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RESEARCH REPORT

Chemical and organic fertilizers affect physiological performance and antioxidant activities in *Myzus persicae* (Hemiptera: Aphididae)

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Abstract

Myzus persicae is a widespread and polyphagous insect that causes severe damages to hundreds of host plants. In the current study, zinc sulfate and vermicompost as chemical and organic fertilizers, were added into cultural soil of *Capsicum annuum* to determine their effects on physiology and antioxidant activities of *M. persicae.* The aphids reared on zinc sulfate-treated culture showed the highest activities of general protease, trypsin, cathepsins, carboxypeptidase and lipase but activities of chymotrypsin and aminopeptidase were the highest in vermicompost-treated culture. Although activities of α-amylase in the fertilizer-treated cultures were higher than control but activities of α- and β-glucosideases showed the highest values in zinc sulfate and vermicompost treatments, respectively. Aspartate aminotransferase and γ-glutamyl transferase showed the highest activity in the aphids reared on the vermicompost-treated culture but alanine aminotransferase activity got the lowest value in fertilizer-treated cultures. Activities of aldolase and lactate dehydrogenase in the fertilizer-treated aphids were higher than those of control and vermicompost-treated aphids, but alkaline phosphatase showed the lower activity although activity of acid phosphatase decreased in vermicompost- treated aphids compared to other treatments. Activities of antioxidant enzymes were found to be the highest in the aphids fed on vermicompost-treated culture including glucose-6-phosphate dehydrogenase, superoxide dismutase, peroxidase and ascorbate oxidase but catalase in vermicompost treatment had lower activity than control and zinc-sulfate treatments. Also, malondialdehyde and RSSR/RSH ratio demonstrated higher values in the aphids fed on zinc sulfate- and vermicompost-treated plants than control, respectively. Finally, the amounts of glycogen and triglyceride revealed the highest values in zinc sulfate-treated plants compared to other treatments. These results indicated significant effects of fertilizers on physiology and antioxidant activity of *M. persicae* which are important to be considered in integrated pest management programs.

Key Words: *Myzus persicae;* fertilizer*;* physiological performance; antioxidant indices

Introduction

The green peach aphid, *Myzus persicae* (Hemiptera: Aphididae), is one of the most important insect pests worldwide because of its severe damages to many crop plants, vegetables and fruit trees as well as transmission of plant pathogenic viruses such as potato leaf roll virus (PLRV), potato virus Y (PVY), pepper mottle virus (Pep MoV), tobacco etch virus (TEV) and cucumber mosaic virus (CMV) (Hill, 1983; Robert *et al.,* 2000). Although population outbreaks of *M. persicae* are somehow suppressed by synthetic insecticides but strong selection pressure of chemical spraying induces resistance to the most registered insecticides so other control methods need to be investigated for appropriate control (Bolandandam *et al.,* 2004).

Although fertilizers are recommended to increase crop yields but they can affect pest populations leading to to use control procedures (Patriquin *et al.,* 1995; Arancon *et al.,* 2006; Edwards *et al.,* 2009). In fact, fertilizers can fluctuate amounts of defensive chemical components in plants which finally change ecological fitness and physiological performance of herbivorous insects (Edwards *et al.,* 2009; Mardani-Talaee *et al.,* 2016). Numerous studies have reported the effects of macronutrient chemical

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fertilizers (such as N, P and K) on population dynamics of insect pests (Lu *et al.,* 2007). Since fertilizers may result in higher growth rate and population increase of herbivorous insects through improving nutritional quality of host plants (Edwards *et al.,* 2009). Moreover, fertilizers residue have raised a great concern of consumers in recent years because of their chemical constituent. So, organic fertilizers may be more appropriate because of their least effects on environment or residual contaminations. In case, vermicompost is an organic fertilizer produced through the interactions between earthworms and microorganisms in a mesophilic process from organic wastes. It reduces pH and C:N ratio in soil, stabilizes the organic matter and makes nutrients readily available to plants (Yardim *et al.,* 2006). Soil amendment with vermicompost have reduced population growth rates in some herbivores such as *Manduca quinquemaculata*, *Acalymma vittatum*, *Diabrotrica undecimpunctata* (Yardim *et al.,* 2006), *Leptinotarsa decemlineata* (Mardani-Talaee *et al.,* 2015), *Pseudococcus* sp., *Teranychus urticae*, *M. persicae* (Arancon *et al.,* 2002, 2006; Edwards *et al.,* 2009; Mardani-Talaee *et al.,* 2016), and *Aphis gossypii* (Razmjou *et al.,* 2011).

In a previous study, we found that vermicompost increased levels of phenolic compounds in the leaves of bell pepper and thereby decreased life table parameters of *M. persicae* (Mardani-Talaee *et al.,* 2016). On the other hand, our results demonstrated an induced resistance in bell pepper cultured in vermisompost-treated culture (Mardani-Talaee *et al.,* 2016). In case, physiological parameters of treated and non-treated aphids by fertilizers must be determined to better understanding of observed changes. So, the current study was conducted to compare potential changes in physiological processes of *M. persicae* induced by fertilizers. In details, a chemical fertilizer (zinc sulfate) and vermicompost (30 %) were separately added into cultural soil of *C. annum* to find their effects on digestion, intermediary metabolism and antioxidant activities of *M. persicae* under greenhouse conditions. These findings will increase our understanding on beneficial or detrimental effects of fertilizers to better management of *M. persicae* in greenhouses.

