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Potential of Methoxyfenozide (an Ecdysone Hormone Analogue) on the Growth Duration and Longevity of Adults of Rice Moth, *Corcyra cephalonica* Staint. (Lepidoptera:Pyralidae) Exposed as First Instar Larvae

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Abstract: *Corcyra cephalonica* first instar larvae when exposed to 1, 5, 10, 15, 20, 25 and 30 ppm concentrations of methoxyfenozide (an ecdysone hormone analogue) caused a significantly ($p < 0.001$) dose-dependent enhancement in the growth duration and a significant ($p < 0.001$; $p < 0.01$) dose-dependent reduction in adult longevity in both the sexes of this pest. The highest concentration i.e. 30 ppm of methoxyfenozide caused 100% larval mortality disrupting the growth and development of the larva. This concentration may be recommended for the complete eradication of *Corcyra cephalonica* in particular and lepidopterous pests in general.

Keywords: *Corcyra cephalonica*, Methoxyfenozide, Growth duration, Longevity, Instar, Larvae

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Introduction

The rice moth, *Corcyra cephalonica* Staint, is one of the most destructive pests of stored cereals and cereal commodities in Asia, Africa, Europe, North America and other tropical and sub-tropical regions of the world (Munro and Thompson, 1929). Its larval stages cause severe damage to rice, gram, sorghum, maize, wheat, ground nut and milled products (Atwal, 1976; Piltz, 1977).

Widely used control methods for stored cereals and cereal products insect pests comprise the application of organochlorines, organophosphates, synthetic pyrethroids and natural plant product. However, the organic synthetic insecticides result into development of resistance in the target pest species (Zettler and Cuperus, 1990; Boyer *et al.*, 2012) and health hazards (Arthur, 1996) in addition to killing of non- target organisms (Fields, 1992; Hagstrum and Subramanyam, 2006). To overcome these problems, a safe and suitable alternatives are required for the management of insect pests of stored cereals and cereal products. One of the alternatives is the inclusion of insect growth regulators (IGRs) (Fox, 1990), which mimic insect's hormone and regulate the insect population through the disruption of moulting and metamorphosis (Williams, 1956; Oberlander *et al*., 1997). The term IGR was coined by Stall (1975) to describe a class of bio-rational compounds. Through greater selectivity of action, these compounds appear to fit the requirements for "Third Generation Pesticides" (Williams, 1967) that disrupt the normal development of several species of insects (Henrick *et al*., 1973).

The IGRs include the compounds that interfere by three different modes of action: (i) juvenile hormone and juvenile hormone analogues that disrupt both insect embryonic development (Williams, 1967; Stall, 1975) and metamorphosis (Stall, 1975; Lim and Lee, 2005; Henrick, 2007) preventing recurrence of pests; (ii) ecdysone hormone and ecdysone hormone analogues (moulting hormone) that regulate moulting and metamorphosis with intimate coordination of juvenile hormone (Nation, 2002); and (iii) chitin synthesis inhibitors that disrupt growth and development by interfering with chitin synthesis (Pener and Dhadiala, 2012).

Methoxyfenozide (RH-2485) is a Lepidopteran-specific suitable ecdysone hormone analogue. It is a member of diacylhydrazine class (Carlson *et al*., 2001), a potent molt- accelerating compound that targets Lepidopteran insects (Enriquez *et al*., 2010) and is harmless to beneficial insects. This compound mimics the natural insect moulting hormone by binding competitively to ecdysteroid receptors in insect cells, thus resulting into prematured larval molt (Chen *et al*., 2019).

Role of ecdysone hormone analogue on the growth duration of insects have also been reported in different insect species like *Spodoptera litura* treated with tebufenozide (Seth *et al*., 2004); *Spodoptera frugiperda* (Zarate *et al*., 2010) and *Trogoderma granarium* and *Tribolium castaneum* exposed to methoxyfenozide (Ali *et al*., 2020). Adult longevity has also been found to be influenced by the exposure of IGR like methoxyfenozide as observed in case of *Spodoptera frugiperda* (Zarate *et al*., 2010); *Spodoptera litura* (Fabricius) (Rehan and Freed, 2015) and *Spodoptera littoralis* (Boisd) (Sabry *et al*., 2017).

