Effect of fenoxycarb, a juvenile hormone analogue, administration to the second instar larvae of rice moth, \textit{Corcyra cephalonica} Staint.

(Lepidoptera: Pyralidae)

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Abstract: The 2\textsuperscript{nd} instar larvae of \textit{Corcyra cephalonica} were exposed to 0.001, 0.005, 0.01, 0.05, 0.10, 0.50 and 1.00 ppm concentrations of fenoxycarb and their insecticidal activities were evaluated. The higher concentrations of this compound severely disrupted the metamorphosis of \textit{C. cephalonica}. The significant difference in larval mortality, pupation, pupal mortality and adult emergence in comparison to their control were observed. At 0.50 and 1.00 ppm concentrations of fenoxycarb there was 100\% suppression of adult emergence. Thus, fenoxycarb at these higher concentrations behaves as insecticides that severely hamper the normal growth, development and metamorphosis of \textit{C. cephalonica}. This juvenile hormone analogue may be used for the effective control of this pest in particular and Lepidopterous pests in general.

Key Words: Second instar larva, metamorphosis, stored cereal pest, fenoxycarb

I. Introduction

There are various species of Lepidoptera known to infest the stored cereal commodities. The rice moth, \textit{Corcyra cephalonica} (Staint.) is one of the major pests of stored cereals and cereal commodities in Asia, Africa, North America, Europe and other tropical and subtropical regions of the world. This moth was first identified and reported by Stainton [1], who named it \textit{Melissoblaptes cephalonica}. Later, Ragonot [2] gave it the generic name \textit{Corcyra}. The only recognized species of this genus is \textit{cephalonica}. Ayyar [3] made the first record of \textit{Corcyra cephalonica}. This moth is believed to be of eastern origin but has become a cosmopolitan species. Its larval stages cause serious damage to rice, gram, sorghum, maize, groundnut, cotton seeds, peanuts, linseeds, raisins, nutmeg, currants, chocolates, army biscuits and milled products [4], [5], [6], [7], [8], [9], [10]. The damage to the stored products could cause weight loss, detriment in quality and reputation.

Being a $r$-selected species, insects are capable of establishing large population within very short time which may manifest the damage more. The loss of weight due to a single larva may be small, only a few milligrams, but with populations measured in millions this would be a remarkable amount. The loss of quality, however, is very important. Therefore it becomes essential to prevent/control the insect’s infestation from the very beginning. Ordinarily, the control measures in stores are based on fumigation with chemicals like hydrogen phosphate. Residues and insect resistance are reasons for potentially limiting the use of fumigation with chemicals in the near future [11]. Now a days, alternative methods are being appreciated. One of the alternatives may be the inclusion of insect growth regulators (IGRs). These compounds are highly effective against various insects attacking stored products and other pests that have become resistant to organic insecticide. There has been a renewed interest in IGRs usage, specifically in the capacity as grain protectant treatment, surface treatments, as well as aerosol and fogging treatments in the interior of food storage structures [12]. There are three types of IGRs: juvenile hormone analogue (agonists), ecdysone agonists and chitin synthesis inhibitors [13].

IGRs with juvenile hormone activity also known as juvenile hormone analogues (JHAs), are nonpoisonous and do not bioaccumulate, therefore they generally do not persist for prolonged periods in the environment. IGRs have been shown to generally have a good margin of safety for most non-target biota, as they display a very low toxicity for humans and other mammals, are readily biodegradable (i.e., very low persistence in the environment), highly toxic to target insects, and leave no hazardous residues, making JHAs very useful in food preservation and storage [14]. Fenoxycarb (a juvenile hormone analogue) is a non neurotoxic carbamate, which was discovered in 1981 and was introduced by R Maag AG [15]. It was the first JHA compound introduced to control agricultural pests [16]. It has shown JHA activities against insects in several orders including Lepidoptera, Coleoptera,
Homoptera, Dictyoptera, Diptera, and Orthoptera [17] (Grenier and Grenier 1993), but also exhibits some non JHA-specific effects on many insects [18] (Retnakaran et al. 1985).

An experiment was conducted to evaluate the effects of fenoxycarb on the life cycle stages of rice moth, *C. cephalonica* when treated as 2nd instar larvae. The objective of this study was to determine the lethal and sublethal effects on survival and development of larvae, pupae and the effects on the growth duration and longevity of adults under laboratory conditions. Such knowledge may devise ways and means for the effective control of *C. cephalonica* in particular and lepidopterous pests in general.

