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Energetic costs of resistance in the *Agonoscena pistaciae* Burckhardt & Lauterer, 1989 (Hemiptera: Aphalaridae), against spirotetramat, acetamiprid and hexaflumuron

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Abstract: The common pistachio psylla, *Agonoscena pistaciae* Burckhardt & Lauterer, 1989 (Hemiptera: Aphalaridae), is a key pest found in pistachio orchards in Iran. This pest has a high potential for developing resistance to insecticides due to its short life cycle and high reproductive potential. Intensive application of insecticides leads to excessive selection pressure followed by resistance to synthetic insecticides in some psylla populations. In this research, effects of four concentrations of three extensively used insecticides (spirotetramat, acetamiprid, and hexaflumuron) on energy resources (like sugar, lipid, glycogen, and protein contents), energy consumption, and cellular energy allocation were investigated in resistant and susceptible populations of the common pistachio psylla in the Kerman province of Iran. Energy resource contents in the susceptible population (133 331.2 mj/insect) were significantly more than in the resistant population (96 253.5 mj/insect), whereas energy consumption in the resistant population (38 630.4 mj energy/h/insect) was higher than in the susceptible population (31 276.8 mj energy/h/insect). Additionally, cellular energy allocation in the resistant population (2 400.9 mj energy/h/insect) was higher than in the susceptible population (2 126.13 mj energy/h/insect). Therefore, stress (especially toxicants) causes variations in metabolism, which influences the growth and reproduction of the pest.

Keywords: cellular energy allocation, common pistachio psylla, energy consumption, energy resource

Introduction

The common pistachio psylla, *Agonoscena pistaciae* Burckhardt & Lauterer, 1989 (Hemiptera: Aphalaridae), is known as the key pest in pistachio trees in Iran (Mehrnejad 2001), causing substantial damages to pistachio orchards annually. Direct feeding of the pest causes reduced plant growth, defoliation, stunting, falling of fruit buds, and poor yield (Burckhardt & Lauterer 1993). Due to its high ability to reproduce and create multiple generations, this pest produces a very large population in most years and causes a lot of damage to pistachio trees. A severe outbreak of this pest not only reduces the current year's yield but also leads to abscission of the following year's flower buds and leaves, and causes weakness in trees (Mehrnejad 2003, Mehrnejad & Copland 2005). Currently, chemical control is the most efficient way to reduce the density of the pest population and inhibit related damage. Several insecticides have been tested against the common pistachio psylla, but due to its high reproductive ability and the existence of several generations of this pest, as well as ambiguous levels of economic loss and extensive fumigations frequently by farmers, continuous use of these toxins over several years has led to resistance, elimination of natural enemies of the pest, a severe outbreak of the pest, and pesticide residues in pistachio trees. Furthermore, insecticides have various

sublethal effects including alteration in developmental rate (Willrich & Boethel 2001), increase and decrease in fecundity (Takada *et al.* 2001), and changes in sex ratio, diapause, and morphology (Croft 1990) of the pest insects.

Currently, some insecticides like spirotetramat, acetamiprid and hexaflumuron are used widely in pistachio orchards (Noorbakhsh *et al.* 2001). Spirotetramat (Movento®) is a tetronic acid derivative and a new cyclic ketoenol compound that is introduced against sucking insect pests in crops. It is a lipogenesis-inhibiting insecticide resulting in lipid content depletion, growth inhibition of younger insects, and a reduction in the reproductive ability of adults (Bruck *et al.* 2009). This compound exhibit toxicity against eggs and nymphs of the susceptible pests (Elizondo & Murguido 2010). Additionally, treated females significantly decrease their fecundity and fertility like in *Bactericera cockerelli* (Šulc, 1909) (Hemiptera: Triozidae) (Tucuch-Haas *et al.* 2020). Acetamiprid is a neonicotinoid insecticide, with systemic, translaminar, and contact activity. This group acts rapidly as an agonist of the nicotinic acetylcholine receptor in the postsynaptic membrane and leads to paralysis and death of treated insects (Elbert *et al.* 1991, Schroeder & Flattum 1984). Hexaflumuron, as an insect growth regulator (a benzoylphe-nylurea) compound, interferes with chitin synthesis and disturbs hormonal balance with the molting process alteration and is a growth inhibition of insects (Oberlander & Silhacek 1998). This insecticide is widely used against homopterous insects, especially *A. pistaciae* (Alimohamadi *et al.* 2014).

The resistance of *A. pistaciae* population to phosalone (Alizadeh *et al.* 2011, Talebi *et al.* 2001), spirotetramat, acetamiprid, and hexaflumuron (Bemani *et al.* 2018) has been determined in some pistachio producing areas of Kerman province.

