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Influence of *Datura stramonium* Leaf Extract on Antioxidants and Activities of Metabolic Enzymes of *Trigonella foenum-graecum* and *Lepidium sativum*

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Abstract

The present investigation aimed to assess the allelopathic effects of aqueous leaf extracts of *Datura stramonium* L. (family: *Solanaceae*) on seed germination, hydroxyl radical (•OH), activities of some metabolic enzymes and antioxidants levels of both *Trigonella foenum-graecum* L. (fenugreek, family: *Fabaceae*)and *Lepidium sativum* L. (cress, family: *Brassicaceae*).The results revealed that *Datura*aqueous leaf extract inhibited seed germination and retarded the activities ofthe following enzymes: glucose-6-phosphate dehydrogenase (G6PD, EC.1.1.1.49), 6 - phosphogluconate dehydrogenase (6PGD, EC.1.1.1.44) and adenosine triphosphatase (ATPase, EC.3.6.1.3). However, *Datura* aqueous leaf extract enhanced NADH-oxidase (EC.1.6.3.1) activity in a concentration-dependent manner. Also, *Datura* leaf extract enhanced production of OH radical and antioxidants including proline, α-tocopherol and reduced glutathione (GSH) but decreased oxidized glutathione (GSSG) content.

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Keywords

Datura stramonium, *Trigonella foenum-graecum*, *Lepidium sativum*, Allelopathy, Hydroxyl radical, Metabolic enzymes, Antioxidants

Introduction

Allelopathy can be defined as negative or positive effect of one plant on other through production of chemicals released in the environment (Thi *et al.,* 2015). Plants with allelopathic capability can fight against weed plants growing along with agricultural crops and affecting growth and crop productivity (Asaduzzaman *et al.,* 2014; Casimiro *et al.,* 2017).

The allelopathic inhibitory effects rise from the action of various groups of allelochemicals (Kil and Shim, 2006). Plants produce allelochemicals as secondary metabolites and they are important for the interaction with the biotic part of the environment (Kroymann, 2011). They affect water relations, photosynthesis, respiration, enzyme activity, stomatal opening, hormone levels, cell division and elongation, mineral availability and permeability of cell membranes (Reigosa *et al.,* 1999). The chemical structure of several allelochemicals has been determined, however the mode of action of some compounds has been illustrated (Vyvyan, 2002). Commercial herbicides of plant origin have been presented onto the market and belong to different chemical groups with novel mechanism of action (Duke *et al.,* 2002). The mechanism of action of such kind of herbicides should be identified due to increasing of herbicide-resistant weeds (Duke, 2012).

The aim of the present work was to compare the allelopathic effect of *Datura* leaf extract on seed germination, activities of some metabolic enzymes andantioxidant compounds in *Trigonella*and *Lepidium*.

Materials and Methods

Collection of experimental plants

Datura stramonium L. was collected from Mansoura, Al-Dakhlia, Egypt during the fruiting season. *Trigonella foenum-graecum* and *Lepidium sativum* were brought from Egyptian Ministry of Agriculture. All chemicals used in this study were of analytical grade. All readings were taken in triplicate and average results are presented.

Preparation of plant powder

Leaves of *Datura stramonium* L. (family: Solanaceae) were air-dried to complete dryness. The air dried leaves were crushed finely by mortar and pestle and thelarger particles were discarded using mesh sieve but the fine powder was retainedfor application. Various concentrations (20, 40, 60, 80 and 100 μg ml^{-1}) were prepared.

*Seed germination of Trigonella*and *Lepidium*

The seeds of *Trigonella* and *Lepidium*were germinated according to El-Shora *et al.,* (2015a). Seeds were surface sterilized with 0.1% $HgCl_2$ for 10 min followed by washing with sterilized distilled water 5 times. The germination test was carried out in sterile Petri dishes (12 cm in size) with filter papers. The extract from each concentration of *Datura* leaves was added to each petri dish enough to wet the seeds. Control seeds were treated with distilled water. The petri dish was set in three replicates andthe various treatments were kept at room temperature range (30-35°C). The experiment was left over period of 10 days to allow the last seed germination and the percentage of seed germination was calculated.

Preparation of enzymes extract from Trigonella and *Lepidium*

Leaves (5 gm) were ground in 100 ml of 50 mM sodium phosphate buffer (pH 7.0) containing 1 mM cysteine using mortar and pestle. The resulting homogenate was centrifugedat 4°C for 15 min at 10,000 g.

The supernatant represented the crude enzyme extract (El-Shora *et al.,* 2016).

Determination of hydroxyl radical (•OH) content

Hydroxyl radical (•OH) was determined according to the method of Halliwel *et al.,* (1988). Leaf sample (5 gm) was incubated in 50 mM phosphate buffer (6.0) containing 20 mM 2-deoxy-D-ribose. Malondialdehyde (MDA) formed was estimated by mixing 0.5 ml of trichloroacetic acid (TCA) with 0.5 ml of incubation medium after being centrifuged and 0.5 ml of 2-thiobarbituric acid. The mixture was boiled for 10 min and cooled under tap water followed by centrifuging and measuring the absorbance at 532 nm.

