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(Original Article)

Toxicological Effects of Certain Insecticides Alone and in Combination with Sub-lethal Concentrations of S-Metolachlor or Myclobutanil on *Culex pipiens* Larvae

Neama S. Saeed*; Sayed A. Ahmed and Ibrahim A. Mohamed

Plant Protection Department, Faculty of Agriculture, Assiut University, Assiut, Egypt.

*Corresponding author email: neama_shaban@aun.edu.eg
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Abstract

The *Culex pipiens* complex is a major and prevalent pest of dipterans for the most part of the world. The addition of sub-lethal quantities of chemical toxicants, such as fungicides and herbicides, can change the toxicity of certain insecticides on mosquito larvae. Hence, the purpose of the current study was to evaluate toxicity of certain insecticides individually and in combination with the sub-lethal concentrations of myclobutanil (1 μg ml^{-1}) or S-metolachlor (0.140 μg ml^{-1}) against *C. pipiens* larvae after 24, 48 and 72 h of exposure. Depending on the combination of the sub-lethal concentrations of S-metolachlor and myclobutanil, as well as the LC_{50} values of each studied insecticide, the insecticides exhibited distinct interaction reactions (e.g., antagonistic and/or synergistic effects) on *C. pipiens* larvae. Permethrin mixed with myclobutanil or S-metolachlor at sub-lethal concentrations produced a synergistic effect and enhanced permethrin's toxicity on *C. pipiens* larvae during exposure durations (except for the sub-lethal concentration of S-metolachlor after 72 h). On the other hand, following exposure times, combining imidacloprid or cyromazine with sub-lethal concentrations of myclobutanil or S-metolachlor was an antagonistic impact and reduced the toxicity of both insecticides on *C. pipiens* larvae. Only after 24 h of exposure did the sub-lethal concentrations of myclobutanil or S-metolachlor increase the lethality of indoxacarb; however, after 48 and 72 h of exposure, they decreased the toxicity of indoxacarb. It will be worthwhile to evaluate the impact of insecticides on fish and mosquito larvae when sub-lethal levels of herbicides and fungicides are present.

Keywords: *Antagonistic effects, Culex pipiens, Pesticides, Synergistic effects, Toxicity*

Introduction

Culex pipiens L. (Diptera: *Culicidae*), is a nuisance dipteran pest that is responsible for transmitting various serious diseases for human such as lymphatic filariasis and various viral encephalitides (Mohamed *et al*., 2023). It is one of the most prevalent mosquito species in temperate areas across the globe (Mohamed *et al*., 2021). In both agricultural and non-agricultural settings, larval stages of *C. pipiens* and other mosquito species inhabit a wide variety of natural and artificial water breeding habitats (Morris *et al*., 2016; Fathy *et al*., 2019; Medeiros-Sousa *et al*., 2020; Multini *et al*., 2021). Several chemical insecticides (e.g., imidacloprid,

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permethrin, cyromazine, malathion and indoxacarb) are frequently and widely used as the main effective strategy for controlling mosquito species at adult and immature stages (Hussain, 2024). Toxicity of some neonicotinoids such as imidacloprid, acetamiprid and clothianidin have been confirmed against different mosquitoes such as *Anopheles coluzzii* (Mouhamadou *et al*., 2019), *C. pipiens* (Hussain, 2024), and *Aedes aegypti* (Tomé *et al*., 2014). Imidacloprid is a neurotoxin compound that targets the nicotinic acetylcholine receptors in insect pests (Elbert *et al*., 1991). The pyrethroid insecticide permethrin have neurotoxic effects on *C. pipiens* larvae as it acts as a blocker to the para-sodium channels in the pest (Taktak *et al*., 2021; Hussain, 2024). Cyromazine is an insect growth regulators (IGRs) compound, which act as a molting disruptor (Hafez and Abbas, 2021). Cyromazine is also induced larvicidal toxicity effects against larvae of *C. pipiens* (Cohen, 1986; Assar *et al*., 2016), and *C. quinquefasciatus* (Hafez and Abbas, 2021). According to Wang *et al.* (2010) and Elghareeb *et al.* (2018), indoxacarb is an oxadiazine that works by blocking neuronal sodium channels in insect pests like mosquitoes.

