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Orijinal araştırma (Original article)

Effects of pyriproxyfen on bioenergetic resources of *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae)

Pyriproxyfenin *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae)'nın biyoenerji kaynakları üzerine etkileri

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Summary

Colorado potato beetles (CPB) overwinters as adults in the soil, thus, their survival is related with their energy reserves. The present study examined the biochemical changes in adults treated with sub-lethal concentrations ($\leq LC_{30}$) of pyriproxyfen. First, both the plants and overwintered adults were sprayed with different levels of pyriproxyfen (0, 250, 500 and 750 µl/l) under field conditions and were tested at 3, 6 and 12 days after treatment. Next, the adults from the first experiments were re-sprayed 1 week later with pyriproxyfen at the same concentrations and were re-sampled at 3, 6 and 12 days after the second treatment. The lipid, sugar, glycogen and protein levels of 4 males and 4 females were determined (mg/g; w/w). Results revealed a significant decline in lipid (87.7%), glycogen (50%) and caloric contents (75%) levels with respect to controls. Sugar and protein levels increased 7.89 and 5.79 times with respect to the controls. The best results were obtained at 250 µl/l of pyriproxyfen. The second round of testing demonstrated the additive effects of pyriproxyfen on bioenergetic reserves. Only the protein level showed a significant difference by sex.

Keywords: Colorado potato beetle, lipids, carbohydrates, proteins, admiral, pyriproxyfen

Özet

Patates böceği, toprakta ergin olarak kışlamaktadır. Bu nedenle, onların hayatta kalmaları enerji rezervleri ile ilişkilidir. Bu çalışmada pyriproxyfenin alt öldürücü konsantrasyonları ($\leq LC_{30}$) uygulanmış erginlerdeki biyokimyasal değişiklikler incelenmiştir. İlk olarak, bitki ve kışlaktaki erginlere tarla şartlarında pyriproxyfen (0, 250, 500 ve 750 µl/l) farklı düzeylerde püskürtülmüş ve uygulamadan 3, 6 ve 12 gün sonra test edilmiştir. Birinci deneyden alınan erginlere 1 hafta sonra tekrar aynı konsantrasyonlarda pyriproxyfen uygulanmış ve 3, 6 ve 12 gün sonunda yeniden örneklenmiştir. 4 erkek ve 4 dişi bireyde lipit, şeker, glikojen ve protein düzeyleri (mg/g; a/a) tespit edilmiştir. Sonuçlar, kontrole göre lipid (% 87.7), glikojen (% 50) ve kalori içeriği (% 75) düzeylerinde önemli bir düşüş göstermiştir. Şeker ve protein düzeyleri kontrole göre 7.89 ve 5.79 kat artmıştır. En iyi sonuçlar, pyriproxyfenin 250 µl/l dozunda elde edilmiştir. Testin ikinci turu pyriproxyfenin biyoenerjik rezervler üzerindeki ilave etkilerini göstermiştir. Sadece protein düzeyi cinsiyete göre anlamlı bir farklılık göstermiştir.

Anahtar sözcükler: Patates böceği, karbonhidratlar, proteinler, admiral, pyriproxyfen

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Introduction

The Colorado potato beetle (CPB) (*Leptinotarsa decemlineata* Say) is native to the United States and was introduced to Iran in 1984 (Nouri-Ghanbalani, 1989). CPB is the most widespread and most serious defoliator insect that attacks potatoes. Heavy defoliation by overwintering adults and later by spring larvae and by second generation of summer adults prior to tuber formation can cause total loss of products (Hare, 1990). Because potato crops are highly susceptible to beetle damage during the early stages of growth and in blooming, management of spring colonization by overwintering adults is critical to minimiz crop losses (Shields & Wyman, 1984).

Under natural conditions in Zanjan, Iran, CPB adults enter diapause in mid-August and emerge from the soil in spring (mid-May; unpublished data). They can, thus, tolerate sub-zero temperatures and lack of food by overwintering. It appears that CPB, like many insects, is anautogenous in the adult stage and requires carbohydrates, proteins and lipids to perform the biological activities necessary for survival and reproduction (Chapman, 1982). During pre-diapause, most insects accumulate enough of reserves to fulfill their needs during diapause. Therefore, period for the total body content of energy reserves (i.e., lipids, proteins and carbohydrates) increases during the pre-diapause period (Lefever et al*.*, 1989 and references cited in). Energy reserves decline during a dormancy and the energy depletion during winter can cause mortality (Hahn & Denlinger, 2011).

