Soilborne pathogens / Agents pathogènes telluriques

Suppression of *Rhizoctonia* and *Pythium* damping-off of radish and cucumber seedlings by addition of fish emulsion to peat mix or soil

Pervaiz A. Abbasi, Kenneth L. Conn, and George Lazarovits

Abstract: The efficacy of fish emulsion in enhancing plant growth and suppressing seedling damping-off diseases caused by *Rhizoctonia solani* and *Pythium aphanidermatum* was investigated on peat mix and soil. Fish emulsion (1%–4%; *m*/*m* peat mix) or equivalent inorganic fertilizer (N–P–K) was incorporated into pathogen-infested peat mix and incubated in plastic bags 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. Negligible protection of seedlings from damping-off occurred in peat mix incubated for 1 day with fish emulsion. After 7 days incubation, however, 70%–80% of the seedlings remained disease-free in peat mix amended with 4% fish emulsion. After 28 days, equivalent levels of disease protection were found with all concentrations of fish emulsion. As the inorganic N–P–K treatment was adjusted to reflect N–P–K levels in the fish emulsion, no disease control was obtained, indicating that disease protection was not due to increased plant nutrition. Incorporation of 0.5% (*m*/*m* soil) fish emulsion into soil 5 days before planting radish provided effective control of damping-off disease. Fish emulsion (2% and 4%; *m*/*m* muck soil) also effectively and consistently suppressed damping-off of cucumber seedlings in muck soil naturally infested with damping-off pathogens. Pasteurization of the peat mix followed by re-infestation with *R. solani* resulted in a much higher level of disease than that obtained in unpasteurized infested peat mix. Addition of fish emulsion resulted in restoration of disease suppression within 7 days. These results suggest that fish emulsion may not be toxic to the pathogens but may create a biological climate in peat substrate or soil that is suppressive to the disease. Fresh and dry mass measurements of plants produced in 4% fish emulsion were 2 to 3 times greater than in nonfertilized peat but were comparable with those receiving equivalent N–P–K. The results suggest that fish emulsion has both nutritive value for plant growth as well as disease suppressive properties. Thus, it may be an ideal product for use in organic or conventional transplant production.

Key words: biological control, *Cucumis sativus*, disease suppression, organic amendment, *Pythium aphanidermatum*, *Raphanus sativus*, *Rhizoctonia solani*.

Résumé : La capacité d'une émulsion de poisson à améliorer la croissance des plantes et à inhiber la fonte des semis causée par le *Rhizoctonia solani* et le *Pythium aphanidermatum* a été étudiée dans un mélange de tourbe et dans du sol. L'émulsion de poisson (1–4 %; *m*/*m* de mélange de tourbe) ou son équivalent sous forme d'engrais inorganique (N–P–K) a été incorporé au mélange de tourbe infesté d'agents pathogènes et incubé durant 1, 7, 14 ou 28 jours dans des sacs de plastique avant que l'on y sème des graines de radis ou de concombre. Quatorze jours plus tard, la fréquence et la gravité de la fonte des semis ont été évaluées sur les plantes. L'incubation du mélange de tourbe durant une journée avec l'émulsion de poisson n'a procuré aux plantules qu'une protection insignifiante contre la fonte des semis. Cependant, après 7 jours d'incubation, 70 à 80 % des plantules n'avaient pas été atteintes par la maladie dans le mélange de tourbe additionné de 4 % d'émulsion de poisson. Après 28 jours, des niveaux équivalents de protection contre la maladie ont été observés pour toutes les concentrations d'émulsion de poisson. Comme le traitement au N–P–K inorganique avait été conçu pour reproduire les niveaux de N–P–K présents dans l'émulsion de poisson et qu'il n'a pas permis de lutter contre la maladie, on suppose que la protection contre la maladie n'est pas due à une meilleure nutrition des plantes. L'incorporation de 0,5 % (*m*/*m* de sol) d'émulsion de poisson dans du sol 5 jours avant de semer des radis a permis de lutter efficacement contre la fonte des semis. L'émulsion de poisson (2 et 4 % *m*/*m* de terre noire) a aussi réduit efficacement et avec constance la fonte des semis de plantules de concombre dans une terre noire

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naturellement infestée par les agents pathogènes de la fonte des semis. La pasteurisation du mélange de tourbe suivie de la réinfestation par le *R. solani* a causé des intensités de maladie plus élevées que celles obtenues avec un mélange de tourbe non pasteurisé et infesté. L'ajout de l'émulsion de poisson a permis de restaurer la capacité de suppression du sol en moins de 7 jours. Ces résultats laissent croire que l'émulsion de poisson n'est peut-être pas toxique pour les agents pathogènes, mais qu'elle crée plutôt un climat biologique dans le substrat tourbeux ou dans le sol qui supprime la maladie. Les poids frais et secs de plantes produites avec 4 % d'émulsion de poisson ont été de 2 à 3 fois plus élevés qu'avec la tourbe non fertilisée, mais ont été semblables aux plantes qui ont reçu l'équivalent de N–P–K. Ces résultats laissent penser que l'émulsion de poisson a, à la fois, une valeur nutritive pour la croissance des végétaux et des propriétés de suppression de maladie. Elle pourrait donc être un produit idéal pour la production biologique ou conventionnelle de plants.

Mots clés : lutte biologique, *Cucumis sativus*, suppression des maladies, engrais organique, *Pythium aphanidermatum*, *Raphanus sativus*, *Rhizoctonia solani*.

Introduction

Seedling damping-off diseases caused by *Rhizoctonia solani* Kühn, *Pythium ultimum* Trow, and *Pythium aphanidermatum* (Edson) Fitzpatrick are significant to many plant species worldwide. Serious losses of young seedlings of several horticultural and vegetable crops have been found in both greenhouse and field production systems (Howard et al. 1994). These pathogens generally attack the juvenile tissues of bedding plants and often cause pre-emergence damping-off (Stephens et al. 1982; Stephens and Powell 1982). Seed and seedling diseases on greenhouse-grown transplants of eggplant, pepper, lettuce, tomato, and zinnia have been reported (Howard et al. 1994; Sherf and MacNab 1986). In the field, such diseases occur primarily in cold and wet soils, where direct-seeded plantings may be affected. Very young seedlings may be killed before or soon after they emerge. Damping-off diseases are also widespread and destructive on seedlings in forest nurseries (Camporota and Perrin 1998; Huang and Kuhlman 1990). Although fungicides are available, they are not always an effective means of control of such diseases. Soil fumigants have been the most effective means of pathogen control. However, such compounds are expensive and can be dangerous to apply. Attempts have been made to find nonchemical treatments for such diseases, including composts and other organic amendments. There is, however, the need to develop additional reduced-risk technologies to control these soilborne plant pathogens.

