

## Antioxidant Defense Response of the Green Peach Aphid, *Myzus persicae* against Secondary Metabolites of the Host Plants Cumin, Anise, and Coriander

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### ABSTRACT

The green peach aphid, *Myzus persicae* is a polyphagous herbivore, attacking apiaceae plants which are rich in defensive secondary metabolites. Thus, *M. persicae* owns a protective antioxidative response to overcome the host defense. The aim of the present study was to investigate the adaptive antioxidative response of *M. persicae* against the secondary metabolites of cumin, anise, and coriander. The dietary antioxidants, ascorbic acid and glutathione and enzymatic antioxidants, superoxide dismutase, catalase, ascorbate peroxidase and glutathione peroxidase within tissues of *M. persicae* were measured every two weeks during the infestation season. The obtained results show that Anise could be a good recommended host in the beginning of the infestation season because it confers escalating levels of ascorbic acid. Coriander and cumin could be a second choice. The variable levels of enzymatic antioxidants during the season indicate the adaptive responses of *M. persicae* against the plant defensive secondary metabolites.

**Keywords:** Adaptive responses, Apiaceae, Enzymatic antioxidants, Herbivorous pests.

### INTRODUCTION

Aphids are important herbivorous pests that affect the crop production by disrupting plant tissues, consuming essential nutrients, transmitting viruses, injecting toxins, and providing a medium for fungal development (Figueroa *et al.*, 1999; Loayza-Muro *et al.*, 2000). Herbivorous insects induce biochemical and physiological changes in their host plants. These changes include the production of Plant Secondary Metabolites (PSM) which in turn stimulate the generation of Reactive Oxygen Species (ROS) in the herbivorous insects (Krishnan *et al.*, 2007; Lukasik *et al.*, 2012). ROS induce oxidative stress and alterations in

radical scavenging enzymes in insects (Felton and Summers 1995).

In response to oxidative stress arising from the ingestion of PSM, herbivorous insects have evolved defense antioxidant mechanisms. The antioxidant systems play important roles in neutralizing the toxicity of ROS. These systems are composed of enzymatic antioxidants and non-enzymatic compounds. The enzymatic antioxidant components such as Superoxide Dismutase (SOD), Catalase (CAT), Ascorbate Peroxidase (APOX) and Glutathione Peroxidase (GPX) protect the tissues against ROS (Lukasik *et al.*, 2011; George and Gatehouse 2013; Jena *et al.*, 2013). The non-enzymatic antioxidant compounds consist of small organic molecules such as Ascorbic

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Acid (ASA) and reduced Glutathione (GSH) (Krishnan *et al.*, 2009).

The host plant has an important effect on the antioxidant systems within the tissue of the herbivore insect. A comparison of antioxidant status of three species of Lepidoptera differing in their preference of the host plant showed a correlation of antioxidant system with their susceptibility to PSM of the host plant (Ahmad and Pardini, 1990). The change of host plant affected the levels of non-enzymatic and enzymatic antioxidants of the pea aphid, *Acyrtosiphon pisum* (Lukasik *et al.*, 2011). Therefore, the oxidative stress may play an important role in interactions between the herbivore insects and their host plants (Lukasik *et al.*, 2012; Lukasik and Goławska 2013).

The green peach aphid, *M. persicae*, attacks the medicinal plants such as cumin (*Cuminum cyminum* L.), anise (*Pimpinella anisum* L.), and coriander (*Coriandrum sativum* L.) in Assiut, Egypt, during the winter season. Since aphids spread greatly in this season, it infects these plants causing damage and loss of production. The role of antioxidative response system, Glutathione S-Transferase (GST) of *M. persicae* against PSM was studied in Brassica plants (Francis *et al.*, 2005), Tobacco and Pepper (Cabrera *et al.*, 2010). There is a lack of physiological studies regarding the role of antioxidative defense in the process of adaptation of *M. persicae* to the medicinally important plants, cumin, anise, and coriander. Thus, the present study aimed at investigating the changes in some antioxidant markers of oxidative stress within the aphid *M. persicae* during the life span of the three medically important plants.

