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Exploiting and Understanding Disease Suppressing Effects of Fish Emulsion for Soil-borne and Foliar Diseases

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ABSTRACT

Organic soil amendments, such as fish emulsion (FE), provide nutrients to crops, alter the biological, chemical, and physical properties of soil, and can suppress plant diseases. FE is a liquid fertilizer used primarily for crop production, but we recently found that FE can also act as a disease management product. As a pre-plant soil amendment, FE provided suppression of seedling damping-off of radish and cucumber under growth room conditions. The damping-off protection increased with the length of incubation of FE in the substrate suggesting that disease control was biologically-based. Microbial activity was also enhanced in the FE-amended soils or substrate. Under micro-plot and field conditions, FE as a pre-plant soil amendment to various soils from commercial potato fields with low to high scab levels also reduced potato scab severity in some soils and increased tuber yield in most soils. In the greenhouse trials, FE added to a sandy-loam soil protected eggplants from verticillium wilt and increased plant biomass. Foliar sprays of diluted FE solutions reduced bacterial spot severity on tomatoes and peppers and increased fruit yield. In the laboratory tests, FE reduced the viability of *Verticillium dahliae* microsclerotia within 1 day after incorporation in a sandy-loam soil indicating that FE contains toxic substances. We detected high quantities of organic acids including some known toxicants such as acetic and formic acids in the FE samples. A mixture of these organic acids at the proportions found in FE provided pathogen or disease suppression depending on the soil and substrate. FE is an excellent model system for development of an organic amendment as fertilizer with disease suppressing effects.

Keywords: biological control, disease management, organic amendment, microbial activity, soil-borne plant pathogen, volatile fatty acid
Abbreviations: BCA, biological control agent; **FE**, fish emulsion; **N-P-K**, N-P_2O_5-K_2O; **VFA**, volatile fatty acid

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INTRODUCTION

Plant diseases caused by soil-borne pathogens and plant-parasitic nematodes are a major factor in crop losses worldwide. Chemical pesticides, particularly broad-spectrum biocides, can provide effective control of such pathogens and pests, but they can be expensive and are becoming undesirable due to increasing public concerns about their impacts on the environment and on human health. On the other hand, the demand for reduced-risk and non-chemical options of managing soil-borne diseases is growing. Organic soil amendments from the agriculture-related industry have been used for centuries as fertilizers, but their effects on plant diseases were also noticed. A general or long-term effect of organic soil amendments is their potential to enhance microbial activity including bio-control agents (BCAs) leading to the establishment of natural disease suppressive conditions (Hoitink and Boehm 1999; McKellar and Nelson 2003; Abbasi *et al*. 2007; Perez *et al*. 2008). Organic amendments increase the activity and diversity of resident microbial communities by increasing the organic matter content of the amended soil (Mäder *et al*. 2002), and thereby may provide a climate more favourable to natural biological control of soil-borne plant pathogens. Also, it appears that the decomposition of organic amendments can result in the generation of compounds that kill plant pathogens (Lazarovits 2001; Abbasi *et al*. 2005; Lazarovits *et al*. 2005; Abbasi *et al*. 2007) and nematode pests (Akhtar and Malik 2000; Abbasi *et al*. 2005). Short-term effects may be more specific to a particular type of amendment that results in an immediate reduction of pathogen inoculum (Tsao and Oster 1981; Tenuta and Lazarovits 2002; Mazzola *et al*. 2007). High nitrogenous and volatile fatty acid (VFA)-containing amendments are excellent examples displaying an

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almost immediate reduction in pathogen populations via short-lived toxic metabolites (Tsao and Oster 1981; Tenuta *et al*. 2002; Conn *et al*. 2005; Lazarovits *et al*. 2005).

Organic matter, an important soil component for improving plant health (Magdoff and Weil 2004), has been severely depleted in many agricultural soils due to intensive cultivation over the years. This has resulted in degradation of soil structure. Organic amendments can play an important role in revitalizing such soils by increasing their organic matter content. They can also retain and provide nutrients to plants, improve soil physical, chemical, and biological properties with greater biological diversity, and increase soil buffering capacity (Grandy *et al*. 2002). Such changes can create conditions that are suppressive to soil-borne plant pathogens (Davis *et al*. 2001; Bailey and Lazarovits 2003), and organic matter-mediated treatments can even suppress foliar plant diseases (Aldahmani *et al*. 2005). Agricultural soils naturally suppressive to soil-borne plant pathogens and nematode pests have been found worldwide (Alabouvette 1999; Weller *et al*. 2002; Westphal 2005). The basis of suppressiveness in most cases has been attributed to biological activity (Weller *et al*. 2002), but soil chemical and physical factors may also play a role. It is possible to artificially create suppressiveness in soils but conditions may be specific and unique for each soil. In a nutshell, organic soil amendments can play an important role in plant or soil nutrition and health.

