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DIFFERENTIAL OXIDATIVE AND BIOCHEMICAL RESPONSES OF TOMATO AND MAIZE LEAVES TO *SPODOPTERA EXIGUA* HERBIVORY

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Abstract

Plants have been interacting with insects leading to complex defense approaches. Plant redox status could be regarded as an indicator for its resistance, which is accomplished by the elimination of generated ROS. In his study, we investigated the relationship between oxidative responses in tomato and maize after *Spodoptera exigua* attack and the plant tolerance. As an indication for plant resistance, fresh and dry biomass of tomato and maize shoot was determined. To investigate the oxidative responses, the concentration of hydrogen peroxide (H_2O_2), phenols and ascorbic acid (ASA) in addition to the activities of ascorbate peroxidase (APX), superoxide dismutase (SOD) and catalase (CAT) were assayed. All data were collected after 2 hours, 2 days and 1 week following infestation. The results indicated that, shoot fresh and dry weight of tomato significantly decreased lower than uninfected controls after 2 d and 1 week of infestation. However, maize shoot fresh and dry biomass decreased than the control only after 1 week of infestation. The infestation increases H_2O_2 and ASA concentration in maize leaves at 2 hours and 2 days after infestation while no change recorded in tomato leaves compared to their corresponding controls. Free phenols content increased in infested tomato leaves more than control. The infestation enhances SOD, CAT and APX activities in tomato and maize leaves. H_2O_2 content in the leaves of studied plants correlated with their differential tolerance responses. Therefore, it could be used as a diffusible signal to activate defensive genes in maize leaves, as recorded by increasing CAT and APX activities.

Key words: Beet armyworm, *Solanum lycopersicum*, *Zea mays*, Antioxidants, Antioxidant enzymes.

Introduction

Plants interact in their surrounding environments with a wide range of herbivore insect with different infestation and strategies lifestyles (Schoonhoven *et al*., 2005). As result plant provides a highly sophisticated defense mechanisms include the regulation of defense signal-transduction pathways and production of defensive compounds (Kessler & Baldwin, 2002; Hilker & Meiners, 2006). These resistance mechanisms are designed in order to protect plants from foreign threats caused by pathogens or insects. Therefore, they can be divided into localized resistance in the damaged tissue or systemic resistance in undamaged tissue to subsequent infestations (Schoonhoven *et al*., 2005; Walters, 2011).

Upon infestation by insects, plants are rapidly increase accumulate of reactive oxygen species (ROS) concentration at attack site, a phenomenon called oxidative burst. It is considered as one of the earliest cellular responses (Hilker & Meiners, 2006). Reactive oxygen species play major role in developmental signaling. Many studies have confirmed that ROS are involved in herbivory-induced responses in plants (Li *et al*., 2002; Leitner *et al*., 2005; Jones & Dangl, 2006; Maffei *et al*., 2007). Being highly reactive, they can destroy lipids, proteins, carbohydrates and DNA and consequently lead to programming cells death (Apel & Hirt, 2004; Bhattacharjee, 2005). On the other hand, plants have developed a complex defense system to scavenging ROS in order to prevent oxidation burst. Therefore, plants also have to keep the balance between production ROS and the production of ROS-detoxifying system to help in reliving the oxidative damage in the plant tissue (Krishnan & Kodrik, 2006; Colodete *et al*., 2015; Arshad *et al*., 2019). Detoxification system include low molecule weight antioxidants and antioxidative enzymes (D'Autréaux & Toledano, 2007). They play an important role in controlling ROS levels amongst others besides having other defensive (Das & Roychoudhury, 2014).

The detoxification of the antioxidant system is created first by the enzymatic actions of SOD reduces superoxide radicals to H_2O_2 (Breusegem *et al*., 2001). The H_2O_2 is then scavenged by CAT and APX into H_2O and O_2 (Breusegem *et al*., 2001). Hydrogen peroxide (H_2O_2) is a ubiquitous, generated as toxic byproducts of aerobic metabolism (Breusegem *et al*., 2001). It involved in signal transduction, the regulation of various biological processes and plays role in mediating immune responses (Bhattacharjee, 2005). Hydrogen peroxide mainly has been investigated on their dual role that depending on their concentration in plants (D'Autréaux & Toledano, 2007). At high concentration plants use it, as one of the oxidative weapons, as second lines of defense against the attacker by purposefully generate ROS (Das & Roychoudhury, 2014). On the other hand, at low/moderate concentration it acts as signaling molecules to control various processes in plant cell (Bhattacharjee, 2005; D'Autréaux & Toledano, 2007; Torresa, 2010; Baxter *et al*., 2014; Das & Roychoudhury, 2014).

However, ROS exerts its effects depending on the cellular concentration as well as its exposure time. Recently, researchers have shown an increased interest in role of production of ROS in plant defense. More recent studies have confirmed that H_2O_2 concentrations are likely to be raised up due to infected by chewing herbivorous insects (Maffei *et al*., 2004; Leitner *et al*., 2005; Maffei & Bossi, 2006). Furthermore, (Maffei *et al*., 2007) showed that H_2O_2 accumulated within 3 hours following chewing

insect *S. littoralis* infestation of lima bean. The finding is consistent with findings of past studies by (Moloi & van der Westhuizen, 2006), which shown a massive accumulation of H_2O_2 in wheat plant after 3 hours of infestation by the Russian wheat aphid. In contrast, the study by (kusnierczyk *et al*., 2008) indicated that H_2O_2 accumulated after 48 hours following *Brevicoryne brassicae* infestation in *Arabidopsis* plant. However, a number of studies show that significant differences do exist, albeit findings are somewhat contradictory. Leitner *et al.*, (2005) found differences suggesting that H_2O_2 not accumulation following infestation by the spider mite *Tetranychus urticae* or *S. littoralis*. However, H_2O_2 accumulated after 24 hours following infected by the chewing insect *Medicago trunculate*.

It is well established that antioxidant system is play a vital role in limiting the oxidative stress by detoxifying ROS, including hydrogen peroxide (Bhattacharjee, 2005; Hilker & Meiners, 2006; Ruuhola & Yang, 2006; Tariq & Shahbaz, 2020). Among the many antioxidant molecules, ascorbic acid and phenolic compounds that considered as secondary metabolites (Golan *et al*., 2017). These antioxidant molecules are generally regarded as defensive molecules (Racchi, 2013). They involved in the defense strategies of plant against insect herbivory. For example, (Eleftherianos *et al*., 2006) found that the level of phenol concentration are increase in maize and barley plant following infestation by *R. padi*, While they caused decrease in phenol levels following infected by *S. avenae*. Other studies suggest that the accumulation of phenolic compounds and ascorbic acid, as well as variation in the activity of antioxidant enzymes, as biochemical plant responses to herbivory, may differ in various plant species (Eleftherianos *et al*., 2006; Golan *et al*., 2017).

