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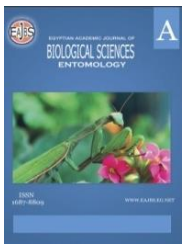

Toxicological Assessment of Neonicotinoid Acetamiprid Against the Confused Flour Beetle, *Tribolium confusum* Du Val (Coleoptera: Tenebrionidae)

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ABSTRACT

The confused flour beetle, *Tribolium confusum,* is a common insect that can be found in stores and homes and attacks a variety of commodities and stored foods. In the past, synthetic chemical insecticides were used to control them, which caused many negative health and environmental effects. Acetamiprid is a neonicotinoid insecticide with a novel mechanism of action, low toxicity for mammals, and a low environmental impact. The effectiveness of that insecticide against *T. confusum* was tested in this study. The toxicological bioassay was performed by contact application with different concentrations of acetamiprid. Our results indicated that the increased concentration and exposure periods significantly enhanced the mortality rate. The mortalities reached 20.6±1.12% and 92.6±1.86% at 1 ppm and 8 ppm concentrations of acetamiprid, respectively, after 14 days of exposure. The effects of acetamiprid on the fecundity and sterility of *T. confusum* have been assessed. Laboratory studies indicated that acetamiprid significantly reduced egg laying and hatching in comparison with the control. All acetamiprid concentrations used resulted in sterility in the treated adults. At the highest concentration (8 ppm), the sterility was 98.21%. The effects of acetamiprid on the activity of the acetylcholinesterase (AChE) enzyme were also investigated. It was observed that AChE from *T. confusum* was almost completely inhibited at the highest concentration. On the contrary, the level of the detoxifying enzyme, glutathione S-transferase, was increased in the treated samples. Acetamiprid's mutagenic effect on *T. confusum* was investigated by applying the median-lethal concentration (LC_{50}), investigating DNA alteration for polymorphic numbers of genetic bands by using RAPD-PCR primers, and comparing it with the control. Results strongly suggest that acetamiprid causes mutagenic effects on *T. confusum*. The results recommended that acetamiprid can be used as a useful tool in pest management programs for *T. confusum* considering its insecticidal effects, which are evident at low applied concentrations and short exposure times.

INTRODUCTION

Tribolium confusum (Coleoptera: Tenebrionidae) is a significant pest in flour mills and cereal processing facilities (Hawkin *et al*., 2011). *T. confusum* infestation is correlated with severe product losses and quality decline (Abdel Rahman *et al*., 2014). It has been reported that members of the genus *Tribolium* secrete certain toxic substances in stored goods that have the potential to cause cancer, posing serious health risks to humans. (Iram

et al., 2013). The use of synthetic insecticides to control insect pests in stored products has been banned or may be restricted worldwide due to issues of food grains' persistent toxicity, insect populations' subsequent development of resistance, effects on non-target organisms, and other negative effects on the environment (Saglam *et al.,* 2013). For instance, phosphine fumigation was frequently used to rid these facilities of insects. However, because reinfestation could occur quickly, this method did not provide long-term protection (Campbell *et al.*, 2010). Some strains of *T. confusum* have been found to be resistant to phosphine as well as contact insecticides like malathion, chlorpyriphos-methyl, and dichlorvos. (Zettler, 1991). Alternatives to phosphine for controlling *Tribolium* species, such as heat treatment and controlled environments, are used (Mahroof *et al.*, 2003; Chiappini *et al*., 2009), but they are expensive and unable to offer long-term protection. High temperatures above 50ºC can only control the adults, not all the life stages of the insect.

An alternative mode of action to organophosphate, carbamate, and pyrethroid insecticides is provided by neonicotinoids (acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid, and thiamethoxam). They are low in toxicity to mammals, have fewer problems with revival, protect the environment, offer options for pest management, and are low in toxicity to natural enemies, among other characteristics. (Jeschke *et al*., 2011; Goulson, 2013). Neonicotinoids are an innovative and unique class of insecticides with exceptional chemical and biological characteristics. In many application methods, they are among the most promising insecticides against lepidopterous pests (Elbert *et al.*, 1998; Hamama *et al*., 2015). Acetamiprid and imidacloprid are novel classes of insecticides with a neurotoxic mode of action. Acetamiprid was used to control all life stages of the asparagus beetle (Kuhar *et al*., 2006). Sublethal concentrations of acetamiprid have transgenerational effects on insect pests (Ullah *et al*., 2020). The reproductive aptitude of *Eriopis connexa* females emerging from fourth-instar larvae survivors were affected by very low concentrations of acetamiprid (Fogel *et al*., 2013). Imidacloprid has been used to treat seeds as coats on a large scale against a variety of pests with success (Pons and Albajes, 2002; Zhang *et al*., 2011). Depending on the concentration, exposure time, and temperature, thiamethoxam was effective against red flour beetles (Arthur *et al.*, 2004). A novel insecticide, chlorantraniliprole, which interrupts normal muscle contraction, is novel in its mode of action. Several pests of fruits and vegetables can be controlled with chlorantraniliprole (Jiang *et al*., 2012). Acetylcholinesterase (AChE) is an enzyme that catalyzes the cleavage of acetylcholine into its constituent compounds, acetate and choline, which play a transmitter role in the neuromuscular junctions (Zhu *et al.*, 2000). Neonicotinoids are nicotinic acetylcholine receptor agonists. Insects' central nervous systems contain nicotinic acetylcholine receptors (nAChRs), which they strongly bind to. At low concentrations, they stimulate the nervous system, but at higher concentrations, they cause receptor blockage, and paralysis, followed by death (Tomizawa and Casida, 2011; Simon-Delso *et al*., 2015).

