

Response of *Chrysodeixis eriosoma* (Doubleday), *Plutella xylostella* L. and the Parasitoid, *Cotesia plutellae* (Kurdjumov) to Feeding Deterrents

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ABSTRACT. *Worldwide interest today is for environmentally safer and selective methods for pest management. Laboratory experiments were conducted at Peradeniya to study the effect of three feeding deterrents; denatonium benzoate (5, 50 and 250 mg/l), azatin EC (0.01, 0.1 and 1 ml/l) and pestistat R (0.1, 1 and 2 ml/l) on 4th instar larvae of important cabbage pests, *Chrysodeixis eriosoma* and *Plutella xylostella* and on the parasitoid of *P. xylostella*, *Cotesia plutellae*. Denatonium benzoate is a synthetic, bitter chemical and the other two are neem derivatives. Leaf area eaten by caterpillars decreased with increased antifeedant concentration. The lowest significant leaf area consumption was observed on 2 ml/l pestistat R. Adult emergence of *P. xylostella* was not observed in all antifeedant treatments. Significantly lower number of parasitic wasps were developed in parasitized *P. xylostella* fed on 0.01 and 0.1 ml/l azatin EC, 0.1 ml/l pestistat R, 50 and 250 mg/l denatonium benzoate compared to control.*

*Results suggest that the 3 antifeedants are effective in managing cabbage pests, *C. eriosoma* and *P. xylostella* and could be used in integrated pest management programmes. Denatonium benzoate assures comparatively more safety to the parasitoid, *C. plutellae*.*

INTRODUCTION

Nearly, 35% of all agricultural crop production in the world is destroyed by insect pests (13%), weeds (10%) and plant pathogens (12%) (Cramer, 1967; Pimentel *et al.*, 1991). Crop loss due to insect feeding is estimated to several billions of dollars each year in the United States alone (Jacobson, 1980) but the greatest yield losses occur in developing countries.

Chemical insecticides have many drawbacks though they have been the backbone of insect pest control since the early 1950s. This created a worldwide interest in search for more environmentally safer, less toxic and selective methods. One such is the use of antifeedants, which may interfere with the processes of host plant selection and feeding

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behaviour and have the potential to prevent crop damage [West and Mordue (Luntz), 1992]. Crude extracts of different plant parts of the neem tree, *Azadirachta indica* A. Juss. especially the seed kernels, possess potent insect antifeedant, repellent, growth and development regulating, and in certain cases toxic properties (Warthen, 1979; Jacobson, 1989; Schmutterer and Ascher, 1987). Neem extracts are known to have antifeedant or growth regulatory effects on over 80 species of insects (Warthen, 1979) because of the presence of azadirachtin. It is a tetranortriterpenoid plant limonoid with antifeedant and growth regulatory properties. It causes interruption of reproduction in insects and acts as an insect repellent.

Denatonium benzoate marketed as Bitrex™ by Macfarlan Smith Ltd, Edinburgh, is an inert, very stable, odourless synthetic chemical with extremely bitter taste. It is commonly used as an additive to prevent people ingesting or chewing a wide variety of hazardous chemical products such as detergents, disinfectants and pesticides. Denatonium benzoate acts as an antifeedant against rabbits (Menu, 1993) and it is used against horses, deer and rats (Andelt, 1994). The effect of denatonium benzoate on insects and their feeding behaviour appear to have received little attention. However, in laboratory experiments, 100–250 mg/l denatonium benzoate (Perera *et al.*, 1995a, b) and 1–5 ml/l pestistat R (Perera *et al.*, 1995b) were shown to reduce the fecundity and lifespan of aphids on Chinese cabbage.

The important pests of cabbage in Sri Lanka are cabbage semiloopers, *Chrysodeixis eriosoma* (Doubleday), (Lepidoptera : Noctuidae) and the diamondback moth, *Plutella xylostella* L. (Lepidoptera : Plutellidae). *C. eriosoma* (generalist leaf feeder) was originally reported in Peradeniya, Sri Lanka in 1919 and became the most predominant and widespread pest of all brassica crops (De Silva, 1960; Hutson, 1924). *P. xylostella* (specialized leaf feeder) is the most destructive pest throughout the world (Telekar, 1992) and became a major cabbage pest in Sri Lanka.

