

REPORT

Short term Scientific Mission in the COST framework

The title: The Use of Entomopathogenic Nematodes in Alginate Gel Formulations for the Control of *Plutella xylostella* in Cruciferous Vegetables

By

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INTRODUCTION

Nematode formulations on the plant did not reach so far a practical use because their persistence on the leaf was too short. In recent years an effort was made to develop an effective formulation for use of the nematode on the plant. This formulation was based on an alginate gel which provided the nematode with persistence for 1-2 days and was edible to the larvae due to the presence of a phagostimulant in the gel (Navon et al., 1998). In the present work the gel formulation was modified for use against *Plutella xylostella* larvae in choice bioassays and plant tests. This report details the experiments with the alginate gel and the implications of the work are discussed.

MATERIALS AND METHODS

Nematodes, insects, plants

The nematode *Steinernema carpocapsae* strain Egypt - S2 was raised on *Galleria mellonella* larvae at room temperature was used in all tests. The diamondback moth, *Plutella xylostella*, was reared on Friesling cabbage in a rearing room (25^o C, 60-80% R.H. and 16 h photophase). 3rd and 4th instar larvae were used for all experiments. The cabbage *Brassica oleraceae* var. capitata was raised in pots and leaves were used for the choice bioassays. In plant bioassays the whole potted plant was sprayed with a 500 ml hand spray bottle.

Alginate gel

The basic alginate gel formulation detailed in Table 1 was used with different phagostimulant mixtures, alginate concentrations and nematode doses.

TABLE 1. Composition of the alginate gel formulation in 40 ml water for tests with *Plutella xylostella* larvae

Material	Amount (mg)	% w/v
Sodium alginate - Protanal SF, "Protan" Drammen Norway	800	2.0
Calcium carbonate	150	0.375
Xanthan gum (Keltrol, Monsanto)	200	0.5
Yeast extract	40	0.1
Methyl para-hydroxy benzoate (Nipagin)	10	0.025
Glucono lactone	400	1

All the ingredients were made by Sigma or Merck. The following steps in gel preparation were used: The calcium carbonate was first mixed into the water followed by sodium alginate, xanthan gum, yeast extract and Nipagin. At this stage infective juvenile nematodes in 1 ml water were mixed in. Then the glucuronolactone used as the gelling agent was mixed in. A kitchen mixer with two propelling wings was used to prepare the gels.

Bioassays

Phagostimulant choice tests in Petri dish bioassays:

The first phagostimulant mixture tested in the alginate gel was an aqueous solution of cabbage leaves (1:1 water:leaf ratio) heated to boiling with water. The second phagostimulant mixture in the gel contained 2% sucrose and 0.5% cellulose powder. Nematode dose in the gel was 1,000/alginate gel. Leaf disks of 18 mm diam. were cut with a cork borer over a sandpaper. In the choice tests two such disks were placed on a moistened filter paper Whatman No. 1. Gel combinations were poured after the addition of the gluconolactone to an 11 cm diam. glass Petri dish. Gel disks of 18 mm diam. and 5 mm thick were cut out and placed each on the leaf disks so that the leaf discs were covered with the alginate layer. Ten larvae of 3rd and 4th instar were placed in the Petri dish (9 cm diam.) and exposed to two different discs for choice tests. The experiment was conducted in a rearing room with ambient temperatures and 40% RH. After 18 h leaf defoliation and after 18 h and 36 h mortality was recorded. Nematode observations were made in the bioassay gel and in the unused gel kept in the refrigerator 15°C.

Effect of humidity

Detached leaves of cabbage were sprayed with the hand sprayer with 0.5% Tween 80 solution followed by a spray of 1% alginate mixture with and without nematodes (500/g gel). The 1% alginate gel was made by dilution of 2% alginate 1:1 water. The xanthan, Nipagin and yeast extract concentration remained as in Table 1. The choice bioassays were conducted in a 100 ml plastic vials (4 cubic cm.). The lid was perforated and a mesh was glued to the hole to avoid larval immigration. The vials with the gel combinations and larvae were placed on cabins at 20° C one at 60% and the other at 85% RH. Defoliation was recorded after 18 h. The larvae were put on fresh cabbage leaves and the mortality was recorded after 36 and 48 h.

Effect of whole plant spray on larval mortality

The potted plant was sprayed with the hand sprayer. The treatments were: 1.) 1% alginate with nematodes 500/g gel; 2.) alginate without nematodes; 3.) no spray control. Fifty larvae were put on each potted plant which was set in a plastic cage with a net cover on one side for ventilation. The experiment was run at 20° C and 85% RH. Mortality was counted after 72 h.

Effect of nematodes in different alginate concentrations

Detached leaves were sprayed with diluted alginate doses with 0.5% Tween 80. The experiment included the following treatments: 1.) 0.25% alginate; 2.) 0.5% alginate; 3.) 0.25% alginate with nematodes; 4.) 0.5% alginate with nematodes; 5.) Control with 0.5% Tween 80. The nematode concentration was 500/g gel. Mortality was counted after 18 h and 58 h. The experiment was conducted in choice bioassays in Petri dish.

