Toxicity, Biochemical Impact, and Inhibition of Glutathione *S*-Transferase of Four Selected Insecticides against *Aphis craccivora* (Hemiptera: Aphididae)[1]

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Abstract The toxicity and biochemical impact of four novel insecticides (sulfoxaflor, spiromesifen, cyantraniliprole, and flonicamid) were evaluated in laboratory bioassays against the cowpea aphid, *Aphis craccivora* Koch (Hemiptera: Aphididae). In addition, the inhibitory effect of the insecticides on glutathione *S*-transferase (GST) was determined using molecular docking analysis. Based on the median lethal concentrations (and associated 95% confidence intervals), spiromesifen (at 1.98 mg/L) and sulfoxaflor (at 3.13 mg/L) exhibited the greatest level of toxicity followed by flonicamid (at 4.02 mg/L) and cyantraniliprole (at 14.93 mg/L). Additionally, sulfoxaflor and cyantraniliprole significantly reduced the activity of α-esterase and cytochrome P450 monooxygenase 48 h after exposure. The insecticidal activity of sulfoxaflor, cyantraniliprole, and spiromesifen was associated with the inhibition of GST activity. The *in silico* studies of the interactions between GST and the insecticides revealed that the proposed binding patterns of sulfoxaflor and spiromesifen had one hydrogen bond with THR 54 and an arene-H contact with HIS 41, respectively. Thus, cyantraniliprole combined with the receptor through two hydrogen bonds (with HIS 53 and ARG 112) and an arene-H contact with HIS 53. Based on these results, spiromesifen and flonicamid have potential for use in aphid management.

Key Words *Aphis craccivora*, toxicity, insecticides, detoxification enzymes, molecular docking analysis

The cowpea aphid, *Aphis craccivora* Koch (Hemiptera: Aphididae), is a polyphagous insect pest that attacks a variety of crops, feeds on all above-ground parts of the plant, and causes significant crop losses. This pest is recognized as a worldwide threat to leguminous plants such as peas, cowpeas, and beans (Obopile and Ositile 2010, Yang et al. 2021). *Aphis craccivora* causes up to 100% yield losses of various legume species (Das 2002) by direct feeding (Shetlar 2021) and transmission of approximately 20 nonpersistent plant viruses (Gadhave et al. 2020). Symptoms include chlorosis and stunting of growth, which can result in delayed onset of flowering and even death of the plant, particularly in the seedling stage (Blackman and Eastop 2006, Keating et al. 2015). In addition, cowpea aphids

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secrete honeydew on plants, which acts as a growth medium for sooty mold that interferes with host plant photosynthetic activity (Powell et al. 2006).

Chemical insecticides have been used primarily for management of *A. craccivora* and *Aphis gossypii* Glover management (Egho 2010, El-Shourbagy et al. 2023, Moustafa et al. 2022a, Yang et al. 2021). However, continuous usage of a single insecticide or a group of insecticides with a similar mode of action can result in development of resistance to the toxicants in populations of the target insects (Wang et al. 2014). Insecticide resistance has been a serious barrier to effective traditional control with chemical insecticides (Li et al. 2019, Moustafa et al. 2024c).

In general, aphid species exhibit the ability to adapt to xenobiotics through different mechanisms; thus, failures of certain chemicals have often been reported (Bass et al. 2014, Simon and Peccoud 2018). Wang et al. (2014) observed that novel insecticides or compounds must be continually developed to help alleviate and manage pesticide resistance phenomena. There is a high demand for new active ingredients possessing favorable toxicological and environmental properties to control insect pests (Awad et al. 2024). Sulfoxaflor, spiromesifen, cyantraniliprole, and flonicamid are four such insecticides and are the subject of this study.