The beet armyworm, *Spodoptera exigua*, is a polyphagous chewing insect that feed on more than 50 plant species from over 10 families such as maize, tomato, alfalfa, cotton, lettuce, etc. (Sertkaya *et al*., 2004; Mardani-Talaei *et al*., 2012). This chewing insect can cause cutting and crushing plant tissues. Therefore, they can lead to serious economic loss due to the significant damage with plant death as well (Leitner *et al*., 2005). It is regarded as a serious defoliator of crops leaves. In five instar larvae, they will feed large irregular holes in the foliages (Schoonhoven *et al*., 2005). In fact, plant responses to insects attack depends on many factors such as the plant species, herbivores number and species and infestation time. These data provided the motivation to make this study, with the aim of verifying the magnitude of some oxidative responses that are involved in the *S. exigua* defense in tomato and maize, two of the world's most important crop plants. To achieve this, we compare the variations in plant pigments content, phenolic compounds, ASA, H_2O_2 and of SOD, CAT and APX activities in response to *S. exigua* infestation in the leaves of tomato and maize plants with three different time periods of infestation.

Materials and Methods

Plant cultivation and insect infestation: This study was carried out at King Abdulaziz University Experimental Station, Saudi Arabia during spring season 2016. During the experiment, the average daily maximum temperature was 32.13ºC and the minimum was 22.08ºC. The seeds of tomato (*Solanum lycopersicum*, cv Super strain B) and maize (*Zea mays*, cv Golden bantam) were brought from USA Seeds Company (Modesto seed Co. Inc.) and germinated in soil mixed of sand and potting soil (2:1), sown in 30 cm diameter plastic pots with ten seeds per pot. Pots were arranged in randomized complete design in three replicates. The seeds were watered with tap water until the fourth leaf appeared.

The *S. exigua* larvae were used in all experiments after they had been reared on lettuce for more than one generation. Early third instars larvae were selected for all insect experiments. Immediately prior to the start of insect infestation, plants were placed into outdoors chamber covered with green a grofabric. Seven-days-old *S. exigua* larvae all about the same size were distributed on the onto plant leaves at approximately five larvae per plant. *S. exigua* larvae were starved for 35 min before the experiments. To investigate the time-course of insect-induced oxidative system plant samples were collected after 2 hours, 2 days and one week after infection. Control groups for each time did not receive any larvae. For all assays, plant leaves were collected after certain time periods and frozen immediately in liquid nitrogen.

Plant growth: At the end of the experimental period, plants were carefully up the shoot region from pots and shoot washed in running tap water. Excess water was removed with blotting paper. Tomato and maize growth was determined by measuring fresh and dry weights in grams (g) of their shoots.

Free and cell wall-bound phenolics determination: The method of Kofalvi & Nassuth (1995).was used to determine the concentration of free and cell wall-bound phenols Half gram of leaf samples was extracted in 50% methanol (1:2 v/v) at 80ºC for 90 min. After centrifugation at 14,000 rpm for 15 min., the supernatant used for free phenols quantitation using the Folin-Ciocalteu's phenol reagent. Bound phenols determined in the pellet after releasing it by sonification with 2 ml of 0.5 N NaOH for 24 h at room temperature, neutralization with 0.5 ml 2 N HCl and centrifugation at 14,000 rpm for 15 min. For bound phenols analysis, 100 µl of the methanol and NaOH extracts was diluted to 1 ml with water and mixed with 0.5 ml 2 N Folin-Ciocalteu's reagent and 2.5 ml of 20% Na_2CO_3. At room temperature, the absorbance of samples was determined at 725 nm after 20 min.

Ascorbic acid determination: The method of Jagota & Dani (1982) was used to determine ascorbic acid concentration. Leaf samples (0.2 g) was homogenized in liquid N_2, suspended in 2 ml of 5% trichloroacetic acid and centrifuged at 5ºC for 15 min. at 10,000 rpm. 0.8 ml of 10% trichloroacetic acid was added for 0.2 ml of tissue homogenate and shaken very well. Then the tubes kept for 5 min on ice. After centrifugation at 5000 rpm for 5 min, 0.5 ml of the extract was diluted to 2.0 ml using double-distilled water. Finally, 0.2 ml of diluted Folin-Ciocaiteu reagent was added, the tubes were vigorously shaken and the absorbance was measured at 760 nm after 10 min.

H_2O_2 Determination: The of H_2O_2 in level plant leaves was analysed by a modified method of Jagota & Dani (1982). 0.1 g leaf samples were extracted with cold acetone. Extracted solution (3 ml) was mixed with 1 ml of 0.5g titanium dioxide in 5 ml H_2SO_4, heated gently until fumes of sulfuric acid appear, then cooled. Then cautiously diluted to about 100 ml with distilled water and filtered. To 1ml of this clear filtrate, 3ml of the extracted solution was added. The absorption of developed color of the supernatant was determined at 415 nm.

ROS-scavenging enzymes activity: For analyzing enzyme activity, half gram of leaves tissues was grounded to a fine powder in liquid N_2 then homogenized in 5 ml of 100 mM potassium phosphate buffer (pH 7.8) containing 0.1 g polyvinylpyrrolidone and 0.1 mM ethylenediamine tetraacetic acid (EDTA). The homogenate was centrifuged at 18,000 rpm for 10 min at 4ºC and the supernatants were collected and used for the antioxidant enzymes assays.

SOD (EC 1.15.1.1): The activity of SOD was measured according to the method described by Misra & Fridovich (1972) following the autoxidation of epinephrine (adenochrome). The reaction medium (2 ml) containing 200 µl 0.5 mM EDTA, 25 mM of sodium carbonate buffer and 100 µl enzyme extract was used for measuring enzyme activity. 100 µl of 15 mM epinephrine was used to initiate the reaction. Epinephrine oxidation was estimated at A_{480} nm ($\varepsilon = 2.5\ mM^{-1}\ cm^{-1}$).

CAT (EC 1.11.1.6): The activity of CAT was analyzed calorimetrically by observing the variation in A_{240} as a result of the decreased H_2O_2 absorption (Zhang & Kirkham, 1996). The final reaction volume was 3 ml containing 2.4 ml 50 mM potassium phosphate buffer (pH 7) and 500 µl of enzyme extract. 100 µl of 10 mM H_2O_2 was used to start the reaction. ($\varepsilon = 39.4\ mM^{-1}\ cm^{-1}$).

APX (EC 1.11.1.11): The activity of APX was determined depending on Zhang & Kirkham (1996). The rate of ascorbic acid oxidation (using H_2O_2 as a substrate) of was determined in reaction mixture contained 50 mM potassium phosphate buffer (pH 7), 5 mM H_2O_2, 0.1 mM Na_2-ETDA, 0.5 mM ascorbic acid and 50 µl enzyme extract. The oxidation rate of ascorbic was monitored from the decline in absorbance at 290 nm. ($\varepsilon = 2.8\ mM^{-1}\ cm^{-1}$).

Statistical analysis

The results of this study statistically analyzed by means of the statistical software package SPSS version 21.0 (SPSS, Chicago, USA). Differences between infested and control (uninfested) plants at the same time point were tested with t-test. Significant increase (*) expressed at $p<0.05$, highly significant increase (**) expressed at $p<0.01$, highly significant increase (***) expressed at $p<0.001$. Differences between different time periods for infested and control plants separately were tested with one-way analysis of variance (*ANOVA*), followed by (Duncan) test at a significance level of 5% ($p<0.05$). All values were expressed as mean three replicates with their standard deviation (SD).

