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Impact of Four Insecticides on the Parasitoid Wasp, *Diaertiella rapae* and its Host Aphid, *Brevicoryne brassicae* under Laboratory Conditions

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Abstract: Four insecticides, Thiomethoxam (Actara 25% WG), Imidacloprid (Confidor 35% SC), Natural oil of Jojoba plant (Nat-1), formulated as 96% FC and Pirimicarb (Aphox 50% DG) were tested against the parasitoid wasp, *Diaertiella rapae* (McIntosh) and its host aphid, *Brevicoryne brassicae* (L). Actara and Aphox scored the highest efficacy against the parasitoid wasp followed by Confidor, then Nat-1. The highest tested concentration (0.125%) induced 56.52, 56.52, 34.78 and 25.53% mortality, after 24 hours, respectively, increased to 57.45, 57.45, 36.17 and 34.78% after 72 hours. Thereafter, no further mortality was detected. In case of treated mummies, the corrected mortality, 9 days post treatment, was 46.24, 56.99, 35.48 and 35.48%, respectively. In case of aphid, the same concentration achieved 100% mortality after 24 hours when Actara and Aphox were applied; and after 48 hours and 5 days when Confidor and Nat-1 formulation were used, respectively.

Keywords: Parasitoid wasp, *Diaertiella rapae*, aphid, *Brevicoryne brassicae*, Actara, Confidor, Aphox, Jojoba oil

INTRODUCTION

Aphid species can attack many field crops, ornamentals, trees and greenhouse plants allover the world, causing leaf-distortion and transfer plant viruses. The noxious aphid species attack cabbage plants is the cabbage aphid, *Brevicoryne brassica* whose colonies resemble of "white ashes". They suck plant sap, causing leaf distortion and poor growth. Cabbage aphids are particularly difficult to control once they get in sheltered parts of the plant, such as cabbage heads or Brussels sprouts. Cabbage aphids can damage crops, but they tend to be more problematic in the fall. They are notorious for transmitting virus diseases, which render some vegetable crops unmarketable. The presence of live aphids, or even dead aphids that have been parasitized, may make the product unmarketable.

Encouraging natural enemies by diversifying the habitat and their food sources, and refraining from the usage of broad-spectrum pesticides was advocated. The primary parasitoid of cabbage aphids is a very small, black wasp, *Diaeretiella rapae*, which lays its eggs inside the aphid. The parasitoid larva feeds inside the aphid, turning it to a bronze colour and killing it. It may take 2-3 weeks from the time the parasitoid lays its eggs inside the aphid until an adult parasitoid emerges from the dead aphid. Generally, there is a lag period between the outbreak of aphids and control by the parasitoid, so some other control measurements should be used, but one that does not harm the parasitoid. A diversified cropping system with several potential aphid hosts can allow *D. rapae* to maintain itself in an area during periods of low levels of aphid abundance on one crop. Many other insects, such as *Aphidoletes aphidimyza*, can also be an effective biological control agent against aphids[16].

The present experiments aimed to evaluate the efficacy of the tested insecticides against the aphid pest, on one hand, and to study their side effects on its specific parasitoid, *Diaertiella rapae* wasp.

MATERIALS AND METHODS

Rearing of the Test Insects:

Aphid: *Brevicoryne brassicae* was collected from cabbage fields at Giza Governorate and allowed to reproduce in the laboratory on cabbage leaves confined in glass jars. Cabbage leaves were renewed every other day with fresh ones. Rearing of aphid culture was carried out at 25±2°C and 70±5% R.H.[6].

Parasitoid: Parasitized aphid's mummies were collected from the cabbage fields and incubated under the previously mentioned laboratory conditions till emergence of the parasitoid, *Diaertiella rapae* (McIntosh) adults. Fresh aphid individuals were placed in glass jars with adult parasitoids (males and females) for 24 hours for parasitism. The parasitized aphid

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individuals were transferred to other glass jars supplied with fresh cabbage seedlings leaves, as food supply till the emergence of parasitoid adults, then stored in a refrigerator at 4°C till come into use[7].

Insecticides:

- Thiamethoxam (Actara): 25% WG. "Syngenta, UK".
- Imidacloprid (Confidor): 35% SC. "Bayer CropScience, Germany".
- Pirimicarb (Aphox): 50% DG. "Syngenta, UK".
- Nat 1: Natural oil of Jojoba plant, formulated as 96% FC. "Egyptian Natural Oils Company-Cairo, Egypt".

