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Efficiency of Azadirachtin, A Chitin Synthesis Inhibitor on Growth, Development and Reproductive Potential of *Tribolium confusum* after Adult Treatment

Najat Aly Khatter
Department of Biology, Faculty of Science for Girls, King Abdul-aziz University, Jeddah, Saudi Arabia

ABSTRACT

The present study was established to evaluate the potential of insect growth regulator on the growth and development of *Tribolium confusum* Azadirachtin, a neem derivative which considered as insect growth regulator due to its chitin inhibitor activity was tested topically at four doses (0.5, 1, 1.5 and 2 µg insect^{-1}) on adults of *Tribolium confusum* (Coleoptera: Tenebrionidae) and first evaluated on fecundity, hatchability and viability of eggs, longevity and morphometric of oocytes. Results show that the compound reduces the longevity and the fecundity. Generally, treatment with azadidrachtin affected growth and development of oocytes and egg-viability as shown by calculating the number of oocytes per ovaries and the size of basal follicle. The width of the follicle has not been affected significantly by these treatments. In the second group of experiments, this IGRs don't induce significant reduction in percentage of hatchability, the hatching rate of eggs laid by F1 females was not affected by the treatment (F = 2.86, DF = 99, p = 0.5) but it affect very significantly the viability rate of eggs laid by first female generation F1 (F = 7.5, DF = 99, p>0.0001). However, the azadirachtin lengthened very significantly the total duration of life cycle. Duration of the embryonic and post embryonic development of eggs laid by treated female increase when the concentration varied from 0 to 2 µg µL^{-1}. This data confirm the efficiency of growth regulators derived from neem plant, on the development of stored product insect pests.

Key words: Azadirachtin, *Tribolium confusum*, eggs, development, vecundity, longevity, morphometric of oocytes, viability

INTRODUCTION

In recent years the use of synthetic organic insecticides in stored product pest control programs around the world has resulted in damage to the environment, pest resurgence, pest resistance to insecticides and lethal effects on non target organisms (Abudulai *et al.*, 2001). Insect growth regulators, botanical insecticides and microbial pesticides are highly effective, safe and ecologically acceptable (Weinzierl and Henn, 1991; Nathan *et al.*, 2005a,b; Nathan and Kalaivani, 2005; Sadeghian and Mortazaienezhad, 2007; Radhakrishnan *et al.*, 2007; Suman *et al.*, 2010).

The common trend in the past to decades toward reducing reliance on synthetic insecticides for control of insect pests in agriculture, stored products, forestry and human health has renewed worldwide interest in the neem derivative, azadirachtin, as an environmentally desirable alternative (Paoletti and Pimentel, 2000; Nderitu *et al.*, 2008). Because of secondary effects of conventional insecticides, the Insect Growth Regulators (IGRs) are receiving more practical allention to provide for safer tools and a cleaner environment.

Among these compounds the neem product, azadirachtin cause abnormal circular deposition and abortive molting in insects by interfering with chitin biosynthesis (Casida and Quistad, 1998). It was found to be effective on several insect species (Khebeb *et al.*, 1997; Ben Jannet *et al.*, 2001; Flamini *et al.*, 2003; Chaieb *et al.*, 2007). These compound have been found to interfere with chitin biosynthesis (Fields *et al.*, 2001; Soltani *et al.*, 1983, 1996a). Other IGRs were reported to inhibit ecdysteroid synthesis (Chebira *et al.*, 2000; Soltani-Mazoun *et al.*, 2000; Al-Fifi, 2006) or mimic the action of 20-hydroxy-ecdysone (Soltani *et al.*, 2002). Saponin extract from alfalfa roots, azadirachtin from the neem seed oil, synthetic ecdysteroid agonist RH-2485 and juvenoid hydroperene disturb the development and reproduction of *Tropinota squalid* (Coleoptera: Scarabeidae) (Hussein *et al.*, 2005; Facknath, 2006). *Tribolium confusum* is a major insect pest of stored grain in all over the world and whose larval development occurred in flour. In additions laboratory and field tests have evaluated the toxicity of several IGRs against some stored product pests (Abo El-Ghar, 1992; Soltani-Mazouni, 1994; Soltani-Mazoun and Soltani, 1995; Soltani *et al.*, 1996b; Oberlander *et al.*, 1997; Peppuy *et al.*, 1998). The aim of the present study was to investigate the effect of azadirachtin on growth and development, adult reproductive potential and egg hatchability of *T. confusum*. This IGR is tested for first time for control insects that develop in stored flour.

