

VOLUME 9

ISSUE 2

2023

ISSN 2454 – 3055



**INTERNATIONAL
JOURNAL OF
ZOOLOGICAL
INVESTIGATIONS**

***Forum for Biological and
Environmental Sciences***

Published by Saran Publications, India



Pyriproxyfen Influenced Reproductive Behaviour of Rice-Moth, *Corcyra cephalonica* Staint. (Lepidoptera: Pyralidae)

Yadav Priyanka* and Tiwari Shri Krishna

Department of Zoology, D.D.U. Gorakhpur University, Gorakhpur 273009, Uttar Pradesh, India

**Corresponding Author*

Received: 29th August, 2023; **Accepted:** 26th September, 2023; **Published online:** 2nd November, 2023

<https://doi.org/10.33745/ijzi.2023.v09i02.109>

Abstract: The rice-moth, *Corcyra cephalonica* Staint. is one of the serious pests of stored cereals and cereal commodities. Application of juvenile hormone analogue, pyriproxyfen, as a safe and suitable alternative to the conventional organic insecticides, affected the growth, development and metamorphosis of this pest that exerted severe effect on their gonadal physiology and hence, their fecundity and fertility was directly influenced in a dose-dependent manner. It was observed that eggs laid by treated females crossed by treated males at 15 ppm concentration of pyriproxyfen show maximum reduction in fecundity i.e. 102.44 ± 6.81 per female and fertility i.e. $31.22 \pm 0.32\%$ in case of 1st instar exposed larvae. Fecundity and fertility was comparatively higher in adults emerged from 2nd, 3rd, and 4th instar larvae, due to their shorter exposure duration. Such finding may be highly useful in the management of *C. cephalonica* in particular and lepidopterous pests in general.

Keywords: Pyriproxyfen, Fecundity, Fertility, Sterility, *Corcyra cephalonica*, Reproductive behaviour

Citation: Yadav Priyanka and Tiwari Shri Krishna: Pyriproxyfen influenced reproductive behaviour of rice-moth, *Corcyra cephalonica* Staint. (Lepidoptera: Pyralidae). Intern. J. Zool. Invest. 9(2): 972-982, 2023.

<https://doi.org/10.33745/ijzi.2023.v09i02.109>



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Introduction

Tremendous use of conventional organic insecticides to control insect pest population has resulted into severe problems of development of physiological resistance (Daglish, 2008; Arthur *et al.*, 2009; Opit *et al.*, 2012), destruction of non-target and beneficial insects as well as environmental threats (Subramanyam and Hagstrum, 1995; Arthur, 1996; Padin *et al.*, 2002). Hence, there is requirement of a safe and suitable alternative for control in stored cereal insect pest

management. One of such alternative is the application of Insect Growth Regulators (Staal, 1972) that affect the growth, development and metamorphosis of insects by influencing their endocrine system and thus break their life-cycle resulting into non-arrival of the pest (Mishra *et al.*, 2013).

Insect pest cause severe loss in stored cereal and cereal commodities throughout the world (Phillips and Throne, 2010). The rice-moth,

Corcyra cephalonica Staint. is one of the notorious pests of stored cereals and their commodities of the world. Their larval stage is destructive and preferably feed on damaged or broken kernels resulting into heavy economic loss. In addition to feeding, they contaminate the entire storage material by their frass i.e. exuviae, egg shells, dead insects, pupal cases and fecal matters (Meena *et al.*, 2014).

Pyriproxyfen is a new juvenile hormone analogue (JHA) that acts as anti-JHs therefore artificially enhances JH levels preventing insect development to the adult stage (Leighton *et al.*, 1981). Their application leads to abnormal embryonic development and failure of pupal formation in insects (Tunaz ang Uygun, 2004). They also severely affect the egg laying capacity of adults and fertility of eggs (Chanbang *et al.*, 2008).

The objective of the present investigation was to break-down the developmental stages of the rice-moth, *Corcyra cephalonica* by influencing their reproductive behaviour through the application of pyriproxyfen so that evolution of new generation may be strictly restricted.

Materials and Methods

Collection and culture of rice-moth, *Corcyra cephalonica*:

Corcyra cephalonica Stainton adults were obtained from Central Integrated Pest Management Centre, Gorakhpur, Uttar Pradesh, India. Culture of rice-moth, *Corcyra cephalonica* was carried out by the method of (Tripathi and Tiwari, 2014).

Insect growth regulator used:

Juvenile Hormone Analogue (Fig. 1) i.e. pyriproxyfen ($C_{20}H_{19}NO_3$), 2-[1-Methyl-2-(4-phenoxyphenoxy) ethoxy] pyridine, used in this study is an aromatic non-terpenoid compound and was supplied by Toronto Research Chemicals Inc. 2 Brisbane Road, Toronto, ON Canada, M3J 2J8.