Preliminary experiments were done using different concentrations of each compound. Three concentration levels (0.125, 0.062 and 0.031%) gave reproducible data were selected. These concentrations were prepared using tap water according to the producing company's recommendations for each of the aforementioned insecticides.

Parasitoid Treatments:

A- Treatment of *D. Rapae* Adults (Direct Treatment): Four groups of test tubes (3x15cm) were used, each group contain three subgroups which comprise 10 pairs of parasitoid adults (males+ females) per each, supplied with honey droplets around the inner surface of the test tubes (as food supply) and a piece of moisten cotton wool (as a source of water). Each subgroup was sprayed with one of the tested concentrations of the four tested insecticides. A fifth group was sprayed with water only as a check. All test tubes were kept under the same laboratory conditions for 9 days under daily examination. The percentages of mortality were recorded after 24, 48 and 72 hours. The LC_{50} and LC_{90} values were calculated after 24 hours.

B- Treatment of *D. Rapae* Adults (Indirect Treatment): Batches of newly formed aphid's mummies, divided into 5 groups, each was divided into three subgroups. The first 4 groups were sprayed, each, with one of the tested insecticides, and the 5th one was used as a check. Subgroups were treated with the aforementioned concentrations. The 5th group was sprayed with water only. This experiment was carried out to examine how far the internal parasitoid could complete its development till emerging from insecticide-treated mummy. Examinations of the test tubes were carried out daily; percentage of mortality, LC_{50} and LC_{90} values were calculated 9 days post treatment.

Aphid Treatment: The following steps were applied for *Brevicoryne brassicae* aphid. Five plastic cups (120ml volume) covered with muslin cloth were used per concentration per insecticide. Each cup comprise a piece of moistened cotton wool for providing moisture and a piece of plant leaf infested with 10 individuals of aphid, sprayed with one concentration of each tested insecticide and placed under the same laboratory conditions. Check cups were sprayed with water only. Percentage of mortality was recorded after 24, 48 and 72 hours. The LC_{50} and LC_{90} values were calculated after 24 hours. The whole experiment was replicated 5 times.

Statistical Analysis: Corrected mortality was carried out using Abbott's formula[1]. All data were subjected to analysis of variance (ANOVA) through SPSS computer program, the mean values were compared using Duncann's Multiple Range test. The LC_{50} and LC_{90} values were calculated by using Finney's equation[8].

RESULTS AND DISCUSSIONS

All used concentrations higher than 0.125% induced 100% mortality for the parasitoid and aphids as well. So that, 1-2 fold dilutions were carried out for another two concentrations. Direct exposure of *D. rapae* adults to the highest concentration level (0.125%) clarified that both Actara and Aphox insecticides were relatively highly toxic after 24h, causing 56.52% mortality to the parasitoid's adults, being significantly higher than the other ones ($F=194.616$, $P=0.000$), Confidor showed moderate toxicity (34.78% mortality) while Nat-1, the natural oil of Jojoba plant, could be classified as a low toxic agent, it gave 25.53% mortality (Table 1). The indirect treatment (treatment of mummies), proved similar results. The emerged parasitoid's adults (9 days post treatment) were markedly affected with the insecticidal treatments. The toxicity could be arranged as follows: Aphox (56.99%) > Actara (46.24%) > Confidor or Nat-1 (35.48%), being significantly different from each other ($F=41.740$, $P=0.000$). These results indicated that Aphox and Actara were more toxic to the newly emerged parasitoid adult than the two other insecticides who gave moderate toxicity. The same trend was observed after 48 and 72 hours, for both direct and indirect treatments (Table 1). Using lower concentration (0.062%), observed mortality 24h post treatment showed that Aphox was still scoring the highest toxicity (56.52% mortality), followed with Actara (36.17%), Confidor (23.91%) then Nat-1 (14.89%), being significantly different of each other ($F=155.545$, $P=0.000$). Forty-eight hours post treatment records revealed, almost, similar figures of mortality, being

Table 1: Corrected percent mortality of *Diaertiella rapae* adult (direct treatment) and mummies (indirect treatment)