Plant health benefits and increased growth responses after application of various by-products of the fish industry are known. Fertilization of crops with fish by-products has a long history (Ceci 1975). Fish meal, the ground and heat-dried fish waste, has been used as a soil amendment with great success in vegetable production systems (Blatt and McRae 1998; Gagnon and Berrouard 1994). Soil amendment of fish meal or fish waste has also been implicated in pathogen or pest suppression (Wilhelm 1951; Akhtar and Mahmood 1995). In the very first report, fish meal as a nitrogen-rich amendment was compared with other soil amendments to suppress inoculum potential of verticillium wilt fungus in naturally infested clay-loam field soil in pot tests (Wilhelm 1951). In the pots amended with 1% fish meal (mass/mass dry soil), none of the tomato (*Lycopersicon esculentum* Mill. cv. 'Bonny Best') seedlings grown for 8 weeks showed verticillium wilt symptoms and all were negative for the fungus when plated on culture medium. Soil amendment of fish solid waste has been shown to reduce the populations of plant-parasitic nematodes (Akhtar and Mahmood 1995). Fish emulsion (FE), the concentrated fraction of the soup left after processing fish into fish meal, is applied to crops in a diluted form (1:100-1000) primarily as a foliar fertilizer (Aung *et al*. 1984), and no studies demonstrating the disease-suppressing effects of FE were reported in the literature prior to ours. In this review, a detailed overview of the work done in our laboratory with FE on plant growth promotion, disease suppression, and mechanisms of disease or pathogen suppression is presented.

FISH EMULSION

FE is made from whole fish or fish waste by cooking and acid processing. The heating process denatures complex proteins, carbohydrates and fats into simplified forms. The oils are then removed to prevent rancidity. Acid processing may involve the addition of sulphuric or phoshoric acids to stabilise the product and prevent premature break-down as well as to provide additional nutrient value. The heating process destroys enzymes and some proteins, micronutrients, vitamins, in FE therefore, essential micronutrients may be supplied with FE for optimal plant growth. Enzymatically-digested hydrolyzed liquid fish may have more nutrients than FE but that can be more expensive. One disadvantage associated with FE is its infamous foul smell though it may be short-lived and gone soon after application.

In our work with FE, the samples made from whole menhaden fish [*Brevoortia tyrannus* (Latrobe)] were provided by Omega Protein, Houston, TX, USA. A nutrient analysis of various FE samples by A&L Canada Laboratories East Inc., (London, ON, Canada) showed that they contained 50-54% dry matter content, 4-6% total nitrogen, 1.5-2% P_2O_5, 1.8-2.7% K_2O, and a C:N ratio of 4-6:1. Density of these FE samples was 1.2 and pH 2.4-2.8. These FE samples also had very high amounts of sulphur (12000-20000 ppm) and that can be explained by acidification of FE with sulphuric acid. According to information provided by Omega Protein, FE contains some heavy metals at non-toxic levels (e.g., chromium at < 4.0 ppm). While the composition of FE is fairly similar, some variability does occur from batch to batch. FE is water-soluble and that makes it an ideal product for application as sprays and with drip irrigation. The concentrated FE is pretty stable, however, once diluted with water FE should be used immediately.

GROWTH PROMOTION AND FERTILIZER EFFECT

The use of FE as a fertilizer is approved by the Organic Materials Review Institute (OMRI) for organic production in the US. The growth promotive effect of FE was determined in growth room trials using radish (*Raphanus sativus* L. cv. 'Early Scarlet Globe') and cucumber (*Cucumis sativus* L. cv. 'Straight Eight') as model systems in the absence of pathogens. Various rates of FE ranging from 1-4% (mass/mass mix) or equivalent N-P-K amounts from inorganic fertilizer source were added to a peat-based substrate in the pots and radish or cucumber seeds planted immediately. Plant fresh and dry weights were determined after 3 or 4 weeks of planting. Fresh and dry weights of radish and cucumber plants produced in FE-amended peat-based mix were heavier than the nonfertilized control plants and similar to the weights and heights as observed with plants fertilized with equivalent N-P-K amounts of inorganic fertilizer (Abbasi *et al*. 2004). Similar growth promotion effects of FE were also seen on tomato, tobacco (*Nicotiana tobacum* L. cv. 'CT 157'), or white Chinese leaf cabbage (*Brassica rapa* var. *chinensis* cv. 'Bok Choy'). These plants were grown for up to 8 weeks in the FE-amended peat-based mix and organic or muck soil.

