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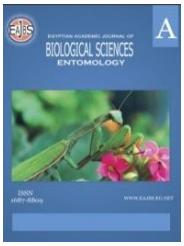

Computational and Toxicological Evaluation of Thiamethoxam as Nicotinic Acetylcholine Receptor Modulator Against Cowpea Aphid, *Aphis craccivora* Koch.

Mohamed N. Abdelmoteleb, Amany Z. Mohamed, Noha A. Genidy and Doaa R. Abdel-Haleem*
Entomology Department, Faculty of Science, Ain Shams University, Cairo, Egypt
***E-mail:** doaaramadan2007@gmail.com

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ABSTRACT

Neonicotinoids, which have a wide range of applications, are systemic insecticides that efficiently control several sucking pests. Recently, thiamethoxam (THIA) has become one of the popular pest control agents. In vivo study, the toxicity of THIA was evaluated against a laboratory strain of cowpea aphid (*Aphis craccivora* Koch.) using the leaf dipping method for 24 and 48 hrs of exposure. In silico study, THIA was docked to acetylcholine binding protein (AChBP) to explain their binding mode of interaction. THIA exhibited good toxicity against adult aphids with LC_{50} of 2.44 ppm at 24 hrs post-treatment, which greatly decreased to 0.75 ppm at 48 hrs post-treatment. THIA showed better binding interaction with AChBP than the co-crystalized ligand (imidacloprid) and good alignment in the active site. THIA indicated different variations in the activities of tested enzymes. Where, aphids treated with LC_{50} of THIA increased the biochemical activities of acetylcholinesterase, antioxidant enzymes and peroxidases while decreasing the activity of alpha esterase, carboxylesterase (CarE), glutathione S-transferase and catalase. Therefore, this compound is efficient against sap-sucking pests with good irreversible and specific binding interaction with nicotinic acetylcholine receptor (nAChR) and, consequently, low mammalian toxicity. So, THIA is considered a favourable agent for the management of insect pests.

INTRODUCTION

In both industrialized and developing nations, the cultivated faba bean is typically utilized as food for humans and animals. It continues to be Egypt's most significant leguminous food crop. (Ebadah *et al*., 2006). The faba bean is among the most important pulse crops and is utilized for both human and animal nutrition due to its high yield potential and grain-rich protein content. The faba bean also contributes significantly to maintaining agricultural productivity and production through crop rationing and nitrogen fixation (Infantino *et al.*, 2006; Torres *et al*., 2006; Braich *et al.*, 2016).

Aphids directly harm plants by sucking sap, and they also cause damage to them indirectly by dispersing viruses and excreting honeydew that promotes the growth of fungus and closes the stomata on leaves (*Capnodium* spp.) (Kitajima *et al*., 2008; Fernandes *et al*., 2012 & 2013; Bachmann *et al*., 2014; *Malaquias et al.*, 2014). Aphids

spread numerous viruses indirectly, including the bean leaf roll virus (BLRV) and the faba bean necrotic yellow virus (FBNYV), which directly injure plants inflicting substantial economic damage (Laamari *et al*., 2008). In addition, in 2015, it was shown that the geminivirus Alfalfa leaf curl virus (ALCV) was widespread by aphids of the species *Aphis craccivora* Koch. 1854 (Hemiptera: Aphididae) (Roumagnac *et al*., 2015; Varsani *et al*., 2017).

Due to the high fertility of aphids and their potential for developing resistance to certain insecticides, using pesticides to control their population is not always successful (Abdallah *et al.,* 2016; Mokbel *et al.,* 2017; Mweke *et al.,* 2019). Selective insecticides, such as neonicotinoids, have recently entered the market to take the place of organophosphates and methyl-carbamates as a result of resistance build-up in insect pests (such aphids) to the bulk of conventional insecticides (Tomizawa *et al*., 2007).

Neonicotinoids are irreversible systemic insecticides with a broad range of applications that can effectively control a variety of sucking pests, including leafhoppers, plant hoppers, whiteflies, and aphids, at very low dosages with low mammalian toxicity (Koch *et al.,* 2005). They selectively attack the insect's central nervous system, where they cause overstimulation, paralysis, and ultimately death (Yamamoto and Casida, 1999). Moreover, neonicotinoids, like thiamethoxam, can have a variety of variable and potentially advantageous effects on plant health, growth, and stress tolerance (Afifi *et al.,* 2015).

First, second, and third-generation neonicotinoids are different in their chemical structures with the same mode of action. All of the chemicals in neonicotinoids have the same mode of action but differ in how they bind to nAChR (competitive modulators to nAChR), according to the Insecticide Resistance Action Committee's (IRAC) 4A classification (Crossthwaite *et al.,* 2017; IRAC 2017). Thiamethoxam (second generation), on which our study is focused, has been sold since 1998 under the trade names Cruser® for seed and Actara® for foliar spray (Mainenfisch *et al*., 2001).