Results

Shoot biomass as affected by *S. exigua* infestation: Tomato and maize plants were infested with *S. exigua* larvae. Shoots biomass were determined after 2 hours, 2 days and 1 week of infestation. Maize showed relatively higher tolerance to *S. exigua* infestation compared to tomato. Depending on the t-test data, shoot fresh weight (FW) and dry weight (DW) of infested tomato plants significantly decreased lower than uninfested plants after 2 days and 1 week of infestation (t-test: FW, P=0.104, 0.031, 0.006; DW, P=0.342, 0.044, 0.001, Table 1). The FW of infested tomato shoots decreased by about 20.14 and 52.37% lower than their corresponding uninfested controls after 2 days and 1 week respectively. While, the DW of infested tomato shoos decreased by about 43.94, and 58.33 % lower than uninfested plants after 2 days and 1 week. In maize, no significant differences recorded in shoot fresh and dry biomass at 2 hours and 2 days after infestation (t- test: FW, P=0.153, 0.940, 0.011; DW, P=0.839, 0.724, 0.016; Table 1). Only after 1 week of infestation, FW and DW of maize shoots decreased by about 222.83 and 24.77 % lower than their uninfested controls respectively. ANOVA analysis showed that increasing infestation time significantly decreased tomato shoot FW and DW (*ANOVA*: FW, F=90.64, P=0.000; DW, F=12.33, P=0.007, Table 1). However, shoot FW and DW of infested maize plants at 2 days and 1week significantly increased higher than that at 2 hours (*ANOVA*: FW, F = 43.57, P = 0.003; DW, F=16.34, P=0.023, Table 1).

Table 1. Shoot fresh and dry weight (g) of tomato and maize plants as affected by *Spodoptera exigua* larvae infestation after different periods of time.

Duration of infestation	Tomato				Maize			
	FW		DW		FW		DW	
	Uninfested	Infested	Uninfested	Infested	Uninfested	Infested	Uninfested	Infested
2 hours	1.76 ± 0.11^{A}	1.58 ± 0.04^{b}	1.76 ± 0.11^{A}	1.58 ± 0.04^{b}	1.76 ± 0.46^{A}	1.58 ± 0.89^{a}	1.76 ± 0.11^{A}	1.58 ± 0.04^{a}
2 days	$1.97 \pm 0.24^{AB,*}$	1.71 ± 0.27^{b}	$1.97 \pm 0.24^{A,*}$	1.71 ± 0.27^{b}	3.63 ± 0.68^{B}	3.67 ± 0.14^{b}	1.97 ± 0.24^{A}	1.71 ± 0.27^{a}
1 week	$2.18 \pm 0.07^{B,**}$	1.04 ± 0.08^{a}	$2.18 \pm 0.07^{A,**}$	1.04 ± 0.08^{a}	$4.95 \pm 0.46^{C,*}$	3.82 ± 0.53^{c}	$2.18 \pm 0.07^{B,*}$	1.64 ± 0.08^{b}

Each value is a mean of three replicates ± standard error. The Different letters represent the statistical significance between different time periods of uninfested (A-C) and infested (a-c) plants at $p \leq 0.05$. Asterisks show significant differences between uninfested and infested plant at each time period (*$p<0.05$; **$p<0.01$; ***$p<0.001$)

Table 2. Plant pigments content (mg/g FW) in tomato leaves as affected by *Spodoptera exigua* larvae infestation after different period of time.

Duration of infestation	Chl a		Chl b		Carotenoid	
	Uninfested	Infested	Uninfested	Infested	Uninfested	Infested
2 hours	1.11 ± 0.10^{A}	$1.68 \pm 0.04^{b,*}$	1.52 ± 0.09^{A}	$1.68 \pm 0.04^{b,*}$	2.79 ± 0.10^{A}	$3.13 \pm 0.06^{a,*}$
2 days	1.86 ± 0.10^{B}	1.69 ± 0.16^{b}	1.86 ± 0.10^{B}	1.69 ± 0.16^{b}	2.92 ± 0.06^{A}	$3.51 \pm 0.17^{b,*}$
1 week	$1.64 \pm 0.10^{AB,*}$	0.96 ± 0.08^{a}	$1.64 \pm 0.10^{AB,*}$	0.96 ± 0.08^{a}	$3.75 \pm 0.05^{B,*}$	2.99 ± 0.11^{a}

Each value is a mean of three replicates ± standard error. The Different letters represent the statistical significance between different time periods of uninfested (A-C) and infested (a-c) plants at $p \leq 0.05$. Asterisks show significant differences between uninfested and infested plant at each time period (*p<0.05; **p<0.01; ***p<0.001)

Table 3. Plant pigments content (mg/g FW) in maize leaves as affected by *Spodoptera exigua* larvae infestation after different period of time.

Duration of infestation	Chl a		Chl b		Carotenoid	
	Uninfested	Infested	Uninfested	Infested	Uninfested	Infested
2 hours	1.85 ± 0.05^{A}	1.68 ± 0.04^{b}	2.29 ± 0.09^{A}	2.40 ± 0.13^{b}	$7.53 \pm 0.10^{A,**}$	6.12 ± 0.24^{a}
2 days	1.88 ± 0.07^{A}	$2.17 \pm 0.06^{c,*}$	2.31 ± 0.11^{A}	$2.69 \pm 0.07^{c,*}$	7.78 ± 0.05^{B}	8.58 ± 0.33^{b}
1 week	$1.99 \pm 0.10^{A,*}$	1.52 ± 0.07^{a}	$2.54 \pm 0.10^{B,**}$	2.06 ± 0.06^{a}	$8.46 \pm 0.13^{C,**}$	6.05 ± 0.30^{a}

Each value is a mean of three replicates ± standard error. The Different letters represent the statistical significance between different time periods of uninfested (A-C) and infested (a-c) plants at $p \leq 0.05$. Asterisks show significant differences between uninfested and infested plant at each time period (*p<0.05; **p<0.01; ***p<0.001)

Plant pigments concentration as affected by *S. exigua* infestation: The statistical analysis showed significant differences in Chl. a and Chl b content at particular variants of the experiment for *S. exigua* infestation in tomato (t-test: Chl a, P= 0.096, 0.017, 0.026; Chl b, P= 0.062, 0.011, 0.006, Table 2) and maize (t-test: Chl a, P=0.069, 0.165, 0.028; Chl b, P=0.026, 0.139, 0.049, Table 3) as well as infestation time in tomato (*ANOVA*: Chl a, F =83.57, P = 0.000, Chl. b, F=32.45, P=0.001, Table 2) and maize (*ANOVA*: Chl a, F =26.174, P = 0.001, Chl. b, F=44.25, P=0.000, Table 3). In infested tomato leaves, Chl. a and Chl. b contents significantly increased after 2 days of infestation by about 15.29 and 16.27 % compared to their uninfested controls respectively; while it reduced after 1 week by about 23.58 and 18.76 % less than controls (Table 2). In maize, *S. exigua* infestation after 1 week significantly reduced Chl. a and Chl. b contents by about 43.34 and 41.66% less than controls respectively (Table 3).