## MATERIALS AND METHODS

### Aphid Collection

Cumin, anise, and coriander were cultivated at the farm of Faculty of Agriculture, Assiut University, Egypt,

during the winter season from December 2013 to March 2014. The maximum temperatures ranged from 24 to 27°C and the minimum ranged from 7 to 9°C with an average of 17°C. The average relative humidity ranged from 51 to 55%. An area of about 630 m<sup>2</sup> was divided into three equal parts. Each part was subdivided into 20 plots of equal size and cultivated with either cumin, anise, or coriander. The normal agricultural practices were performed and no insecticides were used during the study period. Aphids were collected every two weeks during the study period from the beginning of infestation, at the 4<sup>th</sup> week of December, to the end of the season, by the beginning of March 2014. The collected insects were transported to the laboratory for examination. Apterous adults of *M. persicae* were separated and kept in 1.5 mL Eppendorf tubes (200 aphids per tube). The tubes were stored at -20°C.

### Preparation of the Extract

The collected aphids were homogenized in 1.5 mL of 50 mM potassium phosphate buffer (pH 7.4). Then, the homogenates were centrifuged at 3,000×g for 15 minutes. The supernatants were collected and stored at -20°C.

### ASA Assay

ASA concentration was determined using adapted procedure of Omaye *et al.* (1979). First, 100 µL of 5% trichloroacetic were added to 50 µL of the aphid extract and centrifuged at 12,000×g for 15 minutes. Then, 20 µL of the solution were mixed with 480 µL distilled water, 80 µL of 85% H<sub>3</sub>PO<sub>4</sub>, 1.37 mL of 0.5%  $\alpha$ ,  $\alpha$ -dipyridyl, and 280 µL of 1% FeCl<sub>3</sub>. The reaction mixtures were incubated at 42°C for 40 minutes. The absorbance was measured at 525 nm against a control containing K-phosphate buffer (pH 6.5) instead of the aphid extract. Standard curve was applied to calculate the equation

constant. ASA contents were expressed as  $\mu\text{mol mg}^{-1}$  of protein.

#### GSH Assay

Total glutathione content in aphid tissues was determined based on the method of Lukasik *et al.* (2011) modified from Griffith (1980). TCA (50  $\mu\text{L}$  of 5%) was added to 100  $\mu\text{L}$  of aphid extract and centrifuged at  $3,000\times g$  for 15 minutes. Then, 100  $\mu\text{L}$  of the solution were mixed with 400  $\mu\text{L}$  of distilled water, 300  $\mu\text{L}$  of 0.3 mM NADPH, 100  $\mu\text{L}$  of 6 mM DTNB and 10  $\mu\text{L}$  of glutathione reductase (0.1 U  $\text{mL}^{-1}$ ). These mixtures were incubated at room temperature for 5 min. The absorbance was recorded at 412 nm. Standard curve was applied to calculate the equation constant. GSH contents were expressed as  $\mu\text{ mol/mg}$  of protein.

#### APOX Assay

APOX activity was measured as described by Lukasik *et al.* (2011) modified from Asada (1984). Aphid extract (100  $\mu\text{L}$ ) was mixed with 400  $\mu\text{L}$  of distilled water, 300  $\mu\text{L}$  of 2.5 mM in 67 mM potassium phosphate buffer (pH 7) and 500  $\mu\text{L}$  of 30 mM  $\text{H}_2\text{O}_2$ . The decrease in absorbance at 290 nm was recorded for 5 min. Boiled extracts were used as the controls. APOX activity was expressed as  $\mu\text{mol ascorbate oxidized min}^{-1} \text{ mg}^{-1}$  protein. The used extinction coefficient was  $2.8 \text{ mM}^{-1} \text{ cm}^{-1}$ .

#### GPX Assay

GPX activity was determined according to Lukasik and Golawska (2007). Aphid extract (100  $\mu\text{L}$ ) was mixed with 300  $\mu\text{L}$  of distilled water, 200  $\mu\text{L}$  of 1.2 mM Cumene hydroperoxide solution, 200  $\mu\text{L}$  of 1 mM NADPH, 100 $\mu\text{L}$  of 1 mM GSH and 20 $\mu\text{L}$  of glutathione reductase solution (0.1 U  $\text{mL}^{-1}$ ). The absorbance was recorded at 340 nm for 3 min against the control containing 0.4 mL

of 50 mM K-phosphate buffer (pH= 7) with 1mM EDTA, instead of the aphid homogenate. GPX activity was expressed as  $\mu\text{mol NADPH min}^{-1} \text{ mg}^{-1}$  protein using an extinction coefficient of  $0.00373 \text{ mM}^{-1} \text{ cm}^{-1}$ .

