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Comparison of the Suitability of Four Commercial Pistachio Cultivars to the Pistachio Green Stink-bug, *Brachynema germari,* under the Laboratory Conditions

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A B S T R A C T

The pistachio green stink-bug, *Brachynema germari* Kolenati (Hem.: Pentatomidae), is an important pest of pistachio in Iran. In this research, the effects of four commercial pistachio cultivars that are commonly grown in Iran including Kaleghochi, Ahmadaghaii, Ohadi and Akbari were evaluated on the biology (developmental time, mortality rate, longevity) and demographic parameters of *B. germari* under the laboratory conditions at 27.5±1°C, 65±5% RH and 16: 8 (L: D) h, during 2016-2017. The longest and the shortest immature development times were observed on Akbari (38.11 days) and Kaleghochi (25.54 days) cultivars, respectively. The highest and the lowest mortality rates of immature stages were on Akbari and Kaleghochi cultivars, respectively. The gross (*GRR*) and net reproductive rates (R_0) were significantly lower on Akbari compared to other cultivars. The intrinsic rate of increase (r_m) and the finite rate of increase (λ) were significantly different among the studied cultivars. The calculated r_m values were 0.04, 0.06, 0.07 and 0.08 (day^{-1}) on Akbari, Ohadi, Ahmadaghaii and Kaleghochi cultivars, respectively. Also the lowest value of λ was observed on Akbari cultivar that was significantly different from the other three cultivars. Moreover, the longest mean generation time (*T*) was also observed on Akbari cultivar. The reproductive parameters were also significantly different on the studied cultivars and the lowest and the highest values of all parameters were observed on Akbari and Kaleghochi cultivars, respectively. According to these results, it was concluded that among the studied cultivars, Akbari was the less suitable cultivar for pistachio green stink-bug compared to others and it can be used in IPM of this pest.

Introduction

Pistachio (*Pistacia vera* L.) is an economically important nut crop that ranks the first among the agricultural export commodities in Iran (Sheibani *et al*., 1996). The annual exportation of this nut crop reaches to 100,000 tonnes that ranks the second after the oil in revenue income of the country (Anonymous, 2018). Kerman province is the most important pistachio producing province in Iran. The highest acreage of the pistachio producing lands in this province are allocated to Kaleghochi, Ahmadaghaii,

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Ohadi and Akbari cultivars, respectively. Not to mention that these are the most commonly cultivated pistachio cultivars in Iran (Anonymous, 2018). The pistachio trees are attacked by many different insect pests (Esmaeili, 1996). The pistachio green stink-bug, *Brachynema germari* Kolenati (Hem.: Pentatomidae) is one of the key pests of pistachio trees (*Pistacia vera* L.) in Iran which was first reported in 1965 (Mehrnejad, 2001). The adults and nymphs of this insect feed on the developing fruits of pistachio and cause significant damages in the form of pericarp lesions that result to the nut drop (Daane *et al*., 2005). The pistachio green stink-bug is also a vector of the pathogenic fungus, *Nematospora coryli* Peglion that causes the formation of corky appearance and somewhat bitter and distasteful smell of pistachio endocarp (Nyman *et al*., 1967; Michailides *et al*., 1994). In the pistachio producing areas of Iran, this pest is present in the pistachio orchards throughout the year and can produce four to five generations per year in Kerman province (Hashemi-Rad, 1999).

Since the host plant can affect the growth, reproduction and survival of herbivorous insects (Price *et al*., 1980), therefore evaluating the effects of different host plant cultivars on the growth and reproductive characteristics of insect pest will be effective in delineating the unsuitable cultivar that could be used in its management. These effects could be evaluated by calculating the demographic parameters, especially the intrinsic rate of increase of the insect pest (r_m) on different host plants (Southwood and Henderson, 2000). Smith (2005) reminded that the demographic parameters are affected by the quality of host plant and are very useful indicators for evaluation the suitability of host plants to insect pests.

In this research, the effects of four commercial pistachio cultivars that are commonly grown in Iran including Kaleghochi, Ahmadaghaii, Ohadi and Akbari were evaluated on insect biology (developmental time, longevity, mortality rate) and demographic parameters of *B. germari* including gross reproductive rate (*GRR*), net reproductive rate (R_0), innate capacity for increase (r_m), finite rate of increase (λ), mean generation time (T) and doubling time (*DT*) under the laboratory conditions to determine the most unsuitable pistachio cultivar that could be used in IPM program of this pest.

