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The effects of host plants on the feeding indices and chemical activities of elm leaf beetle, *Xanthogaleruca luteola* (Muller) (Coleoptera: Chrysomelidae)

H. Yazdanfar[1*], M. GhodskhahDaryaei[2], J. Jalali Sendi[1]

[1]Department of Plant Protection, Faculty of Agriculture, University of Guilan, Guilan, I.R. Iran.
[2] Department of Forestry, Faculty of Natural Resources, University of Guilan, Guilan, I.R. Iran.

* Corresponding Author: hediyazdanfar@yahoo.com

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ABSTRACT- Elm leaf beetle (ELB), *Xanthoga leruca luteola* (Muller), is considered as one of the most important and destructive phytophagous pests of *Ulmus* species in the north of Iran. In the current study, the effects of three host plants including *Ulmus carpinifolia, U. carpinifolia* var. *umbraculifera,* and *Zelkova carpinifolia*, on feeding indices and biochemical processes of ELB were examined under controlled conditions (25 ± 2 °C; 14:10 LD; 65% RH). The results showed that the highest efficiency of conversion of ingested food (ECI) (23.11±1.36 %) and efficiency of conversion of digested food (ECD) belonged to *U. carpinifolia* (79.9±4.12 %) and the lowest to *Z. carpinifolia* ($p \leq 0.05$). Relative growth rate (RGR) and approximate digestibility (AD) in *U. carpinifolia* var *umbraculifera* and *U. carpinifolia* were similar to each other. The highest consumption index (CI) belonged to *U. carpinifolia* var. *um braculifera* and the lowest to *Z. carpinifolia* (1.93±0.029, 1.47±0.054), respectively ($p \leq 0.05$). Significant differences were found among enzymatic activities of acid (ACP) and alkaline phosphatases (ALP), aspartate aminotransferases (AST and ALT) and lactate dehydrogenase (LDH) in the haemolymph of *X. luteola* larvae reared on three different host plants. The highest activities of LDH (33±0.58), AST (10074.7±25.25) and ALT (1053.75±5.6) were found in larvae fed on *U. carpinifolia* ($p \leq 0.05$). Also, the highest amount of protein and triglyceride (TAG: 126.5±0.866) was found in larvae fed on *U. carpinifolia*. The results show that all enzymatic activities significantly decreased on *Z. carpinifolia* compared with other host plants ($p \leq 0.05$). These results indicated that *U. carpinifolia* is the most appropriate host plant for larvae of *X. luteola*as evidenced by the highest nutritional indices as well as activities of enzymatic component in intermediary metabolism.

INTRODUCTION

In northern Iran, deciduous trees of the family Ulmaceae that include the elms (*Ulmus* spp.) and the zelkovas (*Zelkova* spp.) often grow in Hyrcanian mixed forests as well as many urban areas (Khatamsaz, 1990; Sabeti, 1999). The elm tree is susceptible to more than 80 insect pest species including the elm leaf beetle (ELB), *Xanthogaleruca luteola* (Muller), which is considered as one of the most important and destructive insect pests (Abaei, 1999; Arbab et al., 2001). Pest feeds on elm leaves in larval and adults stages cause defoliation of urban and suburban elm (Behdad, 1988; Abaei, 1999). For the first time, ELB has been reported in 1945 from Iran and it has been considered as one of urban trees' pests (Jalali et al., 2005; Shekari et al., 2008). ELB utilizes various species in both *Ulmus* and *Zelkova* genera as its hosts (Behdad, 1988). The feeding causes deformities in tree crowns and physiological disorder which makes the trees susceptible to several pests, pathological agents and environmental stress (Arbab et al., 2001). Planting species that are less preferred by insect herbivores would decrease the need for insecticide sprays, which would reduce health hazards for both human and environment (Miller and Ware, 1999). Studying the effects of host plants on the digestive performance of insects is important in understanding host suitability of plant infesting insect species (Mardani-Talaee et al., 2014). Data relating to consumed food and its utilization are useful for better comparison across different food materials, larval stages or environmental conditions (Ansari et al., 2012). Differences among efficiencies of food materials are exhibited through food consumption and insect growth (Andreeva, 2010; Ansari et al., 2012).