Determination of reduced GSH and oxidized GSSG contents

Total glutathione and GSSG contents were determined by the method adopted by Anderson (1985). The extract was neutralized in 0.5 ml of 150 mM potassium phosphate buffer (pH 7.5). The reaction mixture of 3 ml consisted of 0.2 ml of 6 mM 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB),0.5 ml of 0.1 M of Na-phosphate buffer (pH 7.5) with EDTA, 1 ml of glutathione reductase (GR)and 0.1 ml of 2 mM NADPH. The absorbance was measured at 412 nm. 2-Vinylpyridine was added to the extract to determine GSSG content. GSH content was calculated by deducting content of GSSG from the total glutathione content.

Determination of proline content

Proline was determined according to Bates *et al.,* (1973). A sample (0.5g) of fresh frozen plant leaves was homogenized in 10 ml of 3% aqueous sulfosalicyclic acid. The homogenate was then filtered using Whatman's No. 2 filter paper. Two ml of the resulting filtrate was put in test tube and mixed with 2 ml acid-ninhydrin and 2 ml of glacial acetic acid. The mixture was heated in a water bath at 100°CforI hr. The reaction mixture was extracted using 4 ml toluene and the chromophore containing toluene was aspirated, cooled to room temperature. The absorbance was measured at 520 nm spectrophotometrically and the proline content was calculated from standard curve of proline.

Determination of α-tocopherol content

α-Tocopherol content was determined by the method adopted by Hira *et al.,* (2001). Leaves (0.5 g) were homogenized in 5 ml of absolute ethanol followed by centrifuging at 10,000 g for 15 min and the supernatant was assayed. The reaction medium consisted of 2.5 ml of

supernatant and 1.5 ml of xylene stoppered and vortexed for 15 min then centrifuged for 15 min. One ml of the xylene layer was mixed with 1.0 ml of 3 mMα, α-dipyridyl reagent, and the absorbance was read at 460 nm. Add 0.5 ml of 5mM $FeCl_3$ reagent to the reaction mixture, mix and the absorbance was measured again at 520 nm.

Preparation of enzyme extract

Five gm of plant tissue fromcontrol and treated plants were homogenized onice in 20 ml extraction buffer composed of 50mM phosphate buffer (pH 7.0) containing 1m Mphenylmethylsulfonyl fluoride, 1mM EDTA and 1% polyvinylpolypirrolidone (PVP). The resulting homogenate was then centrifuged at 10,000g and 4ºCfor 20 min. The supernatantre presented the crude enzyme extract which was used for determination of the enzymes activity.

Determination of glucose-6-phosphate dehydrogenase (G6PD) activity

G6PD (EC.1.1.1.49) activity was determined according to Betke *et al.,* (1967) depending on measuring NADPH production at 340 nm spectrophotometrically. The reaction mixture of 3 ml consisted of0.5 ml of 0.2 mM NADP, 0.5 ml of 0.6 mM G6P, and 1.5 ml of 10 mM $MgCl_2$ in 150 mMTris-HCl buffer (pH 8.0). The reaction was initiated by adding of 0.5 ml enzyme extract. One unit (U) of G6PD activity is defined as the amount of enzyme required to reduce of 1 µmol of NADP per min.

Determination of 6-phosphogluconate dehydrogenase (6PGD) activity

6PGDH (EC.1.1.1.44) was determined according to the method of Papapetridis *et al.,* (2016). The method is dependent on the conversion of NAD^+/$NADP^+$ to NADH/NADPH and measuring absorbance at 340 nm. The reaction mixture of 3 ml consisted of 0.5 ml of 0.4 mM NAD^+ or $NADP^+$, 0.5 ml of 5 mM $MgCl_2$, 1.5 ml of 100 mMTris–HCl (pH 8.0) and the reaction was started by adding 0.5 ml of enzyme extract.

Determination of ATPase activity

ATPase (EC.3.6.1.3) activity was determined according to El-Shora *et al., (*2015b) depending on measuring the released phosphate. The reaction mixture of 3 ml consisted of0.5 ml of 10mM ATP, 1.8 ml of 150mMTricine-KOH buffer (pH 8.0), 0.5 ml of 5mM $MgCl_2$ and 0.2 ml of enzyme extract. The released inorganic phosphate from ATP was determined colorimetrically by ammonium molybdate reagent.

Determination of NADH-oxidase activity

NADH oxidase (EC.1.6.3.1) activity was determined by the method of Morret (1995).

The reaction mixture of 3 ml consisted of 0.3 ml of0.5 mM NADH, 0.2 ml enzyme extract, 2 ml of 100 mMTris-Mes buffer (pH 7.5) and 0.5 ml of 5mM KCN to inhibit oxidase activity. The activity was measured at 340 nm spectrophotometrically.

Statistical analysis

The data is presented as mean value ± SD value. One way ANOVA procedure was used to perform the analysis of variance. Minitab software was used to calculate the significance differences ($P \leq 0.05$) between values.

Results and Discussion

Seed germination

The results in Fig. 1 showed that *Datura* leaf extract reduced seed germination of *Trigonella*and *Lepidium* in a concentration-dependent manner. The percentages of seed germination at 100 µg ml^{-1}were 18.4% and 39.7% for *Trigonella* and *Lepidium*, respectively. Several studies have observed reduction of seed germination under allelopathy (Inderjit and Keating, 1999; Hassannejad and Ghafarbi, 2013, El-Shora and Abd El-Gawad, 2014; El-Shora and Abd El-Gawad, 2015a) but few studies have been carried out to investigate the mechanism of action of allelochemicals (Inderjit and Duke, 2003).

