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The effects of biochemical compounds of different grape cultivars on the digestive physiology of *Lobesia botrana* (Lepidoptera: Tortricidae)

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Abstract

The European grapevine moth, *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae), is an economically important pest of grapevines worldwide, causing severe damage in vineyards. In the present study, the effect of four grapevine cultivars (Askari, Yaghooti, Keshmeshi, and Fakhri) was evaluated on larval weight and enzymatic activity of *L. botrana*. In addition, three major secondary metabolites, including phenolics, flavonoids, and anthocyanins were determined in ripe berries of cultivars, and their correlation with the digestive physiology of *L. botrana* was investigated. Our findings indicated that the fifth-instar larvae of *L. botrana* collected from the Askari cultivar had the highest weight. The enzymatic activities of *L. botrana* were significantly affected by feeding on different grapevine cultivars. The highest amylolytic and proteolytic activity levels were documented in larvae fed on the Fakhri cultivar, while the lowest activities were achieved on the Yaghooti cultivar. Furthermore, the highest catalase and peroxidase activity was observed in the larvae fed on the Yaghooti cultivar. A significant difference in secondary metabolites was quantified among different grapevine cultivars. The highest biochemical compounds of grapevine were detected in the Yaghooti cultivar. Moreover, the larval weight, amylolytic, and proteolytic activity showed a negative correlation with the cultivars' phenols, flavonoids, and anthocyanins contents. Conversely, the antioxidant enzymatic activity (catalase and peroxidase) of the larvae positively correlated with the secondary grapes metabolites. Results revealed that the Yaghooti cultivar, a rich source of biochemical compounds, is not a suitable host plant for larval growth and development of *L. botrana*.

Keywords: Grapevine moth, enzymatic activity, grapevine, plant metabolites, insect–plant interaction

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تأثیر ترکیبات بیوشیمیایی ارقام مختلف انگور بر فیزیولوژی گوارشی *Lobesia botrana* (Lepidoptera: Tortricidae)

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چکیده

خوشه خوار اروپایی انگور، *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae) آفت مهم اقتصادی انگور در سراسر جهان است که باعث خسارت شدید در تاکستان‌ها می‌شود. در مطالعه حاضر، تأثیر چهار رقم انگور (عسکری، یاقوتی، کشمشی و فخری) بر وزن و فعالیت آنزیمی لارو *L. botrana* مورد بررسی قرار گرفت. علاوه بر این، سه متابولیت ثانویه اصلی شامل فنول‌ها، فلاونوئیدها و آنتوسیانین‌ها در حبه‌های رسیده چهار رقم تعیین شد و ارتباط آنها با فیزیولوژی گوارشی *L. botrana* بررسی شد. یافته های ما نشان داد که لاروهای سن پنجم *L. botrana* جمع آوری شده از رقم عسکری بیشترین وزن را داشتند. فعالیت آنزیمی *L. botrana* به طور معنی‌داری با تغذیه از رقم‌های مختلف انگور تحت تاثیر قرار گرفت. بیشترین میزان فعالیت آمیلولیتیک و پروتئولیتیک در لاروهای تغذیه شده از رقم فخری و کمترین میزان فعالیت در رقم یاقوتی مشاهده شد. همچنین بیشترین فعالیت کاتالاز و پراکسیداز در لاروهای تغذیه شده از رقم یاقوتی مشاهده شد. تفاوت معنی‌داری در متابولیت‌های ثانویه در بین رقم‌های مختلف انگور مشاهده شد. بیشترین ترکیبات بیوشیمیایی انگور در رقم یاقوتی شناسایی شد. علاوه بر این، وزن لارو، فعالیت آمیلولیتیک و پروتئولیتیک همبستگی منفی با محتوای فنل، فلاونوئید و آنتوسیانین ارقام نشان داد. در مقابل، فعالیت آنزیم‌های آنتی اکسیدانی، کاتالاز و پراکسیداز لارو با متابولیت‌های ثانویه انگور همبستگی مثبت داشت. نتایج نشان داد که رقم یاقوتی به عنوان منبع غنی از ترکیبات بیوشیمیایی، میزبان مناسبی برای رشد و نمو لارو *L. botrana* نیست.