Larval parasites largely of genera *Diadegma*, *Apanteles* (*Cotesia*) and *Microptelis* are the key species for biological control of *P. xylostella* (Lim, 1986). Magallona (1986) considered that *Apanteles* (*Cotesia*) *plutellae*, which parasitize the 2nd and 3rd larval instars (Magallona and Velasco, 1980) is the predominant biotic mortality agent of *P. xylostella* in the Philippines.

The first objective of this study was to determine whether the bitter-tasting denatonium benzoate has potential as an antifeedant against insect pests of cabbage crops and to compare its effect with neem derivatives, azatin EC and pestistat R. The 2nd objective was to find out the effect of these antifeedants on larval parasitoid, *Cotesia plutellae* Kurdjumov (Hymenoptera : Braconidae) of *P. xylostella* in laboratory experiments.

MATERIALS AND METHODS

P. xylostella and *C. eriosoma* caterpillars were collected from Bandarawela farmer fields and reared in the laboratory in 14×14 cm plastic boxes covered with muslin cloths. The adult moths were placed in 45×45×60 cm rearing cages with a two-month-old cabbage.

plant to lay eggs and were fed with a solution of bee honey. The emerged 1st instar larvae were reared in 14×14 cm plastic containers covered with muslin cloth and were fed with cabbage leaves until they reached the required instar.

C. plutellae cocoons were collected from Bandarawela farmer fields. Cocoons were kept in 24×13 cm transparent plastic boxes covered with muslin cloth and the adults were fed from a plastic pan containing cotton wool soaked in a solution of bee honey. The mated female wasps were used to parasitize *P. xylostella*.

Studies on feeding rate and survival of caterpillars

Fourth instar larvae of *C. eriosoma* and *P. xylostella* were used in experiments. Excised cabbage leaves of exotic F1 (Royal Sluis, Holland) plants grown in 30 cm diameter pots containing normal potting mixture (1/3 top soil, 1/3 cow dung and 1/3 sand) as per recommendations of the Department of Agriculture kept with the cut ends of petioles in water, were sprayed with treatment solutions and allowed to dry before cutting leaf discs (2.5 cm diameter). Treatments used were 5, 50 and 250 mg/l of denatonium benzoate, 0.01, 0.1 and 1 ml/l (0.3, 3.0 and 30 mg/l azadirachtin) azatin EC and 0.1, 1 and 2 ml/l (0.1, 1.0 and 2.0 mg/l azadirachtin) pestistat R. The wetting agent Agral™ was used at 0.1 ml/l to all treatments. Two controls and 5 replicates were used. In control 1, leaf discs were taken from leaves sprayed with distilled water with agral at the rate of 0.1 ml/l and in control 2, distilled water alone was used.

Five 4th instar caterpillars of *C. eriosoma* and *P. xylostella* were provided with three leaf discs in a 12×12 cm petri dish for each treatment. After 24 h, the leaf area consumed by caterpillars was measured using a leaf area meter. The leaf discs were replaced with treated leaf discs every 24 h and the leaf area consumed was recorded until pupation, or until they reach the next instar stage. The larval and pupal survival, time taken for pupation and the number of adult moths emerged were also recorded every 24 h.

Studies on *C. plutellae*

Three hundred 2nd instar *P. xylostella* larvae were parasitized by enclosing them for 12 h with 20 mated female *C. plutellae* in a 45×45×60 cm rearing cage. Another 300 caterpillars were kept separately, away from the parasitic wasps. They were fed with untreated cabbage leaf discs until 85% of the larvae reached the 4th instar stage after which the experiment was commenced.

Fourth instar larvae were fed with cabbage leaf discs treated with same concentrations of antifeedants as in the previous experiment and distilled water+agral control. Three treated leaf discs and 5 *P. xylostella* caterpillars were used in each treatment. The leaf discs were replaced every 24 h period with fresh treated leaf discs. The number of parasitoid cocoons formed, the number of caterpillars which did not form parasitoid cocoons, the number of caterpillars dead before pupation, the number of caterpillars which successfully pupated and the number of parasitic wasps emerged were recorded daily.

