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Situation of the Diamondback Moth (DBM), *Plutella xylostella* and Its Parasitoids in the Cauliflower Fields of Tehran

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Authors' contributions

This work was carried out in collaboration between all authors. Authors GH, HA and AS designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. 'Author JK' and Author FJ' managed the analyses of the study. 'Author AR' managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: The diamondback moth, *Plutella xylostella* (L.) (Lep.: Plutellidae) is one of the most important pests of cruciferous plants. Amongst the most important factors to reduce this pest population are parasitoids. The aim of present study was to identify the parasitoids of the diamondback moth (DMB), computing the density of DBM stages and its parasitoids and their performance in different cauliflower fields of Tehran. Also the objective of this study was to elucidate the reactions of DBM parasitoids to host density. Correlation between different larval densities and parasitism rate of DBM parasitoids were calculated using a recall method in field conditions.

Place and Duration of Study: Place – Department of Plant protection, Faculty of Agricultural Sciences, Shahed university, Tehran, Iran. Duration – June, 2011 to November, 2012.

Methodology: Sampling was carried out every two weeks from late June until early November. From each field 20 plants were selected. All larval instars and pupae on each

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plant were collected, and were counted and recorded separately for each field. Also, collected DBM larvae and pupae were kept on the host plant under controlled conditions ($60 \pm 5\%$ RH, 27 ± 2 °C and 14L:10D photoperiod). In order to do recall of larval parasitoids, different densities of 5, 15, 25 and 35 larvae were placed on each plant. For each density five plants were considered as five replication. Analysis of variance (ANOVA) was used to analyze the data on numbers of DBM larvae and pupa in different regions as well as the mean number of parasitoids and parasitism rate. The means were compared at the 5% level of significance using the Duncan studentized range test.

Results: In all studied areas, the larvae, pupae and the total number of larvae and pupae were determined as 3.05, 2.26 and 7.54 (per plant), respectively. The percentage of parasitism by *D. anurum*, *C. plutellae* and *O. sokolowskii* in all regions and total parasitism by these three species were determined obtained as 12.67, 10.29, 13.27 and 36.23% per plant, respectively.

Conclusion: The number of larval and pupal stages based on unit density, was significantly different between all experimental regions and Shahed university station. The highest percentage of parasitism was caused by *D. anurum* in Tehran. Spraying did not reduce the DBM population in some fields in Tehran, but repeated spraying caused a reduction in the activity of the parasitoids of DBM.

Keywords: *Plutella xylostella*; cauliflower; parasitoid; Iran; parasitism rate; host density; recall method.

1. INTRODUCTION

The diamondback moth, *Plutella xylostella* (L.) (Lep.: Plutellidae) is one of the most important pests of cruciferous plants. The first larval instar has a mining feeding mode. Older Larvae feeds on leaves underlying tissue, as well as veins and epidermis supernatant. Larvae in high densities feed also from stem and petiole [1,2]. The diamondback moth was not considered a serious pest in Iran before 1990. But due to uncontrolled use of pesticides and the increasing under cultivation area of cruciferous plants, the pest density increased and reached to an outbreak stage. Changes such as these have recently happened in China [3,4], Southeast Asia and America [5,6,7,8,9]. Absence of parasitoids, especially larval parasitoids in the cauliflower fields are among factors that have led to the existence of the high density of the diamondback moth [10]. Therefore it seems that identification, introduction and protection of parasitoids are imperative for higher performance of biological control factors [11,12]. More than 150 species of parasitoids on different life stages of *P. xylostella* are known throughout the world. But often controlling the pest is carried out by species belonging to genera *Diadegma*, *Cotesia* and *Oomyzus* in Iran [2]. So far several preliminary studies have been carried out to identify parasitoids of the diamondback moth and their performance in Iran [13,14,15,16,17]. This research identifies diamondback moth parasitoids, calculates the density of DBM stages and its parasitoids and their performance in different cauliflower fields of Tehran. Also the objective of this study was to elucidate the reactions of parasitoids to DBM host density. The correlation between different larvae densities and parasitism rate of DBM parasitoids was calculated using the recall method in field conditions.

