Comparison of Different Delivery System of Trichoderma and Bacillus as Biofertilizer.

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ABSTRACT

The formulation study states that it can be also promoted in liquid formulation which has higher shelf life and stress protection. However in-depth study is required for the commercialization. According to the previous testes the comparison was made between storage at 4 °C, 30 °C, paraffin oil and alginate methods. The study showed that application of alginate formulation and paraffin oil increases the shelf life of Trichoderma, & Bacillus which was used as a biofungicide comparing to the solid formulation of biofungicide used, Application of granulation & alginate formulation of biocontrol agents in the field helped the farmers in promising better yield.

Key words: Trichoderma, Bacillus, formulation, storage at 4 °C, 30 °C, paraffin oil and alginate methods

Introduction

As the agriculture industry becomes more aware of the need to adopt more environmental-friendly approach in providing plant nutrition and sustaining soil fertility, in tandem with increased productivity, bio-fertilizers have a substantial role in it. Biofertilizer products are the most suitable for the small farmers, the large plantation, forestry and recreational industries will be the ones to meet their requirements and expectations. In Malaysia, mycorrhizal products are perceived to be more versatile than the others, and can be tailor-made to address particular problems. This includes the use of mycorrhizal inocula on landscape plants [2]. Currently there are several companies producing mycorrhizal products, through locally developed technologies. Many researches, since the early 1950’s have been conducted on the effectiveness of mycorrhizal inocula on plantation, horticultural and food crops, pastures and lawn, as well as forest trees. The substrates for production of mycorrhizal inocula are easily available, using the following natural resources: sand from ex-mining lands, ground cocoa shells, sweet potato tops, rice husks and rice straw. Generally, the appeal of the mycorrhizal inocula in the Malaysian market is rising. Unless there are other potential uses of the rhizobial inocula, the market for such is limited in Malaysia. This is because no grain legume production is going on in the country and the acreage of rubber and oil palm is slowly decreasing. However, there is potential for use of Azorhizobium inoculum on some N-fixing agroforestry or green manure species including Sesbania rostrata [5].

Biofertilizer products with some potential markets in Malaysia will be based on Azospirillum, the associative nitrogen-fixing bacteria. Research has been initiated in University Putra Malaysia to evaluate the contribution of nitrogen from Azospirillum to oil palm seedlings. Shamsuddin [6]
reported through $^{15}$N-labelling technique, it was measured that up to 89% of the nitrogen requirement of oil palm plantlets inoculated with *Azospirillum* and grown in a liquid media arising from the bacteria. The bacteria probably has great potential in rice production too, as reported for maize, kollar grass, sorghum, millet and sweet potato [6].

Research during the previous two decades has led to the possibility of biological control as an increasingly realistic option for rice disease management. *Trichoderma spp.* has been used for many years as antagonists in the biological control of many fungal plant diseases [7]. *Trichoderma spp.* has been shown to be effective for the control of both brown spot and blast diseases on rice [3]. Pre-sprayed rice plants with spore suspension of *T. harzianum* or *T. viride* at the rate of 5 x $10^5$ and 6 x$10^5$ spore ml$^{-1}$, respectively, showed a significant reduction in the severity of both diseases under greenhouse conditions.

High viability of the bacterial antagonist (*Bacillus megaterium*) on leaf sheath and leaf blade at day 7 after spraying with the formulation was observed (approximately 10$^6$ c.f.u g$^{-1}$ of plant). In a small pilot field study, an aqueous solution of the formulation (3% w/v) applied by spraying at days 1, 5 and 10 after pathogen inoculation of the rice plants was more effective in suppressing rice sheath blight disease than one application of a fungicide (Ipodione) at day 1. Additionally, rice plants sprayed with the aqueous solution of the granule formulation had higher panicle and whole kernel weights than those of fungicide-treated and control (untreated) plants [1].

**Methods and materials**

**Storage at 4 °C, 30 °C temperature:**

The experiment was conducted in the laboratory of Fermentation Technology, School Of BioSciences and BioTechnology, Universiti Kebangsaan Malaysia, with *Trichoderma* & *Bacillus* to determine their shelf-life under different storage temperature 30°C & 4°C on composed oil palm empty fruit bunches EFB. The EFB used for colonization/multiplication of the tow strains, was kept into the polyethylene bag at 150g per bag and sterilized. Strains mass culturing were performed as described by Islam et al. (2002). The inoculated bags (4 x $10^8$ cfu /g for both *Trichoderma* & *Bacillus* were placed at 30°C and 4°C. Data were taken as colony forming unit (cfu/g) of the *Trichoderma* & *Bacillus* colonized EFB (1g mixed with 100 ml sterilized water) up to 210 days after inoculation with 30 days interval. Colony forming unit was counted using four Petri plates for each treatment containing Potato Dextrose Agar & Nutrient Agar media [4].

**Oil Based Formulation of Trichoderma & Bacillus:**

The shelf life study reveal that the survibility of *Trichoderma* & *Bacillus* in paraffin oil was better than other formulation with 4 x $10^8$ Cfu/ml.

**Granulation and Alginate Formulation:**

Dry granules containing the endospores were prepared by dry-granulation using the mixture of each 18 ml bacterial endospores suspension, and 18 ml fungal endospores with 85 g lactose monohydrate, 5 g PVP K-30 and 10 g kaolin or composted EFB. All ingredients were mixed in a planetary mixer (KitchenAid, USA) until they became a damp mass. This mass was passed through a granulator (sieve No.14) and dried in a hot air oven at 60°C for 2 h. The dried granules were screened through another sieve No.16. Dry granules without Inoculums endospores were prepared in an identical way and referred to as ‘control’ granules. [4].

**Results and Discussion**

The shelf life study reveal that the survibility of *Trichoderma viride* & *Bacillus megaterium* in alginate formulation with 1.2 x $10^8$ and 7.2 x $10^8$ Cfu/ml comparing to paraffin oil which was better than other formulation with 1 x $10^8$ and 0.6 x $10^8$ Cfu/ml followed by storage at 4°C with EFB with 1.8 x $10^8$ and 2.0 x $10^8$ Cfu/ml at 210th day for *Trichoderma* and *Bacillus* respectively(Table 1&.2).

There was a steep declain of population level in all formulation . In all the formulation, sedimentation or buoyancy was recorded. The studies about the effects of different environmental factors on mycoparasitic *Trichoderma* & *Bacillus* strains, indicating, that in order to reach effective biological control, it is necessary to broaden our knowledge about the ecophysiology of this genus. Based on our results, competition and mycoparasitism of *Trichoderma* & *Bacillus* can remain active even under environmental conditions unfavorable for mycelia improvement for better stress tolerance. Effective protoplast fusion and genetic transformation. The formulation study states that it can be also promoted in alginate formulation which has higher shelf life and stress protection. However in-depth study is required for the commercialization.

The study showed that application of alginate formulation and paraffin oil increases the shelf life of *Trichoderma*, & *Bacillus* which was used as a biofungicide comparing to the solid formulation of
biofungicide used, Application of granulation & alginate formulation of biocontrol agents in the field helped the farmers in promising better yield.

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References