RESULTS

Feeding rate and survival of *C. eriosoma* caterpillars

Three days after feeding 90% of the larvae were dead in antifeedant treatments. In control treatments 96% of the larvae survived. There were no significant differences on leaf area consumption between the 2 controls. Leaf area eaten from discs sprayed with all concentrations of azatin EC and pestistat R and 250 mg/l denatonium benzoate were significantly lower than the distilled water+agral control on day 1 (Fig. 1). Leaf area eaten decreased as the antifeedant concentration increased, and the differences were not significant except the lowest and the highest concentration of denatonium benzoate on day 1 (Fig. 1).

Leaf area consumed in all antifeedant treatments was decreased further during the second 24 h period and was significantly lower than 2 controls. Decreasing trend in consumption was observed among 3 concentrations but the differences were not significant except the highest and the lowest concentration of azatin EC (Fig. 1). The lowest leaf consumption was observed during the third 24 h period in all treatments. Leaf area consumed on all treatments except 5 and 50 mg/l denatonium benzoate was significantly lower than on the distilled water+agral control. However, leaf feeding in all treatments except denatonium benzoate treatments and 0.01 ml/l azatin EC was significantly lower than the distilled water control. Denatonium benzoate treatments gave the highest consumption followed by azatin EC and pestistat R treatments respectively in all 3 days (Fig. 1).

Feeding rate of *P. xylostella* larvae

Larval death of *P. xylostella* after the 2nd 24 h period (day 2) was 68%. Except 0.01 ml/l azatin EC and 5 and 50 mg/l denatonium benzoate, the differences in leaf area consumption of all other treatments were significantly lower than the distilled water+agral control (Fig. 2). Leaf area consumed decreased further on day 2. Compared to the distilled water+agral control, all the treatments except 0.01 ml/l azatin EC, 0.1 ml/l pestistat R and 5 and 50 mg/l denatonium benzoate showed significantly lower leaf area consumption (Fig. 2).

Leaf area consumed was significantly different between the highest and the lowest concentrations of azatin EC and pestistat R on day 1 and azatin EC and denatonium benzoate on day 2. As the concentration of the antifeedant increases, leaf feeding gradually decreased, irrespective of the antifeedant (Fig. 2). The highest leaf feeding was in the denatonium benzoate followed by azatin EC and pestistat R respectively on day 1 and 2.

Effects of antifeedants on *P. xylostella* and its parasitoid *C. plutellae*

Parasitoid larvae pupated after 2 days of feeding on treated leaf discs and the lowest number of pupal cocoons were observed in the control. The average number of parasitic cocoons formed was significantly less on control than on 0.1 and 1 ml/l azatin

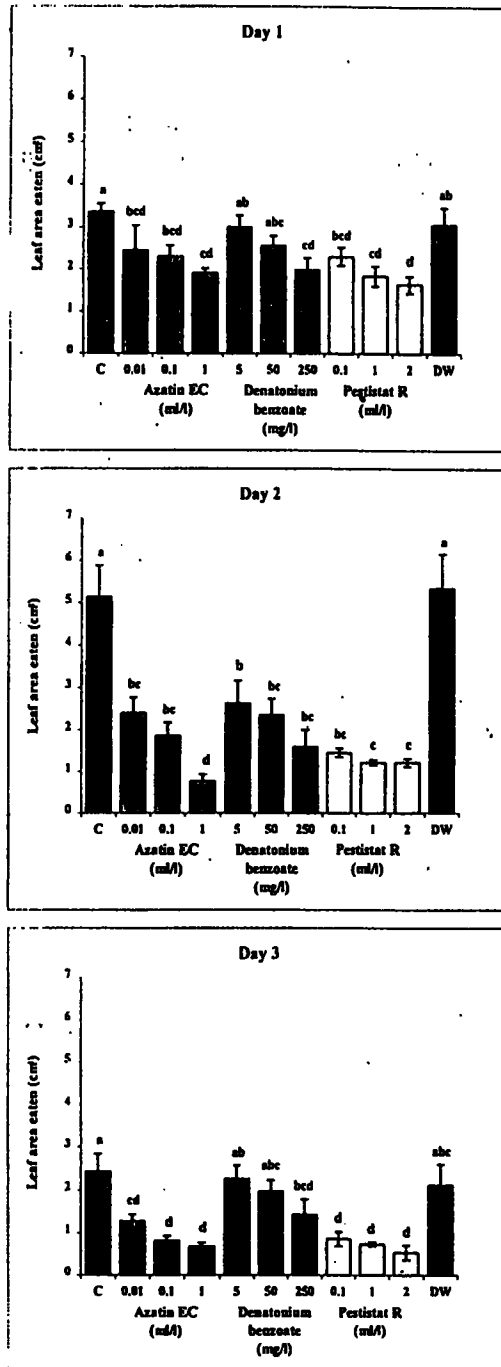


Fig. 1. Mean leaf area eaten by 4th instar larvae (n=5) of *Chrysodeixis eriosoma* feeding continuously on the leaf discs treated with distilled water+agral (C), distilled water alone (DW), azatin EC, denatonium benzoate and pestistat R.