2. MATERIALS AND METHODS

2.1 Studied Regions

In Iran, the maximum cabbage under cultivation is in Tehran province. Just south of this area more than 1000 hectares of farmland have been allocated to the cultivation of cauliflower. Five regions in the south of Tehran were selected for this study including Jahan-Abad, Kahrizak, Shokr-Abad, Palayin and Shahed. One hectare of cauliflower field in the middle of a large field was randomly selected in each region. Spraying operations in regions of Jahan-Abad and Kahrizak were done early in the season every 10 days once, and every 15 to 20 days once in the mid and the late season. Spraying in regions of Shokr-Abad and Palayin was done every 3 to 7 days once early in the season, and was done every 10 days once in the mid and the late season. No spraying against the diamondback moth has been carried out in the Shahed region. Insecticides used were Avaunt[®] Sc150, Consult[®] Ec10%, Deltamethrin[®] EC2.5% (W/V) and Phosalone[®] EC35% (W/V). The insecticides were alternatively used to avoid pest resistance and to make the plants perform better

2.2 Collection and Identification of Parasitoids

2.2.1 Direct collecting

Parasitoids collecting was carried out from different developmental stages of the diamondback moth in the cauliflower fields of south Tehran in 2011. Sampling was carried out every two weeks from late June until early November. From each field 20 plants were selected. All larval instars and pupae on each plant were collected and recorded separately for each farm. All larvae and pupae were placed separately inside a plastic container with a piece of leaf and were transported to the laboratory. Also, collected diamondback moth larvae and pupae were kept on the host plant under controlled conditions ($60 \pm 5\%$ RH, 27 ± 2 °C and 14L:10D photoperiod) until the diamondback moth or parasitoids adults appeared.

2.2.2 Recall collecting

In order to use the recall method of collecting parasitoids, the eggs, larvae and pupae samples of the diamondback moth were collected from cauliflower fields south of Tehran, and were reared in a growth chamber at 25 ± 2 °C and $65 \pm 5\%$ RH and 16:8 L:D. In order to establish a colony, rearing of DBM was done in transparent plastic Plexiglas's cages (80 × 50 × 50 cm). For larval feeding, cauliflower leaves, *Brassica oleracea* var. *botrytis* CV. Daehnfeldt was used.

10% honey solution was used to feed the adults. In order to induce oviposition of the diamondback moth, a piece of aluminium foil impregnated in cabbage leaf extract for 12 h was placed in oviposition cages (30 × 30 × 30 cm). Aluminium foil containing eggs was placed on cabbage leaves after 60 hours, to induce feeding of larvae hatched from the eggs [2]. In order to recall larval parasitoids, different densities of 5, 15, 25 and 35 of larvae were placed on each plant. For each density five plants was considered as five replicates. All larvae were placed on each plant and left for 72 hours [2].

2.3 Parasitoids Identification

Parasitoids emerging from different growth stages of DBM were kept in 75% alcohol until identification could be performed, using diagnosis keys [18]. Also, a number of collected samples were sent to specialists.

2.4 Parasitism Rate Fluctuations

Study on parasitism rate fluctuations of the diamondback moth was carried out in the cauliflower fields, south of Tehran. Four regions with the highest acreage of cauliflower were selected, including Jahan-Abad, Kahrizak, Shokr-Abad and Palayin. For sampling, one hectare of cauliflower field in these regions was selected and sampled once every 14 days. Sampling was done regularly and coincides with cauliflower planting in the region which is from June to December. Sampling was carried out on the diameters of field and from each 10 meters, a plant was randomly selected and sampled. Sampling was not done from plants that were located at the edge of the field. A total of 20 plant samples were taken at each field. All different larval instars and pupae were counted separately on each plant and for each field. Plants without larva were neglected and only plants that contained larvae samples were selected as sample units. All collected larvae and pupae were placed inside a plastic container with a piece of leaf and transported to the laboratory. Larva of each sample was separately placed inside a container with dimensions of 10 × 20 × 5 cm with a piece of fresh leaf. The end of each leaf was placed in wet cotton to prevent desiccation. The old leaves were replaced with fresh ones every two or three days. Also collected pupae of the diamondback moth were put separately inside a lab tube (1 × 10 cm) until the diamondback moth or parasitoids adults appeared.

