needed, and growth were checked after 18-24 hours (Chhonkar, *et al.*, 2007) [4] in which above observation were taken.

Physiological characterization

To determine whether a culture is aerobic or not. Nutrient broth (containing 0.005 per cent bromocresol purple) columns were prepared in culture tubes. Observations were recorded at 48 hr. interval for 7 days. Hanging drop technique was performed for demonstrating motility of bacteria using 12 hr. old nutrient broth culture (Aneja, 2007) [1].

Biochemical characters

(i) Ammonia production: Culture was incubated in peptone broth for 5 days at small piece of filter paper with Nessler reagent was placed in upper part of culture tube. Test tube was warmed on a water bath at 50 - 60^o C. Filter paper shown brown to black, was indicator of ammonia production.

(ii) Urease test: Urease test was performed on urea agar medium**.** Basal medium was poured in 90.0 ml quantities in flasks, autoclaved and cooled to 45 ^{0}C. 10 ml filter sterilized urea solution (20%) was added to each flask, after mixing it was poured in 5 ml culture tubes. After solidification of medium in slanting position, tubes were inoculated with test bacterium, incubated (30 ± 2 ^{0}C) and observations were recorded at regular intervals up to 15 days. Presence of urease gives red colour and for no urease yellow colour.

(iii) Catalase test: Catalase test was performed by adding H_2O_2 to trypticase soy agar slant. Culture medium was poured in culture tubes and sterilized by autoclaving. Test bacterium was inoculated in agar slant and inoculated at 35 ^{0}C for 24-48 hrs. observations were taken by adding 3 - 4 drops of H_2O_2 to flow over the growth of each slant culture and the reaction obtained was recorded. Presence of gas bubbles indicates catalase +ve test.

(iv) Hydrolysis of starch: It was performed by adding soluble starch (0.2 percent) in nutrient agar medium. pH was adjusted to 7.0 - 7.2 with the help of bromothymol blue indicator. The liquid was dispensed in test tube and sterilized. When cooled the liquid was incubated with culture and incubated at 30 ^{0}C. After incubation a small portion of liquid was treated with diluted iodine solution. No change in colour indicate complete hydrolysis, brown colour indicate partial hydrolysis and blue colour indicates that there was no hydrolysis of starch.

(v) Casein hydrolysis: Autoclaved and cooled basal medium was poured into sterilizes Petri plates, inoculated with culture. A single line streak inoculation across the surface of medium was produce and plates incubated for 2 - 3 days at 37 ^{0}C in an inverted position. A clear zone around streak showed a positive reaction while absence of clear area around the growth of an organism is a negative reaction.

(vi) Hydrogen Sulphate production test: Production of H_2S by different isolates was tested with the help of impregnated strips of filter paper with 10% solution of lead acetate and dried. A lead acetate strip was placed at the top of a nutrient broth culture and incubated. Blackening of paper indicated H_2S production.

(vii) Nitrate reduction: Nitrate broth was used with following composition: Peptone - 10.0 g, Beef -extract- 5.0 g, KNO_3 - 1.0 g, Distilled water - 1000.0 ml. The ingredients were dissolved and poured in tubes (5 cm) and autoclaved. The broth was inoculated with the test bacterium and incubated at 25 ^{0}C. Reduction of nitate was checked upto 15 days at regular intervals by adding a few drops of sulfanilic acid (0.8% in 5 M acetic acid) and dimethyl alpha-nephthylamine (0.5% in 5 M acetic acid) to the nitrate broth culture subsequently. Presence of nitrite was detected by the red Colour given by addition of sulfanilic acid, α–naphthylamine reagent to a portion of the liquid.

Determination of specific properties

Nitrogen fixing bacteria - For the assessment of Nitrogen - fixing bacteria, Burk's Nitrogen free medium was used and incubated in 250 ml conical flasks inoculated with culture of test organisms. The flasks were then incubated for 14 days at 30 ^{0}C. After the incubation, the contents of the flasks along with uninoculated contents as control were digested for the

determination of nitrogen by the Kjeldahl's method (Jackson, 1973) [6].

