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Bioassay of nuclear polyhedrosis virus (npv) and in combination with insecticide on *Spodoptera litura* (Fab)

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ABSTRACT

The susceptibility of different stages of Spodoptera litura larvae to NPV was determined by bioassay. The LC_{50} values were found to increase with the age of host. There was 1,500,000 folds increase in LC_{50} value from 2-day-old larvae ($1x10^3$ PIB/ml) to the 8-day-old larvae ($1.5x10^9$ PIB/ml). As the insect grows, there is apparent dilution effect, in that more viruses are required to initiate a lethal infection at later larval age. LT_{50} values increased from 4.4 days for 2 day old larvae to 9.4 days for 8 day old larvae. This suggested that an increase of resistance with age when infected. LT_{50} apparent to be dose dependent. The estimated LT_{50} increased with the increase larval age whereas, it decreased with the increase dose.

The action of nuclear polyhedrosis virus of Spodoptera litura (Fabricius) in combination with sublethal concentration of thiamethoxam (thianocotinyl), diflubenzuron (chitin synthesis inhibitor) and imidacloprid (chloronicotinyl) was investigated on 5 day old larvae of Spodoptera litura. Nuclear polyhedrosis virus with thiamethoxam at 50ppm produced synergistic effect while at lower and higher 50ppm produced additive effect. In the case of diflubenzuron in combination with nuclear polyhedrosis virus, the concentration of diflubenzuron was tested from 1-10ppm have synergistic effect while imidacloprid at the higher concentration 10ppm given antagonistic effect.

Key words: Nuclear polyhedrosis virus, *Spodoptera litura*, biological control,

INTRODUCTION

Spodoptera litura (Fab.) popularly known as tobacco caterpillar or tobacco cutworm is an important polyphagous crop pest of national status in India. It enjoys wide distribution and besides India it is reported from Thailand, Philippines, China, Japan, Vietnam, Indonesia, Australia, Korea, Iran, Egypt, Bahrain, Fiji, and Formosa (Singh and Jalali, 1997) It is reported to feed on 112 species of plants, the outbreak of this pest generally occurs under well rainfall condition after long dry spell. Further, the pest had been reported to develop its resistance to several insecticides like Malathion, Pyrethrum, Lindane and Endosulfan (Ramakrishnan *et al*, 1984).

Baculoviruses, among other insect viruses, are regarded as safe and selective bio-insecticides, restricted to insects only. They have been used worldwide against many insect pests. These viruses with specificity potential that can suppress insect pest of economic importance in agricultural crops, have been indicated their application as microbial pesticides. Susceptibility of many species to baculoviruses has been addressed to decrease with larval age (Hochberg, 1991, Gitanjali *et al*, 1994, Vinay, 1997) and for infected host become to die, the time require to be noticed to increase (Smits and Vlak, 1988). The increase in LC_{50} value of larval maturation of *Spodoptera litura* was reported (Tuan et al, 1998). Earlier, there are several

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investigations on nuclear polyhedrosis virus were carried out (Padmavathamma and Veeresh, 1991; Gitanjali *et al* 1994, Vinay, 1997; Monobrullarh and Nagata, 2000). The present contribution deals with the activity of nuclear polyhedrosis virus on series of 2 to 8 day old larval age of *Spodoptera litura.* The negative effect of their use as microbial control that are long incubation period of the disease with allows the larvae to continue cause damage to crops. Thus, any finding in the early kill of host insect would be important and potentially useful advance. Microbial agent-chemical interaction and their potential advantage have recognized by Steinhaus (1958). This necessitates the exploration of using viruses combined with certain insecticides at low doses than recommended alone to solve many problem due to using insecticides alone or NPV alone. The joint action of nuclear polyhedrosis virus of *S. litura* with a few insecticide was investigated and reported in this contribution

MATERIALS & METHODS

Source of insect and virus:

The egg of *Spodoptera litura* were collected from the castor field (*Ricinus communis*) and was maintained at the laboratory of Entomology Division, IARI, New Delhi, India. Thereafter, it was continued being reared in the cultural room at the temperature of 27± 2^{o}C on the castor leaves. A portion of culture was maintained on artificial diet in order to minimize the natural occurrence of the disease.