Despite the economic importance of ELB, little published information exists on the nutritional indices of this pest on different host plants or on the effect of different host plants on specific activities of enzymatic components of ELB larvae; however, some related studies have been done on the influence of host plants apart from those tested in the current study on feeding indices of *X. luteola*. Miller and Ware (1999), in their studies on ELB, focused on the preference of various elm species and their hybrids for feeding by ELB to select the most resistant species/hybrid of elms for

future elm breeding program. Khalili Mahani (2003), in her study on suitability and food preference based on larval performance, concluded that *U. carpinifolia* is the most suitable host for feeding and reproduction whereas *C. caucasica* is the least suitable one for this pest. Leaf nutritional (minerals and sugars) traits may be associated with host resistance (Bosu and Wagner, 2008; Barbehenn et al., 2014). The larvae fed on host plants with higher level of some minerals such as nitrogen, phosphorus and potassium, had a shorter duration of development as well as a higher survival rate (Yazdanfar and GhodskhahDaryaei, 2016). They showed that *U. carpinifolia* was the most suitable host for survival and development, while *C. caucasica* was the least suitable one for this pest.

Evaluating feeding indices is an important part of feeding ecology clarified by Waldbauer (1968). These indices can provide valuable information about the different impacts of ingredients or total food (Ansari et al., 2011). The aim of this study was to compare food utilization indices for *X. luteola* on different host plants to determine the most susceptible host plants by ELB and the effects of larval feeding on different host plants of ELB on some of enzymatic and non-enzymatic components.

MATERIALS AND METHODS

Host Plants

Three host plant species were used in this study, including: *Ulmus carpinifolia*, *U. carpinifolia* var *umbraculifera*, and *Zelkova carpinifolia*. These plants were selected because they are the main host plants of *X. luteola* in northern Iran. All these plants are distributed throughout the Faculty of Natural Resource in Somesara, north of Iran. These trees were used as a source of leaves for each experiment. Leaves were removed from branches and their petioles were placed immediately in tubes of water and transferred to laboratory. Fresh leaves were daily provided and they were covered with compressed wet sponges to keep their humidity. The younger same size leaves (but not fully expanded) were used for feeding first instar larvae instars, while fully expanded leaves were used for feeding second to third larval instar.

Insect Rearing

The egg clusters ELB with leaf substrate were collected from introduced trees and transferred to laboratory and reared on fresh mentioned host plants. First instar ELB were obtained from the collected egg clusters, and held in a growth chamber at 25 ± 2°C; 14:10 LD; 65% RH. These larvae were subsequently reared on freshly cut foliage of host plants, i.e. *U. carpinifolia, U. carpinifolia* var *umbraculifera,* and *Z. carpinifolia*. Larvae were reared in plastic jars (5×10×20 cm) in which the lid contained holes covered by a fine mesh net for ventilation. In order to prevent the leaves from losing humidity, the bottom of the jars were covered with compressed wet sponges and the leaves containing larvae were vertically placed over the sponges and fresh leaves were provided daily.

Food Consumption and Utilization

One-day-old final instar larvae (3th instar) of ELB were used after several hours of starvation for the food performance test. Third instar larvae were gathered from the host plants and separated into eight replicates (10 larvae in each) and transferred into a plastic jar (5×10×20 cm) with a hole covered by a mesh net for ventilation, containing fresh leaves of each examined plant. The larvae were provided with fresh leaves of the respective food plant on a daily basis until pupation. The weight of the larvae, food given, food left uneaten and faecal matter produced were daily recorded. Concomitantly, a control was run daily throughout the study by keeping weighted leaf in a petri dish and reweighing it after 24 h to assess loss of water/moisture from the food for the purpose of compensation of similar loss from the food offered to the larvae. Nutritional efficiency indices were calculated based on the procedures designed by Waldbauer (1968) using fresh weight basis:

Consumption index (CI) =F/TA
Relative growth rate (RGR) =G/TA
Efficiency of conversion of ingested food (ECI) =WG/FI×100
Approximate digestibility (AD) =FI-WF/FI×100
Efficiency of conversion of digested food (ECD) = WG/FI-WF×100
where:
T = duration of feeding period (days)
A = mean fresh weight of larva during feeding period
F = fresh weight of food eaten
G = fresh weight gain of larva during feeding period
WG = weight gained
FI =weight of food ingested
WF = weight of faces

