SCREENING OF BIOINSECTICIDES AGAINST THE COTTON BOLLWORM ON COTTON¹

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ABSTRACT - Four bioinsecticides and a synthetic pyrethroid were evaluated against the cotton bollworm (Heliothis zea Boddie, 1850), (Lepidoptera: Noctuidae) in laboratory. Treatments were: cypermethrin, Bacillus thuringiensis var. kurstaki Berliner (BT), Steinernema feltiae Weiser, Heliothis Nuclear polyhedrosis Virus (HNPV), Beauveria bassiana (Bals) and the untreated check. The efficiency of the bioinsecticides was also tested in a greenhouse. Treatments were the same but instead of HNPV an experimental formulation of BT (Mycogen®) was used. Mortality rate was recorded at one, two, seven and nine days after treatment. The efficiency of the bioinsecticides was also tested in a greenhouse. Treatments were the same but instead of HNPV an experimental formulation of BT (Mycogen®) was used. Each treatment consisted of four plants (Coker 310) sprayed with insecticide and infested with nine neonate larva. Results were evaluated seven days after treatment. In laboratory cypermethrin and BT showed a significant difference compared to control at day 1, despite concentrations. They did not differ statistically from each other. S. feltiae showed a significant mortality rate to control after day 2. HNPV was highly effective between two and seven days after treatment, killing all larvae. In greenhouse cypermethrin showed a significant difference compared to check.

Index terms: microbial control, pyrethroid, cotton pest management.

INTRODUCTION

The cotton bollworm, Heliothis zea (Boddie, 1850), is a worldwide important insect pest. It is especially noxious to cotton due to the damage of floral buds and because of the increasing difficulties in obtaining an acceptable control. Applications of different chemicals have triggered resistance.
mechanism to most classes of insecticides (Bull et al., 1979; Jackson, 1989). This fact has encouraged a search for alternative methods for management and suppression of the pest including bioinsecticides.

The literature on microbial control of the bollworm is abundant. Much attention has been given to the usage of Bacillus thuringiensis Berliner (BT) because of its safe mode of action producing a crystalline protein (o-endotoxin) capable of paralyzing the larval gut, causing death. The use of the nematode Steinernema feltiae Weiser (=Neoaplectana carpopcapae) has increased tremendously because of its wide host range and ease of mass propagation (Kaya & Burlando, 1989). This nematode is known by its mutualistic relationship with the bacteria Xenorhabdus nematophilus which is ultimately responsible for the death of the larvae. In the past 20 years fungi agents have played an important role in insect control programs. The advantages are both of being saprophotic and able to penetrate directly to the insect’s cuticle although they are limited by the environmental conditions (Ferron, 1981). Interesting results with Beauveria bassiana (Bals) as larvicide for Heliothis spp. were reported by Gardner & Nobleit (1978) and Peku & Grula (1979). Though more than 200 viral diseases are reported in insects of important commodities, only a few have been considered significant for applied control (Yearian & Youngs, 1982). In 1965 a feasible mass production of H. zea Nuclear Polyhedrosis Virus (HNPV) was reported by Ignooff et al. (1965), and in 1976 it was registered under the name Elcartm.

Despite the advantages of using microbial control, chemical insecticides continue to be an indispensable means of controlling insect outbreaks because of their rapid, effective and flexible action in various crop situations. Pyrethroids, for instance, are active against all larval stages of Heliothis spp. The efficacy of pyrethroids used alone (Davis et al., 1975) or mixed with other insecticides (All et al., 1977) for control of bollworm is well established.

This study reports the results of a laboratory experiment treating cotton bollworm larva with four bioinsecticides in three different concentrations compared to a pyrethroid. A greenhouse bioassay was conducted to test the efficiency of four bioinsecticides based in the laboratory results.