The identification and crystallisation of the ligand-free and ligand-bound structures of the acetylcholine binding protein (AChBP) have allowed for a more understanding of the details of the binding site and how they relate to function (Selvam *et al.,* 2015). In order to determine how thiamethoxam binds to the (nAChR) receptors and its in silico mode of action, we will use data about the 3D structure of thiamethoxam and the receptor's structure. The current study aimed to assess the toxicological and biochemical action of specific nicotinic acetylcholine receptor modulators (thiamethoxam) against cowpea aphids and use computer modeling to investigate acetylcholine receptor inhibitors mode of action.

MATERIALS AND METHODS

Molecular Docking Study:

The nicotinic acetylcholine receptors (nAChR) of insects were investigated with regard to their chemical structure and three-dimensional (3D) structure. The 3D structures of nAChR were obtained from the Protein Data Bank website at http://www.pdb.org. *Lymnaea stagnalis* (*L.s.*) acetylcholine binding protein of the great bond snail (*L.s.*-AChBP), was used for the molecular docking studies because the aphid's nAChR still lacks a crystal structure and shares a high degree of homology with the extracellular domain of insect nAChR (Ihara *et al.,* 2008). In fact, *L.s.*-AChBP has been used to investigate how thiamethoxam inhibits nAChR and inhibition mode

X-ray crystal structure of *L.s.*-AChBP (Ihara *et al.,* 2008) was downloaded from Protein Data Bank (PDB ID: 2ZJU). Chemdraw 17.0 (CambridgeSoft) (Perkin Inc.) was used for drawing ligands. The Wave Function Spartan v 14.0 program was used to

optimize the shape and globally decrease the energies of the ligands (tested pesticides) for better fitting and docking results (Adeboye, 2018). The equilibrium geometry experiences 3D protonation, addition of partial charges, and hydrogen atoms for substances under investigation as well as the receptor were performed by using Molecular Operating Environment (MOE software, 2014) (Chemical Computing Group ULC, Canada). According to the ligand preparation protocol, the ligand and thiamethoxam were energy-minimized in the MMFF94x force field to an RMS gradient of 0.05. Using the Triangle Matcher protocol, the MOE-Site Finder application's -spheres were used to specify the active site for imidacloprid placement (Corbeil *et al*., 2012). Imidacloprid and thiamethoxam were docked into the *L.s.*-AChBP binding site, and 30 poses were generated for each molecule in the binding site. The resulting conformations' binding orientations and interactions were investigated, and these conformations were compared.

Insecticidal Assay:

Insects:

The lab strain of the cowpea aphid, *A. craccivora* was obtained from the Central Agricultural Pesticides Laboratory, Dokki, Giza, Egypt. The aphids were reared in the insectary at the Department of Entomology, Faculty of Science, Ain Shams University, under controlled laboratory conditions (22 ± 2 °C, 70 ± 5% R.H., and 12: 12 light: dark photoperiod) without any exposure to insecticides. The insects were kept on metal platforms within chambers and were housed on faba bean seedlings that were grown in plastic pots with a 15 cm diameter. The pots housing the faba bean seedlings were maintained there until needed, free from pesticide contact. This strain established a baseline for our toxicity application and biochemical analysis (Kandil *et al.,* 2022).

Insecticides:

Commercial formulation of thiamethoxam (Actara® 25% WG, Novartis Co.) was obtained from the Central Pesticides Laboratory, Dokki, Giza, Egypt.

Insecticidal Bioassay Test:

With a few minor modifications, (Moores *et al.,* 1996) leaf-dipping bioassay method was used to determine the toxicity of thiamethoxam (Kandil *et al.,* 2022). Fresh faba bean leaves were gathered, cleaned, and then uniformly sliced into discs using a metal tube. The leaf discs were subjected to serial dilutions of the indicated insecticide for around ten seconds, the tested concentrations were as follows: 0.05, 0.1, 0.5, 1, 2, and 4 ppm which were prepared by dilution of thiamethoxam with water as solvent Following that, they were placed upside down on an agar substrate in Petri plates (60 mm in diameter). Ten apterous adults of the *A. craccivora* were transferred to the treated leaf surface, whereas the control group consisted of untreated leaves that had been dipped in water. Each pesticide concentration and control were performed three times. Deaths were noted 24 and 48 hrs later. A correction was made to the mortalities using Abbott's formula (Abbott, 1925). Ldp line software and a probit analysis were used to calculate the LC_{90}, LC_{50}, and LC_{25} values.

Biochemical Assay:

Preparation of samples for biochemical assay:

Following the detection of the LC_{50} values using the adult aphids, the insects were prepared according to (Amin's, 1998) guidelines. In (50 mg/ml) of distilled water, they were homogenized. A chilled centrifuge was used to centrifugal homogenates at 8000 rpm for 15 min at 2 ºC. Then samples were kept until use at -4 ºC.

