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Oxidative stress caused by Basagran® herbicide is altered by salicylic acid treatments in peanut plants

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ABSTRACT

The present work was to study a protective role of salicylic acid (SA) on oxidative stress caused by Basagran® herbicide application. Two peanut cultivars (*Arachis hypogaea* cv. Giza 5 and Giza 6) with different sensitivities to the herbicide were monitored for their antioxidant responses to Basagran® and/or SA treatments. Two weeks after treatment, Basagran® lowered leaf pigments (Chlorophyll a, Chlorophyll b and total Carotenoids) but increased hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) contents indicating occurrence of lipid peroxidation and oxidative stress. Salicylic acid applied prior to low dose Basagran® lowered H_2O_2 and MDA contents in both G5 and G6. Except for SOD which is highly stimulated, POD, CAT and APX activities showed slight changes compared to control in leaves treated with Basagran® ± SA. The extracts tested by DPPH showed increase in total antioxidant activity by 4%–7% in SA + Basagran® treated leaves compared to control. The increased total antioxidant activity was related to the accumulation of amounts of phenolics as a protective action stimulated by SA. Alterations of antioxidant enzymatic system, accumulation of phenolics, increasing the total antioxidant activity by SA provide an evidence of protective action of SA in Basagran® detoxification.

1. Introduction

Under unfavorable conditions, plants suffer from oxidative stress that severely affects plant growth. Many of herbicides which affect photosynthesis cause oxidative stress in plants. Basagran® is one of the post-emergence herbicides used widely as weed killer that controls broadleaf weeds in many perennial grass crops (Dotray and Keeling, 1997; Gnanamurthy and Balasubramaniyan, 1998; Oliveira et al., 2017; Song et al., 2015; Wilcut et al., 1994). Basagran® herbicide [containing 480 g/L of bentazon sodium salt as active ingredient (*a.i.*)] is one of the commercial herbicides available worldwide to control broadleaf weeds in several crops, particularly in the most economically relevant ones such as rice and maize (Oliveira et al., 2017). Basagran® competes with plastoquinone at its binding site on the D1 protein which blocking electron transport from photosystem II (PSII), resulting in inhibition of photosynthesis and oxidative stress followed by cell damage. Dat et al. (1998) reported that excessive production of reactive oxygen species (ROS) causes many injuries in cell organelles and alteration in physiological activities such as photosynthesis.

Salicylic acid is known as a phytohormone functions in abiotic and/or biotic stress regulation in plants. It is involved in the antioxidant responses, to manage the oxidative stress and to avoid oxidative damage in plants. SA, is associated with an enhanced antioxidant system both enzymatic and non-enzymatic (Dat et al., 1998). Moreover, the role of SA in signaling process by altering the enzymatic antioxidant system by stimulating peroxidase (Rao et al., 1997) and inhibiting catalase activities (Larkindale and Huang, 2004). In this respect, the changes in activity of antioxidant enzymes as catalase; in response to herbicides such as paraquat is a way for protection against negative effects in plants (Ananieva et al., 2002). SA plays a certain role in the enzymatic defense mechanisms by changing the antioxidant enzyme activities (Nimchuk et al., 2003).

Polyunsaturated fatty acids are major components of biomembranes which easily peroxidized in response to oxidative stress (Esterbauer et al., 1991). As an indicator for oxidative stress and membrane damage, malondialdehyde (MDA) content is used to analyze the extent of lipid peroxidation (Ohkawa et al., 1979; Yamauchi et al., 2008). Phenolic compounds are a large diverse group of secondary plant metabolites, which considered free radical scavenging molecules (Huang et al., 2006). The levels of their production in the plant are dependent upon environmental factors such as stresses, nutrients, weather, and other growing conditions. A major role of phenolic compounds is to protect

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organisms against oxidative stress induced by free radical species (Cho et al., 2004). Recently, SA was believed to play a role in plant defense mechanism against several abiotic stress conditions (Horváth et al., 2007).

The aim the present investigation was to prove the protective action of SA against Basagran®-induced oxidative stress through alteration of the activities of antioxidant enzymes and the amounts of antioxidant metabolites in peanut cultivars.

2. Materials and methods

2.1. Experiment preparation and treatments

Peanut, *fabaceae* (*Arachis hypogaea*, cv. Giza 5 and Giza 6), seeds were provided by Agriculture Research Center, Ministry of Agriculture, Shandawil, Sohag, Egypt. The two cultivars used in this experiment; G5 and G6, are different in their sensitivity towards Basagran® herbicide. The herbicide was available commercially and used for weed control in the peanut fields. From preliminary experiments, G5 is more sensitive than G6 showing more negative responses towards Basagran® application. The used herbicide was Basagran® (*a.i.* bentazon 48%) [3-(1-Methylethyl)-(1*H*)-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide] which belongs to the benzothiadiazinone group of herbicides. Choosing the doses applied in this experiment was based on the recommended field dose which is (3L ha^{-1}). Five Seeds of each cultivar of peanut were sown in a mixture of sand and clay soil (1:2) in clean plastic 2L-pots; these pots were irrigated by tap water. Four weeks later, plants which have the same growth were chosen, divided into groups (3 pots per group each containing 5 plants) and the experimental plant groups were treated as the following:

Group 1: (C) healthy control plants sprayed with distilled water.
Group 2: (C SA) sprayed by salicylic acid (50 μM).
Group 3: (L BS) sprayed with low dose 35 ppm bentazon; *a.i.* of Basagran® herbicide.
Group 4: (H BS) sprayed with high dose 70 ppm bentazon; *a.i.* of Basagran® herbicide.
Group 5: (C SA + L BS) sprayed by 50 μM SA one day before treatment with 35 ppm of bentazon herbicide.