The greenhouse industry makes extensive use of soilless media consisting mainly of peat-based substrates, bark compost, or a combination of both for transplant production. Even though these substrates are pathogen-free, peat media are generally considered to be disease conducive, whereas compost-amended substrates are considered to be disease suppressive to damping-off pathogens (Nelson and Hoitink 1982; Stephens et al. 1981). This, however, depends greatly upon the composition of the mixes and their biological activity (Chen et al. 1988; Nelson and Hoitink 1982; 1983; Tahvonen 1982). Planting mixes that are naturally disease suppressive have been considered to be rich in microorganisms that induce biological control. The basis for the suppressive effect, however, is not well understood and thus efforts to make disease-suppressive media have been plagued by inconsistency of disease control (Krause et al. 2001). In general, where suppression has been obtained, it has been attributed to the increased microbial activity in the amended substrate (Chen et al. 1988; Hadar and Mandelbaum 1986; 1992; Huber and Summer 1996; Nelson and Hoitink 1983; Papavizas and Davey 1960; Stephens and Stebbins 1985; Tuitert et al. 1998). The steps needed to bring this condition about have not been clearly elucidated.

Fish emulsions or fish soluble nutrients are liquid by-products of the processing of whole menhaden fish (*Brevoortia tyrannus* (Latrobe)) into fish meal. They have been used mainly as fertilizers (Aung and Flick 1980; Aung et al. 1984; Ceci 1975), although other uses have been reported as well (Abbasi et al. 2003; El-Tarabily et al. 2003; Wyatt and McGourty 1990). Foliar sprays of a mixture of fish emulsion and bacteria were used to control moths (Wyatt and McGourty 1990), and we recently demonstrated that diluted solutions of fish emulsion could control bacterial spot of tomato and pepper (Abbasi et al. 2003). There is also a recent report on the use of fish emulsion as a substrate for plant growth promoting rhizobacteria (El-Tarabily et al. 2003). Fish meal, the dried protein obtained from processed fish, has been used as a soil amendment with great success in vegetable production (Blatt and McRae 1998; Gagnon and Berrouard 1994) and to reduce the inoculum density of *Verticillium albo-atrum* Rke. & Bert. (Wilhelm 1951). Fish solid waste has also been known to reduce the populations of plant-parasitic nematodes (Akhtar and Mahmood 1995). However, there were no reports on the use of fish emulsion to control soilborne diseases.

The objectives of this study were to investigate the efficacy of fish emulsion as an amendment to typical planting substrates and soil to suppress seedling damping-off diseases caused by *R. solani* and *P. aphanidermatum*, and to evaluate its ability to support plant growth. A preliminary report on a portion of this work was published previously (Abbasi et al. 2002).

Materials and methods

Plant material, potting mix, soil, and fish emulsion

Radish (*Raphanus sativus* L. 'Early Scarlet Globe'; 94% germination) and cucumber (*Cucumis sativus* L. 'Straight Eight'; 96% germination) seeds were purchased from William Dam Seeds Ltd., Flamborough, Ontario. These seeds were not treated with fungicides. The potting mix used in all the experiments described was a commercial peat-based

Table 1. Analysis of fish emulsion (FE) used in this study.

FE batch	N[a] (%)	NH_4-N (%)	P[b] (%)	K[c] (%)	Na (%)	Ca (%)	Mg (%)	S (ppm)	OM[d] (%)	C:N ratio	Dry matter (%)
2001	4.3	0.6	1.5	1.9	1.2	0.1	0.1	12 700	83	6:1	47.7
2003	6.0	0.4	1.8	2.2	1.4	0.2	0.1	19 900	79	4:1	51.6

Note: Fish emulsion samples were received from Omega Protein and nutrient analyses were performed by A&L Canada Laboratories Inc., London, Ontario. Density of fish emulsion is 1.2.
[a]Total nitrogen.
[b]Phosphate (P) as P_2O_5.
[c]Potash (K) as K_2O.
[d]Organic matter.

mix (Pro-Mix 'BX®', Premier Horticulture Inc., Rivière-du-Loup, Quebec) referred to hereafter as peat mix. The main components of the mix are as follows: Sphagnum peat moss (75%–85% by volume), horticultural grade perlite and vermiculite, macro- and micro-nutrients, dolomitic and calcitic limestone, and a wetting agent. A loamy sand soil (organic matter 1.4%, pH 5, water holding capacity 19%) from a commercial potato field near Alliston, Ontario (site B, Conn and Lazarovits 1999), and a muck soil (organic matter 50.0%, pH 6.8) from Holland Marsh, Ontario, were also used in this study. Fish emulsion samples were provided by Omega Protein, Rockton, Illinois, USA. A nutrient analysis of fish emulsion is given in Table 1.

Fungal cultures and preparation of inoculum

The isolate of *R. solani* used in this study was originally isolated from potatoes and belongs to anastomosis group 4 (AG 4) mating type. *Pythium aphanidermatum* was obtained from Dr. James Traquair, Agiculture and Agri-Food Canada, London, Ontario. Pathogenicity of *R. solani* and *P. aphanidermatum* was tested on radishes and cucumbers, respectively. The organisms were recovered from the infected hosts and maintained on potato dextrose agar (PDA) medium. Inoculum of *R. solani* and *P. aphanidermatum* was prepared in a mixture of soil and finely chopped potato as described previously (Ko and Hora 1971), with some modifications. London clay loam soil (particle size 1–2 mm) was used in this medium. The soil–potato medium (10:1) was moistened and autoclaved for 1 h for 2 consecutive days in 1-L flasks. Flasks were inoculated with 15–20 plugs obtained from the edge of the freshly grown fungal colony. After 10–15 days, when the fungus had completely colonized the soil, the soil inoculum was placed on paper and air dried in a laminar flow hood with sterile air. The soil inoculum of *R. solani* was stored at room temperature and was pathogenic as long as the quantity lasted, which could be up to 1 year. However, the slightly dried soil inoculum of *P. aphanidermatum* had to be used within a week of preparation. Various quantities of the pathogen inocula were added to peat mix in preliminary experiments to determine the amounts required to give approximately 100% disease incidence within 2 weeks after planting.