Genetic variation was screened using random amplified polymorphic DNA analysis by polymerase chain reaction (RAPD-PCR). It provides a useful biomarker assay in ecotoxicology without any prior knowledge of genomic DNA (Rocco *et al*., 2012).

To our knowledge, there is no published information on the toxicity of acetamiprid insecticide on *T. confusum*. Thus, the present study is an attempt to evaluate the toxic effects of acetamiprid on some biological aspects of adults and its latent molecular effects on the larvae of *Tribolium confusum.*

MATERIALS AND METHODS

Insect Maintenance and Bioassay:

A colony of *Tribolium confusum* was obtained from the laboratory of the Grains and Stored Product Pest Research Department, Plant Protection Research Institute, Agricultural Research Center, Ministry of Agriculture and Land Reclamation in Dokki, Giza, Egypt. In the incubator, adult insects were fed organic wheat flour, which was supplemented with 5% (by weight) of brewer's yeast. Flour is sieved to eliminate the larger particles of bran and yeast. The beetles were kept in a dark incubator at 28±2°C and 60-70% RH. Using filter paper (Whatman no.1) that fits into the Petri dish, the contact method of evaluating the insecticidal activity was used. Diluting acetamiprid (Wapkil® 20 SP) in water at six different concentrations: 0, 1, 2, 4, 6, and 8 ppm. One ml of each concentration was distributed on the surface of the filter paper by using a micropipette. All filter papers are placed in plastic Petri dishes (100/15 mm with vents) and labelled according to the used concentration. Control treatments were performed with topical applications of water. After one hour, once the water had been dried, two grams of the mixed wheat flour with yeast were added to Petri dishes, and then 25 unsexed newly emerged adults were deposited and kept at the same temperature and relative humidity in the dark. Five replicates were made for each treatment. The mortality of adults was recorded daily for 14 days after treatment. The accumulated mortalities after fourteen days of the treatment were used to draw the regression line. When prodded three times with a fine brush, adults were considered dead because they lacked appendage movement.

Biological Efficacy:

Pupae were separated into males and females in two different Petri dishes for further experimentation. One adult female and three males were placed in a Petri dish (35/15 mm with vents) containing one gram of mixed flour with yeast (10:1, w/w) and one of each of the six concentrations. The treatment was replicated 10 times. All replicates were left for 4 days to reproduce. After 7 days, a sieve (no. 60 with pores of 250 μm) was used to separate the eggs from the flour. The number of deposited eggs (fecundity), as well as the number of hatched eggs (fertility), were counted to evaluate the effect of the tested compound on *T. confusum*. The formula of Chamberlain (1962) was used to determine the sterility percentage:

$$\text{Sterility percentage} = 100 - \left(\frac{\text{a x b}}{\text{A x B}} \text{ x } 100\right)$$

Where: “a” is the number of eggs produced by each treated female, “b” is the percentage of hatched eggs produced by each treated female, “A” is the number of eggs produced by each control female, and “B” is the percentage of hatched eggs produced by each control female.

Acetylcholinesterase (AChE) Activity Assay:

Adults of *T. confusum* were treated with different concentrations of the acetamiprid (as previously described), and after 48 hours of the exposure, the successive survivors were homogenized in 100 mM phosphate buffer at pH equal to 7.5 and supplemented by 20% glycerol and centrifuged at 14,000 x g for ten minutes at 4-degree centigrade. The supernatant was used for the test. The method described by Ellman *et al*. (1961) was used to measure AChE activity. A mix of 50 mL of 10 mM 5,5'-Dithiobis-2-Nitrobenzoic Acid (DTNB) reagent, 50 mL of 12.5 mM acetylthiocholine iodide, and 800 mL of sodium phosphate buffer (pH 7.5) were added to each aliquot of homogenate from treated and untreated adults. By using an ultraviolet spectrophotometer (Lark LI-UV-2500) against the blank. The sample's optical density was measured at 400 nm following a five-minute incubation period at room temperature.

Glutathione S-transferase (GST) Activity Assay:

After 48 hours, normal and treated adults (as previously described) were homogenized in a 100 mM potassium (K)-phosphate buffer at pH 7.4. The homogenates were centrifuged at 10000 x g for 15 minutes in a cooling centrifuge (4^{o}C). The supernatant was used to measure the GST activity by using 1-chloro-2,4-dinitrobenzene as a substrate, according to Habig *et al*. (1974). The colorimetric test was read at 340 nm.