Materials and Methods

Plant sources, Insect colony and fertilizer treatments

The sandy loam soil collected from a fallow potato field in Ardabil plain (38°14´ N and longitude 48°19´ E) was used to grow *C. annuum* (cv. California Wonder). Seeds of bell pepper, *C. annuum* were grown in an aphid-free greenhouse set at 25 ± 5 ^{o}C, 60 ± 5 % of relative hunidity and a photoperiod of 14:10 (L:D) h. The aphids were collected from a tomato field from Meshkin-Shahr (Ardabil province), Iran and transferred to cultured *C. annuum* at six-leaf stage. To maintain a suitable aphid colony, some aphids were transferred from infested plants to new young plants every week. Experimental treatments here were; i) bell pepper grown in field-collected soil as control treatment; ii) bell pepper grown in the field-collected soil sprayed on 4 - 6 expanded leaves stages of *C. annuum* with zinc sulfate (Provided by Iranian Soil and Water Research Institute in Karaj) at the concentration of 0.001 % (50 mL per plant). iii) bell pepper grown in the same soil amended with 30% of vermicompost (Obtained from cattle manure of Pars Koud Company, Gorgan, Iran) which contained 1.8 % N, 3.9 mg/kg P, pH of 7.3 and EC of 2.2 ds/m before planting.

Sample preparation for biochemical assays

Adults of *M. persicae* from control and fertilized cultures were randomly selected and homogenized in ice cold NaCl solution (0.15 M) in proportion of 20 aphids per ml of saline solution. The samples were then centrifuged at 20000 *g* for 5 min at 4 ºC (Dubovskiy *et al.,* 2008). Supernatants were collected and stored at -20 ºC to onset of the biochemical experiments (< a week).

Determination of digestive enzyme activities

General proteolytic activity

General proteolytic activity in control and treated aphids by fertilizers were determined based on the method of Elpidina *et al.* (2001) using

Table 1 Effects of fertilizers on activities of digestive proteases in M. persicae adults

Treatments	Statistic (Mean±SE U/mg protein)							
	General Protease[1]	Trypsin	Chymotrypsin	Elastease	CathepsinL	CathepsinB	Aminopeptidases	Carboxypeptidases
Control	0.062±0.016 b	4.568±0.336 b	2.818±0.759 c	38.51±1.950 c	2.819±0.459 b	3.885±0.374 c	0.134±0.017 c	1.123±0.125 a
Vermicompost (30%)	0.040±0.008 b	2.829±0.154 b	12.143±0.432 a	60.15±1.890 b	14.97±1.430 b	6.000±1.150 b	5.150±0.797 a	0.657±0.302 b
Zinc sulfate	0.224±0.027 a	14.842±0.996 a	5.25±0.231 b	128.82±0.682 a	23.25±1.280 a	13.280±1.080 a	0.550±0.057 b	1.092±0.0519 a

[1]Activity unit of all enzymes were U per mg protein except for general protease as OD/min. The means followed by different letters in a column are significantly different [p < 0.01, Tukeys (HSD)].

Azocasein (2 %) as substrate. Briefly, 40 µL of azocasein solution, 80 µL of universal buffer [20 mM, (Frugoni, 1957; Mardani-Talaee and Zibaee, 2015)] and 20 µL of sample were incubated for 60 min at 37 ºC. Then, proteolysis was stopped by adding 80 µL of 30 % trichloroacetic acid (TCA) and precipitation was achieved by cooling at 4 °C for 5 min prior to centrifugation at 20,000*g* for 10 min. Finally, an equal volume of NaOH (2 N) was added and absorbance was read at 450 nm (Microplate reader, Awareness Statfax, 3200, USA).

Specific proteolytic activity

Based on Rahbe *et al.* (2003), activities of trypsin, chymotrypsin, cathepsin B, cathepsin L, amino- and carboxypeptidase were determined in control and fertilized-treated aphids. Trypsin and chymotrypsin activities were assayed using 1 mM of BApNA (Nabenzoyl- DL-arginine-*p*-nitroanilide) and SAAPPpNA (*N*-succinyl-alanine- alanine-proline-phenylalanine-*p*-nitroanilide), as substrates, respectively. Substrates (20 µL) were separately added into 50 µL of universal buffer (20 mM, pH 8) and incubation was initiated by adding 10 µL of enzyme solution for 10 min at 30 ºC. The reaction was terminated by adding 100 µL of TCA (30 %) and absorbance was read at 405 nm. Cathepsin B and L activities were determined using Z-Ala-Arg-Arg 4-metjoxy-bnaphtylamide acetate (1 mM) and N-benzoyl-Phe-Val-Arg-p-nitroanilide hydrochloride (1 mM), respectively. Substrates (20 µL) were separately added into 50 µL of universal buffer (20 mM, pH 5) and incubation was initiated by adding 10 µL of enzyme solution for 10 min at 30 ºC. The reaction was terminated by adding 100 µL of TCA (30 %) and absorbance was read at 405 nm. Activities of exopeptidases were determined using hippuryl-L-arginine and hippuryl-L-phenylalanine as the specific substrates (1 mM) of carboxy- and aminopeptidases, respectively. Substrates (20 µL) were separately added into 50 µL of universal buffer (20 mM, pH 7) and incubation was initiated by adding 10 µL of enzyme solution for 10 min at 30 ºC. The reaction was terminated by adding 100 µL of TCA (30%) and absorbance was read at 340 nm.