Biochemical Analysis

Thirty fresh larvae of third instar ELB were killed by freezing, and extracts of the bodies were obtained from the whole body 24h after the treatment. Samples from each treatment were diluted with phosphate buffer (1:1, w/v) and centrifuged for 10 min in 14000 rpm. The supernatant was moved to new tubes and was stored at -30$^{\circ c}$ until used. Each biochemical analysis was repeated 3 times. Alanine amino transferase (ALT) and aspartate amino transferase (AST) activities were measured using Thomas' procedure (1998). The assays were performed with AST and ALT kits (Biochem Co., Tehran, Iran). Reagents 1 and 2 were mixed (4:1), samples were added and absorbance was read at 492 nm. Alkaline (EC 3.1.3.1) and acid (EC 3.1.3.2) phosphatase activity was followed as was described by Bessey et al. (1964). The buffered substrate (phosphate buffer, 0.02 M, pH 7.2) was incubated with samples for 30 min. Alkali was added to stop the reaction and to adjust the pH for determination of the formed product concentration.

The spectral absorbance of p-nitrophenolate was maximal at 310 nm. The molar absorbance of p-nitrophenolate at 400 nm was approximately double

than that of p-nitrophenyl phosphate at 310 nm. On converting the p-nitrophenolate into p-nitrophenol by acidification, the absorption maximum shifted to approximately 320 nm with no detectable absorption at 405 nm.

Protein was measured based on the Biuret's method as was described by Reinhold (1953) using a protein assay kit (Pars Azmon Co, Iran) and measuring the absorbance at 540 nm. Total cholesterol was analyzed by Richmond method (1973). Lactate dehydrogenase (LDH) was measured based on the King's method (1965). Test tubes were prepared containing 1 mL of the buffered substrate and 0.01 mL of the sample. To standardize volumes, 0.2 mL NAD^+ solution was added to the test tubes of the sample group and 0.2 mL of water was added to the test tubes of the control group. Test tube samples were incubated for 15 min at 37°C. The reactions were then arrested by adding 1 mL of color reagent (2, 4-dinitrophenylhydrazine) to each tube, after which incubation was continued for an additional 15 min. The contents were cooled at room temperature, and 10 mL of 0.4 N NaOH was added to each tube to make the solutions strongly alkaline. At 60 s after the addition of alkali to each tube, the intensity of color was measured at 454 nm.

Statistical Analysis

Data obtained in this study were subjected to one-way analysis of variance (ANOVA) and treatments were compared using Tukey's method at $P \leq 0.05$. All statistical analyses were done using SPSSv.16.0 for windows.

RESULTS AND DISCUSSION

Food Consumption and Utilization

Food consumption and conversions of ingested and digested food by *X. luteula* larvae varied significantly among the host plants. The results of the nutritional indices of third instar larvae of *X. luteula* are indicated in Table 1.

The third instar larvae reared on *U. carpinifolia* showed the highest value of efficiency of conversion of digested food (ECD) and the lowest value of ECD was on *Z. carpinifolia* (F= 6292.91; df=2,21; p<0.01). Also, the highest value of efficiency of conversion of ingested food (ECI) was on *U. carpinifolia* compared with other host plants *(*F= 83.64; df=2, 21; p<0.01). The larvae fed on *U. carpinifolia* var *umbraculifera* had the highest consumption index (CI) (F=21.91; df=2, 21; p<0.01). However, the lowest value of CI was observed on *Z. carpinifolia*.

The highest and lowest amount of approximate digestibility (AD) were observed on third in star larvae fed on *Z. carpinifolia* and *U. carpinifolia* var *umbraculifera*, respectively *(*F= 222.31; df=2, 21; p<0.01).

The third instar larvae reared on *U. carpinifolia* and *U. carpinifolia* var *umbraculifera* showed higher relative growth rate *(RGR)* than *Z. carpinifolia.* Based on the analysis, RGR in larvae reared on *U. carpinifolia* var *umbraculifera* and *U. carpinifolia* showed no significant difference (F= 191.51; df=2, 21; p<0.01).

