Philipp. Ent. 13(2): 159-168 ISSN 0048-3753 OCTOBER 1999

FIELD EFFICACY OF NUCLEAR POLYHEDROSIS VIRUS (NPV) AND *BACILLUS THURINGIENSIS* (BT) FOR *SPODOPTERA* CONTROL IN YELLOW GRANEX ONIONS

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ABSTRACT

Field trials in Bongabon, Nueva Ecija showed that NPV and Bt+NPV are more effective against *Spodoptera* larvae than Lambda-cyhalothrin. Highest larval counts were recorded in Lambda-cyhalothrin-treated and control plots. Onion yields were highest in NPV+Bt treated plots followed by Lambda-cyhalothrin-treated plots although the difference between them was not significant. The lowest yield was observed from control plots. These results show that microbials, alone or in combination, are effective alternatives to insecticide application, confirming their positive potential for *Spodoptera* management. The observation was based on two cropping seasons of onion in IPM-CRSP Demo Farm, Bongabon, Nueva Ecija (DS 1997-1998).

Onion yields among the treatments in San Jose, Nueva Ecija also had the same trend with NPV+Bt having the highest yield but not significantly different from the rest of the treatments. However, the cutworm larval densities were too low to demonstrate the effects of the treatments on them and on onion yields.

In a recent outbreak of the armyworm, *S. exigua*, the application of NPV+*Bt* in Bongabon Demo Farm yielded 85% infected larvae collected from the field.

Key words: Nuclear Polyhedrosis Virus (NPV); *Bacillus thuringiensis*; *Spodoptera litura* Control

INTRODUCTION

In the Philippines, a common practice among farmers after harvesting rice particularly in Nueva Ecija, is to grow bulb onions, *Allium cepa* L., as their main second crop. This is followed by eggplant, *Solanum melongena* L., string beans, *Vigna cylindrica* (L.) Skeels and garlic, *A. sativum* L. (IPM-CRSP, 1993).

Onion is one of the Philippines' high-value and most profitable crops. In 1995, about 60% of the rice growing area in Central Luzon was planted to onion. The commonly grown onion varieties are Batanes, Tanduyong, Red Creole and Yellow Granex. The last two are the varieties exported to Japan, Hongkong and other Asian countries (Eusebio, 1996).

During the dry season of 1995, IPM-CRSP gathered data on the major and minor insect pests and their natural enemies on rice-vegetable system. Results of these efforts have led to the identification of *Spodoptera litura* (Fabricius) as one of the pests of onion (Table 1). The common cutworm, *S. litura*, is among the most economically important insect pests in the country. It infests 28 agricultural

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Received 20 May 1999; accepted 23 September 1999

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Table 1. Key insect pests of crops in San Jose, Nueva Ecija, 1994-95.*

PEST/DISEASE	RICE	ONION	EGGPLANT	BEANS	GARLIC
Scirpophaga (stemborers)	XXX				
Nephotettix spp. (Green Leafhopper, GLH)	XX				
Nilaparvata lugens (Brown Planthopper, BPH)	X				
Thrips		XXX	X	XX	XX
Leucinodes orbonalis (Shoot/Fruit Borer)			XXX		
Maruca testulalis (Bean Pod Borer)				XXX	
Spodoptera litura (Common Cutworm)	X	XX	X	XX	
Liriomyza sp. (Leafminer)				XX	
Amrasca biguttula (Cotton Leafhopper)			XX		

*Note: X - minor; XX - potential; XXX - major pest/disease

crops including onion, rice, eggplant and beans as reported by Gabriel (1997). The newly emerged larvae gregariously feed on the soft leaf tissues. The damage done by this pest is exhibited by large feeding holes on the blades of mature and young leaves.

S. litura was more serious on Yellow Granex than on native (Tanduyong) onions very likely because Yellow Granex has broader leaves. Farmers sprayed chemical insecticides four to six times a month during the season upon observing cutworm damage.

