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Evaluation and production of improved formulation of nucleopolyhedrosis virus of *Spodoptera litura*

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Abstract

Larvae of *Spodoptera litura* F. (Lepidoptera Noctuidae), are a pest of many crops, but are susceptible to nucleopolyhedrosis virus (NPV). The virulence of *S. litura* nucleopolyhedrosis virus (spltNPV) proved most effective at 1×10^6 OB's / ml with LT_{50} at 5.09 d when compared with five other doses. The diet surface contamination method was deployed for the production of the virus. It was found that virus yield decreased from 1.57×10^9 to 1.22×10^9 OB's / ml. Laboratory evaluation of different adjuvants showed that jaggery 5% + blue 1% + glycerol 10% + 1×10^6 OB's / ml spltNPV gave LT_{50} value of 5.37 d. Field trials on cabbage, *Brassica oleracea* L. revealed that 2-3 applications of spltNPV at 2.47×10^{11} OB's / ha effectively suppressed *S. litura* larvae better than five other treatments. There were significant differences between farmers practices plot (FPP) (1.52 larvae) and spltNPV plot (SNP) (2.64 larvae) in the number of surviving *S. litura* larvae. But there were no statistical differences between FPP (6.53%) and SNP (5.36 %) in the per cent leaf defoliation by *S. litura* larvae. Benefit : Cost ratio of spltNPV sprayed plot (2.40:1) was lower than FPP (2.78:1). The spltNPV applications were not superior over Indoxacarb 14.5 SC at 1 ml/litre water applications according to experimental data. But in the long run, use of spltNPV is desirable because it is environment friendly, self perpetuating, host specific, virulent and efficient bio-control agent.

Key words: microbial control, *Spodoptera litura*, spltNPV, cabbage, Karnataka, south India.

Introduction

One of the chief constraints in the production of cruciferous vegetables in India is the damage caused by insect pests particularly *Spodoptera litura* F. (Lepidoptera Noctuidae). It is an important, cosmopolitan, polyphagous crop pest. It is reported to feed on 112 species of plants (Shivayogeshwara *et al*., 1991). A nucleopolyhedrovirus (NPV) of *S. litura* (spltNPV) was reported by Ramakrishna and Tiwari (1969). It has emerged as an alternative to chemical insecticides for the management of *S. litura* in tobacco, *Nicotiana tabacum* L. and potato, *Solanum tuberosum* L. cultivated ecosystems (Dhandapani, 1994).

NPVs have disadvantages as well as advantages (Moscardi, 1999). The narrow host range of NPVs generally restricts their effectiveness against the complex of insect pests in fields, where pest species are readily controlled by broad spectrum chemical insecticides (Adams and McClintock, 1991). The slow speed of action against target insect represents another disadvantage of NPVs (Muralibaskaran *et al*., 1997). Mass production of the virus at reasonable costs is an important factor in the development of NPVs into a marketable product (Ignoffo, 1973; Smits and Vlak, 1998). Only the *in vivo* production of the baculoviruses has so far been economically viable due to the high costs involved in the *in vitro* production systems (Shieh, 1989; Kumar *et al*., 2005).

Many factors influence the production and the quality of the virus (Shapiro *et al*., 1981; Shapiro, 1982; 1986). The larval growth rate after virus inoculation will influence the virus yield (Shapiro, 1982). The age of larvae at inoculation, virus dose and incubation temperature significantly influences the larval growth and hence the virus productivity (Carter, 1984; Im *et al*., 1990; Cherry *et al*., 1997). Lack of appropriate formulation technologies for most microbial control agents limit their use. So the goal of this study was to develop a suitable spltNPV formulation.

Materials and methods

Laboratory virulence

Laboratory studies were carried out at the Bio-Control Research Laboratories (BCRL), Bangalore. A *S. litura* NPV (spltNPV) isolated, characterized and identified by BCRL, Bangalore as *Sl*SSR-2 strain was utilized for the study (Anonymous, 2000). An initial serial passage of the isolate was made in *S. litura* using fourth instar larvae fed on semi-synthetic chickpea (*Cicer arietinum* L.) diet surface treated with 1×10^8 OB's / ml (i.e., 3931 OB's / 254.34 mm^2). Dead larvae showing typical disease symptoms were collected in a sterile eppendorf, purified by differential centrifugation method (500 rpm for 10 min further 5000 rpm for 15 min) and occluded body (OB) concentrations were assessed using Neubauer haemocytometer. The stock suspensions were maintained at −4 °C.

A laboratory assay was conducted to evaluate the virulence of BCRL isolates of spltNPV. Serial dilutions of the spltNPV 1×10^6 to 3.2×10^2 OB's / ml were prepared and 10 µl of the suspension was dispended on the semi- synthetic diet filled in 5 ml glass vials. The effective surface area of the diet was 254.34 mm^2 and the ef-

fective concentrations of polyhedral were 39.32, 7.86, 1.57, 0.31, 0.06 and 0.01 OB's / mm^2 of the diet surface. Virus suspension was smeared with a blunt end polished glass rod (6 mm). Third instar *S. litura* of uniform age and size were released on to the diet 15 min after surface treatment. Each dose had 30 third instar *S. litura* (30 larvae × 4 replications). A control with 30 third instar untreated *S. litura* larvae was maintained. The larvae after inoculation were incubated at 25 ± 1 °C in an incubator. Observations on larval mortality were recorded from 2 d through 10 d at 24 h intervals. For determining the efficacy of virus, only time- mortality relationship was considered. The six spltNPV doses were used to calculate LT_{50} in four seasons per year from fresh spltNPV samples. Each season, bioassays were conducted in four replicates of 30 third instar *S. litura* / replicate.

