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Research Article

Developmental and biochemical effects of hexaflumuron and spirodiclofen on the ladybird beetle, *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae)

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Abstract: The ladybird beetle, *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae) is one of the most important natural enemies of the common pistachio psylla, *Agonoscena pistaciae* Burckhardt and Lauterer (Hemiptera: Psyllidae). The effects of hexaflumuron and spirodiclofen were investigated on development and carbohydrates and total lipid contents of 4th instar larvae of *H. variegata*. The insecticides had significant effects on the mortality of eggs, but no significant effects on mortality of larvae or pupae. Hexaflumuron significantly increased the length of development of eggs (2.89 days) and first instar larvae (3.28 days), but had no significant effects on other instars or pupae. Spirodiclofen had no significant effects on developmental stages of *H. variegata*. Glycogen content was significantly reduced by spirodiclofen (17.42 mg/g fresh body weight) and hexaflumuron (16.07 mg/g fresh body weight). Trehalose content in hexaflumuron (1.89 mg/g fresh body weight) and spirodiclofen-treated larvae (2.02 mg/g fresh body weight) was significantly lower than control (8.01 mg/g fresh body weight). Glucose content in spirodiclofen-treated larvae (0.96 mg/g fresh body weight) was significantly higher than in hexaflumuron-treated larvae (0.24 mg/g fresh body weight) and control (0.15 mg/g fresh body weight). Significant reduction in the amount of lipid was observed in spirodiclofen-treated larvae (5.29 mg/g fresh body weight), but not in hexaflumuron-treated larvae (7.11 mg/g fresh body weight). These results suggest that substantial physiological events in the life of larvae are affected in response to the action of the tested insecticides.

Keywords: Carbohydrates, Spirodiclofen, Hexaflumuron, *Hippodamia variegata*

Introduction

Coccinellids have been widely used in biological control for over a century. The Palaearctic coccinellid species, *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae) is a widespread aphidophagous predator (Krafsur *et al*., 1996). This ladybird beetle has been reported as the most important natural enemy of aphids, whiteflies, mealybugs, lepidopteran and coleopteran insects in many countries (Franzman, 2002). The common pistachio psylla, *Agonoscena pistaciae* Burckhardt and Lauterer (Hemiptera: Psyllidae) is one of the most important pests of pistachio trees in Iran. This species is widely distributed throughout the pistachio growing regions of Iran, especially in Rafsanjan, the main pistachio production area of Iran. In autumn, the adult insects enter diapause under the loose bark on the trunks of pistachio trees, soil and leaves and

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overwinter. This pest has a multivoltine life cycle in all parts of Iran. Chemical control is the common method for control of this pest (Mehrnejad, 2003). However, *H. variegata* has been reported as one of the most important natural enemies of *A. pistaciae* (Mehrnejad, 2003). This predatory ladybeetle prefers herbaceous plants, sunny conditions and areas with high densities of the pest (Honek, 1985). The overwintering adults of *H. variegata* migrate to the mountains from late September to late October and remain in the overwintering aggregations for up to 6 months before returning to the lowlands (Hamedi and Moharramipour, 2013).

Since biological control alone does not resolve all pest problems, pesticide application is also needed in integrated pest managements (IPM). Therefore care should be taken using safe pesticides with low side effects. As the pesticides used also affect beneficial insects, the protection of these natural enemies is not easy (Croft, 1990; Bozsik, 1995; Ruberson *et al*., 1998). Only a few studies have been reported the side effects of pesticides on coccinellid predators (De Bach and Rosen, 1991) and further information is needed.

Demographic toxicology is a widespread research method that has been proposed to evaluate lethal and sub-lethal affects of pesticides on various biological parameters of pests and their natural enemies (Stark and Banks, 2003; Desneux *et al*., 2007). Insecticides may have multiple sub-lethal effects on insect life table parameters, such as reduced fecundity (Corrales and Campos, 2004), shortened longevity (Stark and Banks, 2003), prolonged developmental rate (Stark and Banks, 2003), change in sex ratio (Alix *et al*., 2001), effect on behavior (Haynes, 1988) and effect on physiology (Huang *et al*., 2004; Desneux *et al*., 2007; Zhu *et al*., 2012). The sustained effect of insecticides on insect physiology and behavior has been studied at lower doses that are not life endangering (Delpuech *et al*., 1998). Insecticides may be potentially toxic to different instars and stages of insects through diverse effects such as interfering with the function of enzymes, or changing the behavior patterns associated with feeding, migration, reproduction and/or the exchange of chemical information (Lee, 2000). Therefore, studies of sublethal effects of insecticides against insects can influence application of insecticides and potentially reduce negative environmental effects (Zhu *et al*., 2012). Molting and metamorphosis are two critical and important physiological events in the insect life cycles. All insects molt periodically in order to grow but a very few go through either gradual or complete metamorphosis to become an adult. These two events are regulated by the steroid 20-hydroxyecdysone and the sesquiterpenoid juvenile hormone (Nation, 2008). It is obvious that any interference with the homeostasis of these two hormones with exogenous sources of the hormones or synthetic analogs can be exploited as novel insecticide target to disrupt normal development of target pest insect (Aribi *et al*., 2006).

