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RESEARCH REPORT

Investigating the effect of starvation and various nutritional types on the hemocytic profile and phenoloxidase activity in the Indian meal moth *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae)

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Abstract

The defense mechanisms of the insects are based on involvement of the hemocytes and phenoloxidase. Hemocytes are the basic component of the cellular immunity and phenoloxidase as the part of prophenoloxidase (PPO) cascade is the component of both humoral and cellular defense. Nutrition as well as starvation and attack by any organisms can modify these parameters of the innate immunity. In the current study, the effects of the stresses imposed by the starvation or different types of diets were investigated on the important immunity aspects of the indian meal moth larvae. Results showed a decline in the total hemocyte count in hemolymph with the increase in the starvation duration. In the first test, 5th instar larvae were starved for three time intervals including 24, 48, and 72 h and then, the changes in hemocyte number and phenoloxidase activity were studied. In the second experiment, the larvae bred on four diets including diet (A) walnut, diet (B) pistachio, diet (C) pea and raisin, and diet (D) artificial diets were used. The total number of the hemocytes and percentage of each hemocyte were also considered. Larvae were kept in an incubator set at a temperature of 25 ± 1 °C with 45 % of relative humidity (RH), and a constant photoperiod of 14:10 h (L:D) during the tests. The number of the plasmatocytes, one the main immune cells was sharply decreased with prolongation of the starvation duration and finally, their number reached by 134.04 ± 25.25 mm^3 of hemolymph. The number of the granulocytes was also decreased significantly 72 h post-starvation than other treatments. The prohemocytes as the stem cells were initially increased within 24 h, and they were decreased later. The oenocytoids as the key cells involved in the phenoloxidase activity were initially increased significantly within 24 h of starvation compared to the control, but they were decreased significantly after 48 and 72 h reaching the same amount as the controls. Results revealed that the types of the consumed diet influenced the number of cells and phenoloxidase activity. The highest total hemocyte count was related to the diet (C) pea and raisins (2158.18 ± 172.5 mm^3), and the lowest was observed in the larvae fed on the pistachios (924 ± 78.33 mm^3). The number of plasmatocytes, granulocytes, and oenocytoids was the highest in those larvae fed on the diet (C) pea and raisin and diet (D) artificial diet, respectively but the lowest numbers were observed for other treatments. The number of prohemocytes in the larvae fed on different diets did not differ significantly. The phenoloxidase activity was significantly reduced in the fifth instar larvae following starvation. The highest activity of phenoloxidase in feeding treatments was observed in those larvae fed on the artificial diet while the lowest activity was observed in the pistachio-fed larvae. Thus, the amount and type of the diet and the stresses including starvation can determine the immune response of the insects against the entomopathogens.

Key Words: Indian meal moth; immunity; diet; starvation; phenoloxidase

Introduction

Indian meal moth, as a cosmopolitan and polyphagous insect is the pest particularly affecting the dried fruits. The larvae spin a net and feed from the inside, which then includes the larvae and their exudates. They usually exude an unpleasant smell. The contaminants in addition to the direct loss inflict indirect costs, such as reduced quality of the grains and dried fruits (Mohandes *et al.*, 2007), which are considered as the main hosts of the Indian meal moth in the world (Rahrrabe *et al.*, 2020).

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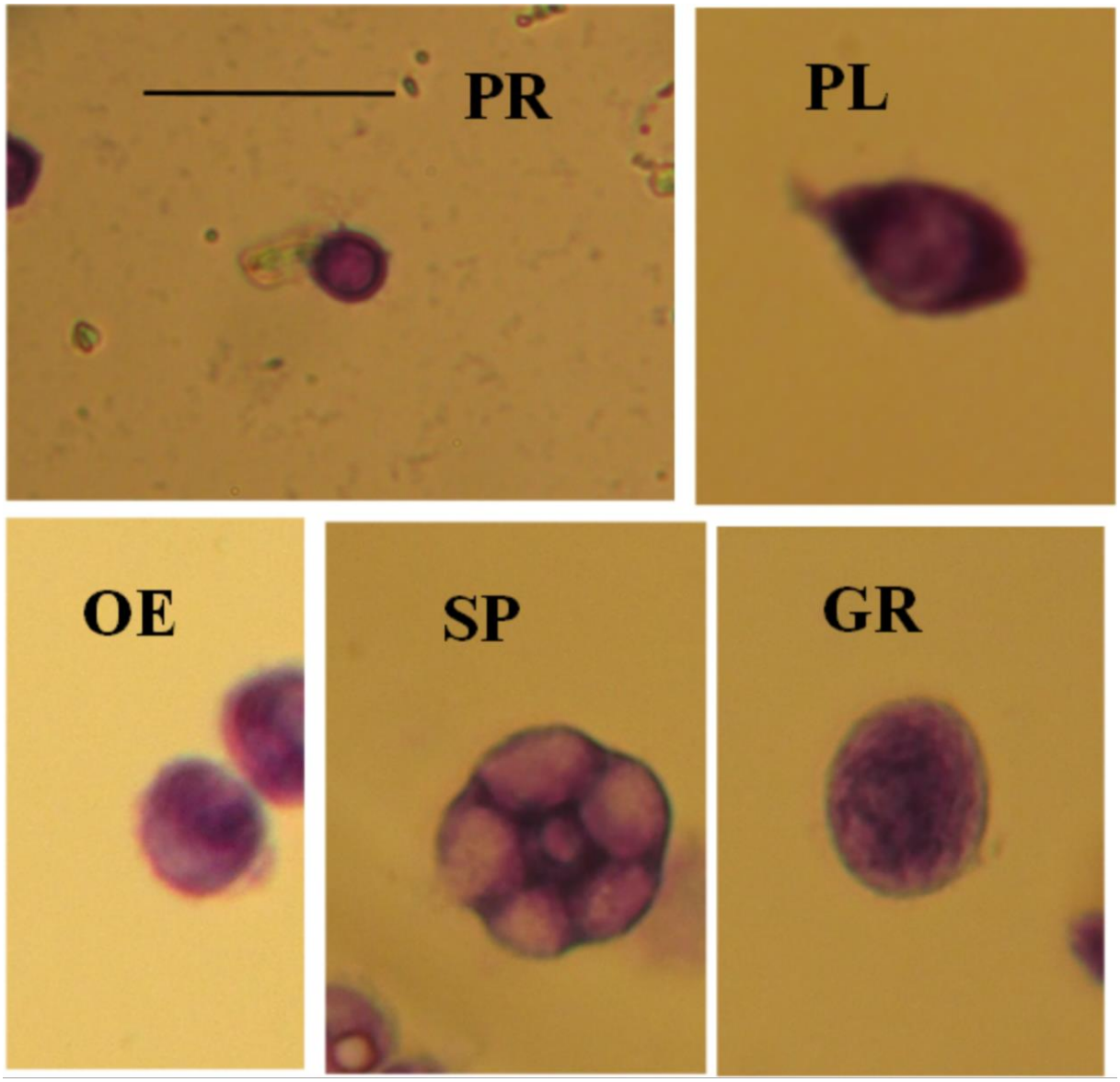