Insecticide resistance studies are not only practically important in pest management programs but are also critical as evolution models of newly adapted phenotypes and their related physiological (and genetic) changes (Raymond *et al.* 2001). The major genes responsible for individual adaptation to a new environment are usually associated with an adaptive cost because they may be at a disadvantage in the previous environment where independent selection pressures shaped dominant phenotypes (Berticat *et al.* 2002). This hypothesis is based on the general view that resource allocation takes place, affecting metabolic or developmental processes and decreasing reproductive potential (Berticat *et al.* 2002).

Overall, insecticide resistance is usually associated with adaptive costs in the absence of insecticides (Coustau & Chevillon 2000). The most common explanation for the effects of insecticide resistance is the existence of resource-based trade-offs. According to the hypothesis; insecticide resistance will decrease the energetic resources of insects, reducing accessibility energy for other biological functions and generating trade-offs between insecticide resistance and essential life requirements (Rivero *et al.* 2011). If energy metabolism does not increase adequately, energy reallocation may deflect energy from other physiological processes involved in insect development, preservation, and reproduction (Chown & Gaston 1999, Harak *et al.* 1999). The insecticide interference with reservation of carbohydrates and proteins in the fat body (Nath 2000, Nath *et al.* 1997). However, these studies are yet to be comparatively applied to insecticide-resistant and insecticide-susceptible populations (Guedes *et al.* 2006).

This study aimed to clarify physiological mechanisms through which the common pistachio psylla becomes resistant to three common insecticides (spirotetramat, acetamiprid, and hexaflumuron). For this purpose, we investigated the effects of four concentrations of these insecticides on several physiological parameters such as body energy

resources (like sugar, lipid, glycogen, and protein), energy consumption, and cellular energy allocation in the fifth instar nymphs of *A. pistaciae* in a resistant population (Rafsanjan) versus susceptible population (Anar) (Bemani *et al.* 2018) in Kerman province, Iran.

Materials and Methods

Five populations of *A. pistaciae* were collected from pistachio orchards of Rafsanjan, Anar, Sirjan, Kerman, and Zarand in Kerman province, Iran, during the summer of 2016. Resistant and susceptible populations were determined using preliminary bioassay and chemical tests. The Anar population (with the LC_{50} values of 11.92, 24.13, and 81.06 mg a.i. L^{-1} for acetamiprid, spirotetramat, and hexaflumuron, respectively) had the lowest LC_{50} values and was used as a susceptible population. The Rafsanjan population (with the LC_{50} values of 40.55, 43.65, and 95.10 mg a.i. L^{-1}, for acetamiprid, spirotetramat, and hexaflumuron, respectively) showed the highest level of resistance (3.4-fold for acetamiprid with confidence limits 95%, 2.27-5.07) based on bioassay (Bemani *et al.* 2018) with high activity of detoxifying enzymes (esterase and glutathione S-transferase) that esterase activity was 1.24-fold in the resistant population as compared to the susceptible population (Bemani *et al.* in press). Resistant and susceptible populations of psylla were routinely reared in plastic boxes (50 × 60 × 80 cm) on young, untreated pistachio leaves under greenhouse conditions at 25 ± 2˚C, 45 ± 5% RH, and a 16:8 L:D photoperiod. One-day-old fifth instar nymphs of *A. pistaciae* were treated with four concentrations (control, LC_{25}, LC_{50}, and LC_{75}) of spirotetramat, acetamiprid, and hexaflumuron. Fifth instar nymphs were used because they were recognizable from the other nymphs and their mortality was low. Sprayed with 1 ml of aqueous emulsions of different concentrations of each insecticide. The spray was applied at 15 mbar using a Potter Precision Spray Tower (Burkard Manufacturing Co. Ltd., Rickmansworth Herts, UK). After 24 hours for acetamiprid and 48 hours for spirotetramat and hexaflumuron (due to their different modes of action), living psyllids were transferred to microtubes and kept in a refrigerator at -80˚C for other experiments.

Preparation of Whole Body Homogenates for Chemical Analysis

Total body sugars (monosaccharides and disaccharides) were measured using a standard method (Warburg & Yuval 1996). Fifth instar nymphs of common pistachio psylla (N=30) from resistant and susceptible populations were carefully weighed and homogenized with 200 µL of 2% Na_2SO_4. chloroform-methanol (1300 µL) (1:2) was added to the homogenate to extract simple carbohydrates of the nymphs. Individual homogenates were centrifuged for 10 minutes at 7150×g. To determine the amount of carbohydrate, 300 µL was taken from the supernatant and mixed with 200 µL distilled water. The sample was made to react for 10 minutes at 90˚C with 1 ml of anthrone reagent (500 mg anthrone dissolved in 500 ml concentrated H_2SO_4). Absorbance was 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) as the standard. This experiment was repeated four times.

Glycogen

Glycogen content was measured from the residual of the centrifugation technique mentioned above. The pellet was washed with methanol, to remove possible remnants of sugar. To extract the glycogen, 250 µl distilled water was added to the pellet and the mixture was heated for 5 minutes at 70˚C. Subsequently, 200 µL of the solution was removed and made to react for 10 minutes at 90˚C with 1 ml anthrone reagent (600 mg anthrone dissolved in 300 ml concentrated 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) as the standard. This experiment was repeated four times.

