Evaluation of indigenous entomopathogenic virus against larvae of *Helicoverpa armigera* (Hubner)

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Abstract

Nuclear Polyhedrosis Virus (NPV) was isolated from larvae of Helicoverpa armigera (Hubner), a voracious polyphagous pest attacking wide variety of economically important crops in Sri Lanka. Effects of this entomopathogenic virus on larval instars of H. armigera were studied under the laboratory conditions. Unformulated Polyhedral Inclusion bodies (PIBs) from ground up virus-killed larvae of H. armigera were used to prepare the inoculums. Constant dose of NPV containing 8 x 10^5 PIBs/ml were fed to different larval instars and the percentage mortality and the approximate time to death was found to be dependent on the age of the larvae. Dose dependent mortality was observed when the 3rd instars larvae treated with serial dilutions of NPV from 10^7 to 10 PIBs/ml.

Keywords: Nuclear Polyhedrosis Virus, *Helicoverpa armigera,* Polyhedral Inclusion bodies

Introduction

Helicoverpa armigera generally known as Legume pod borer or cotton boll worm is a serious pest feeding on the fruiting body of wide variety of economically important crops throughout the world (Fitt, 1989, Reed and Pawer 1982). The wide flight range and the large host plant range have made this pest one of the most difficult and destructive pests (Fitt and Boyan 1990) and farmers solely depend on the synthetic pesticides to control this pest. However failures of this method due to the development of insecticide resistance have been recorded from various parts of the world since mid 1970's (Gunning *et al.*, 1984, McCaffery *et al.*, 1988). In addition to the resistance development the adverse effects of these synthetic pesticides on the environment has initiated the search for other alternative pest control methods which can be incorporated in integrated pest management programmes (Wickramasinghe, 1971).

In a preliminary study on the survey of possible biological control agents of *H. armigera* larvae in Southern Sri Lanka showed that Nuclear Polyhedrosis Virus (NPV) played major role in controlling larval populations under laboratory conditions. The NPV belongs to family Bacculoviridae, which is specific only to Arthropods. Some of these NPVs extracted from various insect pests in the world are already identified as suitable microbial control agents of insects. Some of them are now available as commercial Microbial pesticides in developed world. However it is well known that naturally occurring NPVs which cause epizootics in insects under the local conditions are more effective in controlling insect pests rather than the imported microbial pesticides (Jones, 1988). With the consideration of above facts present study was conducted to study the efficacy of locally isolated NPV on larval instars of *H. armigera.*

Materials and Methods

H. armigera larvae were collected from vegetable fields in Matara district. They were reared in the laboratory until pupation. All the larvae were checked daily for dead or diseased larvae and if any, those were separated for identification of mortality factors.

Laboratory colony of *H. armigera* was started from the healthy pupae and they were maintained on semisynthetic diet developed by Ahmad and McCaffery (1988). Presence of NPV in dead larvae was observed when smears of such larvae were stained by Giemsa stain and observed under the light microscope. Larvae positive with NPV were stored in the freezer for one day and individually homogenized using 10ml of distilled water and filtered through a double layered muslin cloth (Poiner, 1980). Filtrate further diluted adding distilled water up to 100ml and the concentration of Polyhedral Inclusion bodies (PIBs) in the filtrate was counted using an improved Neubaur Haemocytometer. After that, samples were centrifuged and stored in a freezer until the confirmation of identity by the Senior Insect Pathologist, NRI, UK and pathogenecity testing afterwards. Samples identified as Positive with NPVs were pooled together and used for Bioassay studies.

Bioassay studies:

Experiment 1

The test insects were selected on the basis of the age by daily observing their growth and moulting. The Body weight (mg) and Head capsule width (mm) of each test insect was measured as an indicator of their instars stage. Larvae which had similar head capsule width, body weight and same number of moults were put into one instars group. As it was very difficult to measure the weight of newly hatched larvae their weights were not included in this study.