MATERIALS AND METHODS

Rearing of insects: Stock cultures of *T. confusum* were maintained in glass jars (20 cm in height, 10 cm diameter) containing wheat flour. Insects were kept at 30±1°C and 70±5% R.H. under almost continuous darkness. The laboratory experiments were carried out in July 2010 and extended to January 2011.

Toxic material (azadirachtin) and treatments: Azadirachtin was kindly supplied by Dow Elanco (Iniamapolis, USA). Serial dilutions were prepared in acetone and several doses (0, 0,5, 1, 1.5 and 2 μg insect^{-1}) were applied topically on newly emerged adults (24-old). Acetone (1 μg individual^{-1}) was used as control. The bioassay was conducted with four replicates each of 50 adult insects (25 males and 25 females) placed in glass box (20 cm diameter, 5 cm height) containing food (100 g of dry wheat flour).

Measurements of ovaries: Females were sampled from control and treated series at different moments during the adult life (0, 1, 2 and 3 days) and ovaries dissected. The number of oocystes per ovaries and the size of basal oocytes were determined.

Determination of longevity, fecundity and hatchability rate: Newly emerged adults of *T. confusum* were collected from rearing containers control and treated series were reared under standard conditions. The longevity of both males and females, the total number of eggs laid per female (Fecundity and the percentage of egg hatching were determined.

Growth and development: Laid eggs were collected from controls and treated series and placed under standard conditions. The time taken for adult emergence was determined. The number and the body weight of first generation were determined in all series, the survival rate during the post-embryonic development was calculated (number of emerged adults on number of eggs laid×100).

Statistical analysis: Data are presented as the Mean±SD. The age and the number of insects tested per series are given with the results. To detect azadirachtin among treatments, data were subjected to analysis of variance and one way ANOVA test.

RESULTS

Effects on morphometric parameters of ovaries

Number of ovocytes per paired ovaries: Results in Table 1 shown that the number of oocytes by pair of ovaries in treated females was decreased significantly in females (1-3 days), when these females treated with 2 μg of azadirachtin it record (58 to 52.3) when compared with control groups which record a mean varied from (55.9 to 58.3) according to the age of the female.

The oocytes basal size: The length of basal oocytes decreased just significantly according to the age of females descended from treated parents (2 μg). The width has not been affected significantly by this treatment compared with control group which show no difference in width of the basal oocytes size according to the age of the female (Table 2).

Effects on reproductive capacity: Results represented in Table 3 shows the effect of the tested material, azadirachtin on the fecundity of resulted females which indicate a highly significant reduction ($p<0.0001$) of number of eggs laid by each female treated with the higher dose (2 μg μL^{-1}), compared with the control. The number of eggs laid by the first generation females was

Table 1: Effect of azadirachtin on the number of oocytes per paired ovaries in F1(first generation) female adults *of Tribolium confusum* developed from previously treated adults with dose of 2 μg $Insect^{-1}$

Age of females (days)	0	1	2	3
Control	55.9±7.6	59.2±6.9	59.2±8.9	58.3±8.9
Treated females	58±6.6	55.6±6.7	5.7±7.6	52.3±7.15
Anova	F = 3.18	F = 4.13	F = 4.13	F = 16.33
	p = 0.0797ns	p = 0.0431*	p = 0.000***	p = 0.000***

Values represent Mean±SE (n = 50 females per age), ns: No significant difference, *Significant, ***Highly significant at ($p<0.05$)

Table 2: Activity of azadirachtin on the width and the length (mm) of basal oocytes of F1 adult females *of Tribolium confusum* resulted from treated adults (2 μg $Insect^{-1}$)