Lipids

To determine the amount of lipids in psylla nymphs, according to a standard method (Warburg & Yuval 1996), 300 µL of supernatant was taken and evaporated at 35˚C in an oven. Samples from each tube were dissolved in 300 µL H_2So_4. Samples were heated for 10 minutes at 90˚C. Then, they were cooled and stirred. An amount of 2700 µL vanillin reagent (600 mg vanillin + 100 ml distilled water + 400 ml of 85% H_3Po_4) was added to the samples. Tubes were shaken for 30 minutes at room temperature. Absorbance was measured at 530 nm on a spectrophotometer (T60U, Harlow Scientific, USA). The amount of lipid was determined from a standard curve using triolein (Sigma) as the standard. This experiment was repeated four times.

Protein

Total protein content was measured by a standard method (Lowry *et al.* 1951). Fifth instar nymphs of common pistachio psylla (N=30) from two populations (resistant and susceptible) were carefully weighed, and homogenized in 1.5–2 ml of 80% ethanol. Homogenates were centrifuged for 15 min at 12 000 × g. The residue was resuspended in a solution of 1% SDS containing 0.4% sodium hydroxide, 2% sodium carbonate, and 0.18% sodium or potassium tartrate. They were stored at room temperature for 24 hours. Then 150 µl of the sample was mixed with 850 µl of distilled water and then 3 ml of lowry solution was added to each sample. The samples were completely vortexed and after 10 minutes 150 µl of folin solution was added. The absorbance was measured by a spectrometer at 540 nm. Bovine serum albumin was used as the standard. This experiment was repeated four times.

Energy Consumption Assay

Samples were prepared in the same manner as the protein assay and the supernatant was used in this experiment. Energy consumption was determined by measuring mitochondrial electron transport (King & Packard 1975). The homogenizing medium consisted of 0.01 M Tris-HCl buffer (W:V), polyvinylpyrrolidone, magnesium sulphate (153 µl), and 0.2% triton X-100 (W:V). Samples with 200 µl of homogenizing buffer were homogenized and centrifuged for 15 minutes at 10 000 rpm. Forty microliters of the supernatant, 120 µl buffer substrate solution (Tris-HCl buffer, 0.3% triton X-100 and NADH-NADPH), and 80 µl of 8 mM iodonitrotetrazolium chloride were added to microplate wells. Absorbance was read in a microplate reader (Epoch, Gen 5). All chemical compounds except Tris were obtained from Sigma-Aldrich.

Cellular Energy Allocation Estimation

Cellular energy allocation was estimated by a standard method (Gnaiger 1983). Based on the concentrations obtained from each energy source, their equivalent was converted into joule heat by the fuel. The amount of oxygen consumed was determined based on the absorption coefficient of the INT formazan; and since each mole of oxygen is equal to 480 kilojoules of energy consumption, the consumption of energy was calculated. Finally, by determining available energy and energy consumption (through the formula given below), the total pure budget per millijoule for each fifth instar nymph was calculated during the desired period.

$$CEA(mJ\ /\ org) = \frac{(\int_{t_0}^{t_x} E_a dt - \int_{t_o}^{t_x} E_c dt)}{T}$$

Table 1. Effects of acetamiprid on energy resources of resistant and sensitive populations of *Agonoscena pistaciae.* († – four replicates were performed for each concentration and each replication with thirty fifth instar nymphs of the common pistachio psylla, * – means within a column followed by the same lowercase letter are not significantly different among concentrations in each population (LSD's test, $p < 0.05$))

Population	Concentration [mg a.i./L] †	Energy resource [mg x g^{-1}] ± SE			
		Sugar	Glycogen	Protein	Lipid
Rafsanjan	0	3.80 ± 0.53 *a	1.07 ± 0.17	0.18 ± 0.00 a	0.99 ± 0.25
	24	2.84 ± 0.60 ab	0.96 ± 0.04	0.25 ± 0.02 b	0.56 ± 0.19
	40	2.73 ± 0.22 ab	0.79 ± 0.083	0.30 ± 0.00 b	0.46 ± 0.23
	70	1.72 ± 0.20 b	0.61 ± 0.00	0.38 ± 0.03 c	0.16 ± 0.06
Anar	0	4.86 ± 0.10 a	1.32 ± 0.56	0.17 ± 0.00	2.25 ± 0.17 a
	3	3.70 ± 0.33 b	1.08 ± 0.15	0.20 ± 0.04	1.51 ± 0.08 b
	11.9	3.39 ± 0.21 bc	0.92 ± 0.08	0.23 ± 0.02	1.34 ± 0.09 bc
	24	2.87 ± 0.31 c	0.93 ± 0.14	0.25 ± 0.02	1.05 ± 0.00 c