α-Amylase assay

Activity of α-amylase was determined based on the method of Bernfeld (1955) using starch (1 %) and dinitrosalicylic acid (DNS). Briefly, 40 µL of starch solution (1 %), 80 µL of universal buffer (pH 7) and 20 µL of enzyme sample were incubated for 30 min at 35 ºC. Then, 100 µL of DNS was added and the tubes containing reaction mixture were additionally incubated for 10 min at boiling water. Finally, 100 µL of the reaction mixture was poured into wells of microplate and absorbance was read at 540 nm.

α- and β-glucosidase assay

According the method of Silva and Terra (1995), α- and β-glucosidase activities were assayed by adding 20 µL of *p*-nitrophenol α-glucopyranoside for α-glucosidase (5 mM) and *p*-nitrophenol β -glucopyranoside for β-glocusidase (5 mM) (Separately) in 50 µL of universal buffer (pH 7). The incubation was initiated by adding 10 µL of the enzyme solution for 10 min prior to read absorbance at 405 nm.

Lipase assay

Activity of lipase was determined using the method of Tsujita *et al.* (1989) in control and fertilized-treated aphids. Ten microliter of sample and 20 µL of *p*-nitrophenyl butyrate (27 mM) as substrate were added into 50 µL of universal buffer (pH 7), mixed thoroughly and incubated at 37°C. After 1 min, 100 µL of NaOH (1 M) was added and absorbance was read at 405 nm.

Determination of intermediary metabolism in M. persicae

Assay of alanine (EC 2.6.1.1) and aspartate (EC 2.6.1.1) aminotranferases

This is a chlorometric assay using 2,4-dinitrophenyl hydrazine to synthesize pyruvate hydrazine by combination of pyruvate with 2,4-dintitrophenyl puruvate (Thomas, 1998). Based on a kit provided by Biochem Company (Tehran, Iran), reagent A (for AST) and reagent B (for ALT) were incubated separately with reagent D for 5 min. Then, 10 µL of the enzyme solution was added and incubation continued for 60 min. At the end, reagent C was added and absorbance was read at 340 nm. Specific activity was calculated by dividing absorbance with protein content in sample.

Assay of γ-Glutamyl transferase

The assay was done by the method of Szasz (1976) using a kit provided by ZiestChem Diagnostic Company (Tehran-Iran). The reaction mixture consisted 50 µL of buffer reagent, 20 µL of substrate reagent (L-γ-glutamyl-3-carboxy-4-nitrianilide) and 10 µL of the sample. Incubation was prolonged for 3 min and absorbance was read at 405 nm (ZiestChemDiagnostic Co., Tehran-Iran). Specific activity was calculated by dividing absorbance with protein content in sample.

Assay of aldolase

As the instruction of manufacturer, ZiestChem Diagnostics Company (Tehran-Iran), 50 µL of buffer reagent, 25 µL of substrate reagent (Fructose-1,6 di-phosphate), 10 µL of cofactor reagent (NADH) and 20 µL of sample were incubated for 5 min prior to read absorbance at 340 nm (Pinto *et al.,* 1969).

Assay of lactate dehydrogenase

The method of King (1965) was used to evaluate activity of lactate dehydrogenase (LDH). To standardize volumes, 0.2 ml of NAD^+ solution was added to the test tubes and 0.2 ml of water was added to control test tubes, each containing 1 ml of the buffered substrate and 0.01 ml of the sample was also added to the test tubes. Test tube samples were incubated for exactly 15 min at 37 °C and then arrested by adding 1 ml of color reagent (2,4-dinitrophenyl hydrazine) to each tube and the incubation continued for an additional 15 min. Then, the contents were cooled at room temperature, 10 ml of 0.4 N NaOH was added to each tube to make the solutions strongly alkaline. At exactly 60 s after adding of alkali to each tube, the intensity of color was measured at 340 nm.

Table 2 Effect of fertilizers on activities of α- amylase, glucosidases and lipase in *M. persicae* adults

Treatments	Statistic (Mean±SE U/mg protein)			
	α-amylase	α- glucosidases	ß-glucosidases	Lipase
Control	5.870±0.142 b	43.49±2.560 b	6.500±1.480 c	79.790±1.640 b
Vermicompost (30%)	7.120±0.194 a	29.32±1.894 c	25.320±9.120 a	51.19±1.059 c
Zinc sulfate	7.346±0.759 a	65.88±3.730 a	15.723±0.512 b	169.94 ±2.271 a

The means followed by different letters in a column are significantly different [$p < 0.01$, Tukeys (HSD)].