Phosphate solubilizing bacteria - For the assessment of P – solubilizing bacteria, Bunt and Rovira (1955) [3] medium was used. 100 ml of the medium replacing Tricalcium Phosphate by Rock Phosphates equivalent to 50 mg % P_2O_5 in 250 ml conical flasks were inoculated with different isolates. Inoculated flasks were incubated for 15 days at 30 ± 0.2 ^{0}C under static condition and shaken once in 12 hr. along with control un-inoculated flasks. 5 ml of growth medium from each flask was withdrawn aseptically after 15 days and passed through Whatman No. 42 filter paper. The filtrates were assayed for P_2O_5 by Amino-Molybdate Ascorbic acid blue method (Jackson, 1973) [6].

By comparing the results of the above tests as described in Bergey's Manual of Determinative Bacteriology (Holt, *et al.*, 1994) [5], isolates was tentatively identified up to generic level.

Result and Discussion

Microbial population at different location on *Lantana camara* leaf surface

Microbial population of *Lantana camara* leaf surface at different location is presented in Table (1). It is evident from the data that population of bacteria, fungi and actinomycetes decreased significantly in polluted site as compared to unpolluted of *Lantana camara* leaf surface. Bacterial population was reduced to 39.79 per cent followed by actinomycetes population (29.17 per cent) and fungal population (17.39 per cent). Microbial population observed at different locations showed non significant difference. However, interactions among location and pollution also found to be non significant in all cases of microbial population in *lantana camara* leaves.

The reduction in microbial population located in the vicinity of industries and road sites may be attributed to increased absorption of heavy mud, dusts, and traffic fumes. Results are in agreement to the findings of Joshi (2008) [8].

Morphological, Physiological and biochemical characteristics of bacterial isolates

The bacterial isolates found in *Lantana camara* leaf surface from different location have been placed in to five groups on the basis of morphological, physiological and biochemical characteristics (Table 2a & 2b).

Group (A) morphology of the cells was observed as rigid type, dimensions of the different isolates varied between 0.5 µ to 1.5 µ in length and 1.0 µ to 3.0 µ in width, gram negative, colony on nutrient agar medium varied from 5 to 12 mm diameter, colour of the colony, orange, reddish and pink, entire, flat, smooth, muccoid, translucent and opaque. Physiologically they showed positive growth with pressure of oxygen and were motile. Biochemically the isolates hydrolyzed starch and catalase was positive. But, did not produced ammonia, not liquefied casein, not reduced nitrate or starch and not produced H_2S. The isolates resembling in above characters were placed $RRL1_1$, $RSL2_1$, $RRL3_1$, $RSL4_1$, $IRL5_1$ and $ISL5_1$.and tentatively identified as the members of genus *Methylovorus* sp.

Group (B) Cells bearing straight rods, occurring singly in there pairs or in chains, dimensions of the different isolates varied between 0.5 µ to 1.2 µ in length and 2.0 µ to 4.5 µ in width, Gram positive, colony on nutrient agar medium varied in size from 6 to 15 mm, yellow to deep yellow colour, shape of the isolates showed granular, raised, glistening and smooth. Physiologically they showed positive growth in pressure with of oxygen and were non - motile. Biochemically the isolates hydrolyzed urea, produced ammonia, reduced nitrate and was catalase positive. But, did not hydrolyzed starch and not produced H_2S. On the basis of the above characteristics, the isolates tentatively identified as the genus *Aminobacter* sp. This isolate fall in the group was $RRL1_2$, $RSL1_2$, $RRL3_2$, $IRL4_2$, and $ISL6_1$.

Group (C) Cells bearing ellipsoidal shape, diameter ranging between 1.0 µ to 1.5 µ in length and 2.4 to 3.0 in width, Gram negative, size of colony 8 to 12 mm on nutrient agar, white to dull white in colour, shape of colony circular, smooth, slimy and glistening. Aerobic nature of growth and were motile. Produced ammonia and H_2S, failed to NO_3^- reduction but hydrolyzed starch. Cells showed positive growth on specific medium. With above characters organisms were tentatively identified as the genus *Beijerinckia* sp. The following isolates fall in this group was $RSL1_1$, $RSL3_2$ and $IRL5_2$.

