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Combined action of nuclear polyhedrosis virus and neem bitter against *Spodoptera litura* (Fabricius) larvae

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ABSTRACT: Laboratory experiments showed that neem bitter (0.1%), an ethanol-soluble concentrate of neem oil cake when combined with nuclear polyhedrosis virus [$5x10^5$ polyhedral occlusion bodies (POBs/ml)] along with crude sugar (1%) caused a significantly higher mortality of *Spodoptera litura* (Fabricius) larvae. The NPV-neem bitter-crude sugar combination recorded the shortest LT_{50}. The enhanced action was seen even at a lower dose of neem bitter (0.025%) with NPV ($1x10^5$ POBs/ml) and crude sugar (1%). Without crude sugar, the NPV-neem bitter combination was not effective in increasing the mortality. The larval weight and growth rate were significantly reduced in NPV-neem combination.

KEY WORDS: Neem bitter, nuclear polyhedrosis virus, *Spodoptera litura*

The tobacco caterpillar, *Spodoptera litura* (Fabricius) has been controlled with the applications of nuclear polyhedrosis virus on several crops namely cotton, tobacco, banana, cauliflower and mungbean (Jayaraj, 1985). However, the long incubation period and reduced susceptibility in older larvae restrict the use of virus to the early stage larvae. Several methods can be used to enhance the efficacy of baculoviruses. Neem (*Azadirachta indica* A. Juss.) is a rich source of insecticide in the tropics and its potential for the management of several insect pests including *S. litura* has been well documented (Jotwani and Srivastava, 1984). Products of neem are known for their antifeedant properties. The present investigations pertain to the combined use of neem bitter (an ethanol soluble product from neem oil cake) and NPV against larvae of *S. litura*.

MATERIALS AND METHODS

The *Sl*NPV multiplied in fourth instar *S. litura* larvae, was semipurified by differential centrifugation and counts of

POBs standardised with a haemocytometer. Neem bitter (obtained from the Indian Agricultural Research Institute, New Delhi) was first dissolved in small quantities of ethanol and then diluted with distilled water containing Teepol (0.1%) to get the desired strength. NPV suspension was made in distilled water containing Teepol (0.1%) as a surfactant. For treatments with crude sugar, appropriate quantity of crude cane sugar was added and passed through a muslin. Leaves of castor (*Ricinus communis* L.) cut into circular discs (10 cm diam.) (retaining the petiole) were rinsed in distilled water and shade-dried. The leaves were then dipped in different treatments and excess suspension drained off uniformly in all the treatments. For controls, the leaves were dipped in distilled water. With the petioles kept in water taken in 20 ml vials, the leaves were dried under shade. The leaves were then placed inside plastic containers (12 x 20 cm) and second instar larvae of *S. litura* of uniform age and size, starved for 2 h were released on the treated leaves at the rate of 10/treatment. Totally 40 insects were used for each treatment. After 24 h of feeding, the larvae were removed to individual vials containing semisynthetic diet. The leaf area after 24 h of larval feeding was measured in a leaf area meter. Mortality of larvae was recorded daily for 12 days. The mortality percentages were converted to probits and the LT_{50} values were calculated.

Two experiments were conducted, one with NPV at a dose of $5x10^5$ POBs/ml and neem bitter (0.1%) and the other with a lower dose of $1x10^5$ POBs/ml and neem bitter (0.025%) in the Department of Agricultural Entomology, Tamil Nadu Agricultural University. Larval weight was recorded at periodic intervals. Data on moulting were recorded on the 4th and 8th days of inoculation. Mortality was recorded daily up to 14 days. Data in percentage were converted to arcsine values and subjected to analysis of variance and means separated by DMRT. The time-mortality responses from the first experiment were subjected to probit analysis (Finney, 1962).

