www.czasopisma.pan.pl PAN POLSKA AKADEMIA NAUK www.journals.pan.pl

JOURNAL OF PLANT PROTECTION RESEARCH Vol. 54, No. 1 (2014)

DOI: 10.2478/jppr-2014-0012

Cellular energy allocation in the predatory bug, *Andrallus spinidens* Fabricius (Hemiptera: Pentatomidae), following sublethal exposure to diazinon, fenitrothion, and chlorpyrifos

Moloud Gholamzadeh Chitgar[1]*, *Jalil Hajizadeh*[1], *Mohammad Ghadamyari*[1], *Azadeh Karimi-Malati*[1], *Mahbobe Sharifi*[1], *Hassan Hoda*[2]

[1] Department of Plant Protection, Faculty of Agricultural Science, University of Guilan, P.O. Box 1841, Rasht, Iran
[2] Department of Biological Control, Iranian Research Institute of Plant Protection, P.O. Box 145, Amol, Iran

Received: July 13, 2013
Accepted: January 27, 2014

Abstract: It is necessary to study the biochemical changes in insects exposed to toxicants if we want to predict the potential of various chemicals on the natural enemy. Physiological energy, as a biochemical biomarker, may be affected by many pesticides including organophosphate compounds. Therefore, in this study, the sublethal effects of diazinon, fenitrothion, and chlorpyrifos on the cellular energy allocation (CEA) of the predatory bug, *Andrallus spinidens* Fabricius (Hemiptera: Pentatomidae), a potential biological control agent, was studied on 5th-instar nymphs. Among the energy reserves of the *A. spinidens* nymphs, only total protein was significantly affected by pesticide treatments, and the highest value was observed in chlorpyrifos treatment. The energy available (E_a) and energy consumption (E_c) in *A. spinidens* were significantly affected by these pesticides. In exposed bugs, these parameters were affected by fenitrothion and chlorpyrifos more than diazinon. The activity of the electron transport system (ETS) in the Ec assay showed that *A. spinidens* exposed to chlorpyrifos had the highest rate of oxygen consumption. Although, there was no significant change in CEA, the insecticides caused a marked change in the physiological balance of *A. spinidens*. The results suggested that the adverse effect of these insecticides on *A. spinidens* should be considered in Integrated Pest Management (IPM) programs.

Key words: *Andrallus spinidens*, cellular energy allocation, electron transport system, Integrated Pest Management

Introduction

Integrated Pest Management (IPM) programs are used worldwide for controlling different agricultural pests. The use of natural enemy agents in combination with those selected insecticides which have no effect on them, is an important part of the programs (El Wakeil *et al*. 2013). In the rice ecosystem, natural enemies are often important biological agents for control of serious pests such as *Chilo suppressalis* Walker (Pyralidae), *Naranga aenescens* Moore (Noctuidae), and *Mythimna unipunctata* Haworth (Noctuidae). Conservation of natural enemies in the rice field may suppress the pest populations, which in turn will reduce the rate of insecticide applications (Jadhao 2011). Although the pesticides diazinon and fenitrothon are no longer approved for use in the European Union, they were extensively used along with chlorpyrifos, for control of rice pests in northern Iran [Regulation (EC) No. 1107/2009]. Indiscriminate, inadequate and improper use of these pesticides has led to severe problems such as the development of pest resistance, the resurgence of target species, the outbreak of secondary pests, the destruction of beneficial insects as well as health hazards and environmental pollution (Noorhosseini 2010).

Andrallus spinidens Fabricius is a non-specific predator on lepidopteran larvae in rice fields (Manley 1982). Second to 5th-instar nymphs and adults of *A. spinidens* show a predatory activity on such caterpillar pests of rice like *C. suppressalis*, *N. aenescens*, and *M. unipunctata* (Manley 1982). This pentatomid bug has a critical role in the regulation of the rice pest population (Najafi-Navaee *et al.* 1998). There are three factors which should favor *A. spinidens* as potentially useful biological control agent of rice pests: its relatively short life cycle, aggressive feeding behavior, and ability to feed continually for several hours (Manley 1982). This natural enemy may be affected by insecticide sprays in rice fields via direct contact with residues, or indirectly through contaminated food.