Due to severe damage potential of *C. cephalonica*, the present work was designed and conducted to examine into the impact of ecdysone hormone analogue i.e. methoxyfenozide (a safe and suitable alternative) on the growth duration and adult longevity of this pest. Such knowledge may of course devise means and ways for the efficient control of *C. cephalonica* in particular and lepidopterous pests in general.

Materials and Methods

Corcyra cephalonica Staint. adults were obtained from laboratory stock culture maintained on normal dietary medium composed of coarsely ground jowar, *Sorghum vulgare* mixed with 5% (w/w) powdered yeast inside large glass containers (150 mm diameter, 200 mm height) at temperature 26 ± 1^{0}C, relative humidity (R.H.) 93 ± 5% and a light regime of 12 h light and 12 h darkness. Such a standard culture was maintained throughout the year. From this culture, whenever needed, newly emerged males and females were transferred to oviposition glass chambers (35 mm diameter, 200 mm height). Since, *C. cephalonica* individuals do not feed during their adult stage, no food was provided to them 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.

Methoxyfenozide ($C_{22}H_{28}N_2O_3$) (Fig. 1), N-tert-butyl-N′-(3-methoxy-o-toluoyl)-3,5-xylohydrazide, an ecdysone hormone analogue, Catalogue Number M262765, Lot- 16-SCC-7-1 used throughout the experiment, was obtained from Toronto Research Chemicals Inc., 2 Brisbane Road, Toronto, ON, Canada M3J 2J8.

For this study, a stock solution of known

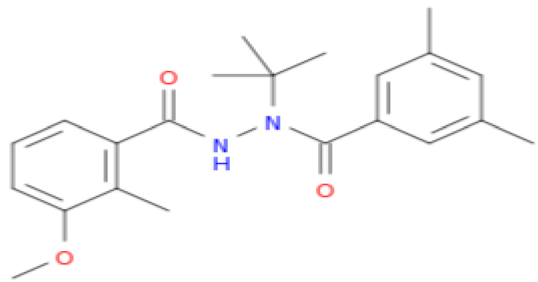

Fig. 1: Methoxyfenozide.

concentrations of methoxyfenozide was prepared in acetone and then adjusted via serial dilutions to achieve its required concentrations. Now, required volume of different concentrations of methoxyfenozide 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, 25 and 30 ppm of methoxyfenozide in dietary media. This treated food was then air dried at room temperature to eliminate completely the acetone. For control purposes, the normal food was thoroughly mixed with a required volume of acetone similar to that of treated food and then air dried in the same way.

For evaluation of growth duration, at each concentration of methoxyfenozide, freshly hatched larvae of *C. cephalonica* were allowed to feed on a normal dietary medium for exactly 4 days and on the 5th day, a group of 25 first instar larvae were transferred to each rearing chamber containing 50 g of dietary medium mixed and treated separately with different known concentrations of methoxyfenozide. On completion of life-cycle, when adults emerged, growth duration of both sexes were recorded (Table 1, Fig. 2). The longevity of adults emerged from these treated food were also recorded from day of emergence to day of death of males and females (Table 1; Fig. 3).

Experiments were replicated six times and the values have been expressed as mean ± SEM in days. Straight line regression equation was applied between different concentrations of methoxyfenozide and their corresponding growth duration of males and females and longevity of male and female adults to observe the significant correlation (Sokal and Rohlf, 1969).

Results and Discussion

Methoxyfenozide exposure to the first instar larvae of *C. cephalonica* caused a significantly ($p < 0.001$) dose-dependent enhancement in the growth duration (the time elapsing between egg laying to adult emergence) of both the sexes and a significantly ($p < 0.001$ and $p < 0.01$) dose-dependent decrease in adult longevity (life span of adults) of both the sexes (Table. 1, Figs. 2, 3). The growth duration of males was found to be 44.00 ± 3.10 days in control which significantly enhanced to 82.00 ± 4.38 days at 20 ppm concentration of methoxyfenozide. While the growth duration of females was recorded to be 47.00 ± 0.63 days in control which enhanced to 88.83 ± 4.03 days at 20 ppm concentration of methoxyfenozide. At a concentration of 25 ppm methoxyfenozide, larval tenure was further enhanced upto a period of 94.00 ± 8.75 days and failed to emerge, so no question of growth duration and adult longevity at this dose level of methoxyfenozide. In addition, larvae exposed to 30 ppm concentration of methoxyfenozide did not survive resulting into 100% larval mortality.