Chemical insecticides have been widely used against the common cutworm as they offer excellent control. However, a report by ESCAP (1987) indicated that at least 449 species of insects and mites have developed resistance to pesticides due to increased or decreased dosage and frequency of application. This jeopardizes the successful and continued use of important chemicals especially when cross and multiple resistance are developed. Pure chemical control may not be feasible anymore in the near future due to environmental and health hazards. For instance, ESCAP (1987) reported that about 54% of occupational poisoning cases are caused by insecticides. Moreover, it is becoming unaffordable to common farmers.

Van Huis (1989) reported a number of entomopathogenic microorganisms (e.g., bacteria, fungi, protozoa, nematodes and viruses) which are potential components in insect pest management. Studies revealed that these entomogenous microbial agents can be potent instruments in reducing insect pest populations below economic threshold. Insect viruses and bacteria in particular have appreciably shown evidences as pest suppressive agents that could complement other control measures.

Moreover, subsequent studies involving simultaneous infection by insect pathogens like nuclear polyhedrosis virus (NPV) mixed with *Bacillus thuringiensis* Berliner have shown a range of interactions. These were exhibited by the susceptible insects that formed a continuous relationship ranging from additive to synergistic interaction. Many of these studies involving simultaneous infections were noted in pathogens like bacteria, fungi, microsporidia and viral interactions. Of these, bacteria and virus interactions have the highest potential as observed by McVay (1977) and Fuxa (1979).

The current study aims to evaluate the potential of NPV and *B. thuringiensis*, alone or in combination, compared to Lambda-cyhalothrin, a chemical insecticide for the management of two species of *Spodoptera* attacking onions.

MATERIALS AND METHODS

Control of Onion Cutworm Using Microbials

The *B. thuringiensis* (Bt) product containing 5% a.i. was produced at the National Institute of Molecular Biology and Biotechnology (BIOTECH), U.P. Los Baños, College, Laguna. The product was developed to control leaffolders, *Marasmia patnalis* (Bradley) and *Cnaphalocrocis medinalis* (Guenee), and was funded by PhilRice, Muñoz, Nueva Ecija. The Bt product also has toxicity against *S. litura*. The nuclear polyhedrosis virus (NPV) was taken from diseased cutworm, *S. litura*, collected from the farmer's field in Palestina, San Jose, Nueva Ecija. The NPV was coded as NPV-CRSP and mass produced in the laboratory for field trials.

Bioassay was conducted at the Insect Pathology Laboratory, Department of Entomology, U.P. Los Baños using natural host through leaf dipping according to the method of Bartelt *et al.* (1990). Fresh and healthy castor leaves were collected from the field and brought to the laboratory. The leaves were thoroughly washed in running (tap) water then rinsed with distilled water and air dried. After a few minutes, the leaves were cut to uniform sizes allocating three cut leaves per bioassay cup per feeding with 10 larvae per cup. The treatments were laid in Completely Randomized Design (CRD) with three replicates having the following concentrations: *B. thuringiensis* product: 8, 9, 10, 11, and 12 gm/L and a control check.

For NPV-CRSP, the following concentrations were used: 8, 9, 10, 11 and 12 ml/L equivalent to 2.8 x 10^7, 3.15 x 10^7, 3.5 x 10^7, 3.85 x 10^7 and 4.2 x 10^7 PIB's/ml and a control check. The natural host leaves were dipped into their assigned concentration, air dried and then placed in the disinfected bioassay cups (7 cm diameter x 6 cm deep). Ten third instar larvae were introduced into individual cups, tightly closed with fine mesh net cover. Thereafter, the larvae were allowed to feed for 12, 24 and 48 hours until enough lethal ingestion was attained. After 48 hours, the larvae were fed with clean leaves until 50% mortality was obtained. The LC$_{50}$ was determined by Probit Analysis based on the method by Finney (1964).