In the peat-based mix, the 2-4% FE rate was optimal and provided sufficient nutrition for plants for up to 6-8 weeks of growth. The plants produced in FE-amended substrate were greener and healthier and did not show any sign of phytotoxicity. For plants growing in soil, 1% FE was often sufficient and rates higher than this were sometimes phytotoxic in sandy-loam soils when not added well before planting of seeds. At least 1-2 weeks a time delay is recommended between the application of FE and planting in sandy-loam soils depending on the nutrient contents of FE particularly N and moisture levels of soil. Nutrient analysis of FE samples should be conducted prior to its application to agricultural soils, and rates should be adjusted based on the N-P-K content of FE to avoid phytotoxicity. Similarly, soil moisture levels of field plots prior to applying FE should be considered to avoid phytotoxicity. High application rates (20,000 L/ha) of FE could cause severe phytotoxicity in relatively dry soil (Abbasi *et al*. 2006).

DISEASE SUPPRESSION

Preliminary studies were conducted in a growth room or greenhouse to determine the effects of FE for suppressing common seedling damping-off diseases and bacterial spot of tomato (processing cv. 'Heinz 9478') or pepper (*Capsicum annum* L. cv. 'Early Niagra') under artificial disease conditions. Several plant bioassays were optimized for this purpose. Based on the results of these preliminary studies, further greenhouse, micro-plot and field trials were initiated targeting some other important soil-borne diseases such as common scab and verticillium wilt, as described below.

Seedling damping-off

Damping-off suppressing effects of FE were assessed using the model systems, radish-*Rhizoctonia solani* Kühn and cucumber-*Pythium aphanidermatum* (Edson) Fitzpatrick or -*Pythium ultimum* Trow, in an artificially infested peat-based mix under controlled environmental conditions (Abbasi *et al*. 2004). FE (1-4% mass/mass mix), or the equivalent inorganic N-P-K fertilizer, was incorporated into pathogen-infested substrate and the mixtures were incubated for 1, 7, 14, and 28 days prior to planting radish or cucumber seeds. Plants were rated 14 days later for incidence and severity of damping-off. Seedlings produced in the peat-based mix incubated for only 1 day with FE were often as highly diseased as the control treatments, although occasionally significant disease reduction was seen (Abbasi *et al*. 2004). In the peat-based mix treated with 4% FE 7 days prior to seeding, 70-80% of the seedlings remained disease-free and when treated 28 days prior to seeding even the 1% rate of FE provided similar high levels of protection. The equivalent levels of inorganic N-P-K treatment provided no disease control (Abbasi *et al*. 2004).

This effect of FE was also tested in an artificially-infested sandy-loam and naturally-infested organic or muck soils. Incorporation of 0.5% (mass/mass soil) FE into a *R. solani*-infested sandy-loam soil 5 days prior to planting radish seed provided effective control of damping-off disease with more than 90% of the seedlings remaining healthy (Abbasi *et al*. 2004). Almost 80% seedlings were healthy in the second planting in the FE-treated soil compared to 25% in the control soil (Abbasi *et al*. 2004). Samples of muck soil naturally infested with *P. ultimum*, *P. irregulare*, and other *Pythium* spp. (Abbasi and Lazarovits 2005, 2006a) were collected from a commercial vegetable field near Holland Marsh, Ontario. These vegetable fields were also heavily infested with the clubroot pathogen *Plasmodiophora brassicae* (Abbasi and Lazarovits 2006b). When cucumber seeds were grown into this infested muck soil, the cucumber seedlings showed a high damping-off incidence and severity. FE (1 and 2% mass/mass soil) provided immediate protection of cucumber seedlings from damping-off in this infested muck soil and disease protection was consistent when planting was delayed for 1, 2, and 4 weeks after adding FE. There was also a significant increase in soil bacteria after addition of FE in muck soil.

The efficacy of FE to suppress damping-off of radish seedlings in peat-based mix may have been due to an increase in the resident microbial activity of the amended mix. Therefore, it is expected that the disease protection can vary from batch to batch of the mix depending on the pathogen and microbial activity for general or specific suppression (Cook and Baker 1983; Hoitink and Boehm 1999). The suppression of *Pythium* or *Phytophthora* damping-off and root rot diseases by organic matter-mediated general microbial activity is a good example of general suppression. Suppression of diseases caused by *R. solani* and *Fusarium oxysporum* is generally considered to be specific through the activities of one or several specific organisms (Cook and Baker 1983; Hoitink and Boehm 1999). A careful analysis of the type of microbial populations induced in the mix after FE amendment can provide further insight into the general or specific nature of disease suppression by FE.

Variability in disease suppression by FE among various batches of peat-based mix needs to be further addressed. In other words, can a disease non-suppressive batch of the mix be made as disease suppressive? To address this concern and for consistent efficacy, a previously described bio-control agent *Trichoderma hamatum* 382 was added to a disease non-suppressive batch of the peat-based mix and damping-off suppression of 1% FE in-combination with the bio-control agent was determined in a growth room bioassay. After 7 days of incubation, radish seedlings had a high incidence and severity of damping-off in the control, *T. hamatum* 382, and FE treatments (**Fig. 1**). However, the combination of *T. hamatum* 382 and FE increased the percentage of healthy seedlings and reduced damping-off severity (**Fig. 1**). Several other *Trichoderma* spp. also effectively enhanced damping-off suppressing efficacy of FE in the peat-based mix (unpublished data).

