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Phytotoxic effect of *Alhagi maurorum* on the growth and physiological activities of *Pisum sativum* L.

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ABSTRACT

The present work was conducted to evaluate the effect of aqueous extract of *Alhagi maurorum* at different rates (1, 5, 10, 15%, w/v) on the growth as well as some physiological parameters of pea (*Pisum sativum* L.). The pot experiment revealed that *Alhagi maurorum* aqueous extract reduced all growth parameters of pea plant along with photosynthesis pigments, insoluble sugars, total carbohydrate, total protein and total phenolics. On the other hand, soluble sugars, soluble protein, proline, flavonoids and antioxidant enzymes increased upon treatment with aqueous solution of *Alhagi maurorum*. The adverse effect of the extract on the growth of treated pea plants especially hydroquinone and sinapyl alcohol that were found in relatively high concentration in the extract were identified and quantified by the GC–MS of the methanolic extract of *Alhagi maurorum*.

1. Introduction

Plants create massive array of secondary metabolites that escape to the environment and affect the growth and development of neighboring plants and other organisms, a phenomenon known as “allelopathy” (Chou, 2006). Allelopathy plays an important role in the agro ecosystems may be involved not only in plant–plant and plant-microorganism's interactions, but also in plant–insects or plant–herbivores communication (Weir et al., 2004). The impact of allelochemical compounds on germination, growth and development of plants is regulated by complexity, interaction and concentration of secondary metabolites (Li et al., 2011; Saleh, 2013). These metabolites are called allelochemicals and are belonging to several chemical classes such as flavonoids, phenolics, alkaloids, terpenoids and cyanogenic glycosides (Mishra, 2015).

Alhagi maurorum plant integrate to family leguminosae and native to north Africa, the middle east and south east Europe (Awmack and Lock, 2002). Moreover, *A. maurorum* distributed and appeared to have wide ecological extent in Egypt, it was noted from the Nile region, oasis, Mediterranean region, Eastern and Western Desert, Red sea coast and Sinai, as well as Saudi Arabia deserts (Boulos, 2009) *Alhagi* species contains many active constituents such as flavonoids, fatty acids, coumarins, sterols, vitamins, and alkaloids (Awaad Amani et al., 2006). Allelopathic inhibition is complex and can include the interaction of different classes of chemical compounds, such as phenolic, flavonoids, terpenoids, alkaloids, steroids, carbohydrates, and amino acids. Mixtures of these different compounds sometime shaving a greater allelopathic effect than individual compounds alone (Einhellig, 1996). In contrast, Chung et al. (2002) argued that the mixture of phenolic compounds was less inhibitory than individual phenolic compounds. Growth et al. (2016) showed that by increasing concentrations of aqueous leaf extracts of *Andrographis paniculata* inhibited the seed germination, shoot length, root length and dry weight of wheat seedlings. At higher concentrations of *Calotropis procera* leaf extracts, a significant reduction in the growth of the test plants were recorded (Barakat and Nazeir, 2016). Singhand Rao (2003) reported that suppress of chlorophylls may decrease the photosynthesis process and substantially decrease all the metabolites such as, total sugars, soluble amino acids and proteins. Hassan and Mathesius (2012) showed that, Flavonoids in plant can be transported within and between tissues and cells, and often are released into the rhizosphere where they are involved in plant to plant

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interactions, specifically allelopathic interference. They can be released by root exudation or through tissue degradation over time, and although both aglycones and glycosides of flavonoids are found in root exudates, their relative role in allelopathic interference, specific activity and selectivity (Hassan and Mathesius, 2012).

The most important hydrolytic enzymes in plant are amylase and protease. Balance between synthesis and proteolysis is the crucial issue that regulates growth and development in all organisms. In plants, protein degradation is essential in some developmental stages such as germination and cell biogenesis. Most investigation of allelopathic showed a highly increased in protease enzyme. Einhellig (1996) reported that this stimulatory effect of protease enzyme emitted from the interaction of the phenolic acids included in the aqueous extract. Antioxidative enzymes are the most important components in the scavenging system of reactive oxygen species (ROS). In which poly phenol oxidase (PPO) and peroxidase (POX) enzymes in turn was experienced in many environmental cases and to emphasize the effects. You can relate its exposure to the modification in genes expression affected by different biotic and abiotic stresses (Jaleel et al., 2009).

On the other hand phenylalanine ammonia-lyase (PAL) consider one of the most important enzymes because PAL is the principle enzyme in the phenyl propanoid pathway. Schuster and Retey (1995) reported that PAL involved in the biosynthesis of the polyphenol compounds such as flavonoids, lignin and coumarin in plants. Madany and Saleh (2015) reported that PAL and POX enzymes in pea seedlings showed a highly significant increase by increasing concentration of *Euphorbia helioscopia* treatments more than non-treated plant.