Assay of acid (EC 3.1.3.2) and alkaline (EC 3.1.3.1) phosphatases

The assays were carried out as described by Bessey *et al.* (1946). The buffered substrate (phosphate buffer, 0.02 M, pH 7.2) was incubated with samples for 30 min. Alkali was added to stop the reaction and to adjust pH for determination of product concentration. The spectral absorbance of *p*-nitrophenolate was maximal at 340 nm. The molar absorbance of *p*-nitrophenolate at 400 nm is approximately double that of *p*-nitrophenyl phosphate at 310 nm. On converting the p-nitrophenolate into p-nitrophenol by acidification, the absorption maximum shifted to approximately 320 nm with no detectable absorption at 405 nm.

Determination of antioxidant activities in M. persicae

Catalase (CAT) activity was measured based on the method of (Wang *et al.,* 2001). Briefly, 100 µL of sample was added into 500 µL of hydrogen peroxide (1 %), incubated at 28 °C for 10 min and the activity was determined as the ΔA at 240 nm/min/mg protein. Superoxide dismutase (SOD) activity was determined based on McCord and Fridovich (1969) as the reduced rate of NBT (Nitro blue tetrazolium) by the superoxide anion due to xanthine oxidation by xanthine oxidase (McCord and Fridovich, 1969). Sample (100 µL) was added into 500 µL of the reaction solution containing 70 µM of NBT; 125 µM of xanthine; both dissolved in PBS and the xanthine oxidase solution [(100 µL; 10 mg of bovine albumin; 100 µL of xanthine oxidase (5.87 units/mL); dissolved in 2 mL of PBS]. The reaction mixture was incubated in darkness for 20 min at 28 °C prior to calculate the activity as differences in absorbance between a sample containing the mixture and a clean reagent mixture at ΔA 560 nm/min/mg protein. Peroxidase activity was assayed based on by the method of Addy and Goodman (1972). The reaction mixture consisted 500 µL of buffered pyrogallol [0.05 M pyrogallol in 0.1 M phosphate buffer (pH 7.0)] and 500 µL of H_2O_2 (1 %) prior to adding 100 µL of enzyme extract. Changes in absorbance was measured at 430 nm for every 30 seconds in 2 minutes. The peroxidase activity was calculated using an extinction coefficient of oxidized pyrogallol (4.5 litres/mol). Ascorbate peroxidase assay was carried out according to Asada (1984). The reaction mixture consisted 100 µL of sample and 250 µL of 67 mM potassium phosphate buffer (pH 7) containing 2.5 mM ascorbic acid and 200 µL of 30 mM H_2O_2. Absorbance was monitored at 290 nm for 5 min. MDA (malondialdehyde) concentration was determined by considering the process of lipid peroxidation due to formation of MDA. Based on Bar-Or *et al.* (2001), Briefly, 100 µL of 20 % trichloroacetic acid was mixed with 100 µL of the sample prior to be centrifuged at 15,000*g* for 10 min at 4 °C. The obtained supernatant was mixed with 100 µL of 0.8 % TBA reagent, and the mixture was incubated at 100 °C for 60 min prior to read absorbance at 535 nm. The MDA concentration is reported as amount of MDA produced per mg protein using a molar extinction coefficient of 1.56×10^5 M-1 cm-1. Activity of glucose-6-phosphate dehydrogenase was measured based on Balinsky and Bernstein (1963). The solution containing 100 µL Tris-HCl buffer (100 mM, pH 8.2), 0.2 mM NADP and 0.1M of $MgCl_2$ was taken in a cuvette along with 100 µL of water and suitable aliquots of enzyme extract. The reaction was initiated by adding 100 of 6 mM glucose-6-phosphate and OD increase was measured at 340 nm. The activity of the enzyme is expressed as µmol/min/mg protein. The concentrations of oxidized (RSSR) and reduced (RSH) thiols were determined based on the method of Khramtsov *et al.* (1997). Initially, RSSRs were decomposed for 20 min by 1 M of hydrochloric acid to form RSH prior to neutralize the mixture pH by sodium hydroxide. Then, fifty microliters of the homogenate was mixed with 500 µL of 0.1 % DNTB solution in PBS, and the mixture was incubated for 10 min at 37 °C. The absorbances of RSH and RSH and RSSR were measured at 405 nm. The concentration of RSSR was calculated as the difference between the final concentration of reduced thiols after reduction by hydrochloric acid (RSH and RSSR) and the initial concentration of one RSH in the sample. The results are presented as the ratio of RSSR to RSH.

Table 3 Effects of fertilizers on activities of transaminases in *M. persicae* adults

Treatments	Statistic (Mean±SE U/mg protein)		
	AST	ALT	γ-GT
Control	0.121±0.020 b	0.182±0.022 a	0.256±0.040 b
Vermicompost (30%)	0.242±0.044 a	0.137±0.027 b	0.680±0.247 a
Zinc sulfate	0.147±0.018 b	0.167±0.023 ab	0.389±0.088 b

AST, aspartate amino transferase; ALT, alanine amino transferase; γ-GT, γ-glutamyl transferase. The means followed by different letters in a column are significantly different [$p < 0.01$, Tukeys (HSD)].