In addition to direct mortality, toxic substances can cause physiological responses, like changes in biochemical contents of the exposed insects. The effect and mode of action of many toxicants can be elucidated by physiological energy as it is assumed that exposure to a contaminant will disturb energy allocation in an organism (Bagheri *et al*. 2010). Total energy reserve in an insect body as energy available (E_a) is the total amount of energy acquired from available total lipid, total protein, glucose, and glycogen content which can be affected by different factors espe-

*Corresponding address:
b_gh.chitgar60@yahoo.com

cially by pesticides (Jabakumar and Jayaraman 1988). Energy consumption (E_c) is measured under saturated substrate conditions. Changes in its activity have to be measured by altering enzyme production in an organism. In fact, E_c is the activity of the electron transport system (ETS). An ETS assay can clarify changes in E_c by interaction of the toxicant with the ETS (Oberdorster *et al*. 1998). Cellular energy allocation (CEA) as a biomarker, has been developed based on the "metabolic cost" hypothesis. This methodology could provide an integrative quantification of the organism energy budget based on a biochemical comparison of the organism's E_c and the energy reserves available for metabolism (De Coen *et al*. 2000). The cellular energy allocation technique (CEA = E_a/E_c) has been used as a reliable specific energy parameter that can measure the effect of toxicants on different energy sources as well as a marker of the available energy content of an organism. CEA is a rapid and instantaneous method for measuring the energy content of an organism (De Coen and Janssen 1997). Several studies provided strong evidence that insecticides affect the CEA and energy reserves (Verslycke *et al*. 2004; Bagheri *et al*. 2010). There is no data from the investigation of insecticides, on CEA of *A. spinidens*. In this study, we examined the sublethal effects of three insecticides, diazinon, fenitrothion, and chlorpyrifos on the CEA of *A. spinidens*. Such information can be used to predict the potential of these pesticides in combination with *A. spinidens*, to control rice pests.

Materials and Methods

Insect rearing

Andrallus spinidens adults and nymphs were collected from the rice fields of Amol, Mazandaran province (Iran), in late September 2012. These insects were reared on last larval instar of *Galleria melonella* Linnaeus (Lepidoptera: Pyralidae) in laboratory conditions of 25±2°C, 60±10% relative humidity (RH) and a photoperiod of 16 : 8 (L : D) h.

Pesticides

The pesticides used in this study were technical material of diazinon (Gyah Corporation, Iran) (99.8% purity), fenitrothion (Pesticides and Agriculture Research Center, Iran) (99.8% purity), and chlorpyrifos (ACO, USA) (99.9% purity).

Chemicals

Vanillin, Anthron, Triton X-100, nicotinamide adenine dinucleotide (NADH), nicotinamide adenine dinucleotide phosphate (NADPH), jodo-nitro-tetrazolium (INT), glycogen, Tris-HCl, glucose, and $MgSO_4$ were obtained from the Merck Company (Germany) and polyvinyl pyrrolidone was purchased from Sigma (USA).

Bioassay

The first, preliminary bioassay was conducted to determine the effective concentrations that caused 10–90% mortality. Each insecticide was bioassayed at serial concentrations in ranges of: 1000–3500, 200–800, and 200–950 ppm a.i. for diazinon, fenitrothion and chlorpyrifos, respectively. These insecticides were diluted in acetone, and 1 µl of each concentration was applied using a micro-applicator on the thoracic dorsum of newly molted 5th-instar nymphs of *A. spinidens*. The control treatment received acetone alone. Forty nymphs of *A. spinidens* were used for each concentration and the control. Mortality was assessed 24 h after treatment and the LC_{30} of each insecticide were calculated.

Biochemical analysis

Determination of available energy

Sample preparation

For the assay, each *A. spinidens* nymph was homogenized in 200 µl of 2% Na_2SO_4 using a plastic pestle. The mixture then had 1,300 µl of chloroform : methanol (1 : 2, v/v) added to it. Next, the mixture was centrifuged at 8,000 × × g for 10 min at 4°C. The supernatant was used as an enzyme solution for assessing total lipid and carbohydrate, and pellet for glycogen content.

For the protein assay, each individual of *A. spinidens* was homogenized in 250 µl of distilled water and centrifuged at 8,000 × g for 10 min at 4°C. Then, supernatant was used for protein determination.

Lipid assay

For lipid assay, 500 µl of supernatant was transferred to the microtube and left to dry at room temperature for 24 h. After this amount of time had passed, 500 µl of H_2SO_4 was added and the mixture was heated in a water bath at 90°C for 10 min. An aliquot of 30 µl was taken from each replicate and added to 270 µl of vanillin reagent (12 mg vanillin in 2 ml distilled water and 8 ml 85% H_3PO_4) in each microplate well. The absorbance of samples was recorded at 530 nm using a microplate reader after the samples were held in the dark for 30 min (Awareness Technology Inc., Stat Fax® 3200). The standard curve for the lipid assay was plotted using cholesterol as the standard (Yuval *et al*. 1998).

Carbohydrate assay

For carbohydrate determination, 300 µl of the supernatant was added to 200 µl distilled water and 900 µl anthron reagent (25 mg anthron dissolved in 25 ml H_2SO_4). The sample was then heated for 10 min at 90°C in a water bath. The absorbance was recorded at 630 nm. The total content of carbohydrates was calculated from a standard curve of glucose (Yuval *et al*. 1998).

Glycogen assay

The pellets resulting from centrifuged samples were washed twice with 500 µl 80% methanol for removing possible remnants of sugar. Distilled water (250 µl) was added to sample and heated at 75°C for 5 min. Then, 900 µl anthron reagent was added to the mixture. After cooling, the absorbance was recorded at 630 nm. Total gly-

cogen was calculated from a standard curve of glycogen (Yuval *et al*. 1998).

