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Lysozyme activity and some fitness parameters of *Helicoverpa armigera* on five maize hybrids

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Abstract: The cotton bollworm, *Helicoverpa armigera* (Hübner), which is known as one of the most important economic pests, can cause severe damage to different crops in Iran and many countries of the world. The effect of five maize hybrids: SC260, SC500, SC700, SC704 and DC370 on lysozyme activity in the hemolymph of sixth instar of *H. armigera* using lytic zone assay and its association with some fitness parameters of this pest was studied in growth chamber (25 ± 1˚C, 65 ± 5% RH and a photoperiod of 16:8 (L : D) h). Our results indicated that except on SC260, the frequency of high immune-activated larvae on the other maize hybrids was lower than that of low immune-activated larvae. The mean lysozyme concentration in *H. armigera* larval hemolymph was the highest on SC260 (0.096 ± 0.01 mg/ml) and lowest on DC370 (0.060 ± 0.007 mg/ml). The longest pupal period was on SC500 (12.00 ± 0.49 days) and the shortest was on SC700 (10.37 ± 0.19 days). Daily and total fecundities of *H. armigera* were the highest on DC370 (61.68 ± 9.85 and 196.89 ± 49.30 eggs, respectively) and lowest on SC260 (20.60 ± 5.88 and 52.71 ± 18.80, respectively). The results of this study can provide fundamental information for management of *H. armigera* on maize hybrids.

Keywords: *Helicoverpa armigera*, lysozyme, fitness parameters, maize hybrid

Introduction

The cotton bollworm, *Helicoverpa armigera* (Hübner) has been reported as an economic pest on field and horticultural crops in Iran (Farid, 1986) and in many parts of the world (Reddy *et al.,* 2004; Subramanian and Mohankumar, 2006). Every year, *H. armigera* can cause an extensive crop loss to various plant species such as cotton, maize, soybean, etc. Although the most common way for controlling *H. armigera* larvae is the application of chemical pesticides, the eradication of non-target organisms, environmental contamination, secondary pests' outbreaks and evolution of insect resistance have been known as the destructive effects of the large scale usage of the broad spectrum insecticides (Gunning *et al.,* 1984).

So far, many studies on different aspects of *H. armigera* immune system have been done in the world (Mackintosh *et al.,* 1998; Ma *et al.,* 2005; Xiang *et al.,* 2006; Liang *et al.,* 2007). Searching on available literature indicated that some works on immune system activity have been done in *Bombyx mori* (Morishima *et al.,* 1994), *Drosophila sp.* (Franc and White, 2000), *Plutella xylostella (L.)* (Schuler and Emden, 2000), *Ephestia kuehniella* Zeller (Rahman *et al.,* 2007) and *Spodoptera exigua* (Hubner) (Hernandez-Martinez *et al.,* 2010). Although all of these studies demonstrated a relationship between immune system activity and measure of resistance to bacteria, the impact of various larval foods on the immune activity was not considered by above-mentioned researchers. To substantiate this idea we targeted lysozyme activity of *H. armigera* in

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response to feeding on various maize hybrids and its association with some fitness parameters.

Knowledge of insect defense mechanisms present in hemolymph can help us select an effective strategy in the pest management programs. Although the insect immune system, as a defense mechanism, can play a main role in insect resistance against diverse pathogens like bacteria, its activation may probably be influenced by different host plants consumed. The insect immune system consists of cellular and humoral immunity (Giannoulis *et al.,* 2006; Feldhaar and Gross, 2008) and when bacterial pathogens attack insects, lysozyme enzymes, as humoral immunity system, come into action and destroy the cell wall of the invading bacteria (Hernandez-Martinez *et al.,* 2010).

Even though insects lack an adaptive immune system, they have an innate immune system that distinguishes and destroys invading pathogens. Most researchers have introduced microorganisms into lab-reared insects via artificial injection and the function of immune system under natural conditions has not been well studied (Freitak *et al.,* 2007). One of the most probable routes of natural infection is via food consumed. However, the role of dietary microbial communities in the development of the insect immune system is largely unknown. Host plants harbor different pathogenic and non-pathogenic microorganisms (Vodovar *et al.,* 2005; Meyling and Eilenberg, 2006). Both the exterior and interior parts of host plants are known to have diverse microbial communities (Monier and Lindow, 2004), which differ between the conspecific plants as well as between different parts of the same plant (Vodovar *et al.,* 2005).