Table 1: Effect of methoxyfenozide on the growth duration and longevity of adults of *C. cephalonica* exposed as first instar larvae

Methoxyfenozide concentration (ppm)	Growth duration# of males (days)	Growth duration# of females (days)	Adult longevity# of males (days)	Adult longevity# of females (days)
Control	44.00 ± 3.10	47.00 ± 0.63	13.66 ± 3.38	6.16 ± 0.75
1	51.50 ± 4.37[b]	54.16 ± 4.71[b]	10.50 ± 1.05[b]	4.66 ± 0.82[b]
5	58.33 ± 2.58[a]	62.83 ± 4.71[a]	9.00 ± 1.55[b]	3.66 ± 0.82[a]
10	64.00 ± 5.90[a]	76.00 ± 3.10[a]	6.66 ± 0.82[a]	2.33 ± 0.82[a]
15	73.16 ± 4.07[a]	80.50 ± 3.78[a]	4.66 ± 0.52[a]	1.50 ± 0.55[a]
20	82.00 ± 4.38[a]	88.83 ± 4.03[a]	2.83 ± 0.41[a]	1.16 ± 0.41[a]
25*	94.00 ± 3.75[a]	94.00 ± 3.75[a]	-	-
30**	-	-	-	-

Values are expressed as mean ± SD of six replicates.

[a] and [b] Significantly different $p < 0.001$ and $p < 0.01$ compared with control values when t-test was applied.

Straight line regression equation was applied between different concentrations of methoxyfenozide and their corresponding growth duration of males and females and longevity of adults to observe the significant correlation:

Growth duration of males	$y = 1.81x + 46.99$;	$r = 0.99$	$p < 0.001$
Growth duration of females	$y = 1.81x + 52.21$;	$r = 0.98$	$p < 0.001$
Longevity of male adults	$y = -0.48x + 11.99$;	$r = -0.97$	$p < 0.001$
Longevity of female adults	$y = -0.23x + 5.21$;	$r = -0.94$	$p < 0.001$

*Larval/pupal tenure- Since adults did not emerge, so, this concentration has not been accounted in correlation.

**100% Larval mortality occurred and hence no question of growth duration and adult longevity at 30 ppm concentration of methoxyfenozide.

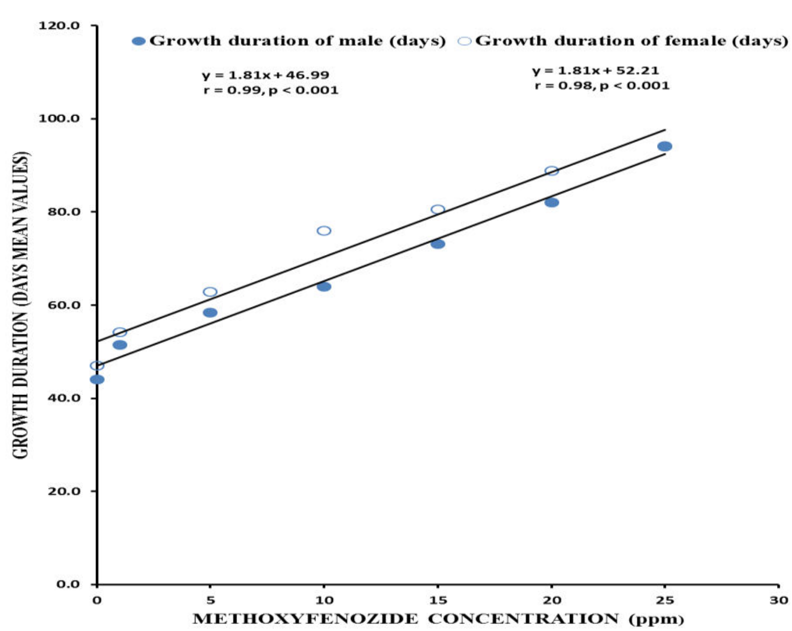

Fig. 2: Graphic representation of effect of methoxyfenozide on the growth duration of rice moth, *C. cephalonica* exposed as first instar larvae (Mean Values).