2.5 Statistical Analysis

Analysis of variance (ANOVA) was used to analyse the data on the number of DBM larvae and pupae in different regions as well as the mean of the number of parasitoids and parasitism rate. The means were compared at the 5% level of significance using the Duncan studentized range test. Pearson correlation was used to determine the relationship between the number of DBM and the parasitism rate [14]. All analyses were done at the 0.05 confidence level. The analyses were done with the SPSS (Statistical Programmed for the Social Sciences) version16 software.

3. RESULTS AND DISCUSSION

3.1 Parasitoids Identification

In the present study, five species of parasitoid wasps were determined. These parasitoids were including the *Diadegma anurum* (Thomson, 1877), *Cotesia plutellae* (Kurdjumov, 1912), *Oomyzus sokolowskii* (Kurdjumov, 1912), *Pteromalus* sp., and *Tetrastichinae* sp. The braconid parasitoid, *C. plutellae*, was reared from the DBM larvae. *D. anurum* as a larval-pupal parasitoid of DBM, and the Chalcidoidea parasitoids, including *O. sokolowskii*, *Pteromalus* sp., and *Tetrastichinae* sp was reared from the DBM pupae.

Table 1. Mean number of density of different stages of the diamondback moth, *P. xylostella* in cauliflower fields south of Tehran

	Shahed	Jahan-Abad	Kahrizak	Shokr-Abad	Palayin	F value (No.)
Larval density	3.40±0.19b	1.79±0.09b	2.53±0.12b	3.85±0.31b	9.67±0.57a	9.74** (50)
Pupal density	2.17±0.11bc	1.67±0.08c	1.72±0.08c	4.03±0.22ab	7.00±0.82a	7.44** (50)
Larval and pupal density	5.57±0.41b	3.33±0.15b	4.25±0.17b	7.88±0.49b	16.67±1.20a	8.97** (50)

* and ** significantly at 5% and 1% level, respectively ^{ns} not significant.
Similar letters in each row show no significant difference between study sites.

Table 2. Mean number of the diamondback moth parasitoids and parasitism rate (%) in cauliflower fields South of Tehran

Species		Shahed	Jahan-Abad	Kahrizak	Shokr-Abad	Palayin	F value (No.)
<i>D. anurum</i>	Mean No. of parasitoid	0.77±0.05a	0.57±0.03ab	0.58±0.03ab	0.50±0.03ab	0.35±0.03b	2.92* (50)
	Parasitism rate	16.96±1.32a	19.31±1.51a	16.00±1.24a	8.41±0.83b	2.70±0.47c	34.84** (50)
<i>C. plutellae</i>	Mean No. of parasitoid	0.43±0.03a	0.31±0.03a	0.29±0.03a	0.26±0.03a	0.22±0.02a	1.40 ^{ns} (50)
	Parasitism rate	12.14±1.53b	17.98±2.25a	12.99±1.85b	6.53±0.95c	1.81±0.30d	15.05** (50)
<i>O. sokolowskii</i>	Mean of No. of parasitoid	0.36±0.03b	0.55±0.03ab	0.67±0.03b	0.56±0.03ab	0.31±0.03b	3.03* (50)
	Parasitism rate	8.40±1.08c	20.19±1.71a	21.48±1.32a	12.59±1.10b	3.69±0.69d	60.85** (50)

* and ** significantly at 5% and 1% level, respectively ^{ns} not significant.
Similar letter in each row show no significant difference between study sites.