#### SOD Assay

SOD content was assayed in accordance with the method of Lukasik *et al.* (2012) based on reduction of NBT. The reaction mixture consisted of 100  $\mu\text{L}$  of aphid extract, 400  $\mu\text{L}$  distilled water and 500  $\mu\text{L}$  of 0.4 mM NBT in 0.2M phosphate buffer (pH 7.8). The increase in absorbance at 490 nm was determined against a blank contained 100  $\mu\text{L}$  of aphid extract, 400  $\mu\text{L}$  distilled water and 500  $\mu\text{L}$  of 0.2M phosphate buffer (pH 7.8). SOD activity was expressed as  $\mu\text{mol NBT min}^{-1} \text{ mg}^{-1}$  protein, using an extinction coefficient of  $12.8 \text{ mM}^{-1} \text{ cm}^{-1}$ .

#### CAT Assay

CAT activity was determined according to Aebi (1984). Fifty  $\mu\text{L}$  of aphid extracts were mixed with 450  $\mu\text{L}$  distilled water, 500  $\mu\text{L}$  of 30 mM  $\text{H}_2\text{O}_2$ . The decomposition of hydrogen peroxide was measured at 240 nm for 3 minutes. CAT activity was expressed as  $\mu\text{mol decomposed H}_2\text{O}_2 \text{ min}^{-1} \text{ mg}^{-1}$  protein, using an extinction coefficient of  $39.4 \text{ mM}^{-1} \text{ cm}^{-1}$ .

#### Protein Assay

The concentration of protein within the tissues of aphids was determined according to the method of Lowry *et al.* (1951).

#### Statistics

All data are reported as means $\pm$ SD,  $n= 3$ , where each replication represents one independent aphid homogenate (200 apterous aphids per tube). Data were



subjected to one-way Analysis Of Variance (ANOVA) followed by the Duncan's multiple-range test. Values followed by different letters are significantly different at  $P \leq 0.05$

## RESULTS

The activities of the antioxidants in the homogenates of *M. persicae* fed on the three host plants, namely, cumin, anise, and coriander are summarized in Table 1. At the beginning of the infestation season by the end of December, ASA level in the tissues of *M. persicae* that fed on cumin was high ( $38.12 \mu\text{mol mg}^{-1}$  protein) and then gradually decreased to its lowest level ( $15.35 \mu\text{mol mg}^{-1}$  protein) by the end of the season in early March. The aphids that fed on anise exhibited escalation of ASA level within their tissues gradually since the end of December and reached the summit ( $63.59 \mu\text{mol mg}^{-1}$  protein) in mid-February, but fell dramatically from the end of February onward ( $24.16 \mu\text{mol mg}^{-1}$  protein). ASA level within tissues of the aphids that fed on coriander was at the top ( $57.9 \mu\text{mol /mg}$  protein) at the beginning of the infestation season, then decreased from mid-January to reach the lowest by mid-February ( $14.98 \mu\text{mol mg}^{-1}$  protein), followed by a gradual rise from end of February. GSH level in *M. persicae* fed on cumin was wobbling as it reached its highest level in mid-January ( $3.18 \mu\text{mol mg}^{-1}$  protein) and declined thereafter down to the minimum level ( $0.53 \mu\text{mol mg}^{-1}$  protein) in mid-February.

Tissues of aphids that fed on anise contained moderate level of GSH ( $1.47 \mu\text{mol mg}^{-1}$  protein) at the beginning of the infestation season and then gradually decreased until the end of January. Gradual increase of GSH was observed again during February. Aphids that fed on coriander had a medium level of GSH ( $1.57 \mu\text{mol mg}^{-1}$  protein) at the beginning of the infestation season, which then escalated strongly to reach its peak at the end of January ( $3.19$

$\mu\text{mol mg}^{-1}$  protein) and then sharply decreased to the end of the season.

