

Role of Exogenous Elicitors in Canola Plant Defense against Cabbage Aphid by Regulating Physiological Balance and Secondary Metabolite Biosynthesis

M. Shahrokhi¹, M. Pahlavan Yali*¹, and M. Bozorg-Amirkalae²

ABSTRACT

Induced resistance in plants can be manipulated using the application of elicitors. In this study, the effects of Salicylic Acid (SA), chitosan, γ -Aminobutyric Acid (GABA), Serenad Aso (*Bacillus subtilis* QST713), and combined application of these elicitors were studied on the resistance of canola to cabbage aphid, *Brevicoryne brassicae* L. (Hemiptera: Aphididae). The number of *B. brassicae* attracted to canola plants treated with different inducers was significantly lower compared to the control in the field experiments. The lowest population density of aphid was observed on plants treated with SA+GABA and SA+chitosan. Moreover, the highest amounts of glucosinolates in canola leaves were recorded in SA+GABA treatment. The contents of sugar, lipid, and glycogen of *B. brassicae* were lowest when fed on the plants treated with SA integrated with GABA or chitosan. Furthermore, the activity of catalase, esterase, peroxidase and glutathione S-transferase enzymes was the lowest in SA+GABA and SA+chitosan. These findings suggest that exogenous application of SA integrated with GABA or chitosan on canola plants are successful in suppressing the *B. brassicae* population and can be considered in integrated management programs against this and other aphids.

Keywords: Amino acid, Antioxidant enzymes, Chitosan, Energy sources, Salicylic acid, Serenade.

INTRODUCTION

The cabbage aphid, *Brevicoryne brassicae* L., is the most abundant and serious aphid species on canola plants during the stages of flowering and podding (Sayed and Teilep, 2013). The common management strategy for this pest mainly depends on chemical control (Shonga and Getu, 2021), but this strategy can result in serious problems such as insecticidal residues in food and the environment, and a rapid increase in the development of resistance genes (Ansari *et al.*, 2014). These problems have encouraged scientists to focus on their attention to other environmentally safe control approaches

such as induced resistance of the plant. Induced response in plants is one of the imperative components of pest control in agriculture that is exploited for regulating the insect population (War *et al.*, 2012).

Salicylic Acid (SA) is an endogenous elicitor and plant growth regulator that generates a wide range of physiological and metabolic responses in plants, thereby influencing their growth processes such as ion uptake, transport, and membrane permeability (Rivas-San Vicente and Plasencia, 2011). Further, SA is a key signaling molecule in systemic acquired resistance with a prominent role in inducing plant tolerance to various abiotic and biotic tensions (Horvath *et al.*, 2007; Kamel *et al.*, 2016).

¹ Department of Plant Protection, Faculty of Agriculture, Shahid Bahonar University, Islamic Republic of Iran.

² Department of Plant Protection, Faculty of Agricultural Sciences, University of Mohaghegh Ardabili, Islamic Republic of Iran.

* Corresponding author; e-mail: pahlavanm@uk.ac.ir



Chitosan, a biodegradable polysaccharide with high molecular weight, is known as an environmentally safe and non-toxic product (Katiyar *et al.*, 2015). It prompts defense responses in the plant, resulting in the formation of some physical and chemical barriers against pest attacks. Chitosan can influence defense mechanisms including different enzymes against unfavorable conditions (Katiyar *et al.*, 2015).

The γ -Aminobutyric Acid (GABA), a natural non-protein amino acid, acts as a signaling molecule or metabolite in some physiological processes of plants under stress conditions (Bao *et al.*, 2015; Zhen *et al.*, 2018). For example, exogenous application of GABA mitigated chilling damage of tomato seedlings by regulating antioxidant enzyme activities and subsequently reducing Reactive Oxygen Species (ROS) (Malekzadeh *et al.*, 2014). It is well known that inducing priming through exogenous application of GABA or its isomer synthetic form, β -Aminobutyric Acid (BABA), could stimulate osmotic balance via synthesis of osmolytes and enhance antioxidant enzymes activity as well as their transcript levels, thus contributing to the reduction of oxidative stress (Vijayakumari *et al.*, 2016).

Serenade ASO is a naturally occurring biological control agent containing *Bacillus subtilis* QST 713 that are not genetically modified. Further, compounds produced by this bacterium prompt the systemic resistance response of the plant as shown by improved peroxidase activity. Serenade ASO may be applied as a foliar spray alone or in alternating spray programs with other crop protection products (Bayer Crop Science Ltd, 2019).

Biotic and abiotic elicitors can affect the amounts of secondary metabolites in plants in response to herbivorous insects (Khoshfarman-Borji *et al.*, 2020). Glucosinolates (GSLs) (a group of naturally occurring thioglucosides) are the main secondary metabolites accumulated in Brassicaceae plants and are essential in the nutrition of the cabbage aphid (Halkier and Gershenzon, 2006). The presence of these metabolites in high amounts causes induction

of resistance in the Brassicaceae plants (Sattari Nasab *et al.*, 2018). Secondary metabolites can affect oxidative stress through producing free radicals and can modify the antioxidant enzyme systems (Zhang and Feng, 2018). The detoxifying enzymes have been demonstrated to be involved in metabolic resistance and are believed to be important factors in determining their resistance to a wide range of toxic chemicals (Francis *et al.*, 2001; Despres *et al.*, 2007).

The knowledge of interactions between insects and plants treated with different biotic and abiotic elicitors is important in pest management. For example, Khoshfarman-Borji reported induction of resistance by combined SA and *Pseudomonas putida* application on the canola plant against *B. brassicae* under laboratory conditions (Khoshfarman-Borji *et al.*, 2020). The present research aimed to compare the impact of SA, chitosan, GABA, and Serenade ASO on population density of *B. brassicae* as well as on the contents of GSLs in canola leaves, energy sources, and antioxidant enzyme activities of this aphid. The information could be used in the pest management programs for *B. brassicae* on the canola.