II. Materials and methods

*Corycra cephalonica* (Staint.) adults were obtained from already existing laboratory stock culture maintained on normal dietary medium composed of coarsely ground jowar (*Sorghum vulgare*) mixed with 5% (w/w) powdered yeast inside large glass containers (150 mm diameter, 200 mm height) at temperature 26 ± 1°C, relative humidity (RH) 93 ± 5% and a light regime of 12 hrs light and 12 hrs darkness. Such standard culture was maintained throughout the year. From this culture whenever needed, newly emerged males and females were transferred to oviposition glass chambers (35 mm diameter, 200 mm height). Since, *C. cephalonica* individuals do not feed during their adult stage, no food was provided to them during their confinement in these vessels. Eggs laid by the females were collected and then placed in glass chambers for hatching. Fenoxycarb (C_{17}H_{19}NO_{3}), Ethyl N-[2-(4- phenoxy phenoxy) ethyl] carbamate, a non terpenoid juvenile hormone analogue, P-686N, used throughout the experiment was obtained from AccuStandard, New Haven, CT 06513, USA.

For the preparation of different concentrations of fenoxycarb in dietary media, a stock solution of known concentration of JHA was prepared by dissolving it in acetone and then adjusted via serial dilutions to achieve its required concentrations. Now required volume of different concentrations of fenoxycarb was thoroughly mixed with the required quantity of normal food (roughly ground jowar mixed with 5% w/w yeast powder) to get different desired concentrations i.e. 0.001, 0.005, 0.01, 0.05, 0.10, 0.50 and 1.00 ppm. This treated food was then air dried to eliminate completely the acetone. For control purposes, the normal food were mixed with a definite volume of acetone similar to that of JHA mixed experimental solution and then air dried in the same way.

To evaluate the toxicity of fenoxycarb on the ontogeny of *C. cephalonica* when exposed as 2nd instar larvae, freshly hatched larvae of *C. cephalonica* were allowed to feed on normal dietary medium (kept inside 250 ml beakers) for exactly 9 days. On 10th day 25, 2nd instar larvae were kept in each beaker containing 50 grams of dietary medium mixed and treated separately with different known concentration of fenoxycarb. Experiment was conducted on seven different concentrations of fenoxycarb (0.001, 0.005, 0.01, 0.05, 0.10, 0.50 and 1.00 ppm). Twenty five 2nd instar larvae were also kept on normal dietary medium. All sets of experiments were kept at the temperature, relative humidity and photophase, as mentioned earlier. After completion of developmental cycle, the percent adult emergence and percent pupal mortality was observed and on that basis percent pupation and percent larval mortality was calculated. The developmental course and external morphology of larvae, pupae and adults were also observed. Adult mortality was also noted up to 24 hrs of emergence. The corrected total mortality was calculated by Abbott’s formula [19]:

\[
\text{Corrected total mortality} = 100 \frac{\% \text{ experimental mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}}
\]

Experiments were replicated six times and values have been expressed as mean ± SEM. Student t-test was applied to observe the significance of difference from their control.

III. Results

Table 1 represents the toxicodynamic effects of fenoxycarb on the ontogeny of rice moth, *Corycra cephalonica* when exposed at 2nd instar larval stage. A significant larval mortality was obtained with the increase of fenoxycarb concentration in the diet. At 0.001 ppm concentration of fenoxycarb larval mortality was 4.00 ± 1.46% while 100% larval mortality was recorded at 1.00 ppm concentration of this compound. As the fenoxycarb concentration increases in the diet, a significant enhancement in pupal mortality occurs. 96.00 ± 1.46% pupation was recorded at 0.001 ppm concentration which decreased to 32.00 ± 4.62 % at 0.50 ppm concentration of fenoxycarb. At the same time 5.56 ± 0.89% pupal mortality was recorded at 0.001 ppm concentration of fenoxycarb, which increased to 100% at 0.50ppm concentration of this compound. A significant reduction in adult emergence was recorded following of increased concentration of fenoxycarb. At 0.001 ppm concentration of fenoxycarb 90.67 ± 1.69% adult emergence was recorded that decreased to 34.00 ± 2.68% at 0.10 ppm concentration of fenoxycarb.

