Changes in the Soil Microflora Induced by Effective Microorganisms

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Abstract

The beneficial effects of effective microorganisms (EM) on plant growth, yield, and quality have been consistently demonstrated. However, there are still questions about which EM cultures, or combinations thereof, are most effective for alleviating certain chemical, physical, and microbiological problems in soils. In the study reported here, EM cultures increased the number of Enterobacter spp. and starch digesting bacteria in soil. A combination of EM 2. 3. 4 markedly suppressed the number of Verticillium, Thielaviopsis, and Fusarium fungal species that are destructive soil borne plant pathogens. Some of the EM cultures significantly increased the population of Trichoderma (EM 2, EM 3, EM 2.3) and Penicillium (EM 3, EM 2. 3, EM 2. 3. 4) species that are known to suppress plant pathogenic fungi in soils: Soil physical properties, including cultivation depth and porosity, were generally improved by EM treatment.

EM 3, EM 4, and EM 3. 4 effectively suppressed nematode damage on tomato plants. With the exception of EM 2, all other EM cultures appeared to either suppress insect damage or heal fruit injuries on tomato caused by insects. Tomato yields obtained with EM 3, EM 4, and EM 2. 3 were comparable to, though less than, the fertilized control. However, the amount of marketable fruit was considerably greater for these EM treatments than for the fertilized plot.

Introduction

Soil microorganisms can have both positive and negative effects on plant growth. They can facilitate nutrient absorption by plants (Bowen and Rovira, 1966); promote plant growth or stimulate seedling development by producing hormone-like substances (Rubenchick, 1963; Mishustin 1970; Brown, 1974); suppress and control plant pathogens and diseases through various antagonistic activities (Marois et al., 1981); or adversely affect plant growth through their pathogenic behavior (Elad, 1985).

A principal goal of nature farming is to produce abundant and healthy crops without using chemical fertilizers and pesticides, and without interrupting the natural ecosystem. Higa (1986; 1988) investigated the effect of effective microorganisms (EM) on different horticultural crops at the University of The Ryukyus. The beneficial effects of EM have been demonstrated when applied to horticultural crops. Questions still remain about which combination of EM cultures changes specific problem soils into healthier and more productive soils, that is, disease-suppressive soils, synthetic soils or zymogenic soils. We need to know which combinations of EM can favorably interact with soil microbial communities and promote beneficial relationships between biotic and abiotic factors which enhance the health and growth of plants.

The purpose of this study was to investigate the effects of EM on the soil microflora, the effects of EM on soil physical and chemical properties, and how the nature farming concept can be successfully applied to modern agriculture.

Materials and Methods

EM 2, EM 3, and EM 4 were obtained from the Horticulture Laboratory, Department of Agriculture, the University of The Ryukyus in Okinawa, Japan. The EM cultures were classified as follows (Higa (1988):

1) **EM 2.** EM 2 is a mixture of more than 10 genera and 80 species of coexisting microorganisms (photosynthetic bacteria, ray fungus, yeast, molds, etc.) that were cultured in a liquid medium controlled at pH 7.0 and stored at pH 8.5. The number of microorganisms in saturated culture solution was 10^9 g^{-1}.
2) **EM 3.** EM 3 consists of 95 % Photosynthetic bacteria that were cultured in a liquid medium and stored at pH 8.5. The number of microorganisms was 10^9 g^{-1}.

3) **EM 4.** EM 4 consists of 90 % *lactobacillus spp*. and microorganisms producing lactic acid, that were cultured in a liquid medium at pH 4.5. The number of microorganisms in the solution was 10^9 g^{-1}.

The experiments were initiated October 23, 1988 at the University of The Ryukyus in Okinawa. Soil was classified as gray upland soil with a pH of 8.3 and had not been cultivated for many years.

To determine the effect of EM on crop production, spinach (var. Radikaru) and tomato (var. Oogata Fukuju) were used.

Prior to planting, the soil was mixed with 1 kg of dry grass m^{-2} which contained 1 % N, and with enough oil meal to supply 3 g N m^{-2}. Soil physical and chemical properties and microbial analysis were determined on soil samples that were taken after the tomato crop was harvested.

Plots were established as a randomized complete block with three replications. Treatments included EM 2, EM 3, EM 4, EM 2.3, EM 2.4, EM 3.4, EM 2.3.4, an unfertilized control (O) without EM, and a fertilized control (OF) without EM. Sufficient levels of N, P, and K were applied to the fertilized control to sustain optimum plant growth. EM cultures were diluted to concentrations of 0.1 % from liquid stock media and watered into the soil at two-week intervals.