Virus yield and time of harvest

Third instar *S. litura* were inoculated at seven different intervals and virus was extracted and harvested and yield recorded eight days later. The larvae were allowed to feed on the diet treated with a viral dose of 1×10^8 OB's / ml (i.e., 3931 OB's / 254.34 mm^2) and incubated at 25 ± 1 °C. The larvae were inoculated at 0, 1, 4, 6, 8, 12 and 24 h. A treatment with harvesting the cadavers from the healthy larvae when died was included as control. Each treatment was replicated three times with 5 larvae per replication. The larval cadavers were collected and immediately frozen. The virus yield from infected and dead larval cadavers was recorded.

Storage of spltNPV

The stability of the spltNPV under storage was studied in the laboratory to determine the effects of storage on the activity of the virus. Three temperatures were selected to represent refrigerated condition (0 ± 1 °C), room temperature (25 ± 1 °C) and high temperature (35 ± 1 °C) was selected. One hundred ml of spltNPV liquid suspension was stored in high density polyethylene bottle containing 1×10^9 OB's / ml under room, refrigerated and high temperature (35 °C) conditions. One ml of spltNPV samples were drawn from the respective bottles at 1, 2, 3 and 6 months storage and the virulence was assessed adopting bioassay method (Finney, 1952) against third instar *S. litura*. The experiment was terminated after 6 months. Observation on larval mortality was recorded at 24 h intervals.

Laboratory evaluation of adjuvants

A suitable UV screener was selected based on the exposure to Sun test machine (Atlas Sun test machine CPS+/XLS+, Atlas material Testing Technology GmoH). The selected spltNPV isolate was irradiated at a concentration of 1×10^9 OB's/ ml (in 0.1% Teepol) by Sun test machine for their persistence under simulated sunlight condition. A set of experiments were conducted with a standardized exposure dose: 500W/ m^2. This dose was selected on the basis of Irradiated Dose (ID_{50}) value. For this purpose, the cited quantity of each adjuvant was prepared and added to 500 µl of the virus in 0.1% Teepol. The suspension was applied on to plastic sheets (6 × 12 cm) using a micropipette .After air drying, treated sheets were irradiated in Sun test machine at 500W/ m^2 for 90 min. At the time of irradiation, 3 sheets could be kept inside the sun test cabinet simultaneously.

After exposure, the virus deposits in each irradiated sheet were eluted with 500 µl of distilled water and the suspension was collected in microfuge tubes, labeled, re-enumerated and kept in a refrigerator. Third instar *S. litura* larvae of uniform age and size were used in these studies. Semi synthetic diet without formaldehyde was prepared and filled in glass vials up to $1/3^{rd}$ height of the vial. The dose of 1×10^9 OB's/ ml was prepared from each treatment and 10 µl of the irradiated virus was applied to the diet surface using a micropipette. The suspension was spread uniformly over the diet surface with a polished blunt end of the sterile 6 mm glass rod. The each dose had 30 larvae. A control with untreated larvae was maintained. The larvae after inoculation were incubated at 25 ± 1 °C in an incubator. The observations on the larval settlements on the diet were checked out from first day and mortalities were recorded from third till the tenth day at 24 h intervals. Each treatment was replicated three times. Median incubatory dose (ID_{50}) of simulated sunlight was calculated for each exposure time.

Pot culture evaluation of adjuvants

Adjuvant that proved most suitable was selected for pot culture experiments under shade and sunlight. Formulations were tested for insecticidal activity after treatments were applied to 30 d old, potted cabbage plants and exposed to natural sunlight and shade conditions (Pots were placed under coconut coir mat). Further, these UV screeners were tested in earthen pot (15 cm Ø × 30 cm height) culture under open and shade conditions with different adjuvant and combinations as given in table 4. One of the adjuvants selected was blue, a liquid detergent. The formulations were sprayed on plants using a litre capacity plastic-hand sprayer.

Field efficacy

Field trials were conducted for the suppression of *S. litura* on cabbage, *Brassica oleracea* L. var. *capitata* at Yellapura village, Doddaballapur Taluk, Bangalore rural district (12°58'35"N 77°36'0"E 980 m asl) during 2009 and 2010. In these villages farmers grow vegetables without using synthetic pesticides and fertilizers. The vegetable growers are recognized and are adopted by the Association for Promotion of Organic Farming (APOP) and the same villages have also been adopted by the Bio-Control Research Laboratories (BCRL), Sriramanahalli. One-month-old seedlings of cabbage were transplanted in 2 × 3 m plots at 60 × 30 cm following the recommended agronomical practices (Package of Practices, 2007) for crop cultivation. The treatments (figure 2) were tested in Randomised Block Design (RBD) with six treatments and three replications per treatment. Pre- treatment observation was recorded at zero d of each spraying on the number of larvae from 25 randomly selected plants and per cent leaves damaged from 25 plants. Treatments were applied to plants with

knapsack sprayer. About 200 ml of test solution was applied to each plant. Post- treatment observations were recorded at 5, 7 and 10 d after each spltNPV application on the number of *S. litura* larvae and healthy leaves from 25 randomly selected plants on each d of observation. Incidence (%) of the defoliated leaves by *S. litura* larvae from each plot on each d of the observation was also recorded.

