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ANTIOXIDANT AND DIGESTIVE ENZYMES ALTERATIONS IN THE CORN APHID, *RHOPALOSIPHUM MAIDIS* (FITCH) (HEMIPTERA: APHIDIDAE) FED ON DIFFERENT VARIETIES OF BARLEY AND WHEAT

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Because of the serious damaging signs induced by aphids on cereal crops, changes in the biochemical parameters of these pests in response to feeding on different varieties of cereal crops require attention. This work addressed the effects of five varieties of barley (Giza 123, Giza124, Giza125, Giza132 and Giza 2000) and four varieties of wheat (Sids 1, Giza 168, Shanduel 1 and Gemieza 11) that are cultivated in Egypt on the apterous adults of the corn aphid, *Rhopalosiphum maidis*. Giza 125 and Giza 132 of barley highly decreased catalase (CAT), superoxide dismutase (SOD) and the total antioxidants compared to other barley varieties. Giza123, Giza 124 and Giza 2000 caused a significant decrease in glutathione-S-transferase (GST) activity. Shanduel 1 and Giza 168 impaired the levels of CAT, SOD and the total antioxidant content in *R. maidis*. GST was in the lowest level in case of Sids 1 and Gemeiza 11 of wheat. Amylase was reduced by three varieties of barley (Giza 123, Giza 125 and Giza 132). The latter two varieties (Giza 125 and Giza 132) decreased the lipase activity in *R. maidis*. Lipase activity did not change in all varieties of wheat-fed *R. maidis* while a single variety (Shanduel 1) had the most negative impact on amylase activity in *R. maidis*. The present investigation emphasized that the cultivation of right field crops can manage aphids that attack them via targeting some metabolic pathways.

Key words: *Rhopalosiphum maidis*,Aphid, Oxidative stress, Digestive enzymes, Cereal crops

1. INTRODUCTION

Crop protection *via* management of plant diseases, weeds and other pests is a key factor in world economy. Insect pests are considered one of the most serious damaging factors to plants (Shanower et al., 1996). Aphids (Hemiptera) are destructive pests of several crops in many regions around the world due to their fast reproduction rate and their polyphagous feeding ability (McGavin, 1993; Piper, 2007). Their destructive effects range from sucking the plant sap to destruction of its biochemical pathways (Singh & Sinhal, 2011). Aphids are characterized by having sucking mouth parts which can be used to suck plant sap and in some cases transmit some plant viruses (Stroyan, 1997; Smith & Chuang, 2014; Yu et al., 2014). A typical life cycle of an aphid starts in spring season with a hatching egg which develops into wingless females on the primary host. These females reproduce by parthenogenesis to produce wingless females (apterous form). When the season ends, they develop wings (alate form) and migrate to a new host plant (the secondary host) where they develop into males and females to reproduce sexually, giving rise to fertilized eggs on the primary host (Dixon, 1971). Cereal grains are considered as the most important source of food worldwide. The whole grain is a rich source of minerals, vitamins, proteins, carbohydrate and lipids (Saldivar, 2010). Antioxidants are key factors in regulation of insect physiology. Reduction of O_2 for energy production releases reactive oxygen species (ROS) which can induce serious destructive actions against lipids, carbohydrates, DNA and RNA and this effect can be lethal to insect tissues. This occurs due to the imbalance between ROS (which serves as oxidants) and the antioxidants in insect body (Felton & Summers, 1995). Carbohydrates and lipids are the primary source of energy in insects. Digestion of these two nutrients to a form that can be absorbed by the midgut cells is a complicated process. Two categories of enzymes participate in carbohydrate digestion depending on the carbohydrate substrate: Des-polymerases (such as α-amylase) and glycosidase which hydrolyze polysaccharides (Terra & Ferreira, 2012). In insects, lipids are present mainly in five different forms triacylglycerols (TAGs), diacylglycerols (DAGs), phospholipids, hydrocarbons, and wax esters (Jurenka et al., 1988). TAGs are the major lipid components in insects and they are hydrolyzed by the action of TAG lipases (Smith et al., 1994). Insecticide use in controlling aphids remains the easiest and cheapest approach. However, excessive use of synthetic insecticides for controlling aphids is unfavorable as they develop resistance to insecticides; in addition to negative effects of insecticides on non-target organisms and hazardous actions to the environment (Tagu et al., 2008). Most crops lack genes to resist aphids, therefore, it was needed to develop promising approaches to use aphid-resistant cultivars. This work aimed to determine the inhibitory effect of different varieties of wheat (*Triticum aestivum)* and barley (*Hordeum vulgare*), that are cultivated in Egypt, on the activities of catalase (CAT), superoxide dismutase (SOD), glutathione S-transferase (GST) and total antioxidant content of the corn aphid, *Rhopalosiphum maidis*. Furthermore, the activities of amylase and lipase digestive enzymes were determined. This study will help in the management of aphids through the selection of field crops that can inhibit the antioxidant and digestive enzymes.

