

## Development of Microbial Inoculants and the Impact of Soil Application on Rice Seedlings Growth

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**Abstract: Problem statement:** The present study was undertaken to assess the Empty Fruit Bunches (EFB) as carrier of Biofertilizer and microbial inoculants interaction. A study in Malaysia had shown a strain of *Trichoderma viride* and *Bacillus megaterium* were isolated from the soil. Questions were raised whether the two inoculants compatible to be mixed in same carrier? It is important to develop delivery system of the inoculants. The present study was undertaken to assess the Empty Fruit Bunches (EFB) as carrier of biofertilizers and microbial inoculants interaction and the impact on rice seedlings. **Approach:** *Bacillus megaterium* and *Trichoderma viride* were grown in their respective broth culture. Cells numbers in broth or suspension were determined. EFB powder 200 g in polypropylene bags was sterilized. Inoculums from broth of suspension were added in EFB using sterilized syringe, moisture level in each packet was maintained by adding respective broth, 1 g was periodically withdraw from fomenters, soaked in 10 mL sterilized distilled water, then serially diluted and plated out, the number of viable cells was expressed as cfu g<sup>-1</sup> dry root. Twenty one-day-old rice seedlings of MR220 cultivar were grown under greenhouse conditions in autoclaved homogeneous soil, rice seedlings were treated with *Trichoderma* and *Bacillus*. Five replicates were used for each treatment. Rice seedlings height (cm), root length (cm), root numbers, root and shoot dry matter, parameters were measured during and after experiment. All data were analyzed at an alpha level of 0.05 or 0.10 using experimental design by completely randomized design model using the analysis of variance module (treatments × 3 R) all treatment means were separated using Fisher's protected least significant difference (LSD) mean separation. **Results:** *Trichoderma* growth in carrier reduces *Bacillus* growth as interaction effect of both in same carrier which indicated that *Bacillus* unable to develop on multi-inoculants biofertilizers with *Trichoderma*. Inoculation of rice seedlings with *Bacillus megaterium* and *Trichoderma viride* produced maximum shoots and roots dry matter, increased significantly root length numbers and seedlings height comparing with control and same action of NPK fertilizers. **Conclusion:** The present study showed the possibility to develop the delivery system of biofertilizers which contained *Bacillus* and *Trichoderma* as mono inoculums not as multi-inoculants due to incompatible of both in same carrier. *Bacillus* and *Trichoderma* also showed positive effect with rice seedlings.

**Key words:** Microbial inoculants, carrier, *Bacillus*, *Trichoderma*, empty fruit bunches

### INTRODUCTION

Microorganisms have long been exploited as inoculants for plants and foodstuffs. Currently, there are numerous inoculants in the market, not only for N<sub>2</sub> fixation and nutrient release, but also for biocontrols and bioremediations and are being produced almost all over the continents. Microbial interactions play very important roles in sustainable agriculture through the integrated nutrient supply<sup>[6]</sup>. It is important to lay major

emphasis on the application of efficient microbial inoculants<sup>[4]</sup>.

Various commercial mono-culture inoculants being produced in India by various government/semi government or private agencies include: *A. rhizobia* and *B. rhizobia*, *Azospirillum*, *Azotobacter*, *Bacillus megaterium*, *Bacillus polymyxa*, mycorrhizal inoculants for forest trees and *Pseudomonas* as Plant Growth Promoting Rhizobacteria (PGPR) and bio control agent.

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Application of efficient inoculants of Rhizobium, Mesorhizobium and Bradyrhizobium<sup>[3]</sup>; Azospirillum; Azotobacter, Pseudomonas and phosphate solubilizing microorganisms<sup>[9]</sup> for the improvement of crop productivity and plant nutrition is well documented. Beneficial interactions of these biofertilizers in both legumes and cereals have been reported<sup>[8]</sup>. *Trichoderma* spp. Have been used for many years as antagonists in the biological control of many fungal plant diseases<sup>[10]</sup>. *Trichoderma* spp. have been shown to be effective for the control of both brown spot and blast diseases on rice<sup>[5]</sup>.

It is important to develop delivery system of the inoculants. The present study was undertaken to assess the Empty Fruit Bunches (EFB) as carrier of biofertilizers and microbial inoculants interaction and the impact on rice seedlings.

## MATERIALS AND METHODS

An isolate of *Trichoderma viride* and *Bacillus megaterium* was obtained from soil samples analysis in fermentation technology lab BioSciences and BioTechnologyschool (UKM).

Rice grains (MR220) were obtained from glass houses UKM.

**Interaction of microbes in EFB carrier:** *Bacillus megaterium* and *Trichoderma viride* were grown in their respective broth culture. Cells numbers in broth pr suspension was determined.

EFB powder 200 g in polypropylene bags was sterilized. inoculum from broth pr suspension was added in EFB using sterilized syringe, moisture level in each packet was maintained by adding respective broth.

About 1 g was periodically withdraw from fomoters, soaked in 10 mL sterilized distal water, then serially diluted and plated out, the number of viable cells was expressed as cfu g<sup>-1</sup> dry root.

**Interaction of microbes with rice seedlings:** Twenty one-day-old rice seedlings of MR220 cultivar were grown under greenhouse conditions in autoclaved homogeneous soil, rice seedlings were treated with *Trichoderma* and *Bacillus*. Five replicates were used for each treatment.

**Plant parameters:** Rice seedlings height (cm), root length (cm), root numbers, root and shoot dray matter, parameters were measured during and after experiment.