It has been reported that the inhibition of seed germination may be due to disruption of mitochondrial respiration (Podesta and Plaxton, 1994).

It has been reported that reserved food mobilization during seed germination was delayed or reduced under stress conditions of allelopathy (Mishra, 2015). It has been suggested that plant extract contains allelochemicals that can prevent seed germination, reduce seedling growth of acceptor plants and influence the antioxidant enzymes activities (Altikat *et al.,* 2013; Ullah *et al.,* 2015).

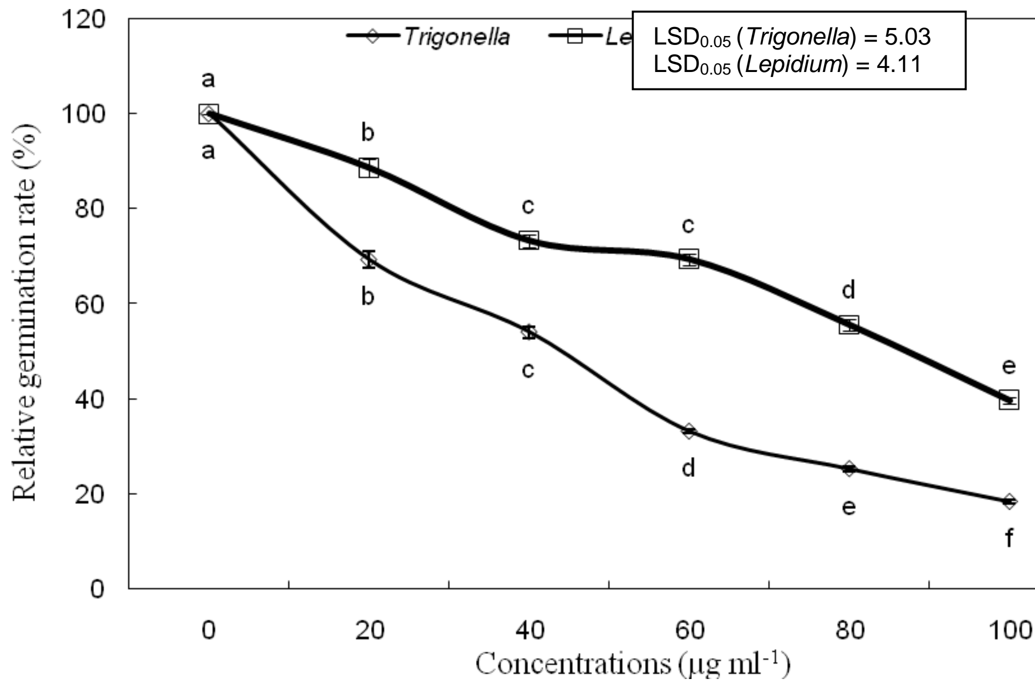

Fig.1 Effect of *Datura* leafextract at different concentrations on relative germination rate in leaves of *Trigonella*and*Lepidium*. Different letters per each line means significance difference (P ≤ 0.05).

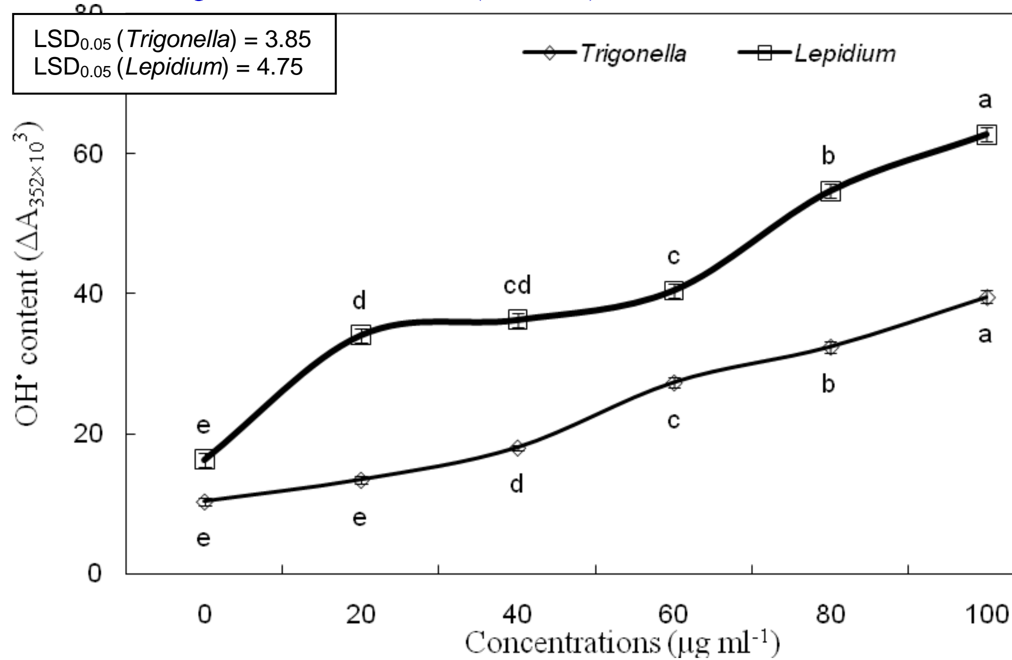

Fig.2 Effect of *Datura* leaf extract at different concentrations on hydroxyl radical (•OH) content in leaves of *Trigonella* and *Lepidium*.
Different letters per each line means significance difference (P ≤ 0.05).

