Article

Reproductive potential of ecdysone hormone in rice-moth, *Corcyra cephalonica* Staint. (Lepidoptera: Pyralidae)

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Abstract
The present study was designed and conducted to evaluate the influence of methoxyfenozide on the reproductive potential of 1st, 2nd, 3rd and 4th instars larvae, as a safe and suitable alternative for the conventional organic insecticide. Results showed that the highest concentration i.e. 15 ppm of methoxyfenozide caused maximum reduction in fecundity i.e. 118.43 ± 6.08 eggs laid/ female and hatchability i.e. 31.52 ± 1.18% when both males and females were crossed emerged from treated culture in case of 1st instar exposed larvae. Such pairs showed maximum sterility also when emerged from 15 ppm concentration of food in 1st instar exposure. Fecundity, fertility and sterility comparatively decreased in 2nd, 3rd and 4thinstar larvae due to their comparatively poor exposure duration. Such knowledge may offcourse be beneficial for the effective control of rice-moth in particular and lepidopterous pest in general in eco-friendly way.

Keywords *Corcyra cephalonica*; hormonal control; methoxyfenozide; fecundity; fertility; sterility.

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1 Introduction

Management of insect pests in stored cereal and cereal products is still a puzzling problem of the world. Besides broad spectrum application of synthetic organic pesticides, increasing population of insect pests are challenging competitor of human beings for food (Udo, 2011). Damage caused by insect pests in storage may account for about 10 – 40% (Raja et al., 2001; Papachristor and Stamopoulos, 2002). The damage to stored products would cause weight loss, detriment in quality and reputation. The loss of weight due to a single larva may be small, only a few milligram but with population measured in millions this would be a remarkable amount (Burkholder and Faustini, 1991; Campbell and Arbogast, 2004). This damage is not only caused by feeding but also by heat produced by the respiratory and metabolic functions of the insects. Accumulation of frass (exuviae, egg shell, dead insects, pupal cases and faecal matter) (El- Mafty et al., 1989; Mondal, 1992) and webbing of food (Hill, 1990) are of great nuisance. Insect recrudescence in stored food causes serious

health risks. Moreover, quinones secreted by some of the pests particularly tenebrionid beetles (Roth, 1943; Mondal 1985, 1992) are actually toxic, allergenic and carcinogenic to human beings (Ladisch et al., 1967).

The rice–moth, *Corcyra cephalonica* Staint. (Lepidoptera: Pyralidae) is one of the most destructive pests of stored cereals and cereal commodities in Asia, Africa, Europe, North America and other tropical and subtropical regions of the world (Munro and Thomson, 1929). Rice, gram, sorghum, maize, wheat and milled products are sererely harmed by its larval phases (Atwal, 1976; Piltz, 1977).

Current control tactics include the use of conventional synthetic insecticides and fumigants (Jackai and Asante, 2003). But their excessive use has some inherent problems like destruction of beneficial insects (Subramanyam and Hagstrum, 1995; Zhang, 2018), environmental risks (Marx, 1977; Pimental, 1983) and development of resistance (Arthur, 1996; Zhang and Liu, 2023). Consequently, there is urgent need to introduce alternative control techniques which are more effective, less persistent, having low toxicity to non-target organisms, more pest specific and relatively safer to the environment.

Insect growth regulators have been receiving a lot of interest in controlling stored product from insects in search of new management measures (Fox, 1990). IGRs retain a novel mode of action, affecting the molting and metamorphosis process of insects (William, 1956; Oberlander et al., 1997). One of the suitable IGR is methoxyfenozide (an ecdysone hormone analogue), a new class of insect growth regulator with novel modes of action. The ecdysone agonists mimics the action of 20- hydroxyl ecdysone causing the treated larvae to enter a premature and lethal molting cycle (Wing, 1988; Wing et al., 1988). Methoxyfenozide belongs to a novel class of IGRs, the molting accelerating compounds or non–steroidal ecdysteroid agonists. These compounds mimic the mode of action of the natural insect molting hormones by true binding on the ecdysteroid receptors of the epidermal cells and inducing precocious molting (Smagghe et al., 2004). They act more slowly than neurotoxin insecticides because they disrupt the hormonal system or the physiological development of insects rather than kills through direct toxic action (Biddinger et al., 2006). The high effectiveness of IGRs on Lepidoptera reproduction has been widely recognized by researchers (El-Sabrout and Zahran, 2016; Hussein and Eldesouky, 2019). The effects of IGR compound on reproductive behaniour can be grouped into many categories as fecundity, fertility and adult sterilization (Mondal and Parween, 2000).

The present study has been designed and conducted to evaluate the effect of methoxyfenozide on the reproductive potential of *C. cephalonica* adults when applied on 1^{st}, 2^{nd}, 3^{rd} and 4^{th} instars larvae.

2 Materials and Methods

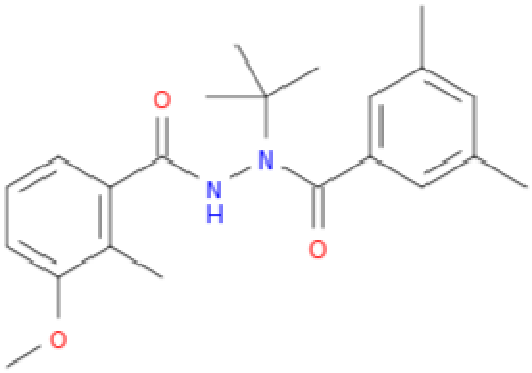
2.1 Collection and culture of rice-moth, *Corcyra cephalonica*

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 (*Sorghum vulgare)* mixed with 5% (w/w) powdered yeast inside large glass containers (150 mm diameter, 200 mm height) at temperature 26 ± 1ºC, relative humidity (R.H.) 93 ± 5% and a light regime of 12 h light and 12 h darkness.

2.2 Insect growth regulator used

Ecdysone hormone analogue i.e. methoxyfenozide used throughout the present study was obtained from Toronto, ON, Canada M3J 2J8.

2.3 Chemical structure of methoxyfenozide



Methoxyfenozide

N-tert-butyl-N'-(3-Methoxy-o-toluoyl)-3,5-xylohydrazide

2.4 Preparation of different concentrations of methoxyfenozide in dietary media

Preparation of different concentrations of food and evaluation of toxicity of methoxyfenozide against developmental cycle of *C. cephalonica* to assess its sublethal concentrations (1, 5, 10 and 15 ppm) of methoxyfenozide that permit the larvae to grow, develop and emerge but cause considerable effect in the gonadial tissues that affect the fecundity, fertility and sterility as well as gonadial biochemistry of adults that can be easily assessed to prove the effectiveness of methoxyfenozide was performed by the method of Singh and Tiwari (2014).

2.5 Evaluation of egg laying capacity and egg hatchability

Evaluation of egg laying capacity and egg hatchability at sublethal concentrations (i.e. 1, 5, 10 and 15 ppm) of methoxyfenozide were performed in the following ways.

The adults emerged from these sublethal doses (that permit the *C. Cephalonica* larvae to grow, develop and emerge but cause significant effect in the immediately sexed and used for mating experiments. For this purpose 4 type of crosses were made as following

a) Normal male x Normal female (Serving as control)

b) Treated male x Normal female

c) Normal male x Treated female

d) Treated male x Treated female

Here treated male and treated female refers to the male and female moths obtained from treated food.