Acetylcholinesterase (AChE) Activity Assay:

As described by (Simpson *et al.,* 1964), acetylcholine bromide (AChBr) was used as the substrate to measure AChE (acetylcholinesterase) activity.

Carboxylesterase Activity Assay:

Simpson *et al.*, (1964) method was followed to determine the carboxylesterase activity, where methyl n-butyrate (MeB) was used as the substrate.

Non-Specific Esterases (alpha esterase) Activity Assay:

Alpha esterase (α-esterase) has been determined using the substrates α -naphthyl acetate, according to (Van Asperen, I962).

Glutathione S- transferase activity assay:

Glutathione S-transferase (GST) uses the glutathione's -SH group as a catalytic intermediate to combine reduced glutathione (GSH) with 1-chloro 2,4-dinitrobenzene (CDNB). S-(2,4-dinitro-phenyl)-L-glutathione, a conjugate, could be detected, according to the procedure of (Habig *et al.,* I974).

Peroxidase Activity Assay:

Peroxidase activity was evaluated using the method described in (Hammerschmidt *et al.,* 1982).

Catalase activity assay:

The catalase activity was measured using the biodiagnostic kit No—CA 2517, which is based on the spectrophotometric method described by (Aebi, 1984).

Antioxidant Capacity Reaction Assay:

To determine the antioxidant capacity, (Prieto *et al.,* 1999) developed a spectrophotometric method**.**

Statistical Analysis:

Mortality was adjusted for control by using Abbott's formula (Abbott, 1925). By applying Ldp line software and probit analysis, LC_{50} values and other parameters were determined. The statistical analysis of the toxicity levels was done using the probit analysis (Finney, 1971) program. All biochemical experiments used 3 repetitions (insect homogenates), and the results of the biochemical analyses were combined from analysis performed in triplicate. One-way analysis of variance (ANOVA) was used to analyze the data using the costat statistical program (Cohort Software, Berkeley). When the ANOVA statistics were significant ($P>0.01$), Duncan's multiple range test was used to compare means (Duncan, 1955) .

RESULTS

Modeling Studies:

The docking result of IMI is shown in Figure (1), where two hydrogen bonds have been formed between (O) from the nitro group of the imidazolidine and Met114, two hydrogen-pi bonds have been formed between (C) atom number 14 of the chloropyridin ring with Trp143, and two pi-pi bonds have been formed between the 6-ring chloropyridin with Tyr185. Co-crystallized IMI and docked THIA have very similar binding mechanisms in the AChBP active site, but thiamethoxam was superior due to its high docking score and the interaction's pi-H bonds, which were superior to pi-pi bonds in IMI and were attached to a crucial amino acid Figure (2). There are two hydrogen bonds between the (Cl) atom (which is attached to the thiazole ring) and Leu112, also there are two hydrogen-pi bonds between (C) atom number 13 (in conjunction between thiazole and oxadiazinane ring) with Tyr192, and two pi-H bonds between the thiazole ring with Met114. Docking energy for IMI and thiamethoxam were -6.49 and - 6.95 kcal/mole, respectively.

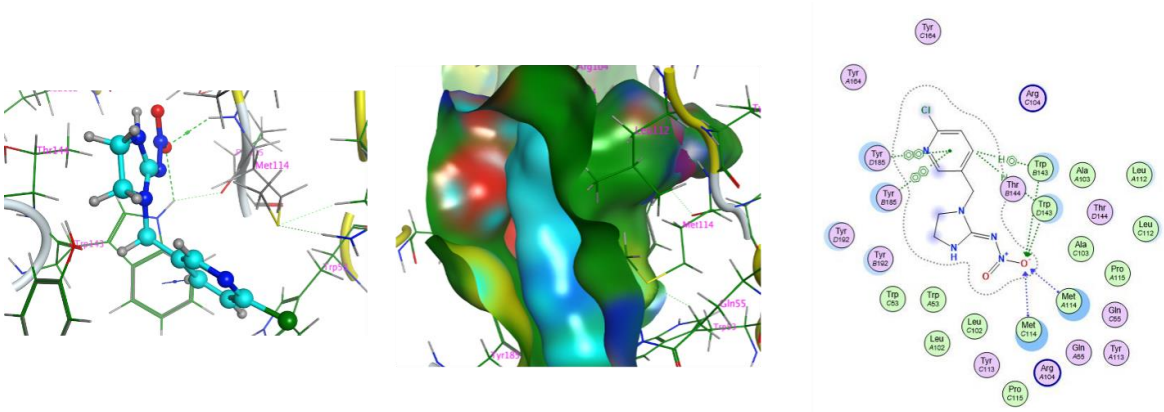

Fig. 1: Shows 2D and 3D configurations of imidacloprid docked to *Lymnaea stagnalis* acetylcholine binding protein.