Validation of spltNPV with Farmer's practices plot

To compare farmer's practices with spltNPV, cabbage fields in Rajankunte, Devanahalli (13°13'57"N 77°41'57"E) and Doddaballapura (12°58'35"N 77°36'0"E) villages at the outskirts (40 km) of Bangalore were selected and observations recorded during 2009-2010. The experiment was conducted in 2 ha plots at each of the three aforementioned locations. Each treatment was applied to 1 ha. In spltNPV sprayed plot (SNP) three rounds of spltNPV at 2.47×10^{11} OB's/ha with jaggery 5% (sugar cake made from sugarcane, *Saccharum officianarum* L.) and glycerol 10% were applied at 15 d intervals during evening when a threshold level of 1 larva/plant was observed. In farmer's practices plot (FPP, 2 km away from the SNP) indoxacarb 14.5 SC (Avant®) at 1 ml/lit of water was applied sequentially. Captan 50 WP (Capaf®) at 2 g/kg of seed was also applied to prevent *Alternaria* leaf spot in SNP and FPP plots. In both the plots first application commenced when the crop was in flowering stage, i.e., 45-d-old plants. Captan was applied to both the plots when the plants were about 20 d old. Since the treatment was common to both the plots applied at early plant age, when infestation by *S. litura* had not occurred, *S. litura* suppression was accounted only due to spltNPV applications.

Pre-treatment counts observed at zero d of each spraying were on the number of larvae / 25 randomly-selected plants and per cent defoliated leaves on 25 plants. Post-treatment counts were observed at 5, 7 and 10 d after each application in each plot (2 × 5 m) on number of infested leaves and *S. litura* larvae on 25 plants / 10 m^2 × 4 plots.

The agronomic practices for both spltNPV sprayed and Farmer's practices plots and yield at harvest (tonnes) from each plot (only marketable cabbage heads are considered as yield) were recorded. The benefit : cost (B : C) ratio was also determined.

Statistical analysis

The data on *S. litura* larval numbers and yields were subjected to Analyses of variance (ANOVA) using SAS software version 6.12 and means were separated by Least Significance Difference tests (LSD). All data in percentages were transformed to angular values and then analyzed. The larval counts were also transformed to square root of $x + 0.5$ values. The Probit analyses (Finney, 1952) to determine LT_{50} values were carried out in a Statistical Package for Social Sciences (SPSS, 1999), version 10.0 and means were computed for field experiments and subjected to paired t-test. For comparisons of LT_{50} and LC_{50} values, χ^2 test was used. The B : C ratio was calculated by dividing the net monetary return by the total added cost due to the treatments.

Table 1. Virulence of spltNPV against *S. litura* 3rd instar larvae.

Treatments	LT_{50} (Days) (Mean ± SD)	Fiducial limit (at 95% CI)	LT_{99} (Days)
1×10^6	5.09 ± 0.04	4.68-5.47	12.16
2×10^5	5.67 ± 0.06	5.23-6.10	14.33
4×10^4	6.38 ± 0.70	5.86-6.95	19.35
8×10^3	7.21 ± 0.60	6.58-8.00	24.19
1.6×10^3	8.57 ± 0.38	7.72-9.95	30.69
3.2×10^2	10.02 ± 0.45	8.82-12.47	35.96

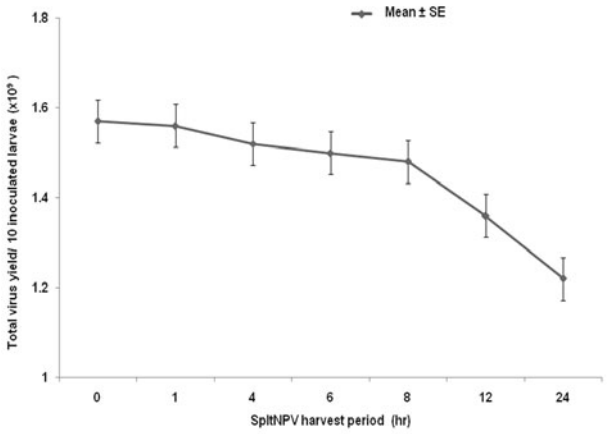

Figure1. Total SpltNPV yield at different harvesting periods.

Results

Laboratory virulence

Bioassays using third instar *S. litura* with spltNPV showed subtle variations in virulence. The lowest LT_{50} value was 5.09 d obtained at 1×10^6 OB's / ml, the next best LT_{50} value of 5.67 d was obtained with 2×10^5 OB's/ml strength. However, with much lower dose of 4×10^4 OB's / ml, the LT_{50} value of 6.38 d was obtained. Thus, the spltNPV obtained from BCRL proved virulent / effective at 1×10^6 OB's / ml based on the LT_{50} value to third instar *S. litura* (table 1).

Virus yield and time of harvest

Studies on the optimization period of harvest revealed that the proportion of dead larvae in samples differed significantly from each period of harvest. The yield per 50 inoculated larvae was $1.57 \pm 0.06 \times 10^9$ OB's / larva at 0 h and $1.22 \pm 0.03 \times 10^9$ OB's / larva at 24 h. The OB's yield was higher when virus infected larvae were utilized as cadavers up to 8 hr after inoculation, beyond which the virus yield started declining (figure1).

Storage stability of spltNPV

Bioassays conducted at four regular intervals against third instar *S. litura* revealed that a virus suspension was formulated with the addition of 10% glycerol and stored at three temperature levels: refrigerated condition (0 ± 1 °C), room temperature (25 ± 1 °C) and high tem-

perature (35 ± 1 °C). Bioassay conducted at regular intervals against third instar *S. litura* revealed that the virus stored under high temperature (35 °C) readily lost its virulence with LC_{50} 0.54-1.54 OB's / mm^2. The LC_{50} under refrigerated condition (0 °C) was 0.04-0.20 ± 0.006 OB's / mm^2 (table 2). However, it was observed that the LC_{50} was 0-3 folds less in formulated spltNPV than unformulated spltNPV. There were significant differences in LC_{50} when spltNPV was stored for different periods under formulated and unformulated conditions (table 2). There were also significant differences in LC_{50} (ANOVA at 3 df, $p < 0.05$) between formulated and unformulated spltNPV at 35 °C (not shown in table 2).

