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Octopamine receptor agonists synergistically increase the selected pesticides' toxicity in *Rhopalosiphum padi*: Perspectives for reducing pesticide use, emergence of resistant strains and environmental impacts

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ABSTRACT

Worldwide, the bird cherry-oat aphid, *Rhopalosiphum padi*, (*R. padi*) affects wheat, sorghum, and other grain crops, and conventional pesticides to control this aphid negatively affects the surrounding environment. Therefore, knowing the entomotoxicity of different chemical compounds against *R. padi* is an important step to control these pests. Thus, we aimed to evaluate the toxicity of different nicotinic acetylcholine receptor modulators (thiamethoxam, imidacloprid, acetamiprid, and sulfoxaflor) and the octopamine receptor agonists' (ORAs hereon) synergistic effect (chlordimeform and amitraz) on the selected pesticides' toxicity against *R. padi* adults. We found that chlordimeform was more effective than amitraz (LC_{50}: 144.01 and 238.33 μg/mL, respectively), after 24-h of exposure. Sulfoxaflor was the most toxic pesticide (LC_{50} values were 4.61 and 0.44 μg/mL), whereas we identified acetamiprid as the least potent one (LC_{50} values were 111.82 and 88.69 μg/ mL). Thiamethoxam was the most effective neonicotinoid pesticide among those we used. Chlordimeform and amitraz had synergized effects with the surveyed pesticides, with amitraz showing the highest synergistic ratio. These findings indicate that ORAs are promising tools to increase the selected pesticides' effectiveness on *R. padi* control, which may contribute to the decrease in the use of generic pesticides, the emergence of resistant strains, and, consequently, their impacts on the environment

1. Introduction

Wheat, *Triticum aestivum* L., is considered one of the most remarkable and secure cereals worldwide (Duan et al. 2017; Peng et al., 2020). However, the bird cherry-oat aphid, *R. padi*, remains one of the most destructive wheat pests, causing direct and indirect damages by feeding and serving as a vector of barley yellow dwarf virus (Li et al., 2018). The outbreaks of *R. padi* cause severe yield losses in wheat (Sallam et al., 2009; Cui et al., 2010; Lu et al., 2016; Li et al., 2018). Furthermore, the insect causes the most damage by transmitting several viruses, especially Barley yellow dwarf virus and Sugarcane mosaic virus, for which it is the most important vector (Snihur et al., 2021). Thus, pesticides are practical tools to control *R. padi* with some of them (e.g., pyrethroids, carbamates, and organophosphates) being extensively used to control it (Wang et al., 2018; Mohammed et al., 2019). Because of their exhaustive and unsupervised application, pesticides' environmental impacts continue to be raised. Thus, new strategies to address the effects of pesticides besides the need for their use have been underscored (Sallam et al., 2009; Gill & Garg, 2014).

In this context, the class of neonicotinoids' mode of action as agonists to the nicotinic acetylcholine receptors (nAChRs) (Ahmed and Matsumura 2012; Deng et al., 2019) allow them to be significantly more utilized in crop protection along with pyrethroids, making them safe pesticides for plant protection compared on previous conventional pesticide groups (Mohammed et al., 2019). In addition, the novel-class of sulfoximine pesticides, such as sulfoxaflor, disturbs the nAChRs in in-

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sects' central nervous system (Sparks et al., 2013; Ahmed and Vogel 2016a; Ma et al., 2019), making them efficient against specific pests. A detailed understanding of sulfoxaflor's effects on *R. padi*'s is an essential approach. However, these recent pesticides' unsupervised application may reduce their effectiveness and allow pest resistance. As discussed by Zuo et al. (2016), insecticide resistance, in addition to being a major cause of pest control failure, can instigate serious environmental concerns and human health effects. Therefore, this reinforces the need to seek new strategies in the control of agricultural pests, such as the bird cherry-oat aphid (*R. padi*).