(%) Concentration	Insecticide	% Corrected mortalities at the indicated post treatment periods			
		Direct treatment			Indirect treatment
		24h	48h	72h	9 days
0.125	Actara	56.52±1.19 a	57.45±1.76 a	57.45±1.45 a	46.24±1.26 b
	Confidor	34.78±1.25 b	36.17±1.68 b	36.17±2.39 b	35.48±1.97 c
	Nat-1	25.53±0.87 c	25.53±0.55 c	34.78±1.13 b	35.48±1.79 c
	Aphox	56.52±1.15 a	57.45±1.53 a	57.45±1.64 a	56.99±1.22 a
F-VALUE		194.616**	119.222**	54.857**	41.740**
0.062	Actara	36.17±1.78 b	45.52±1.50 b	46.81±0.99 b	46.24±0.82 a
	Confidor	23.91±1.40 c	25.53±0.81 c	25.53±1.14 c	35.48±1.22 b
	Nat-1	14.89±0.83 d	23.91±1.55 c	25.53±0.79 c	24.73±0.94 c
	Aphox	56.52±1.59 a	57.45±2.14 a	57.45±2.08 a	46.24±1.11 a
F-VALUE		155.545**	106.452**	144.126**	99.158**
0.031	Actara	14.89±0.83 c	24.91±0.18 b	25.53±1.07 b	24.73±0.51 b
	Confidor	23.91±0.32 b	25.53±0.87 b	25.53±0.81 b	13.98±0.58 c
	Nat-1	4.26±0.27 d	13.04±0.65 c	14.89±0.83 c	3.23±0.55 d
	Aphox	45.65±1.84 a	46.81±0.99 a	46.81±1.48 a	35.48±1.97 a
F-VALUE		290.641**	360.743**	153.853**	161.284**

** = Highly significant.
Means in a column followed with the same letter(s) are not significantly different at 5% level of probability.

significantly different (F=106.452, P=0.000). After 72 hours, Confidor and Nat-1 achieved the same mortality (25.531%) (Table 1).

Regarding indirect treatment, nearly the same trend was observed, the emerged parasitoid's adult was similarly affected, the percentage of mortality was 46.24% for both Aphox and Actara. They were significantly more effective than Confidor (35.48%) and Nat-1 (24.73%) (F=99.158, P=0.000). This result indicated that Aphox and Actara were more toxic to the newly emerged parasitoid adult, while others showed less toxicity (Table 1).

At the lowest used concentration (0.031%), mortality records after 24h showed that Aphox was still the highest toxic one (45.65%), followed with Confidor (23.91%), Actara (14.89%) then Nat-1 (4.26%), being significantly different of each other (F=290.65, P=0.000). After 48 hours, the correspondent figure was Aphox (46.81%), Confidor (25.53%), Actara (24.91%), then Nat-1 (13.04%), being significantly different (F=360.743, P=0.000). After 72 hours, the percent mortality was 46.81% for Aphox, 25.53% for both Actara and Confidor then 14.89% for Nat-1, being significantly different (F=153.853, P=0.000) for direct treatments (Table 1).

In case of indirect treatment, mortality of emerged parasitoid's adults was 35.48% for Aphox, 24.73% for Actara, 13.98% for Confidor then 3.23% for Nat-1, being significantly different of each other (F=161.284, P=0.000). This result also indicated that Aphox and Actara were more toxic to the newly emerged parasitoid adult, while the two other gave less toxic (Table 1).

The LC_{50} values could be arranged as follows: Aphox (0.037) <Actara (0.098) < Confidor (0.756) < Nat-1 (1.236) 24 hours post treatment (Table3). This Indicated that both Aphox and Actara insecticides were found highly toxic to the parasitoid's adult and can be recommended for use, only, at the time of the pest abundance. While Nat-1 and Confidor insecticides may be used but carefully in the abundance of the natural enemies.

The indirect effects on the parasitoid's adults (treated mummies), clarified that the percentage of the adult emergence from the treated mummies 9 days post treatment (no more adult emergence after this date in the check group).

The LC_{50}'s could be arranged in an ascending order as follows: Aphox (0.080) < Actara (0.125) < Confidor (0.218) < Nat-1 (0.262) (Table 3); showing also that both Aphox and Actara was highly toxic.

Treatment of the cabbage aphid, *Brevicoryne brassicae* indicated that, at the highest concentration [0.125%] almost all individuals were died when treated with Aphox or Actara after 24 hours, with Confidor after 48 hs and with Nat-1 after 96hr (Table 2). At the 2nd and