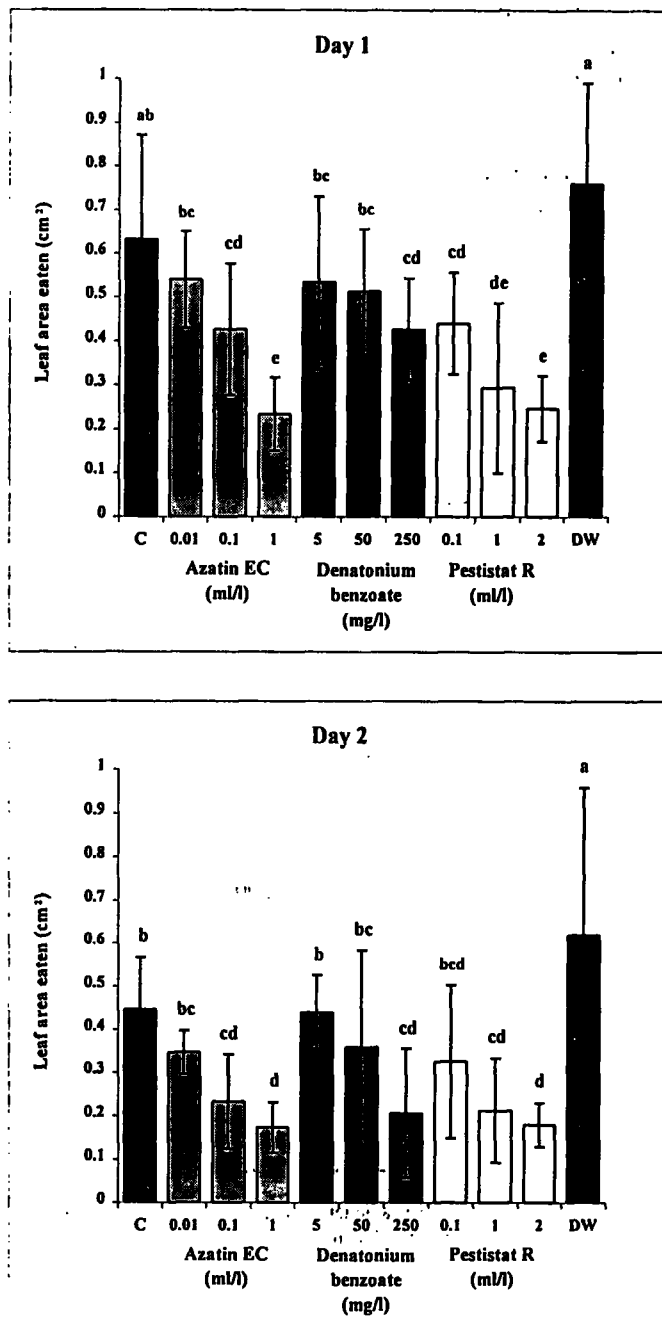


Fig. 2. Mean leaf area eaten by 4th instar larvae (n=5) of *Plutella xylostella* feeding continuously on the leaf discs treated with distilled water+agral (C), distilled water alone (DW), azatin EC, denatonium benzoate and pestistat R.

EC, 1 and 2 ml/l pestistat R and all concentrations of denatonium benzoate. As the concentration of the antifeedant increased, the number of parasitic cocoons formed also increased. There were no significant differences between the percentage cocoons formed on any treatment although a slight decreasing trend in forming parasitoid cocoons with increasing concentrations of azatin EC and pestistat R (Table 1).

Table 1. Average number of unparasitized and parasitized *Plutella xylostella* pupae formed (n=5), average number of parasitic cocoons formed and percentage parasitic cocoons formed on leaf discs treated with distilled water, 0.01, 0.1 and 1 ml/l azatin EC, 0.1, 1 and 2 ml/l pestistat R and 5, 50 and 250 mg/l denatonium benzoate.