One way to avoid the emergence of resistant strains is to invest in the development of studies that seek to increase the efficiency of insecticides against *R. padi*, associating, for example, its uses with synergistic compounds. For many years, synergistic compounds have been used, enhancing the major pesticides' effectiveness, particularly when pesticide resistance emerges (Bao et al., 2016; Wang et al., 2020). For the control of the tobacco budworm *Heliothis virescens* (F.) in cotton plants (Plapp, 1979; Campanhola and Plapp 1987), octopamine receptor agonists (ORAs hereon), such as formamidine, act synergistically with several pesticides. We have recently demonstrated that ORAs are important alternative strategies as synergistic compounds in preventing and controlling *Culex quinquefasciatus* mosquitoes (Ahmed and Vogel 2020). At the time, we showed that the combined use of ORA with pyriproxyfen was the most significant effect. Promising results have also been observed in studies involving Dengue vector *Aedes aegypti* (Ahmed and Vogel. 2015; Ahmed and Vogel, 2016b), *Plutella xylostella* (Deng et al., 2021), and *Drosophila melanogaster* (Ahmed and Vogel, 2020)

So, the ORAs' synergistic action with certain pesticides is a potent control tool on some pests (Liu and Plapp 1990; Prullage et al., 2011; Rodriguez-Vivas et al., 2013; Ahmed and Vogel 2016b; Ahmed and Vogel, 2020). The ORAs, like formamidines, disrupt monoamine-mediated production of cyclic adenosine monophosphate (cAMP) and induce adverse behavioral changes in treated insects, the most critical feature of their insecticidal role (Ahmed et al., 2015; Kita et al, 2017; White et al., 2021). Such features make them essential pest control tools for mosquitoes, locusts, cockroaches, and mites (Ahmed et al., 2015; Monteiro et al., 2019).

Given this scenario, we aim to compare the toxicity of selected neonicotinoids (thiamethoxam, imidacloprid, acetamiprid, and sulfoxaflor) and to evaluate the ORAs' (chlordimeform and amitraz) synergisms with these neonicotinoids' pesticide activity against *R. padi* adults. We hypothesize that chlordimeform (formamidine acaricide and a member of monochlorobenzenes), as well as amitraz (a non-systemic acaricide and insecticide), have a synergistic effect with the surveyed pesticides. In addition, we assume that ORAs can be promising tools to increase the effectiveness of the selected pesticides on *R. padi* control. Therefore, this study is the potential to straighten out the new promising tools to control *R. padi*, reducing pesticide resistance prospective.

2. Material and methods

2.1. *Wheat plants,* R. padi *insects and chemicals*

To conduct acute toxicity tests, we used *T. aestivum* (Sids-12 variety) as a plant species that is a food source for insects, similarly to the study by Peruzzo et al. (2007) and Girvin et al. (2017). It was planted in the farming of Plant Protection Department, Faculty of Agriculture, Assiut University, Egypt. The wheat plants used in this study were 3 weeks old. Wheat plants we used were three weeks old and insects were used from creation in the laboratory from individuals collected in the field. Field wingless strain of *R. padi* was used in all assays. The aphids were reared on water-cultured wheat under controlled environmental conditions (25 ± 1°C and 65 ± 5% RH). As for chemical compounds, sulfoxaflor was purchased from Chem Services (West Chester, PA, USA). thiamethoxam, acetamiprid, and imidacloprid were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) (Table 1).

2.2. *Acute entomotoxicity assay*

We assessed the acute toxicity assay of selected pesticides against *R. padi* adults using the leaf-dip method (Li et al., 2018) (with minor modifications), with the pesticide stock solutions (500, 50, 5, 0.5, and 0.05 μg/mL) being prepared in acetone (P.A.). Briefly, we cut and dipped the leaves into pesticide solutions for 10 seconds and sited in the shaded area to air dry for 2-h. We then placed the leaves with their abaxial surface in a downward position in a petri dish (9.0 cm in diameter). In general, each treatment comprised three replicates of 20 adult aphids. We dipped leaves in acetone to be used as the control. We placed the Petri dishes in climatic chambers at 25°C, 60 ± 5% RH, and at 16: 8 L:D photoperiod. We determined aphid mortalities after 24 and 48-h of exposure to each pesticide, under a stereomicroscope. We considered each aphid adult dead if it did not move when touched with dissecting forceps. We repeated all assays twice.

2.3. *Synergistic action assay*

The synergistic action assay was performed as described above for acute toxicity assay. Each series of synergistic action assays was conducted by evaluating the lethal actions of varying concentrations of each pesticide either alone or co-treated with 10 μg/mL of chlordimeform and 20 μg/mL of amitraz. Importantly, these sublethal concentrations were the maximum sublethal concentration where no mortality was observed by the synergist against *R. padi* adults in preliminary assays. In all experiments, controls received only acetone. All assays were repeated twice. Percentage mortality was recorded after 24 and 48-h of exposure.