MATERIALS AND METHODS

Laboratory test. This experiment was done at the laboratory facilities at the Entomology Department at University of Georgia. The solutions, except for the nematode and the pyrethroid, were tested at a standard dosage, one higher dosage (twice the standard) and one lower dosage (half of the standard). The treatments applied were: Cypermethrin (Cymbush™ 50 EC, ICI Corporation, Wilmington DE) at 7.19 x 10^-5, 0.71 x 10^-5, 7.19 x 10^-4 g/a/ml; B. thuringiensis var. kurstaki (Dipel™ WP, with 2.5 x 10^10 conidium/g, Abbott Laboratory, Chicago IL) at 1.2 x 10^-2, 2.4 x 10^-2 and 0.6 x 10^-2 g/ml; S. feltiae (cultures from Galleria mellonella (L.), University of Georgia) at 5.0 x 10^3, 1.0 x 10^3 and 1.0 x 10^3 infective juveniles/ml; Heliothis NPV (Elcartm with 4 x 10^9 PIB/g, Sandoz Corporation Protection Company, Desplains IL) at 1.5 x 10^3, 3.0 x 10^3 and 7.5 x 10^4 g/ml; B. bassiana [ABG-6178 (experimental formulation) Abbott Laboratory, Chicago IL] at 1.0 x 10^3, 2.0 x 10^3 and 0.5 x 10^2 g/ml and the control. Each treatment consisted of twenty discs of 2.8 cm² of fresh cotton leaf (variety Coker 310) immersed in one of the insecticide solutions for 5 s. After dried out each leaf disc was placed into 1 oz-plastic cup plus one neonate larva. The cups were sealed and kept at 27 °C. After 24 hours all alive larvae were transferred to cubs with Pinto Bean artificial diet. The percentage of larval mortality was recorded at one, two, seven and nine days. Differences between and within treatments means were compared by an Analysis of Variance and separated by Duncan’s multiple range test (SAS, 1982). Based on the results of the laboratory test a greenhouse experiment was conducted using the same formulations.

Greenhouse test. The experiment was conducted in a greenhouse at the Southern Piedmont Extension Station in Watkinsville, Ga. The cotton plants (Coker 310) growing in plastic vases were set in stainless steel trays and watered by dripping irrigation.

Plants were sprayed in a chamber made of plastic walls having at the top a rotatory spray bar equipped with three hollow cone nozzles. A field situation was simulated with the equipment delivering 227 liters of insecticide solution per hectare at a pressure of 1.1 kg/cm² in a speed of 4.8 km/h. The treatments were: Cypermethrin at 0.054 kg a.i./ha; B. thuringiensis at 1.12 kg/ha; B. thuringiensis [(Mycogen™) (experimental formulation)] at 9.35 kg/ha; S. feltiae at 7.00 x 10^9 infective juvenile/ha; B. bassiana (ABG-6178) at 4.2 kg/ha and a larval-infested check.

The sprayed plants were allowed to dry and then infested with ten neonate H. zea larvae distributed in the squares on the upper third canopy. A complete randomized...
block design with six treatments and four repetitions was conducted. Plants were infested twice. The top ten damaged squares were tallied at seven days after infestation. The experiment was repeated four times at seven days interval. Data were analysed by an Analysis of Variance for a complete randomized block design and means were separated at $P = 0.05$ level by Duncan's multiple range test (SAS, 1982).

RESULTS AND DISCUSSION

Laboratory Assay. Statistic analysis is presented in Table 1. The first day showed the prompt effect of cypermethrin causing high mortality. All concentrations had significant differences comparing to check. Even the lowest concentration of cypermethrin was effective showing its characteristic "knock down" effect (Miller & Salgado, 1985). BT was also highly effective and did not differ from cypermethrin which is recommended against the first instar larva. This result coupled with those obtained for *H. zea* in soybean by Ignoffo et al. (1977). *S. feltiae* showed a highly significant difference in comparison with the control. Despite this, it should be considered as an intermediate control agent between the highly effective group (cypermethrin and BT) and the low effective group of insecticides (HNPV and *B. bassiana*). Bari & Kaya (1984) working with *Platyptilia carduidactyla* (Riley) also concluded that *S. feltiae* had a low effect against first instar larva. Tong (1986) obtained the best results against *H. zea* in corn using *S. feltiae* at $4 \times 10^4$ nematodes/ml after ten days. None of the treatments showed any significant difference related to the concentration of the insecticide solutions.

