

Fenugreek Seed Extract Enhanced the Growth of *Vicia faba* and *Zea mays* Seedlings

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HIGHER plants produce many phytochemical constituents that not only play a pivotal role in plants producing them, but also affect their neighboring plant communities. This study was conducted to evaluate the effect of fenugreek seed extract on the growth of faba bean (*Vicia faba*) and maize (*Zea mays*) seedlings. The allelochemical components of fenugreek seed extract were analysed and confirmed the abundance of vanillic, syringic, 4-hydroxycinnamic, sinapic, caffeic, salicylic and p-hydroxybenzoic, ferulic acids as well as coumarin. Fenugreek seed extract up to 1.0 % (w/v) efficiently enhanced the growth as well as the chlorophyll content of faba bean and maize seedlings. Furthermore, elevated levels of the amyolytic and proteolytic activities under these concentrations of fenugreek treatments were associated with a marked accumulation of soluble sugars and proteins. Moreover, the potentiality of fenugreek treatments to accumulate phenolic and flavonoid was associated with stimulation in the activities of phenylalanine ammonia lyase, peroxidase and polyphenol oxidase as well as a marked enhancement in total antioxidant capacity which would improve the plant antioxidant status. Concerning the membrane integrity, fenugreek treatments caused a pronounced retardation in both MDA and H₂O₂ content. Our results elucidated the effect of fenugreek seed extract on the growth and some physiological behaviors of both faba bean and maize seedlings.

Keywords: *Vicia faba*, *Zea mays*, Fenugreek, Phenolic acids, Allelopathy, Antioxidant enzymes.

Introduction

In higher plants secondary metabolites were considered as an ambiguous dispute in plant science. The situation is rapidly changed after the biological activities of these compounds came into light. Recent studies showed that many of these secondary metabolites are essential and beneficial to the plant producing them (Bais et al., 2006). Some of these metabolites are known as allelochemicals, and their action can be beneficial or detrimental to natural or implanted biological communities (Madany & Saleh, 2015). Among these allelochemicals are phenolic and terpenoid constituents that can be synthesized and accumulated in several parts

of the plant, including seeds (Omezzine et al., 2014). These allelochemicals can be released into the environment either by exudation from roots or leaching from the aerial parts and affect the structure of plant community (Candido et al., 2016). Additionally, allelochemicals play a manifold role in communication processes among plant communities and between plants and external invaders by attracting pollinators, warding off microbial infection, deterring herbivores, (Pudelko et al., 2014 and Upadhyay & Dixit, 2015).

Fenugreek (*Trigonella foenum-graecum* L.) is an annual herbaceous legume used anciently in pharmaceutical, human food and animal feed

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purposes (Belguith-Hadriche et al., 2013). Fenugreek seeds characterized by being rich in phytochemical compounds that have antioxidant properties and are traditionally used as a food forage and folk medicine (Kaviarasan et al., 2007). Fenugreek seeds contain 45-60% carbohydrates (mainly mucilaginous fiber: galactomannans); 20-30% proteins (mainly lysine and tryptophan); 5-10% fixed oils (lipids); flavonoids (apigenin, luteolin and quercetin); pyridine-type alkaloids, (mainly trigonelline); steroidal saponins (trigoneoside and furostanol); phenolics (coumarin: scopoletin); steroidal saponinogenins (fenugreekine), as well as volatile oils, vitamins and minerals (Gupta et al., 1986 and Belguith-Hadriche et al., 2013).

Faba bean (*Vicia faba* L.) and maize (*Zea mays* L.) are among the most important cultivated crops in the world as they play important agronomic and socio-economic roles. Faba bean is a multipurpose crop and considers one of the most important legume food in the Mediterranean region (Fernández-Aparicio et al., 2008). In Egypt, faba bean represents one of the most important legume crops occupying about 150,000 ha of the total legume area (Makkouk et al., 1994). Nevertheless, faba bean production has been declined in the last decades due to the climatic changes as well as diseases and pests (Abbes et al., 2006). Moreover, faba bean contributes to the sustainability of cropping systems due to its ability to fix nitrogen through biological nitrogen fixation (Jensen et al., 2010). Maize (*Zea mays* L.) is also considered as an important crop for human consumption. Being an important crop for food and feed in man life, maize is currently the most widely grown crop in the world and has been one of the most valuable subjects for investigation.

The allelopathic effect of several plants upon faba bean and maize growth has been extensively investigated (El-Darier, 2002 and Nasrine et al., 2013). However, to date, there are no reports on the application of fenugreek seed extract on the growth and the underlying biochemical aspects in these crops. The present work was, therefore, undertaken mainly to study the influence of the fenugreek seed extract upon the growth of faba bean and maize seedling as well as the underlying physiological and biochemical parameters.

Materials and Methods

Plant material

Seeds of faba bean (*Vicia faba* L.) and maize (*Zea mays* L.) were kindly obtained from the Department of Vegetables, Agriculture Research Center and used throughout the experiment. Fenugreek (*Trigonella foenum-graecum* L.) seeds were purchased from Agriculture Research Center, Ministry of Agriculture, Giza, Egypt. Fenugreek seeds were ground to a fine powder (0.5 kg), then an extract was prepared by ethanol, evaporated under vacuum at 50°C and the residue was dissolved in 100 ml distilled water that considered as 100% seed extract (w/v).

Pot experiment

The seeds of both faba bean and maize were surface sterilized then sown in plastic pots filled with autoclaved sandy clay (1:2; w/w). The pots were irrigated with half strength Hoagland's nutrient solution containing 0, 0.25, 0.50, 1.0 or 1.50% (w/v) fenugreek seed extract and incubated in growth chamber at 22 ± 2°C with 12 h photoperiod and 75% relative humidity for twenty days.

HPLC analysis

Phenolic compounds of fenugreek seeds were extracted according to the method outlined by Ben-Hammouda et al. (1995). A known dry weight of fine powdered seeds was used to prepare the extract using diethyl ether. After evaporation of diethyl-ether, the residue was re-dissolved in 3 ml of HPLC grade methanol. Seed extract and standard substances were resolved on a Hewlett-Packard HPLC (Model 1100), using ODS C18-hypercil column with 5 µm particle size. The mobile phase was methanol: water: acetonitrile: acetic acid (70:30:1:0.5, v/v) and utilized over 30 min using a UV detector set at wavelength 254 nm.

