Laboratory evaluation of certain bio-pesticides against the larvae of *Helicoverpa armigera* Hubner

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**ABSTRACT**

Applications of *Helicoverpa armigera* Nuclear Polyhedrosis Virus (*Ha*NPV), Quinalphos, *Bacillus thuringiensis kurstaki* (*Btk*) and *Beauveria bassiana* (*B.b.*) as full doses gave hundred percent larval mortality to the test insect, 2\textsuperscript{nd} instar larvae of *Helicoverpa armigera*. Treatment combinations containing half doses of *Ha*NPV + *Btk*, *Ha*NPV + *B.b.*, *Ha*NPV + Quinalphos, *Btk* + *B.b.*, *Btk* + Quinalphos, *B.b.* + Quinalphos, also gave cent percent larval death. Azadirachtin as half dose of application was found to be least effective (50%). However, all the treatment combinations were found effective over the untreated control.

**Key words:** Chickpea, *Helicoverpa armigera*, Interactive effect, Laboratory conditions, Larval mortality, Pesticides, 2\textsuperscript{nd} instar larvae.

**INTRODUCTION**

A large number of insect-pests have been recorded while feeding on chickpea but *Helicoverpa armigera* has attained status of the most serious pest in recent years in terms of economic damage caused to different agricultural crops throughout India including chickpea (Davies and Lateef, 1975; and Lal et al., 1981). Several attempts have been made towards chemical control of the pest using synthetic pyrethroids and other chemical compounds against *H. armigera*. Insecticides are generally preferred for quick action and control, but owing to their continuous, indiscriminate and excessive use, many complex problems have come up, such as development of insecticidal resistance to the pest (Khare et al. 1989; and Dhirgra et al. 1988). During the last three decades attempts have been made to use safer pesticides including neem products and microbial pesticides as treatment combinations. Among microbial pesticides; virus, bacteria and fungus hold some good promises. Nuclear polyhedrosis virus, *Bacillus thuringiensis*, *Metarrhizium anisopliae* and *Beauveria bassiana* are the best known among them (Bull et al., 1976; Nagarare and More, 1998; and Loganathan et al., 2000). The relative specificity, potential activity, environmental safety and immunity to insecticides have made microbial pesticides a favoured component of IPM strategies, and considerable efforts have been made to develop the most promising agents, *Bacillus thuringiensis* and *Helicoverpa armigera* nuclear polyhedrosis virus (*Ha*NPV) into commercially viable products (CAB International, 2015). Keeping in view above facts, several experiments were undertaken to evaluate the bio-control agents such as *Bt*, *B. Bassiana*, *Ha*NPV, the botanical pesticides (Azadirachtin & Neem Seed Kernel Extracts) and a synthetic insecticidal formulation (Quinalphos) against 2\textsuperscript{nd} instar larvae of *H. armigera* under laboratory conditions.

**MATERIALS AND METHODS**

**Laboratory test of insecticides and their formulations**

Studies regarding effectiveness on 2\textsuperscript{nd} larval instars of *H. armigera* were made by using the available commercial formulations (Table-1). The viable spore count in the commercial formulation of *Btk* was around 90-102 billion spores/gm, 1X10\textsuperscript{9} PIB/ml for *Ha*NPV and 1X10\textsuperscript{9} spores/gm for *B.b.*. From stock solution of *B.b.*, dilutions were made in range from 1.0 x 10\textsuperscript{6} to 1.0 x 10\textsuperscript{7} spores/ml. The required concentrations of quinalphos, Azadirachtin were prepared from stock solution. For preparing various concentrations, the required amount of *Btk* and *B.b.* was weighed on a digital electronic balance; and *Ha*NPV, Azadirachtin and quinalphos were measured with the help of pipette (of 0.1 ml capacity); and were dissolved in tap water containing 0.2% Teepol and thereafter homogenous mixture was prepared by stirring the solution with a glass rod. The normal tap water along with 0.2% Teepol was used as the control.

The following formula was used to prepare different concentrations of insecticides (Singh, 2010).

\[
\text{Amount of pesticide} = \frac{\text{Conc. of spray} \times \text{Total spray volume}}{\text{Strength of commercial formulation}}
\]

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Table 1: Details of bio-pesticides, bio-rationals & insecticide used.