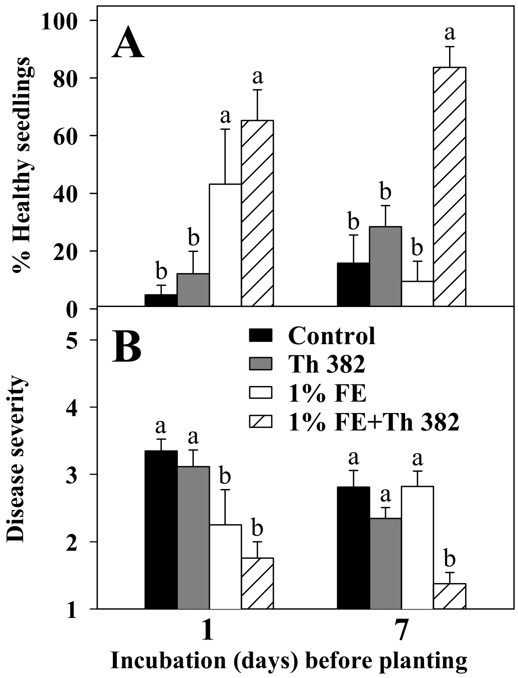

Fig. 1 Effect of fish emulsion (FE) and a bio-control agent *Trichoderma hamatum* 382 (Th 382) as a pre-plant amendment on (A) percent healthy seedlings and (B) the damping-off severity of radish seedlings in a peat-based mix artificially infested with *Rhizoctonia solani*. FE (1% mass/mass mix) and Th 382 (10^6 conidia g^{-1} mix) were mixed into a peat-based mix infested with *R. solani* soil inoculum and incubated at 24±1°C in the dark. After 1 and 7 days, 19 radish seeds were planted in each of five replicate pots and pots were incubated in a growth room. Plants were rated 2 weeks later for damping-off using a 1-5 rating scale. Bars show the means from two experiments, and error bars are standard errors. Bars with the same letter within each planting time do not differ significantly according to (**A**) Fisher's protected least significant difference test or (**B**) Student-Newman-Keuls test at P 0.05.

Common scab and verticillium wilt

Potato (*Solanum tuberosum* L. cv. 'Snowden') has been used as a model system to investigate the effects of various soil amendments on two economically important soil-borne diseases such as common scab [*Streptomyces scabies* (Thaxter) Lambert & Loria = *S. scabiei* (Trüper and dè Clari 1997)] and verticillium wilt [*Verticillium dahliae* Kleb.] (Lazarovits 2001; Lazarovits *et al*. 2005). Both diseases often affect potatoes in the same field. Using these model systems, the effects of FE as a pre-plant soil amendment on verticillium wilt and potato scab in various soils of different characteristics (pH 5.2-7.2, organic matter 1-3.7) were examined under greenhouse, micro-plot, and field conditions (Abbasi *et al*. 2006). Soils for the greenhouse and micro-plot trials were from commercial potato fields in Ontario, New Brunswick, and Prince Edward Island with a history of verticillium wilt and scab.

Under greenhouse conditions, FE (0.5 and 1% mass/mass soil) added to a soil from a commercial potato field in Ontario protected eggplants (*Solanum melongena* L. var. *esculentum* cv. 'Black Beauty') from verticillium wilt and increased (1% only) fresh and dry plant biomass (Abbasi *et al*. 2006). There was a 77-89% reduction in disease incidence and 2.1-2.2 unit reduction in disease severity by the FE treatments over the control. However, only the 1% FE treatment significantly increased the fresh and dry plant biomass which was doubled compared to that of the control. No phytotoxic symptoms as a result of the FE treatments were observed on the eggplants. In greenhouse tests, incorporating FE at a 0.5% (mass/mass) rate to an Ontario potato soil with a history of scab did not reduce disease severity on tubers. The average scab severity on tubers from the untreated control treatment was 1.6. Based on the results of these greenhouse trials, we decided to use a 1% broadcast rate of FE for further micro-plot and field trials. In the greenhouse pot assays, FE (1% mass/mass) restricted the multiplication of root-knot and lesion plant-parasitic nematodes in tomato roots grown in three sandy-loam soils from Ontario fields (unpublished data).