The main purposes of this research on allelopathy include the application of the observed allelopathic effects to agricultural production, reduction of the input of chemical pesticides and consequent environmental pollution, and provision of effective methods for the sustainable development of agricultural production and ecological systems (Jabran et al., 2015). The technological advances made in allelopathy research in recent years have been created, analyzed, and developed by scientific establishments throughout the world, they present exciting and intellectually challenging problems which are solvable using modern techniques (Cheema et al., 2013).

2. Material and methods

Pure strain of pea (*Pisum sativum* L.) seeds was kindly obtained from the Agricultural Research center, Ministry of Agriculture, Giza, Egypt. Wild plants as *Alhagi maurorum* was collected from the field located in EL-Qalyubiyah, Egypt. Seeds of pea under investigation were surface sterilized with 1% of sodium hypo-chloride for 5 min and washed thoroughly with sterilized distilled water. They were then soaked overnight in either (i) distilled water (control) or (ii) different concentrations of *Alhagi maurorum* aqueous extract (1.0, 5.0, 10, 15%, w/v). The seeds were sown in plastic pots (25 cm diameter X 30 cm depth) containing 10 Kg of a mixture of clay/sand (2:1, v/v) soil. Pots were kept under natural condition, with a day/night temperature of 25±2 °C. All pots were arranged in a randomized complete block design with ten replicates pretreatment. Plants were irrigated with tap water when needed to maintain an optimal soil moisture regime throughout the experiment.

Phenolic compounds of *Alhagi maurorum* was extracted according to the method obtained by Santana et al. (2013). A known dry weight of dried powdered sample was extracted by maceration with pure methanol in a closed container and darkness for eight days. The extract was evaporated until dryness in a rotary evaporator. The semi dry methanol crude extract was suspended in water. The crude extract was filtered separately through Whatman No. 41 filter paper to obtained dust free plant crude extract. The residue was re extracted twice follow the same and filtered. The residue was fractionated by chromatography column packed with activated silica gel (60 to 200 mesh).

The analysis was carried out using a GC (Agilent Technologies 7890 A) interfaced with a mass-selective detector (MSD, Agilent 7000) equipped with a polar Agilent HP–5 ms (5%-phenyl methyl poly siloxane capillary colum (30 m x 0.25 mm i. d. and 0.25 μm film thickness). The carrier gas was helium with the linear velocity of 1 ml/min. The identification of components was based on a comparison of their mass spectra and retention time with those of the authentic compounds and by computer matching with NIST and WILEY library as well as by comparison of the fragmentation pattern of the mass spectral data with those reported in the literature.

Pea plants were harvested after 45 days from sowing and their roots washed thoroughly with tap water to remove adhering soil particles. The plants were separated into roots and shoots. The lengths and fresh weights of roots and shoots of pea plants were measured. Plant samples were dried in an oven at 70 °C until constant weight for dry weight measurements. The dried samples were ground to a fine powder and kept under dry condition for biochemical analyses. Other fresh plant samples were quickly frozen and ground to fine powder in liquid nitrogen and stored at −80 °C until used for enzyme assay.

Total photosynthetic pigments were extracted and determined according to the method described by Fadeel (1962). The contents of chlorophylls a and b, as well as carotenoids were calculated using the Equations according to Sestak et al. (1971).

A known weight of dry powdered tissues was boiled in 10 ml of distilled water for one hour on a water-bath. After centrifugation, one ml of the clear extract was hydrolyzed with one ml of 2 N HCl in water-bath to obtain the total soluble reducing sugars. Total carbohydrate content was determined as known weight of dry powdered tissue was boiled in 10 ml of 6 N HCl on water-bath for 2 h. Reducing value of each sugar extract was determined according to the method adopted by Clark and Switzer (1977) using glucose standard curve. Subtracting the total carbohydrate content from the total soluble sugar content gave the insoluble carbohydrate fraction.

Free proline was extracted and determined according to Bates et al. (1973). The absorbance was recorded at 520 nm using toluene as a blank. The proline concentration was determined using a standard curve and calculated as μmol g^{-1} FW.

Protein determination was carried out according to the modified Folin-Lowry method adopted by Hartree (1972). The concentration of protein was determined using bovine serum albumin standard curve, then expressed as μmol g^{-1} FW.