Hints:

- Basagran® contains 48% (w/w) bentazon sodium salt as active ingredient.
- The herbicide was applied by spraying to leaves until run off and SA was sprayed to leaves one day prior herbicide treatments.
- All analyses were done two weeks after treatment.

2.2. Photosynthetic pigments content

Fresh leaf samples (0.1 g) were grinded in 80% acetone at 4 °C and dark conditions. The extract was filtered through double layer nylon and centrifuged at 5000 rpm for 5 min. The supernatant was collected and read using *UV-VIS* spectrophotometer (*T80*, *PG Instruments*, United Kingdom) at 663 and 647nm for Chl a and Chl b, respectively, and at 470 nm for total Carotenoid content. The concentrations for Chl a, Chl b, and the sum of leaf Carotenoids (xanthophylls and carotenes) were given in mg ml^{-1} extract solution according to the following equations (Lichtenthaler and Buschmann, 2001):

$$\text{Chlorophyll a} = 12.25A_{663} - 2.79\ A_{647}$$

$$\text{Chlorophyll b} = 21.50A_{647} - 5.10\ A_{663}$$

$$\text{Carotenoid} = (1000\ A_{470} - 1.82\text{Chl a} - 95.15\text{lChl b})/225$$

2.3. Antioxidant enzymes

2.3.1. Peroxidase (POD) activity

POD (EC 1.11.1.7) activity was analyzed by grinding 1 g of the fresh leaves at 4 °C in 5 mL of phosphate buffer (pH 7.0). The homogenate was centrifuged for 15 min at 15000xg at 10 °C. Supernatants were collected for measuring POD activity. The extracted samples were then mixed with assay mixture and the appearance of the brown color was read using *UV-VIS* spectrophotometer (*T80*, *PG Instruments*, United Kingdom) at 470 nm. The assay mixture for POD activity consists of 40 mM potassium phosphate (pH 7.2), 5 mM guaiacol, 0.1 mM EDTA and 0.3 mM hydrogen peroxide. Then, increase in the absorbance due to oxidation of guiacol (Extinction factor = 26.2 mM cm^{-1}) was analyzed. The enzyme activity was calculated in terms of μmol of guiacol oxidized min^{-1} g^{-1} FW at 25 ± 2 °C (MacAdam et al., 1992; Zhang, 1992).

2.3.2. Catalase (CAT) activity

The activity of CAT (EC 1.11.1.6) was measured by detecting the H_2O_2 disappearance from the assay mixture according to the method of Chandlee and Scandalios (1984). Extraction was done in 50mM phosphate buffer (pH = 7.0). The disappearance of H_2O_2 was measured by using *UV-VIS* spectrophotometer (*T80*, *PG Instruments*, United Kingdom). The decrease in absorbance at 240 nm (Extinction factor = 0.036 mM^{-1} cm^{-1}) of a reaction mixture consisting of 25mM potassium phosphate buffer (pH 7.0), 10mM H_2O_2 and enzyme extract. One CAT unit is defined as the amount of enzyme necessary to decompose 1 μmol min^{-1} H_2O_2 under the above-mentioned assay conditions.

2.3.3. Superoxide dismutase (SOD) activity

SOD (EC 1.15.1.1) enzyme activity was analyzed by modified method of Beauchamp and Fridovich (1971). Samples (0.5 g) were homogenized in the extraction buffer containing 50 mM phosphate, pH 7.8, (w/v), 0.05% b-mercaptoethanol and 0.1% (w/v) ascorbate. The assay mixture in 3mL contained 50mM phosphate buffer (pH 7.8), 9.9 mM L-methionine, 0.025% (w/v) nitroblue tetrazolium (NBT), and 0.0044% (w/v) riboflavin. The photo-reduction of NBT (formation of purple formazan) was read using *UV-VIS* spectrophotometer (*T80*, *PG Instruments*, United Kingdom) at 560nm. When the extract caused 50% inhibition of the photo-reduction of NBT, It is considered one SOD activity unit.

2.3.4. Ascorbate peroxidase (APX) activity

APX (EC 1.11.1.11) activity was determined by the method of Nakano and Asada (1981). One gram of fresh leaves was grinded in 10 ml phosphate buffer (pH 7.0). The homogenate was centrifuged at 4000g for 10 min at 4 °C. The assay medium consists of 3 mL containing 50 mM phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.3 mM ascorbate, 0.06 mM H_2O_2 and 0.1 mL enzyme extract. The decrease in ascorbate concentration was followed by decline in absorbance was measured using *UV-VIS* spectrophotometer (*T80*, *PG Instruments*, United Kingdom) at 290 nm and activity was calculated using the extinction coefficient (Extinction factor = 2.8 mM^{-1} cm^{-1}) for ascorbate.

