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Fenoxycarb - a potent inhibitor of metamorphosis and reproduction in Rice Moth, *Corcyra cephalonica* (Stainton)

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Abstract
Six different concentrations viz. 0.025%, 0.05%, 0.1%, 0.25%, 0.5% and 1.0% of fenoxycarb were topically applied at 2µl dose to the last instar larvae of rice moth, *Corcyra cephalonica*. There was a concentration-based and time-dependent response with respect to various life cycle parameters as quantified in the present work. The larvae treated with 0.5% and 1.0% fenoxycarb did not reach the adult stage as the mortality occurred owing to direct knockdown, incomplete moulting and unsuccessful metamorphosis. At concentration 0.05% and 0.025% fenoxycarb resulted in retardation of metamorphosis and developmental rate of the surviving *C. cephalonica* larva/pupae which, during the course of developmental path, showed morphological abnormalities. The females that emerged from treated stock exhibited malformed reproductive system along with reduced fecundity and hatchability. Furthermore, when the normal eggs were contact exposed to fenoxycarb (0.025% to 1.0%) the hatching was prevented in 37.2%-57.2% eggs; thus indicating the gonadotropic action of the current juvenoid. Therefore, fenoxycarb appears to be a promising agent for the control of *C. cephalonica* with the pronounced effects on the development and reproduction.

Keywords: *C. cephalonica*, Fenoxycarb, Juvenile hormone, Metamorphosis, Insect growth regulators

1. Introduction
Rice moth, *Corcyra cephalonica*, is economically an important stored grain pest in Asia, Africa, North America and Europe [1]. 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, 3]. While feeding the larvae leave silken threads which produce dense webs containing their faecal matter and cast skin which contaminate the grains [4, 5].

The use of conventional organic insecticides to control insect pests has given rise to the problems of development of resistance and accumulation of residue in the environment with adverse ecological and health effects [6]. There is overwhelming evidence that pesticides do pose a potential risk to mammals including humans causing neurodegenerative diseases [7]. A widely used herbicide, Paraquat was found to induce neurodegenerative changes in an insect model *Drosophila melanogaster* [8].

Nowadays, alternative methods are being sought that have more selective modes of action and reduced risks for non-target organisms and the environment. In this direction, progress has been made in the last few decades with the development of natural and synthetic compounds capable of interfering with the processes of growth, development and metamorphosis of the target insects. One of the alternatives may be the inclusion of insect growth regulators (IGRs) in pest control programmes. These compounds are highly effective against various insects attacking stored products and other pests that have become resistant to organic insecticide [9] which include the use of juvenile hormone (JH) and the 20- hydroxyecdysone (20E) that regulate a large number of processes in insects, mostly metamorphosis and reproductive maturation [10]. One of the synthetic formulations of JH is the Fenoxycarb - an insect growth regulator which possesses juvenile hormone activity. It is used to control a wide variety of insect pests like fire ants, fleas, mosquitoes, moths, scale insects and insect attacking olives, cotton and fruits [11]. In many insects it interferes with development by disturbing metamorphosis, leading to supernumerary larval instars, permanent larvae, larva/adult,

larva/pupa or pupa/adult intermediates [12] and inhibitory ovarian development [13]. The present investigation is intended to detect the effects of Fenoxycarb on the *C. cephalonica* keeping in view its potent JHA properties.

2. Materials and methods

For the present work, the culture of *C. cephalonica* was maintained under laboratory condition on dietary medium composed of coarsely ground sorghum, millet and maize. Streptomycin was used as an antibiotic. The rearing jars were maintained at 26 ± 1 °C and 75 ± 5% RH. The eggs of *C. cephalonica* were obtained from Forest Research Institute, Dehradun.

Fenoxycarb, (analytical standard) was obtained from Sigma Aldrich. 0.025%, 0.05%, 0.1%, 0.25%, 0.5% and 1.0% concentrations were prepared from 1% stock solution of fenoxycarb by serial dilution in acetone. 2µl of each concentration was topically applied with the help of micropipette. Moreover, larvae were similarly treated with 2 µl acetone to serve as a parallel control for fenoxycarb.