Sulfoxaflor belongs to the sulfoximines group and provides control over a wide range of plant sap–feeding pests that have developed resistance to other insecticides (Ibrahim et al. 2023, Watson et al. 2021). The sulfoximines group represents a relatively new class of competitive modulators of the nicotinic acetylcholine receptor (nAChR). Spiromesifen is a member of the tetronic derivatives class (IRAC 2022), which affects lipid biosynthesis of the target organism (Bielza et al. 2018). It is highly selective against piercing–sucking insects, with high residual values and is safe for pollinators and predators (Bielza et al. 2018). Cyantraniliprole belongs to the diamide group (IRAC 2022) and is used against aphids and white flies due to its physicochemical properties (El-Hefny et al. 2024, Kandil et al. 2023). It is a potent and selective ryanodine receptor activator that causes mortality from uncontrolled release of calcium ion stores in muscle cells (Kandil et al. 2023). Flonicamid is a selective insecticide that belongs to the pyridine carboxamide group, a new class with biological efficacy for controlling aphids and greenhouse whiteflies (Kodandaram et al. 2017; Moustafa et al. 2024a, 2024b). Its mode of action is by obstructing type A potassium channels, thus preventing insects from moving to and attacking plants (Kodandaram et al. 2017, Moustafa et al. 2024b). Moreover, flonicamid has no cross-resistance with other insecticides such as neonicotinoid insecticides and is a safe for fish and natural predators (Kodandaram et al. 2017).

A comprehensive understanding of resistance mechanisms can help in alleviating the development of strategies for avoiding or mitigating insecticide resistance at a localized level (Siddiqui et al. 2023). In general, insect pests evolve resistance to insecticides via three major mechanisms: metabolic resistance (i.e., enhanced detoxification enzyme activity), target resistance (i.e., decreased target sensitivity), and penetration resistance (i.e., decreased epidermal penetration) (Li et al. 2007). Detoxification enzymes in insects are catalyzed by many exogenous or endogenous compounds. Insects can quickly adapt to environmental stresses such as pesticides, extreme temperatures, and carbon dioxide (Fan et al. 2022, Jeffs and Leather 2014, Zhang et al. 2017) through enhanced biodegradation of xenobiotic

compounds, usually by overproduction of a complex set of detoxifying enzymes such as cytochrome P450 monooxygenases, carboxylesterase (CarE), and glutathione *S*-transferases (GSTs) (Sun et al. 2021). The process of detoxification of xenobiotic agents can be divided into phase I (primary) and phase II (secondary) (Yu 2008). The phase I reactions are usually responsible for reducing the biological activity of the toxic substance through several reactions, one of which is oxidation. Oxidative reactions are carried out by cytochrome P450 monooxygenases, which are considered the most important among the phase I reactions (Lu et al. 2021a). Hydrolysis is carried out by CarE, which splits the ester compounds by adding water to produce acid and alcohol (Stankovic and Kostic 2017). Phase II reactions involve conjugation with endogenous molecules such as glutathione, which is carried out by the multifunctional enzymes GSTs.

Owing to their structure and cost-effectiveness, computational methods and molecular modeling techniques have recently proven to be very useful in biochemical research (Moradi et al. 2019). Docking studies provide prediction of possible molecular interaction of toxicants with enzymes of several significant pathways leading to biomolecule production (Moustafa et al. 2023, Nikita et al. 2021). In addition, docking studies provide a three-dimensional structural explanation of the protein–ligand interaction (Hou et al. 2013) and, thus, are considered the most efficient approach to understanding the molecular interactions between an enzyme and a pesticide. Therefore, this study was focused on evaluating the toxicity and biochemical impact of four novel insecticides (sulfoxaflor, spiromesifen, cyantraniliprole, and flonicamid) against the cowpea aphid, *A. craccivora.* We also used molecular docking analysis to examine the inhibitory effect of these insecticides on GST.

Materials and Methods

***Aphis craccivora* colony.** The *A. craccivora* aphids used in this study were from a laboratory colony that was initiated from a population collected from bean plants cultivated at the Faculty of Agriculture Farm, Cairo University, Giza Governorate, Egypt (30.0220°N; 31.2055°E). The colony was maintained in a rearing room at 20–25°C and 70 $\pm$ 5% relative humidity and a photoperiod of 16 h light and 8 h dark (El-Arnaouty 1991) for more than nine generations without exposure to any insecticide. The colony was maintained on broad bean plants (*Vicia faba* L.) that were grown in plexiglas boxes (30 $\times$ 20 $\times$ 10 cm) on humid pine sawdust (Fouad et al. 2016). The boxes were covered with a noncompacted layer of wet sawdust to facilitate germination and penetration of the seedlings, which occurred about 1 wk after sowing the seeds. When the young plants reached 3–5 cm in height, they were artificially infested with aphids by adding pieces of previously infested green bean plants. One box from the stock colony was used to infest two or three new boxes.

