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Research Article

Variation in populations of common pistachio psyllid, *Agonoscena pistaciae* (Hem.: Psyllidae), with different chemical control levels: narrower wing shape in the stressed environment

Mohammadreza Mostafavi, Mohammadreza Lashkari*, Saeid Iranmanesh, Seyed Mozaffar Mansouri

Department of Biodiversity, Institute of Science and High Technology and Environmental Sciences, Graduate University of Advanced Technology, Kerman, Iran.

Abstract: The common pistachio psyllid, *Agonoscena pistaciae* Burckhardt & Lauterer (Hemiptera: Psyllidae), is a key pest of pistachio trees in Iran. Both psyllid nymphs and adults directly damage pistachio; they extract large quantities of sap from tree and produce honeydew and cause deformation of leaves resulting in great economic damage. Little is known about the intraspecific variation of common pistachio psyllid in regions with stressed conditions, such as areas under high levels of pesticide application. Therefore, this study using geometric morphometrics was designed to 1) evaluate morphological differences in wing shape in populations under different levels of pesticide application in Kerman province as the main pistachio producer in the world, and 2) search for a link between the morphological data and previously studied molecular data. The populations were collected from regions with high and low pesticide applications. The results showed that wing shape ($P < 0.01$) and size ($P < 0.01$) are different between populations exposed to different chemical control programs. Based on the results, narrower wing shape was found in psyllid population in stressed environment (population with extreme chemical control programs). In spite of allometric growth, significant shape differences still remain when the data are adjusted to constant size, which showed the important role of genetic changes in the observed morphological changes. Moreover, links between morphologic and previously studied molecular data were revealed. Based on the results, it appears that an evolutionary resistance process is developing, therefore insecticide resistance management programs, in the regions under investigation, is recommended.

Keywords: Pistachio, geometric morphometrics, biodiversity, pesticide

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Introduction

Pistachio, *Pistacia vera* L., is an important and strategic horticultural product which is cultivated in Asia, Americas, Europe, Africa and Oceania with 76.9%, 20.8%, 1.9%, 0.4% and 0.1% average production, respectively, in the world. Among them Iran, the United States and Turkey are the major producers (FAOSTAT, 2016).

Common pistachio psyllid (CPP), *Agonoscena pistaciae* Burckhardt & Lauterer (Hem.: Psyllidae), is the most serious pest in pistachio producing regions of Iran (Mehrnejad, 2003). The presence of high population density of the psyllid nymphs and adults causes great economic damage by affecting kernel development and yield (Esmaeili, 1983; Samih *et al*., 2005).

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* **Corresponding author**, e-mail: m.lashkari@kgut.ac.ir
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Geometric morphometrics (GM) as a technology in the modern natural sciences has been used to identify the similarities and differences between morphological structures (Adams *et al*., 2004). The method was developed in the late 20th century, following numerous problems in traditional morphometric studies, and was used for distinguishing closely related groups (Adams *et al*., 2004). In such studies investigating aspects such as morphological traits, enzymatic or genetic material directly or indirectly will be discussed and it is determined that the difference between the populations of one species often has a genetic basis. The method has been used to describe the morphometric differences between insect pest populations in Iran (Zahiri, 2003; Mozaffarian *et al*., 2005; Mozaffarian *et al*., 2007a,b; Khaghaninia *et al*., 2008; Sadeghi *et al*., 2009; Khiaban *et al*., 2010a, b; Lashkari *et al*., 2013).

It is shown that the populations of a species can be differentiated by landscape changes such as bottleneck effect and genetic changes by stochastic events (Kim and McPheron, 1993). Insect pests populations are changing rapidly under stressed environmental conditions such as pesticide application (Kim and McPheron, 1993).

A molecular study, using RAPD markers, on some CPP populations collected from pistachio planting areas in Kerman province with different chemical control programs showed differences in genetic structure of the populations under extreme pesticide applications (Karimi Darabi *et al*., 2015). They concluded that heavy chemical control level could probably cause differences among genetic structures of populations (Karimi Darabi *et al*., 2015).