Radish–*Rhizoctonia* bioassay

Air-dried soil inoculum of *R. solani* (0.5%; *m*/*m* peat mix) was added to peat mix in plastic bags and thoroughly mixed. The concentrations of fish emulsion used included 1%, 2%, or 4% (*m*/*m* peat mix). The 1% fish emulsion represented 10 g of factory stock, which was diluted in deionized water, for a total volume of 200 mL. This was then added to 800 g of air-dried peat mix to give a final mass of about 1 kg of moist mix. Based on this final mass of the peat mix, the concentration of fish emulsion was calculated to be 1% (1.25% based on mass of air-dried peat mix). A nonfertilized control and N–P–K fertilizer (equivalent N–P–K of inorganic fertilizers as contained in the fish emulsion) treatments were also included. The amounts of inorganic fertilizer equivalents were calculated based on the N–P–K analysis for the fish emulsion (Table 1) and based on the preliminary growth room experiments (data not shown), assumed that approximately 70% of the N–P–K in the fish emulsion product was immediately available. The quantity of the inorganic fertilizer used per kilogram of moist peat then was 300 mg N, 150 mg P, and 190 mg K for the 1% fish emulsion equivalent treatment. Starter fertilizer (20–20–20) and ammonium sulfate were used in the inorganic fertilizer treatments. The fertilizer was dissolved in 200 mL deionized water and the solution was added to the bag containing the 800 g of peat mix. The contents were then mixed thoroughly, the bags loosely tied, and placed at 24 ± 1 °C in dark for incubation. At day 1, 7, 14, or 28, the various amended potting mixes were dispensed into 10 cm diameter plastic pots and radishes (19 seeds per pot) were planted in five replicate pots per treatment. The peat mix samples amended with fish emulsion were also analysed for pH before planting radish seeds. Pots were arranged in a completely randomized design and incubated in the growth room for disease expression (15 h light at 24 °C and 9 h dark at 19 °C). Pots were watered as required to keep the potting mix moist. Plants were rated 14 days later for pre- and post-emergence damping-off using a 1–5 scale, in which 1 = healthy, 2 = small lesion, 3 = large lesion, 4 = post-emergence damping-off, and 5 = pre-emergence damping-off. Data were also expressed as percent healthy seedlings.

Cucumber–*Pythium* bioassay

Freshly prepared soil inoculum of *P. aphanidermatum* (1%; *m*/*m* peat mix) was added to peat mix in plastic bags and mixed thoroughly. Controls included equivalent N–P–K of inorganic fertilizers as contained in the fish emulsion. Fish emulsion (1%, 2%, or 4%; *m*/*m* peat mix) or inorganic fertilizers were dissolved in water and then incorporated into the peat mix. The contents were mixed thoroughly as described above. Bags were loosely tied and incubated at 24 ± 1 °C in dark. At day 1, 7, 14, or 28, the peat mix from the bags was dispensed into 10 cm diameter plastic pots and cucumbers were planted in five replicate pots per treatment (eight seeds per pot). Pots were arranged in a completely randomized design, watered, and incubated in the growth room as described above. Plants were rated 14 days later for damping-off severity using a 1–4 scale in which 1 = healthy, 2 = yellowing of stem or root, 3 = post-emergence damping-off, and 4 = pre-emergence damping-off. Data were also expressed as percent healthy seedlings.

Effect of pasteurization on disease control

To determine whether the mechanism by which the peat mix amended with fish emulsion suppressed damping-off was biological in nature, peat mix was pasteurized alone or after adding fish emulsion by heating in an oven at 65 ± 2 °C for 5 days. Moisture lost during pasteurization was restored by the addition of sufficient sterile water to restore the mass to that prior to heat treatment. *Rhizoctonia solani* soil inoculum was then added and bags were incubated for 1, 7, 14, or 28 days in the dark. A radish–*Rhizoctonia* bioassay was then performed as described above. Before planting radish seeds, samples from control and amended peat mix were taken for microbial counts based on dilution plating. Total fungi and bacteria were enumerated on Rose Bengal and PDA media, respectively.

Potential of fish emulsion to control damping-off in soil

Fish emulsion (0.5%; *m*/*m* moist soil) and *R. solani* soil inoculum were added to air-dried soil, and the moisture content of the amended soil was adjusted to 10% (*m*/*m* soil) with deionized water. The treated soil (100 g) was dispensed into Magenta jars (Magenta Corporation, Chicago, Illinois) with plastic lids that allowed for aeration, and the jars were incubated at 24 °C in a controlled environment growth room. Five days after incorporation of fish emulsion, 16 radish seeds were planted per jar (three jars per treatment) and incubated at 24 °C in a growth room. Plants were rated for damping-off 14 days later as described above. After the plants were removed from the jars, radish seeds were again planted without further addition of fish emulsion. The jars were incubated in the growth room, and the second set of plants was rated 14 days later.

Efficacy of fish emulsion to control damping-off disease was also determined in muck soil from a commercial field near Holland Marsh, Ontario, naturally infested with *R. solani* and *P. aphanidermatum*. Soil was brought to the laboratory and seeded with radishes and cucumbers to establish disease potential. Damping-off was severe on cucumbers but not on radishes, so only cucumbers were used for subsequent experiments. Fish emulsion (1%, 2%, or 4%; *m*/*m* soil) was incorporated into the infested soil in plastic bags. The contents were mixed thoroughly and loosely tied bags were incubated at 24 ± 1 °C in the dark. At day 1, 7, 14, or 28, the amended soil from the bags was dispensed into 10 cm diameter plastic pots and cucumbers were planted in five replicate pots per treatment (eight seeds per pot). Pots were watered, incubated in the growth room, and rated for damping-off severity as described above.

Effect of fish emulsion on plant growth

The fertilizer effect of fish emulsion on growth of radishes and cucumbers in the absence of a pathogen was compared with its inorganic fertilizer equivalent. Fish emulsion (1%, 2%, or 4%; *m*/*m* peat mix) or inorganic fertilizers were dissolved in water and then thoroughly mixed with peat mix in plastic bags. The contents of the bags were then dispensed into five 10 cm diameter plastic pots. Radish or cucumber seeds (eight per pot) were planted in five replicate pots per treatment. The pots were watered from the top and placed in a growth room as described above. After germination, the seedlings were thinned down to five per pot. Radish roots and shoots were harvested after 28 days, whereas cucumber plants were harvested after 21 days of planting, and their fresh mass was determined. Dry mass measurements were taken after these samples were air dried on a greenhouse bench for 7 days. The concentration of macro- and micro-nutrients in dried radish shoot and root samples was determined by A & L Canada Laboratories Inc., London, Ontario.