Energy reserves correlate positively with the adult size (Lease & Wolf, 2011) and increased size leads to increased overwintering survival (Bosch & Kemp, 2004). It has been reported that some insecticides cause sublethal effects, such as alterations in fecundity and development and changes in sex ratio, diapause, morphology and physiology (Takada et al., 2001; Willrich & Boethel, 2001; Krishna et al., 2007). Some pesticide treatments can affect utilization and storage of carbohydrates, proteins and lipids (Saleem et al., 1998). Juvenile hormones (JH) and analogues (JHA) increase the accumulation of lipids and carbohydrates in fat bodies at low levels and decrease by high titers (Roseler & Roseler, 1988; Cotton, 1989).

Pyriproxyfen (2-[1-methyl-2-(4-phenoxyphenoxy) -ethoxy] pyridine) is a juvenile hormone agonist that interferes with hormonal regulation in susceptible insects and results in disruption of embryogenesis, metamorphosis, and adult formation (Itaya, 1987; Ascher & Eliyahu, 1988; Kawada, 1988; Langley, 1990; Koehler & Patterson, 1991; Dhadialla et al., 1998). It has shown persistent activity against several important agricultural pests (Ishaaya & Horowitz, 1995; Ishaaya et al., 1993), including the CPB (Koopmanschap et al., 1989; De Kort et al., 1997 and refrences cited in; Vermunt et al., 1999; Yi & Adams, 2000). The analogue of JH has a low toxicity on invertebrates and was first registered in Japan in 1991 for control of public health pests (Etebari et al., 2007).

Studies have examined the effects of pyriproxyfen on CPB larvae and adults. Previous studies have shown that treating diapausing CPB adults with pyriproxyfen terminates diapause (Koopmanschap et al., 1989; De Kort & Koopmanschap, 1990; De Kort et al., 1997). In females kept under limited light durations, pyriproxyfen prevents the expression of diapause protein 1 gene in the fat body, but induces the occurence of vitellogenin in the hemolymph (De Kort et al., 1997). This has no direct lethal effect, but induces severe morphological malformations resulting in the inability to emerge as an adult; the insects ultimately die from starvation (Koopmanschap et al., 1989). Since this pest overwinters in soil as an adult, it appears that survival rate correlates with resistance to cold and its level of energy reserves. The present study examined the effects of pyriproxyfen on the bioenergetics resources in adult CPBs.

Materials and Methods

Insect rearing

The fourth instar larvae of second generation CPBs were collected from potato fields in the vicinity of the city of Zanjan in Iran. The larvae were kept on potato plants under natural conditions until the overwintering adults emerged.

Treatments

The commercial compound pyriproxyfen (2-[1-methyl-2-(4-phenoxyphenoxy)-ethoxy] pyridine) used in this study was 10% emulsifiable concentrate (Sumitomo Chemical, Japan). This formulation was considered closest to existing application conditions to produce results comparable to those in the field. Preliminary testing obtained three concentrations that produced less than 30% mortality (250, 500 and 750 µl/l) at 2 weeks after treatment.

Distilled water was used as a control group. The first experiment tested 20 newly-emerged adults (10 males: 10 females); each sex were of approximately the same weight and of the second generation (overwintered adults). They were placed in plastic containers (1 litre) and sprayed using a hand sprayer with sub-lethal concentrations of pyriproxyfen (250, 500, 750 µl/l) in the laboratory. Treated individuals were then transferred into cheese cloth sleeves on field of potato plants sprayed with the same concentrations of pyriproxyfen. The insects were sampled at 3, 6 and 12 days after spraying.

In the second experiment, insects were again sprayed at the previous concentration, 7 days after the first treatment. Sampling was done at 3, 6 and 12 d after the second treatment. Sampled adults after 5 hours (to ensure that gut is empty) were transferred to a deep freezer (-80°C) to be preserved for biochemical analysis. Before analysis, the wings were removed and the wet weights of adults were recorded using a microbalance (0.00).

