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Antifeedant Effects of Denatonium Benzoate and a Neem Derivative on *Myzus persicae* (Sulzer)

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ABSTRACT. *Denatonium benzoate is an extremely bitter, synthetic compound. It is known to act as an antifeedant against mammals, but there seems to be no published information on its effect on insects. In this study, denatonium benzoate (at 0.5, 5, 50, 100 and 250 ppm) was compared with a neem derivative (Pestistat R: at 0.1, 1, 2, 5 ml/l) containing azadirachtin, which is known to act as an antifeedant against insects. Both compounds were tested using Myzus persicae feeding on Chinese cabbage. Pestistat R was found to significantly reduce the lifespan and nymph production of aphids feeding on leaf discs, with higher concentrations of Pestistat R having a greater effect. There was no effect of denatonium benzoate on aphids feeding on leaf discs, but on sprayed whole plants, both aphid lifespan and nymph production decreased with increasing concentration of denatonium benzoate. Measurements of honeydew production indicated that both denatonium benzoate and Pestistat R reduced the feeding rate of the insects, but there was also evidence that, at the highest concentration, Pestistat R also acted as an insect growth regulator. Of the two compounds, Pestistat R was more effective at controlling the aphids; but if denatonium benzoate was used to prevent grazing by mammals, it might also give some protection against insects.*

INTRODUCTION

Antifeedants and growth regulators of plant origin have received considerable attention in recent years due to their effectiveness against a variety of economically important insect pest species (Saxena, 1989). Much of this recent work has been on azadirachtin, a derivative of the neem tree

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Azadirachta indica A. Juss., which inhibits feeding and disrupts growth in insects belonging to various orders (Schmutterer, 1990). Synthetic compounds may also be useful as antifeedants. Denatonium benzoate, marketed as bitrex™ (Macfarlan Smith Ltd, Edinburgh) is an odourless very stable substance with an extremely bitter taste. It is used as an additive to prevent people ingesting or chewing a wide variety of hazardous chemicals such as detergents, disinfectants, and pesticides. Denatonium benzoate has been shown to act as a feeding deterrent against rabbits (Menu, 1993), and it is marketed for use against a variety of mammals including horses, deer and rats. However, the effect of denatonium benzoate on insects and their feeding appears to have received little attention.

The aim of this study was to determine the potential of denatonium benzoate as an insect antifeedant and to compare its effect with a neem derivative. No neem derivatives are licensed for use in the UK at present, but several compounds are being tested. One of these, Pestistat R™ , which contains 0.1% azadirachtin was available for this study. The compounds were tested on *Myzus persicae*, the peach potato aphid. *M. persicae* is an important virus vector, and has the ability to develop resistance rapidly to successive groups of insecticides (Wege, 1994). Therefore, antifeedants may provide an additional means of controlling this pest. *M. persicae* can be reared easily in the laboratory on Chinese cabbage, *Brassica campestris spp. pekinensis* and this plant was used in these experiments. Experiments were carried out to determine the effect of denatonium benzoate and Pestistat R on the survival of aphids and their production of nymphs. Honeydew production was monitored as an index of feeding rate.

MATERIALS AND METHODS

Studies on survival and fecundity

The experiments were carried out using leaf discs of Chinese cabbage (*Var.* Nepos ez F1) and aphids of known age, which were obtained from a culture maintained on Chinese cabbage. All experiments were conducted in the laboratory at a temperature of 18 - 21°C and a day length of 16 - 18 hours.

To determine the effect of the two compounds on the survival and nymph production of *M. persicae*, 20 mm diameter discs of Chinese cabbage leaves were dipped in denatonium benzoate solutions or suspensions of Pestistat R (which is an oil) in water, and were placed in petri dishes

containing hydrogel (Erim™, waterwell), a water absorbent gel which was used to keep the leaf discs fresh. Denatonium benzoate concentrations of 0.5, 5, 50, 100 and 250 parts per million (ppm) and Pestistat R concentrations of 0.1, 1, 2 and 5 ml/l were used. Distilled water was used as the control. The wetting agent agral™ (900 g alkyl phenol ethylene oxide condensate per litre) was added to all solutions, and to the distilled water, at a rate of 0.1 ml/l. Three leaf discs were placed in each dish, and five replicates were used for each treatment. Three one-day old *M. persicae* nymphs were placed on each leaf disc, and the number surviving was recorded each day until all of the original aphids were dead. Once the aphids began to produce nymphs, the number of nymphs produced was also recorded each day, and the nymphs were removed after counting. The leaf discs were replaced with fresh, treated leaf discs every four days. This experiment was carried out twice using Pestistat R and once with denatonium benzoate. A second experiment with denatonium benzoate was carried out using whole plants.