Extraction of Genomic DNA:

For the purpose of extracting genomic DNA, samples of 3rd instar larvae produced from treated adults with LC_{50} (2.43 ppm) and from untreated adults for the control were collected. Following the instructions provided by the manufacturer, DNA was extracted using a QIAamp DNA Mini Kit (QIAGEN, Cat. No. 51304). Using an optical density ratio at 280 nm and 260 nm, a spectrophotometer was used to measure the extracted DNA's purity and concentration. The extracted DNA was placed at -20°C until use in amplification processes.

RAPD-PCR:

The isolated genomic DNA (from treated and untreated larvae) was analyzed by random amplified polymorphic DNA by polymerase chain reaction (RAPD-PCR). Three different primers (Operon primer series A) were used to screen genetic variation in *T. confusum* larvae resulting from treated adults with the LC_{50} of acetamiprid. The used primers in the RAPD-PCR analyses were OP-A07 (5′-GAAACGGGTG-3′), OP-A09 (5′-GGGTAACGCC-3′), and OP-A13 (5′-CAGCACCCAC-3′). DNA was amplified using a 12.5 uL master mix (1.5-unit Taq Polymerase, 10mM dNTPs, and PCR buffer) and 20 ng of the genomic DNA as a template. Ten pmol of the corresponding primer was added and the final reaction volume was adjusted to 25 uL. The amplifications were carried out using a personal thermal cycler (Biometra; Gottingen, Germany) programmed as follows: one cycle at 92^{o}C for three minutes, 45 cycles at 92^{o}C for thirty seconds, 35^{o}C for sixty seconds, and 72^{o}C for two minutes. The reaction was then incubated for a total of 10 minutes at 72°C and another 10 minutes at 63°C. In TAE buffer (0.04 M Tris-acetate, 1 m M EDTA, pH8), 2% agarose gel (1% Nusieve GTG, 1% Seakam L.E., FMC Bioproducts) electrophoresis was carried out. Products from RAPD were stained with 0.2 mg/ml ethidium bromide before being captured on camera under UV light.

Statistical Analyses:

Based on Finney's method (1952), the bioassay results were processed by the computer software Ldp to produce the regression line and detect LC_{25}, LC_{50}, and LC_{95} values.The means of the data in the various experimental treatments were statistically analyzed using ANOVA (LSD). After calculating the mean percentages of the data, the standard error of the mean (SE) was added. A non-significant relationship was presented when $P \geq 0.05$ and a significant relationship was presented when $P < 0.05$.

RESULTS

Toxic Effect of Acetamiprid on *Tribolium confusum* Adults:

A bioassay test was conducted to analyse the toxicity of the neonicotinoid insecticide acetamiprid on the newly emerged adults of *T. confusum* by the contact method. The percentage means of mortalities were calculated and presented in Table (1). All the untreated insects showed a 100% survival rate after the time of the experiment (14 days). The mean percentage of mortalities in the treated adults was significantly ($P < 0.05$) elevated when the concentration of the acetamiprid was increased. The accumulated mortalities after 14 days of exposure were used to produce the regression line. From the regression line in Figure (1), the values of LC_{25}, LC_{50}, and LC_{95} were defined as 1.24, 2.43, and 12.40 ppm,

respectively. In addition, the slope value of the produced line is 2.32±0.1 which indicates the effectiveness of the acetamiprid insecticide.

Table 1: Toxic effect of acetamiprid on *Tribolium confusum* adults as mean percentage mortality at different time intervals**.**

Conc.	Mean percentage mortalities ± SE							
	DAT 1	DAT 2	DAT 3	DAT 4	DAT 5	DAT 6	DAT 7	DAT 14
Control	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
1 ppm	0.8±0.37^{b}	4.6±1.03^{b}	10.2±1.07^{b}	12.4±1.86^{b}	13.75±2.05^{b}	15.2±2.08^{b}	18.2±1.36^{b}	20.6±1.12^{b}
2 ppm	3.6±1.50^{c}	6.8±1.53^{c}	15.6±1.43^{c}	24.6±2.29^{c}	28.2±1.62^{c}	33.4±1.81^{c}	40.6±1.21^{c}	42.2±1.16^{c}
4 ppm	20.6±3.06^{d}	29.48±3.20^{d}	33.4±1.81^{d}	42±1.38^{d}	43.8±2.51^{d}	53.6±1.96^{d}	60.8±2.31^{d}	63.4±1.36^{d}
6 ppm	33.8±2.82^{e}	41.4±3.22^{e}	46.2±1.39^{e}	55.8±2.08^{e}	63.2±2.52^{e}	73±2.41^{e}	80.6±1.4^{e}	81.2±1.88^{e}
8 ppm	61.2±3.54^{f}	64.8±2.75^{f}	70.4±3.19^{f}	72.6±2.25^{f}	83.8±2.76^{f}	87.4±1.6^{f}	91.2±0.73^{f}	92.6±1.86^{f}