2.4. H_2O_2 content

Hydrogem peroxide (H_2O_2) content was determined colorimetrically as described by Jana and Choudhuri (1982). H_2O_2 was extracted by homogenizing 0.5 g leaf tissue with 3 mL phosphate buffer (50 mM pH 6.5). The homogenate was centrifuged at 6000g for 25 min. To determine H_2O_2 level, 3mL of extracted solution was mixed with 1 mL of 0.1% titanium sulfate in 20 % H_2SO_4. The mixture was centrifuged for 15 min at 6000 g. The pellet was then dissolved in 5mL H_2SO_4 (2M) and the intensity of the yellow color of the supernatant at 410 nm was measured using *UV-VIS* spectrophotometer (*T80*, *PG Instruments*, United Kingdom). H_2O_2 level was calculated using the extinction coefficient (E = 0.28 μmol^{-1} cm^{-1}).

2.5. MDA content

Lipid peroxidation was expressed as malondialdehyde (MDA) content and was determined as 2-thiobarbituric acid (TBA) reactive metabolites according to Zhang (1992). One gram of fresh leaves with removed veins was grinded in 5% trichloroacetic acid (TCA) and then centrifuged at 3000g for 10 min. Two mL of the supernatant was mixed with 2 mL of 0.03 thiobarbituric acid (TBA) and incubated for 15 min at 94 °C to develop the $(TBA)_2$-MDA adduct. The mixture was cooled for 5 min using tap water and the absorbance was measured using *UV-VIS* spectrophotometer (*T80*, *PG Instruments*, United Kingdom) at 532 nm. Lipid peroxidation was expressed as nmol $(g\ FW)^{-1}$ by using an extinction coefficient ($E = 155\ mM\ cm^{-1}$).

2.6. Total phenolics content

Total phenolic compounds content of leaves were determined using Folin-Ciocalteau reagents (Singleton and Rossi, 1965). Gallic acid standard solution (2.0 mg/mL) was prepared by dissolving 0.01 g and in 50 mL of distilled water. The solution was diluted to give concentrations of standard solutions of 0.1, 0.2, 0.5, 1.0, 1.5 mg/mL. Leaf samples (0.5 g) were extracted in of 10 ml of methanol. Forty microlitres of extract (in 80% methanol) or gallic acid standard was mixed with 1.8 mL of Folin–Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand at room temperature for 5 min, and then 1.2 mL of sodium bicarbonate (7.5% w/v) was added to the mixture. After standing 60 min at room temperature, absorbance was measured using *UV-VIS* spectrophotometer (*T80*, *PG Instruments*, United Kingdom) at 765 nm. Results are expressed as mg/g gallic acid equivalents.

2.7. DPPH free radical scavenging assay

Leaf samples (0.5 g) were extracted in of methanol (10 ml) and subjected to the DPPH free radical-scavenging activity assay according to Shimada et al. (1992). Each extract (0.2–10 mg/mL) in methanol (2 mL) was mixed with 2 mL of freshly prepared methanolic solution containing 80 ppm of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. The mixture was shaken vigorously and left to stand for 30 min in the dark. The absorbance was then read using *UV-VIS* spectrophotometer (*T80*, *PG Instruments*, United Kingdom) at 517 nm. The percentage of DPPH scavenging activity was calculated as follows:

$$\text{DPPH scavenging ability} = [1- (A_i\text{-}A_j)/A_c]^* 100$$

A_i is absorbance of extract + DPPH, A_j is absorbance of extract + methanol, and A_c is absorbance of DPPH + methanol. A lower absorbance indicates a higher scavenging effect.

2.8. Statistical analysis

The results are reported as mean ± SD of three independent replicates. Statistical analyses of data were carried out by computer using SPSS ver. 22.0 software. One-way ANOVA and least significant differences test (LSD) were used to evaluate the differences among the means. *P*-values less than 0.05 were considered significant.

3. Results

3.1. Photosynthetic pigment contents

Photosynthetic pigment contents (*chl a, chl b* and carotenoids) were analyzed and shown in Table 1. SA spraying to untreated leaves increased amounts of photosynthetic pigments. For example, the *chl a* contents were increased in G5 and G6 leaves by 18% and 15% more than those of the corresponding controls. Moreover, in response to SA treatment, the behavior of increase was also observed in *chl b* and carotenoid contents of G6 leaves, while in G5 leaves, the *chl b* and carotenoid contents were decreased by 27 % and 17% to that of the control. Sum of pigment fractions of SA-treated G5 leaves were almost similar to that of the control while the G6 leaves recorded accumulation in total pigment contents compared to the control. Noticeably, Basagran® herbicide greatly affected the total pigment content and *chl a/b* ratios of Basagran® treated leaves.

High dose of Basagran®, which locally affects leaves causing the occurrence of yellow patches (Fig. 1), caused decrease in G5 *chl a* content by 29% and highly significant decrease in *chl b* and carotenoid content by 47% and 42% to those of the control, respectively. On the other side, G6 leaves responded to Basagran® by reducing the pigment contents depending on its applied dose. Thus, high Basagran® dose caused 23%, 18% and 14% reduction in *chl a*, *chl b* and carotenoid, respectively, compared to those of the control. For *chl b* and carotenoid contents, leaves had amounts almost similar to the corresponding controls as a response to SA + Basagran® spraying. It seemed that mixing SA with Basagran® was powerful and more effective to avoid the harmful effects of Basagran® (Table 1).

