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Effectiveness of F-68 Soil Conditioner

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CONTENTS

SUMMARY

The number and types of microorganisms in F-68 Soil Conditioner were characterized using standard microbiological techniques. F-68 was applied to three soils under laboratory and greenhouse conditions to determine its effect on soil biological properties, chemical properties, and chile (*Capsicum annuum* L) growth. Soil biological and chemical properties evaluated in the laboratory included microbial respiration, dehydrogenase activity, acid phosphatase activity, alkaline phosphatase activity, sulfatase activity, pH, EC, NO_3-N, NH_4-N, and available P. Under greenhouse conditions, F-68's effectiveness was evaluated on soil pH, EC, NO_3-N, NH_4-N, available P, and chile growth. F-68 was applied according to label instructions at one-half the recommended rate, the recommended rate, and double the recommended rate.

The number of bacteria in F-68 was 2.78 x 10^3/ml or 1.05 x 10^7/gal. A wide variety of bacteria were found. This bacteria level was low, considering that the recommended application rate is 1 gal per acre.

F-68 had no effect on soil biological properties, soil chemical properties, or chile growth in either the laboratory or greenhouse experiments. F-68 behaved similarly in all three soils and did not improve soil properties or decrease chile disease incidence.

F-68 cannot be recommended as a soil conditioner to increase nutrient availability, reduce salinity, reduce pH, increase soil biological activity, reduce disease incidence, or increase chile growth. Furthermore, it is unlikely that F-68 could influence soil properties or plant growth given the number of microorganisms contained in the product, the application method, and the soil microbial environment considerations.

EFFECTIVENESS OF F-68 SOIL CONDITIONER

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Using microorganisms to increase agricultural productivity is not new. Microorganisms have been applied to soil to increase nitrogen fixation in legumes since the late 1800s (Alexander, 1977; Tate, 1995). Most microbial inoculants have been applied to soil in traditional agricultural settings to increase nutrient availability, reduce plant disease and nematodes, and protect plants against adverse soil conditions (Metting, 1992; Sylvia et al., 1998). In environmental scenarios, microorganisms have been applied to soils to remediate contaminated soil (Skladany and Metting, 1992; Skipper, 1998).

Successful uses of soil microbial inoculants occur when a specific crop has a specific problem. Explicit microbial inoculants used under these circumstances have proven to be useful. Examples include inoculating soybeans in the Midwest with *Bradyrhizobium japonicum* to increase nitrogen fixation (Graham, 1998), *Phytophthora cactorum* control by *Trichoderma* and *Gliocladium* species (Graham and Mitchell, 1998), and *Gaeumannomyces graminis* control (take-all disease in wheat) by *Fusarium* spp. and fluorescent pseudomonads (Graham and Mitchell, 1998). No scientifically scrutinized microbial inoculants have been found that produce a variety of chemical and biological transformations in

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numerous types of crops under a variety of situations. Inoculating established root systems is difficult and results in a low probability that the inoculated populations will develop (Tate, 1995). Competition of inoculated microorganisms with indigenous microbial populations is the most often cited cause of inoculant failure. The added microorganism must be able to overcome the normal soil community defenses to invasion and cope with soil physical and chemical barriers (Graham and Mitchell, 1998; Tate, 1996). Seed or seedling inoculation has been successful, but only with microorganisms that have specific purposes. F-68 Soil Conditioner (Fenic Co., Mercedes, TX) is commercially sold in New Mexico's Mesilla Valley, Texas, and Mexico. Promotional literature claims that F-68 decreases soil salinity, increases nutrient availability, and reduces disease incidence among other benefits. The New Mexico Chile Commission, through concern generated by Mesilla Valley chile growers, was interested in the effectiveness of F-68 Soil Conditioner. The objective of this research was to determine F-68's effect on soil biological and chemical properties and chile growth.

MATERIAL AND METHODS

Source of F-68 Soil Conditioner

Two batches of F-68 Soil Conditioner (Fenic Co., Mercedes, TX) were obtained from the local distributor (Western Blend Inc., Las Cruces, NM). The first batch had passed the expiration date and was used only to perfect microbiological techniques and gain experience with handling the material. The second batch was obtained on Aug. 8, 1997 and had just been received by the distributor. All experiments were conducted on the fresh batch of F-68. Some chemical properties of F-68 are given (table 1). Chemical analysis was performed by the Soil, Water, and Air Testing Laboratory at New Mexico State University using standard procedures (Page, 1982). Aseptic techniques and unchlorinated water were used in all experiments with F-68. Handling and application rates followed label instructions (F-68 brochure).

