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Research Article

Evaluation of selected insecticides against the cabbage aphid, *Brevicoryne brassicae*, and its parasitoid, *Diaeretiella rapae*

Banafsheh Pooyan[1], Jahangir Khajehali[1*], Mohamadreza Nematollahi[1] and Hamzeh Izadi[2*]

1. Department of Plant Protection, College of Agriculture, Isfahan University of Technology, Isfahan, Iran.
2. Department of Plant Protection, Faculty of Agriculture, Vali-e-Asr University of Rafsanjan, Rafsanjan, Iran.

Abstract: *Diaeretiella rapae* is an important natural enemy in the biological control of the cabbage aphid *Brevicoryne brassicae*. This study evaluated the lethal effects of dichlorvos, dinotefuran, indoxacarb, spinosad, and hexaflumuron on the aphid and its parasitoid. The sub-lethal effects of spinosad and dinotefuran were also examined. Circular leaf discs were soaked in insecticide concentrations, and fourth instar nymphs of the aphid were exposed. For the adult wasps, the bioassay involved contact with insecticide residues. The toxicity of the insecticides against the pest and its parasitoid was ranked as follows: dichlorvos > dinotefuran > spinosad > hexaflumuron > indoxacarb, with significantly higher toxicity against the parasitoid wasp. The highest and lowest selective ratios for the insecticides' safety towards the parasitoid were calculated for hexaflumuron and indoxacarb, respectively. Hexaflumuron and spinosad are the least harmful insecticides to the wasp. The net reproductive rate of dinotefuran was lower than that of the control and spinosad. The values for gross reproductive rate were 41.99 ± 5.34, 27.75 ± 3.3, and 16.97 ± 2.36 offspring/female for the control, spinosad, and dinotefuran treatments, respectively. The intrinsic rate of population increase for *D. rapae* was lowest following exposure to dinotefuran. The percentage of adult *D. rapae* emergence in the control, spinosad, and dinotefuran was 99.6%, 96.8%, and 94.2%, respectively. A Type II functional response was observed for *D. rapae* after exposure to the control, Spinosad, and Dinotefuran treatments. Overall, it was found that hexaflumuron and *D. rapae* are effective options for an integrated pest management program to control the cabbage aphid.

Keywords: The lethal effect, sublethal effects, Functional response, *Diaeretiella rapae*, *Brevicoryne brassicae*, Side-effects

Introduction

Rapeseed, *Brassica napus*, is a valuable oil plant that serves both nutritional and industrial purposes. Iran is known for its favorable climatic conditions, making it an ideal location for rapeseed cultivation. Aphids are the most destructive pests of rapeseed, and the cabbage aphid, *Brevicoryne brassicae* (L.), is the most widely distributed species in Iran. Feeding on young plants can significantly reduce yields by 70-80%. They also decrease the seed oil content by 11%. (Aslam, 2007). The aphid not only reduces the market value of the plant but also

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*Co-corresponding authors: izadi@vru.ac.ir, khajeali@iut.ac.ir
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causes deformation of the leaves and spooning of their margins, leading to decreased yields and ultimately destroying the plant (Kelm *et al*., 1995). The indirect damage caused by aphids is the production of honeydew on the plant, promoting the growth of black fungus that produces fumigation. It also carries approximately 23 types of viral pathogens that affect a wide range of plants (Kelm *et al*., 1995).

The control of cabbage aphids is primarily achieved through the repeated application of insecticides, such as broad-spectrum organophosphates and carbamates. However, this approach has led to the development of resistance to insecticides (Mirmohammadi *et al*., 2009). Recognizing the significance of natural enemies and their crucial role in integrated pest management (IPM) can lead to significant progress in pest control. *Diaeretiella rapae* (McIntosh) (Hymenoptera: Aphidiidae) is a cosmopolitan endoparasitoid of aphids, *B. brassicae* (Kant and Minor, 2017; Nematollahi *et al*., 2014).

Pesticide selectivity is defined as the ability of a pesticide to target a specific pest while minimizing harm to non-target organisms. The selective nature of insecticides can lead to effective pest control while reducing damage to non-target organisms and the environment. The selectivity of insecticides may have an intrinsic origin, which means that the chemical is less toxic to natural enemies than to pests (known as physiological selection). The selectivity of insecticides may be influenced by environmental factors that impact the level of exposure to beneficial insects (ecological selection) (Alix *et al*., 2001; Collier *et al*., 2016). Recently, significant efforts have been made to limit broad-spectrum and non-selective insecticides (Macfadyen *et al*., 2014; Mužinić and Želježić, 2018; Picard *et al*., 2021; Torres and Bueno, 2018).

Several methods are used to study the effects of insecticides on natural enemies. These methods include topical application, pesticide injection, exposure of natural enemies to sub-lethal doses, and field studies to examine changes in natural enemy populations in response to insecticides. Each method provides distinct information regarding the impact of insecticides on natural enemies. Topical application and injection of insecticides provide valuable insights into the direct effects and acute toxicity of these chemicals on insects (Bayram *et al*., 2010). In addition to their direct lethal effects, insecticides can have sub-lethal effects. Sublethal effects refer to the physiological and behavioral impacts on survivors exposed to insecticides for a certain period. In contrast, lethal effects are associated with the acute and rapid consequences of insecticide exposure (Cloyd, 2012).

Given the significance of natural enemies and their contribution to integrated pest management (IPM), this study examines the impact of commonly used insecticides on the cabbage aphid and its parasitoid, *D. rapae*. Currently, the concept of agriculture without the use of pesticides remains an ideal goal that has not been realized. However, utilizing a combination of various methods with an emphasis on biological control offers a potential solution for achieving this goal.