Total microorganisms in the soil were estimated by the plate count method. Bacteria and actinomycete populations were counted on egg albumin agar (Tadao, 1984). Total fungi were counted on rose bengal agar (Martin, 1950). *Azotobacter* were isolated on nitrogen-free mannitol broth agar (Harrigan and Margaret, 1966). *Clostridia* were isolated on media described by Sheldon (1970). *Lactobacillus spp*. were counted on Rogosa agar (Harrigan and Margaret, 1966). *Enterobacter* was counted on MacConkey agar (Harrigan and Margaret, 1966). Starch digesting bacteria were counted using the method of Sheldon (1970). *Agrobacterium*, *Erwinia*, *Pseudomonas*, and *Xanthomonas spp*. were counted on Dl, D3, D4, and D5 selective media, respectively (Kado and Heskett, 1970). *Fusarium* was counted on Komada's medium (Tadao, 1984); *Verticillium* on alcohol agar medium (Mathew and Chester, 1959); and *Thielaviopsis* on RBM2 medium (Tsao, 1964).

Soil bulk density and porosity were determined according to methods described by Henry (1984), using 2 and 4 cm diameter cores from each plot taken to a depth of 10 cm. Soil porosity was calculated from the ratio of pore space and soil volume. Soil aggregation was determined by the pipette method described by Martin and Waksman (1940). Soil phosphorus content was determined by the method of Hormers and Parker (1961).

Results

Change in Soil Microflora

In most cases, the numbers of bacteria, fungi, and actinomycetes increased after the soil was treated with EM cultures, although the numbers of actinomycetes measured in treatments EM 2.3 and EM 2.4 were lower than the unfertilized control (Table 1). It was interesting that the lowest number of actinomycetes occurred when the soil was treated with only fertilizer (OF).

Table 1. Effect of EM Cultures on Numbers of Soil Microorganisms.*

Treatment**	Bacteria *x 10^5*	Fungi *x 10^3*	Actinomycetes *x 10^4*
O	47.8	9.42	17.9
OF	59.4	23.1	8.38
EM2	69.3	39.1	17.8
EM3	79.8	36.9	29.5
EM4	147	35.5	29.6
EM2.3	136	34.7	11.4
EM2.4	112	39.7	11.6
EM3.4	105	11.8	39.2
EM2.3.4	118	14.0	20.2

* Microorganisms per gram of soil (dry weight basis) counted prior to planting tomatoes.

** Unfertilized control without EM (O). Fertilized control without EM (OF)

Generic anaylsis of the bacterial flora in the soil due to EM treatment is shown in Table 2. In most cases EM cultures markedly increased the number of *Enterobacter spp*. and starch digesting bacteria over that of the unfertilized control (O), but had little effect on enhancing the numbers of *Lactobacillus spp*. The highest numbers of *Azotobacter* and *Clustridiurn* species were attained with the fertilized control (OF), while the lowest number of each occurred with the unfertilized control (O). The highest number of *Xanthomonas* and *Erwinia* species were found in the fertilized control (OF), the highest number of *Agrobacterium* specres from treatment with EM 2.3.4, and the highest number of *Pseudomonas* from EM 2.3.

Table 2. Effect of EM Cultures on Generic Composition and Populations of Bacteria in Soil.*

Treatment**	*Entero-bacteria* $X\ 10^3$	*Starch Digesting* $X\ 10^4$	*Lacto-bacillus* $X\ 10^2$	*Azoto-bacter* $X\ 10^2$	*Clostri-dium* $X\ 10^2$	*Xantho-monas* $X\ 10^5$	*Erwina* $X\ 10^3$	*Agro-bacterium* $X\ 10^4$	*Pseudo-monas* $X\ 10^3$
O	239	170	20	5	17	100	404	103	39
OF	224	300	20	700	282	133	800	73	135
EM2	673	400	28	98	61	41	209	106	422
EM3	266	225	18	72	120	22	228	68	52
EM4	273	103	28	700	50	29	164	57	242
EM2.3	724	100	23	100	154	23	431	114	1090
EM2.4	385	150	27	500	191	20	500	64	392
EM3.4	132	219	23	500	40	25	352	41	166
EM2.3.4	415	175	20	500	25	30	382	189	134

* Bacteria per gram of soil (dry weight basis) counted at the second planting of tomatoes.
** Unfertilized control without EM (O). Fertilized control EM (OF).