کلیدواژه‌ها:* *خوشه خوار انگور، فعالیت آنزیمی، انگور، متابولیت‌های گیاهی، تعامل حشره و گیاه

دبیر تخصصی: دکتر فاطمه یاراحمدی

Introduction

Grapevine (*Vitis vinifera* L.) is a species of flowering plant and is considered to be one of the major fruit crops in the world. The grape product has different uses; it is used as fresh fruit, dried fruit, wine, and fruit juice (Torregrosa et al., 2015). Iran is the 11th grape producing country in the world, and has around 316,000 hectares of grape orchards, which yielded about 1945930 tons of fruit in 2019 (Bazgeer et al., 2022).

The European grapevine moth, *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae), causes severe economic damage in vineyards. Neonate larvae of the first-generation feed on unripe berries and cover them with silks. The larvae penetrate berries and continue their development boring into the grape pulps, and piercing several berries. The infected berries turn purple, and then dark brown with the larval frass, rendering them susceptible to fungi species including *Aspergillus* spp., and especially the grey rot fungus *Botrytis cinerea* (Persoon: Fries) (Teleomorph: Botryotinia. fuckeliana Whetzel). The larval infestation leads to severe qualitative and quantitative losses (Benelli et al., 2023).

Plants respond defensively against insect pests' damage through various mechanisms, direct and indirect. Plants' secondary metabolites, such as phenols, are either directly toxic to insects or induce the production of plant toxins. They act as feeding inhibitors or anti-digestives and can cause plant resistance to herbivore pests (War et al., 2012). Plants' resistance traits can delay the growth and development of insects, affect oviposition and feeding, and consequently reduce insect survival (Naseri et al., 2022; Shishehbor & Hemmati, 2021; War et al., 2012). Conversely, herbivore insects can overcome the plant's defense by producing digestive enzymes and metabolizing plant defense compounds. Having information about the digestive enzyme activities of *L. botrana* fed on different grape cultivars can lead to finding the relationship between insects' physiology and the biochemical properties of the host plant, thereby achieving plant tolerance traits (Slansky, 1982). The most important antioxidant enzymes in insects are catalase, peroxidases, and superoxide dismutase (Felton & Summers, 1995). Catalase was the first discovered antioxidant enzyme that decomposes hydrogen peroxide into innocuous products such as oxygen and water (Nandi et al., 2019). Superoxide dismutase is an antioxidant enzyme that catalyzes dismutation of superoxide radicals into H_2O_2 and O_2 (Shankarganesh et al., 2021).

Although grapevine is a globally recognized host to *L. botrana*, there is no published research about the effect of various grape cultivars on the physiological characteristics of this pest. Given the importance of *L. botrana*, the current study was done to evaluate the larval weight, protein content, and digestive enzyme activities in response to secondary metabolites (total phenolic, flavonoid, and anthocyanin content) of different grapevine cultivars. In addition, the correlation between the physiological characteristics of *L. botrana* larvae and grape's secondary metabolites was examined. Our findings may help identify the most resistant cultivar to *L. botrana* damage.

Materials and Methods

Vineyards

The sampling was conducted in 2022 at four vineyards with cultivars commonly cultivated in the Saifabad village, Kamalvand district, Khorramabad, Iran, including Askari, Keshmeshi, Fakhri, and Yaghooti. The geographical characteristics of the selected vineyards are presented in Table 1.

Table 1. Experimental area, with indication of selected vineyards position

Vineyard	Longitude	Latitude	Altitude (m)
Askari	39S 0276219	3696820	1604
Yaghooti	39S 0276234	3696709	1598
Keshmeshi	39S 0276072	3696776	1597
Fakhri	39S 0276125	3696796	1599

Larval sampling from vineyards

The sampling was conducted during the activity period of *L. botrana* third-generation, which occurs in the mid-August when berries are ripe. Fourth and fifth instar larvae are able to build nests, and each larvae is present and active in one nest (Moreau et al., 2008; Moreau et al., 2009). Five days after observing the larval nests, they were sampled and considered as the fifth instar larvae. For each cultivar, 20 fifth instar larvae were collected. The larvae were transported to the laboratory, and their weight was measured individually. Then, these larvae were used to evaluate the enzymatic activity fed on different grape cultivars.

Buffer preparation

The buffer used in amylolytic activity is a combination of 2-succinate-glycine and morpholinoethanesulfonic acid (10 mM). The universal buffer used in enzyme tests related to proteolytic activity consists of 50 mM sodium phosphate-borate (Jafari et al., 2023).