Little is known about the effects of chemical control on the wing shape and size in insects. In a study, effects of some insecticides were investigated on wing formation of *Nilaparvata lugens* Stål (Bao *et al*., 2009). They showed negative effects of sublethal doses of insecticides on the induction of macropterous adults (Bao *et al*., 2009). Despite the economic importance of CPP, little is known about the morphological variation of different populations in Iran, especially the populations of CPP which have been under stressed conditions such as pesticide application; Therefore this study was done (using geometric morphometrics) in order to answer the following questions: 1) With regard to the observed genetic differences between populations under different chemical control programs that was shown by Karimi Darabi *et al*. (2015), would there be morphological difference between the under stressed populations? In other words, would it be possible for us to show a link between molecular and morphometric data?; 2) What would be the wing geometry variations between different populations?

Materials and Methods

Sampling

According to the study of Karimi Darabi *et al*. (2014) and the related information from Agriculture Organization of Kerman Province, Iran, on the chemical control management of the CPP, the following populations with different chemical control managements were used in this study: Kerman (a region with extreme chemical control) and Baft (a region with low chemical control) (Table 1, Fig. 1). The populations were collected exactly from the same region cited in the study of Karimi Darabi *et al*. (2014). Moreover, control populations (population without chemical control) were collected from the same regions (Kerman and Baft), and at the same time, September 2015, to distinguish the interaction effects of geographic and insecticide application components (Table 1, Fig. 1).

Table 1 Collection data for populations of *Agonoscena pistaciae*.

Locality	Treatment	Code	N.	E.	n
Kerman	Extreme chemical control	Ker.	56°57′44″	30°22′29″	30
Kerman- Control	Without chemical control	Ker.C	57°01′56″	30°17′19 ″	30
Baft	Low chemical control	Baf.	56°35′47″	28°58′37″	30
Baft-Control	Without chemical control	Baf.C	56°42′38″	28°58′20″	30