Age of females (days)	Width		Length	
	Control	Azadirachtin	Control	Azadirachtin
0	0.18±0.04	0.19±0.02	0.37±0.5	0.32±0.7
1	0.17±0.05	0.17±0.04	0.32±0.4	0.33±0.2
2	0.16±0.05	0.15±0.03	0.30±0.3	0.32±0.6
3	0.19±0.04	0.16±0.01	0.33±0.6	0.30±0.5
Analysis		F-value		p-value
Anova 2 ways (Width)				
Age factor		3.90		0.005***
Treatment factor		4.20		0.036*
Interaction		2.33		0.046 ns
Anova: 2 ways (Length)				
Age factor		3.32		0.019*
Treatment factor		1.03		0.0301 ns
Interaction		3.31		0.031*

Values represent Mean±SE (n = 50 females per age). ns = No significant difference, *Significant, ***Highly significant at ($p<0.05$)

higher than the one of the females treated especially with the higher doses. The treatment undergone by the parents did not affect the egg laying of the F1 females. The hatching rate of eggs in the control did not differ significantly the one of the eggs laid by the females treated with dose of 0.5 μg μL^{-1} (F = 20.19, DF = 99, p>0.0001). The hatching of eggs laid by the females treated with the doses higher than 0.5 μg μL^{-1} has been reduced more than the one of the eggs laid by the F1 females (Fig. 1). The hatching rate of eggs laid by F1 females was not affected by the treatment (F = 2.86, DF = 99, p = 0.5).

Also data illustrated in Fig. 2 the effect of the tested growth regulator, the neem product (azadirachtin) on the viability or post-embryonic survival rate of eggs laid by the treated females which was significantly reduced (F = 27.04, DF = 99, p>0.0001) different between the control and the treatments.

Effects on growth and development: Results in Table 4 showed the duration of the life cycle, (eggs to adults), did not differ significantly between the control and the treatment with dose of 0.5 μg. However, the azadirachtin lengthened very significantly the total duration of the embryonic and post-embryonic development, when the dose was higher than 0.5 μg μL^{-1}.

The weight of adult males derived from treated adults didn't differ significantly between the control and the treatments (0.5 and 1 μg). This weight decreased just significantly (F = 3.33, DF = 99, p = 0.0218) if the dose of the parents treatment was higher than 1 μg (Fig. 3). The azadirachtin did not affect significantly the weight of the F1 adult females (F = 1.23, DF = 99, p = 0.3011) (Fig. 3). The adult individual (males and females) lived between 8.2 to 8.5 days in the

Table 3: Effect of azadirachtin on the viability rate (Fecundity) of *Tribolium confusum* females

	Doses (μg $Insect^{-1}$)				
Results	0	0.5	1	1.5	2
Treated females	78.0 ±13.7	72.1±13.2	76.0 ±11.7	63.8±2.7	43.0 ±12.3
F1 females	82.4 ±15.2	88.2 ±1.9	87.1 ±5.4	85.3 ±2.1	73.0±7.4
Anova					
F-value	0.35	7.75	4.34	19.46	60.07
p-value	0.573 ns	0.024 ns	0.071 ns	0.002**	0.000***

Values represent Mean±SE (n = 50 females per age), ns = No significant difference, *Significant, ***Highly significant at (p<0.05)

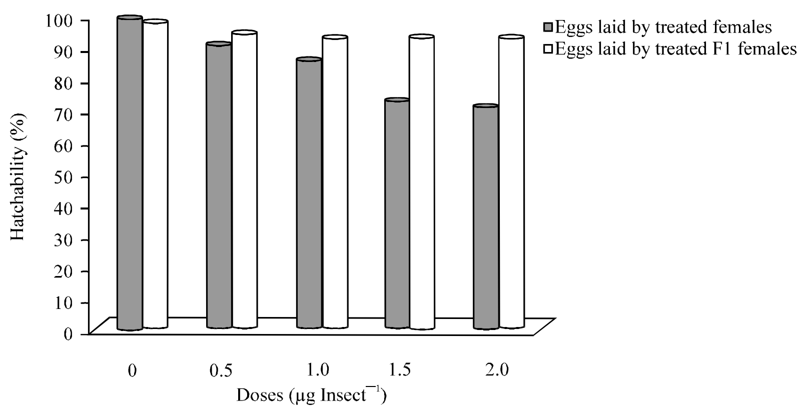

Fig. 1: Hatchability rate of eggs laid by treated females and F1 of *Tribolium confusum*

