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Growth promotional activity of green synthesized nanoparticles on Phyllosphere and Rhizosphere Microflora of *Capsicum baccatum* , *Rosa species* and *Murraya koenigii*.

J.Mani kanta[1], Kumari Neelam[2] S.Anju[3*], J.Sarada[4]

[1, 2, 3,4]Department of Microbiology, *Bhavan's Vivekananda College of sciences, Humanities and Commerce,* Sainikpuri, Secunderabad.

[*]*Corresponding Author: anjunair227@gmail.com[*] Tel.: Tel:040-27111611;ext 214*

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Abstract— Nanoparticle synthesis is identified to occur in nature, in presence of agents like bacteria, fungi, algae and other biologically derived substances that acts as a precursor. These naturally formed nanoparticles could establish in soil and on plants that may result in change of the characteristic features of plant physiology or morphology. Among the various metal nanoparticles, Zinc oxide and Iron nanoparticles have been found to have a profound effect on the plants since their metallic ions act as essential nutrients. These nanoparticles were proved to have significance in promotion of seed germination, impact on the shoot, root and foliage development due to enhanced absorption of these elements. One of the most important requisite for a growing plant is presence of the plant growth promoting rhizobacteria in the soil and surface of plants. The plant growth and susceptibility to pathogens is greatly influenced by microorganisms established on plant parts and rhizosphere. Many plants, with the antagonistic feature of their rhizobacteria against plant pathogens, remain resistant to certain diseases. Hence, one of the reasons for the growth promoting activity of nanomaterials could be due to their influence on the microorganisms. Therefore the present study was carried out to evaluate the effect of zinc oxide and iron oxide nanoparticles on the rhizobacteria and phyllosphere microorganisms of selected plants. Treatment of the Zinc oxide and Iron oxide nanoparticles to the rhizosphere and leaves of *Capsicum baccatum* (Chilli), *Rosa species* (Rose) and *Murraya koenigii* (curry) and *Solanum tuberosum* has indicated a change in the phyllosphere and rhizosphere characteristics and the bacterial consortium that naturally developed on it. A significant change in the phyllosphere and rhizophere bacteria indicated a profound effect of nanoparticles .

Keywords— *Zinc nanoparticles, Iron nanoparticles, Bacillus sps, Phyllosphere,Rhizosphere.*

I. INTRODUCTION

Nanobiotechnology widens the scope of production and application of nanomaterials in various fields and is also a means for technological development. Biological source for nanoparticle synthesis has a greater significance due to the ecofriendly mode of synthesis. Bacteria, fungi, algae and a number of organic substances derived from natural sources act as precursors for synthesis of nanoparticles [1]. Nanoparticles synthesized by biological methods are identified to have less toxicity when compared to nanomaterial prepared by other methods. Therefore the applications of biologically synthesized nanoparticles are in more use due to their altered properties when compared to their elemental forms.

Recent studies have proposed, role of nanoparticles in promoting the plant growth and their effective means of pest control activities. While there has been studies reporting the plant growth promotion, it needs to be noted that these effects are specific to concentration of nanoparticles, plant species and the conditions of growth. Not much has been understood about the mechanisms involved in interactions between plant systems and the nanoparticles. Various parameters are affected in the presence of nanoparticles, which in turn results in either plant growth promotion or inhibition. Exposure of *Allium cepa* to zinc oxide nanoparticles(ZnO) during cultivation was found to show accumulation of these nanoparticles in the roots and was found distributed in the cytoplasm and cell nucleus[2]. A combination of ZnO and FeO nanoparticles on vegetative growth yield was found to be maximum in many species[3].

Among the different parameters that could be considered to enhance the plant growth, the role of plant growth promoting microflora is an essential one. Rhizosphere and phyllosphere microflora are essential for the development of plant and greatly influence the susceptibility and resistance to pests. Phyllosphere microflora are likely to influence the

condition of their hosts. About 14 bacterial isolates obtained from phyllosphere of pearl millet crop were studied for different plant growth promoting features like IAA, ammonia, siderophore production and zinc solubilisation. From the study it has been reported that IAA and ammonia producing strains were highest(100%) when compared to siderophore (57%) production, zinc solubilisation (50%).[4] Inoculation of Bacillus M3 and Pseudomonas BA-8 had been identified to increase the yield, growth and P,Fe,Cu,Zn content of strawberry plant and simultaneously increased the soil P, Fe, Zn , K, Mg availability.[5]