Insect growth regulators often have a lower impact on many beneficial organisms compared to the other insecticides. Recently, insect growth regulators have attracted a considerable attention for their inclusion in IPM programs, but the effects on insects are highly variable depending on the species and studied developmental stage (Nasr *et al*., 2010). Insect growth regulators are more effective on immature stages of insects compared to the mature stages because they are known to disrupt the insect's normal processes of growth and development leading to eventual death. They have a slower toxicity rate resulting in extended time to kill pests. The molting process begins when epidermal cells respond to the hormonal changes by increasing their rate of protein synthesis. The first step of molting is apolysis: the separation of epidermal cells from the inner surface of the old endocuticle and the formation of the subcuticular space. A molting gel (including enzymes) is secreted into this space (Nation, 2008; Aribi *et al*., 2006; Nasr *et al*., 2010).

The benzoylephenylurea hexaflumuron is an insect growth regulator that interferes with chitin synthesis and disrupts hormonal balance with exchanging in molting process and inhibits the

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insect's growth (Oberlander and Silhacek, 1998). This insecticide has been widely used against homopteran pests especially *A. pistaciae*. Spirodiclofen is a broad spectrum acaricide of tetronic acid group which is found to be effective against a variety of insect pests (Bretschneider *et al*., 2003; Nauen *et al*., 2003; Ke *et al*., 2010). This pesticide is widely and effectively used against the common pistachio psylla throughout the pistachio growing regions of Iran.

In the present study, compatibility of hexaflumuron and spirodiclofen and also the effects of sublethal doses of these two pesticides were investigated on biological and biochemical parameters of *H. variegata*. The aims were to characterize the growth and development and the physiological adaptation of *H. variegata* larvae through the sustained action of hexaflumuron at sublethal concentrations.

Materials and Methods

Insects

Adults of *H. variegata* were collected at the beginning of spring from the pistachio gardens in Rafsanjan. Lady beetles were reared in the boxes (20 × 25 × 10 cm) in a growth chamber at 26 ± 20 ºC, 65 ± 5% relative humidity and a photoperiod of 16 h light: 8 h dark. They were fed on a diet of psylla (*A. pistaciae*) and kept for at least 3 weeks to be adapted to the laboratory conditions. Eggs produced by adults of ladybirds were used for biological experiments. For physiological experiments, one-day-old 4th instar larvae were treated with field recommended dose of each pesticide and then transferred individually into the clear Petri dishes (6 cm diameter) and used for homogenization after 24 hr.

Insecticides and bioassay

Commercial formulation of hexaflumuron (Consult, 10% EC [70 mg (AI)/l], Dow AgroSciences) and spirodiclofen (Envidor, 24% SC [96 mg (AI)/l], Bayer Crop Science) were used. Recommended doses of each pesticide (700 and 400 ppm for hexaflumuron and spirodiclofen, respectively) were prepared with distilled water. One-day old eggs with supporter leaf disk were treated by dipping method. Subsequently, eggs were air dried and transferred into Petri dishes. This experiment was repeated six times for each treatment with 20-25 eggs. Treated eggs were monitored daily and numbers of the hatched eggs were counted. In addition, from the hatched eggs, ecdysis, mortality and developmental time of each larval stage were also recorded daily. In the next experiment, in order to know the physiological effects of these two pesticides on the test insect, 1 µl of recommended doses of each pesticide was topically applied to the thorax of the 4th instar larvae of *H. variegata* using automatic microsyringe pump (Stoleting, USA). Distilled water was used as control.