Microbial Field Trials

Field trials of the microbial and chemical insecticide treatments were conducted in Palestina, San Jose and NOGROCOMA Demo Farm, Bongabon, Nueva Ecija. A 4 m x 5 m plot with one meter border was assigned for each treatment and replicated four times laid out in Randomized Complete Block Design (RCBD).

T_1 - Bt product (10 g/L)
T_2 - NPV-CRSP crude extract (10 ml/L)
T_3 - Bt (5 g/L) + NPV-CRSP (5 ml/L)
T_4 - Lambda-cyhalothrin (KARATE™) (1 ml/L)
T_5 - Untreated Control

One month-old Yellow Granex seedlings were transplanted to the field and common farmer's practices for the management of onion were followed except in Palestina, San Jose where rice hull burning was done before transplanting. During the 1997 season, first spraying was done four weeks after transplanting (WAT). In 1998 the same was done two weeks after transplanting. This was followed by biweekly application until harvest. Sampling was done randomly every three weeks by counting the number of larvae per plant with 10 plants per plot. The percentage damage of leaf per plant was also recorded in every sampling. The yields were analyzed to relate the effectiveness of the different treatments. Analysis of variance (ANOVA) was done and mean comparisons were computed using Duncan's Multiple Range Test (DMRT) for the 1997 data and Tukey's HSD for the 1998 data.

RESULTS AND DISCUSSION

LC_{50} Determination

The estimates of median lethal concentration (LC_{50}) of *B. thuringiensis* product and NPV-CRSP crude extract are shown in Table 2. *B. thuringiensis* product has a LC_{50} of 9.75 g/L of 5% a.i. while 9.78 ml/L was determined for NPV-CRSP crude extract equivalent to 3.42 x 10^7 PIBs per Liter with regression equation of $Y = 3.2341 + 1.785x$ and $Y = 0.8648 + 5.922x$, respectively. Moreover, 50% mortality was attained after 5 days for *B. thuringiensis* and 4 days for NPV. Both exerted good fit as shown by chi-square value. However, Ebora (1987) recorded a lower LC_{50} (2.12. X 10^6 PIBs/ml) against the third instar of *S. litura*, 7 days after treatment.

Microbial Field Trials

The comparative effects of NPV-CRSP crude extract and Bt product, whether singly or in combination, and Lambda-cyhalothrin (KARATE ™) on *S. litura* larval populations in Bongabon, Nueva Ecija are shown in Table 3. For the 1997 season, lowest cutworm density was observed in Bt+NPV-treated plots followed by plots treated with NPV alone, Bt alone, Lambda-cyhalothrin and control plots in increasing levels. The same trend was observed in 1998 (Table 4) except that larval densities in the microbial treatments, particularly NPV alone, showed significant differences from Lambda-cyhalothrin 10 and 12 weeks after transplanting (Fig. 1). These data imply that microbials, particularly NPV alone, are comparable to or have a slight advantage over Lambda-cyhalothrin in suppressing cutworm larval populations. In San Jose, Nueva Ecija, where the farmers planted early (November, 1997), cutworm larval densities were very low and did not differ significantly among the treatments, which in effect were not adequate to reflect the advantage of microbials over insecticide applications (Table 5).

Table 2. Estimates of LC_{50} of *B. thuringiensis* and Nuclear Polyhedrosis Virus on third instar larvae of *S. litura.*[1]

Microorganism	LC_{50} (g/L)	LC_{50} (ml/L)	95%Fiducial Limit	Slope+SE	Chi-square
B. thuringiensis	9.75		0.845 - 1.133	0.989 ± 0.07	0.538*
NPV		9.78	0.948 - 1.032	0.990 ± 0.02	0.491*

[1] Bioassayed 3 times, with each assay replicated 3 times with a total of 150 third instar larvae per bioassay.

Table 3. Densities of *S. litura* larvae, observed over 4 sampling periods, NOGROCOMA Demo Farm, Bongabon, Nueva Ecija , Dry Season, 1997.

