Biological Activity of Fenoxycarb, a Juvenile Hormone Analogue on Rice Moth, *Corcyra cephalonica* Staint. (Lepidoptera: Pyralidae)

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**Abstract:** Juvenile hormone agonist, fenoxycarb was exposed to the 3rd instar larvae of rice moth, *Corcyra cephalonica* (Staint) and their insecticidal activity was evaluated. The 3rd instar larvae were exposed to six concentrations of fenoxycarb i.e. 0.001, 0.005, 0.01, 0.05, 0.10 and 1.00 ppm. The results showed that the higher concentrations of this compound disrupt the metamorphosis of *C. cephalonica*. A significant difference in duration of growth, larval mortality, pupation, pupal mortality, adult emergence and their longevity in comparison to their control were observed. At its 1 ppm concentration, fenoxycarb caused 90.00 ± 1.71% larval mortality with a very poor pupation of 10.00 ± 1.71%. But the later get perished and hence failed to emerge. At comparatively higher concentrations i.e. 0.05, 0.10 and 1.00 ppm a dose-dependent supernumerary larvae were formed that after a variable duration of time get perished.

**Key words:** IGR - Toxicity - Insect pest - Ontogeny

**INTRODUCTION**

India is one of the largest grain producers in the world. It produces 200 million MT (metric tons) of wheat and rice annually. According to Nagpal and Kumar [1] post harvest losses in India amount to 12-16 million metric tons of food grain each year, an amount that World Bank estimates could feed one third of India’s poor. At this level considerable losses of food grain are driven by inadequate storage.

Insect infestation in stored products causes serious damage to the infested commodity. Control of insect pests is a puzzling problem since many decades. *C. cephalonica* (Staint.), commonly known as rice moth, is a severe pest of stored cereals and cereal products in Europe, Asia, Africa, North America and other tropical and subtropical regions of the world. 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 [2-9].

Although chemical pesticides are invaluable in controlling insect populations both in the field and storage, their indiscriminate use has resulted in the destruction of beneficial insects and has caused environmental hazards [10, 11, 12]. Moreover insecticide resistance has already developed in many insects which is now a great concern in post-harvest ecosystem throughout the world [13, 14]. There is a need for new alternatives to traditional insecticides used in stored product pest management [14, 15, 16, 17]. In this regard, the insect growth regulators [18] which regulate the insect population through the disruption of moulting and metamorphosis [19] have captured the interest of stored product entomologists. The first use of IGRs against stored product pests was reported by Thomas and Bhatnagar [20].

Juvenile hormones are responsible for the maintenance of the larval state, that is, programming and regulation of metamorphosis [21]. They can function as agonists or antagonists or a mixture of both with natural JHs [22]. They interfere with important biochemical mechanisms such as the secretion and transportation of natural JHs from the secretory site to the target site, degradation, excretion and feedback control [23]. They also act at the genetic level and are associated with transcription of mRNA [24], hence their biological effects are very complex and vary from one analogue to another.

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Fenoxycarb is a non neurotoxic carbamate and exhibits juvenile hormone analog activities on many insects despite being structurally dissimilar to insect juvenile hormone [25, 26]. It was the first JHA compound introduced to control agricultural pests [27]. It has shown JHA activities against insects in several orders including Lepidoptera, Coleoptera, Homoptera, Diptera and Orthoptera [28, 26], but also exhibits some non JHA-specific effects on many insects [23].

Since, there is no published report on the effects of fenoxycarb on rice moth, *C. cephalonica*, the aim of the current study was to determine the effect of fenoxycarb on developmental stages of rice moth, *C. cephalonica* to assess whether fenoxycarb exhibits JHA-specific effects or non JHA-specific effects that are of great importance in stored commodities. Such knowledge may devise ways and means for the effective control of *C. cephalonica* in particular and lepidopterous pests in general.

**MATERIALS AND METHODS**

*Insect-Corcyra 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 (R.H.) 93 ± 5% and a light regime of 12 hrs light and 12 hrs darkness. Such a standard culture was maintained throughout the year.

*Insecticide*- Fenoxycarb (C_{12}H_{19}NO_{12}), Ethyl N-[2-(4-phenoxy phenoxy) ethyl] carbamate, a non terpenoid juvenile hormone analogue, P-686N, Lot-20071 used throughout the experiment was obtained from AccuStandard, New Haven, CT 06513, USA.

**Preparation of Different Dose Levels of Fenoxycarb in Dietary Media:** For this purpose, 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 dose levels i.e. 0.001, 0.005, 0.01, 0.05, 0.10 and 1 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.

