Effect of feeding on four citrus varieties on enzymatic and non enzymatic properties in citrus butterfly Papilio demoleus L.

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The butterfly, citrus swallowtail Papilio demoleus L. is an important pest in nursery and young citrus trees in Iran and worldwide. It has received considerable attention among researchers. Here, we studied the physiological parameters of P. demoleus L. (Lepidoptera: papilionidae) fed on four varieties of citrus (i.e. Citrus sinensis (L.) Osbeck, C. limon (L.) Burm.f., C. sinensis var. valencia, C. paradisi Macfad.). The study was carried out under laboratory conditions (24±2°C, 65±10% RH and 16 h photoperiod). The 5th instar larvae showed highest relative consumption rate (RCR) by feeding on C. limon. The highest relative growth rate (RGR) (1.28±0.03) was recorded with C. sinensis. Efficiency of conversion of ingested food (ECI) (26.21±0.43) and Efficiency of conversion of digested food (ECD) (39.05±0.57) were highest with C. sinensis. The approximate digestibility (AD) recorded the lowest amount on C. sinensis. The highest activities of proteases were observed in the alimentary tracts of the larvae reared on C. sinensis var. valencia and C. paradisi at two different pH, pH 7 and 10. Chymotrypsin, trypsin, aminopeptidase, carboxypeptidase, α-glucosidase and β-glucosidase showed higher activities in larvae reared on C. paradisi. While elastase and lipase showed higher activities in C. sinensis var. valencia, and α-amylase on C. sinensis. The amount of glycogen and triglyceride and total protein were higher in the hemolymph of larvae reared on C. paradisi, C. paradisi and C. sinensis var. valencia, respectively. The highest activities of gamma-glutamyntransferase, lactate dehydrogenase, alkaline aminotransferase, alkaline phosphatase, acids phosphatase were observed on C. paradisi. However, the highest amount of aspartate aminotransferase was observed in C. sinensis var. valencia. Overall, these results indicate that the Citrus limon is an unsuitable host for Papilio demoleus.

Keywords: Citrus swallowtail, Feeding indices, Lemon butterfly

Citrus is one of the important commercial fruit crops of the world, which is cultivated in the tropical and subtropical regions. Among the various insect pests that attack citrus, Papilio demoleus L. (lepidoptera: papilionidae), commonly called citrus swallow tail butterfly, is one of the most economically important pests in many parts of the world. The caterpillars feed voraciously and cause severe damages to the nurseries, young seedlings. In the event of intensive infestation, they defoliate trees, and cause potential threat to the citrus industry. Nutrition is an interaction between physiological processes and ecology. It is directly associated with natural selection as well as the competition for food. In many studies the insect plant relationships, have been investigated in order to quantify the efficiency with which insects use their food plants. The rates of food ingestion, growth and utilization efficiency are important components of herbivores' performance. From a nutritional point of view, utilization efficiency reflects the quality of food consumed. Growth, development and reproduction of insects strongly depend on the quality and quantity of food consumed. The lepidopteran larval midgut has been shown to witness complex proteolytic activities, including trypsins, chymotrypsins, elastases, cathepsin-B like proteases, aminopeptidases and carboxypeptidases that are required for protein digestion. Lepidopteran insects mainly depend on serine proteases for protein digestion.

The alpha-amylase is an enzyme hydrolyzing starch to maltose and glycogen to glucose. In insects, the activity of digestive enzymes, such as proteases and α-amylases depends on the nature of food sources or chemical compounds ingested. Protease and α-amylase activities in crude extracts of larval guts of different lepidopteran species have been described. Insect digestive enzymes have various properties and they could be used to design new techniques for insect control in integrated pest management programs to

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keep the population of a herbivorous pest below an economically important level\textsuperscript{12}.

There are no studies available comparing the food utilization indices of \textit{Papilio demoleus} to citrus varieties\textsuperscript{13}, particularly, food utilization indices, digestive enzyme activities and intermediary metabolism following feeding on various citrus varieties. Therefore, here, we studied the effects of four citrus varieties \textit{viz. Citrus sinensis, C. limon, C. sinensis} var. \textit{valencia, C. paradisi} on physiological performance of the citrus swallowtail \textit{P. demoleus}.