For the experiment with whole plants, three-week old potted Chinese cabbage plants were sprayed once with distilled water or denatonium benzoate solution (0.5, 5, 50, 100 or 250 ppm). Five plants were used for each treatment. Ten one-day old *M. persicae* nymphs were placed on each plant. Aphid survival and number of nymphs produced were recorded daily as for the leaf disc experiment.

Studies on food intake

To measure honeydew production, five 20 mm diameter Chinese cabbage leaf discs were dipped in 50 and 250 ppm denatonium benzoate or 1 and 5 ml/l Pestistat R solutions and placed on hydrogel in 35 mm diameter petri dishes. Distilled water was used for the control treatment, and in all cases agral (0.1 ml/l) was added as a wetting agent. Five two-day old nymphs were introduced on to each leaf disc, then the petri dishes containing the leaf discs were suspended upside down over ashless 125 mm diameter filter paper stained with bromocresol green indicator solution (Banks and Macaulay, 1964). One filter paper was used for each leaf disc. Honeydew droplets caused the paper to change from orange to blue. The number of blue spots on each filter paper and the number of nymphs remaining on each leaf disc were counted after 24 hours and again after 48 hours from the start of the experiment.

The data from all experiments were analyzed using the analysis of variance technique.

RESULTS AND DISCUSSION

There were significant effects of both Pestistat R and denatonium benzoate on aphid mortality and nymph production. The effects on mortality were long term but the treatments could be compared by calculating the mean lifespan of the aphids. In both experiments using Pestistat R, the mean lifespan of the aphids on the leaf discs treated with the two highest concentrations (2 and 5ml/l), was significantly shorter than that on discs treated with distilled water (Table 1). The average lifespan of the aphids on leaf discs treated with 5ml/l Pestistat R was also significantly shorter than on leaf discs treated with 2ml/l Pestistat R in both experiments. In experiment 1, the mean lifespans of aphids on discs treated with the two lower concentrations of Pestistat R were not significantly shorter than in the control. However, in experiment 2 the above parameter at two lower concentrations was significantly different to the control. In experiment 1, treatment with 5ml/l Pestistat R also differed from the other treatments, as the aphid mortality was higher up to the seventh day after introducing aphids (Figure 1). This difference between the 5ml/l concentration and the other concentrations was not so clear in the second experiment (Figure 2). Some of the aphids on all of the treatments survived long enough to produce nymphs. In the first experiment significantly fewer nymphs per adult were produced on leaf discs treated with 2 and 5ml/l Pestistat R than on leaf discs treated with distilled water. In the second experiment, the total number of nymphs produced per aphid was significantly higher on leaf discs treated with distilled water than on all the concentrations of Pestistat R.

There was no significant difference between any of the denatonium benzoate treatments of leaf discs, either in aphid lifespan or number of nymphs produced per adult (Table 2). However, on whole plants, it was possible to relate the lifespan of the aphids and the number of nymphs produced per adult to the denatonium benzoate concentration. The aphids on plants treated with 250 ppm denatonium benzoate had the shortest average lifespan, and this increased as the denatonium of the pesticide decreased. The aphids on plants sprayed with distilled water and the two lowest denatonium benzoate concentrations (0.5 and 5 ppm) lived significantly longer on average than the aphids on plants sprayed with denatonium benzoate at 100 ppm and 250 ppm (Table 2). There was no indication of higher mortality on plants treated with the higher concentrations of denatonium benzoate during the first part of the experiment (Figure 3). The total number of nymphs produced per aphid was significantly higher on plants sprayed with distilled water than on plants sprayed with all of the denatonium benzoate concentrations from 5 to 250 ppm (Table 2).

Table 1. Mean lifespan (days) of *Myzus persicae* and number of nymphs produced per adult on Chinese cabbage leaf discs treated with Pestistat R.

	Pestistat R concentration (ml/l)					
	0	0.1	1	2	5	SED
Experiment 1						
Lifespan	14.8a	14.8a	14.5a	11.4b	6.8c	1.2
Nymphs	37.6a	35.2ab	34.2ab	22.1bc	19.4c	6.6
Experiment 2						
Lifespan	13.4a	9.6a	9.1b	7.6b	5.4c	0.9
Nymphs	27.7a	12.3b	12.1b	10.5b	2.8c	3.7

Values in the same row followed by the same letter are not significantly different ($p>0.05$). SED - Standard Error of the Difference.