Along with above mentioned facts we also found that there were delayed emergences of adults as the concentrations of fenoxycarb increased in diet. At 0.05 and 0.10 ppm many of emerged adults were abnormal.
Degree of abnormalities ranges from no morphological distinguishing clue for male and female, unfolded or twisted wings, twisted legs and abnormally long abdomen in males and too much swollen abdomen in females. Majority of abnormal adults were died within 24 hours of their emergence. However, the normal and quite healthy adults were also emerged along with abnormal ones at all concentrations where adult emergence occurred but their percentages were decreased with increased concentrations (not shown in table). It is noteworthy that females had slightly prolonged growth duration than males in control as well as in treated groups. In addition, the higher concentrations of fenoxycarb i.e. 0.01, 0.05, 0.10, 0.50 and 1 ppm produced giant larvae, supernumerary larvae, larval-pupal intermediates and abnormal pupae. We have considered those larvae as larval-pupal intermediate that were able to form cocoon but failed to form pupae inside cocoon. The abnormal larvae after a variable period of time stopped feeding and eventually died.

IV. Discussion

In the present investigation fenoxycarb, a non terpenoid juvenile hormone analogue, caused a significantly dose dependent enhancement in larval and pupal mortality and a similar associated dose dependent reduction in pupation and adult emergence of C. cephalonica when treated at the later period of 2nd instar larvae in the dietary media. Recently similar findings have reported by Chandra and Tiwari [20] against 3rd instar larvae of Ephesia cautella with methoprene at relatively higher concentration.

Fenoxycarb being a carbamate caused a very different influence on the life-span of C. cephalonica. It showed the potential for prolonging the larval stages and formation of supernumerary larvae or larval-pupal intermediates which were also achieved by Moreno et al. [21] when 0.1, 1 and 10 ppm of fenoxycarb was applied topically to Ephesia kuehniella Zell.Similarly Kostyukovsky et al. [22] reported that 0.1, 0.5, 1, and 2 ppm of pyriproxyfen (a fenoxycarb derivative) caused 100% larval mortality and prolongation of life-span of insecticide susceptible and actelic resistant strain of Tribolium castaneum when treated in food medium from egg laying. Extension of life stages of C. cephalonica, in the present investigation, corresponds to the results of [23] with S-hydropropene on oriental cockroach, [24] with R334 on Bombyx mori, [25] with pyriproxyfen on Plodia interpunctella and [26] with pyriproxyfen on Plutella xylostella. In holometabolous insects, the developmental switch between juvenile and adult forms depends on juvenile hormone (JH), a sesquiterpenoid produced by the corpora allata gland [27]. The presence of JH in pre-final larval instars ensures that the next molt, promoted by ecdysteroids, produces another, only a larger larva [28]. Parthasarathy and Palli [29] also reported that the presence of JHA during the penultimate or final instar larvae of T. castaneum blocked larval-pupal metamorphosis and induced supernumerary larval molts. At an appropriate stage, a natural drop of JH secretion permits metamorphosis. At this critical time if excess of JHA is provided to insect, it may disrupt normal developmental pathway and cause repetition of larval or pupal instars respectively [30], [31] and [32].

Due to increased percentage of larval mortality and prolongation of larval period, there were decreased percentages of pupation with increase in fenoxycarb concentration. In Lepidoptera the low concentration of juvenile hormone coupled with 20-hydroxyecdysone titles promotes larva to pupal moults [33]. Due to excess of JHA in insect body only a small percentage of larvae were able to metabolize this unusual high concentration of JH in its body and got success to reach at pupal stage in dose dependent manner. Decreased pupation along with increased pupal mortality, with increase in concentration of fenoxycarb was also achieved by Moreno et al. [21] on E. kuehniella and Liu and Chen [34] on Chrysoperla rufilabris. Application of IGRs often results in pupal mortality either by direct treatment reported by Soltani et al. [35] or by larval treatment. At the beginning of the pupal stage of holometabolous insects, there is an additional JH-sensitive period for pupal versus adult determination that JH must be absent in epidermal cell obligated to adult development [28]. Hence, the presence of JHA at this critical time, resulted in the production of deformed pupae and adults of C. cephalonica. We found the reduced percentage of emerged adults with increased concentration of fenoxycarb. Fenoxycarb also caused abnormalities in adults such as formation of larvid adult or adults with twisted wings and twisted legs. However the normal and quite healthy adults were also emerged along with abnormal ones at all concentrations where adult emergence occurred but there percentages were decreased with increased concentrations. Abnormalities in adults and decreased adult longevity due to treatment with JHA were also reported by Ghasemi et al. [25] for P. interpunctella. At 0.50 and 1.00 ppm concentrations there was 100% reduction of adult emergence of C. cephalonica. Thind and Edwards [36] also achieved 100% reduction of adult emergence of insecticide susceptible and resistant strains of T. castaneum, Cryptolestes ferrugineus and Oryzaephilus surinamensis at 1 ppm dose level of fenoxycarb, when treated as 24 hrs old larvae. The larvae of C. cephalonica is the most important stage in damaging commodities (as it is the only feeding stage of C. cephalonica and the extension of its development and production of giant larvae would certainly result in more food being consumed. But, at 0.50 and 1 ppm concentration fenoxycarb caused 100% inhibition of the occurrence of adults of this pest. These findings suggest
that fenoxycarb may be considered as a leading compound for the control of rice moth, *C. cephalonica* in particular and Lepidoptera pest in general.

