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Juvenile Hormone Analogue and Management of Rice Moth, *Corcyra cephalonica* Staint. (Lepidoptera: Pyralidae)

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Abstract: A concentration of 25 ppm of pyriproxyfen, when exposed to 1st instar larvae, caused 100% larval mortality but the same dose level when exposed to 2nd, 3rd and 4th instar larvae it resulted comparatively poor larval mortality i.e. 96.00 ± 2.83, 86.40 ± 5.58 and 46.40 ± 3.19%, respectively. In case of 1st instar larval treatment 20 ppm concentration of pyriproxyfen caused 5.60 ± 0.44% pupation but all of them get perished. At a concentration of 15 ppm of pyriproxyfen 20.00 ± 1.18% adult emergence did occur in case of 1st instar treated larvae whereas it resulted into gradually enhanced adult emergence i.e. 42.40 ± 2.19, 64.00 ± 1.21 and 73.60 ± 2.19 % in case of 2nd, 3rd and 4th instar treated larvae, respectively. It is obvious that exposure of pyriproxyfen for longer duration severely affects the growth and development of the larva in comparison to short tenure exposure of JHA. In addition, longer duration exposure of pyriproxyfen resulted in abnormal metamorphosis that produced larval-pupal and pupal-adult intermediates.

Keywords: Stored grain, Pyriproxyfen, Ontogeny, *Corcyra cephalonica*, Instar, Larva

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Introduction

In stored cereals and cereal commodities, damage by insect pests is a severe problem. *Corcyra cephalonica* Stainton is one of the most destructive pest of such cereals and cereal products reported from continents such as Asia, Africa, North America, Europe and other tropical regions causing severe economic losses (Atwal and Dhaliwal, 2008; Jhala *et al.*, 2018). While it is still spreading through the transportation of infested grains and commodities to the other regions of the world (Samodra and Ibrahim, 2006). Its larval stages cause severe damage to rice, maize, sorghum, groundnut, cotton seeds, cocoa beans and millets (Cox *et al.*, 1981; Allotey, 1991). Indiscriminate use of organic insecticides has resulted in development of resistance in many insects (Subramanyam and Hagstrum, 1995; Arthur, 1996), destruction of beneficial insects and has caused environmental hazards (Smith and Bosch, 1967; Marx, 1977; Pimental, 1983). So, there is a dire need for new alternative to traditional insecticides used in stored product pest management (Dunkel and Sears, 1998; Arthur and Phillips, 2003). In this regard, the insect growth regulators which regulate the insect population through the disruption of moulting and metamorphosis (Williams, 1956; Fox, 1990) have captured the interest of stored product entomologists. Pyriproxyfen is one of the JHAs which influences the growth and development of insect pests (Ishaaya and Horowitz, 1992) and thus prevents the arrival of the future generation. It is a pyridine-based juvenile hormone analogue that competes for juvenile hormone binding site receptors in pests, mimicking the action of juvenile hormone and thus maintaining as immature state (Sullivan and Goh, 2008). This compound has a relatively low toxicity for mammals (Miyamoto *et al.*, 1993).

The aim of the current study was to determine that how pyriproxyfen affects the growth and development of rice moth, *C. cephalonica* with special reference to its management in eco-friendly ways.

Materials and Methods

Collection and culture of rice moth, Corcyra cephalonica:

Corcyra cephalonica Stainton adults were obtained from Central Integrated Pest Management Centre, Gorakhpur 273009, Uttar Pradesh, India. A stock laboratory culture was maintained on normal dietary medium composed of coarsely ground jowar, *Sorgham 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 h light and 12 h darkness. Such a standard culture was maintainted throughout the year.

Egg laying and their hatching:

From the above culture, whenever needed, newly emerged males and females were transferred to oviposition glass chambers (35 mm diameter, 200 mm height). Since, *C. cephalonica* individuals were not fed during their adult stage, so they were not given any food during their confinement in these vessels. Eggs laid by the females were collected and then placed in glass chambers (consisting of 250 ml beakers) with the help of zero number camel hair brush for hatching.

