

Soil Microflora in Rhizosphere of *Barringtonia racemosa* (L.) Spreng and *Rauwolfia serpentina* (L.) Benth. ex Kurz from Western Ghats region of Uttara Kannada, Karnataka, India

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DOI: [10.36348/sjpm.2022.v07i07.001](https://doi.org/10.36348/sjpm.2022.v07i07.001)

| Received: 27.05.2022 | Accepted: 30.06.2022 | Published: 05.07.2022

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Abstract

A study was conducted for the isolation and identification of soil micro-flora in rhizosphere of *B. racemosa* (L.) Spreng and *R. serpentina* (L.) Benth. ex Kurz, trees from Western Ghats region of Uttara Kannada, Karnataka, India. Soil samples were collected from rhizosphere of *B. racemosa* and *R. serpentina* plants, during the months of February 2022. Soil microbes were isolated by using soil dilution technique. The total number of bacteria isolated from *B. racemosa* and *R. serpentina* rhizosphere is 2,666,600 and 4,461,160 per gram of soil respectively. The rhizospheric fungal isolates present in *B. racemosa* and *R. serpentina* plants is 3,281,200 and 1,946,200 per gram of soil respectively. In *B. racemosa* rhizosphere, the percentage contribution is *Penicillium* sp with 13.679%, In *R. serpentina* rhizosphere, the highest percentage contribution is *Cladosporium* sp., *Trichoderma* sp. with 17.663% and 17.391% respectively. In rhizosphere of *B. racemosa*, 19 different species belonging to 15 genera were isolated; among which *Penicillium* species is dominating over other fungal species isolated. In rhizosphere of *R. serpentina*, 18 different species belonging to 11 genera were observed; among which *Trichoderma* sp. is dominating over other fungal species isolates. The most frequently isolated fungi from the rhizosphere of *B. racemosa* and *R. serpentina* plants are *Penicillium* sp and *Trichoderma* sp. For the first time the rhizospheric micro-flora i.e., bacteria and fungi was reported from *B. racemosa* soil sample collected from Western Ghats region of Uttara Kannada. For the first time the rhizospheric myco-flora was reported from *R. serpentina* soil samples collected from Western Ghats region of Uttara Kannada. All the bacteria and fungi isolated was new report to Western Ghats region of Uttara Kannada, Karnataka, India.

Keywords: *Barringtonia racemosa*, *Rauwolfia serpentina*, fungal diversity, Western Ghats, Snake bite plants, Rhizosphere fungi.

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INTRODUCTION

Soil contains diverse group of organisms like fungi, bacteria, actinomycetes, protozoa and various decaying material (Wardle *et al.*, 2004, Baldrian, 2003). The soil biomass constitutes microbial world in which larger proportion consists of fungi, they are eukaryotic microorganisms that play fundamental ecological roles as decomposers, mutualists, pathogens, making the availability of nutrient and recycling the different organic materials (Hannula *et al.*, 2017). Distinct microbial communities have been reported in forest floors under different tree species. Soil micro-organisms are the critical link between shifts in the composition of the dominant vegetation and fundamental shifts in ecosystem functioning (Mitchell

et al., 2010). The rhizosphere inhabiting microorganisms compete for water, nutrients, space and sometimes improve their competitiveness by developing an intimate association with plant (Ritz and Young 2004). The rhizosphere microflora includes bacteria, fungi, nematodes, protozoa, algae and micro-arthropods. The distribution of fungi in different types of regions depends majorly on soil structure, vegetation, temperature, humidity etc (Gaddeyya *et al.*, 2012).

Residues of pesticides present in soil can affect species differently, some species can tolerate and degrade the molecules and therefore thrive in the soil (Rohilla and Salar 2012). The rhizosphere microbes plays very important role in improving medicinal values of plants. Recently there is an increasing interests in the

research of the relation between rhizosphere microbes associated with medicinal plant for the improvement of quality of medicines. A large variety of fungi and bacteria is recognized in the rhizosphere soil of medicinal plants that show Significant effect in secondary metabolite alteration and uptake of plant nutrients (Shaikh and Mokat 2022). The study of fungal community in the forest soil of Western Ghat region of Uttara Kannada district is lacking, so our present study aims at soil microflora in rhizosphere of two medicinal plants *B. racemosa* and *R. Serpentina* of Western Ghats region of Uttara Kannada, Karnataka, India.