Preparation of the whole body homogenates for chemical analysis

Total body sugars

Total body sugars (mono and disaccharides) were measured using a method described by Warburg and Yuval (1997). Larvae were carefully brushed to remove contaminating particles, weighed and homogenized in 200 µl of 2% Na_2SO_4. An additional 1300 µl chloroform-methanol (1: 2) was added to the homogenate to extract the simple carbohydrates of the larvae. Individual homogenates were centrifuged for 10 min at 7150 × g. To determine the amount of carbohydrates of larvae, 300 µl was taken from the supernatant and mixed with 200 µl distilled water. The sample was reacted for 10 min at 90 °C with 1ml of anthrone reagent (500 mg anthrone dissolved in 500 ml concentrated (95%) H_2SO_4). Absorbances were measured at 630 nm on a spectrophotometer (T60U, Harlow Scientific, USA). The amount of component was determined from a standard curve by using glucose (Sigma-Aldrich, USA) as standard. This experiment was repeated 6 times with individual larva.

Glycogen

Glycogen content was determined from the pellet resulting from the centrifugation mentioned above

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(total body sugars). The pellet was washed in 400 µl of 80% methanol, thus removing possible remnants of sugar. To extract the glycogen, 250 µl distilled water was added to the washed pellet and the mixture was heated for 5 min at 70 °C. Subsequently, 200 µl of the solution was removed and reacted for 10 min at 90 °C with 1 ml anthrone reagent (600 mg anthrone dissolved in 300 ml concentrated (95%) H_2SO_4). The optical density was read at 630 nm on a spectrophotometer (T60U, Harlow Scientific, USA). The amount of glycogen in the sample was determined from a standard curve by using glycogen (Sigma-Aldrich, USA) as standard. This experiment was repeated 6 times with individual larva.

Lipids

To determine the amount of lipids in larvae, 300 µl of supernatant from Warburg and Yuval (1997) method was taken and evaporated at 35 °C in oven. Sample of each tube was dissolved in 300 µl concentrated (95%) H_2So_4. Samples were heated for 10 min at 90 °C. The samples were than cooled and stirred. 2700 µl of vanillin reagent (600 mg vanillin + 100 ml distilled water + 400 ml 85% H_3Po_4) was added to samples. Tubes were shaken for 30 min at room temperature. Absorbance was measured at 530 nm using a spectrophotometer (T60U, Harlow Scientific, USA). The amount of lipid was determined from a standard curve, using Triolein (Sigma-Aldrich, USA) as standard. This experiment was repeated 6 times with individual larva.

Low molecular weight carbohydrates

Trehalose and glucose were measured using a method described by Khani *et al*. (2007). Larvae were carefully brushed to remove contaminating particles, weighed and homogenized in 1.5-2 ml of 80% ethanol. Homogenates were centrifuged for 15 min at 12000 × g. To determine the amount of sugar alcohols in larvae, the supernatant was taken and evaporated at 40 °C in vacuum drying oven and then resuspended in 1 ml of HPLC grade water. Just before the sample injection, the samples were further cleaned by passing through a 20. m syringe filter. Sugars and alcohol sugars were analyzed by high performance liquid chromatography (Knauer, Berlin, Germany) using a carbohydrate column with 4 µm particle size (250 mm × 4.6 mm, I. D., Waters, Ireland). The eluent was acetonitrile-water (70: 30) and elution speed was 1 ml min^{-1}. Separation was achieved at 40 ± 1 °C. All aqueous solutions were degassed by helium gas. Aliquots of whole body extracts (20 µl) were run along with standards of glucose and trehalose from 1500 to 5500 ppm. This experiment was repeated 6 times with individual larva.

Statistical analysis

The chemical content data were analyzed by one-way analysis of variance (ANOVA) with a post-hoc Tukey's test, using SPSS. The results were expressed as mean ± standard error and considered significantly different at $P < 0.05$. In the biological studies, data were analyzed with Minitab 14 software followed by MSTAT-C to compare effects among treatments. The results were expressed as mean ± standard error.