Apart from larvicides, mosquito larvae that live in aquatic breeding sites are often exposed to large quantities of various chemical pollutants: fungicide, herbicide, microplastic and heavy metal residues; these pollutants are absorbed through erosion, leaching, and spray drift routes, among other mechanisms (Schulz, 2004; Rodney *et al.*, 2013; Bara *et al.*, 2014; Hussain, 2024). Fungicides and herbicides are among the most widely agro-pesticides found as chemical contaminants in aquatic systems worldwide. Later, several studies have indicated that combination of certain insecticides with chemical toxicants may lead additive or synergistic effects of toxicity on aquatic or terrestrial organisms than their individual effects (Bara *et al*., 2014; Hussain, 2024) but in some cases antagonistically effects have been also confirmed (Elghareeb *et al.,* 2018; Hussain, 2024). Exposure the field larvae of *C. pipiens* to combination of malathion with sub-lethal concentrations of six different herbicides evoked synergistic effect and thus increase the toxicity of malathion to the mosquito larvae (Gaaboub *et al.,* 1981). Toxicity of the insecticides abamectin, imidacloprid, sulfoxaflor+spinetoram and indoxacarb on *C. pipiens* larvae appeared to increase when the herbicide glyphosate was combined with these pesticides at relevant environmental concentrations, whereas the toxicity of the herbicide on larvae appeared to decrease when it was combined with spinosad (Elghareeb *et al*., 2018). The interaction between insecticides and other pesticides showed high potential for synergistic or antagonistic effects, which were likely caused by the initiation or inhibition of the pesticides' metabolizing enzymes (Rodney *et al*., 2013).

Triazole fungicide, myclobutanil and chloroacetanilide herbicide S-metolachlor are widely applied to control target pests in several crops worldwide (Lin *et al*., 2014; Chen *et al*., 2017 a&b). Several studies indicated that concentrations of the herbicide S-metolachlor and the fungicide myclobutanil have been commonly and frequently reported in different aquatic systems worldwide, which posed a risk to non-target organisms (Zemolin *et al*., 2014; Kumar *et al*., 2019). Adverse effects of myclobutanil and S-metolachlor have been reported on various aquatic and terrestrial organisms (Chen *et al*., 2017a&b). Myclobutanil can impair Cytochrome P450-mediated detoxification in *Apis cerana cerana* (Han *et al*., 2018), and elicit oxidative stress in *Tetrahymena thermophila* (Huang *et al*., 2016). Myclobutanil at 1000 µg L^{-1} altered behavior and elicited oxidative stress, lipid peroxidation, and potentially apoptosis in larval zebrafish (Kumar *et al*., 2019). Exposure of aquatic and terrestrial insects to multiple chemical pesticides may elicit different interaction responses (e.g., additive, synergistic and antagonistic) (Cedergreen, 2014). Mixing of S-metolachlor with benoxacor caused a synergistic toxicity on *Chironomus riparius* (Bolyard *et al*., 2017). However, there is no information on the combined toxicity effects of insecticides and low concentrations of S-metolachlor or myclobutanil on development stages of mosquitoes include *C. Pipiens*. The purpose of this study is to investigate the lethal toxicity of various insecticides (such as permethrin, imidacloprid, Cyromazine and indoxacarb) on *C. pipiens* larvae under laboratory conditions, either alone or in combination with a sub-lethal concentration of the fungicide myclobutanil and the herbicide S-metolachlor.

Materials and Methods

1-*Culex pipiens* larvae

Larvae of *C. pipiens* complex were collected from wastewater-treatment plants in Arab-El-Madabegh region, Assiut, Egypt. Larvae were maintained in the Environmental Toxicology laboratory conditions (25±2 °C and 12 h/12 h light/dark period) and fed on a mixture of fine grinded bread and dry yeast. Late third to early fourth instar larvae of *C. pipiens* were used in the experiments.

2-Pesticides

Table 1. Description of selected pesticides used against the larvae of *Culex pipiens* in this study

Pesticides	Trade name and formulation	Manufacturer
I. Insecticides		
Permethrin	Ectomethrin® 5% EC	Misr company for pharmaceutical, Egypt
Imidacloprid	Merck Super® 70% WG	Egyptian Seeds and Insecticides Co., Egypt
Cyromazine	Grandair® 75% WP	Dltex Rock Co., Egypt
Indoxacarb	Advance® 15% SC	Astra-Kim Co., Egypt
II. Fungicide		
Myclobutanil	Yemaklunel® 25% EC	Green Arrow Important and Trade Co., Egypt
III. Herbicide		
S-metolachlor	Gardo® 96% EC	Starchem Industrial Chemicals Co., Egypt