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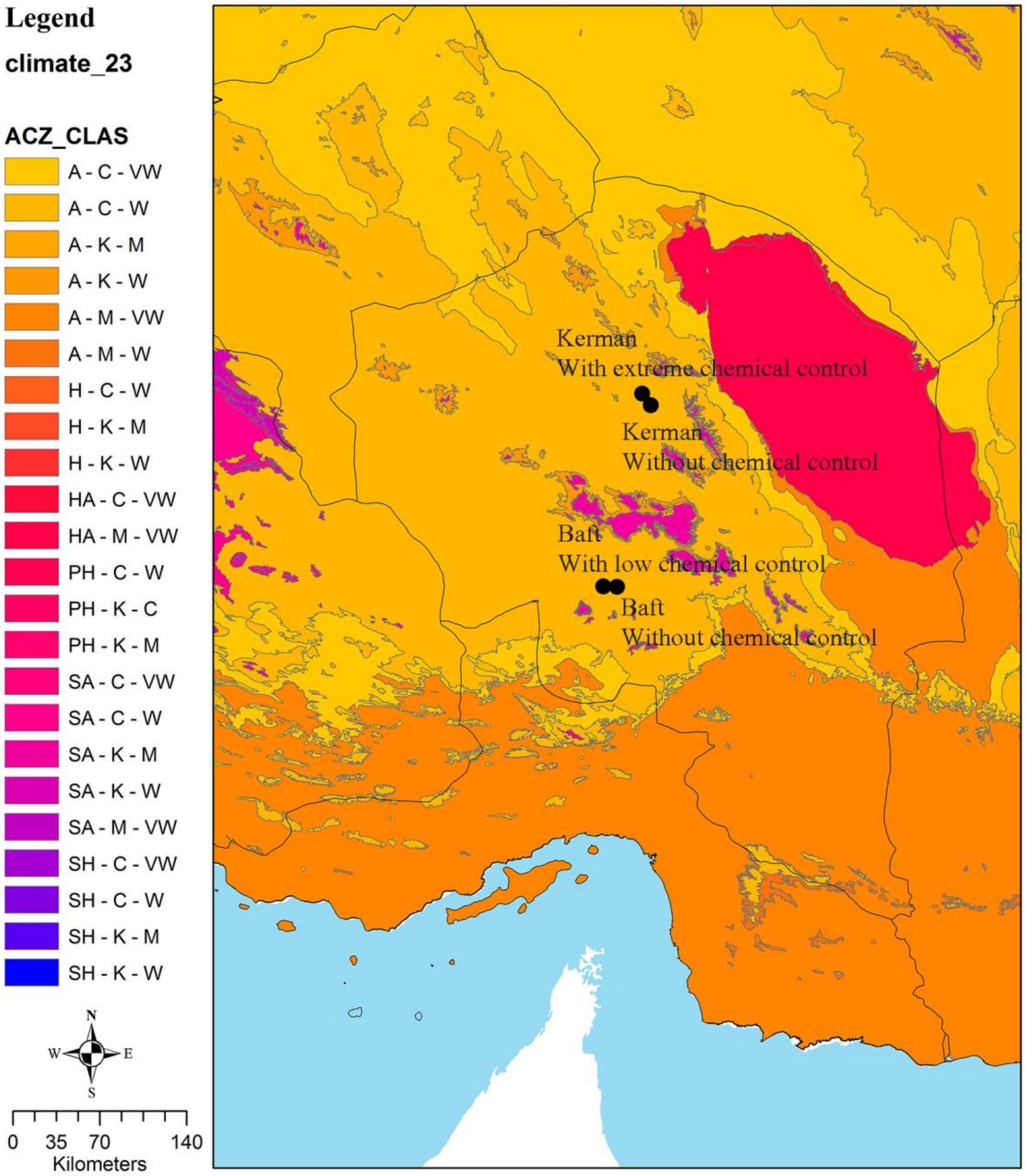

Figure 1 Collecting site map for populations of *Agonoscena pistaciae*.

Preparing microscopic slides and imaging

In this study, forewings were used to compare variation in populations of CPP. First, 30 female insects that had completely intact wings were randomly selected from each population. In this study, only females were selected for analysis, because the number of collected females were more than the males, and also most of them had complete wings, which were suitable for geometric morphometrics analysis. Then, a slide mount of right forewing of each specimen was prepared using Canada balsam. Desired structure images for all samples were prepared by a digital camera (3 megapixels) at 40X magnification.

Data analysis

For morphometric analyses, first, the TPS file (Thin Plate Spline) was built from saved images by software tpsUtil program, version 1.46 (Rohlf, 2010a). Then, a total of 11 homologue landmarks (type 1) according to Lashkari *et al*. (2013) were selected on each image by software tpsDig version 1.40 (Rohlf, 2004). Landmarks' positions are described in Figure 2. Landmark superimposition was done based on Generalized Procrustes Analysis (GPA) procedure in software TpsRelw software (Rohlf, 2010b). Then, shape data i.e. shape variables were produced and extracted. In this study, the Partial Warps (PW) and Centroid Size (CS) were used for investigation of shape and size of wing, respectively.

Two-way MANOVAs were designed for both populations with different chemical control (extreme and low chemical control) and control populations (without chemical control) to detect any significant wing shape differences and their interaction effects. Then Canonical Variate Analysis (CVA) was performed on groups. Regressions of shape on size variables and a MANCOVA was calculated to detect any allometric growth and separate allometric trajectories (Rohlf, 2009). In order to analyse the wing size in different populations, Centroid Size (CS) was calculated in different populations and compared by ANOVA method in software SAS.