At the second day a significant percentage of mortality was observed in the treatment with *S. feltiae* compared to control. Since no further significant increase in the larvae mortality was recorded, an appreciation of the economic damage by the remaining population should be considered

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rate</th>
<th>Control</th>
<th>Number of live larva at day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Cypermethrin</td>
<td>$71.90 \times 10^{-5}$ g ia/ml</td>
<td>20a</td>
<td>Fb</td>
</tr>
<tr>
<td></td>
<td>$7.19 \times 10^{-5}$ g ia/ml</td>
<td>20a</td>
<td>Fb</td>
</tr>
<tr>
<td></td>
<td>$0.71 \times 10^{-5}$ g ia/ml</td>
<td>20a</td>
<td>06</td>
</tr>
<tr>
<td></td>
<td>$2.40 \times 10^{-2}$ g/ml</td>
<td>20a</td>
<td>04</td>
</tr>
<tr>
<td><em>B. thuringiensis</em></td>
<td>$1.20 \times 10^{-2}$ g/ml</td>
<td>20a</td>
<td>04</td>
</tr>
<tr>
<td></td>
<td>$0.60 \times 10^{-2}$ g/ml</td>
<td>20a</td>
<td>04</td>
</tr>
<tr>
<td></td>
<td>$1.00 \times 10^{-3}$ g/ml</td>
<td>20a</td>
<td>10</td>
</tr>
<tr>
<td><em>S. feltiae</em></td>
<td>$5.00 \times 10^{-3}$ g/ml</td>
<td>20a</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>$1.00 \times 10^{-3}$ g/ml</td>
<td>20a</td>
<td>12</td>
</tr>
<tr>
<td>HNPV</td>
<td>$3.00 \times 10^{-3}$ g/ml</td>
<td>20a</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>$1.50 \times 10^{-3}$ g/ml</td>
<td>20a</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>$0.75 \times 10^{-3}$ g/ml</td>
<td>20a</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>$2.00 \times 10^{-2}$ g/ml</td>
<td>20a</td>
<td>18</td>
</tr>
<tr>
<td><em>B. bassiana</em></td>
<td>$1.00 \times 10^{-2}$ g/ml</td>
<td>20a</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>$0.50 \times 10^{-2}$ g/ml</td>
<td>20a</td>
<td>20</td>
</tr>
<tr>
<td>Control</td>
<td>20A</td>
<td>20</td>
<td>A</td>
</tr>
</tbody>
</table>

*Between treatment values in a column followed by the same capital letter are not significantly different (p = 0.05; Duncan’s multiple range test). Within treatment values in a row followed by the same lower case letter are not significantly different (p = 0.05; Duncan’s multiple range test).*
for *S. feltiae* profitable use. Since nematode concentrations did not differ significantly, using the lower concentration (1000 j/l/ml) would be recommended. Similar result was achieved by Bari & Kaya (1984) working with *P. carduidactyla*, who concluded that the lowest effective concentration of *S. feltiae* was 1000 j/l/ml. HNPV was ineffective in the first two observations except for the higher concentration (3.0 x 10^-2 g/ml) on the second day. This was expected since viruses have relative slow action taking several days between virus consumption by the larva and its death (Stancey et al., 1970). According to Alves (1986) the death of small larvae should be expected three days after the ingestion. *B. bassiana* was also totally ineffective to the second day. Pekrul & Grula (1979) state it ideal conditions *B. bassiana* takes at least 18 hours to germinate and begin penetration in the larva cuticle. Effects of the cuticle chemical composition (Ferron, 1981) coupling with the appropriate enzymatic activity of the fungi's hypha (Smith et al 1981) may have a profound effect in the infectious process.