MATERIALS AND METHODS

Study area of Uttara Kannada district lies between 13.9220° N to 15.5252° N latitude and 74.0852° E to 75.0999° E longitude and has total area of 10,291 km². There are variety of soil present in Uttara Kannada which are rich in iron and manganese. Basically they are divided into 2 kinds based on topography; the coastal alluvial soil and the upghat lateritic and granitic soils. The study area comes under taluk Sirsi which has the lateritic soil which is highly leached, reddish brown in colour, shallow to medium in depth and loamy in texture. pH of the soil found to be between 6.3 to 7.3. Rainfall is widely restricted between June to September and has an average rainfall of 2500 to 3500 mm (Fig. A) (Bhat and Murali 2001).

Isolation of Fungi from Soil Samples

Approximately one kg of soil sample was collected (1 meter radius around the tree and the roots) near both the tree respectively and brought to the laboratory for further study. The soil samples were dried, powdered and sieved to get finely powdered soil sample. Soil dilution method is used, to isolate fungi's from the respective samples. One gram of fine soil sample was weighed and dissolved in the 10 ml of distilled water in a test tube and labeled as 10⁻¹. Dilution of 10⁻², 10⁻³, 10⁻⁴ and 10⁻⁵ were prepared by serial dilution method. Approximately 15 ml of PDA (Potato Dextrose Agar) medium is poured in to plates and let it cool. Further it is labeled as 10⁻² to 10⁻⁵. Few drops of inoculums of samples are added to the plate and then the inoculum is spread by gentle rotation of the petridish with the help of an 'L' shaped glass rod. Each dilution of sample is spread over three petridish and each dilution were incubated in an inverted position at 27°C in an incubator for 3 to 7 days. Organism per gram of soil is calculated by formula: No. of colonies × dilution factor /dry wt of soil (Frankland, *et al.*, 1995, Aneja 2008).

% frequency = (No of observations in which a species appeared/ total no of observations) X 100

% Contribution = Total No of colonies of species in all observations taken together/Total no of colonies in all the species X 100

Identification of Fungi

Micro preparation slide of all the fungal species was done and stained with cotton blue and observed under the microscope. Criteria's like shape, size, color, structure of the spores, conidia, mycelium and its branches were considered for the identification. These details were compared with standard works of manual of soil fungi, Manual of *Aspergillus* (Raper and Fennell, 1965), A Manual of *Penicillium* (Raper and Thom, 1949), Soil fungi (Domsch *et al.*, 1980) and Hyphomycetes (Subramanian, 1971).

RESULTS AND DISCUSSION

In the study of *B. racemosa* and *R. serpentina* rhizosphere samples has 29 different fungal species belonging to 20 genera and The total number of bacteria isolated is 2,666,600 and 4,461,160 per gram of soil respectively; So the soil is found to be very fertile. The detailed study of rhizospheric micro-flora is given below i.e.,

The number of fungal isolates present in *B. racemosa* rhizosphere is 3,281,200 per gram of soil, i.e., the 10⁻² dilution have 5,200 fungal isolates per gram of soil, the 10⁻³ dilution have 36,000 fungal isolates per gram of soil, 10⁻⁴ dilution have 240,000 fungal isolates per gram of soil and 10⁻⁵ dilution have 3,000,000. The number of fungal isolates present in *R. serpentina* rhizosphere is 1,946,200 per gram of soil, i.e., the 10⁻² dilution have 4,200 fungal isolates per gram of soil, the 10⁻³ dilution have 32,000 fungal isolates per gram of soil, the 10⁻⁴ dilution have 3,10,000 fungal isolates per gram of soil, the 10⁻⁵ dilution have 1,600,000 fungal isolates per gram of soil (Table 1).

In *B. racemosa* rhizosphere, the percentage contribution of *Penicillium* sp. is highest with 13.679%. Lowest contribution is given by *Absidia* sp. with 0.471%, whereas *Rhizopus stolonifer* and *Phomopsis* sp. contributes 0.943% respectively. In *R. serpentina* rhizosphere, the highest percentage contribution is given by *Cladosporium* sp. and *Trichoderma* sp. with 17.663% and 17.391% respectively; whereas the lowest contribution factor was given by *Lasiodiplodia* sp. with 0.543%. Overall, when both the rhizosphere are considered, the highest percentage of contribution is observed by *Penicillium* sp. with 13.679% and 15.217% and least percentage contribution was observed by *Absidia* sp. with 0.471% and 6.521%. In the *B. racemosa* rhizosphere the highest percentage frequency was shown by *Penicillium* sp. with 21.052%. But In *R. serpentina* rhizosphere the highest percentage frequency was shown by *Cladosporium* sp. and *Trichoderma* with 16.666%. In rhizosphere of *B. racemosa*, 19 different species belonging to 15 genera was observed with *Penicillium* sp. dominating over other species; amongst the isolates, *Penicillium* sp. were 3 (*Penicillium aethiopicum*, *Penicillium globosum*, *Penicillium* sp.), *Botrytis* sp. were 2 (*Botrytis cinerea*,