Results

Wing Shape

Two-way MANOVAs, showed a significant difference in wing shape between the populations with different levels of chemical control (Table 2), but the interaction term was not significant. Allometric analysis showed a significant allometric effect between the populations with different levels of chemical control (Table 2a). Although, allometric slopes did not significantly differ (Table 2b), but there was still significant difference between the wings shape, when size was held constant (Table 2c). Therefore, studied populations had separate allometric directions but were parallel to each other.

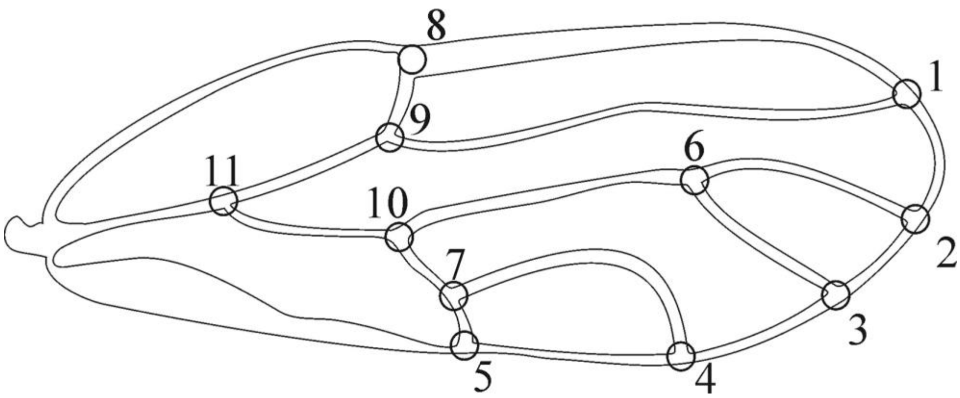

Figure 2 Position of landmarks (circles) in the right forewing of *Agonoscena pistaciae*.

Table 2 Two-way MANOVAs and allometry tests in population with different level of insecticide application.

Source	MANOVA		Allometric growth		Allometric slope		Shape in constant size	
	Wilks' Lambda	P-value	Wilks' Lambda	P-value	Wilks' Lambda	P-value	Wilks' Lambda	P-value
Ker. & Baf.	0.7452	0.0067**	0.5072	0.0019**	0.5976	0.1427	0.5103	0.0278*
Ker.C & Baf.C	0.9099	0.8051	-	-	-	-	-	-
Interaction	0.8101	0.0884	-	-	-			-

See table 1 for abbreviations. * Significant at P < 0.05, ** Significant at P < 0.01.

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Pairwise multiple comparisons showed significant difference between the wing shape of populations with extreme chemical control from Kerman and its control (without chemical control), but, did not show significant difference between populations with low chemical control from Baft and its control (without chemical control) (Table 3). Canonical variate analysis confirmed these results (Fig. 3). Allometric analysis indicated a significant allometric effect between the populations with extreme chemical control from Kerman and its control (without chemical control) (Table 3a). Although, allometric slopes did not significantly differ between these populations (Table 3b), yet significant difference between the wings shape was found, when size was held constant (Table 3c).

Based on the relative warp visualization plot, wings in population of Kerman (extreme chemical application) were narrower than those in its control (Fig. 4B-C), but the wings shape in Baft and its control were relatively similar (Fig. 4C-D).

Wing size

The results of ANOVA showed significant differences between populations ($F = 45.748904$, $P < 0.0000$). Pairwise comparisons between populations (using HSD post-hoc test, alpha = 0.01) showed that Baft-control population (without chemical control) had larger wings than other population, after that, Kerman-control population (without chemical control) and Baft population (Low chemical control) had moderate wing size. Moreover, Kerman population (Extreme chemical control) had smaller wing size (Fig. 5).