Treatment	Number of <i>P. xylostella</i> pupa		Number of Parasitic cocoons Formed on day 2	Percentage (arcsine transformed) Parasitic cocoons Formed	
	Unparasitized	Parasitized			
Control	4.20 a	2.20 a	0.80 d	43.85	(47.99)
Azatin EC					
0.01 ml/l	2.00 cde	0.40 b	1.20 cd	46.38	(52.41)
0.1 ml/l	2.40 bcde	0.40 b	2.00 abc	43.85	(47.99)
1 ml/l	1.20 f	0.20 b	2.40 ab	43.85	(47.99)
Pestistat R					
0.1 ml/l	1.80 de	0.20 b	1.40 bcd	46.38	(52.41)
1 ml/l	1.40 e	0.60 b	2.00 abc	44.07	(48.38)
2 ml/l	1.40 e	0.20 b	2.20 abc	41.31	(43.58)
Denatonium benzoate					
5 mg/l	3.40 ab	0.00 c	2.00 abc	46.38	(52.41)
50 mg/l	3.00 bc	0.20 b	2.20 abc	41.31	(43.58)
250 mg/l	2.80 bcd	0.60 b	2.60 a	46.38	(52.41)
SED	0.53	0.26	0.52	6.93	

Values in the same column followed by the same letter are not significantly different at P=0.05

Values in the parenthesis are the retransformed actual means

SED - Standard Error of the Difference

In the parasitized treatments, some of the larvae turned into pupae without forming parasitized cocoons. Significantly higher average number of *P. xylostella* pupae were formed on the control than all the antifeedant treatments. Pupal cocoons were not formed on 5 mg/l denatonium benzoate and were significantly lower than all the antifeedant treatments and the control (Table 1).

In the unparasitized treatments, average number of *P. xylostella* pupae formed was significantly higher on the control than all the other treatments except 5 mg/l denatonium benzoate. The average number of *P. xylostella* pupae formed between different concentrations of denatonium benzoate and pestistat R was not significant. The average number of *P. xylostella* pupae formed on 1 ml/l azatin EC was significantly lower than 0.01 and 0.1 ml/l azatin EC (Table 1).

The number of adult wasps emerged as well as the percentage of wasp emergence from parasitized larvae was highest in the control and was not significantly different to 5 mg/l denatonium benzoate (Table 2). There was no adult wasp emergence observed on 1 ml/l azatin EC, 1 and 2 ml/l pestistat R and the pupae were dead within their cocoons. The number of wasps emerged on 50 and 250 mg/l denatonium benzoate were significantly less than the 5 mg/l denatonium benzoate. The highest percentage of wasp emergence from larvae was observed in 5 mg/l denatonium benzoate. In all antifeedant treatments percentage wasp emergence decreased as the concentration increased except in 250 mg/l denatonium benzoate (Table 2).

Table 2. Average number adult wasps emerged and percentage of adult wasps emerged from parasitized larvae and from cocoons (n=5) on leaf discs treated with distilled water, 0.01, 0.1 and 1 ml/l azatin EC, 0.1, 1 and 2 ml/l pestistat R and 5, 50 and 250 mg/l denatonium benzoate.

Treatment	Number of wasps emerged	Percentage (arcsine transformed) wasp emergence from larvae	Percentage (arcsine transformed) wasp emergence from cocoons
Control	1.60 a	34.17 a (31.54)	48.48 a (56.06)
Azatin EC			
0.01 ml/l	0.80 b	21.25 bc (13.14)	28.55 ab (22.84)
0.1 ml/l	0.40 b	10.63 d (3.40)	16.21 bc (7.79)
1 ml/l	0.00 c	0.00 e (0.00)	0.00 c (0.00)
Pestistat R			
0.1 ml/l	0.60 b	15.94 bcd (7.54)	22.82 b (15.04)
1 ml/l	0.00 c	0.00 e (0.00)	0.00 c (0.00)
2 ml/l	0.00 c	0.00 e (0.00)	0.00 c (0.00)
Denatonium benzoate			
5 mg/l	1.00 a	23.79 ab (16.27)	31.91 ab (27.94)
50 mg/l	0.60 b	13.16 cd (5.18)	17.46 bc (9.00)
250 mg/l	0.60 b	15.94 bcd (7.54)	22.84 b (15.04)
SED	0.37	5.25	10.47

