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Influence of Grapevine Cultivar on Population Levels of *Lobesia botrana* (Lepidoptera: Tortricidae) and Effectiveness of Insecticides in Controlling This Pest

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Abstract: The European grapevine moth, *Lobesia botrana* (Denis and Schiffermüller) (Lepidoptera: Tortricidae), is the most critical pest of vineyards. In the present study, pheromone-baited traps were applied in 2021 and 2022 to monitor the moth population dynamics and to determine the number of *L. botrana* generations. The number of eggs and larvae was also counted in four vineyards with Askari, Yaghooti, Keshmeshi, and Fakhri cultivars. Moreover, the morphological properties of clusters were evaluated in different grape cultivars to find out the susceptible cultivar to *L. botrana.* In 2022, different insecticides were used in the Askari cultivar vineyard, and larval damage level was assessed. Three generations were recorded in all vineyards each year. The population of males was not affected by the cultivar. In contrast, the population density of eggs and larvae was significantly higher in Yaghooti than in other tested cultivars. It could be attributed to the cluster compactness and thin skin of berries in Yaghooti, which makes it more susceptible to *L. botrana* infestations. In contrast, the lowest eggs and larval population density was reported in the Fakhri cultivar indicating the tolerance of this cultivar compared to the other tested cultivars. The field trial showed that the application of insecticides in the second and third generations reduced the damage level of *L. botrana*. The rotation of insecticides with different modes of action in consecutive generations of *L. botrana* can be used to reduce damage levels.

Keywords: cultivar; pheromone; peak flight; insecticides; generation

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1. Introduction

Grapevine, *Vitis vinifera* L. (Vitaceae), is an economically important fruit plant. There are about 60 species in the genus *Vitis*, and more than 40,000 grapevine cultivars have been identified worldwide [1]. Grapes are divided into wine and table categories. Wine grapes are smaller than table ones, they have numerous seeds and thick skins. Most table grapes are seedless, with less juice, less acidity and sugar, and more pulp with thinner skins [2]. Iran is the most important viticultural region of Asia since about 800–1000 grapevine cultivars are cultivated in Iran [3]; and are classified into three categories, including, table, juices, and dried into raisins [4].

The European grapevine moth, *Lobesia botrana* (Denis and Schiffermüller) (Lepidoptera: Tortricidae), is one of the major pests of grapevine worldwide. The first generation causes damage to the blossoms and newly set berries, while the high damage level is associated with the second and third generations, which cause yield losses [5–7]. Immature berries can become infested with second-generation larvae, which become active after fruit setting and

continue their feeding until the fruit ripens. These larvae not only consume fruit contents but also target the seeds. Additionally, under hot weather conditions, the berries are prone to drying rapidly, creating unfavorable conditions for the growth of saprophytic fungi [8]. Mature berries are infested with third-generation larvae, causing severe direct damage by feeding mature berries, as well as reducing the quantity and quality of the product [9]. These larvae provoke indirect damage by weaving webs, contaminating the product due to fungal infections (the grey mold fungus *Botrytis cinerea* Pers.Fr.), and reducing yield and fruit market value [5,10]. These larvae provoke indirect damage by weaving webs, contaminating the product due to fungal infections (the grey mold fungus *Botrytis cinerea* Pers.Fr.), and reducing yield and fruit market value [11]. The damage rate of *L. botrana* is directly related to the number of generations and the population density within each generation [12]. The number of generations per year reported for this pest differs depending on the climatic conditions and latitude [13,14].

Grape cultivars can affect the susceptibility to *L. botrana* and can be considered in control strategies for this pest. In the susceptible cultivars, the population and infestation level of *L. botrana* were higher than those with less susceptibility [12]. In addition, the egg-laying preference, activity level, and survival of the larvae are the factors that can determine the susceptibility of cultivars to *L. botrana* [12,15,16].

According to the economic damage level of *L. botrana*, chemical control should be considered in an integrated management strategy to reduce population density and fruit damage of this pest. For successful control of the pest, the development stages of *L. botrana* should be monitored in different generations to determine the appropriate time for chemical control. Monitoring is possible by counting the number of male moths captured in pheromone traps, as well as counting the number of eggs and larvae [17]. Pheromone-baited sticky traps with sex attractants are widely used to monitor population dynamics and flight activity of moths [18]. Ioriatti, et al. [19] noted that a prerequisite for successful control of *L. botrana* is monitoring the population with pheromone traps and scouting oviposition of the pest in the field to predict the appropriate time for control. Several insecticides are used against *L. botrana*, which can be applied depending on the generation of the pest and the sensitivity of the cultivar [20]. First-generation larvae of *L. botrana*, especially in grape cultivars with abundant inflorescences, may not require control with chemical insecticides. Grapevine plants can overcompensate for the damage caused by *L. botrana* between flowering and harvest, by increasing the weight and size of healthy berries [19]. According to Ioriatti, et al. [21], for subsequent larval generations, the injury threshold varies between 1% and 5%, or 10% and 15% of damaged clusters depending on the cultivar, grade of bunch tightness, and harvest time.

The effect of various chemical insecticides including methoxyfenozide [22], lufenuron [23], lufenuron + fenoxycarb [24], lufenuron, indoxacarb, spinosad, deltamethrin, cypermethrin, chlorpyrifos [20], and pyrethrin, indoxacarb, spinosad [25] have been investigated on different generations of *L. botrana*. Also, appropriate control of *L. botrana* was achieved by biological insecticides such as *Bacillus thuringiensis* [20,25,26], azadirachtin [20,25,27], kaolin [28], and the entomopathogenic fungus *Beauveria bassiana* (Balsamo) (Ascomycota: Hypocreales) [25].

The current study aimed to determine the male moths' peak flight for each generation using pheromone traps, and the eggs and larvae population dynamics in 2021 and 2022. The research was undertaken in four vineyards to evaluate the susceptibility of four grapevine cultivars to *L. botrana* based on the morphological properties of clusters. Successively, the effectiveness of different insecticides was evaluated to control and reduce damage levels of the second and third generations of *L. botrana*.

2. Materials and Methods

2.1. Vineyards

This research was conducted in four vineyards with Askari, Yaghooti, Keshmeshi, and Fakhri cultivars, in 2021 and 2022. All the vineyards are located in Saifabad village,

Kamalvand district, Khorramabad, Iran. The geographical characteristics of the selected vineyards are shown in Figure 1. For each vineyard, one hectare was examined to carry out the research. In these vineyards, the distance between rows was 3 m and 2 m between grapevines in each row.