Insect growth regulator used:

Pyriproxyfen (Fig. 1), molecular formula-$C_{20}H_{19}NO_3$, 2-[1-Methyl-2-(4-pheoxyphenoxy)-ethoxy]pyridine, an aromatic non-terpenoid juvenile hormone analogue, Catalogue Number P998850, Lot Number 2-SHE-64-1 was obtained from Toronto Research Chemicals Inc. 2 Brisbane Road, Toronto, ON, Canada, M3J 2J8.

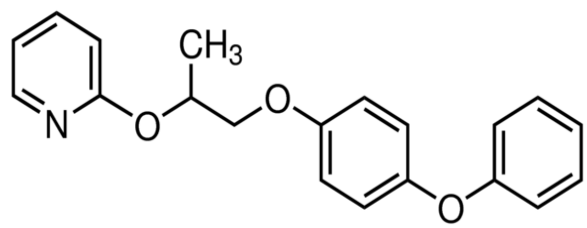

Fig. 1: Chemical structure of pyriproxyfen.

Preparation of different concentrations of pyriproxyfen in dietary media:

For the preparation of different concentrations of pyripoxyfen in dietary media, a stock solution of known concentration of pyriproxyfen was prepared in acetone and then adjusted via serial dilutions to achieve its different required concentrations in acetone. Now required volume of each concentration 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. 1, 5, 10, 15, 20 and 25 ppm of pyriproxyfen. All the six sets of experiments were kept at temperature, relative humidity and photophase, as mentioned earlier. This treated food was then air dried at room temperature to eliminate the acetone. For control purposes, the normal food was thoroughly mixed with the required volume of acetone similar to that of treated food and then air dried in the same way.

Evaluation of toxicity of pyriproxyfen against the developmental stages of C. cephalonica:

To evaluate the toxic effects of various concentrations of pyriproxyfen at different life stages of *C. cephalonica*, four sets of experiments were designed. In each set seven rearing chambers (consisting of 250 ml beakers) containing 50 g of food were used, out of which six rearing chambers contained 1, 5, 10, 15, 20 and 25 ppm pyriproxyfen treated food separately, whereas one chamber contained normal food, serving as control (untreated). In the 1st set of experiment, freshly hatched larvae of *C. cephalonica* were allowed to feed on a normal dietary medium for exactly four days and on the fifth day, a group of 25 first instar larvae were transferred to each rearing chamber (250 ml beaker) containing 50 g of dietary medium mixed and treated separately with different known concentrations of pyriproxyfen and similarly in the 2nd, 3rd and 4th sets of experiment, the second instar, third instar and fourth instar were kept respectively. All the four sets of experiments were kept at the temperature, relative humidity and photophase, as mentioned earlier. On the completion of life-cycle, per cent adult emergence and pupal mortality were observed and on that basis per cent pupation and per cent larval mortality were calculated.

Statistical analysis:

Experiments were replicated six times and values are expressed as mean ± SD. Student's t-test was applied to determine the significant differences between the treated groups and their control. LD_{50} values (µg/larva), 95% confidence limits (lower and upper confidence limits) of LD_{50}, slope values and heterogeneity of pyriproxyfen were calculated by Polo Plus, Probit and Logit Analysis, Version: 2.0, LeOra Software based on probit analysis (Finney, 1952).

Results and Discussion

Effect of pyriproyfen on larval mortality:

The present observation revealed that pyriproxyfen exposure exerts a severe effect on the developmental biology of *C. cephalonica.* As the pyriproxyfen concentration increases, larval mortality increases significantly in all the instars i.e. 1st, 2nd, 3rd and 4th instars. It was found to be 100 % in case of 1st instar which decreased to 96.00 ± 2.83, 86.40 ± 5.58 and 46.40 ± 3.19 % in 2nd, 3rd and 4th instars, respectively at 25 ppm concentration of pyriproxyfen (Table 1). Methoprene at a concentration of 10 and 100 µg caused 11.00 and 16.67% larval mortality respectively in the last larval instar of *C. cephalonica* (Deb and Chakravorty, 1985) and larvae attained extraordinary giant size without undergoing extra moult. Similar observations have also been reported by the action of different JHAs exposed to *Tribolium castaneum* (Ishaaya and Yablonski, 1976), *Tribolium confusum* (Smet *et al.*, 1989) and *Ephestia cautella* (Shaaya and Pisarev, 1986). 4th instar larvae of *Tribolium castaneum* exposed to 10 ppm concentration of pyriproxyfen also resulted 15.00 ± 0.49 % larval mortality (El-barky *et al.*, 2012). Similar observations were also reported in the larvae of *C. cephalonica* following exposure of methoprene (Tripathi and Tiwari, 2014) and fenoxycarb (Singh and Tiwari, 2014). They observed that 20 and 24 ppm concentrations of methoprene and 0.50 and 1.00 ppm concentrations of fenoxycarb behave as severe insecticidal agent for this Lepidopteran pest. Such larval mortality may be due to competing of JHA with JH in binding to the JH receptors or to the JH carrier proteins, injuring the corpora allata cells or interfering with the JH biosynthesis. Juvenile homone analogues have been shown to affect larval growth enhancing their weight and length, larval tenure and lastly lead to death failing their pupation (Ishaaya and Yablonski, 1976; Bakr and Tanani, 2018).

Effect of pyriproxyfen on pupation:

Pyriproxyfen exposure decreased per cent pupation significantly with increase in its concentration. In the present investigation, it was observed that pyriproxyfen caused age dependent reduction i.e. when treated in younger larval stage they cause higher reduction in pupation while

Table 1: Effect of pyriproxyfen on percentage of larval mortality of different larval instars of *Corcyra cephalonica* at temperature 26° ± 1°C, relative humidity (r.h.) 93 ± 5% and a light regime of 12 h light and 12 h darkness

Pyriproxyfen concentrations (ppm)	Percentage Larval Mortality			
	1^{st} instar[#]	2^{nd} instar[#]	3^{rd} instar[#]	4^{th} instar[#]
0	3.20 ± 0.92	3.20 ± 0.92	3.20 ± 0.92	3.20 ± 0.92
1	11.20 ± 1.04[a]	9.60 ± 1.02[a]	7.20 ± 1.02[a]	4.80 ± 0.66[b]
5	20.00 ± 1.66[aa']	16.00 ± 1.72[aa']	10.40 ± 1.12[ab']	7.20 ± 0.72[aa']
10	25.60 ± 3.04[aa']	22.40 ± 1.81[aa']	14.40 ± 1.22[aa']	8.80 ± 0.78[aa']
15	67.20 ± 5.14[aa']	49.60 ± 2.19[aa']	32.00 ± 2.11[aa']	22.40 ± 2.19[aa']
20	94.40 ± 6.22[aa']	88.00 ± 4.00[aa']	72.00 ± 3.82[aa']	32.00 ± 2.83[aa']
25	100	96.00 ± 2.83[aa']	86.40 ± 5.58[aa']	46.40 ± 3.19[aa']

[#]Values are expressed as the mean ± SD of six replicates. [a] and [b] Significantly different $p < 0.001$ and $p < 0.01$, respectively compared with control; [a'] and [b'] significantly different $p < 0.001$ and $P < 0.01$, respectively from the corresponding treated group when t-test was applied.

Table 2: Effect of pyriproxyfen on the percentage pupation of different larval instars of *Corcyra cephalonica* at temperature 26° ± 1°C, relative humidity (r.h.) 93 ± 5% and light regime of 12 h light and 12 h darkness