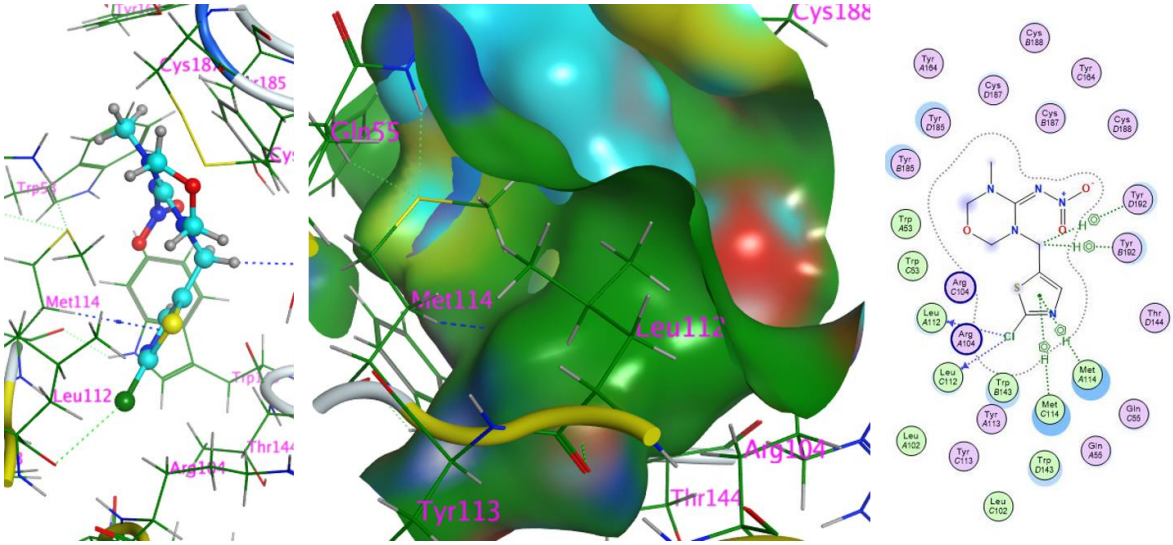

Fig. 2: Shows 2D and 3D configurations of thiamethoxam docked to *Lymnaea stagnalis* acetylcholine binding protein.

Toxicological Study:

In Figure (3), it was noted that the increase in thiamethoxam concentrations greatly elevated the mortality of the aphids. After 24 hrs of exposure, the mortality percent enhanced from 6.67% at 0.05 ppm to 64.40% at 4.00 ppm. Also, the mortalities enhanced with time of exposure, the death percentages of aphids increased after 48 hrs of exposure more than 24 hrs. At a concentration of 2 ppm, the mortality percent of aphids was 40% after 24 hrs of exposure while it increased to 81.34 % at 48 hrs post-treatment.

The data was subjected to probit analysis and the obtained data was tabulated as shown in Table (1). Thiamethoxam exhibited LC_{50} values of 2.44 and 0.75 ppm at 24 and 48 hrs post-treatment, respectively. The slope value was 0.85 after 24 hrs and 1.35 after 48 hrs shown in Table (1) indicating that the bioassay's sample population reacted to the tested pesticides uniformly.

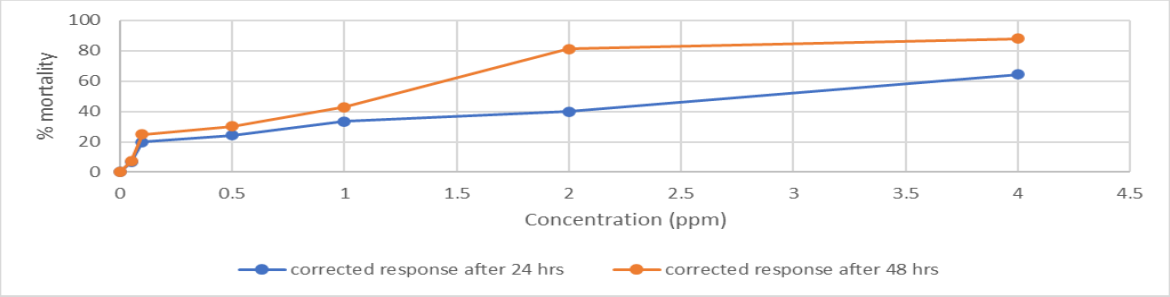

Fig. 3: Mortality of apterous adults of the *A. craccivora* observed against different concentrations of thiamethoxam after 24 and 48 hrs post-treatment.

Table 1: Toxicity of thiamethoxam against apterous adults of the *A. craccivora* after 24 and 48 hrs post-treatment.