MATERIALS AND METHODS

Plant Sources, Induction Treatments, and Experimental Design

The field experiments were conducted during two growing seasons (2020 and 2021) on canola plants grown in a field located at the Chilabad region, Orzueeyeh, Kerman, Iran. Seeds of commercial canola cultivar (Trapper) were obtained from the Agricultural Jihad Office of Kerman, Iran, and were planted for conducting the experiments based on a randomized complete design in October 2020 and 2021. The experimental area was divided into 11 treatment areas including the control. Each treatment consisted of three rows two meters long and one-meter width), which were randomly selected. Rows and plants spacing

were at 1 m and 20 cm, respectively. The field was managed according to the local practice with furrow irrigation (every ten days), and no pesticides were applied. Canola plants were treated with several inducers including solutions of SA (1 mM), chitosan (1.5 mM), GABA (10 mM), and Serenade Aso (*Bacillus subtilis* QST713) (1%) in each year. Also, these inducers were applied in combination: (SA+chitosan, SA+GABA, SA+Serenade, chitosan+GABA, chitosan+Serenade, GABA+Serenade). Control plants were sprayed with distilled water. In combined treatments, sprayings were performed during the day, one of the compounds in the early morning and the other in the evening.

The 300 mL of the treatment solution was sprayed in each plot. The first spraying of all treatments was accomplished in the stage of 4-6 leaves and the second spraying as soon as the first population of *B. brassicae* appeared (March 10 in 2020 and March 19 in 2021).

Determination of Aphid Population Density

To determine the effect of different inducers on the population density of the cabbage aphids on canola plants, 30 plants under each treatment were randomly selected. The sampling was performed seven days after the second spraying at the initial stage of flowering and was weekly continued until the late seed development stage. The cabbage aphids on plants under each treatment were collected by shaking plants on a tray. The collected aphids of each treatment were individually transferred to the laboratory in plastic bags containing labels, and the number of *B. brassicae* per plant was counted using a 20X hand lens.

Determination of Total GLS Contents

The powder (0.1 g) of the treated canola leaves (48 hours after first spraying) was

transferred to a 10 mL glass tube with a lid. Then, GLS was extracted based on the technique explained by Ishida *et al.* (2011), as follows. The 4.8 mL of 80% methanol retained at room temperature was added to the powder in glass tubes. After adding 0.2 mL of 5 mM sinigrin as an internal standard, the tubes were retained at 25°C for 30 minutes and shaken mutually for 30 minutes in a shaker. Afterward, they were centrifuged at 1,600×g for 10 minutes. The supernatant was used as a crude extract.

Colorimetric analysis of the total GLS content was done by simplifying the method performed by Moller *et al.* (1985). To 0.2 mL of crude GLS extract, 0.3 mL of distilled water, and 3 mL of 2 mM palladium chloride reagent, in which 3.54 mg PdCl had been dissolved in concentrated HCL (1.68 mL) and diluted to 1,000 mL with distilled water, were added and mixed. After incubation at 25°C for 1 hour, absorbance was read by a spectrophotometer at 425 nm.

Sample Preparation for Evaluation of Detoxifying Enzyme Activities

The cabbage aphids were randomly selected from each treatment 7 days after the second spraying. To measure the activity of the esterase enzyme, insect samples were homogenized in 200 µL of 0.1M phosphate buffer containing 0.1% triton® X-100 (Sigma Aldrich), and the homogenates were centrifuged at 10,000 rpm for 15 minutes at 4°C. To measure the activity of the peroxidase enzyme, aphids were homogenized in 200 µL of phosphate buffer (20 mM) and then were centrifuged at 2,600 rpm for 5 minutes. at 4°C.

To measure the activity of glutathione S-Transferase (GST) and Catalase (CAT), the aphid specimens were homogenized in 200 µL cold phosphate buffer (10 mM), then, homogenates were centrifuged at 13,000 rpm for 15 minutes at 4°C. The top layer was collected and kept at -20°C before beginning biochemical analyses. All enzymes assays were done in three replications.



Esterase

The activity of this enzyme was measured using a solution containing 10 μL of the enzyme sample, 40 μL of phosphate buffer, 10 μL of β -naphthyl (10 mM in acetone), and 50 μL of fast blue RR salt solution (Sigma Aldrich) in the spectrophotometer at 405 nm (Van Asperen, 1983).

Peroxidase

The reaction was performed in a volume of 0.5 mL containing 225 μL of 225 mM H_2O_2 (Merck Germany) and 225 μL of 45 mM Guaiacol. Also, 50 μL of 50 mM potassium phosphate buffer was applied as a blank. The Guaiacol reaction was read at 470 nm by a spectrophotometer (Bergmeyer, 1974).

Catalase (CAT)

The CAT activity was assessed according to Aebi (1984). For this purpose, 50 μL of the sample, 225 μL of H_2O_2 , and 225 μL of 70 mM potassium phosphate buffer solution with $\text{pH}=7$ were mixed and the absorbance was read at 240 nm by spectrophotometer.

Glutathione S-Transferase (GST)

GST activity was calculated according to Habig *et al.* (1974) method with some changes. Two hundred microliter of the reaction mixture containing 100 μL 1-Chloro-2,4-Dinitrobenzene (CDNB) (1.2 mM), 100 μL reduced glutathione (10 mM) and 15 μL of enzyme sample was poured into a cuvette. The absorbance was read by the spectrophotometer at 340 nm.

Determination of Energy Sources

The amounts of sugar, lipid, and glycogen were measured by the method of Foray *et al.*

(2012). The homogenized adult aphids were centrifuged at 2,600 rpm for 5 minutes at 4°C . Then, the homogenized samples of insects were poured into Falcon tubes and then 22.5 μL of 20% sodium sulfate and 1.88 mL of the methanol-chloroform mixture (1:2 v/v) were added and vortexed. The supernatant containing sugar and lipid was separated using distilled water. The upper part containing sugar was determined using an antron reagent in the spectrophotometer (UV-2100) at a wavelength of 625 nm. The lower part contained lipid, which was measured after adding 98% sulfuric acid (50 μL) and vanillin reagent in a spectrophotometer at a wavelength of 525 nm. Glucose (German Merck) and cholesterol (German Merck) were used to plot the standard curves of sugar and lipid, respectively.