2.4. *Statistical analysis*

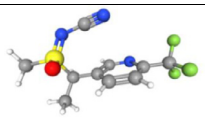
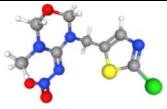
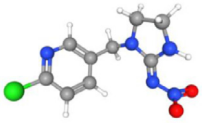
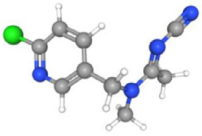
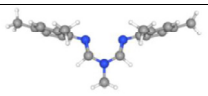
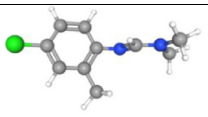
We calculated the corrected mortality based on Abbott's formula (Abbott 1925). We pooled the acute toxicity data (LC_{50}, 95% CL values, slope, X^2, and g values) and analyzed them using IBM SPSS Statistics Desktop for Windows, version 25 (SPSS Inc., Chicago, IL). We determined the statistical differences between LC_{50} estimates using a 95% CI for the ratio of two estimates (Robertson and Preisler, 1992). If the 95% CI for the ratio included 1, then the LC_{50} estimates were not significantly different. We acquired the potency ratio by LC_{50} value of amitraz divided by LC_{50} value of chlordimeform. The toxicity index was determined by the LC_{50} value of the most toxic pesticide by dividing the LC_{50} value of the tested pesticide and multiplying it by 100 for each time-dependent. Furthermore, we calculated the synergistic ratio (SR) by dividing the LC_{50} value of the tested pesticide by the LC_{50} value obtained by the combined "pesticide + synergist". We designed the figures using GraphPad Prism software, version 6.01 (San Diego, CA, USA).

3. Results

The toxicity of ORAs on *R. padi* adults, after 24-h of exposure, is shown in Table 2. Chlordimeform was more potent than amitraz with LC_{50} values of 144.01 and 238.33 μg/mL, respectively. The amitraz potency ratio (PR) was 1.7-fold that of chlordimeform. We show the potency of selected neonicotinoids and sulfoxaflor in Table 3. After 24-h and 48-h of exposure, the most toxic pesticide was sulfoxaflor (LC_{50} values were 4.61 and 0.44 μg/mL, respectively). However, the least potent pesticide was acetamiprid [LC_{50} values were 111.82 μg/mL (after 24-h of exposure) and 88.69 μg/mL (after 48-h)]. The PR increased after 48-h of exposure, and its maximum was 10.5-fold for sulfoxaflor, and the lowest was 1.3-fold for acetamiprid. Synergistic action of chlordimeform and amitraz on the acute toxicity (LC_{50}) of selected neonicotinoids pesticides after 24 and 48-h exposure is presented in Tables 4 and 5, respectively.

The analysis of toxicity for both ORAs (chlordimeform and amitraz) showed that they acted synergistically with the selected pesticides

Table 1
Selected pesticides used in this study[1].

Name	Group	IUPAC name	Molecular formula	3D Conformer	Purity (%)	Molecular weight (g/mol)
Sulfoxaflor	Sulfoximine	[Methyl(oxo){1-[6-(trifluoromethyl)-3-pyridyl]ethyl}-λ⁶-sulfanylidene]cyanamide	$C_{10}H_{10}F_3N_3OS$		99.4	277.27
Thiamethoxam	Neonicotinoid	(NE)-N-[3-[(2-chloro-1,3-thiazol-5-yl)methyl]-5-methyl-1,3,5-oxadiazinan-4-ylidene]nitramide	$C_8H_{10}ClN_5O_3S$		99.5	291.72
Imidacloprid	Neonicotinoid	(NE)-N-[1-[(6-chloropyridin-3-yl)methyl]imidazolidin-2-ylidene]nitramide	$C_9H_{10}ClN_5O_2$		99.5	255.66
Acetamiprid	Neonicotinoid	N-[(6-chloropyridin-3-yl)methyl]-N'-cyano-N-methylethanimidamide	$C_{10}H_{11}ClN_4$		99.5	222.67
Amitraz	Formamidine	N'-(2,4-dimethylphenyl)-N-[(2,4-dimethylphenyl)iminomethyl]-N-methylmethanimidamide	$C_{19}H_{23}N_3$		96.8	293.40
Chlordimeform	Formamidine	N'-(4-chloro-2-methylphenyl)-N,N-dimethylmethanimidamide	$C_{10}H_{13}ClN_2$		99.8	196.67

IUPAC: International Union of Pure and Applied Chemistry.