Materials and Methods

Insect rearing

About 600 adults of *B. germari* were collected from the *Salsola rigid* L., which is the most common out of season non-agricultural host plant of *B. germari*, in the pistachio orchards and their vicinity in March 2017 from Kerman and transferred to the Pistachio Research Center in Kerman. The bugs were reared for one generation in a growth chamber that was set at 27.5 ± 1°C, 65 ± 5% RH and 16: 8 (L: D) h on *Salsola rigid* L, since this weed plant is the first host plant that is available for *B. germari* in their emergence from hibernation. Due to the long life history of this bug, its rearing on the weed host plant was impossible for more than one generation. The plants were replaced every two days until the adult emergence. It took about 40 days from the first instar nymphs to adult emergence. Finally, the adults were used for egg-laying and one-day old cohort eggs were used to construct the life table (Carey, 1993).

Insect biology

To determine the egg incubation period, 200 one-day old eggs were put inside transparent plastic containers of 7×10×17 cm in size covered with 50 mesh nylon screen for ventilation and surveyed every 12 hours and the number of hatched eggs were counted and recorded. To determine the developmental period of nymphal instars on different pistachio cultivar, they were reared in groups of 10 first instar cohort nymphs emerged from the same egg patch inside transparent plastic containers of 10×20×12 cm in size covered with 50 mesh nylon screen for ventilation on the clusters of 20 nuts (25

days old nuts) of the given pistachio cultivar. The nymphal instars were determined on the bases of size and color of the body. The clusters of nuts of four pistachio cultivars that were used in the experiment were collected from the unsprayed orchard in Pistachio Research Center of Rafsanjan. The clusters of pistachio were replaced once every two days and the nymphs were checked daily for their growth stage, survival rate and pre-adult duration and the results were recorded. This experiment replicated four times for each pistachio cultivar.

Demographic parameters

The demographic parameters of *B. germari* were studied on four pistachio cultivars. The nymphs were fed with different studied cultivars in a growth chamber that was set at the above-mentioned conditions. After adult emergence, for each cultivar one pair of adult insects (male and female) transferred into each plastic containers (15×10×7 cm) containing the given pistachio clusters. This experiment conducted in 20 replications for each cultivar. The number of eggs laid in each container was recorded daily until the death of all adults. Based on the obtained data, life table parameters, stable population parameters, and reproductive parameters were calculated by using the Carey (1993) life table construction method.

Statistical analysis

The growth and development, reproductive period and longevity of adult bugs were analyzed on pistachio cultivars by using one-way ANOVA method (SAS Institute Inc. 2004). The means were compared by using Tukeys method in $P<0.05$ level of probability. The population growth parameters of *B. germari* including R_0, r_m, λ, T and DT were calculated by using the equations of Birch (1948) and Carey (1993) on four investigated pistachio cultivars. Moreover, the variances and standard errors of population growth and reproduction parameters were estimated by using Jackknife technique (Meyer *et al*. 1986; Sokal and Rohlf, 1995).

Results

Developmental time and longevity

The effects of four pistachio cultivars on the length of various developmental stages of *B. germari* are given in Table 1. The egg incubation period of this pest was significantly different on the studied cultivars (Turkey's test, $F_{3,685}= 1058.66$, $P< 0.0001$) (Table 1). The longest and the shortest egg incubation period were seen on Akbari and Kaleghochi cultivars, respectively. The longest and shortest nymphal periods also were determined on Akbari and Kaleghochi cultivars, respectively ($F_{3,685}= 40.50$, $P<0.0001$). The total immature development times (egg to adult) were 25.54, 30.53, 35.72 and 38.11 days on Kaleghochi, Ahmadaghaii, Ohadi and Akbari cultivars, respectively. The highest total immature development time was observed on Akbari cultivar that was significantly different from other three cultivars ($F_{3,685}= 67.24$, $P <0.0001$). The longest male longevity was observed on Ohadi cultivar that was significantly different from the other cultivars ($F_{3,685}= 90.02$, $P<0.0001$), whereas the longest female longevity was seen on Akbari cultivar that was also significantly different from the other cultivars ($F_{3,272}= 138.58$, $P<0.0001$). The age-specific survival rates (l_x) of *B. germari* on four studied cultivars are shown in Figure 1 that confirms the mentioned results. The total life cycles of the pest were 82, 92, 104 and 115 days on Kaleghochi, Ahmadaghaii, Ohadi and Akbari cultivars, respectively (Table 1). The oviposition of the new adults was started at 40, 48, 56 and 61 days after the eggs laid on Kaleghochi, Ahmadaghaii, Ohadi and Akbari cultivars, respectively (Figure 1).

