Research Article

Laboratory evaluation of different insecticides against *Brevicoryne brassicae* and its parasitoid, *Diaeretiella rapae* (Hymenoptera: Braconidae: Aphidiinae)

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Laboratory evaluation of different insecticides against *Brevicoryne brassicae* and its parasitoid, *Diaeretiella rapae* (Hymenoptera: Braconidae: Aphidiinae). Pure and Applied Biology. Vol. 9, Issue 1, pp256-268.

<http://dx.doi.org/10.19045/bspab.2020.90030>

Received: 18/07/2019

Revised: 12/09/2019

Accepted: 24/09/2019

Online First: 23/10/2019

Abstract

The toxicity of six insecticides (Advantage[®], Pyramids[®], Polo[®], Imidacloprid[®], Match[®] and Mospilon[®]) on *Brevicoryne brassicae* (Homoptera: Aphididae) and its parasitoid, *Diaeretiella rapae* (Hymenoptera: Braconidae) were evaluated under constant laboratory conditions (25±2 °C; with 65-70% R.H.). All the tested insecticides were lethal against *B. brassicae* with the passage of time at highest concentration. Maximum mortality was observed in Polo[®] and minimum in Mospilon[®] followed by Advantage[®], Pyramid[®], Match[®] and Imidacloprid[®]. All the LC₅₀s were lethal against its parasitoid with maximum mortality at 48 HAT (Hours after treatment) and minimum in all insecticides at 12 hours after treatment. The maximum mortality (100%) were observed at 48 HAT in Polo[®] and Advantage[®] followed by Imidacloprid[®] and Pyramid[®] (93.33%), Match[®] (92.33%) and Mospilon[®] (86.67%). The minimum mortality of *D. rapae* was recorded at 12 HAT in Mospilon[®] (36.67%) as compared to other insecticides. observed harmful (class IV) but only Mospilon[®] was moderately harmful (class III) to *D. rapae*. Maximum survival ship of *D. rapae* was observed in Pyramid[®] after 48 HAT and minimum in Polo[®]. In general, *D. rapae* exhibited the highest sensitivity to all evaluated insecticides. The insecticide Polo[®] showed maximum and minimum mortality of aphid was observed in Mospilon[®] application. In case of parasitoid mortality, the maximum mortality was observed at LC₅₀ of Polo[®] and minimum at Mospilon[®] and trend of parasitoid mortality was that, Polo[®] > Advantage[®] > Match[®] > Pyramid[®] > Imidacloprid[®] > Mospilon[®].

Keywords: *Brevicoryne brassicae*; *Diaeretiella rapae*; Insecticides; Mortality

Introduction

Brassica crops are attacked by number of insect pests including cabbage aphid, *Brevicoryne brassicae* L., (Homoptera:

Aphididae) all over the world [1, 2]. In family Brassicaceae, cabbage aphid has caused substantial losses in many crops which comprise the mustards and crucifers [3-5]. It

prefers feeding on young leaves and flowers and feeding deep into the heads of brussels shoots and cabbage [6-8]. Use of insecticides to control the insect pests in crops is common all over world. Managing population of cabbage aphid is not an easy practice although synthetic insecticides are speciously accessible for usage. However for the control these insect pests, the use of insecticides is fashioned with several disadvantages such as development of insecticide resistance and hazards to non-target organisms including human [9, 10]. Application of synthetic pesticides and botanicals are harmful to natural enemies present in field. Due to use of pesticides, the parasitoid survival range, reproductive rate and suitability of host is reduced. These are great threat to natural enemies as well as for pollinators [11].

For the protection of agricultural and surrounding habitats, evaluation of side effects of insecticides on natural enemies is necessary for pesticide registration [12]. Some selected insecticides have been declared useful to natural enemies. In many studies, insecticides exposures have been assessed against biological control agents like natural enemies [13-16]. Insecticides, acetamiprid moderately harmful to *C. carnea* [17], acetamiprid and abamectin residues showed high toxicity to hatched nymph of *D. brevis* [18]. While Emamectin benzoate was found toxic for the survival of parasitoid, *T. chilonis* [19]. Still it is not clear that which insecticides along with their scope are destructive for other beneficial organism. More considerate of pest natural enemy insecticide collaboration is needed to formulate more effective IPM strategies [16]. In some pest management activities, insecticides that have been used as discriminatory shown unsafe effects on beneficial species [20].