After applying each dose of the fenoxycarb larval mortality, abnormality regarding moulting and metamorphosis were recorded. The adults which emerged after different treatments were paired. Each pair was maintained in controlled condition in separate rearing jars and also provided with food i.e. 10% honey solution soaked in cotton. Fecundity of each treated pair as well as control was recorded. Females were also dissected out within 24 hrs of emergence to expose their reproductive system. The hatched and unhatched eggs were counted and percent hatchability was recorded.

2.1 Egg bioassay

For egg bioassay 1 and 3 day old eggs were harvested using an egg collecting setup. For each concentration of fenoxycarb and control five replicates were run simultaneously. In each replicate 50 eggs were used. For the application of fenoxycarb, a thin film of desired concentration was applied on a Petri dish and the eggs were exposed to it for 10-15 seconds. Then the eggs were transferred to Petri dishes containing food and their hatching was recorded.

2.2 Statistical analysis

Statistical analysis was performed using MS-excel and SPSS (16.0 version). Mortality data were expressed as Mean±SE and data was submitted to analysis of variance (ANOVA) and mean comparison was done by using Duncan multiple range test in the same program. LC_{90} values were determined by probit analysis [14] and percent corrected mortality was calculated using Schneider-Orelli's Formula [15].

$$\text{Corrected Mortality (\%)} = \frac{(\text{Mortality \% in treated plot} - \text{Mortality \% in control plot})}{100 - \text{Mortality \% in control plot}} \times 100$$

3. Results

3.1 Effects on survival rate and mortality

The larval mortality, formation of supernumerary larvae and larval-pupal mosaics by topical application of different concentrations of fenoxycarb on last instar larvae of *C. cephalonica* is shown in Table 1. At 1.0% fenoxycarb 91% larval mortality was recorded after 72 hours and at 0.025% concentration 45% larval mortality occurred and larval mortality in control was recorded as 3%. The regression between concentration and percent larval corrected mortality yields a polynomial correlation (Fig. 1). The same trend was also reflected in pupal mortality. Fig. 2 shows normal larva (A) and morphological malformations (B and C) in last larval instars of *C. cephalonica* treated with different concentrations of fenoxycarb.

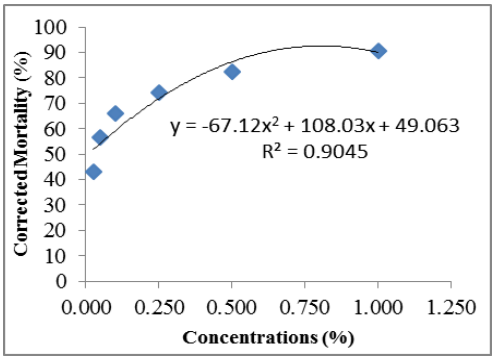

Fig 1: Larval corrected mortality at different concentrations of Fenoxycarb on the last larval instar of *C. cephalonica.*

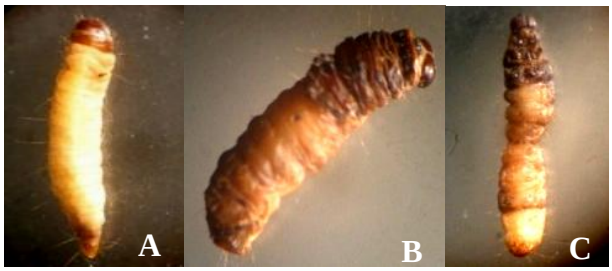

Fig 2: Normal Larva (A) and morphological malformation in last larval instar of *C. cephalonica* treated with different concentrations of fenoxycarb (B and C).

At 1.0% fenoxycarb 100% mortality occurred before pupa formation. Pupae formed at 0.5% concentration did not transform into adults and died in the pupal stage only. At lower concentrations and in control pupae successfully moulted into adults. At 1.0% concentration no adult emergence took place and 0.025% concentration resulted in 78.12% production of adult, out of which 32% were malformed.

3.2 Morphogenetic effects

The treated last instar larvae underwent unsuccessful moulting leading to delayed toxic effects (Table 1). In addition to larval and pupal mortality caused due to acute toxicity several developmental defects were also observed such as formation of supernumerary larvae, larval-pupal mosaics, pupal-adult mosaics.