Protein assay

Total protein was determined according to the Bradford (1976) method, with bovine serum albumin as standard.

Determination of E_c

The E_c was estimated by measuring the ETS according to King and Pakard (1975). For the assay, each *A. spinidens* nymph was homogenized in 100 µl of buffer mixture of 0.2 M Tris-HCl pH 8.5, 30% (w/v) polyvinyl pyrrolidone, 3.6 mg $MgSO_4$ and 0.4% (w/v) Triton X-100 in 10 ml of distilled water. Then 100 µl of diluted buffer (1 : 1) was added and centrifuged at 3,000 × g for 10 min at 4°C. The supernatant was used as an enzyme solution for the energy consumed assay.

In the E_c experiment, 40 µl of the sample was added to 120 µl of the buffered substrate solution [0.13 M Tris-HCl, 0.3% (w/v) Triton X-100 pH 8.5, 11.9 mg NADH (1.7 mM) and 2.1 mg NADPH (250 µM) in 10 ml of distilled water], and 80 µl INT (8 mM). The absorbance was measured at 490 nm continuously for 7 min at 25°C. The aforementioned assays were carried out in triplicate and for all assays appropriate blanks were run. The quantity of oxygen consumed per bug, as derived from the ETS data, was transformed into energetic equivalents. From a theoretical point of view, formation of 2 µl formazan will use 1 µmol of O_2 (484 kJ/mol O_2) (King and Pakard 1975).

Determination of CEA

CEA was calculated after determination of E_a and E_c according to the following formula:

$$E_a = \text{glucose} + \text{lipid} + \text{glycogen} + \text{protein} \quad (\text{mj/mg wet wt/h}),$$

$$E_c = \text{ETS activity} \quad (\text{mj/mg wet wt/h}),$$

$$CEA = E_a/E_c,$$

where: E_a – energy available; E_c – energy consumption; ETS – electron transport system; CEA – cellular energy allocation.

Statistical analysis

The LC_{30} values and 95% confidence intervals were calculated from probit regressions using the POLO-PC computer program (LeOra software 1987). Data were analyzed using one-way analysis of variance (ANOVA) (SAS Institute Inc. 2002). Differences between sample (n = 3) means were evaluated using Tukey's test and were considered significant when the probability $p \leq 0.05$.

Results

The LC_{30} values of each insecticide on *A. spinidens* were presented in table 1. The rank order of the toxicity, from the highest to the lowest, was fenitrothion > chlorpyrifos > diazinon. In this study, there was no significant changes in carbohydrate ($p = 0.72$; $df = 3$; $F = 0.45$) (Fig. 1), lipid ($p = 0.106$; $df = 3$; $F = 2.83$) (Fig. 2), and glycogen ($p = 0.98$; $df = 3$; $F = 4.24$) (Fig. 3) contents in the treated insects when compared with the control. But total protein content of *A. spinidens* was affected significantly by pesticides ($p \leq 0.0001$; $df = 3$; $F = 33.79$) (Fig. 4). All pesticides caused a higher total protein content than the control. The highest values were observed in the chlorpyrifos treatment (667.6±0.01) µg/insect, although there was no statistically significant difference between this pesticide and fenitrothion (645.3±0.02) µg/insect. The E_a of *A. spinidens* was significantly affected by pesticides ($p = 0.003$; $df = 3$; $F = 10.91$) (Fig. 5). The total energy content of fenitrothion-exposed bugs was higher than the E_a in other treatments (85.79±0.04) J/insect. ETS activity in the E_c assay showed a significant difference among all treatments ($p = 0.0054$; $df = 3$; $F = 9.34$) (Fig. 6). *Andrallus spinidens* exposed to chlorpyrifos after 24 h showed the highest rate of oxygen consumption (6.48±0.002) J/insect × min. In this study, no significant difference was observed in the amount of CEA among the treatments (Fig. 7). Although CEA in fenitrothion and chlorpyrifos-exposed bugs was decreased compared to the control, this reduction was not statistically significant ($p = 0.44$; $df = 3$; $F = 0.73$).