Thiamethoxam (ppm)	**24 hrs**	**48 hrs**
LC_{25} (F.l. at 95%)[a]	0.39 (0.07- 0.86)	0.24 (0.11- 0.38)
LC_{50} (F.l. at 95%)[a]	2.44 (1.1-15.96)	0.75 (0.48-1.12)
LC_{95} (F.l. at 95%)[a]	215.76 (25.5-837912.03)	12.35 (6.03-43.70)
Slope ± SE[a]	0.85 ± 0.26	1.35 ± 0.21
P	0.82	0.15
χ^{2b}	1.52	6.70

[a] (F.l.) fiducial limits. The slope of the concentration–inhibition regression line ± standard error. [b] (χ^2) chi square value.

Biochemical Studies:

The activity of the neurotoxic, detoxification enzymes and antioxidant capacity in the tested aphids, was determined by treating aphids with the LC_{50} of the thiamethoxam and collecting samples after 24 hrs of treatment as shown in Table (2) and graphically illustrated in Figure (4). AChE activity slightly increased after treatment by thiamethoxam, with a mean increase value of (100.5 ug AChBr/min/mg protein) with a percentage increase (10.93 %) from the control (90.6 ug AChBr/min/mg protein) with non-significant difference between them at ($p<0.01$)**.**

Thiamethoxam showed a good effect on carboxylesterase activity, with a mean value of (254 ug Meb/min/mg protein) in comparison with the control (282 ug Meb/min/mg protein). Thiamethoxam treatment resulted in a significant decrease in CarE activity (-9.93%). Alpha esterase was the most affected enzyme after treatment with thiamethoxam. Hence, thiamethoxam treatment resulted in a considerable decrease in alpha esterase activity (-30.84%). Where alpha esterase activity diminished to 500 (ug α - naphthol/min/mg protein) after treatment with thiamethoxam compared to the control 723 (ug α - naphthol/min/mg protein). On the other hand, the glutathione S-transferase activity slightly decreased after treatment with thiamethoxam with a mean value (19.6 mmol sub. conjugated/min/mg protein) compared to the control (21.3 mmol sub. conjugated/min/mg protein) and percent change (-7.98%).

Thiamethoxam significantly increased the peroxidase activity as compared to the control (9.1 *m*Δ *O.D.*/min/mg protein), with a mean value of (10.8 *m*Δ *O.D.*/min/mg protein). Thiamethoxam treatments resulted in extremely high peroxidase activity (18.68%) which was significantly different from the control. Thiamethoxam diminished catalase activity with a mean value of (34 *mU*/mg protein) relative to control (37.3 *mU*/mg

protein). This treatment non-significantly reduced the activity of catalase (-8.85%). The thiamethoxam slightly increased the antioxidant activity with a mean increase value (86 mg AAE/ 100 insects) in comparison with (81 mg AAE/ 100 insects) in the control, where thiamethoxam treatment non-significantly increased the enzyme activity with only (6.17%).

According to the biochemical studies, thiamethoxam increased peroxidase, acetylcholine esterase and antioxidant activity compared to control, on the other hand, it inhibited the activity of the detoxifying enzymes alpha esterase, carboxylesterase (CarE) catalase and glutathione s-transferase.

Table 2: Effect of thiamethoxam on acetylcholinesterase, carboxylesterase, alpha esterases, glutathione S- transferase, peroxidase, catalase and antioxidant capacity in the hemolymph of apterous adults of the *A. craccivora* treated with LC_{50} 24 hrs post-treatment.

Enzyme	**Thiamethoxam**		**Control**
	Activity means ± SE*	**%Change**	**Activity means ± SE***
Acetylcholinesterase (ug AChBr/min/mg protein)	100.5 ± 3.3 [a]	10.93%	90.6 ±5.1[a]
Carboxylesterase (ug Meb/min/mg protein)	254 ± 4.5[b]	-9.93%	282±13 [a]
Alpha esterases (ug α - naphthol/min/mg protein)	500 ± 11[b]	-30.84%	723 ± 21[a]
Glutathione S-transferase (mmol sub. conjugated/min/mg protein)	19.6 ± 0.55 [a]	-7.98%	21.3 ± 0.87 [a]
Peroxidase (*m*Δ *O.D.*/min/mg protein)	10.8 ± 1.6 [a]	18.68%	9.1 ± 0.6[b]
Catalase (*mU*/mg protein)	34 ± 1.3 [a]	-8.85%	37.3 ± 3.3 [a]
Total antioxidant capacity (mg AAE/ 100 insects)	86 ±3.5 [a]	6.17%	81±3.1 [a]

-Means bearing different subscripts are significantly different (p> 0.01) Duncan`s multiple range test. *SE standard error.