Laboratory evaluation of adjuvants

Soyaflour 10% + spltNPV (glycerol 10%) indicated efficiency in increasing the virulence of the virus at LT_{50} value of 5.59 d when the adjuvant was applied singly. This was followed by jaggery 5% + spltNPV (glycerol 10%) and boric acid 1% + spltNPV (glycerol 10%) with a LT_{50} value of 5.92 and 6.21 d, respectively. When the adjuvant was combined with blue (1%), the LT_{50} value was 5.37 d for jaggery 5% + blue 1%+ spltNPV (glycerol 10%), followed by soyaflour 10% + blue 1% + spltNPV (glycerol 10%) and boric acid 1% + blue 1% + spltNPV (glycerol 10%) whose LT_{50} were 5.67 and 5.87 d, respectively. The LT_{50} of irradiated (glycerol 10%) and unirradiated virus (glycerol 10%) were 8.37 and 5.09 d, respectively (table 3).

Pot culture evaluation of adjuvants

Based on larval mortality the activity of the virus when applied with adjuvant on cabbage plants was higher under shade than under the direct sun. Under direct sun, the larval mortality was significantly lower compared to under shade conditions. There were statistically significant differences in larval mortality between the sun and shade conditions ($p > 0.05$, df = 3, table 4). Less larval mortality was observed in insecticide treatment under shade (71.25%) compared with spltNPV in combination with blue 1% + jaggery 5% + 10% glycerol (78.75%). A combination of spltNPV (1×10^6 OB's / ml) + blue 1% + jaggery 5% + 10% glycerol under shade recorded significantly the highest larval mortality of 78.75%. The same combination under direct sun recorded a larval mortality of 58.70%. In combination with blue 1% + soyaflour + 10% glycerol, the viral preparation caused the larval mortality of 63.75 % (under shade) and 48.70% (under direct sun). The larval mortality (%) did not differ statistically on the chemical treated plants under shade or direct sun. But larval mortality was significantly lower (26.20%) for virus with Glycerol treated larvae under sun than under shade conditions (31.25%) (table 4). Clearly, the treatment without adjuvant was the most potent as measured by it had the lowest LT_{50}.

Field efficacy

Number of *S. litura* larvae

Indoxacarb 14.5 SC at 1 ml / l proved superior over other chemicals as there were statistically significant differences in the number of surviving larvae 5 d after application (figure 2). Same trend prevailed 7 and 10 d after first application of indoxacarb and other chemicals. The efficacy of indoxacarb 14.5 SC (Avant® India) at 5 d after second application remained superior over spltNPV at 2.47×10^{11} OB's/ha.

Table 2. Comparison of the LC_{50} of unformulated and formulated spltNPV after select storage periods.

Storage period (month)	LC_{50} (OB's / mm^2)					
	Unformulated			Formulated		
	0 °C	25 °C	35 °C	0 °C	25 °C	35 °C
1	0.04[a]	0.21 [a]	1.54 [a]	0.04 [a]	0.09 [a]	0.54 [a]
2	0.05[a]	0.44 [a]	2.55 [b]	0.04 [a]	0.20 [a]	0.86 [b]
3	0.30[b]	0.78 [b]	3.31 [c]	0.09 [a]	0.60 [b]	1.06 [c]
6	0.67[c]	1.55 [c]	4.85 [d]	0.20 [ab]	0.97 [c]	1.54 [d]
SEm ±	0.02	0.04	0.10	0.21	0.18	0.06
CD at 1%	0.12	0.13	0.29	0.68	0.31	0.18

Numbers followed by the same letter are not statistically significant ($p < 0.001$) by LSD.

Table 3. LT_{50} values of SpltNPV in combination with select adjuvants against *S. litura* 3rd instar larvae.

Treatments	χ^2 (n-2)	LT_{50} (Days) (Mean ± SD)	Fiducial limit (at 95% CI)
Soyaflour 10% + 1×10^6 OB's / ml spltNPV (Glycerol 10%)	6.59 n.s.	5.59 ± 0.14	5.15-6.03
Boric acid 1% + 1×10^6 OB's / ml spltNPV (Glycerol 10%)	5.94 n.s.	6.21 ± 0.26	5.69-6.77
Jaggery 5% + 1×10^6 OB's / ml spltNPV (Glycerol 10%)	5.48 n.s.	5.92 ± 0.39	5.47-6.36
Soyaflour 10% + Blue 1%** + 1×10^6 OB's / ml spltNPV (Glycerol 10%)	6.52 n.s.	5.67 ± 0.70	5.23-6.10
Boric acid 1% + Blue 1% +1×10^6 OB's / ml spltNPV (Glycerol 10%)	5.30 n.s.	5.87 ± 0.40	5.42-6.37
Jaggery 5% + Blue 1% + 1×10^6 OB's / ml spltNPV (Glycerol 10%)	6.26 n.s.	5.37 ± 0.63	4.95-5.78
Irradiated virus 1×10^6 OB's / ml (Glycerol 10%)	6.52 n.s.	8.37 ± 0.36	7.67-9.41
Unirradiated virus1 $\times 10^6$ OB's / ml (Glycerol 10%)	6.77 n.s.	5.09 ± 0.71	4.68-5.47

*Blue is a liquid detergent.

Table 4. Pot culture evaluation of different adjuvants on cabbage.