Biochemical assays

Glycogen, sugar and lipid contents were assessed using the method suggested by Van Handel (1988) and modified by Kaufmann & Brown (2008). Four adult beetles from each sex were chosen, the wings were removed and their wet weights recorded on a microbalance (0.00). De-winged adults were placed in a test tube containing 200 µl of 2% Na_2SO_4, the tube was placed in an ice bath and the insects were crushed using an electrical homogenizer (2600 rpm). A solution of 3 ml chloroform and methanol (1:1) was added to the homogenate, which was mixed and centrifuged at 3000 rpm for 1 min in a cooled centrifuge (MIKRO 220R), to separate sugars and lipids from glycogen and protein which were precipitated out in a pellet.

Calculation of lipid and sugar contents

The supernatant produced in previous stage was removed for lipids and sugars measurement. The supernatant was diluted with 2 ml distilled water and centrifuged at 3000 rpm for 1 min to separate the sugars and lipids. The new supernatant was then removed and used to test for lipid content. This fraction was dried, dissolved in H_2SO_4, heated to 100°C for 10 min and mixed with vanillin reagent. The optical densities were read at 530 nm using a single beam spectrophotometer (WPA S2000UV/vis).

To test for sugars, anthrone reagent was added to the remaining fraction, which was then heated to 100°C for 17 min. Samples were placed into a single beam spectrophotometer and the optical densities were read at 625 nm. The amount of average total sugar and lipid content was estimated as mg/g of fresh weight. Glucose was used as the standard by which to quantify the sugar (and glycogen) and soybean oil was used to quantify the lipids.

Estimation of glycogen and protein

The pellet that contain glycogen and protein was immersed in 4 ml distilled water and mixed using a vortex. For glycogen, a portion of the mixture was removed, the anthrone reagent was added and the resulting mixture was heated at 100°C for 17 min. The optical densities were read at 625 nm.

Protein was measured using Bradford's method (Kruger, 1994) with bovine serum albumin as the standard. The optical densities were read at 595 nm. The average total protein and glycogen were estimated as mg/g of fresh weight. Variations in lipid, sugar, protein and glycogen content between treatments and the control were calculated as:

[(mean of item in treatment – mean of it in control) /mean of it in control] × 100%.

Evaluation of caloric content

The caloric content per individual was estimated using the following control values: 4.19 cal/mg for protein, 4.2 cal/mg for carbohydrates and 9.5 cal/mg for lipids (Judd et al., 2010).

Statistical analysis

The general linear model (Statistix 8.0) was used to determine the effects of pyriproxyfen concentration on energy reserves. The experiments were carried out in a completely randomized four level factorial design. The four factors were pyriproxyfen dose (0, 250, 500 and 750 µl/l), sex (2), sampling time (3) and number of treatments (2). The experiments were repeated four times. Multiple comparisons were made using Tukey-Kramer tests.

Results

Total lipid content

The physiological effects of pyriproxyfen on the CPB were estimated and were shown to cause considerable biochemical changes. Table 1 indicates that there was a significant 3-way interaction between pyriproxyfen dose (D), sampling time (S) and treatment time (T) ($p < 0.01$). The highest lipid content was observed for the control at 12 day after the second application (mean: 92.34 $\pm$ 5.3 mg/g) and the lowest was at a dose of 250 µl/l at 6 day after the first spraying (mean: 11.34 $\pm$0.65 mg/g). The table also shows 4 significant two-way interactions that affected lipid content: sex/S, S/D, S/T and D/T ($p < 0.01$).

Pyriproxyfen dose, sampling time and treatment time strongly effected lipid content, as demonstrated by the significant main effects (Table 1). Table 2 shows that the lowest lipid content (20.99 ±2.25 mg/g) was observed for the first treatment at 250 µl/l measured at 6 day after treatment (29.75 ±1.89). Table 2 also indicates that lipid content increased more after the second treatment than that after the first (Table 2). Decreases for doses of 250, 500 and 750 µl/l over the control were observed to be 59.74%, 28.69% and 26.24%, respectively.

