Intrinsic toxicity evaluation of some newer insecticides against beetles of Holotrichia consanguinea Blanch. through adult vial test

Gajanand Nagal, VK Agrawal and AS Baloda

Abstract
A laboratory trial was conducted with seven insecticide molecules to identify the most efficacious compounds against beetles of Holotrichia consanguinea Blanch. during 2016 at RARI, Durgapura. The results of bioassay affirmed bifenthrin 10 EC as the most effective insecticide in terms of toxicity followed by fipronil + imidacloprid 80 WG, clothianidin 50 WDG, imidacloprid 17.8 SL, fipronil 80 WG, imidacloprid 600 FS and quinalphos 25 EC with LC50 values 0.08, 0.20, 0.76, 0.77, 1.44, 1.53 and 36.58 ppm, respectively. The relative toxicity of different insecticides on the basis of LC50 value to beetles by AVT demonstrated that bifenthrin 10 EC, fipronil + imidacloprid 80 WG, clothianidin 50 WDG, imidacloprid 17.8 SL, fipronil 80 WG and imidacloprid 600 FS were 457.25, 182.90, 48.14, 50.58 ppm, respectively. The relative toxicity of different insecticides on the basis of LC50 value to beetles by AVT demonstrated that bifenthrin 10 EC, fipronil + imidacloprid 80 WG, clothianidin 50 WDG, imidacloprid 17.8 SL, fipronil 80 WG and imidacloprid 600 FS were 457.25, 182.90, 48.14, 47.51, 25.40 and 23.91 times more toxic than quinalphos 25 EC.

Keywords: Holotrichia consanguinea, AVT, bifenthrin, imidaclopid

Introduction
White grub belongs to phylum Arthropoda, subphylum Uniramia, class Insecta, order Coleoptera, family Scarabaeidae and subfamily Melolonthinae. The larvae of family Scarabaeidae are recognized as pests of planted crops in many parts of the world and are almost universally known as ‘white grubs’ (Veeresh, 1988) [10]. In India, mostly the white grubs from genera Holotrichia, Brahmina, Leucopholis and Lepidiota are frequently recorded to be the major pests of crops (Kumar, 2015) [4]. Holotrichia is one of the largest genus under subfamily Melolonthinae (Misra and Chandel, 2003) [6]. In Rajasthan, mainly three species viz., Holotrichia consanguinea, Holotrichia serrata and Maladera insanabilis are identified to damage groundnut crop in their larval stages (Mathur et al., 2010) [5]. It is a polyphagous pest both in the grub and adult stage and inflicts heavy damage on various fruit/ forest trees, their nurseries, vegetables, lawns and field crops (Chandel and Kashyap, 1997) [2]. According to Yadava and Sharma (1995) [13], adults of H. consanguinea, occurring in Rajasthan, Gujarat, Uttar Pradesh and Bihar, prefer foliage of ber, neem, jamun and drumstick, sometimes causing severe defoliation. Indiscriminate use of insecticides groups like organochlorine and organophosphates has increased the problems because of their adverse effects on non-target organisms and pollution to the environment. Consequently, less persistent, safer and effective insecticides are needed which can be recommended as an alternative (Nagal and Verma, 2015) [8]. Therefore, keeping the above facts in view, the present investigation was conducted to evaluate some newer insecticides against beetles of H. consanguinea under laboratory conditions.

Materials and Methods
The experiment was carried in the laboratory of Division of Entomology, Rajasthan Agricultural Research Institute, Durgapura, Jaipur, Rajasthan during 2016. The beetles of H. consanguinea were directly collected from light trap as well as different host trees after receiving first shower of monsoon and brought to laboratory. The effectiveness of seven insecticides viz., imidacloprid 17.8 SL (Confidor), Imidacloprid 600 FS (Gaucho), Fipronil 80 WG (Jump), Fipronil + Imidacloprid 80 WG (Lesenta), Quinalphos 25 EC (Kemlox), Bifenthrin 10 EC (Talstar) and Cthionidin 50 WDG (Dantotsu) were tested against the beetles of H. consanguinea through adult vial test under laboratory conditions.
Stock solutions of different used chemicals were developed by dissolving the commercial grade samples in acetone. The chemicals in liquid formulations were measured using pipette whereas the granular formulations were weighed according to dose using electronic weighing balance. The insecticide concentrations (5 for each insecticide) were prepared from the stock solution by serial dilution in the solvent on the basis of the preliminary tests conducted to find 5-95 per cent insect mortality. The serial dilutions started from the highest to the lowest concentration. The serial dilutions were prepared using the equation:

$$C_1V_1 = C_2V_2$$

Where,

- $C_1$ = Initial concentration or Concentration of stock solution,
- $V_1$ = Volume of initial concentration,
- $C_2$ = Final concentration or Concentration of working solution,
- $V_2$ = Volume of final concentration.