Table 1. Properties of F-68 and the Harkey Loam, Belen Clay, and Brazito Sandy Loam soils as collected from the field in July, 1997.

	Soils			
Soil property	Harkey	Belen	Brazito	F-68
Texture	Loam	Clay	Sandy loam	—
Sand (%)	38.4	26.4	59.4	—
Silt (%)	38.0	26.0	26.0	—
Clay (%)	26.6	47.6	14.6	—
pH	7.5	7.4	7.9	7.9
Elect. conductivity (dS m^{-1})	8.2	9.2	2.3	8.5
Sodium adsorption ratio	5.9	5.0	5.2	—
Organic matter (%)	1.0	0.7	0.3	—
Nitrate (mg/kg)	140	345	11	0.8
Phosphorus (mg/kg)	50	37	13	BDL
Potassium (mg/kg)	110	101	45	176
Zinc-DTPA (mg/kg)	0.9	0.5	0.5	—
Manganese-DTPA (mg/kg)	12.4	4.8	4.8	0.1
Iron-DTPA (mg/kg)	13.6	6.7	6.7	0.1
Copper-DTPA (mg/kg)	1.3	1.2	1.2	BDL

— = Not determined
BDL = Below detection limit

Laboratory Characterization of F-68

The numbers and types of microorganisms in F-68 were characterized using standard plating techniques (Wollum, 1982). Dilutions of F-68 from 10^{-1} to 10^{-5} were made in sterile saline and plated on Tryptone Soy Agar, Nutrient Agar, Czapek Dox Agar (Difco, Inc., Detroit, MI), and Soil Extract Agar (Wollum, 1982) with five replications per dilution. Colony development was observed daily, and the plates were counted after 7 days of incubation. Major colony types were observed, but the bacteria were not characterized or identified.

Soils

Three commonly cultivated Mesilla Valley soils (0-15 cm or 0–6 in) were collected in July 1997. The Harkey Loam was collected from an onion field approximately four miles south of Las Cruces. The field had a history of severe *Phytophthora* root rot. The Brazito Sandy Loam was collected from a fallow area at the Fabian Garcia Research Center just west of New Mexico State University. The Belen Clay was collected from a chile field just north of Glass Road (Mesilla, NM). This field had been planted to chile for many years and had a history of severe *Phythophthora* root rot. All soils were sieved through a 2 mm (.08 in) sieve and stored moist at room temperature in plastic bags. Properties of the soils collected are given (table 1). Soil analysis was by the Soil, Water, and Air Testing Laboratory at New Mexico State University using standard procedures (Page, 1982).

Laboratory Experiments

Experiments were designed to determine F-68's effect on soil microbial activities under laboratory conditions. Soils were moistened to approximately field capacity and 100 g (wet weight) of each soil were placed into one .47 *l* (1 pint) jars. Moistening the soils consisted of adding sterile water until the soil became as wet as possible without puddling. Moisture contents were 19.8%, 16.4%, and 8.2% for the Harkey, Belen, and Brazito soils, respectively. Four treatments (F-68 application rates) were applied to each soil with three replications per treatment in a completely random

design. The treatments were one-half the recommended F-68 application rate (One- Half Rate), the recommended rate (Recommend Rate), double the recommended rate (Double Rate), and control (sterile water added in place of F-68). According to label instructions, the recommended F-68 application rate was 9.3 l/ha (1 gal/acre). Dilutions of F-68 were made in sterile water to apply the correct amount of F-68 to 100 g of soil. Biological activity measurements on the treated soils included respiration, dehydrogenase enzyme activity, acid phosphatase activity, alkaline phosphatase activity, and sulfatase activity. Respiration (CO_2-evolution) was measured using the sodium hydroxide trapping method (Anderson, 1982) with daily titrations for one week. Enzyme activity (dehydrogenase, phosphatase, and sulfatase) was determined on the same sample at the conclusion of respiration measurements (Tabatabai, 1982).

Chemical analysis of the soil included soil pH (saturated paste), EC (saturated paste extract), bicarbonate extractable phosphorus or available phosphorus (Olsen and Sommers, 1982), and KCl extractable NH_4-N and NO_3-N (Keeney and Nelso, 1982).