Table 2. Effects of spirotetramat on energy resources of resistant and sensitive populations of *Agonoscena pistaciae*. († – four replicates were performed for each concentration and each replication with thirty fifth instar nymphs of the common pistachio psylla, * – means within a column followed by the same lowercase letter are not significantly different among concentrations in each population (LSD's test, $p < 0.05$))

Population	Concentration [mg a.i./L] †	Energy resource [mg x g^{-1}] ± SE			
		Sugar	Glycogen	Protein	Lipid
Rafsanjan	0	3.78 ± 0.53	1.07 ± 0.17	0.19 ± 0.02	0.99 ± 0.25 *a
	15	3.51 ± 0.27	0.87 ± 0.10	0.22 ± 0.01	0.45 ± 0.17 ab
	43	3.04 ± 0.42	0.81 ± 0.14	0.23 ± 0.02	0.37 ± 0.15 b
	90	3.02 ± 0.24	0.81 ± 0.13	0.25 ± 0.01	0.15 ± 0.10 b
Anar	0	4.86 ± 0.10	1.32 ± 0.56	0.16 ± 0.02	2.25 ± 0.17 a
	10	4.52 ± 0.87	1.19 ± 0.20	0.20 ± 0.01	1.43 ± 0.09 b
	24	4.05 ± 0.07	1.12 ± 0.17	0.22 ± 0.02	1.08 ± 0.07 c
	50	3.93 ± 0.38	1.05 ± 0.25	0.24 ± 0.03	0.90 ± 0.04 c

Statistical Analyses

Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by a post-hoc LSD test (P=0.05). Data were initially tested for normality (Kolmogorov-Smirnov test) and homo-scedasticity (Levene's test) before subjecting them to ANOVA. A Student's t-test was applied to compare the means of two groups when necessary. Results were expressed as mean ± SE and considered significantly different at P<0.05.

Results

Carbohydrate Contents

The total sugars and glycogen levels were not significantly different in resistant and susceptible populations (t=1.98, P=0.1 for sugar and t=0.517, P=0.619 for glycogen) (Fig. 1). In the Anar (susceptible) population, the sugar and the glycogen contents were 1.28 and 1.23 folds more than the Rafsanjan (resistant) population. In the Rafsanjan population, the sugar content was affected by different concentrations of acetamiprid (F=3.554, P=0.042) but not by the two other insecticides (F=0.805, P=0.512 for spirotetramat and F=0.139, P=0.935 for hexaflumuron). However, the glycogen content was not affected by any concentrations of the insecticides in this population (F=2.115, P=0.144 for acetamiprid, F=0.761, P=0.535 for spirotetramat, and F=0.357, P=0.785 for hexaflumuron).

In the Anar population, the sugar content was affected by acetamiprid (F=10.926, P=0.001) but not by the other insecticides (F=0.808, P=0.514 for spirotetramat and F=1.132, P=0.375 for hexaflumuron). Glycogen content, however, was not affected by any concentrations of the insecticides in this

Table 3. Effects of hexaflumuron on energy resources of resistant and sensitive populations of *Agonoscena pistaciae*. († – four replicates were performed for each concentration and each replication with thirty fifth instar nymphs of the common pistachio psylla)

Population	Concentration [mg a.i./L] †	Energy resource [mg x g^{-1}] ± SE			
		Sugar	Glycogen	Protein	Lipid
Rafsanjan	0	3.80 ± 0.53	1.07 ± 0.17	0.19 ± 0.02	0.99 ± 0.25
	60	3.54 ± 1.40	0.88 ± 0.08	0.21 ± 0.02	0.91 ± 0.22
	95	3.26 ± 0.37	0.96 ± 0.17	0.23 ± 0.02	0.77 ± 0.32
	160	3.25 ± 0.20	0.90 ± 0.11	0.27 ± 0.01	0.58 ± 0.21
Anar	0	4.86 ± 0.10	1.32 ± 0.56	0.16 ± 0.02	2.25 ± 0.17
	40	4.11 ± 0.43	0.91 ± 0.18	0.17 ± 0.02	2.01 ± 0.06
	81	4.26 ± 0.38	0.96 ± 0.09	0.18 ± 0.02	1.96 ± 0.05
	130	3.94 ± 0.48	0.95 ± 0.23	0.23 ± 0.04	1.97 ± 0.00

Table 4. Effects of insecticides on energy consumption and cellular energy allocation of resistant and sensitive populations of *Agonoscena pistaciae*. († – four replicates were performed for each concentration and each replication with thirty fifth instar nymphs of the common pistachio psylla)