Total carbohydrates level as a function of pyriproxyfen dose

As shown in Table 1, the carbohydrate content (glycogen and sugar) was strongly affected by pyriproxyfen ($p < 0.01$). There were 2 significant three-way interactions: D/S/T and D/S/sex ($p < 0.05$). The highest glycogen content was observed for 750 µl/l at 12 day after the second spraying in males (mean: 65.47$\pm$5.5 mg/g) and the highest sugar content was observed for 500 µl/l at 12 day after the second spraying (mean: 25.45$\pm$ 1.7 mg/g). The lowest glycogen content was observed for 250 µl/l at 6 day after the first spraying in males (mean: 3.9$\pm$1.64 mg/g) and lowest sugar content was observed for the control (0 µl/l) at 6 day after the first spraying (mean: 2.86 $\pm$ 0.6 mg/g).

Table 1 indicates that pyriproxyfen dose, sampling time and spraying times had strong effects on carbohydrate content as demonstrated by its significant main effects. This JH analogue decreased glycogen content and increased sugar content (Table 2). Low doses of pyriproxyfen significantly decreased the sugar content (Table 2). For doses of 250, 500 and 750 µl, the sugar content increased 43.24%, 160.47%, 37.33% over the control, respectively**.** At doses of 500 and 750 µl/l, the glycogen content increased 40.78% and 45.97% over the control; by contrast, at 250 µl/l, it decreased 47.99%.

Total protein level as a function of pyriproxyfen dose

Table 1 indicates that protein content was strongly affected by pyriproxyfen dose ($p < 0.01$). A 4-way interaction was significant for D, S, T and sex ($p < 0.05$). The highest protein content were observed for 250 µl at 6 day after first spraying in males (mean: 12.52±0.54 mg/g). The lowest protein content was observed for the control at 3 dya after first spraying in females (mean: 2.16 ±0.54 mg/g).

Significant 3-way interactions for protein content (Table 1) were observed for D/S/repeat and D/S/sex ($p < 0.05$) and for the individual effects for D, sex, S and repeat. In all cases, as the pyriproxyfen level decreased, the protein content increased (Table 2). Although high concentrations of pyriproxyfen significantly decreased the protein content, it increased the lipid and glycogen contents, important energy sources for CPBs. The decrease in protein content for 500 and 750 µl/l over the control were 20.74% and 22.64%, respectively; by contrast, for 250 µl/l, protein content increased 10.9%.

Total caloric content

Table 1 indicates that caloric content was strongly affected by D, S and repeat. The highest amount caloric content was observed for the control (0 µl/l) at 12 day in males (mean: 0.96 ± 0.06 cal/mg) and the lowest amount for 250 µl/l at 3 day in females (mean: 0.24 ±0.06 cal/mg). The decreased in caloric content for 250, 500, and 750 µl/l over the control, was 50.79%, 11.11% and 12.7%, respectively.

Table1. Effects of diffrent pyriproxyfen concentrations on bioenergetic resources variations in adults of Colorado potato beetle*, Leptinotarsa decemlineata* (Say) (Mean of square)

Source	df	Lipid	Glycogen	Sugar	Protein	Caloric content
Sex	1	$0.065^{n.s}$	$0.0004^{n.s}$	$0.007^{n.s}$	0.271^{**}	0.034^{*}
Days	2	1.11^{**}	9.091^{**}	0.933^{**}	0.063^{**}	0.54^{**}
Dose	3	1.06^{**}	45.08^{**}	1.513^{**}	0.195^{**}	0.455^{**}
Repeat	1	3.26^{**}	78.75^{**}	0.758^{**}	0.106^{**}	1.563^{**}
sex*days	2	0.095^{**}	$0.299^{n.s}$	$0.08^{n.s}$	$0.014^{n.s}$	$0.016^{n.s}$
sex*dose	3	$0.0005^{n.s}$	$1.003^{n.s}$	$0.035^{n.s}$	$0.015^{n.s}$	$0.0006^{n.s}$
sex*repeat	1	$0.002^{n.s}$	$0.371^{n.s}$	$0.022^{n.s}$	$0.026^{n.s}$	$0.019^{n.s}$
days*dose	6	0.072^{**}	$1.017^{n.s}$	0.087^{**}	0.079^{**}	0.02^{*}
days*repeat	2	0.635^{**}	3.033^{*}	0.446^{**}	0.069^{**}	0.089^{**}
dose*repeat	3	0.249^{**}	12.18^{**}	$0.053^{n.s}$	0.27^{**}	0.131^{**}
sex*days*dose	6	$0.038^{n.s}$	3.037^{**}	0.068^{*}	0.02^{*}	0.022^{*}
sex*days*repeat	2	$0.027^{n.s}$	$1.399^{n.s}$	$0.028^{n.s}$	$0.018^{n.s}$	0.027^{*}
sex*dose*repeat	3	$0.01^{n.s}$	$1.065^{n.s}$	$0.035^{n.s}$	$0.006^{n.s}$	$0.004^{n.s}$
days*dose*repeat	6	0.089^{**}	2.499^{**}	0.126^{**}	0.022^{*}	$0.016^{n.s}$
sex*days*dose*repeat	6	$0.028^{n.s}$	1.637^{*}	$0.026^{n.s}$	0.031^{**}	$0.006^{n.s}$
Error	138	0.019	0.744	0.027	0.009	0.008
CV %		9.34	18.89	18.52	13.39	13.26