**Materials and Methods**

**Plant sources**

The leaves of four host plants used in this study were as follows: \textit{Citrus sinensis} (L.) Osbeck, \textit{C. limon} (L.) Burm.f., \textit{C. sinensis} var. \textit{valencia} and \textit{C. paradisi} (Macfad 1750). All these leaves were collected from Rudsar (37°08′15″N 50°17′17″E), North of Iran.

**Insect rearing**

\textit{Papilio demoleus} L. larvae were collected from infested citrus nursery in Rudsar. They were reared on fresh citrus leaves (various host plants) in growth chamber set at 28±2°C, 65±5% RH and 16 h photoperiod. The emerged adults were placed in cages (50×50×50 cm) with a 10% honey solution on Petri dishes for feeding and citrus leaves were provided for the oviposition. The eggs were separated from the leaves and placed in Petri dishes with a lid in the same condition as above. The F\textsubscript{2} generation of \textit{P. demoleus} was used in all experiments.

**Nutritional indices assay**

Nutritional indices were determined using fifth instars, on different host plants. The larvae were individually transferred into the plastic containers (18×15×7) with a hole on its top covered by fine mesh net for aeration. The experiment was initiated with 40 newly moulted fifth instar larvae divided into four replications of 10 insects each per variety. The weight of the fifth instar larvae, initial food, un consumed food and feces were recorded daily. The quantity of food ingested was determined by subtracting the un consumed food from the total weight of food provided. To calculate the dry weight of larvae, feces and remaining leaves of each variety and sample specimens were weighted, oven dried (until completely dried at 60°C) and then reweighed to establish a percentage of their dry weight.

The nutritional indices were calculated based on the dry weights using the following formulae\textsuperscript{14}.

Consumption index (CI) = E/A; approximate digestibility (AD) = E-F/E; efficiency of conversion of ingested food (ECI) = P/E; efficiency of conversion of digested food (ECD) = P/E-F; and relative growth rate (RGR) = P/A×T. Where A= mean dry weight of insect over unit time, E= dry weight of food consumed, F= dry weight of feces produced, P= dry weight gain of insect, and T= duration of feeding period.

**Biochemical assay**

The guts of fifth instar larvae were used to measure proteolytic and amylolytic activities and the whole body to measure other biochemical assessment as follows: fifth instar larvae (n=5) were randomly selected and reared on different hosts, were cold anesthetized and dissected in saline solution (Nacl, 10 mM) under a stereomicroscope.

**Determination of digestive enzyme activities**

The guts were separated from the larval body, rinsed in ice-cold distilled water, placed in a precooled homogenizer, and ground before centrifugation. Equal portions of larval midgut and distilled water were used to have a desirable concentration of the enzymes w/v. Homogenates were separately transferred to 1.5 mL centrifuge tubes and centrifuged at 13000 rpm for 20 min at 4°C. The supernatants were pooled and stored at−20°C for subsequent analyses. For solubilization of membrane bound enzymes, membrane preparations (via precipitation from primitive centrifuge, above) were exposed to Triton X-100 for 20 h at 40°C, in a ratio of 10 mg of Triton X-100 per mg of protein, followed by centrifugation at 13000 rpm for 30 min. No sediment was visible after the centrifugation of samples. The activity of the enzymes remains stable, at −20°C, for periods of at least one month\textsuperscript{15}. General proteolytic activity of the soluble and membrane proteases in different parts of gut was measured as described by\textsuperscript{16} with slight modification, using azocasein as substrate. The reaction mixture had 20 \(\mu\)L of enzyme, 70 \(\mu\)L of universal buffer (50 mM, pHs 7-10) and 40 \(\mu\)L of substrate. The mixture was incubated for 1 h at 37°C. In order to end reaction, 100 \(\mu\)L of 30% TCA (trichloroacetic acid) were added to it followed by cooling at 4°C for 30 min. The solutions were centrifuged at 13000 rpm for 5 min, after which an equal volume of NaOH was added and absorbance was read at 450 nm. Appropriate blanks in which TCA had been added before to the substrate were prepared for each assay.
Digestive trypsin, chymotrypsin and elastase like activities of the larvae fed on either variety of citrus were estimated using final concentrations of trypsin, chymotrypsin and elastase-like activities (as three subclasses of serine proteinases) were assayed using a concentration 1 mM of BApNA (N-benzoyl-DL-arginine-p-nitroanilide), 1 mM SAAPPnNA (N-succinyl-alanine-alanine-proline-phenylalanine-p-nitroanilide), and 1 mM SAApNA (N-succinyl-alanine-alanine-alanine-proline-p-nitroanilide) as substrates, respectively. The reaction mixture consisted of 50 μL of universal buffer at appropriate pH (pH 7) and 20 mL of soluble starch for 30 min at 37°C. The reaction was terminated by addition of 100 mL DNS and heating in boiling water for 10 min. The absorbance was read at 545 nm. One unit of amylase activity was defined as the amount of enzyme required to produce 1 mg of maltose in 30 min at 37°C.