3-Pesticide bioassays

Larval bioassays were conducted to assess acute toxicity of the four tested insecticides individually and in combination with the sub-lethal concentration of the fungicide myclobutanil (1 µg active ingredient (a.i.) ml^{-1}) or the herbicide S-metolachlor (0.140 µg a.i. ml^{-1}) against the late third to early fourth instar larvae of *C. pipiens* complex, according to the studies of Gaaboub *et al*. (1981), Elghareeb *et al,* (2018), and Hussain (2024). Here, the sub-lethal concentrations of the fungicide myclobutanil (1 µg ml^{-1}) or the herbicide S-metolachlor (0.140 µg ml^{-1}) were chosen according to the studies of Kumar *et al*. (2019) and Battaglina *et al*. (2000), respectively. The sub-lethal concentration of S-metolachlor was approximately 1/10700 from the recommended field rate (1440 mg L^{-1}) of S-metolachlor for maize weeds in Egypt (APC, 2022). The sub-lethal concentration of myclobutanil (1 µg ml^{-1}) was the approximately 1/325 from the recommended field rate (325 mg L^{-1}) of myclobutanil for grape powdery mildew in Egypt (APC, 2022). Furthermore, the tested sub-lethal concentrations of myclobutanil or S-metolachlor did not cause any mortality in larvae of *C. pipiens* after 72 h of exposure in the investigated preliminary experiment.

For acute insecticide toxicity bioassays, six to seven concentrations for each insecticide were prepared in dechlorinated tap water individually and in combination with a chosen sub-lethal concentration of the fungicide myclobutanil (1 µg ml^{-1}) or the herbicide S-metolachlor (0.140 µg ml^{-1}). The tested concentrations were ranged from 0.137 to 70.0 mg a.i. L^{-1} for imidacloprid, 0.703 to 22.5 mg a.i. L^{-1} for permethrin 0.008 to 2.25 mg a.i. L^{-1} for indoxacarb, and 23.43 to 750 mg a.i. L^{-1} for cyromazine, which led to larval mortality ranged from 5% to 99%. Each concentration individually or in combination treatments consisting of three replicates with 10 *C. pipiens* larvae in 100 ml solution per replicate. Controls received only dechlorinated tap water. The mixture of grinded bread and dry yeast was offered to larvae as food. Bioassays were conducted in the Environmental Toxicology laboratory conditions (25±2 °C and 60±5% relative humidity and 12:12 (light: dark) photoperiod). Mortality of *C. pipiens* larvae was recorded after 24, 48 and 72 h of exposure. However, *C. pipiens* larvae were considered dead if they were unrestrained to the touching with a probe or if they could not reach the surface of the water (Hussain, 2024).

4-Data analysis

Values of LC_{50} and LC_{90} and their corresponding 95% fiducial limits (FLs), as well as the slope were estimated for each insecticide alone and in combination with the sub-lethal concentration of the fungicide myclobutanil or the herbicide S-metolachlor, according to Finney (1972) of Probit analysis by using SPSS software version 16.0 (SPSS Inc., Chicago, IL, USA, 2016). Synergistic ratio (SR) was calculated according to the following equation: Synergistic ratio (SR) = the LC_{50} value of the tested insecticide / the LC_{50} obtained for the combined treatment (insecticide + herbicide or fungicide).

Results

1-Toxicity of insecticides individually and in combined with other pesticides to *C. pipiens* larvae.

Tables (2-5) displayed the lethal toxicity of imidacloprid, indoxacarb, permethrin and cyromazine for *C. pipiens* larvae after 24, 48 and 72 hours of exposure, either alone or in combination with the sub-lethal concentrations of the fungicide myclobutanil (1 µg ml^{-1}) or the herbicide S-metolachlor (0.140 µg ml^{-1}).

The sub-lethal concentrations of myclobutanil (1 µg ml^{-1}) or S-metolachlor (0.140 µg ml^{-1}) alone did not result in any mortality to larvae of *C. pipiens*.