Population	Insecticide	Concentration [mg a.i./L] †	Energy consumption [mj energy/h/Insect] ± SE	Cellular energy allocation [mj/Insect] ± SE
Rafsanjan	Acetamiprid	0	38 630.4 ± 6 159.9	2 400.9 ± 401.5
		24	45 676.8 ± 6 965.3	1 939.4 ± 1 104.6
		40	40 320 ± 10 567.7	1 719.1 ± 814.1
		70	39 033.6 ± 3044.7	1 118.2 ± 367.4
	Spirotetramat	0	67 084.8 ± 3 710.5	607.6 ± 124.2
		15	68 336.6 ± 4 643.8	405.8 ± 328
		43	58 828.8 ± 7 071.8	386.5 ± 160.3
		90	56 601.6 ± 7 630	155.4 ± 160.9
	Hexaflumuron	0	67 084.8 ± 3 710.5	607.6 ± 124.2
		60	64 166.4 ± 17 260.4	571.1 ± 624.4
		95	76 761.6 ± 10 786.7	258.3 ± 649.3
		160	73 190.4 ± 3 783.7	205.9 ± 209.3
Anar	Acetamiprid	0	31 276.8 ± 4 877.4	2 126.1 ± 197.1
		3	35 136 ± 7 417	1 699.4 ± 334.3
		11.9	43 449.6 ± 10 935.3	1 167.2 ± 528
		24	48 864 ± 11 186.7	947.9 ± 271.3
	Spirotetramat	0	62 707.2 ± 11 418.6	1471.3 ± 89
		10	67 392 ± 7 928.1	1 096.4 ± 236
		24	64 281.6 ± 10 219	869.9 ± 118.3
		50	69 964.8 ± 7 881.5	561.1 ± 360.6
	Hexaflumuron	0	62 707.2 ± 11 418.6	1471.3 ± 89
		40	71 001.6 ± 9 742.7	1 254.5 ± 212.2
		81	40 704 ± 2 6821.7	1 536.3 ± 938.5
		130	65 702.4 ± 9 120	847.5 ± 237.2

population (F=0.399, P=0.756 for acetamiprid, F=0.122, P=0.945 for spirotetramat and F=0.368, P=0.778 for hexaflumuron) (Tables 1–3).

Lipid Content

The lipid content (t=3.708, P=0.006) steadily decreased in resistant population versus susceptible population (Fig 1). Lipid content was affected by spirotetramat (F=3.533, P=0.043), but not by the two other insecticides (F=2.731, P=0.083 for acetamiprid and F=0.473, P=0.706 for hexaflumuron) in the Rafsanjan population. Lipid content in the Anar population was affected by acetamiprid (F=15.695, P=0.000) and spirotetramat (F=30.892, P=0.000), whereas hexaflumuron did not influence glycogen content (F=1.516, P=0.261).

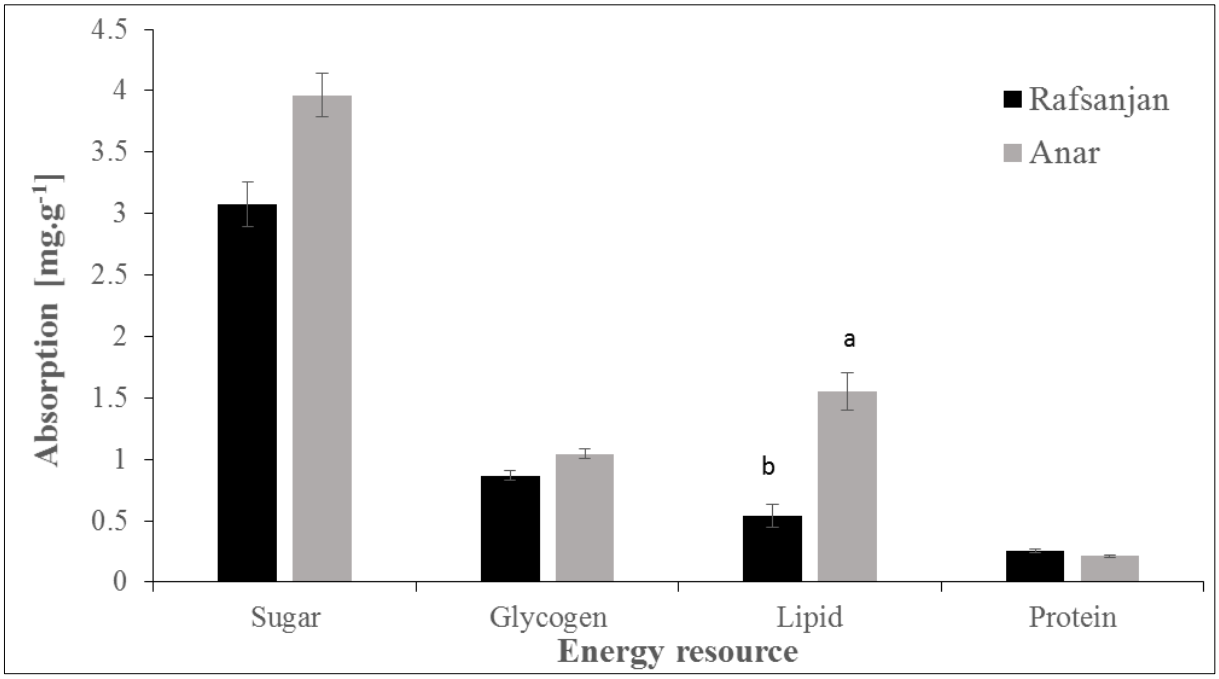

Fig 1. Energy resource content difference between resistance (Rafsanjan) and susceptible (Anar) populations of *Agonoscena pistaciae*.