Results

Effects on development

Egg mortality in spirodiclofen and hexaflumuron treatments was significantly higher than the control ($F_{2,12} = 5.78$, $P < 0.05$) (Table 1). The two insecticides had no significant effect on the mortality of *H. variegata* in the first ($F_{2,10} = 1.67$, $P > 0.05$), second ($F_{2,14} = 3.59$, $P > 0.05$), third ($F_{2,15} = 0.18$, $P > 0.05$) and fourth ($F_{2,15} = 0.18$, $P > 0.05$) larval instars and pupae ($F_{2,12} = 0.41$, $P > 0.05$). As is shown in Table 2, developmental periods of eggs ($F_{2,12} = 3.967$, $P < 0.05$) and first instar larvae ($F_{2,10} = 4.224$, $P < 0.05$) in hexaflumuron-treated were significantly longer than in spirodiclofen-treated larvae and the control but there was no significant difference between spirodiclofen-treated larvae and the control. Hexaflumuron had the maximum inhibitory effect on the eggs and the first instar larvae, but in the second ($F_{2,14} = 0.283$, $P > 0.05$), third ($F_{2,14} = 0.772$, $P > 0.05$) and fourth ($F_{2,14} = 0.725$, $P > 0.05$) instar larvae and pupa ($F_{2,15} = 2.565$, $P > 0.05$) there was no significant difference between developmental period of hexaflumuron treatment and the control.

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Biochemical changes

The effects of spirodiclofen and hexaflumuron were also investigated on some physiological changes of the last instar larvae of *H. variegata* by measuring total simple body sugars, glycogen, trehalose, glucose and lipid contents. As it is evident from Table 3, no significant difference was observed in the total body sugars of the larvae treated with spirodiclofen and hexaflumuron as compared with control ($F_{2,12} = 1.53$, $P = 0.25$), but glycogen content in the larvae treated with spirodiclofen and hexaflumuron was significantly reduced as compared to the control ($F_{2,15} = 11.37$, $P = 0.01$). Lipid content of spirodiclofen-treated larvae was significantly lower than control ($F_{2,11} = 6.19$, $P = 0.016$), whereas there was no significant difference between lipid content of the larvae treated with hexaflumuron and control. The results showed that trehalose content in hexaflumuron and in spirodiclofen treatment was significantly lower than control ($P < 0.05$) (Table 3). Glucose content in the spirodiclofen-treated larvae was significantly different from the larvae treated with hexaflumuron and control.

Table 1 Mortality (mean ± SE) of different stage of *Hippodamia variegata*, when eggs treated with hexaflumuron, spirodiclofen and distilled water as control.

Treatment	% Mortality (mean ± SE)					
	Egg	1st instar larva	2nd instar larva	3ed instar larva	4th instar larva	Pupa
Hexaflumuron	29.2 ± 6.0a	26.3 ± 2.0a	24.3 ± 7.0a	3.0 ± 2.0a	11.5 ± 6.0a	1.7 ± 1.0a
Spirodiclofen	23.2 ± 2.0a	12.9 ± 7.0a	7.2 ± 3.0b	3.3 ± 2.0a	2.3 ± 1.0b	1.5 ± 1.0a
Control	6.0 ± 1.0b	11.5 ± 5.0a	6.2 ± 2.0b	2.7 ± 1.0a	2.3 ± 1.0b	0.0 ± 0.0a

Means with different letters in each column are significantly different at 5% (Tukey's test).

Table 2 Mean developmental time (day ± SE) of different stages of *Hippodamia variegata*, when eggs treated with hexaflumuron, spirodiclofen and distilled water as control.

Treatment	Developmental stage (day ± SE)					
	Egg	1st instar larva	2nd instar larva	3ed instar larva	4th instar larva	Pupa
Hexaflumuron	2.89 ± 0.07a	3.28 ± 0.07a	2.31 ± 0.23a	2.59 ± 0.19a	3.39 ± 0.12a	3.46 ± 0.12a
Spirodiclofen	2.37 ± 0.18b	2.80 ± 0.22b	2.56 ± 0.24a	2.60 ± 0.12a	3.29 ± 0.13a	3.39 ± 0.05a
Control	2.36 ± 0.19b	2.62 ± 0.21b	2.40 ± 0.19a	2.39 ± 0.07a	3.16 ± 0.15a	3.67 ± 0.04a

Means with different letters in each column are significantly different at 5% (Tukey's test).

Table 3 Biochemical contents of *Hippodamia variegata* larvae under hexaflumuron, spirodiclofen and control treatments.

Treatment	Chemical contents (mg/g fresh body weight)				
	Total simple sugars	Glycogen	Glucose	Trehalose	Lipid
Hexaflumuron	15.07 ± 1.07a	16.07 ± 2.4a	0.24 ± 0.06a	1.89 ± 0.37a	7.11 ± 0.3a
Spirodiclofen	18.14 ± 2.20a	17.42 ± 1.6a	0.96 ± 0.12b	2.02 ± 0.29a	5.29 ± 0.2b
Control	19.85 ± 2.27a	29.02 ± 2.1b	0.15 ± 0.01a	8.01 ± 0.18b	7.89 ± 0.4a

Means within a column followed by the same letter are not significantly different ($P > 0.05$, Tukey's test).