Treatment	Mean [1] number of larvae per plant (n = 10 plants)			
	6WAT	8 WAT	10 WAT	12 WAT
Bt	0.98 b	0.38 c	0.08 bc	0.15 ab
NPV	0.78 bc	0.30 cd	0.05	0.05 b
Bt+NPV	0.55 c	0.18 d	0.0 c	0.12 b
Lambda-cyhalothrin	1.45 a	0.58 b	0.18 ab	0.30 ab
Untreated Control	1.55 a	0.72 a	0.22 a	0.42 ab

[1] Means followed by the same letter are not significantly different at 5% level of significance based on LSD. Data analyzed using arcsin of the square root of x transformation.

Table 4. Densities of *S. litura* larvae, observed over 5 sampling periods, NOGROCOMA Demo Farm, Bongabon, Nueva Ecija, Dry Season, 1998.

Treatment	Mean [1] number of larvae per plant (n = 10 plants)				
	4WAT	6WAT	8 WAT	10 WAT	12 WAT
Bt	0.90	0.42	1.58	0.90	0.52 k
NPV	0.22 u	0.32	1.05	0.28 k	0.50 k
Bt+NPV	0.20 u	0.52	0.95	0.30 k	0.82
Lambda-cyhalothrin	0.38	1.80	1.05	1.32	1.32
Untreated Control	1.18	0.92	0.68	0.78	1.18

[1] The means presented in this table were computed from the observed counts (not transformed), but the ANOVA and mean comparisons using Tukey's HSD were performed on log-transformed data.

k - significantly different from Lambda-cyhalothrin

u - significantly different from Untreated Control

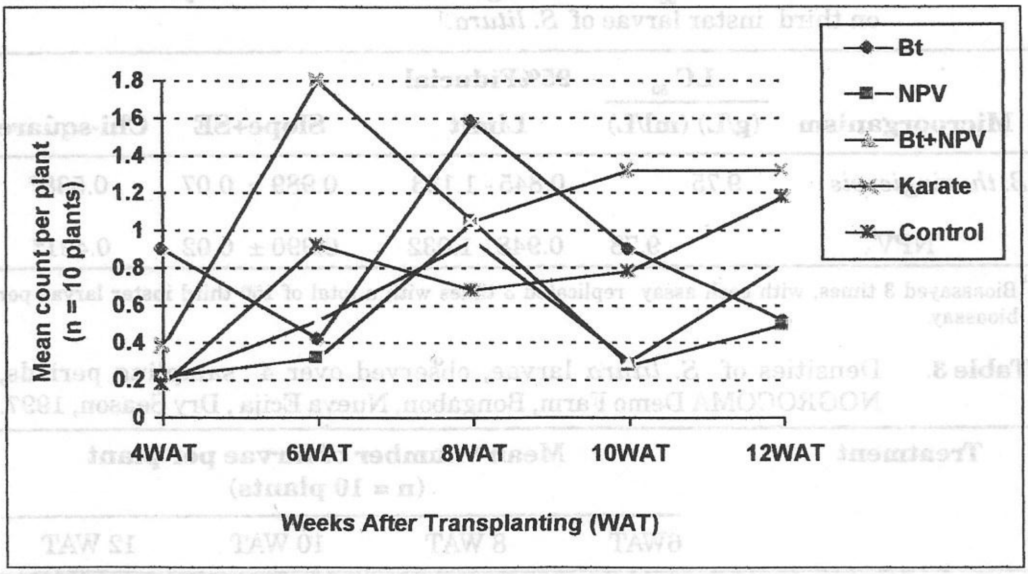

Figure 1. Densities of S. litura larvae in onion field, Bongabon, Nueva Ecija, Dry Season, 1998.

Table 5. Densities of *S. litura* larvae, observed over 5 sampling periods, San Jose, Nueva Ecija, Philippines, Dry Season, 1998.