3.2. Antioxidant enzymes

3.2.1. POD and CAT activities

The results of POD and CAT activities of are presented in Fig. 2 a&b. POD and CAT activities varied greatly among treatments and cultivars. For example, application of high dose of Basagran® caused reduction in POD activity by 12% and 58% in G5 and G6 cultivars respectively. Importantly, it was noticed that more inhibition in CAT activity with the lowest dose of Basagran®. Moreover, a gradual increase in CAT activity was noticed with the increase of Basagran® sprayed dose in G6 cultivar. Application of SA with Basagran® herbicide was able to influence the activities of both enzymes. SA alone was able to induce both enzymes except for CAT in G5 which is significantly inhibited. Application of SA + Basagran® was able to lower the POD but slightly affect CAT activities in both cultivars (Fig 2 a&b).

Table 1
Effect of Basagran® (BS) and SA treatments on pigments contents (mg g^{-1} FW) of *Arachis hypogaea* (cv Giza5 and Giza 6) leaves.

Cultivars	Treatments	Chl *a* [mg g^{-1}(FW)]				Chl *b* [mg g^{-1}(FW)]				Cars [mg g^{-1}(FW)]				*Chl a/b ratio*	*Total* [mg g^{-1}(FW)]
		M	±	SD	%	M	±	SD	%	M	±	SD	%		
Giza 5	Control	1.35	±	0.02	100	0.92	±	0.08	100	0.99	±	0.06	100	1.46	3.27
	Control SA	1.59	±	0.26	118.66	0.67	±	0.13	73.02	0.83	±	0.13	83.75	2.37	3.11
	Low BS	1.28*	±	0.01	95.10	0.58*	±	0.03	62.76	0.71*	±	0.02	70.77	2.21	2.56
	High BS	0.96**	±	0.07	71.69	0.49**	±	0.03	53.05	0.58**	±	0.04	58.04	1.97	2.03
	Mix (SA + Low BS)	1.43	±	0.24	106.51	0.65*	±	0.02	69.94	0.77*	±	0.05	77.34	2.22	2.85
Giza 6	Control	1.71	±	0.05	100	0.76	±	0.03	100	0.90	±	0.03	100	2.27	3.37
	Control SA	1.82	±	0.13	106.42	0.84	±	0.07	111.58	0.99	±	0.09	109.33	2.16	3.65
	Low BS	1.82	±	0.14	106.21	0.82	±	0.07	108.71	1.01	±	0.08	111.93	2.21	3.65
	High BS	1.33**	±	0.22	77.71	0.63*	±	0.10	82.70	0.78*	±	0.13	86.47	2.13	2.73
	Mix (SA + Low BS)	1.84	±	0.24	107.77	0.84	±	0.06	111.32	1.06	±	0.08	117.11	2.19	3.74

Values are means (M) of four replicates ±standard deviation (SD). Statistical significance of differences compared to control: * – significant at $P < 0.05$; ** – significant at $P < 0.01$. Chl – chlorophyll; Cars – carotenoids.