Table 4: Effect of azadirachtin on the duration of the developmental cycle (egg to adult) in *Tribolium confusum*

Doses (µg Insect^{-1})	0	0.5	1	1.5	2
Duration (days)	28.6±1.9	28.4±5.2	30.1±0.5	32.5±0.5	31.5±0.2
Anova	F = 39.11 and p<0.05***				

Values represent Mean±SE (n =100 eggs for each dose), ***Highly significant at (p<0.05)

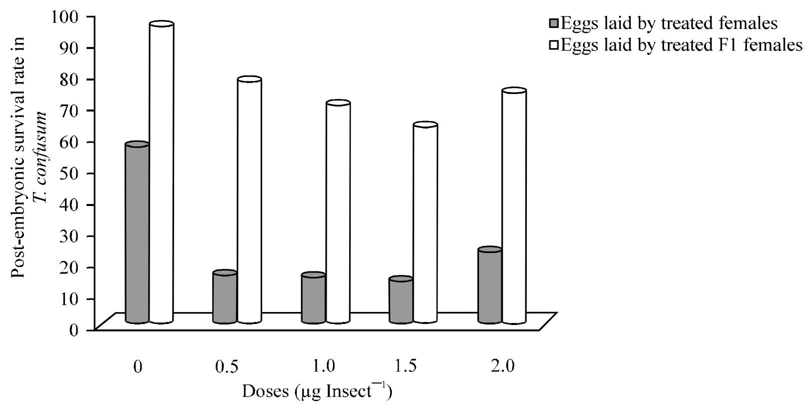

Fig. 2: Post-embryonic survival rate in *Tribolium confusum*

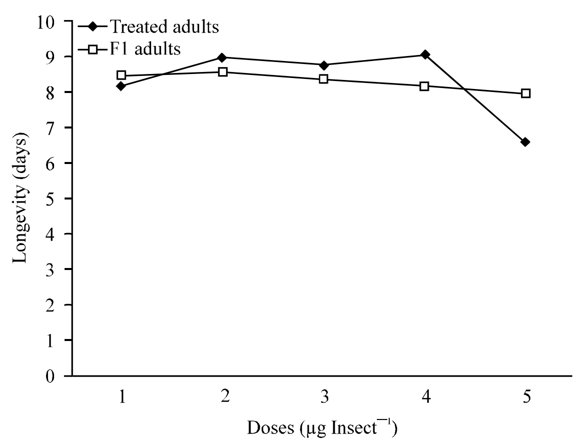

Fig. 3: Weight of adult progeny of *Tribolium confusum* refernined to the dose of parents treatments

control. The azadirachtin reduced significantly (F = 19.63, DF = 29, p = 0.002) the adult longevity when the flour beetle was treated with the highest dose. On the other hand, this growth regulator did not affect the longevity of the first generation adults (Fig. 4).

DISCUSSION

Using of IGRs into management strategies to control *T. confusum* in flour warehouses presents different issues than the use of IGRs in stored grain commodities. The familiarity and visibility of this pest to consumers, its ability to penetrate food packing and the essentially zero tolerance for insects in finished food products are challenges that be faced when using IGRs for any other insecticide. Because IGRs can be considered reduced-risk insecticides, they represent excellent options for control in the warehouses environment. The tests in which *T. confusum* larvae were