2. MATERIAL AND METHODS

2.1. Host plant cultivation, aphid collection and preparation of tissue extract

Different strains of wheat (Sids1, Giza168, Shanduel1, and Gemieza11) and barley (Giza 123, Giza124, Giza125, Giza132 and Giza 2000) were cultivated at Assiut University Farm (Assiut, Egypt) during the winter season starting from November 2016 to April 2017. The corn aphids, *R. maidis* infesting these crops were collected during the infestation peak at the end of February. Apterous adults (approximately 200 aphid) were separated and used for tissue extract preparation. Tissue extract was prepared as described by Abdelsalam et al. (2016). Briefly, *R. maidis* adults (0.1 gm ~200 aphid) were homogenized in 1.0 ml of 50 mM potassium phosphate buffer (pH 7.4). Then, the homogenates were centrifuged at 3000 rpm for 15 min and the supernatants were collected and stored at -20 ˚C till use.

2.2. Antioxidant enzymes and total antioxidant assays

SOD, GST and CAT activities were determined using commercial kits (Biodiagnostics, Cairo, Egypt) according to manufacturer instructions. All enzyme activities were presented as Units (U) / mg protein. Total antioxidant content was determined using commercial kits (Biodiagnostics, Cairo, Egypt) according to method of Koracevic et al. (2001) presented as mM/ mg protein.

2.3. Digestive enzymes assays

Alpha - amylase activity was measured by a commercial kit (Biodiagnostics, Cairo, Egypt) according to manufacturer instructions based on Caraway (1959). Lipase activity was determined using the commercial kit manufactured by Quimica clinica aplicada, Spain.

2.4. Data analysis

Data are presented as mean ± standard deviation (SD) of three independent replicates. Differences among means were analyzed by Duncan's multiple range test at $P \leq 0.05$ using SPSS version 14.0 software (SPSS Inc., Chicago, IL).

3. RESULTS

3.1. Antioxidant enzymes and total antioxidant levels of *R. maidis*

Catalase and SOD activities, and total antioxidant content were significantly reduced ($P<0.05$) in *R. maidis* cultured on Giza 125 variety of barley than those cultured on the remaining four varieties of barely (Giza 123, Giza 124, Giza 132 and Giza 2000). The activity of these biochemical parameters in descending order was Giza 2000, Giza 123, Giza 124, Giza 132 and Giza 125 (Fig. 1A, B, D). This indicates that Giza 125 strain, among the barley strains tested, showed the highest inhibitory effect on CAT, SOD and total antioxidant content in *R. maidis*. Whereas, aphids fed on Giza 125 showed the highest activity of GST. The magnitude of GST activity in descending order was Giza 125, Giza 132, Giza 2000, Giza 124 and Giza 123 (Fig. 1C). These results reflect that Giza 123 strain, among barley strains tested, had the highest inhibitory effect on GST in *R. maidis*. *R. maidis* fed on Giza 168 variety of wheat showed the lowest activities of CAT and SOD, and total antioxidant content compared to those fed on Sids 1, Shanduel 1 and Gemieza 11 varieties of wheat. The activity of these biochemical parameters in descending order was Gemieza 11, Sids 1, Shanduel 1 and Giza 168 (Fig. 2A, B, D). These results indicate that Giza 168 strain of wheat, among the strains tested, showed the highest inhibitory effect on CAT, SOD and total antioxidant content in *R. maidis*. In contrast, wheat Giza 168 variety significantly increased GST activity ($P<0.05$) of *R. maidis* compared to aphids fed on Sids 1, Shanduel 1 and Gemieza 11 varieties of wheat. The activity of GST in descending order was Giza 168, Shanduel 1, Sids 1 and Gemieza 11 varieties (Fig. 2C). This finding clearly showed that Gemieza strain had the most inhibitory effect on GST in *R. maidis* relative to other varieties tested. Giza 168 and Shanduel 1 decreased the total antioxidant content by two folds compared to Sids 1 and Gemieza 11 (Fig. 2D).

