ZANCO Journal of Pure and Applied Sciences
The official scientific journal of Salahaddin University-Erbil
https://zancojournals.su.edu.krd/index.php/JPAS

ISSN (print):2218-0230**, ISSN (online):** 2412-3986**, DOI: http://dx.doi.org/10.21271/zjpas**

RESEARCH PAPER

The effect of Diflubenzuron on Aphis fabae Scopoli and the vitality of their natural predator Coccinella septempunctat (L.)

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A B S T R A C T:

The results of the study of integration between the insect antimoulting compounds like diflubenzuron and the ladybird *Coccinella septempunctata* (L.) to control insects from the Black bean aphid, *Aphis fabae* Scopoli (Hemiptera: Aphididae). The results showed a good effect against insects Black bean aphid, *Aphis fabae* Scopoli. The results also revealed the existence of variation in a predation efficiency of male and female according the treatment method and Diflubenzuron concentration, the highest mean of males predation efficiency reached 52.7 ±4.7 % and for females 55.8 ±4.3 % when treating the plant leaf by Diflubenzuron concentration 0.15% respectively. The results also showed a variation in the mean mortality percentage of *Coccinella septempunctata* male and female and after 5 days from the treatment, highest mortality percentage in male and female were 65.4 ±3.8, 62.5 ±5.3 % respectively, when spraying at concentration of 0.5% on plant leaf with both prey and predator.

KEY WORDS: Insect, Pesticides, ladybird, Black bean aphid and plant production.
DOI: http://dx.doi.org/10.21271/ZJPAS.32.5.18
ZJPAS (2020), 32(5); 179-185.

1. INTRODUCTION

Black bean aphid, *Aphis fabae* Scopoli (Hemiptera: Aphididae) is generally considered a serious pest which cause damages in more than 200 leguminous plants and infesting all plant parts, particularly in northern Europe and worldwide, this pest has holocyclic life cycle with appear in early summer while disappear in winter and overwinter as eggs (Hansen et al., 2008) and (Barnea et al., 2005). This pest causes huge physiological changes in their hosts and reduce plant productivity in many cases (Minks et al., 1987). Many studies attempt to determine the rate of damage after the attacked by black bean aphid or other insect pests and recorded that yield losses can exceed 50% ((Hinz and Daebeler, 1981); (Shannag, 2007); (Ali, 2011) and (Sillero et al., 2017)).

Many Insecticides have been used for control of aphids on legume crops, for example in Iran insecticides like abamectin, imidacloprid and pymetrozine used to prevent these pests (Sabahi et al., 2011), while more recent investigations focused and used diflubenzuron as insecticide against black aphid (Bansal et al., 2012). Also, in Kurdistan region of Iraq crop production have been decreased due to insect pests, thus it is management is important (Ali, 2019). Direct relation present between plant infestation by aphids and predatory insects like *Coccinella septempunctata* which due to volatile compounds induce by plant host (Zhu and Park, 2005).

Diflubenzuron Dimilin is an insecticide of the benzoylurea class which widely have been used for selective control of insect pests (Subrero et al., 2019). A study by (Harðardóttir et al., 2019), explained the mechanism of diflubenzuron action on arthropod pests and confirmed that

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Article History:
Received: 21/12/2020
Accepted: 15/06/2020
Published: 13/10 /2020

diflubenzuron inhibit the production of chitin which used by an insect to build its exoskeleton. Previous study concluded that antimoulting compounds like diflubenzuron and zertel showed a drastic effect on the bioactivity of entomophagous insect (Zaki and Gesraha, 1987). More-ever, diflubenzuron significantly reduce the growth of *Coccinella septempunctata* in cotton fields as has been shown by (Keever et al., 1977).
The present study aimed to determine rate and efficiency of diflubenzuron Dimilin with different concentration against *Aphis fabae* and predator *Coccinella septempunctata.*

MATERIALS AND METHODS

The present study was conducted during 2017 _2018 at the department of Plant Protection _ Faculty of Agriculture and Forestry University of Mosul and carried out under controlled condition (average temperature of 20+ 5 C° and relative humidity 45+ 5%) during the year 2017 - 2018.

