

Effects of Effective Microorganisms™ on the growth of *Brassica rapa*

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Abstract

The effects of Effective Microorganisms™, one type of microbial fertilizer commercially sold, on the growth of Wisconsin Fast Plants (*Brassica rapa*) and on the microbial density in soil were examined for this study. Two controls and three treatments were prepared, and plant height, diameter of stem, biomass of seed pod and plant, and the population of general microorganisms in soil were measured in order to be compared statistically by ANOVA and Dunnett test. Although there was no significant difference in plant height, treatment with EM bokashi plus EM solution resulted in the thickest diameter of stem, followed by the chemical fertilizer. Both seed pod/plant biomass and microbial density showed a maximum response to the EM bokashi plus EM application. This suggests that EM products are effective for the improvement of growth of *Brassica rapa* and for enhancing the increase of microorganisms in soil.

Introduction

Farmers have adopted the strategy of increasing crop yields by applying large amounts of chemical fertilizers and pesticides. At present, however, the negative effects of heavy applications of chemical inputs, in terms of production, environment, and quality deterioration are becoming apparent (Nishio, 1996). In 1989, the National Research Council of the National Academy of Sciences issued a report which raised considerable speculation about the future of the chemical-based agricultural production system (Parr, 1994).

The ultimate goal of sustainable agriculture is to develop farming systems that

are productive, profitable, energy-conserving, environmentally-sound, conserving of natural resources such as soil and water, and that ensure food safety and quality (Parr, 1994). Organic agriculture has much in common with sustainable agriculture. The same stress is placed upon the use of renewable resources, conservation of resources, and the maintenance of environmental quality without using chemical inputs (Poincelot, 1986). For instance, soil amendment with composts is an agricultural practice commonly used to improve soil quality and also to manage organic wastes for a sustainable land use (Piqueres et al, 2005 & Singh, 1995).

Microbial fertilizer is one way Organic Farmers are able to increase yield and quality of crops without a large investment of money and labor (Pham, 2004). Moreover, microbial fertilizer can clean the environment and encourage the productive capacity of land by reducing the amount of chemical fertilizer consumption (Pham, 2004). Lynch and Harper (1985) attempted to increase decomposition of straw by microbial application. EM•1® and EM Bokashi are microbial fertilizers, created in Japan over 25 years ago by Dr. Teruo Higa PhD of University of the Ryukyus in Okinawa and marketed by EMRO (EM Research Organization). The basic purpose of EM is the restoration of healthy ecosystem in both soil and water by using three major genera of microorganisms which are found in nature: phototrophic bacteria (*Rhodopseudomonas*), lactic acid bacteria (*Lactobacillus*), and yeast (*Saccharomyces*). EM•1® contains *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus Fermentum*, *Lactobacillus delbrueckii*, *Saccharomyces cerevisiae*, and *Rhodopseudomonas palustris*. “Bokashi” is a Japanese term generally meaning “obscuring the direct effectiveness”. Today it refers to fermented organic matter because it is used as a product name of organic fertilizer composted from kitchen food

waste. The concentration of nitrogen in normal bokashi is much lower than in chemical fertilizer, ranging from 2 to 5% total nitrogen (Nishio, 1996). EM•1® Bokashi is a product made of organic matter, generally rice bran or wheat bran. In Hawaii, Mil run (left over from flour mill) is used.

The purpose of this research is to observe the effects of *Effective Microorganisms™ on the growth of Wisconsin Fast Plant (*Brassica rapa*) and on the microbial density in soil.

Method

Artificial soil made from mixing equal amounts of small and fine perlite, and fine peat moss was prepared for this study. Soil was sterilized at 180 °C for 24 hours. Twenty five-cm diameter pots were filled with compressed sterilized soil to within 1 cm of the top. Soil in all pots was damp, but not saturated. Pots were divided into five groups of four pots each. Group one was the negative control with only water added throughout the study. Group two was a second control treated with 30ml/week/pot EM solution sterilized by filtering through a 0.45 um syringe filter. Group three was given 30ml/pot of a standard dose of EM solution (0.001%) weekly. Group four was given manufacturer's recommend dose of a granulated slow release fertilizer (13-13-13 containing trace elements); group five was given 30ml/week/pot standard EM solution (0.001%) plus 1gm/pot of an organic additive called bokashi, was scattered over the top of the soil in each pot. The bokashi was added per pot and each pot was allowed to sit for two week before sowing the Wisconsin Fast Plant seeds. Pots in all five groups were sown with seeds, three seeds per pot, and pots were place in trays below four 30 watt Gro-Lux fluorescent lamps that were lit 24 hours per day. After germination, seedlings were thinned to two seedlings per pot. Water in the trays under the pots