Different letters in the same column refer to significant differences (P<0.05)
DAT: Day after treatment

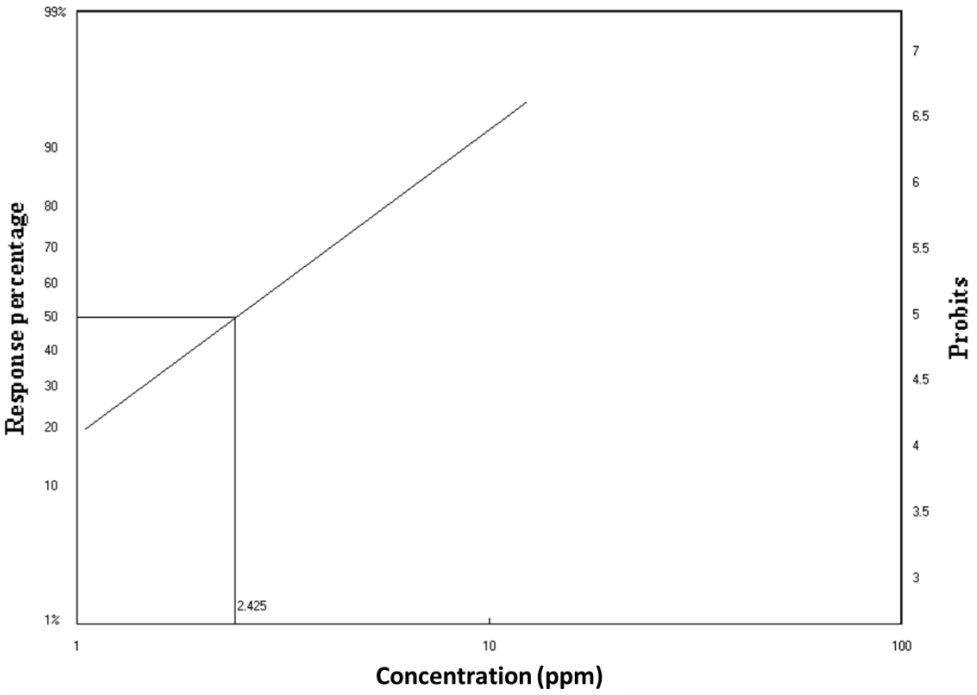

Fig 1: The regression line of the relation between the toxic effect of acetamiprid and the percentage mortalities in *Tribolium confusum* adults.

Effect of Acetamiprid on the Fecundity and Sterility of Adults of *T. confusum*:

Different concentrations of acetamiprid were applied to adult males and females to monitor the number of laid eggs in treated females compared to the untreated ones. As the concentration of the acetamiprid increased, the number of eggs laid by treated females decreased significantly (P<0.05), as shown in Table (2). The reduction percentage of the hatched eggs was significantly (P<0.05) increased as the used concentration of acetamiprid was increased. At the lowest concentration of acetamiprid (1 ppm), the number of laid eggs and the percentage of hatched eggs were non-significantly (P>0.05) different from the control. The sterility percentages increased from 28.78% to 98.21% when the used concentration increased from 1 ppm to 8 ppm, respectively (Table 2).

Table 2: The average number of laid eggs, percentage of hatched eggs, and sterility percentages in treated *Tribolium confusum* females with different concentrations of acetamiprid.

Treatment	Eggs laid/female/7days ± SE	% Egg hatched	Sterility %
Control	20.2±1.07[a]	92.38±2.46[a]	-
1 ppm	16.2±1.16[a]	82.04±3.46[a]	28.78
2 ppm	12±1.1[b]	66.32±6.13[b]	57.12
4 ppm	8.6±0.51[c]	53.98±6.49[b]	75.12
6 ppm	3.8±0.58[d]	26±10.78[c]	94.71
8 ppm	2±0.32[e]	16.7±10.54[c]	98.21

Different letters in the same column refer to significant differences (P<0.05)

Effect of Acetamiprid on the Acetylcholinesterase and Glutathione S-transferase Enzymes in Adults of *T. confusum*:

The acetylcholinesterase (AChE) enzyme has a very important role in transmitting the electrical signal in the insect nervous system. Accordingly, the levels of AChE were measured in the treated adults and compared to their levels in the control samples. Figure (2-A) shows the AChE levels in treated adults with different concentrations of acetamiprid. The analyses of the data indicated that the reduction percentages in the AChE levels were -7.27, -21.21, -46.06, -57.58, and -81.27% in the treated samples with 1, 2, 4, 6, and 8 ppm of acetamiprid, respectively. The activity of AChE was significantly (P<0.05) inhibited considerably in all treated samples except for treated samples with 1 ppm of acetamiprid. On the other hand, insects' detoxification of xenobiotic toxins, such as insecticides, requires glutathione S-transferases (GST). Figure (2-B) shows the GST levels in treated and untreated adults. The GST level was slightly increased from the control level by 5.58, 7.13, and 13.45% in treated samples with 1, 2, and 4 ppm acetamiprid, respectively. While at concentrations 6 and 8 ppm of the acetamiprid, the GST level significantly (P<0.05) increased by 26.92 and 45.17% from the control level, respectively.