To determine the glycogen content, homogenized samples were prepared according to what was mentioned in the first step. The supernatant was removed by a micropipette. After that, 1 mL of antron reagent and 1 mL of 80% methanol were added to the pellet. The sample absorbance was determined in the spectrophotometer at 625 nm, with glucose as the standard.

The amount of protein was determined using Bradford reagent according to Greenfield method (Greenfield, 2018) and its absorption was read at 595 nm.

Statistical Analyses

The normality with the Kolmogorov-Smirnov test was checked for all data, and variables were compared using one-way Analysis Of Variance (ANOVA) in SPSS. Then, multiple comparisons were done using the Tukey HSD post-hoc test (SPSS 2015).

RESULTS

Population Density

The canola plants under different treatments significantly affected the

population density of *B. brassicae* in 2020 ($F= 365.17$; $df= 10, 319$; $P< 0.001$) and 2021 ($F= 956.90$; $df= 10, 319$; $P< 0.001$) (Table 1). The aphid density was significantly lower on canola plants treated with different inducers than on the control plants during both years (Table 1). In 2020, the lowest number of aphids attracted was observed on plants treated with SA+GABA (5.2 ± 0.2) and SA+chitosan (5.7 ± 0.1), and in 2021, the lowest ones were on plants treated with SA+chitosan (8.4 ± 0.1) (Table 1).

Table 1. Population density (mean \pm SE) of *Brevicoryne brassicae* L. on canola plants treated with different elicitors in the field during 2020 and 2021.

Treatments ^a	Sampling year	
	2020	2021
Control	31.1 \pm 0.3a	27.4 \pm 0.2a
Chit	27.2 \pm 0.2b	23.3 \pm 0.3c
GABA	18.1 \pm 0.3d	21.2 \pm 0.4d
Ser	18.7 \pm 3.0d	18.7 \pm 0.2e
SA	8.8 \pm 0.3g	15.7 \pm 0.3g
Chit+ GABA	23.2 \pm 0.3c	26.9 \pm 0.3b
Chit+ Ser	12.1 \pm 0.3f	13.8 \pm 0.1h
GABA+ Ser	12.0 \pm 0.2f	18.4 \pm 0.3e
SA + Chit	5.7 \pm 0.1h	8.4 \pm 0.1j
SA+ GABA	5.2 \pm 0.2h	9.2 \pm 0.1i
SA+ Ser	15.4 \pm 0.3e	17.9 \pm 0.2f

^a SA: Salicylic Acid, Ser= Serenade, GABA= γ -Aminobutyric Acid, Chit= Chitosan. (a-h) Means followed by a different letter within a column are significantly different (Tukey's HSD test; $P< 0.05$).

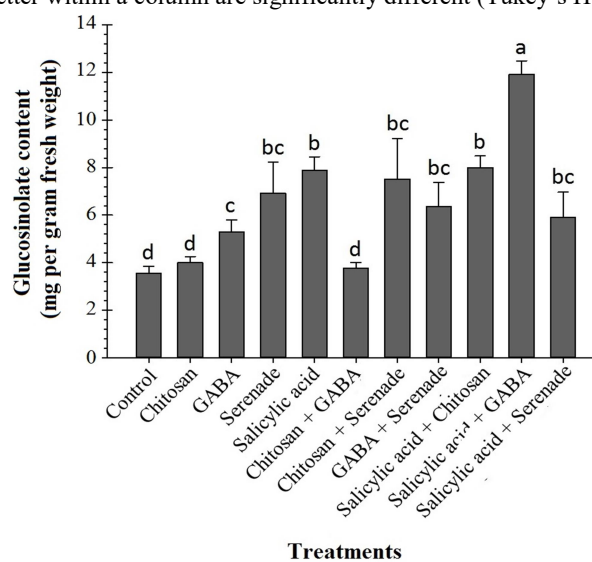


Figure 1. The mean (\pm SE) amount of total glucosinolate in canola leaves treated with different elicitors.



22; $P < 0.001$), Glutathione-S-Transferase (GST) ($F = 66.44$; $df = 10, 22$; $P < 0.001$), and peroxidase ($F = 31.87$; $df = 10, 22$; $P < 0.001$) enzymes in *B. brassicae* feeding on canola plants under different treatments (Table 2).

The highest and lowest CAT activity was observed on the control and SA+GABA, respectively (Table 2). The activity of esterase and peroxidase enzymes was highest on the control and the lowest on SA+GABA and SA+chitosan treatments. GST activity was greatest on control and chitosan, and the least on SA+GABA and

SA+ chitosan treatments (Table 2).

Energy Sources

Analysis of energy sources of *B. brassicae* feeding on plants under the studied treatments showed significant differences in contents of sugar ($F = 31.33$; $df = 10, 22$; $P < 0.001$), lipid ($F = 14.92$; $df = 10, 22$; $P < 0.001$), glycogen ($F = 17.07$; $df = 10, 22$; $P < 0.0001$) and protein ($F = 106.34$; $df = 10, 22$; $P < 0.001$) (Table 3).

Table 2. Mean (\pm SE) activity of detoxification enzymes in *Brevicoryne brassicae* L. fed on canola plants treated with different elicitors.