Treatments	Pretreatment	[x]Mean percent mortality					
		Under shade (DAS)[z]			Under sun (DAS)		
		5th	7th	10th	5th	7th	10th
Jaggery 5% + blue 1% + 1 × 10^6 OB's / ml, spltNPV – Glycerol 10%	20	45.00 [y](42.12)[a]	65.00 (53.76)[a]	78.75 (62.66)[a]	35.00 (36.25)[ab]	48.75 (44.28)[ab]	58.70 (50.06)[a]
Soyaflour 10% + 1 × 10^6 OB's / ml, spltNPV – Glycerol 10%)	20	33.75 (35.48)[b]	47.50 (43.56)[c]	63.75 (53.01)[b]	31.25 (33.98)[b]	42.50 (40.66)[b]	48.70 (44.28)[b]
1 × 10^6 OB's / ml, spltNPV-Glycerol 10%	20	18.75 (25.54)[c]	27.50 (31.61)[d]	31.25 (33.94)[c]	11.25 (19.24)[c]	20.00 (26.48)[c]	26.20 (30.75)[c]
Indoxacarb 14.5 SC, Avant® 1ml / l	20	45.00 (42.12)[a]	55.00 (47.9)[b]	71.25 (56.83)[b]	41.25 (39.94)[a]	52.50 (46.44)[a]	63.75 (53.02)[a]
Control	20	0.00 (0.00)[d]	0.00 (0.00)[e]	0.00 (0.00)[d]	0.00 (0.00)[d]	0.00 (0.00)[d]	0.00 (0.00)[d]
F test		*	*	*	*	*	*
SEm ±		1.253	1.418	1.335	1.381	1.486	1.299
CD at 1%	NS	5.226	5.109	5.562	5.757	6.192	5.415

[x] Mean of 30 larvae/treatment/replication; [y] Figures in the parentheses are Angular transformed values and mean per-cent mortality and means followed by same letters in each column are not significantly different by LSD at 1%; [z] Days after sowing; * significant at 5%.

L e a f d a m a g e

The least leaf damage was observed on plant treated with indoxacarb 14.5 SC (Avant®) at 1 ml / l water, as the per cent infestation of 6.39 was recorded 5 d after first application. This was the minimum leaf damage obtained in the field trial. The test chemicals were applied when cabbage was in 4-5 true leaf stage. Indoxacarb proved superior over other chemicals as there were statistically significant differences ($p < 0.05$, df = 17) in leaf damage after first application. The same trend prevailed in the efficacy of chemicals even after second spray, indoxacarb 14.5 SC (Avant®) proved superior to spltNPV (figure 3).

Validation of spltNPV with Farmer's practices

Three applications of spltNPV at 15 d interval constituted the spltNPV plot (SNP) at 2.47 × 10^{11} OB's / ha and in farmers practices plot (FPP) spraying indoxacarb 14.5 SC (Avant®) at 0.5 ml / l of water sequentially applied for the suppression of *S. litura* at 4-5 true leaf stage. Captan 50 WP (Capaf®) at 2 g / kg of seed was also applied to prevent *Alternaria* leaf spot. However, when data were subjected to "t-test" ("t" $p < 0.05$ at 1 df), there were significant differences between FPP and SNP in the number of surviving *S. litura* larvae but not in the leaf infestation (table 5). The mean number of surviving *S. litura* larvae were in FPP 1.52 larvae / 25 plants after three applications of insecticides compared with 2.64 larvae / 25 plants in SNP. The mean leaf defoliation was 5.36% / 25 plants in FPP compared with 6.53% / 25 plants in SNP. This suggested that based on number of surviving larvae and leaf infestation, the FPP realized better suppression of *S. litura* population than SNP on cabbage. However, when the data were subjected to 't' test, the two plots with respect to number of surviving *S. litura* larvae and leaf infestation were on par ($p < 0.05$, df = 1) and the cabbage yield obtained was 17.5 t / ha in SNP and 21.4 t / ha in FPP.

When percent leaf infestation, mean borer larvae / 25 plants and cabbage yields were compared, there were no statistically significant differences between SNP and FPP for all parameters ($p < 0.05$, df = 17). The benefit : cost (B : C) worked out to be 2.40 in SNP sprayed plot compared with 2.78 in FPP. Part of the added cabbage yields in FPP might have resulted from the added fungicide applications. The data concerning B : C ratio is presented in table 5.

Discussion and conclusions

The use of biological insecticides either solely or in combination in integrated pest management systems is increasingly becoming important (Adams and Bonami, 1991; Cherry *et al*., 1997). Among biologicals, insect baculoviruses have gained prominence (Blissand *el al*., 2000). The use of nucleopolyhedrosis virus (NPV) as a microbial pesticide has received attention (Kamiya *et al*., 2004; Tang *et al*., 2011).

Bioassays using third instar *S. litura* with isolates from five sources were also evaluated. The LC_{50} for the isolates ranged from 0.051 to 1.77 OB's / mm^2. The isolate from BCRL showed the highest virulence with LC_{50} 0.041. The spltNPV from BCRL proved most effective at 1 × 10^6 OB's / ml based on the LT_{50} value (5.09 d) for 3rd instar *S. litura*. A similar trend prevailed in the LT_{99} mortality values (12.16 d). The details of the above two bioassays are to be published elsewhere. Trang and Chaudhari (2002) too used LT_{50} for bioassays of spltNPV against *S. litura* larvae. LT_{50} values were found dose-dependent. The estimated LT_{50} increased with the increase larval age where as it decreased with the increase in dose. Tang *et al*. (2011) also evaluated spltNPV by LT_{50} and LC_{50} using third instar *S. litura*.