SOD level in the aphids that fed on cumin was moderate ( $0.21 \mu\text{mol NBT min}^{-1} \text{mg}^{-1}$  protein) at the beginning of the season and then rose from mid-January ( $0.32 \mu\text{mol NBT min}^{-1} \text{mg}^{-1}$  protein) to settle high during the rest of the season. Aphids that fed on anise reached high level of SOD at the beginning of the infestation ( $0.26 \mu\text{mol NBT min}^{-1} \text{mg}^{-1}$  protein) and then dropped dramatically to minimum in mid-January ( $0.07 \mu\text{mol NBT min}^{-1} \text{mg}^{-1}$  protein). Gradual rise started after that to reach its highest level in mid-February ( $0.39 \mu\text{mol NBT min}^{-1} \text{mg}^{-1}$  protein); and then fell back and continued falling to the minimum by the end of the season. Aphids that fed on coriander contained a high level of SOD at the beginning of the infestation ( $0.32 \mu\text{mol NBT min}^{-1} \text{mg}^{-1}$  protein) and then declined by mid-January ( $0.21 \mu\text{mol NBT min}^{-1} \text{mg}^{-1}$  protein). Sharp rise of SOD to its highest level ( $0.49 \mu\text{mol NBT min}^{-1} \text{mg}^{-1}$  protein) was observed at the end of January, then, declined sharply in mid-February ( $0.17 \mu\text{mol NBT min}^{-1} \text{mg}^{-1}$  protein) and settled down almost to the end of the season.

CAT in aphids that fed on cumin was the highest of all ( $609.56 \mu\text{mol decomposed H}_2\text{O}_2 \text{ min}^{-1} \text{mg}^{-1}$  protein) at the beginning of the infestation season and then gradually declined until mid-February ( $361.39 \mu\text{mol decomposed H}_2\text{O}_2 \text{ min}^{-1} \text{mg}^{-1}$  protein). Re-rise of CAT to  $467.54 \mu\text{mol decomposed H}_2\text{O}_2 \text{ min}^{-1} \text{mg}^{-1}$  protein by end of February was followed with decline by the end of the season ( $432.28 \mu\text{mol decomposed H}_2\text{O}_2 \text{ min}^{-1} \text{mg}^{-1}$  protein).

The aphids that fed on anise contained very low level of CAT ( $123.87 \mu\text{mol decomposed H}_2\text{O}_2 \text{ min}^{-1} \text{mg}^{-1}$  protein) in the beginning of the infestation and then escalated gradually until the end of January ( $433.25 \mu\text{mol decomposed H}_2\text{O}_2 \text{ min}^{-1} \text{mg}^{-1}$  protein). A sharp decreases of CAT was recorded in mid-February ( $156.58 \mu\text{mol decomposed H}_2\text{O}_2 \text{ min}^{-1} \text{mg}^{-1}$  protein). CAT level rose sharply at the end of February ( $453.56 \mu\text{mol decomposed H}_2\text{O}_2 \text{ min}^{-1} \text{mg}^{-1}$  protein) and

**Table 1.** Antioxidants of adults green peach aphid, *M. persicae* fed on different host plants during the infestation season from end of December to beginning of March, 2014, in Assiut, Egypt.<sup>a</sup>