Treatment	Mean number of larvae per plant (n = 10 plants)				
	4WAT	6WAT	8 WAT	10 WAT	12 WAT
Bt	0.05	0.02	0.12	0.68	0.00
NPV	0.05	0.02	0.08	1.32	0.00
Bt+NPV	0.08	0.00	0.08	2.58	0.02
Lambda-cyhalothrin	0.00	0.00	0.20	2.00	0.00
Untreated Control	0.02	0.00	0.52	1.50	0.05

The extent of onion leaf damage due to larval feeding is shown in Tables 6 and 7. In the Bongabon site (Table 7), overall leaf damage levels did not differ significantly among treatments. However, at 12 WAT, Lambda-cyhalothrin-treated plots had the highest leaf damage, followed by control plots, Bt-treated plots, and NPV-treated plots, with NPV+Bt-treated plots having the lowest damage. Percent leaf damage ranged from as low as 11.5% at 4 WAT to as high as 41.75% at 12 WAT in Lambda-cyhalothrin-treated plots. Overall, however, the NPV-treated plots had the lowest leaf damage.

In San Jose (Table 8), the control plots showed the highest leaf damage, although not significantly different, followed by plots treated with Lambda-cyhalothrin, NPV+Bt, and Bt alone, with NPV- treated plots having the lowest damage. As mentioned earlier, the much lower larval densities in the site may only reflect a negligible level of leaf damage, which ranged from as low as 0 at 4 WAT to as high as 21.75% at 10 WAT in NPV+Bt-treated plots.

The higher level of leaf damage in onion plots observed in the Bongabon Demo site could have been due to late planting, with the vegetative stage of the crop coinciding with higher cutworm larval populations in the site.

In the recent outbreak of the armyworm, *S. exigua*, the application of NPV+Bt in Bongabon Demo Farm yielded 85% infected larvae collected from the field.

Onion yields in Bongabon were highest in Bt+NPV- treated plots followed by Lambda-cyhalothrin-treated plots. Although the two treatments did not differ significantly from each other, they were significantly higher than the control (Table 6). Whether the yields were affected by other factors, (e.g, soil-borne diseases, nematodes, and other insects), remains uncertain. It is also likely that the presence of another species, *Helicoverpa armigera,* could have affected the cutworm densities owing to the territorial and solitary behavior of *H. armigera* larvae, exhibiting control of onion leaves over cutworm larvae. Likewise, the timing of sampling could have affected the observed cutworm larval densities. Shifting to night sampling may reflect a different picture of larval populations. In contrast, onion yields in San Jose were very high and did not differ significantly among the treatments (four times as high as that in Bongabon). This could be attributed to early planting, higher plant density per unit area (no furrows between rows and closer spacing), and the sandy loam type of soil. Moreover, the field was subjected to rice hull burning which has been shown to reduce incidence of soil-borne diseases , weeds, and nematodes. In contrast, onion fields in Bongabon Demo farm were planted late (December-January), had lower plant density (plants were arranged in distinct rows with furrows in-between), and the clay loam type of soil. Rice hull burning is not a common practice in Bongabon farms as it is among San Jose farmers. Considering all these factors which could have influenced onion yields in both sites, the effects of NPV and Bt still remain inconclusive. Perhaps, the real potential of Bt and NPV may be realized at higher concentrations, which should be considered in future experiments.

In the Philippines, Gabriel (1968) reported NPV on *S. litura* for the first time. Early laboratory studies on NPV of *S. litura* were conducted by Gabriel and Padua (1974) and Legacion and Gabriel (1975).

The use of the combination of NPV- *B. thuringiensis* was first suggested by McEven and Hervey (1959). Since then, similar combinations have shown promise in several field trials. Stelzer (1965) obtained good results with such a combination

Table 6. Extent of leaf damage caused by *Spodoptera litura* larvae on Yellow Granex onion using Bt, NPV-CRSP, and Lambda-cyhalothrin, Demo Farm, Bongabon Nueva Ecija, DS, 1997-98.