Spodoptera exigua infestation (t-test: tomato, P=0.005, 0.070, 0.005; maize, P=0.044, 0.023, 0.013, Tables 2, 3) and infestation time (*ANOVA*: tomato, F=71.702, P=0.000; maize, F=14.698, P=0.005, Tables 2, 3) significantly alters carotenoids content in tomato maize leaves. In tomato, carotenoids content significantly reduced by about 18.82 and 28.57% less than uninfested controls after 2 hours and 1 week (Table 2). On the other hand, *S. exigua* infestation significantly induced carotenoids content in maize leaves after 2 hours and 2 days (12.23 and 20 % higher than controls); this trend reversed after 1 week of infestation where carotenoid content significantly reduced by about 20.26 % less than unifested control (Table 3).

Phenols concentration as affected by *S. exigua* infestation: No significant differences were observed in the bound phenols level for *S. exigua* infestation (t-test: P= 0.113, 0.103, 0.429, Fig. 1I) in tomato leaves compared to uninfested plants at each observed time (Fig. 1I). By increasing infestation time, bound phenols at 1 week significantly reduced less than 2h and 2 days in infested tomato plants (*ANOVA*: F=8.367, P=0.018, Fig. 1I). In maize, *S. exigua* infestation significantly reduced bound phenols content in infested leaves compared to uninfested controls at all time periods (t-test: P= 0.004, 0.007, 0.0.047, Fig. 1II). Bound phenols content increased significantly by increasing infestation (*ANOVA*: F=15.352, P=0.004, Fig. 1II).

Free phenols content at different periods of herbivory in tomato leaves was significantly different as affected by *S. exigua* infestation (t-test: p=0.007, 0.018, 0.272, Fig. 2I) and infestation time (*ANOVA*: F=33.58, P=0.001, Fig. 2I). At 2 hours and 2 days infested tomato leaves showed huge induction of free phenols content by about 234.44 and 135.44 % respectively higher than uninfested plants, this induction eliminated after one week, where no significant difference was recorded in free phenols content between infested and uninfested tomato leaves (Fig. 2I). By increasing time period, free phenols content decreased in tomato leaves, after 1 week of infestation it was about 52% less than 2 hours and 2 days (Fig. 2I). On the other hand, free phenols content in maize leaves significantly reduced as affected by *S. exigua* infestation at all studied time periods (t-test: P=0.002, 0.049, 0.043, Fig. 2II) reached values of 32.9, 18.32 and 12.59 less than their controls at 2 hours, 2 days and 1 week correspondingly. Depending on ANOVA analysis, free phenols content increased by increasing infestation time in maize leaves reaching its maximum value at 1 week 29.32 % higher than 2 hours (*ANOVA*: F=10.719, P=0.010, Fig. 2II).

Ascorbic acid concentration as affected by *S. exigua* infestation: Ascorbic acid content in tomato shoots did not influenced by *S. exigua* infestation (t-test: P=0.069, 0.599, 0.450, Fig. 3I) compared to unifested plants under all studied time periods, or infestation period (*ANOVA*: F=6.513, P=0.059, Fig. 3I). *Spodoptera exigua* infestation increased ascorbic acid content in maize leaves at 2 hours and 2 days by about 120.75 and 67.71 % higher than uninfested plants respectively (t-tests: P=0.007, 0.012, 0.063, Fig. 3II). ANOVA analysis recorded a significant decrease in ascorbic acid content by increasing infestation time (*ANOVA*: F=122.75, P=0.000, Fig. 3II) in maize leaves.

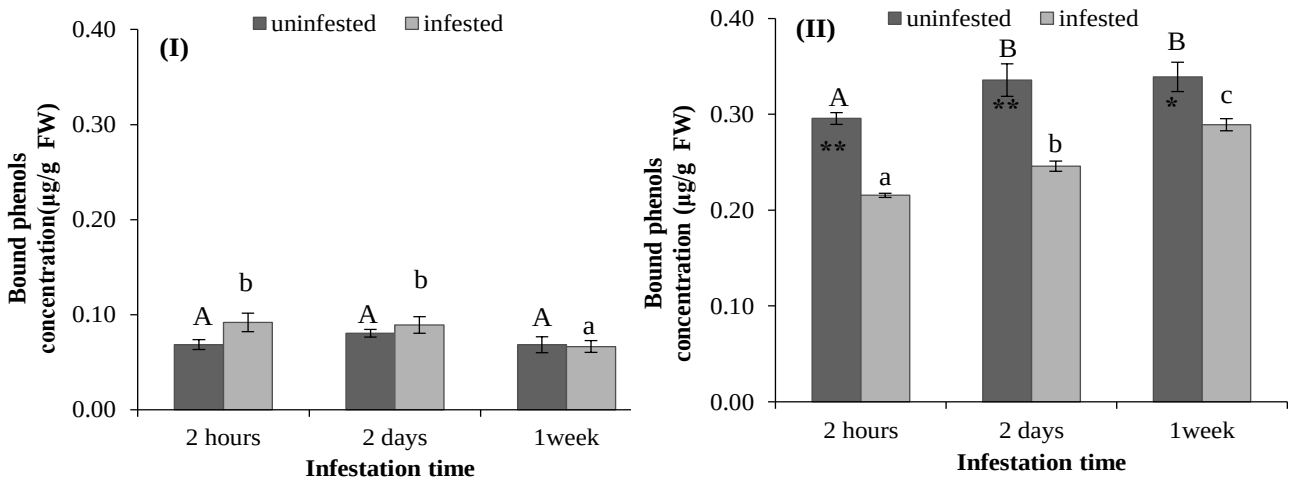

Fig. 1. Bound phenols content (µg/g FW) of (I) tomato and (II) maize as affected by *Spodoptera exigua* larvae infestation after different periods of time. Each point is a mean of three replicates ± standard error. The Different letters represent the statistical significance between different time periods of uninfested (A-C) and infested (a-c) plants at p≤0.05. Asterisks show significant differences between uninfested and infested plant at each time period (*p<0.05; **p<0.01; ***p<0.001).

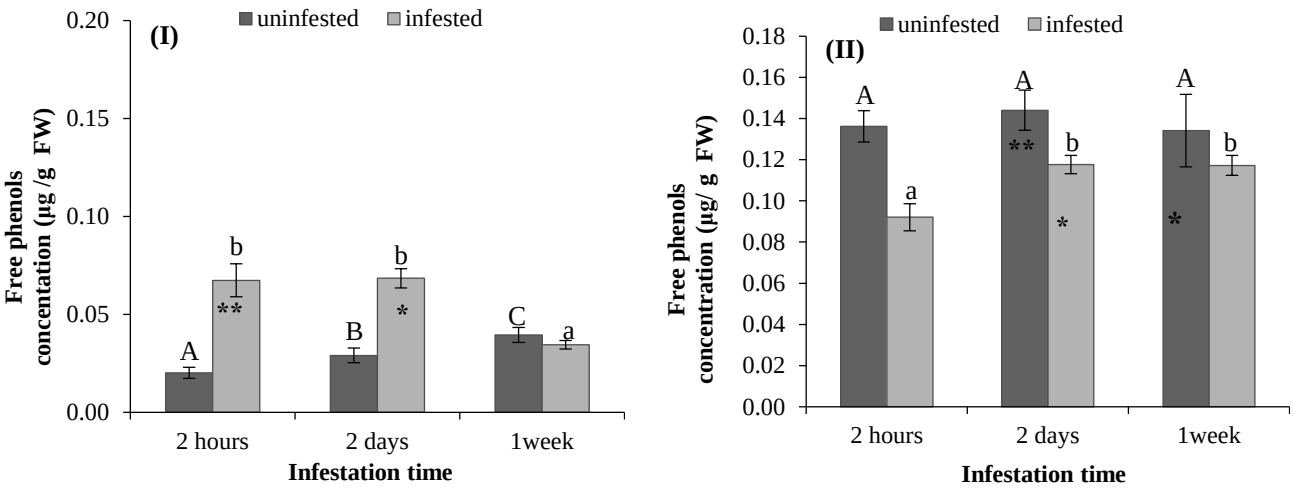

Fig. 2. Free phenols content (µg/g FW) of (I) tomato and (II) maize as affected by *Spodoptera exigua* larvae infestation after different periods of time. Each point is a mean of three replicates ± standard error. The Different letters represent the statistical significance between different time periods of uninfested (A-C) and infested (a-c) plants at $p \leq 0.05$. Asterisks show significant differences between uninfested and infested plant at each time period (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