**Treatment of Larvae with Different dose Levels of Fenoxycarb:** From the laboratory culture, newly emerged males and females were transferred to oviposition glass chambers (35mm diameter, 200 mm height). Eggs laid by the females were collected and then placed in glass chambers (consisting of 250 ml beakers) for hatching.

To evaluate the toxicity of fenoxycarb on the ontogeny of rice moth, *C. cephalonica* when exposed to its 3rd instar larvae, six sets of experiments were designed. In each set seven rearing chambers consisting of (250 ml beakers) 50 gm of food were used, out of which 6 rearing chambers were possessed 0.001, 0.005, 0.01, 0.05, 0.10 and 1 ppm JHA treated food separately, where as one chamber had contained normal food, serving as control. For treatment freshly hatched larvae of *C. cephalonica* were allowed to feed on normal dietary medium for exactly 15 days. On 16th day a group of 25, 3rd instar larvae were kept in each beaker containing above mentioned concentrations in dietary media. All the six sets of experiments were kept at the temperature, relative humidity and photophase, as mentioned earlier. After emergence of moth, the percent adult emergence and pupal mortality were noted and on that basis percent pupal mortality and percent larval mortality were 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 [29].

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

Experiments were replicated six times and data, so obtained, were analyzed statistically.

The growth duration and adult longevity of males and females of *C. cephalonica* were observed and former being counted in days from the time of egg laying to adult emergence and later from the date of adult emergence till death. Straight line regression equation was applied between different concentrations and their corresponding growth duration and adult longevity.

**RESULTS**

Table 1 reveals the mortality in various developmental stages and adult emergence whereas growth duration and longevity of emerged adults of *C. cephalonica* are given in Fig. 1 and 2. The percent larval mortality during the treatment of 3rd instar larvae was 2.00 ± 0.89 in control group, which increased with
Table 1: Toxicity of fenoxycarb on the ontogeny of rice moth, Corcyra cephalonica exposed to its 3rd instar larvae

<table>
<thead>
<tr>
<th>Fenoxycarb concentration (ppm)</th>
<th>Percent(^*) larval mortality</th>
<th>Percent(^*) pupation</th>
<th>Percent(^*) pupal mortality</th>
<th>Percent(^*) adult emergence</th>
<th>Percent(^*) adult mortality</th>
<th>Percent total(^*) mortality</th>
<th>Corrected(^*) total mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>2 ± 0.89</td>
<td>98 ± 0.89</td>
<td>2.03 ± 0.91</td>
<td>96 ± 1.03</td>
<td>-</td>
<td>4 ± 1.03</td>
<td>-</td>
</tr>
<tr>
<td>0.001</td>
<td>4 ± 1.46</td>
<td>96 ± 1.46</td>
<td>4.96 ± 1.76</td>
<td>91.33 ± 2.81</td>
<td>-</td>
<td>8.67 ± 2.81</td>
<td>4.86 ± 2.93</td>
</tr>
<tr>
<td>0.005</td>
<td>5.33 ± 1.33</td>
<td>94.67 ± 1.33</td>
<td>9.16 ± 1.28(^*)</td>
<td>86 ± 1.71(^*)</td>
<td>-</td>
<td>14 ± 1.71</td>
<td>10.42 ± 1.78</td>
</tr>
<tr>
<td>0.01</td>
<td>7.33 ± 1.23(^*)</td>
<td>92.67 ± 1.23(^*)</td>
<td>13.75 ± 1.85(^*)</td>
<td>80 ± 2.53</td>
<td>-</td>
<td>20 ± 2.53</td>
<td>16.67 ± 2.63</td>
</tr>
<tr>
<td>0.05</td>
<td>11.33 ± 1.9(^*)</td>
<td>88.67 ± 1.91(^*)</td>
<td>11.90 ± 2.13(^*)</td>
<td>78 ± 1.71(^a)</td>
<td>21.35 ± 1.10</td>
<td>38.67 ± 1.97</td>
<td>36.11 ± 2.06</td>
</tr>
<tr>
<td>0.10</td>
<td>46 ± 1.71(^*)</td>
<td>54 ± 1.71(^*)</td>
<td>14.80 ± 1.83(^*)</td>
<td>46 ± 1.71(^*)</td>
<td>31.97 ± 2.94</td>
<td>68.67 ± 1.91</td>
<td>67.36 ± 1.98</td>
</tr>
<tr>
<td>1.00</td>
<td>90 ± 1.71(^*)</td>
<td>10 ± 1.71(^*)</td>
<td>100 ± 00(^*)</td>
<td>00</td>
<td>-</td>
<td>100 ± 00</td>
<td>100 ± 00</td>
</tr>
</tbody>
</table>

\(^*\) Values have been expressed as mean ± SEM of six replicates.

a and b significantly different (p = 0.001 and p < 0.05 respectively) from control, when t-test was applied.