Period	Host plant	ASA	GSH	SOD	CAT	APOX	GPX
End of December	Cumin	38.12±5.56 <sup>bc</sup>	1.51±0.02 <sup>cd</sup>	0.21±0.001 <sup>dehgh</sup>	609.56±38.04 <sup>a</sup>	1.62±0.15 <sup>gh</sup>	0.37±0.04 <sup>bcd</sup>
	Anise	35.84±4.43 <sup>bcd</sup>	1.47±0.006 <sup>cd</sup>	0.26±0.04 <sup>cdef</sup>	123.87±11.73 <sup>g</sup>	1.53±0.18 <sup>gh</sup>	0.31±0.05 <sup>d</sup>
	Coriander	57.90±10.85 <sup>a</sup>	1.57±0.23 <sup>c</sup>	0.32±0.08 <sup>bc</sup>	582.59±4.79 <sup>a</sup>	5.81±0.46 <sup>c</sup>	0.54±0.02 <sup>a</sup>
Mid of January	Cumin	35.15±0.97 <sup>bcd</sup>	3.18±0.60 <sup>a</sup>	0.32±0.02 <sup>bc</sup>	482.74±80.99 <sup>b</sup>	2.81±0.43 <sup>efg</sup>	0.35±0.03 <sup>cd</sup>
	Anise	44.58±4.78 <sup>b</sup>	1.08±0.59 <sup>cdef</sup>	0.07±0.03 <sup>j</sup>	348.92±46.30 <sup>cde</sup>	2.19±0.12 <sup>fgh</sup>	0.37±0.02 <sup>cd</sup>
	Coriander	34.58±7.08 <sup>bcd</sup>	2.39±0.45 <sup>b</sup>	0.21±0.05 <sup>dehgh</sup>	338.01±31.26 <sup>de</sup>	3.52±0.46 <sup>ef</sup>	0.54±0.01 <sup>a</sup>
End of January	Cumin	31.17±2.57 <sup>cdef</sup>	1.63±0.39 <sup>c</sup>	0.28±0.02 <sup>cde</sup>	389.21±51.43 <sup>bcd</sup>	7.64±0.12 <sup>b</sup>	0.31±0.08 <sup>d</sup>
	Anise	60.08±8.46 <sup>a</sup>	0.71±0.17 <sup>ef</sup>	0.12±0.06 <sup>ij</sup>	433.25±66.71 <sup>bcd</sup>	6.58±0.86 <sup>bc</sup>	0.23±0.01 <sup>e</sup>
	Coriander	35.40±10.34 <sup>bcd</sup>	3.19±0.32 <sup>a</sup>	0.49±0.08 <sup>a</sup>	472.86±10.72 <sup>b</sup>	13.76±2.63 <sup>a</sup>	0.23±0.03 <sup>e</sup>
Mid of February	Cumin	30.29±3.79 <sup>cdef</sup>	0.53±0.12 <sup>f</sup>	0.31±0.01 <sup>c</sup>	361.39±45.50 <sup>cde</sup>	4.15±0.30 <sup>de</sup>	0.42±0.01 <sup>bc</sup>
	Anise	63.59±7.42 <sup>a</sup>	0.75±0.10 <sup>ef</sup>	0.39±0.04 <sup>b</sup>	156.58±35.01 <sup>g</sup>	3.39±0.24 <sup>ef</sup>	0.50±0.01 <sup>a</sup>
	Coriander	14.98±0.35 <sup>g</sup>	1.28±0.03 <sup>cde</sup>	0.17±0.04 <sup>ghi</sup>	172.53±11.85 <sup>fg</sup>	1.71±0.46 <sup>gh</sup>	0.21±0.021 <sup>e</sup>
End of February	Cumin	25.95±1.09 <sup>def</sup>	1.02±0.04 <sup>cdef</sup>	0.29±0.05 <sup>cd</sup>	467.54±118.78 <sup>b</sup>	2.45±1.35 <sup>fg</sup>	0.14±0.007 <sup>f</sup>
	Anise	24.16±1.86 <sup>efg</sup>	1.15±0.76 <sup>cdef</sup>	0.14±0.001 <sup>hij</sup>	453.56±116.63 <sup>bc</sup>	1.40±0.12 <sup>gh</sup>	0.19±0.04 <sup>ef</sup>
	Coriander	26.99±3.61 <sup>def</sup>	0.92±0.12 <sup>def</sup>	0.20±0.07 <sup>efghi</sup>	261.27±30.08 <sup>ef</sup>	5.39±1.57 <sup>cd</sup>	0.41±0.01 <sup>bc</sup>
Beginning of March	Cumin	15.35±0.65 <sup>g</sup>	0.76±0.01 <sup>ef</sup>	0.25±0.03 <sup>cdefg</sup>	432.28±24.53 <sup>bcd</sup>	0.60±0.36 <sup>h</sup>	0.44±0.04 <sup>b</sup>
	Anise	21.02±4.92 <sup>efg</sup>	0.80±0.06 <sup>ef</sup>	0.07±0.01 <sup>j</sup>	170.00±65.19 <sup>fg</sup>	1.69±0.12 <sup>gh</sup>	0.39±0.082 <sup>bc</sup>
	Coriander	31.58±1.37 <sup>cde</sup>	1.32±0.24 <sup>cde</sup>	0.18±0.003 <sup>ghi</sup>	386.85±34.20 <sup>bcd</sup>	1.17±0.21 <sup>gh</sup>	0.37±0.019 <sup>bcd</sup>

<sup>a</sup> Data for ASA and GSH are mean  $\mu$  mol mg<sup>-1</sup> protein for  $\pm$ SD (n=3). Data for SOD, CAT, APOX and GPX are mean  $\mu$ mol decomposed substrate min<sup>-1</sup> mg<sup>-1</sup> protein  $\pm$ SD (n=3). Values in columns followed by different letters are significantly different at  $P \leq 0.05$  (Duncan multiple range test).