Greenhouse Experiment

The greenhouse experiment was designed to determine F-68's effect on chile growth and soil properties. Freely draining pots (24.8 cm or 9.75 in diameter by 24.8 cm or 9.75 in deep) were filled with soil within one 2.54 cm (1 in) of the top and watered until drainage occurred. All pots were fertilized at the rate of 224 kg N (urea)/ha (200 lb N /acre) and 179 kg P_20_5 (triple super phosphate)/ha (160 lb P_20_5/acre). Chile seeds (Joe E. Parker) were planted on Sept. 2, 1997. Seedlings were thinned to two plants per pot after germination. Four treatments (F-68 application rate) were applied to each soil with four replications per treatment in a randomized complete block design. The F-68 treatments were as previously described for the laboratory experiment. Dilutions of the F-68 were made in sterile water to apply the correct amount of F-68 to each pot. The pots were watered when the rooting zone became dry. Sufficient water was applied to encourage drainage out the bottom of the pots. Temperatures were maintained between 65° and 95°F.

The experiment was terminated the last week of December 1998. Chile plants were cut off at the soil surface, oven dried at

150°F for 3 days and weighed. A soil sample from the top 15 cm (6 in) of each pot was taken with a 2.54 cm (1 in) in diameter soil probe. Soil samples were analyzed for pH, EC, NH_4-N, NO_3-N, and bicarbonate extractable P, as previously described. No attempt was made to diagnose diseased plants.

Statistical Analyses

All statistical analyses were performed using the statistical analysis system (SAS Institute, 1991). Analyses included analysis of variance and least significant differences (LSD) as the mean separation procedure.

Results

Properties of the three soils used are given (table 1). Both the Harkey and Belen soils were saline and had high to excessive nutrient levels.

F-68 was a transparent, green-colored liquid with no odor. The number of viable microorganisms was low (table 2). The Czapek Dox and Soil Extract media gave the highest number of viable bacteria. No fungi developed on the plates. Many different colony types were found, indicating that F-68 was not a pure culture of bacteria or mixture of several bacteria. Eight of the most commonly encountered colony types are given (table 3).

F-68 had no statistically significant (P>0.05) effect on soil chemical or biological activity under laboratory conditions (tables 4 and 5). The interaction of F-68 and soil type was not statistically significant for any of the soil properties. Soil types were signifi-

Table 2. The number of viable bacteria in F-68 as determined on four different media.

Medium	Viable bacteria
	(number/ml)
Tryptone soy agar	1.40×10^3
Nutrient agar	1.86×10^3
Czapek Dox agar	4.70×10^3
Soil extract agar	3.16×10^3
Mean	2.78×10^3

Table 3. Eight of the most common colony morphologies of bacteria found in F-68.

1. Round, shiny, translucent colonies less than 1 mm in diameter.
2. Round, orange, opaque colonies less than 1 mm in diameter.
3. Round, cream-colored colonies less than 1 mm in diameter.
4. Round, shiny, pink, opaque colonies less than 1 mm in diameter.
5. Round, cream-colored colonies with a small indention in the center and 3–5 mm in diameter.
6. Round, orange, shiny colonies with an opaque ring around the center and 3–4 mm in diameter.
7. Irregular, cream-colored colonies with dendritic extensions and 1 cm in diameter.
8. Irregular colonies with a fuzzy haze on the perimeter and 5 mm in diameter.

Table 4. The effect of F-68 and soil type on soil chemical properties under laboratory conditions. Soil type significantly (P<0.05) affected soil chemical properties. Neither the F-68 rate nor the F-68 rate by soil interaction significantly affected soil chemical properties.

F-68 Rate	pH	EC	NO_3 N	NH_4 N	Available P
		dS/m	———	mg/kg	———
			Harkey Loam		
Control	7.7	6.0	115	0.0	37.6
One-half rate	7.6	5.6	131	0.0	37.6
Recommended rate	7.6	6.4	129	0.0	36.5
Double rate	7.7	6.0	135	0.0	44.4
			Belen Clay		
Control	7.7	5.4	312	0.0	30.7
One-half rate	7.8	5.7	316	0.1	31.5
Recommended rate	7.7	6.1	153	0.5	31.9
Double rate	7.7	6.2	294	0.0	33.8
			Brazito Sandy Loam		
Control	8.2	1.7	14	0.0	12.5
One-half rate	8.1	1.6	17	0.0	12.3
Recommended rate	8.1	1.5	17	0.0	12.9
Double rate	8.2	1.5	17	0.0	13.3

cantly different for all soil properties, except NH_4-N and sulfatase. Soil type differences were expected and are of no particular interest to the study unless interactions occurred between soil type and F-68. In general, the Harkey and Belen soils had more biological activity and higher levels of nutrients and salts.