Botrytis sp.) and many other species like *Cladosporium* sp., *Epicoccum catenispodium*, *Geotrichum candidum*, *Geotrichum* sp., *Absidia* sp., *Monilia* sp., *Mucor* sp., *Phoma* sp., *Verticillium* sp., *Phomopsis* sp., *Trichoderma* sp., *Aspergillus flavus*, *Rhizopus stolonifera* and *Sepedonium* sp. In rhizosphere of *Rauwolfia serpentina*, 18 different species belonging to 10 genera were observed with *Trichoderma* sp. and *Cladosporium* sp. is dominating over other species; among the isolates, *Aspergillus* were 4 (*A. niger*, *A.*

flavus, *A. fumigatus*, and *Aspergillus* sp.), *Trichoderma* sp. were 2 (*Trichoderma harzianum*, *Trichoderma viride*.), *Fusarium* sp. were 2 (*Fusarium roseum*, *Fusarium* sp.) and many more species like *Lasiodiplodia* sp., *Colletorichum* sp., *Penicillium aethiopicum*, *Chaetomium* sp., *Penicillium* sp., *Verticillium* sp., *Absidia* sp., and *Chaetomium globosum* were characterized and percentage occurrence of the soil fungi was statistically evaluated (Table 1 and 2) (Fig 1 to 32).

Table 1: Fungal isolates from rhizosphere of *Barringtonia racemosa* plants and *Rauwolfia serpentina* plants of Western Ghat

Sl. No	Area	Dilution factor	No of isolates	Average	Organism per gram of soil
1	<i>Barringtonia racemosa</i>	10^{-2}	<i>Geotrichumcandidum</i> (13)	4	400
			<i>Aspergillus flavus</i> (15)	5	500
			<i>Trichoderma</i> sp (20)	7	700
			<i>Penicillium</i> sp (15)	5	500
			<i>Botrytis cinerea</i> (38)	13	1300
			<i>Sepedonium</i> sp(22)	7	700
			<i>Verticillium</i> sp (19)	6	600
			<i>Mucor</i> sp (9)	3	300
			<i>Phomopsis</i> sp (4)	1	100
			<i>Absidi</i> asp (2)	1	100
2	<i>Barringtonia racemosa</i>	10^{-3}	<i>Penicillium aethiopicum</i> (18)	6	6000
			<i>Rhizopus stolonifer</i> (4)	1	1000
			<i>Botrytis</i> sp (36)	12	12000
			<i>Penicillium</i> sp (11)	4	4000
			<i>Phomas</i> p(20)	7	7000
			<i>Penicillium globosum</i> (10)	3	3000
			<i>Cladosporium</i> sp (9)	3	3000
3	<i>Barringtonia racemosa</i>	10^{-4}	<i>Trichoderma</i> sp (20)	7	70000
			<i>Penicillium aethiopicum</i> (8)	3	30000
			<i>Aspergillus flavus</i> (5)	2	20000
			<i>Monilia</i> sp(10)	3	30000
			<i>Sepedonium</i> sp(6)	2	20000
			<i>Penicillium</i> sp (20)	7	70000
4	<i>Barringtonia racemosa</i>	10^{-5}	<i>Epicoccumcatenispodium</i> (3)	1	100000
			<i>Penicillium</i> sp (12)	4	400000
			<i>Geotrichum</i> sp (36)	12	1200000
			<i>Verticillium</i> sp(21)	7	700000
			<i>Aspergillus flavus</i> (18)	6	600000
5	<i>Rauwolfia serpentina</i>	10^{-2}	<i>Fusarium roseum</i> (4)	1	100
			<i>Penicillium</i> sp (16)	5	500
			<i>Aspergillus niger</i> (5)	2	200
			<i>Cladosporium</i> sp(34)	11	1100
			<i>Aspergillus flavus</i> (15)	5	500
			<i>Trichoderma</i> sp (13)	4	400
			<i>Verticillium</i> sp(7)	2	200
			<i>Absidi</i> asp(10)	3	300
			<i>Colletorichum</i> sp(8)	3	300
			<i>Aspergillus fumigatus</i> (17)	6	600
6	<i>Rauwolfia serpentina</i>	10^{-3}	<i>Penicillium aethiopicum</i> (25)	8	8000
			<i>Fusarium</i> sp(25)	8	8000
			<i>Aspergillus niger</i> (4)	1	1000
			<i>Absidi</i> asp (14)	5	5000