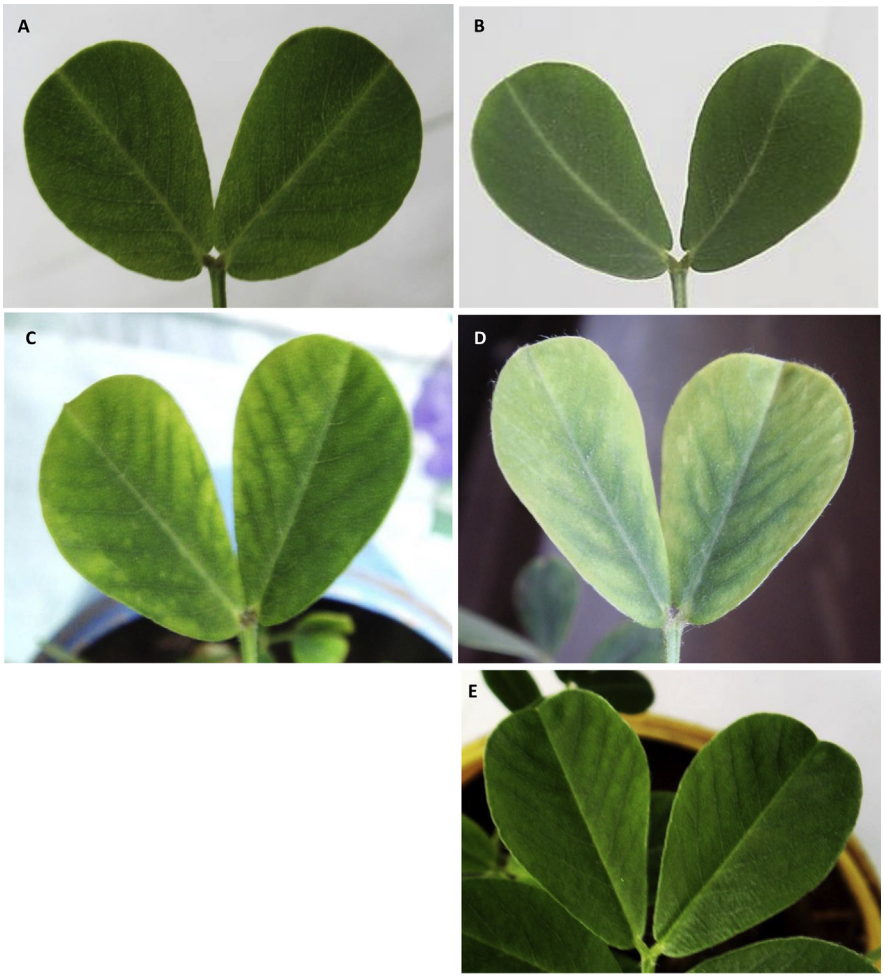

Fig. 1. Effect of Basagran® and SA treatments on leaf morphology of peanut (*Arachis hypogaea,* Giza 6 cultivar). Control (*A*); Control SA (*B*); Low Basagran® (*C*); High Basagran® (*D*); Mix (*E*).

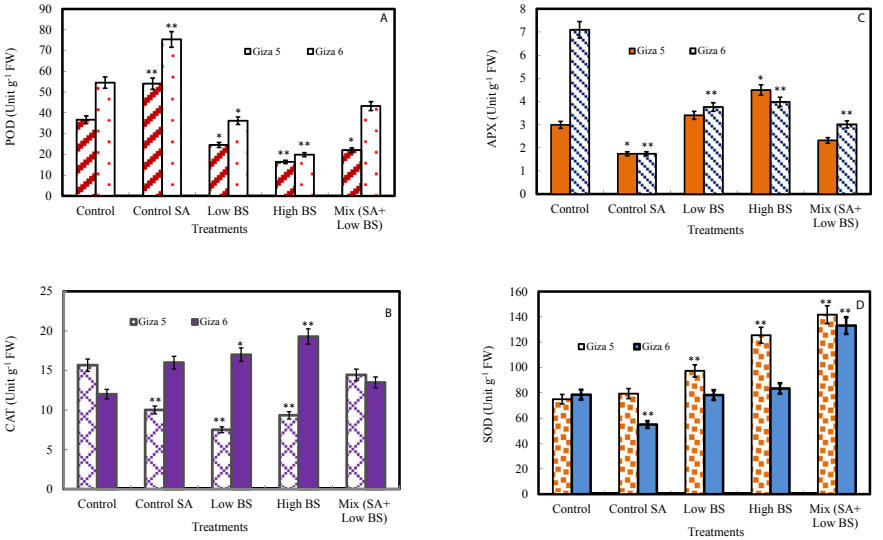

Fig. 2. Effect of Basagran® and SA treatments on antioxidant enzyme activities of peanut (*Arachis hypogaea* L. *cv.* G5 and G5) leaves. Peroxidase (*A*); Catalase (*B*); Ascorbate peroxidase (*C*); Superoxide dismutase (*D*).

3.2.2. APX and SOD activities

Exogenous SA application on leaves caused highly reduction in APX activity of G5 and G6 (42% and 75%) lower compared to their controls, respectively. With Basagran® treatments, APX is up regulated in G5 (Fig. 2c), while is down regulated in G6 compared to the control. On the other hand, with SA application, the changes in SOD activities had no significant values in leaves of G5 but significantly lowered SOD activity in G6, respectively (Fig. 2d). Both Basagran® doses stimulated SOD

activity in G6 cultivar. Basagran® application to G5 leaves resulted in APX and SOD activities stimulation. Importantly, in G6 cultivar, low and high Basagran® doses reduced the activities of APX by 47% and 39%, respectively. APX activity reduced by 50% of the control in leaves sprayed with SA + Basagran® application in G6 cultivar. On the contrary, SOD activity increased by 42% and 37% as a response to SA + Basagran® application in G5 and G6 leaves, respectively (Fig 2 c&d).

3.3. Hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) contents

Application of SA to G5 and G6 leaves showed lower amounts of H_2O_2. Generally, concentration of H_2O_2 in G5 and G6 were increased significantly as a response to Basagran® treatments. The contents were dose dependent (Tables 2& 3). In details, G6 leaves recorded the highest increase in H2O2 (48% higher). Significantly, application of SA prior Basagran® spraying lowered the amount of H_2O_2 in G5 and G6 to be 89% and 89% compared to the control (Tables 2 & 3).

Higher amounts of MDA were produced in response to Basagran® application. High dose of Basagran® caused increase of 177% in G5 leaves and 36% in G6 leaves. Importantly, leaves responded to SA + Basagran® by reducing the amounts of MDA than in both G6 while in case of G5 there is slight increase by 8% but not significant (Tables 2 & 3).

3.4. Phenolic compounds content

Application of SA without herbicide increased the phenolics contents of leaves by 31% and 16% higher compared to the control of G5 and G6 cultivars, respectively. Low and high doses of Basagran® showed induced phenolic contents by 36% and 66 % higher G5 leaves, respectively. On the other side, G6 leaves followed the same behavior of accumulating phenolics content when treated with Basagran® regardless its dose. Obviously, a pronounced increase in phenolic content was observed in both G5 and G6 leaves treated with mixed SA + Basagran® (Table 4).