In agriculture crops, parasitoids and predators generally contribute the reduction

in pest population. Keeping in view the importance of beneficial insects and the ill effects of pesticides to these bio-control agents the present studies were carried out. The aim of present study was to evaluate the side effects of few insecticides used for pest control in canola fields on canola aphid and its parasitoid. The more basic aims were to obtain more data regarding survival ship of parasitoid on the side effects of selected insecticides against parasitoid and prove to investigate the suitability of these insecticides for use in integrated pest management (IPM) programs.

Materials and methods

Aphid parasitoid (*Diaeretiella rapae*)

Population of parasitoids was maintained at 25 ± 2 °C temperature and 65-70% relative humidity in plastics cages. The culture was fed by 10% honey solution and aphid population was offered for maintenance of culture. The reared parasitoids were used for the investigations.

Canola Aphid (*Brevicoryne brassicae*)

Colonies of *B. brassicae* were collected from canola fields and screened out for the presence of parasitoid(s). Aphids were transferred to glass cages containing 10 plants at least. The colony was maintained at 24 ± 2 °C with photoperiod of 16:8 (L: D). Plants were replaced at weekly interval [21].

Insecticides

Different concentrations of insecticides were used against *B. brassicae* under laboratory conditions to check their efficacy. Detail of insecticides used is given in (Table 1).

Bioassays

To check the effectiveness of insecticides leaf dip bioassay method was used. Leaves were dipped in each concentration for 10 seconds and were completely dried for 15 minutes at room temperature. A slightly moistened filter paper was also placed in petri dishes to keep the leaf material turgid. Twenty apterous aphids were released in each petri-dish on these leaflets using fine

camel hair brush. Experiment was repeated three times.

Table 1. Detail of insecticides used in study

Trade Name	Common name	Dose/acre	Concentration used	Group of pesticides	Manufacturer
Advantage® (20 EC)	Carbosulfan	500 ml	0.4 ml 0.2 ml 0.1 ml 0.5 ml 0.25 ml	Carbamate	FMC
Pyramids® (10 SL)	Nitenpyram	200 ml	0.16 ml 0.08 ml 0.04 ml 0.02 ml 0.01 ml	Neonicotinoid	Kanzo Ag
Polo® (500 SC)	diafenthion	200 ml	0.16 ml 0.08 ml 0.04 ml 0.02 ml 0.01 ml	Neonicotinoid	Syngenta, Pakistan
Imidacloprid® (70 WS)	Imidacloprid	80 g	0.064 g 0.032 g 0.016 g 0.008 g 0.004 g	Neonicotinoid	Four Brothers
Match® (0.50 EC)	Lufenuron	200 ml	0.16 ml 0.08 ml 0.04 ml 0.02 ml 0.01 ml	IGRs	Syngenta, Pakistan
Mospilon® (20 SP)	Acetamiprid	125 g	0.1 g 0.05 g 0.025 g 0.0125 g 0.00625 g	Neonicotinoid	Arysta Life Science

Data recording

Data of aphid mortality were recorded at 12, 24 and 48 hours of exposure. Corrected mortality was calculated by using Abbott's formula (Abbott, 1925).

$$\text{Corrected mortality} = 1 - \frac{n \text{ in T after treatment}}{n \text{ in Co after treatment}} \times 100$$

Whereas, T= treated and Co= control

Statistical analysis

Data for percent corrected mortality were analyzed using three factor factorial design under CRD to check the significance of insecticides. LC₅₀ value for each insecticide was calculated by Probit analysis.