At the seventh day HNPV presented a high effective control achieving 100% mortality in treatments. The length of time needed for the virus to infect and kill the larvae was between three and seven days, confirming observations of Stancey et al (1970). The larvae died in the second instar having their body characteristically disrupted, liquified and tarnished. This bioinsecticide may be used in IPM programs for control of the bollworm when time is not a crucial factor. *B. bassiana* reached significant results from the control with the highest concentration being most effective. The mummified bodies showed the characteristic conidia-bearing mycelium covering completely the cadaver. This bioinsecticide had a low effect and little value as a microbial insecticide for control of bollworm in the conditions of this experiment. A similar conclusion was achieved by Tanada & Reiner (1962). No other different results were observed at a nine-day period.

**Greenhouse Test.** The percentage of damaged squares for each of the four tests is shown in Fig. 1. The first test shows a large numerical, but not significant, difference in the percentage of squares damaged between cypermethrin (0%), *S. feltiae* (0%), Mycogen (4.6%) and the control (20.4%). Cypermethrin showed its quick-killing action with a full protection effect. BT and *B. bassiana* showed intermediate results.

In test two, the pyrethroid treatment showed again no damaged squares, being significantly different

![Graphs showing percentage of damaged squares](image)

FIG. 1. Percentage of damage squares by *Heliothis zea* in cotton plants treated with four insecticides in greenhouse. (Columns in a same graphic with the same letter are not significantly different at p = 0.05 using Duncan’s New Multiple Range Test).
compared to the control. Pyrethroids are very effective against all larval stage of Heliothis spp. especially the first instar larva and are currently being employed for late season pest control in cotton (Elliott et al., 1978; Miller & Salgado, 1985). Mycogen (4.6%) had a significant performance compared to the pyrethroid (0%) and the control (30.6%). Also, the treatment with Mycogen (4.6%) showed a significant lower percentage of squares damaged than that with Dipel (30.3%). For BT (30.3%), B. bassiana (30.3%) and S. feltiae (36.9%) the percentage of damage squares was not significantly different as compared with the untreated check (30.6%) representing a whole lack of efficiency. Work by Bull et al. (1979) showed low efficiency of BT against bollworm leading to the conclusion that microbial pesticides may not work well in heavy infestations. This result is also corroborated by Ignoffo et al. (1977) and Bull et al. (1976). On the other hand, Ignoffo et al. (1977) observed a 69-96% reduction in the population larvae of H. zea in soybeans.

In tests 3 and 4 only cypermethrin (0% and 4.6%) gave significant level of protection relative to check (39% and 54.7%, respectively). An aspect to be considered is that the young larval stage is more susceptible to microbial infection than latter stages (Maddox, 1982).

In these experiments, the same plants were infested four times, the larvae surviving on the first insecticide application developed into further instars. This fact may have accounted for the lack of control observed in the treatments except for the cypermethrin which is active against all larval stages (Miller & Salgado, 1985). S. feltiae does not seem to be an appropriate pathogen for insect control above soil. Tanada & Reiner (1962) also concluded that the nematode DD-136 had low efficiency against H. zea when compared with HNPV and BT.

In the experiment the application was directed to the foliage without any protection. Probably, when the leaves dry out, the nematodes cannot move to search for host larvae becoming vulnerable to environment. Bong (1986) reported 70% mortality after second-day application and economic damage was not prevented. Mycogen should be considered for in other tests because it was effective against the cotton bollworm larvae in the first two tests.

CONCLUSIONS

1. Laboratory test at day-1 post applications showed that Cypermethrin and BT were very effective against H. zea larva in all the concentrations used. S. feltiae had a satisfactory result. At day-2 a high larval mortality was recorded for S. feltiae. HNPV was effective only for the highest concentration. At day-7 HNPV was highly effective in all concentrations used. B. bassiana also showed high larval mortality.

2. Test 1 in greenhouse showed no significant results in the percentage of damaged squares between treatments with Cypermethrin, S. feltiae, Mycogen and the control. In test 2 only Cypermethrin and Mycogen showed significant results. In tests 3 and 4 Cypermethrin was the only treatment with significant results compared to check.

REFERENCES


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