Statistical analyses

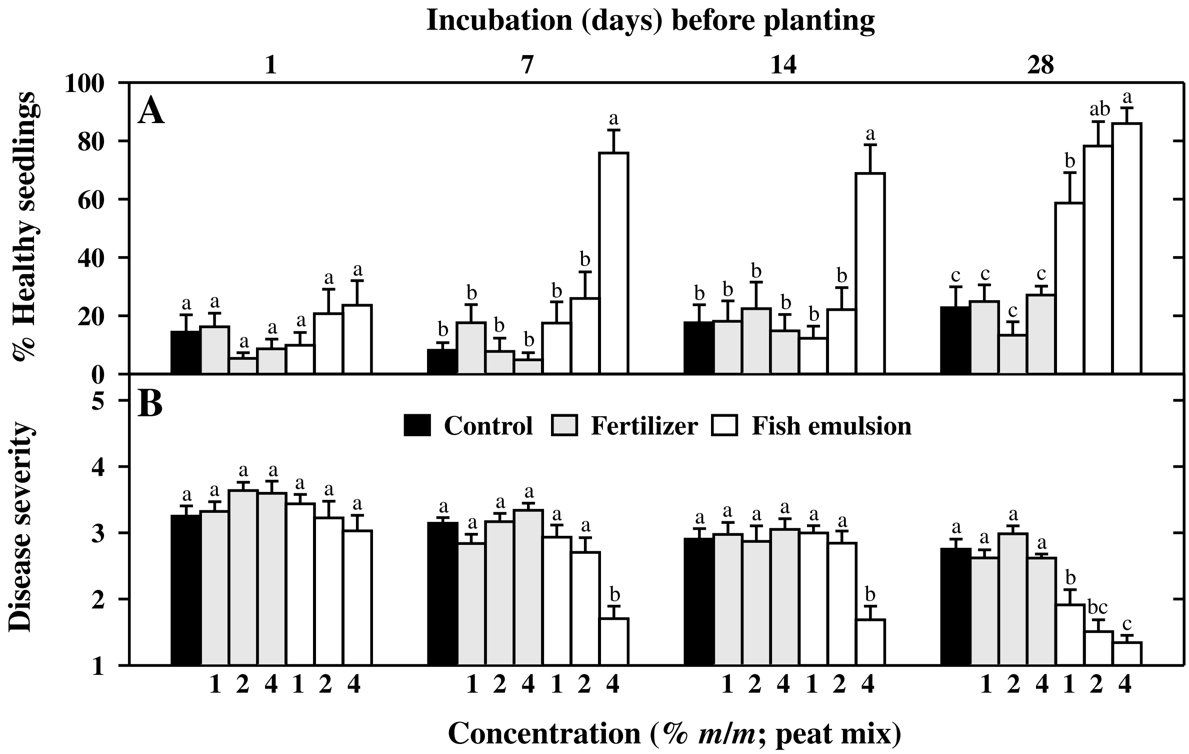
The experiments were done at least twice. The repeated experiments showed similar trends and there were no significant differences between the experiments; hence the data from the repeated experiments were combined and analyzed using MINITAB statistical software version 13.0 (Minitab Inc., State College, Pennsylvania). Analysis of variance (ANOVA) was performed and if *P* values indicated a significant difference ($P \leq 0.05$), means were separated by Fisher's protected least significant different (LSD) test. Since time series experiments were rated separately, each incubation time or day was analyzed separately. Disease severity data were analyzed using nonparametric Friedman's test statistics, and means were separated according to Student–Newman–Keuls (SNK) test.

Results

Effect of fish emulsion on *Rhizoctonia* damping-off of radish seedlings

The percentage of disease-free healthy seedlings and the severity of damping-off on radish seedlings grown in peat mix artificially infested with *R. solani* soil inoculum and amended for various periods of time with fish emulsion or equivalent inorganic fertilizer are shown in Fig. 1. The soil inoculum of *R. solani* was very potent, since more than 75% of the plants were diseased in the nontreated and nonfertilized control treatments. After 1 day of incubation, there were no treatment differences in the percentage of healthy seedlings and disease severity. All had high disease incidence and severity (Fig. 1). When radishes were planted after 7 days of incubation, almost 80% of the seedlings remained disease-free in peat mix amended with 4% fish emulsion compared with fewer than 10% in the untreated or fertilizer equivalent treatments (Fig. 1A). The severity of the disease was also significantly reduced by the 4% fish emulsion treatment (Fig. 1B). After 28 days of incubation, disease protection was also obtained at the lower concentrations (1% and 2%) of fish emulsion but the 4% treatment remained the most effective (Fig. 1). The N–P–K fertilizer treatments provided no disease protection at the rates tested.

Fig. 1. Effect of fish emulsion or fertilizer equivalent on (A) percent healthy seedlings and (B) the *Rhizoctonia* damping-off severity of radish seedlings in a peat-based mix. Fish emulsion or N–P–K fertilizer (1%, 2%, and 4%; *m*/*m* peat mix) and *R. solani* soil inoculum were added to peat mix, mixed thoroughly, and incubated at 24 ± 1 °C in dark. After 1, 7, 14, or 28 days, radish seeds were planted and incubated in a growth room (15 h light at 24 °C and 9 h dark at 19 °C). Plants were rated 2 weeks later for damping-off disease. Means (±SE) are the average of three experiments (n = 15). Means within each planting time followed by the same letter do not differ significantly according to Fisher's protected LSD test (A) or Student–Newman–Keuls (SNK) test (B) at $P \leq 0.05$.



Peat mix samples amended with fish emulsion were tested for alterations in pH prior to planting the radish seeds to determine whether ammonia and nitrous acid may have a role in disease reduction. There was an increase in pH (5.8 to 7.1) after 7 and 14 days of incubation with 4% fish emulsion (Table 2). At such pH levels neither ammonia or nitrous acid would be formed. This was also confirmed by the observations that fish emulsion had no phytotoxic or inhibitory effect on germination and growth of radish seedlings.

Table 2. Effect of fish emulsion on pH of peat mix.

Fish emulsion(%)	pH of peat mix and fish emulsion mixture for incubations of: 1 day	7 days	14 days	28 days
0	6.1	6.2	5.6	5.8
1	6.1	6.2	5.8	5.7
2	6.0	6.5	5.9	5.8
4	5.8	7.1	6.2	6.1

Note: Fish emulsion (1%, 2%, and 4%; *m*/*m* peat mix) was incorporated into peat mix, mixed thoroughly, and incubated at 24 ± 1 °C for 1, 7, 14, and 28 days. pH was determined before planting radishes.

Effect of fish emulsion on *Pythium* damping-off of cucumber seedlings

The effect of fish emulsion on the relative proportion of healthy seedlings and damping-off severity on cucumber seedlings grown in peat mix artificially infested with soil inoculum of *P. aphanidermatum* are shown in Fig. 2. In peat mix that received no treatment (control), fewer than 20% of the plants were healthy after 1, 7, and 14 days of incubation, and about 40% of the plants were disease-free after 28 days of incubation (Fig. 2A). The severity of the disease was gradually decreased over time in the control (Fig. 2B). As seen above for *R. solani* damping-off, there was no disease protection of cucumber seedlings from *P. aphanidermatum* damping-off by any concentration of fish emulsion after 1 day of incubation (Fig. 2). However, almost 60% and 70% of the cucumber seedlings planted into peat mix after 7 days of incubation with the 2% and 4% fish emulsion treatments were free of any damping-off symptoms, respectively (Fig. 2A). The severity of the disease was found to be reduced by 1% fish emulsion after 7 days of incubation (Fig. 2B). Disease protection with all three concentrations of fish emulsion gradually increased with the time of incubation, resulting in a significant increase in healthy seedlings and a reduction in disease severity (Fig. 2). After 14 days, more than 90% of cucumber seedlings were healthy with the 4% fish emulsion treatment (Fig. 2A). All three concentrations of fish emulsion provided highly effective disease control after 28 days of incubation (Fig. 2).