Sl. No	Area	Dilution factor	No of isolates	Average	Organism per gram of soil
			<i>Trichoderma</i> sp (19)	6	6000
			<i>Chaetomium</i> sp(4)	1	1000
			<i>Aspergillus fumigatus</i> (5)	2	2000
			<i>Lasiodiplodiasp</i> (2)	1	1000
7	<i>Rauwolfia serpentina</i>	10 ⁻⁴	<i>Penicillium</i> sp (25)	8	80000
			<i>Trichoderma</i> sp (32)	11	110000
			<i>Chaetomium globosum</i> (12)	4	40000
			<i>Aspergillus</i> sp (5)	2	20000
			<i>Cladosporium</i> sp (18)	6	60000
8	<i>Rauwolfia serpentina</i>	10 ⁻⁵	<i>Aspergillus</i> sp (3)	1	100000
			<i>Penicillium</i> sp (15)	5	500000
			<i>Trichoderma harzianum</i> (18)	6	600000
			<i>Cladosporium</i> sp(13)	4	400000

Table 2: Percentage frequency and contribution of different fungal species from *Barringtonia racemosa* and *Rauwolfia serpentina* rhizosphere soil sample

Sl. No	Species Name	Barringtonia racemosa		Rauwolfia Serpentina		Total	
		A	B	A	B	A	B
1	<i>Absidiasp</i>	5.263	0.471	11.111	6.521	8.187	3.496
2	<i>Aspergillus flavus</i>	15.789	8.962	5.555	4.076	10.672	6.619
3	<i>Aspergillus fumigatus</i>	-	-	11.111	5.978	11.111	5.978
4	<i>Aspergillus niger</i>	-	-	11.111	2.445	11.111	2.445
5	<i>Aspergillus</i> sp	-	-	11.111	2.173	11.111	2.173
6	<i>Botrytis cinerea</i>	5.263	8.962	-	-	5.263	8.962
7	<i>Botrytis</i> sp	5.263	8.490	-	-	5.263	8.490
8	<i>Chaetomium globosum</i>	-	-	5.555	3.260	5.555	3.260
9	<i>Chaetomium</i> sp	-	-	5.555	1.086	5.555	1.086
10	<i>Cladosporium</i> sp	5.263	2.122	16.666	17.663	10.964	9.892
11	<i>Colletorichum</i> sp	-	-	5.555	2.173	5.555	2.173
12	<i>Epicoccumcatenisorium</i>	5.263	0.707	-	-	5.263	0.707
13	<i>Fusarium roseum</i>	-	-	5.555	1.086	5.555	1.086
14	<i>Fusarium</i> sp	-	-	5.555	6.521	5.555	6.521
15	<i>Geotrichumcandidum</i>	5.263	3.066	-	-	5.263	3.066
16	<i>Geotrichum</i> sp	5.263	8.490	-	-	5.263	8.490
17	<i>Lasiodiplodiasp</i>	-	-	5.555	0.543	5.555	0.543
18	<i>Monilia</i> sp	5.263	2.358	-	-	5.263	2.358
19	<i>Mucor</i> sp	5.263	2.122	-	-	5.263	2.122
20	<i>Penicillium aethiopicum</i>	10.526	6.132	5.555	6.793	8.040	6.462
21	<i>Penicillium globosum</i>	5.263	2.358	-	-	5.263	2.358
22	<i>Penicillium</i> sp	21.052	13.679	16.666	15.217	18.859	14.448
23	<i>Phomasp</i>	5.263	4.716	-	-	5.263	4.716
24	<i>Phomopsis</i> sp	5.263	0.943	-	-	5.263	0.943
25	<i>Rhizopus stolonifer</i>	5.263	0.943	-	-	5.263	0.943
26	<i>Sepedonium</i> sp	10.526	6.603	-	-	10.526	6.603
27	<i>Trichoderma harzianum</i>	-	-	5.555	4.891	5.555	4.891
28	<i>Trichoderma</i> viride	10.526	9.433	16.666	17.391	13.592	13.412
29	<i>Verticillium</i> sp	10.526	9.433	5.555	1.902	8.040	5.667