RESULTS AND DISCUSSION

In the first experiment, a combination of NPV, neem bitter and crude sugar recorded significantly higher mortality of second instar larvae of *S. litura* than did either NPV or NPV + neem bitter on the seventh day (Table 1). On the 14th day, neem bitter (0.1%) recorded 92.5 per cent mortality which was on par with NPV + crude sugar. Crude sugar did not enhance the mortality due to NPV. The neem bitter either with or without NPV significantly reduced the larval weight. Maximum effect was, however, observed in NPV + neem bitter + crude sugar. Probit analysis of time-mortality response showed that NPV + neem bitter + crude sugar recorded significantly lower LT_{50} than all other treatments (Table 2).

Since in the first experiment, neem bitter had caused a mortality of 92.5 per cent, a second experiment was conducted with a reduced dose of neem bitter (0.025 %) and NPV (1×10^5 POBs/ml). In this experiment also, a combination of NPV, neem bitter and crude sugar recorded higher mortality of *S. litura* (Table 3). The

data on the leaf consumption showed that neem bitter significantly reduced the feeding rates. Larval weight was reduced significantly due to neem bitter even in the reduced dose (Table 4). Moulting was affected significantly when NPV was administered with neem bitter and crude sugar (Table 5).

Table 1. Combined efficacy of NPV and neem bitter against second instar larvae of *S. litura* (Experiment I)

Treatment	Larval weight (mg) per larva on day		Mean mortality (%) on day	
	3	5	7	14
NPV (5 x 10^5 POBs/ml)	19.1[c]	213.5[c]	62.5[b]	65.0[b]
Neem bitter (0.1%)	7.8[b]	34.9[b]	40.0[c]	92.5[a]
NPV + neem bitter	7.5[b]	30.1[b]	60.0[b]	85.0[a]
NPV + neem bitter + crude sugar (1%)	4.2[a]	11.6[a]	86.7[a]	97.8[a]
NPV + crude sugar	21.2[c]	93.2[c]	52.5[c]	60.0[b]
Control	27.8[d]	260.7[d]	0.0	0.0

In vertical columns, means followed by similar letters are not statistically different (P=0.05) by DMRT

Table 2. Effect of neem bitter on the time-mortality response of second instar larvae of *S. litura* to NPV

Treatment	No. of larvae	Chi^2 (n-2)	Slope "b"	LT_{50} (h)	Fiducial limits
NPV (5 x 10^5 POBs/ml)	40	3.63	5.2	146.6	136.6 - 162.1
Neem bitter (0.1%)	40	8.67	4.8	174.6	161.0 - 188.9
NPV + neem bitter (0.1%)	40	1.58	5.5	148.4	137.0 - 159.1
NPV + neem bitter (0.1%) + crude sugar (1.0%)	40	2.91	2.8	68.8	56.3 - 79.7
NPV + crude sugar (1.0%)	40	0.38	3.6	157.8	141.8 - 187.2

All lines are significantly a good fit (P<0.05)

Table 3. Combined efficacy of NPV and neem bitter against second instar larvae of *S. litura* (Experiment II)

Treatment	Mean leaf area (%) consumed/larva	Mean mortality (%) on 14th day
NPV (1 x 10^5 POBs/ml)	18.9^{c}	42.5^{b}
Neem bitter (0.025%)	12.4^{a}	23.3^{c}
NPV + neem bitter	12.7^{a}	47.5^{b}
NPV + neem bitter + crude sugar (1%)	16.1^{b}	60.0^{a}
NPV + crude sugar (1%)	14.9^{ab}	52.5^{b}
Control	19.4^{c}	0.0

In vertical columns, means followed by similar letters are not statistically different (P=0.05) by DMRT

Table 4. Effect of NPV and neem bitter on the larval weight in *S. litura*

Treatment	Larval weight (mg)*		
	3	5	7
NPV (1 x 10^5 POBs/ml)	13.35^{bc}	110.9^{c}	489.0^{c}
Neem bitter (0.025%)	11.50^{b}	64.8^{b}	288.0^{b}
NPV + neem bitter	6.70^{a}	26.7^{a}	95.8^{a}
NPV + neem bitter + crude sugar (1%)	6.90^{a}	28.1^{a}	80.9^{a}
NPV + crude sugar	13.03^{bc}	111.2^{c}	444.6^{c}
Control	14.58^{c}	113.2^{c}	442.4^{c}