Preparation of different concentrations of pyriproxyfen in food and their evaluation of toxicity against developmental stages of *C.*

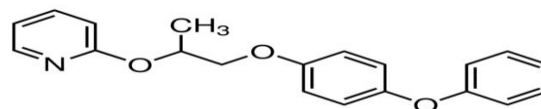


Fig. 1: Chemical structure of pyriproxyfen.

cephalonica to assess its sub-lethal doses (1, 5, 10 and 15 ppm of pyriproxyfen) that permit the larvae to grow, develop and emerge but cause considerable effect in their internal gonadal behaviour that can be easily assessed and detected to prove the effectiveness of this JHA on their fecundity, fertility and sterility, was performed by the method of Tripathi and Tiwari (2014).

Evaluation of egg laying capacity and egg hatchability:

Fecundity and fertility of different crosses at each sub-lethal doses (1, 5, 10 and 15 ppm) of pyriproxyfen were performed. The adults emerged from these sub-lethal doses were immediately sexed and used for experiments. For this purpose, four types of crosses were made as follow:

1. Normal male x Normal female (Served as control)
2. Treated male x Normal female
3. Normal male x Treated female
4. Treated male x Treated female

These pairs were allowed to mate and lay eggs in the oviposition chambers (35 mm diameter, 200 mm height glass tubes) separately. The eggs laid were collected daily till the females died and the collected eggs were transferred to hatching site (glass petri dishes of 100 mm diameter, 10 mm height) and allowed to hatch. The total number of eggs laid per pair (oviposition rate) was recorded and after their hatching the total number of hatched eggs (hatchability) was also recorded. Per cent hatching and per cent sterility were calculated on that basis. Corrected sterility was calculated by Chamberlain's formula (1962) as:

$$\text{Corrected sterility} = 100 \times \frac{A - B}{A}$$

Where, A = % hatch in control; B = % hatch in treated.

At each cross of each concentration in every instar, six pairs of males and females (each pair in separate mating/oviposition chamber) were kept for experimentation up to the concentrations where adults were available.

Statistical analysis:

Experiments were replicated six times and the values have been expressed as the mean $\pm$ SD. Student's t-test was applied to determine the significant differences from their respective control.

Results

Effect of pyriproxyfen on the egg laying capacity of first instar larval treatment:

It was observed that in control cross (Normal male x Normal female) the total egg output was 488.62 ± 6.21 / female. When normal females were crossed with the males emerged from the cultures treated with 1, 5, 10 and 15 ppm of pyriproxyfen, the total number of eggs laid per female was 380.40 ± 6.14 , 260.16 ± 4.94 , 242.51 ± 6.21 and 162.72 ± 4.62 , respectively (Table 1). When normal males were crossed with the females emerged from the cultures, treated with 1, 5, 10 and 15 ppm of pyriproxyfen, the respective number of eggs laid per female was recorded as 325.30 ± 5.24 , 208.42 ± 5.22 , 216.16 ± 6.11 and 2132.56 ± 5.82 , whereas when the crosses were made between the sexes, both emerged from the cultures treated with 1, 5, 10 and 15 ppm of pyriproxyfen concentrations, the number of eggs per female was recorded as 311.20 ± 6.12 , 170.61 ± 6.11 , 188.26 ± 4.62 and 102.44 ± 6.81 , respectively.

Effect of pyriproxyfen on egg hatchability of first instar larval treatment:

The hatchability of eggs collected from control cross was $95.78 \pm 0.34\%$. While the hatchability of eggs, collected from the crosses between normal females and males emerged from the first instar larvae exposed to 1, 5, 10 and 15 ppm concentrations of pyriproxyfen, was found to be 48.62 ± 2.45 , 44.22 ± 2.02 , 40.12 ± 1.22 and 34.66

$\pm 0.22\%$, respectively (Table 1). The hatchability of eggs, collected from the crosses between normal males and the females emerged from the cultures treated with 1, 5, 10 and 15 ppm concentrations of pyriproxyfen, was recorded to be 46.11 ± 2.13 , 43.50 ± 2.44 , 39.00 ± 0.56 and $32.41 \pm 0.31\%$, respectively. The hatchability of eggs, collected from the crosses between males and females both emerged from cultures treated with 1, 5, 10 and 15 ppm of pyriproxyfen concentrations was 42.14 ± 2.32 , 39.21 ± 0.62 , 34.40 ± 0.41 and $31.22 \pm 0.32\%$, respectively.

