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RESEARCH PAPER

Comparative study of microbial inoculants of cultivated and virgin soils of Nilgiri Biosphere for plant growth promotion

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Abstract : In virgin soils, microorganisms and plants live in harmony and both are dependent on each other for their livelihood. Absence of cultivation practices, undisturbed soil condition, high organic matter condition and other favourable conditions enables to flourish beneficial microbes. The research work was started to identify beneficial microbes from undisturbed virgin soils of Nilgiri biosphere with the ability to grow under low pH and under low temeperature conditions. Bio-inoculants viz., *Azospirillum,* Phosphobacteria*, Azotobacter*, *Rhizobium* and pseudomonas were obtained from cultivated and virgin soil samples of Nilgiris biosphere. When compared with type cultures, virgin soil isolates of respective inoculants have recorded better results in promoting plant dry weight in paper towel method. In cross streak assay, selected isolates found to be compatible with each other. In lignite carrier base formulation, the inoculants have reached a maximum population level of 10^7 and phosphobacteria reached 10^8 level. The population remained steady at this level up to 3 months. In the field trial studies conducted, the treatment of *Azospirillum* + *Azotobacter* + Phosphobacteria + *Pseudomonas* + 75% RDF has recorded maximum population of all the inoculants at 45th day after sowing. However, the maximum yield was observed in 100% RDF and bio-inoculant consortium applied treatment. This was closely followed by 75% RDF and bio-inoculant consortium applied plots. The results of the field trial have shown that bio-inoculant consortium along with 75% RDF application will lead to maximum yield with 25% saving in chemical fertilizer application.

Key Words : Bio-inoculants, Virgin soil, *Azospirillum*, *Azotobacter*, Phosphobacteria, *Pseudomonas,* Plant growth promotion

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INTRODUCTION

In virgin soils, microorganisms and plants live in harmony and both are dependent on each other for their livelihood. Absence of cultivation practices, undisturbed soil condition, high organic matter condition and other favourable conditions enables to flourish beneficial microbes. These beneficial microbes form symbiotic relationship with plants (Paola Bonfante and Iulia-Andra Anca, 2010). In undisturbed virgin soils, particularly in

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high density forests, beneficial microbes (*Azospirillum*, Phosphobacteria, *Azotobacter* etc) provides different nutrients, promotes plant growth and enables plants to use water and nutrition from long distances (Mycorrhiza). In case of cultivated soils, microbes are adversely affected by intensive cultivation measures like fungicide application, pesticide application, low organic matter *etc*. (Chen *et al*, 2001).

In this background, a study was undertaken to explore undisturbed virgin soils of forest for survey, screening and selection of beneficial microbes for promoting plant growth, plant nutrition and drought tolerance.

MATERIAL AND METHODS

Details of experiments conducted:

Soil samples were collected from seven places of cultivated region in the Nilgiris biosphere and another set of seven soil samples were collected from uncultivated region of Nilgiris biosphere.

The details of soil samples collected were given in Table A.

The soil samples were serially diluted and plated in respective media for the isolation bio inoculants in Table B.

Further the isolates were characterized and compared with type cultures for plant growth promotion.

Isolates obtained from above soil samples were compared with each other for plant growth promotion through paper towel germination method. In the experiment, surface sterilized radish seeds were inoculated with the isolates of *Azospirillum*, Phosphobacteria, *Azotobacter* and Pseudomonas. For *Rhizobium*, seed of peas were used. Ten days after start of the experiment, plant seedlings were dried in hot air oven at 60°C until constant weights were achieved.

Preparation of consortium of inoculant formulation:

All the four selected inoculants were cultured until late log phase in respective media of the inoculants. The late log phase cultures were mixed with presterilized, pH adjusted lignite carrier material.

Enumeration of inoculant population in carrier based formulation:

The populations of inoculants were enumerated by serial dilution and plate count method. One gram of the carrier based inoculants were serially diluted with sterile distilled water up to 10^9 and one ml from dilutions 10^6 to 10^9 were plated in respective media of the individual inoculants. For each dilution, three replications were made and the population was calculated by calculating average number of colonies from three replications (Table 4).

