FINAL REPORT
(2006-2014)
APPLICATION OF MICROORGANISMS IN AGRICULTURE AND ALLIED SECTORS

Theme of the project	Nutrient management, PGPR and Biocontrol
Title of the project	Application of microorganisms in agriculture and allied sectors (AMAAS)
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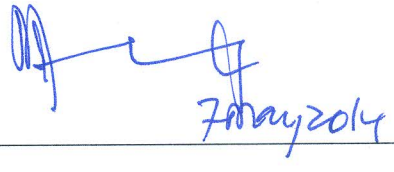
Total budget sanctioned	59.41259 lakhs
Expenditure (Head wise)	Annexure 2
Objectives	Annexure 1
Work done (only in bullet from)	Annexure 1
Significant achievement (only in bullet from)	Annexure 1
Place: Kozhikode Date: 04/05/2014	Signature of PI: 

Objectives:

- To isolate, characterize, evaluate microbes for nutrition mobilization, growth promotion and biological control.
- To screen isolates for the desirable characters.
- To study the compatibility and ecological fitness for development of consortium.
- To studies rhizobacteria mediated induced systemic resistance.
- To study the mechanism of rhizobacteria mediated growth promotion in crop plants.

Practical utility of anticipated results of the project:

- The project aims at better exploitation of indigenously available microbial wealth for crop productivity and cataloguing them
- Development of alternative technologies to chemical fertilizers and pesticides including fungicides for production of cereals, pulses, oilseeds crops, sugarcane, vegetables, spices, medicinal plants and plantation crops.
- Evaluation of PGPR for growth regulation and inducing disease resistance for increasing yields in crops.
- Development of technologies with reduced chemical input for maximum yield and sustainable productivity

Immediate benefits

- To identify microorganisms to control the disease and improve the plant growth in spice crops.
- To identify genes and their products that imparts specific characters to these organisms

Medium term benefits

- Possible patenting of products and processes
- Disease management of spices crop in field conditions.

Long term benefits

- Development of Production technologies involving beneficial microbes and reduced use of chemical input for maximum yield and sustainable productivity

Activity milestones

I year (2006-07)

- Characterization of PGPR for secondary metabolite production, growth promotion and pathogen suppression
- Establishment of rhizobacterial repository
- Evaluation of consortia for compatibility *in vitro.*

II year (2007-08)

- Molecular characterization of short listed isolates and identification of strain using Biolog.
- Population dynamics of PGPR strains in rhizosphere soil and determination of growth promotion
- Field trials with PGPR consortia to reduce fertilizer inputs and disease management.
- Soil analysis for general and specific biochemical parameters related to microbial activity and nutrient transformations

III Year (2008-09)

- Molecular characterization of short listed isolates and identification of strain using Biolog.
- Field trials with PGPR consortia to reduce fertilizer inputs and disease management.
- Mechanism of growth promotion and disease suppression by analyzing the level of defense enzymes and various induction factors.
- Soil analysis for general and specific biochemical parameters related to microbial activity and nutrient transformations
- To develop various formulations of PGPR consortia and studies on shelf life.

IV Year (2009-10)

- Field trials with PGPR consortia growth promotion and disease management
- To develop various formulation of PGPR consortia and studies on shelf life.
- Mechanism of growth promotion and disease suppression by analyzing the level of defense enzymes and various induction factors.

V Year (2010-11)

- Field trials with PGPR consortia to growth promotion and disease suppression
- Mechanism of growth promotion and disease suppression by analyzing the level of defense enzymes and various induction factors
- Compilation of reports and digitization of data

VI Year (2011-12)

- Evaluation of rhizobacterial strains for biocontrol and growth promotion in black pepper– Green house experiment.
- Evaluation of rhizobacterial strains for biocontrol and growth promotion in ginger –Field experiment.
- Effect of rhizobacterial strains on soil physico-chemical, biochemical/ microbial indices.

VII Year (2012-13)

- Development, validation and evaluation of PGPR capsule formulation for growth promotion and soft rot incidence in ginger
- Evaluation of rhizobacterial strains for biocontrol and growth promotion in black pepper and ginger – pot culture experiment.
- Effect of rhizobacterial strains on soil physico-chemical, biochemical/ microbial indices.

VIII Year (2013-14)

- Validation and evaluation of PGPR capsule formulation for growth promotion and soft rot incidence in ginger
- Evaluation of rhizobacterial strains for biocontrol and growth promotion in black pepper and ginger – pot culture experiment.
- Effect of rhizobacterial strains on soil physico-chemical, biochemical/ microbial indices.

Outline of the research

1. Collection of samples from black pepper and ginger growing areas (Kerala & Karnataka)
2. Laboratory investigation: isolation, characterization, identification of bacteria from black pepper and ginger rhizosphere soils
3. Short listing of strains for beneficial agronomic traits such as biocontrol and nutrient mobilization
4. Green house trials for efficacy screening
5. Field evaluation of promising strains to confirm the efficacy
6. Development of PGPR formulation and the evaluation of the formulation in field

Work done (2006-14)

- One hundred and seventy four rhizobacteria have been isolated from black pepper (74) and ginger (100) from different geographical regions such as Peruvannamuzhi and Wayanad in Kerala and Kodagu District in Karnataka State.
- One hundred and seventy-four isolates were tested *in vitro* for beneficial traits, namely mobilization of phosphate, potassium, zinc, silica and ammonia production. These isolates were also tested for growth promotion trait, indole acetic acid (IAA) production. Out of the 174 strains tested, 72 isolates were positive for both IAA and phosphate solubilization (41%) and ammonia production was observed in 124 isolates (71%). Out of 174 isolates only 35 were found to be potassium mobilizers (20%), 26 were zinc mobilizers (15%) and nine were silica (5%) mobilizers.
- One hundred and seventy-four isolates were tested for the production of primary and secondary metabolites. These isolates were tested for Phenazine -1- carboxylic acid (PCA). Only two isolates of black pepper BRB 5 and BRB 28 was positive. The primers were synthesized and tested for the detection of Diacetylphloroglucinol (DAPG), Pyrolnitrin, Pyoluteorin, and Chitinase, and none of the short-listed isolates were positive.
- *In vitro* screening of these isolates was done. Black pepper (BRB 5, BRB 24, BRB 28, BRB 49, BRB 53, BRB 60 and BRB 72) and ginger (GRB 25, GRB 35, GRB 36, GRB 57, GRB 58, GRB 68, GRB 72 and GRB 91) showed more than 50% inhibition against *Phytophthora capsici, Pythium, and Fusarium.*
- Four strains from black pepper and six from ginger were short-listed based on *in vitro* biocontrol assay. The short-listed isolates were identified by phenotypic, biochemical and molecular characterization.
- The identity of eight of these isolates was confirmed by molecular characterization using 16s rDNA sequencing. The black pepper strains belonged to *Pseudomonas aeruginosa* and *Serratia marcescens.* The ginger strains belonged to *Burkholderia pyrrocinia, Bacillus amylolequifaciens, Serratia marcescens* and *Klebsiella* sp. Sequences of the five identified isolates were deposited with National Centre for Biotechnology Information databases.
- Green house and field evaluation of shortlisted 10 strains of PGPR for ginger had been

conducted for two years. Result showed two strain such as GRB35 and GRB 68 were significantly enhance the sprouting per cent and yield apart from reducing soft rot incidence. Hence those two isolates have been selected for further field evaluation to confirm their plant growth promoting efficacy.

- Selected promising PGPR strains namely GRB 35 (*Bacillus amyloliquefaciens*) and GRB 68 (*S. marcescens*) were evaluated in field in comparison with existing biocontrol agent for ginger IISR 51 and chemical fungicides. The result confirmed the efficacy of the selected PGPR strains, GRB 35.
- Developed a novel method of PGPR delivery through encapsulation with long term shelf life. The Biocapsule formulation has been filed for patent: ("A novel method of storing and delivering PGPR/microbes through biocapsules" (Application no.3594/CHE/2013 dated 13/08/2013).
- Isolated and identified a secondary metabolite from GRB 68 (*Serratia marcescens*). LC-MS analysis indicated that the compounds identified belonged to peptides belongs to peptaibols which possess diverse bioactivities (antibacterial, antifungal, and antiparasitic) and are used as biocontrol agents against fungal phytopathogens.
- Developed and commercialized two microbial formulations namely, IISR Biopower (GRB-35; *Bacillus amyloliquefaciens*) for ginger and IISR Biomix (consortium of BRB3-*Micrococcus luteus* and BRB 13-*Enterobacter aerogenes*) for Black pepper.

Isolation of rhizobacteria

Rhizobacteria were isolated from the black pepper and ginger rhizosphere soil collected from Kodagu district of Karnataka and Kozhikode and Wayanad districts of Kerala state. Seventy-four rhizobacterial stains from black pepper and 100 from ginger were isolated. The total collection one hundred and seventy four isolates were stored at – 80°C in IISR repository and are being evaluated for various traits.

Preliminary characterization of rhizobacterial strain

One hundred and seventy four rhizobacteria were preliminarily studied for their phenotypic characters, staining reaction and few biochemical test. Based up on this the isolates were characterized and grouped into eleven genera as shown in Table 1.

In vitro assay for nutrient mobilization traits

One hundred and seventy-four isolates were tested *in vitro* for beneficial traits, namely mobilization of phosphate, potassium, zinc, silica and ammonia production. These isolates were also tested for growth promotion trait, indole acetic acid (IAA) production. Out of the 174 strains tested, 72 isolates were positive for both IAA and phosphate solubilization (41%) and ammonia production was observed in 124 isolates (71%). Out of 174 isolates only 35 were found to be potassium mobilizers (20%), 26 were zinc mobilizers (15%) and nine were silica (5%) mobilizer. The isolates having multiple useful traits are listed below (Table 2).

Identification of strains for specific biocontrol traits

One hundred and seventy-four isolates were tested for the production of primary and secondary metabolites. Among the 174 strains tested eight of the black pepper and seven of the ginger rhizobacteria produced hydrogen cyanide. Ten isolates were short-listed based on the *in vitro* test for antagonism against *Phytophthora, Pythium* and *Fusarium*. These isolates were tested for Phenazine -1- carboxylic acid (PCA). Only two isolates of black pepper BRB 5 and BRB 28 was positive. The primers were synthesized and tested for the detection of Diacetylphloroglucinol (DAPG), Pyrolnitrin, Pyoluteorin, and Chitinase, and none of the short-listed isolates were positive.

In vitro assay for biocontrol traits

One hundred and seventy-four isolates were screened for *in vitro* antagonism against three plant pathogenic fungi namely *Phytophthora*, *Pythium,* and *Fusarium*. Seven isolates from black pepper (BRB 5, BRB 24, BRB 28, BRB 49, BRB 53, BRB 60 and BRB 72) and eight isolates from ginger (GRB 25, GRB 35, GRB 36, GRB 57, GRB 58, GRB 68, GRB 72 and GRB 91) were short-listed (Table 3 &4).

Ginger

Screening of rhizobacteria for growth promoting& diseases suppression in green house

A pot experiment with fourteen treatments and three replications (with three pots) were conducted at IISR, Chelavoor. Three group/set of pots were arranged with 126 pots in each set. First set was used for plant growth promotion study, second for challenge inoculation of bacterial wilt pathogen (*R. solanacearum*) and third set for soft rot pathogen (*Pythium*). In each

set, there were 14 treatments, included 10 selected rhizobacteria, one reported bacterial biocontrol agent (IISR 51), one fungicide (1.2g/L), one bactericide (100mg/L) and one absolute control. The treatments were imposed before planting, by soaking the rhizome in 1% starch solution containing bacterial suspensions (~ x 10^7 CFU/mL) for 1hr. Three pieces of rhizomes (15g) were planted in each pot. The booster dose of treatments was applied during in three regular intervals (30, 60 and 90 days of planting, DAP). Observation of sprouting per cent was recorded on 21st day after planting.

Pathogen was challenge inoculated 30 days after planting in respective set of pots arranged. For the challenge inoculation 24h of *Ralstonia solanacearum* broth in CPG broth (Case peptone Glucose broth) with 1.5 OD was diluted to twice to get a bacterial population of 10^8CU/mL. 500mL of such suspension was poured in ginger pots. For *Pythium* inoculation 10 g of 5 day old mycelia was blended in 100mL sterile distilled water and made up to 1000mL. 100 mL of such mycelia suspension was inoculated in ginger pots on 30DAP. Observation of bacterial wilt and sot rot incidence were recorded. The data recorded was analysed using MstatC software and results are tabulated in Table 5.

Evaluation of rhizobacteria for growth promotion & diseases suppression in field

Field experiment was conducted during 2008-09 and 2009-10 at IISR experimental farm, Peruvannamuzhi with ten short listed isolates. Experiment was set up in RBD, with fourteen treatments and two replications (six beds replication) which included 10 rhizobacteria, two chemical controls, one reported biocontrol for ginger from IISR repository and one absolute control. Ginger rhizome were treated with 1% starch solution containing bacterial suspensions (~ x 10^8 cfu mL^{-1}) for one hour, shade dried for 24 hours and planted. The booster dose was applied during in three regular intervals (30, 60 and 90 DAP).Observation on sprouting was recorded on 21st and 45th day after planting. Disease incidence was recorded throughout the experimental period and data were analyzed using Mstat-C (Table 6).