provided water on demand to all plants. Measurements of the plant height, the diameter of stem, and the biomass (final week) of seed pods and above ground stems, leaves, and pods were taken (Chaudhary & Iqbal, 2006). For plant height (cm), measurement were taken every week from the surface of the soil to the top of flower buds. The diameter of stem was measured (mm) on final week about 3 cm above the surface of soil where they were thickest. The dry weight biomass per seed pod and per plant was weighed with a precision of 0.001 g. Plants were dried at 80 °C for 24 hours before weighing.

Microbial density within the soil was measured on day 35 by performing a basic dilution series on Tryptic soy agar (1.5% tryptone, 0.5% soytone, 0.5% NaCl, and 1.5% agar). This was performed by weighing 1 g of soil from each pot taken from depth of 3 cm and mixing with 9 ml of sterile phosphate buffer (87%Na₂HPO₄, 13% NaH₂PO₄, pH7.0). 100-fold dilutions were performed, with 100 uL samples spread on 15 cm TSA plates (Shipman, 1957, Yanagida & Shinohara, 2005). After incubation, the number of colony-forming units (CFU) per gram was determined to estimate numbers of viable bacterial cells.

Data was compared by analysis of variance (ANOVA) and Dunnett test as a post-hoc test (multiple comparisons) to see the differences between controls and treatment groups.

Results

Table 1: The means of plant height in centimeters with standard deviation in parentheses of each group measured at day 7, 14, 21, 28, and 35.

	Mean (st.dev)				
	Day7	Day14	Day21	Day28	Day35
Group 1 (negative control)	4.15 (1.12)	13.25 (3.77)	19.00 (3.56)	18.42 (6.35)	18.67 (5.92)

Group 2 (control)	4.10 (1.10)	12.75 (3.92)	17.00 (3.54)	17.28 (4.99)	19.25 (5.70)
Group 3 (EM treatment)	4.09 (0.94)	13.75 (3.01)	16.40 (0.54)	15.75 (2.37)	16.80 (2.49)
Group 4 (Chem. Fertilizer)	3.50 (1.22)	10.00 (2.88)	16.00 (2.58)	12.75 (3.30)	15.00 (3.01)
Group5 (EM bokashi)	2.81 (1.31)	8.83 (3.12)	15.5 (6.22)	18.2 (6.22)	18.88 (6.44)

By one-way repeated-measures ANOVA, there was no significant difference ($p=0.162$) of the plant height among those 5 groups throughout this study (Table 1).

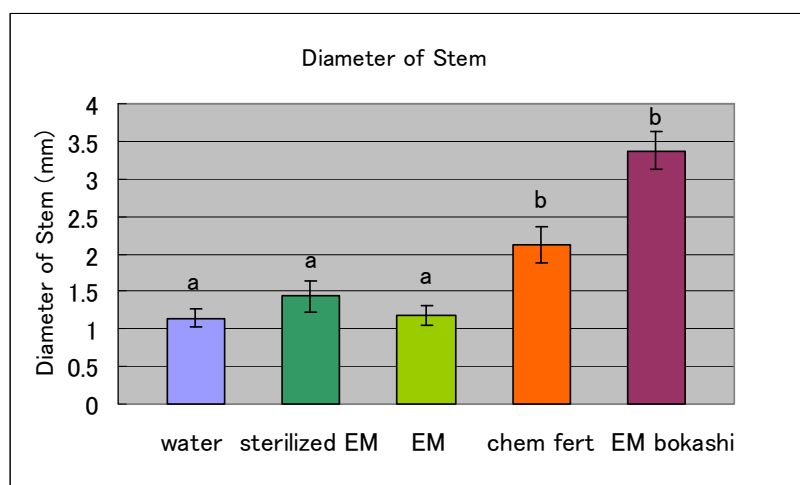


Figure 1 :The average diameters in mm of stems for each group measured at maturity. Values are means with \pm standard deviation lines. Different small letters on top of the bars indicate significant differences ($p<0.05$). There were no significant differences among groups with same letters, but those marked “a” were significantly different from those marked “b”.

