Research Paper

Volume : 3 | Issue : 7 | July 2014 • ISSN No 2277 - 8179

Influence of Host Plants on the Efficacy of Nucleopolyhedro Virus of *Helicoverpa Armigera* (Hubner) (Lepidoptera: Noctuidae)

Zoology

KEYWORDS : Microbial pesticides,Helicoverpa armigera,bioassay,midgut

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ABSTRACT

The comparative efficacy of microbial pesticides on gram pod borer, Helicoverpa armigera and on the effect and dosage of HaNPV on the susceptibility of the borer was studied. Larvae were fed on different host plants treated with HaNPV of three different concentrations. Along with this midgut pH, foliage pH and protease enzyme activity was also observed. The bioassay results clearly correlated the susceptibility of H armigera larvae to both the change in midgut pH as well as protease in the midgut of the larvae on different host plants

Introduction

Agriculture today has not only experienced the green revolution but is also experiencing the most harmful agriculture pests and diseases which have become more and more difficult for the control with our present chemical insecticides. In this context of growing awareness about health hazard on the other have compelled the agricultural entomologist to device newer approach to minimize pest population on the crop plants. Therefore, unilateral approach of controlling of crop pest by synthetic insecticides has dedicated the need for developing cost effective, eco-friendly and safe pest control strategies. In this direction, biopesticides like viruses are increasingly being used as alternatives to chemicals in the managements of noxious insects of crops.

The importance of insect viruses is safe, economic and an effective agent for pest management has been recognized worldwide. In order to extend these viruses, interest in selecting with increased virulence and stability has developed (Brassel and Benz, 1979; Wood *et al.,* 1981). A large number of viruses have been reported to be naturally pathogenic to insects. Among them, only about 35 insect pathogenic viruses of baculovirus group, especially nucleopolyhedrovirus (NPV) has been reported to be prominent. The effective use of baculoviruses and in particular nucleopolyhedrovirus for control of insect pests has been for many years (Hamm, 1994). The effectiveness of baculoviruses in the insects killed or by the survival time of the infected infects. Nucleopolyhedroviruses are studied because of their current or potential use as biological pesticides (Smith, 1975; Doller*et al,* 1983). Although HaNPV has been found to be very effective against the *Helicoverpaarmigera* (Lepidoptera: Noctuidae) on number of crops (Rabindra&Jayaraj, 1990) greater amount of variation in the efficacy of HaPV across the host plants strained its practical utility in the field condition.

Among the most voracious pests are the pod borers *Helicoverpaarmigera*and *Heliothis zea*. These are so well adapted to their new agricultural environment that they are present in almost all warm climates throughout the world. Their main plant hosts are our most important cash and food crop like cotton, maize, sorghum, legumes, tobacco, tomato and many wild fruits. *H. armigera* has infested 181 different host species in 45 families (Manjunath*et al.,* 1989). Owing to the ability of *Heliothines* to feed on all plant structures (Fitt, 1989) it is imperative to understand the influence of host plant phenology on larval susceptibility.

Since food comprises an important component for the polyphagous noctuid, information on the influence of host plants on biopesticide susceptibility of *H. armigera* and the associated biochemical is vital. Early studies showed how vegetative tissue from a number of cultivated hosts like cotton, soybean, and tomato influences the susceptibility of *H. Zea* larvae to HzNPV (Forschler*et al.,* 1992). Determinations of the numeric relationships between baculovirus particles and their insects host have become very important. The importance of these viruses as alternatives to pest managements has been recognized worldwide (National Science Foundation, 1981) and both basic and applied research depends on accurate and precise qualification of the virus host interaction. Along with this, standardization of viral pesticides is essential for commercialization (Dulmage and Burgerjon, 1977). These relationships can be determined by *in vivo*bioassay in the host insect and the interpretation of the result depends on the understanding of biological and stochastic properties.

Hence, the present research work was undertaken to study the comparative efficacy of these biopesticides (microbial pesticides) on gram pod borer, *H. armigera* and on the effect and dosage of HaNPV on the susceptibility of the borer.