F-68 had no statistically significant (P>0.05) effect on soil properties in the greenhouse experiment (table 6). As with the laboratory experiment, soil types were significantly different, but there was no statistically significant interaction of F-68 with soil type. Soil chemical properties were considerably different from those in the laboratory experiment because of extensive leaching of the pots.

Table 5. The effect of F-68 and soil type on soil biological properties under laboratory conditions. Soil type significantly (P<0.05) affected soil biological properties. Neither the F-68 rate nor the F-68 rate by soil interaction significantly affected soil biological properties.

F-68		Soil enzyme activity			
Rate	CO_2	Dhase	Acid-P	Alk-P	Sulfatase
	mg/kg				
			Harkey Loam		
Control	22.4	3.6	47.4	148	10.4
One-half rate	33.2	3.3	54.0	112	9.3
Recommended rate	31.4	3.4	58.1	107	11.1
Double rate	39.1	3.3	51.2	126	10.1
			Belen Clay		
Control	57.6	2.0	33.5	168	10.4
One-half rate	57.0	2.2	37.6	163	9.0
Recommended rate	58.2	2.8	37.8	169	10.7
Double rate	62.3	2.6	35.5	162	11.6
			Brazito Sandy Loam		
Control	51.7	1.9	21.1	94	9.6
One-half rate	44.9	1.6	25.3	85	8.6
Recommended rate	51.7	1.8	23.4	94	8.6
Double rate	56.5	1.8	24.3	96	9.9

Dhase = dehydrogenase
Acid-P = acid phosphatase
Alk-p = alkaline phosphatase

Table 6. The effect of F-68 and soil type on soil chemical properties under greenhouse conditions. Soil type significantly affected (P<0.05) soil properties. Neither the F-68 rate nor the F-68 rate by soil interaction significantly affected soil properties.

F-68			NO_3	NH_4	Available
Rate	pH	EC	N	N	P
		dS/m	mg/kg		
			Harkey Loam		
Control	8.0	2.5	14.6	7.4	40.7
One-half rate	7.8	3.1	14.3	4.1	53.6
Recommended rate	7.8	3.2	19.4	3.8	44.3
Double rate	7.8	3.5	15.2	3.8	44.7
			Belen Clay		
Control	7.8	2.7	17.3	2.6	27.1
One-half rate	7.8	3.6	12.3	2.2	33.3
Recommended rate	7.8	4.0	6.8	1.8	29.6
Double rate	7.8	3.9	20.8	2.5	32.7
			Brazito Sandy Loam		
Control	7.7	1.9	18.7	4.0	22.9
One-half rate	7.8	2.1	18.4	3.2	25.6
Recommended rate	7.9	2.4	17.6	4.7	21.6
Double rate	7.8	2.8	13.1	4.7	26.0

F-68 treatment and soil type significantly affected (P<0.05) chile biomass (table 7), but the interaction of F-68 treatment and soil type was not statistically significant. Chile growth was significantly less in the Brazito Sandy Loam than in the Harkey Loam or Belen Clay. Chile growth was slow in all pots, many seedlings died early in the experiment. The slow growth of the chile was attributed to the low light conditions from September to December. Seedling death was not diagnosed, but damping off and salt stress were at least two of the possible causes. No effect of F-68 was seen on disease incidence during the experiment. Caution must be used when interpreting table 7. Two pots in the control treatment, with Harkey and Belen soils, had about eight times higher biomass production than other pots in these two soils. These two pots skewed the data in favor of the control. Data from these two pots could not be discarded without due cause and were left in the analysis. A subsequent analysis of variance without these two pots indicated no difference between F-68 treatments and the control.

Table 7. The effect of F-68 on chile growth. Chile growth was significantly affected ($P<0.05$) by F-68 rate and soil type, but F-68 by soil interaction was not statistically significant.

F-68 Rate	Dry chile biomass
	(g)
Control	5.08 a*
One half rate	2.90 b
Recommended rate	1.70 b
Double rate	1.09 b

* Means followed by the same letter are not significantly different by the LSD ($P<0.05$).

DISCUSSION

To more broadly test F-68's effectiveness, three soil types were used. Of particular interest was the expectation that F-68 would be more effective on a particular soil type. Thus, soil type differences and the interaction of F-68 and soil type were expected. However, F-68 did not improve soil chemical properties, soil biological properties, or chile growth as claimed in promotional literature. Soil type did not influence F-68's effectiveness. The only consistent response in the experiments was that the three soils were different.

