Potential Use of EM for Control of Phytopathogenic Fungi and Bacteria

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Abstract
Inhibition of fungi and bacteria by Effective Microorganisms (EM) was investigated by a double-layer plate method. Colony diameters of fungi (*Sclerotium rolfsii*, *Pythium* sp., *Rhizoctonia solani, Colletotrichum gloeosporioides, Alternaria* sp., *Thielaviopsis paradoxa, Phytophthora capsici, Aspergillus* sp., *Fusarium moniliforme* and *Fusarium oxysporum fs phaseoli*) and numbers of bacteria (*Xanthomonas campestris* pv *vesicatoria* and *Pseudomonas solanacearum*) were compared with untreated controls after 24 or 48 hours of incubation. Phytopathogenic fungi and bacteria were generally inhibited by EM in this study.

Introduction
Suspensions of Effective Microorganisms or EM are mixed cultures of naturally-occurring beneficial microorganisms that can be applied as inoculants to restore nutritionally and biologically degraded soils (Higa, 1994). As a soil inoculant, EM has been reported to decrease the inoculum density of several crop pathogens (Lin, 1991; Higa and Wididana, 1991). Field observations prompted *in vitro* tests to determine the effect of EM on selected phyiopathogens.

Materials and Methods
Pure cultures of phytopathogenic fungi and bacteria were used for the in vitro tests. The fungi included: *Sclerotium rolfsii*, *Pythium* sp., *Rhizoctonia solani, Colletotrichum gloeosporioides, Alternaria* sp., *Thielaviopsis paradoxa, Phytophthora capsici, Aspergillus* sp., *Fusarium moniliforme*, and *Fusarium orysporum* fs *phaseoli*. Pure cultures of bacteria (*Xanthomonas campestris* pv *vesicatoria* ENA 4347 and *Pseudomonas solanacearum* ENA 2720) were also used.
Mycelial disks of 0.7 cm diameter were used as inoculum from pure cultures of fungi grown on potato dextrose agar (PDA). Each test petri plate was prepared with one layer of PDA with either 1 or 5 percent concentration of EM, dried for 24 hours, and then covered by another layer of PDA without EM on which the inoculum disks were planted. Control plates were similarly prepared but without addition of EM. Each treatment consisted of two plates with EM and one control plate without EM. In each test, three evaluations of mycelial growth were made, beginning at the edges of the disks, after 24 and 48 hours in a BOD incubator at 30^{o}C. Each measurement for either growth period was considered as a replication.
The bacterial inoculum in an 8.5 percent salt solution was standardized at 600 nm with a colorimeter. For each standard bacterial suspension, 0.1 ml of serial dilutions to 10^{-8} were placed into each petri dish with a bottom layer of PDA plus 0.5 or 1 percent of EM supernatant centrifuged at 7500 times gravity for 10 minutes under aseptic conditions. A second layer of dextrose/yeast extract/glutamic acid/peptone agar (DYGS) was added for *X.campestris*; TTC medium (Kelman) was added for P. *solanacearum*. Each treatment consisted of two petri dishes (with EM) and one control (without EM) for each dilution. Each test, conducted at four different times, was considered as a replication. Data were obtained for the number of colonies for each serial dilution after 48 hours of incubation at 30^{o}C.

Results and Discussion
Percent inhibition of mycelial growth of selected phytopathogenic fungi by EM is shown in Table 1. EM at the 1 percent level had little or no effect on the growth of *Aspergillus* sp., *S. rolfsii, Pythium* sp., *R. solani, F. moniliforme*, and *F. oxysporum*. The five percent EM level was more effective in inhibiting fungal growth than the 1 percent level. Mycelial growth of six of the ten phyiopathogenic fungi was reduced by 50 percent or more at 48 hours in media that was amended with concentrated EM solution. *Pythium* sp. was the most sensitive to EM and *Aspergillus* sp. was the least sensitive.

In another test (data not shown), EM inhibited six of the fungi in soil.

Table 1. Percent Inhibition of Mycelial Growth of Selected Phytopathogenic Fungi at Two Levels of EM and after Two Periods of Incubation.

Fungi	1% EM		5% EM	
	Hours of Incubation			
	24	48	24	48
	(% inhibition)			
S. rolfsii	0	0	46	26
Pythium sp.	17	0	100	100
R. solani	11	0	49	50
C. gloeosporioides	33	20	50	20
Alternaria sp.	0	25	0	38
F. moniliforme	0	9	50	55
F. oxysporum	0	10	33	55
P. capsici	42	35	83	57
Aspergillus sp.	0	0	20	0
T. paradoxa	8	33	23	67

Average of 6 replications.

Pseudomonas solanacearum (control, 5.5 x 10^8 CFU) was decreased by 5- and 10-fold in 0.5 and 1.0 percent of added EM, respectively; *Xanthomonas compestris* pv *vesicatoria* was decreased by 15-fold in 0.5 and 1.0 percent of added EM, compared with the control (47 x 10^6 CFU).

The *in vitro* result obtained with *Xanthomonas compestris* pv *vesicatoria* was confirmed by recently conducted field experiments with pepper (*Capsicum annum* ev *margareth*) (Castro t al., 1996). Soils that were infested by *P. solanacearum* and then treated with bokashi, green manure and EM for three consecutive years have since had low levels of bacterial infections when planted with solanaceous crops. These results appear to be related to the *in vitro* effect of EM observed in these tests.

The high concentration of EM needed to inhibit phytopathogenic fungi and bacteria seems somewhat exaggerated when compared with the concentrations commonly applied in the field. However, with several soil applications during the cropping period and the capacity of microorganisms to increase in number, a balance between beneficial and disease-producing microorganisms seems to have occurred.

References

Castro, C. M. S. D. Motta, D. S. Pereira, F. Akiba, and R. D. L. Ribeiro. 1996. Effective microorganisms for control of *Xanthomonas campestris* pv *vesicatoria* in sweet pepper. p. 239-241. In J. F. Parr, S. B. Hornick and M. E. Simpson (ed.) Proceedings of the Third International Conference on Kyusei Nature Farming. U.S. Department of Agriculture, Washington, D.C., USA.

Higa, T. 1994. Effective Microorganisms: A new dimension for nature farming. p.20-22. In J. F. Parr, S. B. Hornick and M. E. Simpson (ed.) Proceedings of the Second International Conference on Kyusei Nature Farming. U.S. Department of Agriculture, Washington, D.C., USA.

Higa, T. and G. N. Wididana. 1991. Changes in the soil microflora induced by effective microorganisms. p. 153-162. In J. F. Parr, S. B. Hornick and C. E. Whitman (ed.) Proceedings of the First International Conference on Kyusei Nature Farming. U.S. Department of Agriculture, Washington, D.C., USA.

Lin, D. L. 1991. Nature farming in Taiwan: Effect of EM on growth and yield of paddy rice. p. 125-131. In J. F. Parr, S. B. Hornick and C. E. Whitman (ed.) Proceedings of the First International Conference on Kyusei Nature Farming. U.S. Department of Agriculture, Washington, D.C., USA.