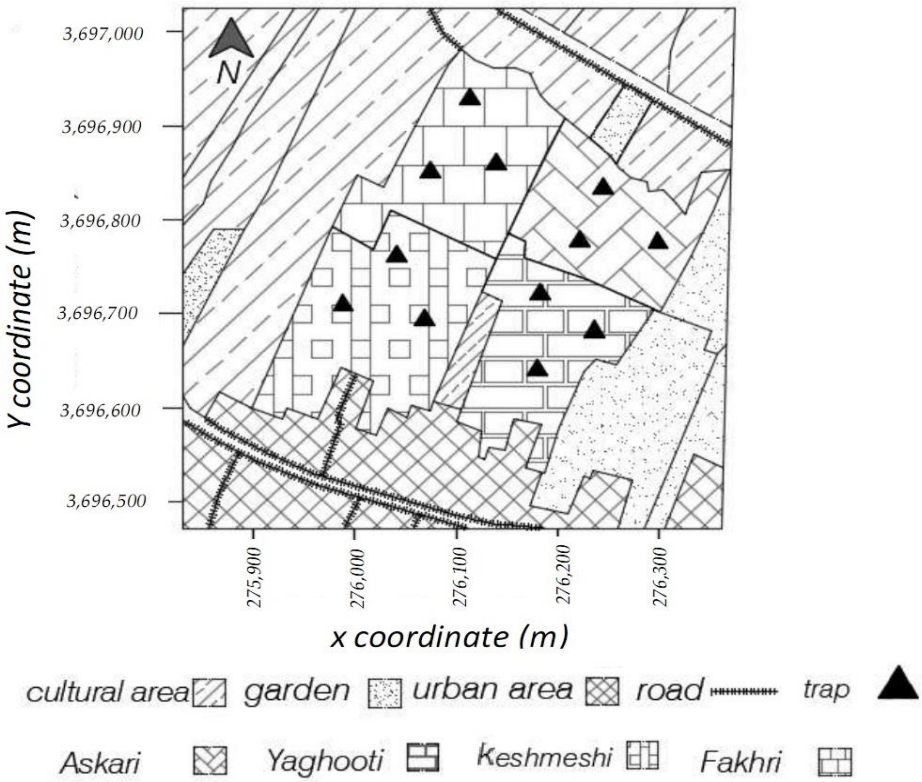


Figure 1. Map of the experimental area with an indication of vineyard position.

2.2. Population Dynamics of Lobesia botrana Different Life Stages

The population dynamics of *L. botrana* males, eggs, and larva was monitored in 2021 and 2022. For monitoring male moths, the sex pheromones of *L. botrana* were obtained from Zist Bani Paya Company (Trifolio®, Lahnau, Germany license) and used in the experiments. In both years, three pheromone-baited sticky traps of the delta type (8 cm height, 12.5 cm length, and 9 cm width) were installed in each vineyard, at a distance of 100 m from each other and 50 m from the edge of the vineyards. Traps were attached in the middle of the grapevine canopy 80 cm from the ground. Pheromone dispenser capsules containing 500 mg of pheromone were placed in the center of the traps. These traps contain female sex pheromones and attract male moths. In both years, the pheromone lures were replaced every month, and the sticky cards of the traps were replaced every 2 weeks. The traps were monitored every five days, and the number of captured male moths in each trap was counted and then removed. Monitoring was conducted from the beginning of April until the end of the harvesting time in mid-September.

To check the number of eggs, a systematic sampling method was performed according to [29]. Three rows were randomly selected, and three grapevines were sampled from each row and three clusters from each grapevine (3 rows × 3 grapevines × 3 clusters = 27 clusters). For the first generation, the clusters were placed in a plastic bag, and the information label (including the location and time) was written and transported to the laboratory. The clusters were examined under a stereomicroscope (SMZ800 Nikon, Tokyo, Japan) in the laboratory, and the egg numbers in each cluster were counted. In the other generations, 27 clusters were monitored using a hand lens (×10 magnification), and the number of eggs was recorded.

To evaluate the larval population in the first to third generation, three rows were randomly selected, and the number of larvae and larval nests were counted in three grapevines from each row [29]. The first-generation larvae feed on flowers, and the fourth and fifth-larval instars build nests in young flower buds [30]. The second-generation larvae penetrate the berries create an entrance hole on each berry and feed on its contents. Therefore, the existence of this hole indicates the presence of one larva in the berry. The third-generation larvae infested the ripening berries [8]. Each *L. botrana* larva develops in one grape bunch, so the damage in each bunch indicates the presence of one larva [31]. In addition, for all generations, the fourth and fifth larval instars build nests, and each larva is present and active in one nest [30]. Therefore, in this study, the live larvae, the presence of a hole in the berries, and the larval nest were considered as the presence of one larva.

The data logger '(Zarin Afzar Co., Mashhad, Iran) was installed in Saifabad village, Kamalvand district on 4 March 2021 and 4 March 2022 to report the temperature and relative humidity for two consecutive years.

2.3. Cluster Analysis

Some morphological properties of clusters in different grape cultivars, at harvest time, were characterized by the Fermaud [16] method. For this purpose, nine clusters (3 grapevines × 3 clusters = 9 clusters) of each cluster were sampled, and the number of berries in each cluster, the cluster weight, and rachis length (total length of the main cluster axis and first ramification) were measured. The compactness index was calculated by dividing the cluster weight by the rachis length. In addition, nine clusters were selected from each cultivar, three berries were separated from each cluster and the berries' weight and skin weight were measured. The skin-to-berry weight ratio is also calculated, indicating the index for the thickness of the berries' skin.

2.4. Application of Insecticides for Controlling Lobesia botrana

The effectiveness of different insecticides in controlling and reducing *L. botrana* population was evaluated based on the method of Civolani, et al. [32] with some modifications. Considering that the Askari was the dominant cultivar in this region, this experiment was conducted in the Askari cultivar vineyard. The experiment was conducted in a randomized complete block design, with three replications (rows) for each treatment. Each row consisted of six grapevines. An untreated row was left between each treatment to separate treatments from each other and also to prevent drift and the possibility of contamination with another treatment. The insecticides used in the experiments are presented in Table 1. The experimental treatments were applied as presented in Table 2.

Table 1. Insecticides used in the vineyards, and their recommended concentrations for controlling *Lobesia botrana* under field conditions.

Insecticides	Formulation	Trade Name	Recommended Concentrations (ppm)	Company
Bacillus thuringiensis subsp. *Kurstaki* (*Bt*)	SC 24%	Biolep	2000	Biorun, Iran
Alpha cypermethrin + teflubenzuron	SC15%	Imunit	750	BASF, Germany
Trichlorfon	SP 80%	Dipterex	1500	Arman Sabz Adineh, Iran
Phosalone	EC 35%	Zolone	1500	Mahan, Iran
Lufenuron + fenoxycarb	EC 10.5%	Lufox	300	Syngenta, Germany

The time of insecticide application to control *L. botrana* second and third generations was considered 12 days after the males' peak flight [20]. Therefore, insecticides were applied on 2 July 2022 (temperature: 29.05 °C, humidity: 17.63%) and 26 August 2022 (temperature: 30.36 °C, humidity: 13.86%) for controlling second and third generations of *L. botrana*, respectively. Spraying insecticides for the second and third generations was performed at 6.00 am using a 20-litre rechargeable knapsack sprayer (Seaflo, Fujian, China). The sprayer tank was washed twice after each treatment to remove the residue of the insecticides [20]. Water was used for the control treatment. Damage assessment was performed based on the

method of Vassiliou [9] with some modifications on the day of spraying, 7 and 14 days after each insecticide application. The systematic sampling method was performed according to [29]. For each treatment, three rows were selected, and three grapevines and three clusters from each grapevine were sampled (3 rows × 3 grapevines × 3 clusters = 27 clusters) to evaluate damage caused by *L. botrana*. The tested clusters were observed for the existence of at least one live larva or larvae nest.

Table 2. The order of spraying insecticides for controlling the second and third generations of *Lobesia botrana* under field conditions.

Treatments	Second Generation	Third Generation
1	Control (water)	Control (water)
2	*Bt*	Alpha cypermethrin + teflubenzuron
3	*Bt*	Trichlorfon
4	*Bt*	Phosalone
5	Lufenuron + fenoxycarb	Alpha cypermethrin + teflubenzuron
6	Lufenuron + fenoxycarb	Trichlorfon

2.5. Statistical Analysis

The data were checked for normality using the Shapiro-Wilk test at $p = 0.05$. The data were normal, and no transformation was performed. The numbers of trapped males, immature stages (eggs and larvae), and clusters' morphological properties were analyzed using a one-way ANOVA. The efficacy of the insecticides over time was analyzed through generalized linear models (GLM). The random variables were time (day), treatments, and generations. Means were compared by Tukey–Kramer (HSD) test at $p = 0.05$, using SPSS software version 16 [33].