Material and Methods

Seeds of host plants of *H. armigera* such as cotton, tomato, chick pea, pigeon pea and field bean were grown in crates in the green house maintained at appropriate atmospheric conditions. Pure cultures of larvae were obtained from BioControl Research Laboratories, Bangalore.

1. Bioassay procedure with host plant foliage

The susceptibility of cotton bollworm *H. armigera* to the *Helicoverpaarmigera* Nuclear Polyhedrosis Viruses (HaNPV) on different host plants was examined. Foliage's of the host plants for bioassays was collected from the plants grown in green house maintained at a constant temperature and humidity. Larvae were first reared on different host plant such as cotton, tomato, pigeonpea, chickpea, and field bean for the first generation to get the purse stock. After obtaining first breading stock these larvae again reared till it molts to the second instar on their respective host plant and were then removed from the host plan after six hours so that larvae begin the inoculation period with empty midguts. Leaves of different host plant were collected and immediately treated with different HaNPV viral suspensions of $1x10^9$, $1x10^7$ and $1x10^5$ polyhedral inclusion bodies. The viral suspension was sprayed uniformly on the lower and upper surface of the leaves using atomiser. These HaNPV sprayed leaves then placed in plastic bowls and 15 starved larvae from each specific host plants were released on these leaves placed in the bowl.

The bowls were covered with muslin cloth to avoid the escape of larva. Concurrently a control was also maintained by taking the same number of larvae grown on the semi synthetic medium diet supplied with proportionate nutrients. Treatments on each host plant species and artificial diet were replicated four times at the ration of 15 larvae per replication. After five days of inoculation on the foliage and artificial diet the rate of mortality was checked at regular interval i.e. after 24hrs, 48hrs, 72hrs, 96hrs and 110hrs after inoculation. A control containing the same number larvae were also reared on different host plants. The difference in the rate of mortality among the larval groups inoculated on different host plant species and artificial diet were compared with ANOVA. Larval mortality within 24hrs was attributed to injury in handling and such larvae were reported with stock culture.

2. Midgut pH of foliage-fed larvae fed on different host plant

Host plants foliages were collected. *H. armigera*larvae were reared on foliages of different hosts plants through sixth star, Cotton (*Gossypiumhirustum)* Tomato (L*ycopersiconesculentum*), ChickPea (*Cicerarietinum*), Pigeon pea (*Cajanuscajan*) and Field bean (*Dolicus lablab*). These sixth instar larvae reared on different host plants were removed from foliages and dissected in ice cold water at room temperature to remove the midgut region of a larva. For each host plant, 2-3larval midgut region was taken and homogenized in a pestle and mortar containing 5ml of double distilled water. Three aliquots of homogenized solution were taken in polyethylene cups and pH was recorded using pH meter. These reading were tabulated and the pH of a larvae was reared on different host plant was taken as the average pH of the three aliquots. In the same was pH of *H. armigera*larva fed on the artificial diet was also recorded.

3. Foliage pH

pH of the leaf surface of different hosts plants was checked. Leaves from the host plant species were excised in the green house. Three leaf samples for each plant species were added to double distilled water at 1g fresh weight per 5ml of water and homogenized for around 5 minutes. Three aliquots of the homogenized solution were collected in polyethylene cups and pH was recorded using pH meter. The readings were tabulated and the average pH of the leaves of the three aliquots was calculated.

4. Enzyme analysis

The enzyme solution was prepared according to the Applebaum et al. (1964) and Ishaaya (1971). Late fifth instar or sixth instar *H. armigera*larvae was dissected in ice and the midgut of each larva was separated. Estranged midgut tissue was transferred to Eppendorf tube and was stored in -40 C. One gram of midgut tissue was suspended in 5ml of 0.1M potassium phosphate buffer, homogenized and centrifuged at 40 C for 15 minutes at 12000 rpm. The supernatant was used as crude enzyme.