Effect of pyriproxyfen on the egg laying capacity of second instar larval treatment:

It was observed that in control cross (Normal male x Normal female) the total egg output was 481.45 ± 5.67 / female. When normal females were crossed with the males emerged from the cultures treated with 1, 5, 10 and 15 ppm concentrations of pyriproxyfen, the respective number of eggs laid per female was 410.15 ± 5.22 , 310.18 ± 5.25 , 258.84 ± 4.89 and 174.46 ± 4.88 , respectively (Table 2). When normal males were crossed with the females emerged from the cultures, treated with the same concentrations i.e. 1, 5, 10 and 15 ppm of pyriproxyfen, the number of eggs laid per female was recorded as 370.52 ± 4.98 , 274.42 ± 4.87 , 232.35 ± 4.67 and 161.37 ± 5.16 , respectively. When males and females, both emerged from the cultures treated with the concentrations 1, 5, 10 and 15 ppm of pyriproxyfen were crossed, the respective number of eggs laid per female was 365.63 ± 5.62 , 221.33 ± 4.18 , 210.25 ± 4.32 and 152.63 ± 4.52 , respectively (Table 2).

Effect of pyriproxyfen on egg hatchability of second instar larval treatment:

The hatchability of eggs collected from control cross was found to be $97.85 \pm 2.34\%$. The hatchability of eggs, collected from the crosses between normal females and males emerged from the cultures treated with 1, 5, 10 and 15 ppm concentrations of pyriproxyfen, was found to be 55.16 ± 1.12 , 52.42 ± 1.34 , 48.67 ± 1.39 and $41.27 \pm 1.21\%$, respectively (Table 2). The hatchability

of eggs, collected from the crosses between normal male and the females, emerged from the cultures treated with 1, 5, 10 and 15 ppm concentrations of pyriproxyfen, was recorded as 51.21 ± 1.31 , 44.72 ± 1.10 , 42.44 ± 1.18 and $34.65 \pm 1.04\%$, respectively (Table 2). The hatchability of eggs, collected from the crosses between males and females both emerged from cultures treated with 1, 5, 10 and 15 ppm concentrations of pyriproxyfen, was 46.62 ± 1.15 , 39.75 ± 1.21 , 36.15 ± 1.22 and $28.42 \pm 0.84\%$, respectively.

Effect of pyriproxyfen on the egg laying capacity of third instar larval treatment:

In third instar larval treatment, the egg laying capacity of control cross (Normal male X Normal female) was found to be 490.51 ± 7.18 / female. When normal females, were crossed with the males emerged from third instar larvae, treated with 1, 5, 10 and 15 ppm concentration of pyriproxyfen, the respective number of eggs laid per female was recorded as 430.26 ± 5.22 , 370.76 ± 4.92 , 310.57 ± 4.92 and 224.18 ± 5.11 , respectively (Table 3). When normal males were crossed with the females emerged from the cultures, treated with 1, 5, 10 and 15 ppm concentrations of pyriproxyfen, the total number of eggs laid per female was 412.14 ± 5.22 , 322.42 ± 4.16 , 268.43 ± 5.82 and 196.56 ± 4.19 , respectively (Table 3). When both the sexes used in the cross were emerged from the cultures treated with 1, 5, 10 and 15 ppm concentrations of pyriproxyfen, the respective number of eggs laid per female was recorded as 392.83 ± 3.92 , 364.61 ± 5.04 , 238.22 ± 5.82 and 173.37 ± 3.92 , respectively.

Effect of pyriproxyfen on egg hatchability of third instar larval treatment:

The hatchability of eggs collected from the crosses between normal male and normal female was $96.69 \pm 1.84\%$. The hatchability of eggs, collected from the crosses between normal females and males emerged from the cultures treated with 1, 5, 10 and 15 ppm concentrations of pyriproxyfen, was found to be 62.74 ± 1.78 , 57.12 ± 1.34 , 51.81 ± 1.11 and $49.11 \pm 2.04\%$, respectively (Table 3). The hatchability of eggs, collected from the crosses

between normal male and the females, emerged from the cultures treated with 1, 5, 10 and 15 ppm concentrations of pyriproxyfen, was observed to be 56.26 ± 1.52 , 49.26 ± 1.17 , 44.15 ± 1.32 and $37.24 \pm 1.97\%$, respectively (Table 3). The hatchability of eggs, collected from the crosses, where both sexes were emerged from the cultures treated with 1, 5, 10 and 15 ppm concentrations of pyriproxyfen, was 47.46 ± 1.61 , 40.66 ± 1.08 , 38.22 ± 1.06 and $31.12 \pm 1.99\%$, respectively.