Repeated field evaluation with selected two rhizobacteria

Based on the green house and field experiments conducted during 2008-09 and 2009-10, *Bacillus amyloliquifaciens* (GRB 35) and *Serratia marcescens* (GRB 68) were found to be

effective for disease control and plant growth promotion. The field experiment using this effective two strains were initiated in IISR experimental farm at Peruvannamuzhi. Experiment was set up in RBD, with six treatments and two replications (six bed replication). Treatment includes two efficient strains (GRB 35 and GRB 68), and an existing biocontrol strain from IISR repository (IISR 51), two chemical control (bactericide and fungicide) and a control. Ginger rhizome were treated with 1% starch solution containing bacterial suspensions (~ x 10^{10} cfu mL^{-1}) for one hour, shade dried for 24 hours and planted. The booster dose was applied during in three regular intervals (30, 60 and 90 DAP). Sprouting was recorded on 45 DAP (Fig.1). Disease incidence was recording at regular intervals (Fig 2). Ginger rhizome yield was recorded after the eighth month (Fig.3)

Identification of bacterial isolates by 16S rDNA sequence analysis and Biolog

The short-listed strains were identified using phenotypic and biochemical characters. The identity of eight of these isolates was confirmed by molecular characterization using 16s rDNA sequencing. The black pepper strains belonged to *Pseudomonas aeruginosa* and *Serratia marcescens.* The ginger strains belonged to *Burkholderia pyrrocinia*, *Bacillus amylolequifaciens, Serratia marcescens* and *Klebsiella* sp. Sequences of the five identified isolates were deposited with National Centre for Biotechnology Information databases (Table 7).The identity of the selected isolates was also confirmed by Biolog based identification tool, using Biolog Microstation System (RDG Laboratories, Hayward, California, USA).

Evaluation of rhizobacterial formulation for ginger

The promising rhizobacterial isolates (GRB 35 and GRB 68) for ginger from earlier study (2011-12) were evaluated in the field for two years (2012-14). The rhizobacterial strain, GRB 35 confirmed its plant growth promoting efficiency besides reducing the soft rot and bacterial wilt in ginger (var. *Mahima*). Field experiment on ginger consisted of treatments involving GRB 35 delivered through various modes The data (Figs 4 & 5) revealed that among the treatments, activated capsule (2 capsule/ 5 kg seed)-T2 followed by non activated capsule (1 capsule/ 5kg seed)-T1 registered the maximum yield (4.7 and 4.0 kg/ bed (1 x 3 m^2), respectively) and the markedly lower soft rot incidence (11 and 12%, respectively).

Development of novel delivery method through encapsulation

For the easy delivery of PGPR, a novel method was developed and named as Biocapsules. The advantage of biocapsules over existing PGPR formulations are long term storage (more than 24 months). The other advantages of PGPR biocapsules are as follows.

- Benign (no harmful by products, less amounts of inorganic and inert material)
- Uses less energy
- Provides all the required conditions
- Cost effective
- Ease of handling during use, storage and transport
- Persistence of the microbial strain in the soil after delivery
- Can be used to deliver all kinds of agriculturally important microorganisms
- Can be used to store pathogenic cultures without loss of virulence

Field evaluation of PGPR biocapsules for ginger

A field experiment for ginger was conducted by using PGPR Biocapsules during 2012-13 at IISR, Kozhikode campus. The observations like sprouting percent, disease incidence and yield were recorded and the results are shown in figures 6 &7. The result confirmed the efficacy of PGPR strain GRB 35 for enhancing sprouting and yield in ginger.

Study on the mode of action of shortlisted rhizobacteria from ginger and black pepper

The antibiotic production of the putative isolates was tested to study the mechanism of biological control. The bioassay of extracted antibiotics was performed on PDA and King's B against various pathogens viz. *Phytophthora capsici* and *Pythium myriotylum*. The selected isolates include GRB 35, GRB 68, GRB 70, BRB 3, BRB 13, and BRB 49. The extract from GRB 68 (*Serratia marcesans)* showed a strong mycelial inhibitory activity against *P.capsici*. The growth of *P.capsici* was completely arrested from the 3rd day of incubation while the control plate showed active growth on further incubation. The active growth towards the negative control zone (methanol alone) in the same plate revealed that the inhibitory activity was due to the action of secondary metabolites from GRB 68. LC-MS analysis indicated that the compounds identified belonged to peptides, which belongs to peptaibol which possess diverse bioactivities, (antibacterial, antifungal, and antiparasitic) and are used as biocontrol agents against fungal phytopathogens.

Black pepper

In vivo evaluation of rhizobacteria for black pepper

Biocontrol and growth promotion aspect

Pot experiment of black pepper variety Panchami carried out using potential rhizobacterial strains and *Trichoderma harzianum* (P26) for three years. The experimental design is a randomized complete block design with nine treatments and six replications including an absolute control and chemical control. Treatments include three rhizobacterial isolates (BRB 21- *Burkholderia*, BRB 28- *Pseudomonas aeruginosa* and BRB 49- *Serratia marcescens*), promising isolates *Pseudomonas* (IISR 6) and *Trichoderma harzianum (*P26) and their consortium (IISR 6+ P26). The chemical treatment included metalaxyl- mancozeb @ 1.25g/l. The growth parameters were recorded at monthly intervals. The pathogen control *Phytophthora capsici* inoculated and recorded the disease incidence and root rot percentage. The details of the treatment are as follows.

1. BRB 21
2. BRB 28
3. BRB 49
4. IISR 6
5. *Trichoderma harzianum* (P26)
6. IISR 6 + P 26
7. Chemical control (Metalaxyl- mancozeb)
8. Pathogen control (*P.capsici*)
9. Absolute control

Results revealed that:

- T6 (IISR 6 + P26) showed less root rot (%) followed by T5 (P26), T4 (IISR 6) and T1 (BRB 21).
- T5 (P 26) showed better root health followed by T6 (IISR 6 + P26) and T1 (BRB 21).
- T5 showed maximum height followed by T1 (BRB 21) (Table 8).

Nutrient mobilization and growth promotion in black pepper

The efficacy of the shortlisted isolates for nutrient mobilization and growth promotion is being studied using variety Panchami of Black pepper. The experimental design is a complete randomized block design with 20 treatments with 6 replications. Three rhizobacterial isolates (BRB 3, BRB 13 and BRB 23) with different combinations of recommended doses of NPK for

black pepper were evaluated. The bacterial suspensions (~ x 10^8 cfu/mL) at the rate of 250mL/pot were drenched at the time of planting and the booster dose was applied twice at 30 days intervals after planting. The growth parameters were recorded at monthly intervals. The plants were uprooted after 150 days of planting and various growth parameters were analyzed. The soil samples were taken 120 days after planting for analysis. The details of treatments are as follows.

T1. Normal soil + Control
T2. Normal soil + BRB 3 (*Micrococcus* luteus)
T3. Normal soil + BRB 13(*Enterobacter aerogenes*)
T4. Normal soil + BRB 23(*Micrococcus* sp.)
T5. 75% N + 100% P + 100% K+ Control
T6. 75% N + 100% P + 100% K+ BRB 3
T7. 75% N + 100% P + 100% K+ BRB 13
T8. 75% N + 100% P + 100% K+ BRB 23
T9. 100% N + 75% P + 100% K+ Control
T10. 100% N + 75% P + 100% K+ BRB 3
T11. 100% N + 75% P + 100% K+ BRB 13
T12. 100% N + 75% P + 100% K+ BRB 23
T13. 100% N + 100% P + 75% K + Control
T14. 100% N + 100% P + 75% K + BRB 3
T15. 100% N + 100% P + 75% K + BRB 13
T16. 100% N + 100% P + 75% K + BRB 23
T17. 100% N + 100% P + 100% K+ Control
T18. 100% N + 100% P + 100% K+ BRB 3
T19. 100% N + 100% P + 100% K+ BRB 13
T20. 100% N + 100% P + 100% K+ BRB 23

The results revealed that:

- T10 (75%N + 100%P + 100%K +BRB3 + BRB13 + BRB23) showed maximum height (Table 7).
- T17 (100%N + 100%P + 75%K + BRB3 + BRB13) showed maximum number of leaves.
- T17 (100%N + 100%P + 75%K + BRB3 + BRB13) showed maximum number of nodes.
- T 25 (100%N + 100%P + 100%K + BRB3 + BRB13 + BRB23) showed maximum fresh weight for leaves, T18 (100%N + 100%P + 75%K + BRB3 + BRB23) and T10 (75%N + 100%P + 100%K +BRB3 + BRB13 + BRB23) showed maximum fresh weight for root and shoot respectively.

- T 25 (100%N + 100%P + 100%K + BRB3 + BRB13 + BRB23) showed maximum dry weight for leaves, T18 (100%N + 100%P + 75%K + BRB3 + BRB23) and T25(100%N + 100%P + 100%K + BRB3 + BRB13 + BRB23) showed maximum dry weight for root and T22 (100%N + 100%P + 100%K + BRB3 + BRB13) showed maximum dry weight for shoot. (Table 8).

- Soil pH varied from 6.76 to 7.66. Conductivity highest in T24 (100% N + 100% P + 100% K+ BRB 13+ BRB 23) and soil organic carbon (SOC) highest in T9 (75% N + 100% P + 100% K+ BRB 13+ BRB 23). (Table 9)

- Mineral N, highest level 1137 mg/kg in T15 (100% N + 100% P + 75% K + BRB 13) Bray- P 17.95 mg/Kg in T23 (100% N + 100% P + 100% K+ BRB 3+ BRB 13) and highest K, 1076 mg/Kg in T9 (75% N + 100% P + 100% K+ BRB 13+ BRB 23). Highest level of Ca 1972.0 mg/kg in T25 (100% N + 100% P + 100% K+ BRB3+ BRB 13+ BRB 23), Mg 230.3 mg/kg in T16 (100% N + 100% P + 75% K+ Control). (Table 10).

- Fe 20.37 mg/kg in T16 (100% N + 100% P + 75% K+ Control) , Mn 31.32 mg/kg in T24 (100% N + 100% P + 100% K+ BRB 13+ BRB 23), Zn 5.84 mg/kg in T17 (100% N + 100% P + 75% K+ BRB 3+ BRB 13) & Cu 2.63 mg/kg in T16 (100% N + 100% P + 75% K+ Control). (Table 11).

- Greatest N mineralization rate 1094 mg/kg in T18 (100% N + 100% P + 75% K+ BRB 3+ BRB 23), increased DOC level 3.45 µg/g in T19 (100% N + 100% P + 100% K+ BRB 13), and increased DON level 102.1 mg/kg in T17 (100% N + 100% P + 75% K+)and T20 (100% N + 100% P + 100% K+ BRB 23) respectively. (Table 12).

- Increased soil respiration rate 93.57 µg CO_2/ g soil was observed in T17 (100% N + 100% P + 100% K+ BRB 3+ BRB 13), Increased C_{MIC} level 70.37 µg/g in T18 (100% N + 100% P + 75% K+ BRB 3+ BRB 23), highest P_{MIC} level 67.40 mg/kg in T9(75% N + 100% P + 100% K+ BRB 13+ BRB 23)and N_{MIC} level 47.73 mg/kg in T12 (100% N + 75% P + 100% K+BRB 3+ BRB 13). (Table 13).

- Increased AcP activity shown by T12 (100% N + 75% P + 100% K+BRB 3+ BRB 13), increased AlkP activity by T14 (100% N + 75% P + 100% K+BRB 13+ BRB 23),AS activity shown by T25(100% N + 100% P + 100% K+ BRB 3+ BRB 13+ BRB 23), Increased BG activity in T23 (100% N + 100% P + 100% K+ BRB 3+ BRB 23), UR activity in T18 (100% N + 100% P + 75% K+BRB 3+ BRB 23)and highest DH activity in T19 (100% N + 100% P + 75% K+ BRB 13+ BRB 23). (Table 14).

Overall, the study indicated that

- Treatment with *Trichoderma harzianum* showed less root rot and better root health in biocontrol experiment.

- Three rhizobacterial isolates (BRB 3, BRB 13 and BRB 23) from black pepper with different combinations of NPK promoted the growth in black pepper.

- The results on growth parameters revealed that the treatments 75% N + 100% P + 100% K+ BRB 3 and 100% N + 100% P + 100% K+ BRB 3 are best.

- In case of consortium experiment good growth obtained in the treatment T10 (75%N + 100%P + 100%K +BRB3 + BRB13 + BRB23), T17 (100%N + 100%P + 75%K + BRB3 + BRB13), T18 (100%N + 100%P + 75%K + BRB3 + BRB23) and T25 (100%N + 100%P + 100%K + BRB3 + BRB13 + BRB23).