3.2 Number of DBM and Its Parasitoids per Plant

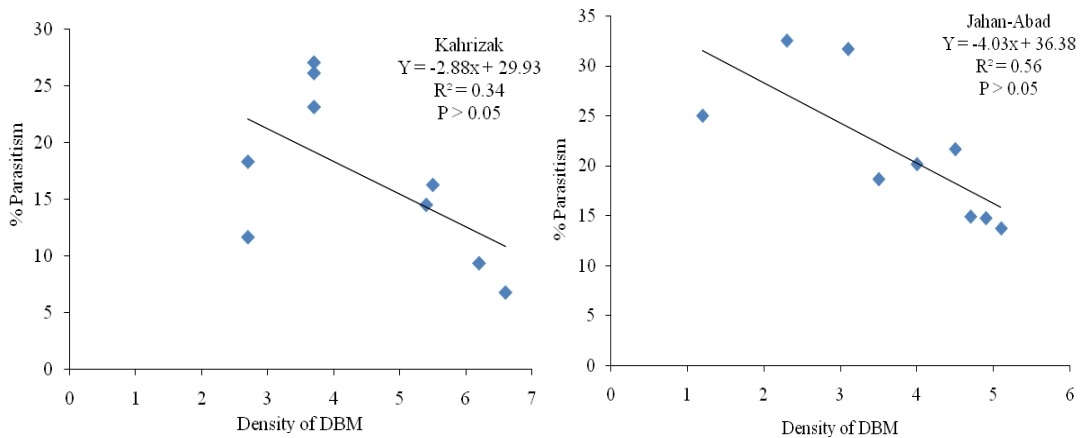
Palayin field had the highest larval density and Jahan-Abad had the lowest larval density per plant (Table 1). Highest and lowest of pupal density per plant was also observed in Palayin and Jahan-Abad, respectively. The larval and pupal density and their total numbers in the Shahed field (no spraying) was two times less than that observed in Palayin and Shokr-Abad farms.

The highest percentage of parasitism by *D. anurum* was observed in Jahan-Abad and the lowest was observed in Palayin farm (Table 2). The highest and the lowest percentage of parasitism by *C. plutellae* were observed in Jahan-Abad and Palayin farms, respectively. The *O. sokolowskii* parasitoid species had the highest activity in Kahrizak and the lowest in Palayin farm. Since activity of these three parasitoids is much less in Palayin and Shokr-Abad farms where the highest spraying occurred, it seems that repeated spraying is a major cause for the reduction of activity of the parasitoids of the diamondback moth in different farms.

3.3 Reactions of DBM Parasitoids to Host Density in Different Regions

3.3.1 Reactions of *D. anurum* to host density

The Regression relationship between pest density and parasitism rate by *D. anurum* in different regions are shown in Fig. 1. As can be seen in the figure, regression relationship between pest density and parasitism rate was not significantly different in different regions, and this indicates that the behaviour of *D. anurum* has no particular dependence on diamondback moth larval density.