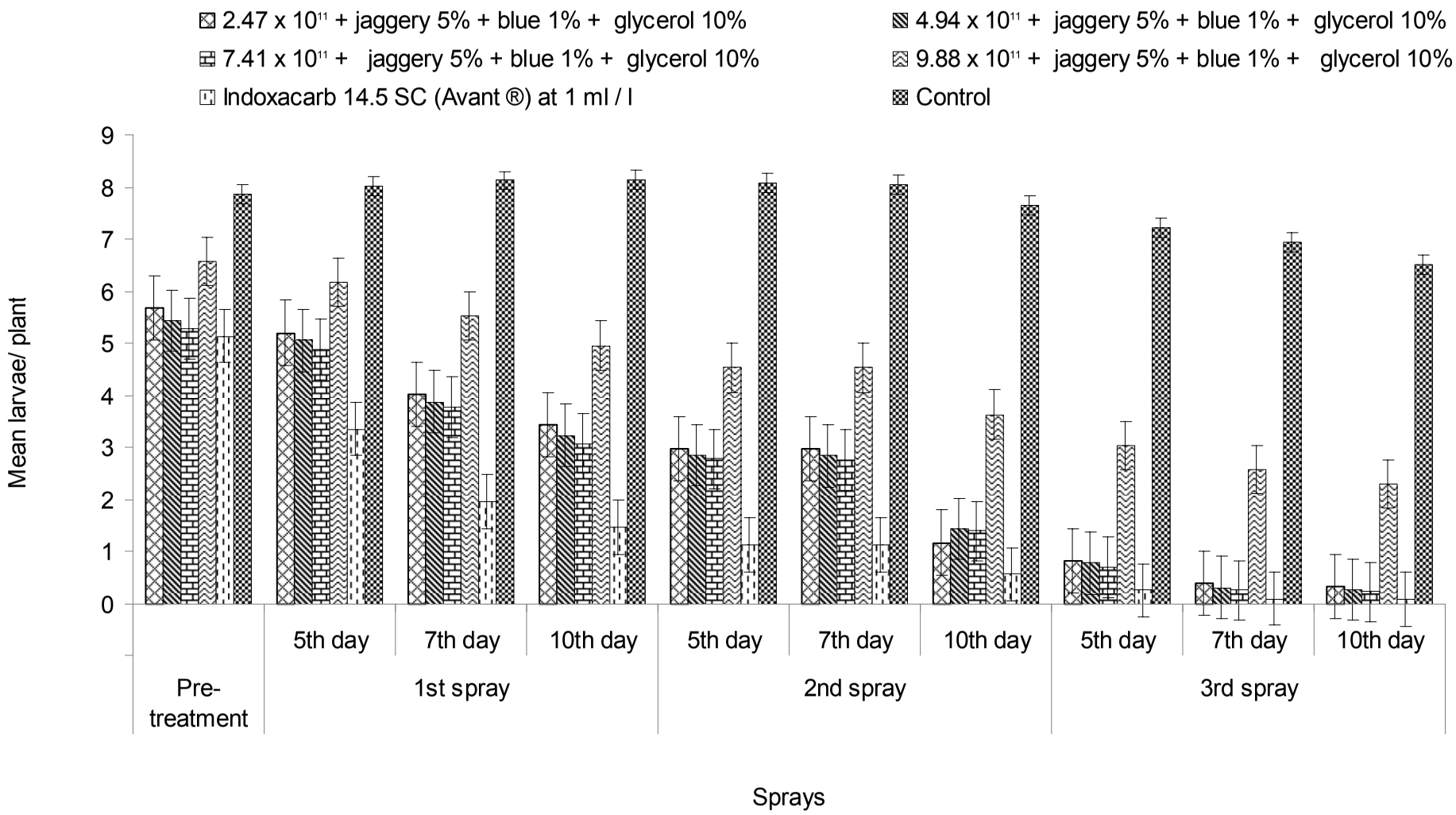

Figure 2. Field efficacy of spltNPV against *S. litura* on cabbage.