then fell in by the beginning of March (170  $\mu\text{mol}$  decomposed  $\text{H}_2\text{O}_2 \text{ min}^{-1} \text{ mg}^{-1}$  protein). Aphids that fed on coriander contained very high levels of CAT (582.59  $\mu\text{mol}$  decomposed  $\text{H}_2\text{O}_2 \text{ min}^{-1} \text{ mg}^{-1}$  protein) in the early infestation, but then CAT level declined by mid-January (338.01  $\mu\text{mol}$  decomposed  $\text{H}_2\text{O}_2 \text{ min}^{-1} \text{ mg}^{-1}$  protein) and rose again by the end of January (472.86  $\mu\text{mol}$  decomposed  $\text{H}_2\text{O}_2 \text{ min}^{-1} \text{ mg}^{-1}$  protein). The level of the enzyme dropped sharply in mid-February (172.53  $\mu\text{mol}$  decomposed  $\text{H}_2\text{O}_2 \text{ min}^{-1} \text{ mg}^{-1}$  protein) followed by a gradual rise until the end of the season.

APOX level in the aphids that fed on cumin was low (1.62  $\mu\text{mol}$  ascorbate oxidized  $\text{min}^{-1} \text{ mg}^{-1}$  protein) at the beginning of the season and then gradually picked up to its highest level by the end of January (7.64  $\mu\text{mol}$  ascorbate oxidized  $\text{min}^{-1} \text{ mg}^{-1}$  protein). Gradual decline to the minimum was observed by the end of the season (0.6  $\mu\text{mol}$  ascorbate oxidized  $\text{min}^{-1} \text{ mg}^{-1}$  protein). Nearly the same behavior occurred for APOX in *M. persicae* that fed on anise. The level of APOX in the aphids that fed on coriander began high (5.81  $\mu\text{mol}$  ascorbate oxidized  $\text{min}^{-1} \text{ mg}^{-1}$  protein) at the start of the infestation and then dropped in mid-January (3.52  $\mu\text{mol}$  ascorbate oxidized  $\text{min}^{-1} \text{ mg}^{-1}$  protein). Sharp increase of APOX to reach top level (13.76  $\mu\text{mol}$  ascorbate oxidized  $\text{min}^{-1} \text{ mg}^{-1}$  protein) at the end of January fell down dramatically in the middle of February (1.71  $\mu\text{mol}$  ascorbate oxidized  $\text{min}^{-1} \text{ mg}^{-1}$  protein). By the end of February, APOX increased (5.39  $\mu\text{mol}$  ascorbate oxidized  $\text{min}^{-1} \text{ mg}^{-1}$  protein), but declined again (1.17  $\mu\text{mol}$  ascorbate oxidized  $\text{min}^{-1} \text{ mg}^{-1}$  protein) at the end of the season.

GPX level in the aphids that fed on cumin was high (0.37  $\mu\text{mol}$  NADPH  $\text{min}^{-1} \text{ mg}^{-1}$  protein) at the beginning of infestation and then gradually decreased, but rose sharply in mid-February (0.42  $\mu\text{mol}$  NADPH  $\text{min}^{-1} \text{ mg}^{-1}$  protein). By the end of February, GPX level fell down to the minimum level (0.14  $\mu\text{mol}$  NADPH  $\text{min}^{-1} \text{ mg}^{-1}$  protein) and rose again strongly at the end of the season (0.44  $\mu\text{mol}$  NADPH  $\text{min}^{-1} \text{ mg}^{-1}$  protein). GPX in

the aphids that fed on anise was high (0.31  $\mu\text{mol}$  NADPH  $\text{min}^{-1} \text{ mg}^{-1}$  protein) at the end of December and rose in mid-January (0.37  $\mu\text{mol}$  NADPH  $\text{min}^{-1} \text{ mg}^{-1}$  protein), then declined by the end of January (0.23  $\mu\text{mol}$  NADPH  $\text{min}^{-1} \text{ mg}^{-1}$  protein). Sharp rise of GPX to its highest level (0.50  $\mu\text{mol}$  NADPH  $\text{min}^{-1} \text{ mg}^{-1}$  protein) was observed in the mid-February. GPX level in the aphids that fed on coriander was very high at the beginning of the infestation (0.54  $\mu\text{mol}$  NADPH  $\text{min}^{-1} \text{ mg}^{-1}$  protein) and continued until mid-January, but fell sharply at the end of January (0.23  $\mu\text{mol}$  NADPH  $\text{min}^{-1} \text{ mg}^{-1}$  protein). GPX level rose strongly at the end of February (0.41  $\mu\text{mol}$  NADPH  $\text{min}^{-1} \text{ mg}^{-1}$  protein).