Materials and Methods

Plant rearing

The wild cabbage, *Brassica oleracea* Acephala was used as a host for growing the cabbage aphid. The cabbage seeds were planted in a seedling tray filled with peat moss. After a few weeks, the seedlings were transferred to the main pot, which contained a soil mixture composed of 50% clay, 25% coco peat, and 25% animal manure. The pots were kept in the Isfahan University of Technology's greenhouse at a temperature of 20 °C and a humidity level of 70%. The pots were watered once every two days.

Insect rearing

The initial colony of cabbage aphids was collected from edible cabbage on a farm located in Abrisham city, Isfahan province, and then reared on wild cabbage, the host plant. The desiccated aphids were brushed off and transferred to a Petri dish, where they were left until the adult wasps emerged. The newly hatched wasps were transferred to pots containing aphids to produce laboratory offspring.

To prevent the wasps from transferring to the other pots, we covered the pots containing parasitoids with transparent talc and a netted roof.

Insecticides used

The commercial formulations of five insecticides, namely indoxacarb, dinotefuran, spinosad, dichlorvos, and hexaflumuron, are summarized in Table 1.

Indoxacarb belongs to the oxadiazine chemical family and operates by blocking voltage-gated sodium channels in insects, leading to neurotoxic effects and death. Indoxacarb is widely used in pest control due to its high insecticidal activity and innovative delivery systems that enhance its effectiveness and safety (Keith D Wing *et al*., 2000).

Dinotefuran is a potent neonicotinoid insecticide that targets insect nicotinic acetylcholine receptors, disrupting neural transmission and effectively managing a variety of pests (Xie and Hou, 2021).

Spinosad is a bioinsecticide derived from the fermentation of the actinomycete Saccharopolyspora spinosa. It is composed primarily of spinosyn A and D and is known for its unique mode of action on the insect nervous system. Spinosad operates primarily by causing neuronal hyperexcitation and modulating nicotinic acetylcholine receptors, leading to paralysis and death in insects (Salgado, 1998).

Dichlorvos is an organophosphorus pesticide widely used to control insect pests in agriculture and industry. Dichlorvos inhibits acetylcholinesterase (AChE), leading to the accumulation of acetylcholine and subsequent overstimulation of nerve cells, which is a common mechanism of organophosphorus pesticides (Mishra *et al*., 2021).

Hexaflumuron is a benzoylphenylurea insecticide known for its high toxicity to various insect species. It functions primarily as a chitin synthesis inhibitor, affecting the growth and development of insects (Perez-Farinos *et al*., 1998).

Aphid acute toxicity tests

Preliminary testing was conducted to determine the range of mortality doses for each insecticide, from zero to one hundred percent. This experiment included one replication with a control treatment and five different concentrations tested. For this experiment, circular leaf discs with a diameter of six cm were prepared from cabbage leaves. The discs were then immersed in insecticide concentrations and distilled water (as a control) for 10 seconds and then air-dried for 30 minutes at room temperature. A 0.8% agar medium was used to preserve moisture in the Petri dishes and promote leaf health. Each leaf disc was placed on the agar surface in a Petri dish. Then, fourth instar nymphs were then placed in each Petri dish. Mortality was recorded 48 hours after the experiment. Aphids that did not move upon being touched with the brush were considered dead. Based on the preliminary test results, the main experiment was conducted using Abbott's Formula (Fleming and Retnakaran, 1985). The experiment included four replications of five concentrations of each pesticide, ranging from 5% (low concentration) to 95% (high concentration) in terms of mortality. A control treatment was also included.

Table 1 The commercial formulations of spinosad, indoxacarb, dinotefuran, dichlorvos, and hexaflumerone.

Pesticide	Trade name	Formulation	Manufacturer	Group in IRAC	Mode of action
Spinosad	Tracer	SC24%	Dow AgroSciences	5 Spinosyns	Disruption of nicotinic acetylcholine receptors
Indoxacarb	Avaunt	SC15%	DuPont	22A Indoxacarb	Blocking of neuronal sodium channels
Dinotefuran	Starkle	SG20%	Mitsui Chemicals	4A Neonicotinoids	Binding to nicotinic receptors and mimics the effects of acetylcholine
Dichlorvos	Dichlorvos Gyah Corp	EC50%	Gyah Corporation, Iran	1B Organophosphates	Acetylcholinesterase inhibitor
Hexaflumuron	Golsam Hexaflumuron	EC10%	Golsam, Iran	15 Benzoylureas	Chitin synthesis inhibitor

Parasitoid acute toxicity test

The bioassay for adult wasps involved exposing them to insecticide residues through contact with a treated surface. Exposure cages were utilized for this purpose." These cages consist of a frame measuring 1.5 × 13 × 13 and two glass plates serving as the floor and ceiling. The dimensions of the glass plates are 13 cm x 13 cm. Six holes were made on each side for ventilation. For the bioassay, various concentrations of insecticides were sprayed onto both glass surfaces, resulting in a deposition of 2.5 mg of pesticide per square centimeter. After the glass surface dried, 30 one-day-old female wasps were released into cages. The wasps were collected using an aspirator at the breeding site. The wasps were anesthetized at a temperature of 4 °C in a refrigerator for three minutes to facilitate the identification and separation of the females. Preliminary testing was conducted to determine the lethal dose range, from zero to one hundred percent, for each pesticide individually. This experiment consisted of one replication with a control treatment and five different concentrations tested. The main experiments were performed with five different concentrations, each replicated three times. Distilled water was used as a control. The mortality rate was recorded every 24 hours.

Evaluation of the insecticides on parasitoids compared to aphid

The selective ratio of the insecticides was calculated using the following formula. In this formula, if the selective ratio for a pesticide is less than one, it indicates that the pesticide is more toxic to the beneficial species (parasitoid) than to the pest. Conversely, if the selective ratio is greater than one, it suggests that the pesticide is less toxic to the beneficial species than to the pest (Anjitha Alexander, 2013; Preetha *et al*., 2010).

$$Selective\ ratio = \frac{LC_{50}\,pest\ species}{LC_{50}\,beneficial\ species}$$

Risk ratio assessment based on comparison with the field recommended dose

The following formula was used for this evaluation. If the ratio is less than 50, it is safe for the beneficial species. If the ratio is between 50 and 2500, the insecticide has a relatively high toxicity to the beneficial species. If the ratio exceeds 2500, the insecticide is extremely dangerous to them (Preetha *et al*., 2010). Risk ratio = LC_{50} beneficial species/Farm recommended concentration.