Fig. 1 Light microscopy pictures of *P. interpunctella* hemocytes stained with Giemsa. PR (Prohemocyte: large nucleus covering whole cell and small cytoplasmic area (arrows). PL (Plasmotocyte spherical with short pseudopodia). OE (Oneocytoid). SP (Spherulocyte), GR (Granulocyte). Scale bar = 10 µm

Biological success of the insects is due to their strong immune system, and the first defensive barrier against the exogenous agents is related to the insect's cuticle, the peritrophic membrane, a curtain around the food, the midgut epithelium, and trachea (Stanley and Miller, 2006; Hillyer, 2016). Understanding the physiological defense features of the insects is considered as an effective step to characterize the genotoxic, physiological and biochemical effects of the infections (Yeh *et al.*, 2005). The immune response of the insects is an important indicator of their sensitivity to various types of contamination caused by the influx of the foreign agents, such as spores of the fungi and bacteria, toxins, diapause, molt, starvation stress, environmental conditions, dietary changes and gender (Mowlds *et al.*, 2008). The cellular immunity is associated with participation of various types of hemocytes to phagocytize, form the nodules, or even encapsulate the invaders depending upon their types (Stanely and Miller, 2006). Humoral immunity includes the production of antimicrobial peptides, reactive oxygen and nitrogen derivatives, as well as coagulation and melanization of hemolymph (Buyukguzel *et al.*, 2007).

Nutrition has been shown to play an important role in the insect growth, development, and immunity (Siva-Jothy *et al.*, 2002; Kang *et al.*, 2011; Myers *et al.*, 2011; Triggs and Knell, 2011; Maggini *et al.*, 2012; Le Gall and Rehmer, 2014). Variation in the usage of energy sources including proteins and carbohydrates could change the immune reactions

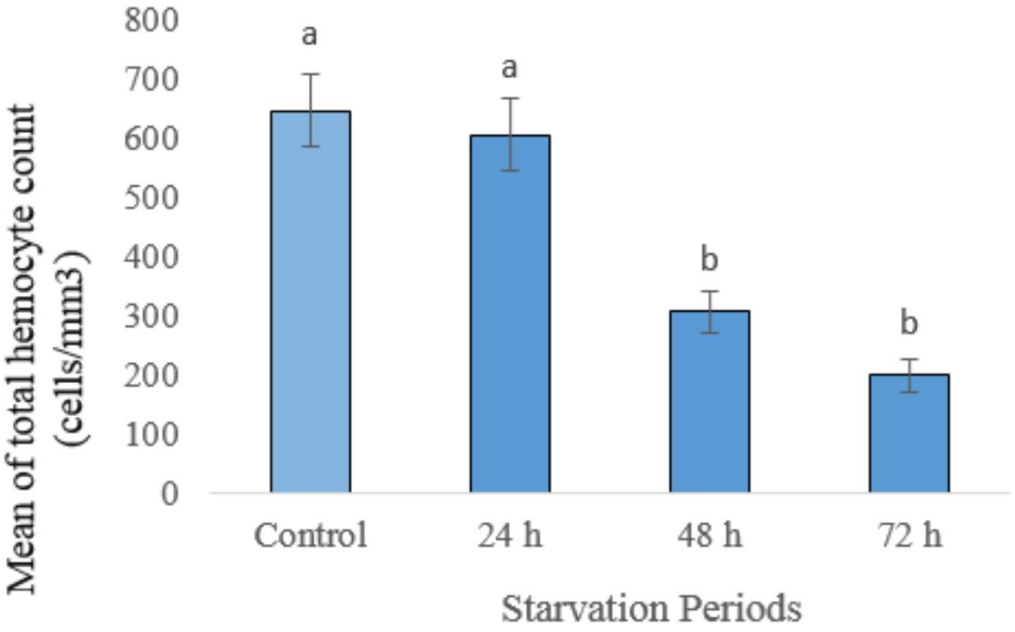

Fig. 2 Effect starvation durations on total hemocyte count of 5th instar larvae of *P. interpunctella* (Different letters show significance using Tukey's test at $p < 0.05$)

and physiological function in the insects (Mason *et al.*, 2014; Vogelweith *et al.*, 2016). If the insects are fed by richer sources of nutrition, they will possess more defensive ability and on the contrary, those that do not have sufficient nutrition or are starved will experience a significant reduction in their number of hemocytes and phenoloxidase enzyme activity making them susceptible to the pathogens (Manjula *et al.*, 2020).