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

Corrected total mortality was calculated by Abbot’s formula (1925).

Fig. 1: Graphic representation of effect of fenoxycarb on the growth duration of the rice moth, Corcyra cephalonica exposed to 3rd instar larvae (Mean±SEM)

Fig. 2: Graphic representation of effect of fenoxycarb on adult longevity of rice moth, Corcyra cephalonica exposed to 3rd instar larvae (Mean±SEM)

Increase in concentration of fenoxycarb in diet. At 1 ppm concentration maximum larval mortality (90.00 ± 1.71) was recorded. As the concentration of fenoxycarb increase in diet, an insignificant reduction in pupation at 0.001 and 0.005 ppm and a significant reduction in pupation at 0.01, 0.05, 0.10 and 1 ppm occurred. The minimum pupation i.e. 10.00 ± 1.71 percent was recorded at 1 ppm dose level.

At the same time percent pupal mortality was increased with increased concentration of fenoxycarb. Reduced percentages of adult emergence were observed with increase in concentration of fenoxycarb. The 100 percent suppression of adult emergence was achieved at 1 ppm, even at 0.10 ppm development of majority of insects was halted at the end of pupal stage (Table 1).
At 0.05 and 0.10 many of emerged adults were abnormal. Degree of abnormality 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 percentage were decreased with increased concentrations (not shown in table).

Figure 1 reveals the growth duration of *C. cephalonica* which was calculated as duration from egg laying to adult emergence. At 0.001 ppm fenoxycarb was not enough to significantly affect growth duration of this insect but at higher concentrations growth duration was significantly increased with increase in concentration. It is noteworthy that females had slightly prolonged growth duration than males in control as well as in 0.001, 0.005 and 0.01 ppm food medium, but there were similar growth duration for males and females at 0.05 and 0.10 ppm. It was observed that adult longevity of both male and females were decreased with increase in concentration of fenoxycarb (fig. 2). As we had not any clue for determining male or female at larval or pupal stages and no any adult was emerged at 1 ppm dose level, their average life span was 102 ± 2.66 days only.

In addition, the higher concentrations of fenoxycarb i.e. 0.05, 0.10 and 1 ppm produced giant larvae, supernumerary larvae and larval-pupal intermediates. We have considered those larvae as larval pupal intermediate that were able to form cocoon but failed to form pupae inside cocoon. These abnormal larvae after a variable period of time stopped feeding and eventually died.

**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 exposed to 3rd instar larvae.

Fenoxycarb 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.* [30] when 0.1, 1 and 10 ppm of fenoxycarb was applied topically to *Ephestia kuehniella* Zell. Kostyukovsky *et al.* [31] 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 actellic resistant strain of *Tribolium castaneum* when treated in food medium from egg laying. Extension of life stages of *C. cephalonica* corresponds to the results of Edward *et al.* [32] with S-hydroprene on oriental cockroach, Sashindran *et al.* [33] with R334 on *Bombyx mori*, Ghasemi *et al.* [34] with pyriproxyfen on *Plodia interpunctella*, Alizadeh *et al.* [35] 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 [36]. The presence of JH in pre-final larval instars ensures that the next molt, promoted by ecdysteroids, produces another, only a larger larva [37]. 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 causes replication of larval or pupal instars, respectively [38, 39, 40].

Due to increased percentage of larval mortality and prolongation of larval period, there were decreased percentages of puation with increase in concentration. In Lepidoptera the low concentration of juvenile hormone coupled with 20-hydroxyecdysone titers promotes larva to pupal moults [41]. 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 puation along with increased pupal mortality, with increase in concentration of fenoxycarb was also achieved by Moreno *et al.* [30] on *Ephestia kuehniella* and Liu and Chen [42] on *Chrysoperla rufilabris*. Application of IGRs often results in pupal mortality either by direct treatment reported by Soltani *et al.* [43] 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 [37]. 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 larvoid adults 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. [34] for P. interpunctella. At 1 ppm dose level there was 100% reduction of adult emergence of C. cephalonica. Thind and Edwards [44] 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. At 1 ppm concentration fenoxycarb completely inhibited 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.

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