Effect of pyriproxyfen on the egg laying capacity of fourth instar larval treatment:

It was observed that in control cross (Normal male x Normal female) the total egg output was 486.71 ± 5.46 / female. When normal females were crossed with the males emerged from the fourth instar larvae treated with 1, 5, 10 and 15 ppm concentrations of pyriproxyfen, the number of eggs laid per female was found to be 452.44 ± 5.84 , 419.41 ± 4.92 , 348.54 ± 5.18 and 242.35 ± 3.98 , respectively (Table 4). when normal males were crossed with the females emerged from the cultures, treated with 1, 5, 10 and 15 ppm concentrations of pyriproxyfen, the respective number of eggs laid per female was recorded to be 431.69 ± 5.45 , 366.26 ± 4.18 , 348.54 ± 5.18 and 242.35 ± 3.98 , respectively (Table 4). When both the sexes used in the cross were emerged from the cultures treated with 1, 5, 10 and 15 ppm concentrations of pyriproxyfen, the respective number of eggs laid per female was observed as 402.92 ± 4.98 , 312.73 ± 4.42 , 177.22 ± 4.61 and 187.86 ± 3.54 , respectively.

Effect of pyriproxyfen on egg hatchability of fourth instar larval treatment:

The hatchability of eggs collected from control cross was found to be $96.67 \pm 2.18\%$. The number of eggs collected from the crosses between normal females and the males emerged from fourth instar larvae, treated with 1, 5, 10 and 15 ppm concentrations of pyriproxyfen, was recorded to be 70.24 ± 1.14 , 68.41 ± 1.72 , 60.65 ± 1.62 and $58.26 \pm 1.32\%$, respectively (Table 4). The hatchability of eggs, collected from the crosses between normal male and the females, emerged

Table 1. Effect of pyriproxyfen on egg laying capacity and their hatchability of *C. cephalonica* as first instar larvae

Pyriproxyfen concentration (ppm)	Crossing sets	Fecundity [#] (Eggs laid/female)	Fertility [#] (Eggs hatched)	% Hatchability [#]	% Observed sterility [#]	% Corrected sterility [*]
Control	N ♂ x N ♀	488.62 ± 6.21	468.00 ± 4.26	95.78 ± 0.34	4.22 ± 0.34	-
1	T ♂ x N ♀	380.40 ± 6.14 ^a	184.95 ± 4.14	48.62 ± 2.45 ^a	51.40 ± 1.62	49.23 ± 1.61
	N ♂ x T ♀	325.30 ± 5.24 ^a	149.99 ± 3.24	46.11 ± 2.13 ^a	53.89 ± 1.42	51.86 ± 1.41
	T ♂ x T ♀	311.20 ± 6.12 ^a	131.14 ± 3.61	42.14 ± 2.32 ^a	57.86 ± 1.84	56.00 ± 0.84
5	T ♂ x N ♀	260.16 ± 4.94 ^a	115.04 ± 2.84	44.22 ± 2.02 ^a	55.78 ± 2.32	53.83 ± 1.31
	N ♂ x T ♀	208.42 ± 5.22 ^a	90.66 ± 3.34	43.50 ± 2.44 ^a	56.50 ± 2.61	54.58 ± 1.21
	T ♂ x T ♀	170.61 ± 6.11 ^a	66.90 ± 2.15	39.21 ± 0.62 ^a	60.79 ± 2.44	59.06 ± 1.44
10	T ♂ x N ♀	242.51 ± 6.21 ^a	97.30 ± 3.62	40.12 ± 1.22 ^a	59.88 ± 2.18	58.11 ± 0.94
	N ♂ x T ♀	216.16 ± 5.11 ^a	84.30 ± 4.11	39.00 ± 0.56 ^a	61.00 ± 2.84	59.28 ± 1.68
	T ♂ x T ♀	188.26 ± 4.31 ^a	64.76 ± 3.14	34.40 ± 0.41 ^a	65.60 ± 2.71	64.08 ± 1.82
15	T ♂ x N ♀	162.72 ± 4.62 ^a	56.40 ± 2.11	34.66 ± 0.22 ^a	65.34 ± 2.34	63.81 ± 2.14
	N ♂ x T ♀	132.56 ± 5.82 ^a	42.96 ± 2.14	32.41 ± 0.31 ^a	67.59 ± 2.18	66.16 ± 2.12
	T ♂ x T ♀	102.44 ± 6.81 ^a	30.96 ± 2.62	31.22 ± 0.32 ^a	69.78 ± 2.31	68.45 ± 1.58

[#] Values are expressed as mean ± SD of six replicates, and significantly different p < 0.001 compared with controls when t-test was applied.

^{*} Calculated by Chamberlain's formula (1962).