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 cite (glass petridishes 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. According to Chamberlain's formula (1962), corrected sterility was calculated as

$$\text{Corrected sterility} = 100 \times \frac{\text{\% hatch in control - \% hatch in treated}}{\text{\% hatch in control}}$$

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 upto the concentrations where adults were available.

2.6 Statistical analysis

Experiments were replicated six times and the values have been expressed as the mean ± SD. Student's *t*-test (Zhang, 2022) was applied to determine the significant differences from their controls.

3 Results

3.1 Effect of methoxyfenozide on the fecundity (egg laying capacity) of the rice-moth, *C. cephalonica* exposed as 1st, 2nd, 3rd and 4thinstar treated larvae (Tables 1, 2, 3 and 4)

Exposure of sublethal concentrations of methoxyfenozide to 1st, 2nd, 3rd and 4th instar larvae, in the present study, caused a significant reduction in egg laying capacity of the rice-moth *Corcyra cephalonica* (Tables 1, 2, 3 and 4).

When normal males were crossed with normal females the total number of eggs laid per female was observed to be 486.45 ± 6.02, 489.16 ± 5.44, 492.41 ± 4.66 and 490.67 ± 5.24 in 1st, 2nd, 3rd and 4th instar respectively. In case of 1st instar larval treatment, when normal males were crossed with the females emerged from the cultures treated with 1, 5, 10 and 15 ppm concentrations of methoxyfenozide, the respective number of eggs laid per female was recorded as 432.14 ± 5.27, 285.19 ± 5.22, 245.32 ± 5.21 and 184.26 ± 4.88, whereas when the crosses were made between the sexes both emerged from the culture treated with 1, 5, 10 and 15 ppm concentrations of methoxyfenozide, the number of eggs laid per female was recorded as 325.36 ± 6.72, 184.48 ± 4.12, 180.18 ±5.03 and 118.43 ± 6.08 respectively (Fig. 1).

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

Methoxyfenozide concentration (ppm)	Crossing sets	Fecundity[#] (Eggs laid/female)	Fertility[#] (Eggs hatched)	% Hatchability[#]	% Observed sterility[#]	% Corrected sterility[*]
Control	N ♂ x N ♀	486.45 ± 6.02	474.22 ± 5.27	97.49 ± 1.24	2.51 ± 0.24	-
1	T ♂ x N ♀	432.14 ± 5.27[a]	228.04 ± 3.84	52.77 ± 2.24[a]	47.23 ± 1.54	45.87 ± 1.31
	N ♂ x T ♀	380.42 ± 5.18[a]	185.15 ± 5.02	48.67 ± 2.68[a]	51.33 ± 1.62	50.08 ± 1.64
	T ♂ x T ♀	325.36 ± 6.72[a]	147.36 ± 5.18	45.29 ± 2.12[a]	54.71 ± 2.04	53.54 ± 2.18
5	T ♂ x N ♀	285.19 ± 5.22[a]	138.06 ± 6.48	48.41 ± 2.11[a]	51.59 ± 2.11	50.34 ± 1.84
	N ♂ x T ♀	230.66 ± 5.91[a]	107.42 ± 4.52	46.57 ± 2.62[a]	53.43 ± 1.98	52.23 ± 2.11
	T ♂ x T ♀	184.48 ± 4.12[a]	78.13 ± 4.88	42.35 ± 1.98[a]	57.65 ± 1.64	56.56 ± 2.34
10	T ♂ x N ♀	245.32 ± 5.21[a]	107.13 ± 5.78	43.67 ± 3.62[a]	56.33 ± 2.12	55.21 ± 2.04
	N ♂ x T ♀	223.33 ± 6.22[a]	92.28 ± 4.82	41.32 ± 3.18[a]	58.68 ± 3.18	57.62 ± 2.27
	T ♂ x T ♀	180.18 ± 5.03[a]	68.74 ± 5.11	38.15 ± 2.96[a]	61.85 ± 2.94	60.87 ± 2.94
15	T ♂ x N ♀	184.26 ± 4.88[a]	68.27 ± 4.52	37.05 ± 1.94[a]	62.95 ± 2.42	61.99 ± 2.51
	N ♂ x T ♀	142.21 ± 5.02[a]	48.52 ± 4.62	34.12 ± 2.26[a]	65.88 ± 2.51	65.00 ± 2.18
	T ♂ x T ♀	118.43 ± 6.08[a]	37.33 ± 3.18	31.52 ± 1.18[a]	68.48 ± 2.16	67.67 ± 2.34

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

[*] Calculated by Chamberlain's formula (1962).

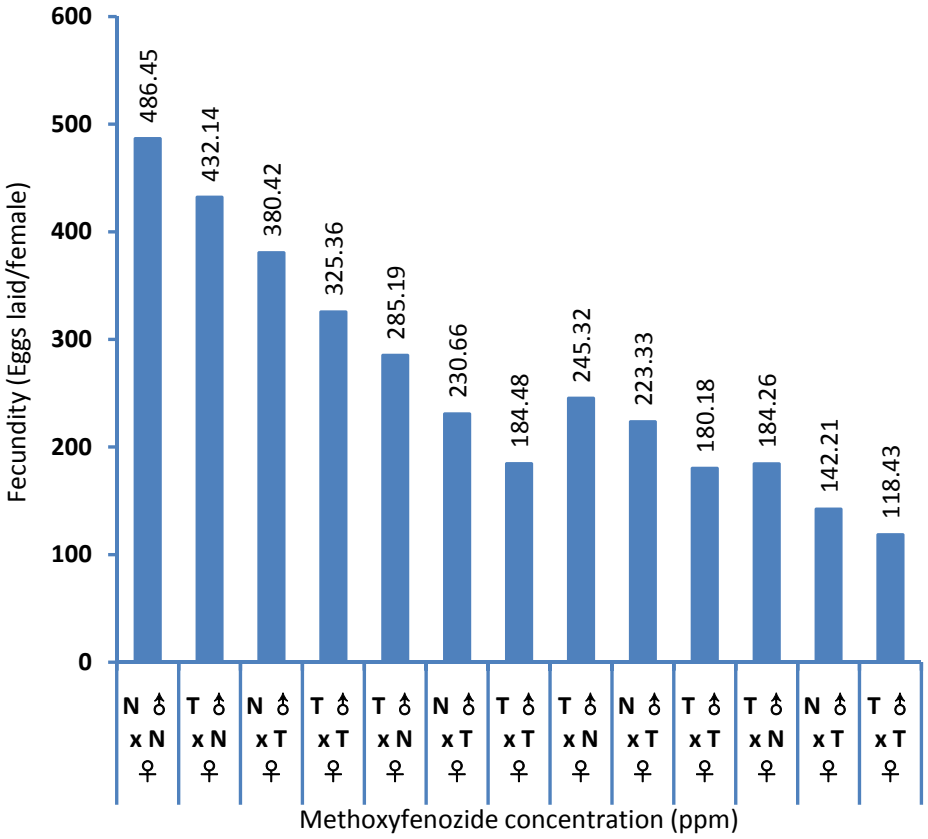


Fig. 1 Effect of methoxyfenozide on the fecundity (Eggs laid/female) of rice-moth *Corcyra cephalonica* exposed to 1st instar larvae.