Extraction and estimation of chlorophyll pigment

Assessments of chlorophyll content were performed during the experimental period. Total chlorophyll, chlorophyll a and b as well as carotenoids from fully expanded fresh leaves were measured spectrophotometrically using 100% acetone, and their concentrations were calculated (Sestak et al., 1971).

Soluble sugars and soluble proteins

Total soluble sugars were analyzed according to the method adopted by El-Tayeb et al. (2006). A known weight of fresh powdered tissues was

boiled in distilled water for 1 h in a water bath, and then centrifuged to obtain the extract. The total soluble sugars were determined using Nelson's reagent (Clark & Switzer, 1977). Soluble protein was extracted by incubating known weight of fresh powdered tissues in 10 mL distilled water for 2 h at 90 °C (El-Tayeb et al., 2007). Proteins determination was carried out according to the modified Folin-Lowry method outlined by Hartree (1972).

Protease and amylase activity

Fresh powdered tissues were homogenized in 20 mM phosphate buffer, pH 7.6 for estimating protease activity. The reaction was initiated by adding 0.5 ml of the crude extract to 2 ml of the substrate solution (20 mM phosphate buffer, pH 7.0, containing 10 mg/ml BSA) and incubated at 40°C for one hour. The resulted soluble peptides were recorded using Folin-Lowry method adopted by Hartree (1972). For amylase extraction, fresh powdered tissues were homogenized in 100 mM acetate buffer, pH 6.0. The amylolytic activity was determined by mixing 0.5 ml of the crude extract with 0.5 ml of 0.5% soluble starch prepared in 0.1M of acetate buffer, pH 6.0, containing 5 mM CaCl₂. The resulting reducing sugars were estimated by the Nelson's method (Clark & Switzer, 1977).

Phenolic and flavonoids content

Total soluble phenolic compounds were extracted with 70% ethanol (Sauvesty et al., 1992). The Folin-Ciocalteu phenol method was used for phenolic estimation (Lowe, 1993). Total flavonoids were extracted and estimated using the method adopted by Sakanaka et al. (2005).

Proline content

Free proline content was determined according to Bates et al. (1973). A known fresh weight of powdered tissue was homogenized in 3% aqueous sulfosalicylic acid. The reaction was initiated by adding acid ninhydrin reagent and glacial acetic acid to the extract in boiling water bath. After cooling, 4 ml toluene was added and mixed well for 20 sec. The absorbance of chromophore-containing toluene layer was recorded at 520 nm against toluene.

Total antioxidant capacity

For extraction of non-enzymatic antioxidants a known weight of liquid nitrogen-powdered tissues was homogenized with pre-chilled 80% ethanol. The total antioxidant capacity was determined by de-colorization of the ABTS^{•+}, 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid), radical (Re et

al., 1999). Ten ml of the extract was mixed with 1 ml of the diluted ABTS^{•+} solution ($A_{734\text{nm}} = 0.700 \pm 0.020$) and O.D. was taken at 734 nm. The TAC was calculated from Trolox standards curve and expressed as $\mu\text{mol Trolox g}^{-1}$ fresh weight.

Malondialdehyde (MDA) and H₂O₂ content

MDA content was determined using the method of Fu & Huang (2001). Fresh powdered sample was homogenized in 4 ml trichloroacetic acid (0.1%; w/v) in an ice bath and the supernatant was used for lipid peroxidation analysis. MDA content was then estimated using thiobarbituric acid (0.5 % in 20% TCA) spectrophotometrically at 532 nm and corrected for nonspecific turbidity at 600 nm.

H₂O₂ was extracted by homogenizing fresh powdered tissues in tri-chloroacetic acid (0.1 %) (Alexieva et al., 2001). The homogenate was centrifuged and 0.5 ml of the supernatant was added to 0.5 ml of phosphate buffer (10 mM, pH 7.0) and 0.2 ml of potassium iodide (5 M). Absorbance was followed for 1 min at 390 nm. The blank consisted of a reaction mixture without potassium iodide, and its absorbance was subtracted from the mixture with H₂O₂ extract.

Activities of antioxidant enzymes

Extraction of peroxidase (POX, EC 1.11.1.7) was carried out according to the method outlined by Kar & Mishra (1976). Based on the method of Wakamatsu & Takahama (1993), the reaction mixture contained the crude enzyme extract and assay mixture (50 mM phosphate buffer, pH 7.2; 0.1 mM EDTA; 5 mM guaiacol; 0.3 mM hydrogen peroxide) and the absorbance was measured at 470 nm then expressed as nmol guaiacol mg protein⁻¹min⁻¹.

Phenylalanine ammonia lyase (PAL, EC 4.3.1.5) activity was assayed using the method outlined by Chandra et al. (2007). The activity was started by mixing enzyme extract and the substrate solution (6 mM of L-phenylalanine in 0.5 mM Tris-HCl buffer, pH 8.0) for two h at 37°C. The absorbance was measured at 290 nm and determined as the rate of conversion of L-phenylalanine to t-cinnamic acid.

Polyphenol oxidase (PPO, EC 1.14.18.1) was extracted as described by Kar & Mishra (1976) with slight modification. According to the method proposed by Nguyen et al. (2003), the assay mixture contained the crude enzyme extract and the substrate solution (0.05 M phosphate buffer, pH 6.0, containing 0.05 M catechol). The mixture was incubated at 30°C

for 30 min and then the absorbance measured at 420 nm then expressed as nmol guaiacol mg protein⁻¹min⁻¹.

Statistical analysis

The experimental design of the greenhouse experiment was performed in a complete-block design with three blocks consisting of five treatments with five plants each. The computer program SPSS (version 18) was used for statistical analyses of studied parameters. A value of $P < 0.05$ was considered to be significant. Five replications were performed for each parameter under analysis, and Student's t-test was used to compare differences between control and experimental values.

Results

The effects of fenugreek seed extract on the growth and some physiological parameters of both faba bean and maize seedlings were investigated. The HPLC analysis of the *T. foenum-graecum* seed extract led to identification of 9 different compounds (Fig. 1). The chromatogram of fenugreek seed extract showed nine phenolic compounds, among these phenolics vanillic acid which represent a major component, where its concentration reached about 244 $\mu\text{g/g}$ dry weight. In addition, syringic,

4-hydroxycinnamic, sinapic and caffeic acids were less dominant and their concentrations ranged from 48-113 $\mu\text{g/g}$ dry weight. Moreover, salicylic, p-hydroxybenzoic and ferulic acids, as well as coumarin were detected in relatively small amounts.