Indoxacarb

The LC_{50} values of individual indoxacarb to larvae of *C. pipiens* were 1.274, 0.036 and 0.009 mg L^{-1}, after 24, 48 and 72 h, respectively (Table 2). When the sub-lethal concentration of myclobutanil was mixed with indoxacarb, the LC_{50} values for exposed *C. pipiens* larvae were 0.740, 0.157 and 0.059 mg L^{-1}, respectively (Table 2). Presence of the sub-lethal concentration of myclobutanil increased the toxicity of indoxacarb on *C. pipiens* larvae by 1.72-fold after 24 h, but it decreased the toxicity of indoxacarb by 4.36 and 6.56-fold after 48 and 72 h, respectively. The same trend was observed when the sub-lethal concentration of S-metolachlor mixed with indoxacarb whereas the LC_{50} values were 0.748, 0.288 and 0.075 mg L^{-1}, respectively (Table 2). These findings showed that after 24 hours of exposure, S-metolachlor increased the toxicity of indoxacarb by 1.70-fold, but after 48 and 72 h, it decreased the toxicity of indoxacarb by 8.00 and 8.33-fold, respectively.

Permethrin

The LC_{50} values of individual permethrin to larvae of *C. pipiens* were 10.899, 2.104 and 0.203 mg L^{-1}, after 24, 48 and 72 h, respectively (Table 3). When the sub-lethal concentration of myclobutanil mixed with permethrin, the LC_{50} values for exposed *C. pipiens* larvae were decreased to 1.362, 0.432 and 0.168 mg L^{-1}, respectively (Table 3). Presence of the sub-lethal concentration of myclobutanil increased toxicity of the insecticide permethrin on *C. pipiens* larvae by 8.00, 4.87 and 1.21-fold, respectively. The LC_{50} values for permethrin in mixture with the sub-lethal concentration of S-metolachlor were 4.929, 1.828 and 1.063 mg L^{-1}, respectively (Table 3). S-metolachlor synergized the toxicity of permethrin by 2.21 and 1.15-fold only after 24 and 48 h of exposure, but it antagonized the toxicity of the insecticide by 5.24-fold after 72 h.

Imidacloprid

The LC_{50} values of individual imidacloprid to *C. pipiens* larvae were 3.565, 0.120 and 0.034 mg L^{-1}, after 24, 48 and 72 h, respectively (Table 4). The LC_{50} values for *C. pipiens* larvae subjected to imidacloprid at sub-lethal concentrations of myclobutanil were increased to 43.285, 7.641 and 3.267 mg L^{-1}, respectively (Table 4). The LC_{50} values for imidacloprid in mixture with the sub-lethal concentration of S-metolachlor were 75.202, 4.030 and 0.970 mg L^{-1}, respectively (Table 4). Presence of either the sub-lethal concentrations of myclobutanil or S-metolachlor decreased the toxicity of the insecticide imidacloprid to *C. pipiens* larvae. Thus, myclobutanil antagonized the toxicity of imidacloprid by 12.14, 63.68 and 96.1-fold, respectively whereas S-metolachlor antagonized the toxicity of the insecticide by 21.1, 33.6 and 28.53-fold after exposure times.

Table 2. Toxicity of indoxacarb alone and mixed with sub-lethal concentration of myclobutanil or S-metolachlor against 4th instar larvae of *Culex pipiens* larvae after 24, 48 and 72 hours of exposure

Pesticides	Time (h)	LC_{50} (mg L^{-1})	Fiducial Limits (FL)	LC_{90} (mg L^{-1})	Fiducial Limits (FL)	Slope ± S.E.	SR_{50}	SR_{90}
Indoxacarb	24	1.274	0.508 - 8.770[a]	61.348	8.872 - 7194.843[a]	0.762 ± 0.077	–	–
	48	0.036	0.014 - 0.063[a]	0.712	0.313 - 4.644[ab]	0.991 ± 0.104	–	–
	72	0.009	0.001 - 0.019[ab]	0.133	0.077 - 0.436[a]	1.115 ± 0.152	–	–
Indoxacarb + myclobutanil	24	0.740	0.494 - 1.275[a]	31.789	12.080 - 137.093[a]	0.785 ± 0.085	1.72	1.93
	48	0.157	0.123 - 0.205[bc]	2.818	1.659 - 5.873[b]	1.022 ± 0.092	0.23	0.25
	72	0.059	0.035 - 0.094[b]	0.487	0.249 - 2.012[b]	1.226 ± 0.080	0.15	0.27
Indoxacarb + S-metolachlor	24	0.748	0.509 - 1.241[a]	4.091	2.201 - 11.030[b]	1.736 ± 0.129	1.70	15.0
	48	0.288	0.145 - 0.864[b]	3.947	1.188 - 54.067[b]	1.128 ± 0.077	0.13	0.18
	72	0.075	0.043 - 0.145[b]	0.829	0.344 - 4.649[b]	1.467 ± 0.146	0.12	0.16

FL [a]: Values in parenthesis for LC_{50} and LC_{90} columns for respective insecticides show upper and lower fiducial limits; the synergistic ratio (SR) was calculated by dividing by the LC for insecticide alone the LC of insecticide + myclobutanil or S-metolachlor. The values with the same letters in the same row each time are not significantly different.