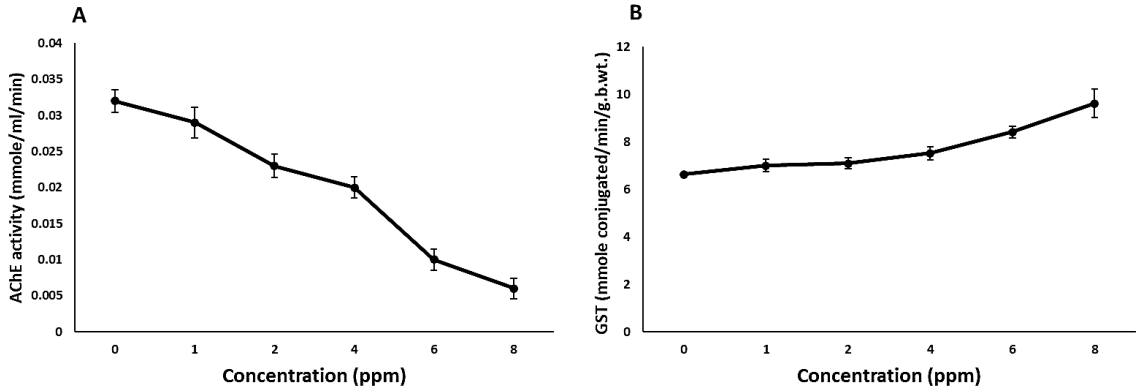

Fig. 2: Effect of different concentrations of acetamiprid on the acetylcholinesterase (A) and glutathione S-transferase (B) enzymes activity in *Tribolium confusum* adults.

Effect of Acetamiprid on the DNA Pattern of *Tribolium confusum* Larvae:

The LC_{50} of the acetamiprid was applied to *T. confusum* adults and the produced larvae from the next generation were used to analyse the DNA patterns. Figure (3) shows the RAPD-PCR profiles of control larvae and larvae resulting from the treatment generated by using three different primers. The data in Table (3) represent the molecular sizes of the DNA bands generated by the three different primers. The corresponding band amount percentage for each band was also presented. The primer OP-A07 generated eight bands in the control larvae, with molecular sizes ranging from 720 to 7778 base pairs (bp). The same

primer generated seven bands in treated larvae (larvae resulted from the treated adults), with molecular sizes ranging from 720 to 5400 bp. Bands numbers 1 and 6, with molecular sizes 7778 and 2351 bp, respectively, are present in the control sample and disappeared in the treated samples. Band number three with a molecular size of 4824 bp is present only in the treated samples. The primer OP-A09 generated eight and six bands in the control and treated samples, respectively, with molecular sizes ranging from 450 to 2114 bp. Bands numbers two and five, with molecular sizes of 1866 bp and 855 bp, respectively, were present only in the control larvae. The primer OP-A13 generated 4 and 5 different bands in the control and treated samples with molecular sizes ranging from 744 bp to 2961 bp and 625 bp to 2961 bp, respectively. Band number five with a molecular size of 625 bp is present only in the treated larvae.