Measurements of shoot and root heights, fresh and dry weight (FW, DW) were recorded to elucidate the effect of the different concentrations of fenugreek seed extract on faba bean and maize seedlings growth (Fig.2). Increasing the concentration of fenugreek seed extract up to 1.0% (w/v) triggered all the measured shoot growth parameters in both faba bean and maize giving maximum enhancement at 0.50% (w/v) ($P < 0.001$), while 1.5 % extract showed a marked retardation in all measured shoot parameters for both plants under investigation. Similarly, root fresh and dry weight of both test plants were improved in response to the different concentrations of fenugreek seed extract reached its maximum value at 0.50 % (w/v). Conversely, the root length of both faba bean and maize did not show a significant effect in response to the different concentrations of the extract (Fig. 2D).

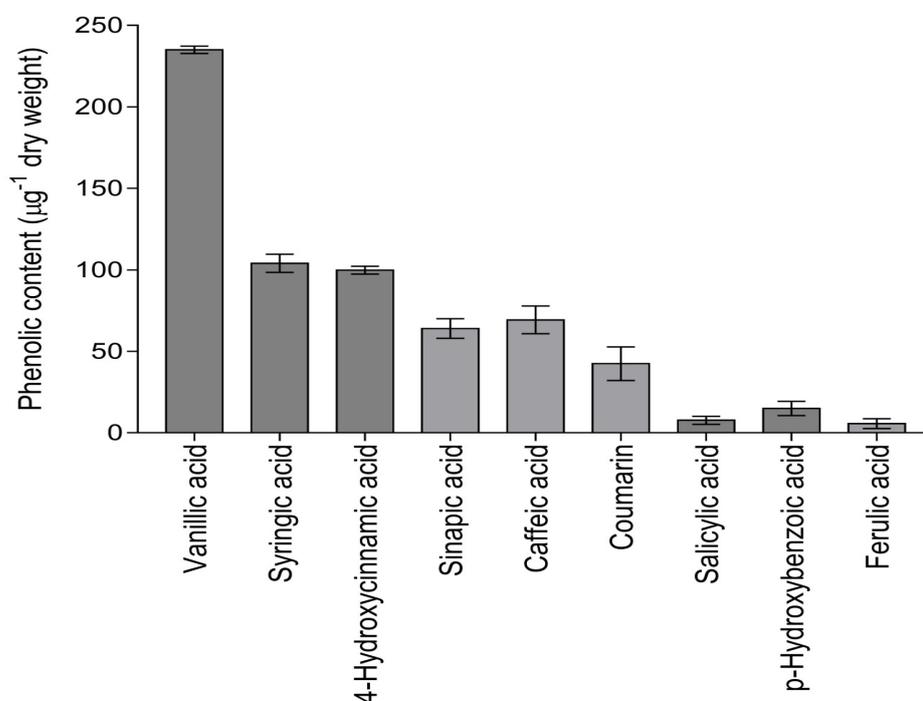


Fig.1. The qualitative and quantitative analysis of phenolic compounds in fenugreek seed extract using HPLC. The bars on each point showed standard error of the means of three independent replications.