Sublethal effects of insecticides on the life table parameters of *D. rapae*

To assess the sub-lethal toxicity of insecticides on adult wasps, approximately 100 specimens were subjected to a sub-lethal concentration of LC_{20} for 24 hours within experimental cages. During this time, the wasps were fed a mixture of cotton soaked in water and honey. Then, twenty female wasps were transferred individually to Petri dishes containing cabbage leaf discs with thirty last-instar aphid nymphs each. A male wasp was placed in each Petri dish and replaced if it died before the female. The wasps were transferred to new Petri dishes every 24 hours until the last parasitoid died. After 7 days, the Petri dishes were inspected for the presence of mummified aphids.

The number of wasps that emerged from parasitized aphids was recorded. For each treatment. The total number of progenies produced by each female, their adult lifespan, emergence percentage, number of progenies, the time required for mummy formation, adult emergence, and sex ratio were all recorded. Life table parameters were calculated using the relevant formulas with the two-sex life table Chi software. Analyses to identify significant differences in parameters were conducted using the Paired Bootstrap Test (Casas *et al*., 2000a).

Sub-lethal effect on the functional response, searching ability, and handling time of the parasitoid wasp

Six-centimeter leaf discs were prepared from cabbage leaves and placed into Petri dishes (100 x15 mm) containing agar. Fourth-instar aphid nymphs with 2, 4, 8, 16, 32, and 64 densities were placed on the leaf discs. Each density was replicated 10 times. Approximately, 100 female wasps were mated for 24 hours in cages with glass plates sprayed with sub-lethal concentrations and equipped with a honey solution. After 24 hours, the cages were placed in icy temperatures for five minutes to anesthetize the wasps and facilitate their movement. A large Petri dish was placed on a container filled with ice, and the wasps were transferred to the dish. After separating the males and females, each female was transferred to a Petri dish with a different density of aphids. Petri dishes containing honey for feeding the wasps and equipped with ventilation holes were placed in an incubator. Each density was offered to the female wasps for five hours. Approximately, 10 days after the experiment, the wasps were removed and the number of mummified aphids in each Petri dish was counted and recorded (Casas *et al*., 2000b). Functional response data analysis was conducted in two steps using SAS software. In the first step, logistic regression was used to estimate the number of parasitized prey (Na) and the initial density of prey (Nt) to determine the type of functional response. The slope of the linear portion of the logistic curve, which represents the ratio of Na to Nt, indicates the type of functional reaction. In the second step, once the type of functional response was determined, the parameters for searching ability and handling time were estimated. Holling and Rogers's models were used to estimate these parameters (Hardy *et al*., 1994). The mentioned models are:

Na = a T Nt / 1 + a Th Nt (Holling's equation)

Na = Nt [1 - exp (a (Th Na - T))] (Rogers's equation)

Na = number of parasitized hosts, N = host density, Th. = handling time, T = test duration, a = search power, and Exp. = basis of the natural logarithm

In addition to evaluating searching ability and handling time, we also calculated the goodness of fit between the functional response data and the model (r^2), as well as the maximum likelihood estimation (T/Th).

Sub-lethal effect on parasitoid behavior

Twenty-one-day-old female wasps were selected and exposed to sub-lethal concentrations for 24 hours in cages with glass plates. During this time, they were fed a honey solution. The control treatments were treated with distilled water. After 24 hours, the surviving female wasps were transferred to Petri dishes containing leaf discs and aphids. The parasitic behavior of the wasps was then monitored for 10 minutes at room temperature. The experiment was repeated ten times. In this experiment, half of the Petri dish was left without leaves while the other half contained leaves and five aphids. The data was analyzed using SPSS 16 software. A comparison of means was conducted using the LSD method at a significance level of 5%. Test observations include the time taken to reach the search area (which contains leaf discs containing aphids), the time spent making contact with the first host, the duration of stay in the search area, and the number of times the ovipositor is inserted into the host (Hardy *et al*., 1994).

Statistical analysis

The LC_{20}, LC_{50}, and LC_{90} values for each pesticide against aphids and parasitoids were calculated using Poloplus software (LeOra Software Company, England). The data was analyzed using SPSS 16 software. The means were compared using the LSD method at a 5% significance level, employing a completely randomized design. Before conducting statistical analysis, the mortality data was normalized using Arcsin√x (angular conversion) to establish variance.

Results

Insecticide's bioassay

Probit analysis, presented in Table 2, indicates that 24 hours after treatment of *D. rapae* with various insecticides, dichlorvos, and hexaflumuron had the highest and lowest toxicity, respectively, with LC_{50} values of 1.3 and 1126.7 mg/kg. Overall, most of the insecticides tested showed high acute toxicity against *D. rapae*, except hexaflumuron. Upon evaluating the effectiveness of insecticides against aphids (as shown in Table 3), dichlorvos exhibited the highest toxicity, with a recorded value of 2.7