Group (D) Cells of this group isolates were short rods, rods, occurring in pairs and chains, individual cells varied from 0.8 µ to 1.2 µ in length and 3.5 µ to 4.0 µ in width, Gram negative, size of colony on nutrient agar medium between 8 to 15 mm diameter, brown and white in colour, shape of these isolates scattered, irregular, raised and wrinkled. Physiologically they showed positive growth with pressure of oxygen and were motile. Biochemically these isolates produced ammonia, urea hydrolyzed and NO_3^- reduced, but H_2S did not produced. Mineral nitrogen free medium showed positive growth of cells. On the basis of the above characteristics, the isolates assembled in one group and tentatively identified as members of genus *Azotobacter* sp. These isolates placed in the group - $RRL2_1$, $IRL5_2$ and $IRL6_2$.

Group (E) Rod shaped cells with round ends, diameter ranging between 2.0 µ to 3.2 µ, Gram negative, colony size on agar 7 to 10 mm, white to shiny white in colour, shape varied raised, glistening, smooth and slimy. Aerobic nature of growth with oxygen and were motile. Urea and starch hydrolyzed and were catalase positive, whereas nitrate reduction was negative. Inorganic phosphate solubilization was observed by cells on medium. The isolates resembling in above characters were placed $RRL2_2$, $ISL4_1$ and $IRL6_1$ and tentatively identified as the members of genus *Pseudomonas* sp.

Less number of bacterial isolates (9) was noted in polluted site as compared to unpolluted sites (12) on *Lantana camara.* Joshi, *et al.* (2008) [9] documented that pollution had direct harmful effect on survival of organisms which might be attributed to reduction in number of isolates observed in present investigations. The dominant genus reported on polluted site were *Aminobacter* and *Pseudomonas*, however, *Beijerinckia* sp. showed their dominancy in polluted sites.

Cultural characteristics and identification of fungal isolates of *Lantana camara*

Seventeen fungal isolates found on leaf surface of *Lantana camara* plant of different location have been placed in to eight different groups based on cultural characteristics (Table 3).

Group (A) colonies showed white to whitish colour on agar medium. Small, globose and bright conidia scattered on vegetative mycelium mostly, hyphae were creeping, septate and forming a flat type, conidiophores erect and arising from short and side branches. Only one isolates *i. e.*, $IRL6_2$ resembling in above characteristics this was placed in a group and tentatively identified as member of genus *Trichoderma* sp.

Group (B) colonies of these group isolates were black to dark brown in colour, mycelium immersed, aerial and partly superficial. Fertile hyphae were creeping, septate and looked like jar shaped structure in branches, conidiophores varied in shape; size & colour laterally formed a jar – shaped. Conidia solitirally, subglobose to globose, subsherical, smooth and black one – celled. On the basis of above characteristics isolates were tentatively identified as to the genus *Nigrospora* sp. and isolates were assembled in this group $RRL1_2$, $ISL4_2$, $IRL6_1$ and $ISL6_2$.

Group (C) colour of these group isolates varied from cottony white to blackish. Conidia, simple, ellipsoidal, fusiform, hyaline, aseptate were scattered on immersed mycelium of substratum. Conidiophores flexous, erect micronematous and unbranched on septate hyphae. *Curvularia* sp. was tentatively identified for these group isolates was $RSL1_1$ and $RRL2_2$.

Group (D) colour of colonies on agar medium varied from bluish gray to white, aerial mycelium consists of septate, Hyphae septate and unbranched. Conidiophores mostly were arise from substrate of aerial Hyphae. Conidia elliptical to subglobose, smooth walled. Mineral phosphate was solubilized by appropriate medium. From above characteristics it may be placed under genus *Penicillium*. These two $RRL2_1$ and $RSL3_2$ isolates were fulfilling these parameters.