Table 1. The immature developmental time, adult longevity and reproduction period (day) of *Brachynema germari* Kolenati four commercial pistachio cultivars.

Developmental stage	Kaleghochi	Ahmadaghaii	Ohadi	Akbari
Egg	4.01±0.03 [d]	5.03±0.03 [c]	6.01±0.04 [b]	7.02±0.03 [a]
Nymph	21.54±0.39 [c]	25.53±0.55 [b]	29.71±0.71 [a]	31.11±1.01 [a]
Egg-to-Adult	25.54±0.40 [d]	30.53±0.55 [c]	35.72±0.71 [b]	38.11±1.01 [a]
Male longevity	34.07±1.04 [c]	36.90±0.92 [c]	54.78±0.99 [a]	48.98±1.24 [b]
Female longevity	55.80±0.57 [d]	61.00±0.72 [c]	67.65±0.82 [b]	75.66±0.74 [a]
Pre-oviposition period	15.02±0.10 [d]	17.11±0.08 [c]	19.35±0.12 [b]	22.58±0.13 [a]
Oviposition period	25.32±0.16 [d]	27.65±0.09 [c]	29.97±0.16 [b]	32.10±0.14 [a]
Post-oviposition period	15.44±0.53 [c]	16.22±0.71 [c]	18.32±0.82 [b]	20.98±0.76 [a]

Mean values followed by the same letter in each row are not significantly different (Tukey test, P<0.05)