The number of fungal species after EM treatment of this soil are shown in Table 3. The highest number of *Trichoderma* species was found after treatment with EM 2.3 and the highest number of *Penicillium* with EM 3. However, the lowest number of specimens in these genera resulted from the fertilizer treatment (OF). The highest number of *Verticillium* species was observed in the fertilized control (OF) and with EM 4. But, the combination of EM 2.3.4 appeared to suppress the numbers of this soil borne plant pathogen. Treatment with EM 2, EM 3, and EM 2.3.4 appeared to suppress *Thielaviopsis*, a potential plant pathogen. The highest number of *Fusarium* species resulted from treatment with the fertilized control (OF), while the combination of EM 2.3.4 markedly suppressed the numbers of this particularly destructive plant pathogen.

Table 3. Effect of EM Cultures on Fungal Populations in Soil.*

Treatment**	*Trichoderma* $X\ 10^2$	*Penicillium* $X\ 10^3$	*Verticillium* $X\ 10^3$	*Thielaviopsis* $X\ 10^3$	*Fusarium* $X\ 10^2$
O	2.77	3.96	32.9	25.0	228
OF	0.77	1.15	38.7	18.8	465
EM2	9.25	1.61	28.6	12.9	110
EM3	5.87	8.61	25.8	10.9	277
EM4	2.73	3.50	38.6	21.0	105
EM2.3	20.4	5.09	24.7	18.4	182
EM2.4	1.18	3.14	22.0	20.8	143
EM3.4	2.75	1.57	22.8	22.8	154
EM2.3.4	0.78	5.10	16.1	16.1	73

* Fungi per gram of soil (dry weight basis) counted at the second planting of tomatoes.
** Unfertilized control without EM (O). Fertilized control without EM (OF).

Change in Soil Physical and Chemical Properties

Soil physical properties were determined one year after treatment with the EM cultures and are shown in Table 4. Cultivation depth and porosity were significantly higher with most EM treatments than with the controls, (O) and (OF). Soil hardness was significantly higher for the unfertilized control, although it was also high for several of the EM treatments. There was little difference in soil bulk density among all treatments.

Soil aggregation was higher for all EM treatments than either the unfertilized control (O) or fertilized control (OF). Soil aggregation actually decreased from the application of fertilizer to this soil.

Table 4. Effect of EM Cultures on Soil Physical Properties.

Treatment*	*Cultivation Depth* *cm*	*Soil Hardness* *kg cm^{-2}*	*Porosity* *%*	*Bulk Density* *g cm^{-2}*	*Aggregation* *%*
O	23.7c**	2.75a	52.9c	1.17ab	70.1
OF	23.9c	1.87bcd	52.7c	1.17a	67.4
EM2	28.5b	1.63e	53.7b	1.17a	71.7
EM3	28.3b	1.74e	53.7b	1.16a	73.2
EM4	32.1b	1.58e	58.7a	1.08b	71.7
EM2.3	27.0b	1.91bcd	56.8ab	1.16a	71.4
EM2.4	29.9b	2.40ab	55.7ab	1.11ab	72.0
EM3.4	30.1b	2.20abc	54.8ab	1.13ab	71.9
EM2.3.4	32.3a	1.77de	52.2ab	1.16a	71.0

* Unfertilized control without EM (O). Fertilized control without EM (OF).

** Means in columns followed by the same letter do not differ significantly at $^{p}0.05$.

There was little difference in the effect of EM treatment, or the controls, on such parameters as soil pH and humus content, or on nutrients such as nitrate, ammonium, and potassium. The most dramatic effect of EM treatments on soil nutrient composition was the increased level of inorganic (plant available) phosphorus which was higher than the unfertilized control (O) in all cases (Figure l).