Determination of storage macromolecules in M. persicae

Triglyceride determination

The amount of triglyceride was measured using a diagnostic kit provided by Pars Azmoon Company (Tehran, Iran) in *M. persicae* adults. Briefly, 10 µL of sample was incubated with 70 µL of reagent solution containing phosphate buffer (50 mM, pH 7.2), 4-chlorophenol (4 mM), adenosine triphosphate (2 mM), Mg^{2+} (15 mM), glycerokinase (0.4 kU/L), peroxidase (2 kU/L), lipoprotein lipase (2 kU/L), 4-aminoantipyrine (0.5 mM) and glycerol-3-phosphate-oxidase (0.5 kU/L) for 20 min at 25 °C (Fossati and Prencipe, 1982). Then, absorbance of sample mixture and reagent was read at 546 nm. The following equation was used to calculate the amount of triacylglyceride:

$$\text{mg/dl} = \frac{\text{OD of sample}}{\text{OD of standard}} \times 0.01126$$

Glycogen determination

Bodies of 10 aphids were immersed in 1 mL of 30 % KOH with Na_2SO_4; covered with foil (to avoid evaporation) and put in boiling water for 30 min. Tubes then were shaken and cooled in ice. Two milliliters of 95 % EtOH was added and the tubes were shaken again and incubated in ice for 30 min. Followed by centrifugation at 22,000*g* for 30 min, supernatant was removed and the pellets (glycogen) were re-dissolved in 1 mL of distilled water before being re-shaken. Incubation continued after adding 5 % phenol for 30 min prior to read absorbance at 492 nm. Standard of glycogen was prepared as 0, 25, 50, 75 and 100 mg/mL to calculate amount of glycogen in sample (Chun and Yin, 1998).

Protein determination

The method of Lowry *et al.* (1951) was used to determine protein concentration in the control and treated aphids (ZiestChem. Co., Tehran, Iran).

Statistical analysis

Normality of data was tested by Kolmogorov-Smirnov method prior to analysis by one-way analysis of variance (ANOVA) followed by comparison of the means with Tukey *post hoc* Honestly Significant Difference (HSD) test at α = 0.05.

Results

Effects of fertilizers on digestive enzymes

Statistical differences were found in activities of general protease, serine proteases, cysteine proteases and exopeptidases (amino- and carboxy-peptidases) in *M. persicae* fed on control and fertilizer-treated *C. annum* (Table 1). Activities of general protease ($F = 27.14$; df = 2, 8; $p < 0.01$), trypsin ($F = 111.95$; df = 2, 8; $p < 0.01$), elastase ($F = 149.79$; df = 2, 8; $p < 0.01$), cathepsin L ($F = 80.84$; df = 2, 8; $p < 0.01$), cathepsin B ($F = 27.88$; df = 2, 8; $p < 0.01$) and carboxypeptidases ($F = 1.86$; df = 5, 17; $p < 0.01$) were the highest in the aphids fed on the zinc sulfate treated-plants while the highest activities of chymotrypsin ($F = 30.69$; df = 2, 8; $p < 0.01$) and aminopeptidase ($F = 36.37$; df = 2, 8; $p < 0.01$) were found in vermocompost treatment (Table 1). For all proteases, the lowest activities were observed in control treatments except for carboxypeptidase (Table 1).

The highest activities of α-amylase ($F = 9.75$; df = 2, 8; $p < 0.01$), α- glucosidase ($F = 9.30$; df = 2, 8; $p < 0.01$) and lipase ($F = 416.68$; df = 2, 8; $p < 0.01$) were found in the aphids fed on *C. annum* treated by zinc sulfate although no significant difference was observed in α-amylase of the aphids fed on both zinc sulfate- and vermicompost-treated plants (Table 2). Aphids fed on control and vermicompost-treated *C. annum* showed the highest and the lowest ß-glucosidase ($F = 3.10$; df = 2, 8; $p < 0.01$) activity, respectively (Table 2). The lowest activities of α-amylase and ß-glucosidase were obtained in control but the aphids fed on vermicompost-treated *C. annum* showed the lowest activities of α-glucosidase and lipase (Table 2).

Effects of fertilizers on intermediary metabolism of M. persicae

Significant differences were found in activities of aspartate amino transferase (AST), alanine amino transferase (ALT) and γ-glutamyl transferase (γ-GT) of *M. persicae* fed on different fertilizer

Table 4 Effects of fertilizers on activities of aldolase, alkaline and acid phosphatase and lactate dehydrogenase in *M. persicae* adults

Treatments	Statistic (Mean±SE U/mg protein)			
	Aldolase	ALP	ACP	LDH
Control	0.424±0.099 b	0.104±0.002 a	0.337±0.033 a	0.259±0.049 b
Vermicompost (30%)	0.773±0.030 a	0.039±0.026 b	0.150±0.012 b	0.796±0.097 a
Zinc sulfate	0.931±0.034 a	0.068±0.017 b	0.353±0.027 a	0.671±0.166 a

ALP, alanine phosphatase; ACP, acid phosphatase; LDH, lactate dehydrogenase. The means followed by different letters in a column are significantly different [$p < 0.01$, Tukeys (HSD)].