Treatments ^a	Catalase (U mg ⁻¹ protein)	Peroxidase (U mg ⁻¹ protein)	Glutathione S-transferase (U mg ⁻¹ protein)	Total esterase (U mg ⁻¹ protein)
Control	1.62 \pm 0.20a	3.13 \pm 0.16a	2.75 \pm 0.11a	0.95 \pm 0.008a
Chit	1.12 \pm 0.10b	2.80 \pm 0.16ab	2.80 \pm 0.16a	0.075 \pm 0.004b
GABA	0.76 \pm 0.08c	2.50 \pm 0.14bc	2.05 \pm 0.08b	0.051 \pm 0.002d
Ser	0.77 \pm 0.05c	2.47 \pm 0.09c	2.12 \pm 0.15b	0.042 \pm 0.002e
SA	0.31 \pm 0.04e	1.52 \pm 0.08e	0.94 \pm 0.10e	0.028 \pm 0.001g
Chit+ GABA	0.97 \pm 0.11b	2.71 \pm 0.17bc	1.82 \pm 0.11c	0.060 \pm 0.003c
Chit+ Ser	0.39 \pm 0.05de	1.47 \pm 0.29de	0.90 \pm 0.07e	0.026 \pm 0.003g
GABA+ Ser	0.67 \pm 0.09c	1.79 \pm 0.08d	1.27 \pm 0.10d	0.042 \pm 0.001e
SA + Chit	0.22 \pm 0.01f	1.31 \pm 0.05f	0.69 \pm 0.00f	0.023 \pm 0.001h
SA+ GABA	0.17 \pm 0.03g	1.22 \pm 0.14f	0.69 \pm 0.03f	0.022 \pm 0.002h
SA+ Ser	0.47 \pm 0.05d	1.67 \pm 0.10de	1.18 \pm 0.05d	0.036 \pm 0.001f

^a SA: Salicylic Acid, Ser= Serenade, GABA= γ -Aminobutyric Acid, Chit= Chitosan. (a-h) Means followed by a different letter within a column are significantly different (Tukey's HSD test; $P < 0.05$).

Table 3. Mean (\pm SE) content of energy resources in *Brevicoryne brassicae* L. fed on canola plants treated with different elicitors.

Treatments ^a	Sugar (mg g ⁻¹ body weight)	Lipid (mg g ⁻¹ body weight)	Glycogen (mg g ⁻¹ body weight)	Protein (mg g ⁻¹ body weight)
Control	0.95 \pm 0.03a	81.2 \pm 4.5a	2.68 \pm 0.18a	11.20 \pm 0.17h
Chit	0.55 \pm 0.03b	79.1 \pm 4.6a	2.69 \pm 0.27a	12.31 \pm 0.15fg
GABA	0.47 \pm 0.06bc	80.3 \pm 5.0a	2.21 \pm 0.15b	12.02 \pm 0.25g
Ser	0.42 \pm 0.02cd	74.0 \pm 2.4ab	1.82 \pm 0.13c	12.51 \pm 0.10f
SA	0.31 \pm 0.03ef	56.3 \pm 7.6de	1.01 \pm 0.07f	16.13 \pm 0.03c
Chit+GABA	0.47 \pm 0.05bc	68.9 \pm 2.9bc	1.62 \pm 0.14cd	13.33 \pm 0.41e
Chit+Ser	0.29 \pm 0.04ef	54.3 \pm 3.5e	1.57 \pm 0.05d	17.01 \pm 0.26b
GABA+Ser	0.35 \pm 0.04de	65.5 \pm 1.5c	1.83 \pm 0.09c	14.77 \pm 0.13d
SA+Chit	0.30 \pm 0.02ef	36.8 \pm 4.7f	1.11 \pm 0.11ef	18.04 \pm 0.15a
SA+GABA	0.27 \pm 0.01f	37.5 \pm 0.5f	1.26 \pm 0.14e	18.01 \pm 0.20a
SA+Ser	0.30 \pm 0.03ef	62.3 \pm 3.0cd	1.67 \pm 0.24cd	16.12 \pm 0.46c

^a SA: Salicylic Acid, Ser= Serenade, GABA= γ -Aminobutyric Acid, Chit= Chitosan. (a-h) Means followed by a different letter within a column are significantly different (Tukey's HSD test; $P < 0.05$).

The greatest and least contents of sugar were observed in the control and SA+GABA treatments, respectively (Table 3). The lipid contents were highest on the control and chitosan+ GABA, and the lowest on SA+GABA and SA+chitosan treatments (Table 3). The glycogen contents were greatest on the control and chitosan, and the least on SA and SA+chitosan treatments (Table 3). The protein contents were lowest on the control and the highest on SA+GABA and SA+chitosan treatments (Table 3).

DISCUSSION

The canola plants treated with the studied inducers negatively affected the population density of *B. brassicae* compared with the control under field conditions. Among individual treatments, SA was more effective than others. This result was consistent with findings of Elhamahmy *et al.* (2016), who demonstrated the foliar application of SA on canola plants results in a diminished population of the cabbage aphid. In addition, Thakur *et al.* (2016) reported that the foliar application of SA was effective on the mustard aphid, *Lipaphis erysimi* Kalt, via positive modulation in the activities of defense proteins. Shi *et al.* (2016) observed that exogenous applications of SA in tomato plants repelled *Bemisia tabaci* (Gennadius) via the release of volatile terpenes such as d-limonene and methyl salicylate. SA elicitor regulates the actions of some enzymes such as peroxidase and polyphenol oxidase, which are involved in induced plant defense against abiotic and biotic stresses (Zhao *et al.*, 2009; War *et al.*, 2011). Both SA and JA defense pathways are vital in activating plant defenses against aphids (Boughton *et al.*, 2005; Selig *et al.*, 2016; Basit *et al.*, 2020; Hanan *et al.*, 2020; Nazir *et al.*, 2020). The synergistic or antagonistic effects of the defense pathways of SA and JA are associated with concentrations, the timing, and level of the interaction between insect species and host plant (Cao *et al.*, 2014; Schweiger *et al.*,