A= % Frequency, B= % Contribution

Rhizosphere of the medicinal plants viz. *Ocimum sanctum* and *Centella asiatica* showed 16–17 species of fungi (Nagsuk, *et al.*, 2003, Sagar and Kumari 2009). Eleven species of rhizosphere fungi isolated from *Santalum album* (Thombre, *et al.*, 2016).

In the present study 19 species was found from *Barringtonia racemose* and 18 species from *Rauwolfia serpentina*. During the study *Aspergillus niger*, *Aspergillus terricola* and *Penicillium* spp. were frequently observed and recorded (Thombre, *et al.*,

2016). In the present study the frequently observed species are *Trichoderma* sp. and *Cladosporium* sp. From *Rauwolfia serpentina* and *Penicillium* sp. from *Barringtonia racemosa*. A novel actinomycete strain, JA03 T, belonging to the genus *Streptomyces*, was isolated from the rhizosphere of *Barringtonia racemosa* (Khaing, *et al.*, 2022). In the present study *Penicillium* sp. were 3 (*Penicillium aethiopicum*, *Penicillium globosum*, *Penicillium* sp.), *Botrytis* sp. were 2 (*Botrytis cinerea*, *Botrytis* sp.) and many other species like (*Cladosporium* sp., *Epicoccum catenispodium*, *Geotrichum candidum*, *Geotrichum* sp., *Absidia* sp., *Monilia* sp., *Mucor* sp., *Phoma* sp., *Verticillium* sp., *Phomopsis* sp., *Trichoderma* sp., *Aspergillus flavus*, *Rhizopus stolonifera* and *Sepedonium* sp. was isolated from *Barringtonia racemosa* and from *Rauwolfia serpentina* *Aspergillus* were 4 (*A. niger*, *A. flavus*, *A. fumigatus*, and *Aspergillus* sp.), *Trichoderma* sp. were 2 (*Trichoderma harzianum*, *Trichoderma* sp.), *Fusarium* sp. were 2 (*Fusarium roseum*, *Fusarium* sp.) and many more species like *Lasiodiplodia* sp., *Colletotrichum* sp.,

Penicillium aethiopicum, *Chaetomium* sp., *Penicillium* sp., *Verticillium* sp., *Absidia* sp., *Penicillium* sp., and *Chaetomium globosum*.

The maximum number of bacteria isolated from rhizosphere of *B. racemosa* is 2,460,000 per gram of soil in 10^{-5} concentration and lowest is 3100 per gram of soil in 10^{-2} ; whereas, in *R. serpentina* highest number of bacteria isolated in 10^{-5} is 4100000 and lowest in 10^{-2} is 5660 per gram of soil respectively.

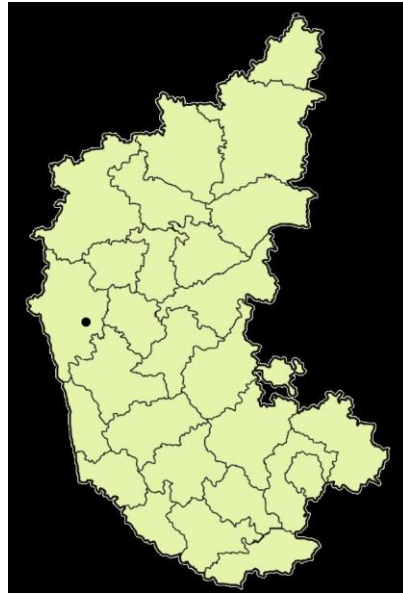
The rhizosphere soils of *Rauwolfia* sp. collected from Western Ghat regions of Karnataka a total of 200 rhizobacteria were isolated, comprising of 15 different bacterial genera (Prasanna Kumar, *et al.*, 2013). In the present study the rhizospheric soil of two medicinal plants *Barringtonia racemosa* and *Rauwolfia serpentina* collected from Uttara Kannada region of Western Ghats has 2,666,600 and 4,461,160 organisms per gram of soil respectively.