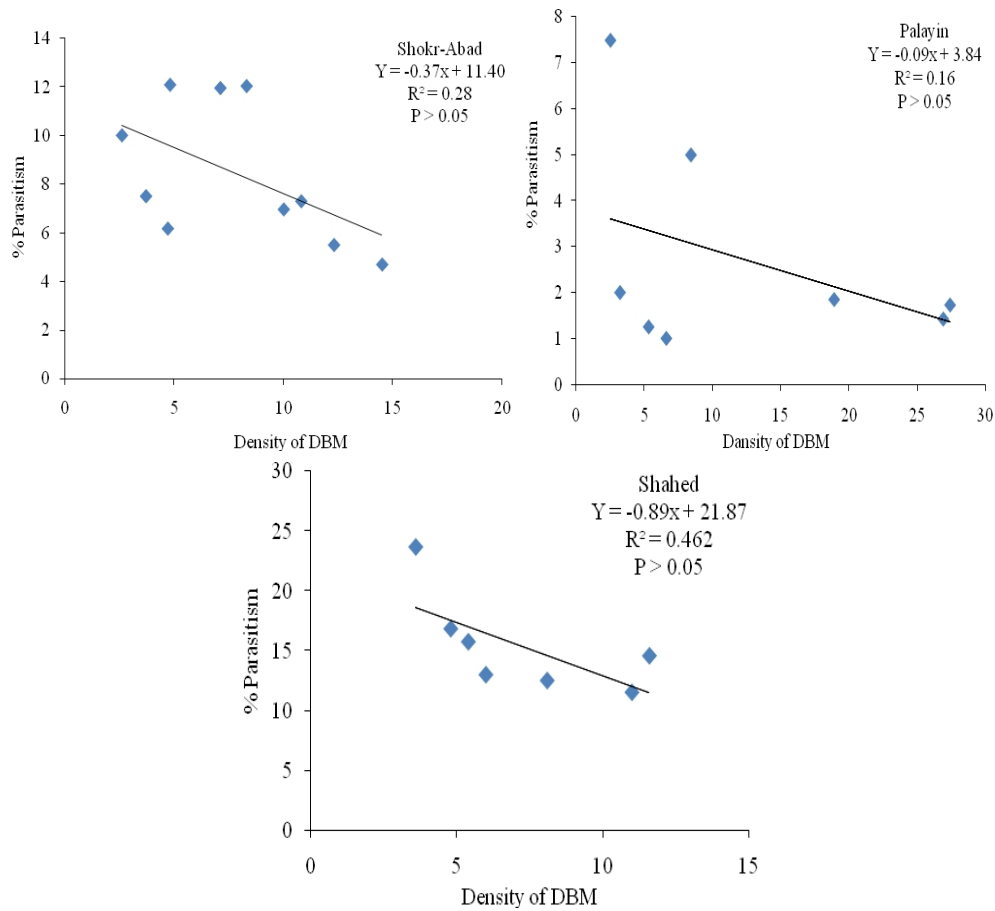


Fig. 1. Regression relationship between DBM density and parasitism rate (%) of *D. anurum* in different studied regions

3.3.2 Reactions of *C. plutellae* to host density

The Regression relationship between pest density and parasitism rate by *C. plutellae* in different regions is shown in Fig. 2. Regression relationship between pest density and parasitism rate in Kahrizak and Shokr-Abad fields was significantly different. Since the R² is small, this model is not a good representative for reactions of parasitoid to pest density. Possibly, other factors apart from pest density have been effective on parasitoid activity.