Larvae of *Samia cynthia* fed on the diet containing cassava leaf have been shown to have an activated cellular defense as evidenced by a higher total hemocyte count (THC) in comparison with the larvae fed on the artificial diet alone (Tungitwitayakul and Tatun, 2017). confirming that the leaf of cassava plant possesses a high percentage of proteins, lipid and carbohydrates (Manjula *et al.*, 2020). Low content of protein in the diet has been shown to significantly influence the immunological challenges and defense power against the foreign agents in the bumblebees (Roger *et al.*, 2017). Angela *et al.* (2011) in a study reported a significant difference in the immune reaction of *Grammia incorrupta* fed on the diets containing different contents of iridoid glycoside.

Results of a study about the diet effects on the honeybee showed that the larvae starved for 7 days had reduced concentration of antimicrobial peptides in the hemolymph (Alaux *et al.*, 2010). Also, results of a research regarding the effects of starvation on the immune characteristics of the 5th instar larvae of *Manduca sexta* (L.) indicated that the amounts of glucose and trehalose were increased. The total hemocytes count was also increased (Adamo *et al.*, 2016). Starvation for 24 and 48 h has been shown to cause significant changes in the count of plasmatocytes and granulocytes and phenoloxidase activity in the larvae of *Yponomeuta malinellus*. (Ajamhassani and Mahmoodzadeh, 2020). Results of the study about the effect of food deprivation on the amount of contamination in the *Galleria mellonella* by *Candida albicans* showed a significant decrease in the density of the hemocytes (Banville *et al.*, 2012). Studies on the flour beetle, *Tribolium castaneum* showed that the susceptibility to the *Beauveria bassiana* was increased by increasing the days of malnutrition (Lord, 2010). Similar results have been reported in other invertebrates e.g., garden snail after starvation on its immunity (Alrawadeh, 2010). Hence, nutrition plays a key role in maintaining the immunity of the garden snail, and continuous starvation for 3 weeks has been found to significantly reduce the total hemocyte count and the number of phagocytic cells, and decrease the activity of the phenoloxidase enzyme.

Based on the reports, macronutrient contents and protein: carbohydrate ratios influence on the life-history traits including pupal and adult weight, and fecundity of the adults in *Plodia interpunctella* (Littlefair and Knell, 2016). Also, larvae of *P. interpunctella* raised on a good-quality diet have been reported to have substantially higher hemocyte count and PO activity in high density (Triggs and Knell, 2011). Thus, it seems that starvation periods or food limitation could influence on the life history-traits and immune function in the natural population of pests, such as *P. interpunctella*. It has been proved that the food depletion could influence on the horizontal transmission of the pathogens by increasing the larval movement (Beisner and Myers, 1999). In addition, the starvation could trigger the virus into an

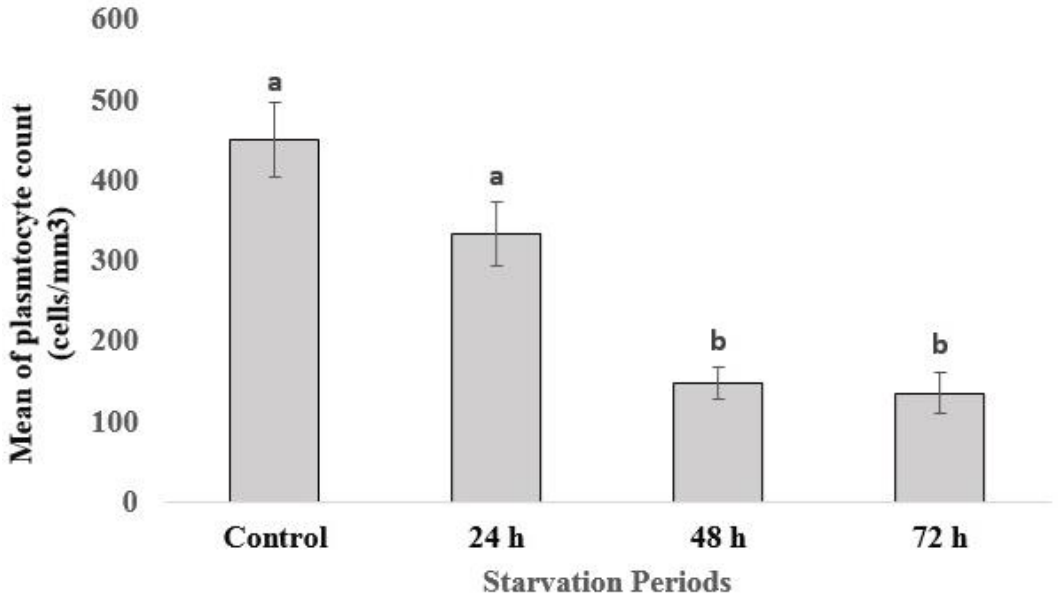

Fig. 3 Effect starvation on plasmatocyte count of 5th instar larvae of *P. interpunctella* (Different letters show significance using Tukey's test at $p < 0.05$)

active form and initiate its activity at high densities of *Malacosoma pluvial* in the field Myers et al, 2011). In contrast, findings of Kang *et al.* (2011) have shown that the starvation of Larvae of *Bombyx mori* at the time of baculovirus exposure had a negative effect on the virus transmission and pathogenesis. Also, no relationship has been found between the PO activity in the hemolymph of the starved larvae of *Lymantria dispar* and larval susceptibility to the baculovirus (Kasianov *et al.*, 2017).

