



Synergistic Effects of Octopamine Receptor Agonists on the selected Pesticides against *Rhopalosiphum padi* Pest on Wheat

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Introduction

- Wheat, *Triticum aestivum* L., is considered one of the most remarkable and secure cereals worldwide.
- 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.
- The outbreaks of *R. padi* cause severe yield losses in wheat.
- The pest causes the most damage by transmitting several viruses, especially Barley yellow dwarf virus and Sugarcane mosaic virus.





Introduction

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- The pest causes the most damage by transmitting several viruses, especially Barley yellow dwarf virus and Sugarcane mosaic virus.





Is there any impact of Octopamine Receptor Agonists on certain selected Pesticides against Rhopalosiphum padi Pest?.



Aims of the study

- To compare the toxicity of selected neonicotinoids (Thiamethoxam, imidacloprid, acetamiprid, and sulfoxaflor).
- To evaluate the ORAs (Chlordimeform and amitraz) synergisms with these neonicotinoids pesticide activity against *R. padi* adults.



Materials and Methods

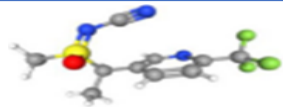
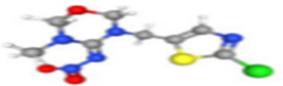
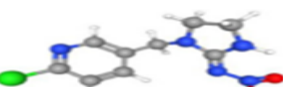
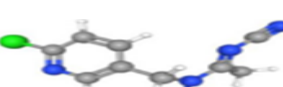

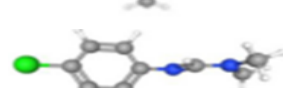
Wheat plants, *R. padi* insects:

- To conduct acute toxicity tests, we used *T. aestivum* (Sids-12 variety) as a plant species that is a food source for insects. 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 ($25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH).



Materials and Methods

Table 1. Selected pesticides used in this study¹.

Name	Group	IUPAC name	Molecular formula	3D Conformer	Purity (%)	Molecular weight (g/mol)
Sulfoxaflor	Sulfoximine	[Methyl(oxo)[1-[6-(trifluoromethyl)-3-pyridyl]ethyl]-λ ² -sulfanylidene]cyanamide	C ₁₀ H ₉ F ₃ N ₂ OS		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 ₁₁ H ₁₂ ClN ₄ OS		99.5	291.72
Imidacloprid	Neonicotinoid	(NE)-N-[1-[(6-chloropyridin-3-yl)methyl]imidazolidin-2-ylidene]nitramide	C ₁₁ H ₁₀ ClN ₄ O ₂		99.5	255.66
Acetamiprid	Neonicotinoid	N-[(6-chloropyridin-3-yl)methyl]-N'-cyano-N-methylethanimidamide	C ₁₀ H ₁₀ ClN ₄		99.5	222.67
Amitraz	Formamidine	N'-(2,4-dimethylphenyl)-N-(2,4-dimethylphenyl)diminomethyl]-N-methylmethanimidamide	C ₁₇ H ₂₀ N ₃		96.8	293.40
Chlordimeform	Formamidine	N'-(4-chloro-2-methylphenyl)-N,N-dimethylmethanimidamide	C ₁₀ H ₁₀ 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>)



Materials and Methods

Acute entomotoxicity assay:

- We assessed the acute toxicity assay of selected pesticides against *R. padi* adults using the leaf-dip method with the pesticide stock solutions (500, 50, 5, 0.5, and 0.05 $\mu\text{g}/\text{mL}$) being prepared in acetone.
- 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 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.



Materials and Methods

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 $\mu\text{g}/\text{mL}$ of chlordimeform and 20 $\mu\text{g}/\text{mL}$ 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.



Materials and Methods

Statistical analysis:

- We calculated the corrected mortality based on Abbott's formula (Abbott 1925).
- We pooled the acute toxicity data (LC50, 95% CL values, slope, X2, and g values) and analyzed them using IBM SPSS Statistics Desktop for Windows, version 25.
- We determined the statistical differences between LC50 estimates using a 95% CI for the ratio of two estimates
- The toxicity index was determined by the LC50 value of the most toxic pesticide by dividing the LC50 value of the tested pesticide and multiplying it by 100 for each time-dependent.
- Furthermore, we calculated the synergistic ratio (SR) by dividing the LC50 value of the tested pesticide by the LC50 value obtained by the combined “pesticide + synergist”.
- We designed the figures using GraphPad Prism software, version 6.01



Results

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)	χ 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



Results

Table 3.