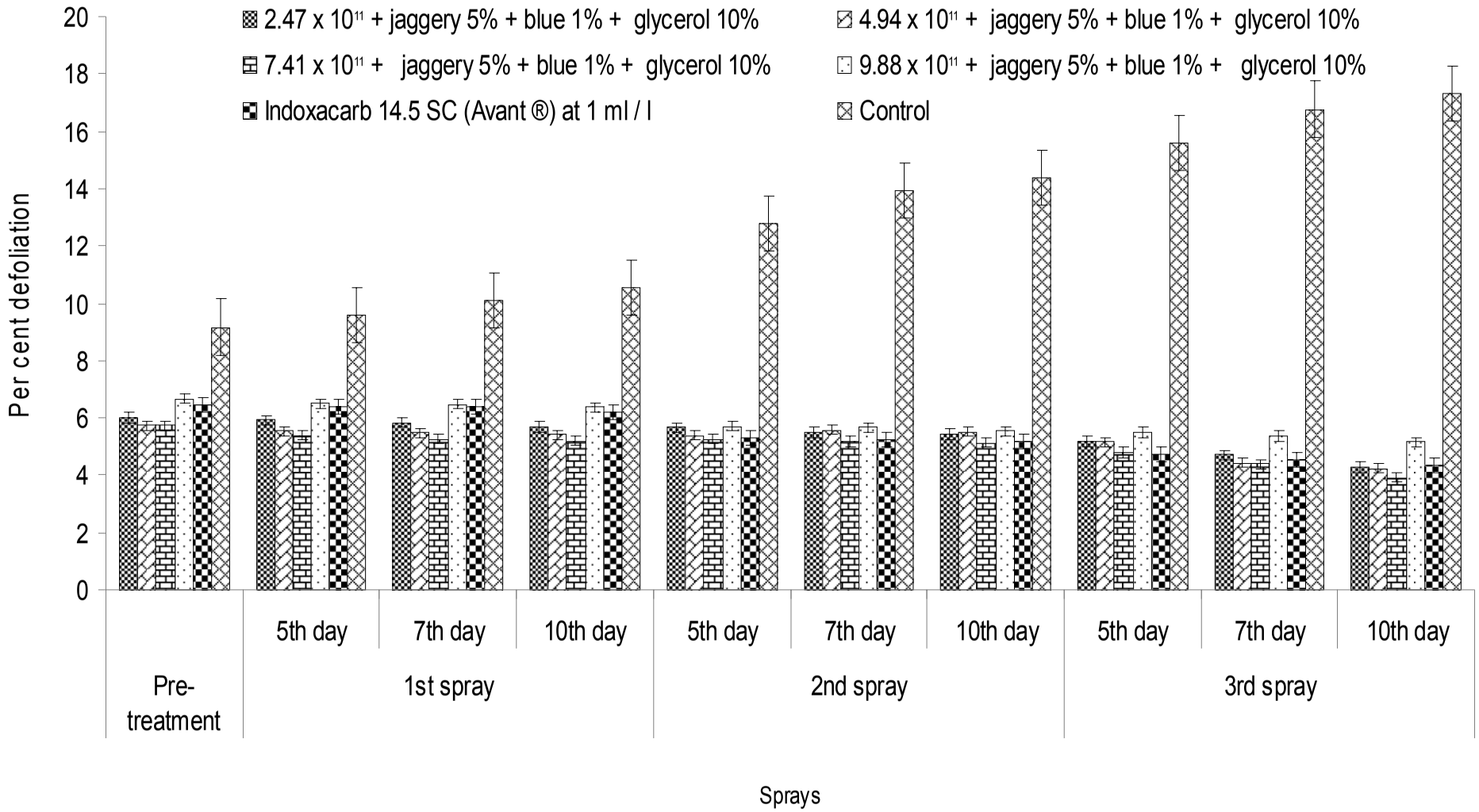

Figure 3. Field efficacy of spltNPV against S. litura leaf defoliation on cabbage.

Table 5. Comparison of different parameters in spltNPV sprayed and Farmers Practice plot during 2009 and 2010. Each mean = 25 plants/plot (5 × 2 m).

[c]Days after spraying		[a]Mean *Spodoptera* larvae / 25 plants	
		spltNPV sprayed plot (SNP)	Farmer's practice plot (FPP)
1st spray[c]	Pretreatment	5.40	5.42
	7th day	3.62	2.72
2nd spray	Pretreatment	2.94	1.11
	7th day	1.16	0.53
3rd spray	Pretreatment	0.79	0.32
	7th day	0.42	0.07
Mean		2.64	1.52
		[b]Mean leaf defoliation (%)	
1st spray[c]	Pretreatment	9.51	9.90
	7th day	6.84	6.78
2nd spray	Pretreatment	5.67	4.92
	7th day	5.42	4.54
3rd spray	Pretreatment	5.19	4.73
	7th day	4.50	4.00
Mean		6.53	5.36
		Plant protection cost (Rs)	
Number of sprays (insecticides and fungicides)		6.5	10
Insecticide used (l/ha)		2.0	8.7
Insecticide cost (Rs/ha)		4,980	11,500
Fungicides used (Kg/ha)		6.8	5.5
Fungicides cost (Rs/ha)		1,530	2,310
% leaf infestation		6.53	4.20
Mean borer larvae		2.64	1.52
Total cost for plant protection (Rs/ha)		6,510	13,810
Cost of cultivation (Rs/ha) (agronomic + plant protection)		102,910	113,210
[d]Yield (t/ha)		17.5	21.4
[e]Gross returns (Rs/ha)		350,000	428,000
Net returns (Rs/ha)		247,090	314,790
B : C ratio		2.40	2.78

[a] mean borer larvae in both plots both the years, t-value = 0.42, table t = 1.74 ($p > 0.05$); [b] mean % leaf defoliation in both plots, t-value = 2.66, table t = 1.74 ($p > 0.05$); [c] spray repeated after 15-d; [d] yield, t-value = 5.53 ($p > 0.05$) table = t-1.74; [e] 1 £ = Rs 20/kg and 1 US$ = Rs 53 (current rate *ie*., September 2012).

Based on LT_{50} and LC_{50} two recombinant forms of spltNPV were recommended for developing commercially viable products.

Collection of spltNPV infected larvae is laborious and time consuming. So, many attempts have been made to optimize the period of harvest as it influence the biological activity of the virus and growth of secondary contaminants (Jayaraj *et al*., 1980). The OB's yield was higher when larvae infected with virus were harvested as cadavers than in other samples harvested at different days after inoculation. Cherry *et al*. (1997) reported yield of $1.40 \pm 0.28 \times 10^9$ OB's / larva of *Spodoptera exigua* (Hubner) and $3.29 \pm 0.46 \times 10^6$ OB's/ larva of *Spodoptera exempta* Walker harvested 5 and 2 d after incubation, respectively.