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

Methoxyfenozide concentration (ppm)	Crossing sets	Fecundity# (Eggs laid/female)	Fertility# (Eggs hatched)	% Hatchability#	% Observed sterility#	% Corrected sterility*
Control	N ♂ x N ♀	489.16 ± 5.44	470.13 ± 5.12	96.11 ± 1.22	3.89 ± 0.24	-
1	T ♂ x N ♀	442.22 ± 5.21[a]	256.35 ± 2.11	57.97 ± 1.16[a]	42.03 ± 0.38	39.68 ± 1.12
	N ♂ x T ♀	397.51 ± 4.34[a]	211.28 ± 2.34	53.15 ± 1.54[a]	46.85 ± 0.33	44.70 ± 1.18
	T ♂ x T ♀	355.23 ± 4.16[a]	167.35 ± 1.72	47.11 ± 1.16[a]	52.89 ± 0.48	50.98 ± 1.04
5	T ♂ x N ♀	310.13 ± 5.11[a]	168.31 ± 3.54	54.27 ± 1.48[a]	45.73 ± 0.52	43.53 ± 1.45
	N ♂ x T ♀	274.44 ± 6.04[a]	129.29 ± 2.70	47.11 ± 1.32[a]	52.89 ± 0.48	50.98 ± 1.28
	T ♂ x T ♀	224.61 ± 6.46[a]	101.91 ± 2.30	45.37 ± 1.45[a]	54.63 ± 0.82	52.79 ± 1.33
10	T ♂ x N ♀	267.72 ± 5.81[a]	133.38 ± 2.62	49.82 ± 1.92[a]	50.18 ±0.84	48.16 ± 1.34
	N ♂ x T ♀	243.34 ± 4.26[a]	111.13 ± 1.84	45.67 ± 1.38[b]	54.33 ± 0.52	52.48 ± 1.17
	T ♂ x T ♀	210.76 ± 5.67[a]	88.08 ± 1.15	41.79 ± 1.14[a]	58.21 ± 1.11	56.52 ± 1.51
15	T ♂ x N ♀	225.82 ± 6.04[a]	98.28 ± 1.12	43.52 ± 1.16[a]	56.48 ± 1.28	54.72 ± 1.18
	N ♂ x T ♀	182.19 ± 5.18[a]	69.63 ± 1.34	38.22 ± 1.34[a]	61.78 ± 1.34	60.23 ± 1.04
	T ♂ x T ♀	141.37 ± 4.71[a]	48.11 ± 1.44	34.03 ± 1.44[a]	65.97 ± 1.12	64.59 ± 1.41

#Values are expressed as mean ± SD of six replicates, [a] and [b] significantly different $p < 0.001$ and $p < 0.01$ compared with controls when *t*-test was applied.

*Calculated by Chamberlain's formula (1962).

Similarly in 2nd instar larval treatment, when normal males were crossed with the females emerged from the cultures treated with 1, 5, 10 and 15 ppm concentrations of methoxyfenozide, the respective number of eggs laid per female was recorded as 442.22 ± 5.21, 310.13 ± 5.11, 267.72 ± 5.81 and 225.82 ± 6.04, whereas when the crosses were made between the sexes both emerged from the culture treated with 1, 5, 10 and 15 ppm concentrations of methoxyfenozide, the number of eggs laid per female was recorded as 355.23 ± 4.16, 224.61 ± 6.46, 210.76 ±5.67 and 141.37 ± 4.71 respectively (Fig. 2).

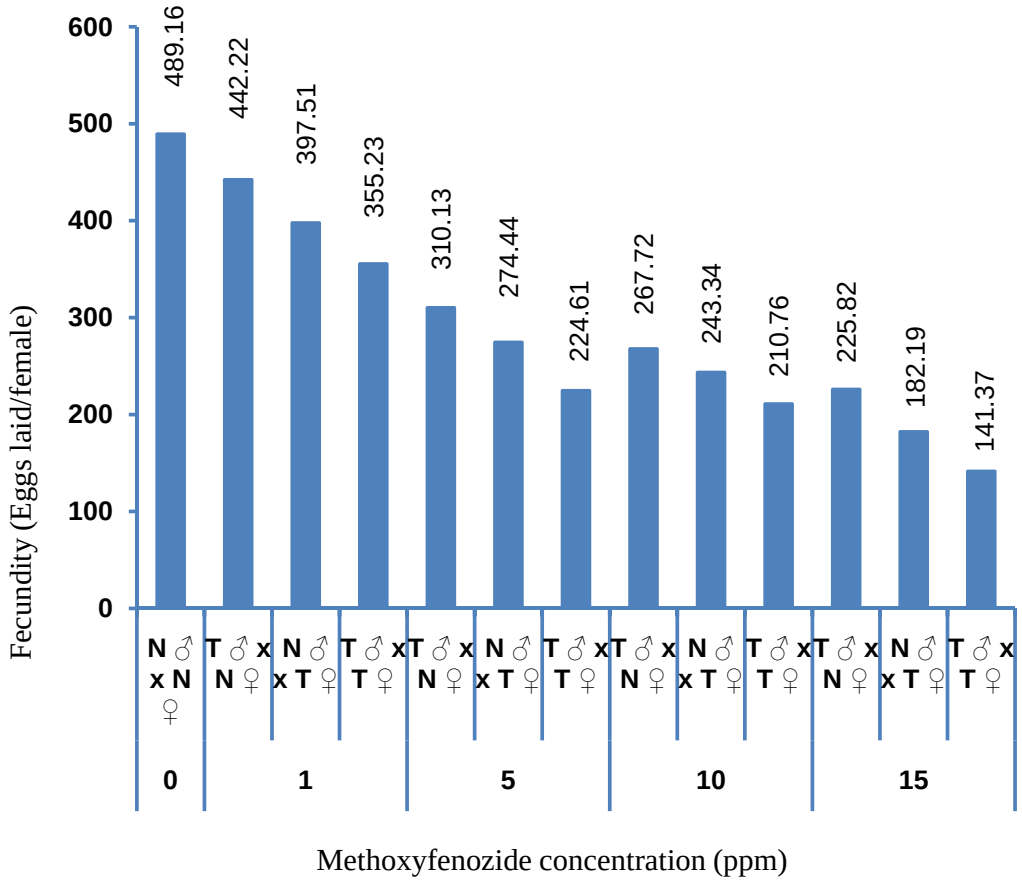


Fig. 2 Effect of methoxyfenozide on the fecundity (Eggs laid/female) of rice-moth *Corcyra cephalonica* exposed to 2nd instar larvae.

In case of 3rd instar larval treatment, when normal males were crossed with the females emerged from the cultures treated with 1, 5, 10 and 15 ppm concentrations of methoxyfenozide, the respective number of eggs laid per female was recorded as 454.15 ± 6.18, 345.15 ± 5.22, 288.42 ± 6.21 and 237.52 ± 5.94, whereas when the crosses were made between the sexes both emerged from the culture treated with 1, 5, 10 and 15 ppm concentrations of methoxyfenozide, the number of eggs laid per female was recorded as 360.13 ± 4.19, 252.26 ± 4.92, 222.21 ± 4.82 and 172.34 ± 5.27 respectively (Fig. 3).

