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Short Communication

Evaluation of resistance inducers in the pistachio trees on population growth and detoxifying enzymes of pistachio psylla, *Agonoscena Pistaciae* (Hemiptera: Psyllidae)

H. Ghasemi Negad Rayeni [1], M. Pahlavan Yali *[2], M. Bozorg-Amirkalaee [3]

1. M.Sc. student, Department of Plant Protection, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran
2. ***Corresponding Author:** Associate Professor, Department of Plant Protection, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran (mrmpahlavan@gmail.com)
3. Ph.D. Graduate of Agricultural Entomology, Department of Plant Protection, Faculty of Agriculture, University of Mohaghegh Ardabili, Ardabil, Iran

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Abstract

Pistachio psylla *Agonoscena pistaciae* Burckhardt & Lauterer is Iran's most important pest of pistachio trees. Due to the negative effects of excessive use of chemical insecticides, alternative strategies are necessary to control this pest. In this research, some resistance-inducing compounds, including chitosan, gamma-aminobutyric acid (GABA), and potassium silicate in pistachio trees, were studied on the percentage of population growth and detoxifying enzymes of this psyllid. Based on the results obtained, 7 days after spraying, the lowest and highest population growth of pistachio psyllid nymphs were observed in chitosan treatment and control, respectively. Also, the average growth percentage of the nymph population after 14 days did not show a significant difference among GABA, chitosan and potassium silicate, but it significantly decreased on these treatments compared to the control. In this study, the highest and lowest activity levels of esterase enzyme in psyllid nymphs were achieved in the control and chitosan treatments, respectively.

On the other hand, the highest level of glutathione S-transferase activity was obtained in chitosan and potassium silicate treatments, and the lowest one was found in the control. The study results revealed that the different inducers could reduce the *A. pistaciae* population growth by generating physiological changes in the host plant and, subsequently the pest insect. Therefore, these compounds could be considered in the integrated management of this pest.

Keywords: Antioxidant, Sap-sucking insects, Physiological changes, Chitosan, Pest management

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گزارش کوتاه انگلیسی

ارزیابی القاگرهای مقاومت در درختان پسته روی رشد جمعیت و آنزیم‌های سم‌زدای پسیل پسته، *Agonoscena pistaciae* (Hemiptera: Psyllidae)

هدی قاسمی نژاد راینی [۱]، مریم پهلوان یلی *[۲]، مریم بزرگ امیرکلائی [۳]

۱- دانشجوی کارشناسی ارشد، گروه گیاه‌پزشکی، دانشکده کشاورزی، دانشگاه شهید باهنر کرمان، کرمان، ایران

۲- * **نویسنده مسوول**: دانشیار، گروه گیاه‌پزشکی، دانشکده کشاورزی، دانشگاه شهید باهنر کرمان، کرمان، ایران (mrmpahlavan@gmail.com)

۳- دانش آموخته‌ی دکتری حشره شناسی کشاورزی، گروه گیاه‌پزشکی، دانشکده کشاورزی، دانشگاه محقق اردبیلی، اردبیل، ایران

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چکیده

پسیل پسته (*Agonoscena pistaciae* Burckhardt & Lauterer) مهم‌ترین آفت درختان پسته در ایران می‌باشد. به دلیل اثرات سوء مصرف بی‌رویه حشره‌کش‌های شیمیایی، روش‌های جایگزین برای کنترل این آفت ضروری می‌باشد. در این تحقیق، کاربرد چند ترکیب القاکننده مقاومت شامل کیتوزان، گاما آمینوبوتیریک اسید و سیلیکات پتاسیم در درختان پسته روی درصد رشد جمعیت و آنزیم‌های سم زدای این پسیل مطالعه شد. بر اساس نتایج حاصل هفت روز پس از پاشش، کمترین و بیشترین رشد جمعیت پوره‌های پسیل پسته به‌ترتیب در تیمار کیتوزان و شاهد مشاهده شد. همچنین میانگین درصد رشد جمعیت پوره‌ها پس از ١٤ روز در میان تیمارهای گابا، کیتوزان و سیلیکات پتاسیم اختلاف معنی‌داری نشان نداد؛ ولی تحت تاثیر این تیمارها در مقایسه با شاهد به‌طور معنی‌داری کاهش یافت. در این مطالعه، بیشترین و کمترین میزان فعالیت آنزیم استراز در پوره‌های پسیل پسته به‌ترتیب در تیمار شاهد و کیتوزان مشاهده شد. از طرفی دیگر بیشترین میزان فعالیت آنزیم گلوتاتیون اس ترانسفراز در تیمار کیتوزان و سیلیکات پتاسیم و کمترین میزان آن در تیمار شاهد مشاهده شد. نتایج این مطالعه نشان داد القاگرهای مختلف با ایجاد تغییرات فیزیولوژیکی در گیاه میزبان و متعاقبا حشره آفت می‌تواند موجب کاهش رشد جمعیت پسیل پسته شود. بنابراین این ترکیبات می‌توانند در مدیریت تلفیقی این آفت مورد توجه قرار گیرند.