Lysozymes are important antibacterial proteins in the insect immune system, which are primarily purified from insect hemolymph (Powning and Davidson, 1976). Lysozyme is known as a bacteriolytic enzyme that cleaves β-1,4-glycosidic linkages between N-acetylglucosamine and N-acetylmuramic acid of the bacterial cell wall peptidoglycan (Dunn, 1986; Jolles and Jolles, 1984). In lepidopteran insects, lysozyme has been reported as a normal component of the serum and plays important roles in immune defense systems (Powning and Davidson, 1973; Jolles *et al.,* 1979). To understand the role of various larval foods on immune system of *H. armigera,* we examined the effects of different maize hybrids on the lytic activity of *H. armigera* larvae. Since activating the immune system implies a considerable expenditure of energy and result in interactions with other fitness parameters (Schmid-Hempel, 2005; Hernandez-Martinez *et al.,* 2010), we studied fitness parameters (such as reproduction and pupal development) of *H. armigera* on various maize hybrids. A similar study has been done on *Spodoptera exigua* Hübner by Hernandez-Martinez *et al.* (2010), but they did not consider the effect of various larval foods on the immune system activity.

The aim of the present research was to determine the effect of various maize hybrids on the activity of lysozyme in *H. armigera* larval hemolymph and its association with some fitness (biological) parameters of this pest. We hope that this information would be useful to introduce a maize hybrid that has a negative influence on the immune system activity of *H. armigera*.

Materials and Methods

Host plants

Seeds of five maize hybrids: SC260, SC500, SC700, SC704 and DC370 were obtained from the Plant and Seed Modification Research Institute (Karaj, Iran) and used to prepare artificial diets according to the methods described by Teakle (1991).

Lytic zone assay

For assessing the immune system lysozyme activity called lytic zone assay (LZA), we used one-day-old sixth instar larvae of *H. armigera* that had fed on various maize hybrids. The larvae were punctured from abdominal prolegs and 1µl of the hemolymph was extracted (200 larvae per hybrid). Petri dishes (diameter 8 cm, depth 1 cm) were filled with 20 ml phosphate buffer saline (PBS) containing 1 mg ml $^{-1}$ of *Micrococcus lysodeikticus* ATCC No. 4698 (Sigma Co., USA) with a final concentration of 1% agar. After

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solidification of the plates, samples of larval hemolymphs were placed individually on determined places over the agar. Finally they were incubated at 37 ˚C for 16 hours. The diameter of the LZA around the samples was measured, then contrasted with lysozyme at various dilutions (0.3, 0.1, 0.03, 0.01 mg ml $^{-1}$) (Roche Diagnostic SL, San Cugat del Vallés) (Hernandez-Martinez *et al.,* 2010). In our experiment 100% of the examined larvae showed lysozyme activity (halo diameters ranged from 1 to 10mm). According to halo diameters around the samples, the larvae were grouped in two categories: the lytic zone diameter of the first category ranged from 1 to 5 mm and the second one varied from 6 to 10mm.

Fitness parameters of *H. armigera*

The first colony of *H. armigera* was acquired from a laboratory culture maintained on cowpea-based artificial diet of the Department of Plant Protection, University of Tabriz, Iran. The neonate larvae were reared for a whole generation on the artificial diets (Teakle, 1991) prepared by different maize hybrids. We started the experiment with the sixth instar larvae of the second generation (nearly 200 larvae for each hybrid). The larvae were kept in plastic containers (diameter 16.5 cm, depth 7.5 cm) with outlets covered by a proper mesh net for larval ventilation until third instar. The older larvae (fourth instar to the end of larval stage) were individually reared in Petri dishes (diameter 8 cm, depth 1 cm) to prevent cannibalism. Observations on pupal period and pupal weight (48 hours after appearance) were monitored up to emerging of adult. To mating adults, we transferred one pair of male and female (five replicates for each maize hybrid) into the plastic containers (diameter 11.5 cm, depth 9.5 cm), which were covered by nylon mesh for ventilation. For feeding adults, 10% of honey solution was used. The number of eggs laid was calculated daily. Eggs were placed in plastic containers (diameter 11.5 cm, depth 9.5 cm), with some moistened cotton until their hatching. The insects used in this experiment were maintained at a growth chamber (25 ± 1˚C, 65 ± 5% RH and a photoperiod of 16:8 (L : D) h). In this experiment, the fitness parameters of *H. armigera* such as pupal weight, pupal period, adult longevity, daily and total fecundity were determined on different maize hybrids.