Values in the same column followed by the same letter are not significantly different at $p=0.05$

Values in the parenthesis are the retransformed actual means

SED - Standard Error of the Difference

Percentage wasp emergence with respect to the cocoons formed was significantly higher on the control than all the antifeedant treatments except 0.01 ml/l azatin EC and 5 mg/l denatonium benzoate. Denatonium benzoate treatments did not show significant differences. Percentage wasp emergence from cocoons was significantly higher on the lowest concentration than the highest concentration of azatin EC (Table 2).

DISCUSSION

Azatin EC, denatonium benzoate and pestistat R consistently gave reductions in leaf feeding with increasing concentration but the neem compounds gave greater reductions. Pestistat R was the more effective antifeedant of the 2 neem derivatives. The leaf feeding also declined as the experiment proceeded. The surfactant agral was found to have no antifeedant effect.

The difference in feeding between the lowest and the highest concentration of each antifeedant was several folds. The decreasing trend in leaf feeding with increase in concentration was observed irrespective of the species and antifeedant, though not significant. The decrease in leaf feeding over time, in all antifeedant treatments, could be attributed to the primary and secondary antifeedancy. Azadirachtin affects the feeding primarily through chemo-reception (primary antifeedancy) but also it reduce the food intake due to toxic effects when consumed (secondary antifeedancy) [Mordue (Luntz) and Blackwell, 1993]. The larvae probably reduced their food consumption at higher concentrations leading to starvation because of primary antifeedancy and finally the death. At lower concentrations since the leaf area consumed was high, due to accumulation of toxic compounds in the gut probably decreased the feeding subsequently (secondary antifeedancy). In denatonium benzoate treatments, reduction in leaf feeding on day 2 was probably because of the bitter taste that remains on mouth parts of caterpillars.

Azatin EC and pestistat R were more effective antifeedants than denatonium benzoate regardless of their growth regulatory effects. Growth regulatory properties of these compounds make them better insecticides. Nasiruddin and Mordue (Luntz) (1994) indicated that treatment with azadirachtin and its analogues in plant protection is most probably a balance between the antifeedant effect and growth regulatory effects, eventually causing death by starvation and the toxic effect if ingested. The effect of azadirachtin varies between species and dose levels. Under laboratory conditions, low doses of azadirachtin (10 and 20 ppm) reduced feeding of final instar *P. brassicae* larvae, which eventually died (Schmutterer, 1992). According to Ruscoe (1972) azadirachtin affects the feeding, growth and development of lepidopterous larvae, *P. brassicae*, *P. xylostella* and *Heliothis virescens*. Arpaia and Van Loon (1993) indicated that 56% antifeedancy was observed on *P. brassicae* larvae by systemic application of 30 ppm azadirachtin. Schmutterer and Doll (1993) have indicated that neem derivatives have other chemicals such as salannin, with antifeedant and insecticidal properties. Pestistat R is a crude neem derivative, which contains other compounds such as salannin and nimbin. The antifeedant and growth regulatory properties of these compounds could have combined the effect with azadirachtin, may explain the lowest leaf area consumption in pestistat R treatments.

Most of the 4th instar *C. eriosoma* and *P. xylostella* larvae died after the 3rd and 2nd day after feeding respectively in all treatments. Larvae exposed to the highest concentration of all antifeedants may have died of starvation because of the low food consumption due to primary antifeedancy. Larvae exposed to the lower concentrations of all of the antifeedants may have died due to the accumulation of toxic compounds in the insect's gut. In addition to these two reasons, larvae exposed to pestistat R and azatin EC may have died due to the growth regulatory effects of azadirachtin.

Among the 3 compounds, those derived from neem had the greatest antifeedant effects. Azatin EC is a purified preparation of neem, which contains comparatively high percentage of azadirachtin (3%) whereas pestistat R is a crude neem preparation, which contains only 0.1% azadirachtin. Pestistat R showed a greater antifeedant effect on caterpillars than the purified neem preparation, azatin EC. This may be due to the other chemicals present in neem, which also possess antifeedant properties. In addition to antifeedant properties, the growth regulatory properties of azadirachtin associated with the neem derivatives probably contribute to the insecticidal activity.