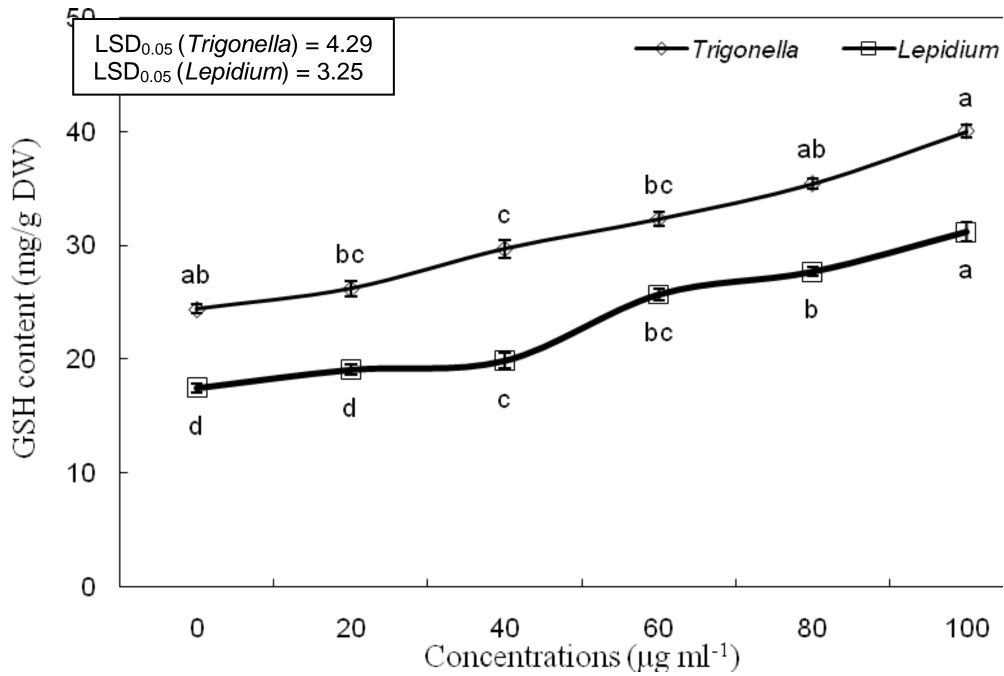

Fig.3 Effect of *Datura* leaf extract at different concentrations on reduced glutathione (GSH) content in leaves of *Trigonella* and *Lepidium*.
Different letters per each line means significance difference ($P \leq 0.05$).

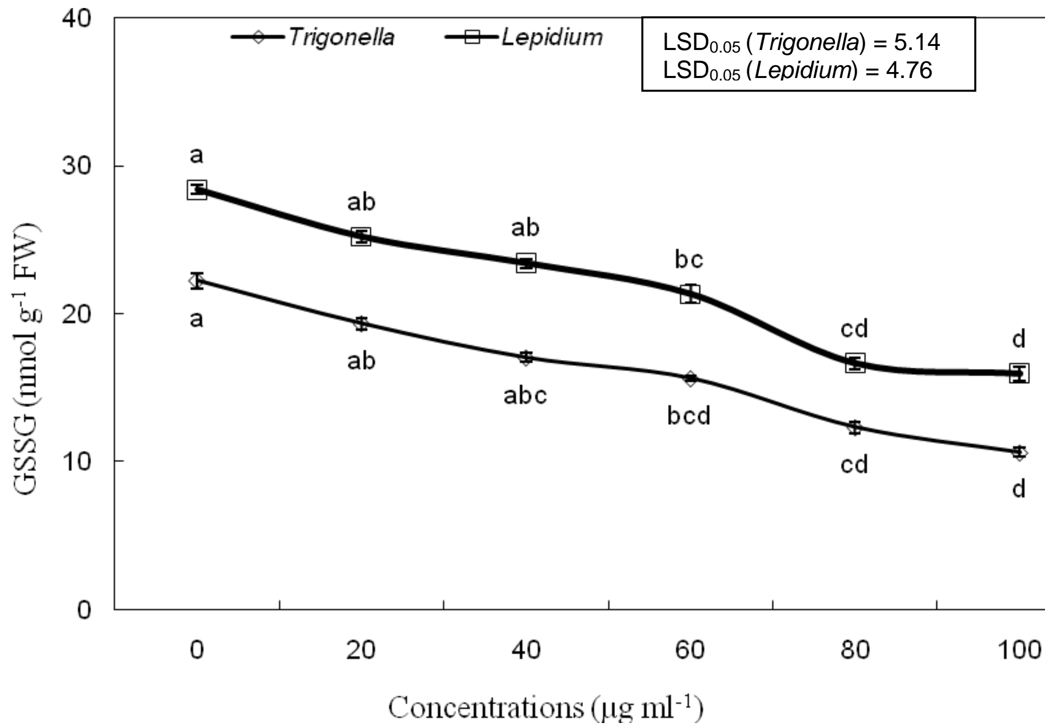

Fig.4 Effect of *Datura* leaf extract at different concentrations on oxidized glutathione (GSSG) content in leaves of *Trigonella* and *Lepidium.*
Different letters per each line means significance difference ($P \leq 0.05$).