Discussion

The results of the present study are similar to those of Karimi Darabi *et al*. (2014), in which the wing shape of CPP population under extreme chemical control (Kerman) was statistically different from others; Moreover, there was not any statistical difference between wing shape of populations under low and without chemical control (Baft and its control population). Considering the following reasons, it seems that the observed variations in the studied populations were developed by the interaction of genetic mechanisms rather than environmental processes: 1) Based on the results, the interaction effect was not statistically significant, this means that the effect of geographic component (Kerman.C & Baft.C) was similar for populations under different chemical controls (Kerman & Baft); 2) the result of allometric relationships showed a significant wing shape difference even after removing wing size. These findings are in agreement with some studies which found a significant allometric growth even after removing size variation in geographical populations (Lashkari and Iranmanesh, 2015; Lashkari *et al*., 2013). They showed differences between wing shape of Asian citrus psyllid populations from Iran and the USA even after removing size variation, which is probably due to genetic factors rather than environmental factors (Dujardin *et al*., 1999); 3) According to these results there appears to be a link between molecular data obtained by the study of Karimi Darabi *et al*. (2014) and morphometric data of the present study. Actually invasive species show rapid evolutionary events (Sakai *et al*., 2001), because they must adapt themselves to different environmental conditions (Kim and McPheron, 1993).

Table 3 MANOVAs and allometry tests between populations with different levels of insecticide application and their control populations.

Source	MANOVA		Allometric growth		Allometric slope		Shape in constant size	
	Wilks' Lambda	P-value	Wilks' Lambda	P-value	Wilks' Lambda	P-value	Wilks' Lambda	P-value
Ker. & Ker.C	0.524654	0.0059**	0.5656	0.01111*	0.7174	0.6195	0.4373	0.0028**
Baf. & Baf.C	0.716770	0.262889	-	-	-	-	-	-

See table 1 for abbreviations. * Significant at $P < 0.05$, ** Significant at $P < 0.01$.

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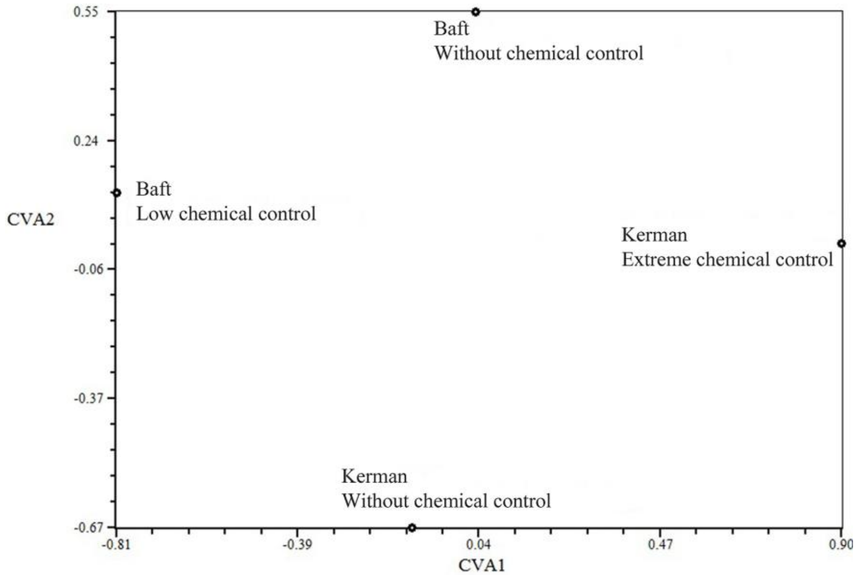

Figure 3 Canonical variate analysis (2D plot of mean scores) in the populations of *Agonoscena pistaciae* with different level of chemical control.