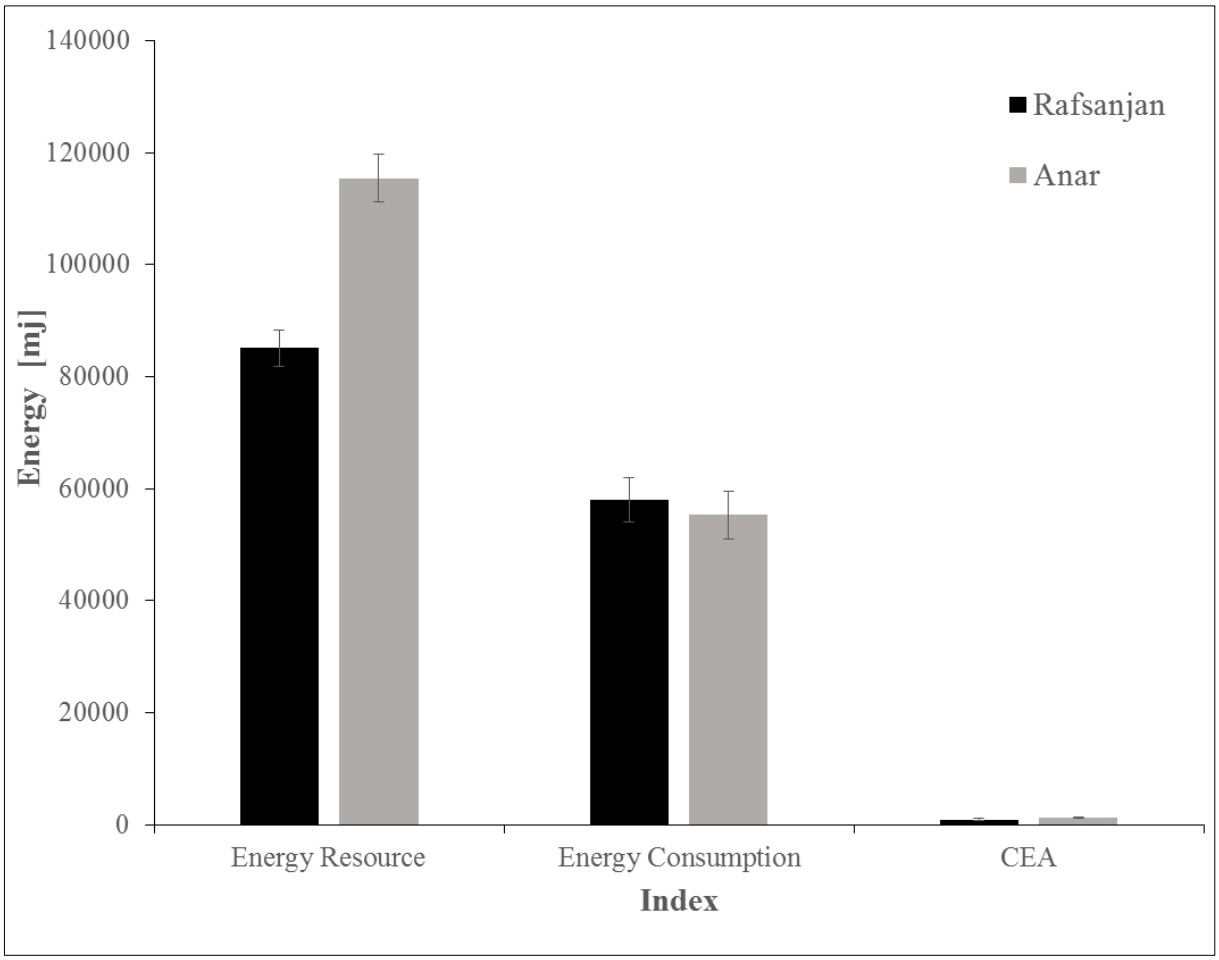

Fig 2. Difference in energy resource, energy consumption and cellular energy allocation content between resistance (Rafsanjan) and susceptible (Anar) populations of *Agonoscena pistaciae*.

Protein Content

There were no significant differences in protein content among the populations (*t*=1.435, *P*=0.201) (Fig. 1). Protein content in the Rafsanjan population was 1.08 fold higher than the Anar population. In the Rafsanjan population protein content was affected by acetamiprid (*F*=16.030, *P*=0.000), whereas spirotetramat (*F*=2.653, *P*=0.096) and hexaflumuron (*F*=3.377, *P*=0.054) did not influence protein content. In the Anar population, protein content was not affected by the insecticides (*F*=2.079, *P*=0.157 for acetamiprid, *F*=2.428, *P*=0.116 for spirotetramat, and *F*=1.206, *P*=0.350 for hexaflumuron).