Insecticides and chemicals. Commercial formulations of sulfoxaflor (Closer 24SC, Shoura Chemicals, Giza, Egypt), spiromesifen (Oberon 24SC, Syngenta Agrosciences, Dielsdorf, Switzerland), cyantraniliprole (Benevia 10OD, FMC Company, Philadelphia, PA), and flonicamid (Teppeki 50WG, Shoura Chemicals) were used in this study. The substrates and reagents required for biochemical studies were procured from Sigma Aldrich (Schnelldorf, Germany).

Bioassays. A leaf disc dipping technique was used to assess the insecticidal activity of the tested insecticides on *A. craccivora* adults (96 h) according to the methods of Dittrich et al. (1990). The leaf discs cut from broad bean (*V. faba*) leaves (30 mm diameter) were immersed for 20 s in serial dilutions prepared in water (five concentrations of 0.75–50 mg/L) of each tested insecticide. Discs were then air-dried for 30 min and placed with adaxial side down on agar (2%, w/v) in the bottom of 30-mm diameter plastic petri dishes. Leaf discs for the control group were immersed in water. For each concentration, there were five replicates, each with 10 adult *A. craccivora.* Percentage mortality was calculated 48 h after treatment; mortality was indicated when individuals failed to walk or move when gently prodded with a soft camel-hair brush. Mortality was corrected for control mortality using Abbott's (1925) formula, and the concentration-mortality responses to each insecticide were determined using probit analysis (Finney 1971) to estimate lethal concentration values. The bioassay was conducted twice.

Detoxification enzymatic activity. Adult *A. craccivora* that survived for 12, 24, 36, and 48 h after initial exposure to the median lethal concentration (LC_{50}) of each insecticide or water control were used to determine the activities of detoxification enzymes. Five replicates were used for each treatment. Thirty milligrams of *A. craccivora* adults was homogenized in 300 μl of phosphate buffer (0.1 M potassium phosphate buffer, pH 7.0), and the homogenates were centrifuged at 12,000 × *g* for 15 min at 4°C. The supernatants were then collected as a source of enzymes and kept at −20°C for analysis.

The enzymatic activity of cytochrome P450 monooxygenase was assayed using *p*-nitroanisole as a substrate according to Hansen and Hodgson (1971). Ninety microliters of the supernatant was added to 100 μl of *p*-nitroanisole (2 mM) and incubated for 2 min, then 10 μl of NADPH (9.6 mM) was added to initiate the reaction. The absorbance was measured at 405 nm for 15 min, and the standard curve was created utilizing *p*-nitrophenol.

The enzymatic activity of α-esterase (CarE) was tested according to Van Asperen (1962) using α-naphthyl acetate (α-NA) as a substrate. The reaction solution was 30 μl of supernatant and α-NA (30 mM) and was incubated for 15 min, after which 50 μl of stopping solution (two parts 1% fast blue b and five parts 5% sodium dodecyl sulfate) was added. The absorbance was measured at 600 nm, and the standard curve was created utilizing α-naphthol.

The enzymatic activity of GST was analyzed according to Habig et al. (1974). Ten microliters of the enzyme solution was mixed with 25 μl of 1-chloro-2,4-dinitrobenzene (30 mM) and 25 μl of glutathione (50 mM). Absorbance was recorded at 340 nm for 5 min using a UV/V spectrophotometer.

Protein content of each supernatant was determined according to Bradford (1976). The absorbance was measured at 595 nm, and the standard curve was created utilizing bovine serum albumin.

Molecular docking analysis. The docking studies considered the entire molecular structure of the insecticides, including cyclic rings, functional groups, and specific atoms that interact with the receptor's active site in various ways, such as hydrogen bonds, hydrophobic interactions, and π-π stacking between the insecticides' rings and the amino acids of the GST enzyme. The protein data bank (PDB; http://www.rcsb.org.pdb) file produced by the Gaussian 09 program was

used to build the structures of the compound. The PDB also provided the crystal structures of GST (PDB ID: 1TU8). ChemDraw 18.0 was used for constructing two-dimensional representations of the insecticides, which were subsequently converted into three-dimensional structures using MOE 2015 software (Molecular Operating Environment, Chemical Computing Group, Montreal, Quebec, Canada). The docking process was validated by generated 10 poses per ligand to explore different potential binding conformations within the active site of the enzyme. The most suitable pose was selected based on several criteria, including the lowest binding energy score (*S*), the number and strength of hydrogen bonds, hydrophobic interactions, and the overall stability of the ligand–receptor complex.