In micro-plots consisting of plastic drainage tiles (25-cm diameter, 25 cm long) buried into a sandy-loam soil, FE was tested in 11 soils from commercial potato fields from three provinces. The 1% FE treatment significantly reduced common scab severity in seven soils with low to medium scab disease pressure and increased total tuber yield by 41-170% in nine soils compared to the controls. Although FE only significantly reduced the petiole infection by *V. dahliae* in one soil, a similar trend in reduced petiole infection was observed in most soils. In field trials at two sites located in grower fields, 1% FE incorporated to a depth of 15 cm (20,000 L/ha) reduced scab severity, increased the percentage of disease-free tubers by 132-366%, and increased marketable tuber yield by two-fold compared to the control at both sites. FE reduced potato scab and verticallium wilt in soils covering the full range of organic matter and pH. Thus, the efficacy of FE to reduce disease was not soil-specific, but was effective in various soils of different characteristics. However, the level of potato scab disease on tubers in the natural soil had an impact on the efficacy of FE as a disease control product. The results indicated that FE may not work in soils showing very high scab severity on tubers.

Uniform incorporation of organic material into field plots may be a key to achieving consistent field efficacy and this may require special equipment. The efficacy of FE was more consistent in micro-plots where FE was incorporated to various soils more uniformly by manually mixing in plastic bags prior to adding to micro-plots. The inconsistent field efficacy and lack of equipment for applying these organic amendments into soil can prevent the wide-spread use of these products. The broadcast rates of FE (20,000 L/ha) that provided effective control of potato scab are not economically feasible and may be too costly for commercial use. However, it may be economical if the disease control effect lasts for multiple years after a single soil application of FE. The costs could possibly be lowered by applying the material in-furrows or in bands most precisely targeting the root zones. Long-term studies with much lower broadcast rates of FE are under way to improve disease suppression and tuber yield by applying the material before planting and after harvesting for several years. FE is a promising material for increasing soil organic matter content and for changing the disease profile of a potato soil.

Bacterial spot

Preliminary trials were conducted under greenhouse conditions to evaluate the effects of FE as foliar sprays on bacterial spot [*Xanthomonas vesicatoria* (Doidge)] of tomato and pepper, and to determine the optimal rates and spray intervals for suppressing bacterial spot on foliage. Under greenhouse conditions, two (one before and one after inoculation) weekly sprays of FE [0.5% volume/volume (v/v) water] consistently suppress bacterial spots symptoms on both tomato and pepper foliage in multiple trials (Abbasi *et al*. 2003). Under field conditions, weekly foliar sprays of a diluted aqueous solution of FE (0.5% v/v) consistently provided effective control of bacterial spot of tomato and pepper foliage (Abbasi *et al*. 2003). The disease incidence on the fruit of these plants was reduced in some years. However, the number of lesions per pepper fruit was significantly less with the FE foliar sprays. FE increased healthy and total fruit yield of tomatoes and healthy fruit yield of peppers in some but not all years. As described below, FE contains large quantities of organic acids that are toxic to pathogens and it is possible that these organic acids may have played a role in disease suppression by foliar sprays of FE on tomato and pepper. FE also contains some residual oil and foliar sprays of fish oils are known to affect foliar diseases (Martin *et al*. 2005; Scherm and Krewer 2008). However, the role of other mechanisms of disease suppression such as induced resistance can not be overlooked and should be investigated.

Our foliar work with FE served as a basis for the widespread use of this organic product in Florida to manage citrus canker and in California for vegetable production as fertilizer as well as to manage plant diseases. A citrus grower in St. Lucie County, Florida sprayed his citrus trees spread over 10,000 acres, and groves that received several applications of FE (Omega Grow) per year were free of citrus canker compared to the surrounding areas that were not sprayed with FE [Fighting canker with fish. Florida Grower, June 2006:16-19]. It is also used on fruit trees, grapes, strawberries as foliar sprays to manage foliar diseases (personal communications/unpublished data). These results of foliar work suggest that disease management programs for bacterial spot and other foliar diseases can be enhanced by including foliar sprays of FE.

MECHANISMS OF DISEASE SUPPRESSION

The mechanisms of disease suppression or disease reduction associated with organic amendments were rarely investigated or understood. Any information on how organic amendments provide disease suppression is essential for optimizing and enhancing disease-reducing efficacy that any organic material may provide. In general, many mechanisms of disease suppression by organic soil amendments have been proposed including biological control by stimulation of resident microorganisms with bio-control activity, induction of systemic resistance in plants, and release or presence of toxic compounds such as ammonia, nitrous acid, and volatile fatty acids (Hoitink and Boehm 1999; Tenuta and Lazarovits 2002; Tenuta *et al*. 2002; Mazzola 2004; Lazarovits *et al*. 2005; Abbasi *et al*. 2007; Mazzola *et al*. 2007). The effects of organic amendment suggest that both chemical and biological components of the amended soils can contribute to disease suppression (Craft and Nelson 1996; Abbasi *et al*. 2002; Bulluck and Ristaino 2002). It is likely that several of these mechanisms may act simultaneously and contribute to disease suppression. Disease suppression by FE has been due to more than one mechanism but which ones play a dominant role is likely to be soil or substrate specific. The rates of amendment used may also have an effect on the prevailing mechanism depending on the soil and substrate.