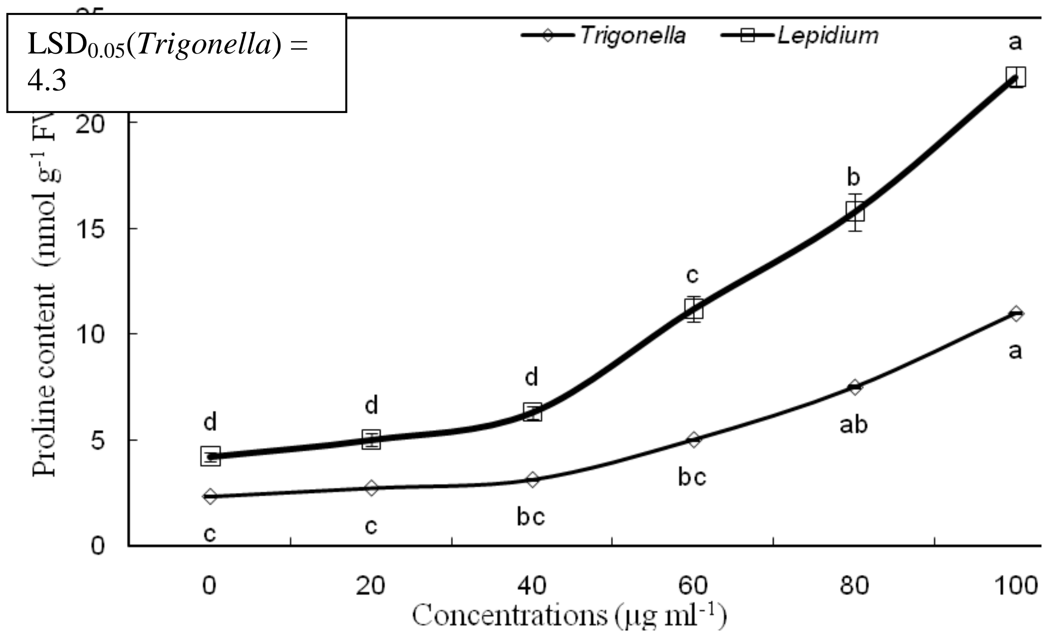

Fig.5 Effect of *Datura* leaf extract at different concentrations on proline content in leaves of *Trigonella* and *Lepidium.*
Different letters per each line means significance difference (P ≤ 0.05).

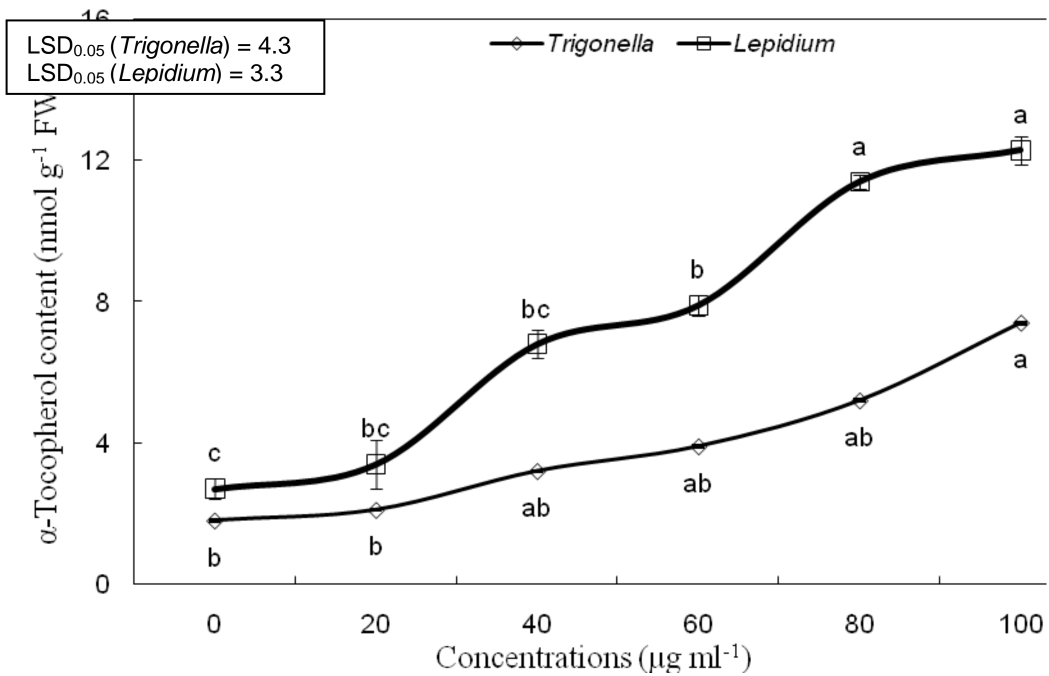

Fig.6 Effect of *Datura* leaf extract at different concentrations on α-tocopherol content in leaves of *Trigonella* and *Lepidium.*
Different letters per each line means significance difference (P ≤ 0.05).