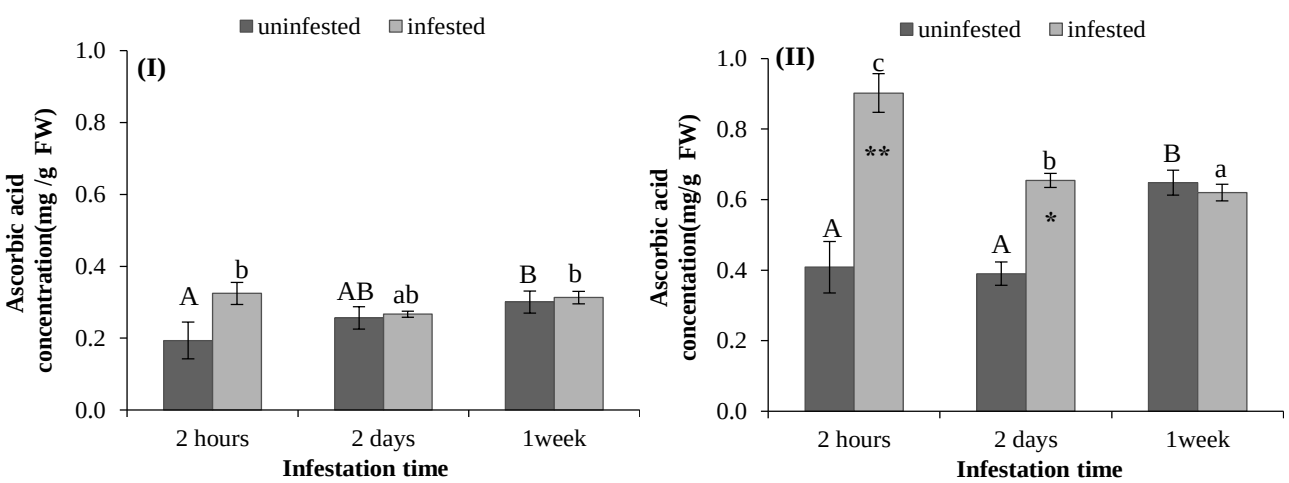

Fig. 3. Ascorbic acid content (mg/g FW) of (I) tomato and (II) maize as affected by *Spodoptera exigua* larvae infestation after different periods of time. Each point is a mean of three replicates ± standard error. The Different letters represent the statistical significance between different time periods of uninfested (A-C) and infested (a-c) plants at $p \leq 0.05$. Asterisks show significant differences between uninfested and infested plant at each time period (*$p<0.05$; **$p<0.01$; ***$p<0.001$).

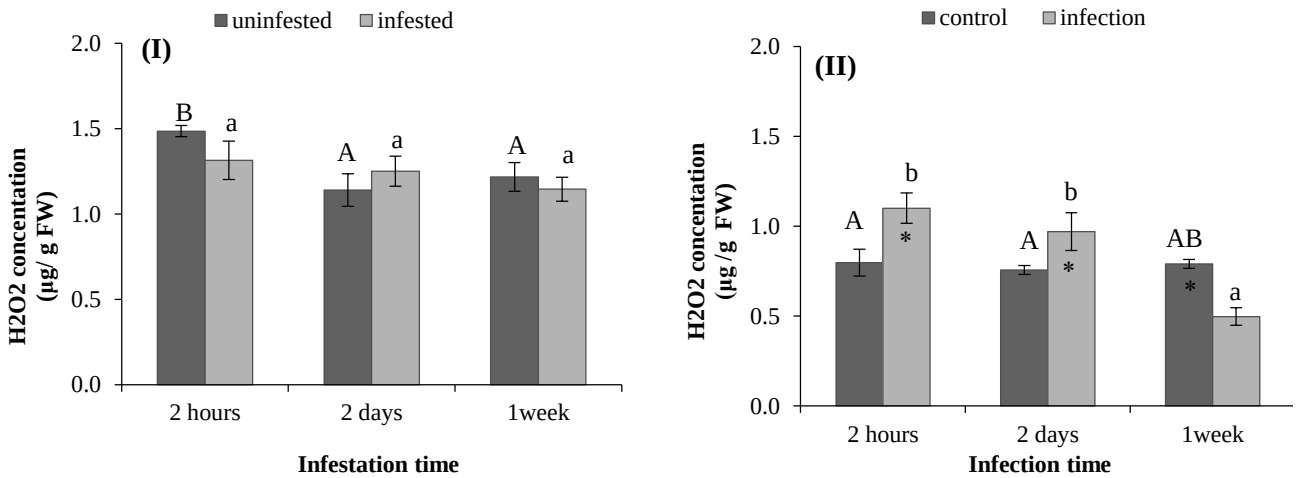

Fig. 4. H_2O_2 content (µg/g FW) of (I) tomato and (II) maize as affected by *Spodoptera exigua* larvae infestation after different periods of time. Each point is a mean of three replicates ± standard error. The Different letters represent the statistical significance between different time periods of uninfested (A-C) and infested (a-c) plants at $p \leq 0.05$. Asterisks show significant differences between uninfested and infested plant at each time period (*p<0.05; **p<0.01; ***p<0.001).