3.5. Total antioxidant activity by DPPH scavenging assay

Basagran® and SA treated as well as control leaf extracts of G5 and G6 were examined for their total antioxidant activities (TAA) by the DPPH free radicals scavenging assay (Tables 5). Applying SA without Basagran® additions significantly increased the G5 leaf in TAA content but not in G6 leaves. Relatively, the trend of results of Basagran® treatments indicated a reduction of TAA in leaves treated with high dose. For example, high dose of Basagran® reduced the TAA by 11 and 9% in G5 and G6 leaves, respectively. It seems that, in most cases, the application of SA to herbicides had adjusted the TAA values to be in the range of the control value or more (Table 5).

4. Discussion

Photosynthesis is the main cause of oxidative stress under unfavorable conditions in plants (Arora et al., 2002; Murata et al., 2007; Rutherford and Krieger-Liszkay, 2001). Herbicides called photosynthesis inhibitors (such as Basagran®) are known to cause oxidative stress and enhance the intracellular formation of reactive oxygen species (ROS) that can lead to damage of macromolecules and a decrease of defense levels in plants (Arora et al., 2002; Galhano et al., 2010). After Basagran® treatments, leaves were mostly patched and sometimes bleached as it is shown in figure (1). Changes in leaf color or bleaching due to stress is mostly related to alterations in photosynthetic pigments contents. Previous reports demonstrate that photosynthetic herbicides caused bleaching due to severe photo-oxidative damage to leaf chloroplasts (Dalla Vecchia et al., 2001), stimulated leaf senescence (Mioduszewska et al., 1998) or oxidative stress (Galhano et al., 2010).

The reduction of chlorophyll content might be a good indicator for monitoring damage to the plant growth and development (Yin et al., 2008). In present study, Basagran® reducing the pigment contents depending on its applied dose in both cultivars. In the presence of herbicides, pigment content was reduced either by pigment degradation or by inhibition or biosynthesis of either chlorophylls or carotenoids (Sandmann et al., 1984). Previously reported, the decreases in chlorophyll content under stress was related to degradation of chlorophyll molecules (Štroch et al., 2008).The main site of action of some herbicides is the inhibition of photosystem II lead to photobleaching of chlorophylls and carotenoids (Fayez, 2000). Therefore, herbicides which block photosynthetic electron transport chain, e.g. Basagran®, led to oxidative process (Galhano et al., 2009). Basagran® acts as an inhibitor of photosynthesis by blocking the electron transfer flow in photosystem II (PS II) (Bagchi et al., 2003). Basagran® competes with quinone B (Q_B) for its binding site in PS II reaction center, thus decreasing the electron flow rate, from H_2O to $NADP^+$ through PS II electron carriers. Blockage of PS II by bentazon induces the production of singlet and triplet chlorophyll energized states, as well as of reactive oxygen species (ROS) like singlet oxygen ($^1O_2$) (Macedo et al., 2008; Pospíšil, 2009; Rutherford and Krieger-Liszkay, 2001), superoxide anion (O_2), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$), have been implicated in a number of physiological disorders in plants (Blokhina et al., 2003; Malenčić et al., 2008; Scandalios, 1993) and promote oxidative damage in proteins and membranes of photosynthetic cells (Pospíšil, 2009).

Recent studies have shown that SA can regulate plant adaptive responses to many biotic and abiotic stresses (Cui et al., 2010; Ding et al., 2002; Zhou et al., 2009). As mentioned in results, SA application to untreated leaves was able to induce formation of photosynthetic pigments in both cultivars due to less degradation because of less oxidative stress. Spraying of SA + Basagran® reduced the injury symptoms in most treatments. Sum of pigment fractions of SA-treated G5 leaves were almost similar to that of the control or higher while the G6 leaves recorded accumulated amounts of total pigments compared to the control. The obtained results are supported by other previous reports. For example, foliar spray of SA to maize leaves increased pigment (*chl a, b* and carotenoids) contents, as well as the rate of photosynthesis (Khodary, 2004). Moreover, exogenous SA application was proved to be equally useful in increasing the pigment contents of wheat seedlings (Hayat et al., 2005). Furthermore, it was suggested that salicylic acid activated the synthesis of carotenoids and xanthophylls (Moharekar et al., 2003).

Content of MDA is considered an indicator of lipid peroxidation and membranes damage (Ohkawa et al., 1979). MDA has been determined as

Table 2
Effect of Basagran® (BS) and SA treatments on hydrogen peroxide (H_2O_2) (nmol g^{-1} FW) and malondialdehyde (MDA) contents (μmol g^{-1} FW) of *Arachis hypogaea* cv. Giza 5 leaves.