کلیدواژه‌ها: آنتی اکسیدان، حشرات مکنده، تغییرات فیزیولوژیکی، کیتوزان، مدیریت آفت

دبیر تخصصی: دکتر سید علی همتی

Introduction

Pistachio psyllid (*Agonoscena pistaciae* Burckhardt & Lauterer) is Iran's most important pest of pistachio trees. Due to the destructive effects of excessive use of chemical insecticides, alternative approaches, such as induced resistance in host plants, are necessary for controlling the damage to this pest (Mahmoudi Maimandi & Ghanbari Adevi 2012). Resistance inducers or elicitors reduce the effect of subsequent pest attacks by affecting insects' feeding, growth and survival (Wu & Baldwin, 2010; War et al., 2012). The elicitors can induce immune responses in plants and positively impact the control of insect pest populations (Pawlowski et al., 2016). The knowledge of elicitor impacts on interactions between insects and plants is useful for pest management. Studies on the use and efficacy of elicitors for population management of *A. pistaciae* are limited. Thus, the objective of the present study was to evaluate the efficiency of three synthetic elicitors (chitosan, GABA, and Potassium Silicate) on the pistachio psylla population under field conditions, as well as to assay the effect of these treatments on detoxifying enzymes of *A. pistaciae*. The information could be used in a comprehensive pest management program against pistachio psylla.

Materials and Methods

Field experiments were conducted on 30-year-old pistachio trees (cv Fandoghi) in a one-hectare orchard located in Sirjan, Kerman, Iran in 2021. In this research, pistachio trees were treated with different inducers including chitosan (0.5%) (Sigma-Aldrich), GABA (10mµ) (Sigma-Aldrich), and potassium silicate (2%) (Merck, Germany). Also, an aqueous solution of distilled water was applied as a control.

Population growth rate of *Agonoscena pistaciae*

To study the effect of different inducers on the percent population growth of *A. pistaciae*, trees almost similar in size and psylla population were considered. 10 trees were selected for each treatment, and there was at least one untreated tree among the tested trees. Then, five branches were designated in the directions of north, south, east, west, and center per each tree and marked by tying the ribbon. The sampling was done one day before the foliar spraying, and 7 and 14 days after one. For this purpose, five leaves from each tree (one leaf per marked branch) were selected, and then these leaves separated and placed in the travel refrigerator with specification labels and transported to the laboratory. The number of nymphs of *A. pistaciae* per leaf was counted and recorded under a stereomicroscope. Finally, the percentage of pistachio psylla population growth was calculated using a linear formula below (Shryock & Siegel, 1971):

$$S= B-A/A\times 100$$

where S is the population growth rate (%), A and B are the pre-spray and post-spray population, respectively.