Table 2. Effect of pyriproxyfen on the egg laying capacity and their hatchability of *C. cephalonica* exposed as second instar larvae

Pyriproxyfen concentration (ppm)	Crossing sets	Fecundity [#] (Eggs laid/female)	Fertility [#] (Eggs hatched)	% Hatchability [#]	% Observed sterility [#]	% Corrected sterility [*]
Control	N ♂ x N ♀	481.45 ± 5.67	471.00 ± 5.18	97.85 ± 2.34	2.15 ± 0.36	-
1	T ♂ x N ♀	410.15 ± 5.22 ^a	226.24 ± 4.52	55.16 ± 1.12 ^a	44.84 ± 2.34	43.65 ± 1.52
	N ♂ x T ♀	370.52 ± 4.98 ^a	189.74 ± 4.37	51.21 ± 1.31 ^a	48.79 ± 1.96	47.66 ± 1.36
	T ♂ x T ♀	365.63 ± 5.62 ^a	170.45 ± 4.84	46.62 ± 1.15 ^a	53.38 ± 3.11	52.36 ± 2.18
5	T ♂ x N ♀	310.18 ± 5.25 ^a	162.59 ± 3.84	52.42 ± 1.34 ^a	47.28 ± 2.04	46.43 ± 1.58
	N ♂ x T ♀	274.42 ± 4.87 ^a	122.72 ± 4.16	44.72 ± 1.10 ^a	55.28 ± 2.18	54.30 ± 1.92
	T ♂ x T ♀	221.33 ± 4.18 ^a	85.76 ± 3.34	39.75 ± 1.21 ^a	61.25 ± 2.68	60.39 ± 2.14
10	T ♂ x N ♀	258.84 ± 4.89 ^a	125.98 ± 3.54	48.67 ± 1.39 ^a	51.33 ± 2.20	50.26 ± 2.32
	N ♂ x T ♀	232.35 ± 4.67 ^a	98.60 ± 2.19	42.44 ± 1.18 ^a	57.56 ± 2.52	56.63 ± 2.48
	T ♂ x T ♀	210.25 ± 4.32 ^a	76.00 ± 2.84	36.15 ± 1.22 ^a	63.85 ± 2.38	63.56 ± 2.72
15	T ♂ x N ♀	174.46 ± 4.88 ^a	71.99 ± 3.16	41.27 ± 1.21 ^a	58.73 ± 3.14	57.82 ± 1.75
	N ♂ x T ♀	161.37 ± 5.16 ^a	55.91 ± 2.14	34.65 ± 1.04 ^a	65.35 ± 3.68	64.59 ± 1.62
	T ♂ x T ♀	152.63 ± 4.52 ^a	43.38 ± 2.22	28.42 ± 0.84 ^a	71.58 ± 2.94	70.96 ± 2.14

[#] Values are expressed as mean ± SD of six replicates, and significantly different p < 0.001 compared with controls when t-test was applied.

^{*} Calculated by Chamberlain's formula (1962).

Table 3. Effect of pyriproxyfen on the egg laying capacity and their hatchability of *C. cephalonica* exposed as third instar larvae

Pyriproxyfen concentration (ppm)	Crossing sets	Fecundity [#] (Eggs laid/female)	Fertility [#] (Eggs hatched)	% Hatchability [#]	% Observed sterility [#]	% Corrected sterility [*]
Control	N ♂ x N ♀	490.51 ± 7.18	474.26 ± 5.18	96.69 ± 1.84	3.31 ± 0.39	-
1	T ♂ x N ♀	430.26 ± 4.84 ^a	269.95 ± 4.68	62.74 ± 1.78 ^a	37.26 ± 1.12	35.11 ± 0.52
	N ♂ x T ♀	412.14 ± 5.22 ^a	231.87 ± 4.99	56.26 ± 1.52 ^a	43.74 ± 1.04	41.81 ± 0.68
	T ♂ x T ♀	392.83 ± 3.92 ^a	186.44 ± 5.04	47.46 ± 1.61 ^a	52.54 ± 1.22	50.92 ± 1.12
5	T ♂ x N ♀	370.76 ± 4.92 ^a	211.78 ± 3.98	57.12 ± 1.34 ^a	42.88 ± 1.32	40.92 ± 1.33
	N ♂ x T ♀	322.42 ± 4.16 ^a	158.82 ± 4.62	49.26 ± 1.17 ^a	50.74 ± 1.16	49.05 ± 0.94
	T ♂ x T ♀	364.61 ± 5.04 ^a	107.59 ± 3.44	40.66 ± 1.08 ^a	59.34 ± 1.44	57.94 ± 1.12
10	T ♂ x N ♀	310.57 ± 4.92 ^a	160.90 ± 3.52	51.81 ± 1.11 ^a	48.19 ± 0.96	46.42 ± 2.18
	N ♂ x T ♀	268.43 ± 5.82 ^a	118.51 ± 3.18	44.15 ± 1.32 ^a	55.85 ± 0.48	54.34 ± 1.84
	T ♂ x T ♀	238.22 ± 4.62 ^a	91.05 ± 2.55	38.22 ± 1.06 ^a	61.78 ± 1.21	60.47 ± 0.96
15	T ♂ x N ♀	224.18 ± 5.11 ^a	110.09 ± 2.42	49.11 ± 2.04 ^a	50.89 ± 0.84	49.21 ± 1.58
	N ♂ x T ♀	196.56 ± 4.19 ^a	73.20 ± 3.00	37.24 ± 1.97 ^a	62.76 ± 0.92	61.49 ± 0.44
	T ♂ x T ♀	173.37 ± 3.92 ^a	53.95 ± 2.92	31.12 ± 1.99 ^a	68.88 ± 1.32	67.81 ± 0.96