mg/kg. On the other hand, hexaflumuron and indoxacarb had the lowest level of toxicity, with an LC_{50} value of approximately 9.0 mg/kg. However, the toxicity of indoxacarb, dichlorvos, and spinosad against the pest was lower than their toxicity against the parasitoid wasp, *D. rapae* (Table 4). Dinotefuran exhibited equal toxicity towards both the aphid and its parasitoid. The toxicity of five insecticides to the parasitoid wasp followed this order: dichlorvos > indoxacarb > dinotefuran > spinosad > hexaflumuron. However, the toxicity of the same insecticides against the aphid pest was in a different order: dichlorvos > dinotefuran > spinosad > hexaflumuron > indoxacarb (Table 4). After evaluating indoxacarb against the cabbage aphid and its natural enemy, it was found that the pesticide was a better fit for the parasitic wasp than for the aphid. This is indicated by the Chi-square values of 0.9 and 0.7, respectively. Specifically, the Chi-square value for the wasp is approximately 0.8-fold less than that of the aphid. After evaluating spinosad's effectiveness against the cabbage aphid and its natural enemy, *D. rapae*, it was found that the pesticide was a better fit for controlling the aphid pest than its parasitic wasp. The chi-square values for the aphid and wasp were 0.4 and 0.5, respectively. This indicates that the chi-square value for the aphid was approximately 1.25-fold less than that of the wasp. In other words, the chi-square values indicate that spinosad is more toxic to *B. brassicae* than to *D. rapae*. When evaluating the toxicity of insecticides against parasitoid wasps, we compared the LC_{50} ratio and the lower and upper 95% confidence limits. Our analysis indicated that there was no significant difference between the LC_{50} values of indoxacarb, dichlorvos, spinosad, and dinotefuran. However, the toxicity of hexaflumuron was significantly lower than that of the other insecticides. Upon evaluating the LC_{50} ratio and the lower and upper 95% confidence limits, it was found that there was no significant difference in the toxicity of the insecticides against the aphid. Specifically, the LC_{50} values of indoxacarb, hexaflumuron, spinosad, and dinotefuran were all found to be similar. The toxicity of dichlorvos was significantly higher than that of the other insecticides. The classifications of the insecticides evaluated in this study, ranging from "unlikely to present acute hazard" to "moderately dangerous," are given in Table 5. Dichlorvos showed the highest risk to *D. rapae*, while spinosad and hexaflumuron were found to be the least hazardous (Table 5).

Table 2 Evaluation of indoxacarb, dichlorvos, spinosad, dinotefuran, and hexaflumuron against the one-day-old female of *Diaeretiella rapae.*

Pesticide	n	Slope ± SE	χ^2 (pdf)	LC_{50} (mg/kg)	Fiducial limits (α = 0.5%)
Indoxacarb	150	1.96 ± 0.30	0.7 (3)	3.1	2.4 - 4.2
Dichlorvos	150	0.20 ± 1.12	0.9 (3)	1.3	0.7 - 2.1
Spinosad	150	0.24 ± 1.45	0.5 (3)	5.9	4.0 - 8.7
Dinotefuran	150	0.34 ± 1.88	0.4 (3)	4.6	3.3 - 6.4
Hexaflumuron	150	0.29 ± 1.82	0.2 (3)	1126.7	842.3 - 1525.0

χ^2 = chi-square; df = degree of freedom, n = number of treated larvae.

Table 3 Evaluation of indoxacarb, dichlorvos, spinosad, dinotefuran, and hexaflumuron against the fourth instar nymphs of *Brevicoryne brassicae*.

Pesticide	Number	Slope ± SE	χ^2 (pdf)	LC_{50} (mg/Kg)	Fiducial limits (α=0.5%)
Indoxacarb	240	0.97 ± 0.13	0.9 (4)	9.0	5.7 - 14.0
Dichlorvos	200	1.45 ± 0.22	1.3 (3)	2.7	1.8 - 3.7
Spinosad	200	2.47 ± 0.41	0.4 (3)	7.9	6.4 - 9.7
Dinotefuran	200	1.28 ± 1.34	1.3 (3)	4.8	3.1 - 7.1
Hexaflumuron	280	1.00 ± 0.14	0.6 (5)	8.9	5.7 - 12.7

χ^2 = chi-square; df = degree of freedom, n = number of treated aphids.

Table 4 Selectivity of indoxacarb, dichlorvos, spinosad, dinotefuran, and hexaflumuron toward *Diaeretiella rapae*, the parasitoid wasp of *Brevicoryne brassicae.*

Pesticide	Target	LC_{50} (mg/kg)	Selective ratio
Indoxacarb	*B. brassicae*	9.0	0.35
	D. rapae	3.1	
Dichlorvos	*B. brassicae*	2.7	0.49
	D. rapae	1.3	
Spinosad	*B. brassicae*	7.9	1.40
	D. rapae	5.9	
Dinotefuran	*B. brassicae*	4.6	1.04
	D. rapae	4.8	
Hexaflumuron	*B. brassicae*	8.9	126.6
	D. rapae	1126.7	

Selective ratio = LC_{50} pest species/LC_{50} beneficial species.

Table 5 Classification of indoxacarb, dichlorvos, spinosad, dinotefuran, and hexaflumuron based on their acute toxicity against *Diaeretiella rapae*, the parasitoid wasp of *Brevicoryne brassicae* (Preetha *et al*., 2010).

Pesticide	Field recommended dose (g/ha)	LC_{50} (mg/kg)	Dangerous rate	Classification	Class
Indoxacarb	200	3.1	64.5	Slightly dangerous	III
Dichlorvos	1500	1.3	1153.8	Moderately dangerous	II
Spinosad	200	5.9	33.9	Unlikely to present acute hazard	U
Dinotefuran	750	4.6	163.0	Slightly dangerous	III
Hexaflumuron	500	1126.7	0.4	Unlikely to present acute hazard	U

Life table parameters of *D. rapae*

Based on the results obtained in Table 6, the average number of male and female offspring produced by each female insect (R0) under laboratory conditions was calculated to be 33 ± 4.13 in the control group. The net reproduction rate under the same conditions with spinosad treatment was estimated to be 21.88 ± 3.21 progeny per female. The net reproduction rate was calculated to be 11.4 ± 2.16 progeny per female with dinotefuran treatment, which was lower compared to the control and spinosad treatments. The comparison of the net reproduction rate among the treatments revealed a significant difference between the control and dinotefuran treatment ($P < 0.05$). However, no significant difference was observed between the control and spinosad treatment ($P < 0.05$).