2014). Activation of the SA pathway is a common mechanism of antibiosis or aphid repellence in resistant host plants, with narrow efficiency in susceptible ones (Morkunas and Gabryś, 2011; Rodriguez *et al.*, 2014). Khoshfarman-Borji *et al.* (2020) concluded that SA in combination with *Pseudomonas putida* (Trevisan, 1889) leads to antibiosis resistance in canola plants to *B. brassicae* under laboratory conditions. The antibiosis resistance can significantly affect biological characteristics of insect pests (Mohammadi *et al.*, 2015). Our study also achieved a new discovery regarding the applications of integrated SA and Gaba/Chitosan leading to the lower population density of this aphid under field conditions. The results suggest the minor suitability of canola plants treated with these compounds compared with the others for this aphid. GABA is a substantial component in plant defense against herbivorous insects (Huang *et al.*, 2011; Mithöfer and Boland, 2012). BABA-induced defense for biotic stress commonly occurs by defense mechanisms regulated through jasmonic acid (Jisha *et al.*, 2018). GABA is also proposed as an inhibitory neuromuscular transmitter performing at GABA-gated chloride channels in insects and could influence ordinary development when ingested through feeding (Shelp *et al.*, 2009). On the other hand, field studies have shown that foliar applications of chitosan caused a reduction of 40% to 70% of *D. citri* individuals (Ramírez-Godoy *et al.*, 2018) and 30% of *Cacopsylla pyricola* (Förster) nymphs (Cooper and Horton, 2017). Chitosan can be involved in the signaling pathway for the biosynthesis of phenolics and inducing immune systems in plants (Katiyar *et al.*, 2015). Reports suggest that Reactive Oxygen Species (ROS), SA, Jasmonic Acid (JA), Abscisic Acid (ABA), ethylene, Nitric Oxide (NO), and Ca²⁺ play an important role in chitosan-mediated signal pathway (Xing *et al.*, 2015). Chitosan has been shown to bind to cell membranes triggering the H₂O₂ production via the octadecanoid pathway, supporting the



biosynthesis of jasmonates, which prompt the expression of defense response genes (Pichyangkura and Chadchawan, 2015). Further, Bistgani *et al.* (2017) reported that chitosan applications caused accumulation of proline in *Thymus daenensis* Celak plants. Foliar chitosan sprays favored an increase in proline synthesis in both flush shoots and leaves of Tahiti lime (Ramírez-Godoy *et al.*, 2018), which could be because of its osmoprotectant function, and led to signal hypersensitive responses against biotic stresses (Qamar *et al.*, 2015). Chitosan can alter the oxidative balance in the cytoplasm as a signaling mechanism to stimulate plant defense reduce and decrease the damages related to biotic stress (Aranega-Bou *et al.*, 2014).

The lower population density of aphids on plants treated with different inducers in this study was related to lower energy reserves (sugar, glycogen, and lipid content) in the cabbage aphids. Variations in biochemical indicators could be used as a suitable indicator of toxicant stress under laboratory conditions. Depletion in the contents of energy reserves was verified in invertebrates exposed to stressful conditions (Jemec *et al.*, 2007). This may be attributed to the increased utilization for energy demand associated with toxic stress (Sancho *et al.*, 2009). Additionally, the studied inducers influenced the levels of glucosinolates in canola. In the current study, the glucosinolates in canola leaves increased significantly under SA+Gaba treatment. In addition, silicon causes an increase in the content of glucosinolates in Brassica species (Smetanska *et al.*, 2007; Sun *et al.*, 2012; Khoshfarman-Borji *et al.* 2020). Furthermore, tritrophic interactions between host plant, pest, and its natural enemy can be affected by the chemicals, leading to the pest population suppression by natural enemies in the resistant plant (Rashedi *et al.* 2019).

In the present study, different inducers especially SA+ Gaba and SA+chitosan appeared to significantly reduce the activity of detoxifying enzymes in *B. brassicae*. Zhang *et al.* (2013) showed that one of the

most effective strategies to inhibit CAT activity in aphids can be the increased production of secondary compounds in host plants. Further, Leszczynski *et al.* (1994) reported GST activity in *S. avenae* and *R. padi* was elevated when fed on a low aphid-resistant wheat cultivar. Thus, some plant allelochemicals inhibit the activity of detoxification enzymes (Jing *et al.*, 2005), increasing the toxicity of co-occurring plant compounds (Herde and Howe, 2014), which lead to reduced population.

In conclusion, canola plants treated with SA treatment, especially in combination with Gaba/Chitosan, were more resistant to *B. brassicae*. These compounds can be considered in the future as a supplementary method in the integrated control programs for the cabbage aphid.

ACKNOWLEDGEMENTS

We appreciate Shahid Bahonar University (Iran) for the financial support of this study.

REFERENCES

1. Aebi, H. 1984. Catalase in Vitro. *Method Enzymol.*, **105**: 121–126.
2. Ansari, M., Moraiet, M. and Ahmad. S. 2014. Insecticides: Impact on the Environment and Human Health. In: “*Environmental Deterioration and Human Health*”, (Eds.): Malik, A., Grohmann, E., Akhtar, R. Springer, Dordrecht, PP. 99–123.
3. Aranega-Bou, P., de la O Leyva, M., Finiti, I., García-Agustín, P. and Gonzalez-Bosch, C. 2014. Priming of Plant Resistance by Natural Compounds. Hexanoic Acid as a Model. *Front. Plant Sci.*, **7**: 1-12.
4. Bao, H., Chen, X., Lv, S., Jiang, P., Feng, J. J., Fan, P. X., Nie, L. L. and Li, Y. X. 2015. Virus-Induced Gene Silencing Reveals Control of Reactive Oxygen Species Accumulation and Salt Tolerance in Tomato by γ -Aminobutyric Acid Metabolic Pathway. *Plant Cell Environ.*, **38**: 600–613.
5. Basit, A., Nazir, T., Hanan, A., Majeed, M. Z., Farhan, M., Dewen, Q. I. U. and Wang,