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

Methoxyfenozide concentration (ppm)	Crossing sets	Fecundity[#] (Eggs laid/female)	Fertility[#] (Eggs hatched)	% Hatchability[#]	% Observed sterility[#]	% Corrected sterility[*]
Control	N ♂ x N ♀	492.41 ± 4.66	475.87 ± 5.24	96.64 ± 1.57	3.36 ± 0.11	-
1	T ♂ x N ♀	454.15 ± 6.18[a]	318.95 ± 5.12	70.23 ± 1.12[a]	29.77 ± 0.94	27.33 ± 0.21
	N ♂ x T ♀	423.22 ± 6.22[a]	247.33 ± 5.34	58.44 ± 1.16[a]	41.56 ± 0.62	39.53 ± 0.19
	T ♂ x T ♀	360.13 ± 4.19[a]	188.42 ± 5.28	52.32 ± 0.68[a]	47.68 ± 0.34	45.86 ± 0.26
5	T ♂ x N ♀	345.15 ± 5.22[a]	211.99 ± 4.18	61.42 ± 1.11[a]	38.58 ± 0.52	36.44 ± 1.12
	N ♂ x T ♀	311.22 ± 5.84[a]	169.77 ± 3.72	54.55 ± 1.52[a]	45.45 ± 0.81	43.55 ± 1.08
	T ♂ x T ♀	252.26 ± 4.92[a]	118.99 ± 3.12	47.17 ± 1.31[b]	52.83 ± 0.72	51.19 ± 1.16
10	T ♂ x N ♀	288.42 ± 6.21[a]	159.29 ± 3.18	55.23 ± 0.94[a]	44.77 ± 0.48	42.85 ± 1.52
	N ♂ x T ♀	256.37 ± 5.76[a]	121.42 ± 3.22	47.36 ± 0.58[a]	52.64 ± 0.49	50.99 ± 1.38
	T ♂ x T ♀	222.21 ± 4.82[a]	91.59 ± 2.94	41.22 ± 0.72[a]	58.78 ± 0.72	57.35 ± 1.29
15	T ♂ x N ♀	237.52 ± 5.94[a]	126.38 ± 3.22	53.21 ± 0.58[a]	46.79 ± 1.10	44.94 ± 1.31
	N ♂ x T ♀	202.11 ± 4.84[a]	85.57 ± 2.98	42.34 ± 0.72[a]	57.66 ± 0.95	56.19 ± 1.04
	T ♂ x T ♀	172.34 ± 5.27[a]	64.52 ± 2.11	37.44 ± 0.55[a]	62.56 ± 1.13	61.26 ± 1.27

#Values are expressed as mean ± SD of six replicates, a andb significantly different $p < 0.001$ and $p < 0.01$ compared with controls when *t*-test was applied.
*Calculated by Chamberlain's formula (1962).

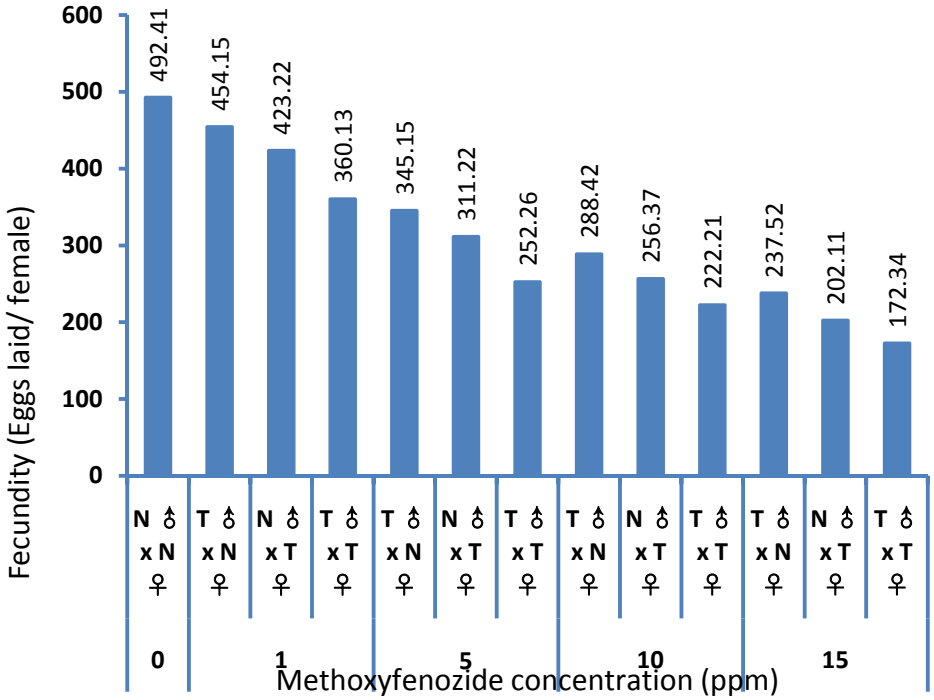


Fig. 3 Effect of methoxyfenozide on the fecundity (Eggs laid/female) of rice-moth *Corcyra cephalonica* exposed to 3rd instar larvae.

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

Methoxyfenozide concentration (ppm)	Crossing sets	Fecundity[#] (Eggs laid/female)	Fertility[#] (Eggs hatched)	% Hatchability[#]	% Observed sterility[#]	% Corrected sterility[*]
Control	N ♂ x N ♀	490.67 ± 5.24	472.12 ± 3.64	96.22 ± 1.14	3.78 ± 0.33	-
1	T ♂ x N ♀	468.41 ± 5.66[a]	337.82 ± 4.22	72.12 ± 1.84[a]	27.88 ± 1.16	25.05 ± 1.21
	N ♂ x T ♀	452.24 ± 5.21[a]	277.40 ± 4.28	61.34 ± 1.49[a]	38.66 ± 1.42	36.25 ± 1.37
	T ♂ x T ♀	382.14 ± 5.19[a]	214.84 ± 5.11	56.22 ± 1.46[a]	43.78 ± 1.38	41.57 ± 1.18
5	T ♂ x N ♀	390.01 ± 6.12[a]	250.31 ± 4.18	64.18 ± 2.14[a]	35.82 ± 1.12	33.30 ± 1.34
	N ♂ x T ♀	350.21 ± 7.44[a]	200.74 ± 4.22	57.32 ± 2.12[a]	42.68 ± 1.36	40.43 ± 1.52
	T ♂ x T ♀	282.73 ± 5.12[a]	144.64 ± 3.51	51.16 ± 2.52[a]	48.84 ± 2.14	46.83 ± 1.21
10	T ♂ x N ♀	360.55 ± 5.44[a]	209.98 ± 4.92	58.24 ± 2.12[a]	41.76 ± 1.82	39.47 ± 0.98
	N ♂ x T ♀	305.31 ± 6.12[a]	154.18 ± 4.66	50.50 ± 1.94[a]	49.50 ± 1.44	47.52 ± 1.52
	T ♂ x T ♀	255.11 ± 5.44[a]	116.36 ± 5.22	45.61 ± 1.48[a]	54.39 ± 1.72	52.60 ± 1.38
15	T ♂ x N ♀	342.24 ± 4.91[a]	190.70 ± 4.92	55.72 ± 1.48[a]	44.28 ± 1.52	42.09 ± 1.62
	N ♂ x T ♀	287.41 ± 5.38[a]	147.56 ± 4.18	51.34 ± 1.52[a]	48.66 ± 1.68	46.64 ± 1.34
	T ♂ x T ♀	233.36 ± 6.72[a]	98.36 ± 3.99	42.15 ± 1.64[a]	57.85 ± 1.34	56.19 ± 1.51

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

* Calculated by Chamberlain's formula (1962).

