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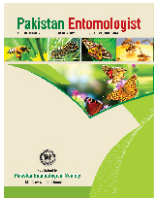

EFFECT OF NEW CHEMISTRY INSECTICIDES ON THE ESTERASE ACTIVITY OF *BREVICORYNE BRASSICAEA* (HOMOPTERA: APHIDIDAE)

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ABSTRACT

Insecticides are widely used to control cabbage aphids attacking on the various agricultural crops. Esterase is one of the enzymes which cause the detoxification of the insecticides leading to the resistance against the insecticides. In the present study four new chemistry insecticides and a plant extract were used against adult of wingless cabbage aphid (*Brevicoryne brassicae)*. Three different concentrations, LC_{40}, LC_{50} and LC_{60} of each insecticide were used. Esterase activity in insects against each insecticide was determined after the 1 hour and 24 hours. Application of LC_{50} of diafentiuron after I hr and 24 hrs, esterase activity was 222.3 µmol/min/mg and 313.33 µmol/min/mg respectively and at LC_{60} after I hr and 24 hrs, the esterase enzymatic activity was 173.66 µmol/min/mg and 293.33 µmol/min/mg respectively. Esterase specific activity of LC_{50} of bifenthrin was 322.5 µmol/min/mg after 1 hr and decreased down to 158.033 µmol/min/mg and at LC_{60} activity was 303.1 µmol/min/mg after 1 hr and decreased down to 185.07 µmol/min/mg after 24 hrs. Higher esterase activity shows higher resistance of insect against insecticide and its fewer efficacies. The results showed that pymetrozine is less effective while bifenthrin is more effective insecticide due to lower esterase activity.

Keywords: *Brevicoryne brassicae*, Esterases, New chemistry insecticides

INTRODUCTION

Brassica crops are considered to be the second most important source for edible oil production. Oilseeds contribute around 10 % in national edible oil production under 482 thousand acres area during 2012-2013 in Pakistan. Its total seed production was 176 thousand tonnes (Anonymous, 2013). Cabbage, rapeseed, mustard, cauliflower and canola are the major Brassica crops of Pakistan. A number of factors are involved for the low yield in Brassica production all around the world. In Pakistan, attack of insect pest on Brassica crop is the major limiting factor for low and deteriorated oil seed production among all factors. A number of aphid species attack on Brassica. More than 4000 aphid species have been described and out of these 250 aphid species are severe pests of different crops and ornamental plants throughout the world (Blackman and Eastop, 2007).

Brevicoryne brassicae (Cabbage aphid) is severe pest of oil seed Brassica and horticultural crops. It feeds almost completely on the phloem sap of the plant (Singh *et al*., 1994; Cole, 1997). *B. brassicae* is blue-grey colored small aphids having black markings and short cornicles (tube like structures at the end of abdomen). Females are grayish green in color having apterous and viviparous (Tariq *et al*., 2010).
Different Pesticides are used to control this pest. These pesticides are extensively used due to easy in handling and have quick results. On the other hand different problems i-e contamination of environment, health of farmers, residues on consumer products and insecticide resistance are caused by indiscriminate use of chemicals (Bullangpoti *et al.,* 2007; Hussain *et al*., 2002).
There are number of factors responsible for the detoxification of insecticides inside the insect body. One of these factors is the enzymes, which are synthesized in the intestine and fat

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body of insects and are the most important constituent of insect metabolism (Yu, 2004). Esterase is one of the most important enzymes which are responsible for the detoxification of insecticides within the insect body. Esterase constitutes widely distributed family of enzymes which hydrolyze specific bond in a variety of compounds. Main objective of the present study was to evaluate the activity of esterase enzymes in cabbage aphid (*B. brassicae*) against commonly used insecticides.

MATERIALS AND METHODS

Field collection and rearing of *Brevicoryne brassicae*

Aphids were collected from the cabbage fields in district Rawalpindi. Equal numbers of apterous adults were transferred individually to each potted host plant. The individual leaves were covered with cone shaped transparencies in order to restrict the movement of aphids. All the plants were covered with a fine net in order to prevent the attack of cabbage aphids by parasites and predators. The potted plants were kept under an LD 16: 8 hours with constant temperature (25 ± 2 ºC) and relative humidity (75 ± 5 %).

Insecticides

Five different insecticides including imidacloprid (Confidor® 20 SL, Bayer Crop Sciences Pvt. Pakistan), bifenthrin (Tallstar, FMC Pvt. Pakistan), diafenthiuron (Polo® 500 SC, Syngenta Pvt. Pakistan), pymetrozine (Chess 25% WP, Bayer Crop Sciences Pvt. Pakistan) and azadirachtin (Neem extract) were used to evaluate the esterase enzymatic activity of cabbage aphid at LC_{40}, LC_{50} and LC_{60} concentrations.