- The green house study on the effects of PGPR, inorganic NPK fertilizers and their combinations on mineral soil nutrient mobilization indicated that BRB 13 (*Enterobacter aerogenes*) and BRB (23) *Micrococcus* sp. applied in combination with 100% NPK or at 75% of either of the nutrients (i.e. 140-50-270 kg ha^{-1} NPK) registered greatest levels of mineral N, Bray P and exchangeable K in soil. Besides, levels of microbial biomass-C, -N, -P, and hydrolytic enzyme activities were consistently higher in treatments with combined application of PGPR + NPK.

- Consortium of three rhizobacterial isolates (BRB 3, BRB 13 and BRB 23) from black pepper with different combinations of NPK was found to promote the growth in black pepper.

Evaluation PGPR consortium for plant growth promotion and biocontrol

Based on the previous year's data, the efficacy of the shortlisted isolates for nutrient mobilization, growth promotion and biocontrol is being studied by using Consortium of PGPR isolates (BRB 3- *Micrococcus luteus*, BRB 13- *Enterobacter* aerogenes., and BRB 23- *Micrococcus* sp.), BRB 21 (*Burkholderia cepacia*) and *Trichoderma harzianum* in varying combinations along with different concentrations of recommended doses of NPK fertilizers for black pepper variety Panchami. Nutrient management studies involve 40 treatments with 5 replications, and biocontrol experiment contains 42 treatments with 4 replications. The bacterial suspensions (~ x 10^8 cfu/mL) at the rate of 250mL/pot were drenched at the time of planting and the booster dose was applied thrice at 30 days intervals after planting. The chemical treatment included metalaxyl- mancozeb @ 1.25g/l. The growth parameters were recorded at monthly intervals.

Green house study on nutrient management

Soil samples for analysis were taken after 120th day of planting. The plants were uprooted 150th day after planting and growth parameters were recorded. The details of treatments for nutrient management study are as follows:

1. CONTROL
2. BRB 21
3. P 26
4. CONSORTIUM
5. BRB 21 + P 26
6. BRB 21 + CONSORTIUM
7. P 26 + CONSORTIUM
8. BRB 21 + P 26 + CONSORTIUM
9. CONTROL + 75% N + 100 PK
10. BRB 21 + 75% N + 100 PK
11. P 26 + 75% N + 100 PK
12. CONSORTIUM + 75% N + 100 PK
13. BRB 21 + P 26 + 75% N + 100 PK
14. BRB 21 + CONSORTIUM + 75% N + 100 PK
15. P 26 + CONSORTIUM + 75% N + 100 PK
16. BRB 21 + P 26 + CONSORTIUM + 75% N + 100 PK
17. CONTROL + 75% P + 100 NK
18. BRB 21 + 75% P + 100 NK
19. P 26 + 75% P + 100 NK
20. CONSORTIUM + 75% P + 100 NK
21. BRB 21 + P 26 + 75% P + 100 NK
22. BRB 21 + CONSORTIUM + 75% P + 100 NK
23. P 26 + CONSORTIUM + 75% P + 100 NK
24. BRB 21 + P 26 + CONSORTIUM + 75% P + 100 NK
25. CONTROL + 75% K + 100 NP
26. BRB 21 + 75% K + 100 NP
27. P 26+ 75% K + 100 NP
28. CONSORTIUM+ 75% K + 100 NP
29. BRB 21 + P 26+ 75% K + 100 NP
30. BRB 21 + CONSORTIUM+ 75% K + 100 NP
31. P 26 + CONSORTIUM+ 75% K + 100 NP
32. BRB 21 + P 26 + CONSORTIUM+ 75% K + 100 NP
33. CONTROL+ 100 NPK
34. BRB 21+ 100 NPK
35. P 26+ 100 NPK
36. CONSORTIUM+ 100 NPK
37. BRB 21 + P 26+ 100 NPK

38. BRB 21 + CONSORTIUM+ 100 NPK
39. P 26 + CONSORTIUM+ 100 NPK
40. BRB 21 + P 26 + CONSORTIUM+ 100 NPK

The results revealed that:

- T20 (CONSORTIUM + 75% P + 100 NK) showed highest growth parameters (Height (figure1 & 2), number of leaves, number of nodes and root length) followed by T19 (P 26 + 75% P + 100 NK) (Table 15).
- T24 (BRB 21 + P 26 + CONSORTIUM + 75% P + 100 NK) showed maximum fresh weight for shoot and leaves. T20 (CONSORTIUM + 75% P + 100 NK) showed maximum fresh weight for root.
- T24 (BRB 21 + P 26 + CONSORTIUM + 75% P + 100 NK) showed maximum dry weight for shoot and leaves. T22 (BRB 21 + CONSORTIUM + 75% P + 100 NK) and T20 (CONSORTIUM + 75% P + 100 NK) showed maximum dry weight for root. (Table 16).
- Soil pH varied from 4.59 to 6.07. Conductivity highest in T35 (P 26+ 100 NPK) and soil organic carbon (SOC) highest in T24 (BRB 21+ P26+ CONSORTIUM+75P +100NK). (Table. 17).
- Mineral N, highest level 98.0 mg/kg in T28 (CONSORTIUM+75K +100NP) and T12 (CONSORTIUM +75N +100PK), Bray- P 1.84 mg/Kg in T39 (P26+ CONSORTIUM+100NPK) and highest K, 528.0 mg/kg in T32 (BRB 21+P26+ CONSORTIUM+75P+100NP).
- Plant N, 4.82% and K 5.03% in T32 (BRB 21+P26+ CONSORTIUM+75P+100NP). Plant P, 0.99%in T20 (CONSORTIUM+75P +100NK) (Table. 18).
- Increased DOC level 1.77 g/kg in T30 (BRB 21+ CONSORTIUM+75K +100NP), and increased DON level 125.3 mg/kg in T35 (P 26+ 100 NPK). (Table. 17).
- Increased AlkP and AS, activity shown by T40 (BRB 21 + P 26 + CONSORTIUM+ 100 NPK), Increased BG activity in T38 (BRB 21 + CONSORTIUM+ 100 NPK), highest UR activity inT35 (P 26+ 100 NPK) and AcP activity in T37 (BRB 21 + P 26+ 100 NPK). (Table. 19).

Green house study on biocontrol

The pathogen control *Phytophthora capsici* (10 sporulated discs of 5mm diameter) inoculated in all treatments except control and recorded the disease incidence and root rot percentage. The details of the treatment are as follows.

1. CONTROL
2. Pathogen control
3. Fungicide control- Metalaxyl
4. BRB 21
5. P 26
6. CONSORTIUM
7. BRB 21 + P 26
8. BRB 21 + CONSORTIUM
9. P 26 + CONSORTIUM
10. BRB 21 + P 26 + CONSORTIUM
11. CONTROL + 75% N + 100 PK
12. BRB 21 + 75% N + 100 PK
13. P 26 + 75% N + 100 PK
14. CONSORTIUM + 75% N + 100 PK
15. BRB 21 + P 26 + 75% N + 100 PK
16. BRB 21 + CONSORTIUM + 75% N + 100 PK
17. P 26 + CONSORTIUM + 75% N + 100 PK
18. BRB 21 + P 26 + CONSORTIUM + 75% N + 100 PK
19. CONTROL + 75% P + 100 NK
20. BRB 21 + 75% P + 100 NK
21. P 26 + 75% P + 100 NK
22. CONSORTIUM + 75% P + 100 NK
23. BRB 21 + P 26 + 75% P + 100 NK
24. BRB 21 + CONSORTIUM + 75% P + 100 NK
25. P 26 + CONSORTIUM + 75% P + 100 NK
26. BRB 21 + P 26 + CONSORTIUM + 75% P + 100 NK
27. CONTROL + 75% K + 100 NP
28. BRB 21 + 75% K + 100 NP
29. P 26+ 75% K + 100 NP
30. CONSORTIUM+ 75% K + 100 NP
31. BRB 21 + P 26+ 75% K + 100 NP
32. BRB 21 + CONSORTIUM+ 75% K + 100 NP
33. P 26 + CONSORTIUM+ 75% K + 100 NP
34. BRB 21 + P 26 + CONSORTIUM+ 75% K + 100 NP
35. CONTROL+ 100 NPK
36. BRB 21+ 100 NPK
37. P 26+ 100 NPK
38. CONSORTIUM+ 100 NPK
39. BRB 21 + P 26+ 100 NPK
40. BRB 21 + CONSORTIUM+ 100 NPK
41. P 26 + CONSORTIUM+ 100 NPK
42. BRB 21 + P 26 + CONSORTIUM+ 100 NPK

Replications: 4
Variety: Panchami
Date of planting: 28-12-2012

Results revealed that:

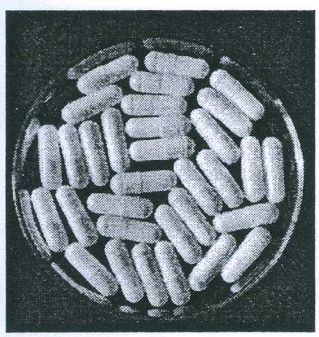
- Treatments with P26 (*Trichoderma harzianum*) shows less root rot index.
- Treatment P26 + consortium shows less root rot and better growth.
- Combined application of BRB21 + P26+ consortium also gives good results.

Overall, the study indicated that

- Treatments involving CONSORTIUM +75%P+100 NK showed better growth parameters.
- PGPR and NPK fertilizers in different combinations enhanced biological activities of soil and thereby promoted the growth of black pepper.

Patents

"A NOVEL METHOD OF STORING AND DELIVERING PGPR/MICROBES THROUGH BIOCAPSULES" (Application no.3594/CHE/2013 dated 13/08/2013)



The novel invention of PGPR formulation as biocapsule is the easy and reliable technology of storing and delivering PGPR bioagents in hard gelatin capsule termed as biocapsule. It is preparation of viable microbial agents in a capsule form. The efficient plant growth promoting rhizobacteria (PGPR) for ginger namely *Bacillus amyloliquefaciens* GRB35 (NCBI -FJ493538), is used in this invention. The viability of the PGPR can be retained for long term (24 months) without losing its beneficial traits.

Process/Products/Technology/Developed

Product: 2

1. Microbial consortium (Talc based formulation) named 'IISR Biomix-BP' for black pepper
2. PGPR strain GRB35-*Bacillus amyloliquefaciens* (Talc based formulation) named 'IISR Biopower-G' for ginger

The business proposal for this product named Biopower- BP has been submitted to the ITMU cell of our institute to facilitate licensing to private industries and the product was showcased during the Horticulture Industry Meet held at IIHR, Bengaluru on 06 March 2012.

Technology:1

1. A novel method of storing and delivering PGPR/microbes through encapsulation (Patent filed)

Publications

1. Aravind R, Bini YK, Kumar A, Anandaraj M (2009) Isolation and characterization of rhizobacteria from black pepper (*Piper nigrum* L.) for biocontrol In: Plant Pathology in the Globalized Era (Eds) D.K. Agarwal, Kalyan K. Mondal. Indian Phytopathological Society, Indian Agricultural Research Institute, New Delhi. p.311.
2. Bini YK, Anandaraj M, Kumar A, Aravind R, Dinesh R (2010) Isolation and characterization of rhizobacteria from ginger (*Zingiber officinale* Rosc.) for biocontrol and growth promotion. In: Indian Phytopathological Society (Southern Zone) Symposium on "Changing plant disease scenario in relation to climate change"

3. Anandaraj M, Dinesh R, Bini YK, Silna N, Kumar A (2011). Evaluation of PGPR for biocontrol and nutrition mobilization in black pepper (*Piper nigrum* L.) In: Proceedings of 2nd Asian PGPR Conference, Beijing, China from August 21-24, 319 p.

4. Bini YK, Anandaraj M, Dinesh R, Silna N, Kumar A (2011). Evaluation of PGPR strains for growth and disease suppression in ginger (*Zingiber officinale* Rosc.) In: Proceedings of 2nd Asian PGPR Conference, Beijing, China from August 21-24, 519 p.

5. Dinesh R, Anandaraj M, Srinivasan V, Hamza S (2012) ENPs in the soil and their potential implications to microbial activity. Geoderma (Elsevier) 173-174: 19-27.

6. Dinesh R, Anandaraj M, Srinivasan V, Kumar A, Bini Y. K, Subila K. P, Aravind R (2013) Combined plant growth promoting rhizobacteria -inorganic fertilization enhances biological quality of soils under ginger (*Zingiber officinale* Rosc.). Agricultural Res., (NAAS-Springer), 2: 346-353.