Sample preparation and biochemical tests

48 h after the spraying, *A. pistaciae* nymphs were randomly prepared from plants under each treatment. To assess the esterase enzyme activity, psyllid samples were homogenized in 0.1 M phosphate buffer (200 µl) comprising 0.1 % triton® X-100 (Sigma Aldrich). The centrifuge of homogenates was completed at 10,000 rpm for 15 min at 4 ˚C. To evaluate the peroxidase (POX) enzyme activity, psyllid samples were homogenized in 20 mM phosphate buffer (200 µl) and then were centrifuged at 2,600 rpm for 5 min at 4◦ C. To measure the activity of glutathione S-Transferase (GST) and catalase (CAT), the psyllid specimens were homogenized in 200 µl cold phosphate buffer (10 mM) and then homogenates were centrifuged (15 min at 4 ˚C) at 13,000 rpm. The top layer was collected and kept at − 20 ◦C pro beginning

biochemical analyses. All enzyme assays were done in four replications.

Esterase measurement

A solution including the 10 µl of prepared enzyme sample, 10 µl of β -naphthyl (10 mM in acetone), 40 µl of phosphate buffer, and 50 µl of fast blue RR salt solution (Sigma Aldrich) was applied for assessment of the esterase activity by the spectrophotometer at 405 nm (van Asperen, 1983).

Peroxidase measurement

The reaction was done in a volume of 0.5 ml comprising 225 µl of 45 mM Guaiacol and 225 µl of 225 mM H2O2 (Merck Germany). In addition, 50 mM potassium phosphate buffer (50 µl) was considered a blank. Guaiacol reaction was measured at 470 nm using a spectrophotometer (Bergmeyer, 1974).

Catalase (CAT) measurement

The CAT activity was assessed based on the method of Aebi (1984). For this purpose, 50 µl of the sample, 225 µl of H2O2, and 225 µl of 70 mM potassium phosphate buffer solution with pH = 7 were mixed and the absorbance was read at 240 nm by spectrophotometer.

Glutathione S-transferase (GST) measurement

The GST activity was evaluated based on Habig et al. (1974) method with some changes. 200 µl of the reaction mixture containing 100 µl 1-chloro2,4-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 at 340 nm by the spectrophotometer.

Data analysis

The normality test with Kolmogorov-Smirnov was done for all data, and variables evaluated using the one-way analysis of variance (ANOVA) according to a completely randomized design using SPSS. After that, the Tukey test was utilized for multiple comparisons among treatments (SPSS ver. 26).

Results

Population growth percentage of *Agonoscena pistaciae*

The average growth percentage of pistachio psyllid population on trees treated with different compounds after 7 and 14 days is shown in Figure 1. According to the results obtained, 7 days after spraying, the lowest average population growth rate of psyllid nymphs was in chitosan treatment (-72.39±12.09%) and the highest in the control (17.34±4.40%) ($P < 0.001$, Figure 1). On the other hand, 14 days after spraying, the average population growth percentage of pistachio psyllid nymphs among treatments of GABA (-11.08±9.15), chitosan (-21.36±10.90) and potassium silicate (-23.11±9.50) did not show a significant difference; but it under these treatments decreased significantly compared to the control (47.07±1.96) ($P < 0.001$, Figure 1).

Antioxidant and detoxifying enzyme activities of *Agonoscena pistaciae*

Catalase enzyme activity in feeding insects on different treatments showed no significant difference ($P > 0.05$). However, there was a significant difference among different treatments in terms of the activity of peroxidase ($P < 0.001$; df = 15 and 3; F = 62.5), glutathione S-transferase ($P < 0.001$; df = 15 and 3; F=179.20), and esterase ($P < 0.05$; df=15 and 3; F=4.16).

Based on the results, the highest level of peroxidase activity was in insects feeding on trees treated with GABA and potassium silicate (0.004±0.0001 U/mg protein) and the lowest on chitosan (0.002±0.0001 U/mg protein). Moreover, the highest and lowest activity of esterase enzyme was observed in insects fed on control (0.046±0.004 U/mg protein) and chitosan (0.034±0.001 U/mg protein) treatments, respectively. On the other hand, the highest level of GST activity was observed in treatments of chitosan (12.95±0.29 U/mg protein) and potassium silicate (12.68±0.48 U/mg protein), and the lowest level of this enzyme was recorded in the control treatment (4.31±0.13 U/mg protein) (Table 1).