Table 3: Bacterial isolates from Soil Samples collected in *Barringtonia racemosa* plants and *Rauwolfia serpentina* plants Field

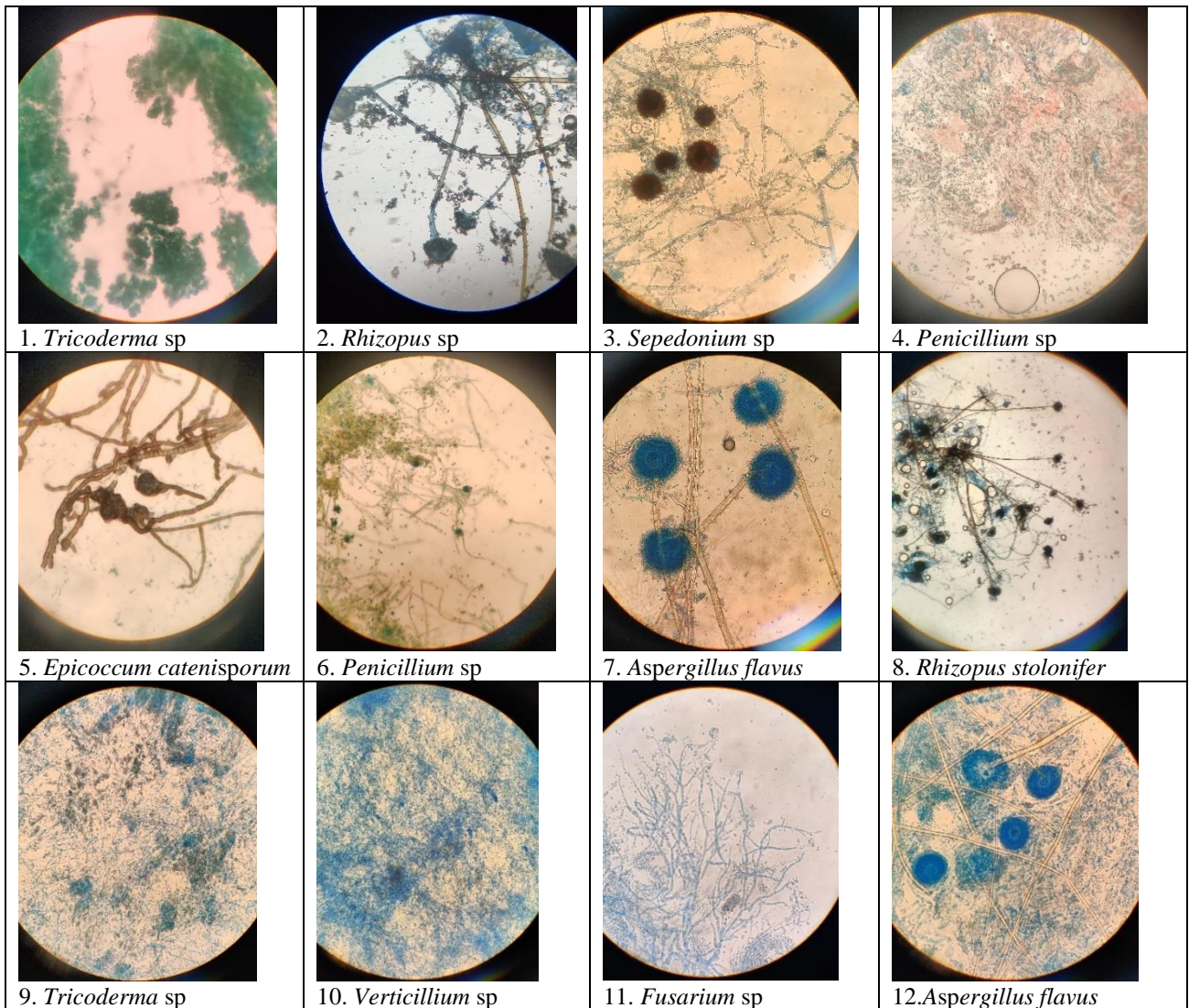
Sl. No	Area	Dilution factor	No of colonies	Average	Organisms per gram of soil
1	<i>Barringtonia racemosa</i>	10^{-2}	94	31	3100
2	<i>Barringtonia racemosa</i>	10^{-3}	136	45.5	45500
3	<i>Barringtonia racemosa</i>	10^{-4}	47	15.8	158000
4	<i>Barringtonia racemosa</i>	10^{-5}	74	24.6	2460000
5	<i>Rauwolfia serpentina</i>	10^{-2}	117	56.6	5660
6	<i>Rauwolfia serpentina</i>	10^{-3}	121	40.5	40500
7	<i>Rauwolfia serpentina</i>	10^{-4}	94	31.5	315000
8	<i>Rauwolfia serpentina</i>	10^{-5}	123	41	4100000