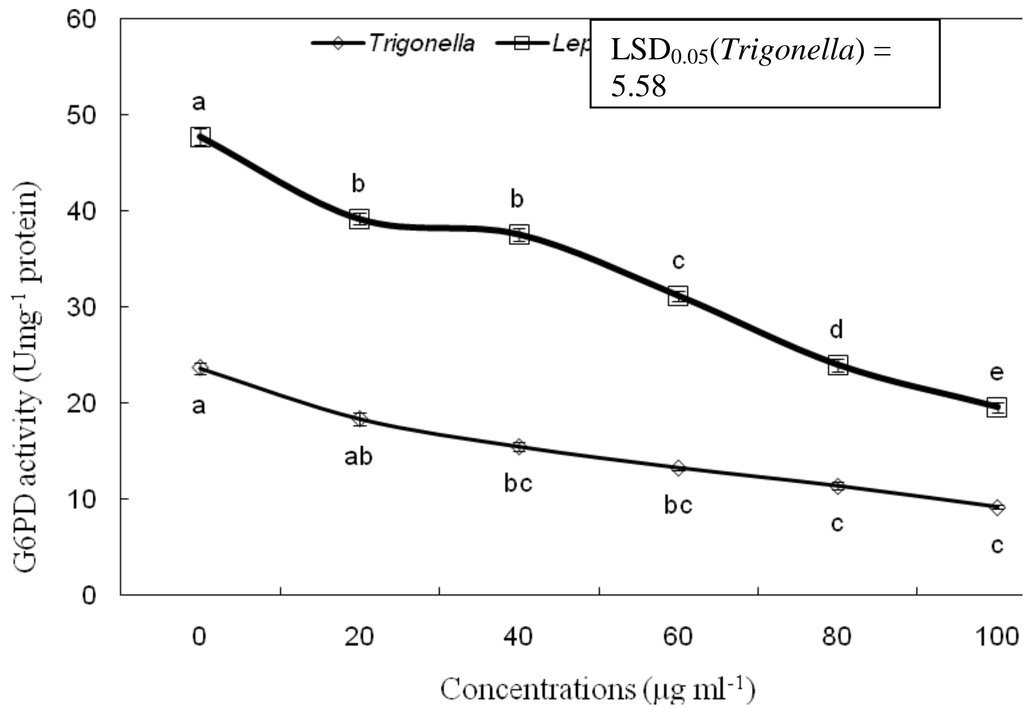

Fig.7 Effect of *Datura* leaf extract at different concentrations on glucose-6-phosphate dehydrogenase (G6PD) activity in leaves of *Trigonella* and *Lepidium.* Different letters per each line means significance difference ($P \leq 0.05$).

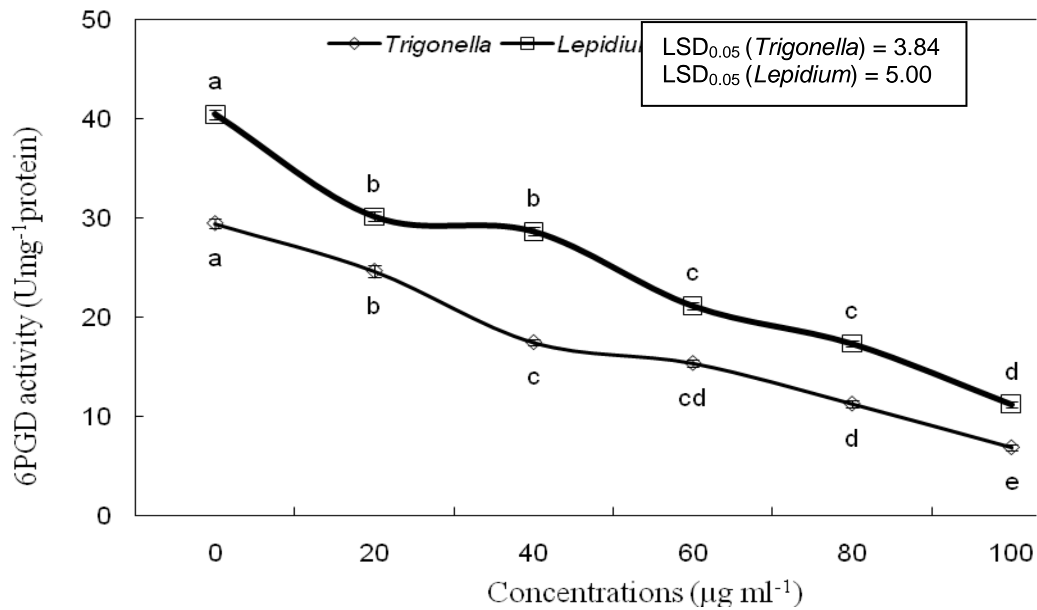

Fig.8 Effect of *Datura* leaf extract at different concentrations on 6-phosphogluconate dehydrogenase (6PGD) activity in leaves of *Trigonella* and *Lepidium.* Different letters per each line means significance difference ($P \leq 0.05$).