Impact of insecticides on *Diaeretiella rapae*
 Newly emerged adult parasitoids were tested against LC₅₀ of each insecticides selected from previous experiment. Detail of LC₅₀ of each insecticide is given in (Table 2).

Table 2. Insecticides with different LC₅₀

Insecticides	LC ₅₀
Advantage® (20 EC)	1.07 ml
Imidacloprid® (70 WS)	0.037g
Pyramids® (10 SL)	0.069 ml
Diafenthuron® (500 SC)	0.033 ml
Lufenuron® (0.50 EC)	0.062 ml
Acetamiprid® (20 SP)	0.08 g

Procedure for bioassay

To check the effect of above mentioned pesticides on the aphid parasitoid, bioassay were carried out into petri dishes. Untreated filter papers were cut into half of the diameter of the petri dish with the help of a scissor. Half of petri dish left without filter paper to provide insecticide free environment to the parasitoids. Systemic insecticidal dilutions (LC₅₀) were sprayed through injector on these filter papers. Five pairs of newly emerged adult parasitoids were released in the petri dishes. Artificial food as cotton soaked with 10 % honey solution was also provided. The % corrected mortality of aphid parasitoids were calculated after 12, 24 and 48 hours using Abbott's formula;

$$\text{Corrected mortality} = 1 - \frac{n \text{ in T after treatment}}{n \text{ in Co after treatment}} \times 100$$

Toxicity class

According to [22] insecticides were classified according to different toxicity classes. These classes were classified on the basis of International organization for biological control (IOBS) and West palaeartic regional section (WPRS) as under,

- Toxicity class I (Harmless) = Mortality (less than 50 %)
- Toxicity class II (Slightly harmful) = Mortality (50-79 %)

- Toxicity class III (Moderately harmful) = Mortality (80-89 %)
- Toxicity class IV (Harmful) = Mortality (more than 90 %)

Statistical analysis

Data for percent corrected mortality were analyzed using three factor factorial design under CRD to check the significance of insecticides. LC₅₀ value for each insecticide was calculated by Probit analysis. All the analysis was performed by using Minitab 16.1 and SPSS 17.0 software.

Results**Percent mortality of *B. brassicae* at different concentrations of insecticides**

Different concentrations of used insecticides showed mortality of *B. brassicae* as shown in (Fig.1). The results showed that all the concentrations of all insecticides had lethal impact to *B. brassicae*. Concentration one of all pesticides showed maximum mortality of *B. brassicae*. Maximum mortality was observed in Polo® and was observed in Mospilon® at first concentration followed by Advantage®, Pyramid®, Match® and Imidacloprid®. In all tested insecticides the minimum mortality were observed at fifth concentration. It is evident from the results that all the insecticides showed maximum mortality by increasing the concentration of the insecticides.

Figure 1. Percent mortality of *B. brassicae* at different concentrations of insecticides

LC₅₀ of different insecticides against *Brevicoryne brassicae*

Lethal concentrations (LC₅₀) of different pesticides against *B. brassicae* are shown in (Table 3). These concentrations showed that the toxicological observations under laboratory conditions. The values for Imidacloprid (70 WS), Lufenuron (0.50 EC), Carbosulfan (20 EC), Difenthiuron (500 SC), Nitanypram (10 SL) and Acetamiprid (20 SP) were 0.037 g, 0.062 ml, 1.07 ml, 0.033 ml, 0.069 ml and 0.08 g respectively. LC₅₀ values of Carbosulfan (20 EC), Difenthiuron (500 SC), Nitanypram (10 SL) and Acetamiprid (20 SP) were observed less toxic to *B. brassicae*. The observation of all insecticides varied significantly as the fiducial limits for tested pesticides overlap each tothers. The regression parameters showed that contribution of Imidacloprid (70 WS),

Lufenuron (0.50 EC) to the mortality of *B. brassicae* was highly significant and positive.