Table 3. Toxicity of permethrin alone and mixed with sub-lethal concentration of myclobutanil or S-metolachlor against 4th instar larvae of *Culex pipiens* larvae after 24, 48 and 72 hours of exposure

Pesticides	Time (h)	LC_{50} (mg L^{-1})	Fiducial Limits (FL)	LC_{90} (mg L^{-1})	Fiducial Limits (FL)	Slope ± S.E.	SR_{50}	SR_{90}
Permethrin	24	10.899	6.252 - 31.400[a]	57.143	22.9494 - 1106.087[a]	1.511 ± 0.188	–	–
	48	2.104	0.294 - 4.882[ab]	70.607	17.567 - 136529.107[a]	0.770 ± 0.095	–	–
	72	0.203	0.000 - 0.727[ab]	18.102	6.894 - 1304.420[a]	0.618 ± 0.109	–	–
Permethrin + myclobutanil	24	1.362	1.000 - 1.737[b]	16.527	11.687 - 26.893[bc]	1.082 ± 0.112	8.00	3.46
	48	0.432	0.251 - 0.617[bc]	3.513	2.753 - 4.825[b]	1.359 ± 0.176	4.87	20.10
	72	0.168	0.048 - 0.317[ab]	1.559	1.128 - 2.134[b]	1.287 ± 0.237	1.21	11.61
Permethrin + S-metolachlor	24	4.929	3.057 - 8.313[c]	26.942	13.875 - 118.765[ab]	1.569 ± 0.117	2.21	2.12
	48	1.828	0.340 - 3.874[ab]	20.249	7.787 - 1193.463[a]	1.165 ± 0.112	1.15	3.49
	72	1.063	0.017- 2.326[a]	22.750	7.177 - 359822.7[a]	0.963 ± 0.142	0.19	0.80

FL [a]: Values in parenthesis for LC_{50} and LC_{90} columns for respective insecticides show upper and lower fiducial limits; the synergistic ratio (SR) was calculated by dividing by the LC for insecticide alone the LC of insecticide + myclobutanil or S-metolachlor. The values with the same letters in the same row each time are not significantly different.

Table 4. Toxicity of imidacloprid alone and mixed with sub-lethal concentration of myclobutanil or S-metolachlor against 4th instar larvae of *Culex pipiens* larvae after 24, 48 and 72 hours of exposure

Pesticides	Time (h)	LC_{50} (mg L^{-1})	Fiducial Limits (FL)	LC_{90} (mg L^{-1})	Fiducial Limits (FL)	Slope ± S.E.	SR_{50}	SR_{90}
Imidacloprid	24	3.565	1.182 - 45.051[ab]	111.643	15.955 - 592669.227[ab]	0.857 ± 0.072	–	–
	48	0.120	0.000 - 0.338[a]	2.499	0.909 - 1178.685[ab]	0.973 ± 0.114	–	–
	72	0.034	0.011 - 0.065[a]	0.394	0.290 - 0.550[a]	1.210 ± 0.201	–	–
Imidacloprid + Myclobutanil	24	43.285	24.780 - 95.645[bc]	314.019	131.400 - 1513.467[b]	1.489 ± 0.130	0.08	0.36
	48	7.641	5.084 - 13.327[b]	44.358	22.723 - 132.689[b]	1.678 ± 0.121	0.02	0.06
	72	3.267	2.066 - 6.215[b]	27.658	12.240 - 123.589[b]	1.381 ± 0.100	0.01	0.01
Imidacloprid + S-metolachlor	24	75.202	49.775 - 129.154[c]	840.554	406.147 - 2384.221[bc]	1.222 ± 0.120	0.05	0.13
	48	4.030	1.584 - 29.549[b]	56.985	12.404 - 28247.692[bc]	1.114 ± 0.093	0.03	0.04
	72	0.970	0.466 - 2.436[c]	14.028	5.588 - 145.380[b]	1.104 ± 0.109	0.04	0.03

FL [a]: Values in parenthesis for LC_{50} and LC_{90} columns for respective insecticides show upper and lower fiducial limits; the synergistic ratio (SR) was calculated by dividing by the LC for insecticide alone the LC of insecticide + myclobutanil or S-metolachlor. The values with the same letters in the same row each time are not significantly different.