Different species act differently while being subjected to the stresses and in particular starvation. Variations in the feeding habit will also cause different effects in various species. Taking into account this theory, the current study was conducted in order to investigate the immune reaction of the Indian meal moth after administrating various diets and starvation periods. But, comprehensive research is needed in this respect to provide new aspects regarding the physiological defense of this important stockpile pest. Understanding the interactions between the insect's immune system and the attacking pathogen is of great importance. *Habrobracon hebetor* and *Venturia canescens* are known to attack and successfully develop within the larvae of several lepidopterous pests of the stored products, mainly pyralids (e.g., *P. interpunctella*) in the stored wheat (Schöller, 1998; Heinlein *et al.*, 2002; Ghimire and Phillips, 2010). In addition, *Plodia* larvae are susceptible to the infection by the *P. interpunctella* granulovirus from genus baculovirus (Sait *et al.*, 1994). So, determining the susceptibility or resistance of the larvae to these parasitoides or pathogens is somehow influenced by the starvation, diets, and other environmental factors.

Materials and methods

Insect Rearing

Adults of *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae) were procured from Plant Protection Research Organisation of Iran (Tehran, Iran). The adults were released in boxes (20 × 15 × 10 cm) for mating in an incubator set at 25 ± 1 °C with 45 % RH, 14:10 L:D. The eggs were collected and the hatched larvae divided into 2 groups. The first group was bred on artificial diet (malts 160 g, wheat germ powder 800 g, glycerol 200 ml and honey 200 ml) for Hemocyte profile light microscopy studies, total hemocyte count, PO activity and the starvation assessments. Second group were bred on four diets including diet (A) walnut, diet (B) pistachio, diet (C) pea and raisin and diet (D) artificial diets for the analysis of immune factors affected by various diet.

Hemocyte profile light microscopy studies

A clean slide was used to make a hemolymph smear after excising one of the larval pro-legs with the help of a microscissor. The slide was left to dry at room temperature for 20 min and then stained with Giemsa (Merck KGaA, Germany) (diluted 9 times with distilled water and filtered before use) for 25 min. It was differentiated in saturated solution of lithium carbonate (Merck KGaA, Germany) for 30 seconds and then washed in distilled water. After drying, permanent microscopy slides were prepared using Canada balsam. Hemocytes were observed under a light microscope (Olympus BH_2) at 40 X magnification (Ghasemi *et al.*, 2013).

Total Hemocyte Counts (THC)

The total hemocyte count was done by collecting the hemolymph of 5th instar larva after

cutting one of the prolegs and diluted with Tyson solution ($NaCl_2$ 72 Mm, Na_2SO_4 9 Mm, Glycerol 43 Mm, Methyl violet 0.06 Mm, Distilled water) (Mahmood and Yusaf, 1985). The THC was counted using a standard Neubauer hemocytometer under a light microscope at 40x magnification. The number of total hemocytes per cubic millimeter (mm^3) was calculated using the following formula of Jones (1962):

$$\frac{\text{Hemocytes in} \times 1\text{mm}^2 \times \text{Dilution} \times \text{Depth factor of chamber}}{\text{No. of squares counted}}$$

Dilution = 10 times
Depth factor of the chamber = 10
No. of squares counted = 5

The effect of starvation on hemocyte number

In order to carry out this experiment, 5th instar larvae (2 old-days) that reared on artificial diet, were starved for three time intervals including 24, 48 and 72 *h*. Each treatment and control included 30 larvae and in total 120 larvae. Each of the larvae experienced starvation individually in a separate petri (total starvation method was used here). The total numbers of hemocytes were counted. Data were analyzed in a complete randomized design with SAS software and the means were compared using Tukey's test ($p < 0.5$). The experiment included four treatments a control and three treatments *i.e.* 24, 48 and 72 *h* starved larvae.

The effect of diet type on hemocyte number

For this purpose, Larvae were bred on four diets including diet (A) walnut, diet (B) pistachio, diet (C) pea and raisin and diet (D) artificial diets were used. (second group of larvae that described above). hatched larvae was transferred individually to separate food-bearing petri. For each treatment, 20 replicates were used (5th instar larvae 2 old-days). (in total 80 larvae). The volume of food in each peti was almost equal about 20 gr. Fresh food was provided for larvae, daily. The total number of hemocytes and percentage of each hemocyte was recorded. Data were analyzed in a complete randomized design with SAS software and averages were compared with Tukey's test ($p < 0.05$).