## DISCUSSION

During the whole vegetation period, cumin, anise, and coriander plants were under the stress of attack by herbivorous insects. A common response of plant tissues to various stress factors including biotic stress is the production of PSM that produce ROS inside aphid's body. On the other hand, antioxidant systems play an important role in scavenging ROS and protecting the insect body (Felton and Summers, 1995; Pardini, 1995; Wang *et al.*, 2012; He *et al.*, 2013). These systems include the dietary antioxidants such as ASA and GSH and enzymatic antioxidants like SOD, CAT, APOX, and GPX. The intensity of oxidative stress in aphids may be conditioned by PSM content.

In the present study, *M. persicae* was a common aphid between the three host plants. The present results showed that ASA contents in *M. persicae* fed on cumin, anise, and coriander vary according to the host plant. *M. persicae* showed a specific preference towards plants with high ascorbate content (Kerchev *et al.*, 2012). The pattern of ASA contents in *M. persicae* fed on cumin and coriander plants were opposite to the pattern of ASA contents in *M. persicae* fed on anise during the season.

The highest ASA contents were recorded for *M. persicae* fed on anise by the end of January and the end of February (maximum infestation). These contents were about two fold or higher in comparison with aphids fed on cumin and coriander. Lukasik *et al.* (2011) noted that the content of ASA in the pea aphid *A. pisum* depends on the host plant species within Fabaceae plants. The variation of ASA content in *M. persicae* may be due to varying ASA contents in their host plants. A relationship was found between dietary ASA and body content of ASA in *Trichoplusia ni* (Timmermann *et al.*, 1999). ASA contents in *M. persicae* fed on cumin, anise, or coriander plants decreased from the end of February until the end of the season. Higher ASA content was found in the young green leaves than the older leaves of two medicinal edible plants, *Oldenlandia corymbosa* and *Dissotis rotundifolia* in Nigeria (Okeri and Alonge 2006). ASA contents in *M. persicae* fed on cumin, anise and coriander were much higher than that were found in the pea aphid, *A. pisum* fed on broad bean, vetch and pea plants and even the cereal aphid (Lukasik *et al.*, 2009; 2011).

The pattern of Glutathione contents in *M. persicae* fed on coriander was opposite to that of *M. persicae* fed on anise in the beginning of the season until the mid-season. By the mid-January, *M. persicae* fed on cumin plant had a higher GSH content than that fed on anise and coriander plants. By the end of February, *M. persicae* fed on anise plant had GSH content higher than that found in aphids that fed on cumin and coriander. The alteration of the diet from different plant sources resulted in the alteration of GSH content in *Lymantria dispar* (Peric-Mataruga *et al.*, 1997). Similar results were recorded by Lukasik *et al.* (2011), where the alteration of the host plants of Fabaceae resulted in alteration in the GSH contents of the pea aphid, *A. pisum*. The regulation of GSH contents in *M. persicae* may be one of the rapid forms of adaptive responses against nutritive and oxidative stress. In the present research, GSH contents in *M. persicae* fed on cumin,

anise, and coriander were much higher than that found in the pea aphid fed on fabaceae plants (Lukasik *et al.*, 2011). Generally, both ASA and GSH contents in *M. persicae* fed on cumin, anise, and coriander decreased by the end of the season compared with the beginning of the season.

Antioxidant enzymes play an important role in the chemical interaction between insects and their host plants, leading to insect adaptation against plant secondary metabolites. The studied host plants were clearly affected the activity of the antioxidant enzymes in *M. persicae*. The present study shows that fluctuations of antioxidant enzymes within the aphid tissues could be attributed to the diversity of PSM in the host plants. SOD activities in *M. persicae* fed on anise and coriander were highly fluctuating with two peaks around mid-season, by the end of January and mid-February. In response to increasing the population size of the herbivore insects, plants may produce higher concentrations of PSM (Ananthakrishnan *et al.*, 1992). The increase in SOD activities is probably associated with the release of superoxide radical as a result of ingesting PSM (Ahmad, 1992). The exposition of some lepidopteran species to Quercetin induced SOD activities in their bodies (Pritsos *et al.*, 1988; Ahmad and Pardini 1990). Moreover, O-dihydroxyphenols increased SOD activity in the cereal aphids (Lukasik, 2007). On the other hand, tannic acid reduced SOD activity in the midgut of grasshoppers (Barbehenn, 2002).