Group (E) colour of colonies on agar medium varied from dark green to gray green, colourless vegetative mycelium consists of septate and branching. Jar shaped hyphae were creeping, branching and septate. Conidiophores erect and short, hemisphered shape, septate and non septate both types occurred which was densely crowded. Varing in size and shape of conidia were spherical to globose and smooth walls, pale green to green colour. inorganic phosphate solubilization was found by isolates on specific medium. Based on the above characteristics these isolates tentatively identified as *Aspergillus* sp. Following isolates assembled in this group $RSL2_1$, $IRL4_1$ and $ISL6_1$.

Group (F) colonies of these group isolates were white to violet, well developed rhizoids present in branches and septa of mycelium, colourless Hyphae spiral and oval shaped, consists of branches and nonseptate. From these points sporangiophores appear erect, straight and unbranched, at the ends of which sporangia are seen containing sporangiophores and sporangia contains spores. Spores were globose and ellipsoidal. Based on the above parameters following these $RSL2_2$ isolates were tentatively identified as to the *Rhizopus* sp.

Group (G) colonies of these isolates were varied from black – green to brown in colour, inverted club shaped, dark brown and oblong ovate conidia scattered on aerial mycelium. Hyphae well developed, creeping, non septate and septate, conidiophores brown, septate, erect, short and irregular. On the basis of above characteristics $RRL3_1$ and $ISL5_1$ isolates were tentatively identified as members of *Alternaria* sp.

Group (H) colonies of the isolate dark brown, mycelium immersed, septate and branched, conidiophores, hyaline, septate and branched only at the base, conidia hyaline, aseptate, straight and smooth. From above characteristics it may be placed under genus *Colletotrichum.* These two $RSL3_1$ and $IRL5_1$ isolates were fulfilling these parameters.

The fungal isolates observed from *Lantana camara* were seventeen. The prominent genera at polluted site were *Aspergillus*. While, *Rhizopus* was found only at unpolluted sites. It might be due to individual fungal species which are highly sensitive or resistant to pollutants could significantly alter ecosystem function. Similar results were reported by Khanna (1986) [10].

Cultural characteristics and identification of actinomycetes isolates of *Lantana camara*

It may be seen from (Table 4) that the twelve isolates taken from the *Lantana camara* plant of different location have been placed in to five different groups based on cultural characteristics.

Group (A) colonies on the agar medium were observed as varied colour, substrate mycelium and substrate Hyphae were observed, short types chains of spores were born on the tips of narrow sporophores directly derived from the substrate hyphae, physiologically they showed anaerobic nature with pressure of oxygen. However, gram positive observed and spore was negative, On the basis of above parameters, the organisms tentatively identified as the genus *Herbidospora* sp. Following isolates belonged to this group were $RRL1_1$, $RSL3_1$ and $IRL5_1$.

Group (B) agar colonies of isolates were showed brown to white in colour, aerial mycelium were stable and branched, longitudinal spores were scattered on aerial mycelium, surface of the spores were smooth. Aerobic nature of growth was observed as positive. However, gram positive observed and spores were negative. Two isolates $RSL1_1$ and $ISL6_1$ resembled above characters and these were placed in a group and tentatively identified as members of genus *Micromonospora* sp.

Group (C) cells colour of this isolate was red, aerobic nature of growth with pressure of oxygen, aerial mycelium were filamentous and fragmenting into coccoid chains & forming bacteria, like coccoid. Gram positive, while spore staining was negative. The isolates $RRL2_1$ resembling in above characters and tentatively identified as members of genus *Nocardia* sp.

Group (D) cell morphology of the isolates were observed on agar medium, orange to deep orange in colour and nature of growth of isolates was aerobic, two types of mycelium were observed one substrate and other aerial type, single conidia were seen singly at the ends of conidiophores on the surface of the mycelium. Two isolates $RSL2_1$, $IRL4_1$, and $IRL6_1$ resembling in above characters, these were placed in a group and tentatively identified as members of genus *Micromonospora* sp.

Group (E) Agar colonies of this isolates appeared dull white to chalky white, physiologically they showed positive growth with pressure of oxygen and produced earthy odour. The isolates showed aerial and substrate mycelium, septate Hyphae were divided in to single conidia chains. Spores were oval to ellipsoidal shape, hairy surface and occurring in chains. Gram and spore staining, both were positive. Based on these characteristics, the isolates tentatively identified for the members of genus *Streptomyces* sp. Assembled isolates were following $RRL3_1$, $ISL4_1$ and $ISL5_1$.