II. RELATED WORK

The susceptibility to pests is controlled in certain plants by the specific microflora with which they are associated .Hence the enhancement of the relevant bacteria or fungi on the plant could improve the plant health due to resistance acquired by the antagonism. One of the studies had revealed production of acyl-homoserine lactones which was responsible for antagonistic activity by a rhizosphere strain *Agrobacterium tumifeciens* that resulted in significant decrease in soil pathogens. [6].*Jatropha curcas* showed increase in its growth by the action of rhizospheric bacterial strains *B.brevis* (MS1), *B.licheniformis* (MS3) *A. Calcoaaceticus* (MS5) which could produce IAA, siderophores, solubilise inorganic phosphate and ACC diaminase.[7]

From the above studies it is seen that plant growth was greatly influenced by phyllosphere and rhizosphere bacteria ,indicating their important role in plant physiology and morphology.The current study focuses on the impact of zinc oxide and iron nanoparticles on the phyllosphere and rhizosphere bacteria of *Capsicum baccatum* (Chilli), *Rosa species* (Rose) and *Murraya koenigii* (curry leaves) and *Solanum tuberosum* (potato).

III. METHODOLOGY

1.Isolation of bacteria

Bacteria have been known to have the ability to transform metal ions of zinc to nanoparticles. To obtain bacteria capable of biologically transform ZnO nanoparticles, various soil samples were screened to identify, reduction of zinc nitrate to zinc oxide nanoparticles. Bacterial isolates were obtained by dilution plate technique on the medium enriched with 1mM concentration of zinc nitrate. [8]

2. Green synthesis of iron oxide nanoparticles.

To synthesize iron oxide nanoparticles various plant extracts that have been not reported earlier studies were subjected to screening and reduction of $FeCl_3$ into iron nanoparticles was studied [9]

3. Synthesis of ZnO nanopartcicles

Potential isolate of *Bacillus sps* was inoculated into Nutrient Broth to obtain 24hr actively growing culture. Extracellular supernatant was obtained by centrifugation of the culture at 10,000rpm for 15 min. 10ml of the supernatant was added to 100ml of 10mM $Zn(NO_3)_2$ solution at room temperature. Formation of ZnO nanoparticles was identified initially by visual color change.[10]

4. Green Synthesis of Iron nanoparticles

5gm of *Azadirachta indica (*Neem*)* flowers were boiled in 50 ml milliQ water for 20 min. The cooled extract was filtered using Whatmann no.1 filter paper. 1ml of flower extract was added to 10ml of 10mM $FeCl_3$ solution and incubated at room temperature. The precipitate formed was centrifuged at 10,000 rpm for 20min.. Formation of FeO nanoparticles was identified initially by visual color change. [11]

5.Characterization of nano particles .

Characterization of biologically synthesized nanoparticles was done by UV – Spectrophotometric analysis. Presence of ZnO and FeO NP's was confirmed by scanning the test solution at a wavelength range of 200-350nm in a UV-Visible spectrophotometer (SYSTRONICS 117)[12]. FTIR analysis has been done for determining the interaction of proteins with ZnO and FeO nanoparticles. The reduced Zinc nitrate and Iron chloride solution was concentrated in the Rotovac for 2hrs and then subjected to FTIR analysis. Spectrum was recorded in the range of 4000-500cm^{-1} using a SHIMADZU Fourier Transform Infrared Spectrophotometer [13]. SEM analysis was done to determine the size and shape of ZnO and FeO nanoparticles. The scanning data was obtained after 10 scans. The size and shape of the ZnO and FeO NP's was determined by Scanning Electron Microscopy using SEM S-3700 with accelerating voltage of 30000 Volts, emission current 82000 nA and a magnification of 5000. [14]

6. Study on phyllosphere and rhizosphere bacteria population.

ZnO and FeO nanoparticles (10mg /ml) was sprayed on dorsal and ventral surface of leaves of *Capsicum baccatum* (Chilli),*Rosa species* (Rose) and *Murraya koenigii* (curry leaves) in potted plants.