treatments (Table 3). AST ($F = 4.73$; df = 2, 8; $p < 0.01$) and γ-GT ($F = 2.13$; df = 2, 8; $p < 0.01$) showed the highest activities in *M. persicae* fed on vermicompost-treated *C. annum* while control and zinc sulfate treatments caused the lowest enzymatic activities (Table 3). In case of ALT, the aphids fed on both fertilizer treatments showed lower activity than control ($F = 0.94$; df = 2, 8; $p < 0.01$) (Table 3). Aldolase ($F = 1.90$; df = 2, 8; $p < 0.01$) and LDH ($F = 6.06$; df = 2, 8; $p < 0.01$) had the highest activities in the aphids fed on fertilizer-treated *C. annum* compared to control while activity of ALP decreased in these treatments ($F = 3.36$; df = 2, 8; $p < 0.01$) (Table 4). Finally, activity of ACP ($F = 20.54$; df = 2, 8; $p < 0.01$) was the highest in *M. persicae* fed on control and zinc sulfate-treated *C. annum* (Table 3).

Effects of fertilizers on antioxidant enzymes

Both control and zinc-sulfate treatments caused the highest activity of catalase in *M. persicae* but the aphids fed on vermicompost-treated *C. annum* had the lowest enzymatic activity (Table 5; $F = 4.10$; df = 2, 8; $p < 0.01$). Activities of peroxidase ($F = 4.90$; df = 2, 8; $p < 0.01$), superoxide dismutase ($F = 5.33$; df = 2, 8; $p < 0.01$), ascorbate oxidase ($F = 6.52$; df = 2, 8; $p < 0.01$) and glucose-6-phosphate dehydrogenase ($F = 8.90$; df = 2, 8; $p < 0.01$) were also the highest in the aphids fed on vermicompost-treated *C. annum* (Table 5). Significant differences were found in the amounts of malondialdehyde (MDA) and oxidized to reduced thiols (RSSR/RSH ratio) in control and fertilizer treatments (Table 6). Adults of *M. persicae* fed on zinc sulfate-treated and control plants showed the highest and the lowest values of MDA, respectively (Table 6; $F = 7.82$; df = 2, 8; $p < 0.01$) while the highest RSSR/RSH ratio was found in the aphids fed on vermicompost-treated *C. annum* (Table 6; $F = 1.84$; df = 2, 8; $p < 0.01$).

Effects of fertilizers on storage macromolecules in M. persicae

Significant differences were found in triacylglyceride (TAG) and glycogen contents of *M. persicae* reared on different fertilizer treatments (Table 7). *M. persicae* fed on *C. annum* treated by zinc sulfate had the highest amount of triglyceride ($F = 4.31$; df = 2, 8; $p < 0.01$) and glycogen ($F = 65.46$; df = 2, 8; $p < 0.01$) compared to other treatments (Table 7).

Discussion

According to the results obtained here, zinc sulfate and vermicompost fertilizers significantly affected physiological performance and antioxidant activities of *M. persicae.* Researches have demonstrated that fertilizer treatment affect fecundity, life table parameters and physiological performance of herbivorous insects (Edwards *et al.,* 2009; Razmjou *et al,* 2011; Mardani-Talaee *et al.,* 2015) because soil amendment with fertilizers increases level of organic matters and soil biological interactions led to fertility and relative host plant resistance to pest damages (Luong and Heong, 2005). Also, organic fertilizers, *e.g.,* vermicompost, may increase amounts of phenolic compounds in host plants which definitely affect biological performance of insects, the phenomenon has been observed in *M. persicae* (Mardani-Talaee *et al.,* 2016).

Adults of *M. persicae* fed on zinc sulfate-treated *C. annum* showed the highest activities of digestive enzymes while the aphids on control and vermicompost treatments had the lowest enzymatic activities except for chymotrypsin, aminopaptidase and β-glucosidase. These results imply on suitability of *C. annum* reared on zinc sulfate cultural soil. The suitability may be created due to higher amounts of nutrients or lower levels of plant secondary metabolites which prevent growth and development of insect via repellency or inhibitory mechanisms on digestive enzymes (Terra and Ferreira, 2005; Nation, 2008). Our previous studies revealed the effect of vermicompost to increase amounts of secondary compounds in *M. persicae* and *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) (Mardani-Talaee *et al.,* 2015, 2016). In details, Mardani-Talaee *et al.* (2015) reported the higher contents of flavonoids, anthocyanins and phenolic compounds in potatoes cultured in the soils containing 30 % of vermicompost. Similar findings were obtained in *C. annum* cultured in vermicompost compared to control

Table 5 Effects of fertilizers on activities of antioxidant enzymes in *M. persicae* adults

Treatments	Statistic (Mean±SE U/mg protein)				
	Catalase	Ascorbate oxidase	GPDH	SOD	PO
Control	0.328±0.081 a	0.778±0.549 c	0.136±0.026 b	0.080±0.002 b	0.003± 0.001 b
Vermicompost (30%)	0.148±0.049 b	7.176±0.463 a	0.447±0.0216 a	1.860±0.310 a	0.013± 0.005 a
Zinc sulfate	0.119 ± 0.021 a	3.704±0.106 b	0.286±0.085 ab	0.834±0.029 ab	0.002± 0.001 b