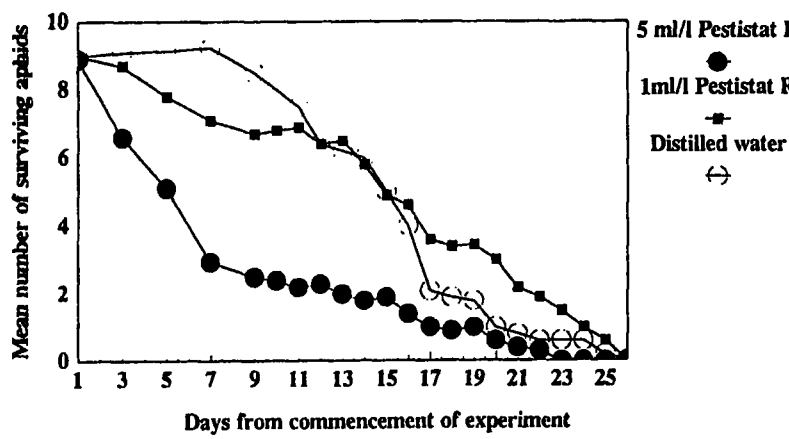

Figure 1. Aphid survival (mean ± SEM, n=5) on Chinese cabbage leaf discs dipped in distilled water, 1ml/l Pestistat R, and 5 ml/l Pestistat R, in experiment 1.

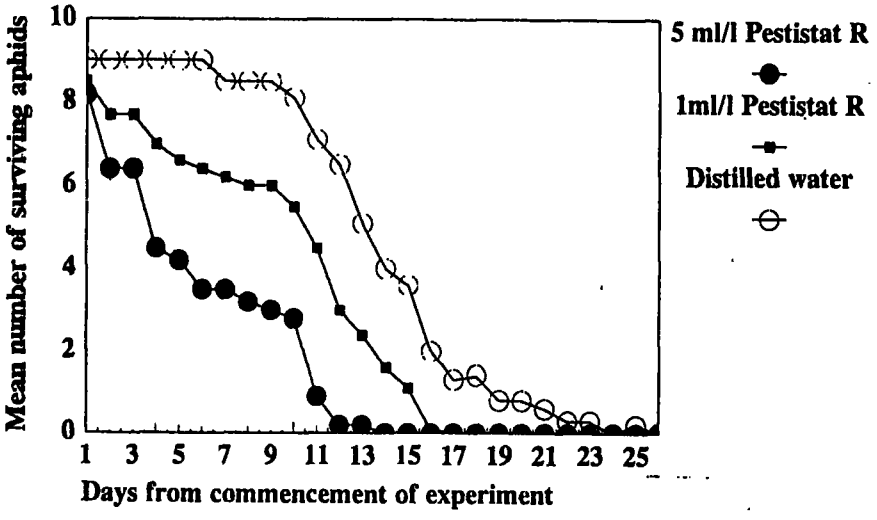

Figure 2. Aphid survival (mean ± SEM, n=5) on Chinese cabbage leaf discs dipped in distilled water, 1ml/l Pestistat R, and 5 ml/l Pestistat R, in experiment 2.

Table 2. Mean lifespan (days) of *Myzus persicae* and number of nymphs produced per adult on Chinese cabbage plants and leaf discs treated with denatonium benzoate.

	Denatonium benzoate concentration (ppm)						
	0	0.5	5	50	100	250	SED
Plants							
Lifespan	15.4a	15.2a	15.0a	13.9ab	10.8b	10.4b	1.6
Nymphs	69.8a	60.5ab	56.6b	45.1c	44.5c	36.5c	5.4
Leaf discs							
Lifespan	12.2a	11.6a	11.3a	12.2a	12.5a	11.3a	0.9
Nymphs	28.3a	31.5a	27.6a	34.0a	31.6a	32.3a	5.7

Values in the same row followed by the same letter are not significantly different ($p>0.05$). SED - Standard Error of the Difference.