Table 5. Toxicity of cyromazine alone and mixed with sub-lethal concentration of myclobutanil or S-metolachlor against 4th instar larvae of *Culex pipiens* larvae after 24, 48 and 72 hours of exposure

Pesticides	Time (h)	LC_{50} (mg L^{-1})	Fiducial Limits (FL)	LC_{90} (mg L^{-1})	Fiducial Limits (FL)	Slope ± S.E.	SR_{50}	SR_{90}
Cyromazine	24	82.060	57.423 - 115.370[a]	750.300	2978.244 - 33648.002[ab]	0.727 ± 0.094	–	–
	48	13.239	5.459 - 22.826[a]	238.900	1022.644 - 10604.218[ab]	0.631 ± 0.095	–	–
	72	13.694	4.022 - 25.495[b]	275.403	145.403 - 990.080[a]	1.011 ± 0.108	–	–
Cyromazine + Myclobutanil	24	337.959	191.496 - 590.278[b]	3316.616	1505.203 - 18103.834[bc]	2.045 ± 1.642	0.24	0.23
	48	83.582	18.835 - 165.172[b]	1375.555	611.886 - 11474.954[bc]	0.666 ± 0.109	0.16	0.17
	72	52.164	4.129 - 114.604[bc]	575.877	268.555 - 5915.749[a]	0.538 ± 0.088	0.26	0.48
Cyromazine + S-metolachlor	24	277.081	136.879 - 523.746[b]	2415.081	1058.165 - 18105.519[c]	2.784 ± 0.835	0.30	0.31
	48	141.536	60.220 - 251.675[b]	928.883	465.615 - 4985.648[c]	0.242 ± 0.088	0.09	0.26
	72	82.598	24.612 - 149.432[c]	641.651	331.861 - 3233.198[ab]	0.321 ± 0.075	0.17	0.43

FL [a]: Values in parenthesis for LC_{50} and LC_{90} columns for respective insecticides show upper and lower fiducial limits; the synergistic ratio (SR) was calculated by dividing by the LC for insecticide alone the LC of insecticide + myclobutanil or S-metolachlor. The values with the same letters in the same row each time are not significantly different.

Cyromazine

The LC_{50} values for cyromazine alone to *C. pipiens* larvae were 82.060, 13.239 13.694 mg L^{-1}, after 24, 48 and 72 h, respectively and the values were increased to be 337.959, 83.582 and 52.164 mg L^{-1}, respectively for the insecticide cyromazine in mixture with myclobutanil while LC_{50} values were 277.081, 141.50 and 82.598 mg L^{-1} for the insecticide cyromazine in mixture with S-metolachlor (Table 5). Consequently, after exposure durations, myclobutanil reduced the toxicity of cyromazine by 4.12, 6.31 and 3.81-fold, whereas S-metolachlor lessened the toxicity of the insecticide by 3.38, 10.69 and 6.03-fold, after 24, 48 and 72 h. (Table 5).

Discussion

In agricultural and urban areas, a variety of agrochemical compounds such as primarily pesticides and pesticides are often identified as important contaminants in aquatic systems across the globe (Schulz, 2004; Bara *et al*., 2014). In aquatic habitats, the immature stages of mosquito species most commonly and continuously exposed to mixtures of chemical toxicant agents, mainly agricultural pesticides, rather than single compounds (Thurman *et al*., 1992; David *et al*., 2010; Nkya *et al*., 2013; Brodeur *et al*., 2014). Larvicides from different insecticide classes (i.e., pyrethroids, organophosphates, neonicotinoids, carbamates and IGR's) are mainly and extensively used to control larvae of mosquito species worldwide. Exposure of mosquito larvae to mixture of an insecticide with sub-lethal concentrations of some chemical toxicants may produce a variety of interaction responses such as additive, antagonistic, or synergistic than their individual effect (Anderson and Zhu, 2004; Poupardin *et al*., 2008; Cedergreen, 2014; Bara *et al*., 2014).