Fig. 2. Effect of fish emulsion (FE) on (A) percentage of healthy seedlings and (B) the *Pythium* damping-off severity of cucumber seedlings in a peat-based mix. Fish emulsion (1%, 2%, and 4%; *m*/*m* peat mix) and *P. aphanidermatum* soil inoculum were added to peat mix, mixed thoroughly, and incubated at 24 ± 1 °C in dark. After 1, 7, 14, or 28 days, cucumber seeds were planted and were incubated in a growth room (15 h light at 24 °C and 9 h dark at 19 °C). Plants were rated 2 weeks later for damping-off disease. Means (±SE) are the average of two experiments (n = 10). Means within each planting time followed by the same letter do not differ significantly according to Fisher's protected LSD test (A) or Student–Newman–Keuls (SNK) test (B) at $P \le 0.05$.

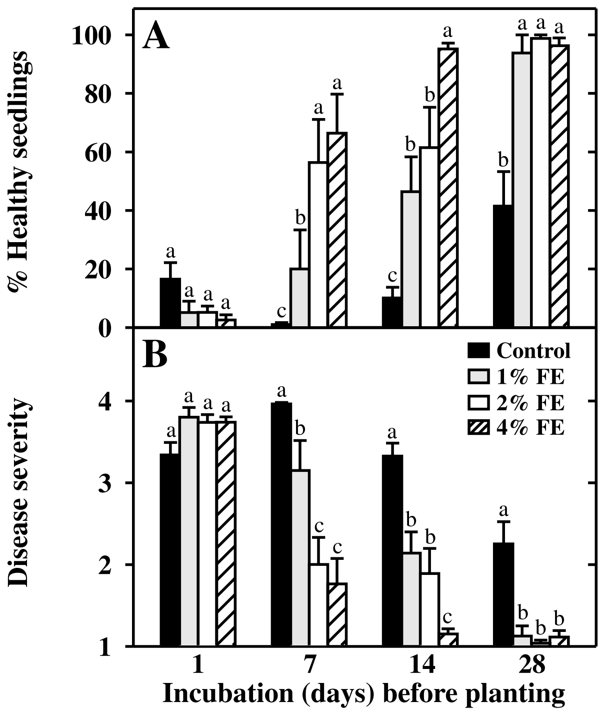
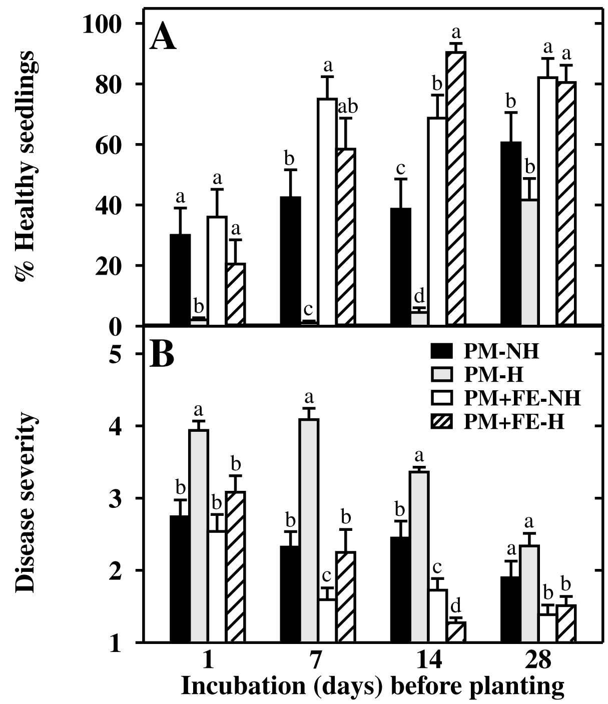


Fig. 3. Effect of heat treatment on the efficacy of fish emulsion (FE) to (A) improve the percentage of healthy seedlings and (B) reduce the *Rhizoctonia* damping-off severity of radish seedlings in a peat-based mix. Pro-Mix (PM) was heated (H) alone or after mixing with FE (4%; *m*/*m* peat mix) at 65 ± 2 °C for 5 consecutive days. Nonheated (NH) PM alone or after adding FE were also included for comparison. *Rhizoctonia solani* soil inoculum was added and the mixture was incubated at 24 ± 1 °C in dark. After 1, 7, 14, or 28 days, radish seeds were planted and incubated in the growth room (15 h light at 24 °C and 9 h dark at 19 °C). Plants were rated 2 weeks later for damping-off disease. Means (±SE) are the average of four experiments (n = 20). Means within each planting time followed by the same letter do not differ significantly according to Fisher's protected LSD test (A) or Student–Newman–Keuls (SNK) test (B) at $P \le 0.05$.



Effect of heat treatment on the efficacy of fish emulsion to control *Rhizoctonia* damping-off of radish seedlings

After 1 day of incubation, the percentage of healthy seedlings was reduced and the damping-off severity was increased in the heated, nonamended (control) peat mix compared with that found in the nonheated, nonamended peat mix (Fig. 3). This increased level of disease in the heated, nonamended mix compared with that in the nonheated control remained until day 28, when disease levels were found to be comparable (Fig. 3). After 1 day incubation, the percentage of healthy seedlings and the disease severity in the nonheated or heated peat mix amended with fish emulsion were comparable to those of the nonheated control but were significantly different from those of the heated control (Fig. 3). Adding fish emulsion to the nonheated peat mix resulted in disease reductions of 100% compared with control (75% healthy vs. 40%) after 7 days of incubation. This relative level of protection was maintained over the entire experiment (Fig. 3A). In the heated peat mix amended with fish emulsion, the damping-off severity was reduced on radish seedlings within 7 days of incubation (Fig. 3B). By 14 days, 90% of radish seedlings were healthy in the heated mix amended with 4% fish emulsion (Fig. 3A).