7. Dinesh R, Anandaraj M, Kumar A, Subila KP, Bini YK, Aravind R (2014) Native multi-trait rhizobacteria promote growth and suppress foot rot in black pepper. J. Spices Aromatic Crops, 23(2) (In press)

Table 1. Preliminary characterization of rhizobacterial strains

Sl. No	Rhizobacterial strains	Number of diverse population	
		Black pepper	Ginger
1	*Pseudomonas sp*	19	33
2	*Serratia sp*	5	18
3	*Enterobacter sp*	5	6
4	*Burkholderia sp*	1	9
5	*Bacillus sp*	10	13
6	*Klebsiella sp*	4	8
7	*Curtobacterium sp*	6	1
8	*Arthrobacter sp*	7	5
9	*Micrococcus sp*	5	5
10	Unidentified	12	2
	Total	74	100

Table 2. *In vitro* assay for beneficial agronomic traits

Isolate Number	Source	IAA	Potassium	Phosphorus	Zinc	Silica	Ammonia
BRB 13	Black pepper	P	P	P	P	N	P
BRB 23	,,	P	P	P	P	N	P
BRB 3	,,	P	P	N	P	N	P
BRB 36	,,	N	P	P	P	N	P
BRB 57	,,	P	P	P	N	N	P
GRB 38	Ginger	P	N	P	P	P	P
GRB 2	"	P	P	P	N	N	P
GRB25	,,	N	P	P	P	N	P
GRB 36	,,	P	N	P	N	P	P
GRB 70	,,	P	P	P	N	N	P

(P- Positive, N- Negative)

Table 3. Antagonistic activity of short-listed strains from black pepper and ginger

Isolate Number	Source	Pathogen inhibition		
		Phytophthora	*Pythium*	*Fusarium*
BRB 5	Black pepper	+	++	+++
BRB 24	,,	+++	++	+++
BRB 28	,,	+++	++	++
BRB 49	,,	+++	++	+
BRB 53	,,	+++	+++	+
BRB 60	,,	+++	++	++
BRB 72	,,	++	+++	+
GRB 25	Ginger	++	++	+++
GRB 35	,,	++	++	++
GRB 36	,,	+++	++	+
GRB 57	,,	+++	+++	++
GRB 58	,,	+++	++	++
GRB 68	,,	+++	++	++
GRB 72	,,	++	++	+
GRB 91	,,	++	++	+

(+ = 50-65% inhibition, ++ = 65-80% inhibition, +++ = >80% inhibition.)

Table 4. *In vitro* screening of ginger rhizobacteria for antagonism against *Pythium* and *P.capsici* (Values in the indices are Arc Sine transformed value)

Isolate Number	Inhibition (%)	
	Pythium sp.	*Phytophthora capsici*
GRB 35 - *Bacillus amyloliquefaciens*	78.51 (64.81)	77.08(61.43)
GRB 57- *Curtobacterium* sp.	85.9 (67.95)	78.75 (62.51)
GRB 58 - *Serratia marcescens*	85.92 (67.99)	75.83 (60.56)
GRB 68 - *Serratia marcescens*	84.07 9 (66.45)	75.00 (60.02)
GRB 91 - *Pseudomonas aeruginosa*	73.93 (58.88)	83.52 (66.08)
GRB25- *Burkholderia* sp.	78.51(62.41)	41.25(39.95)
GRB36- *Klebsiella* sp.	80.00 (63.44)	59.58 (50.52)
GRB38 – *Serratia marcescens*	25.55(30.34)	45.00(42.11)
GRB70 - *Enterobacter* sp.	65.92 (54.76)	40.39 (39.45)
GRB71- *Enterobacter* sp.	6.6 (14.89)	22.35(28.16)
Control	0.0 (4.9)	0.0 (4.9)
LSD	53	43

(Figures in parentheses are Arc Sin transformed values)

Table 5. Evaluation of selected rhizobacteria for enhanced sprouting and disease suppression (bacterial wilt and soft rot) in ginger

Treatment	Sprouting (%)	No. of tillers	Total yield (g/pot)	Individual yield (g/plant)	Bacterial wilt (%)	Soft rot (%)
Burkholderia sp -GRB 25	81.3 (64.6)	3	190.0	84.26	96.3 (78.9)	96.3 (78.9)
Bacillus amyloliquefaciens - GRB 35	96.3 (78.9)	3	365.6	129.9	35.2 (36.4)	48.1 (43.9)
Klebsiella sp -GRB 36	69.9 (56.7)	2	184.4	74.63	66.6(54.7)	66.7 (54.7)
Serratia marcescens -GRB38	96.3 (78.9)	3	358.3	125.6	88.9 (70.5)	61.1 (51.4)
Curtobacterium -GRB 57	88.9 (70.5)	3	334.4	126.1	81.5 (64.5)	92.6 (74.2)
Seratia marcescens -GRB58	88.9 (70.5)	3	334.4	101.1	88.9 (70.5)	92.6 (74.2)
Seratia marcescens -GRB68	96.3 (78.9)	3	384.4	134.1	44.4 (41.8)	57.4 (49.3)
Enterobacter sp. -GRB 70	100 (90.0)	3	380.3	129.4	48.1 (43.9)	70.4 (57.0)
Enterobacter sp. -GRB 71	96.3 (78.9)	3	182.8	63.89	88.9 (70.5)	70.4 (57.0)
Pseudomonas sp. -GRB91	85.2 (67.4)	3	186.7	128.0	53.7 (47.1)	44.4 (41.7)
Pseudomonas aeruginosa - IISR 51	88.9 (70.5)	3	186.1	71.20	81.5 (64.5)	62.9 (52.5)
Streptomycin sulphate 1g/L (seed treatment)	88.9 (70.5)	2	152.8	62.04	72.2 (58.2)	74.1 (59.4)
Metalaxyl- Mancozeb 1.25g/L (Seed treatment+ Soil drench)	88.9(70.5)	3	152.8	56.48	88.9 (70.5)	51.8 (46.1)
Control	66.6 (54.7)	2	134.4	69.44	100 (90.0)	100 (90.0)
LSD	18.4	1	42.14	18.75	16.87	21.93

(Figures in parentheses are Arc Sin transformed values)

Table 6. Management of soft rot *vis-à-vis* rhizome yield by rhizobacterial applications in rhizome and soil- field

Treatment	Sprouting (%)	Soft rot (%)	Rhizome yield (kg $3m^{-2}$)
Bacillus amyloliquefaciens -GRB 35	92.2 (73.9)	38.2 (38.2)	3.8
Seratia marcescens -GRB 68	93.0 (74.7)	26.6 (31.1)	4.1
Curtobacterium sp. -GRB 57	87.2 (69.1)	39.8 (39.1)	3.0
Klebsiella sp -GRB36	80.7 (63.9)	39.8 (39.1)	2.4
Burkholderia sp -GRB 25	76.2 (60.9)	55.6 (48.2)	2.0
Enterobacter sp. -GRB71	86.2 (68.28)	41.4 (40.0)	3.0
Pseudomonas -GRB91	89.5 (71.0)	38.6 (38.4)	3.4
Serratia marcescens -GRB 58	78.4(62.4)	45.8 (42.6)	3.2
Serratia marcescens -GRB 38	84.5 (66.8)	41.8 (40.3)	2.1
Enterobacter sp. -GRB 70	92.0 (73.6)	42.2 (40.5)	3.1
Pseudomonas aeruginosa-IISR 51	76.5 (61.0)	50.4 (45.2)	2.0
Streptomycin sulphate- 100mg/L(seed treatment)	85.5 (67.6)	44.2 (41.7)	3.0
Metalaxyl- Mancozeb – 1.25g/L(Seed treatment+ Soil drench)	86.2 (68.3)	41.3 (41.7)	2.0
Control	84.7 (67.1)	62.8 (52.4)	1.0
LSD	9.0	8.4	1.1

(Figures in parentheses are Arc Sin transformed values)

Table 7. Details of sequence deposited with NCBI

Isolate Number	Source	Identity	Acc. No.
BRB 28	Black pepper	*Pseudomonas aeruginosa*	FJ493536
BRB 49	Black pepper	*Serratia marcescens*	FJ493537
GRB 35	Ginger	*Bacillus amylolequifaciens*	FJ493538
GRB 36	Ginger	*Klebsiella* sp.	FJ493539
GRB 68	Ginger	*Serratia marcescens*	FJ493540

Table 8. Effect of cultures on growth and disease incidence

TREATMENT	ROOT ROT (%)	ROOT HEALTH (%)	HEIGHT
T1	32.77	50.13	332.0
T2	47.77	22.91	294.7
T3	72.50	8.175	161.3
T4	30.41	45.27	275.7
T5	27.91	55.13	361.7
T6	25.27	50.13	313.7
T7	45.13	35.27	313.7
T8	80.00	8.175	228.0
T9	0.810	60.00	239.3
LSD (P<0.05)	33.60	44.21	69.98

T1-BRB 21; T2-BRB 28; T3-BRB 49; T4-IISR 6; *Trichoderma harzianum* (P26); T5-IISR 6 + P 26 T7-Chemical control (Metalaxyl- mancozeb); T8-Pathogen control (*P.capsici*); T9-Absolute control

Table 9. Effect of PGPR in combination with varying rates of NPK on fresh and dry weight of plants

TREATMENT	HEIGHT	NO.OF LEAVES	NO.OF NODES
T1	186.0 e	33.33 d	26.67 e
T2	256.7 cde	33.67 d	36.33 bcde
T3	264.7 cde	38.67 bcd	41.00 abcde
T4	340.0 abc	33.67 d	48.67 abcd
T5	257.0 cde	49.67 ab	39.33 abcde
T6	368.7 a	54.00 a	56.00 a
T7	243.0 de	35.00 cd	32.33 de
T8	315.3 abcd	46.00 abc	45.00 abcd
T9	276.3 bcd	33.33 d	35.00 cde
T10	324.3 abcd	52.33 a	50.67 abc
T11	274.0 bcd	43.33 abcd	43.33 abcde
T12	246.7 de	38.67 bcd	37.33 bcde
T13	263.3 cde	34.33 d	36.67 bcde
T14	253.7 cde	33.33 d	33.33 de
T15	338.0 abc	46.00 abc	51.00 abc
T16	301.7 abcd	44.00 abcd	46.00 abcd
T17	285.7 abcd	46.00 abc	48.00 abcd
T18	354.7 ab	49.67 ab	52.33 ab
T19	254.3 cde	38.00 cd	39.67 abcde
T20	260.3 cde	36.67 cd	35.33 bcde

Mean values followed by the same letter are not significantly different at $P<0.05$

[T1. Normal soil + Control; T2. Normal soil + BRB 3 (*Micrococcus* luteus); T3. Normal soil + BRB 13(*Enterobacter aerogenes*); T4. Normal soil + BRB 23(*Micrococcus* sp.); T5. 75% N + 100% P + 100% K+ Control; T6. 75% N + 100% P + 100% K+ BRB 3; T7. 75% N + 100% P + 100% K+ BRB 13 ; T8. 75% N + 100% P + 100% K+ BRB 23 ; T9. 100% N + 75% P + 100% K+ Control ; T10. 100% N + 75% P + 100% K+ BRB 3; T11. 100% N + 75% P + 100% K+ BRB 13; T12. 100% N + 75% P + 100% K+ BRB 23; T13. 100% N + 100% P + 75% K + Control; T14. 100% N + 100% P + 75% K + BRB 3; T15. 100% N + 100% P + 75% K + BRB 13; T16. 100% N + 100% P + 75% K + BRB 23; T17. 100% N + 100% P + 100% K+ Control; T18. 100% N + 100% P + 100% K+ BRB 3 ; T19. 100% N + 100% P + 100% K+ BRB 13 ; T20. 100% N + 100% P + 100% K+ BRB 23]

Table. 10. Effect of PGPR in combination with varying rates of NPK on leaf, shoot and root weight of black pepper

Treatment	Leaf		Shoot		Root	
	Fresh wt (g)	Dry wt (g)	Fresh wt (g)	Dry wt (g)	Fresh wt (g)	Dry wt (g)
T1	48.33 fg	5.000 e	25.00 efgh	10.00 cd	10.00 f	5.000 b
T2	56.67 de	5.000 e	23.00 fgh	5.000 f	13.33 de	5.000 b
T3	56.67 de	6.667 e	24.33 efgh	8.333 de	11.67 ef	5.000 b
T4	56.67 de	6.667 e	26.67 efg	10.00 cd	16.67 c	5.000 b
T5	61.67 c	11.67 cd	21.67 gh	13.33 ab	11.67 ef	5.000 b
T6	58.33 cd	15.00 b	26.67 efg	13.33 ab	11.67 ef	5.000 b
T7	51.67 ef	10.00 d	30.00 cde	10.00 cd	16.67 c	5.000 b
T8	55.00 de	10.00 d	28.33 def	10.00 cd	16.67 c	5.000 b
T9	45.00 g	13.33 bc	23.33 fgh	10.00 cd	10.00 f	5.000 b
T10	68.33 b	15.00 b	45.00 a	10.00 cd	20.00 b	5.000 b
T11	56.67 de	15.00 b	26.67 efg	10.00 cd	15.00 cd	5.000 b
T12	50.00 fg	15.00 b	23.33 fgh	6.667 ef	15.00 cd	5.000 b
T13	25.00 h	5.000 e	10.00 i	5.000 f	5.000 g	5.000 b
T14	50.00 fg	10.00 d	35.00 bc	6.667 ef	11.67 ef	5.000 b
T15	48.33 fg	10.00 d	26.67 efg	5.000 f	15.00 cd	5.000 b
T16	25.00 h	15.00 b	33.33 bcd	10.00 cd	13.33 de	5.000 b
T17	56.67 de	13.33 bc	36.67 b	10.00 cd	15.00 cd	5.000 b
T18	50.00 fg	10.00 d	28.33 def	10.00 cd	25.00 a	10.00 a
T19	50.00 fg	5.000 e	21.67 gh	5.000 f	20.00 b	5.000 b
T20	45.00 g	5.000 e	20.00 h	5.000 f	15.00 cd	5.000 b
T21	48.33 fg	10.00 d	28.33 def	13.33 ab	10.00 f	5.000 b
T22	60.00 cd	15.00 b	25.00 efgh	15.00 a	10.00 f	5.000 b
T23	50.00 fg	15.00 b	23.33 fgh	10.00 cd	10.00 f	5.000 b
T24	45.00 g	15.00 b	25.67 efgh	11.67 bc	5.000 g	5.000 b
T25	75.00 a	20.00 a	36.67 b	13.33 ab	10.00 f	10.00 a