Enumeration of phyllosphere bacteria before and after treatment with nanoparticles was carried out by Leaf Imprint technique on the Nutrient Agar plates. Colonies formed on the media have been observed for colony

morphology and gram nature has been determined to briefly understand the diversity of the bacteria. [15]

7.Potted plant experiments

Biologically synthesized nano particles (50mg nanoparticles/10mg of soil) were used to enrich the soil planted with Capsicum *baccatum* (Chilli), *Rosa species* (Rose) and *Murraya koenigii* (curry leaves) in pots. Enumeration of rhizobacteria before and after treatment with nanoparticles by dilution plate technique has been carried out to evaluate the effect on microorganisms present in the soil.[16]

IV. RESULTS AND DISCUSSION

Potential bacterial isolate for synthesis of ZnO nanoparticle.

A potential *Bacillus*.sps from the soil samples was isolated which had the ability to reduce the zinc nitrate solution to nano ZnO. This culture has been used in the present study for biological synthesis of ZnO nanoparticle .Fig.1

Synthesis of ZnO and FeO nanoparticles

Addition of 10ml of bacterial supernatant of *Bacillus* species to 100ml of 10mM filter sterilized zinc nitrate solution resulted in white precipitate.50ml of Neem flowers extract when added to 100ml of 10mM iron chloride resulted in greenish black precipitate. Biologically Synthesized ZnO and FeO nanoparticles was identified by white precipitate formation and greenish-black precipitate formation respectively.**Fig.2(a,b)**

Characterization of Zinc and Iron nano particles

UV visible Spectroscopy

Nanoparticle solutions were characterized by determining their absorption maxima. A maximum absorption range observed in between 200-240nm is identification for presence of ZnO nanoparticles. And maximum absorption range observed in 200-250 is identification for presence of iron oxide nanoparticles**. As represented in Fig.3**

FTIR analysis

The stability of the nanoparticles was attributed to the proteins interacting with the particles in the solution. The FTIR analysis has been done in order to figure out the different protein molecules that may have interacted with the nanoparticles. Zinc oxide nanoparticles in the FTIR analysis revealed the peaks at 3909.84, 2924.18, 2081.26, 1639.55, 1627.97 signifying the interaction of the groups C-N stretch, Aromatic groups, Primary and Secondary Amines , Aromatic stretch , Carboxylic acids respectively. These interactions of proteins results in the reduction to ZnO nanoparticles.

Similarly as depicted in . Fig.4,Iron oxide nanoparticles were identified by the formation of peaks observed at 2928.04, 2081.26 ,2067.76, 1842.08,1641.48,1631.83 signifying the interaction of the groups C-N stretch, Aromatic groups, Primary and Secondary Amines , Aromatic stretch and Carboxylic acids

SEM analysis

The morphological characterization of the biologically synthesized metal nanoparticles by SEM analysis has revealed various sizes of nano particles .The images of Zinc oxide nanoparticles after SEM analysis, showed that there was formation of mostly spherical ZnO Nanoparticles of sizes, 91.5nm, 98.2nm, 101nm, 105nm, 121nm and 127nm. Similarly the SEM images of Iron oxide nanoparticles from Fig.5 have shown that there was formation of spherical FeO Nanoparticles of range 48.4nm -124nm.

4. Study on phyllosphere and rhizosphere bacteria

Every plant species is characterized by establishment of microorganisms which are responsible to influence different morphological and physiological traits in them . The rhizosphere and phyllosphere is known to be influenced by a number of factors like nutrient availability, conditions of growth and other environmental factors. Since nanoparticles are being used for different applications in agriculture, the current study is focused on their effect in the plant rhizozphere and phyllosphere. The zinc oxide nanoparticles when applied on the dorsal and ventral surfaces of *Capsicum baccatum , Rosa* species and *Murraya koenigii* to evaluate the effect on the phyllosphere it was found that there was an increase in the phyllosphere organisms on dorsal side and decrease on the ventral side. It was observed that application of ZnO nano on dorsal surface showed increase in the number of phyllosphere bacteria by 50%in *Murraya konini, 18*% in *Rosa* species and 52% in *Capsicum baccatum* . Whereas there was reduction of bacteria on the ventral surface by 86%in *Murraya koenigii* , 53% in *Rosa* species and 42% in *Capsicum baccatum.* Phyllosphere Bacteria were identified by isolation on nutrient agar plates and by gram staining. [Table 1]

However application of iron oxide nanoparticles on the leaves resulted in complete inhibition of phyllosphere microorganisms. Dorsal surface showed reduction in percentage by 84%in *Murraya koenigii* , 80% in *Rosa* species and 69% in *Capsicum baccatum.* In case of ventral surface a lesser reduction was seen that is 72%in *Murraya koenigii* , 57% in *Rosa* species and 71% in *Capsicum baccatum.* [Fig 6]

The nanoparticles when incorprated to the rhizosphere soil of *Capsicum baccatum*, *Rosa* species and *Murraya koenigii* showed increase in bacterial CFU/gm of soil for ZnO and FeO .These metal ions which are considered as micronutrients where found to enhance the population of bacteria in the soil rhizosphere.

