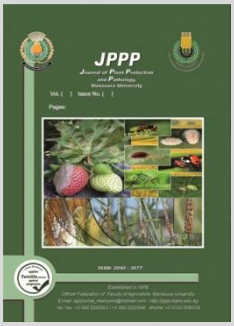
Journal of Plant Protection and Pathology

Journal homepage & Available online at: www.jppp.journals.ekb.eg

Comparative Toxicity, Interspecific Susceptibility, and Enzyme Activity Responses to First- to Fourth-Generation Neonicotinoid Insecticides in *Aphis gossypii* Glover and *Myzus persicae* Sulzer under Laboratory Conditions

Soliman, B. S. M.; M. M. R. Attia; A. A. E. Darwish* and Awatef S. Mansy

Plant protection department, Faculty of Agriculture, Damanhour University, Egypt



Article Information
Received 22 / 10 / 2025
Accepted 5 / 11 / 2025

ABSTRACT

This study evaluated the toxicity of six neonicotinoid insecticides from four chemical generations against *Aphis gossypii* and *Myzus persicae* under laboratory conditions after 24 and 48 hours of exposure. Sulfoxaflor, representing the fourth generation, consistently showed the highest toxicity against both aphid species, with the lowest LC_{50} values and a toxicity index of 100. Second-generation insecticides (clothianidin and thiamethoxam) exhibited moderate to high efficacy, whereas first-generation (imidacloprid and acetamiprid) and third-generation (dinotefuran) compounds showed lower toxic effects. Time-dependent increases in toxicity were observed for most insecticides. Comparative analysis revealed interspecific differences: *M. persicae* exhibited greater tolerance to most compounds than *A. gossypii*, suggesting species-specific resistance profiles. Biochemical assays indicated that several neonicotinoids significantly increased acetylcholinesterase (AChE) and glutathione S-transferase (GST) activity in *M. persicae*, implying adaptive detoxification mechanisms. In contrast, sulfoxaflor significantly inhibited AChE activity, highlighting its distinct neurotoxic mode of action. These findings underscore the importance of chemical innovation and resistance management in aphid control programs. Sulfoxaflor emerged as the most promising candidate due to its high efficacy and novel mechanism, making it suitable for integrated pest management (IPM). However, rotating insecticides and monitoring susceptibility remain essential to delay resistance development.

Keywords: *Aphis gossypii*; *Myzus persicae*; neonicotinoids; toxicity; AchE; GST.

INTRODUCTION

The cotton aphid (*Aphis gossypii* Glover) and the green peach aphid (*Myzus persicae* Sulzer) are globally significant pests that pose serious threats to agricultural productivity due to their rapid reproduction, polyphagy, and ability to transmit a wide range of plant viruses. *A. gossypii* is commonly found infesting cotton, cucurbits, and other economically important crops, causing direct damage through phloem sap extraction and indirect losses through the transmission of viruses such as cucumber mosaic virus and cotton leaf curl virus (Blackman and Eastop, 2000; van Emden and Harrington, 2017). Similarly, *M. persicae* is one of the most damaging aphid species worldwide, attacking over 400 plant species including peach, potato, tobacco, and crucifers, and is known to transmit more than 100 plant viruses (Bass *et al*., 2014; Gadhave *et al*., 2020). Both aphid species have demonstrated remarkable adaptive abilities, especially in their capacity to develop resistance to multiple classes of insecticides. This resistance evolution undermines the efficacy of control strategies and represents a major obstacle for sustainable pest management. Among the most widely used tools for aphid control are neonicotinoid insecticides, which act as agonists of the insect nicotinic acetylcholine receptors (nAChRs), leading to overstimulation of the nervous system, paralysis, and death (Jeschke *et al*., 2011).

Neonicotinoids have been classified into generations based on their chemical structure and development timeline. The first-generation compounds, such as imidacloprid and acetamiprid, were introduced in the 1990s and quickly gained popularity due to their systemic properties and long residual activity. However, resistance to these compounds has been increasingly reported in field populations of aphids worldwide (Denholm *et al*., 2002; Elbert and Nauen, 2000). The second generation, including thiamethoxam and clothianidin, offered some improvements in efficacy and systemic movement within plants, but they too have been affected by cross-resistance in many regions (Bass *et al*., 2015). Dinotefuran, a third-generation neonicotinoid, is characterized by its higher water solubility and faster uptake, which enhances its knockdown effect. More recently, sulfoxaflor, a fourth-generation compound and member of the sulfoximine subclass, has been developed to overcome existing resistance mechanisms, although it shares a similar mode of action and binding site to traditional neonicotinoids (Simon-Delso *et al*., 2015; Sparks *et al*., 2013; Cutler, *et al*. 2013). The increasing frequency of neonicotinoid resistance in *A. gossypii* and *M. persicae* populations necessitates routine monitoring through bioassays. Bioassay techniques provide quantitative data on insect susceptibility, allowing the estimation of lethal concentration values (LC_{50}, LC_{90}) and the detection of shifts in baseline sensitivity that may indicate emerging resistance (IRAC, 2023). These data are essential for informing insecticide rotation strategies and ensuring the continued effectiveness of pest control programs within integrated pest management (IPM) frameworks. In addition to bioassays,

