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Synergistic action of glyphosate on novel pesticides against *Culex pipiens* L. (Diptera: Culicidae) mosquitoes under laboratory conditions

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Abstract

The recognition of how glyphosate affect mosquito ecology and behavior is limited. Thus, in this study, we evaluated the toxicity of six selected pesticides plus combination with glyphosate on 4th instar larvae of *Culex pipiens* after 24, 48, and 72-h exposure. As a result, Sulfoxaflor + spinetoram and abamectin were the most toxic pesticides (LC_{50} was 0.084 and 0.088 ng/ml after 24-h exposure, respectively). After 48-h exposure, emamectin benzoate was the most powerful pesticide (LC_{50} was 0.0036 ng/ml), while sulfoxaflor + spinetoram, abamectin, and spinosad were the modest pesticides. Further, imidacloprid was the lowest toxic pesticide (LC_{50} was 5.57 ng/ml). However, similar trend was achieved after 72-h exposure. In combination with glyphosate, it significantly synergized the selected pesticides except spinosad and emamectin benzoate after 24-h exposure. After 48 and 72-h exposure, glyphosate synergized significantly most of the selected pesticides except spinosad (LC_{50} was 0.052 and 0.01 while synergistic ratio (SR) was 0.38 and 0.30 after 48 and 72-h exposure, respectively). Based on the LT_{50} values, emamectin benzoate and abamectin demonstrated high efficiency against *Culex pipiens* (42.60 and 43.61 hours), and imidacloprid was the least effective (232.08 hours). Whereby, in the combination with glyphosate, sulfoxaflor + spinetoram and emamectin benzoate revealed the high potency against *Culex pipiens* (39.16 and 43.77 hours), and imidacloprid remain with the same trend of efficacy as lowest effective selected pesticide towards *Culex pipiens* (563.30 hours). Further biochemical and molecular biological experiments needs to be done for better understanding of the mechanism of glyphosate on *Culex pipiens* mosquito.

Key words: *Culex pipiens*, herbicides, mosquito control, glyphosate, synergistic ratio (SR)

INTRODUCTION

Over many decades, few species of mosquitoes that intimidated the public and veterinary health have emerged o0r re-emerged in Africa (Ahmed and Matsumura, 2012; Ahmed *et al*., 2015; Ahmed and Vogel, 2015; Ahmed and Vogel 2016a,b,c; Mweya *et al*., 2017). *Culex pipiens* is considered one of the most critical species that threaten and transmitted numerous diseases as a vector for instance, West Nile virus, Bancroftian filariasis, Rift valley fever virus, Usutu virus, and avian malaria (Ahmed and Matsumura, 2012; Mohamed *et al*., 2016; Ahmed and saba, 2016; Nasci *et al*., 2017). However, *Culex pipiens* control is considered a paramount (Badawy *et al*., 2017; Brustolin *et al*., 2017; Tmimi *et al*., 2018). Abundance of control techniques have been utilized, however, the most effective one is chemical control. Consequently, their intensive use has, however, led to the emergence of resistance, particularly through different mechanisms in many insect species and this represents a serious obstacle to their continued and effective uses of many pesticides (Liu *et al*., 2017; Su *et al*., 2017; Xu *et al*., 2017). Thus, it is very important to formulate various strategies to avoid, the development of resistance among major pest insects to pesticides, and the side effects on the environment. On the other side, glyphosate and its mimics inhibit the specific enzyme, 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS), which prohibit the biosynthesizing of three aromatic amino acids (tyrosine, tryptophane, and phenylalanine) which are vital for the processing of plant growth (Ibrahim, 2015; Ibrahim, 2016a; Ibrahim, 2016b). However, negatively toxicological impacts of certain herbicides such as glyphosate, certain ALS-, and ACCase-inhibitors have been confirmed on different insects aquatic animals and these herbicides can affect the survival, ecology, behavior, physiology, reproductive, fecundity, and development of these organisms (Mona *et al*., 2013; Mohamed *et al*., 2016; Jaramillo *et al*., 2017; Kittle *et al*., 2018; Saba *et al*., 2018). Further, Recent studies were conducted to illustrate the dexterous effect of some herbicides on immature and adult stages of different mosquito species in the world. For instance, (Mohamed *et al*., 2016) evaluated the toxicity effects of glyphosate, glufosinate-ammonium, and certain ACCase-inhibitors herbicides on fourth instar larvae of *Culex pipiens* in Egypt, plus, (Saba *et al*, 2018) conducted toxicological and biochemical investigation of certain herbicides including glyphosate on *Culex pipiens*