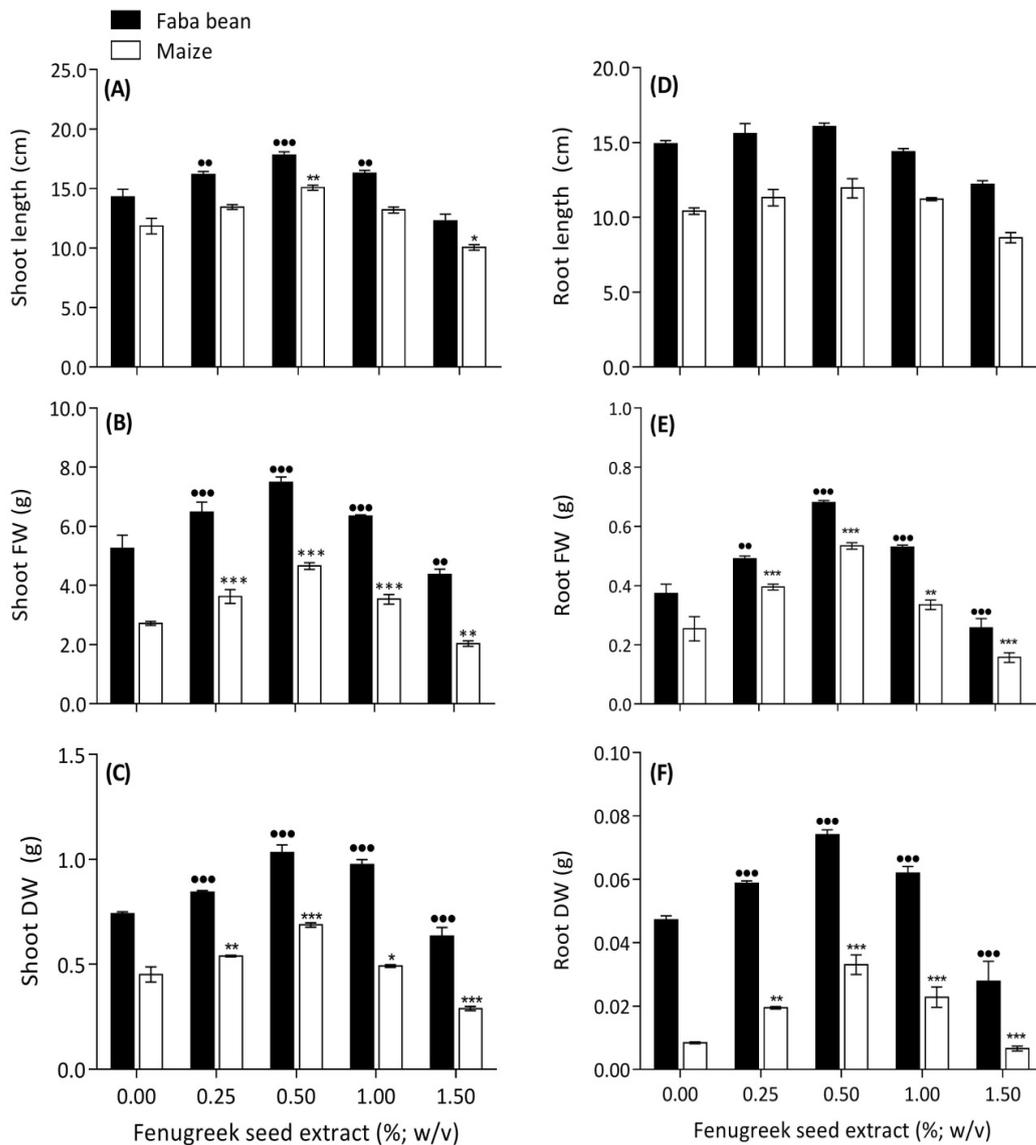


Fig. 2. Effect of different concentrations of fenugreek seed extract upon (A) shoot length (B) shoot fresh weight, (C) shoot dry weight, (D) root length, (E) root fresh weight and (F) root dry weight of faba bean and maize seedlings. The bars on each column showed standard error of the means of five independent replications. The dots and asterisks signs indicate that the mean value of treatments is statistically significantly different from that of the control of faba bean and maize, respectively at $P < 0.05$ (one sign), $P < 0.01$ (two signs) or $P < 0.001$ (three signs) based on Student's t-test.

Increasing the concentration of fenugreek extract had no significant effect on the number of leaves of maize seedlings. On the other hand, concentrations up to 1.0 % increased the number of leaves of faba bean (Fig. 3A). The highest level of fenugreek seed extract (1.5%; w/v) severely retarded the number of leaves ($P < 0.01$) of both maize and faba bean by 36 and 32%, respectively

from the untreated control. Additionally, total leaf area of faba bean and maize seedlings was significantly increased by increasing fenugreek extract concentration (Fig. 3B). Similarly, the 1.5 % fenugreek treatment significantly decreased the total leaf area of both faba bean and maize seedlings by about 51 and 47%, respectively, compared to the control.

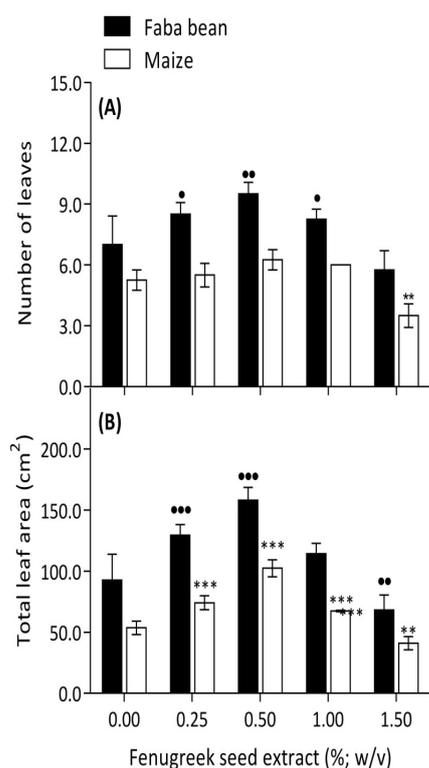


Fig. 3. Effect of different concentrations of fenugreek seed extract upon (A) number of leaves and (B) total leaf area of faba bean and maize seedlings. The bars on each column showed standard error of the means of five independent replications. The dots and asterisks signs indicate that the mean value of treatments is statistically significantly different from that of the control of faba bean and maize, respectively at $P < 0.05$ (one sign), $P < 0.01$ (two signs) or $P < 0.001$ (three signs) based on Student's t-test.

In our study, photosynthetic pigment levels of faba bean and maize seedlings were noticeably enhanced by the different treatments of fenugreek seed extract ($P < 0.001$) except for the highest rate (1.50 %; w/v) that caused a slight inhibition in their pigment levels (Fig. 4). The maximum values of Chl a, Chl b, total pigments and carotenoids in faba bean seedlings were about 66, 111, 82 and 31%, respectively, whereas the magnitude of increase in maize seedlings was about 94, 106, 90 and 63%, respectively, using the 0.5% (w/v) fenugreek treatment relative to their corresponding fenugreek untreated seedlings.

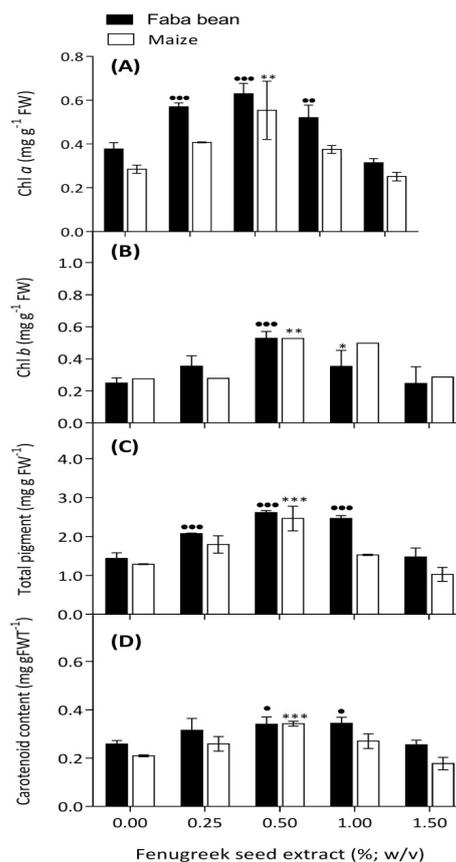


Fig. 4. Effect of different concentrations of Fenugreek seed extract upon the content of (A) *chl a*, (B) *chl b*, (C) total pigment, and (D) carotenoid of faba bean and maize seedlings. The bars on each column showed standard error of the means of five independent replications. The dots and asterisks signs indicate that the mean value of treatments is statistically significantly different from that of the control of faba bean and maize, respectively at $P < 0.05$ (one sign), $P < 0.01$ (two signs) or $P < 0.001$ (three signs) based on Student's t-test.

The difference in amylase and protease activities as well as the content of soluble sugars and soluble proteins between faba bean and maize is clear under the different concentrations of fenugreek seed extract (Fig. 5). A significant improvement in the activities of amylase and protease was found in both test plants until 0.5% (w/v) fenugreek treatment ($P < 0.001$), then the activities decreased afterward (Fig. 5A, B). This behavior was associated with a similar trend in the content of soluble sugars and proteins under the different levels of fenugreek seed extract (Fig. 5C, D). Both faba bean and maize showed a continual increase in soluble sugars and soluble

protein levels reached the maximum value at 0.50% fenugreek concentration. This increment was more noticeable in soluble sugars than in soluble proteins (about 84 and 127% in faba bean and maize, respectively) as compared to the untreated seedlings. Moreover, the highest

level of fenugreek extract significantly inhibit the activities of amylase and protease that is associated with a marked reduction in the content of soluble sugars and soluble proteins in both faba bean and maize seedlings.