The gross reproduction rate (GRR) under laboratory conditions was calculated to be 41.99 ± 5.34 in the control group. The gross reproduction rate under the same conditions with spinosad treatment was estimated to be 27.75 ± 3.3 progeny per female. The gross reproduction rate was calculated for the dinotefuran treatment, yielding 16.97 ± 2.36 progeny per female. This rate was lower compared to the control and spinosad treatments. The comparison of the gross reproduction rate among the treatments revealed a significant difference between the control, spinosad, and dinotefuran treatments ($P < 0.05$). A significant difference was observed between the spinosad and dinotefuran treatments ($P < 0.05$).

The intrinsic rate of population increase (r_m), (Table 6) which refers to the number of females added to the population per individual per day, was calculated as 0.3 ± 0.01 for the control group. The lowest intrinsic rate of population increase was observed in the dinotefuran treatment group, with an average of 0.19 ± 0. The intrinsic rate of population increase in the spinosad treatment was 0.25 ± 0.01. The comparison of the intrinsic population growth rate among the treatments revealed a significant difference between the control, spinosad, and dinotefuran treatments ($P < 0.05$). A significant difference was observed between the spinosad and dinotefuran treatments ($P < 0.05$).

Table 6 Mean (±SE) life-fertility table parameters of *Diaeretiella rapae* under the influence of control, spinosad, and dinotefuran treatment.

Abbreviation	Parameters	Units	Control	Spinosad	Dinotefuran
R_0	Net reproduction rate	offspring/female	40.0 ± 33.1a	21.30 ± 88.2b	11.20 ± 4.2c
GRR	Gross Reproductive Rate	offspring/female	41.5 ± 99.3a	27.30 ± 75.3b	16.20 ± 97.4c
r_m	The intrinsic rate of population increase	female/female/day	0.3 ± 0.01a	0.25 ± 0.01a	0.19 ± 0.01b
λ	Finite population growth rate	time	1.35 ± 0.01	1.280 ± 0.01	1.21 ± 0.02
DT	Doubling time	day	2.30 ± 00.0a	2.80 ± 0.0b	3.60 ± 00.0c
T	Generation time	day	11.61 ± 0.31a	12.31 ± 0.18b	12.48 ± 0.19b

Different lowercase letters within a row indicate significant differences among the treatments.

The finite rate of population increase (λ) indicates the amount of stable population increase each day compared to the previous day. In other words, it shows the daily increase in the insect population compared to the previous day. The finite rate of population increase was calculated to be 1.0 ± 35.0 in the control group, 1.28 ± 0.01 in the spinosad group, and 1.21 ± 0.02 in the dinotefuran group. The rate of increase in the parasitoid population compared to the previous day significantly decreased under the effect of dinotefuran. The finite population growth rate of the treatments showed a significant difference between the control, spinosad, and dinotefuran treatments ($P < 0.05$). A significant difference was observed between the spinosad and dinotefuran treatments ($P < 0.05$).

The average duration of a generation (T), which is the time required for a population to reach R0, was significantly increased with the use of spinosad and dinotefuran treatments compared to the control group. The average length of one generation in the control treatment was 11.61 ± 0.31 days. In the spinosad and dinotefuran treatments, it was calculated to be 12.31 ± 0.18 and 12.48 ± 0.19 days, respectively. The comparison of the average length of one generation (T) among the treatments revealed a significant difference between the control, spinosad, and dinotefuran treatments ($P < 0.05$). However, no significant difference was observed between the spinosad and dinotefuran treatments ($P > 0.05$). Population doubling time (DT) is a parameter that indicates the rate of population growth. In the control, spinosad, and dinotefuran treatments, the DT values were 2.3, 2.77, and 3.55 days, respectively. These results indicate that the insecticides mentioned above reduce the speed of population growth.

Sublethal effects on biological parameters of *D. rapae*

The results presented in Table 7 show the biological characteristics of the parasitoid *D. rapae* under the influence of control, spinosad, and dinotefuran treatments. The emergence of adults decreased under the influence of pesticide treatment compared to the control. The emergence of adult insects under control conditions is almost 100%, whereas, in spinosad treatment, it is less than 96%. Dinotefuran treatment is associated with the lowest emergence rate, which is approximately 94%. Another parameter of the life table is the survival rate, which decreased under the influence of spinosad and dinotefuran. However, the comparison of the average treatments did not show a significant difference between the control, spinosad, and dinotefuran treatments ($P < 0.05$). The maximum lifespan of the female in the control group was estimated to be 16 ± 0.49 days. The use of sublethal concentrations of spinosad and dinotefuran has significantly reduced the lifespan of female insects ($P < 0.05$). The lifespan of parasitoids treated with spinosad and dinotefuran was 14.19 ± 0.41 and 13.33 ± 0.52 days, respectively, and there was no significant difference between the two groups ($P < 0.05$). The fertility of parasitoids, or the number of parasitized aphids per female in the control treatment, was estimated to be 42.9 ± 3.05 aphids. The number of parasitized aphids per insecticide significantly decreased under the influence of spinosad and dinotefuran. The number of aphids killed by the parasitoid was 28.45 ± 2.93 when treated with spinosad and 15.4 ± 2.39 when treated with dinotefuran, which was the lowest among the control and spinosad treatments. The comparison of the number of parasitized aphids per substance in three treatments revealed a significant difference between the control, spinosad, and dinotefuran treatments ($P < 0.05$). A significant difference was observed between the spinosad and dinotefuran treatments ($P < 0.05$).