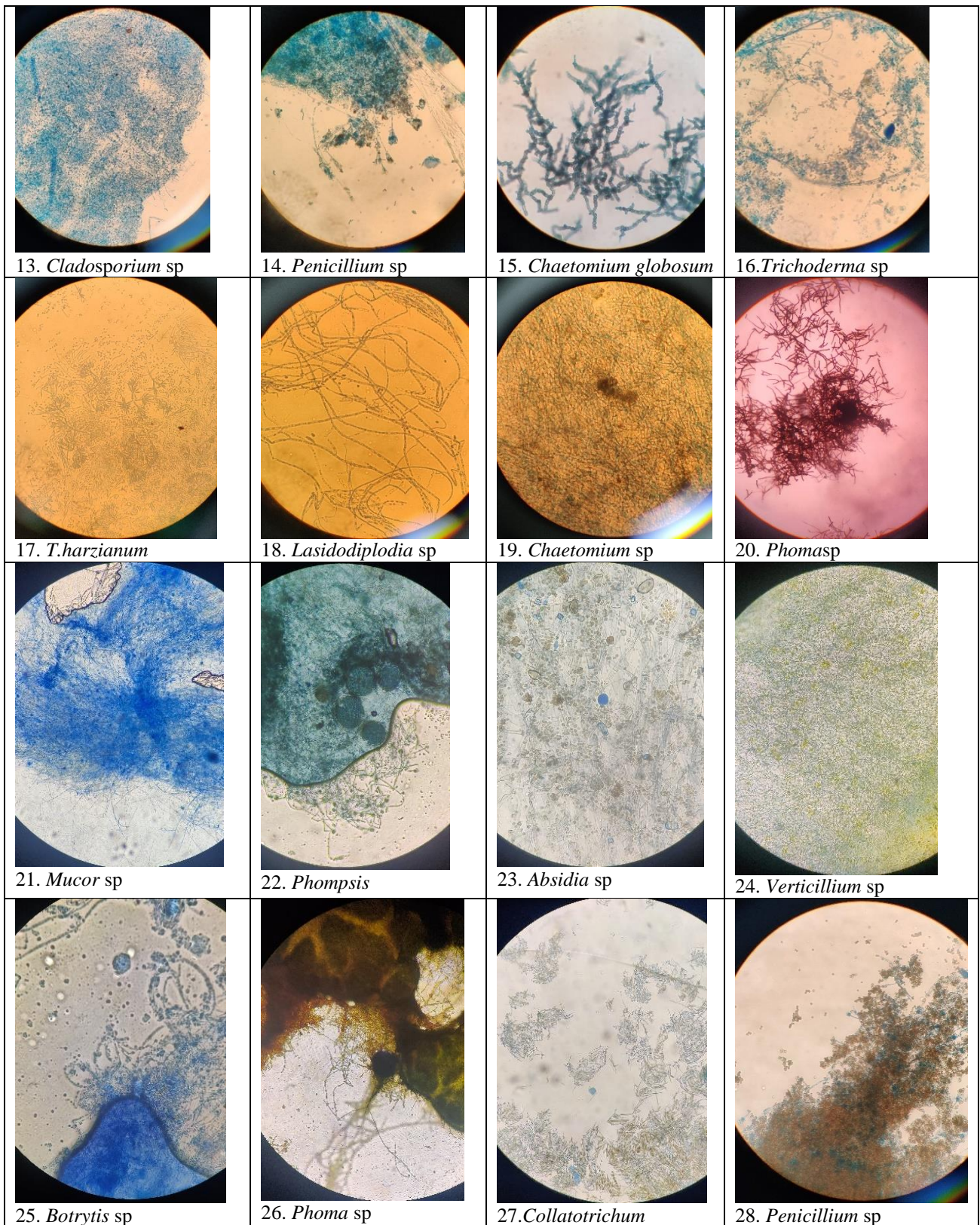
Rhizosphere microflora is known for economic, organic and sustainable inputs to increase the productivity of many crops (Smith, S. E. and Read 1997). In the present study the rhizosphere microflora isolated from *Barringtonia racemosa* and *Rauwolfia serpentina* may increase the organic compounds and productivity of two medicinal plants. The three main categories of plants secondary metabolites namely terpenoids, phenolics and alkaloids used for pharmacological and medicinal purposes (Khare and Pandey 2014). In the present study the rhizospheric microflora may enhance the secondary metabolites in *Barringtonia racemosa* and *Rauwolfia serpentina*.