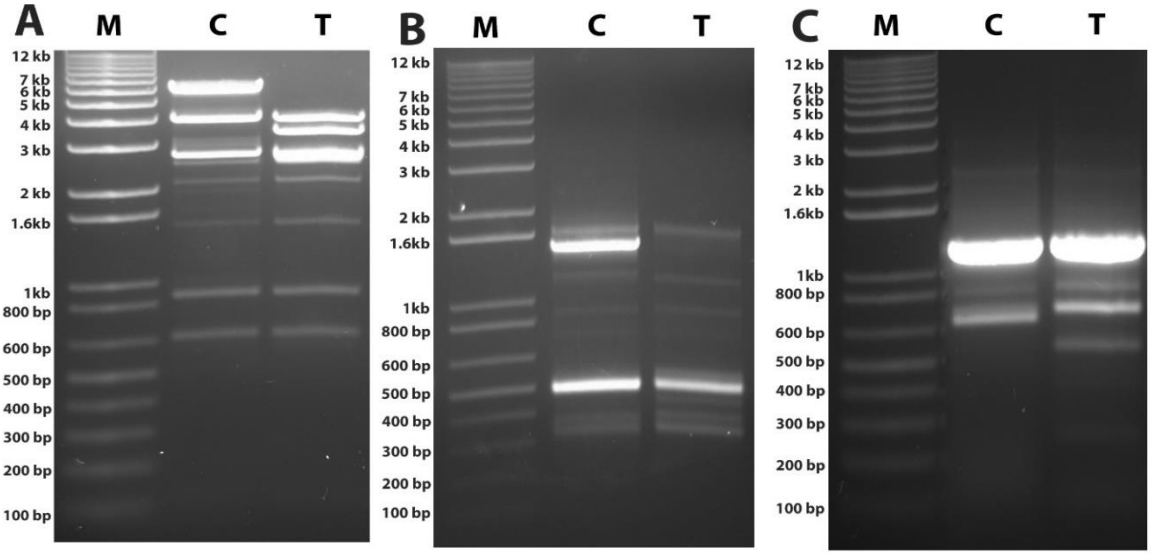

Fig. 3: RAPD-PCR profiles of control larvae (C) and larvae resulted from treated *Tribolium confusum* adults with the LC_{50} of the acetamiprid (T) generated by OP-A07 (A), OP-A09 (B), and OP-A13 (C) primers. Lane M: marker.

Table 3: Molecular size analysis generated by primers OP-A07, OP-A09, and OP-A13. Lane 1: control larvae and Lane 2: larvae resulted from treated *Tribolium confusum* adults with the LC_{50} of the acetamiprid.

Primer	Band No.	Lane 1		Lane 2	
		Molecular size (base pairs)	Band amount %	Molecular size (base pairs)	Band amount %
OP-A07	1	7778	29.5	-	-
	2	5400	20.0	5400	19.8
	3	-	-	4824	16.9
	4	3541	16.7	3541	25.8
	5	2643	3.9	2643	8.7
	6	2351	5.2	-	-
	7	1709	6.1	1709	10.1
	8	934	9.1	934	9.8
	9	720	9.4	720	8.9
OP-A09	1	2114	14.6	2114	13.7
	2	1866	24.5	-	
	3	1425	9.1	1425	16.2
	4	1037	6.7	1037	13.2
	5	855	7.2	-	
	6	582	25.5	582	37.2
	7	477	6.3	477	10.6
	8	450	6.1	450	9.0
OP-A13	1	2961	7.5	2961	5.5
	2	1329	62.4	1329	54.3
	3	931	11.1	931	13.4
	4	744	19.0	744	14.7
	5	-	-	625	12.1

DISCUSSION

Storage is crucial to the supply chain for grains. Between 10 and 20 percent of the overall yield might be lost to grain losses during storage, mostly because of deterioration brought on by insect pests (Phillips and Throne, 2010). More effectively than increasing production, reducing grain and food product losses during storage can help boost food security (Kumar and Kalita, 2017). *Tribolium confusum*, the confused flour beetle, is a significant pest in food plants, warehouses, and side milling and processing facilities. Alternatively, to pyrethroid, carbamate, and organophosphate insecticides, neonicotinoids have a similar mode of action and are considered safer for humans. They bind to nicotinic acetylcholine receptors (nAChRs) in insects' central nervous systems. They stimulate the nervous system at low concentrations, but at higher concentrations, they block receptors, which results in paralysis and death (Tomizawa and Casida, 2005). The current study aimed to determine whether neonicotinoid acetamiprid is effective in controlling adult *T. confusum*. The results of our study showed a positive toxic effect of acetamiprid against *Tribolium confusum* adults in a concentration-dependent manner. The accumulated exposure time was also a positive factor in the observed mortalities. In our experiment, the mortality rate increased as the treatment exposure time increased. Several previous studies reported many types of neonicotinoids as proper insecticides against different pests. In this regard, when Arthur *et al*. (2004) studied the neonicotinoid thiomethoxam against *Sitophilus zeamais*, *Oryzaephilus surinamensis*, *Tribolium castaneum* in maize, and *Sitophilus oryazae,* they found that insecticide concentration, exposure time, and temperature all typically enhanced mortality of all species. Marzouk *et al*. (2016) investigated the effects of the insecticides acetamiprid and imidacloprid against *Bemisia tabaci*, *Spodoptera littoralis*, and *Apis mellifera.* The two compounds produced significant mortalities in the three tested insects. Langdon and Rogers (2017) tested three neonicotinoids (imidacloprid, thiamethoxam, and clothianidin) against *Diaphorina citri* by contact and ingestion methods. The LC_{50} for the tested compounds was very low and ranged from 0.01 to 0.4 ppm. To control adults and larvae of *Trogoderma granarium*, Kavallieratos and Boukouvala (2019) examined the novel neonicotinoid insecticide acetamiprid. Results revealed that acetamiprid caused direct mortality in *Trogoderma granarium* adults that ranged between 87.8 and 96.7%. Abdel-Haleem *et al*. (2020) evaluated the toxicity of the neonicotinoids acetamiprid and on *Culex pipens* larvae and the results showed a highly toxic effect against larvae with LC_{50} values of 0.0093 ppm after 24 hrs of application. In addition, Alhewairini (2020) evaluated the toxicity of acetamiprid on *Rhynchophorus ferrugineus* under laboratory conditions and found that acetamiprid could produce significant toxic effects on both adults and larvae and cause a decline in the population of *Rhynchophorus ferrugineus*.