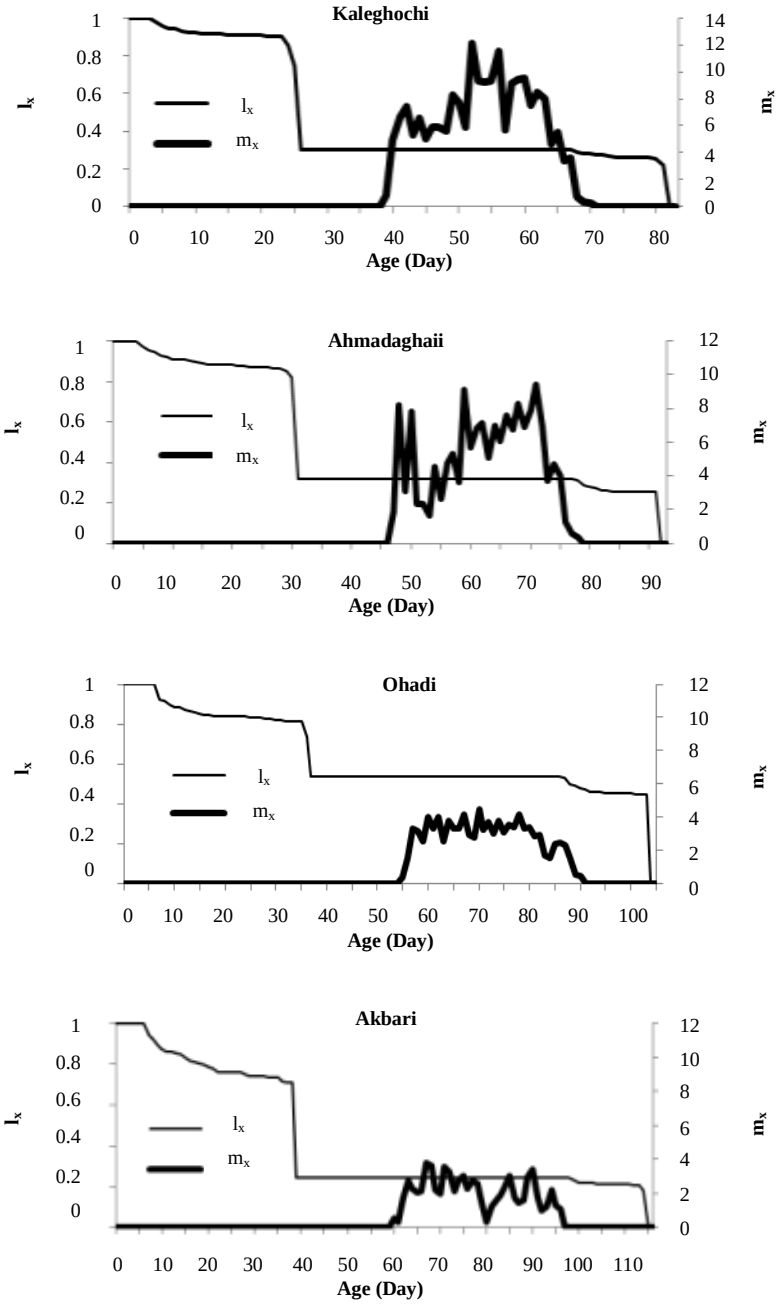

Fig. 1. The age-specific fecundity (m_x) and age- specific survival rate (l_x) *Brachynema germari* Kolenati on four commercial pistachio cultivars.

Egg and nymphal stages mortality rate

The **egg and nymphal stages** mortality rates of *B. germari* on the studied cultivars are shown in Table 2. The highest and the lowest mortality rates in the egg stage were observed on Ohadi (5.88 %) and Kaleghochi (2.06 %) cultivars, respectively. Also, the highest mortality rate in immature stages was observed in the first instar nymph on Ohadi cultivar (0.60 %) and the lowest was on the 5st instar nymph on Kaleghochi cultivar (8.43 %). In addition, the highest and lowest mortality rates from egg to adult stages were observed on Akbari (27.47) and Kaleghochi (9.27) cultivars, respectively.

Table 2. The egg and nymphal mortality rate (%) of *Brachynema germari* Kolenati on four commercial pistachio cultivars.

Cultivar	Egg	Nymph 1st instar	Nymph 2nd instar	Nymph 3rd instar	Nymph 4th instar	Nymph 5th instar	Egg to Adult
Kaleghochi	2.06 (n=194)	3.82 (n=183)	1.73 (n=173)	1.19 (n=168)	0.60 (n=165)	0.61 (n=163)	9.27 (n=194)
Ahmadaghaii	2.70 (n=185)	4.65 (n=172)	3.14 (n=159)	1.98 (n=151)	1.36 (n=146)	1.40 (n=142)	13.51 (n=185)
Ohadi	5.88 (n=187)	5.38 (n=167)	3.94 (n=152)	2.09 (n=143)	1.44 (n=138)	1.49 (n=134)	17.64 (n=187)
Akbari	5.49 (n=182)	8.43 (n=166)	6.66 (n=150)	5.03 (n=139)	3.78 (n=132)	3.14 (n=127)	27.47 (n=182)

Population growth parameters

The population growth parameters of this insect pest on different cultivars are presented in Table 3. There were significant differences among four pistachio cultivars on gross reproductive rate ($F_{3,272}$ = 4.94, P<0.0001), net reproductive rate ($F_{3,272}$= 4809, P<0.0001), the intrinsic rates of increase (r_m) ($F_{3,272}$ = 9317, P<0.0001) and the finite rates of increase (λ) ($F_{3,272}$ =1190, P<0.0001). (Table 3). The r_m of *B. germari* were 0.04, 0.06, 0.07 and 0.08 day^{-1} on Akbari, Ohadi, Ahmadaghaii and Kaleghochi cultivars, respectively. Accordingly, the r_m value on Akbari cultivar was significantly lower than the other three cultivars. The finite rates of increase (λ) were 1.039, 1.060, 1.067 and 1.083 day^{-1} on Akbari, Ohadi, Ahmadaghaii and Kaleghochi, respectively; which was significantly lower in Akbari ($F_{3,272}$ =1190, P<0.0001). The mean generation times (*T*) were 93.70, 79.39, 69.81 and 59.10 days on Akbari, Ohadi, Ahmadaghaii and Kaleghochi, respectively. The longest mean generation time was occurred on Akbari cultivar that was significantly higher than the other three cultivars ($F_{3,272}$ =1.41, P<0.0001). Also there were significant differences among four pistachio cultivars with respect to doubling time (*DT*) ($F_{3,272}$ =7765, P<0.0001). The longest (17.53 days) and the shortest (8.64 days) doubling times were observed on cultivars Akbari and Kaleghochi, respectively.

Table 3. The population growth parameters of *Brachynema germari* Kolenati four commercial pistachio cultivars.