Energy Consumption

Results showed that there was no significant difference in energy consumption among the populations (*t*=0.936, *P*=0.402) (Fig. 2). The content of energy consumption in the Rafsanjan population (38 630.4 mj energy/h/insect) was 1.23 fold higher than the Anar population (31 276.8 mj energy/h/insect).

The content of energy consumption in both Rafsanjan and Anar populations was not affected by the insecticides (*F*=0.204, *P*=0.891 for acetamiprid, *F*=0.958, *P*=0.458 for spirotetramat and *F*=0.296, *P*=0.828 for hexaflumuron in the Rafsanjan) (*F*=0.782, *P*=0.536 for acetamiprid, *F*=0.116, *P*=0.948 for spirotetramat and *F*=0.692, *P*=0.582 for hexaflumuron in the Anar) (Table 4).

Cellular Energy Allocation

Results revealed that there was no significant difference in cellular energy allocation content among the populations (*t*=0.614, *P*=0.572) (Fig. 2). The content of cellular energy allocation in the Rafsanjan population (2 400.9 mj energy/h/insect) was 1.12 fold higher than the Anar population (2 126.13 mj energy/h/insect).

The content of cellular energy allocation in both Rafsanjan and Anar populations was not affected by any of the insecticides (*F*=0.521, *P*=0.680 for acetamiprid, *F*=0.784, *P*=0.536 for spirotetramat and *F*=0.199, *P*=0.894 for hexaflumuron in the Rafsanjan) (*F*=2.243, *P*=0.161 for acetamiprid, *F*=2.831, *P*=0.106 for spirotetramat and *F*=0.390, *P*=0.763 for hexaflumuron in the Anar) (Table 4).

Discussion

In this research, energy resources and energy consumption were investigated in resistant (Rafsanjan) and susceptible (Anar) populations of the common pistachio psylla in the Kerman province of Iran to determine physiological mechanisms associated with resistance to insecticides in this pest.

As a result of the hyperactivity of detoxifying enzymes, some costs will be imposed on insects such as a decline in energy resources (Rivero *et al.* 2011, Sak *et al.* 2006). Data from this study revealed that lipid was the only energy resource with a significant difference in resistant and susceptible psylla populations. So, it can be concluded from our results that the decline in lipid content is a cost of detoxifying enzyme hyperactivity. However, all energy resources (lipid, protein, sugar, and glycogen) were influenced by different concentrations of various insecticides (acetamiprid, spirotetramat, and hexaflumuron) in both populations. Increasing concentrations of insecticides led to a decrease in glycogen, lipid, and sugar contents. A decrease in these energy resources by increasing pesticide doses may also be considered another cost of an increase in detoxifying enzyme activity as well as an increase in xenobiotic stress.

Exposure of the insect body to xenobiotic compounds like pesticides can modify the synthesis of essential metabolites and disrupt their functionality. Although in pest species, resistance to insecticides provides crucial benefits (Berticat *et al.* 2008) but this resistance also imposes several fitness costs (Kliot & Ghanim 2012). However, fitness costs are considered to be a conclusion of trade-offs

in the allocation of energy between fitness and traits underlying insecticide resistance (Ghadamyari *et al.* 2008). Such costs result in impairment of reproductive performance in resistant individuals due to the reallocation of resources from a fundamental physiological process for protection against insecticides, favoring their survival at the expense of their reproduction (Coustau & Chevillon 2000, Guedes *et al.* 2006). Identifying fitness expenses as a consequence of resistance to any insecticide can be profitable for designing an integrated pest management program (IPM) by limiting the distribution of the resistant population (Kliot & Ghanim 2012). Bouabida and colleagues (2017) studied the effects of spiromesifen on *Culiseta longiareolata* Macquart, 1838 mosquitoes. They showed that lipid and protein contents decrease in treating fourth instar larvae, while carbohydrate does not show any changes as compared with control (Bouabida *et al.* 2017). Alimohammadi *et al.* (2014) reported that the fourth instar larvae of *Hippodamia variegate* (Goeze, 1777) showed a reduction in glycogen content after treatment with hexaflumuron and spirodiclofen. Piri *et al.* (2014) revealed that in larvae of *Glyphodes pyloalis* Walker, 1859 treated with spinosad, carbohydrate, lipid, and protein contents decreased with an increase in concentration, (Piri *et al.* 2014). Novais and Amorim (2013) treated the soil invertebrate, *Enchytraeus albidus* Henle, 1837 (Oligochaeta), with dimethoate, atrazine, and carbendazim and found a significant decrease in energy reserves. They reported an increase in energy consumption by increasing both exposure time and concentration of pesticides. Rivero *et al.* (2011) tested energetic resources (lipids, glycogen, and glucose) of larvae and adult females of the mosquito, *Culex pipiens* Linnaeus, 1758 (Diptera: Culicidae), that is resistant to insecticides through two different mechanisms (esterase overproduction and acetylcholinesterase modification). They revealed that insecticide-resistant mosquitoes contain an average of 30% less energetic reserves than their susceptible counterparts through the overproduction of esterases. However, acetylcholinesterase-modified mosquitoes also showed a significant reduction in energetic resources (20% less). Hardstone *et al.* (2010) exemplified this cost by showing the significant reduction of energetic resources (lipid and glycogen) in insecticide-resistant *C. pipiens* through a different detoxification mechanism (cytochrome P_{450}) (Hardstone *et al.* 2010). Sak *et al.* (2006) investigated the changes in total body weight, glycogen, protein, and lipid contents of the endoparasitoid, *Pimpla turionellae* Linnaeus, 1758 (Hymenoptera: Ichneumonidae) reared on *Galleria mellonella* Linnaeus, 1758 (Lepidoptera: Pyralidae). The host was exposed to various sublethal doses of cypermethrin added to the food. Cypermethrin affected the total body weight of larvae, pupae and adult females, but not of males. They showed that levels of glycogen, protein, and lipid in all stages and sexes of the wasp tended to decline with regard to controls.