[1] The information presented was obtained from the compound manufacturers and the PubChem database (https://pubchem.ncbi.nlm.nih.gov/).

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Table 2
Toxicity of octopamine receptor agonists on *R. padi* adults after 24-h exposure.

Compounds	*n*[a]	After 24-h				PR[f]
		LC_{50}[b] (95% CL)[c]	Slope (± SEM)	X^2 (*df*)[d]	g value[e]	
Chlordimeform	360	**144.01** (40.19-164.07)	4.5 (± 0.1)	0.2 (3)	0.02	1.0
Amitraz	360	**238.33** (57.22-452.72)	5.7 (± 0.1)	0.3 (3)	0.03	1.7

[a] *n* = number of adults tested, including control.
[b] Concentration is expressed in μg/mL and the response determined after 24-h exposure
[c] If the 95% CI of the ratio includes a value of one, then the differences between the two LC_{50} values are insignificantly different.
[d] *df* =Degree of freedom.
[e] If the g value < 0.5, the data fit the probit model. Otherwise, the data do not fit the probit model and the analysis is invalid.
[f] Potency ratio= LC_{50} value of amitraz divided by LC_{50} value of chlordimeform after 24-h exposure.

(Fig. 1). Chlordimeform significantly synergized with the selected pesticides. We observed the highest SR when mixing chlordimeform with acetamiprid and the lowest by mixing chlordimeform with sulfoxaflor. We observed the maximum SR after 48-h of exposure (Fig. 1A). Amitraz synergized with the selected pesticides after 24-h and 48-h of exposure (Fig. 1B). We observed the highest SR when mixing amitraz with acetamiprid and the lowest by mixing amitraz with sulfoxaflor. Amitraz was more potent as a synergistic tool than chlordimeform on the selected pesticides' toxicity on *R. padi* adults after 24-h and 48-h of exposure. Furthermore, our toxicity index showed that sulfoxaflor was the most toxic pesticide among all users, whereas thiamethoxam was the most potent one among the neonicotinoids (Fig. 2). We observed this trend when we considered the pesticides alone, the pesticides mixed with chlordimeform, and the pesticides mixed with amitraz (Fig. 2).

Table 3
The difference in the acute toxicity (LC_{50}) of selected nicotinic acetylcholine receptor modulators on *R. padi* adults after 24 and 48-h exposure.

Compounds	n[a]	After 24-h				After 48-h				PR[f]
		LC_{50}[b] (95% CL)[c]	Slope (± SEM)	X^2 (*df*)[d]	g value[e]	LC_{50}[b] (95% CL)[c]	Slope (± SEM)	X^2 (*df*)[d]	g value[e]	
Sulfoxaflor	360	**4.61** (1.61-12.39)	5.6 (± 0.1)	2.6 (3)	0.04	**0.44** (0.059-1.44)	4.6 (± 0.1)	3.4 (3)	0.03	10.5
Thiamethoxam	360	**16.62** (6.04-49.48)	5.7 (± 0.1)	0.3 (3)	0.03	**2.36** (0.39-8.64)	4.5 (± 0.1)	1.9 (3)	0.02	7.0
Imidacloprid	360	**97.23** (38.32-321.41)	5.4 (± 0.1)	1.0 (3)	0.04	**14.33** (3.41-66.65)	4.4 (± 0.1)	0.6 (3)	0.02	6.8
Acetamiprid	360	**111.82** (36.75-567.86)	5.0 (± 0.1)	2.2 (3)	0.03	**88.69** (20.24-93.09)	4.0 (± 0.1)	0.8 (3)	0.01	1.3

[a] *n* = number of adults tested, including control
[b] Concentration is expressed in μg/ml and the response determined after 24 and 48-h exposure
[c] If the 95% CI of the ratio includes a value of one, then the differences between the two LC_{50} values are insignificantly different.
[d] *df* =Degree of freedom.
[e] If the g value < 0.5, the data fit the probit model. Otherwise, the data do not fit the probit model and the analysis is invalid.
[f] Potency ratio= LC_{50} value of each pesticide after 24-h exposure divided by LC_{50} value of the same pesticide after 48-h exposure.

Table 4
Synergistic action of chlordimeform on the toxicity of selected nicotinic acetylcholine receptor modulators on *R. padi* adults after 24 and 48-h exposure.