Impact of insecticides on aphid parasitoid, *D. rapae*

Effect of different insecticides on the mortality of *D. rapae* at different time intervals

All the insecticides showed maximum mortality at 48 HAT (Hours after treatment) and minimum mortality at 12 hours after treatment. Maximum mortality (100%) were observed at 48 HAT in Polo[®] and Advantage[®] followed by Imidacloprid[®] and Pyramid[®] (93.33%), Match[®] (92.33%) and Mospilon[®] (86.67 %) (Fig. 2). The minimum mortality of *D. rapae* was recorded at 12 HAT in Mospilon[®] (36.67 %) as compared to other insecticides. Our findings showed that parasitoids are more sensitive to chemical insecticides than aphids.

Table 3. LC₅₀ of different insecticides against *Brevicoryne brassicae*

Insecticides	LC ₅₀ ± SE	95% CI	χ ²	Regression
Imidacloprid [®] (Imidacloprid 70 WS)	0.037g±0.24	0.27-0.45	43.55**	$Y = -0.38 + 10.25X$
Match [®] (Lufenuron 0.50 EC)	0.062ml±0.08	0.047-0.077	12.39*	$Y = -0.35 + 5.69X$
Advantage [®] (Carbosulfan 20 EC)	1.07ml±1.19	0.54-1.574	8.4 ^{ns}	$Y = 0.04 + 0.043X$
Polo [®] (Difenthiuron 500 SC)	0.033ml±0.007	0.0177-0.048	9.5 ^{ns}	$Y = -0.21 + 6.22X$
Pyramid [®] (Nitanpyram 10 SL)	0.069ml±0.011	0.047-0.92	6.78 ^{ns}	$Y = 0.25 + 3.65X$
Mospilon [®] (Acetamiprid 20 SP)	0.08g±0.12	0.054-0.11	4.662 ^{ns}	$Y = -0.41 + 5.05X$

* Significant (P < 0.05), ** Highly Significant (P < 0.001), ns = Non-Significant (P > 0.05), Y= Percent mortality, X= Insecticides

Figure 2. *D. rapae* mortality at different time intervals against different LC₅₀ of insecticides (Mean ± SEM) mean followed by same letters are not significantly different to each other (Tukey's HSD Test, P≤0.05)

Mortality of *D. rapae* after different exposure and its toxicity class

After the exposure of 12 hours, Imidacloprid (70 WS) and Mospilon[®] (20 SP) were

harmless to parasitoid. Advantage[®] (20 EC), Pyramids[®] (10 SL), Match[®] (0.50 EC) were slightly harmful and Polo[®] (500 SC) was moderately harmful. After 24 hours, all

insecticides were slightly harmful to parasitoid except Polo[®] (500 SC) which was observed harmful. After 48 hours, All the insecticides were observed harmful against parasitoid but only Mospilon[®] (20 SP) was moderately harmful to *D. rapae*. From present study it can be concluded that all insecticides used against *B. brassicae* were harmful to beneficial insect (Table 4).

Survivorship of *D. rapae* at different time intervals

Survivorship of *D. rapae* at different exposures (12, 24 and 48 hours) is shown in

(Fig. 3). The results showed that 12 hours after treatment, maximum survival of parasitoid *D. rapae* was observed in Mospilon[®] treatment. After the exposure of 24 hours, maximum survival was observed in Imidacloprid[®] treatment and minimum in Polo[®] as compared to other treatments. At 48 hours after treatment, the maximum survival was observed in Pyramid[®] treated area and minimum same as 24 hours treatment like Polo[®] as compared to other pesticides treatments.