There was no drastic change in pH of heated and nonheated peat mix, suggesting that ammonia and nitrous acid were not involved (data not shown). Addition of fish emulsion to the nonheated peat mix substantially increased the number of total culturable fungi and bacteria after 1, 2, and 4 weeks of incubation (Table 3). However, these numbers were similar in the control and fish emulsion treatments after 1 day of incubation. Heating of peat mix before or after adding fish emulsion destroyed most of these organisms (Table 3). Samples taken from pasteurized peat mix after 1 day incubation showed no microbial growth on either medium. Fungi and bacteria recolonized the heated peat mix after 7 days of incubation, though bacterial numbers remained lower, whereas fungal numbers were increased by heating in both the control and peat mix amended with fish

Table 3. Effect of pasteurization of peat mix before or after adding fish emulsion on populations of culturable bacteria and fungi.

		Population density for incubations of			
Treatment	Pasteurization	1 day	7 days	14 days	28 days
Total bacterial population (×10^6 CFU/g peat mix)					
Control	–	230±110	640±220	660±110	570±90
Fish emulsion	–	200±100	2100±1100	2800±780	4750±2700
Control	+	0±0	130±70	250±90	390±40
Fish emulsion	+	0.2±0.1	35±5	380±80	1150±140
Total fungal population (×10^4 CFU/g peat mix)					
Control	–	380±130	600±180	2200±1000	200±50
Fish emulsion	–	330±140	1200±300	3150±1100	750±200
Control	+	0±0	150±40	3300±1500	1000±450
Fish emulsion	+	2±1	400±130	4000±400	1750±300

Note: Peat mix was pasteurized alone or after addition of fish emulsion (4%; *m*/*m* peat mix) by heating in an oven at 65 ± 2 °C for 5 days. Treatments were then incubated at 24 ± 1 °C. Samples were taken for microbial counts before planting radishes. Values shown are means ± SE for the average of two experiments (n = 4).

emulsion. These microbial numbers were slightly higher in the peat mix pasteurized after the addition of fish emulsion.

Effect of fish emulsion on radish or cucumber seedling damping-off in soil

The potential of fish emulsion added to a sandy loam soil artificially infested with *R. solani* soil inoculum in improving the percentage of healthy seedlings and reducing the severity of the disease on radish seedlings is shown in Fig. 4. In soils treated with the 0.5% fish emulsion, more than 90% of the radish seedlings remained healthy, as compared with only 10% in the nontreated control. In the second planting, almost 80% of the seedlings were healthy in the fish emulsion-treated soil, whereas 25% were healthy in the control soils (Fig. 4A). The fish emulsion treatment also significantly reduced the damping-off severity in both plantings (Fig. 4B). Rates of fish emulsion higher than 0.5% showed some level of phytotoxicity when added to soil.

Damping-off incidence and severity in naturally infested muck soil was consistently high (Fig. 5). Fewer than 20% of the cucumber plants in the nonamended control were healthy. Unlike in peat mix, all three concentrations of fish emulsion were effective in improving the healthy seedlings and reducing the disease in muck soil 1 day after incubation. However, the efficacy of a low rate (1%; *m*/*m* muck soil) of fish emulsion decreased over time and was similar to the control after 28 days of incubation before planting cucumbers. Higher rates of fish emulsion (2% and 4%) were consistent in reducing the disease throughout. In fact, the effectiveness of higher rates of fish emulsion was increased as the planting of cucumber seeds in the amended soil was delayed 14 or 28 days (Fig. 5). Almost 85% of the cucumber plants were healthy with 4% fish emulsion after 28 days of incubation.

Effect of fish emulsion on plant growth

The fertilizer effect of 1%, 2%, and 4% fish emulsion on growth of radishes and cucumbers in the absence of a pathogen was compared with the N–P–K inorganic fertilizer equivalent. Radish seedlings showed an accelerated growth with the 4% fish emulsion treatment. After 28 days of growth in peat mix, however, the fresh and dry mass of both shoots and roots were similar for plants grown in 4% fish emulsion or equivalent fertilizer (Table 4). Compared with the nonfertilized control plants, these plants were heavier in fresh shoot (145%–150%) and root mass (89%–112%) and in dry shoot (80%–100%) and root mass (62%–67%).

Fig. 4. Effect of fish emulsion (FE) added to soil infested with *R. solani* on (A) the percentage of healthy seedlings and (B) the damping-off severity of radish seedlings. FE (0.5%; *m*/*m* soil) and *R. solani* soil inoculum were incorporated into soil from a commercial potato field. After 5 days, radish seeds were planted and incubated in the growth room (15 h light at 24 °C and 9 h dark at 19 °C). Plants were rated 2 weeks later for pre- and post-emergence damping-off. After the plants from first planting were removed, more radish seeds were planted and incubated in the growth room. Plants were rated 2 weeks later for pre- and post-emergence damping-off. Means (±SE) are the average of two experiments (n = 6). Means within each planting followed by the same letter do not differ significantly according to Fisher's protected LSD test (A) or Student–Newman–Keuls (SNK) test (B) at $P \leq 0.05$.

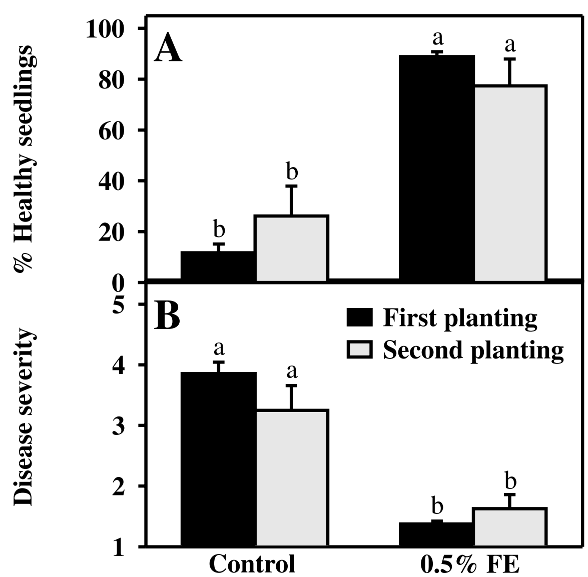
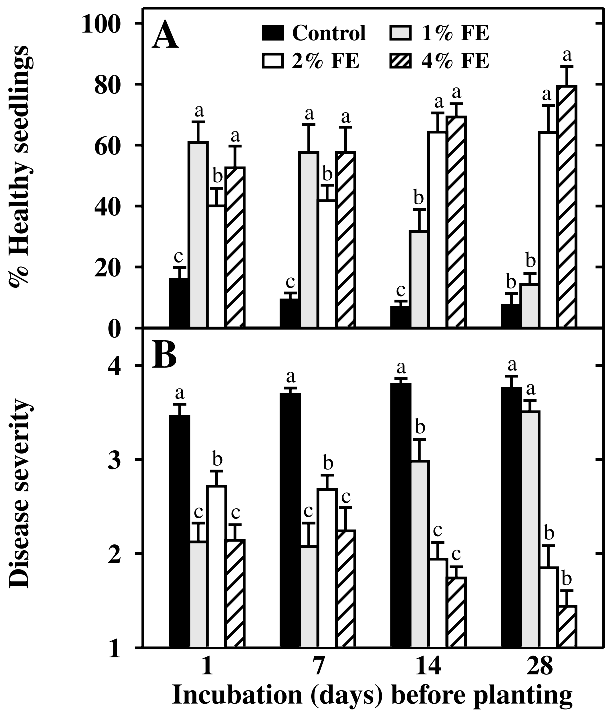