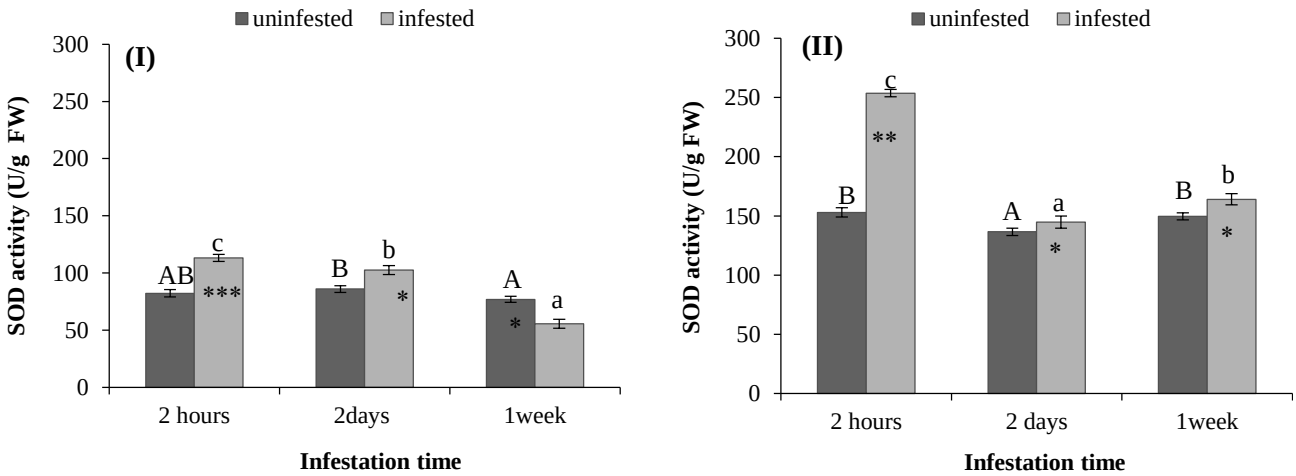

Fig. 5. SOD activity (µg/g FW) of (I) tomato and (II) maize as affected by *Spodoptera exigua* larvae infestation after different periods of time. Each point is a mean of three replicates ± standard error. The Different letters represent the statistical significance between different time periods of uninfested (A-C) and infested (a-c) plants at $p \leq 0.05$. Asterisks show significant differences between uninfested and infested plant at each time period (*p<0.05; **p<0.01; ***p<0.001).

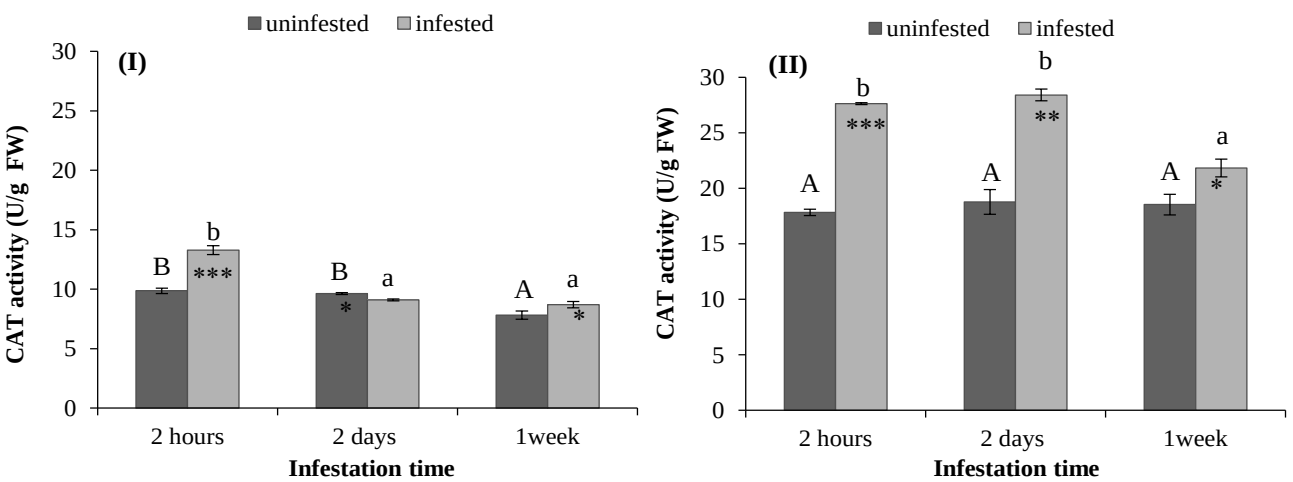

Fig. 6. CAT activity (µg/g FW) of (I) tomato and (II) maize as affected by *Spodoptera exigua* larvae infestation after different periods of time. Each point is a mean of three replicates ± standard error. Each point is a mean of three replicates ± standard error. The Different letters represent the statistical significance between different time periods of uninfested (A-C) and infested (a-c) plants at $p \leq 0.05$. Asterisks show significant differences between uninfested and infested plant at each time period (*p<0.05; **p<0.01; ***p<0.001).

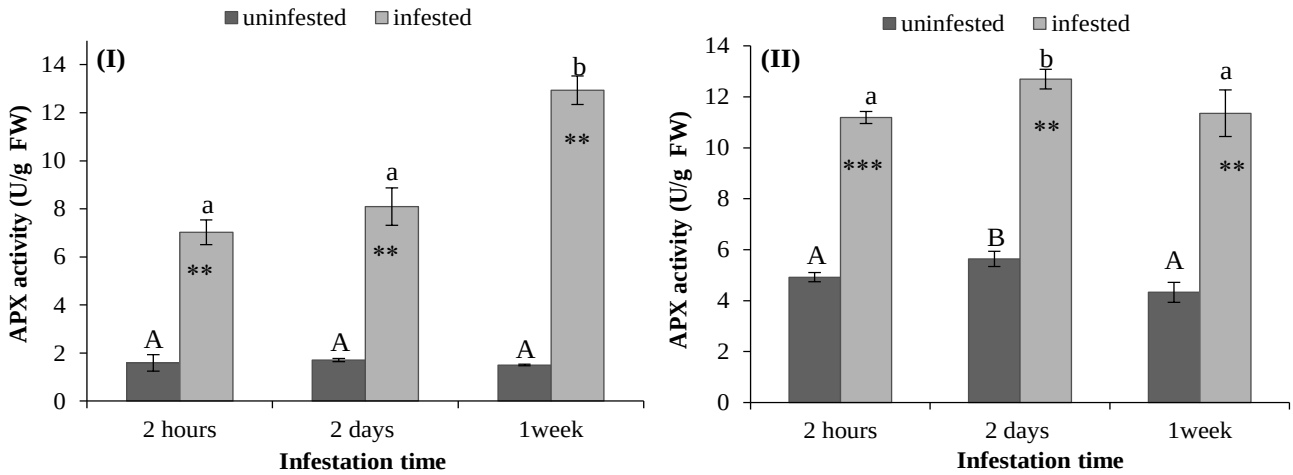

Fig. 7. APX activity (µg/g FW) of (I) tomato and (II) maize as affected by *Spodoptera exigua* larvae infestation after different periods of time. Each point is a mean of three replicates ± standard error. The Different letters represent the statistical significance between different time periods of uninfested (A-C) and infested (a-c) plants at $p \leq 0.05$. Asterisks show significant differences between uninfested and infested plant at each time period (*p<0.05; **p<0.01; ***p<0.001).

Hydrogen peroxide concentration as affected by *S. exigua* infestation: Neither *S. exigua* infestation (t-test: P=0.258, 0.075, 0.0634) nor infestation time (*ANOVA*: F=132, P=1.410) had a significant effect on H_2O_2 content in tomato leaves (Fig. 4I). While in maize leaves, *S. exigua* infestation significantly increases H_2O_2 concentration at 2 hours and 2 days after infestation by about 38.3% higher than their corresponding unifested controls respectively (t-test, P=0.002, 0.034, 0.027, Fig. 4II). ANOVA analysis showed significant reduction in H_2O_2 content in infested maize plants after 1 week of infection compared to 2 h and 2 days (*ANOVA*: F=17.584, P=0.002, Fig. 4II).