**Statistical analyses:** All data were analyzed at an alpha level of 0.05 or 0.10 using experimental design by completely randomized design model using the analysis of variance module (treatments × 3 R) all treatment means were separated using Fisher's protected Least Significant Difference (LSD) mean separation.

## RESULTS

*Bacillus megaterium* and *Trichoderma viride* were being used as monoculture inoculants and being used as biofertilizers with rice seedlings.

The possibility of delivery system and interaction of these tow microbes was also explored.

**Interaction of microbes in EFB carrier:** The interaction of these microbes was studied in EFB carrier, which provides a convenient base to facilitate packaging and application of microbial inoculants. To develop a multi-inoculation biofertilizers, *Bacillus* and *Trichoderma* were grown separately and 20 mL of broth of each inoculant in 200 g of EFB. Microbes in different combination were used. In the monoculture, *Bacillus* count was 8.5 log g<sup>-1</sup> carrier, *Trichoderma* was 9.0 log g<sup>-1</sup> carrier (Table 1). When both of microbial inoculants were mixed together; invariably the counts were more that 2 \* 10<sup>8</sup> cells g<sup>-1</sup> carrier differences in the cell numbers were statically no significant.

The EFB powder-based packets of *Bacillus* initially contained 8.5 \* 10<sup>10</sup> and *Trichoderma* initially contained 9\*10<sup>10</sup> but up 30 days, the number increased to 9.4 and 9.9 for *Bacillus* and *Trichoderma* respectively (Fig. 1). Co culture of *Bacillus* with *Trichoderma* affect the number of cells of *Bacillus* which decreased to 7.0 after 15 days and to 5.0 after 30 days, however this number was more than 2 \* 10<sup>8</sup> cells g<sup>-1</sup> carrier, that may indicated non-compatible of mixed both inoculants at same carrier.

Table 1 interaction of EFB-Based inoculants microbes in carriers using 20 mL of broth inoculums.

Table 1: Log number of CFU

Microbial Inoculants	<i>Bacillus</i> 0	<i>Bacillus</i> 7	<i>Trichoderma</i> 0	<i>Trichoderma</i> 7
<i>Bacillus</i>	9.0	8.6	-	-
<i>Trichoderma</i>	-	-	10.0	9.2
<i>Bacillus</i> + <i>Trichoderma</i>	8.0	6.5	10.0	7.5

CD at 0.05; Microbial Inoculants = NS; Interaction = S; Combinations = S; Day = NS; Initial CFU in broth culture m L<sup>-1</sup>: *Bacillus* 8.5 *Trichoderma* 9.0

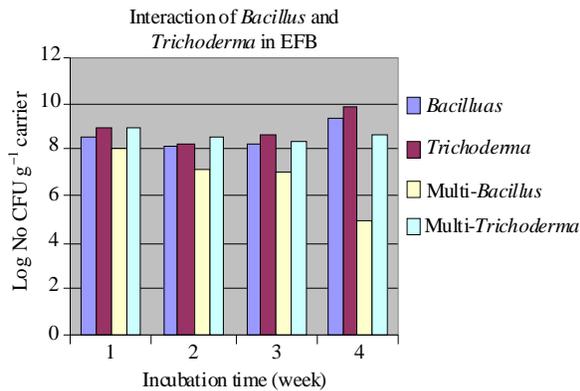


Fig. 1: Interaction of inoculants microbes in EFB

Table 2: Effect of indigenous beneficial microorganisms on rice seedlings growth

Treatment	Root length (cm)	Root Num	Seedlings height (cm)	Shoot DM g 100 g <sup>-1</sup>	Root DM g 100 g <sup>-1</sup>
Control	8.5b	4.8b	10.0b	1.3b	0.7b
NPK	10.5a	5.8a	15.0a	1.8a	0.9a
Trichoderma	11.0a	6.0a	15.5a	1.8a	1.0a
Bacillus	10.7a	6.0a	15.2a	1.6a	0.9a

**Interaction of microbes with rice seedlings:** All treatments effect significantly compare with control increasing root length and numbers, seedlings height, shoot dry matter and root dry matter for more than 20% in all parameters (Table 2) also comparing with the effect of NPK treatment which means that these treatments can be considered same chemicals effective on rice seedlings growth. The percentage of treatments difference from control was positive effect (23.5-29.4%) for root length, (20.8-25.5) root number, (50-55%) seedlings height, (23.1-38.1%) shoot dry matter, (28.6-42.9%) root dry matter (Table 2).

### DISCUSSION

It is important to develop delivery system of the inoculants. The present study was undertaken to assess the Empty Fruit Bunches (EFB) as carrier of biofertilizers and microbial inoculants interaction and the impact on rice seedlings.

In different combinations, determination of cell number showed that co-cultured multiple inoculants of Azospirillum, Azotobacter, Bacillus and Pseudomonas with more than  $2 \times 10^8$  cells g L<sup>-1</sup> of carrier was possible<sup>[8]</sup>. Similarly Apte and Iswaran<sup>[1]</sup> prepared the cultures of Rhizobium with those of *Beijerinckia* and *Azotobacter* sp.; however the ultimate counts after incubation were not determined<sup>[1]</sup>. Rice *et al.*<sup>[7]</sup> also developed a co-cultured inoculant of Rhizobium and Penicillium with good viable counts.

### CONCLUSION

The present study shows the possibility to develop the delivery system of Biofertilizer which contained *Bacillus* and *Trichoderma* as mono inoculants not as multi-inoculants due to incompatible of both in same carrier and that need more studies in granulation technology to produce multi inoculants as next experiments of this project. *Bacillus* and *Trichoderma* also showed appositve effect with rice seedlings which also increase the important for development the delivery system and granulation of those inoculants.

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