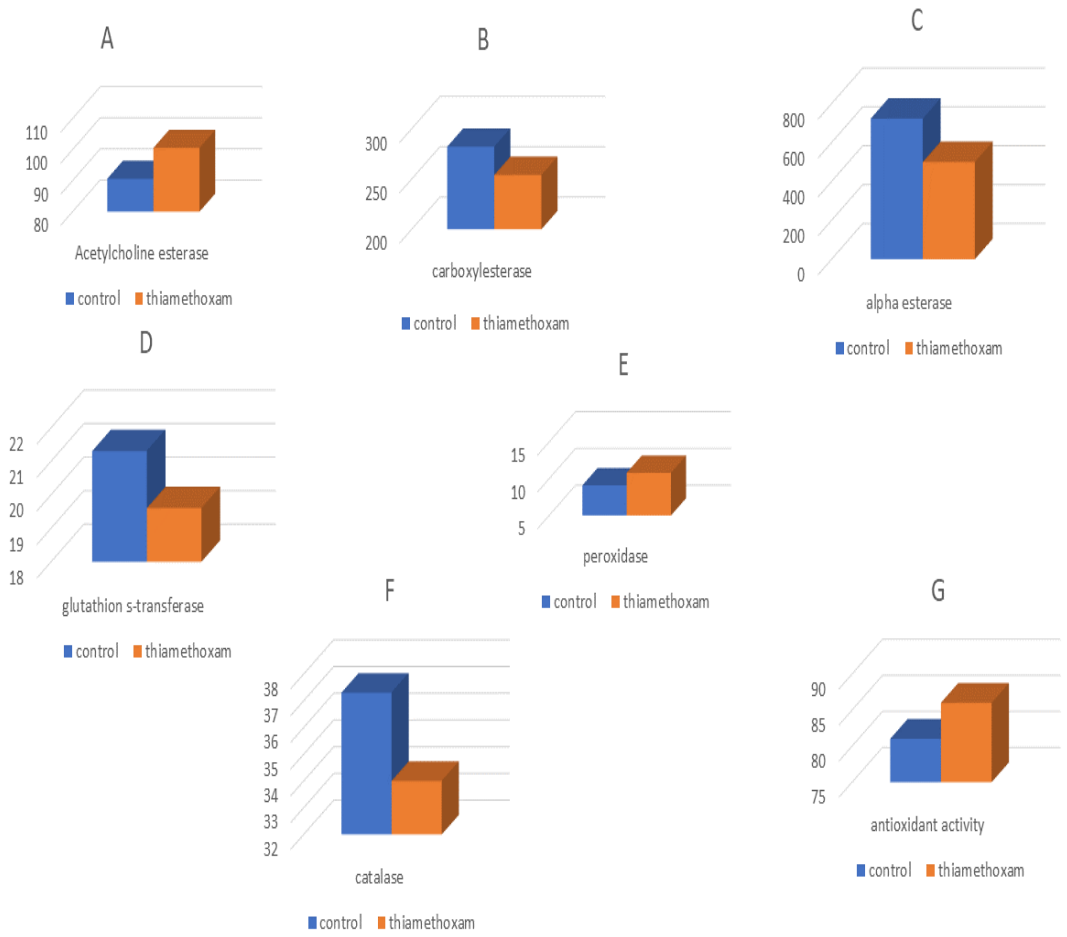

Fig. 4: Effect of thiamethoxam on acetylcholinesterase, carboxylesterase, alpha esterases, glutathione S- transferase, peroxidase, catalase and antioxidant capacity in the hemolymph of apterous adults of the *A. craccivora* treated with LC_{50} 24 hrs post-treatment.

DISCUSSION

Neonicotinoids play a significant role in the management strategy of aphids, which was shown in our results that thiamethoxam effectively reduced aphid populations using low concentration, these results come in harmony with (Nauen and Elbert, 1997) who observed a strong neonicotinoid effect against the susceptible population of *Myzus persicae* and *M. nicotianae*, the green peach aphid. Also, the neonicotinoid group was most effective in causing mortality as indicated by the lower LC_{50} values (0.01-0.04 ppm for imidacloprid; 0.03-0.05 ppm for thiamethoxam) where seven insecticides were tested on adult aphids collected from Nagpur, Amravati, and Wardha districts between 2013 and 2015. Also, George *et al.*, (2019) reported that thiamethoxam effectively diminished the aphid population at low concentrations. Also, agree with Patil *et al.*, (2018), who indicated that imidacloprid had the highest mean% reduction in cowpea aphid population in comparison with control (55.33%), followed by acetamiprid (50.81%) and thiamethoxam (49.92%). As well, according to Choudhary *et al.*, (2017), both imidacloprid and thiamethoxam were successful in controlling cowpea aphids. Thamilarasi, (2016) reported that the mean population of *A. craccivora* was reduced to 7.33 aphids /plant, and *M. persicae* to 4.67 aphids/ plant after the application of THIA for 15 days, which indicates that the treatment with THIA found to be effective against sucking pests which agree with

our results. Moreover, the application of THIA at LC_{50} and LC_{30} showed adverse effects on the biological fitness of the F0 generation of soybean aphids (*Aphis glycines)* at low-lethal concentrations (Zhang *et al.,* 2021).