n.s: Non significant, **and* significantly different at P<0.01 and P<0.05, respectively.

1. Lipid, sugar and protein data transformed to (Log base 10) , glycogen and caloric content to $(\sqrt{x})$ and then were analysed

Table 2. Effects of different concentrations of pyriproxyfen compound on bioenergetic resources variations in adults of Colorado potato beetle, *Liptinotarsa decemlineata* (Say)

Source	Source levels	Mean of lipid (mg/g)*	Mean of glycogen (mg/g)	Mean of sugar (mg/g)	Mean of protein (mg/g)	Mean of caloric content (cal/g)
Sex	Female	35.20±1.53^{ns}	22.88±0.97^{ns}	9.41±0.49^{ns}	4.76±0.11^{b}	490.0±15.97^{b}
	Male	39.18±1.60^{ns}	23.64±1.02^{ns}	9.56±0.52^{ns}	5.7±0.11^{a}	535.6±16.73^{a}
Time spraying	1	26.55±1.54^{b}	16.52±0.98^{b}	8.61±0.50^{b}	5.04±0.11^{b}	378.9±16.08^{b}
	2	47.84±1.59^{a}	30.0±1.01^{a}	10.37±0.51^{a}	5.42±0.11^{a}	646.7±16.62^{a}
Sampling time intervals	3	30.35±1.91^{b}	22.26±1.21^{b}	9.0±0.62^{b}	4.9±0.14^{b}	440.2±19.96^{b}
	6	29.75±1.89^{b}	20.22±1.20^{b}	6.92±0.61^{c}	5.7±0.14^{a}	420.5±19.76^{b}
	12	51.48±1.95^{a}	27.30±1.24^{a}	12.54±0.63^{a}	5.09±0.14^{b}	677.8±20.35^{a}
Concentrations (µl/l)	0	52.14±2.16^{a}	21.21±1.37^{b}	5.92±0.70^{c}	5.69±0.16^{b}	633.1±22.58^{a}
	250	20.99±2.25^{c}	11.03±1.43^{c}	8.48±0.72^{b}	6.31±0.16^{a}	307.8±23.50^{c}
	500	37.18±2.19^{b}	29.86±1.39^{a}	15.42±0.71^{a}	4.51±0.16^{c}	562.3±22.89^{ab}
	750	38.46±2.25^{b}	30.96±1.43^{a}	8.13±0.72^{bc}	4.40±0.16^{c}	548.0±23.50^{b}

* Means within columns followed by the same lower-case letter are not significantly different at the 5% level by Tukey multiple range. n.s.: non significant.

Discussion

The effects of juvenile hormone analogues (JHAs) on metabolic homeostasis and energy metabolism in insects are poorly understood. The most common effect of JHAs treatment is the disruption at the levels of hemolymph and fat body (or whole body) metabolites. The metabolic effects of JHAs might be the result of morphogenetic effects of the compound on the insect. It has been suggested that JHAs may overwhelm the homeostatic mechanisms in insect (Hamock & Quistad, 1981). Little information is available concerning the nature of the physiological and biochemical effects of JHAs on insects.