The isolate of *Spodoptera litura* nuclear polyhedrosis virus (S*l*NPV) was conducted by using early fourth instar larvae that was infected with nuclear polyhedrosis virus by leaf dip feeding method for 24hr, later on provided fresh castor leaves. The larvae exhibiting typical symptoms of polyhedrosis virus were collected and stored in stoppered flask with 1ml of water per larva, they were left to putrefy for about 15 days to enable the release of polyhedra from infected tissue. The putrefied suspension of disease larvae was homogenized using the tissue homogenizer and leave undisturbed for a couple of days to facilitate the polyhedra to settle as a whitish layer at the bottom of flask, after that the dead tissue on the upper layer of flask was removed gently (the polyhedra at the bottom should not be disturbed) and the remains of the polyhedra was filtered by two-layer filter cloth and was centrifuged at 6000 rpm for one hour, the pellets containing pure polyhedral inclusion bodies were collected and resuspended in distilled water and stored at 4^{o}C till further use. The number of PIBs/ml was determined by using Neubaur Hemocytometer (Germany). Appropriate dilutions was made from of stock preparation is 2.2 x10^{11}PIB/ml.

Different concentration of following insecticides were combined with NPV for studying the combination effects:

1. Thiamethoxam: 30, 50, 100, and 150 ppm
2. Diflubenzuron: 1, 3, 5, 7 and 10 ppm
3. Imidacloprid: 1, 3, 5, 7, 10 and 20 ppm.

Bioassay procedure

The larvae used in bioassay were obtained from a laboratory stock with series of 2, 3, 4, 5, 6, 7, 8-day old larval age groups of *Spodoptera litura* which were fed on the castor leaves, the castor leaves were collected from the field must be washed and keep to dry. Leaf dip feeding method was employed to treat the various age stages of larvae for every experiment, before treating of NPV, the larvae were kept starved for 2 hrs. Each treatment was replicated thrice. After 24hr treated with NPV, the larvae were provided fresh castor leaves every day. The mortality was recorded every day after showing the disease symptom. The data was subjected to Probit analysis (Finney, 1952) to determinate LC_{50} of NPV.

The test of effect of insecticidal additives in combination with SlNPV:

PIBs were diluted in various concentration of insecticides. Concentration of each insecticide was tested to find out the sub-lethal dose that can kill not more than 10% of host insect.A constant virus concentration of 2.2x10^{6} PIB/ml was used in these experiments against 5 days old larvae. Each treatment was replicated thrice. Mortality was recorded every 24hrs. The data was

subjected to Probit analysis for calculating LT50 and IRRISTAT program for comparison in the effect of combination of NPV and insecticides. To test the synergism of NPV and the insecticides, the formula of Co-toxicity factor (CTF) was used.

Viral concentration of 2.2 x10 ^{6} PIB/ml which gave approximately 50 per cent mortality to the 5-day old larvae was used in all the experiments. The test solution was repaired by mixing equal volum of insecticide and virus suspension, each having twice the desired concentration.

Five-day old larvae were fed for 24 hrs on castor leaves dipped in suspensions of different combinations of insecticides and virus. The total 60 larvae were used in each of treatment with three replications. In addition to the various combination treatments, virus alone was used in these test as control treatment. All the experiments were kept at the temperature (27±2^{0}C). Observation on mortality for all the treatments was recorded daily up to a period of 10 days.

The mortality due to insecticide, mortality due to virus, a mortality in combination were recorded. Correct mortality was calculated by using Abbott's formula. The Co-toxicity factor to assess the combination effects was calculated according to the following formula

CTF = (OM-ME)/ OMx100

where

OM is the observed percentage mortality producted by the combination,

ME is the expected mortality percentage (i.e., the sum of mortality from each agent).

A = Positive factor of 20 or more (synergism)

B = Negative factor of 20 or more (antagonism)

C = Any value between ± 20(additive to either effect)

A 20 percent difference was allowed to theoretically expected experiments and biological variations. In these experiments, the expected mortality will be the mortality due to either virus and/or insecticide alone and the observed mortality is the mortality in combination.

RESULTS & DISCUSSION

The results of bioassay studies with nuclear polyhedrosis virus on *Spodoptera litura* are presented in Table 1.

Table 1. Calculated LC_{50} values of seven different stages of *S*lNPV treated larvae.