The decrease in glycogen and lipid contents may be due to the utilization of these resources for energy generation as a result of insecticide-induced stress (Sancho *et al.* 1998). Under stress conditions, animals need high energy and the energy demand may have led to the stimulation of protein catabolism. Lohar and Wright (1993) demonstrated lipid depletion in the fat body, oocytes, and hemolymph of *Tenebrio molitor* Linnaeus, 1758 (Coleoptera: Tenebrionidae) females exposed to malathion. They stated that depletion of the lipid content of the body may have been due to the effect of insecticide on the adipokinetic hormone that controls lipid metabolism (Lohar & Wright 1993). Kodrik and Socha (2005) investigated the effect of the permethrin on adipokinetic hormone levels in hemolymph of *Pyrrhocoris apterus* (Linnaeus, 1758) (Hemiptera: Pyrrhocoridae). They found that with increasing insecticide concentration, the amount of adipokinetic peptides in hemolymph increased significantly. Also, in

this study, vibration and dark shock were used as stresses for the insect and it was shown that in both cases, the amount of adipokinetic in haemolymph increased. These results indicate the involvement of adipokinetic peptides in the response of insects to various stresses, including insecticides (Kodrik and Socha 2005).

A negative correlation has been recorded in some (but not all) species between stress resistance in genotypes and their capacity for production in unstressed conditions (Calow & Sibly 1990). The "principle of allocation" is the most important part of evolutionary models of physiological ecology (Calow & Sibly 1990). Stress (especially toxicants) causes metabolism failures and this necessarily limits distribution; within these limits, metabolism is likely to change, which will influence growth and reproduction.

cellular energy allocation (CEA) assay is a method to evaluate the effects of toxic stress on the balance of metabolic in organisms (Novais & Amorim 2013). CEA is also a biological indicator based on comparing available stored energy to metabolism and the amount of energy consumed (De Coen *et al.* 2000). Total energy resources consist of the main sources of energy including carbohydrates, fats, proteins, and in some cases, glycogen (De Coen & Janssen 1997). By measuring the amount of energy resources and the amount of activity of the electron transfer system (energy consumption) in a living person, we can calculate the biological indicator of energy allocation of the cell, which represents the amount of energy available (De Coen & Janssen 1997). In our research, CEA data showed no significant difference between resistant and susceptible populations. Also, CEA was not affected by different concentrations of the three insecticides in any of the populations. In consistent with our results, Novais and Amorim (2013) studied the effects of dimethoate, atrazine, and carbendazim on *E. albidus*; they found no significant reduction in CEA with dimethoate and carbendazim but, CEA was reduced with atrazine. However, Mohammadzadeh *et al.* (2014) revealed that CEA of larvae of *Xanthogaleruca luteola* (Muller, 1766) treated with LC_{50} concentrations of spinosad decreased significantly compared to control. Also, the available energy of the larvae exposed to LC_{30} of spinosad increased significantly, whereas it decreased significantly at LC_{50}. However, the energy consumed was increased at both concentrations significantly (Mohammadzadeh *et al.* 2014).

Several factors may be involved in developing resistance to insecticides. The fat body plays an important role in the intermediary metabolism of insects by storing enzymes involved in the detoxification of toxic molecules, which may also happen in insecticide-resistant populations as observed in *Spodoptera frugiperda* (J. E. Smith, 1797) (Yu *et al.* 2003). The role of carbohydrate reserves in the detoxification of insecticide molecules has been reported by Nath (2000) and Alaoui-Jamali *et al.* (1997). As a result, a higher metabolic rate may be necessary for resistant individuals to preserve their resistance mechanisms (Chown & Gaston 1999, Harak *et al.* 1999, Hostetler *et al.* 1994).

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