- Y. 2020. MAMP-Triggered Resistance Induced by Elicitor Protein PeBA1 Derived from *Bacillus amyloliquefaciens* NC6 in Common Bean (*Phaseolus vulgaris* L.) against Green Peach Aphid (*Myzus persicae* Sulzer). *Not. Bot. Hort. Agrobo.*, **48(2)**: 705-715.
6. Bayer Crop Science. 2019. *Serenade ASO*. EC Regulation No.834/2007 & 889/2008 by IMO Switzerland. www.bayercropscience.ie/sds/serenadeaso.pdf.
 7. Berenbaum, M. R. and Neal, J. J. 1985. Synergism between Myristicin and Xanthotoxin, a Naturally Occurring Plant Toxicant. *J. Chem. Ecol.*, **11**: 1349-1358.
 8. Bergmeyer, H. U. 1974. *Methods of Enzymatic Analysis*. Vol. II, Academ Press, New York, PP. 495-496.
 9. Bistgani, Z. E., Siadat, S. A., Bakhshandeh, A., Pirbalouti, A. G. and Hashemi, M. 2017. Interactive Effects of Drought Stress and Chitosan Application on Physiological Characteristics and Essential Oil Yield of *Thymus daenensis* Celak. *Crop J.*, **5**: 407-415.
 10. Boughton, A. J., Hoover, K. and Felton, G. W. 2005. Methyl Jasmonate Application Induces Increased Densities of Glandular Trichomes on Tomato, *Lycopersicon esculentum*. *J. Chem. Ecol.*, **31**: 2211-2216.
 11. Cao, H. H., Wang, S. H. and Liu, T. X. 2014. Jasmonate- and Salicylate-Induced Defenses in Wheat Affect Host Preference and Probing Behavior but Not Performance of the Grain Aphid, *Sitobion avenae*. *Insect Sci.*, **21**: 47-55.
 12. Cooper, W. R. and Horton, D. R. 2017. Elicitors of Host Plant Defenses Partially Suppress *Cacopsylla pyricola* (Hemiptera: Psyllidae) Populations under Field Conditions. *J. Insect Sci.*, **17(2)**: 1-5.
 13. Despres, L., David, J. P. and Gallet, C. 2007. The Evolutionary Ecology of Insect Resistance to Plant Chemicals. *Trends Ecol. Evol.*, **22**: 298-307.
 14. Elhamahmy, M. A. M., Mahmoud, M. F. and Bayoumi, T. Y. 2016. The Effect of Applying Exogenous Salicylic Acid on Aphid Infection and Its Influence on Histophysiological Traits and Thermal Imaging of Canola. *Cercet. Agron. Mold.*, **XLIX(2)**: 67-85.
 15. Foray, V., Plisson, P. F., Ber-Vener, M. C., Desouhant, E., Venner, S., Menu, F., Giron, D. and Rey, B. 2012. A Handbook for Uncovering the Complete Energetic Budget in Insects: The van Handel's Method (1985) Revisited. *Physiol. Entomol.*, **37**: 295-302.
 16. Francis, F., Haubruge, E., Gaspar, C. and Dierickx, P. J. 2001. Glutathione S-Transferases of *Aulacorthum solani* and *Acyrtosiphon pisum*: Partial Purification and Characterization. *Comp. Biochem. Physiol. Part B.*, **129**: 165-171.
 17. Greenfield, E. A. 2018. *Protein Quantitation*. Cold Spring Harbor Protocols. doi: 10.1101/pdb.prot098202.
 18. Habig, W. H., Pabst, M. J. and Jakoby, W. B. 1974. Glutathione S-Transferases, the First Enzymatic Step in Mercapturic Acid Formation. *J. Biol. Chem.*, **249**: 7130-7139.
 19. Herde, M. and Howe, G. A. 2014. Host Plant-Specific Remodeling of Midgut Physiology in the Generalist Insect Herbivore *Trichoplusia ni*. *Insect Biochem. Mol. Biol.*, **50**: 58-67.
 20. Halkier, B. A. and Gershenzon, J. 2006. Biology and Biochemistry of Glucosinolates. *Annu. Rev. Plant Biol.*, **57**: 303-333.
 21. Hanan, A., Basit, A., Nazir, T., Majeed, M. Z. and Qiu, D. 2020. Anti-Insect Activity of a Partially Purified Protein Derived from the Entomopathogenic Fungus *Lecanicillium lecanii* (Zimmermann) and Its Putative Role in a Tomato Defense Mechanism against Green Peach Aphid. *J. Invertebr. Pathol.*, **170**: 107282.
 22. Horvath, E., Szalai, G. and Janda, T. 2007. Induction of Abiotic Stress Tolerance by Salicylic Acid Signaling. *J. Plant Growth Regul.*, **26**: 290-300.
 23. Huang, T., Jander, G. and de Vos, M. 2011. Non-Protein Amino Acids in Plant Defense against Insect Herbivores: Representative Cases and Opportunities for Further Functional Analysis. *Phytochemistry*, **72**: 1531-1537.
 24. Ishida, M., Kakizaki, T., Ohara, T. and Morimitsu, Y. 2011. Development of a Simple and Rapid Extraction Method of Glucosinolates from Radish Roots. *Breed. Sci.*, **61**: 208-211.
 25. Jemec, A., Drobne, D., Tisler, T., Trebse, P., Ros, M., Sepcic, K., Fournier, D. and Trebse, P. 2007. Comparative Toxicity of Imidacloprid, of Its Commercial Liquid Formulation and of Diazinon to a Non-