Table 1. Effects of diazinon, fenitrothion and chlorpyrifos on the CEA in *A. spinidens* (means ±SE)

Treatment	Glycogen [J/insect]	Lipid [J/insect]	Carbohydrate [J/insect]	Protein [J/insect]	E_a [J/insect]	Consumed energy [J/insect × × min]	CEA [J/insect]
Control	6.14±0.01 a	32.79±0.01 a	30.88±0.02 a	4.72±0.044 b	74.53±0.02 b	4.55±0.005 b	16.35±2.9 a
Fenitrothion	5.408±0.02 a	33.09±0.03 a	36.96±0.02 a	10.32±0.02 a	85.79±0.04 a	5.66±0.001 ab	15.13±1.21 a
Diazinon	3.10±0 a	32.71±0.05 a	29.12±0.03 a	4.88±0.04 b	69.82±0.05 b	4.26±0.004 b	16.36±0.24 a
Chlorpyrifos	4.60±0.002 a	34.77±0.05 a	31.2±0.03 a	10.68±0.01 a	81.26±0.05 a	6.48±0.002 a	12.52±1.6 a

Means ±SE in columns followed by different letters differ significantly ($p \leq 0.05$) according to t-test

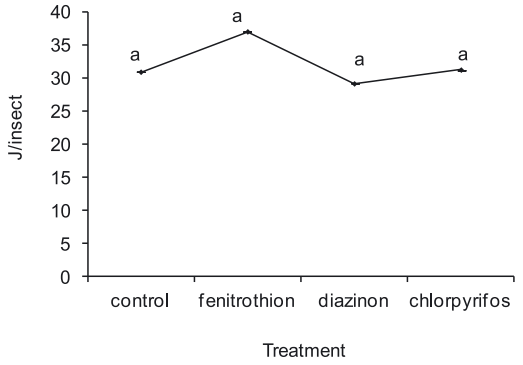

Fig. 1. Carbohydrate content in *A. spinidens* treated with fenitrothion, diazinon, and chlorpyrifos. The points labeled with similar letters are not significantly different ($p \leq 0.05$)

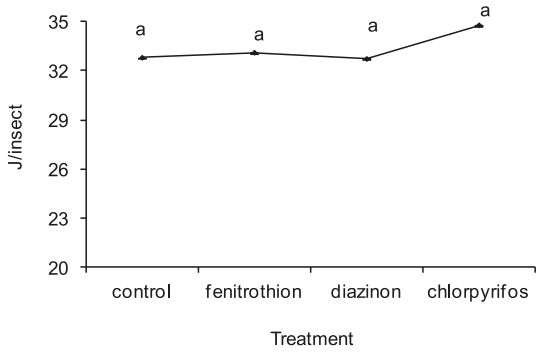

Fig. 2. Lipid content in *A. spinidens* treated with fenitrothion, diazinon, and chlorpyrifos. The points labeled with similar letters are not significantly different ($p \leq 0.05$)

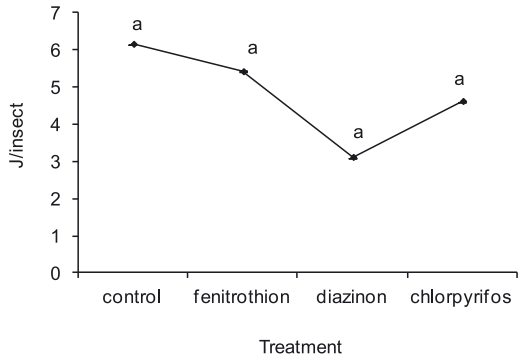

Fig. 3. Glycogen content in *A. spinidens* treated with fenitrothion, diazinon, and chlorpyrifos. The points labeled with similar letters are not significantly different ($p \leq 0.05$)

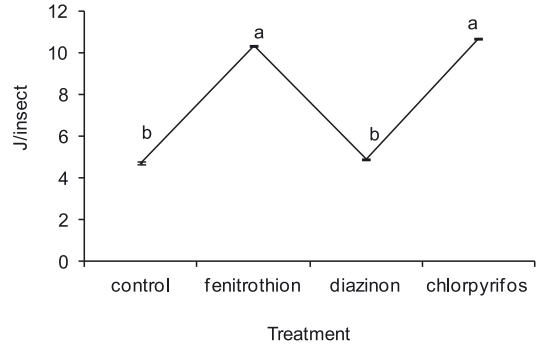

Fig. 4. Protein content in *A. spinidens* treated with fenitrothion, diazinon, and chlorpyrifos. The points labeled with similar letters are not significantly different ($p \leq 0.05$)

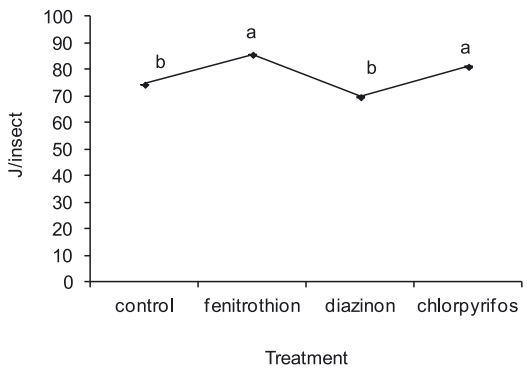

Fig. 5. Energy available (E_a) in *A. spinidens* treated with fenitrothion, diazinon, and chlorpyrifos. The points labeled with similar letters are not significantly different ($p \leq 0.05$)