Phenoloxidase enzyme activity (PO)

To determine the effect of starvation and diet types on the activity of phenoloxidases in hemolymph, hemocyte lysate method was used (Leonard *et al.*, 1985). In this method, for each treatment, 5th instar larval (2 old-days) hemolymph were collected (~ 150 µl hemolymph related to 30 larvae) and centrifuged (Sigma, Germany) at 4 °C and 10,000 g for five minutes. The supernatant was removed and remaining pellet was mixed with 100 µL of phosphate buffer (pH 7) and then homogenized. The solution was centrifuged again at 4 °C and 12.000 g for 15 min, and supernatant was used in PO assay. Then, 25 µL of the supernatant were added to 50 µL of L-DOPA (L-dihydroxy phenyl alanine), a specific substrate of the phenoloxidase enzyme and 50 µL of phosphate buffer. Phenoloxidase activity was measured at 490 nm wavelength (ELX800, USA).

Results

As seen in Fig. 1 we could identify prohemocytes, plasmatocytes, granulocytes, spherulocytes, and oenocitoids in *P. interpunctella*. The smallest cells were round, oval, or elliptical with large nucleus filling the entire cell. The cells with various shapes and sizes and irregular plasma membrane were the plasmatocytes whose nuclei were, with centrally located. The granulocytes were mostly spherical or oval with a peculiar granular packed cytoplasm and a relatively small and centrally located nucleus. The spherulocytes were typically filled with spherules with small centrally located nucleus. The oenocytoids were mostly oval and eccentric nucleus.

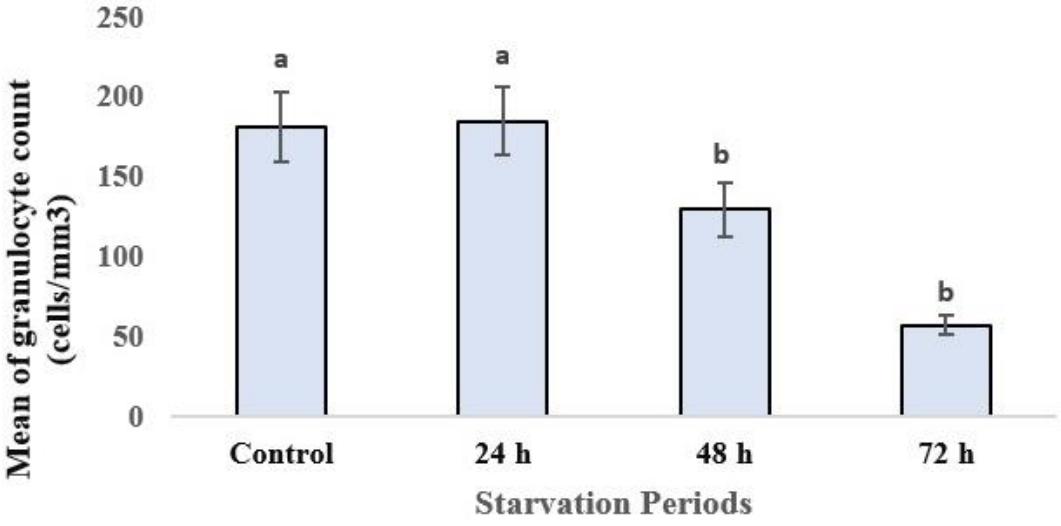

Fig. 4 Effect starvation periods on granulocyte count of 5th instar larvae of *P. interpunctella* (Different letters show significance using Tukey's test at $p < 0.05$)

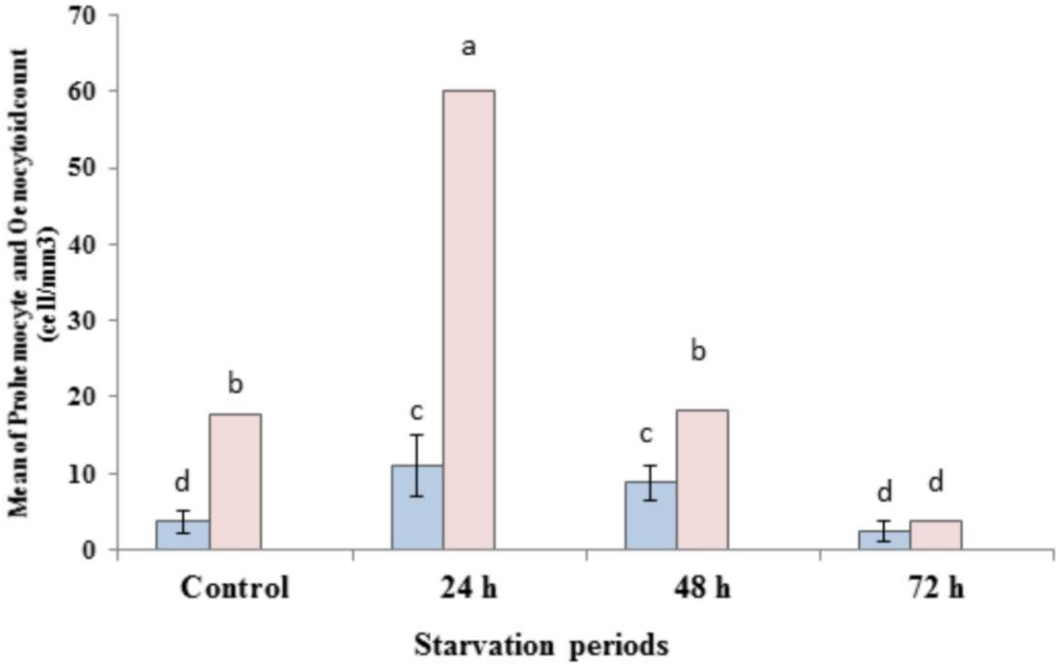

Fig. 5 Effect starvation periods on prohemocyte and oenocytoid count of 5th instar larvae of *P. interpunctella* (Different letters show significance using Tukey's test at $p < 0.05$)

Effect of starvation on hemocyte number

There was no significant changes on hemocyte number at 24 h starvation duration compared to controls ($F = 34.21$, $df_{t,e} = 3, 112$, $p < 0.0001$). However, the cell number significantly decreased after 48 h of starvation. While the total hemocyte count was 647.53 ± 61.78 mm^3, it was significantly reduced to 306.5 ± 34.29 mm^3. The same trend was observed after 72 h of starvation (199.4 ± 29.29 mm^3) (Fig. 2).