Sample collection

The different ages of *Aphis fabae* and *Coccinella septempunctata* were collected from a pea filed (which located in), they were transferred to the laboratory via sterile plastic container with a diameter of 9 cm and examined daily to obtain adult insects of females and males.

Larvicidal activity of Diflubenzuron (Dimilin®) on *Aphis fabae*

Three different concentration of diflubenzuron (0.15, 0.3 and 0.5) have been used each with three replications on twenty immature insects of *Aphis fabae*, 2 ml of each concentration placed on the leaves of pea plant in a covered petri-dish and leaved for about 24 hours under laboratory conditions, finally mortality rate examined and recorded using Abbott equation (Bari et al., 2010).

The effect of diflubenzuron on the mortality of *Coccinella septempunctat*

Three types of treatment have been used each with three concentration of the insecticide diflubenzuron (Dimilin) (0.15, 0.3 and 0.5) on *Coccinella septempunctata*, in order to determine the best efficiency, first treatment included leaf plant, second treatment included leaf plant with the aphids and third treatment included sprinkles each of the plant leaf with both prey and predator. For each treatment 2 ml of each concentration prepared and sprayed. Ten replications of each concentration increased the rate of accuracy and also for the purpose of comparison control treatment has been sprayed with 2 ml of distal water. Finally, all data recorded after 5 days of treatment under laboratory condition and results corrected using Abbott equation. The results were analyzed using the complete global trial design and the Duncan test at a 5% probability level to determine differences between the average (O'Rourke et al., 2005).

RESULTS

Larvicidal activity of diflubenzuron Dimilin on *Aphis fabae*

The highest mortality rate of diflubenzuron Dimilin agansit *Aphis fabae* recorded at the concentration of 0.5 (76.4%), while the lowest effect of this pesticide recorded at the concentration of 0.15 (33.4%) as shown in (Table 1).

Table 1. Effect of the pesticide on *Aphis fabae*

Con.%	% Mortality	
	Rang	Mean ± S.E
0.15	20-53	33.4 ± 8.7 c
0.3	38-71	45.8 ± 7.4 b
0.5	67-92	76.4 ± 6.4 a

The effect of diflubenzuron on males of ladybird under different treatments

In leaf treatment, the results showed that diflubenzuron as an insecticide had a significant effect on efficiency of male ladybird, with increasing the concentration of this pesticide the efficiency of male ladybird decrease, for example the highest efficiency range (43.3-60.5) recorded at the concentration of 0.15. In second treatment when both leaf plant and aphids sprayed with the insecticide, result also showed that the efficiency range increase with the decrease of insecticide concentration as highest efficiency rang was (21.65-64.15) at the concentration of 0.15. this was correct for third treatment as well which

lowest concentration recorded highest efficiency rang (32.5-57.5) as has been shown in (Table 2).

Table 2. Effect of different concentrations of the insecticide and treatment method on predatory efficiency of male ladybird.

Treatments	% Con.	Efficiency		Treatments		Concentrations	
		Rang	Mean ± S.E	Rang	Mean ± S.E	Range	Mean ± S.E
first	0.15	43.3-60.5	52.7 ±4.7 a	40.8-57.75	50.2 ± 4.2 a	35.25-55.8	48.9 ± 4.1 a
	0.3	22.8-69.15	49.2 ±5.8 b				
	0.5	40.8-60.8	48.5 ±4.3 b				
second	0.15	21.65-64.15	43.9 ±5.8 c	13.6-64.15	41.3 ±5.9 b	22.5-56.65	47.65 ± 4.8 a
	0.3	19.15-69.15	45.4 ±6.7 cd				
	0.5	8.3-59.15	34.7 ±8.8 d				
third	0.15	32.5-57.5	50.1 ±5.7 ab	26.35-1.65	46.35 ±4.5 a	23.05-52.7	41.35 ± 6.8 b
	0.3	21.65-60	48.2 ±6.5 b				
	0.5	25-52.5	40.7 ±5.8 c				

* Numbers under the same letter or similar letters do not have significant differences according to Duncan polynomial test at a probability level of 5%

The effect of diflubenzuron on females of ladybird under different treatments

The result in (Table 3), illustrated that with decreasing the concentration of mentioned pesticide the efficiency rang increase. The highest recorded efficiency rang were (50-65.8), (45-57.5) and (43.3-64.15) at concentration of 0.15 for first, third and second type of treatment, respectively.