In present study, toxicity of the tested insecticides on *C. pipiens* larvae was altered by mixing the insecticides with the sub-lethal concentrations of myclobutanil (1 µg ml^{-1}) or S-metolachlor (0.140 µg ml^{-1}) after 24, 48 and 72 h of exposure. Mixing of the sub-lethal concentration of myclobutanil or S-metolachlor with permethrin found to be increased the toxicity of the insecticide permethrin on *C. pipiens* larvae after exposure times (except the sub-lethal concentration S-metolachlor after 72 h). In contrast, adding of the sub-lethal concentrations of myclobutanil or S-metolachlor to imidacloprid and cyromazine caused antagonistic effects for both insecticides imidacloprid and cyromazine increased their toxicity to *C. pipiens* larvae after exposure times. Combination of the sub-lethal concentration of S-metolachlor or myclobutanil with indoxacarb synergized the toxicity of indoxacarb only after 24 h of exposure but antagonized the toxicity of indoxacarb after 48 and 72 h. In accordance with Gaaboub *et al*. (1981), on a field strain of *C. pipiens* larvae, malathion showed synergistic effects when combined with various herbicides (trifluralin, drepamon, oxadiazon, benthiocard, propanil and cremart). Atrazine herbicide also increased the toxicity of some other organophosphate insecticides like diazinon, chlorpyrifos and methyl parathion on *Chironomus tentans* larvae (Jin-Clark *et al*., 2002). In contrast, Boyer *et al*. (2006) indicated that toxicity of the organophosphate temephos against *A. aegypti* larvae was decreased by exposing the larvae to sub-lethal concentration of the herbicide atrazine.

The toxicities of rotenone, carbaryl and temephos insecticides as antagonistic effects were decreased when *A. albopictus* larvae were exposed to low concentrations of specific chemical pollutants, such as pentachlorophenol, a wood-protecting material and benzothiazole, a primary leachate agent of automobile tires. These pollutants can also increase the mosquito *A. albopictus's* tolerance to these insecticides (Suwanchaichinda and Brattsten, 2001, 2002). Mixing sub-lethal concentration of glyphosate with spinosad showed antagonistic effect to *C. pipiens* larvae but it elicited synergistic effects when mixed with abamectin, imidacloprid, sulfoxaflor+spinetoram, and indoxacarb (Elghareeb *et al.,* 2018). Pre-exposure of *A. aegypti* larvae to sub-lethal concentrations of glyphosate and atrazine have been found to decrease the toxicity of propoxur, permethrin, *Bacillus thuringiensis* and imidacloprid and antagonistic interactions might be due to increase the induction of certain detoxification enzymes (i.e., carboxylesterases, Cytochrome P450s and glutathione-S-transferases (GSTs)) that enhanced the metabolism rate of these insecticides (Boyer *et al*., 2006; Poupardin *et al*., 2008; Riaz *et al*., 2009; David *et al*., 2010; Bara *et al*., 2014).

In the present study, the mentioned synergistic interactions among the sub-lethal concentration of myclobutanil or S-metolachlor and permethrin in *C. pipiens* larvae may be resulted from the interference of myclobutanil or S-metolachlor with the metabolic detoxification enzymes of the mosquito larvae, thereby synergizing the toxicity of these insecticides. Khan *et al*. (2013) stated that synergistic action among pesticides mixture might resulted from one toxicant in the pesticide-mixture interferes with the metabolic detoxification factors of the other toxicant, therefor elevating the toxicity effects of the latter toxicant. In mosquito larvae, atrazine exhorted the expression of various detoxification enzymes such as esterases, GSTs and cytochrome P450s (Poupardin *et al*., 2008; David *et al*., 2010) which induced different of important cellular functions (Scott *et al.,* 1999). As a result, after exposure to the herbicide, the mentioned enzymes may be stimulated, which may reduce the toxicity of the insecticides used on larvae and create an antagonistic action that could increase the rates at which larvae survive and emerge as adults. Iwasa *et al*. (2004) stated that combination of propiconazole or triflumizole fungicides with imidacloprid produced a sight increase in toxicity of the insecticide against *Apis mellifera*. The fungicide prochloraz increased the toxicity of certain acaricides (coumaphos, taufluvalinate and fenpyroximate) on *A. mellifera*, likely by inhibiting the detoxicative cytochrome P450-monooxygenase (P450 enzymes) activity in bees (Johnson *et al*., 2013). Triazole fungicides can inhibit cytochrome P450 enzymes of honeybee that could detoxify various synthetic insecticides and other phytochemicals and led to increase the toxicity of these insecticides to honeybees (Mao *et al*., 2017; Almasri *et al*., 2020). Indeed, previous studies have determined that triazoles and neonicotinoids could induce synergistic impact on honeybees (Lv *et al*., 2023). Combinations of myclobutanil fungicide and thiamethoxam insecticide induced synergetic effect on *Danio rerio* embryos that due to oxidative stress and disruption to immune and endocrine systems (Shen *et al*., 2021).