Preparation larval midgut extract

Twenty larvae for each cultivar were anesthetized on ice and dissected under binoculars in cold distilled water. The larval midgut was homogenized on ice. The homogenate was then centrifuged at 15000×g for 10 min at 4 ºC. The supernatant was stored at -20 ºC. Each of the experiments related to digestive enzyme activities was replicated four times.

Protein content measurement

Bradford (1976) method was used to measure total protein content. Bovine serum albumin was used as a standard. The homogenized samples were centrifuged for 5 min at 10000×g at 4 ºC. Then, 10 µl of the supernatant was dissolved in 90 µl distilled water, and 2.5 ml of Coomassie Blue color mixture (10 mg of Coomassie Brilliant Blue powder (G250) in 5 ml ethanol 96%- and 10-ml phosphoric acid 85%) was diluted to a total volume of 100 ml by adding distilled water. The absorbance was measured at 595 nm.

Amylolytic activity assay

The amylolytic activity of *L. botrana* larvae fed on different grapes was evaluated using starch 1% (Sigma Chemical Co., St Louis, USA) as a substance in the buffer containing 10 mM succinate-glycine-2, morpholinoethanesulfonic acid at pH = 10 (Bernfeld, 1955). The solution was incubated at 37 ºC for 30 min. Then, 50 µl of DNSA reagent (3,5 dinitrosalicylic acid) was added and heated in a water bath at 100 ºC for 15 min. The absorbance was measured at 540 nm. The amount of enzyme required for generating one mg of maltose in 30 min at 37 ºC was considered a unit of amylase activity under the assay conditions.

Proteolytic activity assay

The proteolytic activity of *L. botrana* larvae fed on different grapes was evaluated using azocasein protein (1.5%) as a substance in the buffer containing 50 mM sodium phosphate-borate at pH = 11 (Elpidina et al., 2001). The solution containing 50 µl larval extract, and 80 µl substrate in 50 mM universal buffer was incubated at 37 ºC for 50 min. Proteolysis was stopped by adding 100 µl trichloroacetic acid (TCA) 30%, then cooled at 4 °C for 30 min. The solution was centrifuged at 14000×g for 10 min. The absorbance was measured at 440 nm. One unit of proteolytic activity was considered as the quantity of enzyme (mg) that produces an increase in the optical density by 0.1 per minute in 1 mL of the reaction mixture under the assay conditions.

Catalase activity assay

Catalase enzyme activity of *L. botrana* larvae was evaluated using H_2O_2 1% as a substrate (Wang et al., 2001). The solution containing 50 µl larval extract and 500 µl H_2O_2 1% substrate was incubated at 28 ºC for 10 min. The absorbance was measured at 240 nm.

Peroxidase activity assay

Peroxidase enzyme activity of *L. botrana* larvae was evaluated using pyrogallol (0.05 M pyrogallol in 0.1 M phosphate buffer (pH = 7) as a substrate (Addy & Goodman, 1972). The solution was prepared by adding 50 µl larval extract, 250 µL of buffered pyrogallol, and 250 µl H_2O_2 1%. The absorbance was measured every 30s for two min at 430 nm.

Superoxide dismutase activity assay

The superoxide dismutase activity of *L. botrana* larvae was evaluated by adding 50 µl larval extract to 500 µl of the superoxide dismutase mixture. The superoxide dismutase solution was 70 µM nitroblue tetrazolium (NBT), 125 µM xanthine-diluted phosphate-buffered saline (PBS), 100 µl 5.87 U/ml xanthine oxidase solution, and 10 mg bovine serum albumin. Then, the superoxide dismutase solution and 2 mL PBS were added to the mixture and incubated at 28 ºC for 20 min in continuous darkness. The absorbance was measured at 560 nm (Talepout et al., 2021).

Analysis of secondary metabolites in different grape cultivars

Preparation of grape extract

Grape samples (1 g) from each cultivar were homogenized in 10 ml methanol 80%. The contents were passed through the Whatman No.1 filter paper. The sample was extracted using a centrifuge at 15000×g for 5 min. Biochemical properties of grapes of different cultivars were performed in four replications.

Total phenolic content measurement

The total phenolic content in the grapes extract was evaluated using Folin Ciocalteu reagent (Slinkard & Singleton, 1977). For this purpose, 250 µl grape extract was poured into the tube, and mixed with 2.5 ml Folin Ciocalteu reagent. The dilution was kept at room temperature for 15 min, and then 2 ml Na_2CO_3 solution (1 M) was added. After 30 min, the optical density was measured at 765 nm.