Mean values followed by the same letter are not significantly different at $P<0.05$

T1-Control; T2-BRB3 + BRB13; T3-BRB3 + BRB23; T4-BRB13 + BRB23; T5-BRB3 + BRB13 + BRB23; T6-75%N + 100%P + 100%K +Control; T7- 75%N + 100%P + 100%K + BRB3 + BRB13; T8-75%N + 100%P + 100%K + BRB3 + BRB23; T9-75%N + 100%P + 100%K + BRB13 + BRB23; T10-75%N + 100%P + 100%K +BRB3 + BRB13 + BRB23; T11- 100%N + 75%P + 100%K + Control; T12- 100%N + 75%P + 100%K + BRB3 + BRB13; T13-100%N + 75%P + 100%K + BRB3 + BRB23; T14-100%N + 75%P + 100%K + BRB13 + BRB23; T15-100%N + 75%P + 100%K + BRB3 + BRB13 + BRB23; T16-100%N + 100%P + 75%K + Control; T17-100%N + 100%P + 75%K + BRB3 + BRB13; T18- 100%N + 100%P + 75%K + BRB3 + BRB23; T19- 100%N + 100%P + 75%K + BRB13 + BRB23; T20- 100%N + 100%P + 75%K + BRB3 + BRB13 + BRB23; T21-100%N + 100%P + 100%K + Control; T22- 100%N + 100%P + 100%K + BRB3 + BRB13; T23- 100%N + 100%P + 100%K + BRB3 + BRB23; T24- 100%N + 100%P + 100%K + BRB13 + BRB23; T25- 100%N + 100%P + 100%K + BRB3 + BRB13 + BRB23

Table. 11. Effect of PGPR in combination with varying rates of NPK on on soil mineral N, Bray- P, and Exchangeable- K, Ca and Mg

TREATMENT	N (mg/Kg)	P (mg/Kg)	K (mg/Kg)
T1	641.3 n	2.60 q	275.0p
T2	642.8 n	4.90m	193.2q
T3	771.9 f	3.20 o	194.2q
T4	574.7o	3.00 p	194.2r
T5	561.0 p	4.40 n	560.7 b
T6	703.9 j	6.20 l	487.2g
T7	718.2 i	4.40 n	379.2o
T8	751.3 g	8.90 h	484.0 h
T9	690.7 k	6.80 k	410.0 l
T10	730.6 h	9.50 g	439.0 k
T11	817.6 c	7.40 j	512.0 e
T12	912.7 a	8.10 i	397.0m
T13	809.3 d	10.5 f	387.0 n
T14	659.1m	12.5 d	463.5 j
T15	914.9 a	11.3 e	467.2 i
T16	681.1 l	17.4 a	564.0 a
T17	849.4 b	3.00 p	484.0 h
T18	691.0 k	12.8 c	489.2 f
T19	801.5 e	7.50 j	522.0 d
T20	645.5 n	14.1 b	531.0 c

Mean values followed by the same letter are not significantly different at P<0.05

[T1. Normal soil + Control; T2. Normal soil + BRB 3 (*Micrococcus* luteus); T3. Normal soil + BRB 13(*Enterobacter aerogenes*); T4. Normal soil + BRB 23(*Micrococcus* sp.); T5. 75% N + 100% P + 100% K+ Control; T6. 75% N + 100% P + 100% K+ BRB 3; T7. 75% N + 100% P + 100% K+ BRB 13 ; T8. 75% N + 100% P + 100% K+ BRB 23 ; T9. 100% N + 75% P + 100% K+ Control ; T10. 100% N + 75% P + 100% K+ BRB 3; T11. 100% N + 75% P + 100% K+ BRB 13; T12. 100% N + 75% P + 100% K+ BRB 23; T13. 100% N + 100% P + 75% K + Control; T14. 100% N + 100% P + 75% K + BRB 3; T15. 100% N + 100% P + 75% K + BRB 13; T16. 100% N + 100% P + 75% K + BRB 23; T17. 100% N + 100% P + 100% K+ Control; T18. 100% N + 100% P + 100% K+ BRB 3 ; T19. 100% N + 100% P + 100% K+ BRB 13 ; T20. 100% N + 100% P + 100% K+ BRB 23]

Table 12: Effect of PGPR in combination with varying rates of NPK on soil biochemical parameters

TREATMENT	N_{MIN}[a] (mg N/Kg)	DOC (g/kg)	DON (mg N /kg)	SR (µg CO_2)	C_{MIC} (µg/g)	P_{MIC} (mg/Kg)	N_{MIC} (mg N/Kg)
T1	19.6 s	0.019 q	27.67 l	3.100 n	4.39 j	1.110 n	28.50 p
T2	107.8 q	0.042 m	56.00 d	4.533 m	7.39 hi	2.250 n	51.80 l
T3	183.1 m	0.072 c	33.57 k	12.23 k	7.62 hi	1.637 l	59.47 j
T4	147.0 p	0.060 i	36.43 j	12.00 k	4.07j	1.650 l	44.00 n
T5	299.8 g	0.030 n	27.67 l	7.800 l	3.97j	5.700 h	28.50 p
T6	333.2 f	0.057 j	28.33 l	49.63 a	7.88h	6.803 e	75.20 i
T7	454.6 c	0.086 b	50.50 f	18.70 i	15.71cd	7.553 b	77.80 h
T8	628.2 a	0.052 l	53.33 e	12.10 k	11.93e	4.100 i	57.00 k
T9	241.1 i	0.018 s	55.67 d	12.00 k	6.52i	1.450 m	44.10 n
T10	235.4 j	0.068 e	39.47 i	18.70 i	11.10ef	6.250 g	132.2 b
T11	294.7 h	0.056 k	25.20 m	18.80 i	7.52hi	4.100 i	95.50 e
T12	215.6 k	0.063 g	42.00 h	23.13 h	32.66a	6.520 f	121.9 c
T13	98.0 r	0.018 r	28.00 l	16.60 j	11.64e	0.813 o	38.90 o
T14	401.8 d	0.024 p	36.50 j	24.47 g	14.78d	1.710 l	57.00 k
T15	196.0 l	0.066 f	47.63 g	46.33 c	15.85cd	1.167 n	85.60 g
T16	578.2 b	0.025 o	56.00 d	44.33 d	4.44j	7.110 d	49.40 m
T17	176.6 n	0.011 t	61.60 c	35.30 f	9.92g	6.437 f	38.90 o
T18	344.0 e	0.063 h	106.5 a	47.30 b	10.21fg	7.430 c	119.4 d
T19	107.9 q	0.090 a	95.30 b	23.10 h	18.9b	3.967 j	155.6 a
T20	150.0 o	0.069 d	106.4 a	38.50 e	16.78c	10.40 a	93.30 f

[a]N_{MIN}: Total N mineralized; DOC- Dissolved organic C; DON- Dissolved organic N; SR- Soil respiration C_{MIC}- Microbial biomass C; P_{MIC}- Microbial biomass P; N_{MIC}- Microbial biomass N; Mean values followed by the same letter are not significantly different at $P<0.05$

[T1. Normal soil + Control; T2. Normal soil + BRB 3 (*Micrococcus* luteus); T3. Normal soil + BRB 13(*Enterobacter aerogenes*); T4. Normal soil + BRB 23(*Micrococcus* sp.); T5. 75% N + 100% P + 100% K+ Control; T6. 75% N + 100% P + 100% K+ BRB 3; T7. 75% N + 100% P + 100% K+ BRB 13 ; T8. 75% N + 100% P + 100% K+ BRB 23 ; T9. 100% N + 75% P + 100% K+ Control ; T10. 100% N + 75% P + 100% K+ BRB 3; T11. 100% N + 75% P + 100% K+ BRB 13; T12. 100% N + 75% P + 100% K+ BRB 23; T13. 100% N + 100% P + 75% K + Control; T14. 100% N + 100% P + 75% K + BRB 3; T15. 100% N + 100% P + 75% K + BRB 13; T16. 100% N + 100% P + 75% K + BRB 23; T17. 100% N + 100% P + 100% K+ Control; T18. 100% N + 100% P + 100% K+ BRB 3 ; T19. 100% N + 100% P + 100% K+ BRB 13 ; T20. 100% N + 100% P + 100% K+ BRB 23]

Table. 13. Effect of PGPR in combination with varying rates of NPK on soil enzyme activities

TREATMENT	ACP[a] (µmol pNP/gsoil/hr)	ALKP (µmol pNP/gsoil/hr)	AS(µmol pNP/gsoil/ hr)	βG (µmol pNP/gsoil/hr)	UR (mg N/gsoil)	DH (µmol TPF/gsoil/hr)
T1	4.760 fg	12.89 ij	1.397 e	1.780 h	2.077 f	0.437g
T2	4.777 fg	13.32 ghij	1.557 e	1.983 gh	2.497 ef	0.467fg
T3	5.083 fg	13.04 hij	1.570 e	2.147 fgh	2.917def	0.457g
T4	4.750 fg	12.32 j	1.627 e	2.403 efgh	2.450 ef	0.570defg
T5	5.173 fg	14.44 defhi	1.503 e	2.410 efgh	2.327 ef	0.520efg
T6	6.983 de	17.59 ab	1.853 de	3.687 bc	2.847def	0.590defg
T7	5.993 ef	15.20 cdef	1.857 de	3.767 bc	3.827bcd	0.647cdefg
T8	5.450 fg	16.14 bcde	2.647 bc	2.553 defgh	4.410 ab	0.490fg
T9	4.733 fg	12.96 hij	1.397 e	1.957 gh	3.103 cde	0.690cdef
T10	3.450 h	13.62 fghij	2.190 cd	2.200 fgh	3.027 def	0.687cdef
T11	5.507 fg	16.45 bcd	1.527 e	2.897 def	4.237 ab	0.510efg
T12	4.417 gh	14.95 cdefgh	2.907 b	2.650 defg	3.173 cde	0.607cdefg
T13	4.390 gh	15.49 cdef	1.410 e	3.077 cde	2.870 def	0.567defg
T14	10.57 b	14.37 efghi	2.730 b	3.203 cd	2.753 ef	0.590defg
T15	13.05 a	15.82 bcde	2.663 bc	4.303 ab	4.237 ab	0.730cde
T16	9.553 bc	14.37 efghi	2.990 ab	4.153 b	3.033 def	0.757bcd
T17	5.610 fg	14.79cdefghi	1.797 de	2.270 fgh	2.613 ef	0.580defg
T18	9.433 c	15.44 cdef	2.513 bc	4.940 a	4.550 ab	0.943ab
T19	7.497 d	16.62 bc	2.727 b	4.433 ab	3.990 bc	0.810abc
T20	7.977 d	19.12 a	3.403 a	4.260 ab	5.123 a	0.997a

[a] AcP- Acid phosphatase; AlkP-Alkaline phosphatase; AS-Aryl sulphatase; βG-β-glucosidase; UR-urease; DH-dehydrogenase; Mean values followed by the same letter are not significantly different at $P<0.05$

[T1. Normal soil + Control; T2. Normal soil + BRB 3 (*Micrococcus* luteus); T3. Normal soil + BRB 13(*Enterobacter aerogenes*); T4. Normal soil + BRB 23(*Micrococcus* sp.); T5. 75% N + 100% P + 100% K+ Control; T6. 75% N + 100% P + 100% K+ BRB 3; T7. 75% N + 100% P + 100% K+ BRB 13 ; T8. 75% N + 100% P + 100% K+ BRB 23 ; T9. 100% N + 75% P + 100% K+ Control ; T10. 100% N + 75% P + 100% K+ BRB 3; T11. 100% N + 75% P + 100% K+ BRB 13; T12. 100% N + 75% P + 100% K+ BRB 23; T13. 100% N + 100% P + 75% K + Control; T14. 100% N + 100% P + 75% K + BRB 3; T15. 100% N + 100% P + 75% K + BRB 13; T16. 100% N + 100% P + 75% K + BRB 23; T17. 100% N + 100% P + 100% K+ Control; T18. 100% N + 100% P + 100% K+ BRB 3 ; T19. 100% N + 100% P + 100% K+ BRB 13 ; T20. 100% N + 100% P + 100% K+ BRB 23]