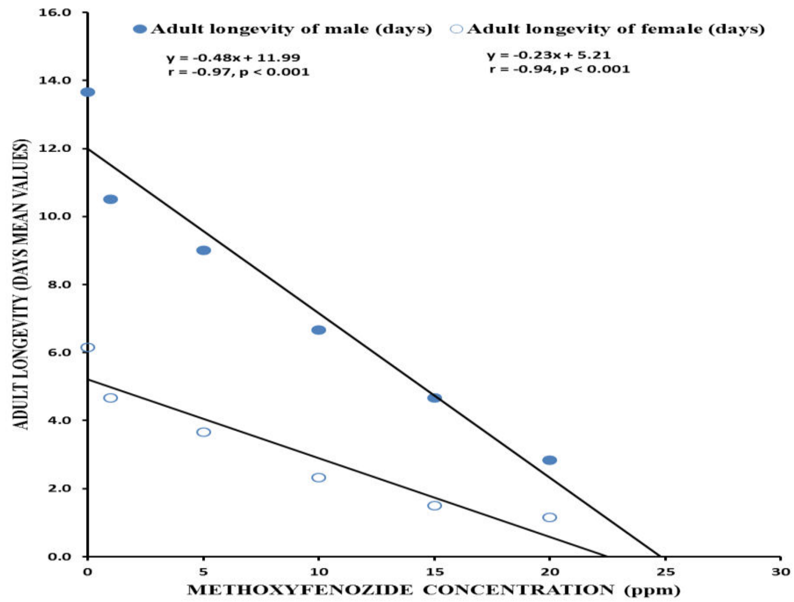

Fig. 3: Graphic representation of effect of methoxyfenozide on adult longevity of rice moth, *C. cephalonica* exposed as first instar larvae (Mean Values).

IGRs as a whole disrupt insect metamorphosis hence, the developmental time for pupation and adult eclosion are considerably increased. A short exposure to IGRs at definite instars of insect larvae would not affect the developmental time but a long exposure usually showed profound effects (Mian and Mulla, 1982).

Earlier findings revealed that RH-5849 (1,2-dibenzoyl-1-tert-butylhydrazine) at a dose level of 4.0 and 5.0 ppm also slightly enhanced the growth duration of *Spodoptera litura* to 5.47 ± 0.02 and 5.42 ± 0.07 days, respectively in comparison to control i.e. 5.08 ± 0.04 days when treated to 6th instar larvae (Seth et al., 2004). They further reported that 0.5, 1.0 and 2.0 ppm concentrations of tebufenozide (RH-5992) enhanced the growth duration of *S. litura* to 5.08 ± 0.02, 5.49 ± 0.66 and 6.09 ± 1.12 days, respectively in comparison to control i.e. 4.89 ± 0.13 (Seth *et al*., 2004). When 5th instar larvae of fall armyworm, *Spodoptera frugiperda* were exposed to 0.24 and 0.35 mg a.i./kg concentration of methoxyfenozide, the duration from the fifth instar to adulthood were 38.8 ± 0.49 and 40.3 ± 0.84 days, respectively in male and 37.0 ± 0.65 and 38.6 ± 0.95 days, respectively in case of female which was more longer period than control i.e. 20.7 ± 1.38 days in males and 17.3 ± 0.92 days in females (Zarate *et al*., 2010). Similar findings have also been reported when larvae of *S. frugiperda* were exposed as neonates to tebufenozide (Chandler *et al*., 1992). Zarate *et al.* (2010) observed that methoxy-fenozide exposed larvae of *S. frugiperda* take 1.6 times longer period to develop into females while 1.7 times longer period to develop into males in comparison to control. They have also mentioned that the possibility of increase in the length of the larval tenure of *S. frugiperda* exposed to methoxyfenozide may be due to the fact that larvae in their study had one or two extra molts. For instance, methoxyfenozide and tebufenozide exposed, *Ostrinia nubilalis* (Hubner) (Trisyono and Chippendale, 1997) and methoxyfenozide treated *S. littoralis* (Adel and Sehnal, 2000) larvae underwent an additional ecdysis into a sixth or seventh instar. Smagghe and Degheele (1994) also reported similar findings in case of *S. exigua* exposed to tebufenozide that resulted into two additional larval moults. In addition, methoxyfenozide induced enhancement in tenure of larval growth of *S. littoralis* has also been reported (Sabry *et al*., 2017). They suggested that methoxyfenozide perturbs the normal hormonal balance of the larva which may cause a crucial disorder in growth and development of *S. littoralis* (Sabry *et al*., 2017). Similar reasons may be assigned in the present investigation, for the dose-dependent enhancement in the growth duration of *C. cephalonica* larvae exposed to different concentrations of methoxyfenozide.