Treatment	Percent (%) Leaf Damage (n= 10 plants)[1]					
	4WAT	6WAT	8WAT	10WAT	12WAT	Overall
Bt	20.5 a	15.0 a	20.8 a	19.5 a	30.5 ab	21.2
NPV	13.5 a	17.2 a	18.0 a	17.0 a	27.0 b	18.6
NPV+Bt	13.5 a	18.2 a	21.0 a	21.5 a	26.2 b	20.1
Lambda cyhalothrin	11.5 a	18.0 a	22.2 a	25.2 a	41.8 b	23.8
Control	20.8 a	18.8 a	25.8 a	25.8 a	34.5 ab	25.1

[1] Means in a column with the same letter are not significantly different at 5% level using DMRT.

Table 7. Extent of leaf damage caused by *S. litura* larvae on Yellow Granex onion using Bt, NPV-CRSP, and Lambda-cyhalothrin, Palestina, San Jose, Nueva Ecija, DS 1997-98.

Treatment	Percent (%) Leaf Damage (n= 10 plants)[1]					
	4WAT	6WAT	8WAT	10WAT	12WAT	Overall
Bt	2.0 b	10.2 a	15.8 a	13.8 a	3.2 a	9.0 a
NPV	2.5 b	6.0 a	13.0 a	13.2 a	3.2 a	7.6 a
NPV+Bt	0.0 b	5.0 a	20.2 a	21.8 a	3.0 a	10.0 a
Lambda cyhalothrin	2.5 b	7.25 a	18.5 a	19.2 a	3.2 a	10.1 a
Control	6.2 a	10.8 a	18.8 a	16.2 a	5.2 a	11.4 a

[1] Means in a column with the same letter are not significantly different at 5% level using DMRT

Table 8. Mean onion yields in two experimental sites, Dry Season, 1998.

Treatment	Mean yields per hectare (t/ha)	
	Bongabon	San Jose
Bt	9.98	33.0
NPV	9.74	39.8
Bt+NPV	11.46 u	45.6
Lambda-cyhalothrin	11.31 u	37.4
Untreated Control	8.41	32.7

u - significantly different from Untreated Control against the Great Basin tent caterpillar, *Malacosoma fragile* as did Steler *et al.* (1975), with the combination against Douglas fir tussock moth, *Orgyia pseudotsugata*. For the trials against the cabbage looper, the NPV-*Bt* combination gave good results (Creighton *et al.* 1970, Vail *et al.* 1972) where a similar combination used against the corn earworm, *Heliothis zea,* proved more effective than the virus alone (Oatman *et al.* 1970). Some workers indicated that a combination of *Trichoplusia ni,* NPV and *B. thuringiensis* showed satisfactory results and was not detrimental to the pathogenic action of NPV. McVay *et al.* (1977) obtained higher mortality among *T. ni* larvae when exposed to NPV- *B. thuringiensis* combination than when exposed to either pathogen alone. The combined NPV and Bt was also found effective against *S. litura* attacking peanut, *Arachis hypogaea* L., as reported by Agsaoay (1998). Mortality rating was 92.22% at 12 days after spraying. All these observations were in line with these studies when the NPV and *B. thuringiensis* combination was used to control *Spodoptera* in the field.

SUMMARY AND CONCLUSION

The combination of NPV + *B. thuringiensis* demonstrated appreciable performance in the field against larvae of *S. litura* and *S. exigua*. Application of NPV alone also gave promising options for *Spodoptera* control. However, further evaluation is necessary for the management of the two species of *Spodoptera* as part of integrated pest management strategies.

ACKNOWLEDGEMENT

This project, Integrated Pest Management Collaborative Research Support Program (IPM-CRSP) is funded by the United States Agency for International Development (USAID) under Grant No. LAG - G - 00 - 93 - 0053 - 00 and the Philippine Rice Research Institute (PhilRice).

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