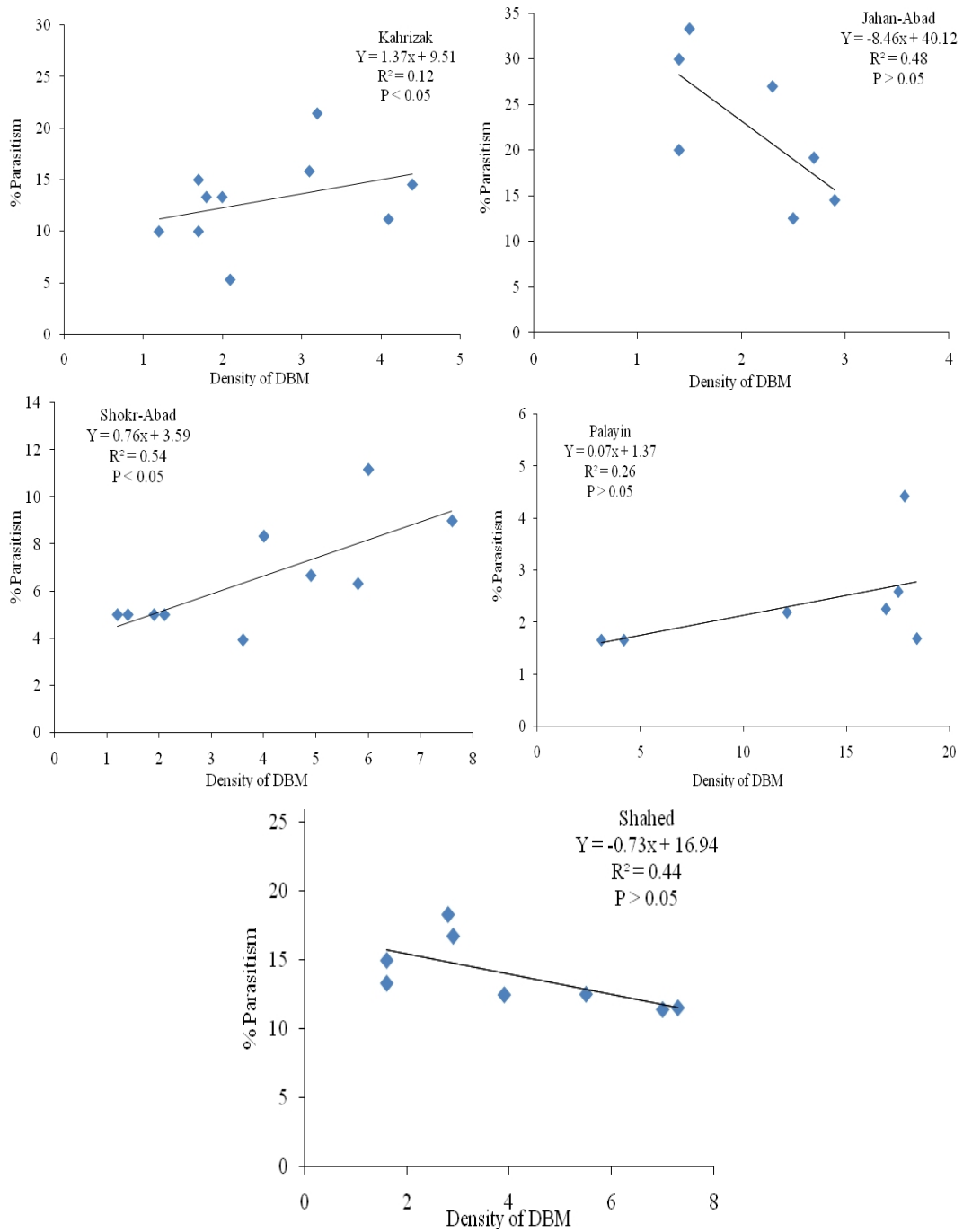


Fig. 2. Regression relationship between density of DBM and parasitism rate (%) of *C. plutellae* in different studied regions

3.3.3 Reactions of *O. sokolowskii* to host density

The Regression relationship between pest density and parasitism rate by *O. sokolowskii* in different regions is shown in Fig. 3. Regression relationship between pest density and parasitism rate was not significantly different in any farms except Shokr-Abad and Jahan-Abad. But regression between parasitism rate of *O. sokolowskii* and pest density was significantly different in Shokr-Abad and Jahan-Abad regions. Since the slope of the regression line is negative, the relationship between pest density and parasitism rate were opposed. Namely, the percentage of parasitism decreased when the pest density increased. Since R^2 is high, this model can explain this relationship.