Phenolic compounds were extracted according to the method outlined by Sauvesty et al. (1992). A known weight of the dried powdered tissues was extracted in 70% ethanol at 40 °C overnight. Each extract was centrifuged for 15 min at 3000 *g*. The clear supernatants were combined, then reduced under low pressure at room temperature, and made up to a known volume with distilled water, then used for determination of phenolic aglycones. This extract contained both phenolic aglycones and glycosides. One milliliter of this extract was hydrolyzed with 1 ml of 2 N HCl in a boiling water bath for 1 h. The mixture was then used for the determination of total phenolics. The Folin–Ciocalteu phenol method (Lowe, 1993) was used for phenolic aglycone determination. Subtraction of phenolic content after and before acid hydrolysis gave the content of phenolic glycosides.

Total flavonoid content was measured using aluminum chloride colorimetric assay (Sakanaka et al., 2005). Dry samples were extracted with methanol, then 0.25 ml of the clear extract was mixed with 1.25 ml of distilled water, followed by addition of 75 μl of 5% (w/v) sodium nitrite solution. After 6 min of incubation, 150 μl of 10% (w/v) aluminum chloride solution was added and the mixture was allowed standing for a further 5 min before addition of 0.5 ml of 1 M sodium hydroxide. The mixture was completed up to 2.5 ml with distilled water. The absorbance was measured immediately at 510 nm and the concentration of total flavonoids was calculated using standard curve of quercetin, then expressed as mg g^{-1} dry weight.

Amylase was extracted by homogenizing known fresh weight in 100 mM acetate buffer, pH 6.0, was used instead of the phosphate buffer. Amylase activity was measured by mixing 0.5 ml of the crude extract with 0.5 ml of 0.5% soluble starch prepared in 0.1 M of acetate buffer, pH 6.0, containing 5 mM $CaCl_2$.The reaction was terminated by $HgCl_2$ after 30 min of incubation at 40 °C. The resulting reducing sugars were estimated by the Nelson's method (Clark and Switzer, 1977).

For Protease extraction, 20 mM phosphate buffer, pH 7.6, with a prechilled pestle and mortar. Proteolytic activity was assayed using bovine serum albumin (BSA) as substrate. The reaction mixture contained 0.5 ml of the crude extract and 2 ml of the substrate solution (20 mM phosphate buffer, pH 7.0, containing 10 mg/ml BSA). After 60 min of incubation at 40 °C, the reaction was stopped by adding 2.0 ml of 10% trichloroacetic acid and heating briefly in boiling water to precipitate undigested albumin. After centrifugation, the concentration of the resulted soluble peptides was measured by the modified Folin-Lowry method adopted by Hartree (1972).

Extraction and assay of PAL (EC 4.3.1.5) were carried out as described by Solecka and Kacperska (2003). A known weight of liquid-nitrogen (LN) fresh powdered tissues was homogenized, in a mortar and pestle, with the extraction buffer (50 mM Tris–HCl buffer, pH 8.9; 5 mM EDTA; 5 mM ascorbic acid). The homogenate was centrifuged at 12,000 *g* for 20 min at 4 °C and the supernatant used as a source of crude enzyme for assaying PAL activity which was expressed as nmol *t*-cinnamic acid g^{-1} FW h^{-1}.

Sample preparation was described by Mukherjee and Choudhuri (1983). POX activity (EC 1.11.1.7) was determined using guaiacol. The reaction mixture composed of 10 mM phosphate buffer (pH 7.0, 10 mM H_2O_2, 20 mM guaiacol) and 0.5 ml crude extract in 3 ml (Malick and Singh, 1980). The increase in absorbance due to the dehydrogenation of guaiacol was monitored at 470 nm (Klapheck et al., 1990) using a spectronic 601 UV spectrophotometer. Enzyme activity is expressed as ABS unit g^{-1} FW h^{-1}.

Polyphenol oxidase (EC 1.14.18.1) was extracted as described by Kar and Mishra (1976) with slight modification. A known weight of LN-frozen tissues was homogenized with ice-cold 0.1 M phosphate buffer, pH 7.0, at 4 °C using a mortar and pestle. After centrifugation at 10,000 *g*, for 10 min at 4 °C, the clear supernatant used for PPO assay. Enzymatic activity was assayed using the method proposed by Nguyen et al. (2003). The assay mixture contained 0.5 ml of the crude enzyme extract and 2.5 ml of 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. PPO activity expressed as ABS unit g^{-1}FW h^{-1}.

2.1. Statistical analysis

All experiments were conducted in a completely randomized design. The computer program SPSS (version 18) was used for statistical analyses of studied parameters. All the data are presented as mean ± SE and subjected to analysis by one-way ANOVA followed by Duncan's multiple range test at $P>0.05$. Where needed, data were transformed by log $(x + 1)$ before statistical analysis.