In the present investigation, the longevity (life span of adults) of *C. cephalonica* adults emerged from control dietary media was recorded as 13.66 ± 3.38 days for males and 6.16 ± 0.75 days for females. With increased concentrations of methoxyfenozide, in the rearing medium, the longevity of adults was found to be significantly reduced for male and female individuals. The longevity of males was recorded as 10.50 ± 1.05 days at 1 ppm concentration of methoxyfenozide, which was significantly reduced to 2.83 ± 0.41 days at 20 ppm concentration of methoxyfenozide. In a similar way, the longevity of females, at 1 ppm concentration of methoxyfenozide was found to be 4.66 ± 0.82 days which was decreased to 1.16 ± 0.41 days at 20 ppm concentration of methoxyfenozide. Similar findings were reported following the action of RH-5849 on 6th instar larvae of male *Spodoptera litura* exposed to 4.0 ppm concentration that reduced male longevity to 5.22 ± 0.28 days than control i.e. 10.15 ± 0.31 (Seth *et al*., 2004). It was observed that 4th instar larvae of *S. littoralis* when exposed to 0.353 ppm concentration of tebufenozide, the adult longevity was found to be reduced to 11.00 ± 0.28 days in comparison to that of control i.e. 12.3 ± 0.1 days (Abdel-Aal, 2012). IGRs are not toxic to the adults themselves, but long exposure to the early larval stages cause disruptions in the organ systems or interrupt adult ecdysis and reduce the adult life-span (Mondal and Parween, 2000). Oral treatment of 100 mg (a.i.)/L concentration of methoxyfenozide had a highly significant effect on the adult longevity of *S. littoralis* i.e. 10.0 ± 0.6 days in comparison to control i.e. 12.3 ± 0.5 days (Pineda *et al*., 2009). Methoxyfenozide reduced the longevity of *S. exigua* males compared with females by upto 17% at the higher concentration i.e. 150 mg (a.i.)/L (Luna *et al*., 2011). Similarly, a reduction in longevity of 19 and 30 % was observed in adults, regardless the sex of *S. littoralis* (Pineda *et al*., 2009) and female of *Grapholita molesta* (Busck) (Lepidoptera: Tortricide) (Reinke and Barrett, 2007), when exposed to methoxyfenozide by ingestion or methoxyfenozide treated surfaces. In contrast, no effects on longevity were observed when adults of *C. pomonella* and *Lobesia botrana* Denis et Schiffermuller were exposed to tebufenozide or methoxyfenozide (Pons *et al*., 1999; Saenz-de-Cabezon *et al*., 2005). When 3rd instar larvae of *Helicoverpa armigera* were exposed to 1.485 μgmL^{-1} of methoxyfenozide, the longevity of male and female was found to be 9.65 ± 0.29 and 7.50 ± 0.73 days, respectively but its higher concentration i.e. 7.749 μgL^{-1} showed more reduction in the longevity of male and female i.e. 8.90 ± 0.77 and 8.30 ± 0.85 days, respectively in comparison to control i.e. 10.20 ± 0.68 days in male and 9.60 ± 0.58 days in female (Zhang *et al*., 2020).

Fifth and sixth instar larvae of *S. litura* exposed to RH-5849 showed a significant reduction in adult longevity but the reason for reduction in adult longevity is still not clear (Seth *et al*., 2004). Pineda *et al*. (2009); Zarate *et al.* (2010) and Luna *et al.* (2011) suggested that the mechanism by which ecdysone agonists can affect adult longevity is unclear because the biochemical target site for these compounds in adult lepidoptera are unknown. Therefore, further research is needed to elucidate the effects of ecdysone agonists on the longevity of adults derived from larvae treated at immature stages.

Conclusion

On the basis of the results of the present study, it may be concluded that initial stage of *C.*

cephalonica larva must be treated with methoxyfenozide to facilitate lenthy duration of exposure for the effective control of this pest in particular and lepidopterous pests in general.

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