3.2. Amylase and lipase activity of *R. maidis*

Apterous forms of *R. maidis* adults fed on three varieties of barley (Giza 123, Giza125, and Giza132) suffered reduced activity of amylase (Fig. 3A). Among these three varieties, two only (Giza 125 and Giza 132) induced significant decrease in the activity of lipase (Fig. 3B). Feeding on wheat varieties produced different pattern in enzyme activity where Shanduel 1 reduced amylase activity (Fig. 4A) and none of the used varieties affected the activity of the lipase enzyme (Fig. 4B).

4. DISCUSSION

Some varieties of barley and wheat used in the present study induced significant decrease in some antioxidants including CAT, SOD and total antioxidants while other varieties targeted GST. Insects use CAT and SOD to respond the oxidative stress induced by ROS (Barbehenn, 2003) and use GST against plant allelochemicals (Vanhaelen et al., 2001). Plants respond rapidly to stress by several defense reactions such as "oxygen burst" which involves the production of ROS like superoxide radical n (O_2^-) and hydrogen peroxide H_2O_2 (Kuzniak and Urbanek, 2000; Mittler, 2002). Hydrogen peroxide plays a critical role in plant defense as it causes direct toxicity against insect pests, and acting as signal inducer of defense genes (Kuzniak and Urbanek, 2000). In response to oxidative stress originating from host plants, insects have evolved several mechanisms to act against ROS as they possess a complex system of antioxidants.

Xenobiotics such as plant allelochemicals are overcome by different defense systems in most insect species. GST in insects is necessary in the detoxification process of many chemicals including allelochemicals of plant origin. GST activity elevated in the green peach aphid, *Myzus persicae*, in response to secondary metabolites from different host plants (Francis et al., 2005).

Digestion of insect diet after ingestion is the process by which insects treat their diet to release energy stored in it. Understanding such process helps in establishment of the possible target in controlling insects (Wu et. al., 2014). Biotic agents, such as the host plant when they can impair such processes they will be suitable for cultivation rather than those which are not affecting digestive pathways. Barley-fed *R. maidis* showed reduced activity of amylase in three varieties of barley (Giza 123, Giza 125, and Giza 132) and two only (Giza 125 and Giza 132) induced a significant decrease in the activity of lipase. Similarly, wheat-fed *R. maidis* showed the lowest activity of amylase in case of Shanduel 1 variety while lipase activity was unchanged. The reduced activities might arise from the antifeedant activity of these varieties or due to the lower carbohydrate and lipid content or even due to the presence of inhibitors in the genome of some plant varieties. Alpha - amylase is the digestive enzyme which catalyzes α-1,4 glucan bonds in starch and glycogen (Terra & Ferreira, 2012). This step is the starting point in carbohydrate metabolism and when retarded it causes serious symptoms in insect biological activities. Some varieties of wheat and barley are characterized by having inhibitors to α-amylase in their genomes (Franco et al., 2000; Bandani, 2005) which might cause the reduced levels of aphid amylase. This is supported by the fact that there is no evidence on antifeedant activity and differences in the carbohydrate content between the varieties of wheat and barley. Like amylases, lipases are involved in the catabolic reactions of lipids in insects. Due to lack of lipase inhibitors in the genomes of wheat and barley, excluding the antifeedant activity of these plants, it is likely to conclude that the fluctuation in lipase activity due to different varieties of barley and wheat arise from the composition of the nutrients received by the phytophagous aphids from the plant.