Determination of larval enzyme activity

Larval enzyme activity was determined using casein as substrate and suspending 0.1M potassium phosphate buffer werein larval gut extract were used to determine the activity and standard curve was plotted.

Enzyme assay: (Eugchi and Iwamoto Casein digestion method)

Regents required:

1. 1% casein solution: 1g casein was suspended in 100ml of 0.1M potassium phosphate buffer and was incubated in boiling water bath for 15-20 minutes. (Note: casein is stable for 1 week when stored at refrigerator.)
2. 5% trichloroacetic acid (TCA): To 500g of trichloroacetic acid 222ml of water was added to get 100% solution.
3. 0.1 Potassium phosphate buffer (pH 7.6): to 86.6ml of dipotassium hydrogen phosphate (K2HPO4 – 1M) 13.4ml of potassium dihydrogen phosphate (KH2PO4 - 1M)was added and volume was made upto the required quantity with distilled water after adjusting the pH to 7.6.
4. 0.5N Sodium hydroxide: 0.5g of sodium hydroxide in 100ml of distilled water.
5. Borate buffer: 6.18g of boric acid (0.1M) added to 950ml of distilled water. Adjusted the pH to 11.3 with 10M NaOH. Make up to one litre of distilled water.
6. Folic-Ciocalteau regent: Commercial available regent is diluted by 1:1 ratio.

Procedure

The protease activity was studied by following the method of Eguchi and Iwamoto (1976) with slight modifications.One ml of 1% casein and 1ml of borate buffer (pH 11.3) were incubated with 0.5ml of enzyme solution for 30minutes at room temperature. After digestion, intact casein was precipitated with 2ml of 10% TCA and centrifuged at 3000rpm for 10min.One ml of supernatant was taken along with 2ml of 0.5N NaOHabd 0.5ml of Folin's reagent was added and the mixture was incubated for 30 minutes at room temperature. Optical density was measured at 600nm using UV-Vis spectrophotometer 118. The enzyme activity was calculated by following method.

Enzyme activity= Standard X Optical density X Dilution factor X Dilution for 20%homogenate

Results

The effects of host plants on the susceptibility of *H. armigera* to HaNPV were examined in laboratory bioassay. Host plant factor to large extent governs important biological parameters related to life cycle of the pest and the susceptibility to various bio pesticides as well.

The percentage of larval mortality on 5th day after inoculation on different host plants is given in tables 1 to 5

Table 1. Percent mortality of H. armigera larvae fed on HaNPV treated cotton plant on 5th day after inoculation

Trial	T_1-1x10^5	T_2-1x10^7	T_3-1x10^9
R_1	23	46.6	64.3
R_2	26.6	40	80
R_3	40	40	60
R_4	33.3	76.9	80
AVERAGE	30.7	50.9	71

Table 2. Percent mortality of H. armigera larvae fed on HaNPV treated tomato plant on 5th day after inoculation

Trial	T_1-1x10^5	T_2-1x10^7	T_3-1x10^9
R_1	72.7	83.3	100
R_2	80	84.6	100
R_3	71.4	85.7	100
R_4	71.6	80	100
AVERAGE	75.5	83.4	100

Table 3. Percent mortality of H. armigera larvae fed on HaNPV treated chick pea plant on 5th day after inoculation

Trial	T_1-1x10^5	T_2-1x10^7	T_3-1x10^9
R_1	80	73.3	100
R_2	80	73.3	100
R_3	73.3	93.3	100
R_4	60	66.6	100
AVERAGE	73.3	76.7	100

Table 4. Percent mortality of H. armigera larvae fed on HaNPV treated field bean plant on 5th day after inoculation

Trial	T_1-1x10^5	T_2-1x10^7	T_3-1x10^9
R_1	75	77.7	100
R_2	77.7	88.8	100
R_3	66.6	100	100
R_4	90	77.7	100
AVERAGE	77.3	86	100

Table 5. Percent mortality of H. armigera larvae fed on HaNPV treated synthetic diet plant on 5th day after inoculation