Table 3. Effect of different concentrations of insecticide and treatment method on predatory efficiency of female ladybird.

Treatments	% Con.	Efficiency		Treatments		Concentrations	
		Rang	Mean ± S.E	Rang	Mean ± S.E	Range	Mean ± S.E
First	0.15	50-65.8	55.8 ±4.3 a	49.1-63.3	54.2 ±3.2 a	48.6-60.5	54.1 ±1.8 a
	0.3	37.5-65.8	52.1 ±9.1 b				
	0.5	40.8-65.8	54.6 ±6.6 a				

Second	0.15	43.3-64.15	55 ±6.5 a	30.8-60.8	48.75±7.5 b	30-61.1	48.85 ± 8.8 b
	0.3	13.3-61.5	45.5 ±8.7 d				
	0.5	25.8-60.8	45.7 ±9.4 d				
Third	0.15	45-57.5	51.5 ±2.7 b	36.1-53.8	49.7 ±3.8 b	34.1-58.3	49.65 ±4.7 b
	0.3	34.15-57.5	48.9 ±5.8 c				
	0.5	24.15-60.8	48.9 ±6.1 c				

* Numbers under the same letter or similar letters do not have significant differences according to Duncan polynomial test at a probability level of 5%

The effect of diflubenzuron on males and females of ladybird under different treatments

The results in (Table 4 and 5) showed killing rate of this insecticides with different methods of treatment on the predatory efficiency of both males and females ladybird, killing rate increased with high concentration for all three treatment methods and there were significant differences in average mortality depending on the treatment method and the used concentration.

for male ladybird the highest mortality percentage was (57-71%) at the diflubenzuron concentration of 0.5 (third treatment methods), while for female ladybird mortality percentage was (42.5-71%) at same concentration of diflubenzuron (third treatment methods).

Table 4. Effect of different concentrations of insecticide and treatment method on the mortality of ladybirds' male

Treatments	**% Con.**	**Mortality%**		**Treatments**		**Concentrations**	
		Rang	**Mean ± S.E**	**Rang**	**Mean ± S.E**	**Range**	**Mean ± S.E**
First	0.15	0-28.5	17.1 ±4.2 h	19-33	26 ±2.8 c	14-42.5	30.2 ±5.25 c
	0.3	0-43.5	25.6 ±3.3 g				
	0.5	28.5-42.5	36.9 ±4.5 e				
second	0.15	0-28.5	22.8 ±2.5f	33-42.5	36.7 ±2.3 b	28-52	38.6 ±4.3 b
	0.3	14-57	39.7 ±6.5 d				
	0.5	42.5-57	48.3 ±4.1 c				
third	0.15	14-71	51.1 ±6.3 b	33-66	54.8 ±1.7 a	47-52	48.2 ±1.4 a
	0.3	28.5-71	51.1 ±5.1 b				
	0.5	57-71	65.4 ±3.8 a				

* Numbers under the same letter or similar letters do not have significant differences according to Duncan polynomial test at a probability level of 5%

Table 5. Effect of different concentrations of insecticide and treatment method on the mortality of ladybirds' female