Lipids

Pyriproxyfen, sampling time, spraying time and their interactions decreased the lipid content of CPBs. Numerous studies have shown that excessive accumulations of lipids in the fat body of allatectomized insects (Bailey et al., 1975; Steele, 1985; Downer, 1985; Beenakkers et al., 1981; Cymborowski, 1992) and that corpus allatum (CA) hormone accelerates lipid release from tissue. Morohoshi hypothesized that increasing the secretion of corpus allatum hormone increased active energy metabolism and lipid release from the fat body and other tissues in response to the accelerated metabolism (cited in Mandal, 1982).

Locusts, at 10 day after emergence adults, undergo an extended period of somatic growth and lipid accumulation that allows for storage of fuel for migratory flight. During this period, the CA remains inactive (Johnson & Hill, 1975). Upon activation of the CA, lipid accumulation ceases and vitellogenesis begins (Hill & Izatt, 1974). Contrarily, lipids accumulate in the response to high titers of JH in the larval stage of some insects. Decreased JH titers trigger pupation and decrease utilization of stored lipids (Nijhout &

Williams, 1974). In the mosquito (*Aedes taeniorhynchus*) removing of the CA does not cause lipids to accumulate (Van Handel & Lea, 1970).

These results are contrary to what should be expected for allatectomized insects. The mechanism how JH effects lipid accumulation, is not clear (Stanley-Samuelson & Nelson, 1993). Allatectomy increases the activity of a number of fat body enzymes involved in lipogenesis (Walker & Bailey, 1971). Patel (2005) reported that when in the aquatic, invertebrate, *Hydropsyche contubernalis* L. (Trichoptera) were under stress, adipokinetic hormones were activated which in turn increased lypolysis activity in fat body adipocytes.

The results of the present study are similar to those reported for different insects for various JH mimics. Pyriproxyfen decreased lipid content in *Corcyra cephalonica* (Mandal & Chaudhuri, 1992), *Spodoptera littoralis* (cited in Hamadah et al., 2012), *Plodia interpunctella* larvae (Ghasemi et al., 2010), *Eurygaster integriceps* nymphs (Zibaee et al., 2011), *Bombyx mori* larvae (Etebari et al., 2007), *Schistocerca gregaria* (Hamadah et al., 2012) and *Brachynema germari* Kol (Bagheri et al., 2010). Lipid levels in the sixth instar larvae of *Choristoneura fumiferana* were depleted as a result of fenoxycarb treatment (Mulye & Gordon, 1993). This JHA impairs lipid synthesis in the fat body, suppress both the synthesis of fatty acids and their subsequent esterification into complex lipids (Mulye & Gordon, 1993).

The lipid content in the pupae of *Spodoptera littoralis* was decreased after larval treatment with mevalonic acid as a JH precursor (Ghoneim, 1994). It should be mentioned that, in some insect species, instead of inhibiting lipid content, JH activates this metabolite (Amer, 1990; Ghoneim, 1994). In some cases, the increase or decrease lipid content was dose-dependent; carbohydrates content in the fat body are increased with a decrease in JH level or from high titers (Cotton, 1989); this is the opposite of the results for *Schistocerca gregaria* (Hamadah et al., 2012).

Other probable functions of JHA is to decrease AKH release from the central nervous system (CNS) resulting in the increase in the AKH content of the CNS upon the accumulation rather than stimulation of AKH synthesis (Kodrik et al., 2010). The most important function of AKHs is the control of energy metabolism and mobilization of different kinds of energy reserves (lipids, carbohydrates and/or certain amino acids) (Gade et al., 1997). The decrease in total lipid content is duration dependent. It may be a result of the activation of alternate metabolic pathways to energy from the fat body during insecticidal stress (Kalimuthu & Pandian, 2010). Pyriproxyfen could affect the lipid reserves at all concentrations.

Carbohydrates

Results showed significant differences between carbohydrate content, sampling time and repeat spraying. Total body glycogen content increased, except in CPBs treated with the 250 µl/l (low) pyriproxyfen concentration. Total sugar content also increased over that of the control. It appears that the increase or decrease in total sugar content may be in response to the hormonal effects of pyriproxyfen and interference with energy cycles and energy consumption. This is especially significant for the effects of concentration and lag phase effects (sampling interval), which was confirmed by the hormonal effects of pyriproxyfen on CPBs.