Field Trial studies with developed consortium of inoculants:

A field trial study with the newly developed consortium of inoculants on carrot crop was conducted in Nanjanad farm. The treatment details are described below:

Table A : Characters of soil samples collected from different locations

Sr. No.	Location	Cultivated soil		Virgin soil	
		Soil type	pH	Soil type	pH
1.	Woodhouse	Laterite soil	4.7 to 5.3	Laterite soil	5.1
2.	Nanjanad	Laterite soil	4.5 to 5.2	Laterite Soil	4.9
3.	Emerald	Alluvial soil	4.6	Alluvial soil	4.9
4.	Madikarai	Red loamy	4.5	Red loamy	4.7
5.	Bikkatti	Laterite soil	4.6	Laterite soil	4.7
6.	Kundha	Red laterite	4.7	Red laterite	4.9
7.	Lovedale	Red laterite	4.8	Red laterite	5.1

Table B : Inoculants and respective media of plating

Sr. No.	Bio inoculant	Media
1.	*Azospirillum* (Azos)	Nitrogen free Bromothymol semi soil broth
2.	Phosphobacteria (Phos)	Pikovaskya
3.	*Azotobacter* (Azoto)	Wasksman 77
4.	*Rhizobium* (Rhiz)	Yeast Extract Mannitol Congored Agar Medium
5.	*Pseudomonas* (Pseuds)	King's B

Treatment Details:

T_1 : Control

T_2 : *Azosprillum* + *Azotobacter* + 75% RDF

T_3 : Phospobacteria + 75% RDF

T_4 : Pseudomonas + 75% RDF

T_5: *Azosprillum* + *Azotobacter* + Phosphobacteria 75% RDF

T_6 : *Azosprillum* + *Azotobacter* + *Pseudomonas* 75% RDF

T_7 : Phospobacteria + *Pseudomonas* + 75% RDF

T_8 : *Azosprillum* + *Azotobacteria* + Phosphobacteria + *Pseudomonas* + 75% RDF

T_9 : *Azosprillum* + *Azotobacter* + Phosphobacteria + *Pseudomonas* + 100% RDF

T_{10} : 100% RDF alone

Field trial details:

Crop : Carrot
Variety : F1 Hybrid
Date of sowing : February 14
No. of Treatments : 10
No. of replications : 3
Design : RBD

The trial was conducted with Randomized Block Design and three replications. The inoculants were cultured in their respective broth, mixed in carrier as consortium of inoculants and applied as seed treatment and soil application. Fertilizers were applied as per the treatment schedule.

Crop growth and soil inoculant population of the plants in the field trial were observed in 30 days interval. Yield of the crop was observed at the harvest after hundred days of sowing.

Soil Microbial population:

Soil microbial population was recorded before bio-inoculant application (at the time of sowing) and after bio-inoculant consortium application at 45th day.

RESULTS AND DISCUSSION

From the soil samples of cultivated region, seven isolates of *Azotobacter*, *Azospirillum*, Phosphobacteria, *Rhizobium* and *Pseudomonas* were isolated. Another set of seven isolates were isolated from uncultivated virgin soil samples. All the isolated were purified by repeated culturing and maintained in slants for further studies.

Population observation of bio-inoculants in the cultivated and virgin soils has revealed the influence of cultivation on bio-inoculants. In general, the population of the bio-inoculants are higher in virgin, uncultivated soils than in cultivated soils.

Isolates from cultivated soils and virgin soils were compared for plant growth promotion through paper towel method (Table 2).

Results of the experiment have indicated that virgin soil isolates are better in promoting plant growth when compared to cultivated soil isolates. Among the different isolates studied, *Azospirillum* isolate from virgin soil of Madikarai, *Azotobacter* isolate from virgin soil of Woodhouse forest region, *Pseudomonas* isolate from virgin soil of Woodhouse forest region and *Rhizobium* isolate from virgin soil of Lovedale have performed well when compared with other isolates and type cultures of respective inoculants.