Total flavonoid and anthocyanin content measurement

The total flavonoid and anthocyanin content in the grapes was evaluated as described by (Kim et al., 2003). For this purpose, 2 g grape samples of each cultivar were homogenized in acidified ethanol (1 acid acetic: 100 ethanol w: w). The samples were centrifuged at 12000×g for 15 min, and the contents were filtered through the Whatman No.1 filter paper. The extract was heated in a water bath at 80 ºC for 5 min, then kept at room temperature for 1 h. Subsequently, the absorbance was measured at 415 nm for total flavonoid and 520 nm for total anthocyanin. The standard curve was made utilizing quercetin standard solution for flavonoid, and cyanidin standard solutions for anthocyanin.

Brix percentage measurement

The grapes were crushed in a mortar and passed through the Whatman filter paper No. 1. The total soluble solids (refractometer) were attained on a glass prism and the soluble solid content was determined in the juice using a digital refractometer (Atago, RX-50000, Japan) (Hoehn et al., 2003).

Statistical analysis

The normality of the data was checked using the Shapiro-Wilk test. The data of *L. botrana* larval weight, enzymatic activity, and secondary metabolites of grape cultivars were analyzed using one-way analysis of variance. Means were compared using the Tukey–Kramer (HSD) test at $P < 0.05$. The correlation between secondary metabolites of different grape cultivars with physiological characteristics of *L. botrana* was evaluated through Pearson's correlation test. All analyses were performed using SPSS software version 16.0

Results

Larval weight

The weight of *L. botrana* larvae differed significantly among the cultivars ($F_{3,12}$ = 275.150; $P < 0.001$). The highest weight was obtained in the larvae fed on Askari cultivar (9.984 mg), while the lowest weight was related to the larvae fed on Yaghooti cultivar (6.044 mg) (Figure 1a).

The protein content and enzymatic activities

Regarding the protein content of *L. botrana* larvae fed on different grape cultivars, there was a significant difference between the cultivars ($F_{3,12}$ = 24.396; $P < 0.001$), and the protein content ranged from 0.4338 to 0.4908 µg/g (Figure 1b). Significant differences in amylolytic enzyme activity were observed among the larvae reared on different grape cultivars ($F_{3,12}$ = 10.143; P < 0.001), and the highest activity was related to the larvae reared on Fakhri cultivar (1.420 µg/g). The lowest level of amylolytic enzyme activities was reported in the larvae fed on Yaghooti (1.137 µg/g) and Askari (1.172 µg/g) cultivars (Figure 1c). The activity of proteolytic enzymes in *L. botrana* larvae was significantly differed among the cultivars ($F_{3,12}$ = 36.762; P < 0.001). The larvae reared on Fakhri cultivar (0.336 µg/g) exhibited the highest proteolytic enzyme activity, while the lowest activity level was related to larvae fed on Yaghooti cultivar (0.173 µg/g) (Figure 1d).

Catalase enzyme activity in *L. botrana* larvae showed significant difference among the grape cultivars ($F_{3,12}$ = 9.288; P < 0.001). The highest catalase enzyme activity was reported in the larvae reared on Yaghooti cultivar (0.00649 U/mg protein), while the lowest activity level was related to the larvae fed on Askari cultivar (0.00284 U/mg protein) (Figure 2a). Concerning the peroxidase enzyme activity of *L. botrana* larvae, there was a significant difference among different grape cultivars ($F_{3,12}$ = 4.094; P = 0.032). The highest peroxidase enzyme activity was reported in Yaghooti cultivar (0.0265 U/mg protein), while the lowest activity level occurred on Fakhri cultivar (0.00696 U/mg protein) (Figure 2b). Concerning the superoxide dismutase enzyme activity of *L. botrana* larvae fed on different grape cultivars, there was no significant difference between different cultivars ($F_{3,12}$ = 0.101; P = 0.958) (Figure 2c).