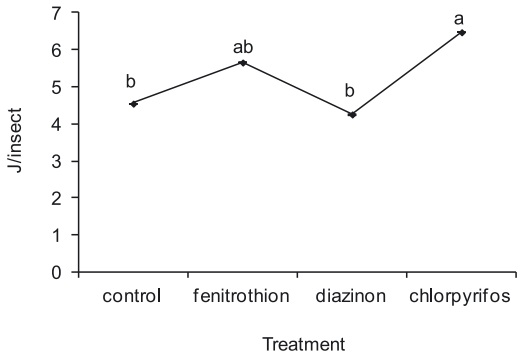

Fig. 6. Energy consumption (E_c) in *A. spinidens* treated with fenitrothion, diazinon, and chlorpyrifos. The points labeled with similar letters are not significantly different ($p \leq 0.05$)

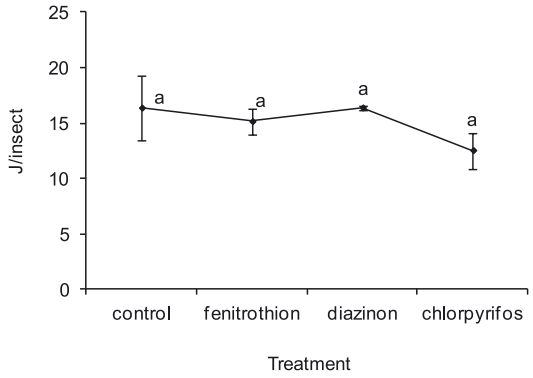

Fig. 7. Cellular energy allocation (CEA) in *A. spinidens* treated with fenitrothion, diazinon, and chlorpyrifos. The points labeled with similar letters are not significantly different ($p \leq 0.05$)

Discussion

Carbohydrate, protein, and lipid which are the major components of the insect body, play an important role in body construction and energy metabolism (Shoba *et al*. 2011). These constituents are affected by many factors especially by pesticide (Jabakumar and Jayaraman 1988). Investigations on the effects of pesticides have revealed that pesticides interfere with energy reserve metabolism in different species (Babu *et al*. 1988).

According to the results, the exposed insect showed no significant changes in carbohydrate contents. Investigations on the effects of pesticides have also revealed

that pesticides interfere with carbohydrate metabolism in different species (Babu *et al*. 1988). Either an increase or decrease in the carbohydrate contents has been observed after treatment with different pesticides. Some authors have reported elevated carbohydrate contents in some insect species as a response to the action of different pesticides, while others have reported the opposite. Tebufenozide treatment induced *Schistocerca gregaria* Forskal (Orthoptera: Acrididae) nymphs to gain more carbohydrates. Also, pyriproxyfen treatments of nymphs resulted in a reduced carbohydrate content in the fat body of 1-day old adults (Tanani *et al*. 2012). When compared to all the other treatments, we found that the fenitrothion treatment slightly increased carbohydrate contents, although this increase was not statistically significant among treatments. Fenitrothion induced an increase in blood glucose in the freshwater fish, *Sarotherodon mossambicus* Peters (Perciformes: Cichlidae), and this induction was associated with a decrease in hepatic glycogen (Koundinya and Ramamuthi 1979). Remia *et al*. (2008) reported that the elevation of carbohydrates might be due to the stress induced by the insecticides on the physiology of organisms with the help of corticosteroids.

Total lipid content in *A. spinidens* nymphs was not significantly affected by pesticides. Because of its high-energy content, lipid is the primary stored nutrient in insects and most other animals (Downer and Matthews 1976). Lipids play an important role in maintaining the integrity of cell structure and functions. Many studies have documented that lipid contents are affected by pesticide application. Fluctuation of lipid content in different species of insects treated with different toxicants has been reported by several investigators. Some authors found a significant decrease in the amount of lipid in the exposed insect (Verslycke *et al*. 2004; Bagheri *et al*. 2010; Shoba *et al*. 2011), while others have reported the opposite result. It is reported that a decrease in lipid contents may be induced by their metabolic activity for the energy production during pesticide stress. In this study, in the case of chlorpyrifos, pesticide was observed to slightly increase lipid contents, although this increase was not statistically significant among treatments. Chlorpyrifos treatment showed a significant increase in lipid content in resistant and susceptible strains of *Trogoderma granarium* E. (Coleoptera: Dermestidae) (Abdul Mujeeb *et al*. 2011). Some research showed that toxicants can influence lipid synthesis rather than lipid consumption. For example, after allatectomy of *Melanoplus differentialis* Thomas (Orthoptera: Acrididae) lipids accumulated in the fat bodies (Cymborowski 1992).