In vertical columns, means followed by similar letters are not statistically different (P=0.05) by DMRT

Table 5. Effect of NPV and neem bitter on the development of *S. litura* larvae

Treatment	Per cent larvae in instar	
	IV (Day 4)	IV (Day 8)
NPV (1 x 10^5 POBs/ml)	62.5^{bc}	74.1^{bc}
Neem bitter (0.025%)	85.0^{c}	65.7^{b}
NPV + neem bitter	37.5^{ab}	10.3^{a}
NPV + neem bitter + crude sugar (1%)	20.0^{a}	3.7^{a}
NPV + crude sugar	62.5^{bc}	88.0^{cd}
Control	90.0^{c}	97.3^{d}

In vertical columns, means followed by similar letters are not statistically different (P=0.05) by DMRT

Data from the above experiments have proved that NPV in combination with neem bitter and crude sugar caused significantly higher mortalities than NPV or either a combination of NPV with neem bitter or crude sugar. Neem showed an antifeedant effect against *S. litura* as evidenced by the reduced leaf consumption. Reduced leaf consumption would have resulted in reduced NPV intake. Addition of crude sugar along with neem bitter, however, should have nullified this antifeedant effect and improved the virus intake. Neem bitter had caused a stress on the larvae as seen by both reduced larval weight and moulting which probably increased the susceptibility of the larvae to NPV.

There seems to be no earlier studies on the interaction of neem products and NPV against *S. litura*. El Salamouny *et al.* (1997), however, reported a dose-dependent increase in potency of nuclear polyhedrosis virus against *Spodoptera littoralis* (Boisduval) when Neem Azal-T was added to the virus. Azadirachtin was also shown to synergise the effects of NPV in the gypsy moth larvae by Shapiro *et al.* (1994). Helpap and Zebity (1986) reported the interaction of *Bacillus thuringiensis kurstaki* (Dipel 0.1%) and extracts of neem seed kernels against *Spodoptera frugiperda* (J. A. Smith). They found that the Dipel which is normally effective against *S. frugiperda* only at high concentration, caused high mortality even at lower doses when used in combination with methanolic or water extract of neem seed kernels.

REFERENCES

El Salamouny, H. J., El Nagar, S. and El-Sheikh, M. A. K. 1997. Increasing the susceptibility to nuclear polyhedrosis viruses by synergistic additives, pp. 289-292. In: *British Crop Protection Council Proceedings No. 68 : Microbial Insecticides : Novelty or Necessity.* British Crop Protection Council, Surrey, U. K.

Finney, D. J. 1962. "Probit Analysis. A Statistical Treatment of the Sigmoid Response Curve", 2nd Ed. Cambridge University Press, London.

Helpap, C. and Zebity, C. P. W. 1986. Combined application of neem seed extracts with *Bacillus thuringiensis* products in the control of *Spodoptera frugiperda* and *Aedes togoi*. *Journal of Applied Entomlogy*, **101**: 515-524.

Jayaraj, S. 1985. Role of insect pathogens in plant protection. *Proceedings of the Indian National Academy, B;* **51**: 19-35.

Jotwani, M. G. and Srivastava, K. P. 1984. A review of neem research in India in relation to insects, pp. 43-56. In: *Natural pesticides from the neem tree [Azadirachta indica (A. Juss)] and other tropical plants.* Proceedings of the Second International Neem Conference, Federal Republic of Germany, 25-28 May 1983, Eschborn, 1984.

Shapiro, M., Robertson, J. L. and Weep, R. E. 1994. effect of neem extract upon the gypsy moth (Lepidoptera: Lymantriidae) and its nuclear polyhedrosis virus. *Journal of Economic Entomology*, **87**: 356-360.