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Discussion

Our results confirm that benzoylephenylurea, hexaflumuron had both ovicidal and larvicidal effects and also affected the developmental period of the eggs and the first instar larvae without affecting other stages. Previous studies with different chitin synthesis inhibitors such as lufenuron, flufenoxuron and hexaflumuron also indicated ovicidal activity of this group of insect growth regulators (Ascher *et al*., 1983; Liu and Chen, 2001). Since longevity of the eggs and the first instar larvae of *H. variegata* in hexaflumuron treatment were significantly higher than spirodiclofen and control, it can be concluded that using hexaflumuron against psylla has different effects on the coccinellid biology. It has been revealed that some insect growth regulators have negative impacts on several species of ladybirds such as *Stethorus punctum* (LeConte) (Biddinger and Hull, 1995*), Chilocorus nigritus* (Fabricius) (Magagula and Samways, 2000); *Harmonia axyridis* (Pallas) (Youn *et al*., 2003; James, 2004). From our results it is clear that the egg stage is the most sensitive stage of *H. variegata* to both hexaflumuron and spirodiclofen. The data obtained with hexaflumuron and spirodiclofen on *H. variegata* larvae showed low toxicity of these insecticides against different larval stages and pupa.

A lot of studies have been performed to compare toxicity and biochemistry mechanisms in pest insects and natural enemies (Biddinger and Hull, 1995; Alix *et al*., 2001; Desneux *et al*., 2007). Use of insecticide could be effective on the level of proteins, lipids and carbohydrates. In this study, treatment of the forth instar larvae with hexaflumuron and spirodiclofen significantly reduced glycogen content of *H. variegata*. One of the most important constituents of insect and most of other arthropod cuticle is chitin. The starting point for synthesis of this polysaccharide is glucose, which may comes from a storage form such as trehalose or glycogen. Chitin is based on N-acetyl glucosamine polymerization (Cohen and Casida, 1983; Nation, 2008; Nasr *et al*., 2010). The mode of action of chitin synthesis inhibitors is different from other group of insecticides. Hexaflumuron is one of these groups, which can have an effect on physiology and biochemistry of insects (Retnakaran *et al*., 1985). As a chitin synthesis inhibitor, hexaflumuron is known to disrupt chitin synthesis pathway resulting in reduction of glycogen and/ or trehalose contents. In this research, glycogen and trehalose contents were reduced 1.8 and 4.23 times, respectively by hexaflumuron. Reduction of glycogen and trehalose contents was 1.7 and 3.96 times, respectively in spirodiclofen treatment. In agreement with our results, Gordon and Burford (1984) reported that when 4th instar larvae of *Aedes aegypti* (Linnaeus) (Diptera: Culicidae) were treated by methoprene, glycogen content decreased. Garcia *et al*. (1998) showed that precocene II decreased glycogen content of *Stomoxys calcitrans* (Linnaeus) (Diptera: Muscidae). Behroozi Moghadam *et al*. (2011) found that treatment of the last instar larvae of *Ocneria terebinthina* Strg. (Lepidoptera: Lymantriidae) with chlorfluazuron (a chitin synthesis inhibitor) significantly reduced glycogen content.

Our results indicate that hexaflumuron has no significant effect on the level of glucose and lipid contents but spirodiclofen reduced lipid level. Lipids and carbohydrates are both essential sources of energy and metabolic demands and take part in constitution of different parts of body (Zhou *et al*., 2004). For example, numerous kinds of proteins are present in enzymes structure, carbohydrate in skin structure and lipids in cell wall. Spirodiclofen belongs to a new chemical class, the tetronic acid (Bretschneider *et al*., 2003; Nauen *et al*., 2003; Ke *et al*., 2010). It's mode of action is inhibition of lipid biosynthesis; therefore this result seems to be reasonable. Probably, spirodiclofen increases lipase enzyme activity and consequently lipid metabolism resulting decrease in lipid content. Spiromesifen, another insecticide from the class of spirocyclic tetronic acids acts as an inhibitor of acetyl-CoA-carboxylase, a lipid metabolism enzyme.