3. Results

GC–MS analysis of methanolic extract of *A. maurorum* plant revealed qualitative and quantitative variations in its phenolic profile. Twenty-one phenolic compounds were identified and quantified and their peaks were represented in the chromatogram (Fig. 1) and listed in Table 1. These bioactive compounds include simple phenols: hydroquinone (1) and levorphanol (11) as well as phenolic acids: syringic (10), m-salicylic (18), vanillic (20) and mandelic acids (21). Additionally, some phenylpropanoids were found in the extract that include sinapyl alcohol (2), m-coumaric acid (4), 6-Hydroxyflavone (7), and 5,7,3',4'-Tetrahydroxyflavonone (17).

The GC–MS of the methanolic extract of *A. maurorum* shoot showed different phenolic compounds. Among these phenolics, hydroquinone (1) and sinapyl alcohol (2) that are the major components as their concentrations represent 33.3 and 25.8%, respectively. In addition, 3,5-di-t- butylcatechol (3), m-coumaric acid (4), phenol, 2,5-bis(1-methylpropyl) (5), phenol, 2-(1,1-dimethylethyl)–4,6-dimethyl (6), 6-hydroxyflavone (7), phloroglucino (8), phenol, 2,5-di-tert-butyl (9), cyanidin cation (13), scytalone (14), 3,4-dihydroxyphenylacetic acid (15), apigenin 7-glucoside (19) were found in moderate concentrations as their concentrations ranged from 1.07 to 5.31%. Moreover, syringic acid (10), levorphanol (11), hippuric acid, o-hydroxy (12), 4-tert-octyl-o-cresol (16), m-salicylic acid (18), vanillic acid (20) and mandelic acid, 3,4-dihydroxy (21) were less

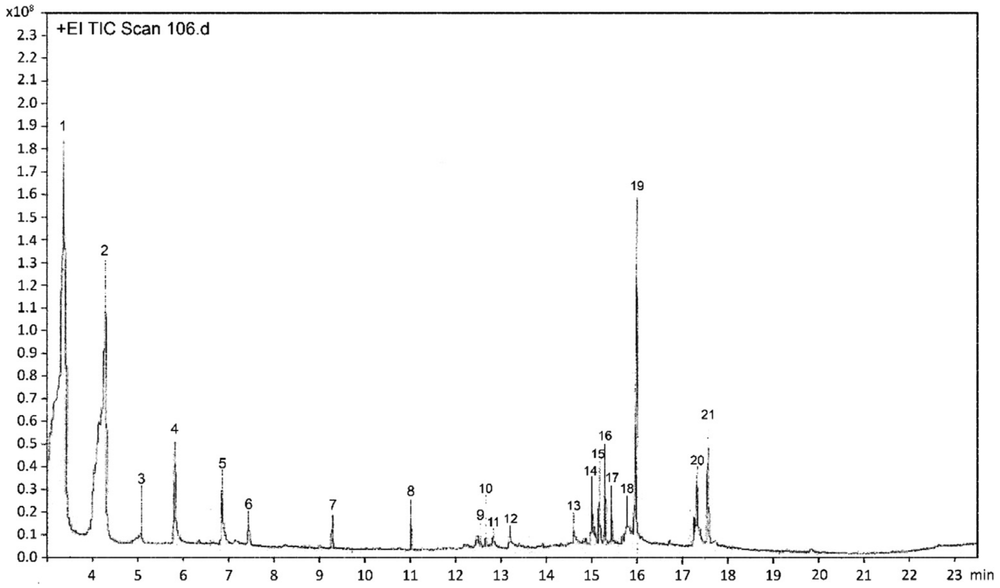

Fig. 1. GC/MS chromatograms of phenolic compounds of *Alhagi maurorum* aqueous extract.

Table 1
GC/MS Analysis of chemical constituents in *Alhagi mauroruma* aqueous extract. The results shown out of 21 phenolic compounds.

Peak NO.	RT (min)	Area%	Name
1	3.140	33.323654	Hydroquinone
2	4.260	25.766109	Sinapyl alcohol
3	5.037	1.10249	3,5-di-t- Butylcatechol
4	5.943	5.317698	m-Coumaric acid
5	6.768	1.912562	Phenol, 2,5-bis(1-methylpropyl)
6	7.493	3.473842	Phenol, 2-(1,1-dimethylethyl)−4,6-dimethyl
7	9.349	2.155763	6-Hydroxyflavone
8	11.242	1.695593	Phloroglucino
9	12.635	1.079657	Phenol, 2,5-di-tert-butyl
10	12.708	0.722286	Syringic acid
11	12.98	0.575606	Levorphanol
12	13.334	0.962996	Hippuric acid, o-hydroxy
13	14.675	1.235138	Cyanidin cation
14	15.081	1.523026	Scytalone
15	15.206	1.856977	3,4-Dihydroxyphenylacetic acid
16	15.692	0.456163	4-tert-Octyl-o-Cresol
17	15.949	11.1717	5,7,3',4'-Tetrahydroxyflavonone
18	16.682	0.404454	m-Salicylic acid
19	17.476	4.156624	Apigenin 7-glucoside
20	19.672	0.376131	Vanillic acid
21	23.737	0.741084	Mandelic acid, 3,4-dihydroxy

dominant or found in trace quantities represent only from 0.320 to 0.962% in the total phenolic profile.