The supernumerary larvae were identified as being generally larger with prolonged larval developmental duration as compared to that of normal larvae. These larvae did not form pupae and eventually died after spending a long duration in the larval stage (Fig. 3). The regression between concentration and percent formation of supernumerary larvae yields a polynomial correlation (Fig. 4).

Fig 3: Supernumerary larvae of *C. cephalonica* treated with different concentrations of Fenoxycarb**.**

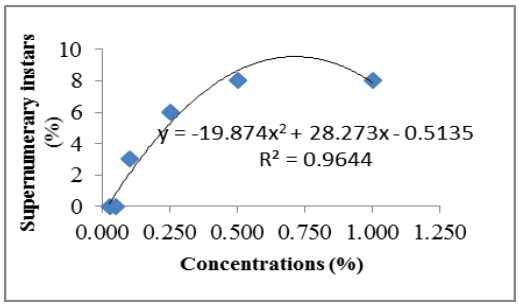

Fig 4: Supernumerary instars at different concentrations of Fenoxycarb on the last larval instar of *C. cephalonica*.

0.025% concentration of fenoxycarb produced 23% larval-pupal mosaics whereas at 1.0% concentration only 1% larval-pupal mosaics produced as most of the mortality occurred at larval and pre-pupal stages. The larval-pupal mosaic were characterized by ruptured skin, incomplete pupal case wherein some larval parts like thoracic legs were exposed while other dorsal parts were covered with pupal skin. Fig. 5 shows normal Pupa (A) Larval-pupal mosaics (B and C) and Pupal-adult mosaics (D and E).

Table 1: Larval mortality, larval mortality due to abnormal moulting and total larval mortality following the topical application of different concentrations of Fenoxycarb on last larval instars of *C. cephalonica*

Concentrations (%)	Larval Mortality (%) Mean±SE	Larval mortality due to abnormal moulting		Total Larval Mortality (%) Mean±SE
		Larvae moulted to Supernumerary instar (%) Mean±SE	Larval- pupal Mosaic (%) Mean±SE	
Control	03.00±1.22^{a}	-	-	03.00±1.22^{a}
0.025	45.00±1.58^{b}	-	23.00±1.22^{d}	68.00±1.22^{b}
0.050	58.00±2.54^{c}	-	20.00±1.30^{d}	78.00±1.30^{c}
0.100	67.00±1.22^{d}	03.00±1.04^{ab}	19.00±1.87^{d}	89.00±1.60^{d}
0.250	75.00±1.44^{e}	06.00±1.87^{bc}	15.00±1.58^{c}	96.00±1.70^{e}
0.500	83.00±2.54^{f}	08.00±1.22^{c}	08.00±1.04^{b}	99.00±0.54
1.000	91.00±1.87^{g}	08.00±0.94^{c}	01.00±0.77^{a}	100.00±00

Means in the same column followed by the same letters do not differ significantly at P <0.05 (Duncan test)
100 insects (5 replicates of 20 each) were treated at each concentration.

Fig 5: Normal Pupa (A) Larval-pupal mosaics (B and C) and Pupal-adult mosaics (D and E) of *C. cephalonica* formed at different concentrations of Fenoxycarb.

At 0.1%, 0.05%, 0.025% concentrations 9.09%, 9.09%, and 12.50% pupal-adult mosaics were formed (Table 2). In some of the mosaics, thorax and abdomen were fused and wings were underdeveloped. These mosaics died after surviving for some time. Malformed pupae did not form a proper pupal covering and failed to emerge as mature adults i.e. complete inhibition in emergence of adults occurred.