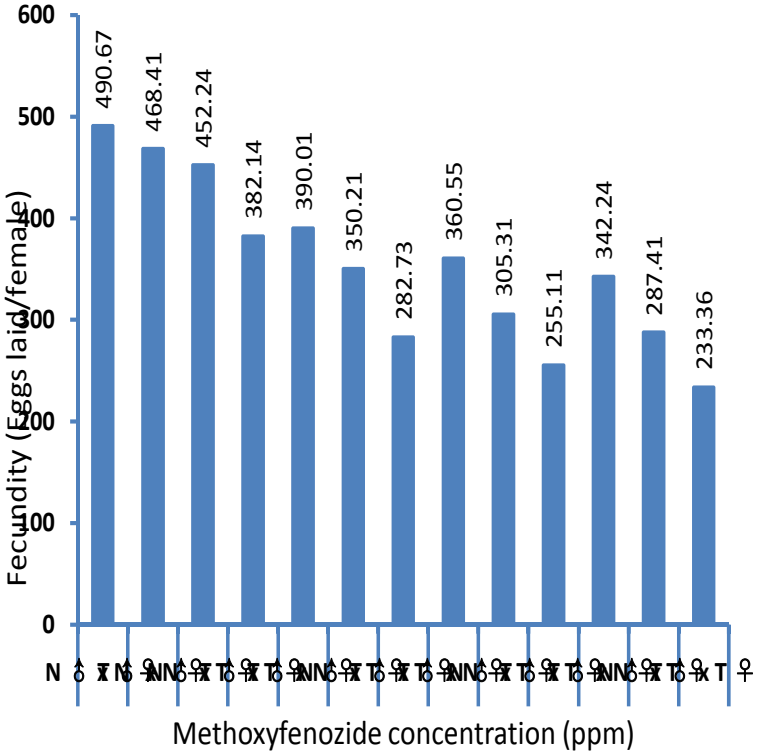


Fig. 4 Effect of methoxyfenozide on the fecundity (Eggs laid/female) of rice-moth *Corcyra cephalonica* exposed to 4th instar larvae.

In 4th instar larval treatment, when normal males were crossed with the females emerged from the cultures treated with 1, 5, 10 and 15 ppm concentrations of methoxyfenozide, the respective number of eggs laid per female was recorded as 468.41 ± 5.66, 390.01 ± 6.12, 360.55 ± 5.44 and 342.24 ± 4.91, whereas when the crosses were made between the sexes both emerged from the culture treated with 1, 5, 10 and 15 ppm concentrations of methoxyfenozide, the number of eggs laid per female was recorded as 382.14 ± 5.19, 282.73 ± 5.12, 255.11 ± 5.44 and 233.36 ± 6.72 respectively (Fig. 4).

3.2 Effect of methoxyfenozide on the hatchability of rice-moth, *C. cephalonica* exposed as 1st, 2nd, 3rd and 4th instar treated larvae (Tables 1, 2, 3 and 4)

When females emerged from first instar control larvae were crossed with the males, emerged from cultures treated with 1, 5, 10 and 15 ppm concentrations of methoxyfenozide, the hatchability was found to be 52.77 ± 2.24, 48.41 ± 2.11, 43.67 ± 3.62 and 37.05 ± 1.94% respectively. The hatchability of eggs collected from crosses between normal males and the females emerged from the cultures treated with 1, 5, 10 and 15 ppm of methoxyfenozide, was recorded to be 48.67 ± 2.68, 46.57 ± 2.62, 41.32 ± 3.18 and 34.12 ± 2.26% respectively. When both the sexes used in the cross, were emerged from the cultures treated with 1, 5, 10 and 15 ppm concentration of methoxyfenozide, the hatchability of eggs was recorded to be 45.29 ± 2.12, 42.35 ± 1.98, 38.15 ± 2.96 and 31.52 ± 1.18% respectively (Fig. 5).

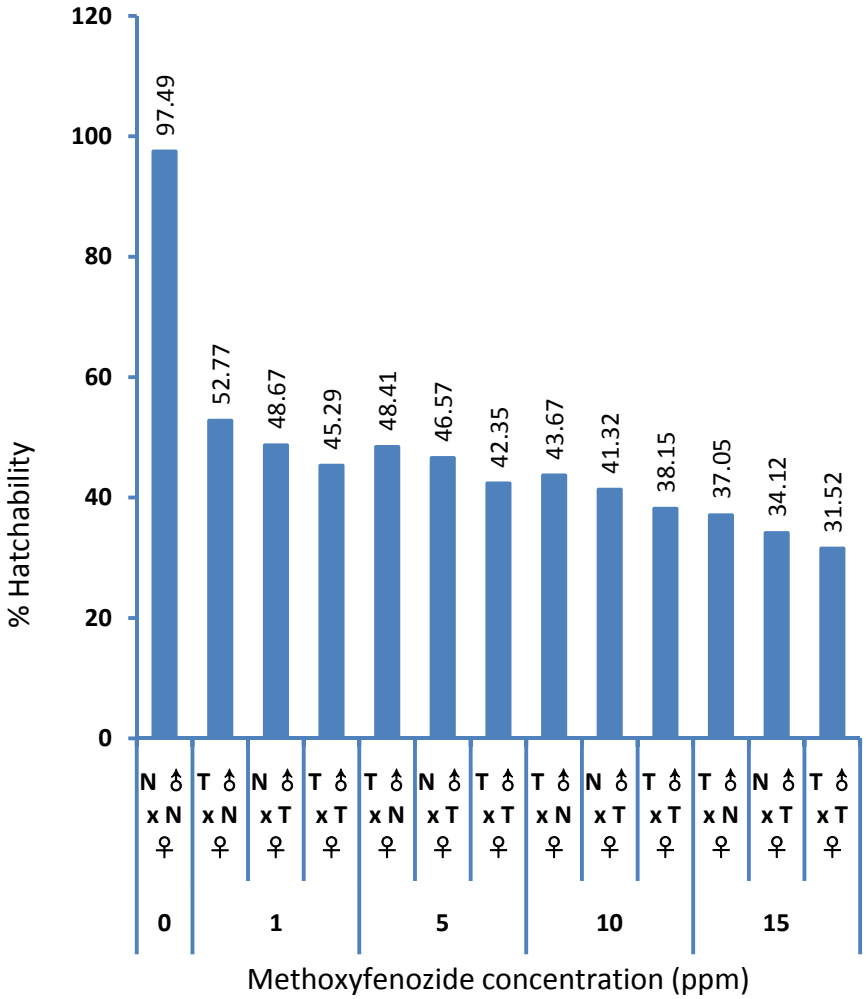


Fig. 5 Effect of methoxyfenozide on the % hatchability of rice-moth *Corcyra cephalonica* exposed to 1st instar larvae.

Similarly females emerged from 2nd instar control larvae were crossed with the males, emerged from cultures treated with 1, 5, 10 and 15 ppm concentrations of methoxyfenozide, the hatchability was found to be 57.97 ± 1.16, 54.27 ± 1.48, 49.82 ± 1.92 and 43.52 ± 1.16% respectively. When both the sexes used in the cross, were emerged from the cultures treated with 1, 5, 10 and 15 ppm concentration of methoxyfenozide, the hatchability of eggs was recorded to be 47.11 ± 1.16, 45.37 ± 1.45, 41.79 ± 1.14 and 34.03 ± 1.44% respectively (Fig. 6).