The sex ratio did not show a significant difference from the control due to the use of insecticides. Spinosad and dinotefuran have extended the duration of premature growth and development compared to the control group. The duration of growth and development before puberty was estimated to be 9.75 ± 0.18, 10.55 ± 0.18, and 11.27 ± 0.22 days in the control, spinosad, and dinotefuran treatments, respectively. A comparison of the duration of growth and development before puberty in three treatments revealed a significant difference between the control, spinosad, and dinotefuran treatments ($P < 0.05$). A significant difference was observed between the spinosad and dinotefuran treatments ($P < 0.05$). The average daily parasitism rates were calculated as 5.6 ± 0.43, 3.95 ± 0.26, and 2.55 ± 0.24 for the control, spinosad, and dinotefuran treatments, respectively. The use of insecticides has significantly reduced the average parasitism per day ($P < 0.05$).

Discussion

Despite their undeniable drawbacks, insecticides remain the most important strategy for controlling pests (Bueno *et al*., 2017). To mitigate the economic impact of pests, over three billion tons of various insecticides are used worldwide each year. Despite the widespread use of insecticides, pests remain a major concern in the agricultural industry and are responsible for destroying over 40% of crops (Pimentel, 2009). The most comprehensive approach to controlling pests with reduced application of insecticides is known as integrated pest management (IPM). In integrated pest management (IPM), various pest control strategies, such as the use of insecticides and biological control agents, are combined (Pimentel, 2009). A prerequisite for the simultaneous utilization of biological control agents and insecticides is the selectivity of the pesticide toward the natural enemy. A selective pesticide is a chemical that has a higher toxicity against pests than against their natural enemies (Smith, 1991).

Our results indicate that indoxacarb is highly toxic to the pest (*B. brassicae*), but it also exhibits significant toxicity toward the parasitoid (*D. rapae*). Indoxacarb is an oxadiazine insecticide that acts as a neuronal sodium channel blocker, effectively controlling various pests such as lepidopteran, homopteran, and coleopteran (Wing *et al*., 2000). Furthermore, certain studies indicate the high toxicity of this pesticide towards specific parasitoids. For instance, a concentration of 53 mg a.i./liter of indoxacarb resulted in 100% mortality of adult *Cotesia plutellae* (Kurdjumov), which is a larval endoparasitoid of the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae). When exposed to indoxacarb at its field rate concentration, both *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae) and *Oomyzus sokolowskii* (Kurdjumov) (Hymenoptera: Eulophidae) experienced 100% mortality after 72 hours (Cordero *et al*., 2007).

However, the field rate of indoxacarb did not prove to be toxic to *Trichogramma* nr. *brassicae* (Hymenoptera: Trichogrammatidae) (Hewa-Kapuge *et al*., 2003). When *T. chiloni* eggs were treated with the recommended concentrations of indoxacarb in the field, the insecticide was found to be relatively toxic (Sattar *et al*., 2011). The risk level associated with indoxacarb has been identified as high for the parasitoid wasp, *T. chilonis*, based on a comparison with the recommended field concentration. Additionally, it has been classified as a moderately hazardous insecticide for the parasitoid, *T. brasiliensis* (Shankarganesh *et al*., 2013). The selectivity ratio of indoxacarb indicates that this pesticide is less toxic to the parasitoid *T. chilonis* than to the spotted bollworm *Earias vittella* Ishii. Therefore, it can be incorporated into integrated pest management programs (Sampathkumar and Krishnamoorthy, 2013).

Table 7 Mean (±SE) biological parameters of *Diaeretiella rapae* under the influence of sub-lethal concentrations of spinosad and dinotefuran.

Parameters	Control	Spinosad	Dinotefuran
Adult emergence (%)	99.7 ± 0.0a	95.8 ± 0.0a	94.2 ± 0.0a
Survival rate	100.0 ± 0.0a	96.0 ± 0.0a	92.0 ± 0.0a
Sex ratio	77.0 ± 0.0a	76.0 ± 0.0a	74.0 ± 0.0a
Female longevity (day)	16.5 ± 0.0a	14.0 ± 19.4b	13.0 ± 33.5b
The number of parasitized aphids per female	43.2 ± 9.0a	28.2 ± 45.9b	15.2 ± 4.4c
Premature developmental time (day)	9.0 ± 75.3a	10.0 ± 55.2b	11.0 ± 27.2c
Average parasitism per day	5.0 ± 6.4a	3.0 ± 95.3b	2.0 ± 55.2c

Different lowercase letters within a row indicate significant differences among the treatments.

Based on the results of the current research, dichlorvos, which had the lowest LC_{50}, exhibited the highest toxicity against the pest (*B. brassicae*) and its parasitoid (*D. rapae*). Dichlorvos is an insecticide belonging to the organophosphate class, which functions as an inhibitor of acetylcholinesterase. Based on the pesticide's mode of action, it is not expected to have selective toxicity towards beneficial insects. In a study, dichlorvos was found to cause 100% mortality in larvae of *Itoplectis maculator* (F.) (Hymenoptera: Ichneumonidae) after 8 hours of exposure. *Itoplectis maculator* is a parasitoid that preys on various butterfly and moth pupae (Aydoğdu and Güner 2013).

Our results indicate that spinosad is more toxic to the pest than to its parasitoids. Spinosad is a broad-spectrum insecticide made up of a natural mixture of two macrocyclic lactones, spinosyns A and D. It is commonly used to control a wide range of pests. This insecticide targets nicotinic and GABA-gated ion channels (Salgado, 1998; Salgado *et al*., 1997). *Trichogramma inyoense* Pinto and Oatman (Hymenoptera: Trichogrammatidae) and *Microplitis mediator* (Haliday) (Hymenoptera: Braconidae), egg and larval parasitoids of *Mamestra configurata* Walker (Lepidoptera: Noctuidae), respectively showed high susceptibility to different concentrations of spinosad (Mason *et al*., 2002). An evaluation of imidacloprid, lambda-cyhalothrin, and spinosad against the aphid parasitoid *Aphidius colemani* Viereck (Hymenoptera: Braconidae) revealed that the wasp was more susceptible to spinosad (D'Ávila *et al*., 2018).