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mosquitos under laboratory conditions. In this particular, persistent of glyphosate in water and in aquatic habits as well can reduce the population of mosquitoes that feed on these aquatic plants and organic matters (Serandour *et al*., 2011). Further, glyphosate convinced certain potential effect on larvae of *Aedes aegypti* and the sublethal concentrations of glyphosate can mutate the larval development time, life cycle, and sex ratio of emerging *Aedes aegypti* and *Aedes albopictus* adults (Serandour *et al*., 2011; Morris *et al*., 2016). In this regard, many different types of efforts and strategies have been made to bring down the use of the classical insecticide of the pest control programs. For example, minimizing the percentage used in terms of the spray areas, using mixtures of insecticides with different modes of action, using the rotation of pesticides that have different mode of action to reduce the outbreak of resistance issue, using the right insecticide formulation, and targeting only the critical time or area of vulnerability (Ahmed and Matsumura, 2012; Ahmed and Vogel, 2015; Morris *et al*., 2016). Interestingly, there are numerous examples of successful applications of mixtures of pesticides in resistance management (Ahmed and Matsumura, 2012; Ahmed and Vogel, 2015; Ahmed and Vogel, 2016b). To date, no reliable data are available on the synergistic effects of glyphosate in combination with other pesticides on *Culex pipiens* mosquito. Therefore, the present study seeks to evaluate first, the toxicity of certain novel pesticides that have different mode of action on *Culex pipiens* larvae, second, the synergistic action of glyphosate on the activity of these pesticides on the 4th instar larvae of *Culex pipiens* mosquitoes under laboratory conditions.

MATERIALS AND METHODS

Mosquitoes:

Field strain: Culex pipiens larvae were collected from Arab El-Madabegh region (Assiut Governorate, Egypt) then transferred directly to the plant protection research building, Department of Plant Protection, Faculty of Agriculture, Assiut University for conducting the experiments.

Chemicals:

Glyphosate 48% WSC, mixing of sulfoxaflor (30% WG) + spinetoram (10% WG), abamectin 1.8% EC, spinosad 24% SC, Emamectin benzoate 5% SG, indoxacarb 30% WG, and imidacloprid 25% WP were obtained from Central Agricultural Pesticides Laboratory (CAPL) in Dokki, Giza, Egypt.

Toxicological bioassay:

Standard procedures of the toxicological bioassay were conducted according to WHO (1981) and Mohamed *et al*., (2016) with little modified. Briefly, five concentrations of each tested pesticide were set by using tap water. Three replicates were prepared for each concentration. However, a set of ten early 4th instar larvae of *Culex pipiens* with uniform size were devolved to different plastic cups which containing 100 ml of appropriate concentrations of each tested pesticide. Controls were only contained tap water. Mixture of dry yeast and grinded bread were given as a vital source of food. The bioassay was conducted and kept under laboratory conditions at 25±2 °C and 60±5% relative humidity. Mortality percentage was determined after 24, 48, and 72-h of exposure. However, 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.

Synergistic Action Bioassay:

The synergistic action bioassay was carried as described above. In this regard, controls were applied concurrently with each spectrum of tests. Synergism was determined by testing the lethal action (LC_{50}) of varying concentrations of test insecticide alone or co-administered with 10 µg/ml of glyphosate dissolved in 1 ml of acetone. Preliminary tests have been specified that 10 µg/ml of glyphosate was sublethal concentration at which mortality was not occurred. Five concentrations of each pesticide were used for all bioassays, and all bioassay tests were held at 25^{o}C. Mortality percentage was recorded after 24, 48, and 72 hours of exposure.

Data analysis:

LC_{50} and LC_{90} values and their corresponding 95% confidence limits (CLs), and the slope values were determined using IBM SPSS statistics 23 program (SPSS Inc., Chicago, IL). All figures were designed by using GraphPad Prism 6.07 software (San Diego, CA, USA). Toxicity index was calculated as follows:

Toxicity index= [(LC_{50} value of the most toxic tested pesticide / LC_{50} value of the tested pesticide) × 100].

RESULTS AND DISCUSSIONS

Toxicity of the tested pesticides on the 4th instar larvae of *Culex pipiens* after 24, 48, and 72-h exposure was exhibited in tables 1-3. Sulfoxaflor + spinetoram and abamectin were the most toxic pesticides (LC_{50} was 0.084 and 0.088 ng/ml after 24-h exposure, respectively). Further, spinosad and emamectin benzoate were the moderate toxic pesticides (LC_{50} was 0.10 and 0.16 after 24-h exposure, respectively). Whereas, indoxacarb and imidacloprid were the least potent pesticides (LC_{50} was 9.26 and 14.12 ng/ml after 24-h exposure, respectively). In contrast, after 48-h exposure, emamectin benzoate was the most powerful pesticide (LC_{50} was 0.0036 ng/ml), while sulfoxaflor + spinetoram, abamectin, and spinosad were the modest pesticides. Further, imidacloprid was still the lowest toxic pesticide ((LC_{50} was 5.57 ng/ml). However, the same trend was observed after 72-h exposure.