Data analysis. Probit analysis (version 1.5, U.S. Environmental Protection Agency; http://www.epa.gov/nerleerd/stat2.htm) was used to calculate the LC_{50} values of the tested insecticides against *A. craccivora* adults according to Finney (1971). Enzymatic activity data were coded, entered, and processed using SPSS (version 22, IBM, Armonk, NY). Results were tested for satisfying assumptions of parametric tests, and continuous variables were subjected to Shapiro–Wilk and Kolmogorov–Smirnov tests for normality. Enzymatic activity data were subjected to an analysis of variance. The posthoc analysis was performed using a Tukey pairwise comparison, and differences were considered significant a $P < 0.05$. All analyses were conducted with Minitab (version 14.0), and data were visualized with RStudio (version 2022.02.4, Posit PBC; https://posit.co/products/open-source/rstudio/).

Results

Insecticide toxicity to *A. craccivora*. At 48 h following initial exposure, the LC_{50} values were 1.98–14.93 mg/L, estimated as 1.98 mg/L for spiromesifen, 3.13 mg/L for sulfoxaflor, 4.02 mg/L for flonicamid, and 14.93 mg/L for cyantraniliprole (Table 1). Based on nonoverlapping 95% confidence intervals, toxicity of the four insecticides to *A. craccivora* adults followed a descending order of spiromesifen = sulfoxaflor > flonicamid > cyantraniliprole.

Impact of insecticides on detoxification enzyme activity in *A. craccivora*. Sulfoxaflor and cyantraniliprole significantly reduced the activities of α-esterase (143.35 ± 24.33 and 141.62 ± 29.62 μmole/mg of protein, respectively) and cytochrome P450 monooxygenase (0.47 ± 0.06 and 0.45 ± 0.13 μmole/ml/mg of protein, respectively) 48 h after treatment. In comparison, the activities of α-esterase and cytochrome P450 monooxygenase in the control group were 226.72 ± 13.33 and 0.74 ± 0.05, respectively (Table 2; Fig. 1). Flonicamid also significantly reduced ($F = 1.47$, $P = 0.283$) the activity of α-esterase at 24 and 36 h after treatment (126.16 ± 24.35 and 120.55 ± 12.42 Mmole/mg of protein, respectively), but no significant effect ($F = 1.47$, $P = 0.283$) was recorded for cytochrome P450 monooxygenase activity 24 h after treatment compared with the control group. Spiromesifen exhibited no significant effects on either α-esterase or cytochrome P450 monooxygenase (Table 2; Fig. 1). All but flonicamid significantly reduced GST activity ($F = 2.06$, $P = 0.043$) (Table 2; Fig. 1).

Molecular docking analysis. Molecular docking was performed for the tested insecticides against the active site of GST (PDB ID: 2IMK). Docking studies were conducted to discern the precise binding modes and intricate interactions between

Table 1. Toxicity of four chemical insecticides against *Aphis craccivora*.*

Insecticide	LC_{50} (mg/L) (95% CL)	LC_{90} (mg/L) (95% CL)	Slope (Mean ± SE)	χ^2
Sulfoxaflor	3.13 (2.18–4.29)	13.11 (10.58–17.32)	1.49 ± 0.24	2.10
Spiromesifen	1.98 (1.48–2.60)	10.70 (7.19–19.70)	1.74 ± 0.22	1.96
Cyantraniliprole	14.93 (10.53–21.50)	280.05 (108.50–1884.10)	1.41 ± 0.21	1.79
Flonicamid	4.02 (3.16–5.10)	20.61 (14.37–34.95)	1.80 ± 0.20	0.56

* LC_{50}, median lethal concentration; LC_{90}, concentration lethal for 90% of the population; CL, confidence limit.

the compound of interest (the insecticide) and the key amino acids present in the target receptor.