Discussion

In the current research, pistachio trees treated with the studied inducers negatively affected the population growth percentage of *A. pistaciae* nymphs compared with the control. Different inducer compounds may trigger different or similar resistance pathways in the plant and change the level of plant secondary compounds. Among tested treatments, chitosan was more effective in controlling the pest population 7 days after spraying. The high effect of chitosan in inducing resistance can be related to the high phenol contents in the plant. Also, chitosan treatment can increase some other secondary metabolites, such as glucosides, which are involved in regulating plant reactions and have a protective and defensive role (Cheng et al., 2006).

Activity levels of detoxifying enzymes in insects are believed to be important factors in determining their resistance to a wide range of toxic chemicals (Despres et al., 2007). In the present study, there was no significant difference in the catalase enzyme activity in psyllid nymphs fed on different treatments. As a result, this enzyme did not play an important role in the detoxification mechanism of toxic substances produced in pistachio trees after treatment with the tested compounds.

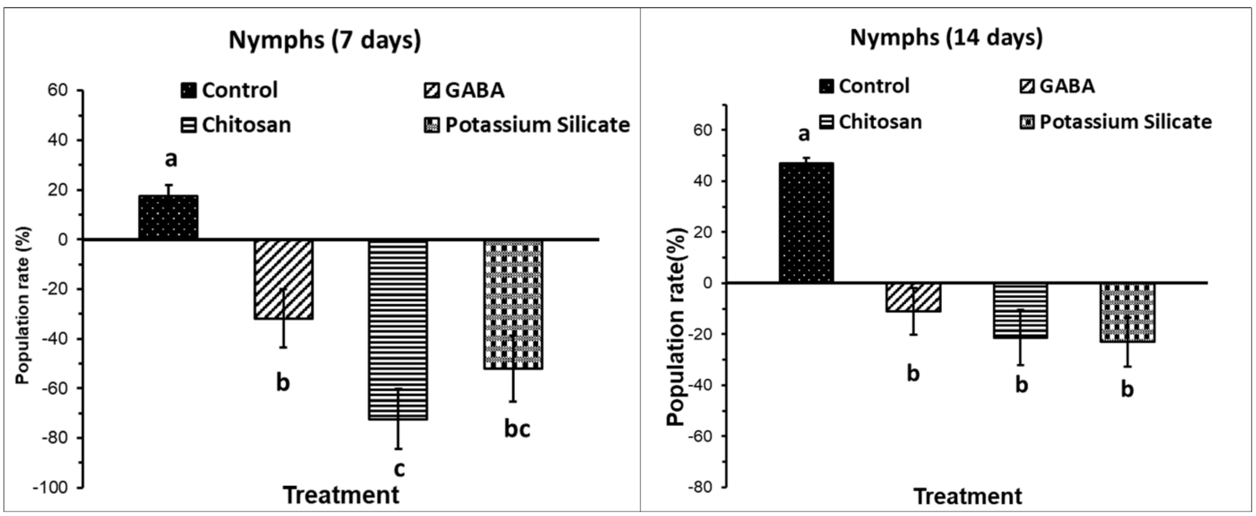

Figure 1. Mean population growth percentage of *Agonoscena pistaciae* nymph stage, under different treatments (7 and 14 days after spraying) (Tukey, *P* < 0.05)

Table 1. Comparison of the mean (± SE) activities of antioxidant and detoxifying enzymes in *Agonoscena pistaciae* fed on pistachio trees treated with different compounds.

Treatments	Catalase (U/mg protein)	Peroxidase (U/mg protein)	Glutathione S-transferase (U/mg protein)	Total Esterase (U/mg protein)
Control	2.26±0.06a	0.003±0.0001b	4.31±0.13c	0.046±0.004a
GABA	2.22±0.11a	0.004±0.0001a	7.55±0.23b	0.044±0.001ab
Chitosan	1.93±0.17a	0.002±0.0001c	12.95±0.29a	0.034±0.001b
Potassium silicate	2.20±0.12a	0.004±0.0001a	12.68±0.48a	0.043±0.002ab

Means followed by a different letter within a column are significantly different (Tukey's HSD test; *P* < 0.05). GABA= Gama amino butyric acid.