The plasmatocyte numbers after 48 *h* reduced significantly (174.15 ± 20.85 mm^3) and more significantly after 72 *h* (134.04 ± 25.25 mm^3) whereas in control it was (450 ± 46.65 mm^3). ($F = 19.34$, $df_{t,e} = 3, 112$, $p < 0.0001$). In other words, plasmatocytes were significantly reduced in larvae that had a longer starvation than the controls (Fig. 3). Of the with the same trend of reduction was also seen in the number of granulocytes ($F = 11.32$, $df_{t,e} = 3, 112$, $p < 0.0001$). The number was as low as 75.33 ± 6.02 mm^3 after 72 h starvation (Fig. 4). On the other hand the number of prohemocytes and oenocytoids behaved somehow differently where it was initially increased after 24 of starvation ($F = 74.5$, $df_{t,e}=3, 231$, $p < 0.0001$), but decreased gradually after 48 h and reaching the amount of control after 72 *h*. (Fig. 5).

Effect of diet types on hemocyte number

Diets showed a significant effect on all hemocyte numbers except prohemocytes. The type of food provided to the larvae could affect the number of hemocytes of Indian meal moth larvae. The highest total hemocyte count ($F = 20.60$, $df_{t,e} = 3, 72$, $p < 0.0001$) was related to diet (C) pea and raisins (2158.18 ± 172.5 mm^3). The total hemocyte counts in those larvae that were provided with diet (D) artificial diet, diet (C) walnuts and diet (B) pistachios were 1078 ± 128.1, 963 ± 116 and 924 ± 78.33 mm^3, respectively (Table 1).

Table 1 Effect of diet on hemocyte number of 5th instar larvae of *P. interpunctella*

diet[m]	THC[n]	PL	GR	OE	PR
A	963 ± 116.5 b	552.7 ± 78 b	332 ± 53.6 b	16.5 ± 4.4 b	13.2 ± 6.4 a
B	924.5 ± 87.4 b	521.3 ± 52 b	377 ± 57.6 b	25.3 ± 5.8 b	10 ± 3.7 a
C	2158 ± 172.6 a	1463 ± 152.6 a	555.5 ± 64.6 a	71.5 ± 12.3 a	20 ± 5.5 a
D	1078.4 ± 128.4 b	506 ± 72.5 b	497.5 ± 71.4 a	34 ± 7.7 b	6.6 ± 4 a

Diet[m]: A= walnut, B=pistachios, C= pea and raisin, D= artificial diet
THC[n]= all hemocyte numbers are in mm^3 hemolymph
(Different letters in each column show significance using Tukey's test at $p < 0.05$)

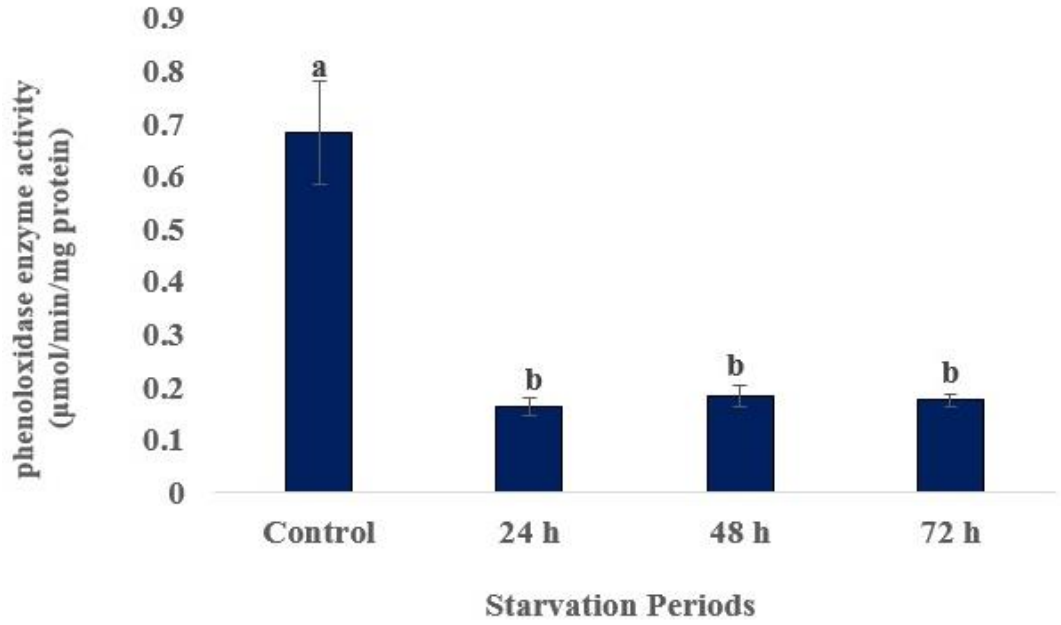

Fig. 6 Effect of starvation periods on phenoloxidase activity of 5th instar larvae of *P. interpunctella* (Different letters show significance using Tukey's test at $p < 0.05$)

As it is shown in Fig. 6. the highest total number of plasmotocytes (F = 23.17, $df_{t,e}$ = 3, 72, p < 0.0001) and oenocytoids (F = 8.79, $df_{t,e}$ = 3, 72, p < 0.0001) were observed in those larvae that fed on diet (C) pea and raisins. The number of these cells in the larvae fed on other diets were not different significantly. (Table 1).

