

# **EFFECT OF A MICROBIAL INOCULANT ON GROWTH AND CHLOROPHYLL LEVEL OF LETTUCE AND RADISH SEEDLINGS: A PRELIMINARY STUDY**

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## **INTRODUCTION**

Microorganisms are often used in organic agriculture, as they are useful for eliminating problems associated with chemical fertilizers and pesticides (Higa, 1991; Parr et al 1994 cited in Higa and Parr, 1994). A microbial culture named "Effective Microorganisms" (EM) was developed by Professor Teruo Higa of the University of Ryukyus, Japan after he began his EM technology research in 1984, with the purpose of improving soil quality, soil health, and the growth, yield and quality of plants (Higa and Parr, 1994).

EM consists of around 80 species of selected beneficial microorganisms including lactic acid bacteria, yeasts, photosynthetic bacteria, and actinomycetes, among other types of microorganisms such as fungi (Xu, 2000). These produce a wide range of benefits arising from the increased microbial diversity in the soil as well as the individual effects of the particular types of microorganisms. Benefits to soil and plant health include the fixation of atmospheric Nitrogen, decomposition of organic wastes and residues, suppression of soil borne pathogens, and the increased availability of plant nutrients (Higa and Parr, 1994). It has been shown that the application of EM can improve photosynthetic efficiency and capacity due to an increase in nutrient availability, as well as increase root mass (Fujita et al., 1997 cited in Yamada and Xu, 2000).

EM bokashi is an organic fertilizer used for soil and plant application to promote growth and increase yield, and is made from a solution of EM1 and molasses, usually added to bran or straw and then fermented (Daly and Okuda, 1998). It can also be made with oilseed cake, rice husk and bran, and fish processing waste (Xu, 2000). EM bokashi has been found to be a rich nutrient source (Xu et al., 2000), as the addition of EM greatly accelerates the breakdown of the organic matter in the bokashi (Attanayake et al., 1993). In this study the effect of EM bokashi on the growth and chlorophyll level of radish and lettuce seedlings will be measured over time, comparing this with the effect of PastureAid, a microbial fermentation product containing dead microorganisms that promotes growth by stimulating microbial activity.

## **MATERIALS AND METHODS**

### **Materials and Treatments**

Radish seeds were sown into 45mL cell transplant trays, with 60 cells per tray. They were grown in a seedling mix containing 60% peat and 40% pumice, with Osmocote Mini (18-2.6-10) 100g, Dolomite 400g, and Hydraflo II 100g. Lettuce seeds were sown into trays using the same potting mix. The treatments for both the radishes and lettuces were designed as follows: (1) Pasture-Aid (equivalent to 20 L per hectare (by surface area) applied directly after sowing by watering can); (2) EM Bokashi (equivalent to 5 tonne/hectare (by volume) mixed into the potting

mix, and the equivalent volume of water to the Pasture-Aid treatment; (3) Pasture-Aid + EM Bokashi (combination of treatments 1 and 2 with the same volume of water as other treatments); (4) Control (received equivalent water to other treatments). There were 5 trays for each treatment, with each tray as a replicate.

### **Measurement of Fresh and Dry Weights, Chlorophyll Levels, and Length.**

Chlorophyll levels and length of radish leaves were measured at each harvest, 3 and 6 weeks after the radishes were sown. Chlorophyll was measured using a spadmeter, randomly selecting 5 plants from each tray, then measuring the chlorophyll of the largest leaf and a cotyledon leaf. The length of the longest leaf was measured only at the first harvest, using a ruler to measure every radish. At each harvest half of the plugs were pulled out, and plugs with more than one or no seedlings were discarded. The shoots were then cut off and placed in paper bags for measuring fresh weight of each tray. 10 were randomly selected to wash clean with water and place the roots and bulbs in separate paper bags for weighing. After fresh weights were obtained, the roots, shoots and bulbs were placed in an oven at around 70° until they lost all their water content, and dry weights were taken. The length and chlorophyll level of the lettuces were not measured. Dry and fresh weights of shoots and roots of lettuces were taken in the same manner as for the radishes only for one harvest, 3 weeks after they were sown.