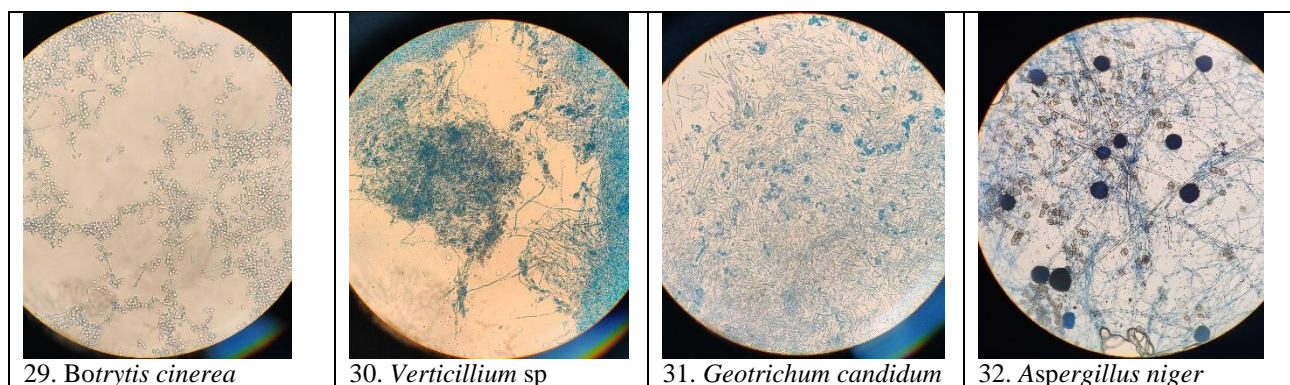
Essential oils generally consisting of monoterpenes, phenylpropanoids and sesquiterpenes are used as antimicrobials, fragrances and flavours and antioxidants (Khare and Pandey 2014, Shaikh, and Nadaf 2013) studied that *Pseudomonas fluorescens*, *Trichoderma viride* and *Bacillus megaterium* in alone and combined treatments showed maximum enhancement (22.27%) of essential oil as compared to untreated plant in *Ocimum tenuiflorum* (Khare and Pandey 2014). In the present study *Trichoderma viride* isolated from two medicinal plants may increase the essential oil concentration in *Rauwolfia serpentina*.



A. Map of karnataka pointing study region, Sirsi







CONCLUSION

For the first time the study of primary screening, and characterization of microflora, isolated from two soil samples collected from rhizosphere of *B. racemosa* and *R. serpentine* in two different locations in Western Ghats, Uttara Kannada District, Karnataka, India was done. In *B. racemosa* rhizosphere, 19 different species belonging to 15 genera was observed. Among which *Penicillium* sp. is dominating over other species. In *R. serpentine* rhizosphere, 18 different species belonging to 10 genera were observed. Among which *Trichoderma* sp. and *Cladosporium* sp. are dominating over other species. *Penicillium* sp. is seen to a larger extent in both *B. racemosa* and *R. serpentine* rhizosphere over other species. The second dominant species in both the rhizosphere is *Trichoderma* sp.

The medicinal properties of plants can be specified on the antimicrobial, antipyretic and antioxidant activity of the biologically active compounds. This rhizosphere containing the distinctive microflora with fungi and bacteria responsible for increases the fertility of the soil. Our result also reveals that different microflora isolated from two different samples increases the fertility of the soil. However, increasing the quality of medicinal plants is dependent on inoculating efficient and specific rhizosphere fungi for a particular plant. In our study also, different rhizosphere fungi may increase the quality of two medicinal plants tested. The extracts from *B. racemosa* and *R. serpentina* plants are recognized to have medicinal properties and can be potential source for treating snakebite victims. So the effect of rhizosphere microflora on increasing the secondary metabolites in two medicinal plants tested need further research.

ACKNOWLEDGEMENT

Authors wish to thank the Management of St. Joseph's University, Bengaluru for Providing Laboratory Facilities and Constant encouragement. We express our gratitude to all those have supported their might for the carrying out of the project.

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