* **Corresponding author.**
E-mail address: adnandarwish2012@yahoo.com
DOI: 10.21608/jppp.2025.434862.1393

enzymatic analyses have become an essential tool for understanding the biochemical mechanisms underlying insecticide resistance in aphids. Studies of detoxification enzymes (such as esterases, cytochrome P450 monooxygenases, and glutathione S-transferases) can reveal metabolic resistance pathways that reduce the efficacy of neonicotinoids. By comparing enzyme activity levels in susceptible and resistant populations, researchers can gain valuable insights into the adaptive responses of aphids to chemical pressure. Such knowledge enhances resistance monitoring efforts and supports the development of more targeted and sustainable pest management strategies. The present study was therefore conducted to evaluate the susceptibility of *A. gossypii* and *M. persicae* to six neonicotinoid insecticides representing four different chemical generations: acetamiprid and imidacloprid (first generation), thiamethoxam and clothianidin (second generation), dinotefuran (third generation), and sulfoxaflor (fourth generation). Using standardized bioassay protocols, the study aims to generate up-to-date LC_{50} values for laboratory populations of the two aphid species and provide essential baseline data for resistance management and future policy development regarding the use of neonicotinoids in aphid control programs. Additionally, the study aims to conduct enzymatic assays in both aphid species to assess potential biochemical mechanisms contributing to insecticide resistance.

MATERIALS AND METHODS

Insect collection and rearing

Two aphid species were used in this study: *A. gossypii* (cotton aphid) and *M. persicae* (green peach aphid). Colonies of both species were collected from untreated cotton and peach orchards, respectively, located in the Nubaria region, Egypt, during the 2025 growing season. Aphid populations were maintained for several generations under laboratory conditions (25 ± 2 °C, 65 ± 5% RH, and a 16:8 h L:D photoperiod) on cucumber seedlings (grown in plastic pots in mesh-covered cages), to reduce field-related physiological variability.

Insecticides Tested

The tested neonicotinoid insecticides were categorized according to their generational development as follows:

- **First generation**: Imidacloprid (Confidor 20% SL, Bayer CropScience) and Acetamiprid (Mospilan 20% SP, Nippon Soda).
- **Second generation**: Thiamethoxam (Actara 25% WG, Syngenta) and clothianidin (Supertox-1® 48% SC, provided by Jiangsu Jiag Chemical Industry Co. Ltd, China).
- **Third generation**: Dinotefuran (MTI 446 4% SG, Mitsui Chemicals).
- **Fourth generation**: Sulfoxaflor (Closer 24% SC, Corteva Agriscience).

The toxicity test

Toxicity bioassays were conducted following a modified leaf-dip method adapted from IRAC Method No. 019, with adjustments suited for each aphid species. Fresh cucumber (*Cucumis sativus*) leaves were selected as the substrate due to their broad surface and suitability for both aphid species. Leaves were washed thoroughly using mild soap and distilled water to eliminate naturally occurring pests and residues, then allowed to air dry. Uniform discs (6 cm in diameter) were excised using a metal cutter. The leaf discs were dipped for 10 seconds in one of five concentrations of each insecticide, prepared as serial dilutions in distilled water with 0.05% nonionic surfactant (Tween-80) to ensure uniform wetting. Discs were then allowed to air dry on sterile filter paper. Each treated disc was placed abaxial side up in a 9-cm Petri dish containing 1.5% agar to maintain moisture. For each replicate, 20 apterous adult aphids (2–3 days old) were gently transferred to the disc using a fine camel-hair brush. Each treatment was replicated four times. Control treatments involved leaf discs dipped in distilled water containing only the surfactant.

Concentration Ranges

Based on preliminary trials and previous LC_{50} reports (e.g., Elbert *et al*., 2008), the concentration ranges (in mg/L) for each insecticide were as follows (that will able to mortality ranged between 10 and 90%):

- **Acetamiprid**: 5, 10, 15, 30, 60
- **Imidacloprid**: 2, 4, 8, 16, 32
- **Thiamethoxam**: 1, 2, 4, 8, 16
- **Clothianidin**: 0.75, 1.5, 3, 6, 12
- **Dinotefuran**: 5, 10, 20, 30, 50
- **Sulfoxaflor**: 0.5, 1, 2, 4, 8

Mortality Assessment and Statistical Analysis

Mortality was assessed after 24 and 48 hours of exposure. Aphids were considered dead if they showed no movement upon gentle probing with a fine brush. Mortality data were corrected using Abbott's formula (Abbott, 1925) when control mortality exceeded 5%. Probit analysis (Finney, 1971) was conducted using LdP-Line software (Ehab Software, http://www.ehabsoft.com/ldpline/) to estimate (LC_{25}, LC_{50} and LC_{90}) and their 95% confidence limits. Additionally, toxicity index values (TI) were calculated using the formula of Sun (1950) allowing for a comparative assessment of relative toxicity among tested compounds as follows;

$$\mathbf{TI = (A / B) \times 100}$$

Where; **A:** LC_{50} **of most toxic compound and B:** LC_{50} **of tested compound**

Biochemical enzyme assays following LC_{50} pesticide exposure

To investigate the biochemical response of *A. gossypii* and *M. persicae* to selected insecticides, enzyme activity assays were performed after exposure to LC_{50} doses. Individuals from both aphid species were exposed to six insecticides at their respective LC_{50} concentrations using the leaf-dip method. Discs of cucumber leaves (*C. sativus*) were immersed in pesticide solutions, then air-dried, and placed in Petri dishes. Aphids were transferred onto the treated leaf discs and maintained under controlled laboratory conditions (25 ± 1 °C, 65 ± 5% RH, and a 16:8 h light:dark photoperiod). A parallel control group was treated with distilled water only. After 24 hours of exposure, the surviving individuals were collected, homogenized in ice-cold phosphate buffer (0.1 M, pH 7.0), and centrifuged to obtain supernatants used for enzyme assays.