α-Amylase activity was assayed by the dinitrosalicylic acid (DNS) procedure17, with 1% soluble starch as substrate. The amount of 10 μL of the enzyme was incubated with 50 μL of universal buffer (pH 7) and 20 mL of soluble starch for 30 min at 37°C. The reaction was terminated by addition of 100 mL DNS and heating in boiling water for 10 min. The absorbance was read at 545 nm. One unit of amylase activity was defined as the amount of enzyme required to produce 1 mg of maltose in 30 min at 37°C.

α- and β-glucosidase activities were assayed with a little change by incubating 15 μL enzyme solution with 30 μL of p-nitrophenyl-α-D-glucopyranoside (pNaG) (5 mM), p-nitrophenyl-β-D-glucopyranoside (pNBG) (5 mM) and 50 μL of universal buffer (50 mM, pH 7) at 37°C for 10 min. Two exopeptidases in the midgut of P. demoleus were obtained using hippuryl-Larginine and hippuryl-L-phenylalanine for carbox-y- and aminopeptidases, respectively. The reaction mixture consisted of 50 μL of universal buffer (pH 7), 20 μL of each substrate, and 10 μL of enzyme solution. The reaction mixture was incubated at 30°C for a period of 0 to 10 min and the absorbance was read at 340 nm. Lipase assays were carried out as described by. About 10 μL of gut extract and 20 μL of p-nitrophenyl butyrate (50 mM) as substrate were incubated with 50 μL of universal buffer (50 mM, pH 7) and incubated at 37°C after 7 min, the absorbance was read at 492 nm.

Determination of some enzymatic activities and components in hemolymph

Estimation of acid phosphatases (ACP) and alkaline phosphatases (ALP) were carried out as per procedure21. About 50 μL universal buffer (pH 5 for ACP and pH 8 for ALP) 20 μL of p-nitrophenolphosphate as substrate and 10 μL enzymes were mixed together, after 1 min, the absorbance were read at 405 nm. Alkaline aminotransferase (ALT) and aspartate aminotransferase (AST) were measured using22 procedure. This assay was done by AST and ALT kit (Biochem Co., Tehran, Iran). The absorbance was read at 492 nm. The lactate dehydrogenase activity was measured23. About 100 μL of substrate buffer (first solution) and 10 μL enzyme were added to each tube and incubated at 37°C for 5 min, after that, 25 μL of (2,4-dinitrophenyl hydrazine) from the second coloured solution was added to each tube, after 1 min, the absorbance was read at 340 nm.

Gamma-glutamyltransferase activity was measured24. This assay was done by (Zist Chem Diagnostic Co., Tehran, Iran). The reaction mixture consisted of 100 μL of buffer reagent, 25 μL of substrate reagent (L-γ-glutamyl-3-carboxy-4-nitrianiilide) and 10 μL of the sample. Absorbance was read at 545 nm after incubation for 1 min.

Determination of storage components of fat body

Triacylglyceride determination

Measurement of triacylglyceride was done using a diagnostic kit manufactured by Pars Azmoon Co. (Tehran, Iran). The reagent solution contained phosphate buffer (50 mM, pH 7.2), 4-chlorophenol (4 mM), adenosine triphosphate (2 mM), Mg 2+ (15 mM), glycerokinase (0.4 kU/L), peroxidase (2 kU/L), glycerol (2 kU/L), 4-aminoantipyrine (0.5 mM), and glycerol-3-phosphate-oxidase (0.5 kU/L). Samples (10 mL) were incubated with 20 mL of distilled water and 70 mL of reagent for 20 min at 25°C. Optical densities (ODs) of samples and reagent, as a standard, were read at 546 nm. Protein concentrations were assayed according to the method26. In this assay, 20 μL of the sample was added to 50 μL of reagent, and the mixture was incubated for 15 min prior to reading the absorbance at 545 nm (recommended by Ziest Chem. Co., Tehran, Iran).

