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USE OF ARTIFICIAL DIET FOR TESTING TOXICITY OF NPV ON *HELICOVERPA ARMIGERA*

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ABSTRACT
Helicoverpa armigera is common pest of many vegetables and all leguminous plants etc. Being a polyphagous pest, it feeds on every plant parts causing variety of damages and decreases crop production. Present experiment was setup to verify the specific effect of NPV (Nuclear polyhydrosis virus) on target insect pest reared on artificial diet. This method will eliminate the possible effect of other natural diet related issues to infer the mortality of *H. armigera* by NPV. Third and fourth instars were feed with artificial diet as control and same inoculated with NPV. The mortality was observed for ten days. Then the data was analyzed statistically to determine the variations in toxicity of NPV and natural death. Larval mortality in NPV infected artificial diet was significantly high as compared to artificial diet (control).

KEY WORDS: artificial diet, *Helicoverpa armigera*, nuclear polyhydrosis virus.

INTRODUCTION

Helicoverpa (often called *Heliothis* or Cotton ball worm) is a serious pest of different crops. There are two pests species of Helicoverpa i.e. Budworm or *Helicoverpa punctigera* and Cotton Boll worm or corn earworm or H. armigera. *H. armigera* undergoes six larval instars and has egg, larvae, pupae and adult stages. Sometimes the pupae remain in a state of suspended development that is diapauses. *H. armigera* feeds on leaves, flower, buds, developing pods, fruits, and seeds. This species are wide spread throughout tropical and subtropical regions mainly in Europe, Asia, Africa, and Australia.

H. armigera is a major pest causing reduction in yield of crops ranging from 40-50% (Rai et al., 2003). On an average a single larva of *H. armigera* per plant of pigeon pea could reduce the yield to an extent of 138.5 kg/ ha (Reddy et al., 2001). It is a major polyphagous of agricultural crops worldwide. The immature stages feed and forage on crops at all stages on plant development, damaging fruiting and non-fruiting structures. In India losses of the pigeon pea and chick pea alone exceeds three hundred million dollars (Sharma, 2000) and in Australia losses due to *H. armigera* is about twenty five million dollars (Twine et al., 1989). In India and Pakistan, the polyphagous larvae have been recorded infesting cotton crops, pulses, sunflower, peas, wheat, tobacco, tomato etc. (Sharma, 2000, Ahmed et al., 1992). It is one of the important pests of cotton causing extensive damage to the fruits about 50-60% (Saleem, and Younas, 1982). Therefore, it is important to develop necessary and efficient pest control program and IPM strategies. Among many control measures use of pathogens as virus, fungi and bacteria are some that infect and kill *Helicoverpa*. The two commercially available pathogen to control Helicoverpa are bacterial toxin from *Bacillus thurinogenesis* (Bt) and NPV. NPV belongs to a group of insect diseases called baculoviruses that infect and kill the larvae of moths. The commercially available NPVs only kill helicoverpa larvae. They do not harm humans, wildlife, or other insects (Vyas and Yadav, 1992). One of the key differences between NPV and a conventional insecticide is that NPV is applied as a live disease. Helicoverpa larvae have to eat NPV particles to become infected. These particles are called polyhedral inclusion bodies (PIBs). It is one of the important biological options of IPM and its several attractive features like specificity, ecological safety, and recyclability. In present study, bio-efficacy of NPV was evaluated on *H. armigera* reared on artificial diet. This could help in commercial mass production of NPV.

MATERIALS AND METHODS

The larvae were collected from the chickpea field which were of third, fourth and fifth instar. The larvae were transferred individually into the vials containing artificial diet. Artificial Diet was formulated with some modification (K. A. et al., 1998) as mentioned in Table No. 1. All ingredients of fraction A of was mixed with 390 ml of D/W in the blender for 2-3 min. Fraction B was boiled in remaining 390 ml D/W. Fraction A and fraction B were mixed in the blander for 1-2 min. Finally, fraction C was also added to the mixture of A and B. Again, blender was run for 2-3 min. Formaldehyde solution was added at the end in the mixture. The NPV infected diet was also prepared in the same way as described above. In addition to all ingredients, 1.5 ml of NPV was added into the mixture of the diet at the end (Qayyum et al., 2015). One milliliter of the formulated product contained 1.2 X 10^9 PIB's (Polyhedral inclusion

bodies). *H. Armigera* Nucleus polyhyrdess virus (NPV) was provided by NHRDF (Regional Research Station) Nashik, M.S. India. The 5.0 gm was poured in the plastic vials, allowed to cool, and solidified. The lids of the plastic vials were perforated for the circulation of air and can be stored in the refrigerator up to 2 weeks. The larvae were transferred individually into the vials containing artificial diet. Larvae were seemed to feed on the diet after few hours. There was increase in size of the larvae within 10-12 days. The larvae stopped feeding. Began wandering in the vial and burrowed into the remaining diet to form "pupation cell". Slowly the larvae become shorter, fatter and grub like. Once the pupae were formed, they left undisturbed for 2-3 days until the soft newly formed cuticle (pale yellow–green) was harden (red-brown). The pupae were removed carefully from the vials using blunt forceps. Deformed pupae were discarded. Insect rearing cages are used for adult emergence and development.