Ages (days)	LC_{50} (PIB/ml)	95% limit		Slope	Intercept	χ^2	df
		Lower	Upper				
2	1x10^{3}	2.1x10^{2}	3.7x10^{3}	0.26228	0.07103	1.963	3
3	5.3x10^{3}	8.58x10^{2}	9.4x10^{3}	0.20408	-0.42901	3.332	3
4	4.1x10^{4}	4.7x10^{3}	1.6x10^{5}	0.26731	0.35152	2.504	4
5	3.9x10^{6}	8.84x10^{5}	1.4x10^{7}	0.3279	0.07834	0.732	3
6	1.4x 107	2.0x106	6.9x107	0.264	0.110	1.175	3
7	1.2x108	3.4x107	3.6x108	0.338	0.057	1.336	3
8	1.5x10^{9}	2.3x10^{8}	8.1x10^{9}	0.21475	0.03406	0.941	3

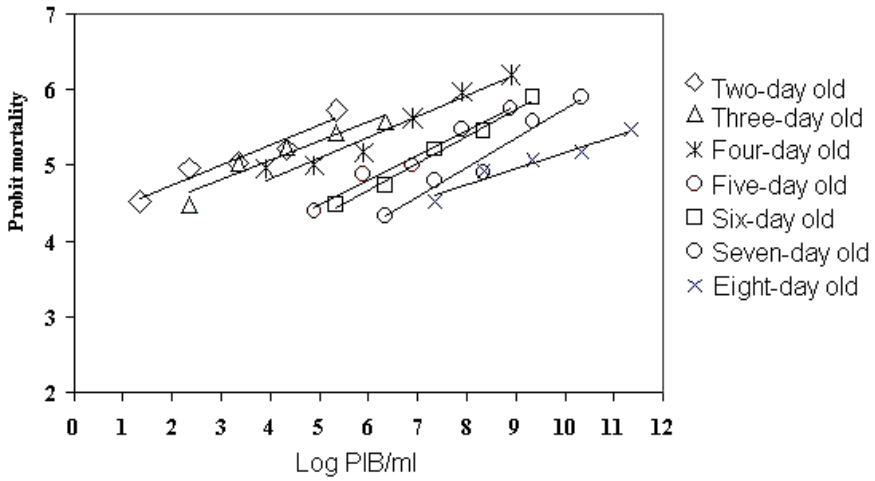

Fig 1. Regression line of SlNPV against different larval age group of S. litura

In the seven groups of larvae tested, the finding showed that LC_{50} of 2,3,4,5,6,7 and 8-day old larvae are $1x10^3$, $5.3x10^3$, $4.1x10^4$, $3.9x10^6$, $1.2x10^7$, $1.4x10^8$ and $1.5x10^9$ PIB/ml respectively. Slopes for concentration mortality relationship for larvae of various ages are showed in Fig1. The shift in position of the Probit lines reflects the increase in LC_{50} with age.

It was found that on the basis of LC_{50} the two-day old larvae was about 5.3, 41, 3900, 14000, 120000 and 1500000 time more susceptible to NPV than 3, 4, 5, 6, 7 and 8-day old larvae, respectively. Similar observation was made in *Heliothis zea* found that the slopes of the dosage mortality response were similar for larvae up to 7-day old, the Probit mortality lines are not parallel (except 8-day old larvae) suggesting that some degree of the resistance in larvae older than 8-day old larvae. The results agree with finding of Whitlock (1977) in *Heliothis armigera* and differ from those of Gitanjali *et al* (1994) and Monobrullah and Nagata, (2000). In the later, it was concluded that responses of larvae of all instars were similar since the line of different instars were parallel. However, the overall susceptibility of the younger larvae was much greater than that of the older ones. The increase LC_{50} in this result showed that there are some mechanisms of the development resistance of *Spodoptera litura* over many generations.

Lethal time data for concentration of S/NPV causing greater than 50% mortality. Table 2 showed that the LT_{50} values were dependent upon the age of larvae tested and the concentration used. The LT_{50} for the two-day old larvae was 5.4 to 4.4 days at the lowest and highest concentration, respectively, as compare to 3-day old larvae. LT_{50} was different from 8.0 to 5.4 days against the same concentration. Similar trend was found in the 4, 5, 6, 7 and 8-day old larval age.