The concentrations thus prepared were used to carry out Adult Vial Test (AVT) or residue film bioassay procedure which was developed by Plapp et al. (1987) [9] was used to evaluate the intrinsic toxicity of the new chemical molecules against beetles of test insect. Glass vials (20 ml) were washed with detergent and water, rinsed with acetone, and oven dried at 60ºC before treatment. The interior surface of the vials was coated with the appropriate insecticide solution by pipetting 0.5 ml of the insecticide solution into the vials using a micropipette. Vials used in the control were treated with only acetone. A residue of insecticide material was left on the interior surface of the vials after evaporation of the acetone. Vials were used the same day of treatment. One beetle was placed in insecticide treated and untreated vials. For each concentration a total of 50 beetles were used.

Mortality counts were taken after 24 hours of treatment. Moribund beetles unable to move or having uncoordinated movements were considered as dead. All assays were conducted at ambient temperature (24 °C). Corrected per cent mortalities were calculated from mortality data by using Abbott’s formula (Abbott, 1925) [1]. The corrected per cent mortality data thus obtained for different concentrations of each insecticide were subjected to probit analysis as per the method given by Finney (1971) [3]. The heterogeneity, LC50 and LC90 values and their fiducial limits were obtained for the different insecticides. The regression equations thus worked out were used for working out standard mortality response curves for each insecticide. The relative toxicity values of different insecticides by the residue film method of bioassay were worked out by taking LC50 and LC90 values of the least toxic compound as unity.

Results and Discussion

Bioassay studies carried out to determine the order of toxicity of seven insecticides following residue film method against the beetles of *H. consanguinea* on the basis of LC50 values revealed bifenthrin 10 EC, fipronil + imidacloprid 80 WG,clothianidin 50 WDG, imidacloprid 17.8 SL, fipronil 80 WG, imidacloprid 600 FS and quinalphos 25 EC to be in the decreasing order of toxicity with respective LC50 values of 0.08, 0.20, 0.76, 0.77, 1.44, 1.53 and 36.58 ppm. The adults of *H. consanguinea* when laid inside the glass vials with residue film of imidacloprid 17.8 SL at concentrations ranging from 0.25 to 4.0 ppm experienced mortality in the range of 22.0 to 86.0 per cent with 2.0 per cent mortality in control treatment where the glass vials were treated with acetone only. After correcting the mortality data using Abbott’s correction and subjecting it to probit analysis, a median lethal concentration (LC50) of 0.77 ppm was obtained. Its lower and upper fiducial limits were 0.55 and 0.98 ppm, respectively. The concentration of this insecticide required to kill 90 per cent of the test population (LC90) was estimated to be 5.18 ppm with 3.75 and 6.48 ppm being its fiducial limits (Table 1). The probit kill (Y) was linearly related with log concentration (X) by the regression equation $Y = 2.04 + 1.56X$. No significant heterogeneity was recorded in the data as the calculated chi-square ($\chi^2_{cal} = 0.31$) was less than the tabulated value ($\chi^2_{tab} = 7.82$) at 5 per cent rejection level and 3 degrees of freedom (Fig. 1.a). Similarly, results of LC values, fiducial limits, regression equation, slope of concentration-mortality curve and heterogeneity for other insecticides are shown in Table 1 and Fig. 1.b to g.

The slope of the concentration-response (mortality) curve is a measure of variability in response to treatment within the insect population tested. As the value of slope decreases, less change in response is seen per unit change in concentration of the insecticide and reverse is the case with increase in the slope. Against the beetles, highest slope (1.96) was recorded with fipronil 80 WG (Fig. 1.c), whereas, quinalphos 25 EC had the lowest slope (1.30) in concentration-mortality curve (Fig. 1.e). The present finding corroborates with the finding of Wang et al. (2005) [11] who reported LC50 value of clothianidin was lower than imidacloprid against adults of *Anoplophora glabripennis* (Coleoptera: Cerambycidae). Yadava (1981) [12] also evaluated different 17 insecticides against adults of *H. consanguinea* fed on twigs of *karonda* under laboratory conditions and observed that carbaryl 0.2 per cent was the best followed by monocrotophos 0.05 per cent and dimethoate 0.05 per cent resulting in to 100, 60 and 60 per cent mortality, respectively. Nagal and Agrawal (2019) [7] also reported that bifenthrin 10 EC was most effective insecticide in terms of toxicity against the adults of *Holotrichia consanguinea* Blanch, under laboratory condition through leaf dip method.

The relative toxicity of different insecticides to adults of *H. consanguinea* by leaf dip method is given in table 2. The relative toxicity of adults of *H. consanguinea* calculated on the basis of LC50 value by leaf dip method bifenthrin 10 EC, fipronil + imidacloprid 80 WG, clothianidin 50 WDG, imidacloprid 17.8 SL, fipronil 80 WG and imidacloprid 600 FS were 457.25, 182.90, 48.14, 47.51, 25.40 and 23.91 times more toxic than quinalphos 25 EC. At LC90 level, the order of toxicity was also in the similar trend.