GPDH, glucose-6-phosphate dehydrogenase; SOD, superoxide dismutase; PO, peroxidase. The means followed by different letters in a column are significantly different [$p < 0.01$, Tukeys (HSD)].

and zinc sulfate treatments (Mardani-Talaee *et al.,* 2016). Moreover, Stevenson *et al.* (1993) found that phenolic compounds were responsible for development retardation of *Spodoptera litura* (Lepidoptera: Noctuidae) reared on wild ground nut. Haukioja *et al.* (2002) reported changes of consumption rate in *Epirrita autumnata* (Lepidoptera: Geometridae) due to presence of phenolic compounds. Finally, Edwards *et al.* (2009) highlighted the role of phenolic substances to alleviate feeding performance in sap sucking insects.

Insects are depend on several processes involved in intermediary metabolism to gain their required energy for biological activities such as flight, reproduction and etc. Intermediary metabolism relies on activities of transaminases to process amino acids for energetic demands, tissue construction and lipid oxidation to provide energy and metabolic water, besides processing of glucose via glycolysis and krebs cycle (Nation, 2008). ALT and AST are the two important enzymes in transaminase mechanisms of insects that catalyze alanine cycle in proline metabolism and facilitate conversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate, respectively (Nation, 2008). These two enzymes are involved in proline metabolism and providing some components for krebs cycle. Although γ-GT is a transaminase but it transfers γ-glutamyl moiety of glutathione to a receptor for glutamate formation so it is important in γ-glutamyl cycle to synthesize and degrade glutathione and xenobiotic compounds (Tate and Meister, 1985). In our study, activities of AST and γ-GT significantly increased in the aphids fed on vermicompost-treated *C. annum* compared to control and zinc sulfate treatments while activity of ALT decreased in both fertilizer treatments compared to control. The lower activity of ALT in fertilized-treated aphids may be attributed to non-dependence of energy production via proline metabolism or protein shortage in hemolymph and fat bodies because of disordered protein digestion. The second reason seems to be more important because ALT is crucial to convert amino acids to each other and keto-acid. Meanwhile, increased activities of AST and γ-GT may be due to energy demand via Krebs cycle by aspartate and α-ketoglutarate conversions to oxalate and glutamate as well as detoxification of secondary metabolites entered into hemolymph.

Aldolase is an isomerase which make sugars available in initial steps of glycolysis (Pinto *et al.,* 1969). LDH involves in pyruvate conversion to lactate in anaerobic conditions and utilizes electrons to provide NADH from NAD^+ (Nation, 2008). Also, the enzyme is considered as an index of tissue damage in clinical chemistry (Kaplan and Pesce, 1996). The higher activities of aldolase and LDH in the aphids fed on fertilized-treated plants refer to glycolysis cycle for energy demand from one hand and potential tissue damages due to increased levels of plants' secondary metabolites. Mardani-Talaee *et al.* (2015) reported higher activity of LDH in *L. decemlineata* adults fed on potatoes cultured in vermicompost (30 %) with regard to increased levels of secondary metabolites in the treated plants.

Acid and alkaline phosphatases are the two hydrolytic enzymes that detach phosphate groups from different molecules such as nucleotids, proteins and alkaloinds in acid and alkali media. The higher activities of these enzymes indicate digestion efficacy and nutrient absorption in midgut and appropriate transfer of dietary nutrients to fat bodies (Senthil-Nathan *et al.,* 2006). Lower activities of these enzymes mainly ALP in fertilized-treated aphids indicate disturbance of dietary utilization of ingested food because of disordered performance of digestive enzymes. On the other hand, it can be concluded that impaired digestive process led to lower availability of nutrients in aphids mainly storage in fat bodies which affect activities of given phosphatases.

Antioxidant system is recruited to protect animal cells against ROS (Reactive Oxygen Species) by decrease level of lipid peroxidation, DNA and protein damage (Felton and Duffey, 1991; Dubovskiy *et al.,* 2008). Several enzymes are involved in antioxidant defense system including

Table 6 Effects of fertilizers on RSSR/RSH Ratio and malondialdehyde (MDA) in *M. persicae* asults

Treatments	Statistic (Mean±SE)	
	MDA (nM/mg protein)	RSSR/RSH Ratio
Control	0.674±0.157 b	0.977±0.132 b
Vermicompost (30%)	3.699±1.22 ab	1.626±0.773 a
Zinc sulfate	4.783±0.467 a	0.5200±0.0560 b

The means followed by different letters in a column are significantly different [$p < 0.01$, Tukeys (HSD)].