Acetylcholinesterase (AChE) activity was determined according to Moores *et al*. (1996), mixed-function oxidase (MFO) activity was assayed as per Hansen and Hodgson (1971), carboxylesterase (CarE) activity was

measured using naphthyl acetate following Van Asperen (1962), and glutathione-S-transferase (GST) activity was assessed following Habig *et al*. (1974).

RESULTS AND DISCUSSION

Toxicity of six neonicotinoid insecticides against *A. gossypii*

The toxicity of six neonicotinoid insecticides (representing four successive chemical generations) was evaluated against the cotton aphid *A. gossypii* after 24 and 48 hours of exposure (Tables 1-2 and Figs. 1-2). The tested compounds included: imidacloprid and acetamiprid (first-generation neonicotinoids), thiamethoxam and clothianidin (second generation), dinotefuran (third generation), and sulfoxaflor (fourth generation).

After 24 Hours

Sulfoxaflor, representing the fourth generation, demonstrated the highest toxicity with the lowest LC_{50} value (1.394 mg/L), serving as the reference standard (T.I. = 100%). Among the earlier generations, second-generation clothianidin and thiamethoxam followed with LC_{50} values of 3.209 and 4.081 mg/L, respectively. Imidacloprid, a first-generation compound, showed moderate toxicity (LC_{50} = 7.547 mg/L), while acetamiprid and dinotefuran (first and third generations, respectively) recorded the least toxicity values (13.89 and 19.502 mg/L). The toxicity index ranked the compounds as follows: sulfoxaflor (100%) > clothianidin (43.44%) > thiamethoxam (34.16%) > imidacloprid (18.47%) > acetamiprid (10.04%) > dinotefuran (7.15%).

This ranking highlights a progressive increase in efficacy with chemical evolution, with newer-generation compounds generally exhibiting greater toxicity. The relatively high potency of sulfoxaflor may be attributed to its distinct binding mode to nicotinic acetylcholine receptors (nAChRs), overcoming resistance mechanisms associated with traditional neonicotinoids.

After 48 Hours

After 48 hours of exposure the toxicity for all compounds was increased, reflecting either enhanced penetration or delayed action. Sulfoxaflor remained the most toxic (LC_{50} = 1.026 mg/L), reinforcing its status as a fast-acting and potent aphicide. Clothianidin and thiamethoxam also improved in efficacy (LC_{50} = 2.232 and 2.933 mg/L, respectively), while imidacloprid showed a moderate shift (LC_{50} = 5.512 mg/L). Acetamiprid and dinotefuran remained the least effective, albeit with some improvement (LC_{50} = 10.541 and 14.735 mg/L, respectively). The updated toxicity indices were: sulfoxaflor (100%) > clothianidin (45.97%) > thiamethoxam (34.98%%) > imidacloprid (18.61%) > acetamiprid (9.73%) > dinotefuran (6.96%). These results indicate that while time-dependent increases in efficacy were noted for most compounds, the relative order of toxicity remained largely consistent, particularly for the most and least potent insecticides.

A generational comparison underscores clear differences in toxicological performance. First-generation neonicotinoids (imidacloprid and acetamiprid) consistently showed moderate to low efficacy, possibly due to widespread resistance in *A. gossypii* populations resulting from long-term and repeated usage. Second-generation compounds (thiamethoxam and clothianidin) offered improved activity, suggesting partial circumvention of resistance pathways. The third-generation Dinotefuran, despite its novelty, exhibited lower than expected toxicity, potentially indicating cross-resistance. Sulfoxaflor, the fourth-generation sulfoximine, emerged as the most effective compound across all durations, likely due to its unique binding properties and limited prior exposure in the field.

The findings confirm that chemical innovation within the neonicotinoid class contributes significantly to improved aphid control. However, the variable efficacy among compounds from different generations signals the need for careful rotation and judicious application in resistance management programs. The poor performance of acetamiprid and Dinotefuran (even after 48 hours) raises concerns regarding their continued standalone use against *A. gossypii*. Meanwhile, sulfoxaflor, due to its high efficacy and possibly novel mode of action, could be a valuable candidate in integrated pest management (IPM) strategies, particularly in fields experiencing reduced sensitivity to earlier neonicotinoid generations.

These observations align with the findings of Sparks *et al*. (2013) and Babcock *et al*. (2011), who noted sulfoxaflor's efficacy against sap-feeding insects and its potential in managing resistant aphid populations. Nonetheless, regular monitoring of susceptibility levels and avoidance of overreliance on a single compound are essential to prolong efficacy and delay resistance onset.