V. Conclusion and Future Scope

Nanoparticle synthesis by biological means is economical and less time cosuming. Diverse natural sources needs to be explored to standardize controlled synthesis of nanoparticles that are ecofriendly. ZnO and FeO nanoparticles synthesized in the current study are found to be promoting the growth of rhizosphere microflora. However the impact on the phyllosphere is found to be varied. Further characterization of the microorganisms and standardization of concentrations of nanoparticles , could help to figure out the specific bacterial population that gets enhanced or inhibited in the presence of the nanomaterials.

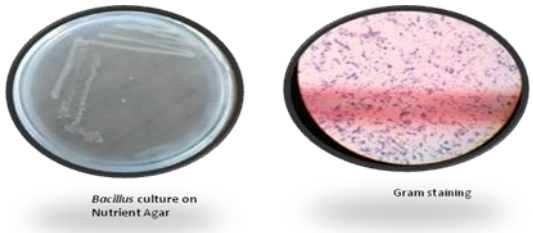


Figure 1. *Bacillus* isolate –Synthesizing ZnO Nano

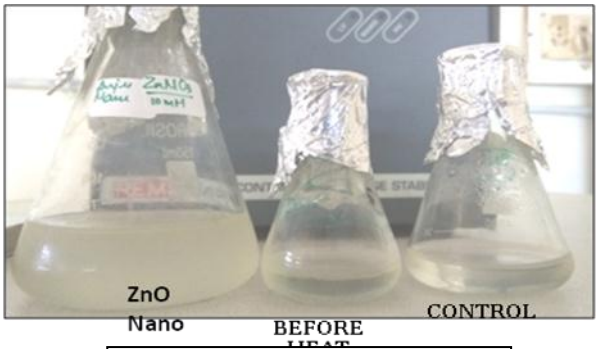


Figure 2.(a) ZnO synthesis

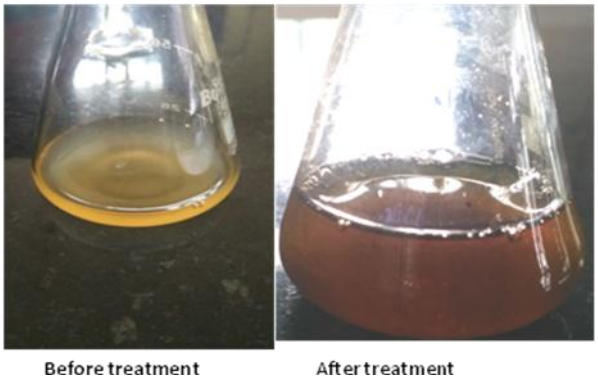


Figure 2.(b) FeO synthesis

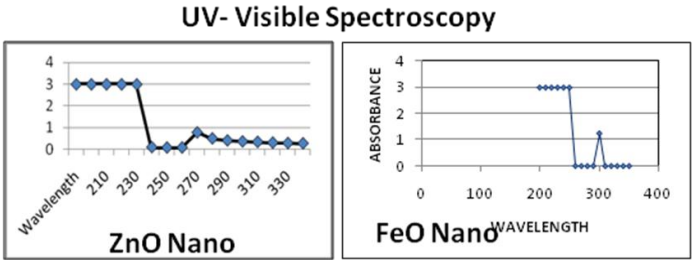


Figure 3. UV-Visible Spectroscopy of ZnO and FeO nano

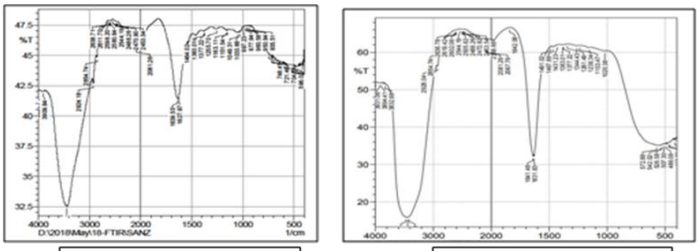


Figure 4. FTIR Analysis of ZnO and FeO nano

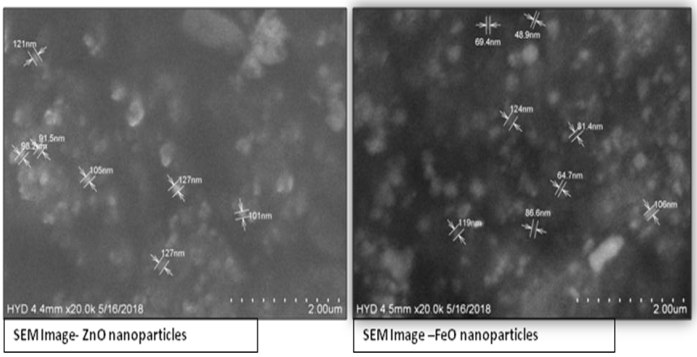


Figure 5. SEM Analysis of ZnO and FeO nano

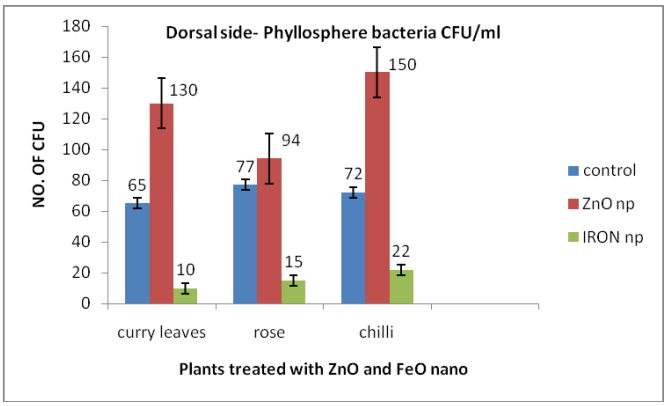


Figure 6.(a) Nanoparticles treated on dorsal side of leaves

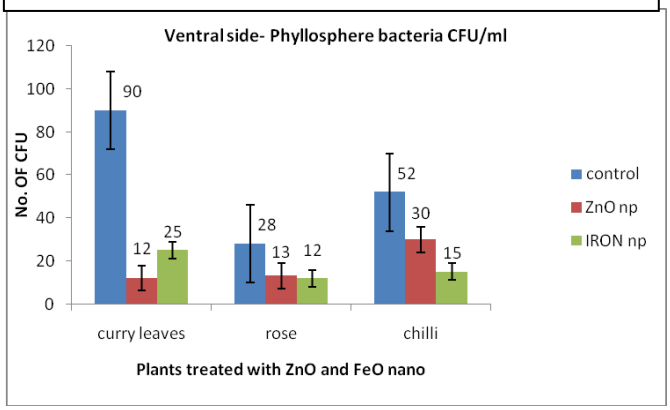


Figure 6.(b) Nanoparticles treated onVentralside of leaves