Compounds+chlordimeform[a]	n[b]	After 24-h				After 48-h			
		LC_{50}[c] (95% CL)[d]	Slope (± SE)	X^2 (*df*)	g value[e]	LC_{50}[c] (95% CL)[d]	Slope (± SE)	X^2 (*df*)	g value[e]
Sulfoxaflor	360	**2.26** (0.57-6.81)	5.0 (± 0.1)	2.8 (3)	0.04	**0.15** (0.010-0.54)	3.9 (± 0.2)	2.6 (3)	0.05
Thiamethoxam	360	**5.43** 1.86-14.84)	5.7 (± 0.2)	0.2 (3)	0.02	**0.55** (0.12-1.48)	4.8 (± 0.2)	0.4 (3)	0.01
Imidacloprid	360	**25.57** (8.71-88.41)	5.3 (± 0.1)	0.8 (3)	0.01	**2.89** (0.68-9.25)	4.9 (± 0.1)	3.7 (3)	0.02
Acetamiprid	360	**20.55** (5.77-91.45)	4.7 (± 0.1)	3.2 (3)	0.03	**10.11** (2.61-39.91)	4.7 (± 0.1)	1.4 (3)	0.03

[a] Concentration of chlordimeform was 10 μg/mL and adults were exposed to insecticide and synergist simultaneously.
[b] *n* = number of adults tested, including control
[c] Concentration is expressed in μg/mL and the response determined after 24 and 48-h exposure
[d] If the 95% CI of the ratio includes a value of one, then the differences between the two LC_{50} values are insignificantly different.
[e] If g value is < 0.5, the data fit the probit model. Otherwise, the data do not fit the probit model and the analysis is invalid.

Table 5
Synergistic action of amitraz on the toxicity of selected nicotinic acetylcholine receptor modulators on *R. padi* adults after 24 and 48-h exposure.

Compounds+amitraz[a]	n[b]	After 24-h				After 48-h			
		LC_{50}[c] (95% CL)[d]	Slope (± SE)	X^2 (*df*)	g value[e]	LC_{50}[c] (95% CL)[d]	Slope (± SE)	X^2 (*df*)	g value[e]
Sulfoxaflor	360	**1.00** (0.25-2.78)	5.1 (± 0.1)	0.8 (3)	0.05	**0.053** (0.001-0.19)	3.2 (± 0.3)	0.8 (3)	0.02
Thiamethoxam	360	**2.70** 1.00-6.43)	5.8 (± 0.2)	1.4 (3)	0.08	**0.19** (0.17-0.65)	4.2 (± 0.2)	0.3 (3)	0.03
Imidacloprid	360	**9.00** (2.83-28.24)	5.3 (± 0.1)	0.4 (3)	0.03	**0.98** (0.28-2.52)	5.2 (± 0.1)	0.7 (3)	0.07
Acetamiprid	360	**9.70** (0.17-48.06)	5.3 (± 0.1)	5.7 (3)	0.01	**4.74** (1.58-13.03)	5.5 (± 0.1)	2.9 (3)	0.03

[a] Concentration of amitraz was 20 μg/mL and adults were exposed to insecticide and synergist simultaneously.
[b] *n* = number of adults tested, including control
[c] Concentration is expressed in μg/mL and the response determined after 24 and 48-h exposure
[d] If the 95% CI of the ratio includes a value of one, then the differences between the two LC_{50} values are insignificantly different.
[e] If g value is < 0.5, the data fit the probit model. Otherwise, the data do not fit the probit model and the analysis is invalid.

4. Discussion

Neonicotinoid pesticides have been widely applied to control piercing-sucking insect pests in various crops. This study tested the comparative effectiveness of selected neonicotinoid and sulfoximine groups on *R. padi* adults. We found that all the tested pesticides were potent upon *R. padi* adults. In agreement with our findings, Mohammed et al., (2019) found the imidacloprid was effective on *R. padi* adults by using the leaf dip method and the LC_{50} value was 15.46 ppm after 24-h post-treatment. Daniels et al. (2009) revealed that body water contents of famished *R. padi* fed on thiamethoxam-exposed wheat were significantly lower compared to aphids feeding on distilled-water treated wheat (74.5 ± 0.23 and 75.6 ± 0.18%, respectively), revealing thiamethoxam's adverse effects on the aphids' fitness. Furthermore, the reproductive maturity of aphids that emerged on thiamethoxam-treated wheat was significantly smaller. The exposed *R. padi* females' longevity and fecundity to different sublethal doses (L_{10}, L_{20}, and L_{30}) of dinotefuran decreased significantly, with their behavioral patterns also changing abnormally

