

The Biological Control of Pea Root Rot and Damping off on Lettuce by Effective Microorganisms.

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1. Abstract

Pea root rot *Aphanomyces euteiches* is an important pea (*Pisum sativum*) disease world wide. Previous trials with suppressive composts had shown a suppression of pea root rot by up to 57 percent. A verified bioassay was used to measure the level of biological control offered by two concentrations of effective microorganisms (EM) (a commercial mixture of microorganisms including yeasts, fungi, bacteria and Actinomycetes), with water and water + molasses controls, against three inoculation levels (0, 5×10^2 , 5×10^3 spores/ml) of *Aphanomyces euteiches* zoospores. There was no significant difference between the EM treatments, water or water + molasses. There was a highly significant difference in infection levels between the three zoospore inoculum levels. Previous trials with composts have indicated that the presence of *Acremonium* spp., *Fusarium* spp., and *Paecilomyces* spp. improved disease suppression in the composts while *Penicillium* spp. increased disease development. This may have a bearing on the failure of EM to control pea root rot.

Control of damping off in organic production systems is difficult, due to the prohibition of synthetic fungicides. A successful biological control agent would be of considerable benefit for organic agriculture. A trial was set up to evaluate the potential of EM for control of damping off in lettuce. Soil, with a history of high levels of damping off, from the Biological Husbandry Unit at Lincoln University, Canterbury, New Zealand, and a control of commercial seed raising mix was placed into 7.5 cm pots and planted with ten lettuce seeds (*Lactuca sativa*, cv. Red Sails). Treatments were EM at standard and double strength, water + molasses, water, bokashi (a compost fermented with EM), and bokashi plus EM. The peat based seed raising compost received a water treatment and EM treatment. A very low rate of emergence of lettuce seedlings in all treatments appeared to be caused by high temperature dormancy. The trial was repeated in lower temperatures without success. The number of weed seedlings from the first trial were analysed but no clear trend was apparent.

Keywords.

Effective microorganisms, EM, lettuce, *Lactuca sativa*, pea, *Pisum sativum* pea root rot, *Aphanomyces euteiches*, biological control, damping off.

2. Introduction

EM is a water based mixed culture of microorganisms, including yeasts, lactic acid bacteria, photosynthetic bacteria, Actinomycetes, and fermenting fungi which include *Aspergillus* spp. and *Penicillium* spp, developed by Professor Teruo Higa of the University of Ryukyus, Japan, (Higa 1994).

Pea root rot is an important pea disease world wide, and has also caused severe crop losses in Canterbury and Nelson, New Zealand (Manning & Menzies 1980). There is a lack of suitable control measures. To

date neither chemical and biological control measures nor disease resistant pea cultivars exist (Cerenius et al. 1992, Roa et al. 1995). Disease avoidance is practised, forcing growers to use rotations of six years or more.

Some composts can suppress soil borne diseases (Craft & Nelson 1996, Hoitink & Grebus 1994). Walter et al (1995a) recorded suppression of up to 57 % in the level of *A. euteiches* on peas grown in manure based composts. As some of the microorganisms in EM are present in composts it indicated that EM may be able to reduce pea root rot levels. A verified bioassay was used to assess the effect of EM on pea root rots.

Damping off is the generic name for a group of pathogens that attack seeds or young seedlings and include *Pythium* spp. and *Rhizoctonia solani* (Stewart 1997). Damping off is a widespread problem for horticulture and agriculture. It also can be suppressed by composts (Craft & Nelson 1996). Damping off can be successfully controlled by fungicides, particularly as seed dressings (Omar & Rahhal 1993, Paradela et al. 1995). This however is not an option for organic farmers. There is also increasing pressure from consumers to reduce the levels of agrichemicals used in food production (Ball 1997). A biological control agent for control of damping off would be of considerable use to both organic producers and non-organic producers wishing to reduce agrichemical use in their production systems.

3. Methods

3.1 Pea Root Rot

There were twelve treatments consisting of two strengths of EM (EM1 & EM2), two controls (molasses + water control (Mol), and water control (H₂O)) and three concentrations of *A. euteiches*, (Ae2, Ae1, Ae0) with five replicates and four pea plants per replicate.

EM1 solution was prepared by mixing molasses from F.E.L. Ltd. Timaru, New Zealand and EM stock solution from T. Higa, I.N.F.R.C. Japan, with untreated water, in a ratio of 1:1:1000 EM stock solution:molasses:water. For EM2 a ratio of 2:2:1000 was used. This mixture was fermented in a closed container at room temperature for a minimum of 24 hours before use and was discarded after five days. It was kept in the dark at room temperature.

Molasses solution was prepared by dilution with water in a ratio of 1:1000 molasses:water, and kept in the same conditions as the EM solutions.

A. euteiches spore suspensions were prepared according to the methods of Walter *et al* (1995a), in three concentrations, Ae0 = 0 zoospores/ml water, Ae1 = 5×10^2 zoospores/ml water, Ae2 = 5×10^3 zoospores/ml water.