Table 4. Percentage mortality of *D. rapae* after different exposure and its toxicity

Treatments	12 HAT	24 HAT	48 HAT
Advantage[®] (20 EC) (Carbosulfan)	66.67 (II)	73.3 (II)	100 (IV)
Imidacloprid (70WS) (Imidacloprid)	46.67 (I)	66.66 (II)	93.3 (IV)
Pyramids[®] (10 SL) (Nitenpyram)	60 (II)	66.6 (II)	93.3 (IV)
Polo[®] (500 SC) (Diafenthiuron)	83.3 (III)	96.67 (IV)	100 (IV)
Match[®] (0.50 EC) (Lufenuron)	56.6 (II)	66.67 (II)	92.33 (IV)
Mospilon[®] (20 SP) (Acetameprid)	36.67 (I)	63.33 (II)	86.67 (III)

Toxicity class I (Harmless) = Mortality (less than 50 %), Toxicity class II (Slightly harmful) = Mortality (50-79 %)
 Toxicity class III (Moderately harmful) = Mortality (80-89 %), Toxicity class IV (Harmful) = Mortality (more than 90%), HAT = hours after treatment

Figure 3. Survivorship of *D. rapae* feeding on *B. brassicae* at different time intervals

Discussion

All the insecticides showed lethal impact against *B. brassicae* at all concentrations. At highest concentration of all insecticides, the *B. brassicae* mortality was maximum. Maximum mortality was observed in Polo[®] and minimum in Mospilon[®] at the highest concentration followed by Advantage[®], Pyramid[®], Match[®] and Imidacloprid[®]. Our results are also similar with [23] who investigated nine different insecticides, each have two concentrations against *Lipaphis erysimi* and found that all the concentrations effective against tested insect. Our findings are also correlates with [24] Razzaq *et al.* (2010) who observed that imidacloprid and dimethoate caused more than 60 % mortality against *L. erysimi* seven days after application. Our results are similar with [25, 26] who observed that imidacloprid was most effective as compared to endosulfan against *L. erysimi* with the passage of time interval. According to [27] imidacloprid and dimethoate induced 73 % and 62 % reduction in population of *P. horticola*. Our achievements are also similar with [28] and [29]. They observed same results as present study. In our study all tested insecticides showed maximum mortality after 48 hours and these results are similar with [30] and [31]. Our results are also similar with [32] [33] they observed that all the tested insecticides reduced the aphid population significantly. According to this finding pyramid and imidacloprid showed maximum mortality with the increase in exposure time. In present study, LC₅₀ values of Carbosulfan (20 EC), Difenthiuron (500 SC), Nitapyram (10 SL) and Acetamiprid (20 SP) were less toxic to host. The observation of all pesticides varied significantly as the fiducial limits for tested pesticides overlap to each other's. The regression parameters showed that contribution of Imidacloprid (70 WS) and Lufenuron (0.50 EC) showed highest mortality. These results are similar to the

results of [34] against mites [35, 36] against aphids and [37] against whiteflies. These all of sucking arthropods who observed that LC₅₀ value of 0.24 micro liter/ml is highly effective for neonicotinoids.

Sadeghi *et al.* [38] calculated the different other LC₅₀ values for *A. pisum* with the passage of time. Application of imidacloprid decreased the egg laying capacity of *M. persicae* according to [39]. [40] reported that new chemistry insecticides and botanicals are highly effective to reduce the population of *B. brassicae* and recommended for alternative management. Our results emphasized with [41] who used advantage[®] against mealybug (*P. solenopsis*) with different concentrations and observed the mortality of mealybug. Our results are also accordance with [42] who observed the toxicity of different insecticides against *L. erysimi* and reported that advantage and imidacloprid showed maximum mortality against *L. erysimi*. In earlier studies, the synthetic insecticides are more successful against aphids (*S. avenae* and *R. maidis*) for its reduction in population [42-46].

Impact of insecticides on *D. rapae*

In present study the determined LC₅₀ values were tested against parasitoid to check out the impact against non-target organisms because non selective insecticides decrease the population of natural enemies which results that pest population increased [47]. Due to this incidence of pest population increased, because parasitoids and predators are closely in related with insecticides via its host, direct impact and by consumption of pollen and nectars. The insecticides application may cause the mortality against target as well non target organisms [48]. In many studies, pesticides exposures assessed against biological control agent like natural enemies [13-15, 49].