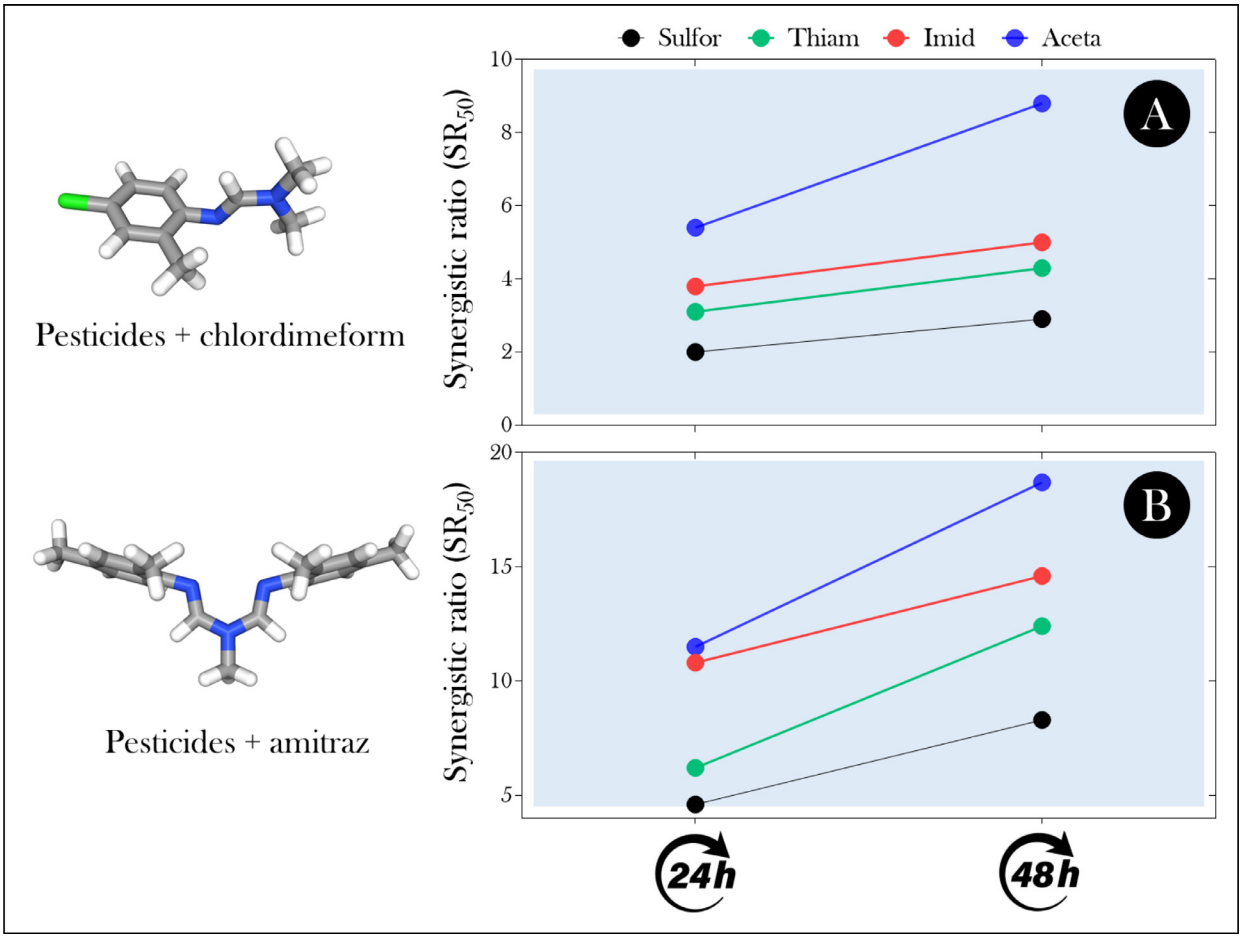

Fig. 1. Time-dependent changes in the synergistic ratio (SR_{50}) as calculated from LC_{50} values from Tables 3–5 for combined treatments with (A) chlordimeform, and (B) amitraz on *R. padi* adults as assessed 24 and 48-h after their initial exposure. Thiam: thiamethoxam, Imid: imidacloprid, Aceta: acetamiprid, Sulf: sulfoxaflor, Chlor: chlordimeform, Ami: amitraz.