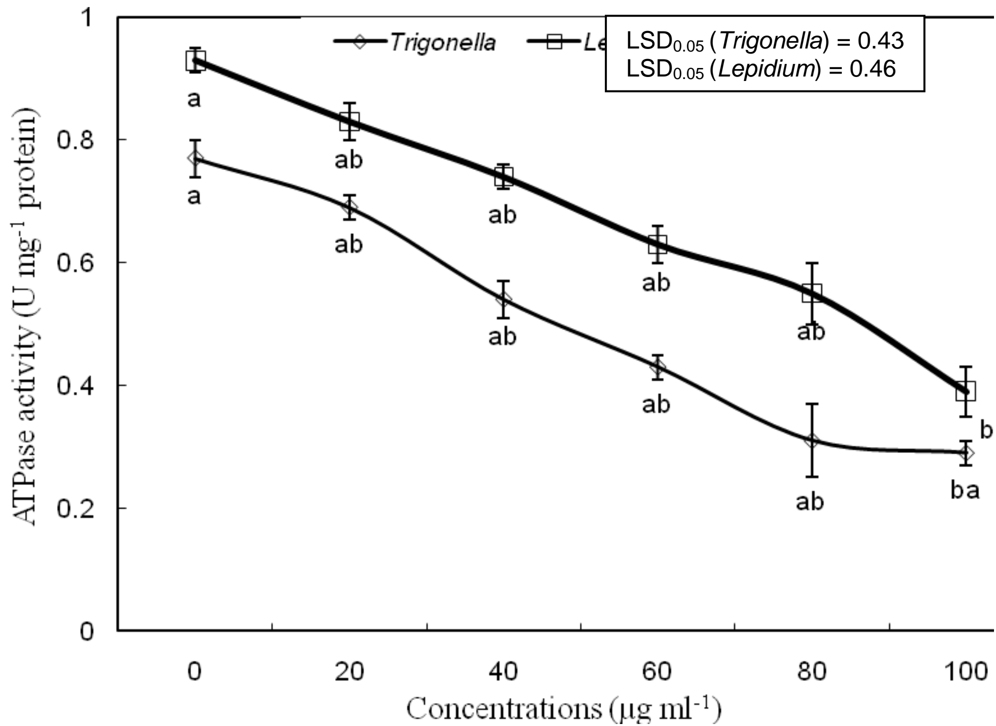

Fig.9 Effect of *Datura* leaf extract at different concentrations on plasma membrane ATPase activity in leaves of *Trigonella* and *Lepidium.*
Different letters per each line means significance difference (P ≤ 0.05).

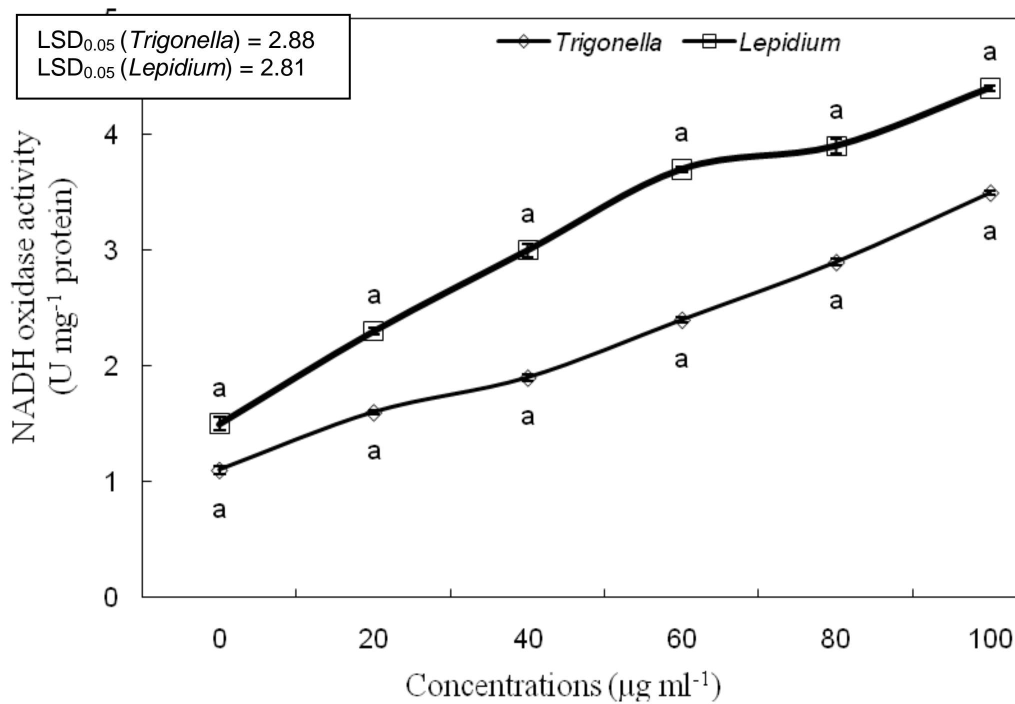

Fig. 10 Effect of *Datura* leaf extract at different concentrations on NADH oxidase activity in leaves of *Trigonella* and *Lepidium.* Different letters per each line means significance difference (P ≤ 0.05).

Hydroxyl radical (OH.) content

The treatment of *Trigonella* and *Lepidium* plants with *Datura* leaf extract increased$^{\bullet}$OH content with increasing the concentration particularly in *Lepidium* (Fig. 2).$^{\bullet}$OH is reactive radical that reacts with biomolecules in cells including lipids, DNA and proteins in its vicinity at diffusion controlled rates. This radical has a very short half-life and consequently reacts with biomolecules at the site of its formation (Betteridge, 2000). Thus, it is more deleterious to biological systems compared to other ROS (Halliwell and Gutteridge, 2007). $^{\bullet}$OH induces oxidative damage to lipids by lipid peroxidation which starts by addition of $^{\bullet}$OH to a double bond or through elimination of a hydrogen atom by $^{\bullet}$OH from a methylene group of polyunsaturated fatty acids.