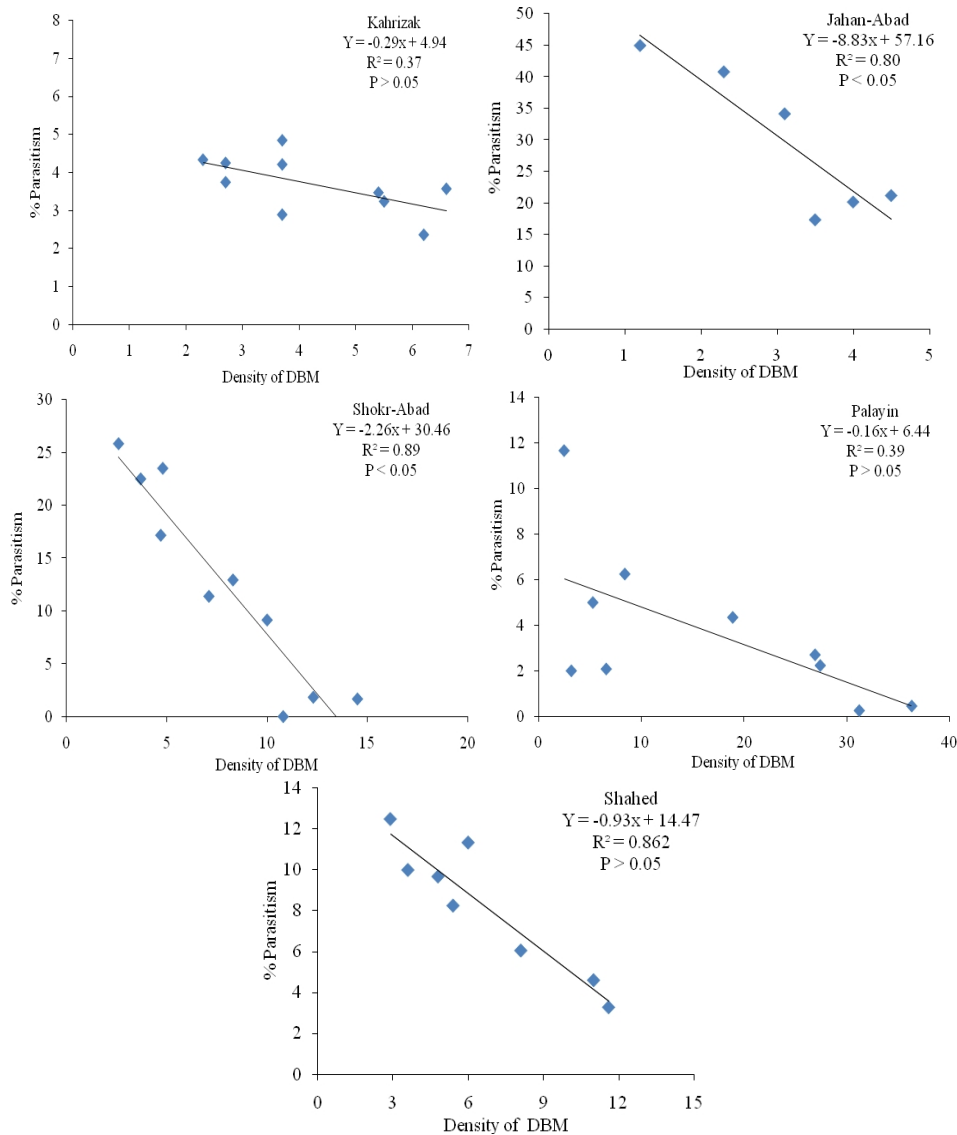


Fig. 3. Regression relationship between the DBM density and parasitism rate (%) of *O. sokolowskii* in different studied regions

3.4 Correlation between Different DBM Larval Densities and Parasitism Rate In The Recall Method

According to Table 3, there are significant differences in the regression between different densities of larvae and the percentage of parasitism of *D. anurum* on 11. Aug., 1. Sep. and 1. Nov. ($P = .05$). The slope of the regression line is negative in the relationship between density and parasitism rate, which show that there is an inverse relationship between density and the percentage of parasitism. Given that the correlation coefficient has a positive value on these three dates ($R^2 \geq 0.7$), it can be concluded that parasitism by this parasitoid decreased with the density increase of the diamondback moth larvae.

Regression between different densities of larvae and the percentage of parasitism of *C. plutellae* has shown significant difference on the three dates at the end of the study. The slope of the regression line is positive between different densities of larvae and parasitism rate, which shows that the percentage of parasitism by this species has increased with a density increase of the pest. Given that correlation coefficient has a positive value in these three dates ($R^2 \geq 0.7$), it can be concluded that the percentage of parasitism by this parasitoid has increased with a density increase of the diamondback moth larvae.

Regression between different densities of larvae and the percentage of parasitism of *O. sokolowskii* has shown significant difference on all dates analysed. The slope of the regression line is negative in the relationship between density and parasitism rate which show there is an inverse relationship between the density of larva and the percentage of parasitism. Given that the correlation coefficient has a positive value on all dates ($R^2 \geq 0.7$), it can be concluded that the percentage of parasitism by this parasitoid has decreased with a density increase of the diamondback moth larvae.

Regression relationship between percentage of parasitism by all three parasitoids and different densities of larvae did not show significant differences.

Table 3. Correlation coefficient (Pearson correlation) between DBM larva density and parasitism rate (%) of parasitoids of with recall method in different sampling times

Parasitoid	Sampling times				
	11 Aug.	1 Sep.	21 Sep.	20 Oct.	1 Nov.
<i>D. anurum</i>	-0.81(13.44)*	-0.83(14.78)*	-0.39(1.99) ^{ns}	-0.41(2.09) ^{ns}	-0.86(19.78)*
<i>C. plutellae</i>	0.9(1.24) ^{ns}	-0.67(6.34) ^{ns}	0.85(17.70)*	0.94(52.33)**	0.85(17.39)*
<i>O. sokolowskii</i>	-0.73(8.29) ^{ns}	-0.91(31.57)*	-0.72(7.72) ^{ns}	-0.79(11.58)*	-0.88(23.47)*
Total	-0.66(6.06) ^{ns}	-0.68(19.81)*	-0.24(0.97) ^{ns}	0.41(2.12) ^{ns}	0.17(0.65) ^{ns}

* and ** significantly at 5% and 1% level, respectively.
^{ns} not significant.