Treatments	**% Con.**	**Mortality%**		**Treatments**		**Concentrations**	
		Rang	**Mean ± S.E**	**Rang**	**Mean ± S.E**	**Range**	**Mean ± S.E**
first	0.15	28.5-57	37 ±6.8 e	37.5-53	49.1 ±3.2 a	19-42.5	31 ±8.3 c
	0.3	14-71	45.5 ±9.5				

			d				
	0.5	27-71	45.4 ±3.8 b				
second	0.15	14-28.5	19.5 ±4.1 h	28-42.5	32 ±3.3 b	23.5-47	40.4 ±4.5 b
	0.3	14-42.5	28 ±3.5 g				
	0.5	28.5-71	48 ±7.6 c				
third	0.15	14-71	37 ±8.8 e	37.5-66	49 ±6.4 a	52-61.5	58.6 ±2.4 a
	0.3	42.5-57	48 ±4.2 c				
	0.5	42.5-71	62.5 ±5.3 a				

* Numbers under the same letter or similar letters do not have significant differences according to Duncan polynomial test at a probability level of 5%

DISCUSSION

Originally, insecticides have a great role in the managements of insect pests, however, the concentration and treatment methods of insecticide critical as adverse effects of insecticide on soil characteristics has been reported (Khudhur and Sarmamy, 2019). Diflubenzuron was first discovered as larvicidal after ingestion, however more comprehensive studies determined that this pesticide could also prevent hatching of eggs after direct contact of eggs or after treatment method of females (Grosscurt, 1976) and (Singh, 2015). A study by (Mulder and Gijswijt, 1973), explained the mechanism of action by diflubenzuron on larva and observed the histological changes could be resulted due to this insecticide which reported that diflubenzuron blocking of the formation of the cuticle (Mulder and Gijswijt, 1973). Additional biochemical studies resulted in a number of hypotheses about the primary mode of action of diflubenzuron in insects and explanations of the mode of action of diflubenzuron based on activation of chitinase, phenoloxidase, or on effects on ecdyson-metabolizing enzymes have probably to be considered as secondary (Verloop and Ferrell, 1977) and (Kumar et al., 2019). Many investigations have shown azadirachtin and diflubenzuron to be more toxic towards earlier instar larvae than later instar larvae when targeting a variety of other insect pests including semilooper, *Achaea janata* (Linnaeus), tobacco leaf eating caterpillar, *Spodoptera litura* (Fabricius), sweetpotato whitefly, *Bemisia tabaci* (Gennadius) and root weevil, Diaprepes abbreviates (Mule and Patil, 2000) and (Kumar et al., 2005).

From the present study highest mortality rate of diflubenzuron (Dimilin®) agansit *Aphis fabae* recorded at the concentration of 0.5 (76.4%), while the lowest effect recorded at the concentration of 0.15 (33.4%), thus with increasing concentration the effects of present pesticide has been increased directly on *Aphis fabae* and this result is agree with previous findings by Grosscurt (1978), which confirmed that insecticide action increase with increasing concentration of diflubenzuron. Another method to control fly, larvae by oral intake of diflubenzuron has been explained by (Grosscurt, 1978) , which mixed into poultry or cattle feed or into mineral blocks, in this application of diflubenzuron as a food additive, *Stomoxys calcitrans* seems to be more susceptible than *Musca domestica*.

In France a study done by Schwenke (1979), which concluded that difiubenzuron has been registered against *Thaumetopoea pityocampa* (Denis & Schiffermüller) at a concentration of 150 g considered as a great deal of field management for *T. pityocampa* . Other studies determined that some of the insecticides elicited a drastical reduction of the fecundity, especially in ladybirds fed with contaminated aphids (e.g. with teflubenzuron, fenoxycarb and flufenoxuron), moreover, chlorfluazuron was the most dangerous one for almost all larval stages (Olszak et al., 1994).

CONCLUSION

Concentration of the insecticide diflubenzuron was critical as highest mortality rate of

diflubenzuron on *Aphis fabae* recorded at the concentration of 0.5 (76.4%), while the lowest mortality rate recorded at the concentration of 0.15 (33.4%). The effects of diflubenzuron on ladybird were differ depended on treatment methods, concentration and sex of ladybird, for example highest recorded efficiency rang were (50-65.8), (45-57.5) and (43.3-64.15) at concentration of 0.15 for first, third and second type of treatment, respectively.

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