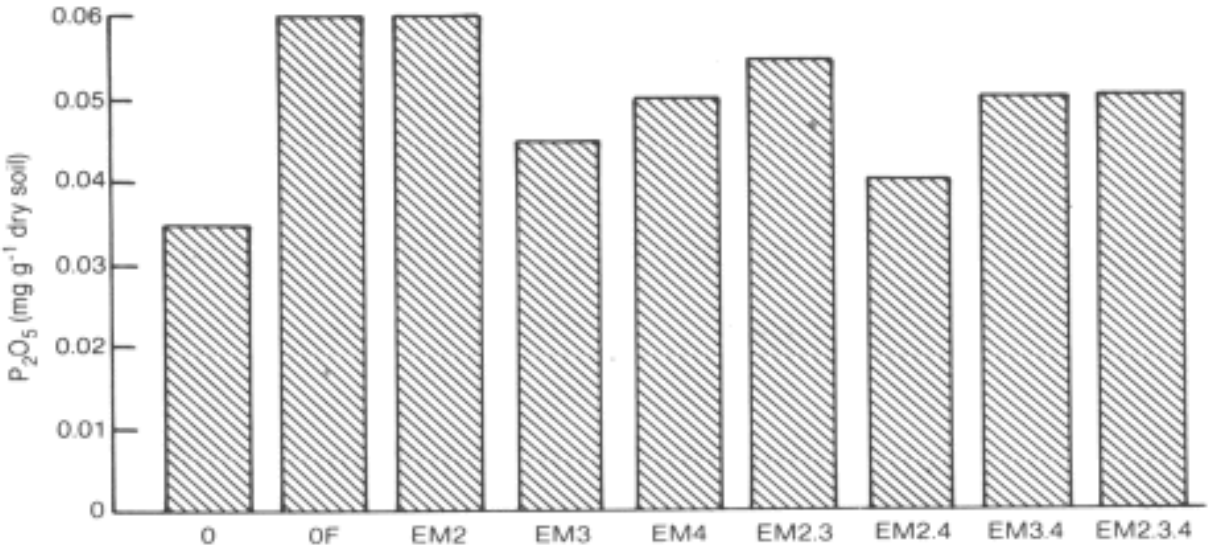

Figure 1. The Effect of EM Cultures on Soil P_2O_5 Content.

Tomato and Spinach Production

Tomato yields for the first crop showed no significant difference between the EM treatments and the unfertilized control (O). However, yields were significantly higher with the fertilized control (OF) than the EM treatments (Figure 2 and Table 5). The number of nematode galls on tomato plants grown in the control plots, both fertilized and unfertilized, and with EM 2 were higher than for the other treatments. EM 3, EM 4, and EM 3.4 were particularly effective in suppressing nematode damage.

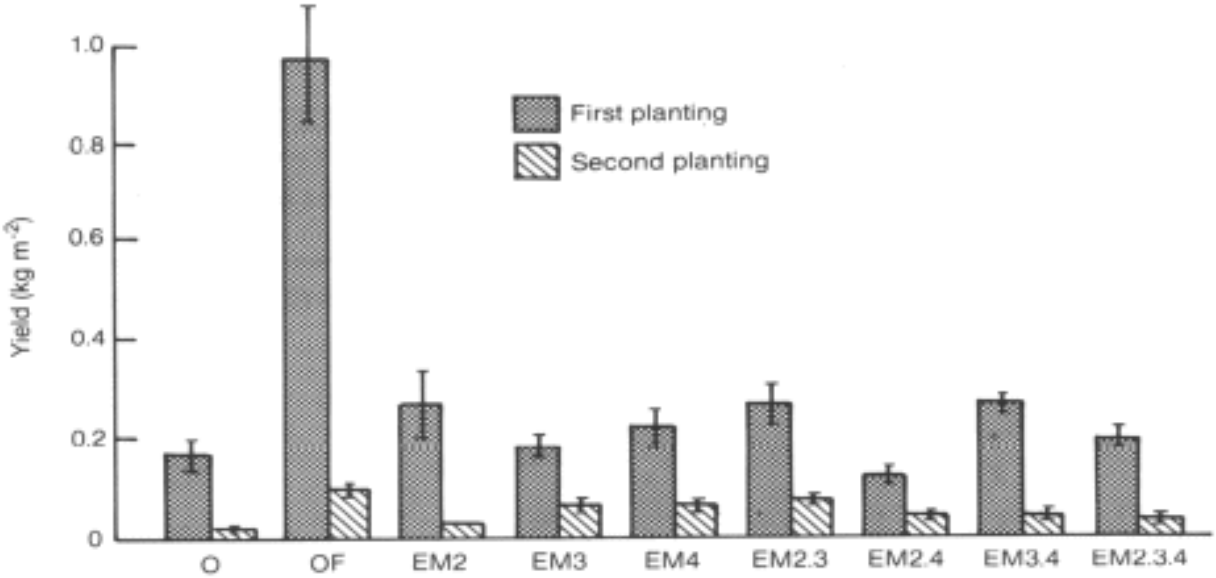

Figure 2. The Effect of EM Cultures on the Yield on Tomatoes.

Table 5. Effect of EM Cultures on Yield and Quality of A Tomato Crop.