Different varieties of plants vary in their tolerance to environmental factors suggesting they may be different from each other in their response to aphid attack and their effect on aphid physiology (Zare, 2012). Forty nine out of hundred thirty six varieties of barley showed resistance to the aphid *R. padi* (Hsu & Robinson, 1962). Two wheat varieties (hard red and soft white winter wheat) were resistant to *R. padi* (Grivin et al., 2017). Different plant stages and feeding sites of wheat also affected the fecundity of *R. padi* (Leather & Dixon, 1981). The effect of some members of family Fabaceae on the pea aphid, *Acyrthosiphon pisum*, has been reviewed by Lukasik et. al. (2011). The ascorbate concentration was higher for aphids fed on *Pisum sativum*. The content of antioxidant, GSH, was in the highest levels within the tissues of *A. pisum* fed on *Vicia faba*. *Pisum sativum* - fed aphids had three - fold - lower activities of the antioxidant enzymes than those fed on *V. faba* (Lukasik et al., 2011). Aphids belonging to the same species are also affected by the host plant. *Myzus persicae* and *Myzus persicae nicotianae* are a species and subspecies of the same organism. *Myzus persicae* showed low performance than *M. persicae nicotianae* on tobacco (Nikolakakis et al., 2003) and this occurs through the enzymatic detoxification system which plays an important role in the ability of *M. persicae nicotianae* to avoid the tobacco defenses leading to higher performance. Esterases, GST and cytochrome P-450 monooxygenases play a acritical role in removal of xenobiotics in *M. persicae nicotianae* (Cabrera et al., 2010).

5. CONCLUSION

Our results showed that different plant varieties affected the antioxidant response and digestive enzyme activity in *R. maidis*. We assumed that low content in antioxidants and reduced digestive enzyme activities can be considered as biomarkers of impaired physiological status in this aphid species. Giza 125 and Giza 132 of barley reduced the total antioxidant content, lipase and amylase in *R. maidis*. Feeding on wheat varieties reduced both the total antioxidant content and the amylase only in Shanduel 1 variety. The present investigation emphasized that the cultivation of right field crops can manage aphids that attack them via targeting some metabolic pathways.

6. ACKNOWLEDGEMENT

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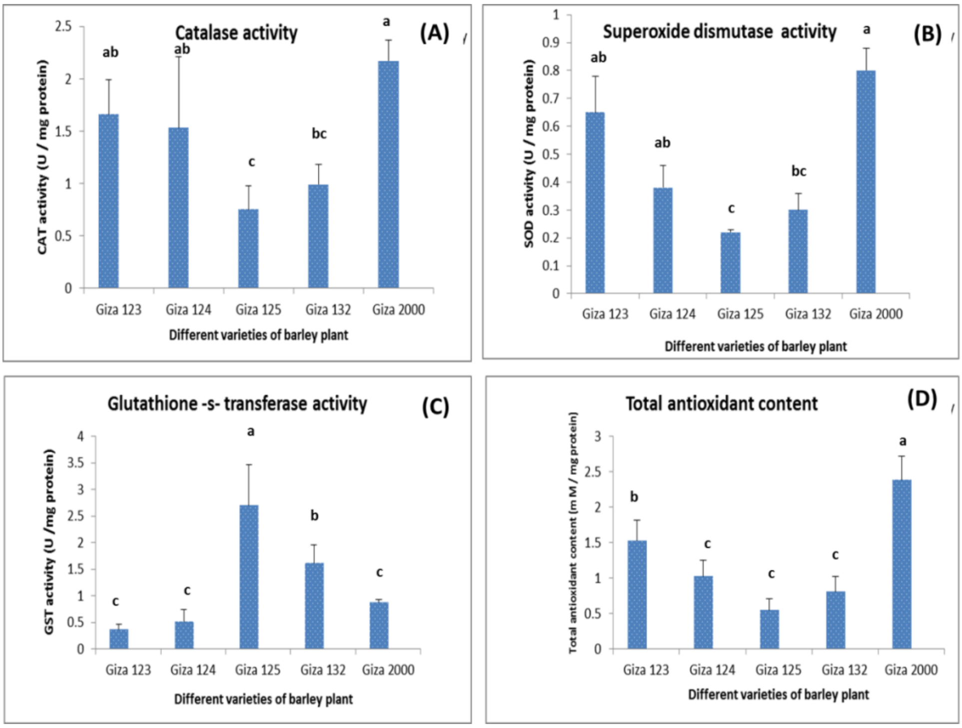


Figure 1. Activity of antioxidant enzymes and content of total antioxidants within the whole homogenate of the corn leaf aphid, *R. maidis* collected from different varieties of barley plant (Giza 123, Giza 124, Giza 125, Giza 132 and Giza 2000). (A) Catalase (CAT), (B) Superoxide dismutase (SOD), (C) Glutathione S- Transferase (GST), (D) Total antioxidant content. Data are presented as mean ± SD, n=3. Values in columns followed by different letters are significantly different at $P \leq 0.05$ (Duncan's multiple range test). Enzyme activities are presented as units / mg protein while total antioxidants are presented as mM/ mg protein.