The changes in growth criteria of 45-day-old *pisum sativum* plants in response to different concentrations of *A. maurorum* aqueous extract at the rates: 1, 5, 10 and 15%; (w/v) were recorded (Figs. 2 and 3). The obtained results showed that the height of shoot and root as well as their fresh and dry weights, number of leaves per plant, area of leaves per plant, fresh and dry weight of pods significantly decreased by increasing *A. maurorum* aqueous extract levels, the maximum reduction was observed in the plant subjected to 15% *Alhagi* concentration, as their values were decreased by 57.21%, 71.56% in length, 70.73%, 70.83% in fresh weight, 93.18%, 65% in dry weight for shoot and root respectively, 14% in number of leaves and 86% in area of leaves as compared with the untreated control plants. On the other hand, fresh and dry weight of pods decreased 100% as compared with non-treated plant. Our results showed that, the highest concentration of *Alhagi* aqueous extract 15% (w/v) suppress all growth criteria in tested pea plants as compared with the non-treated plant.

Carotenoids and total pigment contents of 45-day old pea plant were significantly reduced by increasing the concentration of *A. maurorum* aqueous extract level up to 15% (w/v) as compared with untreated control plants (Fig. 4). The highest inhibitory effect of *Alhagi* on carotenoids and total pigments were recorded in plant subjected to 15% (w/v) *Alhagi* aqueous extract and estimated by 56.72 and 64.1% respectively, as compared with untreated control plants.

The pattern of changes in the amount of various carbohydrate fractions in the shoots of 45-day-old pea plant treated with different concentrations of *A. maurorum* aqueous extract were investigated (Fig. 5A). The data clearly show that by increasing the concentration of *Alhagi* up to 15% (w/v) lead to very little drop rate is almost negligible in total carbohydrates content to about 4.53%, relative to the untreated plants. This response was mainly due to a significant increase in soluble sugar contents over the control plants. In which amount of increasing soluble sugars in treated pea plant under different concentrations of *A. maurorum* aqueous extract 1, 5, 10 and 15% (w/v) were 10.30, 15.88, 22.47 and 30.93% respectively, as compared with control plants. On the other hand, the insoluble sugar contents markedly decreased by raising *Alhagi* aqueous extract concentrations. The maximum inhibition in insoluble sugar was 26.01, 37.71 and 45.58% at 5, 10 and 15% (w/v) from *Alhagi* aqueous extract as compared with untreated plants. *A. maurorum* aqueous extract influences the protein content of *Pisum sativum* shoots (Fig. 5B). The amount of total protein decreased by raising *A. maurorum* aqueous extract up to 15% (w/v) that reached to 8.95% as compared to their respective control. In which 1% (w/v) from *Alhagi* aqueous extract has a slight undetectable decrease on pea plants the amount of decreasing was 2.19% relative to those of the control plant. This response mainly due to a significant increase in the soluble protein contents over the control pea plants. The amount of increasing in the soluble protein of pea plants treated with different concentrations of *Alhagi maurorum* extract 1, 5, 10, 15% (w/v) calculated by 17.5, 18.47, 28.34 and 33.44% respectively to control pea plant. On the other hand, insoluble protein in treated plant significantly decreased by increasing *A. maurorum* aqueous extract up to 15% (w/v) that reached to 38.29% as compared to their respective non-treated pea plants.

A highly significant increase in the phenolic aglycone content in pea shoots was observed by increasing the *Alhagi* concentrations up to 15% (w/v) that reached 82.97% (Fig. 5C). In addition, the phenolic glycoside content was progressively decreased by raising the *Alhagi* concentrations up to 15% (w/v), in which reduced by about 93.29% as compared to their respective control plants. This response reflected a lower significant depression of total phenolic contents in pea shoots as compared with control plants, the amount of decreasing in total phenolics due to treatment with *A. maurorum* concentrations 1, 5, 10, 15% (w/v) were 8.1, 15.15, 27.47 and 28.48% respectively, as compared with untreated control plants.