Table 14. Effect of bacterial isolates in combination with different nutrient levels on plant growth parameters

TREATMENT	HEIGHT (cm)	NO.OF LEAVES	NO.OF NODES
T1	127.7 ab	21.67 cd	22.33 ef
T2	147.3 ab	22.33 cd	26.67 bc
T3	151.7 ab	22.67 cd	24.67 cde
T4	127.0 ab	22.00 cd	22.67 ef
T5	140.3 ab	24.67 abcd	26.00 bcd
T6	123.7 ab	31.00 abc	27.00 bc
T7	146.3 ab	24.67 abcd	25.00 cde
T8	149.0 ab	26.00 abcd	28.33 b
T9	124.0 ab	21.00 cd	22.67 ef
T10	168.3 a	22.00 cd	21.00 f
T11	123.3 ab	33.00 ab	28.67 b
T12	121.0 ab	24.33 abcd	27.00 bc
T13	81.67 b	16.00 d	16.00 g
T14	182.7 a	18.00 d	28.67 b
T15	128.0 ab	21.67 cd	21.00 f
T16	120.0 ab	23.33 bcd	35.67 a
T17	179.0 a	33.33 a	33.33 a
T18	180.0 a	25.33 abcd	26.33 bcd
T19	128.0 ab	18.33 d	26.67 bc
T20	139.3 ab	17.33 d	23.33 def
T21	147.7 ab	19.33 d	28.33 b
T22	152.0 ab	23.67 abcd	33.67 a
T23	125.7 ab	19.33 d	25.00 cde
T24	122.3 ab	22.33 cd	26.00 bcd
T25	164.3 a	24.00 abcd	33.33 a
L.S.D	25.91	2.58	2.63

Mean values followed by the same letter are not significantly different at $P<0.05$

Table 15. Effect of bacterial isolates in combination with different nutrient levels on plant growth parameters

TREATMENT	Height (cm)	No. of leaves	No. of nodes	Root length
T1	179.3	20.33	20.33	26.33
T2	201.2	31.67	33.33	29.67
T3	171.2	28.00	28.67	25.00
T4	168.0	27.00	28.67	29.33
T5	174.0	28.67	30.00	28.67
T6	259.0	45.33	48.00	27.33
T7	197.0	37.67	38.00	32.00
T8	178.2	27.67	30.33	23.33
T9	213.0	32.00	33.33	23.67
T10	243.0	43.33	46.67	28.33
T11	215.0	32.67	34.33	24.33
T12	245.3	36.33	37.33	22.67
T13	245.3	37.00	38.33	25.67
T14	216.0	35.67	37.33	23.33
T15	207.0	34.00	36.67	21.67
T16	246.0	35.33	37.00	24.00
T17	212.3	29.67	30.33	23.00
T18	217.0	42.33	43.67	29.33
T19	298.0	40.67	42.67	27.33
T20	300.0	48.00	50.00	31.33
T21	260.0	38.00	39.33	25.67
T22	264.0	42.67	42.67	23.33
T23	219.0	34.00	36.33	26.00
T24	296.0	45.00	47.33	26.00
T25	204.0	33.33	33.33	19.33
T26	221.3	33.33	34.00	22.00
T27	235.3	36.33	37.33	25.00
T28	269.0	38.00	40.00	26.00
T29	249.0	35.33	38.67	29.00
T30	270.0	38.00	39.67	28.67
T31	284.0	33.33	35.00	24.00
T32	291.0	41.00	46.33	23.00
T33	202.3	32.00	32.67	24.00
T34	249.0	41.00	41.33	19.33
T35	186.7	29.33	33.00	18.33
T36	221.0	34.33	36.00	26.33
T37	238.3	37.00	38.33	25.00
T38	251.0	40.33	45.00	25.00
T39	243.7	38.00	41.33	26.00
T40	248.3	40.00	42.67	23.67
L.S.D (0.05)	1.720	8.757	9.169	4.103

[T1-CONTROL; T2-BRB 21; T3-P 26; T4-CONSORTIUM; T5-BRB 21 + P 26; T6-BRB 21 + CONSORTIUM; T7-P 26 + CONSORTIUM; T8-BRB 21 + P 26 + CONSORTIUM; T9-CONTROL + 75% N + 100 PK; T10-BRB 21 + 75% N + 100 PK; T11-P 26 + 75% N + 100 PK; T12-CONSORTIUM + 75% N + 100 PK; T13-BRB 21 + P 26 + 75% N + 100 PK; T14-BRB 21 + CONSORTIUM + 75% N + 100 PK;T15- P 26 + CONSORTIUM + 75% N + 100 PK; T16-BRB 21 + P 26 + CONSORTIUM + 75% N + 100 PK; T17-CONTROL + 75% P + 100 NK; T18-BRB 21 + 75% P + 100 NK; T19-P 26 + 75% P + 100 NK; T20-CONSORTIUM + 75% P + 100 NK; T21-BRB 21 + P 26 + 75% P + 100 NK; T22-BRB 21 + CONSORTIUM + 75% P + 100 NK; T23-P 26 + CONSORTIUM + 75% P + 100 NK; T24-BRB 21 + P 26 + CONSORTIUM + 75% P + 100 NK;T25- CONTROL + 75% K + 100 NP; T26-BRB 21 + 75% K + 100 NP; T27-P 26+ 75% K + 100 NP; T28-CONSORTIUM+ 75% K + 100 NP; T29-BRB 21 + P 26+ 75% K + 100 NP; T30-BRB 21 + CONSORTIUM+ 75% K + 100 NP; T31-P 26 + CONSORTIUM+ 75% K + 100 NP; T32-BRB 21 + P 26 + CONSORTIUM+ 75% K + 100 NP;T33- CONTROL+ 100 NPK; T34-BRB 21+ 100 NPK;T35- P26+ 100 NPK; T36-CONSORTIUM+ 100 NPK; T37-BRB 21 + P 26+ 100 NPK; T38-BRB 21 + CONSORTIUM+ 100 NPK; T39-P 26 + CONSORTIUM+ 100 NPK; T40-BRB 21 + P 26 + CONSORTIUM+ 100 NPK]

Table 16. Effect of bacterial isolates in combination with different nutrient levels on fresh and dry weight of plants

TREATMENT	Fresh weight (g)			Dry weight (g)		
	Shoot	Leaf	Root	Shoot	Leaf	Root
T1	23.33	41.67	10.00	5.333	8.333	1.500
T2	41.67	56.67	15.00	7.000	10.00	2.667
T3	30.00	50.00	16.67	5.333	9.333	2.900
T4	40.00	63.33	18.33	6.333	11.00	2.500
T5	35.00	50.00	11.67	5.667	8.000	2.800
T6	41.67	56.67	13.33	7.667	12.00	3.333
T7	41.67	71.67	11.67	7.667	12.33	3.333
T8	38.33	61.67	11.67	5.667	11.00	3.000
T9	35.00	61.67	11.67	7.000	11.00	2.933
T10	43.33	63.33	16.67	7.667	13.33	3.733
T11	33.33	50.00	13.33	7.333	10.33	2.700
T12	31.67	53.33	15.00	6.000	11.00	2.800
T13	40.00	63.33	16.67	6.667	12.00	2.933
T14	36.67	55.00	15.00	6.000	11.33	3.000
T15	33.33	55.00	13.33	6.333	10.67	3.067
T16	38.33	51.67	18.30	7.667	11.33	3.333
T17	38.33	61.67	10.00	6.333	11.00	3.000
T18	43.33	68.33	20.00	7.667	13.00	3.633
T19	40.00	65.00	20.00	7.667	16.33	3.600
T20	45.00	71.67	23.33	8.000	15.33	3.833
T21	38.33	61.67	21.67	7.333	12.33	3.633
T22	38.33	63.33	20.00	8.000	13.00	4.000
T23	41.67	58.33	23.33	7.333	11.67	3.000
T24	46.67	80.00	16.67	8.667	17.67	3.000
T25	31.67	50.00	11.67	5.333	8.333	1.967
T26	33.33	51.67	18.33	5.000	10.67	2.133
T27	35.00	56.67	18.33	6.000	11.33	2.367
T28	35.00	65.00	18.33	6.333	11.67	2.333
T29	36.67	56.67	20.00	7.333	12.00	2.233
T30	36.67	61.67	20.00	5.667	14.67	2.033
T31	36.67	58.33	20.00	5.333	11.00	2.233
T32	40.00	63.33	20.00	6.000	11.33	1.967
T33	28.33	53.33	10.00	5.000	9.333	2.867
T34	30.00	63.33	10.00	5.667	9.333	2.833
T35	23.33	45.00	8.333	4.667	7.667	2.533
T36	26.67	53.33	10.00	4.333	8.333	2.867
T37	31.67	58.33	15.00	4.667	9.000	3.000
T38	40.00	63.33	11.67	6.667	10.67	3.733
T39	30.00	51.67	10.00	5.333	9.000	3.000
T40	33.33	55.00	11.67	6.000	10.67	3.667
L.S.D	8.022	11.39	3.782	1.423	2.922	0.6418

Table 17. Effect of bacterial isolates in combination with different nutrient levels on soil physico-chemical parameters, organic C, dissolved organic C and N

TREATMENT	pH	O.C (%)	DOC(g/kg)	DON(mg/kg)
1	5.553	2.033	0.5667	16.57
2	5.797	2.333	0.3967	21.33
3	5.773	2.600	1.470	18.37
4	5.763	3.100	1.040	32.17
5	5.890	3.100	0.8933	30.73
6	5.960	2.900	1.420	26.63
7	6.067	2.800	1.140	21.00
8	5.967	2.833	0.9500	15.53
9	5.320	1.933	1.533	21.33
10	4.787	2.100	1.623	22.47
11	4.803	2.400	1.370	37.90
12	5.310	4.000	1.243	22.50
13	5.333	3.100	1.133	28.33
14	5.220	3.500	1.113	22.47
15	5.090	3.400	1.420	14.67
16	5.120	2.900	1.257	28.00
17	5.150	2.033	1.183	29.30
18	4.990	2.233	1.030	19.50
19	4.940	2.433	1.237	46.27
20	4.987	3.200	0.9100	30.83
21	5.103	2.933	1.150	48.97
22	5.197	3.533	0.9400	54.63
23	4.880	3.667	0.7967	40.63
24	5.213	4.033	1.193	46.37
25	5.117	2.100	1.097	30.83
26	4.927	2.300	1.423	39.37
27	4.590	2.600	1.240	71.50
28	5.253	2.533	1.697	19.70
29	5.133	2.733	1.367	54.70
30	5.050	2.933	1.773	58.83
31	4.850	3.100	1.217	36.53
32	4.753	3.600	1.093	44.83
33	5.037	1.800	0.7967	39.37
34	5.033	3.200	1.297	64.53
35	4.693	3.533	1.437	125.3
36	5.170	3.800	0.9900	29.57
37	5.060	3.133	1.633	36.47
38	5.640	3.000	1.497	48.67
39	4.867	2.933	1.487	96.67
40	4.913	2.900	1.513	60.30
L.S.D (0.05)	0.0514	0.1259	0.0514	0.4847

T1-CONTROL; T2-BRB 21; T3-P 26; T4-CONSORTIUM; T5-BRB 21 + P 26; T6-BRB 21 + CONSORTIUM; T7-P 26 + CONSORTIUM; T8-BRB 21 + P 26 + CONSORTIUM; T9-CONTROL + 75% N + 100 PK; T10-BRB 21 + 75% N + 100 PK; T11-P 26 + 75% N + 100 PK; T12-CONSORTIUM + 75% N + 100 PK; T13-BRB 21 + P 26 + 75% N + 100 PK; T14-BRB 21 + CONSORTIUM + 75% N + 100 PK;T15- P 26 + CONSORTIUM + 75% N + 100 PK; T16-BRB 21 + P 26 + CONSORTIUM + 75% N + 100 PK; T17-CONTROL + 75% P + 100 NK; T18-BRB 21 + 75% P + 100 NK; T19-P 26 + 75% P + 100 NK; T20-CONSORTIUM + 75% P + 100 NK; T21-BRB 21 + P 26 + 75% P + 100 NK; T22-BRB 21 + CONSORTIUM + 75% P + 100 NK; T23-P 26 + CONSORTIUM + 75% P + 100 NK; T24-BRB 21 + P 26 + CONSORTIUM + 75% P + 100 NK;T25- CONTROL + 75% K + 100 NP; T26-BRB 21 + 75% K + 100 NP; T27-P 26+ 75% K + 100 NP; T28-CONSORTIUM+ 75% K + 100 NP; T29-BRB 21 + P 26+ 75% K + 100 NP; T30-BRB 21 + CONSORTIUM+ 75% K + 100 NP; T31-P 26 + CONSORTIUM+ 75% K + 100 NP; T32-BRB 21 + P 26 + CONSORTIUM+ 75% K + 100 NP;T33- CONTROL+ 100 NPK; T34-BRB 21+ 100 NPK;T35- P26+ 100 NPK; T36-CONSORTIUM+ 100 NPK; T37-BRB 21 + P 26+ 100 NPK; T38-BRB 21 + CONSORTIUM+ 100 NPK; T39-P 26 + CONSORTIUM+ 100 NPK; T40-BRB 21 + P 26 + CONSORTIUM+ 100 NPK]