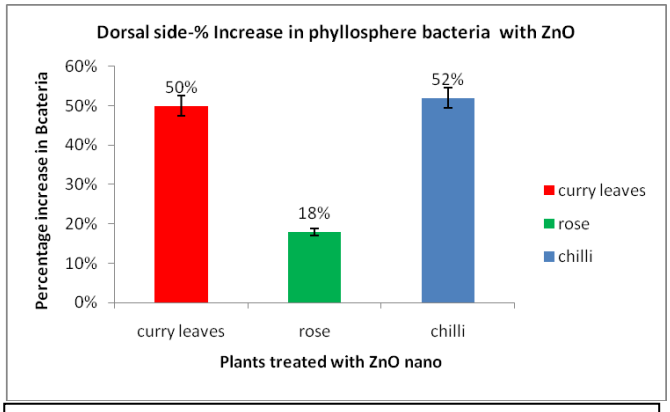


Figure 6.(c) Effect of ZnO nano on Dorsal phyllosphere

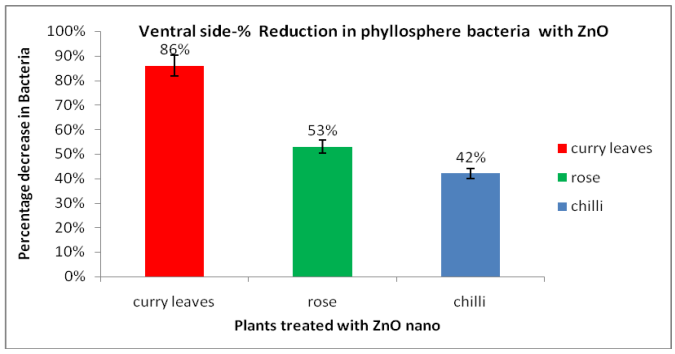


Figure 6.(d) Effect of ZnO nano on ventral phyllosphere

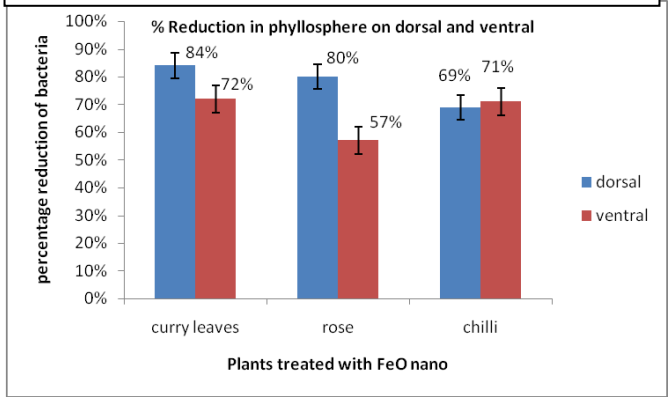


Figure 6.(e) Effect of FeO nano on Phyllosphere

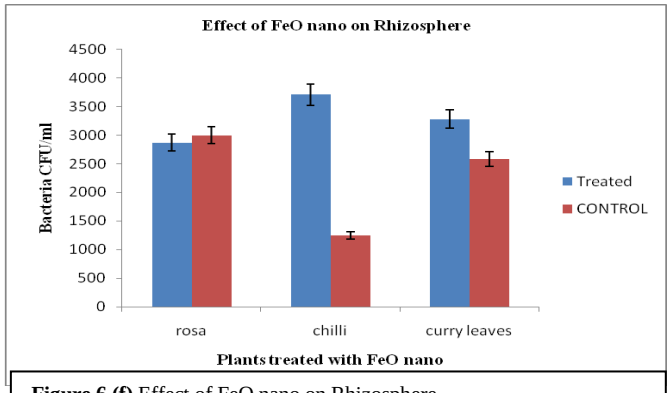


Figure 6.(f) Effect of FeO nano on Rhizosphere

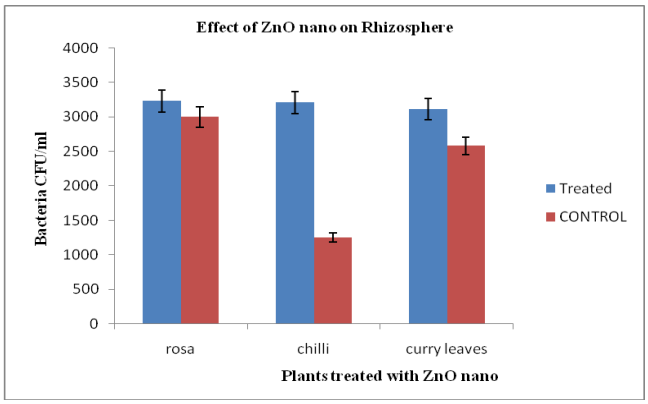


Figure 6.(g) Effect of ZnO nano on Rhizosphere

Table 2 [a]: Phyllosphere of *Rosa species*

Table:1 Most Commonly Found Rhizosphere and Phyllosphere Micro organisms in *Solanaceae family , Rosa sp., Murraya koenigii.*