Statistical analysis

The data obtained in this research were analyzed by one-way analysis of variance (ANOVA) using Minitab ver. 16 and the differences of the means were compared by the LSD (least significant difference) test.

Results

Lytic zone diameters of *H. armigera* on various maize hybrids

In this study determining the lysozyme activity of *H. armigera* larval hemolymph on various maize hybrids indicated that all larvae showed the lytic activity. On the basis of the lysozyme activity, the larvae were grouped into two categories; Group 1 with halo diameters of 6 to 10 mm (high immune-activated larvae), group 2 with halo diameters of up to 5 mm (low immune-activated larvae). Frequency of the larvae having lytic zone diameters for each category is shown in Fig. 1. The frequency of high immune-activated larvae on maize hybrids (SC500, SC700, SC704 and DC370) was lower than those of low immune-activated larvae but, it was the reverse on SC260. More than 60% of the larvae reared on SC500, SC700 and DC370 had halo diameters up to 5 mm. Results therefore indicate that different hybrids can affect the lysozyme activity in *H. armigera* larval hemolymph.

Lysozyme concentration in *H. armigera* larval hemolymph

The impact of various maize hybrids on lysozyme concentration in larval hemolymph of *H. armigera* indicated significant differences among five maize hybrids (Fig. 2). The results indicated that the mean lysozyme concentration was the highest on SC260 (0.096 ± 0.01 mg ml^{-1}) and lowest on DC370 (0.060 ± 0.007 mg ml^{-1}).

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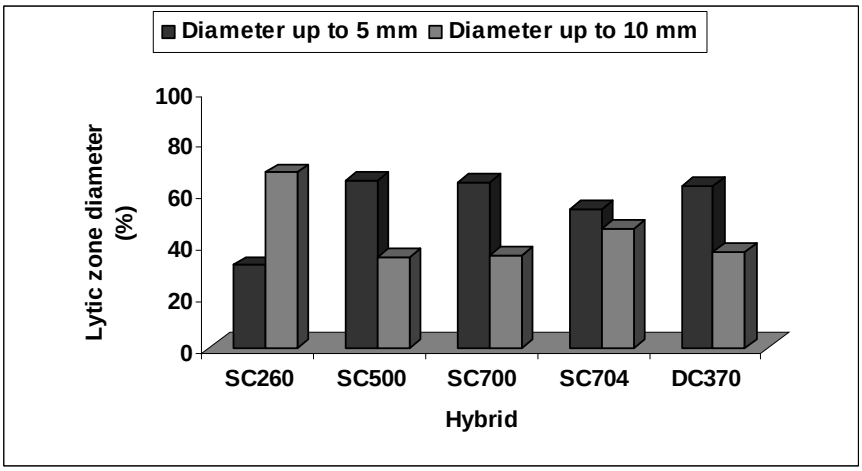

Figure 1 Effect of various maize hybrids on frequency of the lytic zone diameters of *Helicoverpa armigera*.

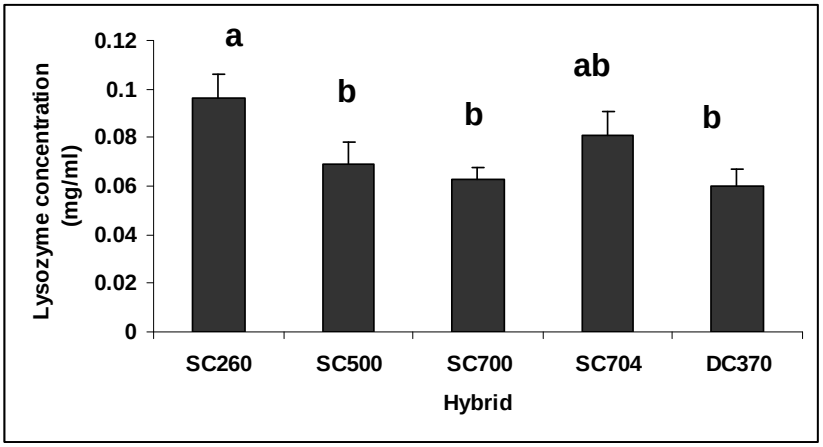

Figure 2 Mean lysozyme concentration (mg/ml) in *Helicoverpa armigera* larval hemolymph on various maize hybrids. Bars represent standard error of the means (LSD, $P < 0.05$).