Interestingly, glyphosate significantly synergized the selected pesticides except spinosad and emamectin benzoate after 24-h exposure, meanwhile, after 48 and 72-h exposure, glyphosate significantly synergized most of the selected pesticides except spinosad (LC_{50} was 0.052 and 0.01, while SR was 0.38 and 0.30 after 48 and 72-h exposure, respectively) and the most toxic pesticides after 48-h were emamectin benzoate (LC_{50} = 0.00074 ng/ml and SR was 4.86), whereas glyphosate tremendously synergized indoxacarb after 72-h (LC_{50} = 0.013 ng/ml and SR was 10.77) (Tables 4-6).

According to the slope values of the tested pesticides, *Culex pipiens* showed relative high homogeneity response to imidacloprid and indoxacarb pesticides (4.11 and 3.67) after 24-h exposure, and demonstrated heterogeneity to sulfoxaflor + spinetoram (0.63). However, after 48-h exposure, *Culex pipiens* displayed heterogeneity response to emamectin benzoate (0.54). The same trend was recognized after 72-h exposure. Further, based on the slope values of the synergistic action of glyphosate on each pesticides, *Culex pipiens* revealed homogeneity restraint towards spinosad and indoxacarb (4.12 and 4.09) meanwhile it demonstrated heterogeneity response to sulfoxaflor + spinetoram (0.57). After 48-h exposure, slightly changes occurred to the response and *Culex pipiens* evidenced heterogeneity towards emamectin benzoate (0.53). The same tendency was perceived after 72-h exposure.

Figure 1 showed the time-dependent changes in the SR of LC_{50} values as affected by glyphosate. The most observed trends were 1) the most well-defined time-dependent decrease in SR was noticed on imidacloprid; 2) there were noticeable time-dependent increases in SR on indoxacarb and to a lesser extent sulfoxaflor + spinetoram; 3) relatively consistent levels of SR were found on abamectin; 4) glyphosate did not synergize spinosad.

The toxicity index of selected pesticides was exhibited in Figure 2 (A-C). In this interim, for LC_{50} values of pesticides alone after 24-h exposure, the values were 100, 95.45, 84, 52.50, 0.91, and 0.60 for sulfoxaflor + spinetoram, abamectin, spinosad, emamectin benzoate, indoxacarb, and imidacloprid, respectively, whereas after 48-h exposure were 18.96, 10.59, 18, 100, 0.33, and 0.07, respectively. The same trend was completely evidence after 72-h exposure.

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Table 1: Lethal toxicity of selected pesticides on the 4th instar larvae of *Culex pipiens* after 24-h of exposure.

Compounds	LC_{50} [a] (95% CL)	LC_{90} [a] (95% CL)	Slope (± SE)	*n* [b]
Sulfoxaflor 30% WG+Spinetoram 10% WG	0.084 (0.0044-0.35)	9.01 (0.30-12.70)	0.63 (± 0.10)	180
Abamectin 1.8% EC	0.088 (0.027-0.96)	29.06 (18.54-37.11)	2.31 (±0.08)	180
Spinosad 24% SC	0.10 (0.059-0.78)	18.33 (2.99-40.85)	1.87 (±0.09)	180
Emamectin benzoate 5% SG	0.16 (0.072-0.95)	15.11 (10.34-23.66)	1.09 (±0.04)	180
Indoxacarb 30% WG	9.26 (2.33-15.84)	50.60 (13.05-75.98)	3.67 (± 0.16)	180
Imidacloprid 25% WP	14.12 (11.46-22.89)	98.80 (60.33-127.06)	4.11 (± 0.20)	180

[a] Concentrations are expressed in ng/ml and the response determined after 24-h.
[b] *n*, no. of larvae tested including control.

Table 2: Lethal toxicity of selected pesticides on the 4th instar larvae of *Culex pipiens* after 48-h of exposure.