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Treatment with spiromesifen causes a significant decrease in total lipids. Several studies have shown the effectiveness of spiromesifen against whiteflies and mites (Kontsedalov *et al*., 2008). Zera and Zhao (2004) showed that juvenile hormone analogue, methoprene caused 36% reduction in lipase enzyme activity and lipid metabolism in *Gryllus firmus*.

Results of this experiment revealed that there was no significant difference in the total body sugars when fourth instar larvae of *H. variegata* were treated with hexaflumuron and spirodiclofen. Parveen and Miyata (2000) showed that chlorfluazuron as insect growth regulator had no effect on total carbohydrate and lipid in ovariole when last instar larvae of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) were treated. Behroozi Moghadam *et al*. (2011) reported that chlorfluazuron and pyriproxyfen did not affect total carbohydrate and lipid contents in white leaf borer, *O. terebinthina*. Zhu *et al*. (2012) found that the treatment of *S. litura* larvae with sublethal concentrations of hexaflumuron increased the content of total carbohydrate and trehalose in hematoplasma. The content of glyceride in hemolymph was significantly higher at 24 hours post–treatment.

In conclusion, the results of evaluating the effects of hexaflumuron and spirodiclofen on *H. variegata* suggested that substantial physiological events in the life of larvae are involved in responding to the action of these insecticides.

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اثرات رشدی و بیوشیمیایی هگزافلومورون و اسپیرودیکلوفن روی کفشدوزک *Hippodamia variegata* (Coleoptera: Coccinellidae) (Goeze)

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چکیده: کفشدوزک *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae) یکی از مهم‌ترین دشمنان طبیعی پسیل معمولی پسته، *Agonoscena pistaciae* Burckhardt and Lauterer (Hemiptera: Psyllidae) می‌باشد. اثرات هگزافلومورون و اسپیرودیکلوفن روی رشد و نمو و میزان قندها و چربی کل لاروهای سن ۴ این کفشدوزک مورد بررسی قرار گرفت. اثر هر دو آفت‌کش روی مرگ‌ومیر تخم‌ها معنی‌داری بود ولی هیچ‌کدام اثر معنی‌داری روی مرگ‌ومیر مراحل لاروی و شفیرگی نداشتند. هگزافلومورون به طور معنی‌داری طول دوره جنینی (۲/۸۹ روز) و لارو سن یک (۳/۲۸ روز) را افزایش داد ولی روی طول دوره رشدی سایر سنین لاروی و شفیرگی اثری نداشت. اسپیرودیکلوفن هیچ‌گونه اثری روی طول دوره مراحل مختلف رشدی کفشدوزک *H. variegata* نداشت. میزان گلیکوژن به‌وسیله اسپیرودیکلوفن (۱۷/۴۲ میلی‌گرم بر گرم وزن تازه) و هگزافلومورون (۱۶/۰۷ میلی‌گرم بر گرم وزن تازه) به‌طور معنی‌داری کاهش یافت. میزان ترهالوز در لاروهای تیمار شده به‌وسیله هگزافلومورون با ۱/۸۹ میلی‌گرم بر گرم وزن تازه و در لاروهای تیمار شده به‌وسیله اسپیرودیکلوفن با ۲/۰۲ میلی‌گرم بر گرم وزن تازه به‌طور معنی‌داری کمتر از شاهد (۸/۰۱ میلی‌گرم بر گرم وزن تازه) بود. میزان گلوکز در لاروهای تیمار شده به‌وسیله اسپیرودیکلوفن با ۰/۹۶ میلی‌گرم بر گرم وزن تازه به‌طور معنی‌داری از لاروهای تیمار شده به‌وسیله هگزافلومورون (۰/۲۴ میلی‌گرم بر گرم وزن تازه) و شاهد (۰/۱۵ میلی‌گرم بر گرم وزن تازه) بیشتر بود. اسپیرودیکلوفن میزان چربی لاروها (۵/۲۹ میلی‌گرم بر گرم وزن تازه) را به‌طور معنی‌داری کاهش داد ولی اثر هگزافلومورون بر میزان چربی (۷/۱۱ میلی‌گرم بر گرم وزن تازه) معنی‌دار نبود. نتایج نشان دهنده تغییرات فیزیولوژیکی مهم در رشد و نمو لاروهای کفشدوزک به‌دنبال تیمار شدن با حشره‌کش‌های مورد آزمایش می‌باشد.

واژگان کلیدی: کربوهیدرات‌ها، اسپیرودیکلوفن، هگزافلومورون، *Hippodamia variegate*

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