3. Results

3.1. Population Dynamics of Lobesia botrana Different Life Stages

In 2021, three flight peaks were recorded corresponding to three generations of *L. botrana*. In all cultivars, capture started on 4 April 2021, and after that, the number of captured male adults in the traps increased, and on 19 April 2021, the flight peak of the first generation occurred at the time of the bud burst. At this date, each trap caught a mean number of 59.00 ± 7.09, 65.33 ± 7.53, 63.33 ± 8.33, and 63.66 ± 5.33 males per trap in Askari, Yaghooti, Keshmeshi, and Fakhri vineyards, respectively. After that, the population of male adults gradually decreased, indicating the end of the first-generation population. In mid-June, the number of males captured in the traps increased again, indicating the occurrence of the second generation at the time of ripening. On 18 June 2021, the mean number of 49.00 ± 4.72, 50.00 ± 7.09, 56.33 ± 4.17, and 53.33 ± 14.53 males per trap were reported in the traps installed in Askari, Yaghooti, Keshmeshi, and Fakhri vineyards, respectively. In August, the number of captured males increased, and the flight peak of the third generation was observed on 7 August 2021. At this date, the mean number of 37.66 ± 9.53, 47.66 ± 3.84, 43.66 ± 2.96, and 56.66 ± 4.40 males per trap were captured in each trap in Askari, Yaghooti, Keshmeshi, and Fakhri vineyards, respectively (Figure 2; Table 3).

In 2022, similar to 2021, three flight peaks were observed corresponding to three generations of *L. botrana*. In all cultivars, males captured started on 11 April 2022, and after that, the number of captured male adults in the traps increased, and after that the number of captured male adults in the traps increased, and on 6 May 2022, the flight peak of the first generation occurred. At this date, each trap caught a mean number of 27.33 ± 4.33, 23.67 ± 4.66, 22.00 ± 3.00, and 28.66 ± 4.09 males per trap in Askari, Yaghooti, Keshmeshi, and Fakhri vineyards, respectively. The number of males captured in the traps increased again, indicating the second flight on 20 June 2022. The mean number of 57.33 ± 4.33, 53.33 ± 8.25, 59.33 ± 4.05, and 56.66 ± 6.00 males per trap were reported in the traps installed in Askari, Yaghooti, Keshmeshi, and Fakhri vineyards, respectively.

In the mid-August, the flight of third-generation moths takes place. On 14 August, the mean number of 110.67 ± 5.78, 105.00± 5.00, 105.00 ± 7.63, and 113.33 ± 8.82 males per trap were captured in each trap in Askari, Yaghooti, Keshmeshi, and Fakhri vineyards, respectively. There was a sudden increase in the number of caught males from 3 September to 13 September 2022, and after that, the population decreased. It was not considered a generation (Figure 3; Table 3).

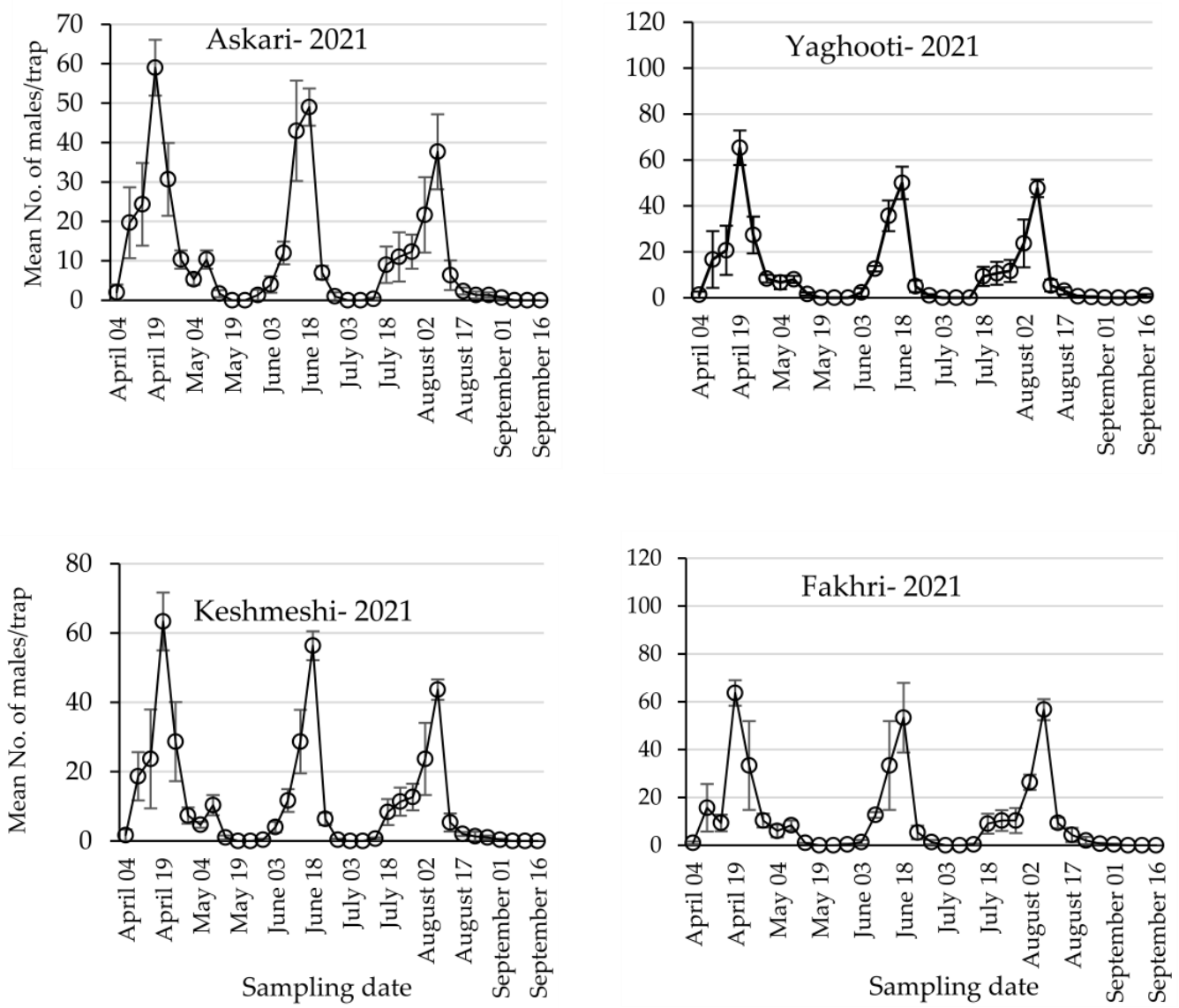


Figure 2. Mean number (±SE) of *Lobesia botrana* adult males captured per trap in the untreated vineyards (2021).

Table 3. Mean number (±SE) of males/trap/in different varieties at the start of each generation.