The tested compounds had energy scores from −5.63 to −7.42 kcal/mol (Table 3). Sulfoxaflor had one hydrogen bond with THR 54, and spiromesifen had an arene-H contact with HIS 41 (Fig. 2). The interaction between flonicamid and GST was stabilized via a pair of hydrogen bonds (with SER 12 and ILE 55). In contrast, cyantraniliprole combined with the receptor through two hydrogen bonds (with HIS 53 and ARG 112) and H-arene contact with HIS 53 (Fig. 2). *S*-hexylglutathione (GTX) has an energy score of −7.40 kcal/mol and had two H-bonds, with GLU 116 and ILE 55 (Fig. 2).

Discussion

Monitoring insecticide resistance is crucial for assessing insecticide efficacy and selecting the effective insecticides for insect pest management (Sabra et al. 2023). The results of our laboratory study showed that spiromesifen and sulfoxaflor were the most toxic to *A. craccivora* adults, with spiromesifen being 7.5 times more toxic than cyantraniliprole. Bouabida et al. (2017) attributed the high toxicity of spiromesifen to its effect on lipid synthesis through inhibiting acetyl-CoA carboxylase.

Cyantraniliprole is well known for its effectiveness against a wide range of insect pests, including lepidopterans, dipteran leafminers, aphids, leafhoppers, psyllids, beetles, whiteflies, thrips, and weevils (Mantzoukas et al. 2022), whereas spiromesifen has been widely used for controlling sucking insects (de Little and Umina 2017). Our findings agree with those of Ahmed et al. (2018), who reported LC_{50} values of spiromesifen against adults of *A. craccivora* as 0.073 and 0.063 mg/L at 24 and 48 h after exposure, respectively. Others reported LC_{50} values for flonicamid and sulfoxaflor against *A. craccivora* as 0.079 and 0.46 mg/L, respectively, after 24 h (Batana et al. 2023, Manjarika et al. 2018). In addition, Patil et al. (2017) found that spiromesifen had higher toxicity against aphids than did cyantraniliprole, and Mishra and Pandey (2023) reported that neonicotinoid insecticides, including thiamethoxam, were effective. However, Moustafa et al. (2022a) found that sulfoxaflor was more effective for decreasing the number of *A. craccivora* adults than were other insecticides, including acetamiprid, thiamethoxam, imidacloprid, azadirachtin, and thiocyclam, until 7 d after application under field conditions.

Table 2. Enzyme activities (mean ± SD) of α-esterase, glutathione *S*-transferase (GST), and cytochrome P450 monooxygenase of *A. craccivora* after 12, 24, 36, and 48 h of exposure to sulfoxaflor, spiromesifen, cyantraniliprole, and flonicamid.*

Enzyme	Treatment	12 h	24 h	36 h	48 h
α-Esterase (Mmole/mg of protein)	Control	182.33 ± 8.32ab	191.1 ± 24.79a	193.85 ± 8.11a	226.72 ± 13.33a
	Sulfoxaflor	139.61 ± 31.24ab	162.62 ± 8.96ab	170.91 ± 19.58a	143.35 ± 24.33b
	Spiromesifen	210.6 ± 17.67a	223.76 ± 19.27a	172.95 ± 15.48a	158.14 ± 27.34ab
	Cyantraniliprole	159.88 ± 26.31ab	180.99 ± 10.59ab	181.93 ± 11.5a	141.62 ± 29.62b
	Flonicamid	134.28 ± 23.45b	126.16 ± 24.35b	120.55 ± 12.42b	159.72 ± 15.65ab
GST (Mmole/ml/mg of protein)	Control	15.45 ± 1.44a	15.35 ± 1.74a	13.19 ± 0.94a	12.09 ± 1.3a
	Sulfoxaflor	12.93 ± 0.88ab	10.95 ± 2.25ab	8.6 ± 1.73b	5.91 ± 1.34b
	Spiromesifen	12.15 ± 1.65ab	11.8 ± 0.45ab	7.96 ± 0.92b	8.61 ± 0.76b
	Cyantraniliprole	7.51 ± 1.19c	6.13 ± 0.55c	6.94 ± 0.8b	7.94 ± 0.64b
	Flonicamid	10.86 ± 0.64bc	9.86 ± 0.72bc	9.62 ± 1.37ab	8.98 ± 0.34ab
Cytochrome P450 monooxygenase (Mmole/ml/mg of protein)	Control	0.55 ± 0.1b	0.61 ± 0.08a	0.59 ± 0.05a	0.74 ± 0.05a
	Sulfoxaflor	0.83 ± 0.08a	0.66 ± 0.15a	0.49 ± 0.04ab	0.47 ± 0.06b
	Spiromesifen	0.44 ± 0.04b	0.52 ± 0.04a	0.33 ± 0.007b	0.61 ± 0.03ab
	Cyantraniliprole	0.62 ± 0.12ab	0.57 ± 0.06a	0.68 ± 0.09a	0.45 ± 0.13b
	Flonicamid	0.48 ± 0.01b	0.46 ± 0.05a	0.52 ± 0.06ab	0.57 ± 0.02ab

* Within a column, means with different letters are significantly different ($P < 0.05$).