The fat bodies of fifth larvae of each variety were cut and immersed in 1 mL of 30% KOH and Na2SO4. Tubes containing the samples were covered with aluminium foil (to avoid evaporation) and were boiled for 20-30 min. Tubes were shaken and cooled in ice. Two milliliters of 95% EtOH was then added to precipitate glycogen from the digested solution. The samples were then shaken again and incubated in ice.
for 30 min. Tubes were centrifuged 13000 rpm for 30 min. Supernatant was removed and pellets (glycogen) were re-dissolved in 500 μL of distilled water before being shaken. Then, it was added to reaction mixture in 5% phenol and 500 μL sulfuric acid. Incubation was performed in an ice bath for 30 min. Standards and samples were read at 492 nm and distilled water was used as a control27.

Chemical analysis of the leaves

Leaves from all four citrus trees were collected during the experimental period in September 2016, dried in the shade, and prepared for chemical analysis. For phosphorous, calcium and potassium, dry ashing and mixing with HCl was first performed. Total organic nitrogen and protein contents were determined by micro-Kjeldahl method, potassium content was measured by flame photometry, using a lithium internal standard, and phosphorus content was determined using the colorimetric method with blue coloured acid ascorbic and read at 880 nm. For measuring calcium, the compleximetric method was used28.

Statistical analysis

Nutritional indices of P. demoleus reared on different hosts were analyzed with one-way ANOVA using the statistical software Minitab ver. 14.0 (Minitab Inc., Philadelphia, PA, USA; http://www.minitab.com; 1994) to determine similarities and significant differences. Statistical differences among the means were assessed using an LSD test at a=0.01. Data were tested for normality before analysis. The data from other experiments were subjected to analysis of variance (ANOVA) using SAS software. The least significant differences among treatments were compared using Tukey’s multiple range tests (SAS Institute, Cary, NC, USA; 1997). Differences among means were considered significant at P ≤0.01.

Results

The results of the nutritional indices of fifth instar larvae of P. demoleus are shown in Table 1. The nutritional indices of fifth instar larvae were significantly different on various host plants (P <0.01). The larvae reared on Citrus limon showed the highest value of CI (3.34±0.04 %) (F= 1170; df=3; P<0.0001), RCR (15.54±0.66%) (F= 158.87; df= 3; P <0.01) and AD (91.18±0.43%) (F= 529.72; df=3; P<0.0001) except in RGR. However, the lowest value of CI, RCR and AD was noticed in C. paradisi (1.22±0.02, 4.60±0.20 and 71.17±0.41, respectively). On the other hand, the highest value of ECD (F= 568.45; df=3; P <0.0001) (39.05±0.57) and ECI (F= 364.52; df=3; P <0.0001) (26.21±0.43) were observed in C. sinensis. While the highest and lowest value of RGR (F= 11.21; df=3; P <0.0001) were observed in C. sinensis and C. paradisi (1.28±0.03 and 0.99±0.03, respectively).

Biochemical assessments

The results of biochemical assessments of P. demoleus larvae feeding on different hosts are depicted in Table 2. The larvae reared on Citrus sinensis var. valencia (F=14.22, df=3, P <0.0003) (0.05±0.001) and C. paradisi (F=3.55, df= 3, P >0.0477) (0.029±0.03 U/mg) showed the highest levels of proteolytic activity at pH 7 and pH 10, respectively. It was observed that larvae that fed on C. paradisi had the highest activity of chymotrypsin and trypsin (U/mg), (F= 8.21, df=3, P <0.0080) and (F= 12.66, df=3, P <0.0005) (0.32±0.08 and 0.55±0.06 U/mg, respectively). The larvae reared on C. sinensis var. valencia (0.34±0.08 U/mg) showed the highest activity of elastase (F=4.26, df=3, P <0.0290), and the those feeding on C. limon showed the lowest activity of trypsin and elastase (0.12±0.05 and 0.26±0.04 U/mg, respectively). As for carbohydrases, the highest levels of amylase activity (F= 13.67, df=3, P <0.0016) were found on the larvae that fed on C. sinensis (0.38±0.01 U/mg), and the larvae feeding on C. limon had the lowest activity of amylase (0.08±0.01 U/mg).