Table 1. Comparative toxicity of neonicotinoid insecticides against apterous adult *A. gossypii* after 24-hour laboratory exposure

Treatments	LC_{25} (ppm) ± 95% CL	LC_{50} (ppm)± 95% CL	LC_{90} (ppm)± 95% CL	Slop ± SE	Chi^2	Toxicity index
Sulfoxaflor	0.631(0.467-0.788)	1.394(1.168-1.636)	6.285(4.854-9.023)	1.96±0.196	4.195	100
Thiamethoxam	1.564(1.132-1.987)	4.081(3.364-4.958)	25.245(17.63-42.585)	1.619±0.171	0.559	34.16
Imidacloprid	3.475(2.709-4.22)	7.547(6.408-8.871)	32.953(25.26-47.37)	2.002±0.184	0.263	18.47
Clothianidin	1.227(0.893-1.554)	3.209(2.647-3.907)	19.938(13.84-33.97)	1.615±0.171	0.197	43.44
Dinotefuran	9.224(7.244-11.07)	19.502(12.77-22.77)	80.889(60.98-121.54)	2.074±0.213	3.676	7.15
Acetamiprid	6.539(5.002-7.982)	13.89(11.83-16.18)	58.14(44.87-83.77)	2.061±0.206	0/537	10.04

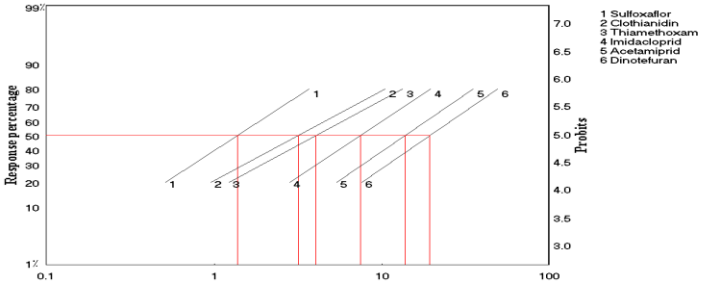


Fig. 1. Toxicity lines of six neonicotinoid insecticides against apterous adult *A. gossypii* after 24-hour laboratory exposure

Table 2. Comparative toxicity of neonicotinoid insecticides against apterous adult *A. gossypii* after 48-hour laboratory exposure

Treatments	LC_{25} (ppm)± 95% CL	LC_{50} (ppm) ± 95% CL	LC_{90} (ppm) ± 95% CL	Slop ± SE	Chi^2	Toxicity index
Sulfoxaflor	0.481(0.338-0.612)	1.026(0.844-1.216)	4.328(3.278-6.579)	2.05±0.244	0.0053	100
Thiamethoxam	1.154(0.805-1.496)	2.933(2.39-3.54)	17.271(12.59-27.18)	1.664±0.174	1.202	34.98
Imidacloprid	2.592(1.997-4.22)	5.512(4.662-8.871)	23.121(18.613-47.37)	2.058±0.173	0.433	18.61
Clothianidin	0.889(0.627-1.146)	2.232(1.826-2.685)	12.837(9.396-20.035)	1.687±0.174	0.222	45.97
Dinotefuran	6.855(5.166-8.431)	14.735(12.501-17.165)	63.031(48.51-91.535)	2.03±0.208	6.309	6.96
Acetamiprid	4.905(3.527-6.19)	10.541(8.76-12.38)	45.096(34.89-65.38)	2.03±0.221	1.621	9.73

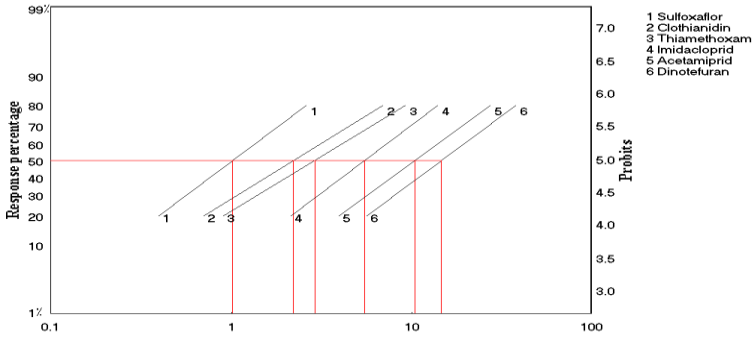


Fig. 2. Toxicity lines of six neonicotinoid insecticides against apterous adult *A. gossypii* after 48-hour laboratory exposure

Toxicity of six neonicotinoid insecticides against *M. persicae*

The present laboratory bioassay revealed clear variations in the susceptibility of *M. persicae* to six insecticides belonging to four different generations of neonicotinoids (Tables 3-4). Based on LC_{50} values after 24 and 48 hours, sulfoxaflor (a fourth-generation sulfoximine) demonstrated the highest toxicity with LC_{50} values of 0.943 ppm at 24 hours and 0.818 ppm at 48 hours, and thus was assigned a toxicity index (T.I.) of 100 %. Among the neonicotinoids, clothianidin (second generation) ranked second in toxicity with LC_{50} = 3.237 and 2.41 ppm after 24 and 48 hours, respectively. This was followed by thiamethoxam (second generation) and imidacloprid (first generation), with moderate toxicity levels (T.I. = 15.65 % at 24h and 17 % at 48h for thiamethoxam; 9.23–10.37 % for imidacloprid). In contrast, dinotefuran (third generation) and acetamiprid (first generation) showed markedly lower toxicity, with LC_{50} values exceeding 17 ppm at 24 hours and 12 ppm at 48 hours, indicating weaker efficacy against *M. persicae* under laboratory conditions.

These findings indicate that insecticides of the second generation (clothianidin and thiamethoxam) were more toxic than those from the first and third generations, a result that may be attributed to their better translaminar activity and higher affinity to nicotinic acetylcholine receptors (Jeschke *et al*., 2011). On the other hand, acetamiprid and dinotefuran showed delayed and reduced action, possibly due to lower systemic movement or rapid detoxification by *M. persicae*, which is known for its high detoxifying enzyme systems and genetic plasticity (Bass *et al*., 2014).

A comparison between 24- and 48-hour exposures revealed that toxicity generally increased with time for all compounds, especially for less effective insecticides like acetamiprid and dinotefuran, suggesting a cumulative toxic effect or delayed mortality, which is typical for some neonicotinoids (Nauen *et al*., 2003).