Table 2: Calculated LT_{50} values of *S. litura* on different stages

Age (days)	Concentration	LT_{50}	95% limit		Slope	Intercept	χ^2	Df
			Lower	Upper				
2	$2.2x10^1$	5.9	5.6	6.3	5.908	-0.009	9.772	4
	$2.2x10^2$	5.4	5.1	5.8	5.111	0.105	17.717	4
	$2.2x10^3$	5.2	4.9	5.6	5.296	0.168	14.842	4
	$2.2x10^4$	4.9	4.6	5.2	5.818	0.289	14.359	4
	$2.2x10^5$	4.4	4.1	4.6	7.907	0.401	4.722	4
3	$2.2x10^2$	8.0	7.3	9.2	5.410	-0.536	7.071	4
	$2.2x10^3$	6.3	6.0	6.7	6.658	-0.086	5.671	4
	$2.2x10^4$	5.7	5.4	6.0	6.546	0.048	4.566	4
	$2.2x10^5$	5.4	5.0	5.7	6.618	0.136	4.572	4
	$2.2x10^6$	5.0	4.7	5.3	7.050	0.114	7.667	4
	$7.9x10^3$	6.9	6.6	7.3	9.499	-0.034	14.866	3
	$7.9x10^4$	6.7	6.4	7.1	9.135	0.384	10.581	3
4	$7.9x10^5$	6.2	5.9	6.5	10.556	0.176	5.771	3
	$7.9x10^6$	5.9	5.7	6.2	10.671	0.339	14.228	3
	$7.9x10^7$	5.6	4.7	5.8	11.071	0.425	17.743	3
	$7.9x10^8$	5.3	5.1	5.5	15.099	0.213	6.131	3
5	$7.9x10^4$	8.6	8.1	9.6	7.653	-0.424	4.768	4
	$7.9x10^5$	7.7	7.2	8.3	6.459	-0.202	3.568	4
	$7.9x10^6$	6.9	6.6	7.3	7.931	-0.021	2.039	4
	$7.9x10^7$	6.3	5.9	6.6	8.621	0.154	1.121	4
	$7.9x10^8$	5.8	5.5	6.0	9.424	0.278	4.144	4
6	$2.2x10^5$	9.6	8.6	11.9	5.452	-0.606	12.46	4
	$2.2x10^6$	7.8	7.4	8.3	8.038	-0.214	9.713	4
	$2.2x10^7$	6.8	6.5	7.2	8.414	0.016	9.511	4
	$2.2x10^8$	6.3	5.9	6.6	8.524	0.234	4.584	4
	$2.2x10^9$	5.5	5.1	5.8	8.83	0.31	3.160	4
7	$2.2x10^6$	8.7	8.2	9.6	8.64	-0.49	1.214	4
	$2.2x10^7$	7.6	7.2	8.2	7.724	-0.183	6.341	4
	$2.2x10^8$	6.9	6.5	7.3	8.34	-0.314	8.442	4
	$2.2x10^9$	6.2	5.8	6.2	8.59	0.25	3.203	4
	$2.2x10^{10}$	5.8	5.5	6.1	0.026	0.203	3.642	4
8	$2.2x10^7$	9.4	8.9	10.0	6.375	-0.265	9.570	5
	$2.2x10^8$	8.1	7.7	8.5	6.85	0.017	7.121	5
	$2.2x10^9$	7.7	7.3	8.0	7.07	0.091	8.564	5
	$2.2x10^{10}$	7.4	7.0	7.7	7.18	0.156	5.399	5
	$2.2x10^{11}$	6.8	6.5	7.2	7.346	-0.26	8.329	5

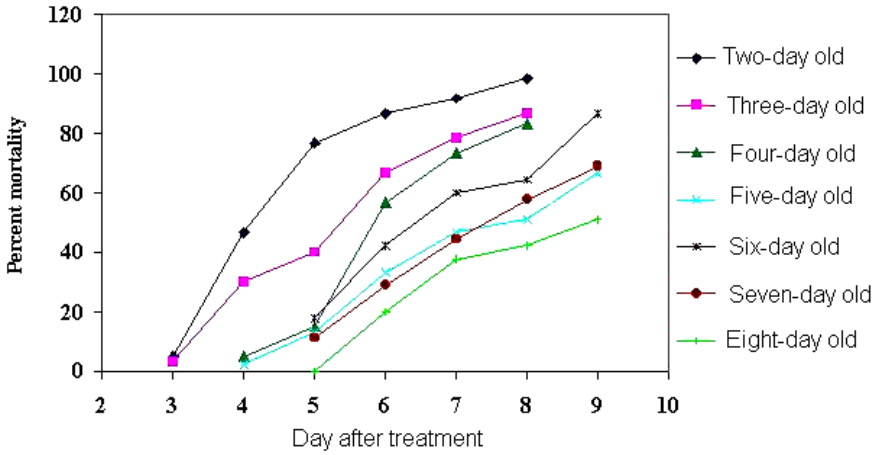

Fig. 2. Time mortality curves for *S. litura* larvae treated with NPV at different age.