Treatment*	Plant Height *cm*	Root Freash Weight *g*	Fusarium** *X 10²*	Galls/plant *No.*	Gall Weight *g*	Yield *% of control*
O	70.0	13.4	228	83.0	1.20	100
OF	161	37.9	465	13.7	2.69	598
EM2	84.9	23.9	100	14.6	3.12	162
EM3	87.2	21.7	277	7.4	0.72	108
EM4	90.0	19.2	105	5.0	0.87	133
EM2.3	97.1	24.5	183	10.0	2.57	164
EM2.4	69.2	18.2	143	10.7	2.50	70.1
EM3.4	81.0	19.8	154	3.9	0.73	162
EM2.3.4	79.8	17.3	73	11.2	2.05	117

* Unfertilized control without EM (O). Fertilized control without EM (OF).
** Fusarium fungi per gram of soil (dry weight basis).

Table 6 shows the indirect effect of EM treatments on covering tomato fruit injuries caused by green june bug. The percentage of fruit damaged by the insect on control plots and with EM 2 were higher than for the other treatments. The other EM cultures appeared to either suppress insect damage or heal fruit injuries caused by the insects. The lowest fruit yield was on the unfertilized control (O) plots, and the highest yield was from the fertilized control (OP). Among the EM treatments, the highest yields were obtained with EM 3, EM 4, and EM 2.3. While these yields were somewhat less than the fertilized control, the number of marketable tomatoes was considerably higher for these EM treatments than for the fertilized plots.

Table 6. Indirect Effect of EM Cultures on Covering Fruit Injuries by Insects.

Treatment*	*Production* *g cm⁻²*	*Marketable Fruit* *No.*	*Fruit Damage* *No.*	*Fruit Damage* *%*
O	110	15	69	82.1
OF	875	55	114	67.5
EM2	212	12	57	82.6
EM3	616	66	27	29.0
EM4	558	69	30	30.3
EM2.3	697	60	30	33.3
EM2.4	366	42	12	22.2
EM3.4	366	30	45	60.0
EM2.3.4	312	30	14	31.8

* Unfertilized control without EM (O). Fertilized control without EM (OF).

The effect of continuous cropping and EM treatments on spinach production is reported in Figure 3. Continuous cropping tends to decrease spinach production. The highest production was achieved on the fertilized control (OF) and the lowest on the unfertilized control (O).