The results of present study were that all the insecticides showed maximum mortality at 48 HAT (hours after treatment) and

minimum mortality at 12 hours after treatment. Imidacloprid[®] was showed 44.67%, 66.67% and 93.33% mortality after 12, 24 and 48 hours treatment respectively. These results are similar to [17, 48] who observed that imidacloprid under laboratory conditions were harmful to *T. chilonis*. Present study found that Match[®] (Lufenuron) was showed 56.67 %, 66.67 % and 92.33 % mortality after 12, 24 and 24 hours treatment respectively. These findings correlate with the findings of [50] which concluded that lufenuron and imidacloprid showed negative impact on emergence of *T. chilonis*. According to [51] imidacloprid is highly effective against whitefly parasitoid *E. mundus*.

In present findings Mospilon[®] (Acetameprid) after time interval of 12, 24 and 48 hours, treatment showed 36.67 %, 63.33 % and 86.67 % mortality respectively. These results are similar to [52] who found that different insecticides along with acetamiprid and imidacloprid showed more than 61 % mortality of *Encarsia* sp. a parasitoid of whitefly. According to findings of [53] that acetamiprid is highly toxic with the passage of time and imidacloprid showed drift toxicity with time intervals due to sunlight effect. Our result are also revealed with the results of [54] who observed that in cotton field of Arizona, the population of predatory arthropods reduced with the application of acetamiprid. According to [55, 56] who observed that *A. colemani* is highly sensitive to acetamiprid and indoxacarb and showed maximum mortality.

Present findings resulted that Advantage[®] (Carbosulfan) showed 66.67%, 73.33 % and 100 % mortality after 12, 24 and 48 HAT respectively. In treatment where Polo[®] (Diafenthiuron) was applied the mortality was 83.33%, 96.67 % and 100 % at 12, 24 and 48 HAT respectively. These results are similar with the results of [57] who observed that neonicotinoids showed adverse effects to

natural enemies. According to [58] who reported that *C. carnea* against carbamate and organophosphate group of insecticides are most sensitive to these pesticides.

The present study resulted that after the exposure of 12 hours the insecticides, Imidacloprid (70 WS) and Mospilon[®] (20 SP) were harmless to parasitoid. Advantage[®] (20 EC), Pyramids[®] (10 SL), Match[®] (0.50 EC) were slightly harmful to parasitoid and Polo[®] (500 SC) after 12 hours was moderately harmful for *D. rapae*. After 24 hours treatment all the applied pesticides were slightly harmful to parasitoid except Polo[®] (500 SC) which observed harmful for parasitoid. After 48 hours treatment the used pesticides change the toxicity class. All the insecticides were observed harmful to parasitoid, only Mospilon[®] (20 SP) was moderately harmful to parasitoid. These results are correlated with [59] who observed that acetamiprid revealed great change in toxicity ranging from harmless to harmful against *A. colemani*.

Conclusion

It was concluded that all insecticides showed maximum mortality with the passage of time. The insecticide Polo[®] showed maximum and minimum mortality of aphid was observed in Mospilon[®] application. In case of parasitoid mortality, the maximum mortality was observed at LC₅₀ of Polo[®] and minimum at Mospilon[®] and trend of parasitoid mortality was that, Polo[®] > Advantage[®] > Match[®] > Pyramid[®] > Imidacloprid[®] > Mospilon[®]. LC₅₀ values of different insecticides showed 70 to 80 % mortality of parasitoid which showed that parasitoids are more sensitive to insecticides. Maximum survival of parasitoid after 12, 24 and 48 hours were observed in Mospilon[®], Imidacloprid[®] and Pyramid[®] respectively. The toxicity exposure of these insecticides decreased when time period increased. This information is useful for the farmers as well as researcher which insecticide is better and cause minimum

mortality and maximum survival of parasitoid when application of spray is necessary.

Authors' contributions

Conceived and designed the experiments: MA Aqeel, Performed the experiments: MJ Hussain, Analyzed the data: ABM Raza & SK Abbas, Contributed materials/ analysis/ tools: MA Aqeel & ABM Raza, Wrote the paper: MJ Hussain & SK Abbas.

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