Year	Generation	Askari	Yaghooti	Keshmeshi	Fakhri	$F_{3,8}$, p
2021	First	59.00 ± 7.09	65.33 ± 7.53	63.33 ± 8.33	63.66 ± 6.33	0.135, 0.937
	Second	49.00 ± 4.72	50.00 ± 7.09	56.33 ± 4.17	53.33 ± 6.00	0.353, 0.788
	Third	37.66 ± 9.52	47.66 ± 3.84	43.66 ± 2.96	56.66 ± 6.00	1.691, 0.246
2022	First	27.33 ± 4.33	23.66 ± 4.66	22.00 ± 3.00	28.66 ± 4.09	0.582, 0.643
	Second	57.33 ± 4.33	53.33 ± 8.25	59.33 ± 4.05	56.66 ± 6.00	0.178, 0.908
	Third	110.67 ± 5.78	105.00 ± 5.00	105.00 ± 7.63	113.33 ± 8.82	0.360, 0.784

No significant differences were observed in each row using the Tukey–Kramer (HSD) test at $p = 0.05$.

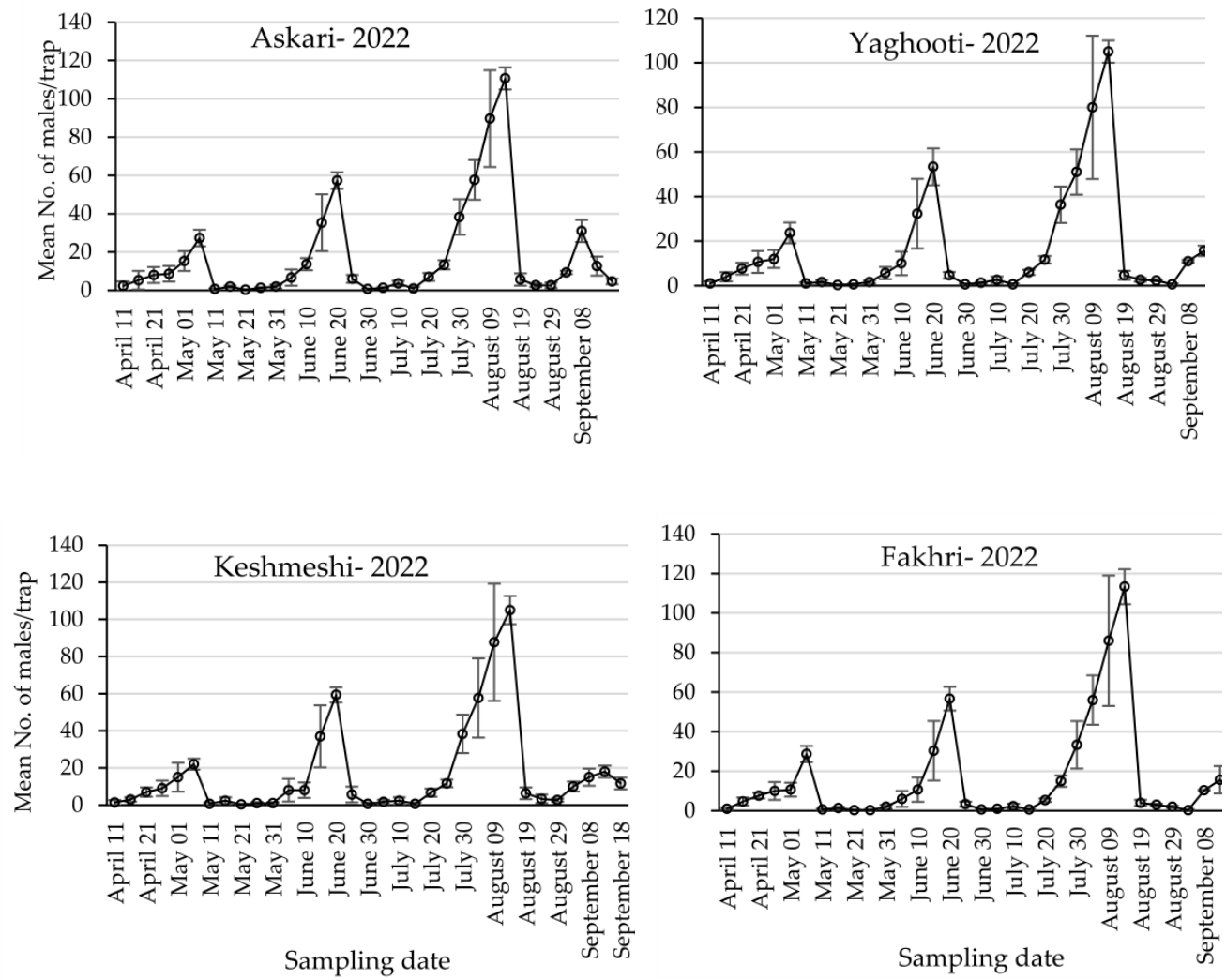


Figure 3. Mean number (±SE) of *Lobesia botrana* adult males captured per trap in the untreated vineyards (2022).

In 2021, the mean number of eggs and larvae was assayed in Askari, Yaghooti, Keshmeshi, and Fakhri vineyards. The maximum number of eggs in the first generation was on 29 April 2021 for Askari, Yaghooti, and Fakhri cultivars and on 24 April 2021 for Keshmeshi cultivars. The second generation of *L. botrana* laid a mean number of 2.11 ± 0.61, 2.22 ± 1.31, 1.33 ± 0.29, and 1.77 ± 0.55 eggs per cluster in the Askari, Yaghooti, Keshmeshi, and Fakhri vineyards, respectively. The highest number of eggs of the second generation in the Askari cultivar was observed on 28 June 2021; in the rest of the cultivars, it was recorded on 3 July 2021. Female moths started laying eggs for a third generation in mid-August, and for all examined cultivars, the maximum number of eggs was observed on 17 August 2021. In 2021, the maximum number of larvae for the first generation was reported on 9 May 2021 for the Yaghooti cultivar (2.44 larvae per cluster). In the second generation, the larvae hatched in early July, and the highest number of larvae was observed in Yaghooti (2.11 larvae per cluster), followed by Keshmeshi (2.00 larvae per cluster) cultivars. The third generation of larvae reaches its peak from late August to early September (Figure 4).

In 2022, the first generation of *L. botrana* laid a mean number of 1.11 ± 0.35, 1.55 ± 0.50, 1.66 ± 0.53, and 1.11 ± 0.31 eggs per cluster in Askari, Yaghooti, Keshmeshi, and Fakhri vineyards, respectively. The egg-laying occurred in mid-May to late May. Females of *L. botrana* started laying eggs for the second generation in late June, and the maximum

number of eggs was observed for Keshmeshi (1.66 eggs per cluster), followed by Yaghooti (1.55 eggs per cluster) cultivars. Eggs of the third generation were observed in mid-August. For all the examined cultivars, the first-generation larvae hatched from eggs on 26 May 2022 and continued to late June. The maximum number of larvae was reported in the Yaghooti cultivar (2.00 larvae per cluster). Second-generation larvae hatched in early July and continued to early August. Third-generation larvae were present in vineyards from late August to mid-September (Figure 5).