Polybags (size 3) were filled with 1200 g (\pm 4 g) of soil taken from a field that had been under organic management for 6 years, permanent pasture for eight years, and clear of peas for 12 years. The polybags were placed individually in two litre containers to retain surplus water. 130 ml of water was added to the soil in each polybag to field capacity.

13 ml of EM1, EM2, Mol or H₂O treatments, diluted in 100 ml of water, were applied to the polybags two days before planting. The polybags were immediately covered with newspaper and a clear polythene sheet to reduce moisture loss, and kept at room temperature until planting.

Peas seeds were surface sterilised in 0.3% sodium hypochlorite for three minutes, rinsed in sterile water, planted in sterile sand and grown under artificial illumination at room temperature for five days. The

seedlings were carefully removed from the trays, and adhering sand particles were rinsed off with distilled water. The pea roots were inoculated with the *A. euteiches* zoospore suspension by dipping them up to the epicotyl for three seconds, and then immediately planted.

The polybags were transferred to a glasshouse and arranged in four blocks for each of the four treatments EM1, EM2, Mol, or H₂O to minimise cross contamination. The blocks were rotated round the glasshouse bench, and the internal arrangement of the polybags in the blocks was rotated every four days. The two litre containers were kept topped up with one centimetre of water.

The EM1, EM2, Mol, or H₂O treatments were applied at a rate of 13 ml per pot to the pea foliage and soil immediately surrounding the plants. This is equivalent to the recommended EM field application rate of 10,000 l/ha (Anon 1995). Treatments were applied immediately after planting and at 4, 8, 14 and 18 days.

At day 24 disease severity index (DSI) (Sherwood & Hagedorn 1958) and pea shoot wet weight were measured. These two measurements were combined into a die-off rate calculated by; die-off rate = $(1 - \text{wet weight}_{\text{fungus}} / \text{wet weight}_{\text{H}_2\text{O}}) * \text{DSI}_{\text{fungus}} / 100$. Data was analysed using analysis of variance.

3.2 Damping-Off

Soil with a history of severe post seedling emergence damping-off was taken from a polytunnel at the Biological Husbandry Unit (BHU), Lincoln University, Canterbury, New Zealand. The BHU has been under organic management for 20 years. The soil was thoroughly mixed with a spade. 7.5 cm plastic plant pots were filled with the soil to one centimetre below the rim. The pots were then placed in trays to catch excess water.

There were six treatments consisting of two concentrations of EM (EM1 & EM2), two bokashi applications (bokashi & bokashi + EM1), and two controls (molasses + water & water control)

There were 16 replicates per treatment, with ten lettuce seeds per replicate.

EM1, EM2 and molasses & water were prepared as for the pea root rot trial.

Bokashi was prepared by finely chopping lucerne (*Medicago sativa*) hay, then applying EM1 solution, prepared as above, onto the pile of chopped lucerne with a watering can until the moisture level was about 30-40%. The mixture was then placed in a pile on the ground, covered with hessian sacking and fermented for five days. 2.6g of bokashi was applied to each pot and mixed into the top half of the soil.

Bokashi & EM1 treatment was a combination of the EM1 treatment and bokashi treatment.

After filling the pots with soil and applying the bokashi as described above, 7.5 ml of EM1, EM2, Mol, or H₂O was added to 125 ml of water and applied to the pots, to bring the soil up to field capacity. The pots were left for two days and then sown with 10 lettuce seeds. The seeds were covered with one centimetre of soil from the initial mixed soil sample and an additional 125 ml of water with 7.5 ml of EM1, EM2, Mol, or H₂O was applied.

The pots were placed in the same polytunnel from where the soil was collected, in a fully randomised block design. Two layers of Mikroclima® knitted frost protection fabric were placed over the pots to increase the humidity. Further treatments of 7.5 ml of EM1, EM2, Mol, or H₂O were applied at 4, 8 and 14 days, to the soil surface. This rate is equivalent to the recommended EM field application rate of 10,000 l/ha (Anon 1995).

On the second trail two additional treatments were added. These were Commercial seed raising mix with water control and Commercial seed raising mix with EM1.

The second trial was established with fresh soil and all equipment was sterilised. All other procedures were the same as the first trial except for the following. The pots were placed in a well ventilated cloche 1.2 m wide and 40 cm high, covered with clear polythene sheet. After eight days and no seedling emergence, the pots were moved into a building with restricted amounts of natural light, and at room temperature. A further ten seeds were added to each pot and mixed into the top 0.5 cm of the soil. Treatments were applied at 2 days prior to first seeding, at seeding, 4, 8 (second seeding), 12, 16, and 20 days.

The lettuce seed was tested for germination rate by placing 50 seeds on moist blotting paper in a 10 cm diameter by 5 cm high opaque plastic container with an air tight lid, and kept at 30°C for four days.

4. Results

4.1 Pea Root Rot

There was no significant difference ($P>0.05$) between the EM1, EM2, H₂O, or Mol treatments. There was a highly significant difference ($P<0.001$) between the three Ae treatments, Table 1.