Fig. 5. Effect of fish emulsion (FE) on (A) the percentage of healthy seedlings and (B) the damping-off severity of cucumber seedlings in muck soil naturally infested with damping-off pathogens. FE (1%, 2%, or 4%; *m*/*m* soil) was incorporated into infested muck soil from a commercial field. After 1, 7, 14, and 28 days, cucumber seeds were planted and incubated in the growth room (15 h light at 24 °C and 9 h dark at 19 °C). Plants were rated 2 weeks later for damping-off. Means (±SE) are the average of three experiments (n = 15). Means within each planting time followed by the same letter do not differ significantly according to Fisher's protected LSD test (A) or Student–Newman–Keuls (SNK) test (B) at $P \leq 0.05$.



Similarly, the mass of cucumber plants produced in fish emulsion or peat mix amended with equivalent inorganic fertilizer increased proportionally as the concentration of fish emulsion or N–P–K fertilizer increased (Table 5). Fresh and dry mass measurements of plants produced in 4% fish emulsion treatment were three and two times greater, respectively, than those of the nonfertilized control plants.

Radish shoot and root samples were analysed for concentration of macro- and micro-nutrients. Total nitrogen and iron contents were higher in shoot tissues of radish grown in peat mix amended with 4% fish emulsion or equivalent fertilizer as compared with the nonfertilized control treatment (Table 6). Sodium content was higher in both shoot and root samples of radishes produced in peat mix amended with fish emulsion. The concentration of all other elements analysed was similar in samples from fish emulsion, fertilizer, and control treatments.

Discussion

This study demonstrated that use of fish emulsion as an amendment to peat mix or soil suppressed damping-off diseases caused by *R. solani* and *P. aphanidermatum*. The suppressive effect started to appear 5–7 days after addition of fish emulsion, indicating that an incubation period was required before disease protection occurred. There was no protection of the seedlings in the peat mix by fish emulsion after 1 day of treatment, indicating that fish emulsion has no immediate or direct impact on the pathogens. However, there is a need to further evaluate what effect fish emulsion has on the fate of the pathogen populations in the amended mix. Similar results were reported in a study by Theodore and Toribio (1995). They reported that suppression of *P. aphanidermatum* in composts prepared from sugarcane factory residues was not immediate, but rather occurred within 14 days after infestation.

Addition of composts and other organic materials to potting substrates or agricultural soils has been reported to suppress damping-off diseases caused by *R. solani* and *Pythium* spp. (Chen et al. 1988; Hadar and Mandelbaum 1986; 1992; Huber and Summer 1996; Nelson and Hoitink 1983; Papavizas and Davey 1960; Tuitert et al. 1998). Most of these studies attributed the suppression of disease to increased microbial activity in the amended substrate. Although dark Sphagnum peat-based mix has been considered conducive to damping-off and less supportive of microbial

Table 4. Effect of fish emulsion or fertilizer equivalent added to peat mix on growth of radishes.

	Fresh mass/plant (g)		Dry mass/plant (g)	
Treatments	Shoot	Root	Shoot	Root
Control	2.0±0.1 b	6.5±0.7 b	0.3±0.0 b	0.6±0.1 b
4% fertilizer	5.0±0.1 a	12.4±0.6 a	0.5±0.0 a	0.9±0.0 a
4% fish emulsion	5.1±0.1 a	13.8±0.6 a	0.5±0.0 a	0.9±0.0 a

Note: Means followed by the same letter in each column do not differ significantly according to Fisher's protected LSD test at $P \leq 0.05$. Fish emulsion or fertilizer equivalent (4%; *m*/*m* peat mix) was incorporated into peat mix, mixed thoroughly, and radish seeds were immediately planted. Radishes were harvested after 4 weeks and fresh and dry mass measurements were determined. Values shown are means ± SE for the average of two experiments (n = 50).

Table 5. Effect of fish emulsion or fertilizer equivalent added to peat mix on growth of cucumbers.

Treatments	Fresh mass/plant (g)	Dry mass/plant (g)
Control	3.3±0.2 d	0.4±0.0 d
1% fertilizer	5.5±0.1 c	0.6±0.0 c
2% fertilizer	7.2±0.2 b	0.7±0.0 b
4% fertilizer	9.1±0.2 a	0.8±0.0 a
1% fish emulsion	5.4±0.1 c	0.6±0.0 c
2% fish emulsion	7.1±0.2 b	0.7±0.0 b
4% fish emulsion	10.3±0.3 a	0.9±0.0 a

Note: Means followed by the same letter in each column do not differ significantly according to Fisher's protected LSD test at $P \leq 0.05$. Fish emulsion or fertilizer equivalent (1%, 2%, and 4%; *m*/*m* peat mix) was incorporated into peat mix, mixed thoroughly, and cucumber seeds were immediately planted. Cucumbers were harvested after 3 weeks and fresh and dry mass measurements were determined. Values shown are means ± SE for the average of two experiments (n = 50).

Table 6. Nutrient analysis of radish plants grown in peat mix amended with fish emulsion or equivalent inorganic fertilizer.

	Concentration in shoot tissue			Concentration in root tissue		
Nutrients analyzed	Control	Fertilizer	Fish emulsion	Control	Fertilizer	Fish emulsion
N (%)	3.0±0.3 b	5.6±0.1 a	5.6±0.1 a	1.7±0.0 a	2.6±0.1 a	2.5±0.1 a
P (%)	0.6±0.0 a	1.0±0.0 a	0.7±0.0 a	0.7±0.0 a	0.7±0.0 a	0.7±0.0 a
K (%)	3.8±0.2 a	4.8±0.1 a	4.1±0.1 a	3.7±0.1 a	3.8±0.1 a	3.4±0.0 a
S (%)	0.9±0.0 a	1.4±0.0 a	1.2±0.0 a	0.8±0.0 a	0.7±0.0 a	0.8±0.0 a
Mg (%)	0.7±0.0 a	0.6±0.0 a	0.6±0.0 a	0.2±0.0 a	0.2±0.0 a	0.2±0.0 a
Ca (%)	3.3±0.1 a	2.7±0.0 a	2.6±0.0 a	0.5±0.0 a	0.4±0.0 a	0.4±0.0 a
Na (%)	0.3±0.0 b	0.4±0.0 b	1.2±0.1 a	0.2±0.0 a	0.3±0.0 a	0.8±0.0 a
Fe (µg/g)	73±5 b	110±3 a	130±6 a	46±1 a	49±1 a	50±1 a
Al (µg/g)	14±1 a	16±0 a	20±1 a	11±1 a	12±1 a	13±1 a
Mn (µg/g)	170±8 a	170±12 a	170±11 a	25±2 a	27±1 a	27±0 a
B (µg/g)	63±2 a	71±3 a	55±1 a	20±0 a	20±0 a	18±0 a
Cu (µg/g)	9±0 a	15±3 a	16±3 a	9±2 a	13±4 a	12±4 a
Zn (µg/g)	140±11 a	130±3 a	160±6 a	62±1 a	61±1 a	60±1 a