In this study, glycogen content was not significantly affected by pesticides. According to the obtained values, glycogen appeared to decrease in the treated insects compared to the control, although this reduction was not statistically significant among treatments. Glycogen is an important nutrient reserve in animal tissue and it is used as an immediate energy source when required by any animal. Glycogen is an essential component of the normal metabolism (Thunberg and Manchester 1972). Many authors have reported that glycogen content can be affected in the pesticide-exposed insect. Shoba *et al*. (2011) reported a decrease in the glycogen content of *Sphaerodema rusticum* Fabricous (Hemiptera: Belostomatidae) exposed to phytopesticide nimbecidine. It is suggested that this macromolecule assisted both aerobic and anaerobic glycolysis in providing energy to the exhausted insects. But the insecticidal toxicity no longer allow the synthesis of this macromolecule and the quantity of this macromolecule was reduced. Nath (2000) revealed a significant decrease in fat body glycogen on exposure to organophosphorus insecticides. In agreement with our results, Bagheri *et al*. (2010) reported no significant decrease or increase of glycogen content in *Brachynema germari* Kol. (Hemiptera: Pentatomidae) in relation to juvenoid pyriproxyfen. This means that the reservoir in the exposed insect was not affected by pesticide treatment.

Proteins are the most important organic constituents of animal tissues and play an important role in energy production. These macromolecules are the basic structural elements of insect muscles, glands, and other tissues, making up about 20 percent of some tissues such as flight muscles (Landa *et al*. 1991). In our experiments, pesticides caused a significant difference in protein contents compared to the control. From among the treatments, fenitrothion and chlorpyrifos increased protein contents twice more than the control. The highest value was observed in the chlorpyrifos treatment. It can be concluded, that increased protein contents after pesticide treatments could be due to outside stress or a response to suppress the stress. It is reported, that tissue proteins in animals under toxic stress play a role in the activation of a compensatory mechanism (Downer 1982) and increased whole body protein content occurs in response to some insecticides (Neoliya *et al*. 2005). Zhao and Jones (2012) demonstrated that insects respond to a variety of chemical and physical stresses by a rapid increase in the synthesis of a set of conserved polypeptides, collectively referred to as heat shock proteins (Hsps). The results of this study are consistent with the results of Abdul Mujeeb *et al*. (2011), who reported that *T. granarium* larvae showed a significant increase in total protein contents when exposed to chlorpyrifos. The researchers demonstrate that an increase in protein contents may be due to increased protein biosynthesis as a result of enzyme induction, to counter the toxic effect of the pesticide (Shahid Ali *et al*. 2013). In addition, elevated protein contents could be attributed to a possible conversion under stress conditions due to pesticides. A raised level in the soluble protein may be related to the increased activities of various enzymes (Saleem *et al*. 1998). Thus, different stresses can affect the amount of total protein in an insect. It appears that any possible protein loss is compensated by an increase in the tissue protein synthesis.

Results of the present research showed that E_a of *A. spinidens* was significantly affected by pesticides. Among the treatments, fenitrothion and chlorpyrifos showed the highest E_a value. Studies by many authors showed that insecticides may interfere with available energy. In agreement with our results, the total energy content of chlorpyrifos-exposed *Neomysis integer* Leach (Crustacea: Mysidacea) was higher than the E_a in the control (Verslycke *et al*. 2004). Bagheri *et al*. (2010) reported that pyriproxyfen affects the E_a amount in *B. germari*. En-

ergy availability can limit the ability of organisms to survive under stressful conditions (Marron *et al*. 2003).

In the case of E_c, there were significant changes in treated bugs. The highest value was observed in the chlorpyrifos treatment. It can be concluded that chlorpyrifos-treated bugs had a higher rate of oxygen consumption than those exposed to other treatments. Verslycke *et al*. (2004) reported similar results in chlorpyrifos-treated *N. integer*. Also, increased rates of oxygen consumption have been reported for *N. americana* exposed to naphthalene (Smith and Hargreaves 1984) and *Americamysis bahia* Haworth (Crustacae: Mysidae) exposed to the thiobencarb, endrin, fenthion, and DEF (McKenney 1998). Application of pyriproxyfen on *Aulacophora nigripennis* Motschulsky (Coleoptera: Chrysomelidae) also resulted in an increase in an insect's respiration rate (Watanabe and Tanaka 2009).

The present results indicate that a significant difference in the case of CEA among treatments was not observed. This parameter was calculated as the ratio of the E_a (sum of protein, sugar, and lipid reserve) to the E_c (as derived from the ETS activity). Thus, a decline in CEA indicates either a reduction in available energy or an increase in energy expenditure; both result in a lower amount of E_a for growth or reproduction (Verslycke *et al*. 2004). In this study CEA value in chlorpyrifos-treated bugs was the least, although there was no significant difference among treatments. The increase in E_c in chlorpyrifos-exposed bugs was the determining factor for the observed decline in CEA. Verslycke *et al*. (2004) reported that chlorpyrifos exposure has significant effects on the energy expenditure/acquisition of *N. integer*.