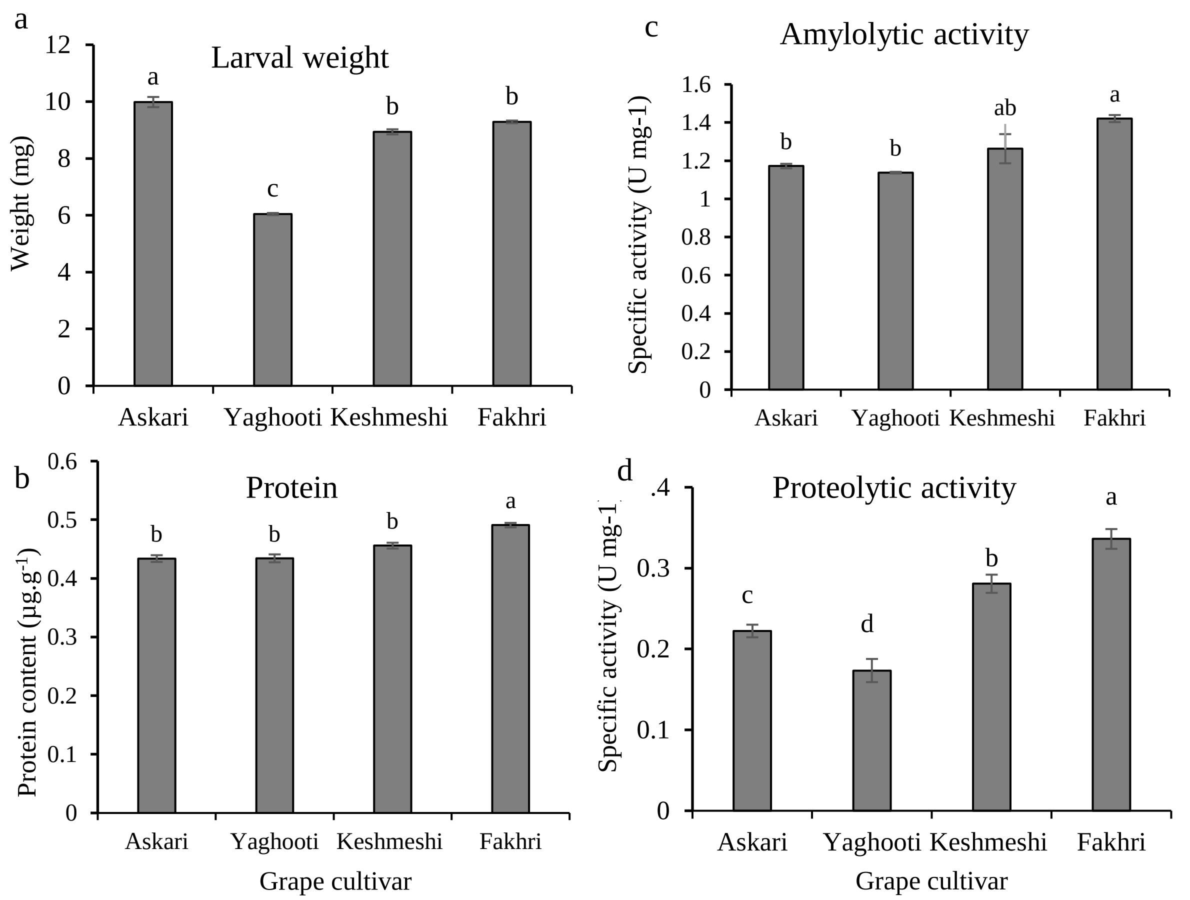

Figure 1. Mean (±SE) of (a) larval weight, and (b) protein content, (c) amylolytic, and (d) proteolytic activity of midgut extracts from *Lobesia botrana* larvae fed on different grape cultivars. Means followed by the same letter are not significantly different using Tukey-Kramer (HSD) test at *P* = 0.05.

Secondary metabolites of different grape cultivars

Significant differences were observed in the contents of secondary metabolites among the grape cultivars ($P < 0.001$). The highest phenol content was observed in the Yaghooti cultivar (0.503 µg/g), while the lowest was found in the Fakhri cultivar (0.116 µg/g). Moreover, the highest flavonoid content was observed in the Yaghooti cultivar (0.970 µg/g), while the lowest one was related to the Fakhri cultivar (0.498 µg/g). The highest anthocyanin content was determined in the Yaghooti cultivar (3.252 µmol/g). The highest Brix percentage was observed in the Fakhri cultivar (25.972%), while the lowest Brix was related to the Askari cultivar (13.352%) (Table 2).