A comparison was made between the LC_{50} values of indoxacarb, dichlorvos, spinosad, dinotefuran, and hexaflumuron against one-day-old female *D. rapae* and fourth instar nymphs of *B. brassicae* (see Fig. 1). Based on the results, it was found that dinotefuran exhibited high toxicity towards both the aphid and its parasitoid. This pesticide is a neonicotinoid that functions as an agonist of insect nicotinic acetylcholine receptors (Tomizawa and Yamamoto, 1993). The evaluation of dinotefuran against *Leptomastix dactylopii* Howard (Hymenoptera: Encyrtidae), a parasitoid of the citrus mealybug *Planococcus citri* (Risso) (Homoptera: Pseudococcidae), showed a highly detrimental effect on the adult parasitoids (Cloyd and Dickinson, 2006). The harmful impact of dinotefuran on the adult stage of three egg parasitoids that prey on the rice yellow stem borer, *Scirpophaga incertulas* (Walk). viz. *Trichogramma*, *Telenomus*, and *Tetrastichus* sp. were demonstrated (Rahaman and Stout, 2019). In this study, hexaflumuron was the only pesticide that exhibited significantly higher toxicity against the targeted pest than its parasitoid wasp (see Fig. 1). Interestingly, hexaflumuron was approximately 126 times more toxic to the aphid than to the wasp. The substantial difference in the toxicity of hexaflumuron against the aphid and its parasitoid may be related to the low penetration rate, change in the site of activity, and/or an increased detoxification rate of this pesticide.

The same was conceivable for more selectivity of spinosad toward *T. brassicae* than its host, *T. absoluta* (Nozad-Bonab *et al*., 2021). As an insect growth regulator (IGR), this insecticide is a chitin synthesis inhibitor. In this regard, the sublethal effect of hexaflumuron against *Habrobrachon hebetoll* (sei) (Hymenoptera: Braconidae) has been studied, with results showing significant adverse effects of pesticides on biological parameters at the immature stage (Dastjerdi *et al*., 2009). In another study, hexaflumuron showed significantly higher toxicity against *Adelphocoris lineolatus* (Goeze) (Hemiptera: Miridae) compared to its parasitoids, *Peristenus spretus* (Chen & van Achterberg) (Hymenoptera: Braconidae) (Liu *et al*., 2015).

The development, survival, and reproduction of insect populations are commonly determined by analyzing life table parameters (Ning *et al*. 2017). Demographic toxicology is an ecotoxicological approach that incorporates life table parameters into the field of toxicology. A reproductive life table is an age table that shows the reproductive, survival, and mortality rates of a population. However, in the current study, the sublethal effects of her two insecticides, spinosad and dinotefuran, were evaluated against the parasitic wasp D. rapae, and the results showed a higher sublethal effect for dinotefuran. For example, the net reproductive rate of wasps with this insecticide application reached the lowest level, about four times lower than that of the control, and he was two times lower than that of spinosad. Furthermore, our analysis revealed that both spinosad and dinotefuran treatments resulted in a significant decrease in parasitoid female longevity, as well as a decrease in performance measures such as the number of parasitized aphids per female and the average parasitism of the aphid when compared to the control group. Additionally, our findings indicate that the gross and net reproductive rates in the control group were significantly higher than those observed in the spinosad and dinotefuran treatment groups. These results suggest that the insecticides have negative side effects on the parasitoid population.

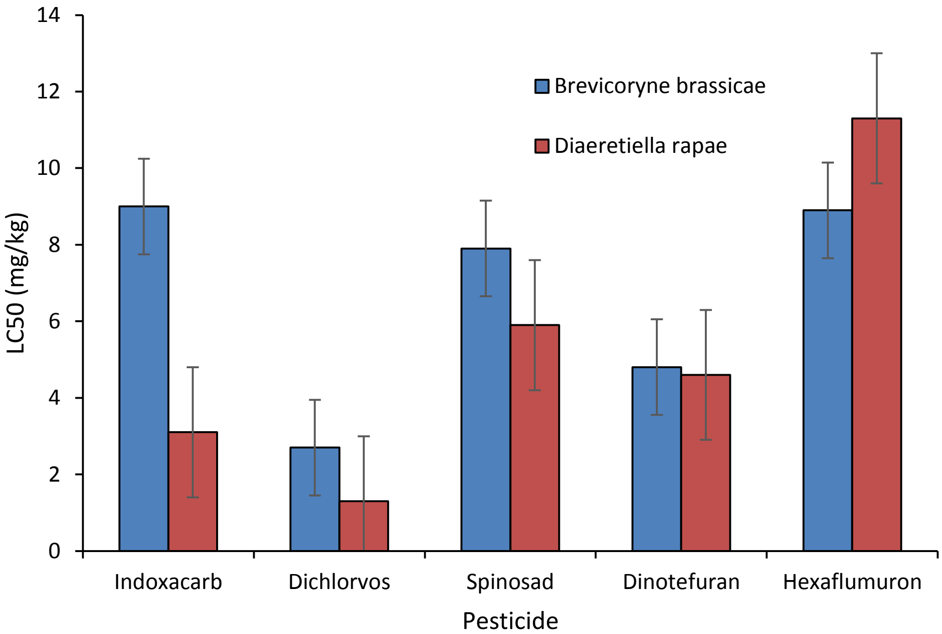

Figure 1 A comparison between LC_{50} values of indoxacarb, dichlorvos, spinosad, dinotefuran, and hexaflumuron against the one-day-old female of *Diaeretiella rapae* and the fourth instar nymphs of *Brevicoryne brassicae*. Note: The LC_{50} value of hexaflumuron must be multiplied by 100.