Denatonium benzoate was less effective than the neem compounds and was found to act as an antifeedant. This again indicates that some insects show an aversion to bitter tasting compounds. Ramaswamy *et al.* (1992) recorded aversion to bitter tasting compounds, including denatonium benzoate in *H. virescens*. It may also be due to chronic toxic effects such as accumulation in the gut. The trend of decreased feeding with increasing concentration suggests that leaf damage could be decreased by using higher concentrations of denatonium benzoate. Since denatonium benzoate has only antifeedant effects, it could be used as an environmentally safe supplementary chemical in integrated pest management programmes.

Effects of antifeedants on *P. xylostella* and its parasitoid, *C. plutellae*

The earlier parasitic cocoon formation by parasitoids exposed to all the denatonium benzoate concentrations and 2 higher concentrations of azatin EC and pestistat R than the control may be due to the stress conditions imposed by the restriction of food consumption because of the antifeedant effects. The greater mortality of unparasitized larvae exposed to antifeedants corresponds to lower numbers of *P. xylostella* pupae formed. Therefore, the significant differences between all antifeedant treatments except 5 mg/l denatonium benzoate and the control indicates that larval deaths occurred due to primary and/or secondary antifeedancy because of the small body size of the larvae. The comparatively higher larval death exposed to the neem derivatives than to denatonium benzoate may be because of the growth regulatory effects of azadirachtin. Pestistat R being a crude neem derivative, higher larval deaths and non-significant differences among concentrations could be expected. Significantly higher larval deaths observed in the highest concentration of azatin EC, compared to 2 lower concentrations may be because of the relatively high azadirachtin content. Ruscoe (1972) also demonstrated that growth disruption effects of azadirachtin on last instar larvae of *P. xylostella* and the treated insects developed at much slower rate than the untreated insects. Adult emergence in antifeedant treatments was not observed as it caused feeding reduction and death due to starvation.

The percentage of parasitic cocoons formed did not show significant differences among all the treatments but there were significant differences in the percentage wasp emergence. This indicates that the effects of antifeedants on the parasitoids were more pronounced during the pupal stage where drastic physiological and morphological changes occur. The wasp emergence from cocoons was not significantly affected by the lowest concentration of azatin EC and denatonium benzoate. According to Schmutterer (1988), in spite of the sensitivity of insects of most orders to azadirachtin, neem products are selective as they do not harm important natural enemies of pests. Schmutterer (1992) also surmised that final instar larvae of *P. brassicae* was affected by the low doses of azadirachtin under laboratory conditions but its parasitoid *Apanteles glomeratus* was not harmed. Therefore, delayed spraying in the field could avoid the destruction of the parasitoid but not the host. Likewise, in this experiment, treatments were imposed on the final instar *P. xylostella* larvae and antifeedant treatments where *C. plutellae* adults survived and emerged were fatal to unparasitized moth larvae.

The high percentage of parasitization observed (41–46%) could be attributed to the fact that the parasitization of *P. xylostella* larva was carried out under laboratory conditions in a restricted environment (Table 1). Another possibility is that the nutritional condition of the host plant, in this case leaf discs, also could affect the parasitization rate. Oji (1992) surmised that the parasitization rarely exceeds 60% in *C. plutellae*. Talekar (1992) indicated that in Taiwan 57% of *P. xylostella* were found to be parasitized of which only 11% was due to *C. plutellae* and other 46% from *Diadegma Semiclausum*. Morallo and Sayaboc (1992) also indicated that parasitization of *P. xylostella* by *C. plutellae* in Baguio city in the Philippines was as low as 17.4% in farmers field and 36.5% in demonstration fields.

CONCLUSIONS

It can be concluded that both neem derivatives reduced feeding to a greater extent than denatonium benzoate. In all 3 antifeedant treatments, the reduction in food intake was proportional to concentration and elapsed time. Further, the antifeedants containing azadirachtin should be sprayed at later larval stages in order to reduce damage to *C. plutellae* while controlling *P. xylostella*, although the strategy would carry the risk of greater damage to the crop. However, denatonium benzoate could be used in integrated pest management programmes at an earlier stage because it is less harmful to *C. plutellae*. It may in fact be best to use denatonium benzoate at an early stage of the crop in order to allow the bitter taste to wear off before harvest.

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