Role of breakdown products

The microbial breakdown products of some organic amendments can be lethal to some pathogens and pests. Nitrogen transformation products, such as ammonia and nitrous acid are produced after addition of higher rates (2-10% by weight) of high nitrogen-containing organic amendments such as chicken manure, meat and bone meal, chitin and chitosan, and soy meal to soil (Tenuta and Lazarovits 2002; Lazarovits *et al*. 2005). Ammonia at neutral or acid pH levels is converted to ammonium. However, at pH levels above 8.0 ammonium is converted to the volatile gas ammonia and while ammonium is not toxic, ammonia is very toxic (Tenuta and Lazarovits 2002). In the course of nitrification ammonium is converted to nitrite which at neutral pH is not toxic but at low pH (5.0 or less) is converted to nitrous acid which is a very potent biocide (Tenuta and Lazarovits 2002). The presence of these active products is highly dependent on soil organic matter content and soil pH and its buffering capacity (Tenuta and Lazarovits 2002).

Fish meal, a high nitrogenous amendment, when incor-

Table 1 Concentration of organic acids in fish emulsion (FE) samples determined with chemical suppression ion exclusion chromatography and conductivity detection.*

FE†	Concentration of organic acids (mmol/L)							
	Glycolic acid	**Formic acid**	**Acetic acid**	**Propionic acid**	***n*-butyric acid**	***iso*-butyric acid**	***iso*-valeric acid**	**Total**
A	346	28	95	12	22	4	2	509
B	768	20	198	45	46	9	5	1091

* FE samples were diluted 10 and 100 times before analyses and particulates removed by centrifugation for 10 min at 10 600 g.
† A = Omega Protein, USA, B = Fish Fertilizantes, Brazil

porated into a sandy-loam soil did not immediately affect the germination of *V. dahliae* microsclerotia, but the effect started to appear after 6 days (Abbasi *et al*. 2009a). The pH of the soil amended with 1% fish meal was increased from 5.6 in the control to 8.7 after 6 days (Abbasi *et al*. 2009a). This indicates that a reduction in the viability of *V. dahliae* microsclerotia in the fish meal-amended sandy-loam soil was certainly due to ammonia toxicity. The release of nitrous acid in the fish meal experiments was not followed. Nitrous acid is released late after 2-3 weeks of adding nitrogenous amendment in a sandy type soil when soil pH drops below 5.0. On the contrary, disease or pathogen suppression by FE in various soils or substrates (Abbasi *et al*. 2004, 2006, 2009a) was certainly not due to toxicities of ammonia or nitrous acid. The rates of FE used in sandy-loam soil were not high enough to generate ammonia at the toxic concentration, and even high rates of FE could not have generated a toxic concentration of ammonia due to the high buffering capacity of peat-based mix and muck soil. The pH of the FE-treated peat-based mix or muck soil did not substantially increase or decrease compared to their respective controls.

Role of organic acids

Some organic amendments, for instance liquid swine manure, contain VFAs which can be toxic to pathogens in low pH soils (Tenuta *et al*. 2002; Conn *et al*. 2005). Volatile toxic compounds can also be produced by creating anaerobic and reducing conditions in the soil by incorporating fresh organic material such as broccoli or grass (Blok *et al*. 2000). Covering of the amended plots with plastic sheets enhances the concentration and the longevity of the toxicants in the soil profile (Blok *et al*. 2000).

In our study with FE, the viability of *V. dahliae* microsclerotia was significantly reduced within 1 day after incorporation of FE in a sandy-loam soil, and within 3 days more than 98% of microsclerotia did not germinate after exposure to 2% FE (Abbasi *et al*. 2009a). This immediate kill of microsclerotia indicated that FE contains toxic substances. We then analysed FE samples for the presence of any organic acids and found that it contained high concentrations of organic acids including some known toxicants (Abbasi *et al*. 2009a). Glycolic, acetic, formic, *n*-butyric, and propionic acids were the major organic acids detected in FE. We also compared FE samples from two different sources (Omega Protein USA and Fish Fertilizantes Brazil) for the contents of organic acids. Both samples had a similar chromatogram but comparatively the FE samples from the Brazil had a very high concentration of organic acids (**Table 1**). These organic acids are likely present in FE samples from other sources as well and they may be produced during anaerobic decomposition of sugars or proteins present in the FE.