Compounds	LC_{50} [a] (95% CL)	LC_{90} [a] (95% CL)	Slope (± SE)	*n* [b]
Sulfoxaflor 30% WG+Spinetoram 10% WG	0.019 (0.00032-3.53)	4.34 (0.15- 7.92)	2.34 (± 0.09)	180
Abamectin 1.8% EC	0.034 (0.012-0.74)	17.70 (8.04-28.33)	1.98 (±0.09)	180
Spinosad 24% SC	0.020 (0.012-0.13)	4.02 (0.70-8.55)	2.17 (±0.09)	180
Emamectin benzoate 5% SG	0.0036 (0.0012-0.073)	11.50 (7.62-29.11)	0.54 (±0.04)	180
Indoxacarb 30% WG	1.09 (0.81-5.37)	30.77 (24.26-41.61)	2.41 (± 0.11)	180
Imidacloprid 25% WP	5.57 (1.09-17.92)	86.53 (23.83-115.88)	2.58 (± 0.12)	180

[a] Concentrations are expressed in ng/ml and the response determined after 48-h.
[b] *n*, no. of larvae tested including control.

Table 3: Lethal toxicity of selected pesticides on the 4th instar larvae of *Culex pipiens* after 72-h of exposure.

Compounds	LC_{50} [a] (95% CL)	LC_{90} [a] (95% CL)	Slope (± SE)	*n* [b]
Sulfoxaflor 30% WG+Spinetoram 10% WG	0.0048 (0.0014-0.014)	0.84 (0.18-4.50)	2.99 (± 0.10)	180
Abamectin 1.8% EC	0.0056 (0.0013-0.036)	10.52 (2.07-31.58)	2.46 (±0.10)	180
Spinosad 24% SC	0.0030 (0.00098-0.0084)	0.36 (0.078-7.56)	3.23 (±0.12)	180
Emamectin benzoate 5% SG	0.00079 (0.00041-0.0017)	10.42 (0.21-25.01)	0.61 (±0.04)	180
Indoxacarb 30% WG	0.14 (0.038-0.78)	21.96 (5.48-22.03)	3.08 (± 0.13)	180
Imidacloprid 25% WP	2.07 (0.071-7.08)	72.19 (6.87-82.16)	3.72 (± 0.13)	180

[a] Concentrations are expressed in ng/ml and the response determined after 72-h.
[b] *n*, no. of larvae tested including control.

Table 4: Toxicity of selected pesticides and the synergistic action of glyphosate on each pesticide on 4th instar larvae of *Culex pipiens* after 24-h exposure

Pesticides	*n*[a]	LC_{50} [b] (95% CL)	LC_{90} [b] (95% CL)	Slope (± SE)	SR_{50} [C]
Sulfoxaflor 30% WG + Spinetoram 10% WG	180	0.046 (0.0021-0.53)	8.16 (0.25-15.74)	0.57 (± 0.09)	1.83
Abamectin 1.8% EC	180	0.036 (0.0097-0.67)	64.41 (2.39-123.05)	3.31 (± 0.09)	2.44
Spinosad 24% SC	180	0.53 (0.26-4.17)	8.51 (1.86-21.27)	4.12 (± 0.21)	0.19
Emamectin benzoate 5% SG	180	0.48 (0.16-2.09)	14.99 (6.89-26.81)	3.05 (± 0.04)	0.33
Indoxacarb 30% WG	180	4.52 (0.041-6.67)	41.14 (9.38-61.12)	4.09 (± 0.12)	2.05
Imidacloprid 25% WP	180	3.37 (1.89-9.56)	86.98 (52.07-92.19	3.42 (± 0.11)	4.19

[a] *n*, no. of larvae tested including control.
[b] Concentrations are expressed in ng/ml and the response determined after 24-h of exposure.
[C] SR, synergistic ratio. Calculated by dividing LC_{50} for the pesticide by LC_{50} of the pesticide + glyphosate.
*Concentration of glyphosate was 10 µg/ml and larvae exposed to pesticides and glyphosate simultaneously.

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Table 5: Toxicity of selected pesticides and the synergistic action of glyphosate on each pesticide on 4th instar larvae of *Culex pipiens* after 48-h exposure

Pesticides	*n*[a]	LC_{50}[b] 95% CL	LC_{90}[b] 95% CL	Slope (± SE)	SR_{50}[C]
Sulfoxaflor 30% WG + Spinetoram 10% WG	180	0.0061 (0.0017-0.021)	1.96 (0.35-4.89)	3.85 (± 0.09)	3.11
Abamectin 1.8% EC	180	0.012 (0.0082-0.17)	15.03 (7.13-27.78)	3.91 (± 0.10)	2.83
Spinosad 24% SC	180	0.052 (0.031-0.35)	7.24 (1.28-52.95)	3.66 (± 0.10)	0.38
Emamectin benzoate 5% SG	180	0.00074 (0.00015-0.0011)	8.48 (2.98-14.67)	0.53 (± 0.03)	4.86
Indoxacarb 30% WG	180	0.35 (0.42-5.77)	36.29 (3.94-51.36)	3.76 (± 0.10)	3.11
Imidacloprid 25% WP	180	2.86 (0.93-6.46)	61.70 (56.01-84.89)	3.98 (± 0.10)	1.95