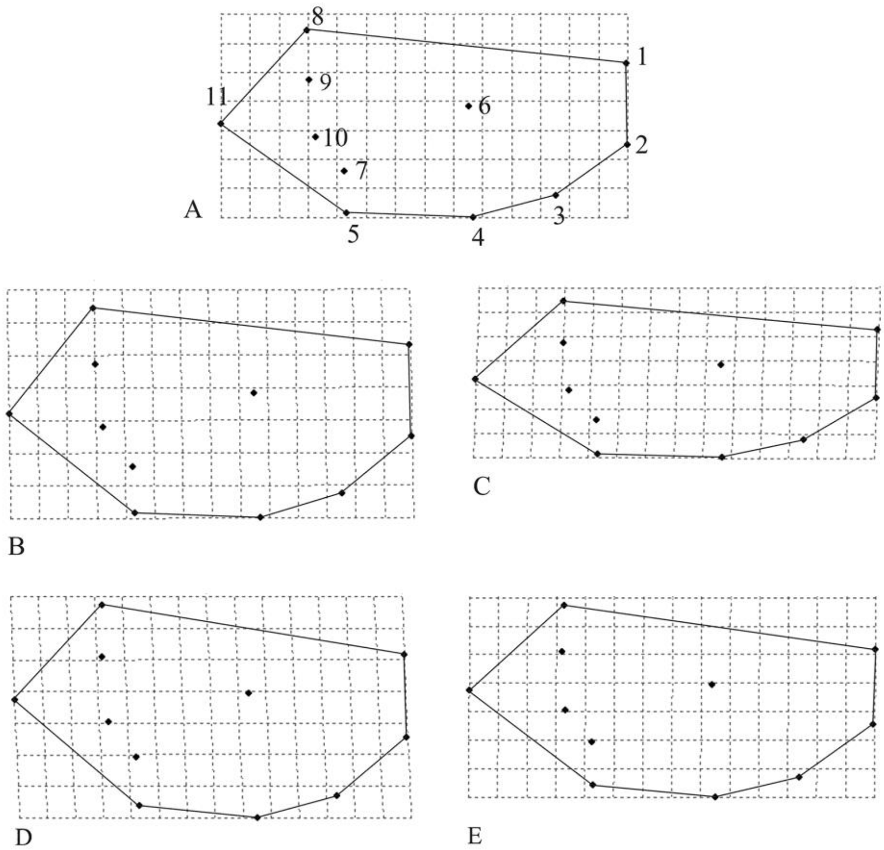

Figure 4 Predicted wing shape of forewings in the populations of *Agonoscena pistaciae* with different levels of chemical control. A: The consensus configuration, B: Kerman-control population (without chemical control), C: Kerman population (Extreme chemical application), D: Baft-control population (without chemical control), E: Baft population (Low chemical control).

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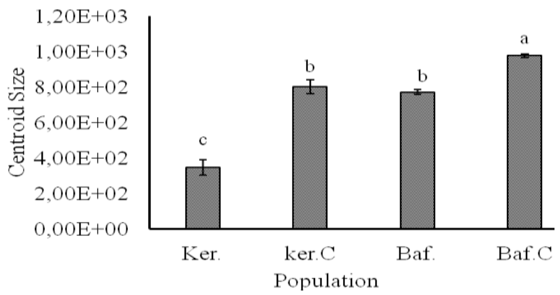

Figure 5 Wing size comparison of forewings in the populations of *Agonoscena pistaciae* with different levels of chemical control. See table 1 for abbreviations.

The visualization plot (Fig. 4) showed that the population from a region with severe chemical control (Kerman) has narrower forewing than the populations exposed to low levels of chemical or none at all. It seems that the populations with narrower wings act better at flight rather than populations with wider wing shape (Bai *et al*., 2016). Totally, the long-winged adults have long-distance migration ability and can migrate to new areas (Bao *et al*., 2009). So, the individuals under extreme chemical control in this study (Kerman), with narrower and longer wig shape probably have flight abilities for long-distance migration; this in turn may make difficulties in the control of this pest.