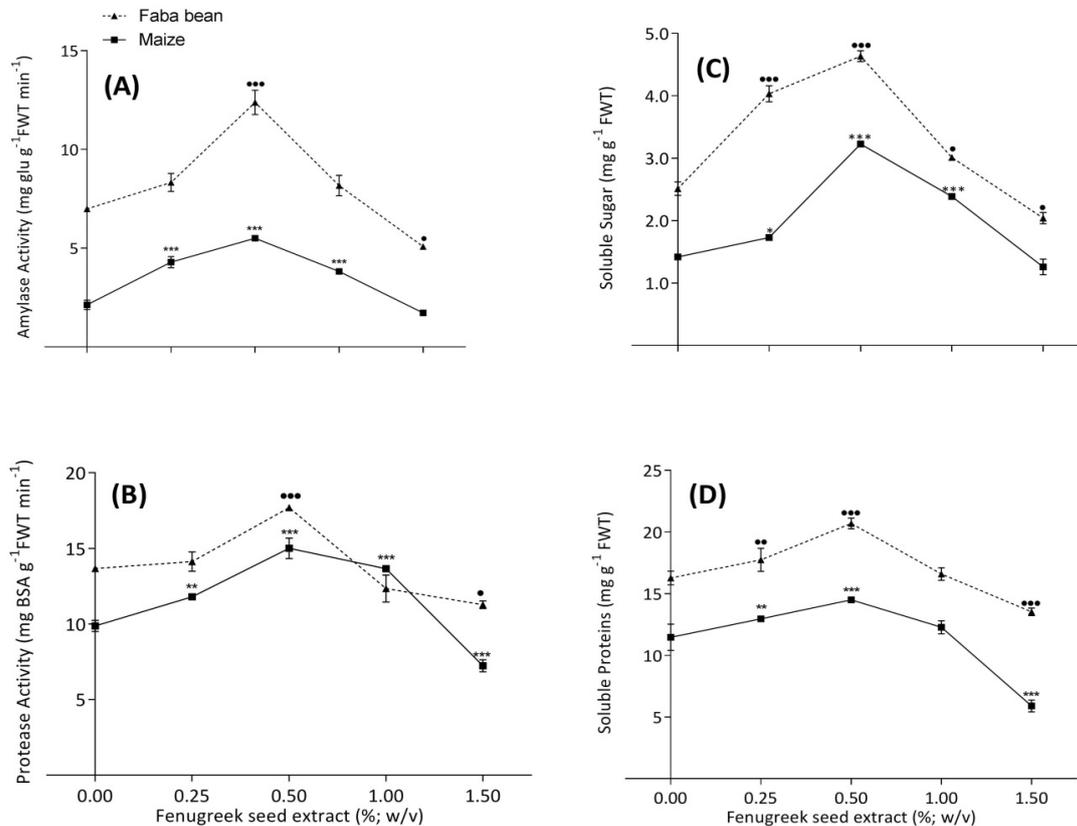


Fig. 5. Effect of different concentrations of fenugreek seed extract upon (A) amylase activity (B) protease activity, (C) soluble sugar content, (D) soluble protein content of faba bean and maize seedlings. The bars on each point showed standard error of the means of five independent replications. The dots and asterisks signs indicate that the mean value of treatments is statistically significantly different from that of the control of faba bean and maize, respectively at $P < 0.05$ (one sign), $P < 0.01$ (two signs) or $P < 0.001$ (three signs) based on Student's t-test.

During this study, we found a significant difference in soluble phenolic content between the two test plants under different fenugreek treatments (Fig. 6A). For example, 0.5% (w/v) fenugreek extract significantly ($P > 0.001$) enhanced the accumulation of soluble phenolic in faba bean and maize seedlings (about 99% and 122%, respectively) as compared with their counter control. A similar pattern of flavonoids and proline content changes was observed in both target plants (Fig. 6B, C). Conversely, 1.5% (w/v) fenugreek seed extract markedly retard the accumulation soluble phenolics, flavonoids and proline in faba bean and maize seedlings compared to untreated control seedlings.

Total antioxidant capacity as well as H_2O_2 content were estimated to shed the light on the oxidative status of the plants under investigation after treatment with the different levels of fenugreek seed extract (Fig. 7A, B). Moreover, the integrity of membranes was estimated by evaluating lipid peroxidation as MDA content (Fig. 7C). Exposing faba bean and maize seedlings to 0.25 and 0.50% (w/v) caused an increase in their total antioxidant capacity, compared to control values (Fig. 7A). This increment was more pronounced in faba bean than maize-treated seedlings. Further increase in the concentration of fenugreek seed extract caused a

decrease in the total antioxidant capacity of both test plants. The content of H_2O_2 significantly decreased in faba bean and maize in response to treatment with fenugreek seed extract (Fig. 7B). This decrease was substantial and more profound with further increase in extract concentration down to 1.50% (w/v) in both faba bean and maize seedlings. Moreover, when the fenugreek seed extract increased, the levels of MDA significantly reduced in both faba bean and maize seedlings reaching the maximum rate of decrease at the highest level of fenugreek seed extract and were 51% and 69% of those in the control, respectively (Fig. 7C).