[a] *n*, no. of larvae tested including control.
[b] Concentrations are expressed in ng/ml and the response determined after 48-h of exposure.
[C] SR, synergistic ratio. Calculated by dividing LC_{50} for the pesticide by LC_{50} of the pesticide + glyphosate.
*Concentration of glyphosate was 10 µg/ml and larvae exposed to pesticides and glyphosate simultaneously.

Table 6: Toxicity of selected pesticides and the synergistic action of glyphosate on each pesticide on 4th instar larvae of *Culex pipiens* after 72-h exposure

Pesticides	*n*[a]	LC_{50}[b] 95% CL	LC_{90}[b] 95% CL	Slope (± SE)	SR_{50}[C]
Sulfoxaflor 30% WG + Spinetoram 10% WG	180	0.00054 (0.000053-0.0021)	0.20 (0.038-5.86)	3.21 (± 0.11)	8.89
Abamectin 1.8% EC	180	0.0019 (0.00082-0.027)	9.36 (4.11-17.08)	2.93 (± 0.12)	3.42
Spinosad 24% SC	180	0.010 (0.0058-0.063)	1.89 (0.34-5.33)	2.82 (± 0.10)	0.30
Emamectin benzoate 5% SG	180	0.00023 (0.00012-0.0037)	1.71 (0.037-4.13)	0.67 (± 0.04)	3.43
Indoxacarb 30% WG	180	0.013 (0.0029-0.72)	6.50 (1.36-16.17)	2.79 (± 0.12)	10.77
Imidacloprid 25% WP	180	0.90 (0.35-2.23)	26.90 (8.64-79.13)	3.87 (± 0.13)	2.30

[a] *n*, no. of larvae tested including control.
[b] Concentrations are expressed in ng/ml and the response determined after 72-h of exposure.
[C] SR, synergistic ratio. Calculated by dividing LC_{50} for the pesticide by LC_{50} of the pesticide + glyphosate.
*Concentration of glyphosate was 10 µg/ml and larvae exposed to pesticides and glyphosate simultaneously.