Table 2: Showing pupation, pupal mortality, pupal-adult mosaics, adult emergence and adult malformation, following the topical application of different concentrations of Fenoxycarb on last larval instars of *C. cephalonica*

Concentrations (%)	Pupae Formed (%) Mean±SE	Pupal Mortality (%)	Pupal-adult Mosaics (%) Mean±SE	Adult Emergence (%)	Malformed Adult (%)
Control	97.00±1.22^{f}	01.03	-	98.96	-
0.025	32.00±0.63^{e}	09.37	12.50	78.12	32.00
0.050	22.00±.089^{d}	18.18	09.09	72.72	37.50
0.100	11.00±1.18^{c}	36.36	09.09	54.54	66.66
0.250	04.00±1.00^{b}	75.00	-	25.00	100.00
0.500	01.00±0.77^{a}	100.00	-	-	-
1.000	-	-	-	-	-

Means in the same column followed by the same letters do not differ significantly at P <0.05 (Duncan test)

Adults with abnormalities like crumpled and totally malformed wings and a marked reduction in size were also observed. Apart from this, the deformity in legs was also noticeable. The regression between concentration and percent total larval corrected mortality yields a polynomial correlation (Fig. 6).

3.3 Total mortality upto adult emergence

Total mortality upto adult emergence following the application of different concentrations of fenoxycarb on last larval instar of *C. cephalonica* is shown in Table 3. The LC_{90} value of fenoxycarb is found to be 0.08%. The regression between concentration and percent total corrected mortality yields a

logarithmic correlation (Fig. 7).

Table 3: Total larval mortality and total mortality upto adult emergence following topical application of Fenoxycarb on *C. cephalonica*

Mortality (%)	Conc. (%)	Mortality (%) (Mean±SE)	Corrected Mortality (%)	LC_{90} (%)	Regression Equation	95% Confidence limit		Variance (F)
						Lower	Upper	
Total Larval Mortality (%)	control	03.00±1.22[a]	-	0.121	y = -71.87x² + 98.88 + 72.12 R² = 0.834	-00.40	06.40	736.5
	0.025	68.00±1.40[b]	67.01			64.07	71.93	
	0.050	78.00±1.30[c]	77.31			74.38	81.62	
	0.100	89.00±1.60[d]	88.65			84.52	93.48	
	0.250	96.00±1.70[e]	95.87			91.11	100.00	
	0.500	99.00±0.54[ef]	98.96			97.48	100.00	
	1.000	100.00±00[f]	100			100.00	100.00	
Total Mortality upto Adult Emergence (%)	control	04.00±1.30[a]	-	0.08	y = 7.031ln(x) + 104.5 R² = 0.850	00.38	07.62	789.3
	0.025	75.00±1.60[b]	73.96			70.61	79.39	
	0.050	84.00±1.37[c]	83.33			80.17	87.83	
	0.100	94.00±1.87[d]	93.75			88.81	99.19	
	0.250	99.00±1.00[e]	98.58			96.22	99.80	
	0.500	100.00±00[e]	100			100.00	100.00	
	1.000	100.00±00[e]	100			100.00	100.00	

LC_{90}= lethal concentration that kills 90% of the treated insects. Means in the same column followed by the same letters do not differ significantly at P <0.05(Duncan test)

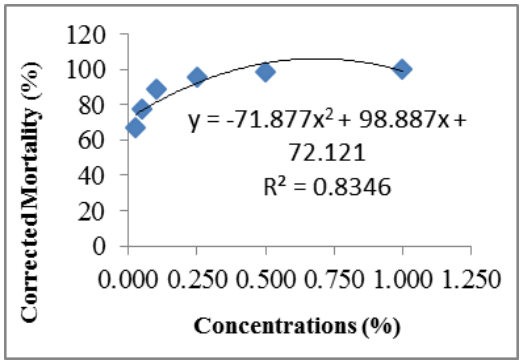

Fig 6: Total larval corrected mortality at different concentrations of Fenoxycarb on the last larval instar of *C. cephalonica.*

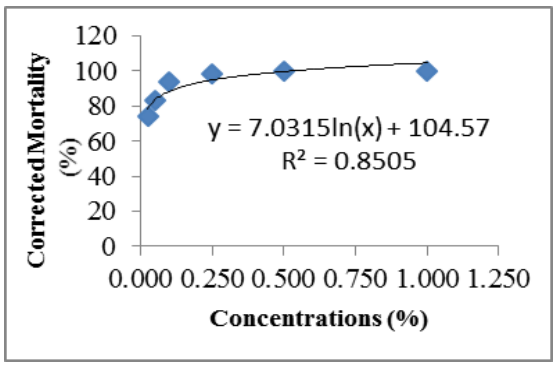

Fig 7: Total corrected mortality upto adult emergence at different concentrations of fenoxycarb on the last larval instar of *C. cephalonica*.