A constant dose of NPV was prepared by the serial dilution of viral stock solution. Then it was fed to larvae by applying $8X10^4$ PIBs in 0.1ml of distilled water on the top of the 2 X 2 X 2mm cubes of semi synthetic diet using a micro applicator. When the water on the top of the diet was evaporated, each cube was carefully put into individual 25ml plastic containers with lids. 10 larvae from each instars group individually introduced into these plastic containers and allowed to feed on the diet. Except for the first instars larvae, all the other larvae had fed on the given diet cube within 36 hours. Control larvae fed on the diet treated with distilled water. After the feeding of experimental diets, they were individually transferred into newly labelled plastic containers with fresh diet and observed daily. Incubation time was calculated from the time they started feeding on the treated diet and the time of death. 3 replications were done.

Experiment 2

Laboratory reared 3rd Instars larvae were selected to study the efficacy of various concentrations of NPVs on *H. armigera*. In general toxicology studies, 3rd instars stage of *H. armigera* larvae is considered as the most suitable size as they are in the middle of their larval period and they have suitable body weight and size for such studies (McCaffery *et al.*, 1988). Five fold dilutions of viral stock solutions were prepared and using a micro applicator, 0.1 ml of the test concentrations was applied to the top of the 2 X 2 X 2mm diet cube. Control larvae were treated with 0.1ml distilled water. Each diet cube was put into 25ml plastic containers and after the test insects were put into each container, the lids were closed and they are allowed to feed on the diet. Within 24 hour period larvae had consumed the diet cube and the next day they were transferred into new containers with fresh cube of diet. Larvae were observed daily for their mortality and the data were analysed using the probit analysis method.

Results

A weight related susceptibility to constant dose of virus stock solutions were observed for *H. armigera* larvae (Table 1) 1st and 2nd instars larvae were highly susceptible to the experimental dose of NPV and mortality occurred within a short period. With increasing weight percentage mortality steadily decreased while the incubation time to gradually increased. Although the percentage survival steadily

increased in higher instars (Figure 1) only the 4th and 5th instars larvae produced some pupae. Among them most of the pupae were deformed.

Table 1. Percentage mortality and Incubation period of different instars of *Helicoverpa armigera* larvae treated with constant dose (8X10^5 PIBs /ml) of Nuclear Polyhedrosis virus preparation

Larval instars	No. of larvae	Mean larval weight(mg) ±SE	Mean head capsule width(mm) ±SE	% mortality	Incubation period (Days)	% pupae
1	30		0.205 ± 0.22	100	2.5	-
2	30	2.66 ± 0.66	0.442 ± 0.04	100	4.0	-
3	30	22.25 ± 2.19	0.812 ± 0.06	93	6.5	-
4	30	56.20±1.80	1.367 ± 0.12	77	7.0	57
5	30	128.90±18.0	2.343 ± 0.16	50	9.0	33
6	30	344.80±40.4	2.607 ± 0.14	43	11	-

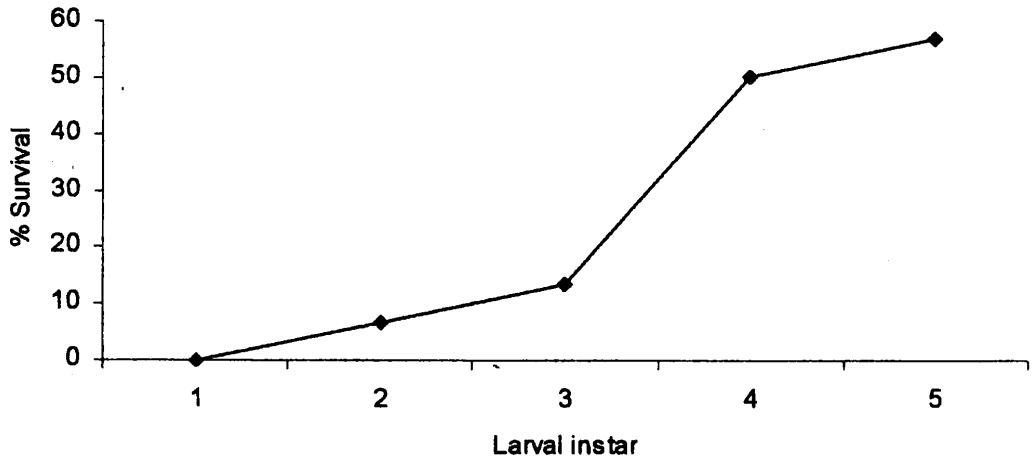

Fig. 1: Percentage survival of various instars of *Helicovera armigera* larvae treated with constant dose of NPV

Dose dependent mortality was observed when the 3rd Instars larvae (mean body weight 22.25 ± 2.19 mg and mean head capsule width 22.25 ± 2.19 mm) were treated with various concentrations of PIBs of nuclear polyhedrosis virus (Figure 2). In addition, higher doses killed these larvae within short periods compared to the lower doses (Table 2).