Aminopeptidase and carboxpeptidase had the highest activity in those larvae that were reared on

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**Table 1 — Nutritional indices of different varieties of citrus on Papilio demoleus larvae**

<table>
<thead>
<tr>
<th>Variety</th>
<th>% ECI</th>
<th>% ECD</th>
<th>% AD</th>
<th>RGR*(mg/day)</th>
<th>CI *(mg/day)</th>
<th>RCR*(mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrus paradise</td>
<td>23.04±0.39&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.42±0.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>71.17±0.41&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.99±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.22±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.60±0.20&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>C. limon</td>
<td>7.95±0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.13±0.27&lt;sup&gt;d&lt;/sup&gt;</td>
<td>91.18±0.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.19±0.03&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.34±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.54±0.66&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C. sinensis</td>
<td>26.21±0.43&lt;sup&gt;c&lt;/sup&gt;</td>
<td>39.05±0.57&lt;sup&gt;c&lt;/sup&gt;</td>
<td>66.55±0.41&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.28±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00±0.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.83±0.16&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>C. sinensis var.valencia</td>
<td>15.53±0.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.29±0.54&lt;sup&gt;c&lt;/sup&gt;</td>
<td>83.51±0.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.22±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.64±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.97±0.36&lt;sup&gt;b&lt;/sup&gt;</td>
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</tbody>
</table>

<sup>*ECI, efficiency of conversion of ingested food; ECD, efficiency of conversion of digested food; AD, approximate digestibility; RGR, relative growth rate; CI, consumption index; RCR, Relative growth rate. Means followed by different letters in the same columns are significantly different (LSD, P <0.01) |
C. paradisi leaves (F= 11.44, df=3, P < 0.0029) (0.33±0.15) (F= 244.19 df=3 P < 0.0001) (0.51±0.05), respectively. The activity of aminopeptidase in other varieties showed no significant differences. Carboxypeptidase was more active in the larvae that fed on C. sinensis (0.03±0.01). The larvae fed on C. paradisi (0.29±0.01) showed the highest activity of α-glucosidase (F= 15.38, df=3, P < 0.0002) and β-glucosidase activity (F= 34.37, df=3, P < 0.0001) (0.23±0.1). The lipase (0.05±0.01%) (F=8.22; df=3; P < 0.0031) was most active in those larvae that fed on C. sinensis var. valencia.

Our observation on non-enzymatic compounds showed that those larvae reared on C. paradisi had the highest levels of triglyceride level (3.10±0.16%) and those fed on C. sinensis (1.87±0.01%) had the lowest value (F= 41.89, df=3, P < 0.0001). Glycogen content was maximal in the larvae fed on C. paradisi. The highest total protein was recorded in larvae that fed on C. sinensis, var. valencia (F= 3.41; df=3; P < 0.0732) (15.04±0.77%). As illustrated in Table 2, the highest activity level of Gamma-glutamyl transferase (F=89.54; df=3; P < 0.0001) and lactate dehydrogenase (F=18.78, df=3, P < 0.00001) were found on C. paradisi (0.58±0.01 and 0.09±0.006 U/mg, respectively) and the lowest in C. sinensis var. valencia and C. sinensis, respectively. The larvae that fed on C. paradisi had the highest ALT (F= 20.56, df=3, P < 0.0001), ALP (F= 59.24, df=3, P < 0.0001) and ACP (F= 18.01, df=3, P < 0.0001) values (0.15±0.02, 0.11±0.04 and 0.13±0.03 U/mg), respectively. The larvae reared on C. sinensis var. valencia (0.06±0.01 U/mg) showed the highest levels of AST and was lowest in C. sinensis (0.02±0.02 U/mg), (F= 17.91, df=3, P < 0.0001).

Chemical analyses of citrus leaves

The results of leaf analysis demonstrated that phosphorus content were highest in C. sinensis and C. sinensis var. valencia (0.51±0.01 and 0.51±0.02) and lowest in C. paradisi (0.48±0.01) (F=15.56; df=2; P <0.01), respectively. The highest potassium content was C. sinensis (2.44±0.04) and the lowest in C. limon (0.79±0.02) (F=10.41, df=3, P < 0.002). Calcium content was highest in C. sinensis var. valencia (3.19±0.48) and the lowest in C. paradisi (1.26±1.14) and the other two varieties were not significantly different from each other (F=6.57; df=3; P > 0.0150), while the highest nitrogen content was observed in C. sinensis var. valencia (5.36±0.24) and the lowest in C. limon (1.8±0.68) (F=5.88; df=3; P > 0.0202). Similarly, protein content was highest in C. sinensis var. valencia and lowest in C. limon (33.54±0.52 and 11.24±2.57), respectively (Fig. 1).