(Deng et al., 2019). Moreover, Li et al., (2018) demonstrated imidacloprid's sublethal concentrations of LC_{10}, LC_{20}, and LC_{25} values on *R. padi* adults, which had a significantly lower pre-adult survival rate. Nonetheless, the pesticide prolonged the development duration, pre-oviposition period, and first instar nymphs' adult longevity, and the LC_{20} concentration also extended the adult oviposition period. The IPP-10, a novel neonicotinoid pesticide recently developed in China, has a detrimental effect on the total time and bout duration of xylem and phloem ingestion, but it significantly delays the total time and bout duration of phloem salivation (Cui et al, 2010). Thus, there are clear indications that effective integrated resistance management (IRM) plans need to combine pesticides with different modes of action (Bernard and Philoge'ne 1993) and is synergistic increases the selected pesticides' toxicity. No one showed ORAs' effectiveness as synergistic tools on *R. padi*'s control, though it was already observed for other pests.

The ORAs are potent tools as synergistic action when they are mixed with certain pesticides on some pests. Shojaei et al., (2018) observed the synergistic effects of the amitraz and imidacloprid mixing at all lethal concentrations (LC_{10}-LC_{90}), especially upon one-day-old apterous adults of *Aphis gossypii*. Those researchers found that the highest SR in the mixture of amitraz with malathion (LC_{90}) and imidacloprid (LC_{10}) was 1.5 and 3.09-fold, respectively, based on tested compounds. Ahmed and Matsumura (2012) showed that the amitraz's synergistic effects on the two major pesticide classes (pyrethroids and neonicotinoids) were more significant than that of chlordimeform on *Aedes aegypti* mosquitoes. After 24-h post-treatment, the synergism of formamidines was the maximum on imidacloprid. Ahmed and Vogel (2015) revealed that amitraz and chlordimeform were highly synergistic with nitenpyram, a neurotoxin neonicotinoid pesticide with SRs of 8.6 and 4.8-fold, respectively, on the fourth instar larvae of *Aedes aegypti* after 24-h post-treatment. Liu and Plapp (1990) found that cypermethrin's synergism occurred in house flies treated simultaneously to cypermethrin plus formamidines and exposed to cypermethrin even if exposed before or after to a formamidine.

However, the SR was up to 11.8-fold and was more significant in susceptible than in resistant individuals. Further, they stated that amitraz's monomeric derivative form (BTS 27271) was the most active synergist, followed by chlordimeform and amitraz. Prullage et al., (2011) found that SRs between fipronil alone and fipronil+amitraz were, respectively, > 7.3, 137, and 97 at 6, 24, and 48-h of exposure on the adult ticks of *Rhipicephalus sanguineus*. Also experimenting with this species in *vivo* experiments, and Rodriguez-Vivas et al., (2013) emphasized that mixtures of cypermethrin+piperonyl butoxide (PBO) (80.6-97.3%), and cypermethrin+amitraz (87.0-89.7%) were more effective than cypermethrin alone (76.3-80.5%). In their experiment, the mixture of cypermethrin+amitraz+PBO had the highest efficacy, with more than 95% of the control specimens persisting for 28 days after exposure. However, ORAs' synergistic effect neonicotinoid pesticides are likely to inactivate the detoxification enzymes, enhancing the penetration or uptake, and/or depression of the nervous system.