Interestingly, while sulfoxaflor consistently maintained its top rank in toxicity for both aphid species, the order of effectiveness among neonicotinoids differed. In *A. gossypii*, imidacloprid and thiamethoxam were more effective than clothianidin, whereas in *M. persicae*, clothianidin showed superior toxicity. This discrepancy may be due to species-specific differences in detoxification enzymes, receptor sensitivity, or cuticle penetration rates. It has been reported that *M. persicae* harbors more P450 and esterase-based resistance mechanisms compared to *A. gossypii*, potentially altering the relative toxicity (Puinean *et al*., 2010).

The data strongly suggest that sulfoxaflor is the most promising alternative among the tested compounds against *M. persicae*, followed by second-generation neonicotinoids like clothianidin. The observed interspecies and intergenerational variations in toxicity highlight the importance of species-specific evaluations and the need to rotate between different generations to delay resistance development.

Table 3. Comparative toxicity of neonicotinoid insecticides against apterous adult *M. persicae* after 24-hour laboratory exposure

Treatments	LC_{25} (ppm)± 95% CL	LC_{50} (ppm) ± 95% CL	LC_{90} (ppm) ± 95% CL	Slop ± SE	Chi^2	Toxicity index
Sulfoxaflor	0.476(0.346-0.595)	0.943(0.784-1.105)	3.452(2.721-4.88)	2.27±0.257	3.98	100
Thiamethoxam	2.349(1.791-2.9)	6.027(4.985-7.47)	36.122(24.327-64.62)	1.648±0.175	1.749	15.65
Imidacloprid	3.702(2.75-463)	9.5(7.87-11.58)	56.95(39.47-97.08)	1.648±0.172	2.535	9.23
Clothianidin	1.094(0.746-1.435)	3.237(2.614-4.035)	25.446(16.46-49.44)	1.431±0.165	2.491	29.13
Dinotefuran	7.972(6.12-9.695)	17.17(14.67-20.04)	73.78(55.87-110.07)	2.024±0.209	5.708	5.49
Acetamiprid	7.096(5.155-8.926)	17.864(14.88-21.56)	103.229(71.46-179.99)	1.682±0.191	1.949	5.28

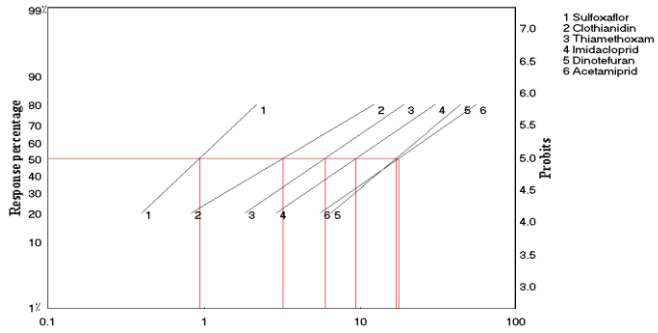


Fig. 3. Toxicity lines of six neonicotinoid insecticides against apterous adult *M. persicae* after 24-hour laboratory exposure

Table 4. Comparative toxicity of neonicotinoid insecticides against apterous adult *M. persicae* after 48-hour laboratory exposure

Treatments	LC_{25} (ppm) ± 95% CL	LC_{50} (ppm) ± 95% CL	LC_{90} (ppm) ± 95% CL	Slop ± SE	Chi^2	Toxicity index
Sulfoxaflor	0.41(0.289-0.522)	0.818(0.668-0.964)	3.035(2.425-4.186)	2.25±0.254	3.1	100
Thiamethoxam	1.811(1.298-2.294)	4.811(3.949-5.92)	31.05(20.97-55.79)	1.582±0.175	1.01	17.00
Imidacloprid	3.125(2.289-3.942)	7.886(6.532-9.51)	45.783(32.63-74.48)	1.678±0.172	2.237	10.37
Clothianidin	0.856(0.571-1.14)	2.41(1.936-2.95)	17.237(11.86-30.08)	1.5±0.168	1.84	33.94
Dinotefuran	6.215(4.59-7.73)	13.528(13.53-15.81)	59.298(59.3-85.96)	1.997±0.207	7.077	6.05
Acetamiprid	4.824(3.167-6.4)	12.831(10.37-15.52)	82.334(57.42-143.42)	1.587±0.19	4.27	6.38

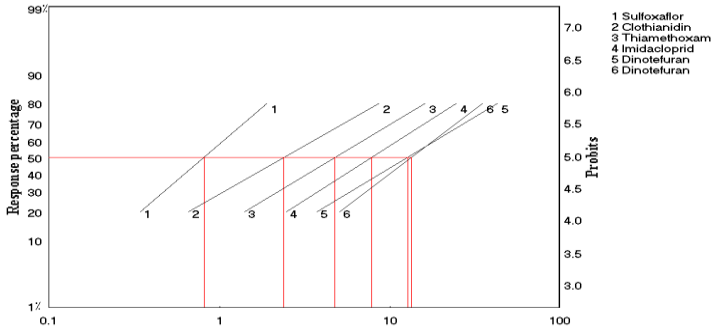


Fig. 4. Toxicity lines of six neonicotinoid insecticides against apterous adult. *M. persicae* after 48-hour laboratory exposure

Interspecific differences in susceptibility between *A. gossypii* and *M. persicae*