Pyriproxyfen concentrations (ppm)	Percentage Pupation			
	1^{st} instar[#]	2^{nd} instar[#]	3^{rd} instar[#]	4^{th} instar[#]
0	96.80 ± 3.35	96.80 ± 3.35	96.80 ± 3.35	96.80 ± 3.35
1	88.80 ± 3.35[a]	90.40 ± 2.19[b]	92.80 ± 1.79[b]	95.20 ± 1.79[c]
5	81.60 ± 5.37[ab']	84.00 ± 2.83[ab']	89.60 ± 2.19[bb']	92.80 ± 1.79[cb']
10	75.20 ± 3.66[aa']	77.60 ± 2.19[aa']	85.60 ± 2.19[aa']	91.20 ± 1.79[ca']
15	32.80 ± 2.35[aa']	50.40 ± 2.19[aa']	68.00 ± 2.83[aa']	77.60 ± 2.19[aa']
20	5.60 ± 0.44[aa']	12.00 ± 1.04[aa']	28.00 ± 2.83[aa']	68.00 ± 2.83[aa']
25	00	4.00 ± 0.41[aa']	13.60 ± 1.04[aa']	53.60 ± 2.19[aa']

[#] Values are expressed as the mean ± SD of six replicates. [a, b] and [c] Significantly different $p < 0.001$, $p < 0.01$ and $p < 0.05$, respectively compared with control; [a'] and [b'] significantly different $p < 0.001$ and $p < 0.01$, respectively from the corresponding treated group when t-test was applied.

when treated in older stage they showed comparatively poor reduction in pupation. Most pronounced impact of this JHA was found in 1^{st} instar larvae treated with highest concentration of its 25 ppm but the same concentration has poor effect in 4^{th} instar larvae which was recorded to be 53.60 ± 2.19. (Table 2). Similarly, Loschiavo (1977) reported that 20 ppm of methoprene and hydroprene prevented pupation of *Tribolium castaneum* and substantially reduced the adult emergence in *T. confusum.* Aribi *et al.* (2006) also observed that when the JHA, pyriproxyfen was applied to newly ecdysed pupae of *T. molitor*, the ecdysteroid hemolymph titers were inhibited significantly. Thus, *Tenebrio in vivo* and *in vitro* showed that pyriproxyfen caused a blockage of ecdysteroid production. These observations indicate that as the percentage of larval mortality and prolongation of larval period increased, there were decreased percentages of pupation with increase in JHA concentration. Due to excess of JHA in the 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 (Singh and Tiwari, 2014). Elevated level of JHA in larval body suppresses the activity of natural ecdysone hormone consequently ecdysis of the larva does not occur (Slama *et al.*, 1974).

Effect of pyriproxyfen on pupal mortality:

Pyriproxyfen induced severe stress were observed in the growth and development of *C. cephalonica.* At higher concentration 20 ppm of pyriproxyfen

Table 3: Effect of pyriproxyfen on the percentage pupal mortality of different larval instars of *Corcyra cephalonica* at temperature 26° ± 1°C, relative humidity (r.h.) 93 ± 5% and a light regime of 12 h and 12 h darkness

Pyriproxyfen concentrations (ppm)	Per cent Pupal Mortality			
	1^{st} instar[#]	2^{nd} instar[#]	3^{rd} instar[#]	4^{th} instar[#]
0	00	00	00	00
1	4.50 ± 0.24^{a}	4.40 ± 0.19^{a}	2.60 ± 0.11	00
5	8.82 ± 0.22^{a}	5.60 ± 0.34^{a}	4.40 ± 0.23	00
10	25.70 ± 2.19$^{aa'}$	17.50 ± 2.11$^{aa'}$	7.40 ± 0.52$^{aa'}$	3.50 ± 0.12$^{aa'}$
15	35.90 ± 2.93$^{bb'}$	23.10 ± 1.04$^{ab'}$	14.10 ± 0.26$^{ab'}$	5.20 ± 0.14$^{cb'}$
20	100	36.70 ± 3.45$^{aa'}$	46.70 ± 2.$^{41aa'}$	7.00 ± 0.11$^{ca'}$
25	-	100	78.30 ± 3.12$^{aa'}$	10.45 ± 0.14$^{ca'}$

[#] Values are expressed as the mean ± SD of six replicates. $^{a, b}$ and ^{c} Significantly different $p < 0.001$, $p < 0.01$ and $p < 0.05$, respectively compared with control; $^{a'}$ and $^{b'}$ significantly different $p < 0.001$ and $p < 0.01$, respectively from the corresponding treated group when t-test was applied.