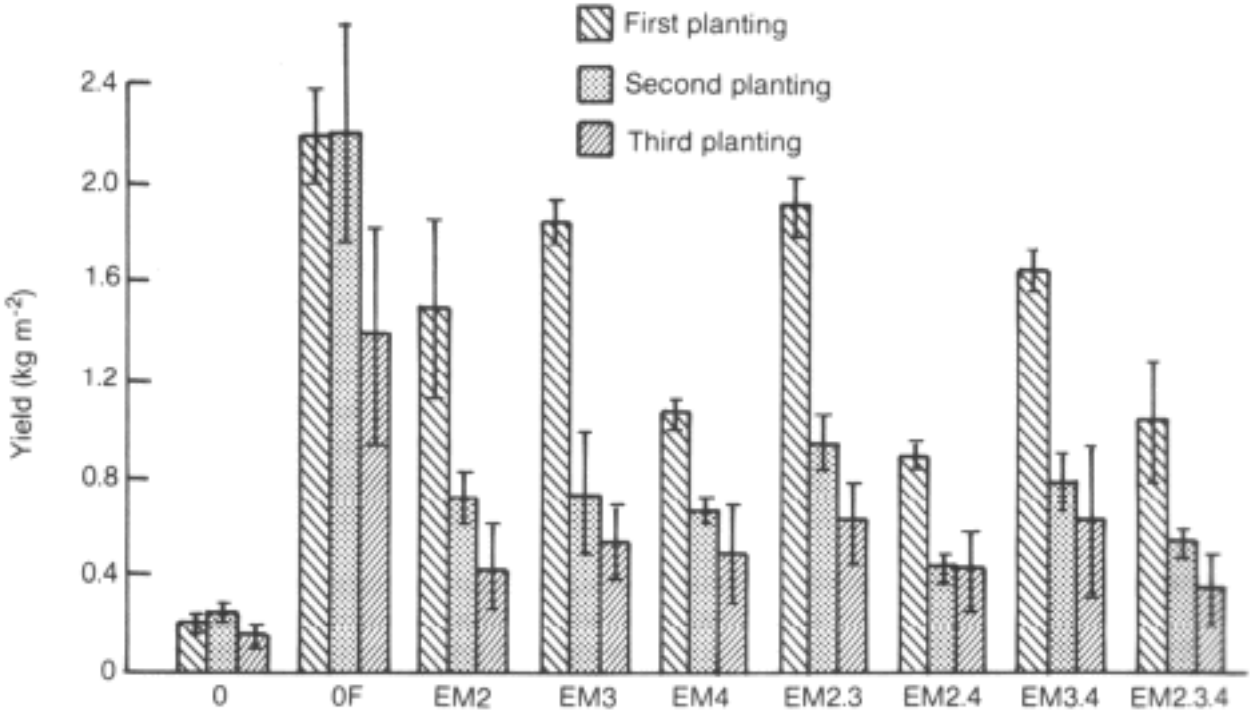

Figure 3. The Effect of EM Cultures on the Yield of Spinach.

Discussion

The lowest number of actinomycetes occurred in soil treated with fertilizer (OF) suggesting that these microorganisms may somehow have been suppressed, either directly or indirectly, by the fertilizer components. Beliaev (1958) found that continuous application of ammonium fertilizer without lime can suppress the actinomycetes since the ammonium is oxidized to nitric acid by microbial action. The resultant decrease in soil pH can cause unfavorable growth conditions.

The generic analysis of the bacterial flora (Table 2) showed that fermentative bacteria such as *Enterobacter*, starch digesting bacteria, *Azotobacter*, and *Clostridia*, are present in soil treated with EM and the fertilized control (OF), but to a lesser extent in the unfertilized control (O). This may have been due to the effect of some specific nutrient requirement for the growth of fermentative bacteria. Gyllenberg (1956) reported seasonal variations in which the relative abundance of Aa grouping bacteria increased, with a corresponding decrease in the abundance of Ba grouping bacteria. It remains unexplained whether the increase in the relative abundance of the Aa grouping bacteria was accompanied by the accumulation of specific nutrients such as amino acids.

There is not a clear relationship between EM treatments and the number of soil disease bacteria, e.g., *Xanthomonas*, *Erwinia*, *Agrobacterium*, and *Pseudomonas*, as shown in Table 2. But in the preliminary experiment it appeared that treatment with EM 4 is associated with a rather low population of disease bacteria.

The effect of EM on fungal populations in soil (Table 3), indicated that soil treated with only fertilizer had low numbers of *Penicillium* and *Trichoderma*. These beneficial fungi can play an important role in inhibiting or suppressing soil-borne microbial plant pathogens through their antagonistic activities. Large numbers of fungal disease pathogens were found in both of the control treatments.

The effect of EM on soil physical properties suggests that EM can induce plant roots to penetrate soil more effectively. Soil treated with EM becomes more friable and porous, less compact, and

promotes deeper cultivation. Microorganisms, particularly fungi, can bind soil particles into more stable aggregates. Bacteria can synthesize cementing agents in the form of gums and polysaccharides that also help to promote good aggregation. Lynch (1981) found that *Azotobacter chroococcum*, *Lipomyces starkeyi*, and *Pseudomonas spp*. can promote the stabilization of soil aggregates.

Insoluble soil phosphorus compounds (both organic and inorganic) are largely unavailable to plants, however, many microorganisms can solubilize these compounds and make them available for uptake. Martin (1961) found that one-tenth to one-half of the bacterial isolates he tested were capable of solubilizing calcium phosphate. Fungal species of the genera *Pseudomonas*, *Mycobacter*, *Micrococcus*, *Flavobacterium*, *Penicillium*, *Sclerotium*, *Aspergillus*, and others are also known to solubilize insoluble phosphates to plant-available forms.

EM treatment has an indirect effect on covering or healing tomato fruit injuries caused by green june bug (figeater). Fruit damage was greatest for the controls and for the EM 2 treatment. However, fruit damage was considerably less with the other EM cultures compared with the controls. These results are probably soil specific. Soils that do not have a good fermentation potential can produce malodors and attract harmful insects that prefer to lay their eggs in that soil. Nevertheless, it is noteworthy that three of the EM treatments, EM 3, EM 4, and EM 2.3 produced yields that were comparable to, though less than, the fertilized control. These three EM cultures also produced a greater amount of marketable fruit than the fertilized control indicating a beneficial effect of EM on fruit quality. The actual role of EM in covering tomato fruit injuries needs further investigation to determine precisely what relationships and mechanisms are involved in this process.

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