According to the experimental results, THIA was efficient against adult aphids. It was suggested that one of the reasons for the high efficiency of THIA is that, THIA was converted to clothianidin (a neonicotinoid pesticide) during the metabolism process and its good translocation in insects and plants (Meredith *et al.,* 2002), which enhances the THIA toxicity. Where, THIA is a pro-insecticide, being activated post-ingestion because the THIA is rapidly converted to clothianidin in insects and plants. This hypothesis has been previously illustrated where, it was noted that the toxicity of THIA increased when metabolized in *Spodoptera frugiperda* and *Periplaneta americana* after 24 hours of treatment (Nauen *et al.,* 2003; Mota-Sanchez *et al*., 2006; Benzidane *et al*., 2010).

Modelling research demonstrated how neonicotinoids work by clearly demonstrating how they interact with AChBP. Although there is no correlation between AChE and neonicotinoid toxicity, AChE was identified to study how this enzyme reacts to neonicotinoids. The docking studies showed that THIA had more effective bonding interactions than ligand (IMI) and this confirmed its high extremital potency. Although both IMI and THIA were targeted at the same active site they showed different binding modes with nAChR. These differences between THIA and IMI are primarily caused by their different pharmacological characteristics (Foster *et al.*, 2008; Shi *et al.,* 2011).

Neonicotinoids are the only insecticides that increase AChE activity (Samson-Robert *et al.,* 2015). In our result, it was found that AChE activity increased after treatment with thiamethoxam which agreed with (Boily *et al.,* 2013) on honey bees and agree with (Abdel-Haleem, *et al*., 2018) on houseflies, but disagreed with the findings of (Győri *et al.,* 2017) and (Grünewald and Siefert 2019), both of which claimed that neonicotinoids reduce AChE activity.

One of the primary biochemical resistance of insects against insecticides is detoxification (metabolic resistance). The main enzymes involved in the metabolism or detoxification of toxins include esterases (α and β), glutathione s-transferase, and CarEs (Li *et al.,* 2007; Kaleem Ullah *et al.,* 2023). Insecticides can be metabolized by detoxifying enzymes, primarily GSTs, and CarEs, into low- or non-toxic compounds (Enayati *et al.,* 2005; Yan *et al.,* 2009; Feyereisen, 2011). Esterase-mediated pesticide resistance has been detected in more than 30 insect pest species, according to (Hemingway *et al.,* 1998). It was noted in our results that α -esterases activity and carboxylesterase decreased after thiamethoxam treatment which disagrees with (Shehawy and Alshehri, 2015) who discovered that imidacloprid treatment dramatically increased the α -esterases activities in a laboratory strain of cowpea aphid, also our results disagree with (Abdel-Haleem *et al.,* 2020) who indicated that the third larval instar of *Cx. pipiens* treated with THIA showed a significant increase in the activity of the carboxylesterase and (α and β) esterases. The activity of general esterases clearly correlated with IC_{50}, indicating that these enzymes may contribute significantly to neonicotinoid resistance.

Carboxylesterase activity declined post-treatment with THIA, which agrees with Zhou *et al.,* (2019) who found that CarE activity demonstrated a general trend of early upregulation and subsequent suppression following THIA application by LC_{10} and LC_{25} values. The activity peaking in *Sogatella furcifera* was detected at 6 h. Also, CarE expression in silkworms was reduced at low doses of acetamiprid. Similarly, in *Oxycarenus hyalinipennis*, the acetamiprid suppressed CarE activity (Malik *et al.,* 2019), which agrees with our results. A previous study indicated that after 48 hrs of treatment with cycloxaprid and imidacloprid (LC_{50}), the GST activity in *Aphis craccivora* was greatly enhanced, while the CarE activity was suppressed, however, the observed

difference was not significant (Wu *et al.,* 2016) in which agree with our finding in decreasing CarE activity but disagree with our finding in which GST level was greatly reduced. These results indicate that CarE may have a significant impact on how insects react to the stress of neonicotinoid application.

GSTs are a class of enzymes that perform several functions in insecticide detoxification. They are present in many aerobic organisms and play a major role in the phase II metabolism of xenobiotic substrates (Liu *et al*., 2014; Wang *et al.,* 2019). GSTs facilitate the coupling of electrophilic xenobiotics with reduced glutathione (GSH), increasing their water solubility and making it easier for excretion (Kostaropoulos *et al.*, 2001; Ranson *et al.*, 2005; Li *et al.,* 2009; Hu *et al.,* 2020). Because GST enzymes are soluble and persistent, they are found in a variety of insects, and exposure to xenobiotics increases the production of these enzymes (Yu, 1996).