Antioxidant enzymes activity as affected by *S. exigua* infestation: Generally, *S. exigua* infestation affected the activity of the antioxidant enzymes more than the level of the antioxidant compounds (phenols and ASA). *Spodoptera exigua* infestation increased SOD activity by about 37.44 and 19.25% in infested tomato leaves at 2 hours and 2 days higher than uninfested controls; while at 1 week, SOD activity significantly inhibited compared to uninfested plants (t-test: P=0.000, 0.022, 0.028, Fig. 5I). Increasing infestation time inhibit gradually SOD activity in tomato leaves reached its lowest value after 1 week (*ANOVA*: F=211.47, P=0.000, Fig. 5I). SOD activity in maize leaves varied significantly in response to *S. exigua* infestation (t-test: P=0.002, 0.046, 0.033, Fig. 5II) and infestation period (*ANOVA*: F=529.930, P=0.000, Fig. 5II). Under all studied periods, the activity of SOD in infested maize leaves was significantly higher than that in uninfested leaves, the response was the strongest after 2 hours of infestation (65.91% higher than its corresponding control) (Fig. 5II). Higher time periods (2 days and 1 week) showed significantly lower SOD activity compared to that at 2 h in infested maize leaves (Fig. 5II).

CAT activity in tomato leaves less affected by *S. exigua* infestation compared to maize (Fig. 6). In infested tomato leaves, CAT activity significantly increased by about 34.64% higher than uninfested plants only at 2 hours of infestation, then significantly reduced at 2 days and 1 week (t-test: P=0.000, 0.011, 0.042, Fig. 6I). CAT activity reduced after 2 days and 1 week in infested tomato leaves compared to 2 hours (*ANOVA*: F=170.69, P=0.000, Fig. 6I). At all observed time periods, CAT activity enhanced in infested maize leaves higher their corresponding uninfested controls (t-test: P=0.000, 0.008, 0.048, Fig. 6II). CAT activity on infested maize leaves was about 55.04, 51.34 and 36.14% higher than their corresponding uninfested controls at 2 hours, 2 days and 1 week respectively. Increasing infestation time significantly reduced CAT activity in infested maize leaves at 1 week lower than 2 h and 2 days (*ANOVA*: F=122.36, P=0.000, Fig. 6II).

APX activity markedly increased (four and half folds) in tomato leaves infested by *S. exigua* compared to uninfested plants already after 2 hours of insect feeding (t-test: P= 0.007, 0.006, 0.001 Fig. 7I). This response persevered during the following infestation time periods and was the highest after 1 week (*ANOVA*: F=72.172, P=0.000, Fig. 7I). A comparable response was verified in maize, where APX activity in infested maize leaves was about two and half folds that in uninfested control after the 2 hours of the infestation (t-test: P= 0.000, 0.002, 0.002, Fig. 7II). As inferred from ANOVA analysis, the strongest induction in APX activity in maize leaves recorded after 2 days of *S. exigua* feeding compared to 2 hours and 1 week (*ANOVA*: F=5.943, P=0.038, Fig. 7II).

Discussion

Many studies have exposed a comprehensive plasticity of defense responses to herbivore attack (Karban & Baldwin, 2007). Stimulated plant tolerance may depend on the redox status of the host tissue that vary according to the plant species and kind of herbivory. In this study, tomato and maize fresh and dry weight results indicated relatively different tolerance degrees to *S. exigua* infestation. Maize showed higher tolerance to *S. exigua* infestation compared to tomato. Insect feeding in most cases leads to the reduction of major cell processes involved in growth and photosynthesis in order to

promote plant fitness and defensive mechanisms (Appel *et al*., 2014; Zhou *et al*., 2015). As a defense mechanism, infested plants tend to use its food resources in building up defense compounds instead of using them in growth and development (Mole, 1994). In some cases the defense compounds showed autotoxicity characters against different enemies in which increasing of defense against one insect enhances susceptibility to another (Kessler & Baldwin, 2002; Frost *et al*., 2008).

Photosynthesis rate and potential photosynthetic productivity is mainly indicated by leaf chlorophyll content (Zarco-Tejada *et al*., 2002; Mao *et al*., 2007). In the current study, *S. exigua* infestation negatively affected Chl. a, Chl. b and carotenoids content in tomato and maize only after 1 week of *S. exigua* infestation. In agreement with our results, Golan *et al*., (2014) demonstrated that *Coccus hesperidum* infestation can negatively affect the chlorophyll and carotenoid content. Similarly, *Hypericum sampsoni* leaves accumulated less chlorophyll pigment under *Thrips tabaci* damage (Dai *et al*., 2009). Other studies recorded that chlorophyll loss was correlated with feeding time and the degree of feeding damage (Goławska *et al*., 2010; Huang *et al*., 2014). The changes in pigments contents often considered as a comparatively delayed mechanism of photosynthetic adaptation (Anderson *et al*., 1995). In this study, the reduction in the chlorophyll content in response to *S. exigua* infestation was dependent on the host plant. It was observed that the reduction in Chl a, and Chl b level in tomato leaves was twice that in maize. Similarly, Heng-Moss *et al*., (2003) recorded that different wheat lines accumulated various concentrations of total chlorophyll and carotenoid as affected by aphid feeding. The degradation of plant pigments is a complex phenomenon that often accompanies insect infestation of plants and varies by plant species (Ni *et al*., 2001).

Phenolic compounds are important secondary metabolites found in plants and play a key role in plant growth. Many studies assumed that the alternations in phenolic compounds in plant tissue, in response to insect attack, is an adaptive mechanism for optimizing the capability of whole individual plants (War *et al*., 2012). The reduction of the bound and free phenols content in maize leaves at all observed time periods due to *S. exigua* infestation, that correlated with infestation tolerance, probably due to other phenolic transformation, not investigated in the current study. For instance, oxidation of phenols mediated by polyphenol oxidases (PPOs) and peroxidases (PODs) is a known mechanism of plant resistance against insects herbivory (Chrzanowski *et al*., 2003). Quinones produced from these transformation can cause direct toxicity to herbivores or at least inhibit the protein digestion in it (War *et al*., 2012). Similar results were recorded by Golan *et al*., (2017) where total phenols content decreased in orchid leaves as affected by grape mealybug. Induction of free phenols in infected tomato leaves more than control at 2 hours and 2 days after infestation is not correlated with insects tolerance and vanished after 1 weeks, which may reveal less significance of this mechanism in tomato as affected by *S. exigua* feeding. The explanation could be that, the enzymes required to activate phenols defensive properties e.g. PPOs or PODs are not activated by *S. exigua* in tomato leaves. Our results support this idea in part, where H_2O_2 content in tomato leaves did not affect by *S. exigua* infestation and PODs use H_2O_2, as a co-substrate, and O_2, to help in removing toxic ROS that inhibit tomato growth.