Table 4: Effect of pyriproxyfen on the percentage adult emergence of different larval instars of *Corcyra cephalonica* at temperature 26º ±1°C, relative humidity (r. h.) 93 ± 5% and a light regime of 12 h light and 12 h darkness

Pyriproxyfen concentrations (ppm)	Percentage Adult Emergence			
	1^{st} instar[#]	2^{nd} instar[#]	3^{rd} instar[#]	4^{th} instar[#]
0	96.80 ± 3.35	96.80 ± 3.35	96.80 ± 3.35	96.80 ± 3.35
1	79.20 ± 5.93^{a}	86.40 ± 3.58^{b}	90.40 ± 2.19^{a}	95.20 ± 1.79^{a}
5	76.00 ± 6.32$^{ac'}$	79.20 ± 1.79$^{aa'}$	87.20 ± 1.79$^{ac'}$	92.80 ± 1.79^{a}
10	52.80 ± 3.03$^{ca'}$	57.60 ± 3.58$^{bc'}$	79.20 ± 4.38$^{ca'}$	88.00 ± 2.04$^{aa'}$
15	20.00 ± 1.18$^{aa'}$	42.40 ± 2.19$^{cb'}$	64.00 ± 1.21$^{bc'}$	73.60 ± 2.19$^{ac'}$
20	00	6.40 ± 0.44$^{cc'}$	25.60 ± 1.62$^{aa'}$	63.20 ± 1.79$^{cc'}$
25	-	00	12.00 ± 1.14$^{aa'}$	48.00 ± 2.83$^{c'}$

[#] Values are expressed as the mean ± SD of six replicates. $^{a, b}$ and ^{c} Significantly different $p< 0.001$, $p <0.01$ and $p < 0.05$, respectively compared with control; $^{a', b'}$ and $^{c'}$ significantly different $p < 0.001$, $p < 0.01$ and $p < 0.05$, respectively from the corresponding treated group when t-test was applied.

this stress led to failure of pupation leading to 100% pupal mortality in 1^{st} instar, but in 2^{nd} instar also 100% pupal mortality was observed at 20 ppm and at a highest concentration of its 25 ppm. In case of 4^{th} instar larval treatment, 1 and 5 ppm concentrations of this JHA pupal mortality did not occur (Table 3). Similar findings were also observed in Indian meal moth, *Plodia interpunctella* exposed to 0.02 ppm concentration which caused 6.25 ± 2.20 % abnormal pupae after treatment of 10-day old larvae, while this abnormality was 80.00 ± 2.20 % on the dose level of 0.3 ppm of pyriproxyfen (Ghasemi *et al.*, 2010). Pyriproxyfen, in the present investigation, showed the potential for extending life stages which is in conformity with the results of (S) hydroprene exposed *Blatta orientalis* (Edwards *et al.*, 1995) and Juvenoid, R394 treated with *Bombyx mori* (Sashindran *et al.*, 2007). The prolongation of larval and pupal duration may be due to the persistence of JH in the hemolymph where it is only in the absence of JH that ecdysone could be activated and lead to the formation of the next instar (Kuwano *et al.*, 2008). In the pupal stage, JH is not produced by the insects and only ecdysone is produced (Chapman, 1998). Therefore, by the addition of external juvenile hormone analogue during the larval period the insect is unable to

Table 5: LD_{10}, LD_{50} and LD_{90} values, Confidence limits (LCL and UCL) of LD_{10}, LD_{50} and LD_{90}, Slope Values and Heterogeneity of pyriproxyfen to the first, second, third and fourth instar larvae of *C. cephalonica*