Moreover, the lower levels of antioxidant and detoxifying enzymes (peroxidase and esterase) in *A. pistaciae* feeding on plants treated with chitosan (especially after 7 days) indicate that the induced plant defense in pistachio trees causes the creation of compounds that can lead to inhibiting or reducing the activities of the detoxifying enzymes (Lukasik & Golawska, 2007). In this study, the treatment of pistachio trees with gamma-aminobutyric acid (GABA) and potassium silicate caused a significant decrease in the growth of the psyllid population compared to the control. GABA acts as an internal mediator in various plant physiological processes, such as activating the jasmonic acid pathway by producing defensive compounds (Bown et al., 2002). It also stimulates the phenylalanine ammonia-lyase enzyme, which activates the phenylpropanoid pathway and causes the synthesis of defense compounds in the plant (Aghdam et al., 2016).

Additionally, the significant difference in the growth percentage of the pest population on the potassium silicate treatment compared to the control could be due to the decrease in sodium and increase in potassium inside the aerial parts of the plant (Hassanvand & Rezaii Nejad, 2016). Soluble silicon can induce chemical defenses against insect herbivory by increasing the production of defensive enzymes (Reynolds et al. 2009). The increase in plant resistance may be related to the high level of activity of hormones and enzymes in the plant that act as signaling molecules during the period of growth and adaptation of the plant against stresses (Koo, 2018; Koo et al., 2020).

On the other hand, after 14 days, the growth of the nymph population showed no significant difference between chitosan, GABA and potassium silicate treatments. One of the main mechanisms of resistance in herbivores is the decomposition of plants' toxic compounds by their body's detoxifying enzymes (Navarro-Roldán et al., 2020). Therefore, it can be assumed that the insect can neutralize the toxic effects of secondary metabolites to a great extent using some detoxifying enzymes such as GST enzyme in chitosan treatment. Moreover, the GST enzyme in pear psylla, *Cacopsylla bidens* (Sulc) under chitosan treatment was significantly increased compared to control (Ziaaddini et al., 2022). GSTs detoxify secondary oxidation products created from ROS that react with intracellular macromolecules (Hayes and McLellan 1999; Lei and Zhu-Salzman 2015). However, the difference in population growth percentage of *A. pistaciae* nymphs fed on studied treatments was significant compared to the control. It can be concluded that the insect is probably unable to complete the decomposition of toxic chemicals due to the high amounts of defensive compounds in plants treated. In another study, GST enzyme activity increased in whitefly *Bemisia tabaci* (Gennadius) fed on cucumber plants after induction of resistance compared to control plants, while the activity of the esterase enzyme in this insect was reduced compared to the control (Lin et al., 2019).

The significant differences in the activity of detoxifying enzymes in psyllid fed on trees treated with the studied compounds indicate that different treatments lead to the induction of higher or lower levels of secondary metabolites in the plant, which, possibly in some cases, the insect can break down some of these compounds by the activity of detoxifying enzymes in response to the effects of plant secondary metabolites.

Conclusion

This research showed that the population of *A. pistaciae* on pistachio trees treated with different inducers was significantly lower compared to control trees. Among them, trees treated with chitosan after 7 days were less attractive to the pistachio psylla due to a strong immune system in the plant and generating some secondary metabolites which can lead to inhibiting or reducing the activities of antioxidant and detoxifying enzymes, including peroxidase and esterase in the insect. However, the growth population percentage of nymphs (after 14 days) on trees treated with chitosan did not show a significant difference compared to GABA and potassium silicate, which was probably due to the high activity of glutathione S-transferase enzyme in insects feeding on this treatment to detoxify the defense compounds of the plant. On the other hand, because of the higher antioxidant activity of peroxidase enzyme in the feeding psyllids on trees treated with GABA and potassium silicate compared to chitosan, similar results in insect population growth were observed after 14 days. Therefore, the increase in activity of antioxidant and detoxifying enzymes is one of the adaptation procedures of psyllids against plant defense responses. Our findings suggest that the inducer compounds have different effects on the resistance of pistachio trees to *A. pistaciae*. However, the tested compounds caused the plant to be unfavorable to this pest. These compounds can be used in integrated management programs against this psyllid in pistachio orchards.

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