Compatibility studies with in the bio inoculants:

All the four chosen bioinoculants of *Azospirillum*, Phosphobacteria, *Azotobacter* and *Pseudomonas* were tested for compatability in common growth medium. Nutrient agar medium was prepared and plated in petriplates. The log phase cultures of selected inoculants were streaked by cross streak method to test compatibility of inoculants. The isolate Pseudomonas was streaked in straight line. All other inoculants were streaked in right angles to Pseudomonas inoculant.

Table 1 : Population of the inoculants in uninoculated soil samples

Sample	Cultivated soil					Vrigin soil				
	Azos	Azoto	Phos	Rhiz	Pseuds	Azos	Azoto	Phos	Rhiz	Pseuds
Woodhouse	$3x10^2$	$1x10^4$	$3x10^3$	$5x10^1$	$2x10^2$	$7x10^2$	$4x10^4$	$5x10^3$	$7x10^1$	$7x10^2$
Nanjanad	$7x10^2$	$9x10^3$	$8x10^3$	$1x10^2$	$9x10^1$	$9x10^2$	$5x10^3$	$7x10^3$	$9x10^1$	$2x10^2$
Emerald	$9x10^2$	$2x10^3$	$9x10^3$	$6x10^1$	$7x10^2$	$3x10^3$	$7x10^3$	$3x10^3$	$8x10^1$	$9x10^2$
Madikarai	$6x10^2$	$5x10^3$	$7x10^3$	$3x10^1$	$4x10^1$	$9x10^2$	$5x10^3$	$2x10^3$	$7x10^1$	$3x10^2$
Bikkatti	$7x10^2$	$8x10^3$	$5x10^3$	$9x10^1$	$6x10^2$	$8x10^2$	$9x10^3$	$5x10^3$	$3x10^2$	$7x10^3$
Kundha	$5x10^2$	$9x10^3$	$8x10^3$	$6x10^1$	$7x10^2$	$1x10^3$	$7x10^3$	$7x10^3$	$8x10^1$	$2x10^1$
Lovedale	$2x10^2$	$2x10^3$	$2x10^3$	$4x10^1$	$1x10^3$	$8x10^2$	$7x10^3$	$2x10^3$	$7x10^1$	$3x10^2$

Table 2 : Population of the inoculants in uninoculated soil samples

Sample	Cultivated soil					Vrigin soil				
	Azos	Azoto	Phos	Rhiz	Pseuds	Azos	Azoto	Phos	Rhiz	Pseuds
Woodhouse	$3x10^2$	$1x10^4$	$3x10^3$	$5x10^1$	$2x10^2$	$7x10^2$	$4x10^4$	$5x10^3$	$7x10^1$	$7x10^2$
Nanjanad	$7x10^2$	$9x10^3$	$8x10^3$	$1x10^2$	$9x10^1$	$9x10^2$	$5x10^3$	$7x10^3$	$9x10^1$	$2x10^2$
Emerald	$9x10^2$	$2x10^3$	$9x10^3$	$6x10^1$	$7x10^2$	$3x10^3$	$7x10^3$	$3x10^3$	$8x10^1$	$9x10^2$
Madikarai	$6x10^2$	$5x10^3$	$7x10^3$	$3x10^1$	$4x10^1$	$9x10^2$	$5x10^3$	$2x10^3$	$7x10^1$	$3x10^2$
Bikkatti	$7x10^2$	$8x10^3$	$5x10^3$	$9x10^1$	$6x10^2$	$8x10^2$	$9x10^3$	$5x10^3$	$3x10^2$	$7x10^3$
Kundha	$5x10^2$	$9x10^3$	$8x10^3$	$6x10^1$	$7x10^2$	$1x10^3$	$7x10^3$	$7x10^3$	$8x10^1$	$2x10^1$
Lovedale	$2x10^2$	$2x10^3$	$2x10^3$	$4x10^1$	$1x10^3$	$8x10^2$	$7x10^3$	$2x10^3$	$7x10^1$	$3x10^2$