G5 Treatments	H_2O_2 [nmol g^{-1} (FW)]				MDA [μmol g^{-1} (FW)]			
	M	±	SD	%	M	±	SD	%
Control	38.46	±	2.56	100	21.25	±	4.14	100
Control SA	35.77*	±	3.89	93.01	20.34	±	3.54	95.72
Low BS	53.65**	±	3.41	140.1	48.13**	±	4.16	226.49
High BS	56.64**	±	3.34	147.27	58.95**	±	9.67	277.36
Mix (SA + Low BS)	34.23*	±	4.17	89.00	23.15	±	4.33	108.94

Values are means (M) of four replicates ±standard deviation (SD). Statistical significance of differences compared to control: *– significant *at P 0.05*; **– significant at *P 0.01*.

Table 3
Effect of Basagran® (BS) and SA treatments on hydrogen peroxide (H_2O_2) (nmol g^{-1} FW) and Malondialdehyde (MDA) contents (μmol g^{-1} FW) of *Arachis hypogaea* cv. Giza 6 leaves.

G6 Treatments	H_2O_2 [nmol g^{-1} (FW)]				MDA [μmol g^{-1} (FW)]			
	M	±	SD	%	M	±	SD	%
Control	29.33	±	3.49	100	39.22	±	3.58	100
Control SA	25.83*	±	3.66	88.07	33.14**	±	2.46	84.50
Low BS	35.86*	±	3.75	122.26	43.47*	±	5.43	110.84
High BS	43.47**	±	5.64	148.21	53.46**	±	2.66	136.31
Mix (SA + Low BS)	26.18*	±	3.82	89.26	35.48*	±	3.65	90.46

Values are means (M) of four replicates ±standard deviation (SD). Statistical significance of differences compared to control: *– significant *at P 0.05*; **– significant at *P 0.01*.

Table 4
Effect of Basagran® (BS) and SA treatments on phenolic compounds contents (μg g^{-1}FW) of *Arachis hypogaea* cv. Giza 5 and Giza 6 leaves.

Treatments	Phenolics [μg g^{-1}(FW)]							
	Giza 5				Giza 6			
	M	±	SD	%	M	±	SD	%
Control	25.94	±	2.23	100	45.13	±	5.23	100
Control SA	29.36**	±	0.91	131.18	52.47**	±	5.43	116.26
Low BS	35.16**	±	4.23	135.54	50.93**	±	0.82	112.85
High BS	43.12**	±	1.12	166.23	57.46**	±	4.79	127.32
Mix (SA + Low BS)	49.28**	±	4.23	189.98	53.85**	±	7.42	11932

Values are means (M) of four replicates ±standard deviation (SD). Statistical significance of differences compared to control: *–significant *at P 0.05*; **– significant at *P 0.01*.

Table 5
Effect of Basagran® (BS) and SA treatments on DPPH scavenging assay of leaf extracts (Relative %) of *Arachis hypogaea* cv. Giza 5 and Giza 6 leaves.

Treatments	DPPH assay (%)					
	Giza 5			Giza 6		
	M	±	SD	M	±	SD
Control	76.05	±	0.39	83.6	±	4.17
Control SA	83.65**	±	1.17	83.6	±	3.00
Low BS	74.13	±	0.83	79.25	±	2.82
High BS	65.35**	±	0.01	74.22*	±	0.00
Mix (SA + Low BS)	80.35**	±	0.30	91.88*	±	4.90

Values are means (M) of four replicates ±standard deviation (SD). Statistical significance of differences compared to control: *– significant *at P 0.05*; **– significant at *P 0.01*.

an oxidative stress marker (Yamauchi et al., 2008). In this work, accumulation of MDA was observed by Basagran® application in peanut cultivars. The obtained values of MDA of G5 leaves treated with high dose of basagran recorded highly significant increase reached almost 3 fold of the control. Accumulation was more in G5 than G6 indicating G5 was more sensitive cultivar towards Basagran® stress. These results probably suggest that the other site of action for Basagran® is on the membranes where ROS and/or free radicals are involved in lipid peroxidation. Also application of Basagran® ± SA, lowered MDA contents in both cultivars. This indicates a protective role of SA to avoid oxidative stress by lowering MDA contents and consequently lipid peroxidation. MDA causes adverse effects in plants, thus, free MDA has been determined in various sources as an oxidative stress marker (Yamauchi et al., 2008). Previously, Galhano et al. (2010), reported increase of MDA in *Anabaena*, a photosynthetic algae, when subjected to Basagran®. Moreover, Radwan (2012) reported increase in MDA content as a result of herbicide application to maize leaves indicating oxidative stress and confirmed protective action of SA against herbicide toxicity.

In this experiment, higher amounts of H_2O_2 than control were observed in Basagran® treated leaves. In response to Basagran® herbicide, H_2O_2 accumulated in both G5 and G6 leaves. Previously, it was found that broad bean treated with herbicides showed a significant increase in H_2O_2 content (Hassan and Alla, 2005). High content of H_2O_2 obtained in paraquat treated seedlings was due to enhanced energy dissipation through photorespiration in stressed plants (Ananieva et al., 2002). In this work, treatment with SA prior Basagran® led to production of significantly lower amounts of H_2O_2 in both cultivars. To support, it was reported that, pre-treatment of barley seedlings with SA prior to paraquat improved the rate of photosynthesis due to decreased production of H_2O_2 by SA, or herbicide may fail to penetrate the chloroplasts and to cause chloroplast destruction and hence paraquat is rapidly detoxified (Ananieva et al., 2002). Increasing SOD activity is also another reason lead to increase the level of H_2O_2 in Basagran® + SA treated leaves.