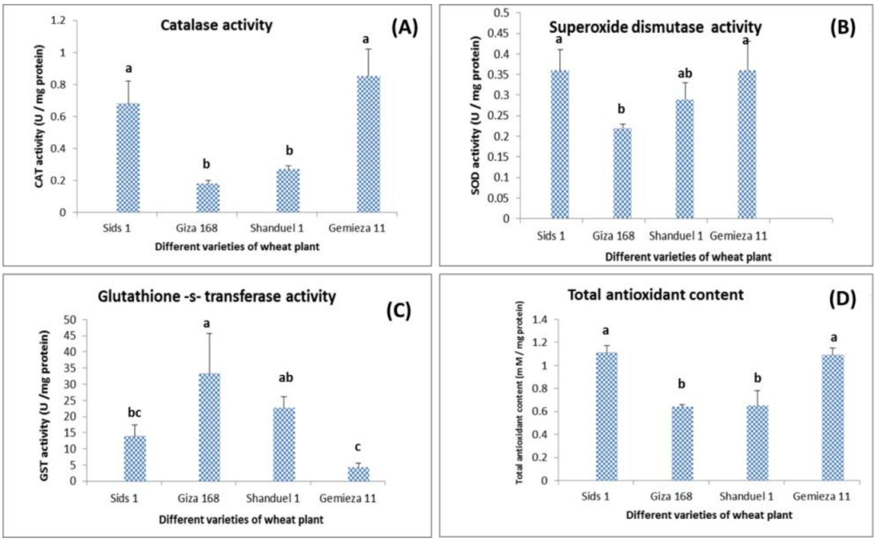
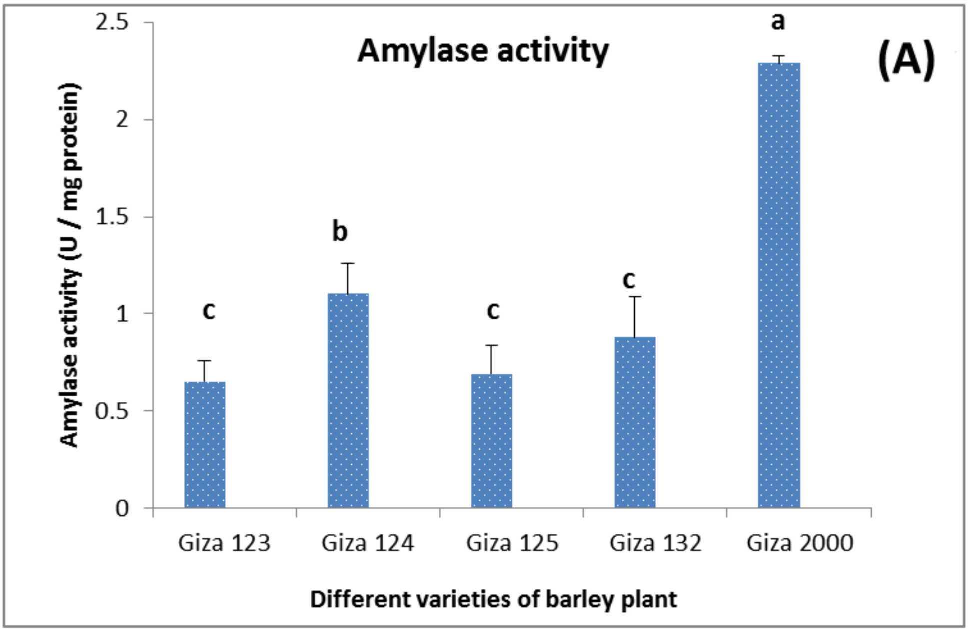


Figure 2. Activity of antioxidant enzymes and content of total antioxidants within the whole homogenate of the corn leaf aphid, *R. maidis* collected from different varieties of wheat plant (Sids1, Giza168, Shanduel1, and Gemieza11). (A) Catalase (CAT), (B) Superoxide dismutase (SOD), (C) Glutathione –S-Transferase (GST), (D) Total antioxidant content. Data are presented as mean ± SD, n=3. Values in columns followed by different letters are significantly different at $P \leq 0.05$ (Duncan's multiple range test). Enzyme activities are presented as units / mg protein while total antioxidants are presented as mM/ mg protein.