The depletion of carbohydrates in whole body tissue may a response to insect hyperactivity caused by pesticide treatment (Singh, 1986). Insecticides may affect the utilization of carbohydrates, proteins and lipids (Saleem et al., 1998). It has been suggested that the decrease in carbohydrates, may be the result of the production of extra energy to combat insecticidal stress (El-Sheikh et al., 2005). Several reports confirm a fall in carbohydrate levels in insects in response to toxicity stress (Bais et al., 1995). A decrease in carbohydrate content after treatment with IGRs can be attributed to their anti-feeding action (Salem, 1994), to a decrease in trehalose activity (El-Shiekh, 2002 cited in Tanani et al., 2012), or to their effects on carboxylase activity (Mukherjee & Sharma, 1996). Pyriproxyfen strongly decreased the carbohydrate content in the hemolymph of 1-day old adults of *Schistocerca gregaria* (Forsk.), but the low concentration

prompted an increase in 4-day old adults (Tanani et al., 2012). Pyriproxyfen prevented *S. gregaria* nymphs from attaining a normal carbohydrate content in the hemolymph.

Proteins

Pyriproxyfen and interacted factors such as sampling time, spraying times and sex affected the total protein content. The results indicated that the total protein content decreased at all levels except those treated at the 250 µl/l (low) concentrations. Interaction effects between all factors were significant. A previous study found that CPB adults treated with pyriproxyfen showed a gradual increase in total free amino acid concentration in the hemolymph up to 20 d of adult life when induced vitellogenin synthesis and cessation of diapause protein synthesis occurred when insects were held for short-day conditions after treatment (Yi & Adams, 2000). Another study (De Kort et al., 1997) reported that pyriproxyfen had a little effect on total protein concentration of the hemolymph, but did affect protein composition.

It appears that the increase for 250 µl/l and decrease at other levels, is in response to the effects of pyriproxyfen on hormones; changes in total protein content may be related to the synthesis, uptake and degradation of major proteins. For example, in *Locusta migratoria migratorioides*, increased ploidy levels in methoprene-treated trophocytes appeared to be in response to stimulation of protein synthesis (Cotton, 1989).

Numerous studies have shown that allatectomy of adult female *Periplaneta americana* (Thomas & Nation, 1966), of male and female of *Gryllotalpa gryllotalpa* (Mandal, 1982) and inactivation of the CA (by the application of precocene) in adult *Locusta* (Gellissen & Wyatt, 1981) and *Drosophila melanogaster* (Landers & Happ 1980; Wilson et al., 1983) decreased whole body protein levels and that methoprene-treated *L. migratoria migratorioides* (Cotton, 1989) increased production of larval proteins. Pyriproxyfen decreased total protein content in *E. integriceps* (Zibaee et al., 2011).

In *E. integriceps*, JHs at very low concentrations, may regulate the manufacture of larval proteins by the fat body and vitellogenin in adult females (Cotton, 1989). In contrast to the results of allatectomy, the application of JHA inhibited protein synthesis in the fat body of *Aedes aegypti* larvae and pupae (Gordon and Burford, 1984), *Culex tarsalis* larvae (Himeno et al., 1979) and *D. melanogaster* (Breccia et al., 1976). The results of the present study support those of several previous studies on JHAs.

This study confirmed that pyriproxyfen could be effective on the major biomolecules of carbohydrates, proteins and lipids in the whole body of overwintered adult CPBs. Although it is known the calories available through that the survival of other species overwinter is associated with the level of stored energy, the next step is to examine the effects of pyriproxyfen on the fitness of overwintered adults and the relationship between energy reserves and dormant adult vitality.

Caloric contents

This approach adds up the bioenergetics sources like proteins, carbohydrates and lipids. Caloric contents related to the quantity changes of lipids, carbohydrates and proteins regardless of their physiological roles . So, the variation of bioenergetics in treated CPB tend to change of caloric contents in this research. In the other word, the most reduction of caloric contents, nearly 50%, observed in 250 µl/l level of pyriproxyfen, because of the reduction effects of this analogue on carbohydrates and lipids.

In animal species, changes in this measurement are often associated with contents of constitutive items, which varies seasonally with food supply, reproductive condition and the extent of storage for overwintering. Large caloric reserves could provide greater potential energy for egg production, oviposition, survival, and flight capacity (Magna-relli & Modi 1988; Harre et al., 2001).

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