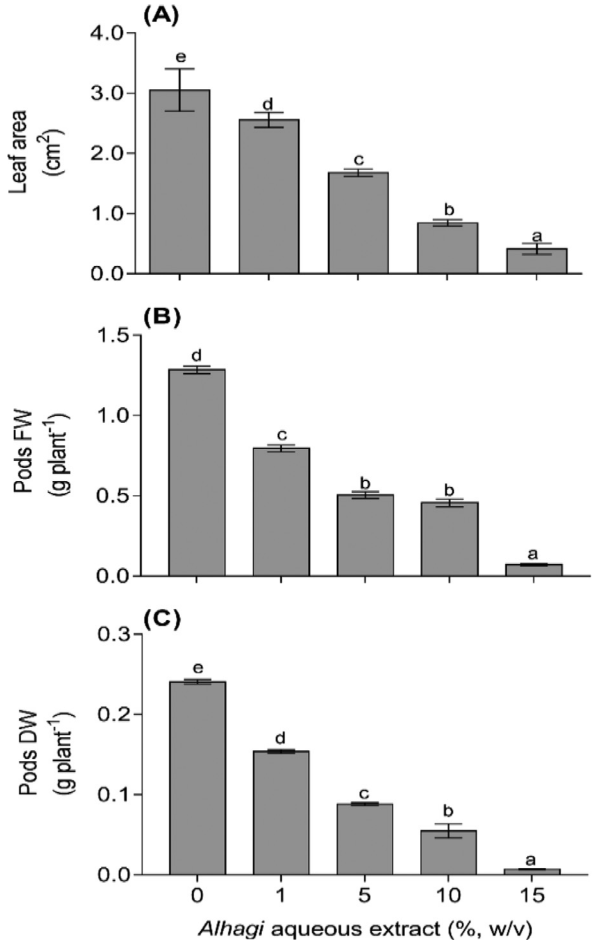

Fig. 2. Phytotoxic effect of different concentrations of *Alhagi* aqueous extract upon **(A)** height, **(B)** fresh weight biomass, **(C)** dry weight biomass of both shoot and root of 45-days-old pea plants. All the data are the mean of three replicates ($n = 3$), vertical bars show standard error (±SE) and same letters indicate no significant difference ($P \leq 0.05$) as analyzed by Duncan test (lower and upper-case letters are used for shoot and root sets, respectively).

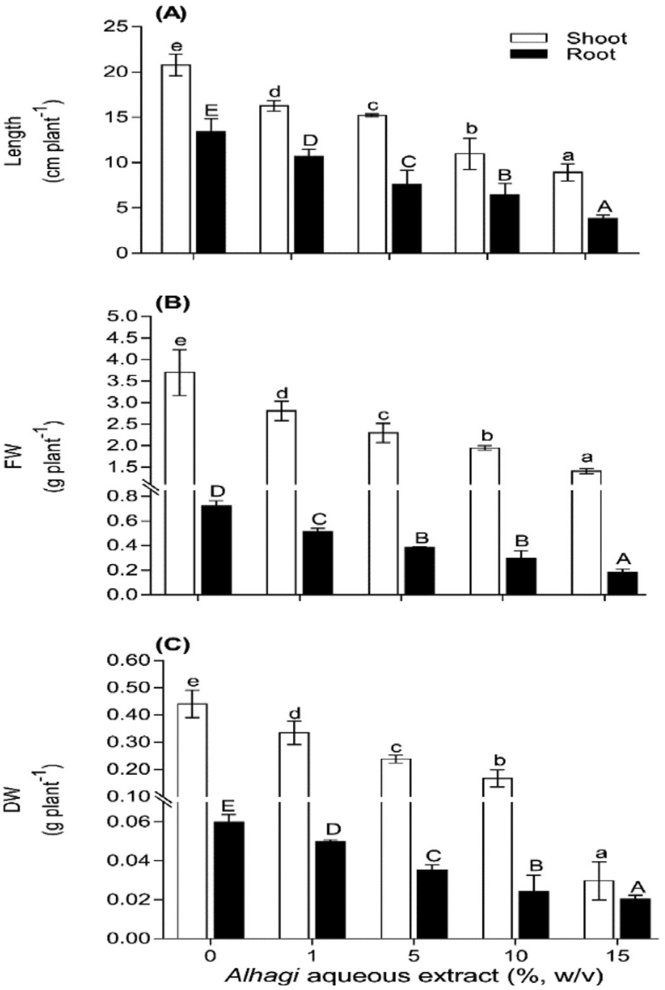

Fig. 3. Phytotoxic effect of different concentrations of *Alhagi* aqueous extract (**A**) leaf area, (**B**) pods fresh weigh, (**C**) pods dry weigh of 45-days-old pea plants. All the data are the mean of three replicates (n = 3), vertical bars show standard error (±SE) and same letters indicate no significant difference ($P \leq 0.05$) as analyzed by Duncan test.