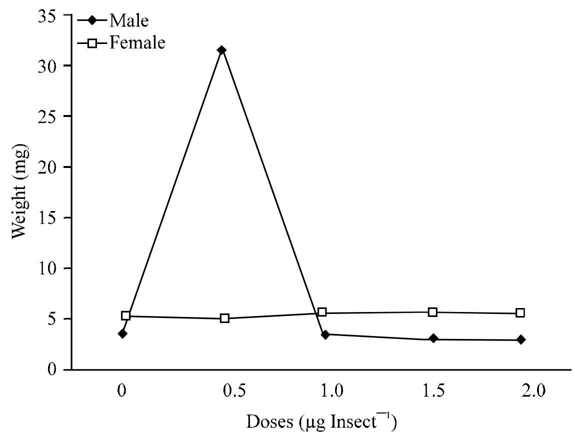

Fig. 4: Effects of azadjrachtin treatments (µg Insects^{-1}) on adult longevity of *Tribolium confusum*

exposed on concrete treated with azadirachtin show the potential of using IGRs to control this species in food warehouses, retail stores and urban storage situations. Arbogast *et al.* (2002) showed reduction in population of several stored-product pests including *T. confusum*, when hydroprene was applied as a spot-treatment in a botanical warehouses. In laboratory tests, exposure of *T. confusum* adults prolonged the development time of the eggs and caused mortality in the dose-dependent manner similar to the results described for larval exposure (Mohandass *et al.*, 2006).

The results of our study confirm the efficiency of the growth regulators, derived of the neem plant, azadirachtin on the development of insect pest of stored products whose known as a steroid like tetra-notriteroenoid, isolated in 1968 from neem seeds (Butterworth and Morgan, 1971) along with about 40 biner principles components belonging to diterpenoid, triterpenoids and flavinoid groups from different parts of neem tree. The structural geometry of azadirachtin was revealed by Nakanishi (1975). These neem compounds have shown broad range of bioactivity against insects such as reproductive fitness oviposition, hatchability, antifeedent, repellent, metamorphosis disruption and death (Su and Mulla, 1998; Kumar and Parmar, 1998; Mulla and Su, 1999; Mital and Subbarao, 2003; Garcia *et al.*, 2006; Abdullah and Subramanian, 2008), these findings were in agreement of present results. Feng and Ishman (1995) showed the development of resistance in Peach potato aphid *Myzans persicae* to pure azadirachtin over 4 generation but the same did not happen with neem extract. The multiple mode of action or complexity of mechanism may be responsible for avoiding the development of resistance in insects against mixture of neem compounds. Hami *et al.* (2005) study the comparative toxicity of three ecdysone against insecticide against the Mediterranean flour moth, *Ephestia kuehniella*. They concluded that using bisacylhdrazine derivatives are non steroidal ecdysteroid agonists that mimic the action of moulting hormone. Also, our findings agree with that obtained by Arthur *et al.* (2009) who concluded that pyriproxyfen gave effective residual control of primary stored-product insect species by inhibition emergence of exposed larvae and Sengottayan *et al.* (2009) concluded that the neem based insecticide (Parker oil and Neema) containing low lethal concentration, can be used effectively to inhibit the growth and survival of *Nilaparavata lugens*.

Kumari (2010) found that IGRs cause a reduction in chitin, also defects in the mobility (leg movement) was observed, these observations also detected during our laboratory experiments. In contrast, Athanassiou *et al.* (2005) found that neemazal was not very effective against *T. confusum* where adult mortality was very low. It is clear from many investigations that azadirachtin-based insecticides can be used with success for protection of stored products, Kavallieratos *et al.* (2007) found that the use of these substances is not comparable to the use of traditional grain protectants.

In summary, there are many opportunities for the increased use of IGRs in management programs to control stored product insects. In storage sites where the primary pest is *Tribolium confusum* and control is targeted towards the larval and adult stages, IGRs used alone may be sufficient to give complete control.

CONCLUSION

In this study, we have demonstrated that azadirachtin has a latent effect on *T. confusum* progenies as manifested by the reduced growth and development of immature and mature stages and lesser production of adults. The present results imply that the use of natural products like azadirachtin as pesticide would help controlling the storage pests in an environment-friendly way.

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