The plants treated with EM bokashi ($p=0.000528$) showed the thickest diameters of mature stems (3.38mm), followed by group 4 treated with chemical fertilizer ($p=0.0210$, 2.13mm). However, stem diameters of plants treated with sterilized EM ($p=0.251$) and with EM solution alone ($p=0.849$) were not significantly different from those given water only (Figure 1) .

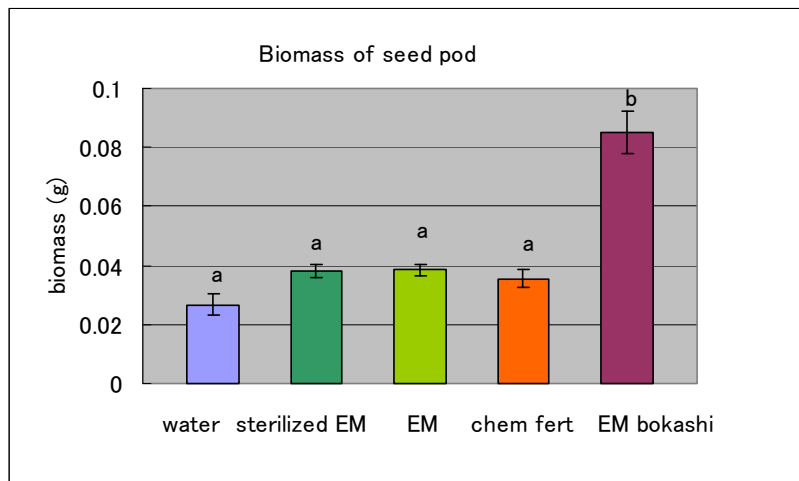


Figure 2 : The average dry weight biomass (grams) of seed pod for each group measured at maturity. Values are means with \pm standard deviation lines. Different small letters on top of the bars indicate significant differences ($p < 0.05$). There were no significant differences among groups with same letters, but those labeled “a” were significantly different from those labeled “b”.

The plants treated with EM bokashi ($p = 0.000246$) showed the heaviest biomass of seed pods (0.0852g) and were significantly heavier than the other four groups. Pod weights of groups with sterilized EM ($p = 0.0948$), with EM solution ($p = 0.0552$), and with chemical fertilizer ($p = 0.136$) were not significantly different from pod weights from plants given water only (Figure 2).

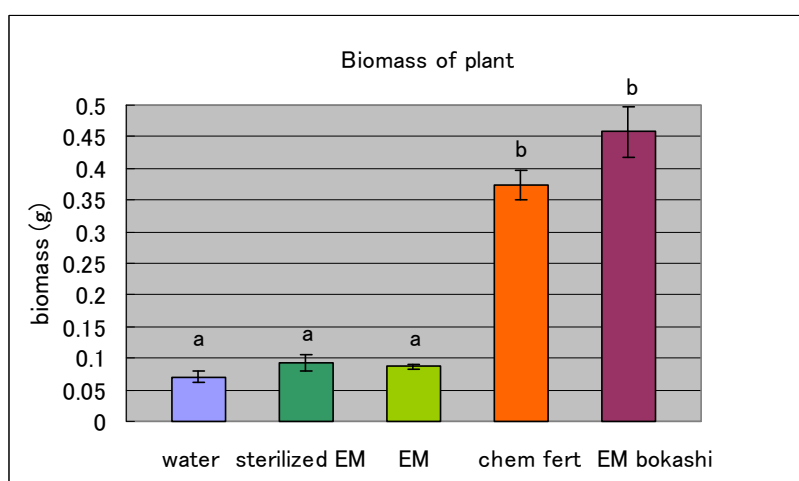


Figure 3 : The average dry weight (gram) of biomass of plants for each group measured at maturity.