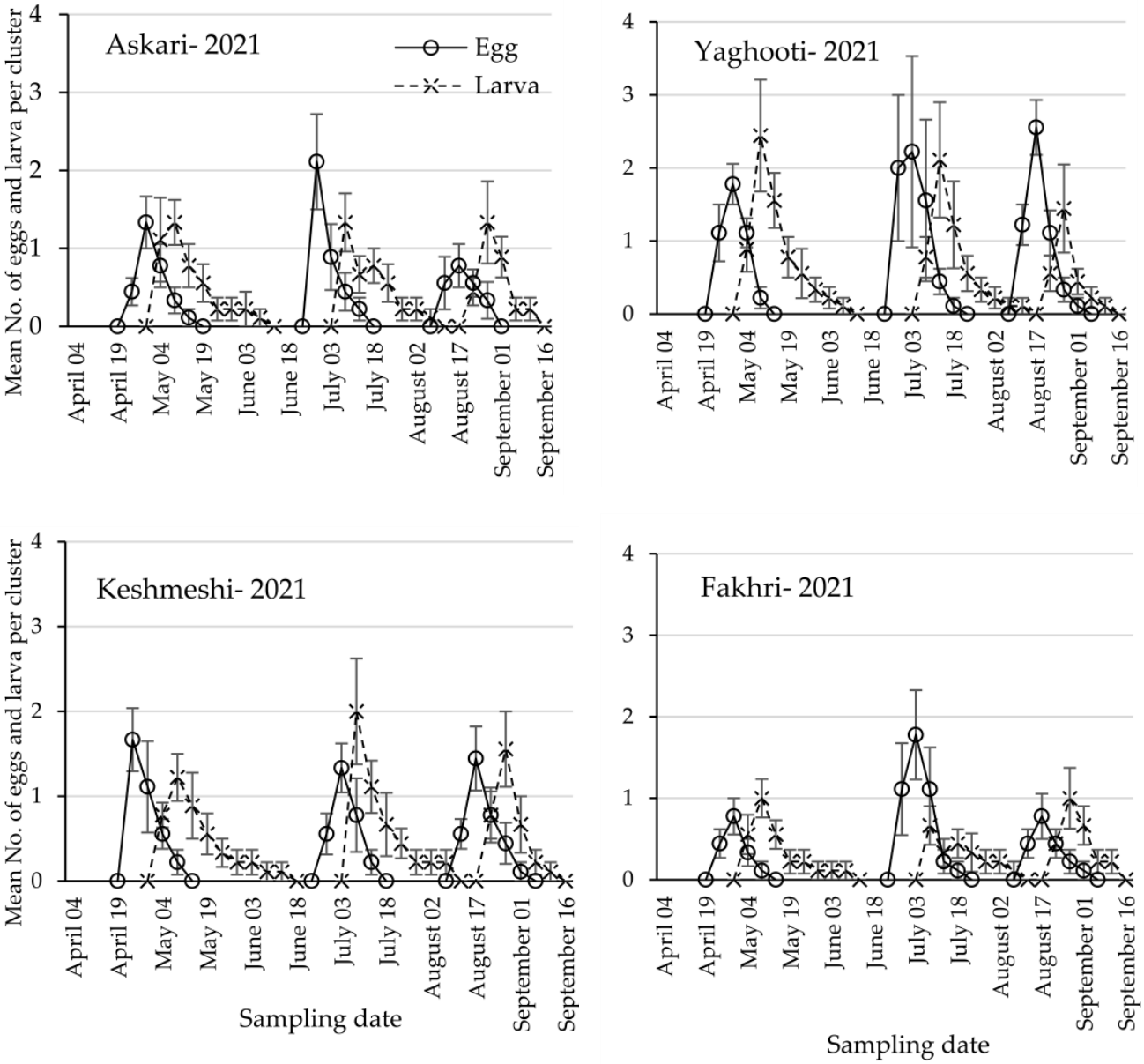


Figure 4. Mean number (±SE) of *Lobesia botrana* eggs and larvae in the untreated vineyards (2021).

The mean number of eggs laid by *L. botrana* in 2021 ($F_{3,8}$ = 4.534; p = 0.039) and 2022 ($F_{3,8}$ = 6.195; p = 0.018) was significantly different among cultivars. The highest number of eggs in both years was observed in the Yaghooti cultivar, while the lowest number of eggs was related to the Fakhri cultivar. The larval population was also significantly different among cultivars in 2021 ($F_{3,8}$ = 4.287; p = 0.044) and 2022 ($F_{3,8}$ = 3.942; p = 0.054). The highest number of larvae in both years was found in the Yaghooti cultivar, and the lowest was related to the Fakhri cultivar (Figure 6).

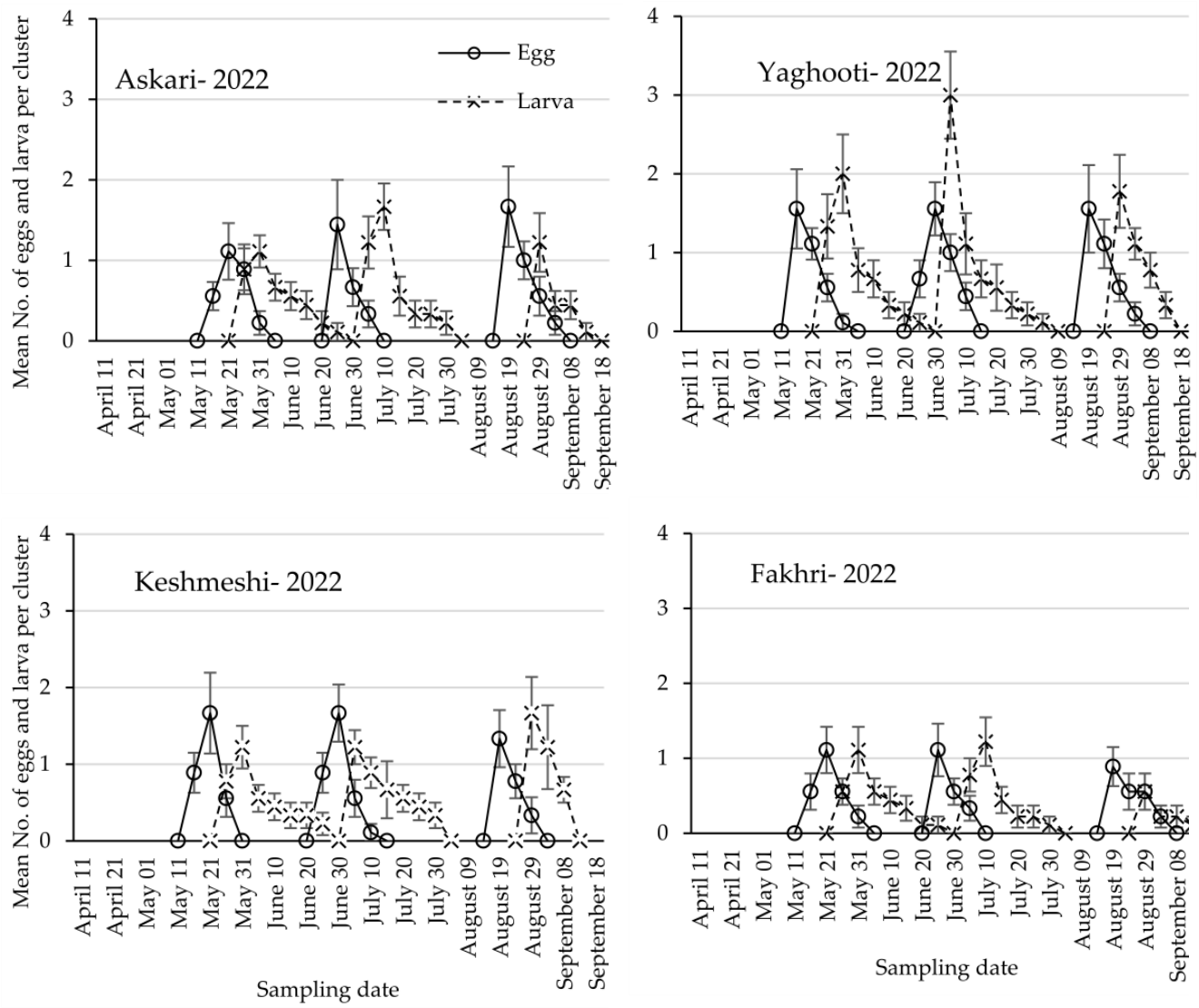
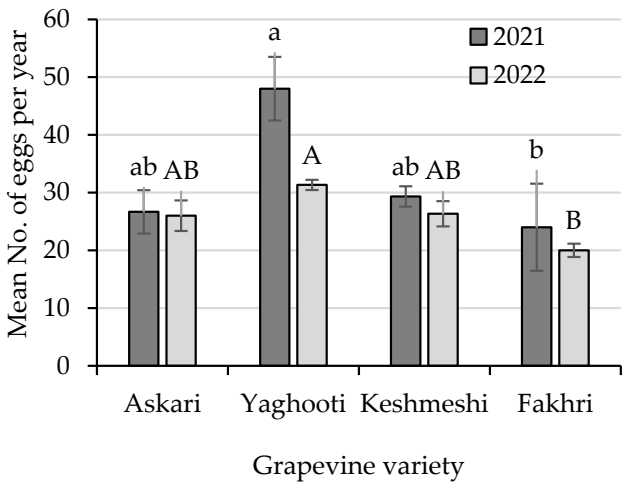