Total flavonoids showed a marked accumulation in treated pea shoots under upon treatment with *Alhagi* aqueous extract (Fig. 6). Our data showed that different concentrations of *A. maurorum* aqueous extract caused a significant increase by about 50, 162, 281 and 337% for the concentrations of 1, 5, 10 and 15% (w/v) respectively, as compared with their untreated pea plants.

The change in proline content of pea plant in response to treatment with different concentrations of *A. maurorum* aqueous extract was studied (Fig. 5). Our data clearly demonstrated a highly significant accumulation of proline under different concentrations of *Alhagi maurorum* aqueous extract as compared to control plants. The maximum value was detected in plants treated with 15% (w/v) *A. maurorum* aqueous extract which was calculated by137% as compared with the control plants.

The activity of protease enzyme showed progressive, highly significant increase by raising the *Alhagi* concentrations up to 15% (w/v) and reached to about 75% as relative to the control plant (Fig. 7). On the other hand, other *Alhagi* treatments 1, 5 and 10% (w/v) recorded 15.63, 34.38 and 50% respectively, as compared to non-treated plants. Similarly, amylase activity in pea plants showed a noticeable increase in those treated with *A. maurorum* concentration, where the maximum value was observed at 15% (w/v) *Alhagi* treatment, which increased by more than 200% over the control plants. However, the plants treated with other concentrations of *Alhagi* aqueous extract 1, 5 and 10% (w/v) showed a marked increase in amylases activity by about 56.25, 139.5 and 191.7%, respectively relative to the non-treated plants.

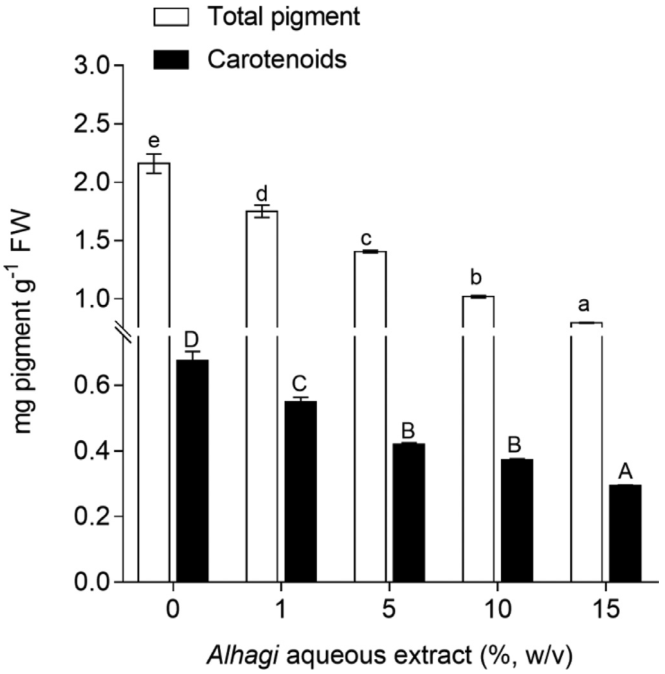

Fig. 4. Phytotoxic effect of different concentrations of *Alhagi* aqueous extract upon photosynthetic pigments (mg g^{-1} FW) of 45-day-old pea plants. All the data are the mean of three replicates (n = 3), vertical bars show standard error (±SE) and same letters indicate no significant difference ($P \leq 0.05$) as analyzed by Duncan test (lower and upper-case letters are used for total pigment and carotenoids, respectively).

The effect of different concentrations of *A. maurorum* aqueous extract on the activities of the antioxidant enzymes were investigated (Fig. 8). Regardless the lower rates of *Alhagi*, the activity of peroxidase (POX) showed a dramatic enhancement in response to treatment with higher concentrations of *A.maurorum* reaching about 48.27 and 55.17% at 10 and 15% (w/v), respectively (Fig. 8C). Also, *Alhag*i treatments caused a significant increase in both phenylalanine ammonia lyase (PAL) and polyphenol oxidase (PPO) enzymes over those of the non-treated pea plants (Fig. 8A and B). The maximum increment of PAL found at the highest concentrations of the aqueous extract 10 and 15% (w/v) that recorded about 113 and 153% for pea plants relative to their respective control. Similarly, PPO showed a marked improvement in response to treatment with *Alhag*i water extract. There was an increase in the PPO content upon raising the rate of aqueous extract. Where the rate of increases over control were about 19.23 and 34.62% respective to 1 and 5% (w/v) *A. maurorum* aqueous extract whereas the concentration 10% (w/v) gives 100% increases over control plants. the maximum enhancement observed at the highest rate of the extract 15% (w/v) where the rate of increases reached about 142% as compared with non-treated plants. The rate of increasing of antioxidant enzymes (PAL and PPO) exceeded 100% increase over the untreated control pea plants at the highest level of *A. maurorum* aqueous extract.