- Target Arthropod, the Microcrustacean *Daphnia magna*. *Chemosphere*, **68**: 1408–1418.
26. Jing, L., Fang, Y., Ying, X., Wenxing, H., Meng, X., Syed, M., Syed, N. and Fang, C. 2005. Toxic Impact of Ingested Jatropherol-I on Selected Enzymatic Activities and the Ultrastructure of Midgut Cells in Silkworm, *Bombyx mori* L. *J. Appl. Entomol.*, **129**: 98–104.
27. Jisha, K. C., Shackira, A. M. and Puthur, J. T. 2018. GABA/BABA Priming Causes Signaling of Defense Pathways Related to Abiotic Stress Tolerance in Plants. In: “*Neurotransmitters in Plants Perspectives and Applications*”, (Eds.): Ramakrishna, A. and Roshchina, V. V. CRC Press, Taylor and Francis Group, Boca Raton, PP. 219–230.
28. Kamel, S. M., Mahfouz, H. M., Blal, H. A., Said, M., Mahmoud, M. F. 2016. Effects of Salicylic Acid Elicitor and Potassium Fertilizer as Foliar Spray on Canola Production in the Reclaimed Land in Ismailia Governorate Egypt. *Cercet. Agron. Mold.*, **XLIX(1)**: 81–89.
29. Katiyar, D., Hemantaranjan, A. and Singh, B. 2015. Chitosan as a Promising Natural Compound to Enhance Potential Physiological Responses in Plant: A Review. *Indian J. Plant Physiol.*, **20**: 1–9.
30. Khoshfarman-Borji, H., Pahlavan Yali, M. and Bozorg-Amirkalae, M. 2020. Induction of Resistance against *Brevicoryne brassicae* by *Pseudomonas putida* and Salicylic Acid in Canola. *Bull. Entomol. Res.*, **110**: 597–610.
31. Leszczynski, B., Matok, M. and Dixon, A. F. G. 1994. Detoxification of Cereal Plant Allelochemicals by Aphids: Activity and Molecular Weights of Glutathione-S-Transferase in Three Species of Cereal Aphids. *J. Chem. Ecol.*, **20**: 387–394.
32. Malekzadeh, P., Khara, J. and Heydari, R. 2014. Alleviating Effects of Exogenous Gamma Aminobutyric Acid on Tomato Seedling under Chilling Stress. *Physiol. Mol. Biol. Plants*, **20**: 133–137.
33. Mithöfer, A. and Boland, W. 2012. Plant Defense against Herbivores: Chemical Aspects. *Annu. Rev. Plant Biol.*, **63**: 431–450.
34. Moller, P., Ploger, A. and Sørensen, H. 1985. Quantitative Analysis of Total Glucosinolate Content in Concentrated Extracts from Double Low Rapeseed by the Pd-Glucosinolate Complex Method. In: “*Advances in the Production and Utilization of Cruciferous Crop*”, (Ed.): Sørensen, H. Martinus Nijhoff/DR W. Junk Publishers, Dordrecht, PP. 97–110.
35. Mohammadi, S., Seraj, A. and Rajabpour, A. 2015. Effects of Six Greenhouse Cucumber Cultivars on Reproductive Performance and Life Expectancy of *Tetranychus turkestanii* (Acari: Tetranychidae). *Acarologia*, **55**: 231–242.
36. Morkunas, I., Gabryś, B. 2011. Phytohormonal Signaling in Plant Responses to Aphid Feeding. *Acta Physiol. Plant.*, **33**: 2057–2073.
37. Nazir, T., Hanan, A., Basit, A., Majeed, M. Z., Anwar, T., Nawaz, I. and Qiu, D. 2020. Putative Role of a Yet Uncharacterized Protein Elicitor PeBb1 Derived from *Beauveria bassiana* ARSEF 2860 Strain against *Myzus persicae* (Homoptera: Aphididae) in *Brassica rapa* ssp. Pekinensis. *Pathogens*, **9**: 1–13.
38. Pichyangkura R, Chadchawan S 2015. Biostimulant Activity of Chitosan in Horticulture. *Sci. Hortic.*, **196**: 49–65.
39. Ramirez-Godoy, A, Vera-Hoyos, M. D. P., Jimenez-Beltran, N. and Restrepo-Diaz, H. 2018. Application of Foliar Synthetic Elicitors for the Management of *Diaphorina citri* (Hemiptera: Liviidae) Populations in Tahiti Lime (*Citrus latifolia* Tanaka). *Hortscience*, **53**: 1012–1020.
40. Rashedi, A., Rajabpour, A., Rasekh, A. and Zandi-Sohani, N. 2019. Interactions between Host Plant, *Aphis fabae*, and Its Natural Enemies, *Orius albidipennis* and *Lysiphlebus fabarum* in a Tritrophic System. *J. Asia-Pacific Entomol.*, **22**: 847–852.
41. Qamar, A., Mysore, K. S. and Senthil-Kumar, M. 2015. Role of Proline and Pyrroline-5- Carboxylate Metabolism in Plant Defense against Invading Pathogens. *Front. Plant Sci.*, **6**: 1–9.
42. Rivas-San Vicente, M. and Plasencia, J. 2011. Salicylic Acid beyond Defense: It’s Role in Plant Growth and Development. *J. Exp. Bot.*, **62**: 3321–38.
43. Rodriguez, P. A., Stam R., Warbroek T. and Bos J. I. B. 2014. Mp10 and Mp42 from the Aphid Species *Myzus persicae* Trigger Plant Defenses in *Nicotiana*