Toxicity bioassay

Leaf dip bioassay was carried out to determine the lethal concentrations (LC_{50}) of the test insecticides. Cabbage leaves were dipped in the aqueous solution of any of the tested insecticide for about 10 second and allowed to dry on slightly moistend filter paper in labeled Petri dishes (60 mm diameter). Ten apterous adults of *B. brassicae* were placed on the treated leaf surface, while leaves dipped in water serve as controls. Five replicate batches of aphids (i.e, 50 insects) were used for each insecticide concentration and six concentrations were used for each insecticide. Mortality was accessed 24, 48 and 72 hours after treatment. Three different concentrations i.e., LC_{40}, LC_{50} and LC_{60} were used to observe the esterase enzyme activity of *B. brassicae.*

Enzyme extraction

For carboxylic esterase, aphids treated with LC_{40}, LC_{50} and LC_{60} of insecticides were grinded in 3 ml of ice–cold sodium phosphate buffer (0.04 M, pH 7.0) in a tissue grinder. The homogenates were centrifuged at 10,000g for 15 min at 4 ºC. The resulting supernatants were used as the enzyme sources. Aphids treated with water were used as control. The samples were taken after 1h and 24 h.

Enzyme assays

Esterase assays were performed by mixing 1 ml of enzyme stock solution (10 µl of supernatant of aphid homogenate in 990 µl phosphate buffer (40 mM, pH 6.8) into 5 ml of substrate solution (30 mM α-naphthyl acetate). Mixture was incubated at 30°C for 20 min and then 1 ml of staining solution (Fast blue BS salt (1%), phosphate buffer (0.04 M) and SDS 5% w/v) was added and again incubated for 20 minutes. After that absorbance was taken spectrophotometrically at 590 nm to find esterase activity.

Enzyme Activity

Enzymatic activity of esterase was measured as follow:

$$\text{Enzyme Activity} = \frac{\text{Absorbance at 590nm} \times \text{Standard factor} \times \text{D.F}}{20}$$

Statistical analysis

LC_{40}, LC_{50} and LC_{60} values of the insecticide were determined with R version 2.9.0 (R Development Core Team, 2009).

RESULTS

Lethal concentrations of insecticides

Lc_{40}, LC_{50} and LC_{60} values of each insecticide were given in Table 1. LC_{50} values of imidachloprid, diafenthiuron, azadiractin, pymetrozine and bifenthrin were 32.26, 13.31, 13.60, 316.79 and 9.74 ppm, respectively.

Esterase activity in *B. brassicaea* in control treatment

The esterase enzymatic activity in control treatment was 250 µ mol/min/mg protein after 1h and 24 hr (Table 2 & 3).

Esterase activity in *B. brassicaea* after the application of imidacloprid

Esterase enzymatic activity at $LC_{40,}$ LC_{50} and LC_{60} after 1 h of application were measured 253.86, 275.13 and 164.66 µmol/min/mg, respectively (Table 2). Esterase enzymatic activity at $LC_{40,}$ LC_{50} and LC_{60} after 24 h of application were measured 221.59, 389.83 and 349.31 µmol/min/mg, respectively (Table 3).

Esterase activity in *B. brassicaea* after the application of diafenthiuron

Esterase enzymatic activity at $LC_{40,}$ LC_{50} and LC_{60} after 1 h of application were measured 234.31, 222.30 and 173.66 µmol/min/mg, respectively (Table 2). Esterase enzymatic activity at $LC_{40,}$ LC_{50} and LC_{60} after 24 h of application were measured 208.5, 313.33 and 293.33 µmol/min/mg respectively (Table 3).

Esterase activity in *B. brassicaea* after the application of pymetrozine

Esterase enzymatic activity at $LC_{40,}$ LC_{50} and LC_{60} after 1 h of application were measured 312.66, 484.20 and 322.33 µmol/min/mg, respectively (Table 2). Esterase enzymatic activity at $Lc_{40,}$ Lc_{50} and LC_{60} after 24 h of application were measured 344.26, 383.33 and 392.80 µmol/min/mg, respectively (Table 3).

Esterase activity in *B. brassicaea* after the application of bifenthrin

Esterase enzymatic activity at $LC_{40,}$ LC_{50} and LC_{60} after 1 h of application were measured 212.76, 222.50 and 203.10 µmol/min/mg, respectively (Table 2). Esterase enzymatic activity at $LC_{40,}$ LC_{50} and LC_{60} after 24 h of application were measured 185.40, 158.03 and 185.07 µmol/min/mg, respectively (Table 3).

Esterase activity in *B. brassicaea* after the application of azadirachtin

Esterase enzymatic activity at $LC_{40,}$ LC_{50} and LC_{60} after 1 h of application were measured 294.00, 254.33 and 254.43 µmol/min/mg, respectively (Table 2). Esterase enzymatic activity at LC_{40}, LC_{50} and LC_{60} after 24 h of application were measured 313.73, 332.23 and 303.30 µmol/min/mg,

Table 1

Lethal concentration (LC_{40}, LC_{50} and LC_{60}) values of insecticides against *B. brassicaea*.