References


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Table 1: Effect of fenoxycarb on the ontogeny of rice moth, Corcyra cephalonica exposed as 2\textsuperscript{nd} instar larvae

<table>
<thead>
<tr>
<th>Fenoxycarb concentration (ppm)</th>
<th>Percent(^a) larval mortality</th>
<th>Percent(^d) pupation</th>
<th>Percent(^e) pupal mortality</th>
<th>Percent(^a) adult emergence</th>
<th>Percent(^d) adult mortality</th>
<th>Percent(^a) total mortality</th>
<th>Corrected(^*) total mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.67 ± 1.33</td>
<td>97.33 ± 0.93</td>
<td>1.37 ± 0.84</td>
<td>95.33 ± 1.23</td>
<td>-</td>
<td>4.00 ± 1.03</td>
<td>-</td>
</tr>
<tr>
<td>0.001</td>
<td>4.00 ± 1.46</td>
<td>96.00 ± 1.46</td>
<td>5.56 ± 0.89(^b)</td>
<td>90.67 ± 1.69(^c)</td>
<td>-</td>
<td>9.33 ± 1.69</td>
<td>5.56 ± 1.76</td>
</tr>
<tr>
<td>0.005</td>
<td>6.00 ± 0.89(^d)</td>
<td>94.00 ± 0.89(^d)</td>
<td>13.48 ± 1.74(^e)</td>
<td>81.33 ± 1.98(^f)</td>
<td>-</td>
<td>18.67 ± 1.98</td>
<td>15.28 ± 2.06</td>
</tr>
<tr>
<td>0.01</td>
<td>14.67 ± 1.98(^e)</td>
<td>85.33 ± 1.98(^e)</td>
<td>24.99 ± 2.67(^d)</td>
<td>64.00 ± 2.30(^d)</td>
<td>-</td>
<td>36.00 ± 2.30</td>
<td>33.33 ± 2.41</td>
</tr>
<tr>
<td>0.05</td>
<td>22.00 ± 2.00(^e)</td>
<td>78.00 ± 1.98(^e)</td>
<td>27.35 ± 2.81(^f)</td>
<td>56.67 ± 2.81(^f)</td>
<td>17.40 ± 2.62</td>
<td>53.19 ± 2.23</td>
<td>51.23 ± 2.32</td>
</tr>
<tr>
<td>0.10</td>
<td>43.33 ± 3.78(^f)</td>
<td>56.67 ± 3.78(^f)</td>
<td>40.00 ± 3.38(^f)</td>
<td>34.00 ± 2.68(^f)</td>
<td>21.44 ± 4.25</td>
<td>73.29 ± 2.46</td>
<td>72.17 ± 2.56</td>
</tr>
<tr>
<td>0.50</td>
<td>68.00 ± 4.62(^e)</td>
<td>32.00 ± 4.62(^e)</td>
<td>100(^\circ)</td>
<td>-</td>
<td>-</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>1.00</td>
<td>100(^\circ)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

\(^#\) Values have been expressed as mean ± SEM of six replicates.
\(^a, b, c\) and \(^d\), significantly different (p < 0.001, p < 0.01, p < 0.05 and p < 0.1 respectively) from control, when \(t\)-test was applied.

Total mortality includes larval mortality, pupal mortality and adult mortality.

\(^*\)Corrected total mortality was calculated by Abbott’s formula (1925).