As shown in Tables 1–4 and Figs. 3 and 4, the comparative toxicity data of six neonicotinoid insecticides against *A. gossypii* (cotton strain) and *M. persicae* (green peach aphid) revealed clear interspecific differences in susceptibility. Notably, *M. persicae* exhibited higher LC_{50} values for four of the tested insecticides (thiamethoxam, imidacloprid, acetamiprid, and clothianidin) at both 24 and 48 hours post-treatment, indicating a relatively higher tolerance or reduced susceptibility compared to *A. gossypii*. In contrast, *A. gossypii* showed higher LC_{50} values only for sulfoxaflor and dinotefuran, suggesting that these two compounds were more toxic to *M. persicae*. These findings suggest that *M. persicae* populations may have developed greater tolerance to a broader range of neonicotinoid insecticides, while *A. gossypii* may exhibit specific resistance patterns to certain active ingredients. Such host-dependent variations in susceptibility highlight the importance of tailoring insecticide strategies based on pest identity and prior exposure history.

Biochemical responses of *M. persicae* to neonicotinoid insecticides

The activity of acetylcholinesterase (AChE) was significantly influenced by exposure to the tested neonicotinoid insecticides (F = 6.6224, LSD = 1.8847) (Table 5). Most treatments, notably thiamethoxam, clothianidin, and acetamiprid, significantly increased AChE activity compared to the control (13.11, 12.79, and 13.02 vs. 10.32 mOD.min–1.mg–1protein, respectively), suggesting a compensatory overexpression or enzyme induction. This aligns with findings by Abdelmoteleb *et al*., (2023), who reported enhanced AChE activity in aphids exposed to thiamethoxam, possibly as a neurotoxic response. Neonicotinoids are the only insecticides reported to increase AChE activity (Samson-Robert *et al*., 2015). Interestingly, sulfoxaflor led to a significant reduction in AChE activity (8.61), which may indicate a distinct mode of action or a higher inhibitory potency at the neural level. This inhibitory effect supports previous observations that sulfoximines, while structurally related to neonicotinoids, exhibit a different interaction profile with nicotinic acetylcholine receptors (Sparks *et al*., 2013).

As shown in Table 5, the glutathione S-transferase (GST) activity, an important detoxification enzyme, was markedly upregulated in all insecticide-treated groups compared to control (F = 10.9568, LSD = 1.0149). The highest levels were observed in aphids exposed to sulfoxaflor and thiamethoxam (9.63 and 9.57, respectively), both significantly higher than the control (6.33). These results are

consistent with the detoxification mechanisms reported in resistant *M. persicae* strains, where increased GST activity facilitated neutralization of oxidative byproducts from pesticide metabolism. The variation among compounds could reflect different capacities to induce oxidative stress, with Sulfoxaflor possibly eliciting stronger oxidative responses, triggering GST-mediated detoxification pathways.

Carboxylesterase (CES) activity was significantly suppressed by most neonicotinoids (F = 11.7685, LSD = 0.00729), with clothianidin showing the lowest CES activity (0.045) followed by sulfoxaflor and thiamethoxam (0.051 and 0.052, respectively), all significantly different from the control (0.0698). These reductions suggest an inhibition of general esterase pathways, a finding that mirrors those of (Zhao *et al*., 2016), who reported esterase suppression in aphids subjected to sublethal doses of systemic insecticides. Notably, dinotefuran and acetamiprid maintained CES activity closer to the control, suggesting either a reduced inhibitory capacity or possible alternative detoxification routes. These differences underline the heterogeneity of metabolic responses among neonicotinoids.

Unlike the other enzymes, MFO activity did not exhibit significant variation among treatments (F = 1.2533, LSD = 0.11302), although minor differences were observed. The highest MFO activity was associated with *Thiamethoxam* (2.34), and the lowest with clothianidin (2.21), but these differences lacked statistical significance. This might suggest that the CYP450 monooxygenase system was not strongly induced or inhibited within the 24-hour window post-exposure, or that baseline MFO activity in *M. persicae* is relatively stable across different neonicotinoid exposures. Nonetheless, some studies have reported upregulation of P450 genes such as CYP6CY3 in resistant aphid populations exposed to imidacloprid and related compounds (Puinean *et al*., 2010), indicating that longer exposure or higher doses may be needed to observe a robust response in MFO levels.

Table 5. Effect of different neonicotinoid insecticides on the activity of detoxification and neural enzymes in green peach aphids, *M. persicae* after 24 Hours of exposure to LC_{50} values

Treatments	AchE	GST	CES	MFO
Control	10.32±0.71bc	6.33±0.31c	0.0698±0.006a	2.22±0.42b
Sulfoxaflor	8.61±1.01c	9.63±0.65a	0.051±0.0047bc	2.27±0.06ab
Thiamethoxam	13.11±1.47a	9.57±0.47a	0.052±0.0066bc	2.34±0.13a
Imidacloprid	11.63±1.17ab	8.83±0.47ab	0.055±0.0055b	2.26±0.054b
Clothianidin	12.79±1.18a	9.44±a0.27b	0.045±0.0035c	2.21±0.04b
Dinotefuran	11.82±1.96ab	8.53±1.26b	0.065±0.0024a	2.28±0.06ab
Acetamiprid	13.02±1.09a	8.81±0.62ab	0.057±0.0042b	2.27±0.104ab
F values	6.6224	10.9568	11.7685	1.2533
LSD	1.8847	1.0149	0.00729	0.11302

AchE=Acetyl cholinesterase ($mOD.min^{-1}.mg^{-1}$protein); GST=Glutathione-S-transferase ($\mu mol.min^{-1}$ mg^{-1} protein); CES =Carboxylesterase ($mol.min^{-1}.mg^{-1}$protein); MFO= Mixed function oxidase ($mOD.min^{-1}.mg^{-1}$protein)

Effects of neonicotinoid insecticides on enzymatic activity in cotton aphid after 24 hours exposure

The results presented in Table 6 demonstrate that all tested neonicotinoid insecticides significantly influenced the activity of key detoxification and nervous system-related enzymes in the cotton aphid (*Aphis gossypii*) following 24 hours of exposure to their respective LC_{50} values.