Apparently the genetic variations between the studied populations affected the observed morphological differences, and may also have changed their resistance to the insecticide, which is suggested for future studies. Certainly, these changes can affect the status of control management programs. Based on the results the wing shape was statistically different in CPP population with extreme chemical control, but it was not different in population with low chemical control. Therefore, reduced use of pesticides is recommended in the regions, especially Kerman. Knowing the natural resistance of pests to pesticides allows us to use strategies to prevent or delay the development of resistance to pesticides (REX Consortium, 2013). Significant changes in the size and shape of the wing in the studied populations indicate that resistance to pesticides is underway.

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تنوع در جمعیت‌های پسیل معمولی پسته، *Agonoscena pistaciae* (Hem.: Psyllidae)، مرتبط با سطوح کنترل شیمیایی متفاوت: شکل بال باریک‌تر در شرایط استرس‌زا

محمدرضا مصطفوی، محمدرضا لشکری*، سعید ایرانمنش و سیدمظفر منصوری

گروه تنوع زیستی، پژوهشگاه علوم و تکنولوژی پیشرفته و علوم محیطی، دانشگاه تحصیلات تکمیلی صنعتی و فناوری پیشرفته، کرمان، ایران.

* پست الکترونیکی نویسنده مسئول مکاتبه: m.lashkari@kgut.ac.ir

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چکیده: پسیل معمولی پسته، *Agonoscena pistaciae* Burckhardt & Lauterer (Hem.: Psyllidae)، یک آفت کلیدی درختان پسته در ایران است. پوره‌ها و حشرات بالغ به‌طور مستقیم به پسته آسیب می‌زنند؛ آن‌ها با استخراج مقدار زیادی از شیره گیاهی باعث تولید عسلک و پیچیدگی برگ‌ها می‌شوند و خسارت اقتصادی ایجاد می‌کنند. شناخت چندانی درباره تنوع درون گونه‌ای پسیل معمولی پسته در مناطق با شرایط استرس‌زا، مانند نواحی تحت کاربرد شدید آفت‌کش‌ها وجود ندارد. بنابراین، این مطالعه با کاربرد روش ریخت‌سنجی هندسی طراحی شد تا ۱) تفاوت‌های ریخت‌شناختی در شکل بال در جمعیت‌های تحت سطوح مختلف کاربرد حشره‌کش‌ها در استان کرمان، به‌عنوان عمده‌ترین تولیدکننده پسته دنیا و ۲) ارتباط بین داده‌های ریخت‌شناختی و مولکولی (حاصل مطالعات گذشته) را بررسی کند. در این مطالعه، جمعیت‌های موردنظر از نواحی مختلف با سطوح شدید و کم کاربرد آفت‌کش جمع‌آوری شدند. نتایج نشان داد که شکل بال ($P < ۰/۰۱$) و اندازه بال ($P < ۰/۰۱$) بین جمعیت‌های مختلف با سطوح مختلف کنترل شیمیایی متفاوت است. براساس نتایج، بال باریک‌تر در جمعیت‌های پسیل پسته موجود در شرایط استرس‌زا دیده شد (جمعیت‌های با کنترل شیمیایی شدید). با وجود رشد معنی‌دار آلومتریک، زمانی‌که اندازه بال ثابت نگه داشته شد، شکل بال هنوز به‌طور معنی‌داری ثابت بود، که اهمیت نقش تغییرات ژنتیکی در تغییرات ریخت‌شناختی مشاهده شده را نشان می‌دهد. به علاوه، نتایج ارتباط بین داده‌های ریخت‌سنجی و مولکولی (حاصل از مطالعات گذشته) را نشان داد. براساس نتایج، ممکن است یک فرایند تکاملی مقاومت به حشره‌کش‌ها در حال گسترش باشد، بنابراین برنامه‌های مدیریت مقاومت به حشره‌کش‌ها در برخی از مناطق مورد بررسی توصیه می‌شود.

واژگان کلیدی: پسته، ریخت‌سنجی هندسی، تنوع زیستی، آفت‌کش