Note: Means followed by the same letter in each row for shoot or root tissue do not differ significantly according to Fisher's protected LSD test at $P \leq 0.05$. Fish emulsion or fertilizer equivalent (4%; *m*/*m* peat mix) was incorporated into peat mix, mixed thoroughly, and radish seeds were immediately planted. Radishes were harvested after 4 weeks and analysed for macro- and micro-nutrients. Values shown are means ± SE for the average of two experiments ($n = 4$).

activity (Nelson and Hoitink 1982), most of the testing done was with immediate planting, that is, 1 day after adding biological control agents in the mix (Krause et al. 2001). The results may have been different if the authors had increased the incubation time before planting radish seeds. In general, disease suppression has been attributed to the increased biological activity and the microbial carrying capacity of the mix. Results of this study also indicated that the suppressive effect of fish emulsion was dependent on biological activity or microbial carrying capacity of the peat mix. The disease-suppressive effect of the peat mix amended with fish emulsion was enhanced over time and corresponded with increased microbial activity in the amended mix. Seven days after incorporation of fish emulsion to peat mix, the populations of culturable fungi and bacteria had increased. This incubation time also corresponded to the initiation of the disease suppression by the amended peat mix. When the fish emulsion was assayed for culturable microbes, no colonies were recovered on growth medium (unpublished data). Thus, the fish emulsion does not seem to be a source of suppressive microbes.

The biological nature of disease suppression was further evident from our experiments with pasteurized peat mix. Pasteurizing peat mix led to a much higher incidence of disease of radish seedlings than that in the nonheated control. This suggests that peat mix has some natural disease-suppressive component that begins to reappear in pasteurized peat mix after 28 days of incubation. Addition of fish emulsion enhanced the disease suppressive effect of unpasteurized peat mix within 7 days of incubation. Pasteurization obviously destroyed the microbial activity. Samples taken from pasteurized peat mix after 1 day incubation showed no microbial growth on various culture media. However, fungi and bacteria recolonized the heated peat mix after 7 days of incubation. On the other hand, when peat mix amended with fish emulsion was pasteurized, the disease suppressive effect of peat mix was restored within 7 days of incubation and was maximized after 14 days. This effect of heat treatment on disease suppression by the peat mix amended with fish emulsion needs to be further explored. Further studies are also required to determine whether fish emulsion favours any specific kinds of microorganisms or whether it generally enhances the microbial number in the peat-based substrates. Also, it will be interesting to know the disease suppressive efficacy of fish emulsion in other substrates, for instance, in compost-amended substrates, etc.

Lower rates of fish emulsion gave comparable disease suppression in loamy sand soil and peat mix or muck soil. It is very unlikely that nitrogen transformation products such as ammonia and nitrous acid (Tenuta and Lazarovits 2002a) or volatile fatty acids (Tenuta et al. 2002) had a role in disease suppression in this study. The presence of enough volatile fatty acids or generation of enough ammonia and nitrous acids sufficient to kill the damping-off pathogen was not possible at the low rates of fish emulsion used in this growth chamber study. Although rates higher than 1% (*m*/*m*) in soil may generate enough ammonia for pathogen disinfestation (unpublished data), it may be phytotoxic and economically nonviable. However, this may not be the case with peat-based substrates or muck soil, which have high organic matter content that has been shown to prevent ammonia accumulation (Tenuta and Lazarovits 2002b). The low rate of fish emulsion did not completely eradicate the pathogen in the soil but established biological conditions that prevented infection.

In addition to disease protection, fish emulsion also enhanced the growth of radishes and cucumber plants. The fertilizer effect of fish emulsion was comparable to that of its equivalent inorganic fertilizer. The mass of these plants produced in fish emulsion or equivalent inorganic fertilizer-amended peat mix increased proportionally as the concentration of fish emulsion or N–P–K fertilizer increased. Fresh and dry mass measurements of plants produced in peat mix amended with fish emulsion were significantly greater than those of the nonfertilized control plants. This is not surprising, since use of fish emulsion or fish soluble nutrients as fertilizer is well known (Aung and Flick 1980; Aung et al.

1983, 1984; Ceci 1975; Emino 1981). Nutrient analysis of radish plants treated with fish emulsion showed similar contents of the majority of mineral elements to that of N–P–K fertilizers. It is important to point out here that the N–P–K inorganic fertilizer equivalent treatment provided no disease protection against *R. solani* damping-off, indicating that the disease reduction by fish emulsion was not due to plant nutrition.

Information to assist in the consistent use of fish emulsion for disease control or plant nutrition is still lacking. First, the rates at which fish emulsion provides disease suppression in loamy sand soil, muck soil, or peat-based substrates are very different. The rates that are effective in peat mix may be highly phytotoxic in soil if not added sufficiently ahead of planting. Second, we have found that the efficacy of fish emulsion may be impacted by batch to batch variability in the peat-based substrates. This is important because disease suppression by fish emulsion appears to depend primarily on the microbial carrying capacity of the peat mix. While the composition of fish emulsion is fairly similar, some variability does occur from batch to batch. For instance, batches of fish emulsion received in 2001 and 2003 had different amounts of sulfur (12 700 vs. 19 894 ppm, respectively) and total nitrogen (4.3% vs. 6%, respectively). While the 2003 batch of fish emulsion was as effective in disease suppression as the 2001 batch, the rates may need to be adjusted to avoid phytotoxicity (unpublished data).

In conclusion, fish emulsion had no direct toxicity to the damping-off pathogens but appeared to create a biological climate that was suppressive to disease initiation. The rates used were highly economical and the practise appears to be environmentally benign. Incorporating fish emulsion to peat-based substrate 7–14 days before planting provides a potentially safe way to generate healthy seedlings. It can also be used as an organic fertilizer. This study indicates that fish emulsion may provide an ideal base to deliver effective biological control agents under both greenhouse and field settings. Currently, studies are underway to further enhance the disease suppression by formulating effective biocontrol agents with fish emulsion in peat-based substrates. Our aim is to minimize the incubation time required for maximum disease suppression by fish emulsion amendments.

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