Twelve isolates of actinomycetes, corresponding to five genus were noted from *Lantana camara* leaf surface at polluted and unpolluted sites. The genera *Herbidospora* was found at both site but, dominant at unpolluted site. It might be due to the harmful effect of pollutants on survival of this species (Brighigna, *et al.,* 1999) [2]. In our study the genera *Nocardia* was observed only at unpolluted site and *Microbispora* only at polluted sites. It was documented by (Khanna, 1986) [10] that the plants at polluted site exposed to microbial flora different from those at the unpolluted site. This might have been an important factor for the altered microbial population

recorded at polluted site. However, many of alteration specific to hosts. Our findings are in agreement with (Rai and Pathak, 1981) [11].

Table 1: Effect of pollution on microbial population cm^{-2} of *Lantana camara* leaf surface at different locations

Location	Bacteria (x 10^7)			Fungi (x 10^5)			Actinomycetes (x 10^3)		
	UPS	PS	Mean	UPS	PS	Mean	UPS	PS	Mean
L1	8.8	5.8	7.3	4.7	4.2	4.5	2.1	1.4	1.8
L2	11.0	5.8	8.4	4.1	3.0	3.6	2.7	2.1	2.4
L3	10.4	6.3	8.4	4.5	4.1	4.3	2.3	1.7	2.0
L4	12.8	6.0	9.4	4.2	2.8	3.5	3.0	1.6	2.3
L5	8.0	7.8	7.9	5.4	4.5	5.0	2.2	1.8	2.0
L6	7.6	3.7	5.7	4.5	4.2	4.4	1.9	1.6	1.8
Mean	9.8	5.9	7.8	4.6	3.8	4.2	2.4	1.7	2.0
	L	P	L x P	L	P	L x P	L	P	L x P
S.Em. ±	0.47	0.16	1.26	0.25	0.08	0.86	0.17	0.05	0.86
C.D. 5%	NS	0.33	NS	NS	0.17	NS	NS	0.12	NS
C.V. %	8.71			8.54			11.87		

UPS – Un-polluted site, PS – Polluted site.

Table 2(a): Cultural characteristics and identification of bacterial isolates of *Lantana camara*.

Location	Isolate no.	Morphological						Physiological	
		Shape and arrangement of cells	Size	Gram stain	Colony on agar			O_2 relation	Motility
					Size, mm	Colour	Shape		
RRL1	1^A	Rigid type	1.6 x 1.5	–	10	Orange	Entire, flat, smooth, mucoid	Aerobic	+
	2^B	Rods, singly in pairs	1.2 x 4.5	+	6	Yellow	Granular, raised, glistening	„	–
RSL1	1^C	Ellipsoidal, singly in pairs	1.0 x 2.5	–	8	White	Circular, slimy & smooth	„	+
	2^B	Straight rods, singly in chains	1.0 x 3.5	+	12	Yellow	Raised, glistening, smooth	„	–
RRL2	1^D	Cylindrical rods, singly, pairs	1.0 x 4.0	–	15	Brown	Scattered and irregular	„	+
	2^E	Rods, round ends	2.0	–	10	Shiny white	Raised, glistening, smooth	„	+
RSL2	1^A	Rigid type	0.8 x 2.5	–	8	Orange	Entire, flat, muccoid, transl.	„	+
RRL3	1^A	Rigid type	0.6 x 1.2	–	5	Reddish	Entire, flat, smooth, opaque	„	+
	2^B	Rod singly in pairs	0.7 x 2.5	+	10	Deep yellow	Raised, glistening, smooth	„	–
RSL3	1^A	Rigid type	1.0 x 2.5	–	11	Orange	Entire, flat, smooth, mucoid	„	+
	2^C	Ellipsoidal, singly in chains	1.5 x 2.4	–	12	White	Circular, smooth, glistening	„	+
IRL4	1^A	Rigid type	1.2 x 2.0	–	7	Pink	Entire, smooth, translucent	„	+
	2^B	Rods, pairs	0.5 x 2.0	+	15	Yellow	Smooth, raised, granular	„	–
ISL4	1^E	Rods, round ends	3.2	–	4	Shiny white	Raised, glistening, slimy	„	+
IRL5	1^A	Rigid type	1.5 x 1.0	–	5	Reddish	Entire, flat, smooth, opaque	„	+
	2^C	Ellipsoidal, single short chain	1.0 x 3.0	–	8	White	Circular, smooth, slimy	„	–
ISL5	1^A	Rigid type	0.5 x 3.0	–	12	Reddish	Entire, flat, smooth, muccoid	„	+
	2^D	Rod, singly in chains	1.2 x 3.5	–	10	White	Irregular and scattered, raised	„	+
IRL6	1^E	Rod, round ends	2.5	–	7	White	Raised, glistening, smooth	„	+
	2^D	Rod, singly in pairs	0.8 x 4.0	–	5	Brown	Irregular, scattered, wrinkled	„	+
ISL6	1^B	Straight rods, singly in pairs	0.7 x 3.0	+	8	Yellow	Granular, raised, glistening	„	–