Fig. 8. Shows relationship between larval mortality, supernumerary instar, larval-pupal mosaic, pupation and total mortality (%) at different concentrations of fenoxycarb on the last larval instar of *C. cephalonica*.

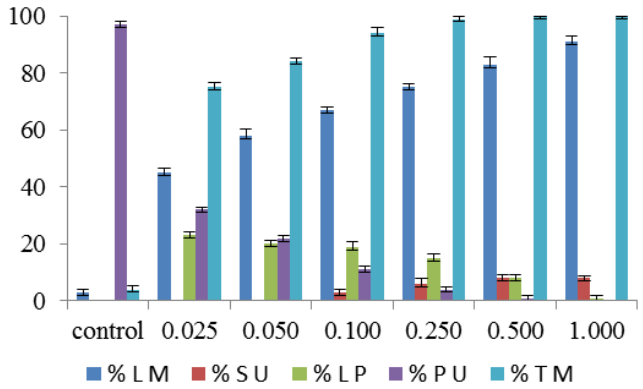

Fig 8: Relation between % larval mortality, % supernumerary larval instars, % larval pupal mosaics, % pupation and % total mortality upto adult emergence at different concentrations of Fenoxycarb on the last larval instar of *C. cephalonica*.

3.4 Effect on the ovarioles of *C. cephalonica*

The female reproductive system of *C. cephalonica* consists of a pair of ovaries. Each ovary is composed of four ovarioles, divided into apical part called germarium and basal part vitellarium that contain immature eggs. In case of control group (Fig. 9A) ovarioles showed progressive and orderly development of ova along the entire length. The affected ovarioles exhibited random, unorganized and uneven development of oocytes with several empty spaces. In many cases the immature, small ova were interposed between large ova and vice versa along the length of ovariole (Fig. 9B-D).

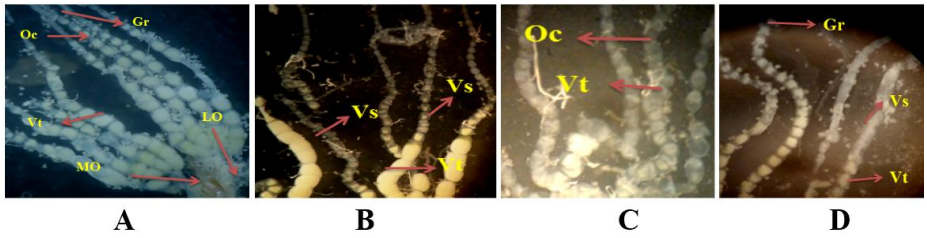

Fig 9: Ovarioles of female emerged from Control (A), 0.025% (B) 0.050% (C) and 0.100 (D) Fenoxycarb treated larvae of *C. cephalonica.*Gr = Germarium, Vt = Vitellarium, VS = Vacant Space, LO = Lateral oviduct, MO = Median oviduct and Oc = oocyte.

3.5 Egg bioassay

The effect of fenoxycarb on hatchability of different stages of eggs of *C. cephalonica* is shown in Fig. 10. Hatchability in case of one day-old eggs treated with concentrations 0.025%, 0.05%, 0.1%, 0.25%, 0.5% and 1% were 62.8%, 57.6%, 51.2%, 46.4%, 44.4%, and 42.8% respectively, and in control it was recorded as 95.2%. Significant difference was observed in percentage of eggs that hatched in control and in 0.025% concentration. When 3 day-old eggs were treated, no significant differences between treatments were observed.