Table 18. Effect of bacterial isolates in combination with different nutrient levels on soil major nutrients in soil and plant

TREATMENT	SOIL NUTRIENTS			PLANT NUTRIENTS		
	N(mg/kg)	P(mg/kg)	K(mg/kg)	N (%)	P(%)	K(%)
1	37.00	1.183	297.7	3.040	0.063	3.733
2	42.00	1.077	311.3	3.133	0.390	3.670
3	43.00	0.6300	257.0	3.037	0.350	3.510
4	72.33	0.8133	351.0	3.400	0.470	3.870
5	51.00	0.9633	305.3	2.500	0.280	3.343
6	38.33	0.9667	310.3	2.890	0.350	3.420
7	68.33	0.4667	318.0	3.087	0.353	3.593
8	52.00	0.9767	312.3	3.250	0.370	3.447
9	39.33	1.313	296.0	2.650	0.333	3.513
10	44.00	1.693	324.7	2.750	0.353	3.543
11	45.33	0.8900	404.0	3.540	0.360	4.527
12	98.00	1.213	445.0	3.640	0.687	4.467
13	70.00	1.283	354.0	3.533	0.643	4.337
14	71.33	0.6700	349.3	3.800	0.773	4.680
15	92.00	1.310	445.7	3.033	0.570	4.150
16	70.33	0.7400	353.3	3.380	0.653	4.227
17	38.00	0.7367	310.0	3.583	0.653	4.393
18	72.33	0.9000	344.3	3.753	0.657	4.250
19	73.33	1.597	443.3	3.183	0.560	4.350
20	95.33	1.450	465.7	3.253	0.990	4.350
21	76.00	1.363	358.0	4.040	0.630	4.333
22	78.33	1.427	369.3	4.140	0.643	4.263
23	93.33	1.347	457.3	4.037	0.637	4.113
24	81.33	1.097	371.3	4.367	0.640	4.480
25	40.33	0.9800	321.0	3.600	0.653	4.070
26	82.33	0.8933	353.7	3.827	0.940	4.027
27	83.33	0.9000	359.3	4.083	0.983	4.193
28	98.00	1.517	513.7	4.260	0.973	4.050
29	76.33	0.8400	466.7	3.660	0.647	4.180
30	89.33	1.360	472.0	3.763	0.727	4.163
31	95.00	0.8400	501.3	4.533	0.740	4.163
32	78.67	1.053	528.0	4.827	0.757	5.033
33	43.33	1.437	352.0	4.533	0.530	4.917
34	59.33	1.157	422.3	4.333	0.670	4.680

35	61.00	1.670	448.7	4.200	0.620	4.760
36	32.33	1.320	495.7	4.373	0.673	4.817
37	73.33	1.473	412.0	4.537	0.537	4.967
38	74.67	1.667	422.0	4.167	0.583	4.840
39	94.33	1.843	487.3	4.253	0.573	4.917
40	89.00	0.8867	490.7	4.650	0.593	4.943
L.S.D (0.05)	1.267	0.2517	15.58	0.1028	0.0163	0.0514

[T1-CONTROL; T2-BRB 21; T3-P 26; T4-CONSORTIUM; T5-BRB 21 + P 26; T6-BRB 21 + CONSORTIUM; T7-P 26 + CONSORTIUM; T8-BRB 21 + P 26 + CONSORTIUM; T9-CONTROL + 75% N + 100 PK; T10-BRB 21 + 75% N + 100 PK; T11-P 26 + 75% N + 100 PK; T12-CONSORTIUM + 75% N + 100 PK; T13-BRB 21 + P 26 + 75% N + 100 PK; T14-BRB 21 + CONSORTIUM + 75% N + 100 PK;T15- P 26 + CONSORTIUM + 75% N + 100 PK; T16-BRB 21 + P 26 + CONSORTIUM + 75% N + 100 PK; T17-CONTROL + 75% P + 100 NK; T18-BRB 21 + 75% P + 100 NK; T19-P 26 + 75% P + 100 NK; T20-CONSORTIUM + 75% P + 100 NK; T21-BRB 21 + P 26 + 75% P + 100 NK; T22-BRB 21 + CONSORTIUM + 75% P + 100 NK; T23-P 26 + CONSORTIUM + 75% P + 100 NK; T24-BRB 21 + P 26 + CONSORTIUM + 75% P + 100 NK;T25- CONTROL + 75% K + 100 NP; T26-BRB 21 + 75% K + 100 NP; T27-P 26+ 75% K + 100 NP; T28-CONSORTIUM+ 75% K + 100 NP; T29-BRB 21 + P 26+ 75% K + 100 NP; T30-BRB 21 + CONSORTIUM+ 75% K + 100 NP; T31-P 26 + CONSORTIUM+ 75% K + 100 NP; T32-BRB 21 + P 26 + CONSORTIUM+ 75% K + 100 NP;T33- CONTROL+ 100 NPK; T34-BRB 21+ 100 NPK;T35- P26+ 100 NPK; T36-CONSORTIUM+ 100 NPK; T37-BRB 21 + P 26+ 100 NPK; T38-BRB 21 + CONSORTIUM+ 100 NPK; T39-P 26 + CONSORTIUM+ 100 NPK; T40-BRB 21 + P 26 + CONSORTIUM+ 100 NPK]

Table 19. Effect of bacterial isolates in combination with different nutrient levels on soil enzyme activities

Treatment	AlkP[a] (µmol PNP/g/hr)	AcP(µmol PNP/g/hr)	βG(µmol PNG/g/hr)	AS(µmol PNP/g/hr)	UR(mg N/g soil)
1	9.867	3.133	0.8800	5.230	3.270
2	10.33	4.897	0.9733	6.720	3.887
3	10.27	4.500	1.027	5.517	3.320
4	10.57	5.927	0.9633	6.547	4.443
5	10.33	5.227	0.8700	6.947	3.743
6	10.47	5.400	0.7033	6.430	4.230
7	10.23	4.753	0.9300	6.510	3.393
8	10.50	6.500	0.9400	7.400	3.183
9	9.900	5.333	0.6133	6.990	2.660
10	10.90	4.600	0.3700	8.947	3.530
11	11.00	3.667	0.2933	8.917	2.130
12	11.03	5.467	0.4133	9.257	2.763
13	11.20	4.600	0.5633	10.52	2.450
14	11.40	4.563	0.7033	9.263	2.797
15	11.53	4.217	0.5133	10.06	2.797
16	12.30	3.857	0.3733	9.227	2.343
17	11.03	5.140	0.4933	7.623	3.387
18	12.70	5.647	0.3933	7.410	3.463
19	12.43	3.883	0.3700	8.833	4.477
20	13.35	5.087	0.4500	9.320	2.450
21	12.80	5.327	0.4433	6.773	3.530
22	12.73	5.433	0.5300	6.477	3.480
23	12.43	4.297	0.3933	6.377	1.990
24	12.93	6.420	0.6433	6.710	2.343
25	11.40	3.760	0.5433	7.673	2.790
26	12.57	4.673	0.4233	6.513	2.560
27	13.40	3.720	0.3767	6.887	3.067
28	16.40	4.320	0.4067	8.843	2.343
29	12.80	4.427	0.4433	7.817	2.443
30	12.80	4.170	0.5433	6.787	3.037
31	12.53	5.327	0.4433	7.813	3.880
32	16.37	5.360	0.3400	7.233	3.387
33	12.93	5.247	0.5300	7.970	3.530
34	15.53	5.087	0.4500	6.803	3.060
35	14.60	3.830	0.2933	8.780	4.720
36	15.67	4.293	0.3633	9.257	3.530
37	16.27	6.737	0.4133	8.950	3.530
38	14.70	5.383	1.163	8.910	2.343

39	13.87	6.080	0.7100	8.520	3.037
40	16.63	4.617	0.4233	12.52	2.693
L.S.D(0.05)	0.2056	0.1149	0.0514	0.1359	0.0727

[a]AlkP-Alkaline phosphatase; AcP- Acid phosphatase; βG-β-glucosidase; AS-Aryl sulphatase; UR-urease

[T1-CONTROL; T2-BRB 21; T3-P 26; T4-CONSORTIUM; T5-BRB 21 + P 26; T6-BRB 21 + CONSORTIUM; T7-P 26 + CONSORTIUM; T8-BRB 21 + P 26 + CONSORTIUM; T9-CONTROL + 75% N + 100 PK; T10-BRB 21 + 75% N + 100 PK; T11-P 26 + 75% N + 100 PK; T12-CONSORTIUM + 75% N + 100 PK; T13-BRB 21 + P 26 + 75% N + 100 PK; T14-BRB 21 + CONSORTIUM + 75% N + 100 PK;T15- P 26 + CONSORTIUM + 75% N + 100 PK; T16-BRB 21 + P 26 + CONSORTIUM + 75% N + 100 PK; T17-CONTROL + 75% P + 100 NK; T18-BRB 21 + 75% P + 100 NK; T19-P 26 + 75% P + 100 NK; T20-CONSORTIUM + 75% P + 100 NK; T21-BRB 21 + P 26 + 75% P + 100 NK; T22-BRB 21 + CONSORTIUM + 75% P + 100 NK; T23-P 26 + CONSORTIUM + 75% P + 100 NK; T24-BRB 21 + P 26 + CONSORTIUM + 75% P + 100 NK;T25- CONTROL + 75% K + 100 NP; T26-BRB 21 + 75% K + 100 NP; T27-P 26+ 75% K + 100 NP; T28-CONSORTIUM+ 75% K + 100 NP; T29-BRB 21 + P 26+ 75% K + 100 NP; T30-BRB 21 + CONSORTIUM+ 75% K + 100 NP; T31-P 26 + CONSORTIUM+ 75% K + 100 NP; T32-BRB 21 + P 26 + CONSORTIUM+ 75% K + 100 NP;T33- CONTROL+ 100 NPK; T34-BRB 21+ 100 NPK;T35- P26+ 100 NPK; T36-CONSORTIUM+ 100 NPK; T37-BRB 21 + P 26+ 100 NPK; T38-BRB 21 + CONSORTIUM+ 100 NPK; T39-P 26 + CONSORTIUM+ 100 NPK; T40-BRB 21 + P 26 + CONSORTIUM+ 100 NPK]