[#] Values are expressed as mean ± SD of six replicates, and significantly different p < 0.001 compared with controls when t-test was applied.

^{*} Calculated by Chamberlain's formula (1962).

Table 4. Effect of pyriproxyfen on the egg laying capacity and their hatchability of *C. cephalonica* exposed as fourth instar larvae

Pyriproxyfen concentration (ppm)	Crossing sets	Fecundity [#] (Eggs laid/female)	Fertility [#] (Eggs hatched)	% Hatchability [#]	% Observed sterility [#]	% Corrected sterility [*]
Control	N ♂ x N ♀	486.71 ± 5.46	470.52 ± 4.18	96.67 ± 2.18	3.33 ± 0.42	-
1	T ♂ x N ♀	452.44 ± 5.84 ^a	317.79 ± 4.12	70.24 ± 1.14 ^a	29.76 ± 0.86	27.34 ± 1.12
	N ♂ x T ♀	431.69 ± 5.45 ^a	263.98 ± 4.06	61.15 ± 1.42 ^a	38.85 ± 0.52	36.74 ± 1.61
	T ♂ x T ♀	402.92 ± 4.98 ^a	212.22 ± 3.94	52.67 ± 1.86 ^a	47.33 ± 0.39	45.52 ± 1.44
5	T ♂ x N ♀	419.41 ± 4.92 ^a	286.92 ± 4.92	68.41 ± 1.72 ^a	31.59 ± 0.41	29.23 ± 1.35
	N ♂ x T ♀	366.26 ± 4.18 ^a	216.64 ± 4.64	59.15 ± 1.47 ^a	40.85 ± 0.88	38.81 ± 1.78
	T ♂ x T ♀	312.73 ± 4.42 ^a	150.95 ± 3.85	48.27 ± 1.55 ^a	51.73 ± 1.52	50.07 ± 2.04
10	T ♂ x N ♀	348.54 ± 5.18 ^a	211.39 ± 3.65	60.65 ± 1.62 ^a	39.35 ± 1.34	37.26 ± 0.96
	N ♂ x T ♀	206.17 ± 4.14 ^a	102.69 ± 2.84	49.81 ± 1.54 ^a	50.19 ± 1.12	48.47 ± 1.14
	T ♂ x T ♀	177.22 ± 4.61 ^a	79.24 ± 2.52	44.71 ± 0.86 ^a	55.29 ± 1.96	53.75 ± 1.38
15	T ♂ x N ♀	242.35 ± 3.98 ^a	141.19 ± 3.18	58.26 ± 1.32 ^a	41.74 ± 0.84	39.73 ± 1.52
	N ♂ x T ♀	223.27 ± 4.06 ^a	116.41 ± 3.44	52.14 ± 1.14 ^a	47.86 ± 0.55	46.06 ± 1.48
	T ♂ x T ♀	187.86 ± 3.54 ^a	73.77 ± 2.41	39.27 ± 1.21 ^a	60.73 ± 1.06	59.38 ± 1.96

[#] Values are expressed as mean ± SD of six replicates, and significantly different p < 0.001 compared with controls when t-test was applied. ^{*} Calculated by Chamberlain's formula (1962).

from the cultures treated with 1, 5, 10 and 15 ppm concentrations of pyriproxyfen, was observed to be 61.15 ± 1.42, 59.15 ± 1.47, 49.81 ± 1.54 and 52.14 ± 1.14%, respectively (Table 4). The

hatchability of eggs, collected from the crosses between males and females both emerged from cultures treated with 1, 5, 10 and 15 ppm concentrations of pyriproxyfen, was found to be

52.67 ± 1.86, 48.27 ± 1.55, 44.71 ± 0.86 and 39.27 ± 1.21%, respectively.