Figure 5. Mean number (±SE) of *Lobesia botrana* eggs and larvae in the untreated vineyards (2022).



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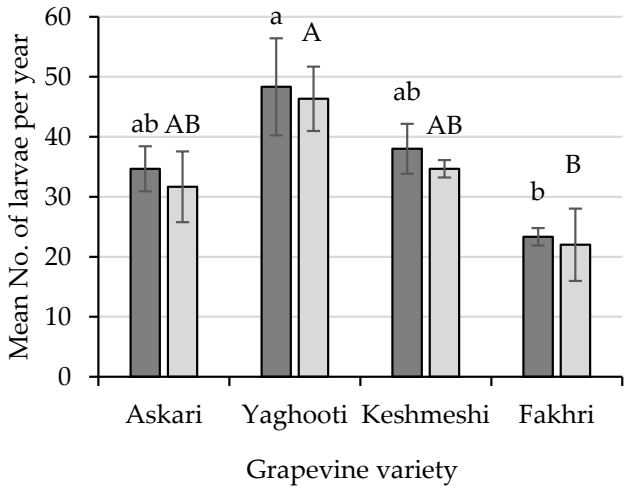

Figure 6. Mean number (±SE) of eggs, and first instar larvae in different varieties in the experimental years. Means followed by the same lowercase letters for 2021, and uppercase letters for 2022 are not significantly different using the Tukey–Kramer (HSD) test at $p = 0.05$.

In 2021, there was a small amount of rain. The relative humidity increased in mid-April and reached about 49.38%, but after that, it had a decreasing trend, so the percentage of humidity reached less than 30% from mid-May 2021. In 2022, a similar trend was reported, and the rainfall was very low. The relative humidity increased at the end of April and was reported above 60% for a week. But after that, there was a decreasing trend, so the percentage of relative humidity reached less than 30% from early May and remained at this level until the end of September (Figure 7).

3.2. Cluster Analysis

There were significant differences among cultivars in the cluster weight ($F_{3,32} = 11.111$; $p < 0.001$), rachis length ($F_{3,32} = 6.229$; $p < 0.001$), compactness index ($F_{3,32} = 14.428$; $p < 0.001$), berries skin weight ($F_{3,32} = 56.665$; $p < 0.001$), and skin to berry weight ratio ($F_{3,32} = 21.058$; $p < 0.001$). The lowest cluster weight was observed in the Fakhri cultivar, while there were no significant differences between the Askari, Yaghooti, and Keshmeshi cultivars in cluster weight. The rachis length was significantly higher in the Askari cultivar, followed by the Keshmeshi cultivar. The highest compactness index was reported in the Yaghooti cultivar, followed by the Keshmeshi cultivar. However, the compactness index was lowest in the Fakhri cultivar. The highest skin weight was related to Fakhri (0.29 ± 0.01 g), while the lowest amount was related to the Yaghooti cultivar (0.201 ± 0.004 g). Fakhri cultivar (0.15 ± 0.00), followed by Askari (0.13 ± 0.00) has the highest skin weight to berry weight ratio (Figure 8).

3.3. Application of Insecticides for Controlling *Lobesia botrana*

The results of the effect of different insecticides in reducing the damage level of *L. botrana* are presented in Table 4. The main effect of treatments was significant, while time and generation were not significant. The associated interaction of time with treatment on damage level was significant. However, the interactions of time with generation and time × treatment × generation were not significant (Table 4).

The highest percentage of *L. botrana* damage (28.39 ± 1.23%) in the second generation was reported in the control plots, 14 days after foliar spraying; however, there was no significant difference among the other treatments. For controlling the third generation of *L. botrana*, there was no significant difference between treatments one day after the application of insecticides. Seven days after foliar spraying insecticides, the highest damage (19.75 ± 1.23%) was reported in the control. The damage percentage increased significantly

with time, and 14 days after foliar spraying, *L. botrana* caused 29.62 ± 2.13% damage in the control plots (Table 5).

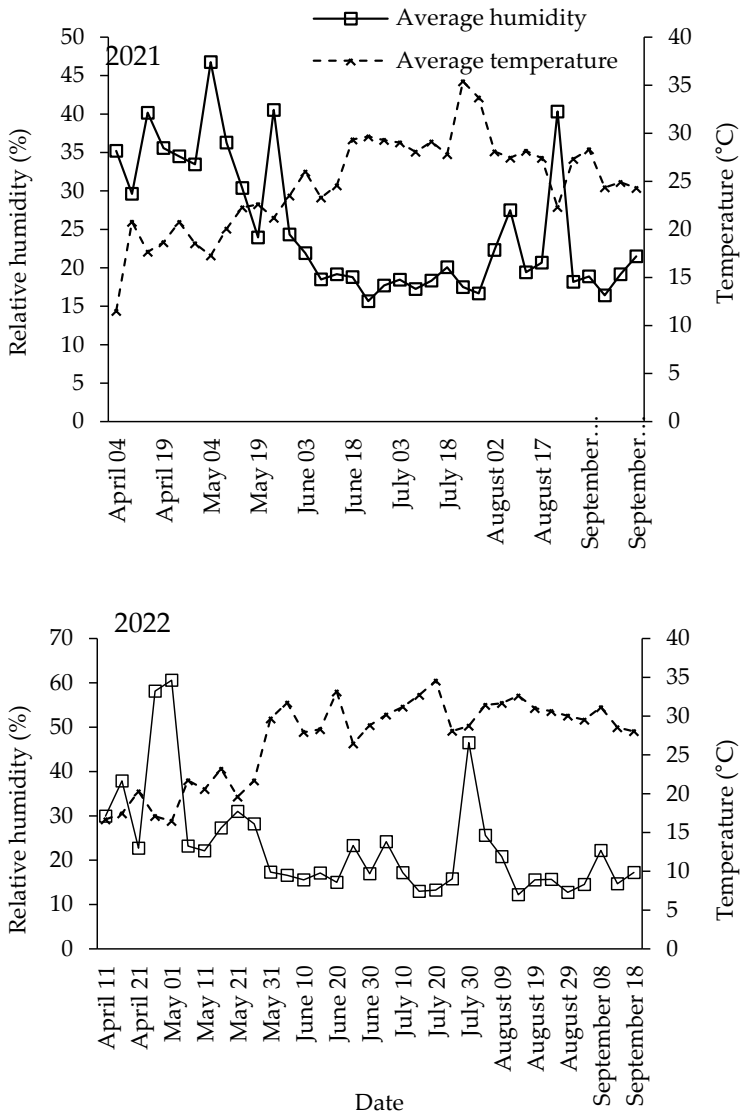

Figure 7. The mean temperature, and relative humidity in Saifabad village, Kamalvand district in 2021 and 2022.