Discussion

Different host plants affect insect physiology and have significant impact on its performance. In the present study, feeding on different citrus varieties led
Digestive enzymes activity of insects depends on the quality of food sources\textsuperscript{10}. Proteinases are among the main groups of hydrolytic enzymes involved in digestive processes of ingested food\textsuperscript{15}. In the current research, higher protease activities were seen at two different pH., i.e., pH 7 and pH 10 in C. sinensis var. valencia and C. paradisi-fed larvae, respectively. The highest values of ECI and ECD were found in both individuals fed on C. sinensis, indicated the ability of these insects to convert ingested food into body mass or finally into their growth. The larvae feeding on C. sinensis also showed the highest RGR value, suggesting a direct relationship of RGR to ECI and ECD values. Hence, we may suggest that C. sinensis is a suitable variety for larvae of P. demoleus. In the current study, the highest values of CI and RCR were recorded in the larvae that fed on C. limon demonstrating confrontation of larvae to lower food quality. Hence, larvae utilize various mechanisms of nutritional compensatory via increase of feeding period to gain required nutrients\textsuperscript{33}.

It is reported that the lepidopterous larvae that feed on nutrient rich food, increase growth rates and complete the developmental period faster than those feeding on low nutrient food\textsuperscript{34}. The duration of the feeding period may be considered a key factor in the RGR and RCR values. Our results of fifth instar larvae showed that the RCR and RGR values were the highest on C. limon and C. sinensis, respectively, and lowest on C. paradisi. The present results indicated that C. paradisi was a low nutrient food for the larvae, and as a result the larvae need a longer period for development, and to complete immature stages. Conversely, C. limon and C. sinensis are among the high nutrient foods for the larvae, and a shorter period of development was needed for completion of their immature stages.

Digestive enzymes activity of insects depends on the quality of food sources\textsuperscript{10}. Proteinases are among the main groups of hydrolytic enzymes involved in digestive processes of ingested food\textsuperscript{15}. In the current research, higher protease activities were seen at two different pH., i.e., pH 7 and pH 10 in C. sinensis var. valencia and C. paradisi-fed larvae, respectively.
which might have been due to the high protein content of the diet\textsuperscript{35}. The highest chymotrypsin and trypsin activities were seen in \textit{C. paradisi}-fed larvae compared with other varieties. The larvae feeding on \textit{C. sinensis} \textit{var. valencia} showed the highest activity of elastase, while the lowest activities were recorded in \textit{C. sinensis} and \textit{C. limon}. Lectins are carbohydrate-binding proteins distributed in different species of plants\textsuperscript{36}. They play different roles in plants; many of them have a direct inhibitory effect on some digestive enzymes of higher animals and insects, including δ-amylases\textsuperscript{36}, and esterases and proteases\textsuperscript{38}.

Initial digestion of carbohydrates is made by alpha-amylase. The oligosaccharides are then digested to monomers by the activities of glycosidases\textsuperscript{15}. In the current study, activity of alpha-amylase in the larvae of \textit{P. demoleus} was the highest on \textit{C. paradisi}, but the lowest activity was seen in \textit{C. limon}. According to chemical analyses of leaves, we observed that the varieties \textit{C. paradisi} and \textit{C. limon} had the highest and lowest levels of protein, respectively. In contrast, activity of α and β-glucosidases were the highest in the larvae fed on \textit{C. paradisi} but the larvae fed on \textit{C. limon} and \textit{C. sinensis} \textit{var. valencia} showed the lowest activity. While, there was no significant difference in β-glucosidase between three varieties of \textit{C. sinensis} \textit{var. valencia}, \textit{C. sinensis} and \textit{C. limon}. It is believed that activities of glucosidases depend on the amount of secondary compounds, mainly phenol. Therefore, the higher activities of glucosidases in \textit{C. paradisi} may reflect the presence of higher amount of these compounds, indicating unsuitability of the variety for \textit{P. demoleus}\textsuperscript{39}.