It deserves mention that the data given in Tables 1 to 4 indicated that the egg laying capacity (fecundity) and hatchability (fertility) is low when both the sexes of pair were treated. However, the rate of fecundity and fertility was decreased when only female of the pair was treated, in comparison to those crosses where only male of the pair was treated. It was observed that at least one treated adult of the pair either male or female will cause the pair had low fecundity and fertility. In addition, it is also noticeable that in the present investigation fecundity as well as fertility of the eggs was lowest in the first instar larval treatment which gradually increased in second, third and fourth instar larval treatments. Lowest fecundity as well as fertility, in the first instar treated larvae, was of course due to their lengthy tenure of exposure to pyriproxyfen. In the successive instars (second, third and fourth instars) the tenure of pyriproxyfen exposure decreased and hence, their fecundity and fertility were less suppressed in comparison to first instar exposed larvae.

Discussion

Findings of the present investigation suggest that pyriproxyfen (a JHA) caused an adverse effect on the reproductive behaviour of rice-moth, *Corcyra cephalonica*. It was observed that as the concentration of pyriproxyfen increases, a significant reduction occur in egg laying capacity of *C. cephalonica* in each instars that is 1st, 2nd, 3rd and 4th capacity of *C. cephalonica* (Tables 1-4). It further deserves mention that highest adverse effect on fecundity was observed when treated females was crossed with treated males both emerged from 15 ppm concentration of pyriproxyfen in 1st instar exposed larvae. Fecundity in *C. cephalonica* adults emerged from 2nd, 3rd and 4th instar pyriproxyfen exposed larvae show comparatively mild effect possibly due to their decrease in duration of pyriproxyfen exposure period which is longer in 1st instar and comparatively shorter in 2nd, 3rd and 4th instar larval treatment. Similar affirmation have been

reported in *T. castaneum* and *T. confusum* following treatment with concentrations of methoprene (Loschiavo, 1976) and *R. dominica* (Daglish and Palvirenti, 1998; Chanbang *et al.*, 2008). Pyriproxyfen induced reduction in fecundity of *Plodia interpunctella* has also been recorded when their 10-day old larvae were exposed to different concentrations of pyriproxyfen (Ghasemi *et al.*, 2010). They reported that lower concentration that is 0.02 ppm of pyriproxyfen showed mild reduction (261.5 ± 2.38) in fecundity while its higher concentration showed comparatively higher reduction in fecundity and its highest concentration that is 0.3 ppm caused severe adverse effect on its reproductive behaviour resulting into very poor fecundity, which is only 47.8 ± 2.38 in comparison to 294.7 ± 2.38 in control.

Other JHAs, like fenoxycarb (Singh and Tiwari, 2014) and methoprene (Tripathi and Tiwari, 2014) have also been reported to decrease the fecundity and fertility rate with increase in their concentrations exposed to all the instars. There are several mechanisms by which JHA could have suppressed egg production in insects. JHAs exposure to males disrupt spermatogenesis and functioning of accessory glands in some insects and the degree to which the target tissue is affected differs with the species (Dumser and Daney, 1974). Studies suggest that JHAs affect the development of oviducts in female (Koeppe *et al.*, 1985), follicular growth (Koeppe *et al.*, 1980), oocyte maturation (Koeppe *et al.*, 1985) and also the functioning of accessory glands (Koeppe *et al.*, 1985).

JHAs treatment produced severe disorders in the ovaries of *T. molitor* including cell death in the germarium, resorption of oocytes in the previtellarium and vitellarium, formation of compound egg chambers and undue proliferation of follicular cells sometimes resulting in malformation of the whole ovaries (Metwally *et al.*, 1972). It was observed that high dose levels of pyriproxyfen may damage the reproductive

system of female *S. litura*, resulting in the reduced viability of their eggs and also inhibit the production of JH and interrupt the uptake of vitellogenin by oocytes, resulting in oosorption (Xu *et al.*, 2015). These investigations show that decrease in ovarian growth and oogenesis is responsible for the decline in fecundity. JHAs affect morphology of genitalia also (DeVries and Brown, 1977).

Hatchability in the present study also followed the same pattern as that of fecundity, that is the lowest fertility was obtained in the crosses between normal females and treated males (Tables 1-4). The present findings revealed a dose-dependent inhibitory effect of pyriproxyfen in the fertile eggs of *C. cephalonica*.