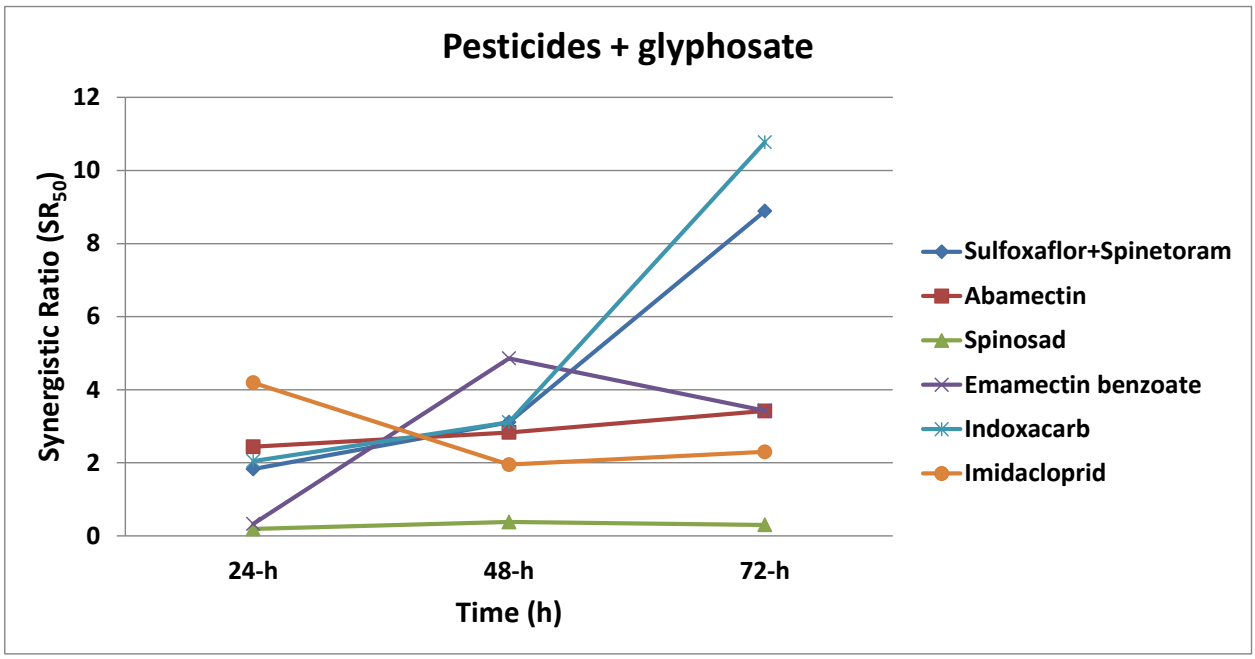

Fig. 1: Time-dependent changes in the synergistic ratio (SR_{50}) as calculated from LC_{50} values from Tables 4-6 for the combined treatments of pesticides and glyphosate as determined after 24, 48, and 72-h exposure.

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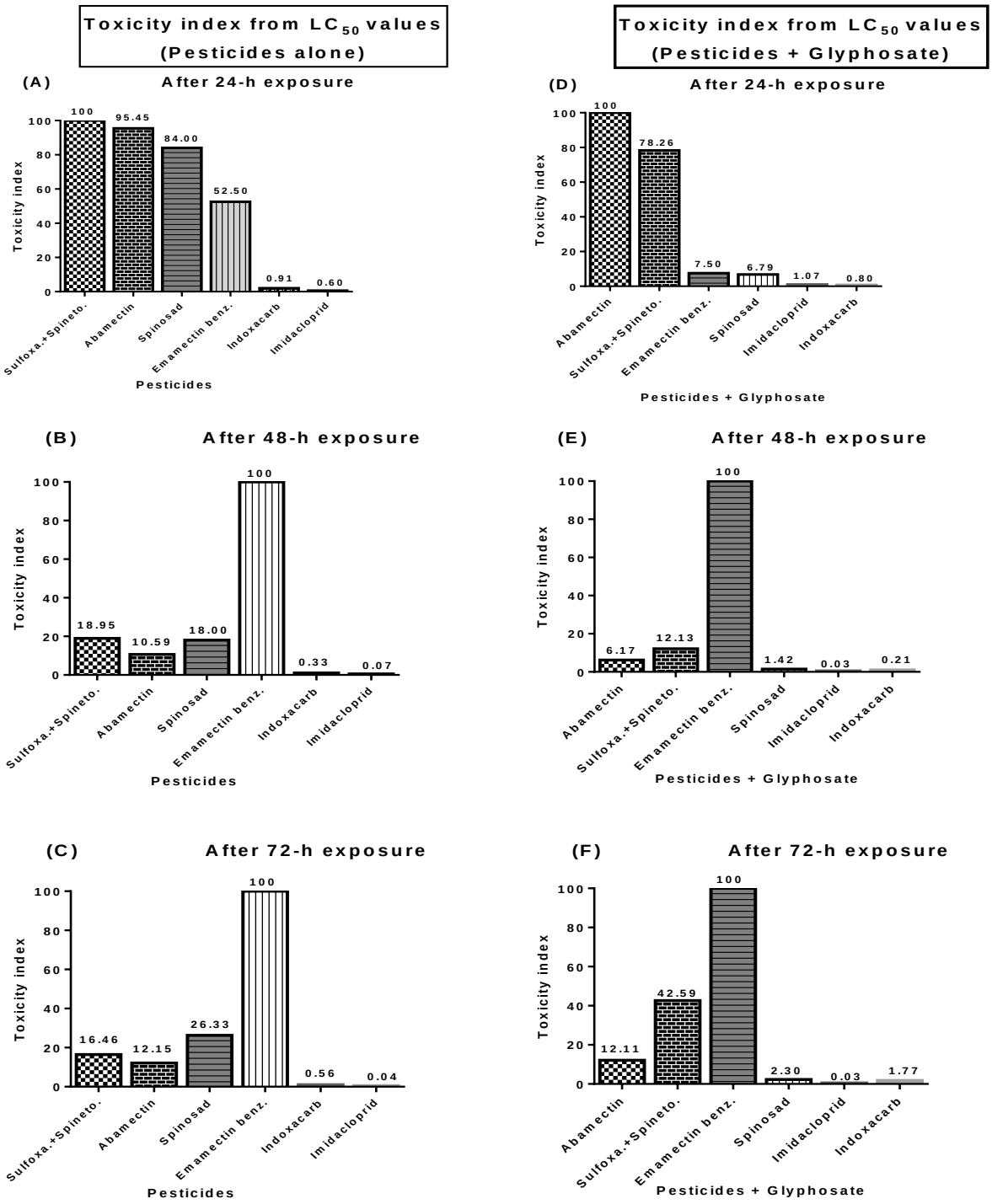

Fig. 2: Toxicity index of pesticides (A, B, and C) and pesticides + glyphosate (D, E, and F) on 4th instar larvae of *Culex pipiens* after 24, 48, and 72-h exposure. Toxicity index = [(LC_{50} of the most toxic tested pesticide / LC_{50} of the tested pesticide) ×100].