Taking the number of granulocytes into consideration the diet (C) pea and raisin and diet (D) artificial diet significantly (F = 66.5, $df_{t,e}$ = 3, 72, p < 0.0001) increased its number. However, there was no differences between other diet types as far as granulocytes were concerned (Table 1).

Effect of starvation and diets on the activity of phenoloxidase enzyme

Our results indicated that by prolonging the starvation time, significant reduction in the activity of the phenoloxidase enzyme are expected (F = 25.4, $df_{t,e}$ = 3, 112, p < 0.0001). (Fig. 6). We observed that the nutrition can affect the activity of the phenoloxidase enzyme. The highest PO activity in larvae fed on diet (D) artificial diet (F = 32.61, $df_{t,e}$ = 3, 112, p < 0.0001) was significantly higher than that in larvae fed on other diets. (Fig. 7).

Discussion

Beeman *et al.,* (1983) in a research studied the hemocytes of *P. interpunctella*. They identified six different types of hemocytes: prohemocytes, plasmatocytes, granulocytes, oenocytoids, spherulocytes, and granulocytophagous cells. In the present research, five various types of hemocytes were found in the hemolymph of *P. interpunctella*. Existence of these hemocytes (prohemocytes, plasmatocytes, granulocytes, oenocytoids, and spherulocytes) has already been reported by other researchers in the hemolymph of more insects particularly Lepidoptera. e.g., *B. mori*, (Liu *et al.*, 2013), *Eupholidoptera smyrnensis* (Ozturk *et al.*, 2018) and *Zeuzera pyrina* (Ajamhassani, 2019) and many other lepidopteran species.

Physicochemical properties of the insect's hemolymph are significantly influenced by the stress conditions (Mowlds *et al.*, 2008; Duarte *et al.*, 2020). Once there is a weakness in the immunity of the insects there will be a chance of more susceptibility to the routine control practices of the pests (Yeh *et al.*, 2005; Ajamhassani, 2015). The hemocytes or insect's blood cells are the most important hemolymph factors that undergo the environmental changes ,such as temperature, population density, starvation, nutrition, gender and the effects by foreign agents e.g., pollutants, insecticides ,and spore of entomopathogenic fungi and bacteria Lee et al, 2008, Siva-jothy and Thompson 2002, Ghasemi *et al.*, 2014; Pourali and Ajamhassani, 2018). It has been proved that the immune potential is parallel to the hemocyte number (Li *et al.*, 2019). So, new hemocytes as the key factors in the innate immunity are always produced to replace the damaged hemocytes in order to maintain the homeostasis that is essential in the hemolymph (Nakahara *et al.,* 2003).

The decreased number of hemocytes can be deadly to the insects, as the immune system weakens and makes them helpless against any control methods including the microbiological and chemical controls (Zhu *et al.*, 2012; Ajamhassani *et al.*, 2013). On the other hand, different insects show varied immune levels, which is based on the type and

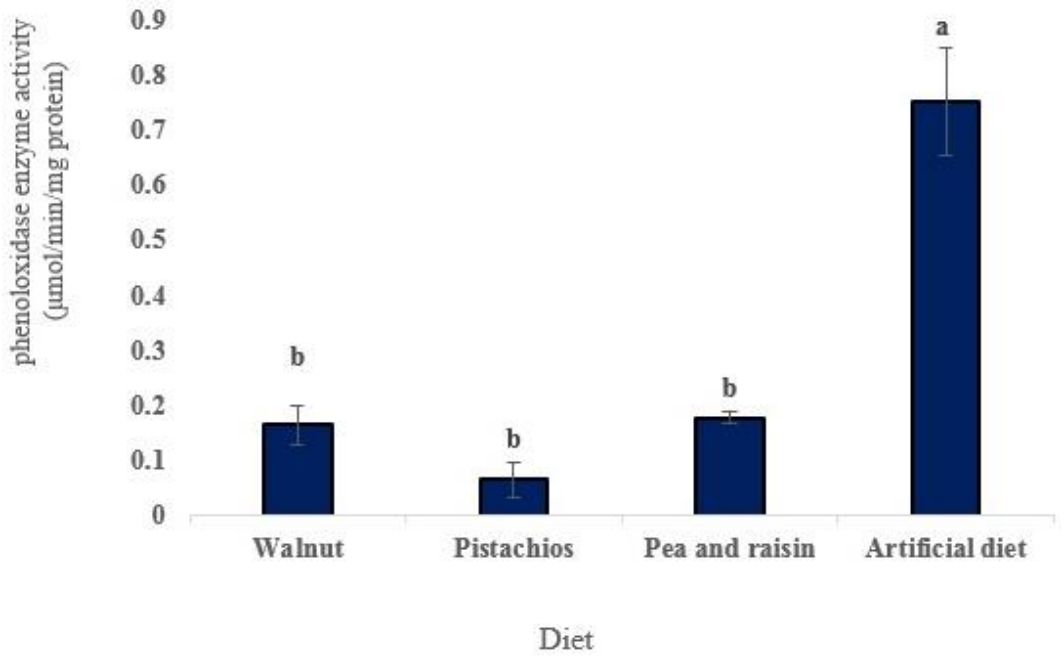

Fig. 7 Effect of diet on phenoloxidase activity of 5th instar larvae of *P. interpunctella* (Different letters show significance using Tukey's test at $p < 0.05$)

percentage of the infection. The insects physiology can act in its best condition if the nutrition is available to them adequately (both quantitatively and qualitatively).