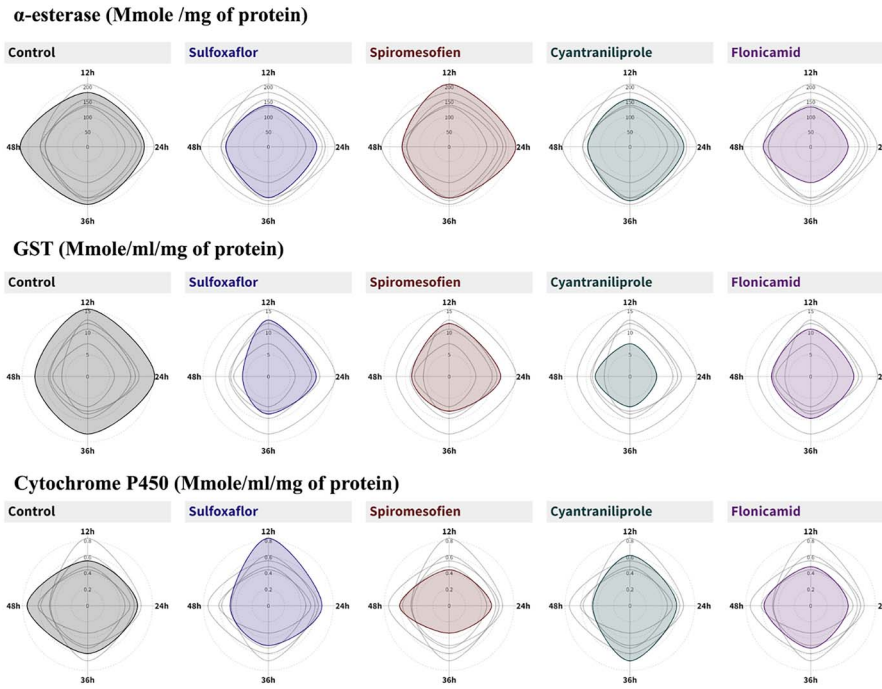

Fig. 1. Radar chart representing the activities of α-esterase, glutathione *S*-transferase (GST), and cytochrome P450 monooxygenase of *A. craccivora* at 12, 24, 36, and 48 h following treatment with sulfoxaflor, spiromesifen, cyantraniliprole, and flonicamid.

Insects are exposed to a remarkable array of natural and synthetic xenobiotics, including chemical insecticides (Lu et al. 2021b). To cope with these xenobiotics, insects have evolved a range of adaptation mechanisms, including behavioral and physiological changes (Moustafa et al. 2022b) leading to avoidance and tolerance of xenobiotics. One of these adaptation mechanisms is detoxifying enzymes, which enhances the insect's metabolic capacity to counteract pesticides (Fan et al. 2023).

The ability of insects to detoxify chemicals is reflected by the response of detoxification enzymes to insecticides (Tang et al. 2021). Metabolic detoxification is a multistep process involving enzymatic hydrolysis and conjugation of lipophilic compounds with water-soluble and excretable metabolites (Koirala et al. 2022). In phase I, carboxylesterase and cytochrome P450 oxidase enzymes convert the lipophilic xenobiotics to more hydrophilic products (Cruse et al. 2023). As revealed in our results, α-esterase and cytochrome P450 monooxygenase activities significantly decreased in *A. craccivora* adults 48 h after treatment with sulfoxaflor and cyantraniliprole. Conversely, cytochrome P450 monooxygenase and CarE activities were elevated after treatment with sublethal concentrations of cyantraniliprole in the leafhopper *Laodelphax striatellus* (Fallén) (Wang et al. 2022).