## **RESULTS**

### **Lettuce shoot and root weights**

EM Bokashi tended to have a great effect on both the shoot and root growth compared to both the control and PastureAid. PastureAid did not prove to be effective alone, however it tended to increase the effect of EM Bokashi on shoot growth although this was not a statistically significant result

### **Lettuce Shoot Fresh Weight**

Treatment 3 Weeks (g fresh weight)

Control 3.17b

PastureAid 2.72b

EM Bokashi 5.34a<sup>\*\*\*</sup>

EM Bokashi + PastureAid 5.80a<sup>\*\*\*</sup>

<sup>\*\*\*</sup>: Highly statistically significant compared to control,  $p < 0.01$

<sup>\*\*</sup>: Statistically significant compared to control,  $p < 0.05$

<sup>\*</sup>: Weakly statistically significant compared to control,  $p < 0.1$

Column values sharing the same letters are not significantly different at  $P = 0.05$  according to Pearson's test.

### **Lettuce Shoot Dry Weight**

Treatment 3 Weeks (g dry weight)

Control 0.37b

PastureAid 0.32b

EM Bokashi 0.54a<sup>\*\*\*</sup>

EM Bokashi + PastureAid 0.57a\*\*\*

### **Lettuce Root Dry Weight**

Treatment 3 Weeks (g dry weight)

Control 1.48a

PastureAid 1.72a

EM Bokashi 2.03a\*

EM Bokashi + PastureAid 1.81a

### **Radish shoot and root weights**

The EM Bokashi treatments and the EM Bokashi + PastureAid treatments tended to be significantly different from the other treatments although not significantly different from each other. Differences appear to be much more dramatic at 6 weeks where the EM Bokashi probably started to have a greater effect on the shoot, root and bulb growth.

### **Radish Root Fresh Weight**

Treatment 3 Weeks (g fresh weight) 6 Weeks (g fresh weight)

Control 0.814b 1.540b

PastureAid 0.746b 1.470b

EM Bokashi 1.694a\*\*\* 3.489a\*\*\*

EM Bokashi + PastureAid 1.580a\*\*\* 3.056a\*\*\*

### **Radish Bulb Fresh Weight**

Treatment 3 Weeks (g fresh weight) 6 Weeks (g fresh weight)

Control 0.564a 0.539b

PastureAid 0.602a 0.598b

EM Bokashi 0.854a 1.080a\*\*\*

EM Bokashi + PastureAid 1.03a\* 1.366a\*\*\*

### **Radish Roots Dry Weight**

Treatment 3 Weeks (g dry weight)

Control 0.465a

PastureAid 0.762a

EM Bokashi 0.464a

EM Bokashi + PastureAid 0.532a

### **Radish Shoots Dry Weight**

Treatment 3 Weeks (g dry weight) 6 Weeks (g dry weight)

Control 0.166b 0.190b

PastureAid 0.176b 0.187b

EM Bokashi 0.264a\*\*\* 0.334a\*\*\*

EM Bokashi + PastureAid 0.256a\*\*\* 0.297a\*\*\*

### Radish Bulbs Dry Weight

Treatment 3 Weeks (g dry weight) 6 Weeks (g dry weight)

Control 0.082a 0.092c

PastureAid 0.066a 0.107bc

EM Bokashi 0.070a 0.152ab\*\*\*

EM Bokashi + PastureAid 0.080a 0.176a\*\*\*

### Radish Chlorophyll Level

The EM Bokashi and EM Bokashi + PastureAid treatments have higher chlorophyll level readings than the control or PastureAid only treatment. The PastureAid treatment appears to have had a higher chlorophyll level than the control for two of the measurements. EM Bokashi + PastureAid appears to be the most effective in increasing the chlorophyll level but this is not statistically significant from the EM Bokashi only treatment. By the 12/12/01 no differences were statistically significant although plants that received EM Bokashi still tended to have higher levels (5 - 8 % higher than control). The standard deviations at this date were 1.5 times on average higher than on the 29/12/01 most likely due to older plants having greater variation between plants in "greenness". This variation could hide actual effects of a treatment on chlorophyll levels.