The rearing cage consist of cylindrical iron frame (50 cm x 30 cm) having two rings (above and below) with a black cloth enclosing the frame. The cloth cover is open at both the ends; this can be tied with the rubber bands. The cage can be open or close with zip at vertical slit. A petri plate having cotton soaked with water and honey was placed at the centre of the cage. The pupae were kept in the plate with soil in it. Based on the optimum conditions of the temperature and humidity above 70^{o}C, adult emerged out within 12-14 days. (Abdida and Ghulam, 2000). Moths were provided with sucrose solution (honey) soaked in cotton. After few days, moths laid eggs on the inner surface of the cloth of the cage. The pale yellow colored eggs were collected with the help of brush very carefully and transferred on the fresh cabbage leaves or pigeon pea leaves. Than these leaves were kept in a beaker and was tied with muslin cloth. It was observed daily. After the emergence of the larvae, they were placed on artificial diet. After 3-4 days when larvae attain second or third instars, were shifted to the NPV infected artificial diet. The larvae were allowed to feed for some days. Regular examinations of the larvae were carried out. Fifty larvae with three replication were released on NPV infected diet also a set of control was maintained to compare the mortality due to NPV. T-test statistical analysis was applied to evaluate the variation in mortality.

Table No. 1 : Ingredients used in formulating artificial diet

Fraction A	
Chick flour	105.0 gm
Methyl parahydroxy benzoate	2.0 gm
Sorbic acid	1.0 gm
Streptomycin	0.25 gm
Fraction B	
Agar agar	12.75gm
Fraction C	
Yeast powder	40 gm
Ascorbic acid	3.25 gm
Multi vitamin (capsules)	2
Vitamin E (capsules)	2
Distilled water	780 ml
10% Formaldehyde	2.0 ml

RESULTS AND OBSERVATIONS

The artificial diet formulated in the laboratory had supported the growth of larvae. It had all necessary constituents, which are required for normal growth of the larvae. The constituents like Streptomycin, Methyl para hydroxyl benzoate and sorbic acid has ability to reduce spillage by preventing the growth of fungus. The diet had the solution of multi vitamin capsules, which supported the larval development. The formaldehyde acts as preservative in the diet, which prevents the fungal growth and maintains the good quality of diet for long time. Agar had provided the solidify base for the larval growth. So all the above constituents had prevented the spoilage of the diet. This was the reason for successful growth of the larvae. During present experimentation, Toxicity test proved significant variations among control i.e. larvae reared on artificial diet and NPV inoculated artificial diet. The data was analyzed by large sample t - test, where H0: there is no significant difference between average mortality with NPV and without NPV diet, Against H1: there is significant difference between average mortality with NPV and without NPV diet at 5% as well as 1% L.O.S. The L.O.S. at 1% is 2.58, at 5% is 1.96. The calculated value was 9.632, which is grater than the table value, therefore we reject H0. These observations clearly conclude that all mortality in NPV inoculated diet is due to the

Proliferation of NPV infection in the *H. armigera* larva and no other factors are responsible other than the inoculated NPV for mortality of *H. armigera*.

In case of use of natural diet, there is a chance of pre exposure to some other chemicals and factors, which may cause larval mortality and not the NPV. Therefore, it raises difficulties in inferring the causes of larval mortality. Thus, use of specific artificial diet to identify and study entomocidal property of various chemicals or pathogens is indispensible. Artificial diet use in present study for rearing *H. armigera* is the effective way for the above purpose, as it is cheap and constituents required for making it are easily available and can be easily formulated.

Table 2: Mortality of *H. armigera* with artificial diet (control) and artificial diet inoculated with NPV.

Treatment	Set	Total mortality %	
Artificial diet with NPV	Set 1	70	Mean: 67.333 SD: 3.055
	Set 2	68	
	Set 3	64	
Artificial diet	Set 1	6	Mean: 5.33 SD: 1.154
	Set 2	6	
	Set 3	4	

Mortality was recorded for 216 Hrs.

Infection of NPV occurs when larvae feeds on the NPV infected diet. It may start accumulating in the gut wall, reproduce rapidly in the internal tissue causing toxicity. No mortality was seen within 0-24 hours, (Table No. 2) this might be due to the less no. of PIB'S in the body. (Knaak and Fiuza 2005). After 24 hours, feeding rate goes on decreasing and showed less movement. This may be due to the initial invasion of virus body in gut wall. Within 48-192 hours, rapid mortality was seen. This may be due to the large number of PIB's which might had blocked the respiratory system or had damaged the tissues and ultimately caused the death (Duraimurugan et al., 2009). The infected larvae became pale, turned black with loose skin, bodies liquefied and were disintegrated. This NPV insect pathogen thus proves to be effective way of controlling *Helicoverpa*. Availability of this natural enemy and reduction in the application of broad-spectrum chemical pesticide increases the potential for IPM. Use of artificial diet in mass rearing of *H. armigera* and NPV at such economically feasible system may help farmers to use such natural pathogen at local level. In future, this study can be extended to know effective artificial diet formulation for mass production of other lepidopteron pest larvae.

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