Table 3 : Comparison of isolates through paper towel germination method

Sample	Plant Dry Weight (mg per plant) - Cultivated soil isolates					Plant Dry Weight (mg per plant) - Vrigin soil isolates				
	Azos	Azoto	Phos	Rhiz	Pseuds	Azos	Azoto	Phos	Rhiz	Pseuds
Woodhouse	20.9	26.4	18.5	15.0	28.1	21.2	29.5	19.4	16.0	30.9
Nanjanad	21.5	25.3	19.6	16.2	27.3	22.9	28.2	20.6	17.3	28.2
Emerald	20.3	24.0	17.3	17.3	25.6	22.8	25.3	19.4	18.2	27.5
Madikarai	25.3	23.9	18.5	15.9	26.5	26.2	27.2	19.8	16.9	29.3
Bikkatti	23.0	28.0	19.0	18.3	27.3	24.2	29.0	20.30	19.2	30.2
Kundha	24.9	26.5	18.9	14.9	26.5	25.0	28.3	19.2	15.0	29.8
Lovedale	21.6	23.1	19.0	18.2	25.9	23.7	25.2	21.0	20.7	27.2
Type culture	20.1	23.2	16.5	17.2	24.2	20.1	23.2	16.5	17.2	24.2

Table 4 : Enumeration of inoculant population in carrier based formulation

Days after inoculation	*Azospirillum*	Phosphpbacteria	*Azotobacter*	*Pseudomonas*
0	$3.33 x10^6$	$4.00 x10^6$	$6.33x10^6$	$3.00 x10^6$
15	$5.00 x10^7$	$7.33 x10^7$	$5.66 x 10^7$	$6.33 x 10^7$
30	$7.00 x10^7$	$5.33 x 10^8$	$6.66 x 10^7$	$7.00 x 10^7$
45	$6.33 x10^7$	$7.66 x 10^8$	$5.33 x 10^7$	$6.33 x 10^7$
60	$5.33 x10^7$	$7.00 x 10^8$	$5.00 x 10^7$	$6.66 x 10^7$
75	$4.00 x10^7$	$6.00 x 10^8$	$5.66 x 10^7$	$6.00 x 10^7$
90	$4.33 x10^7$	$5.66 x 10^8$	$5.00 x 10^7$	$5.66 x 10^7$

Likewise all the inoculants were checked for compatibility. All the four inoculants were found compatible with each other and no inhibition zone was observed.

Enumerations of the population have revealed that the population of all the four inoculants was in the level of 10^6 at the time of inoculant preparation (Table. 4).

After fifteen days, the inoculant populations have raised to 10^7 levels. At 30 days after inoculant preparation, phosphobacteria population has raised to 10^8 level where as the population of other inoculants remained at 10^7 level up to 90 days after inoculant preparation.

Observations of the plant growth have shown the beneficial effects of the bio-inoculants application on plant growth (Table. 5).

Among the treatments studied, application of Azos + Azoto + Phospho + Pseudomonas + 100% RDF have recorded maximum plant height on the days of observation. Treatment Azos + Azoto + Phospho + Pseudomonas + 75% RDF have recorded on par results with the treatment 100% RDF alone on all the days of observation. The same trend was recorded in the yield of the crop. Highest yield was recorded in the Azos + Azoto + Phospho + Pseudomonas + 100% RDF applied plots, where as 75% recommended dose of fertilizers and bio-inoculants consortium applied plots have recorded on par results with 100% RDF alone applied plots.

Bio-inoculant consortium application have shown considerable difference in the population of the inoculants in the soil (Table. 6).

Before inoculant application and at the time of sowing, the population of the inoculants are very low. The population of the inoculants have increased considerably at the time of peak vegetative growth.

Table 5 : Plant biometric observations

Treatments	Plant height (in cm)			Yield per plot (in Kgs)	Yield per ha (in Kgs)
	30 days after sowing	60 days after sowing	90 days after sowing		
T_1 – Control	15	25	34	2.10	31500
T_2 – Azos + Azoto + 75% RDF	17	28	37	2.70	40500
T_3 – Phospo + 75% RDF	18	26	36	2.75	41250
T_4 - Pseudomonas + 75% RDF	18	27	35	2.80	42000
T_5 - Azos + Azoto + Phospho 75% RDF	20	30	38	2.89	43350
T_6 - Azos + Azoto + Pseudo 75% RDF	21	30	37	2.90	43500
T_7 - Phospo + Pseudomonas + 75% RDF	17	27	35	2.60	39000
T_8 - Azos + Azoto + Phospho + Pseudomonas + 75% RDF	20	32	39	3.15	47250
T_9 - Azos + Azoto + Phospho + Pseudomonas + 100% RDF	23	35	42	3.25	48750
T_{10} - 100% RDF alone	21	32	38	3.05	45750
C.D. (P=0.05)	0.0825	0.1083	0.0800	0.0111	166.71
S.E.±	0.0393	0.0515	0.0381	0.0053	79.35