Effect of combination of NPV with insecticide

Preliminary studies with sublethal dosages with Actara 25WG, Diflubenzuron 25 WP and Imidacloprid 17.8% EC produced negligible mortality. So, these sublethal dosages were selected for the combination studies. Optimum concentration of Actara (30, 50, 100 and 150ppm), Diflubenzuron (1, 3, 5, 7, and 10ppm), Imidacloprid (1, 3, 5, 7, 10 and 20ppm) were used against 5-day old larvae.

The result of comparative effectiveness of various additives on the pathogenicity of *S*lNPV is presented in Table 3, 4, and 5.

From the lowest up to highest concentration of Actara, the mortality increased from 65% to 83.3% as compared to 48.5% of virus alone which was significantly different at 1% level. However, there was no significant difference among other insecticide combination treatments except at treatment in lowest and highest concenttration of Actara.

Table 3. Combination effect of NPV with Actara against 5-day old larvae.

Treatment	Concentration of insecticide (ppm)	Concentration of NPV (PIB/ml)	% Mean of mortality
NPV alone	0	2.2×10^6	48.3 a
NPV + Actara	30	2.2×10^6	65.0 b
NPV + Actara	50	2.2×10^6	70.0 bc
NPV + Actara	100	2.2×10^6	73.3 bc
NPV + Actara	150	2.2×10^6	83.3 c
F			38.18**
CV (%)			14.50

** Significant at 1% and the values with same alphabet is not significantly different.

The combination of NPV and Diflubenzuron was also found to increase the mortality even with lower concentration. The data showed that the mortality increase from 43.3% up to 93.3% at the lowest to highest concentration as compared to 43.3% mortality due to virus alone. There is significant difference between the treatments and virus alone as well as between different treatments at 1% level (Table 4).

Table 4. Combination effect of NPV with Diflubenzuron against 5-day old larvae.

Treatment	Conc. of insecticide (ppm)	Con. of NPV (PIB/ml)	% Mean of mortality
NPV alone	0	2.2×10^6	43.3 a
NPV + Diflubenzuron	1	2.2×10^6	65.0 b
NPV + Diflubenzuron	3	2.2×10^6	66.7 b
NPV + Diflubenzuron	5	2.2×10^6	70.0 b
NPV + Diflubenzuron	7	2.2×10^6	73.3 b
NPV + Diflubenzuron	10	2.2×10^6	93.3 c
F			8.93**
CV (%)			13.5

** Significant at 1% and the values with same alphabet is not significantly different.

In case of combination of NPV and Imidacloprid, the mortality increases the concentration from 1 to 7 ppm and decrease when applied at the higher concentration (10 and 20 ppm) (Table 5). There is significant difference between treatments and virus alone at 1% level.

Table 5. Combination effect of NPV with Imidacloprid against 5-day old larvae.

Treatment	Conc. of insecticide (ppm)	Con. of NPV (PIB/ml)	% Mean of mortality
NPV alone	0	2.2×10^6	46.6 a
NPV + Imidacloprid	1	2.2×10^6	73.3 b
NPV + Imidacloprid	3	2.2×10^6	80.0 bc
NPV + Imidacloprid	5	2.2×10^6	81.7 cd
NPV + Imidacloprid	7	2.2×10^6	88.3 d
NPV + Imidacloprid	10	2.2×10^6	48.3 a
NPV + Imidacloprid	20	2.2×10^6	45.0 a
F			127.5**
CV (%)			7.4

** Significant at 1% and the values with same alphabet is not significantly different.

Combination effect of *S*/NPV with sublethal doses of insecticides

Larval mortality in *S*/NPV + insecticide mixture was generally synergistic when it was fed to 5-day old larvae with Actara and Diflubenzuron and with lower concentration of Imidacloprid.

In 5-day old larvae, the additive effect was strong with NPV + Actara (100-150ppm) with positive factor of 14.6 and 10.7, respectively, followed by NPV+ Diflubenzuron (1ppm to 10ppm), all the treatments were synergistic effect with positive co-toxicity factor of 23.7 to 50.1. In the case of NPV+ Imidacloprid mixture, the effect was synergistic at the lower concentration (1 up to 7ppm) with co-toxicity factor of more than 20 i.e. 38.8-50.0, but it became antagonistic at higher concentrations of 10 and 20ppm (Table 6) with negative co-toxicity factor of -7.8 and -26.0, respectively.