- benthamiana* through Different Activities. *Mol. Plant-Microbe Interact.*, **27**: 30–39.
44. Ronald, S. F. and Laima, S. K. 1999. *Phenolics and Cold Tolerance of Brassica napus*. Department of Plant Agriculture, Crop Sciences Division, University of Guelph, Guelph, Ontario, Canada.
 45. Sayed, A. M. M. and Teilep, W. M. A. 2013. Role of Natural Enemies, Climatic Factors and Performance Genotypes on Regulating Pests and Establishment of Canola in Egypt. *J. Basic Appl. Zool.*, **66**: 18-26.
 46. Sancho, E., Villarroel, M. J., Andreu, E. and Ferrando, M. D. 2009. Disturbances in Energy Metabolism of *Daphnia magna* after Exposure to Tebuconazole. *Chemosphere*, **74**: 1171–1178.
 47. Sattari Nasab, R., Pahlavan Yali, M. and Bozorg-Amirkalae, M. 2018. Effects of Humic Acid and Plant Growth-Promoting Rhizobacteria (PGPR) on Induced Resistance of Canola to *Brevicoryne brassicae* L. *Bull. Entomol. Res.*, **23**: 1–11.
 48. Schweiger, R., Heise, A. M., Persicke, M. and Müller, C. 2014. Interactions between the Jasmonic and Salicylic Acid Pathway Modulate the Plant Metabolome and Affect Herbivores of Different Feeding Types. *Plant Cell Environ.*, **37**: 1574–1585.
 49. Selig, P., Keough, S., Nalam, V. J. and Nachappa, P. 2016. Jasmonate-Dependent Plant Defenses Mediate Soybean Thrips and Soybean Aphid Performance on Soybean. *Arthropod Plant Interact.*, **10**: 273–282.
 50. Shelp, B. J., Allan W. L. and Faure, D. 2009. Role of γ -Aminobutyrate and γ -Hydroxybutyrate in Plant Communication. In: “*Plant-Environment Interactions*”, (Ed.): Baluška, F. Springer-Verlag, Heidelberg, Berlin, PP. 73–84.
 51. Shi, X., Chen, G., Tian, L., Peng, Z., Xie, W., Wu, Q. and Zhang, Y. 2016. The Salicylic Acid-Mediated Release of Plant Volatiles Affects the Host Choice of *Bemisia tabaci*. *Int. J. Mol. Sci.*, **17**: 1-11.
 52. Shonga, E. and Getu, E. 2021. Efficacy of Plant-Derived and Synthetic Insecticides against Cabbage Aphid, *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae) and Their Effect on Coccinellid Predators. *Ethiop. J. Sci.*, **44**: 27–37.
 53. Smetanska, I., Krumbein, A., Schreiner, M. and Knorr, D. 2007. Influence of Salicylic Acid and Methyl Jasmonate on Glucosinolate Levels in Turnip. *J. Hort. Sci. Biotechnol.*, **82**: 690–694.
 54. SPSS. 2015. *SPSS 22.0 for Windows*. SPSS Inc., Chicago, IL.
 55. Sun, B., Yan, H., Zhang, F. and Wang, Q. 2012. Effects of Plant Hormones on Main Health-Promoting Compounds and Antioxidant Capacity of Chinese Kale. *Food Res. Int.*, **48**: 359–366.
 56. Thakur, T., Sangha, M. K., Arora, R. and Javed, M. 2016. Effect of Foliar Spray of Elicitors on Status of Defense Proteins in Relation to Mustard Aphid Infestation in Crop *Brassica* Cultivars. *J. Appl. Nat. Sci.*, **8(4)**: 2242-2248.
 57. van Asperen, K. 1983. A Study of Housefly Esterase by Means of a Sensitive Colorimetric Method. *J. Insect Physiol.*, **8**: 401–416.
 58. Vijayakumari, K., Jisha, K. C. and Puthur, J. T. 2016. GABA/BABA Priming: A Means for Enhancing Abiotic Stress Tolerance Potential of Plants with Less Energy Investments on Defense Cache. *Acta Physiol. Plant*, **38**: 230–244.
 59. War, A. R., Paulraj, M. G., War, M. Y. and Ignacimuthu, S. 2011. Role of Salicylic Acid in Induction of Plant Defense System in Chickpea (*Cicer arietinum* L.). *Plant Signal Behav.*, **6**: 1787–1792.
 60. War, A. R., Paulraj, M. G., Ahmad, T., Buhroo, A. A., Hussain, B., Ignacimuthu, S. and Sharma, H. C. 2012. Mechanisms of Plant Defense against Insect Herbivores. *Plant Signal Behav.*, **7**: 1306–1320.
 61. Zhang, M., Fang, T., Pu, G., Sun, X., Zhou, X. and Cai, Q. 2013. Xenobiotic Metabolism of Plant Secondary Compounds in the English Grain Aphid, *Sitobion avenae* (F.) (Hemiptera: Aphididae). *Pestic. Biochem. Physiol.*, **107**: 44-49.
 62. Xing, K., Zhu, X., Peng, X. and Qin, Sh. 2015. Chitosan Antimicrobial and Eliciting Properties for Pest Control in Agriculture: A Review. *Agron. Sustain. Dev.*, **35**: 569–588.
 63. Zhang, L. B. and Feng M. G. 2018. Antioxidant Enzymes and Their Contributions to Biological Control Potential of Fungal Insect Pathogens. *Appl. Microbiol. Biotechnol.*, **102**: 4995-5004.
 64. Zhao, L. Y., Chen, J. L., Cheng, D. F., Sun, J. R., Liu Y. and Tian Z. 2009. Biochemical



- and Molecular Characterizations of *Sitobion avenae*-Induced Wheat Defense Responses. *Crop Prot.*, **28**: 435–442.
65. Zhen, A., Zhang, Z., Jin, X. Q., Liu, T., Ren, W. Q. and Hu, X. H. 2018. Exogenous GABA Application Improves the NO₃ – N Absorption and Assimilation in Ca(NO₃)₂-Treated Muskmelon Seedlings. *Sci. Hortic.*, **227**: 117–23.

تأثیر کاربرد خارجی القاگرها روی دفاع فیزیولوژیکی و شیمیایی گیاه کلزا نسبت به شته مومی کلم

چکیده

م. شاهرخی، م. پهلوان یلی، و م. بزرگ امیرکلانی

مقاومت القایی در گیاهان می تواند با کاربرد القاگرها تغییر یابد. در این مطالعه، اثر اسید سالیسیلیک، کیتوزان، گاما-آمینو بوتیریک اسید (گابا)، سرناد آسو (*Bacillus subtilis* QST713) و کاربرد تلفیقی این القاگرها بر مقاومت کلزا نسبت به شته مومی کلم، *Brevicoryne brassicae* L. (Hemiptera: Aphididae) بررسی شد. در آزمایش های مزرعه ای تعداد شته جلب شده به گیاهان کلزا تیمار شده با القاگرهای مختلف در مقایسه با شاهد به طور معنی داری کمتر بود. کمترین تراکم جمعیت شته در گیاهان تیمار شده با اسید سالیسیلیک + گابا و اسید سالیسیلیک + کیتوزان مشاهده شد. همچنین بیشترین مقدار گلیکوزینولات در برگ های کلزا تیمار شده با اسید سالیسیلیک + گابا ثبت شد. محتویات فنل، لیپید و گلیکوژن بدن شته با تغذیه روی گیاهان تیمار شده با اسید سالیسیلیک + گابا و اسید سالیسیلیک + کیتوزان کمترین مقدار بود. همچنین، کمترین میزان فعالیت آنزیم های کاتالاز، استراز، پراکسیداز و گلوکاتینون S-ترانسفراز این شته در تیمارهای اسید سالیسیلیک + گابا و اسید سالیسیلیک + کیتوزان به دست آمد. نتایج این تحقیق نشان داد که تیمار خارجی گیاه کلزا با اسید سالیسیلیک همراه با گابا یا کیتوزان به طور موفقیت آمیزی باعث کاهش جمعیت شته مومی کلم شده و می تواند در برنامه های مدیریت تلفیقی علیه این شته و سایر شته ها مورد توجه قرار گیرد.