Ascorbic acid contributes in the regeneration of vitamin E which act as cofactor for enzymes involved in phytohormones and flavonoids biosynthesis, and therefore help in protecting cells from oxidative injuries (Goggin *et al*., 2010; Łukasik *et al*., 2012). Accordingly the plant mutants with inhibited ASA levels are hypersensitive to environmental stresses (Conklin *et al*., 1996). In a plant-herbivory interaction study (Schlaeppi *et al*., 2008) find that, *S. littoralis* larval weight was significantly enhanced by feeding on vtc1-1 (ASA deficient) mutants of *Arabidopsis* compared with larvae nurtured on the wild-type plants. In the current study, ASA content in tomato shoots did not influenced significantly by *S. exigua* infestation or infestation period. However, *S. exigua* infestation increased ASA content in maize shoot at 2 hours and 2 days' time periods. Similar results shown by (Suza *et al*., 2010) who recorded an enhancement in ASA level in *Arabidopsis* leaves, but low ASA content in tomato. These results indicate that the ASA regulation is complex mechanism and varies between different plants. Patykowski and Urbanek (2005) recorded similar levels of ASA in both tolerant and sensitive tomato cultivars infested by *Botrytis cinerea*, therefore they proposed that ASA was not pivotal for tomato tolerance.

H_2O_2 can play a dual role in plant cells. At low levels, it performs as a second messenger that could act as a signal molecule inducing of antioxidant system or other parallel interactive signaling pathways (Leoân *et al*., 2001; Kawano, 2003). At high levels it causes cell injury as a toxic free radical and may lead finally to cell death (Vandenabeele *et al*., 2003). Herbivory by chewing insects leads to an oxidative damage accompanying by H_2O_2 induction (Lamb & Dixon, 1997), giving rise to both local and systemic responses (Orozco-Cárdenas & Ryan, 1999). In this study, *S. exigua* infestation induced early production of H_2O_2 at higher concentrations in maize leaves compared to tomato. Moloi & van der Westhuizen (2006) find that RWA infestation causes early production of H_2O_2 at higher levels in tolerant than in sensitive plants. In maize leaves, the accumulation to H_2O_2 at high levels at the early stage of the *S. exigua* infestation (2 h and 2 days) could be regarded as a plant defense approach against subsequent secondary invasion by microbial pathogen as explored in other investigation by Orozco-Cárdenas *et al*., (2001). Plant can also counterattack herbivore feeding activity directly through oxidative damage to insect digestive system by the plants alimentary and antioxidant compounds (Orozco-Cárdenas & Ryan, 1999). Furthermore, transient accumulation of H_2O_2 at the early stage of herbivory attack could be used as signaling molecule to up-regulate genes encoding defense proteins used in other defensive pathway (Mur *et al*., 2005), such as glutathione peroxidase, glutathione S-transferase, and ubiquitin (Levine *et al*., 1994) as well as jasmonic acid biosynthesis (Browse & Howe, 2008; Bruinsma *et al*., 2009), volatile products (Mithofer *et al*., 2004) and phytoalexin (Devlin & Gustine, 1992; Mithofer *et al*., 2004) accumulation. The reduction in H_2O_2 content in infested maize leaves later on after 1 week could be a direct result of increasing the antioxidant capacity recorded by increasing CAT and APX activity.

Many inducible defense responses could be generated in plants due to herbivore attack (Karban & Baldwin, 2007). Plant redox status could be regarded as an indicator for its resistance, which is accomplished by the elimination of generated ROS (Peleg-Grossman *et al*., 2014). CAT, SOD, and APX are the most important enzymes in the antioxidant system where it play a significant role in the induced defense response by inhibiting efficiently ROS from damaging the living tissues (War *et al*., 2012). The results of our study strongly indicate an alter in the oxidative status of maize and tomato plant due to *S. exigua* attack. SOD catalyses the conversion of O_2^- to H_2O_2, therefore it can neutralize oxidative damage generated by O_2^- over accumulation in the plasma membrane due to herbivory attach (Río *et al*., 2002; Halliwell, 2006). The results of this study suggest correlation between SOD activity and the defense strategy of maize to the *S. exigua* infestation compared to tomato. Similarly, Ni & Quisenberry (2003) found resistant wheat (Halt) and resistant oat (Border) showed higher induction of SOD activity following RWA feeding. Plant resistance to many abiotic stresses conditions has also been associated with high SOD activity (Hernández *et al*., 2001; Schutzendubel *et al*., 2001). Generally, H_2O_2 accumulation is a consequence of SOD catalytic action, however Zhao *et al*., (2016) observed that after 1 week of *S. exigua* infestation this trend is changed where H_2O_2 levels was opposite that of SOD activity.

At all studied time periods, CAT activity in infested maize leaves was significantly increased compared to uninfested controls due to *S. exigua* infestation, while it significantly enhanced in infested tomato leaves only at 2 hours. The current data indicated that CAT could be implicated in the defense response against herbivore. Catalase is a considerable constituent of the cell protective antioxidant system (Mhamdi *et al*., 2012; Nicholls, 2012; Afiyanti & Chen, 2014), and it plays a vital role in preserving the induced defense response (Mittler, 2002). For example, resistant alfalfa plants showed higher activities of CAT than susceptible plants in response to spotted alfalfa aphid attack. *Spodoptera exigua* infestation induced APX activity in both test plants at all observed time periods. The induction in APX activity due to *S. exigua* infestation in tomato leaves is twice higher than that in maize. This stimulation in APX activity indicated an adaptive role of APX in plant resistant to high amounts of ROS generated due to insects infestation. Aphids induced a considerable enhancement in APX activity in cotton and eucalypt leaves (Gomez *et al*., 2004). APX activity induced also by *Helicoverpa zea* infestation (Bi *et al*., 1997).

Depending on the data of this study, antioxidant enzymes play a secondary role in plant defense against S. *exigua* infestation, just to regulate H_2O_2 (ROS) concentration in the host plant from increasing to toxic levels and keep acritical value that is needed for up-regulating other defense mechanism. H_2O_2 seems to play a vital role in plant defense response against *S. exigua* infestation. It may up-regulate many tolerance mechanisms (discussed earlier). It may initiate redox-sensitive defense elements in JA pathway by shifting the redox status in tissues of infested plants, and then influence plant tolerance (under investigation). Supporting to our hypothesis, the data from this study showed that the activity of antioxidant enzymes induced to comparable values in both tomato and maize, while H_2O_2 content in maize is significantly enhanced due to *S. exigua* herbivory which correlated with the tolerance level of the host plants. This tendency is constant with that of the differential plant tolerance observed in biomass data, i.e., that *S. exigua* infestation reduced tomato biomass to higher levels compared to maize. In tomato, *S. exigua* infestation enhance SOD activity but this is not correlated with increased H_2O_2 due to the induction of APX activity which consumed H_2O_2 and did not stimulate the efficient tolerance mechanism which make tomato more susceptible to *S. exigua* infestation compared to maize. The huge induction in APX activity in tomato (450% of corresponding control) compensates the absence of CAT activity in scavenging H_2O_2.

Conclusion

In conclusion, these results clearly demonstrate that, maize plant showed relatively higher tolerance to *S. exigua* infestation more than tomato plants. These differential tolerance responses correlated with H_2O_2 level in test plants. The accumulation of H_2O_2 resulted from the catalytic action of SOD. H_2O_2 may regarded as a diffusible signal in maize leaves, causes the activation of defense genes as observed by increasing CAT and APX activities. Furthermore, accumulated H_2O_2 could play a curial role as a signaling molecule to activate other resistance pathways to insect infection. Taken together we could conclude that oxidative system in maize induced and enhance plant defense against *S. exigua* infestation more than tomato.

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