The soils chosen for the experiments were ideal because they had problems that F-68 claims to improve. The Belen and Harkey soils were saline and had severe problems with *Phytophthora* wilt. The Brazito soil had low levels of P and K. If F-68 was effective, soil biological activity and chile growth should improve with F-68 application. Additionally, a response parameter would be expected to increase with increasing rates of F-68 application.

The Belen and Harkey soils did not need fertilizer applied before the greenhouse experiment because they already had excessive levels of NO_3-N and high levels of P and K. However, the soil analysis was not complete by the time the greenhouse experiment started. Leaching during the greenhouse experiment considerably lowered the soil's salinity and NO_3-N levels to more typical levels

by the end of the experiment, regardless of F-68 treatment. F-68 had no effect on soil salinity or soil pH in the laboratory experiment where leaching did not occur or early in the greenhouse experiment before leaching had removed some of the excessive salts. Also, it is unclear how adding microorganisms could "correct salinity in the soil," "restore the productivity of soils affected by excessive salinity," or "reduce the soil alkalinity" (F-68 brochure). In order to influence salinity, microorganisms would need to take salts from the soil solution by absorption, adsorption, or chemical precipitation. The occurrence of these mechanisms appears remote when considering the number of indigenous soil microorganisms, the amount of microorganisms added by F-68, and the amount of salt in these soils.

The nutrient status of the soils was not enhanced with F-68. According to promotional literature, F-68 releases blocked fertilizer nutrients and increases residue decomposition in "nonorganic" farming scenarios. Microorganisms function by producing enzymes that catalyze reactions. Respiration (CO_2 evolution) and dehydrogenase are measures of general microbial activity. If F-68 microorganisms release blocked fertilizer nutrients or increase decomposition, an increase in general microbial activity would occur and more nitrogen and phosphorus would be made available. Specific enzymes such as the acid and alkaline phosphatases and sulfatase also would increase because these enzymes are specifically involved in the release of phosphorus and sulfur from unavailable phosphorus and sulfur in soil. Because F-68 had no influence on general microbial activity or specific enzyme activities, an increase in plant nutrients, particularly phosphorus, would seem unlikely.

All plants showed signs of disease during growth. Promotional literature (F-68 brochure) states that F-68 combats soil fungi known to be harmful to crops and is effective for controlling *Phytophthora* on peppers. No response was seen in the vigor of chile vegetative growth. If F-68 was effective in combating disease, disease should have been greatest in the control and least in the Double Rate treatment.

Microorganisms introduced to the soil ecosystem have a hard time competing with indigenous microorganisms and establishing themselves in an environment that typically has 10^7–10^9 bacteria/g of soil (Alexander, 1977; Paul and Clark, 1989; Sylvia et. al, 1998;

Tate, 1995). Many soil fungi, protozoa, nematodes, and viruses prey on bacteria and are also present in the range of 10^6–10^8 microorganisms/g of soil. Before introduced bacteria can carry out a particular function, they must be able to survive and multiply in an environment that is extremely competitive and hostile to laboratory grown microorganisms. The use of introduced microorganisms to impart some beneficial reaction in agricultural scenarios is best achieved when the microorganisms are added directly to seed or at seeding (Kloepper, 1992). Additionally, high numbers of organisms are typically added with the expectation that most will die and a few may survive to carry out the desired reaction (Graham and Mitchell, 1998). For example, the number of *Rhizobium* in legume inoculants generally is greater than 10^9/g, and the inoculant is applied directly to the seed or planting furrow, giving populations of 10^4–10^9 bacteria/seed (Paul and Clark, 1989).

The difficulty with establishing an effective microbial population by spreading a liquid that contains a low level of microorganisms on an acre of soil is demonstrated below. A typical loam soil has a bulk density of 1.35 g/cm^3 and a typical bacterial population of 1 x 10^7/g. Also, most of the active roots are contained in the top foot of soil. The dry weight of this acre of soil, 1 foot deep is 6,674,619 lb and contains 1.7 x 10^{16} bacteria. The F-68 used in the experiments contained 2.78 x 10^3 bacteria/ml or 1.05 x 10^7 bacteria/gal. Thus, one gallon of F-68 applied to one 1 acre of soil and penetration of 1 foot deep would result in 241 bacteria/acre foot or one F-68 bacterium competing with 1.58 x 10^9 indigenous soil bacteria. The likelihood that F-68 can influence soil properties appears remote.

We conclude that F-68 had no influence on soil chemical or biological properties or chile growth under the conditions of the experiments described above. Furthermore, it is unlikely that F-68 could influence soil properties or plant growth given the number of microorganisms contained in the product, the application method, and soil microbial environment considerations.

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