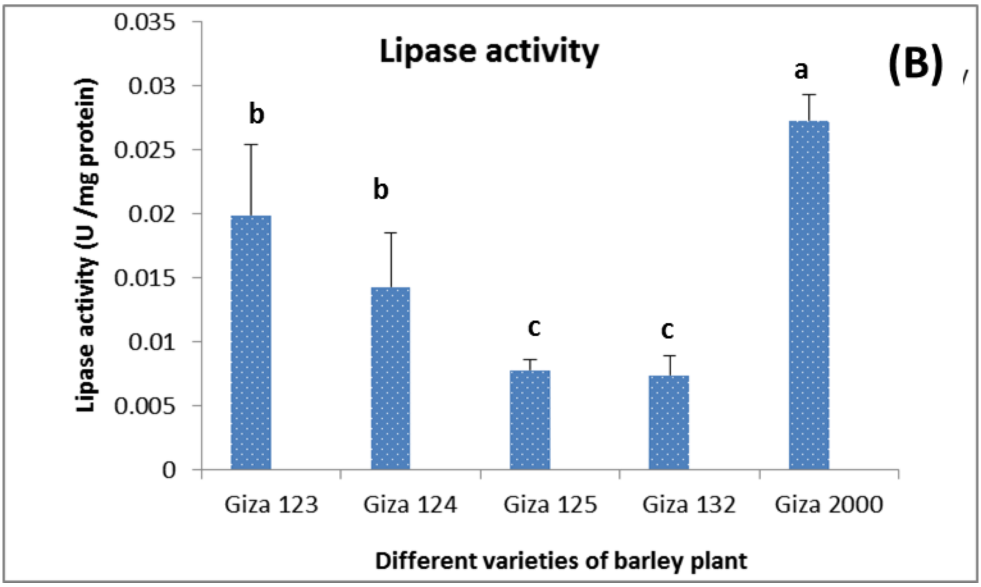


Figure 3. Activity of amylase and lipase within the whole homogenate of the corn leaf aphid, *R. maidis* fed on different varieties of barley plant. (A) Amylase activity, (B) Lipase activity. Data are presented as mean ± SD, n=3. Values in columns followed by different letters are significantly different at $P \leq 0.05$ (Duncan's multiple range test).