Radish Leaf Chlorophyll Level (Spadmeter measurement)

Treatment 3 Weeks(Largest Leaf) 3 Weeks(Cotyledon Leaf) 6 Weeks(Largest Leaf)

Control 30.6b 13.1c 27.6a

PastureAid 32.0ab 18.1b 27.0a

EM Bokashi 33.3ab 23.7a 29.1a

EM Bokashi + PastureAid 34.8a 23.7a 29.8a

Column values sharing the same letter are not significantly different at  $P = 0.05$  according to Fisher's pairwise comparisons test.

## DISCUSSION

There has been a great deal of research conducted on the effect of EM bokashi on plant growth and photosynthesis (Fujita et al., 1997; Xu et al., 1997 cited by Yamada and Xu, 2000). Such research has shown that the addition of EM bokashi leads to more vigorous growth with greater root mass and activity, and a higher rate of photosynthesis compared to the addition of chemical fertilizers (Fujita et al., 1997 cited by Yamada and Xu, 2000). These results are suggested by (Kato et al., 1997 cited in Yamada and Xu, 2000) to be due to the continual nutrient supply from the bokashi. It is suggested by (Yamada and Xu, 2000) that EM contains phytohormones or other biologically active substances that cause the delay of senescence of plants. (Yamada and Xu, 2000) believe that the increase in the development of roots from the added bokashi may also help maintain a higher rate of growth and photosynthetic activity. The results from this preliminary study are consistent with these findings, with higher root, shoot and

bulb growth with the application of EM bokashi, and more dramatic results occurring at the later stage where the bokashi may have had a greater effect due to the sustained nutrient release. The increase in chlorophyll levels with EM bokashi treatments is consistent with other studies, and the cotyledon leaves of control plants were noted to be generally yellow with senescence beginning at the later date, when treated plants appeared to be much greener.

(Yamada and Xu, 2000) propose that the effect of EM bokashi can be attributed to biologically active substances along with the sustained release of available plant nutrients from the decomposition of organic materials. The results from this preliminary study show that EM bokashi has an effect on growth and chlorophyll levels, and especially so with the addition of PastureAid, although this has little effect on its own. The reason for this effect may have been that PastureAid accelerated the effect of the EM bokashi, but the mechanisms for this are unknown. Future studies could concentrate on measuring the effect of the ingredients of bokashi with and without the inoculation of EM, and trialling different ingredients of bokashi to determine the most effective ingredients for agricultural use.

## REFERENCES

Attanayake, K.B.; Piyadasa, E.R.; Ratnayake, A.D.A.; Sangakkara, U.R. 1993. The role of Effective Microorganisms in releasing nutrients from organic matter. Second Conference of Effective Microorganisms (EM) Proceedings. pp 7-14.

Daly, M.; Okuda, A. 1998. (Unpublished). New Zealand EM Application Manual. 6pp.

Higa, T; Parr, J.F. 1994. Beneficial and Effective Microorganisms for a Sustainable Agriculture and Environment. International Nature Farming research Center, Japan.

Xu, H. 2000. Soil-root interface water potential in sweet corn as affected by organic fertilizer and a microbial inoculant. In Xu, H.; Parr, J.F.; Umemura, H. (eds) Nature Farming and Microbial Applications. Pp139-156 The Haworth Press Inc. New York.

Xu, H.; Wang, R.; Mridha, A.U. 2000 Effects of Organic Fertilizers and a Microbial inoculant on Leaf Photosynthesis and Fruit Yield and Quality of Tomato Plants. In Xu, H.; Parr, J.F.; Umemura, H. (eds) Nature Farming and Microbial Applications. Pp 235-243. The Haworth Press, Inc. New York.

Yamada, K.; Xu, H. 2000. Properties and applications of an organic fertilizer inoculated with Effective Microorganisms. In Xu, H.; Parr, J.F.; Umemura, H. (eds) Nature Farming and Microbial Applications. Pp 255-268 The Haworth Press, Inc. New York.