The spltNPV virus suspension stored at refrigerated condition (0 ± 1 °C) up to 6 months did not show much variation in virulence. But, when stored under room (25 ± 1 °C) and higher temperatures (35 ± 1 °C), the virulence of viral suspension varied with LC_{50} values as low as 0.04. Mehrvar *et al*. (2008) also reported LC_{50} values of NPV of *Helicoverpa armigera* (Hubner) as low as 0.023 from Bangalore, South India. Stability of the spltNPV quickly decreased at > 60 °C and during prolonged exposure to high temperatures (Im *et al*., 1990). The virulence of spltNPV started declining at 50 °C and was completely inactivated at 90 °C (Ebora and Cadapan, 1987). The mortality of 3rd instar larvae treated with spltNPV at 1.0×10^6 OB's / ml after different storage periods was 83.3% 4 years after storage, 96.7% after 2 years and 100% after 6 months storage (Su, 1992). Nucleopolyhedrosis viruses are relatively stable in the environment as the virions are occluded in the polyhedral bodies. They get inactivated by exposure to UV rays (Morris, 1971).

Effect of UV rays on the pathogenicity of the spltNPV was determined in laboratory. The virus was progressively inactivated with prolonged exposure to UV source. Sunscreens are often added to formulations capable of selectively absorbing the damaging wavelength (Jones *et al*., 1997). Cantwell *et al*. (1967) reported that *Trichoplusia ni* (Hubner) NPV was completely inactivated by direct sunlight within 3 h. When nucleopolyhedrosis viruses are applied in the field, they are inactivated by the UV rays (2537 to 3200 Å). Pawar and Ramakrishnan (1977) tested the stability of spltNPV.

Complete inactivation of the virus was noticed after 15 min exposure to UV light from 10 cm. The virus at low concentrations was considerably inactivated at 8 h exposure to sunlight.

The addition of folic acid, pyridoxine, riboflavin, charcoal, black ink and urea provided protection to spltNPV from the far and near ultraviolet light. With far ultraviolet, protection was concentration-dependent and folic acid and charcoal resulted in 100% *S. litura* larval mortality (Yelshetty *et al*., 2009). Although the relative efficacy factor was low in direct exposure to sunlight compared with a germicidal lamp, riboflavin and folic acid protected the virus by factors of 4.9 and 3.6, respectively (Ramakrishnan and Chaudhary, 1991). A study conducted to select an adjuvant to protect virus from UV rays showed that spltNPV without any adjuvant proved the most effective (LT_{50} at 5.09 d), followed by Jaggery 5% + Blue 1% + 1×10^6 OB's / ml spltNPV (Glycerol 10%) with a LT_{50} of 5.37 d. This clearly showed that the use of adjuvants may not be beneficial under sunlight conditions.

Further, to check the effects of UV rays under natural conditions, application of the formulated virus suspension was carried under shade and direct sunlight conditions. The effect of the UV rays on larval mortality was more (78.75%) under direct sunlight than shade (58.70%). Field evaluation of spltNPV under field conditions at Yellapura village, Doddaballapur revealed that 2-3 applications of spltNPV at 2.47×10^{11} OB's / ha suppressed *S. litura* larvae. Even if indoxacarb 14.5 SC (Avant®) at 0.5 ml / l is applied, at least 2-3 applications are required to protect the crop from *S. litura* damage. The results are in agreement with the results of Suhas *et al*. (2009).

Field tests were conducted by Kumari and Singh (2009) to evaluate the effect of spltNPV alone and in combination with endosulfan and neemarin (neem seed kernel extract) on cabbage. Treatment with spltNPV (500 LE / ha) + endosulfan (625 ml / ha) was better in reducing the larval numbers and in increasing yields more than other treatments. However, considering B : C ratio, treatment with endosulfan (1250 ml / ha) alone was found to be the most beneficial compared with other treatments. A Supreme court of India order dated 14th May 2011 banned the production, use and sale of endosulfan all over India under section 12 of the Insecticides Act, 1968 (Government of India, 2011). So an alternative insecticide to endosulfan is desirable.

SpltNPV was also evaluated based on leaf defoliation. Indoxacarb 14.5 SC (Avant®) at 0.5 ml / l recorded the lowest percentage leaf defoliation of 6.39% at 5 d after application. The efficacy of spltNPV at 2.47×10^{11} OB's / ha was on par with indoxacarb at 5 d after application of the test chemicals. The advantage of spltNPV over other chemicals is that it is eco-friendly (Groner, 1987). The results were suggested based on number of surviving *S. litura* larvae and percent leaf infestation, the FPP realised better suppression of *S. litura* population than SNP.

For validation of spltNPV with farmers practices in large plots, one hectare plot of cabbage was selected. The results suggested that based on the number of surviving larvae and percent leaf defoliation, the FPP realised better suppression of *S. litura* population than SNP. However, when the data were subjected to t-test, there were significant differences between FPP (indoxacarb 14.5 SC application) and SNP as far as the number of surviving larvae but there was no difference in the per cent leaf defoliation. The costs incurred in the SNP and FPP plots are indicated in table 5. The initial cost was lower in SNP compared with FPP. However, increased cabbage yields and added Rs 7,300 (= 13,810 − 6,510) spent in FPP resulted in higher yields and added Rs 78,000 (= 428,000 − 350,000). Therefore, FPP proved economical over SNP. But in long term, spltNPV being environmental friendly and self perpetuating biological formulation, it is desirable.

Researchers have also evaluated the effect of spltNPV alone and in combination with endosulfan and neemarin (neem seed kernel extract) on cabbage. Treatment with NPV-S (500 LE / ha) + endosulfan (625 ml / ha) was better in reducing the *S. litura* larval numbers and in increasing cabbage yields than other treatments. Based on B : C ratio, endosulfan at 1, 250 ml / ha alone was found to be the most beneficial compared with other treatments (Kumari and Singh, 2009). However, the vegetable growers of Yellapura were convinced that the spltNPV can be incorporated with other pest management practices.

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