Table 1. Effect of treatments on die-off rate of peas (analysis of variance)

	Ae0	Ae1	Ae2	Total	S.E for Total
EM1	0.00	2.52	1.32	1.28	0.354
EM2	0.00	3.46	2.08	1.85	0.403
H ₂ O	0.00	3.62	1.48	1.70	0.442
Mol	0.00	2.99	1.17	1.39	0.411
Total	0.00	3.15	1.51	1.55	
S.E. For Total	0.000	0.291	0.152		

4.2 Damping-Off

Neither trial had successful lettuce germination. A variation was noticed between weed seed in the pots in the first trial. These were counted and analysed by analysis of variance with a Bonferroni pairwise comparison of means at $P<0.05$, Figure 1.

The lettuce seed germination test gave a 94 % germination rate.

5. Discussion

5.1 Pea Root Rot Trial

There was no evidence of disease suppression by the EM treatments.. The high level of significance between the three Ae levels ($P<0.001$) indicates the test worked correctly. It has been suggested that perhaps alternative preparations of EM e.g. EM5 (EM fermented with vinegar and alcohol) or EM Fermented Plant Extracts (EM fermented with fresh weeds) (Anon 1995) would be more appropriate in this situation.

Previous trials with composts by Walter et al (1995b) have indicated the presence of *Acremonium* spp., *Fusarium* spp., and *Paecilomyces* spp. improved disease suppression in the composts while *Penicillium* spp. increased disease development. EM contains *Penicillium* spp. (Anon. 1995) which may be counteracting the beneficial effects of the other constituents of EM which may have a bearing on the failure

of EM to control pea root rot. Further trials using EM5 and EM-FPE would be beneficial, and more detailed work on the mode of action of EM on *A. euteiches* is needed to clarify this work.

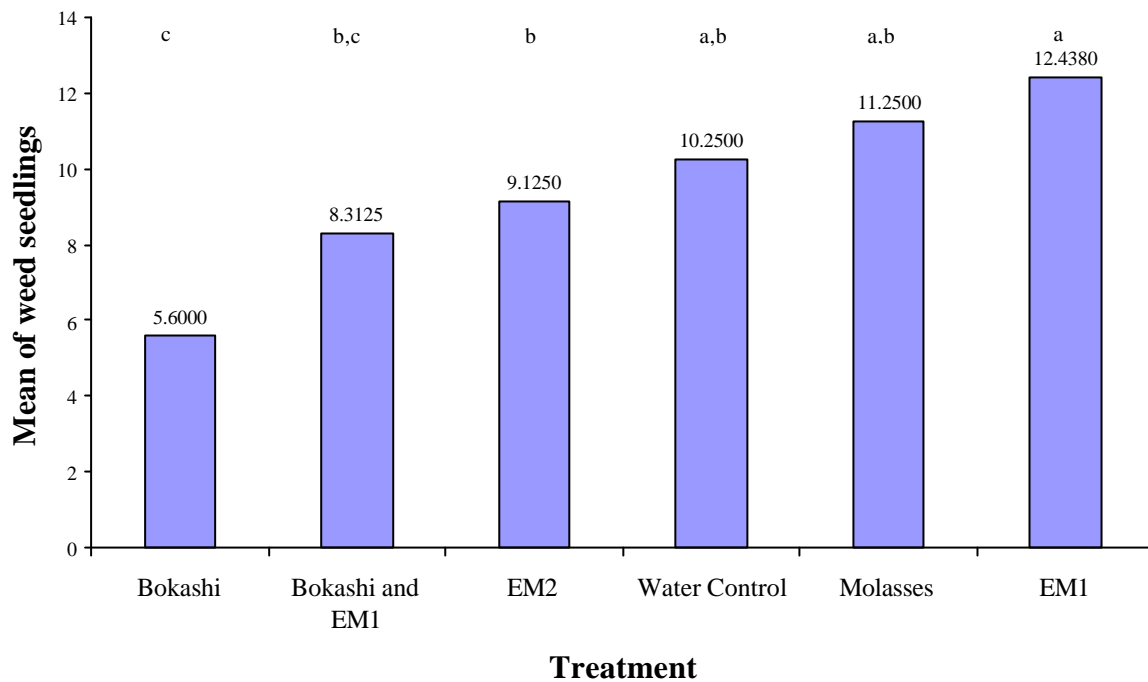


Figure 1. Mean number of weed seedlings for six treatments. Treatments with the same letter above them are not significantly different ($P < 0.05$) analysis of variance with Bonferroni pairwise comparison of means.

5.2 Damping-Off Trial

The 94 % germination rate for the lettuce seed and the satisfactory germination of lettuce seed from the same batch as used in the trial but planted elsewhere, suggested high temperature dormancy may be responsible for the lack of lettuce germination in the trial. Damping off is predominately a disease of spring, when there are lower light levels and temperatures. The trial has therefore been postponed until next spring.

The weed emergence data is not conclusive, Figure 1 . The bokashi treatment is the only one significantly different from the controls. It is possible that the extra organic material in the bokashi treatments decreased the amount of soil, and hence the amount of weed seed, in the germination zone. Further work would be required to confirm and explain these results.

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