Parameter	Kaleghochi	Ahmadaghaii	Ohadi	Akbari
Gross reproductive rate (*GRR*)	204.15±0.01 [a]	163.03±0.01[b]	102.09±0.01[c]	77.82±9.25 [d]
Net reproductive rate (R_0)	61.72±0.05 [a]	51.70±0.05 [b]	1.68±0.004 [d]	18.93±4.63 [c]
Intrinsic rates of increase (r_m) (day^{-1})	0.08±1.89 [a]	0.07 ±1.76 [b]	0.06 ±1.29 [c]	0.04 ±1.83 [d]
Finite rates of increase (λ) (day^{-1})	1.08±2.04 [a]	1.067±1.88 [b]	1.060±1.37 [c]	1.039±8.67 [d]
Mean generation time (*T*) (day)	59.10±0.003 [d]	69.81±0.004 [c]	79.39±0.004 [b]	93.70±1.29 [a]
Doubling time (*DT*) (day)	8.64±0.002 [d]	10.60±0.002 [c]	11.76±0.002 [b]	17.53±0.008 [a]

Mean values followed by the same letter in each row are not significantly different (Tukey test, P<0.05)

Reproductive parameters

The reproductive parameters of *B. germari* on four pistachio cultivars are given in Table 4. There were significant differences on the gross fecundity rate ($F_{3, 272}$= 4.21, P<0.0001), gross fertility rate ($F_{3,272}$= 1.00, P<0.0001) and net fertility rate ($F_{3,272}$= 3152, P<0.0001) of *B. germari* on four investigated pistachio cultivars. The lowest (R_0 = 18.930) and the highest (R_0 = 61.72) net fecundity rate of this pest was observed on Akbari and Kaleghochi cultivar that were significantly different ($F_{3,272}$= 1406, P<0.0001) (Table 4). The lowest daily fecundity or the number of eggs laid per female per day (0.77 eggs) was observed on Akbari cultivar and the highest was on Kaleghochi cultivar (2.85 eggs) ($F_{3,272}$= 6647, P<0.0001) (Table 4). In addition, the lowest and highest daily fertility or the number of fertile eggs laid per female per day were observed on Akbari (0.07 eggs) and Kaleghochi (2.74 eggs) cultivars, respectively ($F_{3,272}$= 1234, P<0.0001).

Table 4. The reproductive parameters of *Brachynema germari* Kolenati four commercial pistachio cultivars.

Parameter	Kaleghochi	Ahmadaghaii	Ohadi	Akbari
Gross fecundity rate	378.07±0.02 [a]	301.92±0.01 [b]	200.17±0.01 [c]	158.83±3.70 [d]
Gross fertility rate	362.94±0.02 [a]	280.78±0.01 [b]	187.46±0.01 [c]	14.93±9.25 [d]
Net fecundity rate	114.25±0.10 [a]	95.74±0.09 [c]	107.49±0.08 [b]	38.64±1.85 [d]
Net fertility rate	109.68±0.10 [a]	89.04±0.08 [c]	100.67±0.08 [b]	3.63±4.05 [d]
Eggs/ female/ day	2.85±0.001 [a]	2.06±0.001 [b]	1.61±0.001[c]	0.77±0.0004 [d]
Fertile eggs/female/day	2.74±0.001 [a]	1.92±0.001 [b]	1.51±0.001[c]	0.07±4.28 [d]

Mean values followed by the same letter in each row are not significantly different (Tukey test, P<0.05)

Discussion

The quality of host plants strongly affects the biology and demography of herbivore insects especially their development time, longevity, survival, mortality, reproductive characteristics and population growth parameters (Price, 1998; Smith, 2005). Many researchers have demonstrated significant differences of growth period in many insect pests on different host plant cultivars (Greenberg *et al*., 2001; Tsai and Wang, 2001; Kumral *et al*., 2007; Pourbehi *et al*., 2010; Esmaeili *et al*., 2013; Goodarzi *et al*., 2015; Razazzian *et al*., 2015). Basirat *et al*. (2016) concluded that the mortality rate of immature stages of *Arimania komaroffi* Ragonot (Lepidoptera: Pyralidae) on Kaleghochi, Ahmadaghaii and Ohadi cultivars was significantly different. Razazzian *et al*. (2015), who studied the mortality rate of egg, larva and pupa of *Plodia interpunctella* Hüb., found that there were significant differences among four pistachio cultivars of Kaleghochi, Ahmadaghaii, Ohadi and Akbari with respect to these parameters. Our results are in agreement with the results of these researches.