The following conclusions were drawn from the present investigation:

- On the basis of LC50 values, bifenthrin 10 EC was found to be most toxic for the beetles of *H. consanguinea* under laboratory conditions through adult vial test.
- Fipronil + imidacloprid 80WG and clothianidin 50WDG were proved to be the second most effective insecticide.
- The data from this study indicate that bifenthrin 10 EC and Fipronil + imidacloprid 80 WG were most effective against *H. consanguinea* beetles at relatively low rates, so it might further be tested to manage this pest on their host trees under field conditions.
Acknowledgement
Authors are highly grateful to the Department of Science and Technology (DST), New Delhi for providing the INSPIRE fellowship to carryout and accomplish the present mission.

A. Imidacloprid 17.8 SL
B. Imidacloprid 600 FS
C. Fipronil 80 WG
D. Fipronil + Imidacloprid 80 WG
E. Quinalphos 25 EC
F. Bifenthrin 10 EC
**Table 1**: Bio-efficacy of different newer insecticides against beetles of *Holotrichia consanguinea* Blanch through adult vial test

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Concentrations used (in ppm)</th>
<th>LC50 (in ppm)</th>
<th>LC90 (in ppm)</th>
<th>Slope (b ± SE)</th>
<th>Heterogeneity (χ²cal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imidacloprid 17.8 SL</td>
<td>0.25, 0.5, 1.0, 2.0, 4.0</td>
<td>0.77 (0.55 – 0.98)</td>
<td>5.18 (3.75 – 6.48)</td>
<td>1.56 ± 0.22</td>
<td>0.31</td>
</tr>
<tr>
<td>Imidacloprid 600 FS</td>
<td>0.625, 1.25, 2.5, 5.0, 10.0</td>
<td>1.53 (1.08 – 1.98)</td>
<td>11.29 (8.26 – 14.32)</td>
<td>1.30 ± 0.21</td>
<td>0.51</td>
</tr>
<tr>
<td>Fipronil 80 WG</td>
<td>0.3125, 0.625, 1.25, 2.5, 5.0</td>
<td>1.44 (1.12 – 1.76)</td>
<td>6.47 (5.11 – 7.84)</td>
<td>1.96 ± 0.23</td>
<td>0.46</td>
</tr>
<tr>
<td>Fipronil + Imidacloprid 80 WG</td>
<td>0.0625, 0.125, 0.25, 0.5, 1.0</td>
<td>0.20 (0.15 – 0.25)</td>
<td>1.12 (0.86 – 1.38)</td>
<td>1.73 ± 0.22</td>
<td>0.51</td>
</tr>
<tr>
<td>Quinalphos 25 EC</td>
<td>12.5, 25.0, 50.0, 100.0, 200.0</td>
<td>36.58 (24.09 – 49.07)</td>
<td>361.46 (231.46 – 491.47)</td>
<td>1.30 ± 0.21</td>
<td>0.14</td>
</tr>
<tr>
<td>Bifenthrin 10 EC</td>
<td>0.003, 0.006, 0.0125, 0.025, 0.05</td>
<td>0.08 (0.05 – 0.10)</td>
<td>0.58 (0.42 – 0.75)</td>
<td>1.48 ± 0.21</td>
<td>0.38</td>
</tr>
<tr>
<td>Clothianidin 50 WDG</td>
<td>0.3125, 0.625, 1.25, 2.5, 5.0</td>
<td>0.76 (0.55 – 0.97)</td>
<td>5.06 (3.79 – 6.32)</td>
<td>1.58 ± 0.22</td>
<td>0.54</td>
</tr>
</tbody>
</table>

Heterogeneity: Significant at P= 0.05, 3df (χ²cal = 7.82); SE= Standard error;
Figures in parentheses are fiducial limits.

**Table 2**: Relative toxicity of different newer insecticides to beetles of *Holotrichia consanguinea* Blanch

<table>
<thead>
<tr>
<th>Insecticides</th>
<th>LC50 (ppm)</th>
<th>Relative toxicity according to LC50</th>
<th>LC90 (ppm)</th>
<th>Relative toxicity according to LC90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imidacloprid 17.8 SL</td>
<td>0.77</td>
<td>47.51</td>
<td>5.18</td>
<td>69.78</td>
</tr>
<tr>
<td>Imidacloprid 600 FS</td>
<td>1.53</td>
<td>23.91</td>
<td>11.29</td>
<td>32.02</td>
</tr>
<tr>
<td>Fipronil 80 WG</td>
<td>1.44</td>
<td>25.40</td>
<td>6.47</td>
<td>55.87</td>
</tr>
<tr>
<td>Fipronil + Imidacloprid 80 WG</td>
<td>0.20</td>
<td>182.90</td>
<td>1.12</td>
<td>322.73</td>
</tr>
<tr>
<td>Quinalphos 25 EC</td>
<td>36.58</td>
<td>1.00</td>
<td>361.46</td>
<td>1.00</td>
</tr>
<tr>
<td>Bifenthrin 10 EC</td>
<td>0.08</td>
<td>457.25</td>
<td>0.58</td>
<td>623.21</td>
</tr>
<tr>
<td>Clothianidin 50 WDG</td>
<td>0.76</td>
<td>48.14</td>
<td>5.06</td>
<td>71.43</td>
</tr>
</tbody>
</table>

**References**

6. Misra SS, Chandel RS. Potato white grubs in India. Technical bulletin No. 60. CPR, Shimla, India 2003, 47.