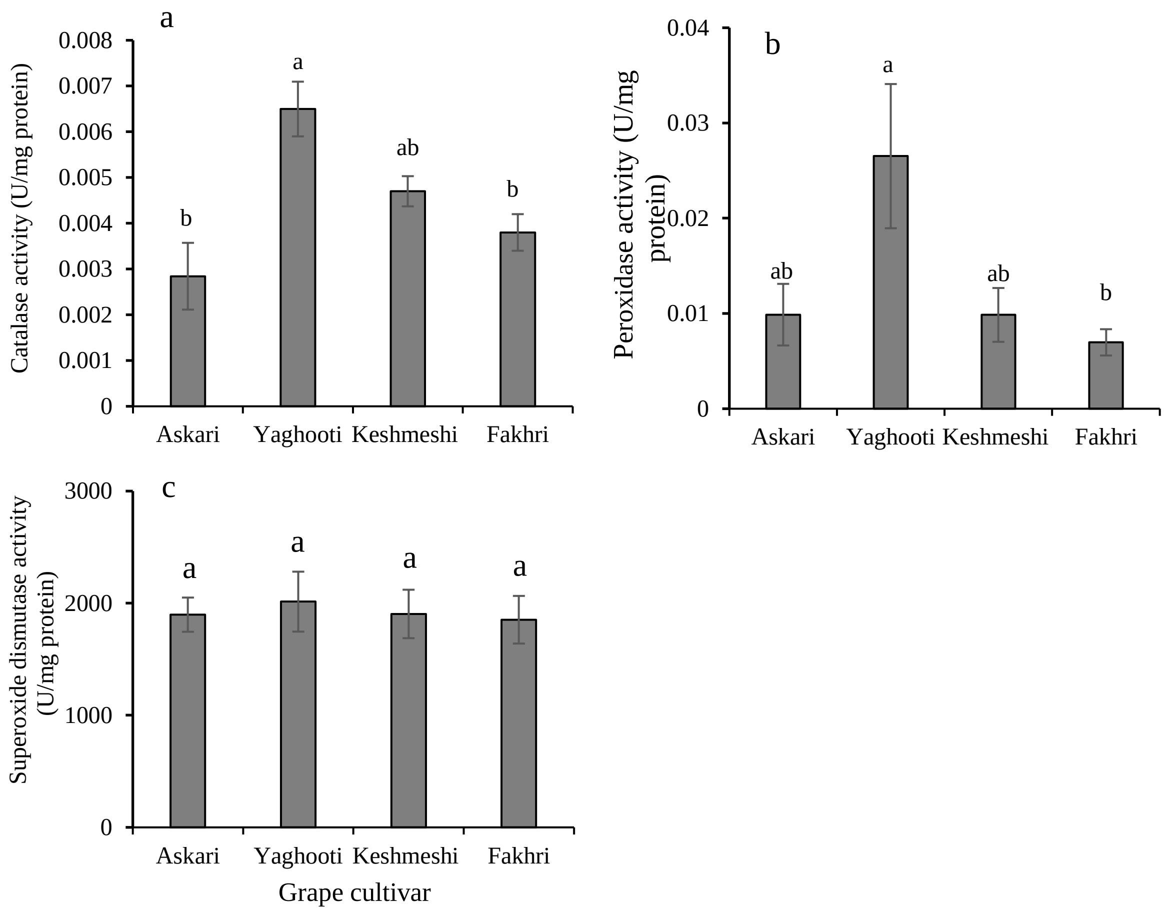

Figure 2. Mean (±SE) of antioxidant enzymes; (a) catalase, (b) peroxidase and (c) superoxide dismutase activity of midgut extracts from *Lobesia botrana* larvae fed on different grape cultivars. Means followed by the same letter are not significantly different using Tukey-Kramer (HSD) test at *P* = 0.05.

Table 2. Mean (±SE) of phenols, flavonoids, and anthocyanins total contents of different grape cultivars

Cultivars	Total phenols (µg/g)	Total flavonoids (µg/g)	Total anthocyanin (µmol/g)	Brix (%)
Askari	0.180 ± 0.006b	0.6917±0.036b	0.048 ± 0.003c	13.352 ± 0.062d
Yaghooti	0.503 ± 0.017a	0.970±0.042a	3.252 ± 0.258a	20.852 ± 0.002c
Keshmeshi	0.165 ± 0.015bc	0.771±0.014b	0.597 ± 0.033b	25.232 ± 0.097b
Fakhri	0.116 ± 0.003c	0.498±0.038c	0.046 ± 0.002c	25.972 ± 0.241a
$F_{3,12}$	220.958	31.798	137.835	187.100
P	< 0.001	< 0.001	< 0.001	< 0.001

Means followed by the same letter are not significantly different using Tukey-Kramer (HSD) test at $P < 0.05$.