Insecticide	Instars	Effective doses (µg/larva)		95% Confidence limits of LD_{50}		Slope values	Heterogeneity
				LCL	UCL		
Pyriproxyfen	First	LD_{10} LD_{50} LD_{90}	6.043 11.165 20.629	3.811 9.228 17.690	7.685 12.750 26.719	4.807 ± 0.496	3.73
Pyriproxyfen	Second	LD_{10} LD_{50} LD_{90}	6.696 12.878 24.768	4.077 10.805 21.074	8.553 14.519 33.492	4.807 ± 0.416	2.97
Pyriproxyfen	Third	LD_{10} LD_{50} LD_{90}	10.071 16.700 27.692	7.491 15.176 24.294	11.788 18.102 35.181	5.835 ± 0.633	2.20
Pyriproxyfen	Fourth	LD_{10} LD_{50} LD_{90}	10.129 29.601 86.506	7.281 25.278 57.340	12.070 39.562 198.485	2.752 ± 0.482	0.40

LCL = Lower Confidence Limit, UCL = Upper Confidence Limit

exude excess JH. Therefore, the presence of JHA may lead to inhibition of ecdysone production, leading to discrepancy in later development as was shown in *T. molitor* (Aribi *et al.*, 2006). Decreased pupation along with increased pupal mortality with increase in concentration of pyriproxyfen was also achieved in *Tenebrio molitor* (Aribi *et al.*, 2006) and in *Trogoderma granarium* (Swidan *et al.*, 2016). Various studies have reported increased pupal mortality with increase in its concentration as observed in case of *Ephestia kuehniella* Zell. exposed to fenoxycarb (Moreno *et al.*, 1992), *Ephestia cautella* exposed to methoprene (Chandra and Tiwari, 2013) and *C. cephalonica* exposed to fenoxycarb (Singh and Tiwari, 2014). In holometabolous insects, at the beginning of the pupal stage there is an additional JH-sensitive period for pupal versus adult determination that JH must be absent in epidermal cell obligated to adult development. Hence, the presence of JHA during this critical time, resulted in the production of deformed pupae and adults of *C. cephalonica* (Nijhout, 1994).

Effect of pyriproxyfen on adult emergence:

Pyriproxyfen significantly influenced the developmental stages of life in this moth. In 1st instar larvae, 1 ppm concentration of pyriproxyfen caused 79.20 ± 5.93% adult emergence which decreased to 20.00 ± 1.18% at 15 ppm concentration of this JHA. Adult emergence was found to be nil at 20 and 25 ppm concentrations of pyriproxyfen in 1st and 2nd instar, respectively. These concentrations are extremely fatal to the larvae of *C. cephalonica* while the same concentrations have mild effect when exposed to 3rd and 4th instar larvae (Table 4). Similarly, Ghasemi *et al.* (2010) achieved that 0.3 ppm concentration of pyriproxyfen resulted in decreased emergence of normal adults, so that some adultoids were emerged from 10-day old treated larvae of *Plodia interpunctella* which was more pronounced in 0.16 ppm concentration. These adultoids showed abnormalities in legs and wings thus, the adults were unable to move their legs and wings. Chandra and Tiwari (2013) examined that 12 and 14 ppm concentration of methoprene (JHA) against 3rd instar larvae of Almond moth, *Ephestia cautella* caused 100 % suppression of adult progeny. Our results are in conformity with methoprene and hydroprene treated *Tribolium castaneum* and *Tribolium confusum* (Loschiavo, 1977), hydroprene exposed *Tribolium castaneum* and *Tribolium confusum*

(Arthur, 2003), pyriproxyfen fed 4th instar larvae of *Tribolium castaneum* (El-barky *et al.*, 2012) and pyriproxyfen treated *Tribolium granarium* (Swidan *et al.*, 2016).

LD_{50} values (μg/larva), 95% confidence limits (lower and upper confidence limits) of LD_{50}, slope values and heterogeneity of pyriproxyfen to the first to fourth instar larvae are presented in Table 5. It is evident from the table that pyriproxyfen is more effective on the first instar larvae than second, third and fourth instar larvae.

Conclusion

The results of the present study suggest that exposure of pyriproxyfen to the early larval instar of rice moth *C. cephalonica* may lead to its successful control in eco-friendly way.

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