Conclusion

In an integrated control program, it was necessary to utilize some insecticides which have minimal toxicity to the natural enemies of the pests. The pesticides used in this study were organophosphate insecticides that are widely used to control rice lepidopter pests in Iran. Among the pesticides used in this study, fenitrothion and chlorpyrifos caused considerable effects on *A. spinidens* nymphs compared to the effect of the pesticide diazinon. The results showed that the E_a and E_c in exposed bugs were affected by fenitrothion and chlorpyrifos more than diazinon. Diazinon is commonly used in all of Iran's rice fields to control pests. Because the pesticide is extensively used, in comparison with the two other pesticides, it seems that bugs exposed to diazinon are more compatible with this pesticide. In addition, data obtained from LC_{30} values confirmed the low toxicity of this pesticide on *A. spinidens* nymphs compared with other pesticide treatments. Although there was no significant change in CEA, these insecticides markedly changed the physiological balance of *A. spinidens*. This fluctuation can potentially limit predator efficiency. In our study, proteins were the fraction that was most strongly affected by pesticides, therefore, protein metabolism might be an important endpoint to measure in toxicant-exposed bugs. This finding provides appreciable evidence that fenitrothion, chlorpyrifos, and diazinon are not suitable for use in the IPM of rice pests with *A. spinidens*. It is recommended that where the use of these pesticides is essential for control of rice pests, they should be used with caution. June and July are the times with the highest population of *A. spinidens* in rice fields. During these months the excessive use of pesticides should be avoided. The results suggest that the adverse effect of these insecticides on *A. spinidens* should be noted in IPM programs.

Acknowledgments

The authors express their gratitude to the Research Council of the University of Guilan and the Ministry of Science, Research and Technology for financial support during the course.

References

Abdul Mujeeb K., Latif M., Ayaz Ilyas M., Shahid Ali S. 2011. Effect of organophosphate insecticide chlorpyrifos on some biochemical components of *Trogoderma granarium* larvae Everts. Sci. Int. (Lahore) 23 (3): 229–233.

Babu P.R.A., Reddy G.R., Babu G.R.V., Chetty C.S. 1988. Glycolitic oxidation in freshwater fish, *Sarothe-rodon mossambicus* during benthiocarb exposure. Curr. Sci. 57 (11): 591–594.

Bagheri F., Talebi K.H., Hosseininaveh V. 2010. Cellular energy allocation of pistachio green stink bug, *Brachynema germari* Kol. (Hemiptera: Pentatomidae) in relation to juvenoid pyriproxyfen. Afr. J. Biotechnol. 9 (35): 5746–5753.

Bradford M. 1976. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72 (72): 248–254.

Cymborowski B. 1992. Insect Endocrinology. Polish Scientific Publisher PWN, Warszawa, 234 pp.

De Coen W.M., Janssen C.R., Giesy J.P. 2000. Biomarker applications in ecotoxicology: Bridging the gap between toxicology and ecology. p. 13–25. In: "New Microbiotests for Routine Toxicity Screening and Biomonitoring" (C.R. Janssen, G. Persoone, W.M. De Coen, eds.). Springer-Verlag, Berlin, 550 pp.

De Coen W.M., Janssen C.R. 1997. The use of biomarkers in *Daphnia magna* toxicity. Cellular energy allocation: a new methodology to assess the energy budget of toxicant-stressed Daphnia populations. J. Aquat. Ecosyst. Stress Recovery 6 (1): 43–55.

Downer R.G., Matthews J.R. 1976. Patterns of lipid storage and utilization in insects. Am. Zool. 16 (4): 733–745.

Downer R.G. 1982. Fat body and metabolism. p. 52–60. In: "The American Cockroach" (W.J. Bell, K.G. Adiyodi, eds.). Chapman & Hall, NY, 529 pp.

El Wakeil N., Gaafar N., Sallam A., Volkmar C. 2013. Side effects of insecticides on natural enemies and possibility of their integration in plant protection strategies. p. 1–56. In: "Insecticides: Development of Safer and More Effective Technologies Agricultural and Biological Sciences (S. Trdan, ed.). InTech Open Access Publisher, 548 pp.

Jabakumar S.R.D., Jayaraman F.A.Z. 1988. Changes in protein, lipid and carbohydrate content in fresh water fish, *Lepidocephalicythys thermalis* during short-term sublethal exposure of malathion. Ann. Zool. 26 (1): 82–89.

Jadhao M. 2011. A preliminary study of the predatory natural enemy complex of rice ecosystem in Vidarbha region of Maharashtra, India. Int. Ref. Res. J. 2 (22): 25–27.

King F., Packard T.T. 1975. Respiration and the activity of the respiratory electron transport system in marine zooplankton. Limnol. Oceanogr. 20 (5): 849–854.

Koundinya P.R., Ramamurthi R. 1979. Effect of organophosphate pesticide (fenitrothion) on some aspects of carbohydrate metabolism in a freshwater fish, *Sarotherodon* (*Tilapia*) *mossamicus* (Peters). Experientia 35: 1632–1633.

Landa V., Sula J., Marec F., Matha V., Soldan, T. 1991. Methods for assessing exposure of insects. p. 249–266. In: "Methods for Assessing Exposure of Human and Non-Human Biota" (R.G. Tardiff, B. Goldstein, eds.). John Wiley & Sons Ltd., Chichester, New York, 417 pp.