The lipidic cell membrane is weakened by oxidative stress because of various free radicals peroxiding it. Pesticides cause oxidative stress in the cell, which produces several Reactive Oxygen Species (ROS) free radicals (Cortés-Iza *et al.,* 2018). Atoms or molecules containing unpaired electrons are considered free radicals (Abdollahi *et al.,* 2004; Cortés-Iza *et al.,* 2018). The pursuit of electronic stability by free radicals results in attacks on other molecules, altering chemical structures and affecting biomolecular functioning. Exposure to ROS can lead to modifications that cause genomic DNA mutations, negatively affect protein activity, damage cellular membranes, and eventually lead to cell death. The enzyme glutathione peroxidase is responsible for preventing the oxidation of lipids and proteins (Koirala *et al.,* 2022).

In our results we found that peroxidase (POD) activity increased after thiamethoxam treatment, also, (Zhou *et al.,* 2019) found that the exposure of *Sogatella furcifera* to thiamethoxam initially tended to promote upregulation of POD's activities before inhibiting it after the insecticide's concentration increase from LC_{10} to LC_{25}. According to these findings, POD and CAT activities in insects are linked to insect resistance (Zhou *et al.,* 2019) which agrees with our deduction.

CONCLUSION:

Thiamethoxam was docked to the acetylcholine binding protein (AChBP) to demonstrate its interaction mode. 24 hours after treatment, THIA showed good toxicity against adult aphids; and after 48 hours, its toxicity significantly increased. Compared to the co-crystallized ligand imidacloprid, THIA exhibited preferable binding interaction with AChBP and excellent alignment in the active site. Strategies based on the rotation of new modes of action groups may weaken the strength of selection for novel resistance mechanisms. Aphids treated with THIA exhibited a decrease in the activity of glutathione S-transferase, alpha esterase, carboxylesterase (CarE), and catalase, and an increase in the biochemical activities of acetylcholinesterase, antioxidant enzymes, and peroxidases. THIA has minimal mammalian toxicity and is effective against sap-sucking pests due to its strong, permanent binding affinity with nAChR.

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ARABIC SUMMARY

التقييم الحاسوبي والسمي للثياميثوكسام كمعدل لمستقبلات الأسيتيل كولين النيكوتينية ضد حشرة مَنْ اللوبيا، *أفيس كراسيفورا* كوخ

محمد ناصر الدين محمد عبد المطلب – أماني زكي محمد– نهى عونى جنيدى – دعاء رمضان عبد الحليم

قسم علم الحشرات- كلية العلوم- جامعة عين شمس- القاهرة- مصر

مبيدات النيوكوتينويد، التي لها مجموعة واسعة من التطبيقات، هي مبيدات حشرية جهازية تتحكم بكفاءة في عدد من الآفات الماصة. في الآونة الأخيرة، أصبح الثياميثوكسام (THIA) أحد عوامل مكافحة الآفات الشائعة. في دراسة على الجسم الحي، تم تقييم سمية THIA ضد السلالة المختبرية لحشرة من اللوبيا (*Aphis craccivora* Koch) باستخدام طريقة غمس الأوراق لمدة 24 و48 ساعة من التعرض. في دراسة السيليكو، تم ربط THIA ببروتين ربط الأسيتيل كولين (AChBP) لشرح طريقة التفاعل المرتبطة بها. أظهرت THIA سمية جيدة ضد حشرات المن البالغة مع LC_{50} بمقدار 2.44 جزء في المليون بعد 24 ساعة من العلاج، والتي انخفضت بشكل كبير إلى 0.75 جزء في المليون بعد 48 ساعة من العلاج. أظهرت THIA تفاعلًا ملزمًا أفضل مع AChBP مقارنةً بالمركب المتبلور المشترك (IMI) ومحاذاة جيدة في الموقع النشط. أشارت THIA إلى اختلافات مختلفة في أنشطة الإنزيمات المختبرة. حيث أدى علاج المن بـ THIA باستخدام جرعة 2.44 جزء في المليون التي تؤدي لقتل نصف العدد بعد 24 ساعة من التطبيق إلى زيادة النشاط الكيميائي الحيوي لأنزيمات antioxidant enzymes AChE وperoxidase، بينما انخفض نشاط إنزيم alpha-esterase و (CarE) وglutathione s-transferase وcatalase. هذا المركب فعال ضد الآفات الماصة للعصارة مع تفاعل جيد لا رجعة فيه ومحدد مع nAChR، وبالتالي، سمية منخفضة للثدييات. لذلك، تعتبر THIA عاملاً مناسبًا لمكافحة الآفات الحشرية.