Toxicity index for selected pesticides in combination with glyphosate was showed in Figure 2 (D-F). After 24-h exposure, the values were 100, 78.26, 7.50, 6.79, 1.07, and 0.80 for abamectin, sulfoxaflor + spinetoram, emamectin benzoate, spinosad, imidacloprid, and indoxacarb, respectively. After 48-h exposure, the values were 6.17, 12.13, 100, 1.42, 0.03, and 0.21, respectively. Similar direction was observed after 72-h exposure.

Based on the LT_{50} values (Table 7-8), emamectin benzoate and abamectin demonstrated high efficiency against *Culex pipiens* (42.60 and 43.61 hours), and imidacloprid was the least effective (232.08 hours). Whereby, in the combination with glyphosate, sulfoxaflor + spinetoram and emamectin benzoate revealed the high potency against *Culex pipiens* (39.16 and 43.77 hours), and imidacloprid remain with the same trend of efficacy as least effective selected pesticide towards *Culex pipiens* (563.30 hours).

In spit of the fact that exposure of mosquito larvae to herbicides is considered prevalent, our knowledge and understanding of how these pesticides affect mosquito still limited. (Zahran, 2010) found that emamectin benzoate was the third most toxic tested compound against *Culex pipiens* after deltamethrin and Bti among the six tested compounds after 72-h exposure (LC_{50} was 0.07 ppm). In the same trend, emamectin benzoate was showed potency on *Culex quinquefasciatus* female and the LC_{50} was 0.27 µg/ml which considered the second most toxic compound among the 25 tested compounds (Shah *et al*., 2016).

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In general, the mechanism of how herbicides affected insects and other animals is still obscure. Scientists suggested that the herbicides can interfere directly and indirectly on other target sites on these organisms that due to disrupt effect on their physiological, genetic, and biochemical systems which lead to abnormal growth and death at the end (Lanctot *et al*., 2013; Lanctot *et al*., 2014; Mohamed *et al*., 2016; Saba *et al*., 2018). In this regards, glyphosate had been indicated as an inhibitor to the cytochrome P450 family enzymes and other enzymes that play an essential role through their disruption of the thyroid hormones pathway in the growth and development of animals (Lanctot *et al*., 2013; Kruger *et al*., 2014; Ibrahim, 2015; Ibrahim, 2016a,b).

Table 7: LT_{50} and LT_{90} with 95% confidence limits of selected insecticides at 0.01 ng/ml of 4th instar larvae of *Culex pipiens*

Pesticides	*n*[a]	LT_{50}[b] 95% CL	LT_{90}[b] 95% CL	Slope (± SE)
Sulfoxaflor 30% WG + Spinetoram 10% WG	180	66.48 (47.15-292.26)	337.87 (135.61-397.45)	1.82 (± 0.72)
Abamectin 1.8% EC	180	43.61 (27.98-51.02)	1619.08 (983.67-1892.23)	0.82 (± 0.68)
Spinosad 24% SC	180	57.17 (40.09-168.39)	298.74 (125.47-205.27)	1.78 (± 0.71)
Emamectin benzoate 5% SG	180	42.60 (30.62-57.40)	143.57 (88.92-78.63)	2.43 (± 0.71)
Indoxacarb 30% WG	180	97.80 (75.42-132.92)	191.15 (113.15-692.74)	4.42 (± 1.74)
Imidacloprid 25% WP	180	232.08 (189.66-356.42)	642.88 (576.71-709.24)	2.90 (± 1.33)

[a]*n*, no. of larvae tested including control.
[b] LT_{50} and LT_{90} values are expressed in hours.