In phase II, GSTs conjugate the xenobiotic metabolites with concomitant antioxidant activity against the stress induced by organic hydroperoxides (Zhang et al.

Table 3. Docking interaction data calculations of sulfoxaflor, spiromesifen, flonicamid, cyantraniliprole, and *S*-hexylglutathione (GTX) inside glutathione-*S*-transferase (PDB ID: 2IMK) active spots.

Compound	Energy Score (kcal/mol)	Affinity Bond Strength (kcal/mol)	Affinity Bond Length (A° from main residue)	Amino acids	Ligand	Interaction
Sulfoxaflor	−5.63	−0.6	3.78	THR 54	N 3	H-acceptor
Spiromesifen	−7.42	−0.6	3.58	HIS 41	6-Ring	pi-H
Flonicamid	−5.72	−1.9	2.90	SER 12	O 11	H-acceptor
		−2.9	3.27	ILE 55	N 22	H-acceptor
Cyantraniliprole	−6.83	−0.7	3.65	HIS 53	Cl 42	H-donor
		−1.5	2.82	ARG 112	O 8	H-acceptor
		−0.6	4.23	HIS 53	C 27	H-pi
GTX	−7.40	−14.8	2.88	GLU 116	N 1	H-donor
		−0.8	4.14	ILE 55	S 28	H-acceptor

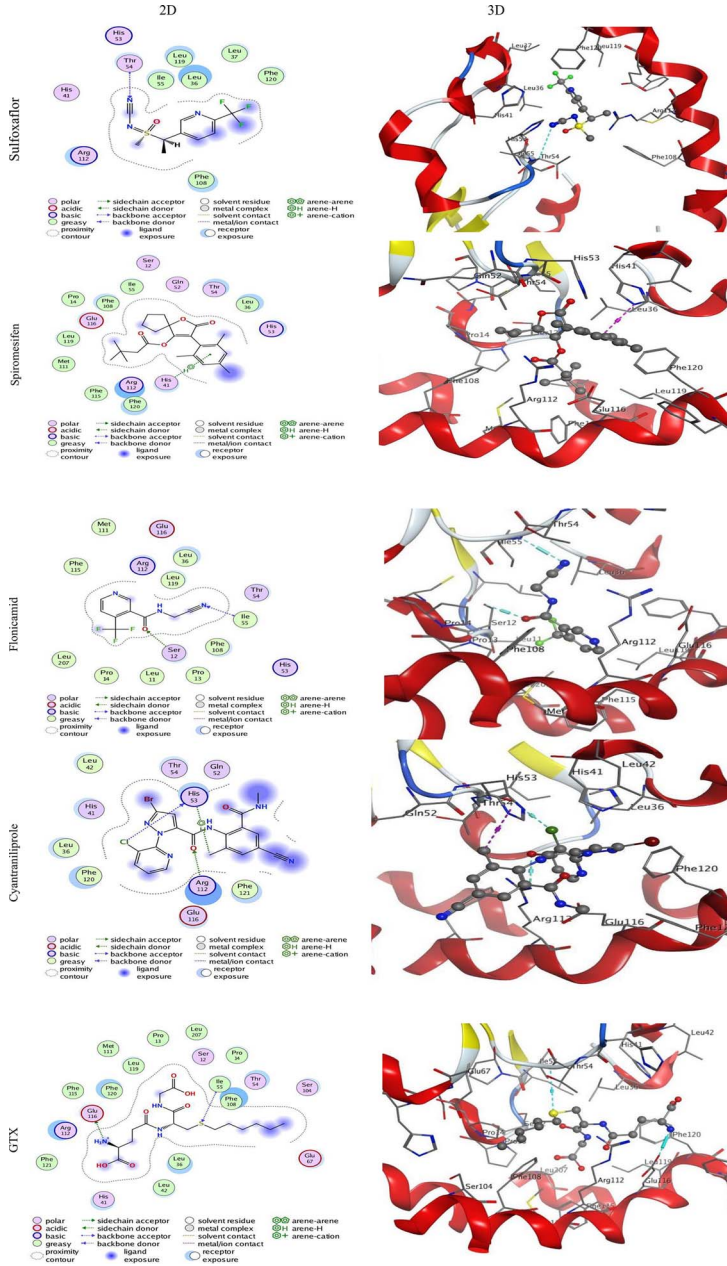

Fig. 2. Two- and three-dimensional interactions of sulfoxaflor, spiromesifen, flonicamid, cyantraniliprole, and *S*-hexylglutathione (GTX) in the active site of glutathione-*S*-transferase (PDB ID: 2IMK). Hydrogen bonds are displayed in cyan, and H-pi-bonds are displayed in dark magenta.