AchE activity was significantly altered by the tested insecticides compared to the control (F = 76.255, LSD = 0.7616). Clothianidin and thiamethoxam showed the highest AchE activity (14.4 ± 1.08 and 14.0 ± 0.04, respectively), suggesting an overstimulation or compensatory upregulation of this enzyme in response to neurotoxic stress. In contrast, sulfoxaflor and dinotefuran significantly suppressed AchE activity (9.12 ± 0.24 and 9.01 ± 0.56), possibly indicating a stronger inhibitory interaction with the enzyme. These variations align with previous findings suggesting differential modulation of AchE by neonicotinoids, depending on their molecular affinity to nicotinic acetylcholine receptors (Elbert *et al*., 2008; Simon-Delso *et al*., 2015).

GST activity, which plays a pivotal role in detoxification of xenobiotics, was markedly increased in insects treated with sulfoxaflor (11.79 ± 0.89) and thiamethoxam (10.07 ± 0.49), relative to the control group (7.08 ± 0.64), with significant variation among treatments (F = 41.2762, LSD = 0.78107). This enhanced GST activity likely reflects an induced metabolic response aimed at neutralizing oxidative stress or facilitating conjugation of reactive intermediates. Similar enzymatic induction by neonicotinoids has been reported in aphids and other hemipterans, often correlating with sublethal stress responses (Shi *et al*., 2012; Wu *et al*., 2021).

Carboxylesterase activity was significantly reduced in most insecticide treatments compared to the control (F = 31.6815, LSD = 0.00607). Clothianidin (0.041 ± 0.002) exhibited the most pronounced inhibitory effect, while dinotefuran (0.065 ± 0.002) caused a relatively moderate reduction. Given the role of CES in the hydrolysis of ester-containing insecticides, such reductions could indicate either direct inhibition or depletion of enzymatic pools due to metabolic overuse. These findings support earlier observations by Sparks and Nauen (2015) who noted that CES inhibition can be a marker of neonicotinoid-induced toxicity in susceptible insect species.

MFO activity also varied significantly across treatments (F = 12.3772, LSD = 0.09676). Thiamethoxam (2.46 ± 0.1) and sulfoxaflor (2.40 ± 0.1) led to significantly elevated MFO levels, suggesting the induction of cytochrome P450 enzymes, which are commonly associated with insecticide detoxification and metabolic resistance. In contrast, dinotefuran and acetamiprid exhibited lower MFO activities, more comparable to the control. The elevation of MFO by some compounds highlights their potential to trigger resistance-related metabolic pathways, in agreement with studies on P450 overexpression in neonicotinoid-exposed aphids (Bass *et al*., 2011; Bingham *et al*., 2007).

Table 6. Effect of different neonicotinoid insecticides on the activity of detoxification and neural enzymes in cotton aphid, *A. gossypii* after 24 Hours of exposure to LC_{50} values

Treatments	AchE	GST	CES	MFO
Control	9.93±0.19d	7.08±0.64e	0.075±0.009a	2.14±0.034d
Sulfoxaflor	9.12±0.24e	11.79±0.89a	0.048±0.007c	2.4±0.1ab
Thiamethoxam	14±0.04a	10.07±0.49b	0.049±0.004c	2.46±0.1a
Imidacloprid	11.96±0.53c	8.15±0.32cd	0.051±0.003c	2.32±0.07bc
Clothianidin	14.4±1.08a	10.14±0.35b	0.041±0.002d	2.31±0.05bc
Dinotefuran	9.01±0.56e	7.42±0.34de	0.065±0.003b	2.2±0.03d
Acetamiprid	12.75±0.14b	8.42±0.49c	0.054±0.003c	2.22±0.03cd
F values	76.255	41.2762	31.6815	12.3772
LSD	0.7616	0.78107	0.00607	0.09676

AchE=Acetyl cholinesterase (mOD.min^{-1}.mg^{-1}protein); GST=Glutathione-S-transferase (µmol.min^{-1} mg^{-1} protein); CES =Carboxylesterase (mol.min^{-1}·mg^{-1}protein); MFO= Mixed function oxidase (mOD.min^{-1}.mg^{-1}protein)

The biochemical responses of *Myzus persicae* and *Aphis gossypii* to neonicotinoid insecticides displayed notable similarities and distinct species-specific patterns. In both aphid species, acetylcholinesterase (AChE) activity was significantly elevated by *Clothianidin* and *Thiamethoxam*, suggesting a conserved compensatory mechanism in response to neurotoxic stress. However, *Sulfoxaflor* markedly reduced AChE activity in both species, reinforcing its divergent neurophysiological mode of action despite being grouped with neonicotinoids. This duality implies that while certain compounds elicit generalizable enzyme responses, others like sulfoxaflor induce unique inhibitory effects.