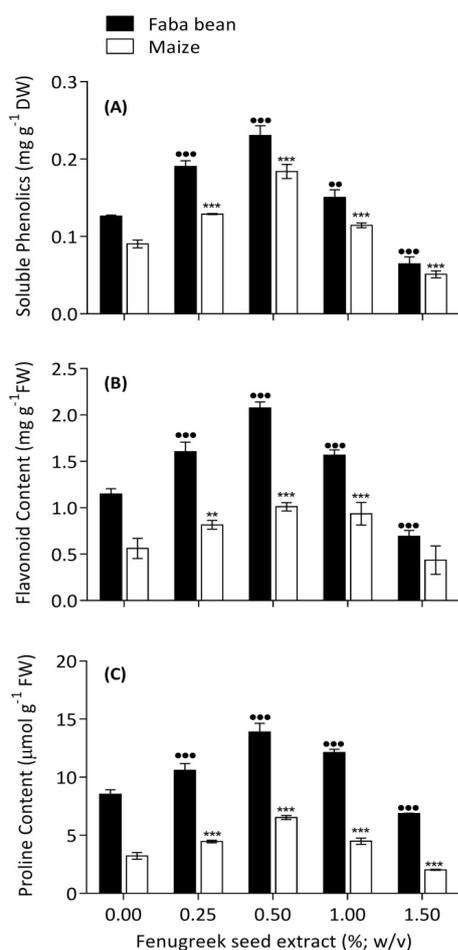


Fig.6. Effect of different concentrations of fenugreek seed extract upon (A) soluble phenolic content (B) flavonoid content, and (C) proline content of faba bean and maize seedlings. The bars on each column showed standard error of the means of five independent replications. The dots and asterisks signs indicate that the mean value of treatments is statistically significantly different from that of the control of faba bean and maize, respectively at $P < 0.05$ (one sign), $P < 0.01$ (two signs) or $P < 0.001$ (three signs) based on Student's t-test.

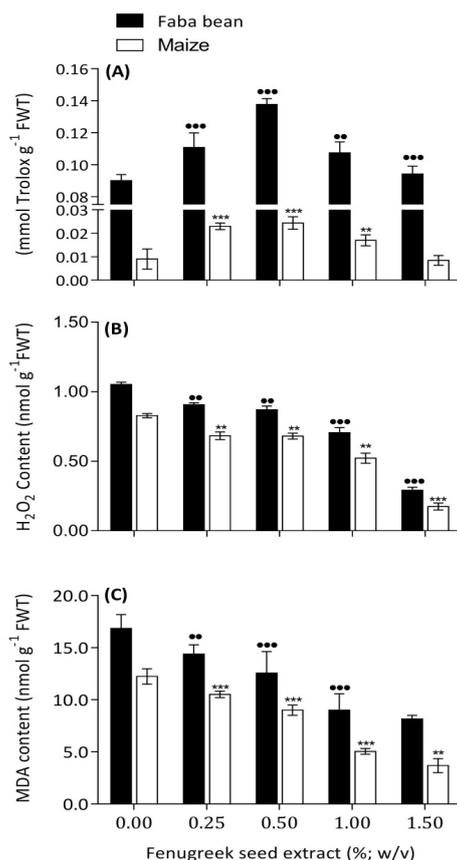


Fig.7. Effect of different concentrations of fenugreek seed extract upon (A) total antioxidant capacity (B) H_2O_2 content and (C) MDA content of faba bean and maize seedlings. The bars on each column showed standard error of the means of five independent replications. The dots and asterisks signs indicate that the mean value of treatments is statistically significantly different from that of the control of faba bean and maize, respectively at $P < 0.05$ (one sign), $P < 0.01$ (two signs) or $P < 0.001$ (three signs) based on Student's t-test.

The activities of POX, PAL and PPO from control and fenugreek-treated seedlings were characterized (Fig. 8). In faba bean, the activities of POX, PAL and PPO exhibited a noticeable improvement at 0.50% fenugreek concentration ($P < 0.001$), compared to the control. However, it increased to its maximum (97, 63, 44 % of the control, respectively) ($P < 0.01$) at 0.50% then significantly inhibited ($P < 0.01$) at 1.50% of fenugreek extract. A similar trend was observed in maize seedlings except for PAL activity which didn't show a significant increase upon treatment with different levels of fenugreek seed extract.

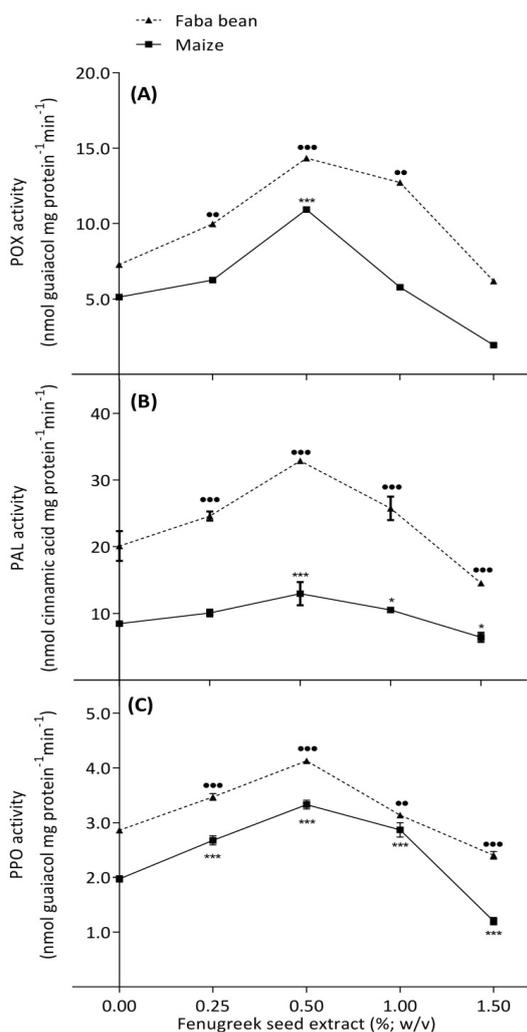


Fig. 8. Effect of different concentrations of fenugreek seed extract upon (A) POX activity (B) PAL activity and (C) PPO activity of faba bean and maize seedlings. The bars on each point showed standard error of the means of five independent replications. The dots and asterisks signs indicate that the mean value of treatments is statistically significantly different from that of the control of faba bean and maize, respectively at $P < 0.05$ (one sign), $P < 0.01$ (two signs) or $P < 0.001$ (three signs) based on Student's *t*-test.

Discussion

Higher plants contain many phytochemical constituents (allelochemicals) that are known to be biologically active compounds. Several studies heavily discussed the impact of these phyto-allelochemicals upon different crop plants including faba bean and maize (El-Darier, 2002; Madany & Saleh, 2015 and Salama & Rabiah, 2015). However, to date, the influence of fenugreek upon these plants is unaddressed. The results of

the HPLC analysis revealed the presence of four hydroxycinnamic acid derivatives (caffeic acid, ferulic acid, 4-hydroxycinnamic acid and sinapic acid) and four dihydroxybenzoic acid derivative (salicylic acid, syringic acid, *p*-hydroxybenzoic acid and vanillic acid) as well as coumarin.