In our study, the longest nymphal period was observed on Akbari cultivar indicating that this cultivar is an unsuitable host plant compared with the other three pistachio cultivars for the nymphal growth of *B. germari*. Basirat *et al*. (2016) concluded that *A. komaroffi* had the longest immature development time on Ahmadaghaii cultivar. This contradiction may be related to the different insect species that were used in these two different studies, which feed from different parts of the pistachio plants. The larvae of *A. komaroffi* feed from pistachio fruit hull, while the nymphs of *B. germari* feed on the fresh pistachio kernel. In addition, Basirat *et al*. (2016) reported that the longest male longevity of *A. komaroffi* was on Ohadi cultivar that was significantly different with Kaleghochi and Ahmadaghaii cultivars; but, the female longevity of this pest was not significantly different on three cultivars. The results of our study

also were similar with the results obtained by these researchers concerning the male longevity of *A. komaroffi*, but there were no similarity on the female longevity. In addition, our results indicated that pre-oviposition, oviposition and post-oviposition period of *B. germari* were significantly different on four investigated pistachio cultivars. Pourkhatoon *et al*. (2016) showed that the total pre-oviposition period of *B. germari* on artificial diet was longer than natural diets, probably because the artificial diet lacks some essential nutrients required for the insect,s growth and development.

Among the population growth parameters, the intrinsic rate of increase (r_m) is an important parameter for assessing the suitability of different host plants or plant cultivars for an insect pest (Salas *et al*., 1993). This parameter has been frequently used by different researchers to assess the sensitivity or resistance of host plants to different pests (Smith, 2005; Flores *et al*., 2013; Marouf *et al*., 2013; Razazzian *et al*., 2015). Our results showed that the r_m of *B. germari* was significantly different on four pistachio cultivars and the lowest value of this parameter was observed on Akbari cultivar. Also the lowest number of eggs laid per female per day and the longest mean generation time of *B. germari* were observed on Akbari cultivar. Mehrnejad (2001) has reported that the intrinsic rate of increase of *Agonoscena pistaciae* Burckhardt and Lauterer (Hem.: Aphalaridae) was significantly higher on Kaleghochi cultivar compared to Ahmadaghaii and Ohadi. Also the highest daily fecundity of *A. pistaciae* was on Akbari cultivar. Basirat *et al*., (2016) also found that the intrinsic rate of increase in *A. komaroffi* was significantly different among Kaleghochi, Ahmadaghaii and Ohadi cultivars. Our results are not in complience with the results reported by Mehrnejad (2001) and Basirat *et al*. (2016) concerning the different effects of different pistachio cultivars on the biology and demographic parameters of *B. germari*. These differences might be due to the differences in plant structure and primary and secondary metabolites of the fresh pistachio fruit kernel in different pistachio cultivars that were studied in this research.

The higher reproductive parameters and the shorter generation time of an insect on a host plant can indicate the suitability of that host for that plant pest (Price, 1998). In this research, the lowest values of the reproductive parameters and the shortest generation time of *B. germari* were observed on Akbari cultivar. Nadernejad *et al*. (2013) concluded that the amount of phenolic, flavonoid and anthocyanin compounds in pistachio kernel were different on Kaleghochi, Ahmadaghaii and Ohadi cultivars. The differences in the level of these plant compounds of different pistachio cultivars might have affected the reproductive parameters of *B. germari* on the studied cultivars. Basirat *et al*. (2016) reported that the gross fecundity rate of *A. komaroffi* was significantly different on three pistachio cultivars crop. The reason for these differences could be assigned to the differences in feeding habits of these two insect pests. *B. germari* feeds from fresh pistachio kernel, whereas *A. pistaciae* sucks the plant sap from the leaves of pistachio tree.

In this study the lowest reproductive parameters, the lowest survival rate, the lowest intrinsic rate of increase and the longest mean generation time were observed in Akbari cultivar. Terefore, Akbari cultivar is identified as less suitable, whereas Kaleghochi cultivar was more suitable pistachio cultivar for *B. germari*. These findings could provide useful information for developing an efficient integrated pest management program (IPM) for this important pest of pistachio trees.

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