Table 2: Effect of the different tested insecticides on *Brevicoryne brassicae*

(%) Concentration	Insecticide	% Corrected mortality after the indicated hours		
		24h	48h	72h
0.125	Actara	98.96±0.30 a	---	---
	Confidor	79.17±0.44 b	98.93±0.06 a	---
	Nat 1	58.33±0.44 c	78.50±0.46 b	86.96±0.49
	Aphox	98.96±0.03 a	---	---
F-VALUE		310.102**	197.931**	---
0.062	Actara	79.17±1.21 b	94.62±0.31 a	97.83±0.17 b
	Confidor	89.58±0.20 a	94.62±0.51 a	98.91±0.07 a
	Nat 1	58.33±0.34 c	78.50±0.32 c	91.30±0.34 c
	Aphox	79.17±0.43 b	89.25±0.32 b	98.91±0.10 a
F-VALUE		382.222**	416.642**	338.797**
0.031	Actara	73.96±0.33 a	89.25±0.27 a	96.74±0.13 a
	Confidor	68.75±0.14 b	87.10±0.29 b	94.75±0.66 b
	Nat 1	47.92±0.59 c	67.74±0.69 c	72.83±0.62 c
	Aphox	68.75±0.80 b	88.17±0.72 ab	97.83±0.34 a
F-VALUE		481.660**	366.403**	587.676**

** = Highly significant.
Means in a column followed with the same letter(s) are not significantly different at 5% level of probability.

Table 3: LC_{50} and slope values for the tested insecticides

Insecticide	*Diaertiella rapae* adults 24h post treatment			*Brevicoryne brassicae* 24h post treatment			*Diaertiella rapae* mummies 9 days post treatment		
	LC_{50}	LC_{90}	Slope	LC_{50}	LC_{90}	Slope	LC_{50}	LC_{90}	Slope
Actara	0.098	0.426	2.010	0.0110	0.112	1.266	0.125	2.852	0.943
Confidor	0.756	241.372	0.512	0.0210	0.387	0.582	0.218	3.253	1.092
Nat 1	1.236	45.648	0.818	0.0400	17.834	0.436	0.262	2.297	1.359
Aphox	0.037	29.586	0.442	0.0024	0.070	2.305	0.080	2.075	0.905

the 3rd concentrations, complete mortality was achieved after 4 and 5 days post treatment, respectively (Table 2). The LC_{50} value could be arranged as follows: Aphox (0.0024) <Actara (0.0110) < Confidor (0.0200) < Nat-1 (0.0210) (Table 3).

Discussion: Information about the side effects of such insecticides on natural enemies is scarce. The obtained results clarify that the tested insecticides were harmful to our parasitoid. These are in accordance with those obtained by Sterk *et al.*[14] who evaluated the effects of some pesticides against non-target beneficial arthropods. They reported that, neonicotinoids, imidacloprid and thimethoxam, were very toxic to the whitefly parasitoid, *Encarsia formosa*.

Results about the highly toxic effect of Aphox and Actara insecticides are matches with those obtained by Torres and Ruberson[15], who study the toxic effects of Thiamethoxam and Imidacloprid to the parasitoid, *Podisus nigrispinus* (Dallas) nymphs, and their efficacy against whitefly and cotton aphid. They reported that aphid treatment with doses over 1mg (a.i.) per plant retained aphid infestation lower than 10% up to 61 days of plant age. They concluded that preservation can be more successful when they are used at doses bellow 1mg (a.i.) per plant due to their short residual effect. In case of aphids,The obtained esults were matched with those results obtained by Nucifora[12] when applied Imidacloprid to control *Aphis gossypii* associated with small colonies of *Myzus persicae* and *Toxoptera aurantii* infestation, he mentioned that Imidacloprid activity was very satisfactory. Our results are in accordance with those of Kharboutli and Allen[9] when compared some insecticides efficacy to control cotton aphid, *Aphis gossypii*, they reported that Actara insecticide gave quick and substantial aphid control, at the 2nd day post treatment when applied (0.023lb ai/acre). Also, with those of Laskowski[10],

when Imidacloprid was used at 4 and 40g a.i./ha against pea aphid, he revealed that significant effect on the life history and population dynamics were recorded, Imidaclopride induced high mortality after application, lasted up to the 9th day. Similarly, Babu and Santharam[2,3] and Babu and Sharma[4,5], reported that Confidor caused significant reduction in *Rhopalosiphium maidis* aphid population at all tested concentrations. When they used Actara against aphids of wheat, it induced up to 95% reduction in aphid population and no phytotoxicity was observed. Results are matched also those found by Workman *et al.*[17] when tested 12 recommended insecticide against lettuce aphid, they mentioned that Confidor induced 100% mortality to treated aphid within 48 hours at the rate of 300ml/ha. The same conclusion was mentioned by Royer *et al.*[13], when evaluate the planting date and different rates of Imidacloprid for management of cereal aphids.

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