In this study the density of larvae, pupae and both stages in total were determined in studied regions as 3.05, 2.26 and 7.54 (per plant), respectively. The calculated density of larvae and pupae and total different growth stages in the Karaj region [14], was 29.01, 10.68 and 37.09 (per plant), respectively. In another study, the density of larvae and pupae was calculated as 0.60 and 5.10 (per plant), respectively [19]. Among parasitoids of the diamondback moth, the highest density was belonged to *Diadegma*, *Cotesia* and *Oomyzus* in Iran [2]. The percentage of parasitism in all regions by *D. anurum*, *C. plutellae* and *O. sokolowskii* and total parasitism by these three species were determined as 12.67, 10.29,

13.27 and 36.23 percent (per plant), respectively in this study. Golizadeh [14]; Mitchell et al. [20] and Berlandier and Cousins [21] have reported the percentage of parasitism by *D. anurum* to be 42.39, 35 to 76 and 39%, respectively. The percentage of parasitism by *C. plutellae* is 13.64, 72 and 30.84% in Karaj, India and China, respectively [22,14]. The percentage of parasitism by *O. sokolowskii* was reported as 11 to 15 and 37.10% in different regions [23,24]. Parasitism rate by the whole complex of parasitoids was calculated as 90 to 100% and 83 to 92% [25,26]. According to obtained information, it can be seen that spraying operations in different study areas have not reduced the population of the diamondback moth, but instead activity of all three parasitoid species were reduced. So it can be argued that the diamondback moth probably has a type of resistance to different pesticides used in these regions. The results of this study showed that any relationship was not observed between the diamondback moth density and the parasitism rate by different parasitoids in cauliflower fields in the south of Tehran. Different densities of eggs of *Empoasca decipiens* have a significant negative effect on the parasitism rate by *Anagrus atomus*, resulting in higher parasitism at lower densities [27]. *D. anurum* is not present in high numbers in natural conditions in Karaj, and this is probably due to resistance to insecticides in the DBM moth and sensitivity of the *D. anurum* parasitoids to pesticides in the DBM [22]. Parasitism rate of the alfalfa leaf weevil by *Oomyzus incertus* is higher at higher density of the pest than at lower density [28]. Golizadeh [14] calculated the regression relationship between pest density and parasitism rate, and concluded that the percentage of parasitism of the diamondback moth is independent of the different density of its pests during the season. The results of the pest density regression and parasitism rate with the recall method of parasitoids at different densities of pest showed that parasitism was decreased with increasing pest density of *D. anurum* and *O. sokolowskii* parasitoid species. But percentage of parasitism by *C. plutellae* was increased with increasing pest density. Hasanshahi et al. [17] studied density of *P. xylostella* on eight cauliflower cultivars and showed that Buris and Snow crown cultivars had the lowest infestation and had a kind of resistance to DBM. Hasanshahi et al. [29] studied parasitism activity of *D. anurum* on different cauliflower cultivars, and it was shown that the highest parasitism rates were on the Buris and White cloud cultivars ($19.92 \pm 1.06\%$ and $16.20 \pm 1.49\%$, respectively) and the lowest parasitism rates were on the Snow crown and SG cultivars (3.42% and 5.00% , respectively) during the season. The effect of different cauliflower cultivars on biological parameters of the *Brevicoryne brassicae* (L.) (Hem: Aphididae) was studied under controlled conditions. The results showed that using different cauliflower cultivars affected the adult biological parameters [30]. Askarianzadeh et al. [31] studied density of *P. xylostella* on different canola cultivars, and Jahan et al. [32] studied the *B. brassicae* and its parasitoid *Diaeretiella rapae* density on eight cauliflower cultivars. They showed that using Smilla, Buris and SG cultivars probably increase the *D. rapae* efficiency in controlling the *B. brassicae*. In our study, plant resistance changed with plant phenology. Other studies also showed that plant resistance changes with plant phenology and effectiveness on insect biological parameters [33,30].

4. CONCLUSION

The number of larval and pupal stages based on unit density, between all experimental regions and Shahed University station (no spraying) were significantly different. The highest percentage of parasitism was caused by *D. anurum* in Tehran. The spraying did not reduce the population of diamondback moth in some farms in Tehran. Also, the repeated spraying operation is a major cause in the reduction of the activity of the parasitoids of DBM.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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