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

Compounds	n^a	After 24-h				After 48-h				PR ^f
		$LC_{50}^{b(95\% CL)^c}$	Slope(\pm SEM)	$\chi^2(df)^d$	g value ^e	$LC_{50}^{b(95\% CL)^c}$	Slope(\pm SEM)	$\chi^2(df)^d$	g value ^e	
Sulfoxaflor	360	4.61 (1.61-12.39)	5.6 (\pm 0.1)	2.6 (3)	0.04	0.44 (0.059-1.44)	4.6 (\pm 0.1)	3.4 (3)	0.03	10.5
Thiamethoxam	360	16.62 (6.04-49.48)	5.7 (\pm 0.1)	0.3 (3)	0.03	2.36 (0.039-8.64)	4.5 (\pm 0.1)	1.9 (3)	0.02	7.0
Imidacloprid	360	97.23 (38.32-321.41)	5.4 (\pm 0.1)	1.0 (3)	0.04	14.33 (3.41-66.65)	4.4 (\pm 0.1)	0.6 (3)	0.02	6.8
Acetamiprid	360	111.82 (36.75-567.86)	5.0 (\pm 0.1)	2.2 (3)	0.03	88.69 (20.24-93.09)	4.0 (\pm 0.1)	0.8 (3)	0.01	1.3



Results

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



Results

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

Results

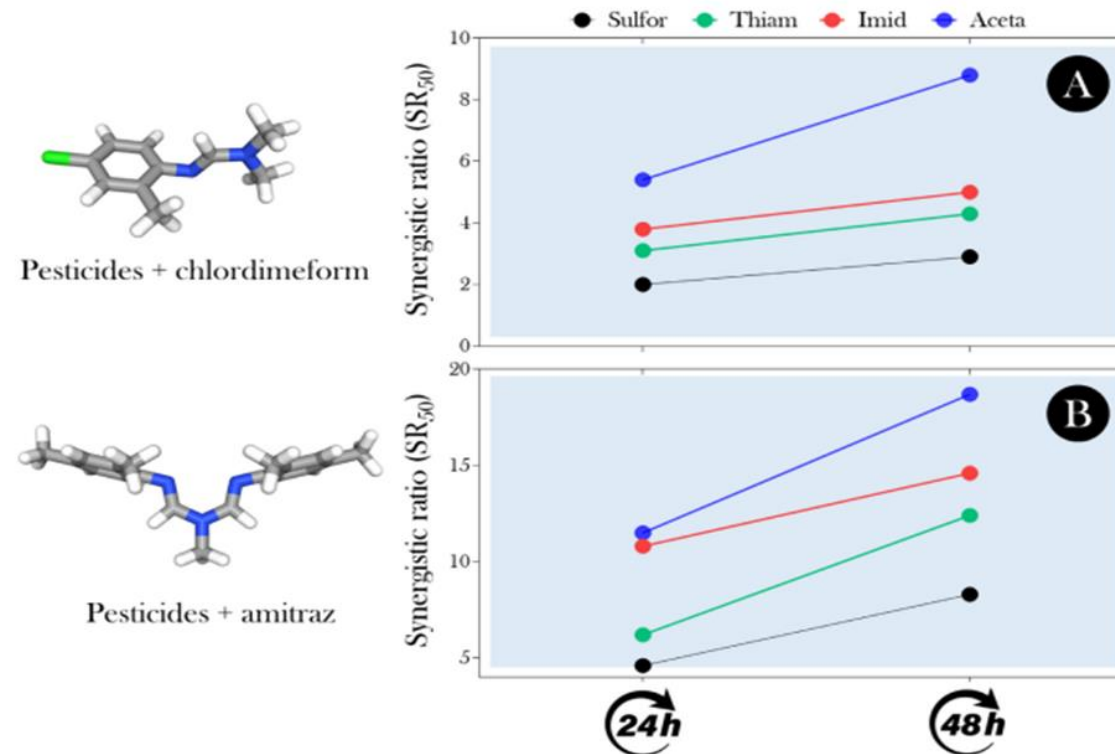


Fig.1. Time-dependent changes in the synergistic ratio (SR₅₀) as calculated from LC₅₀ values from **Tables 3-5** for combined treatments with (A) chlordimeform, and (B) amitraz on *R.padi* adults as assessed 24 and 48-h after their initial exposure. Thiam: thiamethoxam, Imid: imidacloprid, Aceta: acetamiprid, Sulf: sulfoxaflor, Chlor: chlordimeform, Ami: amitraz.