LeOra software. 1987. POLO-PC: a user guide to probit or logit 786 analysis. Berkeley (CA): LeOra software.

Manley G.V. 1982. Biology and life history of the rice field predator *Andrallus spinidens* F. (Heteroptera: Pentatomidae). Entomol. News 93 (1): 19–24.

Marron M.T., Markow T.A., Kain K.J., Gibbs A.G. 2003. Effects of starvation and desiccation on energy metabolism in desert and mesic *Drosophila*. J. Insect Physiol. 49 (3): 261–270.

McKenney C.L. 1998. Physiological dysfunction in estuarine mysids and larval decapods with chronic pesticide exposure. p. 465– 476. In: "Microscale Testing in Aquatic Toxicology: Advances, Techniques, and Practice" (P.G. Wells, C. Blaise, eds.). CRC Press, Boca Raton, Florida, 720 pp.

Najafi-Navaee A., Saeb H., Osco T. 1998. Biology and ecology of *Andrallus spinidens* F. as the predator of rice, cotton and maize pests. p. 49. In: 13th Iranian Plant Protection Congress, Karaj, Iran, 23-27 August 1998, 262 pp.

Nath B.S. 2000. Changes in carbohydrate metabolism in hemolymph and fat body of the silkworm, *Bombyx mori* L. exposed to organophosphorus insecticides. Pestic. Biochem. Physiol. 68 (3): 127.

Neoliya N.K., Singh D., Sangawan R. 2005. Azadiractin influences total head protein content of *Helicoverpa armigera* Hub. larvae. Curr. Sci. 88 (12): 1889–1990.

Noorhosseini Niyaki S.A. 2010. Decline of pesticides application by using biological control: the case study in North of Iran. Middle-East J. Scientific Res. 6 (2): 166–169.

Oberdorster E., Rittschof D., McClellan-Green P. 1998. Induction of cytochrome P450 3A and heat shock protein by tributyltin in blue crab, *Callinectes sapidus*. Aquat. Toxicol. 41 (1–2): 83–100.

Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC.

Remia K.M., Logaswamy S., Logankumar K., Rajmohan D. 2008. Effect of an insecticides (Monocrotophos) on some biochemical constituents of the fish *Tilipia mossambica*. Poll. Res. 27 (3): 523–526.

Saleem M.A., Shakoori A.R., Mantle D. 1998. In vivo ripcord induced macromolecules abnormalities in *Tribolium castenium* larvae. Pakistan J. Zool. 30 (3): 233–243.

SAS Institute, 2002. SAS/STAT user's guide. SAS Institute Inc., Cary, NC Inc.

Shahid Ali N., Shahid Ali S., Shakoori A.R. 2013. Effects of sublethal doses of malathion on biochemical components of malathion-resistant and -susceptible adults of *Rhyzopertha dominica*. Pakistan J. Zool. 45 (1): 203–212.

Shoba V., Elanchezhiyan C., Hemalatha S., Selvisabanayakam S. 2011. Sublethal effect of phytopesticide nimbecidine on biochemical changes in the adult male insect *Sphaerodema rusticum* (Heteroptera: Belostomatidae). Int. J. Res. Pharm. Sci. 2 (1): 12–17.

Smith R.L., Hargreaves B.R. 1984. Oxygen consumption in *Neomysis americana* (Crustacea: Mysidacea) and the effects of naphthalene exposure. Mar. Biol. 79 (2): 109– 116.

Tanani M.A., Ghoneim K.S., Hamadah Kh.Sh. 2012. Comparative effects of certain igrs on the carbohydrates of hemolymph and fat body of the desert locust, *Schistocerca gregaria* (Orthoptera: Acrididae). Fla. Entomol. 95 (4): 928–935.

Thunberg L.V., Manchester K.L. 1972. Effect of denervation on the glycogen content and on the activities of enzyme glucose and glycogen metabolism in rat diaphragm muscle. Biol. J. 128 (4): 128–789.

Verslycke T., Roast S.D., Widdows J., Jones M.B., Janssen C.R. 2004. Cellular energy allocation and scope for growth in the estuarine mysid *Neomysis integer* (Crustaycea: Mysidacea) following chlorpyrifos exposure: a method comparison. Exp. Marine Biol. Ecol. J. 360 (1): 1–16.

Watanabe M., Tanaka K. 2009. Hormonal control of diapause and overwintering traits in a leaf beetle, *Aulacophora nigripennis*. Physiol. Entomol. 25 (4): 337–345.

Yuval B., Kaspi R., Shloush S., Warburg M.S. 1998. Nutritional reserves regulate male participation in Mediterranean fruit fly leks. Ecol. Entomol. 23 (2): 211–215.

Zhao L., Jones W.A. 2012. Expression of heat shock protein genes in insect stress responses. Minireview 9 (1): 93–101.