Effects of Various Host Plants on Some Biochemical Compounds

The results of the activities of ALT and AST, acid (ACP) and alkaline (ALP) phosphatase, as well as LDH in the haemolymph of larvae reared on *U. carpinifolia*, *U. carpinifolia* var *umbraculifera* and *Z. carpinifolia* are shown in Table 2. The activities of mentioned compounds of the third instar larvae of *X. luteola* were significantly different on various host plants. The activity of ALT and AST showed the highest activity on *U. carpinifolia* compared to other host plants (F= 1482.83; df = 2, 9; p<0.01) and (F= 2370.13; df=2, 9; p<0.01), respectively.

Also, the activities of LDH in the larvae of *X. luteola*vwere was the highest on *U. carpinifolia*v (F=207.07*;*df=2, 9; p<0.01). The enzymatic activity of ALP and ACP showed the highest activity on *U. carpinifolia* var *umbraculifera in* comparison with other host plants (F= 156; df=2, 9; p<0.01) and (F=5771.4; df=2, 9; p<0.01), respectively.

Effects of Various Host Plants on Storage Components of The Fat Body

Significant differences were found among the amount of triglyceride and total protein in the larvae reared on three different host plants.

The results showed that the highest amount of triglyceride was found in the fat body of larvae fed on *U. carpinifolia* and the lowest amount was on *Z. carpinifolia (F=815.73;*df=2, 9; p<0.01). Also, the amount of total protein in the larvae of *X. luteola* was the lowest on *Z. carpinifolia (F=1472.4;*df=2, 9; p<0.01). Components of host plant quality directly affect potential and achieved herbivore fecundity. It may influence survival, growth, fecundity and development time (Awmack and Leather, 2002; Ansari et al., 2011). Host plant quality can also affect the insects' enzymatic and non-enzymatic activities. In the current study, nutritional indices of third instar larvae of *X.luteola* on different host plants were investigated. The nutritional indices, especially ECI and ECD values, of *X.luteola* reared on various host plants were significantly different, suggesting that the host plants had different nutritional values. ECI measures the overall conversion of ingested food into biomass and ECD measures the efficiency with which assimilated food is converted into insect biomass (Waldbauer, 1968). The highest ECI value of *X. luteola* was on *U.carpinifolia*, indicating that it was more efficient in the conversion of ingested food to biomass. The larvae fed on *Z. carpinifolia* had the lowest value of ECD, which indicates that these larvae were apparently not as efficient in turning digested food into biomass. It is well understood that the degree of food usage depends on the digestibility of food and the efficiency with which digested food is turned into biomass (Batista Pereira et al., 2002). The decrease in dietary utilization shows that the decrease in

Table 1. Nutritional indices of 3rd instar larvae of *X.luteula* on tree host plants

Treatment	CI±SEM(g/g/day)	RGR±SEM(g/g/day)	ECI (%)±SEM	AD (%)±SEM	ECD (%)±SEM
U.carpinifolia	1.67±0.059[b]	0.38±0.015[a]	23.11±1.36[a]	29.78±0.066[b]	79.9±4.12[a]
*U.C.*var*umbra culifera*	1.93±0.029[a]	0.38±0.008[a]	19.5±0.33[b]	29.53±0.043[b]	66.15±1.12[b]
Z.carpinifolia	1.47±0.054[c]	0.13±0.005[b]	8.7±0.25[c]	33.61±0.253[a]	25.9±0.65[c]

Within columns, mean standard error (±SEM) followed with at least one similar letter are not significantly different at p≤0.05; CI: consumption index; RGR: relative growth rate; ECI: Efficiency of conversion of ingested food; AD: approximate digestibility; ECD: Efficiency of conversion of digested food

Table 2. Effects of host plants on specific activities of enzymatic in intermediary metabolism of *X.luteola* 3rd larvae

Host plants	ALP(IU/L)±SEM	ALT(IU/L)±SEM	AST (IU/L)±SEM	LDH (IU/L)±SEM	ACP(IU/L)±SEM
U.carpinifolia	8.75±0.25[b]	1053.75±5.6[a]	10074.7±25.25[a]	33±0.58[a]	384±2.041[b]
*U.C.*var*umbraculifera*	10.25±0.25[a]	919.5±10.75[b]	9537.5±89.29[b]	24.25±0.75[b]	872.5±6.61[a]
Z.carpinifolia	4.25±0.25[c]	532.75±1.03[c]	5301.25±6.57[c]	16.75±0.25[c]	276.25±2.13[c]

ALP, alkaline phosphatase; ACP, acid phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase. Mean standard error (±SEM) followed by different letters in the same column are significantly different (P<0.05, Tukey'spost-hoc honestly significant difference).

nutritional values may be due to behavioral and physiological effects (Nathan et al., 2005).