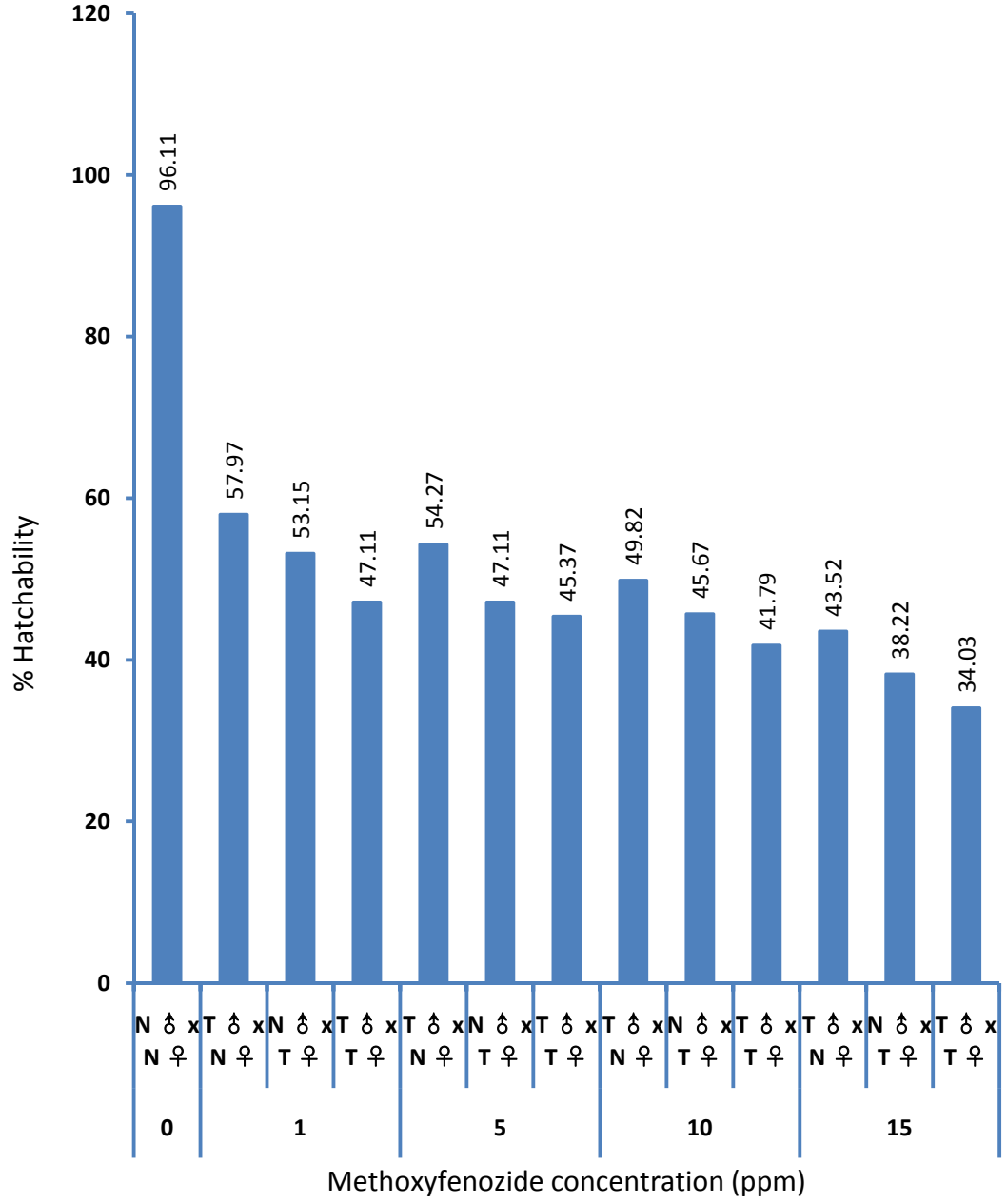


Fig. 6 Effect of methoxyfenozide on the % hatchability of rice-moth *Corcyra cephalonica* exposed to 2nd instar larvae.

When females emerged from 3rd instar control larvae were crossed with the males, emerged from cultures treated with 1, 5, 10 and 15 ppm concentrations of methoxyfenozide, the hatchability was found to be 70.23 ± 1.12, 61.42 ± 1.11, 55.23 ± 0.94 and 53.21 ± 0.58% respectively. When both the sexes used in the cross, were emerged from the cultures treated with 1, 5, 10 and 15 ppm concentration of methoxyfenozide, the hatchability of eggs was recorded to be 52.32 ± 0.68, 47.17 ± 1.31, 41.22 ± 0.72 and 37.44 ± 0.55% respectively (Fig. 7).

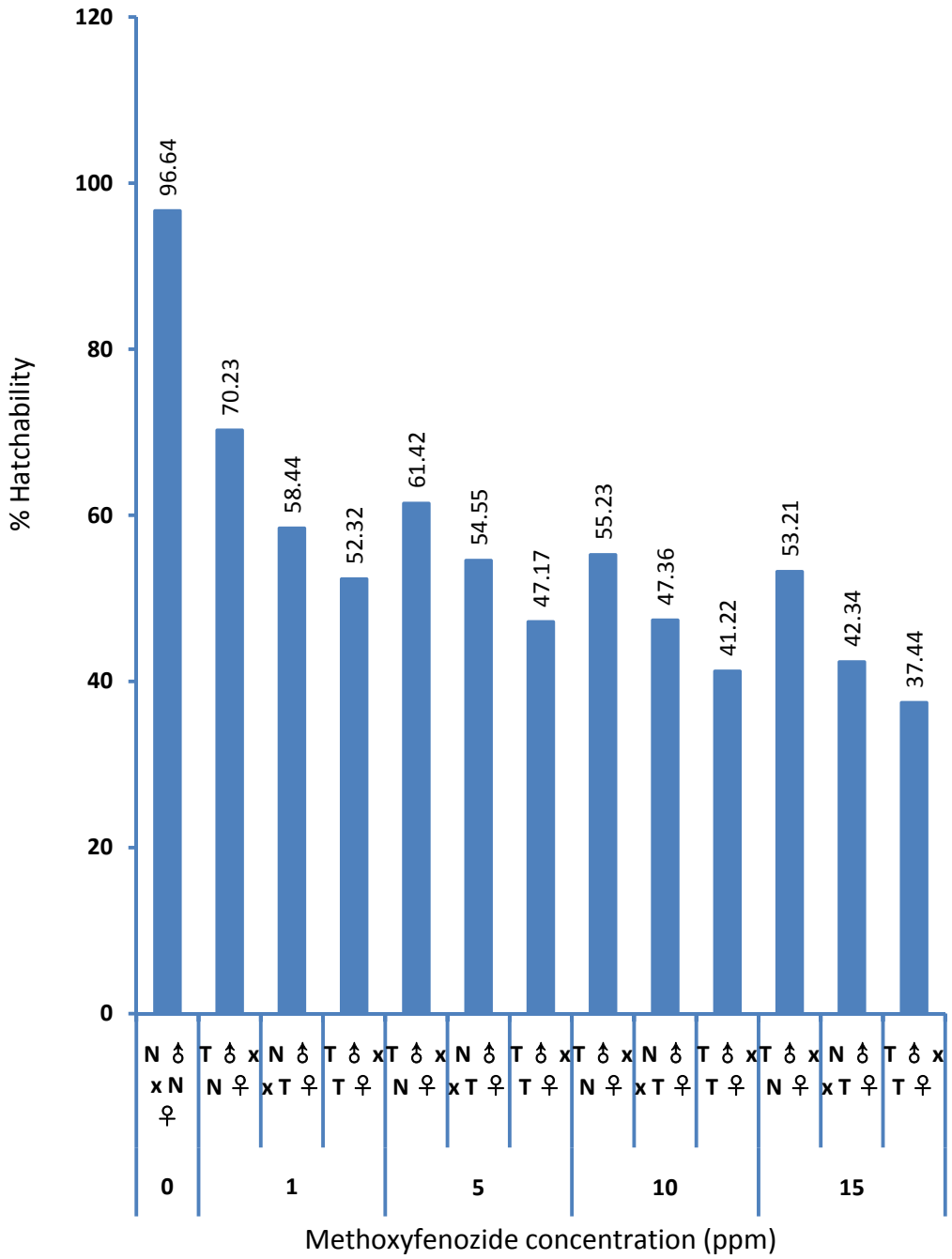


Fig. 7 Effect of methoxyfenozide on the % hatchability of rice-moth *Corcyra cephalonica* exposed to 3rd instar larvae.