Reduced hatching of eggs were reported El-barkey *et al.* (2012) in *Tribolium castaneum* when treated with pyriproxyfen. Similarly, Ali *et al.* (2020) observed that when *Tribolium castaneum* and *Tribolium confusum* were exposed to IGRs treated diet the pyriproxyfen was the most effective IGR in suppressing the egg hatching. Most IGRs are potential ovicides and are reported to affect hatchability, either by direct treatment of the eggs or as a delayed effect by treating the parents at any stage of their life-cycle (Mondal and Parween, 2000). Similar findings have also been reported for hydroprene and methoprene treated *Sitophilus granarius* (Nordlander, 1975), hydroprene treated *C. maculatus* (Rup and Chopra, 1984) and *R. dominica* when their eggs were exposed to methoprene (Chanbang *et al.*, 2008). Reduced hatching of eggs were also reported by Mojaver and Bandani (2010) in *E. integriceps* and by Ghasemi *et al.*, (2010) in *P. interpunctella* when treated with pyriproxyfen. Our results regarding fecundity and fertility are in conformity with Ghasemi *et al.* (2010), Abdel-Aal (2012) and El-barkey (2012) as have reported in case of *P. interpunctella*, *S. littoralis* and *T. castaneum*, respectively.

Reduction in the percentage of egg-hatch obtained by Abdel-Aal (2012) may be attributed to sterilization of either eggs and sperms; or may be

due to inability of the sperms to be transferred to females during copulation (Ismail, 1980). Moursy and Bartlett (1991) reported that pyriproxyfen decrease the number of spermatophores transferred to *P. gossypiella*. In addition, Riddiford and Williams (1967) and Riddiford (1970) in their study demonstrated that the failure of eggs to hatch was apparently due to ovicidal activity of JHAs which block embryonic-larval transition and thus prevented further development of the insects. These compounds (IGRs) have ability to disturb the growth and maturation of the gonads in both sexes (Metwally *et al.*, 1972; Parween, 1996) resulting in production of non-viable eggs. Reduction in fertility could be caused by sterilizing eggs, reducing survival of the viable eggs. This reduction may be either due to an effect on some later steps in the differentiation and functioning of follicular cells (Gelbic and Sehna, 1973) or derangement of humoral control of oviposition (Matolín and Gelbic, 1975).

Pyriproxyfen induced sterility has also been observed in all the cross sets of each instar (Tables 1-4). This sterility was found to be most severe in the eggs obtained from the cross of treated males and treated females in 1st instar and comparatively it becomes milder in the rest instars i.e. from 2nd to 4th.

In *Endopterygota* insect exposure to juvenoids may cause female sterility when applied at a certain stage of oocyte development (Riddiford, 1970). Taher and Cutkomp (1983) demonstrated that the sterility of female seems to be attribute chiefly to a delay or reduction of ova giving opportunities not for retention but for possible resorption of eggs in ovaries. Similarly, Gardner (1991) observed the effect of fenoxycarb on egg eclosion of *Spodoptera frugiperda*. An ovicidal effect of methoprene has been reported by Mian and Mulla (1982) on *O. surinamensis* and *R. domonica* and also reported by Walker and Bowers, (1970); Staal (1975) and Chanbang *et al.* (2008) in young eggs which were more sensitive than older eggs. Qian *et al.* (2020) suggested that pyriproxyfen exposure could influence ovarian

tissue development following a decline in number of oocytes and oögonia in the ovaries of pyriproxyfen-fed silkworms.

It is evident from the study of Sláma *et al.* (1974) and Mkhize (1992), if adult population were treated with high dosages of JHAs, female beetles thus deposit defective eggs of very low or zero hatchability. Sterility in insects might be due to distorted gonads, inhibition of vitellogenin in the ova of treated females, reduction of the number of spermatophores in treated males and extrusion of ovipositor in the adult females (Mondal and Parween, 2000). Methoprene and R08-9801, completely inhibited yolk absorption by oocytes in *Blatella germanica* and formed empty cavities in oocytes. JHAs cause protein degradation and the degeneration of the nuclei in oocyte, subsequently rendering the eggs sterile (Maiza *et al.*, 2004). It may be concluded that high dose levels of pyriproxyfen in the present study may affect the reproductive physiology of the gonads of female *C. cephalonica* resulting in the reduced viability of their eggs.

Acknowledgements

Authors are thankful to Prof. Veena B. Kushwaha, Head, Department of Zoology D.D.U. Gorakhpur University, Gorakhpur for providing laboratory facilities, to Prof. Rajendra Singh, former Head, Department of Zoology, D.D.U. Gorakhpur University Gorakhpur for critical review of the manuscript and to Prof. Ravi Kant Upadhyay, Prof. V. K. Singh, Prof. Keshav Singh and Dr. Sushil Kumar for their kind co-operation and valuable suggestion. Thanks are also due to Toronto Research Chemicals Inc. 2 Brisbane Road, Toronto, ON Canada, M3J 2J8 for providing pyriproxyfen.

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