Trial	T_1-1x10^5	T_2-1x10^7	T_3-1x10^9
R_1	40	66.6	100
R_2	33.3	57.1	100
R_3	46.6	53.3	100
R_4	46.6	66.6	100
AVERAGE	41.6	60.9	100

At 1x10^5 POB/ml on cotton and synthetic diet larvae mortality was not significantly showing 30.7% and 41.6% mortality

respectively on 5th day after inoculation and it was significantly different from that of other plants. Percent mortality on larvae on tomato and chick pea was on par with each other but are statistically different form cotton field bean and synthetic diet at $1x10^5$ POB viral suspension and showed 75.70% and 73.30% mortality. The field bean which shown 77.3% mortality was significantly different from that of cotton and the diet but were on par with that of tomato and pea plant. This indicate that the host plant cotton influence the mortality of *H. armigera* and cotton fed larvae were less susceptible at $1x10^5$ concentration compare to other plants. The percent mortality of larvae was highest on field bean followed by tomato chick pea and synthetic diet and cotton.

At the highest concentration of $1x10^9$ larvae mortality on cotton was significantly different from all other host plants showed 71% mortality but was no significant different in larvae mortality b/w any of the other host plant.

From the result it was found that in all viral concentration such as $1x10^51x10^7$ and $1x10^9$ larvae fed cotton were significantly less susceptible showing respectively 31%, 51% and 71% than those on tomato, chick pea and field bean. Larvae fed on HaNPV treated chick pea were moresusceptible than those on cotton but less susceptible than those on tomato and field bean. It showed 73.3%, 76.6% and 100% mortality at $1x10^5$, $1x10^5$ and $1x10^5$ concentrations, respectively.

Rate of enzyme activity in the midgut of larvae

Protease enzyme in the midgut plays a major role in the susceptibility of the larvae to HaNPV on host plants. The protease activity in each host plant specific larvae were calculated and expressed in terms of µ moles/g of tyrosine liberated.

Table 6 shows the amount of tyrosine liberated in the larva fed on different host plants. Cotton fed larvae liberated maximum amount of tyrosine of 290 µmoles/g compared to that of other host plant specific larvae. Larvae on tomato generated 240 µmoles/g, chickpea 275 µmoles/g and on the diet 263 µ moles/g of tyrosine. Field bean showed maximum of 116.4 µmoles/g of tyrosine.

Table 6. Influence of midgut protease on larval susceptibility of H. armigera

Host plant	Mean midgut pH	OD @ 660nm	µmoles/g tyrosine liberated
Cotton	7.82	1.45	290
Chickpea	7	1.37	275
Ciet	6.87	1.31	263
Tomato	6.82	1.21	240
Field bean	6.61	0.58	116

From the result it shows that cotton which was less susceptible had liberated 290 µmoles/g of tyrosine compared to others. This indicates that an increase in the amount of tyrosine liberated makes the larvae less susceptible

Discussion

The host plant plays an important role in mediating the susceptibility of lepidopteran larvae to baculoviruses (Fuxa, 1982). Viral susceptibility in *H. zea* and *H. virescens* is significant affected by interspecific in the host plant. Forschler et al.(1992) reported the susceptibility for H zea with larvae least susceptibility on cotton. An array of factor may be responsible for enhance larvae susceptibility to HzNPV. Herbivory by *H. zea* significantly increase folian enzyme and secondary metabolites but decrease certain essential nutrients in soybean (Bi et al, 1994)

Duffey et al.(1995), reported that insect suffering from nutritional stress are generally more susceptibility to disease. It is also possible that this condition could affect the virion itself. The interaction may involve levels of leaf phenolics directly. (Felton et al, 1987). The factor responsible for the reduced susceptibility of *H. armigera* fed dosed with HaNPV on cotton foliage are most likely the result of the interaction among the midgut condition, foliar constitute and viral but not a direct interaction between plant and virus.