Table 6 : Bio-incoulant population in soil before and during cultivation

Treatment details	Bio-inoculant population before sowing				Bio-inoculant population 45th day after sowing			
	Azos	Azoto	Phos	Pseuds	Azos	Azoto	Phos	Pseuds
T_1 – Control	$2x10^1$	$1x10^1$	$1x10^2$	$1x10^1$	$3x10^2$	$2x10^2$	$8x10^2$	$9x10^1$
T_2 – Azos + Azoto + 75% RDF	$4x10^1$	$2x10^1$	$2x10^2$	$4x10^1$	$5x10^3$	$3x10^3$	$2x10^1$	$3x10^1$
T_3 – Phospo + 75% RDF	$2x10^1$	$1x10^2$	$4x10^2$	$6x10^1$	$8x10^1$	$5x10^1$	$4x10^3$	$5x10^3$
T_4 - Pseudomonas + 75% RDF	$3x10^1$	$2x10^1$	$3x10^2$	$1x10^1$	$5x10^1$	$7x10^2$	$8x10^1$	$3x10^3$
T_5 - Azos + Azoto + Phospho 75% RDF	$2x10^1$	$1x10^2$	$3x10^2$	$5x10^1$	$7x10^2$	$3x10^2$	$5x10^3$	$5x10^2$
T_6 - Azos + Azoto + Pseudo 75% RDF	$2x10^1$	$5x10^1$	$7x10^1$	$4x10^1$	$2x10^3$	$3x10^3$	$2x10^2$	$2x10^3$
T_7 - Phospo + Pseudomonas + 75% RDF	$3x10^1$	$4x10^1$	$6x10^2$	$2x10^2$	$8x10^1$	$4x10^1$	$2x10^3$	$5x10^3$
T_8 - Azos + Azoto + Phospho + Pseudomonas+75% RDF	$3x10^1$	$3x10^1$	$3x10^2$	$7x10^1$	$3x10^3$	$7x10^3$	$7x10^3$	$2x10^3$
T_9 - Azos + Azoto + Phospho + Pseudomonas+100% RDF	$1x10^1$	$5x10^1$	$7x10^1$	$5x10^1$	$5x10^2$	$4x10^3$	$3x10^3$	$7x10^2$
T_{10} - 100% RDF alone	$3x10^1$	$4x10^1$	$5x10^1$	$7x10^1$	$6x10^1$	$7x10^1$	$7x10^1$	$3x10^1$

Among all the treatments studied, application of T8 - Azos + Azoto + Phospho + Pseudomonas + 75% RDF has recorded the maximum population of all the inoculants applied, Where as the lowest was recorded in 100% RDF alone applied treatment.

Cultivation practices has strong influence on type and population of microflora in the soil. Many researchers have found that apart from climatic factors, soil type, soil organic matter content, cultivation practices and moisture content influences type and population of the microflora in the soil (Makarov, 2007; Diaz-Ravina *et al*., 1992; Karaguyshieva and Illyaletdinov, 1957).

In the present study, the presence of higher bio-inoculants population in uncultivated virgin soils, correlates with the findings of above reports. The results indicates the presence of diverse microflora in virgin soils. The undisturbed nature of forest soils without the addition of any nutrients externally, might have contributed the diverse beneficial microflora to flourish. The findings of Chernova *et. al.* (2019) and Panaiyadiyan P. and S.R. Chellaia (2011) on abundance of microbial prokaryotic taxonomic structure in tropical forest eco systems is in line with the findings of this study.

The present study also indicated the ability of bio-inoculants isolated from virgin soils in promoting plant growth promotion under field conditions. Similar findings in the virgin soil isolates for the efficiency of phosphate solubilization were reported by Kaur and Kaur (2020).

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