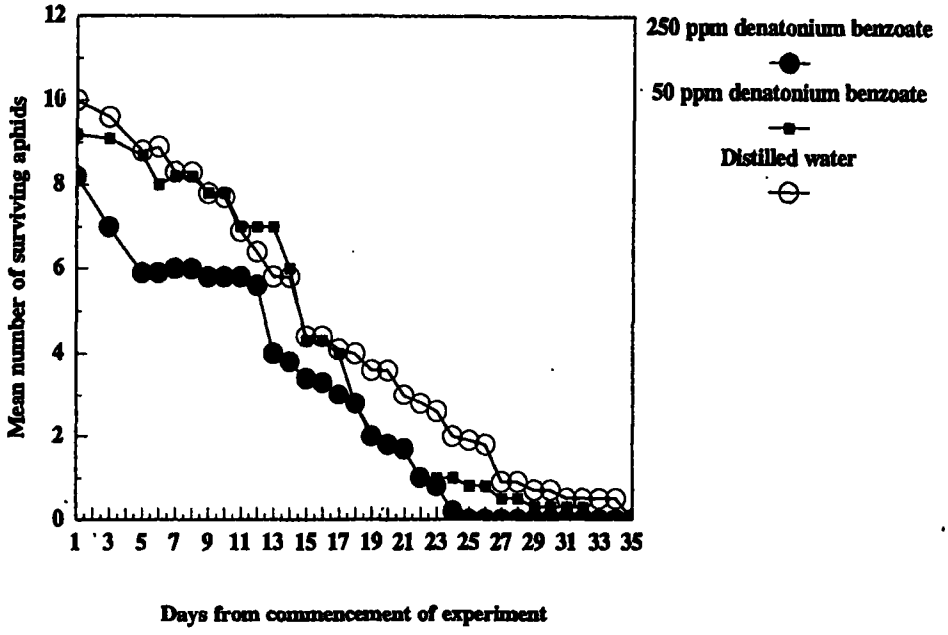

Figure 3. Aphid survival (mean ± SEM, n=5) on Chinese cabbage plants sprayed with distilled water, 50 ppm denatonium benzoate, and 250 ppm denatonium benzoate.

The aphids on leaf discs treated with Pestistat R and denatonium benzoate produced fewer honeydew spots than aphids on leaf discs treated with distilled water (Table 3). The average number of honeydew spots produced during the first 24 hours was significantly lower on leaf discs treated with 1 and 5 ml/l Pestistat R and 50 ppm denatonium benzoate than on leaf discs treated with distilled water. However, after the second 24 hours, only aphids on leaf discs treated with 5 ml/l Pestistat R produced significantly fewer honeydew spots than on the control discs. Aphids on leaf discs subjected to all the other treatments produced less honeydew spots than the control, but the differences were not significant.

Table 3. Mean number of honeydew spots produced by *Myzus persicae* feeding on Chinese cabbage leaf discs during two 24 h periods.

Day	Control	Pestistat R concentration(ml/l)		Denatonium benzoate concentration (ppm)		SED
		1	5	50	250	
1	13.6a	8.9b	8.9b	8.8b	10.3ab	2.1
2	12.8a	8.6ab	6.6b	9.1ab	9.2ab	2.7

SED - Standard Error of the Difference.

The results of the experiment using Pestistat R on leaf discs and denatonium benzoate on whole plants suggest that both compounds would significantly reduce the infestation rate of aphids. The measurements of honeydew production showed that both compounds act as antifeedants. However, Pestistat R may also have a growth regulatory effect. In the first experiment the number of surviving aphids declined more rapidly on leaf discs treated with 5ml/l Pestistat R during the first seven days of the experiment than during the following days. This suggests that Pestistat R at the higher concentration was more effective against nymphs (normally *M. persicae* nymphs become adults 8 - 10 days after their birth) than adults. This may be due to an effect on the moulting process, as is typical of neem products (Nisbet *et al.*, 1994).

The effect of denatonium benzoate on aphid survival and nymph production was only seen on sprayed whole plants. The reason for the difference between the results from whole plants and leaf discs may be because denatonium benzoate can be taken up systematically by sprayed plants (Ondruskova *et al.*, 1992; Menu, 1993). This might increase the effectiveness of the denatonium benzoate. However, if denatonium benzoate is systematic in sprayed plants, it would not be possible to keep the bitter taste from edible parts of crops. Consequently, it would probably not be of any use on crop plants. These results indicate that Pestistat R would be the more effective of the two compounds to control aphids infestations, but the results also suggest that if denatonium benzoate was used against mammals, for example to protect ornamental plants, it would also give some protection

against insect pests. However, firm conclusions regarding the effectiveness of these compounds cannot be made until they are tested in the field.

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