Our investigation revealed that the doubling time (DT) of the parasitoid population increased from 2.3 days in the control group to 2.8 days in the spinosad treatment group and 3.6 days in the dinotefuran treatment group. On the other hand, the generation time (T) was only slightly affected by the insecticides. Similar results were obtained in a previous study that evaluated the effects of abamectin, indoxacarb, chlorantraniliprole, and spinosad on the parasitoid *Trichogramma brassicae*, which is a natural enemy of the tomato leaf miner *Tuta absoluta* (Ribeiro *et al.* 2021).

The insecticide dinotefuran belongs to the neonicotinoid class of broad-spectrum insecticides, which irreversibly inhibit nicotinic acetylcholine receptors. Our study found that the reproductive performance of the parasitoid was significantly reduced by dinotefuran exposure. This is consistent with the results of a review of multiple studies, which suggests that the sublethal effects of insecticides may vary based on factors such as their mode of action, timing, and method of application (Teder and Knapp, 2019).

In summary, our study demonstrates that the sublethal effects of insecticides can have significant impacts on the growth, development, and performance of parasitoids, in addition to their direct detrimental effects. These findings highlight the potential incompatibility of insecticides with biological control methods. Therefore, when evaluating the efficacy of insecticides, it is crucial to consider not only their direct effects on target pests but also their unintended effects on non-target organisms, particularly biological control agents. The sublethal effects of insecticides on parasitoids have been reported for various species, emphasizing the need for further research in this area. For example, in agreement with our results, sublethal effects of chlorpyrifos, dimethoate, malathion, and methidathion have been demonstrated on the longevity and fecundity of the parasitoid, *Aphytis melinus* (Hym.: Aphelinidae)(Rosenheim and Hoy, 1988). Evaluation of maximum and half field rates of pyrethrins, the mixture of azadirachtin and pyrethrins, and clothianidin on the egg parasitoid, *Trissolcus japonicas* significantly decreased the survival, longevity, and reproduction rate of the female wasp.

After evaluating the lethal and sublethal effects on biological control agents, it has been determined that hexaflumuron is the most suitable insecticide for integrated pest management of aphids, in combination with the parasitoid wasp. This is due to its acceptable toxicity against the pest and its safe impact on the parasitoid wasp.

Conflict of interest

The authors declare no conflicts of interest.

Data availability statement

Data will be available on reasonable request

Author contributions

Pooyan, Khajehali, and Neamatollahi: Methodology, Software, Data curation and Izadi: Writing- Original draft preparation

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ارزیابی حشره‌کش‌های منتخب علیه شته کلم *Brevicoryne brassicae* و پارازیتوئید آن *Diaeretiella rapae*

بنفشه پویان[۱]، جهانگیر خواجه‌علی[۱*]، محمدرضا نعمت‌اللهی[۱] و حمزه ایزدی[۲*]

۱- گروه گیاه‌پزشکی، دانشکده کشاورزی، دانشگاه صنعتی اصفهان، اصفهان، ایران.
۲-گروه گیاه‌پزشکی، دانشکده کشاورزی، دانشگاه ولی‌عصر رفسنجان، رفسنجان، ایران.
پست الکترونیکی نویسندگان مسئول مکاتبه: izadi@vru.ac.ir, khajeali@iut.ac.ir
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چکیده: زنبور *Diaeretiella rapae* یکی از دشمنان طبیعی مهم در کنترل بیولوژیکی شته کلم *Brevicoryne brassicae* است. این مطالعه به بررسی اثرات کشندگی دی‌کلرووس، دینوتفوران، ایندوکساکارب، اسپینوساد و هگزافلوموورون روی شته و پارازیتوئید آن می‌پردازد. اثرات زیرکشندگی اسپینوساد و دینوتفوران نیز مورد بررسی قرار گرفت. دیسک‌های دایره‌ای برگ در غلظت‌های مختلف حشره‌کش آغشته شدند و پوره‌های سن چهارم شته در معرض آن‌ها قرار گرفتند. برای زنبورهای بالغ، زیست‌سنجی شامل تماس با باقی‌مانده‌های حشره‌کش بود. سمّیت حشره‌کش‌ها برای آفت و پارازیتوئید آن به‌صورت زیر رتبه‌بندی شد: دیکلروس> دینوتفوران> اسپینوساد> هگزافلومرون> ایندوکساکارب و با سمّیت قابل‌توجهی بالاتر برای زنبور پارازیتوئید. بیش‌ترین و کم‌ترین نسبت انتخابی برای ایمنی حشره‌کش‌ها نسبت به پارازیتوئید به‌ترتیب برای هگزافلوموورون و ایندوکساکارب محاسبه شد. هگزافلوموورون و اسپینوساد کم‌ضررترین حشره‌کش‌ها برای زنبور هستند. نرخ خالص تولیدمثلی دینوتفوران کم‌تر از شاهد و اسپینوساد بود. مقادیر نرخ ناخالص تولیدمثلی برای تیمارهای شاهد، اسپینوساد و دینوتفوران به‌ترتیب ۵/۳۴ ± ۴۱/۹۹، ۳/۳ ± ۲۷/۷۵ و ۲/۳۶ ± ۱٦/۹۷ فرزند/ماده بود. نرخ ذاتی افزایش جمعیت برای *D. rapae* در دینوتفوران کم‌ترین بود. درصد ظهور *D. rapae* بالغ در شاهد، اسپینوساد و دینوتفوران به‌ترتیب ۹۹/٦، ۹٦/۸ و ۹٤/۲ درصد بود. واکنش تابعی نوع دو برای *D. rapae* پس از قرار گرفتن در معرض تیمارهای شاهد، اسپینوساد و دینوتفوران مشاهده شد. به‌طورکلی، مشخص شد که هگزافومرون و زنبور پارازیتوئید گزینه‌های مؤثری برای یک برنامه مدیریت تلفیقی برای کنترل شته کلم هستند.

واژگان کلیدی: اثر کشنده، اثرات زیرکشنده، واکنش تابعی، *Diaeretiella rapae*، *Brevicoryne brassicae*، عوارض جانبی