The higher CI achieved by larvae fed on *U. carpinifolia* var *umbraculifera* (Table 1) suggests that the rate of intake relative to the mean larval weight during the feeding period was the highest on this host plant. The approximate digestibility (AD), by larvae feeding on *Z. carpinifolia* was higher than for those feeding on two other host plants while RGR on *Z. carpinifolia* was the lowest. Third larvae instar of *X.luteola*fed on *Z.carpinifolia* had the highest AD and the lowest RGR and ECD. It seems that increasing in AD could not offset the decrution in ECD, which accordingly resulted in a decreased growth rate. Growth decrease is a general response of herbivore insects because of changing to a new host plant (Grabstein and Scriber, 1982; Sheppard and Friedman, 1990; Lazarevicand Peric-Mataruga, 2003). The results show that larvae reared on *U. carpinifolia* have the highest amounts of ECI, ECD and RGR which make them a more suitable host plant for *X. luteola* larvae than the others. Previous work (KhaliliMahani et al., 2003) indicated that when *X. luteola* larvae have been fed on *U. carpinifolia*, its intrinsic rate of increase and net reproduction rate were higher than three other treatments.

Triglyceride and total protein are the two main storage macromolecules in insect fat bodies. As variable amounts of these molecules enist in host plants, the use of different plants as feeding source of insects could result in a gain in various levels of storage macromolecules in insects. As it has shown in Table 3, the highest amount of TAG and total protein were found in the larvae fed on *U. carpinifolia* although no significant difference was observed in the amount of total protein between *U. carpinifolia* and *U. carpinifolia* var *umbraculifera.* Regarding insect energy demands, lipids are considered to be the most important class of nutrients because of their use as fuel in many physiological processes such as flight and reproduction.

However, proteins are mainly used in tissue repair and for other enzymatic processes. Therefore, larvae fed on *U. carpinifolia* might be far better in reproduction or other activities when they reach the adult stage. These results are in line with what has been obtained in nutritional indices.

Table 3. Effect of different host plants on the amount of triglyceride and total protein of *X.luteola* 3rd larvae

Host plants	Total protein(g/dl)± SEM	TAG (mg/dl)±SEM
U. carpinifolia	0.48±0.0057[a]	126.50±0.866[a]
U. c. var *umbraculifera*	0.467±0.0047[a]	104.25±1.43[b]
Z. carpinifolia	0.132±0.0047[b]	70±0.408[c]

TAG, triglyceride. Mean standard error (±SEM) followed by different letters in the same column are significantly different (P<0.05, Tukey's post-hoc honestly significant difference).

It was found that various host plants have significant differences on ALT and AST activities in the haemolymph of *X. luteola*. The highest activity occurred in the larvae fed on *U. carpinifolia*. These two enzymes are the most important components of amino acid catabolism participating in transfering of an amino group from one amino acid to α- keto acid (Nation, 2008). The ALT and AST work as a main link between the carbohydrates and protein metabolism and are converted during various physiological processes (Nation, 2008). Higher activities of these enzymes in larvae fed on *U. carpinifolia* may indicate the presence of higher amounts of protein in this host plant, for possible use of amino acids in tissue, development, excretion, and energy demands. These two enzymes are

the transaminases that are present in the haemolymph and fat bodies of insects (Nation, 2008). As shown in Table (2) (Ansari et al., 2011), the highest activities of ACP were found in the larvae fed on *U. carpinifolia* var *umbraculifera*, but the highest activities of the ALP were found in the larvae fed on *U. carpinifolia*. ALP and ACP are kinds of hydrolyzing enzyme and are responsible for transferring different kinds of phosphate from diverse groups of molecules including nucleotides, proteins and alkaloid (Zibaee and JalaliSendi, 2011). Several phenomena, such as the efficiency of digestion and transportation of nutrients in the midgut as well as haemolymph, significantly affect activities of these enzymes (Zibaee and JalaliSendi, 2011).