To sum up, the sub-lethal concentrations of the herbicide S-metolachlor or the fungicide myclobutanil altered the toxicity of the insecticides imidacloprid, cyromazine, indoxacarb and permethrin towered mosquito larvae. Even though several of these mixtures showed synergistic effect and made the tested pesticides more toxic to mosquito larvae, there may be a risk to fish, tadpoles and aquatic predatory insects. Furthermore, the presence of S-metolachlor and myclobutanil residues in aquatic mosquito breeding sites may boost mosquito population resistance development and reduce the toxicity of imidacloprid and cyromazine insecticides against mosquito larvae. To comprehend how the sub-lethal concentration of pesticide changed the toxicity of larvicides, more studies still is required.

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التأثيرات السـامة لبعض المبيدات الحشـرية بمفردها ومخلوطة مع التركيزات تحت المميتة من ميكلوبوتانيل أو أس- ميتولاكلور على يرقات بعوض *Culex pipiens*

نعمة شعبان سعيد *، سيد عاشور احمد، ابراهيم عبد الوهاب محمد

قسم وقاية النبات، كلية الزراعة، جامعة أسيوط، مصر.

الملخص

يُعد بعوض *Culex pipiens* من الآفات الحشـرية الخطيرة التابعة لرتبة ذات الجناحين وهو سـائد الانتشـار في معظم أنحاء العالم. إن إضـافة تركيزات تحت مميتة من المواد الكيميائية السامة، مثل مبيدات الفطريات ومبيدات الحشـائش، يمكن أن تغير سـمية بعض المبيدات الحشـرية على يرقات البعوض. ومن ثم، هدفت هذه الدراسـة إلى تقييم السـمية الحادة لعدد من المبيدات الحشـرية بشكل فردي أومخلوطة مع التركيز تحت المميت للمبيد الفطري ميكلوبوتانيل (1 ميكروجرام/ مل) أو مبيد الحشـائش أس- ميتولاكلور (0.140 ميكروجرام/ مل) ضد يرقات بعوض *Culex pipiens* بعد 24، 48، 72 سـاعة من المعاملة. واسـتناداً إلى قيم التركيزات نصـف المميتة (LC_{50}) للمبيدات الحشـرية المختبرة بشـكل فردي أومخلوطة مع التركيز تحت المميت لمبيدى ميكلوبوتانيل أو أس- ميتولاكلور، أحدث الخلط تغيرات مختلفة في سـمية المبيدات الحشـرية (تنشـيطية و/ أو تثبيطيه) ضـد يرقات البعوض بعد فترات المعاملة. فقد أدى خلط التركيز تحت المميت لمبيدى ميكلوبوتانيل أو أس- ميتولاكلور مع المبيد الحشـري بيرميثرين إلى إحداث تأثير تنشـيطي وزيادة في سـمية مركب بيرميثرين ضـد اليرقات بعد فترات المعاملة (باسـتثناء التركيز تحت المميت للأس- ميتولاكلور بعد 72 سـاعة). على النقيض من ذلك، فإن خلط التركيز تحت المميت لمبيدى ميكلوبوتانيل أو أس- ميتولاكلور مع المبيدين الحشـريين إيميداكلوبريد وسـيرومازين كان له تأثير مضـاد وقلل من سـمية كلا المبيدين الحشـريين ضـد يرقات البعوض بعد فترات المعاملة. كما أدت إضـافة التركيز تحت المميت لمبيدى ميكلوبوتانيل أوأس- ميتولاكلور إلى المبيد الحشـري اندوكسـاكارب إلى زيادة سـميته فقط بعد 24 سـاعة من المعاملة، لكن الخلط أحدث تأثير تضـادي وخفض سمية مركب اندوكساكارب ضد اليرقات بعد 48، 72 ساعة من المعاملة.

سيكون من المفيد تقييم تأثيرات المبيدات الحشرية على يرقات البعوض والأسماك في وجود تركيزات تحت مميتة من مبيدات الفطريات ومبيدات الحشائش.

الكلمات المفتاحية: *السمية، تأثيرات تثبيطية، تأثيرات تنشيطية، مبيدات الآفات، Culex pipiens.*