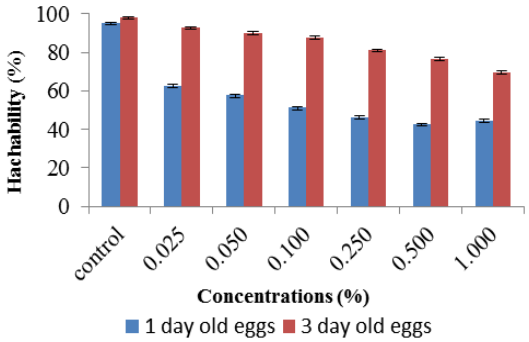

Fig 10: Percent eggs hatched at different concentrations of Fenoxycarb on the last larval instar of *C. cephalonica*.

3.6 Effects on fecundity and hatchability

At 0.050% and 0.025% concentrations adults laid an average number of 35.4±0.37 and 56.6±0.51 eggs, whereas 258.4±0.24 eggs were laid in case of control. Hatching did not take place in eggs laid by treated adults and in case of control 97.05% hatching took place (Table 4).

Table 4: Showing fecundity and hatchability of female adult emerged from the last instar larvae of *C. cephalonica* treated with Fenoxycarb

Concentrations (%)	Number of eggs laid Mean±SE	Number of eggs hatched Mean±SE	% Hatching
Control	258.4±0.24^{c}	250.8±0.37	97.05
0.025	56.6±0.51^{b}	Nil	Nil
0.050	35.4±0.37^{a}	Nil	Nil
0.100	Nil	Nil	Nil
0.250	Nil	Nil	Nil
0.500	Nil	Nil	Nil
1.000	Nil	Nil	NIL

Means in the same column followed by the same letters do not differ significantly at P <0.05 (Duncan test)

4. Discussion

In the present study we revealed the effects of Fenoxycarb on developmental parameters in *C. cephalonica.* Juvenile hormone analogues (JHAs), are widely used for the control of insect pests and are reported to kill eggs and larvae of various insect species. Fenoxycarb a potent JHA causes insects from reaching the reproductive stage. Their disruptive effect seems to be the presence of large amount of juvenoids overpowering the homeostatic effects. A second mechanism can be the imperfect mimicking ability of the JH leading to its antagonistic action leading to the disruption of the moulting and slowing down the process of development [16].

In this study fenoxycarb treated larvae showed varying degrees of inhibitory and delayed effects, pre-pupal mortality and failure to ecdyse to adult. Fenoxycarb has the potential for increasing the period of the larval stage and also results in the formation of supernumerary larvae or larval-pupal intermediates. Similar types of effects were also observed in case of administration of anti JH precocene analog (7-ethoxy 6-methoxy-2, 2-dimethyl chromene) to fifth and last larval instars of S*podoptera litura* [17]. Another category of IGR viz chitin synthesis inhibitor Flucycloxuron, (Andalin) successfully hampered the postembryonic development. The inhibition of chitin synthesis by Andalin has manifested in *Dysdercus koenigii* [18].

JHAs have profound effect on the embryogenesis of insects. They block embryonic development by inhibiting the differentiation of the embryos [19, 20], usually before or at blastokinesis [21, 22, 23]. The present result demonstrated that the application of fenoxycarb to eggs of *C. cephalonica,* resulted in reduced hatchability. The hatching rate of *C. cephalonica* eggs laid by treated female was decreased with increased concentration of fenoxycarb. Similar results have been reported when fenoxycarb tested on eggs of *C. rufilabris* [24]. Vasuki [25], Ali *et al*., [26], Su and Mulla [27] and Suman *et al*., [28] have suggested that larvicidal and ovicidal efficacies are governed by the type and concentration of different classes of IGRs. Jacob and Qamar [29] also reported the lethal and ovicidal effects of different essential oils against *C. cephalonica,* which seem to coincide with fenoxycarb.

5. Conclusion

On the basis of overall findings, it can be concluded that fenoxycarb is toxic to *C. cephalonica*, as it mimics the action of JH and maintains the insect in an immature state. This action keeps the insects from moulting successfully or reproducing normally. Fenoxycarb caused mortality in larvae and produced abnormal adults and it also affected the fecundity of the *C. cephalonica.* Thus fenoxycarb may be considered as a leading target compound having the potential to control *C. cephalonica* and can therefore form an important component of various Integrated Pest Management (IPM) programs for other such insects.

6. Acknowledgement

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