2023). Additionally, GST is involved in the establishment of defense mechanisms against insecticides (Kostaropoulos et al. 2001) and is usually regarded as the target in devising new insecticides (Wang et al. 2014). Accordingly, GST is a promising candidate for developing a biosensor for detecting insecticide levels. Therefore, GST could be an indicator of the adaptation of insects to xenobiotics. In this context, our findings revealed that all the tested insecticides except flonicamid significantly inhibited the activity of GST in adult *A. craccivora* (Table 2; Fig. 1).

These insecticides remain effective against this insect pest. In general, the inhibitory effect of pesticides on detoxification enzyme activity is a positive marker that can delay resistance against toxic compounds (Pengsook et al. 2022). In contrast, high detoxification enzyme activity suggests the involvement of those enzymes in insecticidal stress, thereby altering the susceptibility of the insects to the insecticide (Fan et al. 2023). Therefore, it is important to elucidate the mode of action of insecticides to provide opportunities for the development of environmentally friendly insecticides. New formulations of pesticides could be used to explore alternatives to traditional pesticides (El-Hefny et al. 2024). The *in vitro* detoxification enzyme inhibition assays indicated that the four insecticides had an inhibitory effect on GST activity, suggesting the possibility of discovering new insecticidal synergists that act by interfering with conjugation-mediated detoxification in insects (Wang et al. 2016). In this respect, molecular docking analysis is a promising method for the effective identification of potential biosensing enzymes for detecting pesticides electrochemically. In this study, we evaluated the interaction between the target enzymes and the tested insecticides. Our results indicated that all the tested insecticides had good energy scores; spiromesifen had an energy score of −7.42, which is very close to that of GTX ligand (−7.40).

The high binding affinity of an insecticide may be attributed to its molecular structure. Currently, pesticide development is related to the development of sustainable agricultural and molecular docking technology (Hou et al. 2023). Combined studies involving molecular docking and pharmacophore with active site of the nAChR homology model were used to clarify the binding affinity of about 78 neonicotinoid insecticides with various structures tested on *A. craccivora* (Crisan et al. 2022) for the purpose of designing environmentally friendly insecticides against this insect pest. Other molecular docking studies explored that the bioactive compounds (cymbodiacetal, proximadiol, geranylacetone, and rutin) that are responsible for the insecticidal and acetylcholinesterase (AChE) inhibitory effect of an extract of West Indian lemon grass, *Cymbopogen citratus* Stapf, that exhibited different levels of binding affinity ranging from −8.148 to −9.407 kcal/mol (Johnson et al. 2021). In addition, Moustafa et al. (2023) suggested that inhibition of cytochrome P450 could be a vital mechanism via which *C. citratus* and citral act on *Spodoptera littoralis* Boisduval. Mattar et al. (2022) found that the best ligands for AChE in the beetle *Rhipibruchus picturatus* (F.) are the sesquiterpenoid molecules, including 1-epi-cadinol, which is the main constituent of *Schinus areira* L. (Anacardiaceae) essential oil.

In conclusion, over the past 30 yr, pyrethroids and neonicotinoid synthetic insecticides, such as cypermethrin, deltamethrin, alphacypermethrin, and lambdacyhalothrin, have been widely employed to control sucking insects, including aphids. However, synthetic chemicals pose health risks due to toxic residues, especially on regularly

harvested leafy vegetables, and kill the natural enemies of *A. craccivora,* leading to a resurgence of the pest and the need for greater use of insecticides. Therefore, use of new insecticides not only mitigates the development of insect resistance but also contributes to achieving more effective pest control. This study shows the impact of four insecticides on adult *A. craccivora*. Spiromesifen was more toxic to *A. craccivora* adults. Additionally, the interaction between these insecticides and the GST target site was assessed. Spiromesifen had the lowest energy score of −7.42 compared with the other insecticides, suggesting that inhibition of GST could be a vital mechanism of spiromesifen. This may be a starting point for the development of new insecticides that can be used to overcome insecticide resistance.

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