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Fitness parameters of *H. armigera*

Table 1 summarizes the effect of different maize hybrids on fitness parameters of *H. armigera*. In this study**,** pupal weight, pupal period, adult longevity, daily and total fecundity were recorded on five maize hybrids. Data analysis indicated that there were no significant differences in pupal weight and adult longevity of *H. armigera* reared on maize hybrids. However, pupal period, daily fecundity and total fecundity were significantly different. The longest mean pupal period was on SC500 (12.00 ± 0.49 days), while the shortest was on SC700 (10.37 ± 0.19 days). The results indicated that daily and total fecundities of *H. armigera* were the highest on DC370 (61.68±9.85 and 196.89 ± 49.30 eggs, respectively) and lowest on SC260 (20.60 ± 5.88 and 52.71 ± 18.80, respectively).

Table 1 Biological parameters (mean ± SE) of *Helicoverpa armigera* reared on various maize hybrids.

Hybrid	Pupal weight (mg)	Pupal period (day)	Adult longevity (day)	Daily fecundity (egg/female/day)	Total fecundity (egg/female)
SC500	193.82 ± 9.78a	12.00 ± 0.49a*	9.64 ± 1.36a	39.50 ± 4.11abc	85.50 ± 14.30bc
SC700	177.84 ± 5.80a	10.37 ± 0.19c	10.11 ± 0.82a	56.45 ± 10.8ab	80.29 ± 19.30bc
SC704	177.84 ± 6.66a	11.21 ± 0.22b	11.32 ± 0.81a	33.68 ± 7.76bc	143.89 ± 32.00ab
SC260	176.67 ± 20.4a	11.56 ± 0.41ab	8.00 ± 1.32a	20.60 ± 5.88c	52.71 ± 18.80c
DC370	195.38 ± 8.10a	11.00 ± 0.19bc	10.33 ± 1.08a	61.68 ± 9.85a	196.89 ± 49.30a

The means followed by different letters in each column are significantly different (LSD, $P < 0.01^{*}$; $P < 0.05$).

Discussion

Insects have a variety of immune systems to effectively defend themselves against invading pathogens (Freitak *et al.,* 2007). Although a lot of agents are involved in this defense, phenoloxidase (Shrestha and Kim, 2007) and lysozyme enzymes (Dunn, 1986) have a main role in challenging with bacterial pathogens. Consciousness of the insect immune status can be useful to select the best means to control insect pests.

Various maize hybrids as larval food source did not have any significant effect on pupal weight and adult longevity of *H. armigera*. However, pupal period and fecundity of *H. armigera* on five maize hybrids were significantly affected by five hybrids tested in this research. The increase in pupal period observed in *H. armigera* reared on SC500 may be due to unsuitability of this hybrid for the pupal development. However, no clear relationship was found between lytic activity of the larvae fed on SC500 and its duration in pupal stage.

Females of *H. armigera* emerged from the larvae fed on SC260 had the lowest daily and total fecundity. The percentage of high immune-activated larvae and the lysozyme concentration of *H. armigera* larval hemolymph were higher on SC260 than on the other maize hybrids. Therefore, fitness cost related to fecundity of *H. armigera* was the highest on this maize hybrid, which is in agreement with the findings of Herandez-Martinez *et al.* (2010), who reported lower fecundity of immune-activated *S. exigua* than non-immune-activated insects.

Our study indicated for the first time that different maize hybrids consumed by *H. armigera* larvae can induce its immune defense response with related fitness costs. Potential interference or overlapping of different plant secondary compounds with various bacterial communities present in these plants (Freitak *et al.,* 2007) may explain the observed variations in lysozyme activity of *H. armigera* larval hemolymph reared on five different maize hybrids. However, according to Freitak *et al.* (2007) using artificial diet instead of intact host crops can overcome obvious variations between influences caused by bacterial community of seed surface and different plant secondary compounds.

The insect alimentary canal, especially midgut may play an efficient role in recognition of foreign microorganisms and mounting of protective responses. Furthermore, according to Hernandez-Martinez *et al.*, (2010) immune defense system of the hemolymph may be influenced even if the foreign microorganisms never enter the hemocel (Freitak *et al.,* 2007). Thus, the midgut deserves notice not only as digestion and assimilation organ, but also as an organ of defense system.