Table 2. Larval mortality and percentage pupation of 3rd instars *H. armigera* larvae treated with various concentrations of Nuclear Polyhedrosis virus

No. of PIBs /ml	No. treated	% mortality	Incubation period (days)	% pupae
2 X 10^7	30	100	5.5	-
4 X 10^6	30	100	5.5	-
8 X 10^5	52	92	6.5	-
1.6 X 10^5	55	87	6.5	14
3.2 X 10^4	60	63	9.6	68
6.4 X 10^3	60	33	11	75
1.24 X 10^3	65	23	15	80
2.48 X 10^2	40	05	16	84
4.96 X 10	35	03	16	88
Control	40	00	00	98

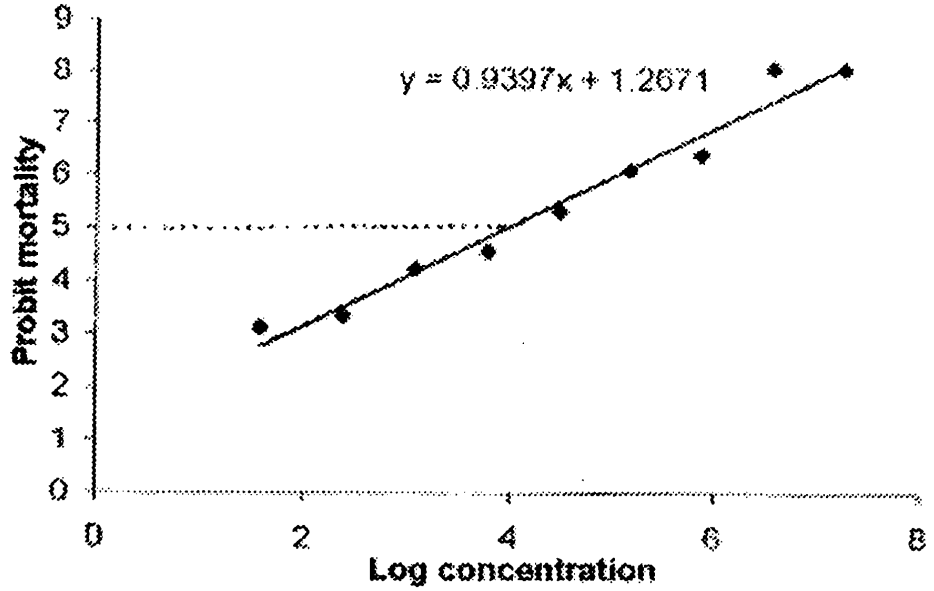

Fig. 2: Dosage mortality curve of 3rd instars larvae of *Helicovera armigera* exposed to NPV

Discussion

The efficacy of entomopathogenic microorganims on its target insects depends on their pathogenicity and the physiological conditions of the target organism. Age and weight of the insect larvae were important factors, which determine their response to pathogens (Entwistle and Evans, 1985). An inverse relationship between the mortality due infection of virus and the larval age has been demonstrated in this study. This also agrees with the fact that susceptibility of insect larvae may increase with the increasing larval weight and age. This may be due to the fact that in higher larval instars, their bigger body masses are capable of resisting the invasion of the given dose of viruses into their vital body organs. However when the bigger larvae were treated with the virus, they did not pupate properly and produced malformed pupae. This may indicate the disruption of larval pupal transformation by these viruses which need further investigation.

As with chemical insecticides, dose dependent mortality was observed when the 3rd instars larvae were treated with various concentrations of virus preparations. With the higher doses, larval mortality occurred within a very short period. This study indicated that the virus induced mortality of *H.armigera* larvae decreased with the increasing larval age and incubation period shorten with the higher doses. Therefore, in order to achieve effective and economical control, it is necessary to target application of viruses to early instars with effective doses and otherwise it will not produce the expected results under the field conditions.

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