Females emerged from 4th instar control larvae were crossed with the males, emerged from cultures treated with 1, 5, 10 and 15 ppm concentrations of methoxyfenozide, the hatchability was found to be 72.12 ± 1.84, 64.18 ± 2.14, 58.24 ± 2.12 and 55.72 ± 1.48% respectively. When both the sexes used in the cross, were emerged from the cultures treated with 1, 5, 10 and 15 ppm concentration of methoxyfenozide, the hatchability of eggs was recorded to be 56.22 ± 1.46, 51.16 ± 2.52, 45.61 ± 1.48 and 42.15 ± 1.64% respectively (Fig. 8).

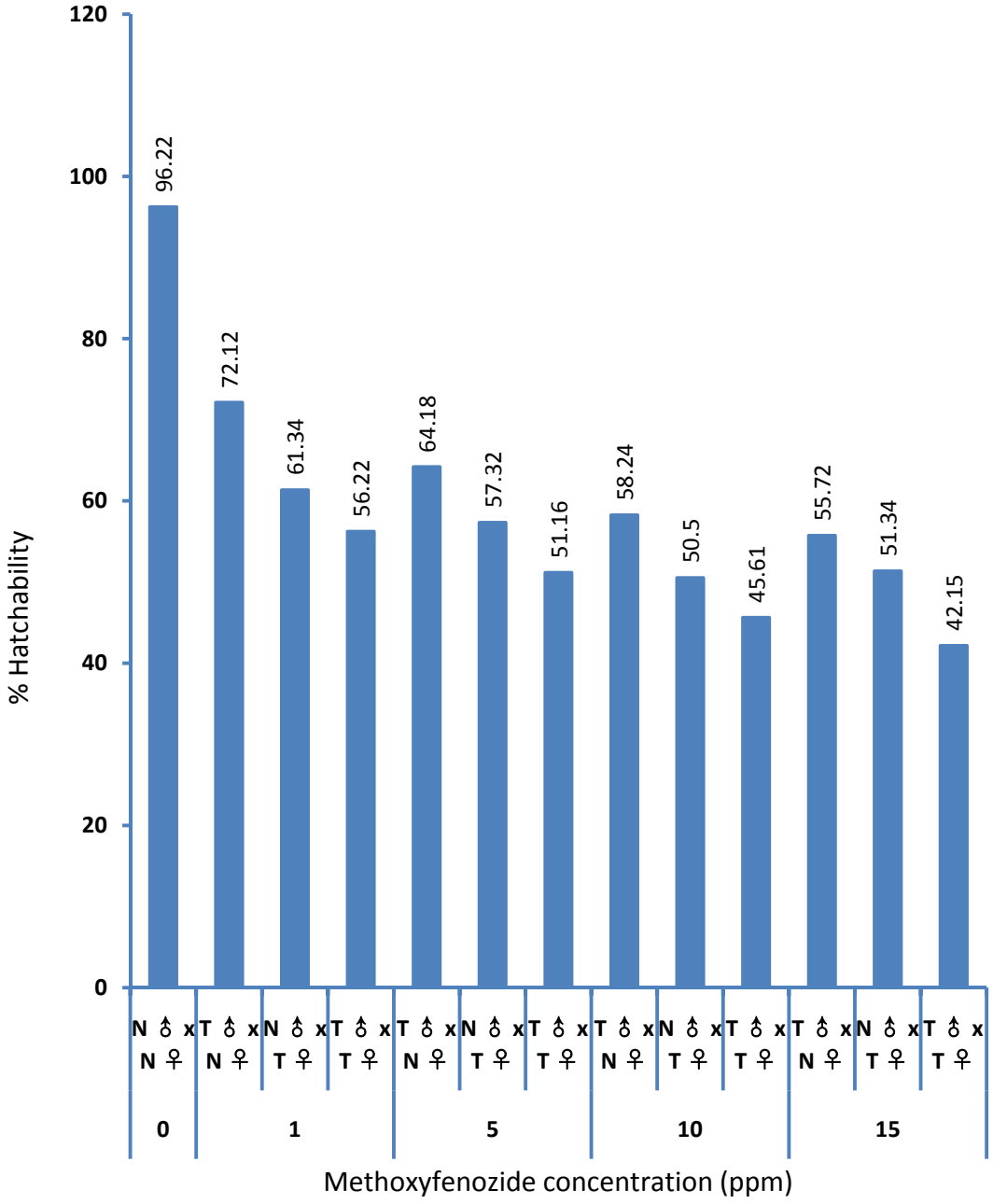


Fig. 8 Effect of methoxyfenozide on the % hatchability of rice-moth *Corcyra cephalonica* exposed to 4th instar larvae.

3.3 Effect of methoxyfenozide on the sterility of rice-moth, *C. cephalonica* exposed as 1st, 2nd, 3rd and 4th instar treated larvae (Tables 1, 2, 3 and 4)

When females emerged from first instar control larvae were crossed with the males, emerged from cultures treated with 1, 5, 10 and 15 ppm concentrations of methoxyfenozide, the sterility was found to be 47.23 ± 1.54, 51.59 ± 2.11, 56.33 ± 2.12 and 62.95 ± 2.42% respectively. The sterility of eggs collected from crosses between normal males and the females emerged from the cultures treated with 1, 5, 10 and 15 ppm of methoxyfenozide, was recorded to be 51.33 ± 1.62, 53.43 ± 1.98, 58.68 ± 3.18 and 65.88 ± 2.51% respectively. When both the sexes used in the cross, were emerged from the cultures treated with 1, 5, 10 and 15 ppm concentration of methoxyfenozide, the sterility of eggs was recorded to be 54.71 ± 2.04, 57.65 ± 1.64, 61.85 ±2.94 and 68.48 ± 2.165% respectively (Table 1)

Similarly, when females emerged from 2nd instar control larvae were crossed with the males, emerged from cultures treated with 1, 5, 10 and 15 ppm concentrations of methoxyfenozide, the sterility was found to be 42.03 ± 0.38, 45.73 ± 0.52, 50.18 ± 0.84 and 56.48 ± 1.28% respectively. When both the sexes used in the cross, were emerged from the cultures treated with 1, 5, 10 and 15 ppm concentration of methoxyfenozide, the sterility of eggs was recorded to be 52.89 ± 0.48, 54.63 ± 0.82, 58.21 ± 1.11 and 65.97 ± 1.12% respectively (Table 2).

When females emerged from 3rd instar control larvae were crossed with the males, emerged from cultures treated with 1, 5, 10 and 15 ppm concentrations of methoxyfenozide, the sterility was found to be 29.77 ± 0.94, 38.58 ± 0.52, 44.77 ± 0.48 and 46.79 ± 1.10% respectively. When both the sexes used in the cross, were emerged from the cultures treated with 1, 5, 10 and 15 ppm concentration of methoxyfenozide, the sterility of eggs was recorded to be 47.68 ± 0.34, 52.83 ± 0.72, 58.78 ± 0.72 and 62.56 ± 1.13% respectively (Table 3).