Insecticides	LC (ppm)			Slope ±SE	N
	LC_{40} (Fuditial limit)	LC_{50} (Fuditial limit)	LC_{60} (Fuditial limit)		
Imidacloprid	9.24 (3.34- 48.27)	32.26 (9.91- 61.99)	112.69 (26.378- 303.7)	0.47 ±0.08	300
Diafenthiuron	4.08 (1.749-8.57)	13.31 (6.34- 31.56)	43.46 (19.53-136.67)	0.49 ±0.09	300
Azadirachtin	5.34 (2.82-20.46)	13.60 (5.73-98.47)	34.63 (11.36- 86.82)	0.62±0.16	300
Pymetrozine	112.41 (55.90-256.82)	316.79 (160.50 -1009)	819.84 (461.27-1163.7)	0.61 ±0.17	300
Bifenthrin	2.32 (.80-5.45)	9.75 (4.13-26.71)	40.91 (16.31-71.49)	0.41±0.08	300

Table 2

Mean esterase activities (µmol/min/mg) in *B. Barassicae* at LC_{40}, LC_{50} and LC_{60} of insecticides after 1 hour.

Insecticide	Control	LC_{40} ppm	LC_{50} ppm	LC_{60} ppm
Imidachloprid	250	253.86	275.13	264.66
Diafenthiuron	250	234.31	222.30	173.66
Pymetrazine	250	312.66	484.20	322.33
Azadirachtin	250	294.00	254.33	254.43
Bifenthrin	250	212.76	222.50	203.10

Table 3

Mean esterase activities (µmol/min/mg) in *B. Barassicae* at LC_{40}, LC_{50} and LC_{60} of insecticides after 24 hour.

Insecticide	Control	LC_{40} ppm	LC_{50} ppm	LC_{60} ppm
Imidachloprid	250	221.56	389.83	349.31
Diafenthiuron	250	208.50	313.33	293.33
Pymetrazine	250	344.26	383.33	392.80
Azadirachtin	250	313.73	332.23	303.30
Bifenthrin	250	185.40	158.03	185.07

respectively (Table 3).

DISCUSSION

The toxicity of insecticide varied according to the species of insects, insect age and the chemical structure of the insecticide. The results of the present study revealed that bifenthrin was more toxic against *B. brassicaea* at LC_{40}, LC_{50} and LC_{60} than imidachloprid, diafenthiuron, azadirachtin and pymetrozine. In another study, imidacloprid was tested against *M. persicae* and *A. gossypi* and their LC_{50} values were 21.25 ppm and 22.34 ppm, respectively (Khalequzzaman and Nahar, 2008). Diafenthiuron showed LC_{50} values of 52.75 ppm against radish aphid (Halder *et al*., 2011). Cowpea aphid (*Aphid craccivora*) treated with pymetrozine which is a new chemistry insecticide demonstrated LC_{50} values of 28.6 ppm (Mokbel, 2007). The insecticides with lower LC_{50} values were more effective (Ahmad and Aslam, 2005).

Detoxification enzymes are generally demonstrated as the enzymatic defense against foreign compounds and play important roles in maintaining their normal physiological functions. The suppression of these enzymes after the application of insecticide indicated that these are not responsible for the detoxification of insecticide and may be increase the susceptibility of insect pest to these insecticides (Li and Liu, 2007).

Variation in esterases activity depends upon the insecticide. Specific activity of esterase fluctuated up and down from the normal count due to destruction caused by application of insecticide. Effect of imidacloprid on carboxylesterase activity extracted from two populations of cotton aphid and melon aphid showed esterase activity of 124.6 µmol/min/mg and 71.6 µmol/min/mg, respectively (Tabasian *et al*., 2010). A population of *M. persicae* had the highest esterase activity of 214.2 µmol/min/mg protien (Srigiriraju, 2009). Mujeeb and Shakoori (2012) determined the specific activity of esterase of FSS-11 strain of red flour beetle (*Tribolium castaneum*) at 4^{th}, 6^{th} instar larvae, newly emerged beetles and 15 days old beetles and reported a decrease of 45, 33,55 and 17% after application of sublethal doses of Fury a synthetic pyrethroid. It was also observed that specific activity of esterases of Pak strain of *T. castaneum* increased 9% after treatment with Fury. Increasing esterase activity showed resistance in red flour beetle against insecticide. In conclusion pymetrozine proved to be less effective insecticide against adult wingless cabbage aphid because their esterase enzymatic activity was higher from normal and aphid showed high resistance against this insecticide. In case of bifenthrin, a decrease in enzymatic activity from normal after 24 h indicates that it is more effective insecticide.

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