Correlation between secondary metabolites of grape cultivars with physiological characteristics of *L. botrana* larvae

The correlation analysis of *L. botrana* larval weight and digestive enzymatic activities with secondary metabolites of different grapevine cultivars is presented in Table 3. Larval weight showed a negative correlation with grapevine phenolic content ($r = -0.924$; $P = 0.001$), flavonoids ($r = -0.737$; $P = 0.001$), and anthocyanin contents ($r = -0.967$; $P = 0.001$). There was no significant correlation between larval weight with the Brix of grapevines ($P > 0.05$). The protein content of *L. botrana* larvae was negatively correlated with grapevine phenolic content ($r = -0.567$; $P = 0.022$), and flavonoid contents ($r = -0.755$; $P = 0.001$). However, the protein content of larvae was positively correlated with grapevine Brix value. In addition, both amylolytic and proteolytic activity of larvae were negatively correlated with grapevine phenol, flavonoids, and anthocyanin contents. While, amylolytic ($r = 0.587$; $P = 0.017$), and proteolytic activity ($r = 0.608$; $P = 0.012$) of the larvae were positively correlated with grapevine Brix value. Catalase and peroxidase were positively correlated with grapevine phenol, flavonoids, and anthocyanin contents. In contrast, there was no significant correlation between catalase and peroxidase with grapevine Brix value ($P > 0.05$). There was no significant correlation between superoxide dismutase enzymatic activity and the secondary metabolites of grapes ($P > 0.05$) (Table 3).

Discussion

In the current study, the digestive physiology of *L. botrana* in response to feeding on different grape cultivars was evaluated. The insect body weight is considered one of the leading biological indices of population dynamics, indicating the suitability of a plant host. The larval weight increases by feeding on more nutritious food (Hemati et al., 2012). In this study, the highest larval weight was reported in the larvae fed with the Askari cultivar. In addition, the food consumed affects the activity of digestive enzymes and consequently the insect growth and development (Hosseininejad et al., 2015). The types and levels of digestive enzyme activities in insects can be related to the nutritional quality of host plants and their biochemical composition, which ultimately affects the life cycle of the insects (Borzoui & Naseri, 2016). Amylase metabolizes carbohydrates, including starch and glycogen, in the insects gut to regulate larval energy metabolism (Dastranj et al., 2018). Our findings revealed that the larvae's amylase activity differed among the tested cultivars. The high Brix percentage was observed in the Fakhri cultivar, indicating that an increase in amylase activity in the larvae fed on this cultivar may be due to its high sucrose level. The digestive performance of insects fed on suitable food diets leads to survival and more significant growth and development of the individual (Debnath et al., 2020).

Table 3. Correlation coefficients between grapes secondary metabolites with physiological characteristics of *Lobesia botrana* larvae fed on different grape cultivars

Parameter	Total phenols	Total flavonoids	Total anthocyanin	Brix
Larval weight	-0.924 (0.001) **	-0.737 (0.001) **	-0.967 (0.001) **	-0.175 (0.516)
Protein	-0.567 (0.022) *	-0.755 (0.001) **	-0.469 (0.067)	0.695 (0.003) **
Amylolytic activity	-0.561 (0.024) *	-0.661 (0.005) **	-0.498 (0.050) *	0.587 (0.017) *
Proteolytic activity	-0.781 (0.001) **	-0.779 (0.001) **	-0.674 (0.004) **	0.608 (0.012) *
Catalase	0.676 (0.004) **	0.562 (0.023) *	0.790 (0.001) **	0.312 (0.240)
Peroxidase	0.705 (0.002) **	0.606 (0.013) *	0.749 (0.001) **	-0.095 (0.726)
Superoxide dismutase	0.185 (0.493)	0.034 (0.901)	0.073 (0.789)	-0.009 (0.974)

Correlations were evaluated based on Pearson's correlation test at $P < 0.05$. The number in parenthesis is P value. *, **: Correlation is significant at the 0.05 and 0.01 level (2-tailed).

High amylase activity in *Callosobruchus maculatus* F. (Coleoptera: Chrysomelidae: Bruchinae) fed on Acauã and Tapaihum cultivars of cowpea led to higher population growth and development (Silva et al., 2017).According to our results, the amylase effectiveness in breaking down and digesting food increases with the rise in the sugar content of the host plant. In addition, the highest level of protein content was observed in the larvae fed on the Fakhri cultivar. This result is in agreement with Borzoui et al. (2017), who found that the lowest protein content in pupae from larvae of *Sitotroga cerealella* Olivier that had fed on sorghum, could be attributed to the lowest amylolytic activity.