Many researches have demonstrated that the susceptibility of lepidopteran insects to Bt can be affected by different factors including the insect host (Schuler and van Emden, 2000; Broderick *et al.,* 2003; Rahman *et al.,* 2007), Bt strain (Slamti and Lereclus, 2002) and climatic conditions (Mostafa *et al.,* 2005). Phytochemicals are also known as one of the main factors affecting the susceptibility of lepidopteran insects to Bt (Broderick *et al.,* 2009). Furthermore, larval enteric bacteria influence susceptibility of lepidopteran insects to Bt, and the extent of this effect may differ

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across the lepidopteran species (Broderick *et al.,* 2009). In some cases these factors may have an interaction, for example, the composition of enteric bacteria can be changed with host plant diet (Broderick *et al.,* 2004). According to Broderick *et al.* (2006) the enteric bacteria may delete an immunological barrier, such as defensive enzymes or antimicrobial peptides. Thus as both basic and applicable knowledge, consumed host crops such as maize hybrids may potentially increase the susceptibility of *H. armigera* to Bt by altering the community of enteric bacteria. Such information would be useful to plan approaches for controlling this economic pest by harnessing their indigenous microbial communities or combining Bt with bacteria that can induce septicemia (Broderick *et al.,* 2006). Whether this immune measure can predict resistance to Bt, remains to be examined.

Based on our findings since more than 60% of *H. armigera* larvae that fed on SC500, SC700 and DC370 had halo diameters lower than 5 mm (low immune-activated insects) and also because the lysozyme concentration in larval hemolymph was the lowest in amount on these hybrids, it is therefore suggested to design strategies for successful use of Bt products in IPM programs of *H. armigera* on these maize hybrids.

In conclusion, according to the results of this study it is clear that the lysozyme activity and some related fitness parameters of *H. armigera* can be affected by different maize hybrids. For a better conceiving of the insect-plant interactions, basic biochemical researches for the identification of secondary chemicals and microbial communities present in maize hybrids, which influence the susceptibility of *H. armigera* to Bt are necessary. Future works should also be focused on study of the effect of various maize hybrids (as larval food in both natural and artificial diets conditions) on the pathogenicity of Bt products against *H. armigera*.

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فعالیت آنزیم لیزوزیم و برخی پارامترهای زیستی *Helicoverpa armigera* روی پنج هیبرید ذرت

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چکیده: کرم غوزه پنبه (Hübner) *Helicoverpa armigera* به عنوان یکی از آفات اقتصادی مهم خسارت شدیدی به محصولات کشاورزی مختلف در ایران و بسیاری از کشورهای جهان وارد می کند. تاثیر پنج هیبرید ذرت شامل SC260، SC500، SC700، SC704 و DC370 روی فعالیت آنزیم لیزوزیم همولمف لارو سن ششم *H. armigera* و رابطه آن با برخی پارامترهای زیستی آفت در اتاقک رشد (دمای ۱ ± ۲۵ درجه سلسیوس، رطوبت ۵ ± ۶۵ درصد و دوره ۱۶ ساعت روشنایی و ۸ ساعت تاریکی) بررسی شد. نتایج نشان داد که فراوانی لاروهای با فعالیت بالای سیستم ایمنی به جز هیبرید SC260 روی سایر هیبریدهای ذرت کمتر از حشرات دارنده فعالیت پایین سیستم ایمنی بودند. میانگین غلظت لیزوزیم در همولمف لارو روی SC260 بیشترین (۰/۰۱ ± ۰/۰۹۶ میلی‌گرم بر میلی‌لیتر) و روی DC370 کمترین (۰/۰۰۷ ± ۰/۰۶۰ میلی‌گرم بر میلی‌لیتر) بود. طولانی‌ترین دوره شفیرگی روی SC500 (۰/۴۹ ± ۱۲/۰۰ روز) و کوتاهترین آن روی SC700 (۰/۱۹ ± ۱۰/۳۷ روز) بود. باروری روزانه و کل *H. armigera* روی DC370 بیشترین (به ترتیب ۹/۸۵±۶۱/۶۸ و ۴۹/۳۰ ± ۱۹۶/۸۹ تخم) و روی SC260 کمترین تعداد (به ترتیب ۵/۸۸ ± ۲۰/۶۰ و ۱۸/۸۰ ± ۵۲/۷۱ تخم) بود. نتایج این پژوهش می تواند اطلاعات پایه ای را به منظور مدیریت *H. armigera* روی هیبریدهای ذرت ارائه نماید.

واژگان کلیدی: *Helicoverpa armigera* ، لیزوزیم، پارامترهای زیستی، هیبریدهای ذرت

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