Regarding the importance of feeding for the organisms including the insects, as well as, the existence of differences among the species, herein, it was attempted to show the role of feeding in causing these differences. Hence, in the current study, two sets of experiments were performed that may influence the immune system of our model. Hence, the starvation was administered in different time intervals to assess its effects on the insect hemocyte count in one set of experiments. It was observed that the nutrition is very important in the studied insect, as both the cell counts and the production of phenoloxidase were significantly influenced. Thus, it seems that the activity of PPO cascade is related to the number of hemocytes. However, it is assumed that the feeding rapidly increases the level of phenoloxidase activity and hemocyte density, which is related to the energy obtained from nutrition (Siva-jothy et al, 2002). As we know, the immune responses by the insects are dependent on the amount of energy received through feeding that is essential for homeostasis and immune activities (Banville *et al.*, 2012).

Our results indicated that despite experiencing short-term starvation for 3 days, the larvae of *P. interpunctella* showed significant immune responses to this stress. It was indicated that all the larvae survived after overcoming the starvation and continued the feeding until they became pupa and adult (the data were observational and not statistical). Similar to our results, Siva-Jothy *et al.*, (2002) indicated that short-term nutrient deprivation influenced the immune function in *Tenebrio molitor* L. Starvation causes a significant decrease in the phenoloxidase activity and on the contrary, it increases immediately after access to the food. Also, 24 h exposure to a supplemented diet has been shown to increase the immune capacity of *Drosophila melanogaster* larvae in the face of parasitoid infusion into hemocoel (Vass and Nappi, 1998). In our study, starved larvae survived and fed on the diet 72 h after the starvation. Similar results have also been observed in *Dendrolimus pini* larvae characterized by the ability to survive without food for up to one month (Lukowski *et al*, 2020). In contrast, food limitation has been shown to sharply reduce the larval survival, development rate, larval and pupal size, fecundity rate, and phenoloxidase activity in the *M. pluvial* (Myers *et al*, 2011).

Regarding the effect of different diets on the immune function, it was found that the changes in the number of hemocytes and phenoloxidase activity were sharply increased by feeding on the diets C and D. This is probably due to the presence of the considerable amounts of macromolecules involved in the growth and immunity, namely carbohydrates, proteins, and lipid in these diets compared to other diets. It has been well established that the carbohydrates are an important macronutrient necessary for the activities with high-energy needs, such as movement, somatic maintenance, and growth (Maklakov et al, 2008). In addition, proteins are essential for different developmental stages and reproduction of the adults (Bowen *et al.*, 1995; Crutz and Hay, 2000; Simpson and Raubenheimer, 2012). Increased

dietary protein has been reported to elevate the level of hemocytes and enzymes in the hemolymph leading to the high immune level (Lee et al, 2008, Graham et al, 2014). Fatty acids as a rich source of energy are critical for the ecdysis. Also, they are essential in the insects that have non-feeding adult stages, such as some Lepidopteran species (Stockhoff, 1993).

For investigating the effect of various diets on the immune function, we must define what is variation in the quantity of macronutrients in each diet, but it is noteworthy that the exact contents of different macronutrients in the diets (e.g., carbohydrates, proteins, and lipids) are also unknown. On the other hand, the effects of the diet types should also be considered with respect to their total calories (Littlefair and Knell, 2016).

Similar results have been reported in the Larvae of *Hyposidra talaca.* They showed significantly higher THC when reared on the artificial diet (containing a percentage of protein) in comparison with the natural diet (tea leaf) (Ghosh *et al.*, 2018). Shikano *et al.*, (2010) found that *Trichoplusia ni* larvae fed on a good quality host plant showed increased hemocyte number in their hemolymph and were more resistant against the nucleopolyhedral virus. In the larvae of *Spodoptera littoralis*, immune parameters, such as lysozyme-like antibacterial activity were higher following feeding on the high-protein diet (casein) compared to the larvae grown with poor quality proteins (zein). These larvae also had a more melanized cuticle, and thus nutrition was also effective as a key factor in the melanization (Lee *et al.,* 2008). In addition, the macronutrient composition and zinc supplementation in the diet have been shown to have positive effect on the immune-tolerance in *Spodoptera littoralis* larvae exposed to the entomopathogenic nematode of *Mesorhabditis belari*, symbiotic bacteria, and its metabolites (Manjula *et al.*, 2020). In a study to evaluate the changes in immune function of the *Eupoecilia ambiguella* larvae under the effects of diets, it was found that the larvae fed on high-sugar grapes showed a higher immunity against *Bacillus cereus*, *Beauveria bassiana* and *Serratia marcescens* (Vogelweith *et al.*, 2016).

In conclusion, results of the present study and studies by several authors including Alaux *et al.,* (2010) on the honey bees indicated that the immunity of the insects is dependent on the quality and quantity of the foods they receive. Therefore, depriving the pest insects from obtaining enough nutrition alters their immunity function as a result of which the microbial agents can efficiently kill them. Therefore, if any degradable material and in-minute quantities are included in our microbial control measures to weaken the immune system of the pests, then their efficiency will be certainly enhanced.

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