The highest activity of TAG-lipase observed in the larvae that fed on \textit{C. sinensis} \textit{var. valencia}, which may be due to the presence of higher amount of sterols\textsuperscript{15}. Intermediary metabolism is a complicated phenomenon in which energy is provided for biological activities via several processes, such as detoxification of xenobiotics, etc.\textsuperscript{29}. The aminotransferases are enzymes that catalyze the reaction between an amino acid and a keto acid. This reaction removes the amino group from the amino acid, leaving a keto acid and converting it into an amino acid. These enzymes serve as a link between the carbohydrates and protein metabolism and are altered during various physiological processes\textsuperscript{40}. Our results showed that various host plants had significant impact on ALT and AST activities in \textit{P. demoleus}. The highest activities were found in the larvae fed on \textit{C. paradisi} and \textit{C. sinensis} \textit{var. valencia}. In the present study, the highest activities of ALP were found in the larvae that fed on \textit{C. paradisi} and the highest activity of ACP was found in the larvae that fed on \textit{C. paradisi} and \textit{C. sinensis} \textit{var. valencia}. ALP and ACP hydrolyze phosphate groups of several molecules, including nucleotides, proteins and alkaloids in alkaline and acidic conditions through dephosphorylation\textsuperscript{41}. According to the results of chemical analyses of the leaves, \textit{C. paradisi} and \textit{C. sinensis} \textit{var. valencia} leaves had the highest level of protein (Fig. 1).

GGT is an enzyme that transfers gamma-glutamylmoiety of glutathione to form glutamate. It plays a crucial role in gamma-glutamyl cycle for the synthesis and degradation of glutathione and xenobiotic compounds\textsuperscript{42}. Larvae that fed on \textit{C. sinensis} and \textit{C. paradisi} exhibited the highest activity of GGT. These findings indicated the presence of glutathione or other xenobiotic compounds such as glycoalkaloids in the diet components to be degraded by GGT.

LDH is an important enzyme involved in carbohydrate metabolism through glycolysis cycle. Pyruvate is the final product of glycolysis, which transfers to Krebs cycle for ATP synthesis under oxygen sufficient conditions. If there is a shortage in oxygen, it converts to lactate. So, it must be degraded by LDH. This enzyme is an indicator of tissue exposure to chemical stress\textsuperscript{43}. The highest amount of LDH was obtained in the larvae that fed on \textit{C. paradisi}, where the highest amount of glycogen was found. These findings demonstrated that larvae that fed on \textit{C. paradisi} gain higher levels of polysaccharides that are stored as glycogen. These larvae may depend on glycogen rather than TAG for their biological activities, and this dependence might have caused higher activities of LDH. Glycogen, triglyceride and total proteins are the three main storage macromolecules in insect fat bodies. Because there are variable amounts of these molecules in host plants, utilization of different plants might lead to a gain in various levels of storage macromolecules in insects.

In this study, we observed that the larva that was reared on \textit{C. paradisi} had the highest total protein (Fig. 1). TAG and protein are the two major storage macromolecules in fat bodies of insect. Their amounts depend on developmental stages and nutrient status of insect\textsuperscript{29}. Regarding insect energy demands, lipids are considered to be the most important nutrient because
they are used in tissue repair, energy demands, and enzymatic processes. In the current study, the highest amounts of TAG was observed in the larvae that fed on C. paradisi and we also observed that the larvae that was reared on C. sinensis var. valencia had the highest total protein, which corresponds with other results (Fig. 1), showing that these varieties have the highest protein content.

In this study, we found that the chemical compounds present in the host plant can play an important role in the feeding activity, digestive enzyme activity and chemical compounds stored in the plant when fed upon by pests. Based on our results, C. sinensis and C. paradisi had the highest nutritional indices and the highest activity of digestive enzymes to make them the preferred hosts for P. demoleus. The host C. limon had the lowest activity of digestive enzymes and nutritional indices, identifying it as an unsuitable host.

Physiological findings on the larvae of P. demoleus fed on different citrus varieties highlighted the value of nutrient requirements in its development. In fact, differences in the amounts of nutritional indices may be due to physiological and behavioural changes along with developmental stages. The present study revealed that C. limon was less suitable host for P. demoleus. Even if these varieties prove to be the less suitable citrus varieties under field conditions, then it may be considered in the IPM program to increase efficiency of current control approaches. The understandings of differences in food quality among citrus varieties, presence of secondary components, and possible inhibitors from a wider range of citrus varieties are necessary for designing the stable crop production systems. In the future, extraction and identification of chemical compounds responsible for the resistance of C. limon could be a promising way to provide new varieties via biotechnological procedures.

References