Table 4. ANOVA parameters for main effects and associated interactions for clusters damaged by *Lobesia botrana* in vineyards treated with different insecticides, per generation.

Source	df	Mean Square	*F*	*p*
Time	2	4.699	0.617	0.543
Treatment	5	766.245	100.547	<0.001 **
Generation	1	4.572	0.600	0.441
Time × Treatment	10	155.896	20.457	<0.001**
Treatment × Generation	5	13.108	1.720	0.141
Time × Treatment × Generation	12	6.732	0.883	0.567
Error	72	7.621		
Total	108			

**: significance at $p < 0.001$.

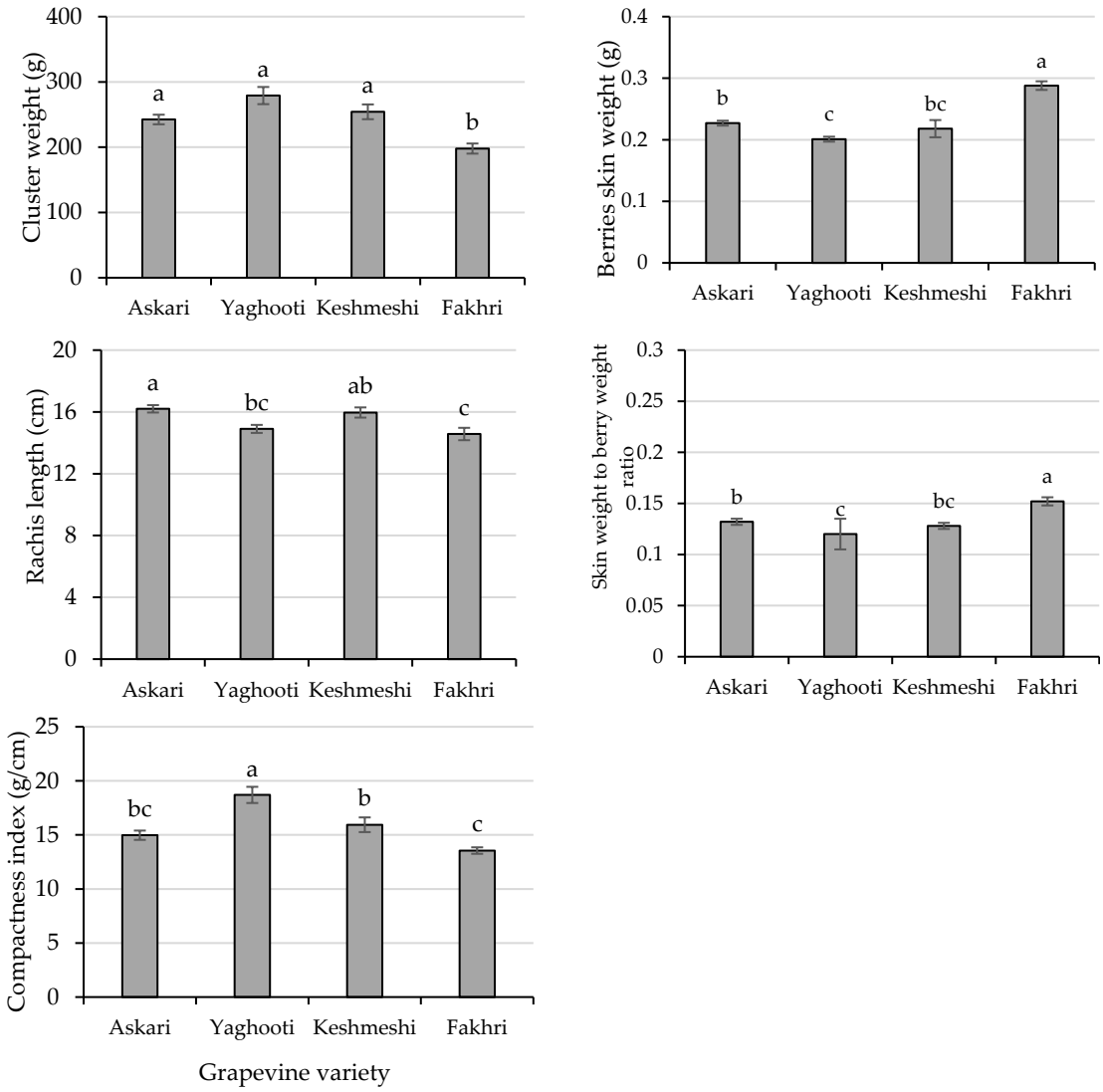

Figure 8. Morphological properties (mean ± SE) of berry clusters of different grape cultivars. Means followed by the same lowercase letters are not significantly different using the Tukey–Kramer (HSD) test at $p = 0.05$.

Table 5. Mean (±SE) percentage of clusters damaged by *Lobesia botrana* in the vineyard treated with different insecticides, per generation.

Time after Spray (d)	Generation	Treatments					
		1	2	3	4	5	6
1	Second	8.64 ± 1.23 a	7.40 ± 0.00 ab	8.64 ± 1.23 a	3.70 ± 0.00 b	3.70 ± 0.00 b	4.93 ± 1.23 ab
	Third	7.40 ± 2.13	2.46 ± 1.23	3.7. ± 0.00	4.93 ± 3.26	7.40 ± 2.13	6.17 ± 3.26
7	Second	22.22 ± 2.13 a	3.70 ± 0.00 b	1.23 ± 1.23 b	4.93 ± 3.26 b	2.46 ± 2.46 b	2.46 ± 1.23 b
	Third	19.75 ± 1.23 a	1.23 ± 1.23 b	1.23 ± 1.23 b	2.46 ± 1.23 b	4.93 ± 1.23 b	6.17 ± 1.23 b
14	Second	28.39 ± 1.23 a	0.00 ± 0.00 b	3.70 ± 0.00 b	3.70 ± 2.10 b	1.23 ± 1.23 b	2.46 ± 1.23 b
	Third	29.62 ± 2.13 a	1.23 ± 1.23 b	1.23 ± 1.23 b	1.23 ± 1.23 b	2.46 ± 1.23 b	2.46 ± 1.23 b

Treatment 1: Control, Treatment 2: *Bt* and alpha cypermethrin + teflubenzuron, Treatment 3: *Bt* and trichlorfon, Treatment 4: *Bt* and phosalone, Treatment 5: lufenuron + fenoxycarb and alpha cypermethrin + teflubenzuron, Treatment 6: lufenuron + fenoxycarb and trichlorfon. Means followed by the same lowercase letters in each row are not significantly different using the Tukey–Kramer (HSD) test at $p = 0.05$.