The joint action of these phyto-allelochemicals of fenugreek seed extract was observed on the magnitude of growth stimulation or retardation of faba bean and maize seedlings that was depended on their amount present in the different fenugreek treatments. Improvement of all measured growth criteria in the roots and shoots of the both target plants treated with the lower rates of fenugreek seed extract (0.25, 0.50 and 1.0 %; w/v) indicates that these treatments had no harmful effects. On the other hand, a deleterious effect was observed at the highest level of fenugreek extract that diminished all the measured growth parameters of faba bean and maize seedlings. In a previous work, it was found that faba bean seeds primed with 1.0 mM coumarin showed a marked improvement in the length of both plumule and radical as well as their fresh and dry weights of seedlings and this could be attributed to the elevated levels of endogenous phyto-hormones (IAA, GA3, and ABA) (Saleh et al., 2014). Additionally, tomato plants treated with salicylic acid exhibited a significant improvement in their shoot and root heights as well as their fresh and dry weights (AL-Wakeel et al., 2012). Moreover, salicylic acid used as a spray solution to the shoots of soybean significantly increase the growth of the shoots and roots under greenhouse or field conditions (Li et al., 2014).

Conversion of inorganic molecules or ions into organic bio-molecules is a biologically important process that is mediated by chlorophyll pigment through photosynthesis. Therefore, photosynthesis is one of the most crucial indicators of physiological activities in higher plants. Therefore, impairing the plant's photosynthetic capacity could affect its carbon fixation and carbohydrate status. The different levels of fenugreek seed extract improve the levels of chlorophyll a and b as well as carotenoids in the seedlings of both faba bean and maize except for the highest rate (1.5 %; w/v). This may be attributed to the beneficial effect of some of phenolic acids in the extract like salicylic acid as it plays key roles in the regulation of plant growth and development as well as the responses to

environmental stresses (AL-Wakeel et al., 2012). The effects of exogenously applied salicylic acid on plant physiological processes under optimal environmental conditions are controversial (Janda et al., 2014). In this regard, foliar spray of *Torreyia grandis* with salicylic acid significantly enhanced its chlorophyll content (Li et al., 2014). Moreover, an earlier study reported that the increase or decrease of chlorophyll content in cowpea under salicylic acid treatment was concentration-dependent and genotype specific (Chandra & Bhatt, 1998). Also, chlorophyll levels were significantly improved in tomato leaves treated with salicylic acid (AL-Wakeel et al., 2012). In this context, Li et al. (2014) revealed the increase in chlorophyll content in *Torreyia grandis* under salicylic acid treatment to the increase in the activity of certain enzymes, that involved in chlorophyll biosynthesis or reducing chlorophyll degradation, leading to increased net photosynthesis under salt stress tolerance.

In plants, some developmental stages such as germination and cell biogenesis are regulated by the degradation of carbohydrates and proteins (Palma et al., 2002). It is well known that the activity and function of certain enzymes can be altered by phenolic phytochemicals after passing through the plant cell membrane (Li et al., 2011). Our results showed that fenugreek seed treatment enhanced the accumulation of soluble sugars and protein in both faba bean and maize seedlings. This enhancement may be ascribed to the improvement in both amylolytic and proteolytic activities in response to some phytochemicals in the extract. Also, increment of soluble sugars could be attributed to the enhancement of photosynthetic pigments and stimulation of Rubisco activity (Khodary, 2004). Furthermore, coumarin was found to induce the biosynthesis of amylase in wheat grains (Saleh & Abu El-Soud, 2015). Moreover, the application of salicylic acid stimulated the accumulation of soluble sugars and proteins in faba bean plants (Orabi et al., 2013). Also in a previous study, we found that application of 1.0 mM coumarin significantly enhance the activities of both amylase and protease as well as the accumulation of soluble sugars and proteins in *Vicia faba* seedlings under salt stress (Saleh et al., 2014). Other study showed that ferulic acid increased the activity of proteases in mung bean hypocotyls (Singh & Kaur, 2014). Similarly, pretreatment of cucumber seedlings with 0.5 mM ferulic acid had protected them from dehydration stress and resulted in accumulation of soluble sugars in their leaves (Li et al., 2013).

One of the most important metabolic changes occurs in plants is the accumulation of phenolics, flavonoids and proline. Phenolics, flavonoids and proline are of great interest to scientists due to their pivotal role in plants such as pigmentation, growth and reproduction. Lower levels of fenugreek seed treatments exhibited a conspicuous accumulation of soluble phenolics in plants under study. This accumulation may be due to an increased enzyme activity of PAL which regulates the phenolic biosynthesis in plants through phenylpropanoid pathway, suggesting a shift from sucrose production to processes of defense and repair (Cheynier et al., 2013). Similarly, lower concentration of fenugreek seed extract improved the accumulation of flavonoids and proline in both test plants. In addition to phenolics, flavonoids are considered as secondary ROS-scavenging system in plants protecting them against various environmental disturbances (Fini et al., 2011). Proline is a solute that improves the protection against a variety of abiotic stresses and its accumulation provides precursors necessary for phenolic biosynthesis in the shikimic acid pathway via increasing the ratio of NADP⁺/NADPH that in turn promotes the oxidative pentose phosphate pathway (Cheynier et al., 2013). These accumulative effects of phenolics, flavonoids and proline under fenugreek seed treatments could be attributed to the phytochemicals present in the extract. In this context, treatment of tomato with salicylic acid showed a pronounced accumulation of both phenolic and flavonoid contents (AL-Wakeel et al., 2013). In a previous work, we found that wheat seedlings treated with coumarin exhibited a significant increase in phenolic, flavonoids and proline (Saleh & Madany, 2015). Similarly, salicylic acid induced a two-fold increase in proline content at the vegetative stage of tomato plants (Umebese et al., 2009). In accordance with our results, chickpea and cucumber seedlings treated with ellagic and ferulic acids has markedly enhanced proline accumulation (Abu El-Soud et al., 2013 and Li et al., 2013)