The highest amount of LDH was obtained in the larvae fed on *U. carpinifolia*, where the highest amount of triglyceride was detected. These findings indicated that larvae fed on *U.carpinifolia* may depend on TAG for their biological activities, and this dependence may cause higher activities of LDH.

CONCLUSIONS

In this survey, the effect of three host plants on nutritional indices and some enzymatic and non- enzymatic activities of *X. luteola*larvae have been investigated. The results show that the highest amount of most of nutritional indices such as ECD, ECI and RGR was obtained in the larvae fed on *U. carpinifolia* which is making it the most susceptible host plant to *X. luteola* compared to the other host plants. On the other hand, the highest activity of most of the enzymes in intermediary metabolism, amount of TAG and total protein of *X.luteola* larvae was observed in larvae fed on *U. carpinifolia*.

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اثرات گیاهان میزبان روی شاخص‌های تغذیه و فعالیت‌های شیمیایی سوسک برگ‌خوارنارون *Xanthogaleruca luteola* (Muller) (Coleoptera: Chrysomelidae)

هیدی یزدانفر[1]*، مهرداد قدس خواه دریایی[2]، جلال جلالی سندی[1]

[1]گروه گیاهپزشکی، دانشکده کشاورزی، دانشگاه گیلان، گیلان، ج. ا. ایران.
[2]گروه جنگلداری، دانشکده منابع طبیعی، دانشگاه گیلان، گیلان ، ج. ا. ایران.

*نویسنده مسئول

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واژه‌های کلیدی:
سوسک برگ‌خوار نارون
شاخص‌های تغذیه
متابولیسم های حدواسط
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چکیده- سوسک برگ‌خوار نارون (*Xanthogaleruca luteola* (Muller)) ،به عنوان یکی ازمهمترین و مخرب‌ترین آفات گیاه‌خوارگونه‌های نارون (*Ulmus* spp.) درشمال ایران، معرفی شده است. در این تحقیق تاثیر سه گونه‌ی میزبان ازخانواده‌ی نارون شامل نارون چتری (*Ulmus carpinifolia* var. *umbraculifera*) ، اوجا (*Ulmus carpinifolia*) و آزاد (*Zelkova carpinifolia*)روی شاخص های تغذیه و فعالیت های بیوشیمیایی آفت در شرایط استاندارد آزمایشگاهی (دمای ۲±۲۵ درجه سانتیگراد، ۱۴ ساعت روشنایی - ۸ ساعت تاریکی و رطوبت نسبی ۶۵ درصد) بررسی شد. نتایج نشان داد که بالاترین مقدار برای کارایی تغییر مواد غذایی خورده شده (ECI) و بالاترین کارایی تغییر مواد غذایی هضم شده (ECD) مربوط به درخت اوجا و کمترین مقدار این دو شاخص مربوط به گونه آزاد می باشد. نرخ نسبی رشد (RGR) و هضم تقریبی (AD) روی اوجا و نارون چتری مشابه بود. بالاترین مقدار شاخص مصرف (CI) به نارون چتری و کمترین مقدار آن به آزاد تعلق داشت. اختلاف معنی داری میان فعالیت های آنزیمی اسید فسفاتاز (ACP)، آلکالین فسفاتاز (ALP)، اسپارتات آمینوترانسفرازها (AST و ALT) و لاکتات دی هیدروژناز (LDH) در همولنف لاروهای سوسک برگ‌خوار نارون که از سه میزبان مختلف تغذیه کرده بودند، مشاهده شد. بالاترین فعالیت آنزیمی AST، LDH و ALT در لاروهایی مشاهده شد که از برگ های اوجا تغذیه کرده بودند. در ضمن، بالاترین مقدار پروتئین و تری گلیسیرید (TAG) نیز در لاروهایی اندازه گیری شد که از برگ های همین میزبان (اوجا) تغذیه کرده بودند. نتایج نشان داد تمامی فعالیت های آنزیمی به طور معنی داری روی میزبان آزاد کاهش می یابد. این نتایج نشان می دهند با توجه به بالاترین مقدار شاخص های تغذیه و فعالیت های آنزیمی، اوجا مناسب ترین میزبان برای سوسک برگخوار نارون است.