4. Discussion

Our results showed that *L. botrana* population density of males captured per trap was different over the two-year period, which indicates the importance of monitoring the pest during its life cycle. Monitoring can determine the occurrence of pests, as well as population

outbreaks [34]. Sex pheromones are appropriate tools to understand whether a pest species is present in a field and its population is at the level of economic damage [15,18]. Sciarretta, et al. [35] reported that the information obtained from the distribution of *L. botrana* males in the field based on flight models (126 ± 20 m) can be used to develop sampling plans, which indicates the optimal trap distance in this field. They found that pheromone traps should be placed about 115 m apart. We installed pheromone-baited traps at a distance of 100 m from each other.

According to pheromone-baited traps, the activity of *L. botrana* started in early April and continued until early September 2021 and mid-September 2022. For both tested years, three generations of *L. botrana* occurred during the grape cultivation season. The number of generations of this pest depends on factors such as temperature, humidity, photoperiod, and food quality, among which the temperature and light period are more critical factors [36]. In general, the number of generations of *L. botrana* differed due to different weather conditions in different geographical places. *Lobesia botrana* has two generations per year in cold regions of Europe (Switzerland) [37], three generations in Egypt [38], Israel [15], Mardin, Turkey [39], fourth generations in southern Europe [14], and four and five generations occur in hotter areas of Spain [40,41], and California [42]. van der Geest and Evenhuis [43] reported that *L. botrana* in temperate climate regions has two to four generations per year. According to our results, in both years, three generations of *L. botrana* occurred per year in the climatic conditions of Lorestan province, Khorramabad.

The population density of male moths was similar in four vineyards with different cultivars indicating that *L. botrana* was attracted to the traps baited with the female sex pheromone rather than the type of the cultivars or volatile compounds released from them. Contrary to our results, Sharon, et al. [15] noted that grapevine cultivar significantly affected the number of trapped males. According to their findings, for all phenological stages of the grapes, Carignan and French Colombard cultivars attracted the most and Cabernet Sauvignon the fewest males and females of *L. botrana*. They concluded that *L. botrana* can distinguish between different cultivars according to the volatile compounds of each cultivar. To this extent, the type of cultivar can influence the distribution pattern of this insect. Previous studies have also shown that females, in different phenological stages of grapevines, can discriminate between host grapes for oviposition [15,44]. With regards to the low-density insect population in the Fakhri, it can be considered as the least susceptible cultivar to *L. botrana* infestations. While Yaghooti was a significantly more preferred cultivar for *L. botrana* oviposition. The volatile compounds secreted from some cultivars stimulate *L. botrana* females to locate suitable hosts for feeding and oviposition [12]. Therefore, the appropriate monitoring can be performed based on *L. botrana* host-plant preference in vineyards. In this way, the pest population size in different cultivars is determined more precisely, and different control tactics are utilized on the preferred cultivars [15].

According to the relative humidity data in 2021, the mean relative humidity on 14 April 2021 increased and reached 40.17%, which was one of the wettest days in this season. Again, on 4 May, 24 May, and 22 August 2021, the relative humidity increased and was 46.75, 40.54, and 40.33%, respectively. Our results showed that higher relative humidity adversely influenced the egg density by decreasing the oviposition process. However, high relative humidity did not have much effect on the larval population density. It is speculated that the larvae are protected from severe weather while developing inside the berries or active in the larval nests. A similar trend was observed in 2022. This is in agreement with Torres-Vila, et al. [45] who illustrated that climate change influences insect growth, development, and reproduction. Low relative humidity and high temperature resulted in *L. botana's* better reproductive performance, but on the contrary, rainfall and low temperature decreased the egg-laying.

Grapevine cultivars with compact clusters are more susceptible to *L. botrana* and infested more by the larvae [16]. According to our results, the higher eggs and larval population was recorded in the Yaghooti cultivar. This is very likely due to the cluster compactness in this cultivar. In contrast, in cultivars with loose clusters, the larvae do

not have a suitable shelter and are more exposed to unfavorable weather conditions or predation [16]. Moreover, the skin thickness of berries affects their susceptibility to *L. botrana* infestation [12]. It has been proved that *L. botrana* attacks more ripened grapes with thinner skin [26]. Tonina, et al. [46] noted that *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) infestation varied across grapevine cultivars. They preferred thin-skinned fruits for oviposition and larval development. Females had a better performance for egg-laying in Yaghooti than in the other cultivars tested, which is in accordance with the thinner skin of berries in this cultivar.

The second and third generations of *L. botrana* cause severe damage to grapevine cultivars [5–7]. Therefore, the use of insecticides as a means of a controlling agent can reduce *L. botrana* population and damage level. The appropriate time for chemical control can be determined according to the peak flight of generations. At peak flight, male and female mating takes place, and the most significant number of eggs are laid, which can be used to determine the best time to spray insecticide in vineyards [47]. Irigaray, et al. [27] revealed that the application of insecticides to control the second and third generations of *L. botrana* should be at the beginning of the flight peak to affect egg-laying and egg-hatching. According to Ioriatti, et al. [19], the first generation of *L. botrana* does not need to be controlled because the damage to the first generation is during the flowering time, and this damage is somehow compensated by increasing the quantity and quality of the grapes. Therefore, in our study, insecticides were applied against the second and third generations of *L. botrana* after observing the peak flight of males. The performance of different insecticides to control *L. botrana* depends on several factors, including cluster or berry stage, pest population density, and weather conditions [20]. In this research, the use of insecticides decreased larval damage compared to the control. Sáenz-de-Cabezón, et al. [23] exposed *L. botrana* to lufenuron, a chitin synthesis inhibitor. They found that lufenuron decreased the fecundity and fertility of adults, as well as larval activity to perforate berries. Lufenuron has larvicidal activity and can be considered in integrated pest management programs for grapevines. In our study, lufenuron + fenoxycarb was used against the second generation of *L. botrana* in treatments 5 and 6. The results indicated that this treatment significantly declined larval damage of the second generation compared to the control. Given the mode of action of lufenuron, it can reduce population level by preventing the hatching of eggs and disrupting the larval emergence from eggs. In the other research, two *B. thuringiensis* strains, vars kurstaki, and aizawai, protected bunches from the infestations of *L. botrana* second generation [26]. According to our results, *Bt* was effective in controlling the second generation of *L. botrana* in treatments 2, 3, and 4, and it was able to reduce larval damage compared to the control. The excessive use of chemical insecticides to control populations of *L. botrana* has caused the development of pesticide resistance. Moreover, unsuccessful control of this pest by synthetic insecticides leads to the selection of resistant populations to insecticides, which has been reported as a case for indoxacarb in Emilia-Romagna [32]. In addition, using chemical pesticides with the same mode of action can develop resistance in insect populations. Therefore, an alternation between insecticides with different modes of action can overcome resistance issues [48]. Vassiliou [20] reported that rotation between conventional and biological insecticides could effectively control the *L. botrana* population under Cyprus field conditions. The rotation strategy is using one insecticide in one generation and a different one in the next generation of insect pests. The performance of the rotation strategy is better than other strategies, including sequential and mixture use strategies for pesticide resistance management [49]. Therefore, in our study, the rotation of insecticides with different modes of action was applied to reduce the infestations of *L. botrana*.

5. Conclusions

Lobesia botrana had three generations per year in the grapevines of Khorramabad, Iran. The population of eggs and larvae is affected by the cultivar, and the compactness of clusters can cause a preference for *L. botrana* egg-laying and larval hatching. However, the

effect of other factors, such as volatile compounds released from host plants, on oviposition preference should be investigated in future research. Proper use of insecticides in rotation can be recommended as an integrated pest management strategy to delay the development of resistance. In addition, integrating insecticide applications with other control techniques such as mass trapping and a mating disruption program using pheromones can be recommended to reduce the *L. botrana* population.

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