The market for neonicotinoids as neurotoxic insecticides has significantly expanded. They have undergone successful testing for the control of pyrethroid-resistant agricultural pests. It has been discovered that some neonicotinoids are effective against a wide variety of stored product insect species, including the rusty grain beetle, *Cryptolestes ferrugineus*, the lesser grain borer *Rhyzopertha dominica*, and the larger grain borer *Prostephanus truncatus* (Nayak and Daglish, 2006; Daglish and Nayak, 2012; Athanassiou *et al*., 2013). In the current research, we focused on the adult stage of *T. confusum*, which is considered the most destructive stage for stored products. Our results were promising to control the adult infestation by significantly lowering the treated pest number. Several previous authors reported the highly toxic effects of members of neonicotinoids against the adult stage of the stored product pests. Tsaganou *et al*. (2014) found that adults of *Prostephanus truncatus* and *Rhyzopertha dominica* were effectively controlled by brief exposures to thiamethoxam. Following that, Tsaganou *et al*. (2021) found that as the

pesticide concentration increased, there was a decrease in the production of offspring and an increase in the mortality of adults in seven significant beetle species that are significant pests of stored grains. Using thiamethoxam in storage to prevent insect attack as an alternative to standard insecticides such as organophosphorus chemicals and pyrethroids. Recently, Su *et al*. (2022) demonstrated that acetamiprid and dinotefuran were toxic to *Chrysopa pallens* and had negative effects on its growth and development.

Also in the current work, adults that received treatment with different concentrations of acetamiprid had concentration-dependent levels of fecundity and fertility. According to our findings, as the concentration of acetamiprid increased, the number of eggs deposited by treated females decreased significantly. The obtained results agree with many authors' findings on different insect pests after insecticide treatments. Ahmad *et al*. (2013) reported a potential reduction in the fertility and fecundity of the treated females of *Helicoverpa armigera* with different concentrations of the neonicotinoid imidacloprid. Similarly, Rodríguez-González *et al*. (2016) found that imidacloprid has toxic effects on adults and a reduction in egg hatchability of *Xylotrechus arvicola* beetles. In another study, after sublethal exposure of three strains of the bed bug *Cimex lectularius* to a pyrethroid-neonicotinoid combination insecticide (Temprid), the egg-hatching rate steadily declined by 73% in all tested strains (Crawley *et al*., 2017). When Zhang *et al*. (2022) examined acetamiprid against *Aphis glycines*, they reported a highly toxic effect in the adult stage and a reduction in the fecundity of the survival beetles. Ramachandran *et al*. (2022) discovered that treating *Tribolium castaneum* with essential oils of *Callistemon citrinus* resulted in reductions in fecundity.

Treatment with the neonicotinoid could induce declines in fecundity due to changes in spermatogenesis, sperm motility, oogenesis, ovulation, egg fertilization, or changes in mating behavior following the exposure, which negatively impacts their fecundity (Haynes *et al*., 1988; Jones *et al*., 2015)

Acetylcholinesterase (AChE) is a crucial enzyme in the nervous system. It contributes significantly to neurotransmission at cholinergic synapses by converting acetylcholine quickly into choline and acetate. Instead of being protonated, the electronegative tip of neonicotinoids is made of a nitro or cyano pharmacophore, giving them potency and selectivity by binding to a specific cationic subsite of the insect receptor. Because they bind at a specific location, the post-synaptic nAChR, and because their mode of action differs from that of carbamates and organophosphates, they are essential for controlling insecticide resistance (Tomizawa and Casida, 2009). Our results revealed that acetamiprid inhibits the AChE isolated from *T. confusum*. The concentration of the inhibitor had an effect on AChE enzyme activity; the higher the concentration, the more pronounced the inhibition of AChE. That result was in line with Sami *et al*. (2018), who observed that saponins isolated from a medicinal plant, *Azadirachta indica*, inhibited AChE in *T. castaneum* but did not inhibit AChE in *Apis mellifera*. Similarly, acetamiprid inhibits the AChE activity in both sexes of *Blatella germanica* (Morakchi *et al*., 2005), *Musca domestica* (Ahmed *et al*., 2002), and *Pyrausta sticticalis* (Leonova and Slynko, 2004).

Glutathione S-transferase (GST) is essential for both endogenous and exogenous chemical detoxification, as well as intracellular transport, hormone production, and defence against oxidative stress (Hayes *et al*., 2005). Insects' detoxification of xenobiotic toxins, such as insecticides, is greatly aided by GST. Accordingly, the level of GST was measured in treated samples and compared to the untreated samples. Our results indicated that the GST level was increased by a small amount in the treated samples with low and moderate concentrations of acetamiprid. While at high concentrations, the GST level was significantly increased. These results agree with Badawy *et al*. (2015), who found that acetamiprid induced oxidative stress in *Apis mellifera* by elevating the GST activity. Lotfy and Embaby

(2020) reported a significant increase in the GST level in *Spodoptera littoralis* larvae after 24 hours post-treatment with thiacloprid neonicotinoids. Also, Abdel-Haleem *et al*. (2020) found a significant elevation of the GST level in *Culex pipiens* larvae treated with thiamethoxam or acetamiprid.