Cell damage occurs in consequent of adverse environmental stimuli which disrupts the normal homeostasis of affected cells. Its deleterious effect can be monitored by tracing total antioxidant capacity and H₂O₂ content as well as, estimating the end product of lipid peroxidation (MDA) (Rao et al., 1997 and Hodges et al., 1999). Lower levels of fenugreek seed extract significantly

increased the total antioxidant capacity and retarded both MDA and H_2O_2 of faba bean and maize. This behavior could be due to the potentiality of phytochemicals in the extract to induce the accumulation of cellular antioxidants such as phenolics and flavonoids. Depending on their structure, phenolics and flavonoids were found to constrain lipid peroxidation, as they can trap the lipid alkoxy radical (Milić et al., 1998). They also can act as direct scavengers for ROS, where they have the ability to donate electrons or hydrogen atoms (Duthie & Crozier, 2000 and Michalak, 2006). In this context, alleviation of oxidative stress by application of phenolic acids like ferulic and cinnamic acids is mediated by decreasing H_2O_2 , MDA contents and increasing total antioxidant capacity of seedlings (Sun et al., 2012 and Li et al., 2013). In accordance with these results, ellagic acid was found to increase the total antioxidant capacity and decrease both lipid peroxidation and H_2O_2 content of chickpea seedlings (Abu El-Soud et al., 2013). They revealed the enhanced antioxidant capacity to the marked increase in flavonoids content under ellagic acid treatments. Moreover, Li et al (2014) reported that the salicylic acid treatment significantly reduced the increase in the MDA content in *Torreya grandis* seedlings under salt stress. Also, we found that wheat seedlings treated with coumarin significantly exhibit higher values of total antioxidant capacity (Saleh & Madany, 2015).

Among oxido-reductases are polyphenol oxidases (PPOs) which are copper-containing enzymes found in thylakoids of plastids in plants and enhances the oxidation of the O-diphenol compounds into highly reactive quinones (Araji et al., 2014). Meanwhile, PAL is the key enzyme of phenylpropanoid pathway that plays a crucial role in the biosynthesis of phenolics in plants. Also, Peroxidases play a pivotal role in plant cell including detoxification of H_2O_2 and formation of ROS (Kösesakal & Ünal, 2009). This improvement may shed the light on the effective role of phenolics found in fenugreek seed extract in improvement plant antioxidant status. In the present study, the activities of POX, PAL and PPO enhanced significantly in both faba bean and maize seedlings in response to lower levels of fenugreek treatments. The enhanced activities of the measured antioxidant enzymes could be explained by expression of antioxidant enzymes transcripts directly due to treatment

with fenugreek seed extract which is enriched in phenolic acids. This improvement could enhance the plant antioxidant reserve by scavenging H_2O_2 and producing oxidized substrates utilized for many physiological processes (Michalak, 2006). Moreover, enhanced POX activity could protect membrane integrity, leading to an increase in the amount of photosynthetic pigments and the net photosynthetic rate (Li et al., 2014). A number of reports are available that indicate an increased activity of POXs in response to phenolic compounds. For example, seedlings treated with ellagic, ferulic or cinnamic acids under both normal or abiotic stress conditions was found to significantly enhance POX activity (Li et al., 2011; Abu El-Soud et al., 2013 and Singh & Kaur, 2014). Also, we found that coumarin seed pretreatment caused obvious stimulation in the activities of both PAL and POX in wheat seedlings under salinity stress (Saleh & Madany, 2015). Moreover, Plants treated with salicylic acid exhibited a noticeable increase in the activities of PPO, PAL and POX that in turn, could induce the plant resistance against several pathogenic invaders (Mandal et al., 2009; AL-Wakeel et al., 2013 and Li et al., 2014). Additionally, plants treated with caffeic, chlorogenic, vanillic and ferulic acids significantly enhanced POX activity (Garg & Garg, 1989; Devi & Prasad, 1992 and Politycka et al., 2003).

In conclusion, our study showed that fenugreek seed extract enhanced the growth as well as the relying physiological and biochemical processes of both *Vicia faba* and *Zea mays*. The results of this study showed that these phytochemicals have the potentiality to enhance the plants antioxidant enzyme mechanisms that could avoid membrane oxidative damage under different environmental stresses. Another divaricate of fenugreek extract is the induction of peroxidase, phenylalanine ammonia lyase and polyphenol oxidase activities that were associated with accumulation of phenolics and flavonoids that may improve the antioxidant status either directly by acting as antioxidant molecules or indirectly by serving as substrates for peroxidase, and hence helps in scavenging of H_2O_2 . Therefore, these results can provide a useful guide for the conservation of these plants when exposed to such stresses.

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مستخلص بذور الحلبة يحسن نمو بادرات الفول والذرة

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تنتج النباتات العديد من المركبات الكيميائية التى لا تقتصر اهميتها على النبات نفسه بل يتعدى تأثيرها إلى المجتمعات النباتية المجاورة. أجريت هذه الدراسة لتقييم التغيرات الحيوية والفسيلوجية المصاحبة للمعاملات المختلفة من مستخلص بذور الحلبة (0,25 و 0,50 و 1,0 و 1,5 %) وتأثيره هذه المعاملات على نمو بادرات الفول والذرة. أظهر الفصل الكروماتوجرافى لمستخلص بذور الحلبة باستخدام جهاز الفصل الكروماتوجرافى السائل ذو الكفاءة العالية (HPLC) وجود تسعة مركبات فينولية تختلف فى كمياتها وهى: vanillic, syringic, 4-hydroxycinnamic, sinapic, caffeic, salicylic and p-hydroxybenzoic coumarin

اظهرت الدراسة ان التركيزات من 0,25% الى 1,0% ادت إلى تحسن ملحوظ فى نمو المجموع الجذرى والخضرى بالإضافة إلى تحسين محتوى الكلوروفيل لنباتات الفول والذرة وصاحب هذا التحسن زيادة فى أنشطة انزيمات البروتينيز والاميليز مما ادى إلى زيادة واضحة فى محتوى البروتينات والسكريات الذائبة. على الجانب الاخر، اظهرت الدراسة زيادة ملحوظة فى أنشطة انزيمات الـ PAL و الـ POX و الـ PPO مما ادى بدوره إلى زيادة فى محتوى الفينولات والفلافونيدات وصاحب ذلك تعزيزاً واضحاً فى القدرة المضادة للاكسدة لبادرات النباتات محل الدراسة. وفيما يتعلق بسلامة الغشاء الخلوى، تسببت معالجات مستخلص بذور الحلبة فى تقليل ملحوظ للـ MDA و H_2O_2 .

إذا قد تسلط هذه النتائج الضوء على الدور الفعال للفينولات الموجودة في مستخلص بذور الحلبة في تحسين النمو والعمليات الفيسيولوجية لاثنتين من اهم محاصيل العالم الا وهما الفول والذرة.