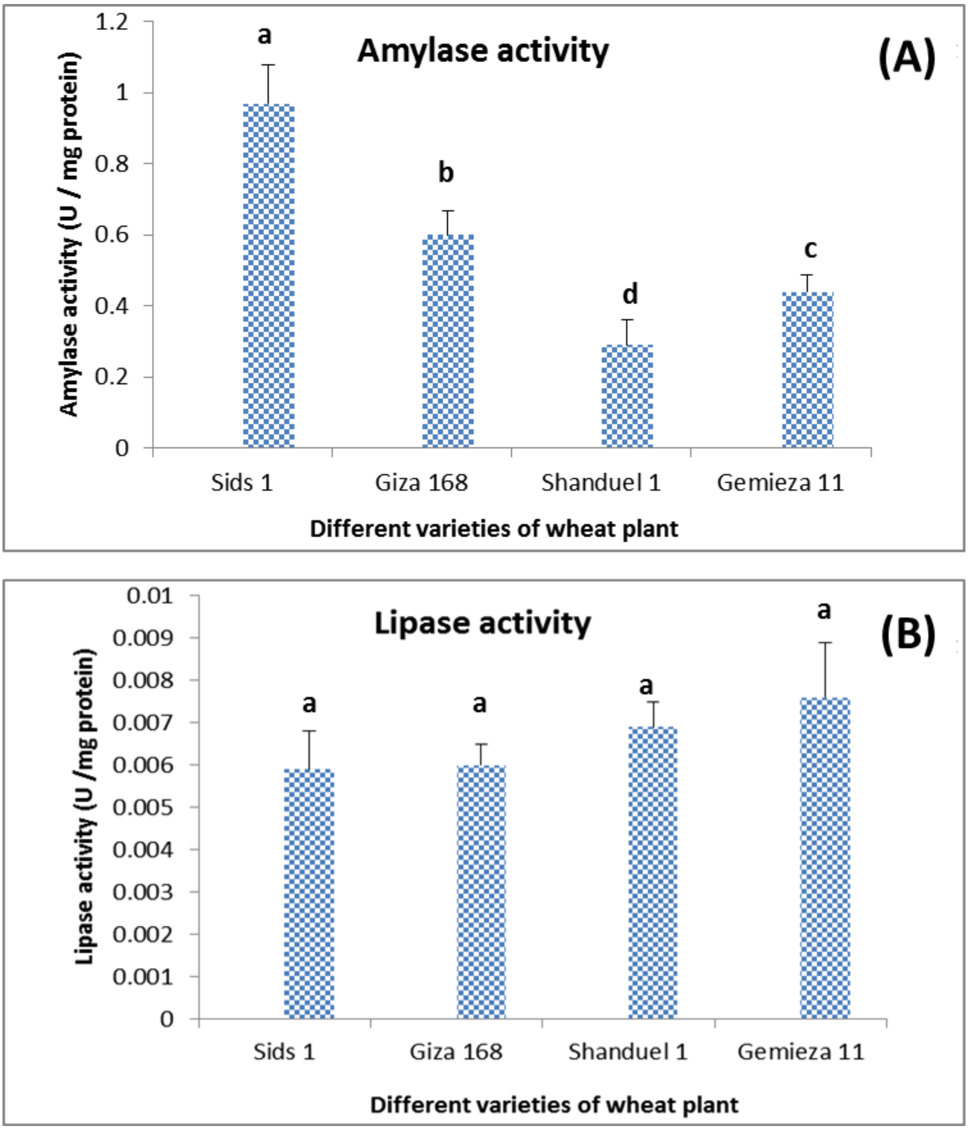


Figure 4. Activity of amylase and lipase within the whole homogenate of the corn leaf aphid, *R. maidis* fed on different varieties of wheat plant. (A) Amylase activity, (B) Lipase activity. Data are presented as mean ± SD, n=3. Values in columns followed by different letters are significantly different at $P \leq 0.05$ (Duncan's multiple range test).

التغيرات في الإنزيمات المضادة للأكسدة والهاضمة لحشرة مَنَّ الذرة المغتذية علي أصناف مختلفة من نباتي الشعير والقمح

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أجريت هذه الدراسة لمعرفة أكثر السلالات من نباتي القمح والشعير تأثيرا علي حشرة مَنَّ الذرة والتي تعتبر من اخطر الآفات الحشرية إضرارا بمحاصيل الحبوب في جمهورية مصر العربية. تمت دراسة التغيرات البيو كيميائية لحشرة مَنَّ الذرة بعد تغذيتها على أصناف مختلفة من نباتي القمح والشعير وذلك لمساعدة المزراعين في اختيار أكثر الأصناف مقاومة لهذه الحشرة. استخدمت في هذه الدراسة خمسة أصناف من الشعير (Giza 123, Giza 124, Giza 125, Giza 132, Giza 2000) وأربعة أصناف من القمح (Shanduel 1, Gemeiza 11, Sids 1, Giza 168). تبين من الدراسات البيوكيميائية أن مضادات الأكسدة الكلية وإنزيمي (CAT, SOD) قد انخفضت معدلاتها في حشرة المَنَّ بعد تغذيتها علي صنفي Giza 125, Giza 132. وأيضا انخفضت معدلات إنزيم GST في المَنَّ المُغتذي علي أصناف Giza 123,Giza 124,Giza 2000. علي الجانب الآخر انخفضت معدلات مضادات الأكسدة الكلية وانزيم CAT عند تغذية المَنَّ علي نبات القمح من صنف Shanduel 1 وانخفضت معدلات إنزيم SOD عند التغذية علي صنف Giza168 وأما صنفا Sids1 وGemeiza11 فثبطا معدلات GST. وعند دراسة تأثير الأصناف علي الإنزيمات الهاضمة لحشرة مَنَّ الذرة تبين أن انزيم Lipase لم يتغير معدل نشاطه في كل أصناف القمح وانخفض في صنفي Giza132, Giza 125 لنبات الشعير. أما انزيم Amylase فقد انخفضت معدلاته في ثلاثة أصناف من نبات الشعير (Giza 132, Giza 125, Giza 123) وفي نوع واحد من القمح (Shanduel1). تظهر الدراسة الحالية أن زراعة محصول الحقل الصحيح يمكن من السيطرة على حشرات المن التي تهاجمه من خلال استهداف بعض المسارات الأيضية.