+ Positive, – Negative.

Table 2(b): Bio-chemical characteristics and identification of bacterial isolates of *Lantana camara*.

Location	Isolate no.	Ammonia production	Urease test	Catalase test	Starch hydrolysis	Casein hydrolysis	H_2S production	Nitrate reduction	Growth on N free media	Growth on PSB media	Identified Genus
RRL1	1^A	–		+	+	–	–	–	–	–	*Methylovorus*
	2^B	+	+	+	–		–	+	–	–	*Aminobacter*
RSL1	1^C	+	+	–		–	+		–	–	*Beijerinckia*
	2^B	+	+	+	–		–	+	+	–	*Aminobacter*
RRL2	1^D	+	+				–	+	+	–	*Azotobacter*
	2^E		+	+	–			–	–	+	*Pseudomonas*
RSL2	1^A	–		+	+	–	–	–	–	–	*Methylovorus*
RRL3	1^A			+	+	–	–	–	–	–	*Methylovorus*
	2^B	+	+	+	–		–	+	–	–	*Aminobacter*

RSL3	1^{A}	–		+	+	–	–	–	–	–	*Methylovorus*
	2^{C}	+	+	–		–	+		+	–	*Beijerinckia*
IRL4	1^{A}	–		+	+	–	–	–	–	–	*Methylovorus*
	2^{B}	+	+	+	–		–	+	–	–	*Aminobacter*
ISL4	1^{E}		+	+	–			–	–	+	*Pseudomonas*
IRL5	1^{A}	–		+	+	–	–	–	–	–	*Methylovorus*
	2^{C}	+	+	–		–	+		+	–	*Beijerinckia*
ISL5	1^{A}	–		+	+	–	–	–	–	–	*Methylovorus*
	2^{D}	+	+				–	+	+	–	*Azotobacter*
IRL6	1^{E}		+	+	–			–	–	+	*Pseudomonas*
	2^{D}	+	+				–	+	+	–	*Azotobacter*
ISL6	1^{B}	+	+	+	–		–	+	–	–	*Aminobacter*

+ Positive, – Negative.

Table 3: Cultural characteristics and identification of fungal isolates of *Lantana camera.*