Females emerged from 4th instar control larvae were crossed with the males, emerged from cultures treated with 1, 5, 10 and 15 ppm concentrations of methoxyfenozide, the sterility was found to be 27.88 ± 1.16, 35.82 ± 1.12, 41.76 ± 1.82 and 44.28 ± 1.52 respectively. When both the sexes used in the cross, were emerged from the cultures treated with 1, 5, 10 and 15 ppm concentration of methoxyfenozide, the sterility of eggs was recorded to be 43.78 ± 1.38, 48.84 ± 2.14, 54.39 ± 1.72 and 57.85 ± 1.34 respectively (Table 4).

4 Discussion

The present investigation shows that egg laying capacity of the rice-moth, *Corcyra cephalonica* decreases significantly with the increase in the concentration of methoxyfenozide (Tables 1, 2, 3 and 4). The maximum inhibition of egg laying capacity was observed in the crosses between treated males and treated females while it was minimum in the crosses between treated males and normal females. Reduction in fecundity was found to be maximum i.e. 118.43 ± 6.08 in crosses between treated males and treated females emerged from 15 ppm concentration of methoxyfenozide while it was minimum in the crosses between treated males and treated females i.e 432.14 ± 5.27 at 1 ppm concentration of 1st instar exposed larvae. While potential of methoxyfenozide comparatively decreases gradually in 2nd, 3rd and 4th instar exposed larvae due to their shorter duration period of their exposure. Similar results have also been reported by Seth et al. (2004) in *Spodopteralitura* treated with moulting hormone RH-5849; Soltani-Mazouni et al. (2012) in *Ephestiakuehniella* Zeller exposed to methoxyfenozide and Bhagyalakshmi (2022) in *C. cephalonica*treated with ecdysone hormone RH-5849.

Methoxyfenozide arrest in ovarian development resulting into delaying of transition from vitellogenesis to choriogenesis has been observed in codling moth, *Cydia pomonella* and *Bombyxx mori* (Sun et al., 2003; Swevers and Iatrau, 2003) and *Spodoptera littoralis* (Boisd.) (Sabry et al., 2017). In addition, decrease in amount of yolk granules leading to wrap the egg chorion which probably results into defective eggs that lose

their viability as compared to normal eggs (Sabry et al., 2017). Smagghe et al. (1996) also suggested that ecdysone analogue methoxyfenozide might arrest the ovarian growth of *Cydia pomonella* or block sperm transfer to female, that may be due to oogenesis and vitellogenesis already started at the late period of pupal stage or during the onset of adult stage (Web et al., 1999). Methoxyfenozide affect the mating ability also of males surviving from treated larvae and either have decreased size of the testes in comparison to control testes or delaying spermatogenesis, as well as reduce the number of spermatocytes and spermatogonia. Similar explanations were also reported by Seth et al. (2004) regarding the concept of reduction in fecundity, sperm production and transfer of sperms resulting from *S. littoralis* larvae treated with sublethal concentrations of methoxyfenozide and tebufenozide. Our findings are in accordance with the results of RH-5849 exposed *S. litura* (Seth et al., 2004); methoxyfenozide exposed *E. kuehniella* (Sultani-Mazouni et al., 2012) and RH-5849 exposed *C. cephalonica* (Bhagyalakshmi, 2022).

Reproductionin insects is mainly controlled by the corpus allatum hormone, which is also responsible for protein metabolism and its specifically needed for egg maturation. According to research, IGRs can make treated insects less fertile or sterile (Metwally et al., 1972). Insects that have been exposed to IGRs may grow into morphologically malformed adults that are either non-viable or have a diminished potential for reproduction (Williams and Amos, 1974). Seth et al. (2004) reported that larval exposure to both RH-5849 and tebufenozide leads to an impaired rate of release of sperm from the testes, and disruption of the rhythm of sperm release. Tebufenozide also is known to almost completely inhibit the release of sperm from testes in the adult male gypsy moth, *Lymantria dispar*, resulting in sterility (Giebultowicz et al., 1993).

In the present investigation, the hatchability of eggs collected from the different crosses was also recorded to be reduced with increase in the concentration of methoxyfenozide in each instar. The maximum reduction of hatchability i.e. 37.33 ± 3.18 was observed at 15 ppm concentration of methoxyfenozide in the crosses between treated males and treated females and minimum reduction i.e. 228.04 ± 3.84 was recorded at 1 ppm concentration of this ecdysone in the crosses between treated males and normal females (Table 1, 2, 3 and 4). Similar result have also been reported in RH-5849 and tebufenozide (RH-5992) exposed *Spodoptera litura* (Seth et al., 2004); and methoxyfenozide treated *S. litora* (Shahout et al., 2011) methoxyfenozide fed *Spodoptera littoralis* (Sabry et al., 2017; El- Sabrout et al., 2019) and RH-5849 exposed *C. cephalonica* (Bhagyalakshmi, 2022).

Duration period of exposure of IGR to the larvae are important factor in the hatchability of eggs. Maturation of insect eggs is dependent, basically on the materials taken up from the surrounding hemolymph and by the material synthesized by the ovary (Indrasith et al., 1988). Proteins, lipids and carbohydrates, are some of these materials, and they are all necessary for embryogenesis (Kanost et al., 1990). They supposed that methoxyfenozide could interfere with the accumulation proteins in the eggs, which might result in the reduction of fecundity and fertility in *S. litura.* Also, the significant decline in egg fertility may in small part be explained by the fact that methoxyfenozide has been reported to be ovicidal (Trisyono and Chippendale, 1997, 1998). In addition, when the larvae were fed on diet treated with sublethal dosages of its analogue tebufenozide, fertility was also reduced in adult tufted apple bud moth, *Platynota idaeusalis* (Walker), corn earworm *Helicoverpazea* (Boddie) and codling moth *Cydia pomonella* (Carpenter and Chandler, 1994; Brown, 1996; Biddinger and Hull, 1999).

The sterility in *C. Cephalonica* is recorded in terms of inhibition of egg laying capacity and reduced egg hatchability. Present findings (Table 1, 2, 3 and 4) clearly indicate that significant sterility can be achieved by treating larval stages of this moth with sublethal concentrations of 1, 5, 10 and 15 ppm of methoxyfenozide in rearing medium and maximum sterility can be achieved by treating first instar larvae with 15 ppm concentration of this IGR.

Sterility in insects might be due to (i) distorted gonads (ii) inhibition of vitellogenesis in the ova of treated females (iii) reduction of the no. of spermatophores in treated males and (iv) extrusion of ovipositor in the adult females (Mondal and Parween, 2000). Treatment of immature stages with IGRs produced various degrees of ovarian abnormalities and adults with such abnormality were generally sterile (Deb and Chakravorty, 1981; Mkhize and Gupta, 1982). The permanent ovarian abnormalities led to female sterility (Metwally and Land, 1972; Deb and Chakravorty, 1981). The sterility indices were pronouncedly dose-dependent and the egg viability increased by the decreasing dose level of methoxyfenozide and vice-versa. Our findings are in conformity with the results of Bhagyalakshmi (2022) who reported similar observation of sterility in RH-5849 exposed *C. cephalonica*.

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