<table>
<thead>
<tr>
<th>Composition</th>
<th>Trade Name</th>
<th>Strength</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear Polyhedrosis Virus (NPV)</td>
<td>Biovirus-H</td>
<td>Polyhedral Inclusion Bodies (PIB) count-1x10^6 PIB/ml</td>
<td>M/S Biotech International ltd., New Delhi</td>
</tr>
<tr>
<td>of Helicoverpa armigera, Strain No. BIL/HV-9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacillus thuringiensis var. kurstaki, Strain No. Z-S2, Serotype: H-3a, 3b</td>
<td>Biolep</td>
<td>50000 IU/mg of product</td>
<td>M/S Biotech International Ltd., New Delhi</td>
</tr>
<tr>
<td>Beauveria bassiana, Strain No. NBRI-9947</td>
<td>Daman</td>
<td>1x10^6 CFU/gm of product</td>
<td>M/S International Panacea Ltd., New Delhi</td>
</tr>
<tr>
<td>Quinalphos</td>
<td>Vazra</td>
<td>25 EC</td>
<td>M/S Cheminova India Ltd., Mumbai</td>
</tr>
<tr>
<td>Azadirachtin</td>
<td>Neemarin</td>
<td>1500 ppm</td>
<td>M/S International Panacea Ltd., New Delhi</td>
</tr>
<tr>
<td>Neem Seed Kernel Exports (NSKE)</td>
<td>Neemarin</td>
<td></td>
<td>From local market</td>
</tr>
</tbody>
</table>

Preparations of neem seed kernel extract (NSKE):
After hand pounding of 50gm of neem seed it was tied loosely in a thin muslin cloth and thereafter submerged in 1 litre of water for overnight. The soaked material was pressed well repeatedly to prepare a suspension where un-dissolved hard material was removed. In such a neem extract, 10 grams of ordinary washing soap was dissolved for making a spray solution.

Sterilization and test of insecticide and bio-pesticides:
Before conducting the experiment, all the glass-wares were thoroughly sterilized to avoid viral infections. In order to make plastic vials duly cleaned and sterilised, such vials were submerged in water for an hour and later on washed with tap water. Then plastic vials were rinsed with 0.1% mercuric chloride solution (to avoid microbial contamination) and thereafter cleaned with distilled water. Then such vials were submerged in sodium hypochlorite solution (0.2%) for at least 6 hours. Thereafter such vials were rinsed in fresh water and dried under direct sunlight for completing the standard process of sterilization. Second instar larvae of H. armigera were used as test-insects, and bio-potency of various concentrations of Bt, B.b, Ha NPV, Azadirachtin, NSKE and Quinalphos were examined against this pest under laboratory conditions.

Chickpea leaves were washed with running tap water and dried in the shade under fan for 5 minutes and then dipped into the respective concentrations of 0.005%, 0.001%, 0.002%, 0.004%, and 0.006% for Quinalphos; 0.005%, 0.05%, 0.10%, 0.15% and 0.20% for Bt; 1.0x10^6, 3.0x10^6, 5.0x10^6, 7.0x10^6, and 1.0x10^7 spores/ml for B. bassiana; 1.0x10^6, 3.0x10^6, 5.0x10^6, 7.0x10^6, and 1.0x10^7 PIB/ml for HaNPV; 0.001%, 0.003%, 0.005%, 0.01%, and 0.015% for Azadirachtin; and 0.05%, 0.15%, 0.30%, 0.50%, and 0.7% for NSKE and left for drying in the shade under fan again for 5 minutes and then placed into plastic vials.

Twigs of chickpea leaves were covered with wet cotton swab, for prevention of leaves from drying. During the month of March, 2nd instars larvae of H. armigera were kept on starvation for 24 hours and then 10 larvae were released individually in plastic vials (with a perforated lid for aeration) to avoid cannibalism. The vials were labelled properly and treatments were replicated thrice along with the control. The test larvae were allowed to feed on treated leaves for 24 hours and thereafter fresh leaves were provided to them. After removing the excreta and old food, the fresh food was given on daily basis to the test larvae. The larval mortality was observed in various treatments after one day. A larva was considered as dead if it was unable to move. The % mortality was calculated by using the following formula as suggested by Finney, 1971:

\[
\text{Per cent mortality} = \frac{\text{Number of dead larvae}}{\text{Number of tested larvae}} \times 100
\]

Thus data on mortality of H. armigera larvae were collected and subjected for statistical analyses. The compatibility test of bio-pesticides (Btk, HaNPV and B. bassiana), insecticide (Quinalphos) and bio-rationals (Azadirachtin and NSKE) as alone and in treatment combinations were accomplished at Entomological laboratory. Different treatments were applied against 2nd instars larvae of H. armigera and thereafter data on mean larval mortality (%) were recorded up to 10 days regularly.

RESULTS AND DISCUSSION
Interactive effect of bio-pesticides, bio-rationals & insecticide under laboratory conditions: Mortality after 1st day of treatment was recorded as the highest with quinalphos as 53.33%. Such results are in agreement with Bajya et al. (2015) as they concluded that the quinalphos 25% EC @ 1000 gm/ha is the most effective pesticide against the pest. Next effective treatments were ½ quinalphos + ½ NSKE (36.67%), ½ quinalphos (30%), and ½ quinalphos + ½ Azadirachtin (30%). While treatments with HaNPV, ½ HaNPV + ½ Btk, ½ HaNPV + ½ B.b., ½ HaNPV, Btk, ½ Btk + ½ B.b., Btk, B.b. exhibited no mortality. Other effective treatments showed mortality in a range from 26.67% to 10.0% (Table-2).