Acetic, formic, *n*-butyric, and propionic acids were the major toxic volatile organic acids in FE (Abbasi *et al*. 2009a). All these organic acids, except glycolic and formic acids, have also been found in liquid swine manure and were considered to be a major factor associated with disease reduction by liquid swine manure (Tenuta *et al*. 2002; Conn *et al*. 2005). FE in contrast contains high levels of formic acid and formic acid was found to be 7 times more toxic to pathogens such as *V. dahliae* compared with acetic acid (Tenuta *et al*. 2002). The rapid reduction in the viability of *V. dahliae* microsclerotia in a sandy-loam soil with increasing concentrations of FE was likely due to the toxicity of these organic acids. Similarly, when microsclerotia were treated in solution assays with the mixture of organic acids at the proportions found in FE, the viability of microsclerotia was significantly reduced and was comparable to that seen with equivalent rates of FE (Abbasi *et al*. 2009a). The viability of microsclerotia was increased when the treatment solutions (FE and mixture of organic acids) were buffered to pH 6.0 with NaOH-citrate buffer. This clearly suggests that organic acids in FE play an important role in pathogen suppression. The biological activity of VFAs can be enhanced under acidic conditions (Tenuta *et al*. 2002). It is therefore possible that VFA toxicity may have been one of the mechanisms of disease reduction by FE in the low pH soils (Abbasi *et al*. 2006).

Glycolic acid was found in both FE samples at very high concentrations (**Table 1**). Glycolic acid is naturally found in sugar beets, sugar cane, and unripe grapes, and is also manufactured synthetically. Its presence in FE at very high concentrations needs further investigations. Although glycolic acid has been patented as a plant growth promoting agent (Kinnersley 2002), it has antimicrobial properties and is widely used as an ingredient in skin care products for controlling microbial induced facial blemishes. In growth room assays, glycolic acid at the concentrations much lower to that found in FE enhanced the percentage of healthy cucumber seedlings in a muck soil infested with *Pythium* spp. (Abbasi *et al*. 2009b). A mixture of organic acids equivalent to the proportions found in FE and acetic and formic acids were toxic to *P. ultimum* in solution assays (Abbasi *et al*. 2009a). The increasing concentrations of a mixture of organic acids also suppressed damping-off of cucumber seedlings in a muck and sandy-loam soils but not in a peat-based mix. Similarly, comparable rates of FE also provided immediate protection of cucumber seedlings from damping-off within one day after incorporation of FE in an infested muck soil (Abbasi *et al*. 2004).

Damping-off suppression of radish and cucumber seedlings in a peat-based mix was achieved only after planting was delayed for a week or more after adding FE to the pathogen-infested mix (Abbasi *et al*. 2004, 2009a). It is less likely that organic acids from FE played a role in delayed suppression of damping-off in a peat-based mix. Immediate pathogen or disease suppression in sandy-loam or muck soils suggests that organic acids may play a major role. FE contains large quantities of organic acids that are toxic to plant pathogens and may have a role in disease suppression depending on the soil and substrate. In order to maximize the pathogen or disease suppression effect of organic acids, FE should be applied to the soils with low pH and low buffering capacity.

Role of biological control

Biological control involves managing pathogens and pests such that their populations or activities are reduced to keep crop losses below economic thresholds. It can be accomplished by general microbial activity (general suppression) or the activity of specific organisms (specific suppression). Biological control at some form and level is always active in natural soils where it prevents most soil-borne plant pathogens and pests from causing diseases. One of the long-term effects of incorporating organic amendments such as FE into agricultural soils is to establish disease suppressive conditions by inducing natural biological control. Organic matter can act as a substrate for growth of antagonists, but it also can lead to enhance enzyme activities required for biological control (Downer *et al*. 2001; White and Traquair 2006). FE has been used as a nutrient base for plant growth promoting rhizobacteria to enhance radish growth (El-Tarabily *et al*. 2003).

In our studies, FE stimulated the microbial activity in amended soils or substrates, specifically those of *Trichoderma* spp. often associated with disease suppressive composts (Abbasi *et al*. 2004). In peat-based mix, FE did not have any direct toxicity to damping-off pathogens but appeared to act by creating a biological climate that was suppressive to disease initiation (Abbasi *et al*. 2004). The delayed improvement of disease control with time corresponded with increased microbial activity in the amended mix. In peat-based mixes where we observed the best control, the populations of culturable fungi and bacteria had increased significantly by 7 days after incorporation of FE to peat-based mix. This incubation time also corresponded to the initiation of the disease suppression by the amended peat-based mix. In the field plots, FE soil amendment generally enhanced the activity of both fungi and bacteria but any specific role this enhanced microbial activity might have played in disease suppression needs to be determined. We have isolated and identified several strains of soil bacteria from FE-amended field plots based on antimicrobial activity against known pathogens in agar plate tests in the laboratory and plant bioassays in the growth room.