Plant	Rhizosphere micro flora	Phyllosphere micro flora
Solanaceae family	*Chryseobacterium spp Massilia spp,Agrobacterium tumefaciens,Pseudomonas spp,Lysobacter capsici,Ralstonia solanacearum,*Trichoderma,*Penicillium,.Fusarium,* Phoma	*Azotobacterchroococcum,Azoto-bacter indicum,Klebsiella, Cladosporium,Aspergillus, Alternaria,Colletotrichum Curvularia*
Rosa sp.	*Acetobacter,Acinetobacter, Methylococcus,Micrococcus, Planococcus*	*Pantoea agglomerans, Klebsiella terrigena, Erwinia rhapontici, Rahnella aquatilis, Nigrospora oryzae, Aureobasidium spp, Acremonium spp, Nodulisporium sp, Gliocladium virens, Cladosporium sp*
Murraya koenigii	*Cupriavidus,Bradyrhizobium,* Rhizobium,*Burkholderia,Cell vibrio*	*Enterobacter clocae, Pseudomonas aeruginosa, Aspergillus oryzae, Colletotrichum gloeosporioides*

Table 2 [b] Phyllosphere of *Capsicum baccatum*

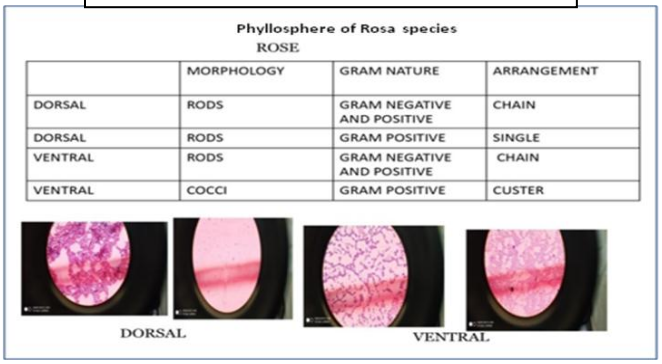
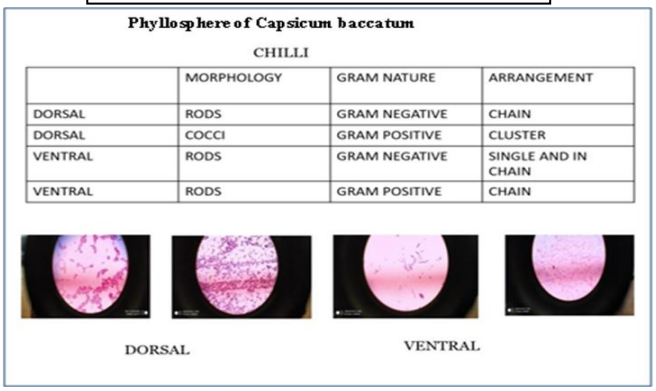
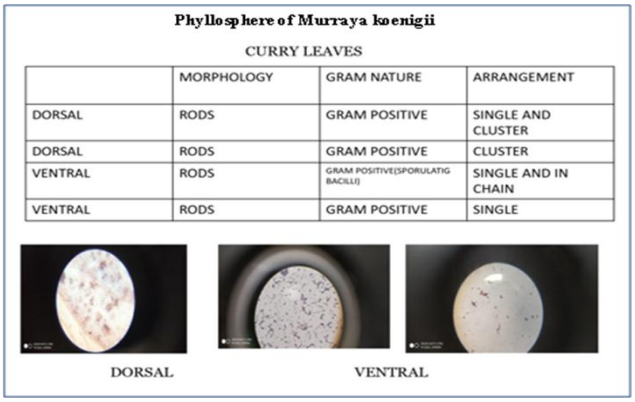


Table 2[c]: Phyllosphere of *Murraya koenigii*





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AUTHORS PROFILE

Dr. J.Sarada, Ph.D from Osmania University, in 1995. She has 18years of teaching experience and research in industry.She is currently working as Assistant Professor,Dept.of Microbiology, Bhavan's Vivekananda College, Sainikpuri,Secunderabad. She is a member of AMI and IWSA. She has more than 20 research publications in reputed scopus and web of Science indexed journals and 1 text book and 1 book chapter in the Springer Book edition and has presented 40 papers in various International and National Conferences. Her research work foucuses mainly in the fields of Quorum Sensing, Microbial enzymes, Nanobiotechnonology, Biofilms and Bioinformatics.

Mrs. S.Anju, M.Sc,NET, currently working as Assistant Professor,Dept.of Microbiology, Bhavan's Vivekananda College, Sainikpuri,Secunderabad, has teaching experience of 15 years. She is a member of AMI and has 6 research publication in international journals and 2 book chapters in the Springer Book editions. Her areas of interest in research work includes Nanobiotechnonology, Biofilms and Bioinformatics.