After 3rd day, all the treatments were found effective and significantly superior over untreated check (0.0%). Cent
percent larval mortality was registered with quinalphos treatment followed by \( \frac{1}{2} \) Quinalphos + \( \frac{1}{2} \) NSKE (66.67%), \( \frac{1}{2} \) Quinalphos + \( \frac{1}{2} \) Azadirachtin (63.33%). All other treatments showed larval mortality in a range from 56.67% to 26.67%. Ahmed et al. (2015) and Zahra et al. (2014) concluded also that the different neem insecticides had widely varying adverse effects on the fitness parameters of *H. armigera*.

After 5th day of treatment, 100% mortality was registered with quinalphos followed by \( \frac{1}{2} \) HaNPV + \( \frac{1}{2} \) Quinalphos (96.67%), \( \frac{1}{2} \) Btk + \( \frac{1}{2} \) Quinalphos (93.33%) and Btk (86.67%). This is in agreement with Chandra et al. (1999) as they reported that all the concentrations of *Bt* products had adverse effect on growth and development of *H. armigera*. Similarly, Chandrasekran et al. (2015) used different spray concentrations of *Bt* products under lab conditions and found that the growth rate of the pest was declined significantly. Other treatments showed mortality varying from 83.33% to 46.67%. The mortality increased with increase of date of exposure for all the treatments. Jahkan and Suman (2015) have also confirmed similar results after using HaNPV and quinalphos in tomato crop. This is in agreement with Sharma et al. (2015) as they reported that incidence of *H. armigera* could be reduced by using combinations of neem oil and HaNPV with narrow range of contact and systemic insecticides.

After 7th day of treatment onwards all the treatments were found significantly superior over untreated check (0.0%) and 100% mortality was registered with HaNPV, \( \frac{1}{2} \) Btk + \( \frac{1}{2} \) B.b., \( \frac{1}{2} \) B.b. + \( \frac{1}{2} \) Quinalphos, \( \frac{1}{2} \) HaNPV + \( \frac{1}{2} \) B.b., B.b., \( \frac{1}{2} \) HaNPV + \( \frac{1}{2} \) Quinalphos, \( \frac{1}{2} \) Btk + \( \frac{1}{2} \) Quinalphos, Btk, \( \frac{1}{2} \) HaNPV + \( \frac{1}{2} \) Btk and Quinalphos treatments. This is in agreement with Kumar et al. (2015) that the *H. armigera* on chickpea could be effectively managed by the combination of Btk and HaNPV. Other treatments indicated mortality rate from 96.67% to 50.0%. There was no mortality in untreated check. Such results are in conformity with Rahman et al. (2014) that the lowest pod infestation was obtained after using treatments of HaNPV and Btk.

The efficacy for all the treatment combinations (after 10th days) is arranged here in decreasing order in percentage as: HaNPV (100.0), \( \frac{1}{2} \) HaNPV + \( \frac{1}{2} \) Btk (100.0), \( \frac{1}{2} \) HaNPV + \( \frac{1}{2} \) B.b. (100.0), \( \frac{1}{2} \) HaNPV + \( \frac{1}{2} \) Quinalphos (100.0), Btk (100.0), \( \frac{1}{2} \) Btk + \( \frac{1}{2} \) B.b. (100.0), \( \frac{1}{2} \) Btk + \( \frac{1}{2} \) }
Quinalphos (100.0), B.b. (100.0), \( \frac{1}{2} \) B.b. + \( \frac{1}{2} \) Quinalphos (100.0), Quinalphos (100.0), \( \frac{1}{2} \) Btk + \( \frac{1}{2} \) NSKE (96.67), \( \frac{1}{2} \) HaNPV + \( \frac{1}{2} \) NSKE (90.0), \( \frac{1}{2} \) Btk + \( \frac{1}{2} \) Azadirachtin (90.0), \( \frac{1}{2} \) HaNPV + \( \frac{1}{2} \) Azadirachtin (86.67), \( \frac{1}{2} \) Quinalphos + \( \frac{1}{2} \) Azadirachtin (80.0), \( \frac{1}{2} \) Quinalphos + \( \frac{1}{2} \) Azadirachtin (76.67), \( \frac{1}{2} \) B.b. + \( \frac{1}{2} \) NSKE (73.33), NSKE (73.33), \( \frac{1}{2} \) Btk (66.67), \( \frac{1}{2} \) B.b. + \( \frac{1}{2} \) Azadirachtin (66.67), \( \frac{1}{2} \) Azadirachtin + \( \frac{1}{2} \) NSKE (66.67), \( \frac{1}{2} \) B.b. (63.33), Azadirachtin (63.33), \( \frac{1}{2} \) HaNPV (60.0), \( \frac{1}{2} \) Quinalphos (60.0), \( \frac{1}{2} \) NSKE (60.0) and \( \frac{1}{2} \) Azadirachtin (50.0).

REFERENCES