ascorbate peroxidases (APX), superoxide dismutases (SOD), catalases (CAT), peroxidases (POX), glutathione-S-transferase (GST), glucose-6-phosphate dehydrogenase and nonenzyme antioxidants such as ascorbic acid, thiols, and α-tocopherol (Fridovich, 1978; Dubovskiy *et al.,* 2008). Functionally, SOD converts superoxide radical O_2^- into H_2O_2; CAT and POX convert H_2O_2 into H_2O; GST removes the products of lipid peroxidation or hydroperoxides from cells; thiols are important to protect cells against hydroxyl radical (OH^-); nitroxyl radical (NO^-) and superoxide radical O_2^- (Udupi and Rice-Evans, 1992; Dubovsky *et al.,* 2008). Besides, thiol ratio (RSSR/RSH) is an important index to highlight the lower amounts of reduced SH-groups (RSH) and the higher amounts of oxidated SH-groups (RSSR) due to oxidative stress (Dubovsky *et al.,* 2008). Meanwhile, increased amount of MDA is an evidence of oxidative stress in an organism (Dubovsky *et al.,* 2008). Peroxidase, SOD, ascorbate oxidase and GPDH had the highest activity in the aphids fed on vermicompost treated plants but activities of ascorbat oxidase and catalase were the highest in control and zinc sulfate treatments. Although the highest amount of MDA was found in Zinc sulfate treatment but the aphids fed on vermicompost-treated plants showed the highest value of RSSR/RSH ratio. Enhanced activity of SOD in *M. persicae* reared on vermicompost indicated the higher amount of H_2O_2. Meanwhile, CAT is known to be inhibited by accumulation of superoxide anions in oxidative stressed organisms so the lower activity in vermicompost treatments may be attributed to possible destruction of aphid tissues mainly gut due to increased amounts of secondary metabolites (Kono and Fridovich, 1982; Pardini *et al.,* 1988).

In glycolysis, glycerol-3-phosphate shuttle transfers the reducing equivalents from cytoplasmic pool of NADPH to the mitochondrial membrane (Nation, 2008). Then, NADPH is re-oxidized by transferring electrons across the mitochondrial membrane rather than NADH itself (Nation, 2008). Moreover, Ascorbate peroxidase enzymatically removes H_2O_2 with the concurrent oxidation of ascorbate (Asada, 1992). NADPH is the final reducing equivalent in GSH-GSSG system to equilibrate chemical constitutions between dehydroascorbate and ascorbate. So, the higher activity of ascorbate oxidase should be compensated with increase in glucose-6-phosphate dehydrogenase activity. Our results on the highest activities of both enzymes in the aphids fed on vermicompost-treated plants indicate more reliance of aphids to provide energy from glycolysis and to maintain the balance between produced oxidative reagents.

Malondialdehyde (MDA) is an organic compound which naturally exist in biological media indicating oxidative stress. MDA is produced due to lipid peroxidation of polyunsaturated fatty acids. In fact, reactive oxygen species produced in cells degrade polyunsaturated lipids and produce MDA which is a reactive electrophile species leading to toxic stress in cells (Wang *et al.,* 2001; Dubovsky *et al.,* 2008). In our study, adults of *M. persicae* fed on Zinc sulfate and vermicompost showed higher amounts of MDA compared to control. It can be concluded that oxidative stress in fertilizer-treated aphids caused a high level of lipid peroxidation which is accompanied with the lower amounts of storage triglyceride mainly in vermicompost.

Our results demonstrated the highest RSSR/RSH ratio in the aphids fed on vermicompost-treated *C. annum*. Alteration of balance between oxidized thiols in an organism indicates the higher activity of radical oxidative species. Wang *et al.* (2001) believe higher ratio of oxidized to reduced thiols is accompanied with increase in lipid peroxidation processes led to higher amount of MDA. Their conclusion is corresponds with our findings on the aphids fed on vermicompost-treated plants with the highest RSSR/RSH ratio and MDA concentration.

Insects are highly dependent on their stored nutrients to survive in environment (Nation, 2008). Dietary food is transferred to fat bodies and stored as three macromolecules, protein, glycogen and triglyceride (Nation, 2008). Amounts of the two measured macromolecule, glycogen and triglyceride, were the highest in the aphids fed on Zinc sulfate-treated plants compared to vermicompost and control. In fact, *C. annum* reared in soil containing zinc sulfate is an appropriate host

Table 7 Effect of fertilizers on storage macromolecules in *M. persicae* adults

Treatments	Statistic (Mean±SE mg/mL)	
	TAG	Glycogen
Control	1.136±0.029 ab	0.034±0.025 b
Vermicompost (30%)	0.032±0.032 b	0.042±0.009 b
Zinc sulfate	4.783±0.015 a	0.510±0.001 a

The means followed by different letters in a column are significantly different [$p < 0.01$, Tukeys (HSD)].

plant for *M. persicae* because of nutrient quality and lack of feeding inhibitors mainly secondary metabolites. In other two treatments, mainly vermicompost, shortage of nutrients due to impaired digestive process led to utilize storage glycogen and triglyceride to support energetic mechanisms via glycolysis or β-oxidation of lipids. The conclusion can be supported by altered activities of intermediary enzymes highlighted earlier.

In summary, significant differences were observed in digestion, intermediary metabolism and antioxidant activities of *M. persicae* fed on *C. annum* cultured in control and fertilizer-treated soils. The physiological findings here and life table parameters reported in our previous study imply on negative effect of vermicompost on ecological fitness of *M. persicae*. So, it is recommended to use vermicompost as a culture substrate in greenhouses infested by *M. persicae* to suppress population outbreaks and to alleviate production costs due to unnecessary control procedures.

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