It appears that starvation does not have a significant impact on the depletion of protein. Consequently protein does not appear to serve as a source of energy. The influence of host plant on the susceptibility of larval population to viral epizootics also may depend upon the role of host plants plays in altering larval resistance during the period of viral incubation following inoculation. There may also be host plant species specific variation in the prevalence of GmNPV and in the rate which virus contaminated foliage is consumed.

Keating et al.(1988) reported a significant positive correlation between susceptibility to niuclearpolyhedrosis virus and leaf tissue pH. Lower mortality rates were strongly associated with food material which lowered larval midgut pH levels. Stiles and Paschke (1980), concluded that decreasing midgut pH increasing the susceptibility of mosquito species.Nagata and Kobayashi (1990), showed that the storage protein concentration in the larval haemolymph was influenced by dietary protein levels. Chatterjee (1992), reported that the enzyme activity was found to be higher in the larval stage of larval development.

Sarangi (1986) reported that midgut protease activity varied significantly in bivoltine, multivoltine races and crossbreed variety which may possibly be correlated to the different in their silk output. Jadhav and kallapur (1988) also showed sex affect the enzyme significantly in bivoltine.General property of Lepidoptera midgut proteinases is that maximum activity occurs at pH value equal to or greater than 10 as proved in *H. zea*. From the investigation it is clear that a change inmidgut pH and protease enzyme activity influence the susceptibility of *H. armigera* larvae.

In conclusion,the comparative efficacy of microbial pesticides on gram pod borer, *H. armigera* and on the effect and dosage of HaNPV on the susceptibility of the borer was studied.In material and methods larvae were fed on different host plants treated with HaNPV of three different concentrations. Along with this midgut pH, foliage pH and protease enzyme activity was also observed. The bioassay results clearly correlated the susceptibility of *H armigera* larvae to both the change in midgut pH as well as protease in the midgut of the larvae on different host plants.

REFERENCE

Bi J L, Felton G W, Muller A J, 1994. Induced resistance in soybean to Helicoverpazea. Role of plant protein quality. J. Chem, Ecol, 200, 183-198 | Chatterjee S N, 1992, Hierarchical clustering of 54 races and strain of the mulberry silkworm Bombyxmori significance of biochemical parameters. Theor. Appl. Genet, 85, 394-402 | Duffey S S, Hoover K, Bonning B and Hammock B D, 1995. The impact of host plant on the efficacy of biculoviruses. Rev PesticToxicol, 3. 137-275 | Felton G W, Duffey S S, Vail P V, Kaya H K, and Maining J, 1987, interaction of nuclear polyhedrosis virus with catechols potential incompatibility for host resistance against noctuid larvae, J ChemEcol, 13, 947-957. | Forschler B T, Young S Y and Feltor, 1992, Diet and the susceptibility of Helicoverpazea to a nuclear polyhedrosis virus, Env. Ento 21, 1220-1223 | Fuxa J R, 1982, Prevalence of viral infection in population of fall armyworm, Spodopterafrugiperda in southeasternlousiana, Env. Ento, 11, 239-242 | Jadhav G and kallapur V L, 1988. Influence of age sex and feeding on the protease activity of certain tissues of fifth instar silkworm, Bombyxmori. Entomon, 13, 289-293 | Keating S T, Yendol W G, and Schutz J C, 1988. Relationship between susceptibility of gypsy moth larvar to a baculovirus and host plant foliage constituents. Environ Entomo, 17, 952-958 | Nagata M and Kobayashi M, 1990, effect of nutrition on storage protein concentrations in the larval haemolymph of silkworm, bombymori L. J SericSciJpn, 59(6), 469-474. | Sarangi S K, 1986, studies on midgut protease activity during fifth instar development of the silkworm. Bombyx mori. Entomon, 11, 165-169 | Stiles A L and Paschke J D 1980, midgut pH in different instars of three Aedes species and the relationship between pH and susceptibility of larvae to a nuclear polyherosis virus. J Invertbe. Patho, 35, 58-64