Results

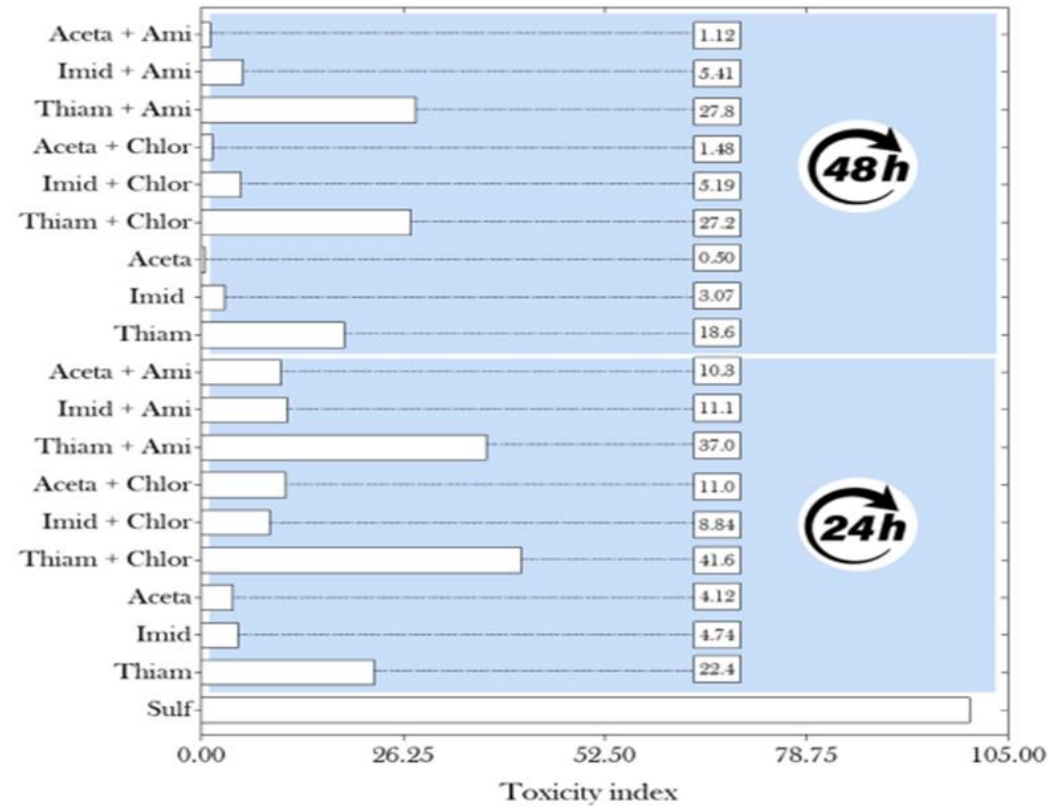


Fig.2. Toxicity index of pesticides alone, pesticides + chlordimeform, and pesticides + amitraz on *R.padi* adults after 24 and 48-h of exposure. Toxicity index = $[(LC_{50} \text{ of the most toxic tested pesticide} / LC_{50} \text{ of the tested pesticide}) \times]$. Thiam: thiamethoxam, Imid: imidacloprid,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



Summary

- Thiamethoxam, imidacloprid, acetamiprid and sulfoxaflor were toxic pesticides.
- Thiamethoxam was the most effective neonicotinoid pesticide.
- Chlordimeform and amitraz had synergized effects with the surveyed pesticides.
- Amitraz showing the highest synergistic ratio.

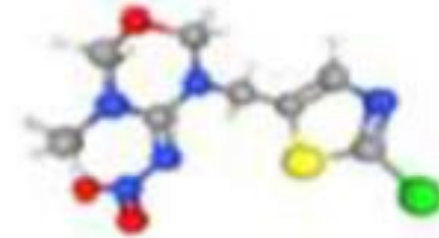
Conclusion



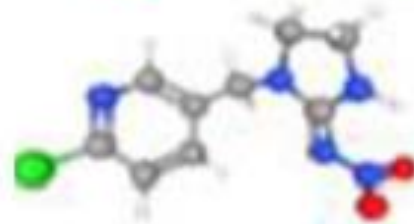
AMITRAZ

AND

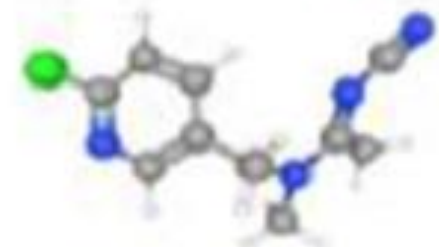
CHLORODIMEFORM



Thiamethoxam



Imidacloprid



Acetamiprid



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 (chlorfomeform and amitraz) on the selected pesticides' toxicity against *R. padi* adults. We found that chlorfomeform was more effective than amitraz (LC_{50} : 144.01 and 238.33 $\mu\text{g}/\text{mL}$, respectively), after 24-h of exposure. Sulfoxaflor was the most toxic pesticide (LC_{50} values were 4.61 and 0.44 $\mu\text{g}/\text{mL}$), whereas we identified acetamiprid as the least potent one (LC_{50} values were 111.82 and 88.69 $\mu\text{g}/\text{mL}$). Thiamethoxam was the most effective neonicotinoid pesticide among those we used. Chlorfomeform 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.

Acknowledgements





Thank you for your attention