Values are means with \pm standard deviation lines. Different small letters on top of the bars indicate significant differences ($p < 0.05$). There were no significant differences among groups with same letters, but those labeled “a” were significantly different from those labeled “b”.

The plants treated with EM bokashi ($p = 0.000151$) showed the heaviest biomass (0.458g) followed by the plants treated with chemical fertilizer ($p = 0.00126$, 0.373g). Plants treated with EM solution ($p = 0.581$) and sterilized EM ($p = 0.419$) showed no significant difference from the control group with only water added (Figure 3).

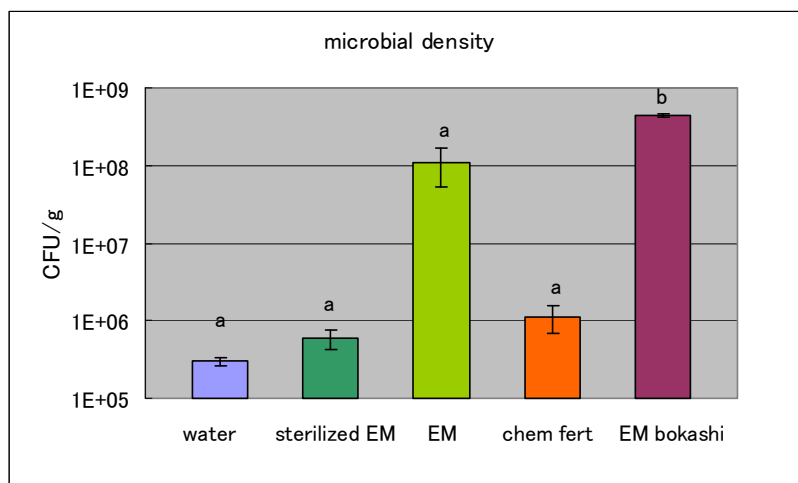


Figure 4 : The average microbial density in soil of each group measured when the plants were matured. Values are means with \pm standard deviation line. Different small letters on top of the bars indicate significant differences ($p < 0.05$). There were no significant differences among groups with same letters, but those labeled “a” were significantly different from those labeled “b”.

EM solution increased the overall densities of viable bacteria in the soil, but was statistically insignificant ($p = 0.0945$) compared to the control group with sterilized EM ($p = 0.999$) and with the treatment received chemical fertilizer ($p = 0.987$). Only EM bokashi plus EM solution significantly increased the microbial density (435000000CFU) in the soil ($p = 0.000992$) (Figure 4).

Discussion

None of the treatments caused significant ($p > 0.05$) differences of plant height from the control plants, whereas chemical fertilizer and EM plus bokashi showed the significant differences on the other measurements. Both chemical fertilizer and EM bokashi plus EM solution generated thicker stems and heavier plant biomass than controls and EM solution only. Since treatment with EM bokashi plus EM solution resulted in the maximum biomass of seed pod, it also enhanced the increase of seed yield. Moreover, EM plus bokashi had the highest microbial densities through either a stimulation of the soil microflora, or an input of the compost microflora or a combination of both. To clarify this point, according to Piqueres et al (2005), an arithmetic density of bacteria in soil-bokashi mixture could be easily calculated based on the known number of bacteria in the natural soils (approximately 0 since it was sterilized by heating) and pure bokashi (about 600000 CFU per gram on TS agar). EM solution (30 ml of 0.001%) was added weekly to the treatment, but it does not contain microorganisms that are cultivable on TSA. Therefore, since the observed soil-bokashi mixtures densities (435000000 CFU per gram) were considerably higher than calculated densities (600000 CFU per gram), it could indicate a stimulation of the microflora. Actually, the treatment with EM solution showed 110000000 CFU per gram on TS agar, indicating that it enhances the growth of other microorganisms.

Over all, EM solution did increase the soil microbial density, but did not significantly change the growth of *Brassica rapa* over the controls. EM combined with organic matter such as EM bokashi or other compost which is an important source of nutrients usable by microorganisms for improving the plant growth did increase plant

growth biomass. When EM solution was used with EM bokashi, it enhanced the plant growth equally to using chemical fertilizer (13-13-13 slow release).

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