Table 6 . Interaction of *S*INPV with different insecticides and additive effect against 5-day old larvae of *S. litura*

Concentration of insecticide	Concentration of NPV	NPV alone mortality	Combination of insecticide and NPV		Co-toxicity factor	Combination effect
			Expected mortality (V + I)	Observed mortality in combination		
Actara (30 ppm)	$2.2x10^6$	60.0	60.0	65.0	8.3	Additive
Actara (50 ppm)	$2.2x10^6$	60.0	60.0	73.3	22.1	Synergism
Actara (100 ppm)	$2.2x10^6$	60.0	68.3	78.3	14.6	Additive
Actara (150 ppm)	$2.2x10^6$	60.0	78.3	86.7	10.7	Additive
Diflubenzuron (1 ppm)	$2.2x10^6$	43.3	43.3	65.0	50.1	Synergism
Diflubenzuron (3 ppm)	$2.2x10^6$	43.3	51.6	66.7	29.2	Synergism
Diflubenzuron (5 ppm)	$2.2x10^6$	43.3	56.6	70.0	27.3	Synergism
Diflubenzuron (7 ppm)	$2.2x10^6$	43.3	56.6	73.3	29.5	Synergism
Diflubenzuron (10 ppm)	$2.2x10^6$	43.3	61.6	93.3	40.0	Synergism
Imidacloprid (1 ppm)	$2.2x10^6$	36.7	38.4	53.3	38.8	Synergism
Imidacloprid (3 ppm)	$2.2x10^6$	36.7	40.0	58.3	45.6	Synergism
Imidacloprid (5 ppm)	$2.2x10^6$	36.7	40.0	60.0	50.0	Synergism
Imidacloprid (7 ppm)	$2.2x10^6$	36.7	41.7	63.3	51.7	Synergism
Imidacloprid (10 ppm)	$2.2x10^6$	36.7	43.4	40.0	-7.8	Antagonism
Imidacloprid (20ppm)	$2.2x10^6$	36.7	45.0	33.3	-26.0	Antagonism

Although many of insect viruses have been reported to affect pests of economical crops, few have given economic control to date. The negative aspects of their use as microbial control are long incubation period of the disease which allows the larvae to continue cause damage to crops. Thus, any finding in the early kill of host insect would be an important and potentially useful advantage.

All NPV insecticide mixture tested in the present study show additive and synergistic effect against 5-day old larvae at lower concentration of insecticides except antagonism effect at higher concentration of Imidacloprid (10 and 20ppm)

Larval mortality was also rapid in most of treatments as compared to NPV alone (except Imidacloprid at higher concentration).

Vinay (1997) found that NPV + Diflubenzuron has additive effect against 5 and 8-day old larvae and synergistic effect in case of 10-day old larvae.

These results conflict with sole report of Mohamed *et al* (1983) describing that NPV – Diflubenzuron combination was antagonistic after 7 days of treatment when the third stage larvae were exposed and additive at pupation stage. In the present study, most of mixtures resulted in faster mortality of *S. litura* with differed significantly from that of NPV alone.

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SUMMARY IN VIETNAMESE

Kết qủa nghiên cứu NPV và hiệu qủa của sự kết hợp NPV với thuốc hoá học để phòng trừ sâu ăn tạp *Spodoptera litura*

Sử dụng NPV để phòng trừ sâu hại là một trong những biện pháp tối hảo trong chương trình IPM, NPV là một trong những nhóm phụ của họ Baculovirus được xem như một loại thuốc sinh học với tính chuyên biệt cao đối với nhiều loại sâu hại thuộc họ Lepidoptera. Nồng độ sử dụng tùy thuộc nhiều vào độ tuổi của sâu. Sâu 2 ngày tuổi sẽ bị nhiễm nhanh gấp 1.500.000 lần so với sâu ở 8 ngày tuổi, thời gian yêu cầu để 50% sâu chết gia tăng với tuổi sâu. Sự kết hợp NPV với một số thuốc hóa học ở liều lượng cực nhỏ cho thấy có kết qủa tốt như trong trường hợp NPV kết hợp với Actara và Diflubenzuron cho tác dụng bổ trợ tăng phần trăm tỷ lệ chết của sâu và thời gian gây chết được rút ngắn hơn so với sử dụng NPV đơn độc. Tuy nhiên đối với sự kết hợp NPV và Imidacloprid cho tác dụng bổ trợ ở nồng độ thấp (từ 1-7ppm) và tác dụng đối kháng nếu nồng độ Imidacloprid trên 7ppm. Từ các kết qủa nhận được cho thấy tiềm năng sử dụng NPV để phòng trừ *Spodoptera litura* trên các loại rau màu là có triển vọng tốt phù hợp với chương trình an toàn lương thực thực phẩm.