Table 8: LT_{50} and LT_{90} with 95% confidence limits of selected insecticides in combination with glyphosate at 0.01 ng/ml of 4th instar larvae of *Culex pipiens*

Pesticides	*n*[a]	LT_{50}[b] 95% CL	LT_{90}[b] 95% CL	Slope (± SE)
Sulfoxaflor 30% WG + Spinetoram 10% WG	180	39.16 (27.84-50.66)	122.85 (80.85-450.59)	2.58 (± 0.72)
Abamectin 1.8% EC	180	56.42 (48.09-71.43)	204.39 (189.66-367.43)	0.50 (± 0.67)
Spinosad 24% SC	180	65.75 (52.90-72.62)	890.49 (734.23-1004.12)	1.13 (± 0.69)
Emamectin benzoate 5% SG	180	43.77 (28.65-66.35)	190.89 (100.38-484.99)	2.00 (± 0.70)
Indoxacarb 30% WG	180	78.33 (64.71-132.24)	155.45 (104.52-654.28)	4.31 (± 1.28)
Imidacloprid 25% WP	180	563.30 (467.84-681.65)	1359.98 (1154.27-1583.74)	0.93 (± 0.85)

[a]*n*, no. of larvae tested including control.
[b] LT_{50} and LT_{90} values are expressed in hours.

Further, herbicide formulations contained some chemical additives such as surfactants that considered highly toxic on different aquatic animals and insects (Tu *et al*., 2001; Mohamed *et al*., 2016; Saba *et al*., 2018). Moreover, (Janssens and Stoks, 2017) revealed that the formulation of roundup was more toxic on insects than their active ingredient if tested alone. Certain herbicides such as Fluazifop-p-butyl and fenoxaprop-p-ethyl exhibited high toxicity against C*ulex pipiens* larvae with LC_{50} values of 2.69 and 2.05 µg/ml after 24-h exposure, respectively (Mohamed *et al*., 2016).

Particularly, the treatment of *Aedes aegypti* larvae with glyphosate resulted in increase of the induction of certain enzymes such as P450s, GSTs, and carboxylesterase in larvae which resulted in confessing the larvae tolerance to the tested insecticides that commonly used in the field of mosquitoes control, propoxur, B.t., permethrin, and imidacloprid (Raiz *et al*.,2009; David *et al*., 2010).

No reliable data are available on the synergistic effect of glyphosate in combination with other pesticides on *Culex pipiens* mosquito. However, certain studies have been done on the compatibility of glyphosate-pesticides combination on some pests. (Xiao-yan *et al*., 2016) stated that addition of glyphosate to acephate pesticide improved cotton aphid control compared with acephate alone. In contrast, addition of glyphosate to carbosulfan, the rest of the pesticides such as endosulfan, imidacloprid, or lambda-cyhalothrin did not affect the aphid control when compared with the insecticide alone treatments. In the same trend, (Scroggs *et al*., 2005) evaluated 15 different insecticides and they demonstrated that insecticides acephate, acetamiprid, bifenthrin, cyfluthrin, cypermetherin, dicrotophos, dimethoate, emanectin benzoate, imidacloprid, indoxacarb, lambda-cyhalothrin, methoxy-fenozide, spinosad, thiamethoxam, and zeta-cypermethrin which applied in mixture with glyphosate resulted in no reduction in visual weed control or biomass of tested weeds in comparison with glyphosate alone. Further, (Pankey *et al*., 2004) revealed that lambda-cyhalothrin, acephate, dicrotophos, dimethoate, imidacloprid, oxamyl, and endosulfan did not affect efficacy of glyphosate on control of selected weeds such as pitted morningglory, prickly sida (*Sida spinosa* L.), and redweed (*Melochia corchorifolia* L.); however, mixing of lambda-cyhalothrin or fipronil with glyphosate minimized the control of hemp sesbania by 19 and 9%, respectively, when compared with glyphosate alone. Further, (Mascarenhas and Griffin, 1997) demonstrated that mixing of imidacloprid to glyphosate decreased barnyardgrass control, in addition, mixing chlorpyrifos, fipronil, methamidophos, and imidacloprid with glyphosate lessened the pitted morningglory control when compared to the glyphosate alone. Also, (Pankey *et al.*, 2004) stated that the mixture of glyphosate with dicrotophos and imidacloprid improved cotton aphid and thrips control respectively compared to insecticide alone. Furthermore, (Sparks *et al.*, 2003) reported that *Helicoverpa zea* Boddie control was not minimized by the mixture of glyphosate and emamectin. However, the reasons behind the synergistic action of glyphosate on the activities of the selected pesticides may be due to the increase of theses pesticides penetration or uptake, inactivation of detoxification enzymes, and/or generic sensitization of the nervous system of *Culex pipiens*.

Overall, this study emphasized on the potency of selected pesticides on the 4th instar larvae of *Culex pipiens* and the synergistic action of glyphosate on the tested pesticides. Further biochemical and molecular biological investigation should be proceeded to get better understanding about the functional role of glyphosate in changing the behavior and ecology of *Culex pipiens* mosquito. Furthermore, field and semi-field experiments should be done to illustrate the efficacy of these pesticides alone or in combination with glyphosate under field conditions.

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