On the other hand, the decrease in energy sources in the insects' bodies can be achieved by reducing protease enzyme activity. The high level of proteolytic activity in *Helicoverpa armigera* (Hubner) larvae fed on chickpeas compared to the other host plants can be due to the high protein content in this plant or the larval response to protease inhibitors secreted from plants to inhibit protease activity (Hemati et al., 2012). Naseri et al. (2010) reported that the larvae of *H. armigera* that have fed on some soybean cultivars (L17, M4, and Sahar) begin to hyper-produce proteases in the midgut cells, in contrast to the synthesis of protease inhibitors. Insects synthesize and secrete more proteases in the defense response of the plants and the secretion of protease inhibitors (Hosseininejad et al., 2015). In our study, high proteolytic activity was reported in the Fakhri cultivar which could be attributed to the high protein content and the presence of protease inhibitors within this cultivar. Proteases can break down proteins into amino acids essential for insects' growth and development (Jafari et al., 2023).

Secondary metabolites of plants are defense mechanisms of the host plant that can inhibit the digestive enzymes of insect pests and turn the plant into an unsuitable host for the insect. In contrast, insects store energy reserves such as protein, lipid, and sugar content for growth and development (Borzoui et al., 2017). Therefore, the lowest proteolytic activity was observed in the Yaghooti cultivar which may be as a result of the high secondary metabolites including phenols, flavonoids, and anthocyanin, found in this cultivar. Shishehbor & Hemmati (2021) reported that the high phenols content in the Mashhad bean cultivar, among 11 tested cultivars, significantly decreased the nutritional performance of *Spodoptera littoralis* (Boisd) larvae. The growth rate and larval weight decreased in cultivars with higher phenolic compounds. According to our results the larvae that fed on the Yaghooti cultivar had the lowest weight, which could be due to the presence of the highest levels of secondary metabolites such as phenols, flavonoids and anthocyanins in this cultivar. Borzoui et al. (2017) found a positive correlation between amylolytic activity and the survival rate, fecundity, and fertility of *S. cerealella*, suggesting that larvae feeding on suitable grains have a high level of amylase enzymes and more growth and development. In the other research, Babamir-Satehi et al. (2022) revealed that the total phenol content of sugarcane cultivars was negatively correlated to amylolytic activity. They showed that increasing the phenol concentration in the sugarcane cultivars caused a reduction in the nutritional performance and digestive enzyme secretion of *Sesamia cretica* Lederer larvae. According to our findings, amylolytic and proteolytic activity of the larvae negatively correlated with secondary metabolism in grapevines.

Feeding a diet containing PPA (*Polygonum persicaria* L. lectin) increased catalase activity in *Sitophilus oryzae* L. adults, leading to oxidative stress in this pest. The absorption of PPA increases concentrations of superoxide and hydrogen peroxide radicals, which activates these enzymes (Khoobdel et al., 2022). Biotic and abiotic stresses may increase antioxidant enzymes level in insects' body. The superoxide dismutase activity increased in *Acerophagus papayae* (Noyes and Schauff) (Encyrtidae: Hymenoptera) when temperature increased from 25 to 34 °C (Shankarganesh et al., 2021). Antioxidants in date palm plants induced after the infestation of *Rhynchophorus ferrugineus* (Oliver), and resulted in elevated anti-oxidase activities in this herbivorous insects (Manzoor et al., 2022). In the current study, the highest levels of antioxidant enzymes were observed in the larvae fed on the Yaghooti cultivar, indicating that the secondary metabolites of this cultivar induce oxidative stress in *L. botrana* larvae. Therefore, catalase, and peroxidase plays an influential role in preventing and reducing the suffering of the larvae due to oxidative damage caused by feeding on plant secondary metabolites.

Conclusion

The physiochemical properties of insect pests are affected by the nutritional quality of host plants. Our results found evidence of reduced fitness and higher oxidative stress in larvae fed on the Yaghooti cultivar. Yaghooti, as an unsuitable host, contains some inhibitory compounds caused adverse effects on *L. botrana* and reduced larval weight. Knowing about the secondary metabolites of plants and insect digestive enzymes can be used as a strategy for insect pest control using plant inhibitors. Moreover, further experiments on food and oviposition preference as well as life history of *L. botrana* on different grapevine cultivars are required, which could help in development of cultivars resistance to this pest.

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