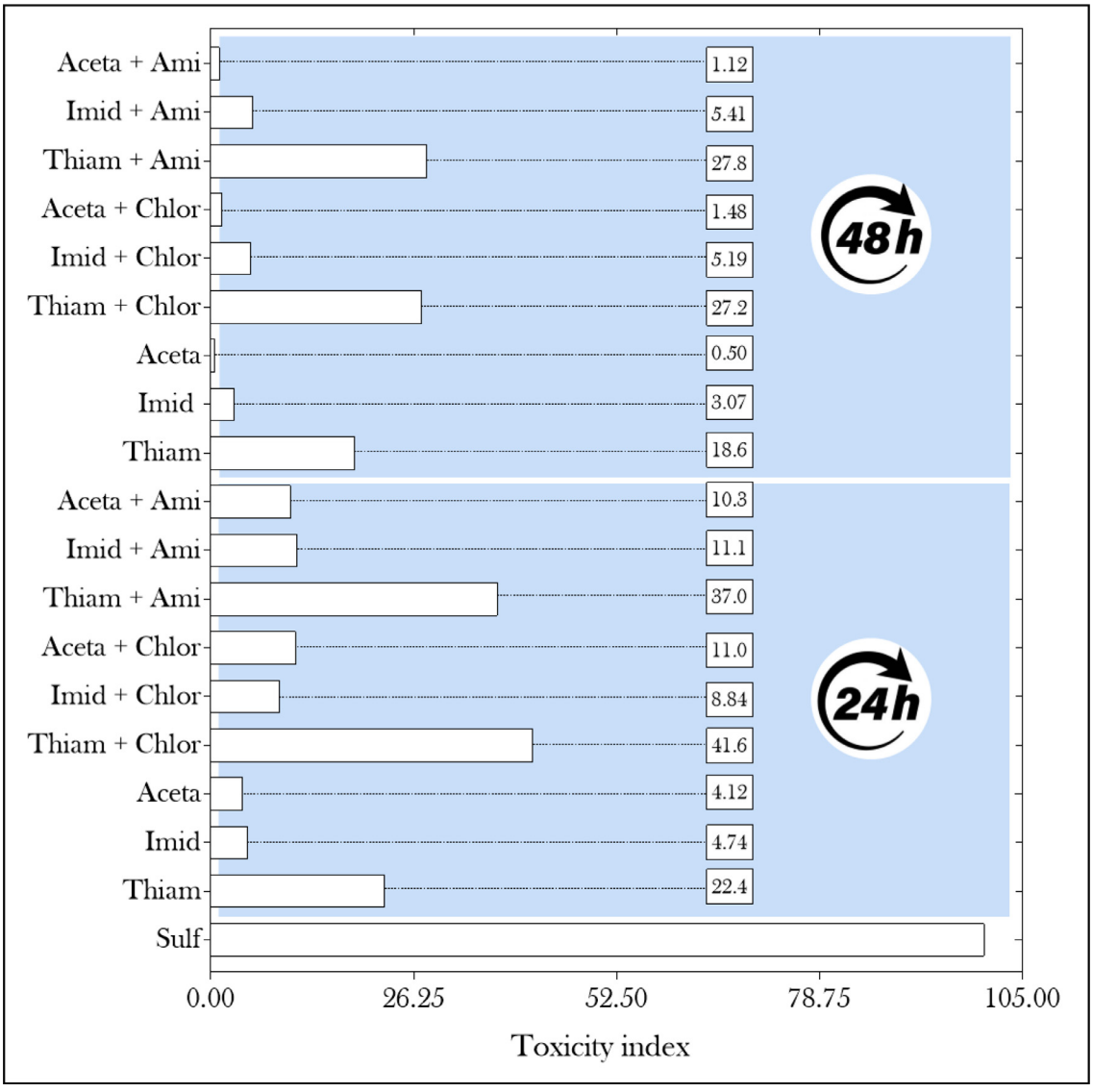

Fig. 2. Toxicity index of pesticides alone, pesticides + chlordimeform, and pesticides + amitraz on *R. padi* adults after 24 and 48-h of exposure. Toxicity index = [(LC_{50} of the most toxic tested pesticide/LC_{50} of the tested pesticide) × 100]. Thiam: thiamethoxam, Imid: imidacloprid, Aceta: acetamiprid, Sulf: sulfoxaflor, Chlor: chlordimeform, Ami: amitraz.

5. Conclusion

In conclusion, our study confirms the hypothesis that chlordimeform and amitraz had synergized effects with the surveyed pesticides, with amitraz showing the highest synergistic ratio. These findings indicate that ORAs are promising tools to increase the selected pesticides' effectiveness on *R. padi*'s control. In the perspective of ORAs' effectiveness, it is worth noting that they reduce the number of pesticides required to control *R. padi* adults. Thus, we encourage future research involving physiological, biochemical, and molecular biology to broaden our knowledge about the mechanisms of action of the evaluated pesticides, especially those that explain the greater entomotoxic efficiency of ORA's.

Ethics statement

No specific permits were required for the described experiments, as none of the experiments involved any endangered or protected vertebrates. The *R. padi* used in this study was originally collected from the Plant Protection Department's farms in the Faculty of Agriculture, Assiut University, Egypt.

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Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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