In the present study, CAT activities were generally very much higher than that found within the cereal aphids studied by Lukasik and Golawska (2007). The expected reasons for this result could be attributed to the extreme polyphagous habit of *M. persicae*. Also, the host plants cumin, anise, and coriander belong to Apiaceae which are rich in photodynamic pro-oxidants such as furanocoumarins and *B*-carboline alkaloids that induce CAT activity (Lee and Berenbaum, 1993). CAT activities in *M. persicae* may reflect its role in scavenging



ROS in this extremely polyphagous aphid. Low concentration of hydroxamic acid in wheat cultivars induced the activity of CAT enzyme in the cereal aphid, unlike high concentration that inhibited the activity (Loayza-Muro *et al.*, 2000).

APOX is a component of ascorbate–recycling system. In the present study, APOX activities were generally low. This result is consistent with the above mentioned results showing that CAT activity was very high. Therefore, ascorbate recycling system may not have a main role in detoxifying peroxides in *M. persicae*. GPX activity patterns in *M. persicae* were fluctuating during the season. The inhibition of APOX and GPX activity was recorded in herbivores fed on food with tannic acid (Barbehenn, 2002). Kinetin induced significant increase of GPX, SOD and CAT activities in the mustard aphid (Rup *et al.*, 2006).

The present results give the evidence that the host plants affected the antioxidant defense systems of the aphid *M. persicae*. Anise could be a good recommended host in the beginning of the infestation season because it confers escalating levels of ASA. Coriander could be another choice because it also confers high GSH levels to *M. persicae* in the same period. The fluctuating levels of enzymatic antioxidants during the season may indicate the adaptive response of *M. persicae* against PSM. The possibility that the aphids may move between the three hosts is not excluded, therefore, the enzyme activities patterns were complicated. The expected reason for various concentrations during the season is the release of plant secondary metabolites as a plant defense against the aphid infestation. However, these metabolites are numerous, thus, future laboratory studies using specific pure metabolites are required. Future studies such as laboratory experiments using artificial diets with pure secondary metabolites are required to confirm the defensive response of GST of *M. persicae* fed on cumin, anise, and coriander. The role of GST activity of *M. persicae* was confirmed as a defense against PSM of brassica plants (Francis *et*

*al.*, 2005). In contrast, there was no significant change in GST activity of *M. persicae* fed on tobacco and pepper (Cabrera *et al.*, 2010). Investigating the involvement of aphid Myrosinase (Francis *et al.*, 2002) Monooxygenase and Esterases detoxification systems (Cabrera *et al.*, 2010; Ramsey *et al.*, 2010) in adapting *M. persicae* to cumin, anise, and coriander is crucial. The study is a field investigation; therefore, it gives an insight into the effects of three PSM rich host plants on the polyphagous aphid *M. persicae*.

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### واکنش دفاعی آنتی اکسیدانی شته سبز هلو *Myzus persicae* در برابر متابولیت های ثانویه گیاه میزبان شامل زیره، رازیانه و گشنیز

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#### چکیده

شته سبز هلو (*Myzus persicae*) گیاهخواری چند غذایی (polyphagous) است که به گیاهان چتریان (apiaceae) که سرشار از متابولیت های دفاعی ثانویه هستند حمله می کند. از این قرار، *M. persicae* برای غلبه بر دفاع میزبان، واکنشی حفاظتی و آنتی اکسیداتیو دارد. هدف پژوهش حاضر، بررسی واکنش سازگاری و آنتی اکسیداتیو *M. persicae* در برابر متابولیت های ثانویه گیاهان زیره، رازیانه، و گشنیز بود. به این منظور، هر دو هفته یک بار در طی دوره آلودگی، آنتی اکسیدان های تغذیه ای، اسید اسکریک و گلوکوتایون و آنتی اکسیدان های آنزیمی، سوپراکسید دیسموتاز، کاتالاز، آسکوربات پراکسیداز، و گلوکوتایون پراکسیداز دریافت های *M. persicae* اندازه گیری شد. نتایج نشان می دهد که رازیانه می تواند میزبان خوب و قابل توصیه ای برای مرحله آغازین فصل آلودگی باشد زیرا منجر به افزایش مقدار اسید اسکریک می شود. گشنیز و زیره را می توان انتخاب دوم قلمداد کرد. گفتنی است که تغییرات سطح آنتی اکسیدان های آنزیمی در طی فصل حاکی از واکنش سازگاری *M. persicae* در برابر متابولیت های ثانویه دفاعی گیاه است.