Location	Isolate no.	Characteristics					Growth on PSF media	Identified Genus
		Colour	Mycelium	Hyphae	Conidiophores	Conidia		
RRL1	1^{A}	Whitish	Vegetative	Creeping, septate, forming a flat	Erect, arising from short, branched side branches	Small, globose, bright colour	–	*Trichoderma*
	2^{B}	Black	Immersed	Creeping	Laterally, jar-shaped	Solitary, subsherical, black	–	*Nigrospora*
RSL1	1^{C}	Cottony white	Immersed in natural substratum	Septate	Flexous, erect, unbranched	Simple, fusiform, ellipsoidal	–	*Curvularia*
RRL2	1^{D}	Bluish gray	Septate	Septate	Arise from the substrate	Smooth-walled, elliptical	+	*Penicillium*
	2^{C}	Blackish	Immersed, substratum	Septate	Micronematous, flexous	Hyaline, aseptate	–	*Curvularia*
RSL2	1^{E}	Dark green	Vegetative, colorless	Branched, septate	Varying in shpe, size, colour & margins	Non septate, usually enlarging & broadening	+	*Aspergillus*
	2^{F}	Violet	Septate	Branch, nonseptate	Erect, straight	Globose, ellipsoidal	–	*Rhizopus*
RRL3	1^{G}	Dark green		Well developed, branched	Sparingly septate & noded	Ovate, elongate, club-shaped	–	*Alternaria*
RSL3	1^{H}	Brown	Immersed, hyaline		Septate, cylindrical, integrated	Aseptate, hyaline, thin walled	–	*Colletotrichum*
	2^{D}	White	Aerial	Unbranched	Mostly as branches of aerial hyphae,	Elliptical to subglobose	+	*Penicillium*
IRL4	1^{E}	Dark green	Vegetative, consists of septate, branched	Branched	Smooth walls, coarse, short, flask shaped	Globose, cleistothecium presents, heads blue green	+	*Aspergillus*
ISL4	1^{B}	Black	Partly superficial	Creeping, jar shape		Solitirally, globose, smooth	–	*Nigrospora*
IRL5	1^{H}	Dark grown	Immersed, septate, branched		Hyaline, septate, branched at the base	Hyaline, aseptate, straight, smooth	–	*Colletotrichum*
ISL5	1^{G}	Black	Immersed	Branched, septate	Erect, simple, flexous, septate	Very short chains, curved, smooth	–	*Alternaria*
IRL6	1^{B}	Dark brown	Aerial	Fertile hyphae creeping, septate,	Laterally, jar-like	One-celled, subglobose, smooth	–	*Nigrospora*
ISL6	1^{E}	Gray green	Aerial	Jar shape,creeping	smooth wall, colourless	Elliptical, green, smooth	+	*Aspergillus*
	2^{B}	Dark brown	Immersed	Septate, branched	Terminally	Smooth, black, one cell	–	*Nigrospora*

+ Positive, – Negative.

Table 4: Cultural characteristics and identification of actinomycetes isolates of *Lantana camera* plants.

Location	Isolate no.	Morphological	Staining		Cultural		Identified Genus
			Gram stain	Spore stain	Colour	Growth	
RRL1	1^{A}	Short chains of spores are borne on the tips of narrow sporophores directly derived from the substrate hyphae	+	–	Orange	Anaerobic	*Herbidospora*
RSL1	1^{B}	Stable, branched mycelium carries in characteristics longitudinal spores on aerial mycelium, surface smooth	+	–	Brown	„	*Microbispora*
RRL2	1^{C}	Filamentous, fragmenting into coccoid chain	+	–	Red	„	*Nocardia*
RSL2	1^{D}	substrate mycelium, septate Hyphae in single conidia	+	–	Deep orange	„	*Micromonospora*
RRL3	1^{E}	Aerial mycelium, septate hyphae divided in a single chains	+	+	Dull white	„	*Streptomyces*
RSL3	1^{A}	Short chains of spores are borne on the tips of sporophores derived from the substrate hyphae	+	–	Pale pink	Anaerobic	*Herbidospora*
IRL4	1^{D}	Septate hyphae, aerial mycelium single conidia	+	–	Orange	Aerobic	*Micromonospora*

ISL4	1^{E}	Mature umbels of spore chains whorls are shown	+	–	Chalky white	„	*Streptomyces*
IRL5	1^{A}	Substrate mycelium, chains of spores are borne on the tips of sporophores	+	+	Light red	Anaerobic	*Herbidospora*
ISL5	1^{E}	Spores were oval to ellipsoidal shape, hairy surface and occurring in chains.	+	–	Chalky white	„	*Streptomyces*
IRL6	1^{D}	Nonseptate hyphae, aerial mycelium, conidia single	+	+	Pale pink	„	*Micromonospora*
ISL6	1^{B}	Stable, branched mycelium carries in characteristics longitudinal spores on aerial mycelium, surface smooth	+	–	Brown	„	*Microbispora*

+ Positive, – Negative.

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