The antioxidant enzyme system including POD, SOD, APX and CAT enzymes plays an important role in scavenging ROS providing a balanced redox status of an organism (Fecht-Christoffers et al., 2003). In this work, a variable change in the activities of antioxidant enzymes was detected. These changes were including the induction of some enzymes and/or inhibition of others. For example, Basagran® application to G5 leaves resulted in APX, SOD activity stimulation and POD and CAT inhibition. CAT activity was able to be inhibited by other herbicides as atrazine, thus the decrease in CAT activity by atrazine was able to explain the accumulated levels of H_2O_2. APX and CAT represent the major enzymes of H_2O_2 degradation (Vanacker et al., 1998). Also, POD and APX enzyme had an important role in preventing H_2O_2 accumulation (Hassan and Alla, 2005). Compounds (as SA) that reduce the damaging effects of certain stresses, may manage antioxidant status through alteration of antioxidant enzymes. Previously reported, SA mediate the acclimation of plants to environmental stress, and they may interact with other cellular metabolites and environmental factors in the regulation of stress responses (Yordanova and Popova, 2007). In this work, the activities of analyzed enzymes were altered significantly in case of application of SA prior to Basagran® application. This indicates a kind of management of antioxidant enzyme system by SA to control oxidative stress. Previously reported, alteration of APX and CAT activities is considered one of defense mechanisms, which coupled with the inhibition of photosynthetic activity suggesting a plasticity and diversity of responses to different oxidative stresses (Janicka et al., 2008; Rizhsky et al., 2002). The present results were supported by Janda et al. (1999), who reported pre-treatment with SA for one day provided protection in maize plants against stress and induced increased antioxidant activity. Moreover, it

was found that pre-treatment with SA effectively retarded the rapid, stress-associated decreases in SOD, CAT, and APX activities (Kim et al., 2003). Furthermore, elevated POD activity in plant tissues has been used as a biomarker for various contaminant stresses (Song et al., 2007). The increase in POD activity in wheat leaves as a result of the haloxyfop herbicide treatment is probably due to the peroxidation of the membrane lipids, POD may be a sensitive monitor indicating the damage of plant that was contaminated with the herbicide.

Obviously, in this work, SOD activity was stimulated in both G5 and G6 cultivars in response to SA + Basagran. Similar stimulation in SOD has been observed in tomato under salt stress (Molina et al., 2002) and in wheat plants under herbicide stress (Agarwal et al., 2005).Enhanced levels of SOD in plants have been correlated with tolerance to oxidative stress (Van Breusegem et al., 1999). In addition, over production of SOD in plant chloroplasts has been found to increase protection against herbicides (Cui et al., 2010; Iannelli et al., 1999; Van Camp et al., 1996).

Application of SA ± Basagran® herbicide increased the phenolics content of leaves in both cultivars. It was reported that, phenolic compounds known to have high antioxidant activity (Ali et al., 2007). The change in phenolics content was previously explained on basis of altering the activity of enzymes involved in phenolic compounds synthesis (Ali et al., 2007). Moreover, it was reported that phenolic compounds are induced as a result to SA pretreatment as a response to photosynthetic inhibitor herbicides (such as bentazon), generation of high levels of reactive oxygen species decreased the non-enzymatic antioxidant defense system in *S. japonica* (Kumar et al., 2010).

To confirm the change in the antioxidant activity in response to Basagran® and SA treatments, the TAA by using DPPH of leaf extracts was analyzed. Applying SA without herbicide increased the TAA. The total antioxidant activity (TAA) was lowered greatly by herbicide spraying. TAA was dose-dependent. Similarly, it was reported lowered antioxidant activity due to bentazon treatment in some photosynthetic organisms (Galhano et al., 2010). Similarly, decreasing TAA capacity of *S. japonica* was due to overloading of high levels of ROS produced by herbicide application (Kumar et al., 2010). SA application ± Basagran® increased greatly the TAA in both cultivars. Closer results were obtained in case of SA treated cucumber plants, the TAA recorded noticeably higher levels (Radwan et al., 2007).

5. Conclusion

This work discussed Basagran® herbicide induced oxidative stress in peanut cultivars and the use of SA to reduce the injuries which resulted from Basagran® application. Leaf pigment contents were severely reduced by Basagran® treatment. Spraying Basagran® altered POD, CAT, APX, and SOD activities. SA ± Basagran® changed the antioxidant status to protect peanut plants from cellular damage caused by Basagran® induced oxidative stress.

5.1. Summary statement

Salicylic acid is a new way to avoid herbicide injuries in peanut crops. Basagran® herbicide causes severe oxidative stress in peanut leaves and treatment with salicylic acid can protect plants from such injuries by Basagran® detoxification. The work provides a possibility of using SA against abiotic stress.

Declarations

Author contribution statement

Deya Radwan: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed analysis tools or data; Wrote the paper.

Asmaa Mohammed: Performed the experiments; Contributed analysis tools or data.

Khalaf Fayez: Analyzed and interpreted the data; Contributed analysis tools or data.

Abdelrahaman Mahmoud: Analyzed and interpreted the data.

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The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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