For glutathione S-transferase (GST), both aphid species exhibited a strong induction under *Sulfoxaflor* and *Thiamethoxam* exposure, indicating elevated detoxification efforts possibly linked to oxidative stress. This pattern underscores GST's critical role as a frontline defense enzyme against neonicotinoid-induced toxicity. Interestingly, *Aphis gossypii* showed slightly higher GST induction values than *M. persicae*, suggesting potential interspecific variability in oxidative stress handling or GST gene regulation.

Carboxylesterase (CES) activity was consistently suppressed by most neonicotinoids in both species, particularly under *Clothianidin* and *Sulfoxaflor*, aligning with the hypothesis of esterase inhibition as a marker of neonicotinoid exposure. Notably, *Dinotefuran* maintained relatively higher CES activity in both aphids, suggesting a milder impact on esterase function or a shift toward alternative detoxification mechanisms.

Unlike the other enzymes, mixed-function oxidase (MFO) activity exhibited a more variable profile. While *M. persicae* showed no significant changes in MFO activity, *A. gossypii* responded to *Thiamethoxam* and *Sulfoxaflor* with significant increases, indicating a species-dependent activation of cytochrome P450-mediated detoxification. These differences may reflect disparities in basal P450 expression levels or in the regulatory pathways triggered by neonicotinoids.

Together, these findings highlight both conserved and divergent biochemical strategies employed by the two aphid species in coping with neonicotinoid-induced stress, and may provide insights into their differential susceptibility and resistance development.

CONCLUSION

The study demonstrated significant variations in the toxicity of six neonicotinoid insecticides—representing four chemical generations—against two aphid species: *Aphis gossypii* and *Myzus persicae*. Sulfoxaflor (a fourth-generation sulfoximine) consistently exhibited the highest toxicity across both species and time intervals, highlighting its potential as a powerful tool in aphid management. Second-generation compounds, particularly clothianidin and thiamethoxam, showed enhanced efficacy over first- and third-generation neonicotinoids, likely due to improved receptor affinity and reduced cross-resistance. *M. persicae* displayed higher tolerance to several neonicotinoids compared to *A. gossypii*, suggesting interspecific differences in detoxification capacity and resistance mechanisms. Biochemical assays supported these findings, revealing elevated AChE and GST enzyme activities in response to several treatments, with sulfoxaflor uniquely reducing AChE activity, indicating a distinct mode of action. These results emphasize the importance of selecting insecticides based on pest species and resistance profiles. Moreover, the use of newer-generation compounds like sulfoxaflor, alongside rotation strategies, can help sustain effective aphid control and delay resistance development in integrated pest management (IPM) programs.

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مقارنة السمية، والحساسية بين الأنواع، واستجابات النشاط الإنزيمي لمبيدات النيونيكوتينويد من الجيل الأول إلى الرابع على حشرة من القطن (*Aphis gossypii* Glover) وحشرة من الخوخ الأخضر (*Myzus persicae* Sulzer) تحت الظروف المعملية

بلال سليمان محمد سليمان، محمد مبروك رجب عطية، عدنان عبد الفتاح السيد درويش وعواطف سعد منسي

قسم وقاية النبات، كلية الزراعة، جامعة دمنهور، مصر

الملخص

في هذه الدراسة تم تقييم سمية ستة مبيدات حشرية من النيونيكوتينويد (من أربعة أجيال كيميائية) ضد حشرتي من القطن (*Aphis gossypii* Glover) وحشرة من الخوخ الأخضر (*Myzus persicae* Sulzer) تحت الظروف المعملية بعد ٢٤ و٤٨ ساعة من التعرض. أظهر سلفوكسافلور، الذي يمثل الجيل الرابع، أعلى سمية باستمرار ضد كلا نوعي المن، مع أدنى قيم LC_{50} ومؤشر سمية ١٠٠ . أظهرت مبيدات الحشرات من الجيل الثاني (كلوثيانيدين وثياميثوكسام) فعالية تتراوح بين متوسطة وعالية، بينما أظهرت مركبات الجيل الأول (إيميداكلوبريد وأسيتاميبريد) والجيل الثالث (دينوتيفوران) تأثيرات سمية أقل. لوحظت زيادات في السمية مرتبطة بالوقت لمعظم المبيدات الحشرية. كشف التحليل المقارن عن اختلافات بين الأنواع: أظهرت *M. persicae* قدرة أكبر على تحمل معظم المركبات مقارنةً بـ *A. gossypii*، مما يشير إلى أنماط مقاومة خاصة بالأنواع. أشارت الاختبارات الكيميائية الحيوية إلى أن العديد من مبيدات النيونيكوتينويدات زادت بشكل ملحوظ من نشاط أستيل كولينستراز (AChE) وجلوتاثيون S-ترانسفيراز (GST) في *M. persicae*، مما يدل على وجود آليات تكيفية لإزالة السمية. في المقابل، ثبّط السلفوكسافلور نشاط إنزيم الأسيتيل كولينستريز (AChE) بشكل ملحوظ، مما يُبرز آلية تأثيره العصبي السمية المتميزة. تُؤكد هذه النتائج أهمية الابتكار الكيميائي وإدارة المقاومة في برامج مكافحة المن. برز السلفوكسافلور كأكثر المرشحين واعدًا نظرًا لفعاليته العالية وآليته المبتكرة، مما يجعله مناسبًا للإدارة المتكاملة للآفات (IPM). ومع ذلك، لا يزال استخدام المبيدات الحشرية بالتناوب ومراقبة حساسية الآفات أمرًا ضروريًا لتأخير تطور المقاومة.