RESULTS

The results of the choice test are summarized in Figure 1. Of the leaf with the alginate disk 15% were defoliated and close to 40% of the leaf in the untreated control. The presence of phagostimulants in the gel somewhat increased defoliation (42%) but without significant difference from the alginate treatment (33%). Furthermore, the effect of cabbage extract phagostimulants mixture in the gel (44%) was similar to that of the control leaf (46%). The defoliation caused by phagostimulants in the gel in the presence of nematodes was somewhat higher (15%) than without the phagostimulants (8%). These records suggest that the phagostimulants did not contribute to reducing leaf feeding as a result of nematode effect on the larvae. Therefore, the use of the cabbage extract in the gel for pest control seems unjustified. On the other hand, the nematode reduced defoliation to about one third of that in the control leaf, indicating that the pathogenic effects of the nematodes as a layer on the leaf minimized leaf damage.

Figure 2 shows that similarly to the use of the cabbage extract (Fig. 1) the sucrose-cellulose phagostimulant mixture did not increase defoliation and again, the presence of nematodes in the gel reduced defoliation to 30-40% of the level in the control leaf. Figure 3 shows that at 80% humidity defoliation in leaves with alginate or alginate with nematodes was higher than in 60%. But, there were exceptions where defoliation of the control leaf was smaller than in the alginate spray and this is explained by the fact that the control leaf at the start of the experiment were somewhat dry and less tasteful than in the alginate treatment where the leaf freshness was high and supported larval feeding. In Figure 4 more consistent result were obtained showing that both 60% and 80% mortality constantly increased from 48 to 72 h. Also, at 80% humidity the mortality was superior to that of 60% humidity, where 60% of the larvae were dead after 72 h.

The experiment with whole plant spray showed the following mortality records: 1.) Alginate with nematodes - 57.8%; 2.) alginate without nematodes - 11.4%; 3.) control - 11.4%. Mortality reached more than 50% but the cover of the leaves with the spray was partial and spraying with the hand sprayer was problematic mainly because the gel formulation was too viscous, still without a surfactant to improve leaf wetting.

Fig. 5 shows that after 18 h mortality was close to nil. Still the 0,25% alginate concentration already caused about 40% mortality, better than the record of 0.5% alginate in the gel. After 58 h 0.25% alginate dose was superior to that of 0.5% alginate. The treatment of control and alginate without nematodes ranged 1-20%. The superior effect of the 0.25% alginate can be explained by the better cover of the leaves with this alginate dose, and probably the availability of the nematodes to the larvae with this concentration was better than with 0.5% alginate.

DISCUSSION

The present results show that nematodes in the alginate gel reduced defoliation to a significant low level, although mortality became apparent only after 48-72 h. This means that the protection of the plant with nematodes in gel formulation against leaf damage caused by the larvae is useful and probably with significant economic value. The use of phagostimulants in the high alginate level of 2% did not contribute to the activity of the nematodes in the gel or increase leave consumption by DBM and therefore there is no justification to include

it in the alginate formulation. Larval mortality started after 48 h but it increased within 72 h and therefore effects of the nematodes against the DBM larvae has to be evaluated 3-4 days after nematode application. The superior nematode effect at 80% RH compared with 60% suggests that use of the gel formulation should be used in agricultural crops in geographical regions with high humidity or in sheltered crops with humidity exceeding 60%. The waxy layer of the cabbage required the addition of a surfactant. Tween 80 proved to be compatible with the gel and provided adequate leaf wetting. The Tween 80 itself did not avoid rapid evaporation of the water whereas the gel formulation (diluted to 0.5%) remained on the leaf for 72 h, mostly on the leaf underside where DBM larvae infest the plant. The addition of Tween 80 to the gel formulation at intensive mixing produced high level of foam. However, this effect did not avoid the cause of high mortality (94%) after 58 h. Nevertheless, it should be evaluated to what extent the foam effects larval mortality, and if yes, how to avoid the production of the foam.

The use of high alginate concentration (2%) seems problematic because: 1.) a special spraying machinery has to be developed for spreading large gel drops on the leaf; 2.) Gelling time is a limiting factor at a daily routine field work; 3.) Costs of high alginate concentration can exceed economic value of nematode usage. As opposed to the high alginate level in the formulation, the experiments with lower alginate concentration (0.25%) suggests the following points: 1.) the gel is sprayable with a hand sprayer and possibly with similar sprayers such as knapsack and motor-driven sprayers used in conventional plant protection programs. 2.) There is no time limitation in spraying because the gel is already set without additional changes in the gel consistence. 3.) Mortality of larvae with 0.25% alginate showed better effect than with the higher concentration 4.) The use of the low alginate formulation can reach acceptable costs for IPM with the nematodes.

Future work should focus on controlled field/glasshouse experiments to evaluate the persistence of nematodes in different temperature/humidity combinations and to evaluate the economic return in control efficacy by using the nematodes in low alginate concentrations to control DBM larvae.