Similarly, significant increases in soil bacteria were seen after addition of FE in a muck soil suggesting that FE may enhance a biological climate that is suppressive to plant pathogens. As a first step toward creating disease suppressive conditions for any plant-pathogen interaction, it is essential that we identify the microbial communities playing a role in disease suppression by addition of FE. In our attempts to define what changes in bacterial communities occurred following FE additions in muck soil, the FE-amended muck soil were characterized based on DNA analysis of the chaperonin-60 sequence and high-throughput sequencing and sequences were compared to reference sequence database. The FE library showed a relatively lower proportions of Actinobacteria and Deltaproteobacteria and correspondingly higher proportions of Betaproteobacteria and Firmicutes (Lazarovits *et al*. 2009). A lack of reference sequences of relevant taxa in the data base was the greatest hindrance for identification of muck soil library sequences. Similarly, soil contains a lot of uncultured organisms, such as the Acidobacteria, for which little sequence data is available. The identification of microbial species based on sequencing is dependent on the availability of sufficient reference sequences.

Although FE amendment resulted in significant increases in the microbial activity in the amended soil or substrate, it is not that simple to readily identify the key organisms involved in the activity. Such information would assist in selecting and enriching suppressive microflora in the rhizosphere. Although characterization and comparisons of microbial communities in rhizosphere samples of healthy and diseased plants are now possible with advances in molecular technologies, we are still a long way from establishing those individual BCAs for real and long-lasting disease suppression in the field. FE may provide that ideal base to enrich effective BCAs under both greenhouse and field settings. Meanwhile, soil management practices such as with organic amendments will continue to play an important role in improving soils' biological buffering capacity by maintaining the abundance and diversity of resident soil microbial communities for natural biological control. There are always long-term benefits associated with the addition organic materials to soils. For instance in The Netherlands, organic farming systems resulted in lower levels of both nitrate and total soluble nitrogen in the soil, an increase in the number and diversity of bacteria, and species richness in both bacteria and nematode communities (van Diepeningen *et al*. 2006). There can be other possible mechanisms of disease suppression such as an increase in plant tolerance or resistance involved in FE-mediated suppression of soil-borne plant pathogens.

CONCLUSIONS AND FUTURE WORK

FE is an excellent organic product with both plant growth-promoting and disease suppressing effects. Our studies suggest that biological control and organic acids play a role in FE-mediated disease suppression depending on substrates and soils. The use of FE for disease control or plant nutrition in organic farming is of paramount importance, but for maximum benefits several factors should be considered. First, the rates at which FE provides disease suppression in sandy-loam soil, muck soil, or peat-based substrates are very different. The rates that are effective in peat-based mix can be highly phytotoxic in a sandy-loam soil if not added sufficiently ahead of planting. Second, the efficacy of FE may be impacted by batch to batch variability in the peat-based substrates. This is important because disease suppression by FE appears to mainly depending primarily on the microbial carrying capacity of the peat-based mix. In suppressive batches of the peat-based mix, the disease suppression can be enhanced by the FE amendment over time, whereas in non-suppressive batches, the disease suppression by FE can be achieved by adding known biocontrol agents in the mix. Immediate pathogen or disease suppression in sandy-loam or muck soils suggests that organic acids from FE may play a major role.

Third, the soil incorporation of the organic material is not always uniform and this is one of the contributing factors to inconsistent field efficacy. The growers need to have an access to the proper equipment to apply the material where it needed the most for any desired results. In order for this technology to be adopted for crop production and disease management, the performance of this product should be eye catching to growers and in some cases comparable to chemical pesticides. Consistency in field efficacy of organic soil amendments may stimulate conventional growers to adopt and integrate such practices as well. FE is a safe and pathogen-free liquid organic product, though not odourless, that can be adopted more rapidly and can be readily added to the planting rows by manure injectors or even by irrigation systems.

Fourth, the broadcast rates of FE that provided effective control of potato scab are not economically feasible and may be too costly for commercial use unless applied in furrows or bands. Lower rates applied more frequently may provide longer lasting disease suppression and that may be economically feasible. This can create a disease suppressive condition in fields where the practice has been used for years. An organic soil amendment applied once at higher rate may be economical if its disease control and yield effects last for more than 2 years. There is also real concern about pollution and ground water contamination with high rates of application. Applying FE in the fall immediately after harvesting a crop when the soil pH is the lowest may provide better control of the plant pathogens affected by organic acids than application in the spring, prior to the planting.

The use of organic amendment for managing soil-borne diseases and enhancing soil fertility will continue to expand as an alternative to agrochemicals especially in organic production systems. There are potential benefits of FE for organic crop production systems where growers heavily rely on organic residue management to control diseases caused by soil-borne plant pathogens. There is a great need to focus on the soil management practices that improve soil quality and health. The practices such as organic soil amendments can maintain the abundance and diversity of resident soil microbial communities. Organic amendments can also be useful for building high populations of resident beneficial organisms while decreasing populations or activities of plant pathogens and nematode pests.

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