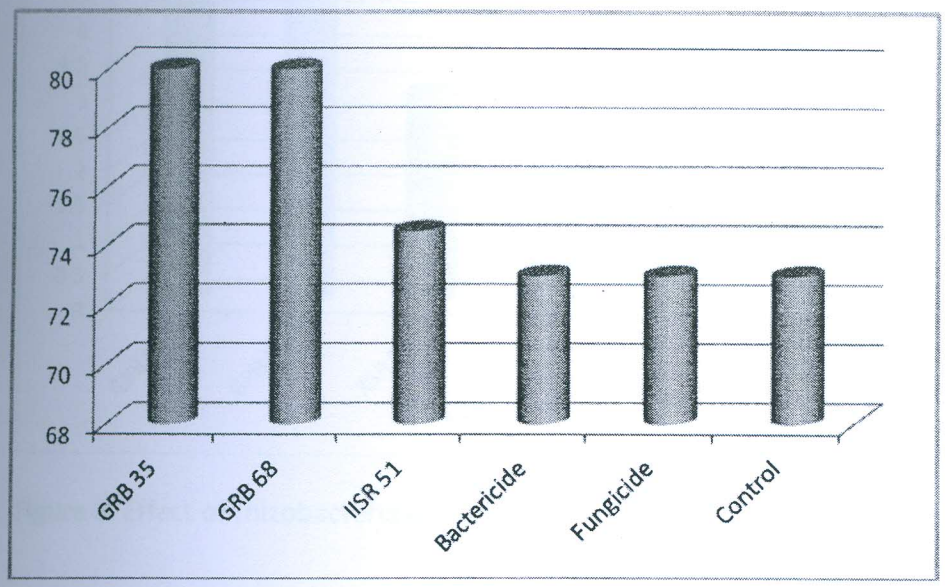


Figure 1. Effect of rhizobacteria on sprouting (%) in ginger

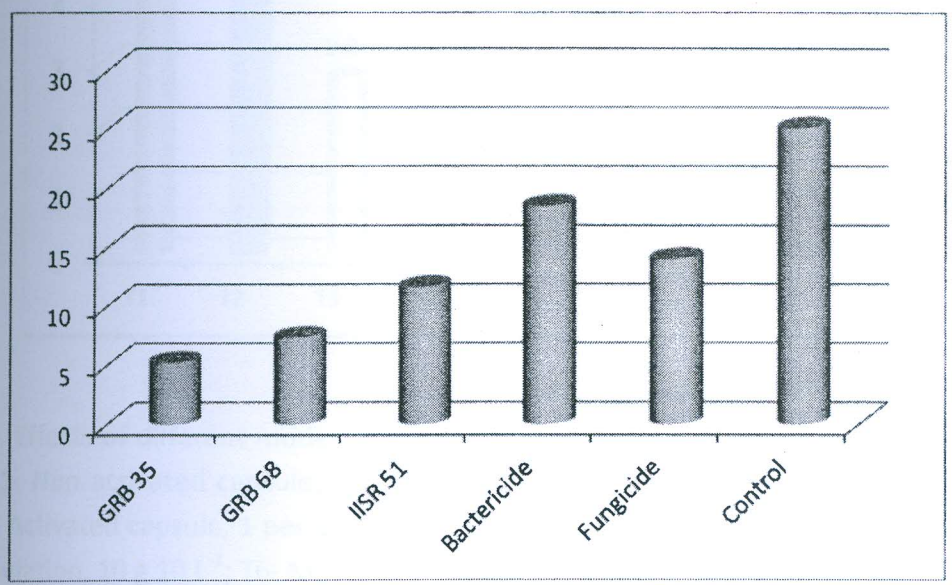


Figure 2. Effect of rhizobacteria on soft rot disease (%) in ginger

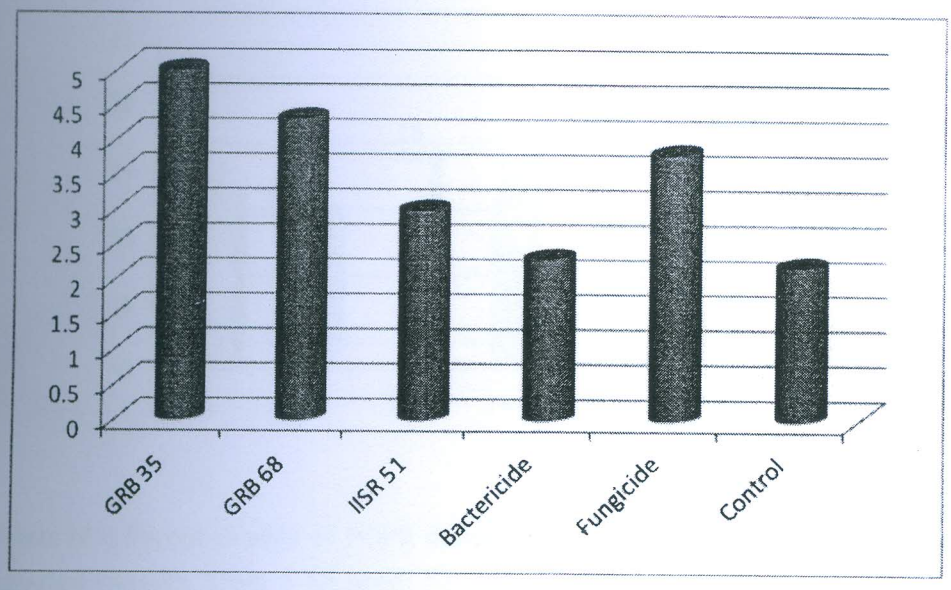


Figure 3. Effect of rhizobacteria on yield (kg bed^{-1} of 3 x 1 m^2) of ginger

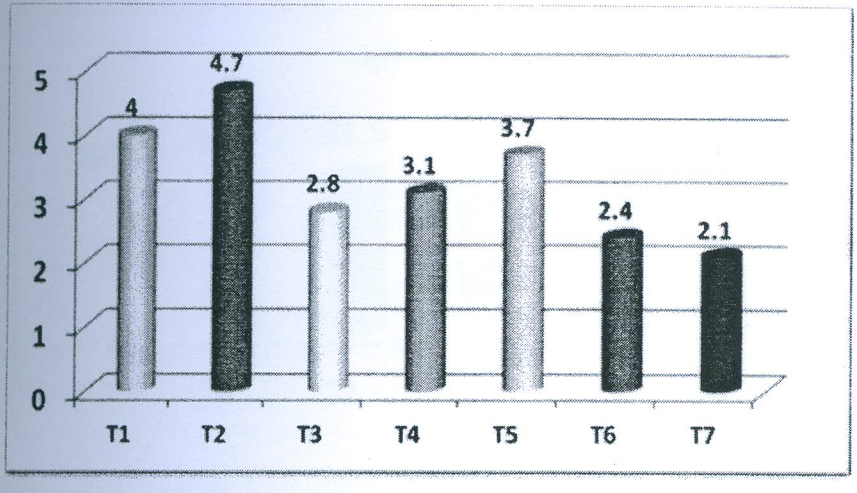


Figure 4. Effects of different modes of PGPR delivery on yield (kg bed^{-1} of 3 x 1 m^2) of ginger (T1- Non activated capsule, 1 per 5 kg seed; T2- Activated capsule, 2 per 5 kg seed; T3- Activated capsule, 1 per 10 kg seed; T4- Activated capsule, 1per 5kg seed; T5- Talc formulation, 10 g 10 L^{-1}; T6- Metalaxyl- Mancozeb, 1.25g L^{-1}; T7- Absolute control)

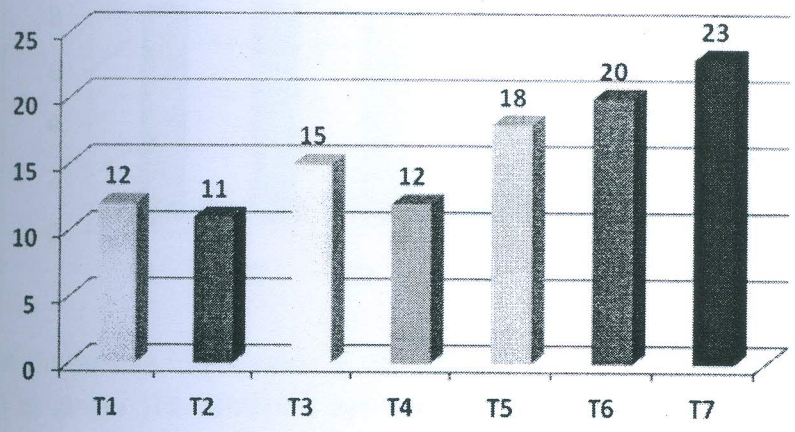


Figure 5. Effects of different modes of PGPR delivery on soft rot incidence (%) in ginger (T1- Non activated capsule, 1 per 5 kg seed; T2- Activated capsule, 2 per 5 kg seed; T3- Activated capsule, 1 per 10 kg seed; T4- Activated capsule, 1per 5kg seed; T5- Talc formulation, 10 g 10 L-1; T6- Metalaxyl- Mancozeb, 1.25g L-1; T7- Absolute control)

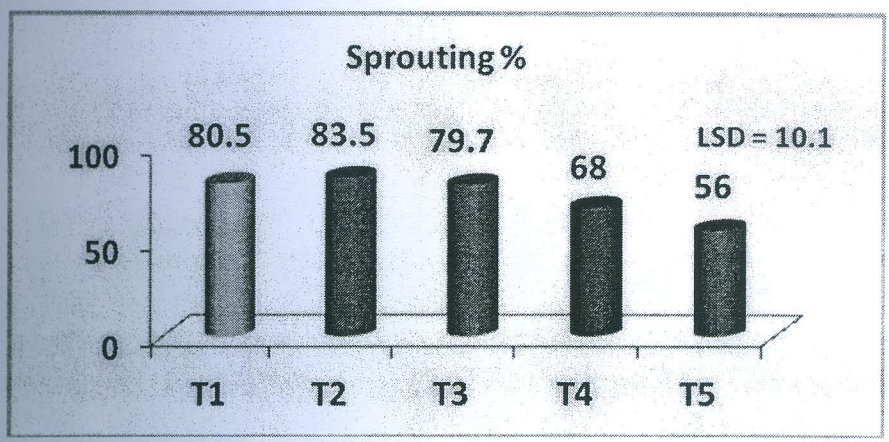


Figure 6. Efficacy of Biocapsule formulation of PGPR strain IISR GRB35 for enhancing sprouting in ginger (T1- GRB35 – cell suspension; T2- Biocapsule (single dose); T3- Biocapsule (Double dose); T4- Fungicide (Metalaxyl-Mancozeb); T5- Control -without PGPR)

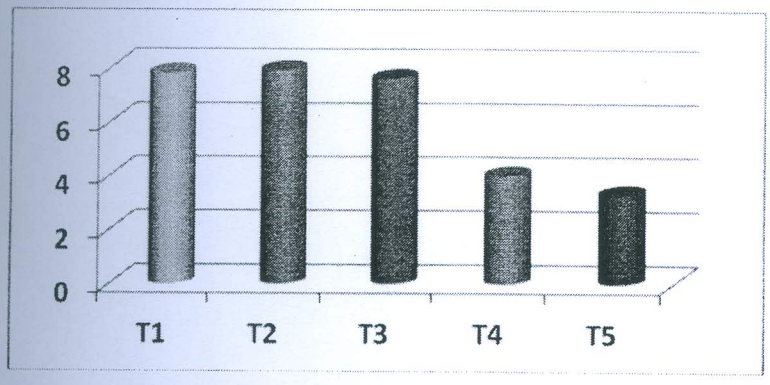
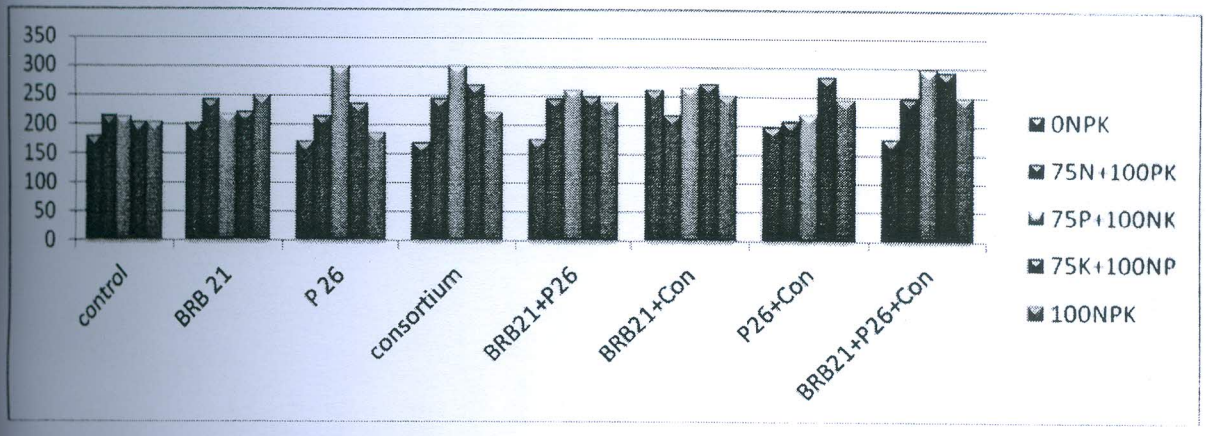
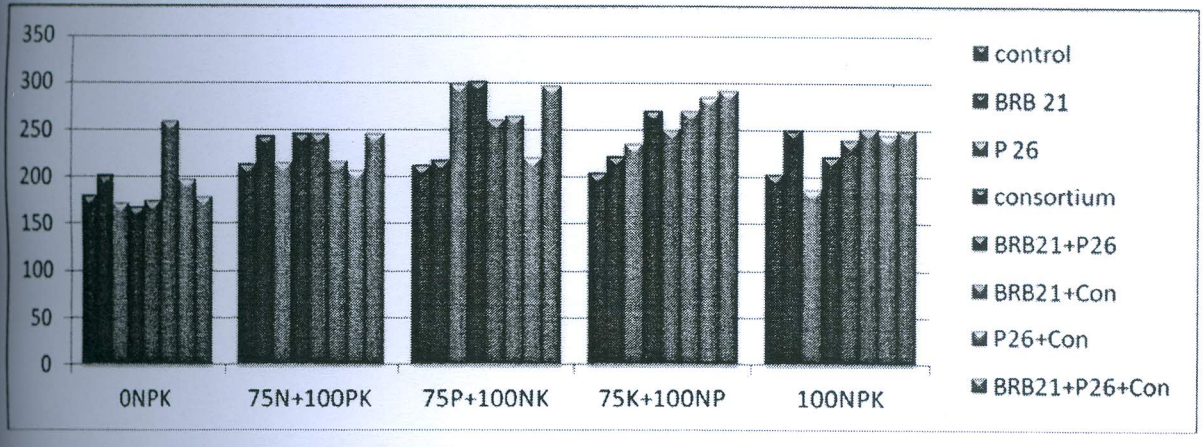


Figure 7. Efficacy of Biocapsule formulation of PGPR strain IISR GRB35 for enhancing yield ((kg bed^{-1} of 3 x 1 m^2) in ginger (T1- GRB35 – cell suspension, T2- Biocapsule (single dose), T3- Biocapsule (Double dose), T4- Fungicide (Metalaxy mancozeb)





Figures 8 & 9. Average height of the plants at 150 DAP