GSH and GSSG contents

The results in Fig. 3 indicate that GSH content in *Trigonella* was higher than *Lepidium* in control as well as treated plants. The results are in harmony with those of El-Shora and Abd El-Gawad (2015a) who reported an increase in GSH in *Cicer arietinum* under allelopathic effect of *Portulaca oleracea* root extract. On the other hand, GSSG content (Fig. 4) was reduced continuously in both plants with increasing *Datura* leaf extract concentration. It was noticed that the decrease of GSSG in *Trigonella* was higher than in *Lepidium*. At 100 µg ml^{-1} GSSG levels were 10.6 and 15.9 nmol g^{-1} for *Trigonella* and *Lepidium,* respectively.

GSH playsan essential role in preserving cellular redox status (Rennenberg, 1980). GSSG can be reduced back to GSH by glutathione reductase (GR) presence of NADPH (RicdeVos *et al.,* 1994) and the reduction process is dependent on the intercellular GSH/GSSG ratio. Changes in the redox ratio of glutathione depend on GSH biosynthesis, GSH catabolism and GSH concentration (Mullineaux and Rausch, 2005).

Proline content

The proline content (Fig. 5) increased in both treated plants gradually in a concentration-dependent manner. At 100 µg ml^{-1} the proline contents were 11.0 and 22.2 nmol g^{-1} FW for *Trigonella* and *Lepidium*, respectively. The increase in proline content under allelopathy in this investigation is consistent with that reported by El-Shora and Abd El-Gawad (2015a). Proline accumulation is associated with the oxidation of NADPH and thus increases the ratio of NADP+/NADPH which enhances the oxidative pentose phosphate pathway for providing the precursors for biosynthesis of phenolic antioxidants through shikimic acid pathway (Cheynier *et al.,* 2013).

α-Tocopherol (vitamin E) content

α-Tocopherol level (Fig. 6) increased gradually in both treated plants depending on the concentrations of *Datura* leaf extract, however. α-Tocopherol content was higher in *Lepidium* than *Trigonella* under all tested concentrations.α-Tocopherol plays an essential role in plant growth, development and protection against oxidative damage.α-Tocopherol is located in chloroplast envelope, thylakoid membranes and plastoglobuli. This antioxidant scavenges$^{\bullet}O_2$ and $^{\bullet}$OH as photosynthesis-derived ROS and prevents lipid peroxidation by scavenging lipid peroxyl radicals produced in thylakoid membranes (Munné-Bosch, 2005). It is possible that the changes in α-tocopherol level may result from altered expression of some genes and it is assumed that the increased α-tocopherol content supports plant stress tolerance (Fahad *et al.,* 2014).

G6PD and 6PGDactivities

The activities of G6PD (Fig. 7) and 6PGD (Fig. 8) in the two tested plants were inhibited by *Datura* leaf extract in a concentration-dependent manner. The two enzymes from *Trigonella* seem likely to be more susceptible to *Datura* leaf extract than those of *Lepidium*. These results are in agreement with the results of other investigators (El-Shora and Abd El-Gawad, 2015b; Abd El-Gawad and El-Shora, 2017) who observed inhibition of G6PD under allelopathy effect. The inhibition of the two enzymes in the present work indicates that allelochemicals in *Datura* leaf extract possibly expressed an inhibitory effect on the respiration of the two plants under investigation.

ATPase activity

ATPase generates proton electrochemical gradient and thus provides the driving force for uptake of ions and metabolites through the plasma membrane (Palmgren, 2001). ATPase activity was inhibited in both tested plants (Fig. 9) depending on the concentration of *Datura* leaf extract; however the ATPase activity of *Lepidium* was higher than that of *Trigonella* under all tested concentrations. ATPase activities at 100 µg ml^{-1} were 0.39 and 0.29 units mg^{-1} protein for *Lepidium* and *Trigonella*, respectively. In support, ATPase activity was reduced in *Portulaca oleracea* under allelopathy effect of

Trichoderma africanum L (El-Shora *et al.,* 2015b). The inhibition of ATPase might result in reduction ofwater and mineral uptake by roots and thus affect plant functions including photosynthesis, respiration and protein synthesis leading to growth reduction (El-Shora *et al.,* 2015a). In addition, the inhibitory effect of alllelopathy on respiration process might cause a reduction of ATP production and this consequently affects other cell processes which are energy-dependent (Bogatek *et al.,* 2002).

NADH-oxidase activity

The results in present investigation show enhanced NADH-oxidase activity (Fig. 10) in the two tested plants by *Datura* leaf extract. The increase in the enzyme activity was dependent on the extract concentration. NADH-oxidase activities at 100 µg ml^{-1} were 3.5 and 4.4 units mg^{-1} protein for *Trigonella* and *Lepidium*, respectively. Similar results for the enhancement of NADH-oxidase under allelopathy were reported by El-Shora *et al.,* (2015b). The increase of NADPH oxidase activity seems likely to be due to increased $H_2O_2$and $^{\bullet}O_2$ production (Gulzar and Siddiqui, 2016).

In conclusion, *Datura* leaf extract seems to exert its allelopathic effect by its allelochemicals which enhance production of reactive oxygen species (ROS). The later attack the metabolic enzymes and consequently results in the production of antioxidant compounds to scavenge ROS for cell protection.

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