Genome-wide DNA variation strategies induced by toxicants have all been successfully assessed with RAPD analysis in previous work, making RAPD-PCR-based analytical assays highly essential. The RAPD assay has shown promise in the detection of genomic instability exhibited as point mutations, genetic rearrangements, chromosomal deletions, and insertions (Baeshin *et al*., 2009). The RAPD-PCR method reveals modifications such as variations in band intensity and gains or losses of DNA bands, which are referred to as diagnostic markers (Lalrotluanga *et al*., 2011). The present results suggest that RAPD-PCR detected specific genomic alterations between the treated samples compared to the control. Based on the obvious results, we discovered that acetamiprid has a mutagenic effect in the subsequent generation of *T. confusum* following the treatment. On a variety of insect pests treated with insecticides, the obtained results are consistent with those of numerous authors (Atienzar *et al*., 2002; Ciabatti *et al*., 2006; Fleurat-Lessard and Pronier, 2006).

Conclusion

The increased concentration and exposure periods significantly enhanced the mortality rate. In addition, the fecundity and fertility of *Tribolium confusum* were affected. All the acetamiprid concentrations used resulted in sterility in the treated adults. AChE from *T. confusum* was almost completely inhibited at the highest concentration, and the level of the detoxifying enzyme, glutathione S-transferase, was increased in the treated samples. Acetamiprid also has a mutagenic effect on *T. confusum.* These results suggest that acetamiprid can be used efficiently to control *T. confusum*. In the future, further studies on the toxic effects of acetamiprid on the gonads of adult males and its deleterious effects on sperm formation will be needed to provide a further reference for the use of neonicotinoids in controlling stored grains.

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ARABIC SUMMARY

التقييم السمي لأسيتاميبريد النيونيكوتينويد على خنفساء الدقيق المتشابهة *Tribolium confusum* (Coleoptera: Tenebrionidae)

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إن خنفساء الدقيق المتشابهة، هي حشرة شائعة يمكن العثور عليها في المتاجر والمنازل وتهاجم مجموعة متنوعة من السلع والأطعمة المخزنة. كان يتم استخدام المبيدات الحشرية الكيميائية الاصطناعية للسيطرة عليها في الماضي، مما تسبب في العديد من الآثار الصحية والبيئية السلبية. أسيتاميبريد هو مبيد حشري نيونيكوتينويد له آلية عمل جديدة، وسمية منخفضة للثدييات، وتأثير بيئي منخفض. وقد تم اختبار فعالية هذا المبيد الحشري ضد خنفساء الدقيق المتشابهة في هذه الدراسة. قد تم إجراء الفحص البيولوجي السمي وذلك عن طريق التطبيق باستخدام تركيزات مختلفة من الأسيتاميبريد. وقد أشارت النتائج إلى أن زيادة التركيز وفترات التعرض قد عززت بشكل كبير معدل الإماتة. وقد بلغت الوفيات 1.12±20.6 في المائة و92.6±1.86 في المائة عند تركيزات 1 جزء في المليون و8 جزء في المليون من الأسيتاميبريد، على التوالي، بعد 14 يوما من التعرض للمبيد. وقد تم تقييم آثار الأسيتاميبريد على خصوبة وعقم خنفساء الدقيق المتشابهة. وقد أشارت الدراسات المختبرية إلى أن الأسيتاميبريد قلل بشكل كبير من وضع البيض وفقسه مقارنة بالعينات المرجعية. وأن جميع تركيزات الأسيتاميبريد المستخدمة قد أدت إلى العقم لدى البالغين المعالجين. وعند أعلى تركيز (8 جزء في المليون) كان العقم 98.21 ٪. كما تم التحقيق في آثار الأسيتاميبريد على نشاط إنزيم الأستيل كولين استريز (AChE). وقد لوحظ أن الإنزيم قد تم تثبيطه بالكامل تقريبا عند أعلى تركيز. وعلى العكس من ذلك، قد تم زيادة مستوى إنزيم إزالة السموم، Glutathione-S-transferase، في العينات المعالجة. وقد تم دراسة تأثير الأسيتاميبريد في احداث طفرات وراثية على خنفساء الدقيق المتشابهة من خلال تطبيق التركيز نصف المميت، ودراسة التغيير في الحمض النووي للحزم الجينية باستخدام تقنية RAPD-PCR، ومقارنتها مع العينات المرجعية. إن النتائج تشير بقوة إلى أن الأسيتاميبريد يسبب طفرات وراثية على خنفساء الدقيق المتشابهة. وأوصت النتائج بأن الأسيتاميبريد يمكن استخدامه كمبيد مفيد في برامج مكافحة الآفات تبعاً إلى آثاره الحشرية، والتي كانت واضحة في التركيزات المطبقة المنخفضة وأوقات التعرض القصيرة.