4. Discussion

The qualitative and quantitative analysis of phenolic profile of *Alhagi maurorum* extract revealed the presence of twenty-one phenolic compounds and some unidentified compounds. These bioactive phenolics in *A. maurorum* extract may be the cause of adverse effect on the growth of pea plants when treated with aqueous extract of hydroquinone and sinapyl alcohol that were present in relatively high concentration. Hydroquinone, represents 33% of our phenolic profile and considered as reducing agent that is soluble in water. As a

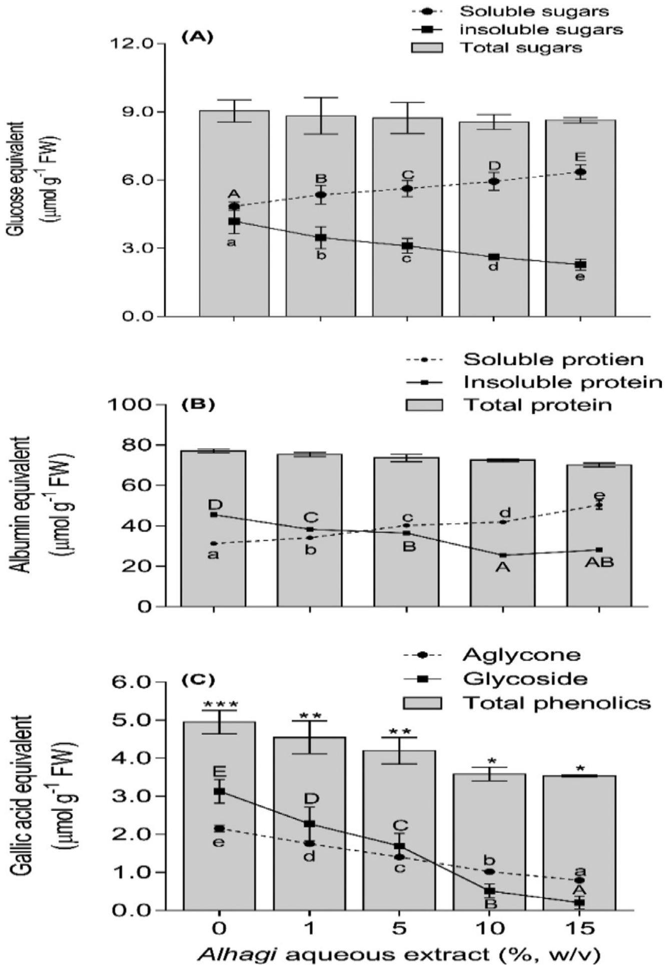

Fig. 5. Phytotoxic effect of different concentrations of *Alhagi* aqueous extract upon (**A**) sugar content, (**B**) protein content and (**C**) phenolic content of 45-day-old pea plants. All the data are the mean of three replicates ($n = 3$), vertical bars show standard error (±SE) and same letters indicate no significant difference ($P \leq 0.05$) as analyzed by Duncan test (lower and upper-case letters are used for different sets while asterisks used for the total phenolic set).

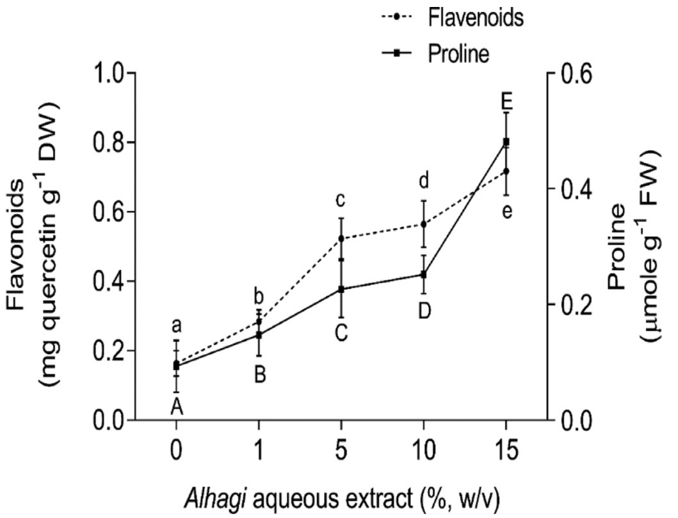

Fig. 6. Phytotoxic effect of different concentrations of *Alhagi* aqueous extract upon flavonoids and proline contents of 45-day-old pea plants. All the data are the mean of three replicates ($n = 3$), vertical bars show standard error (±SE) and same letters indicate no significant difference ($P \leq 0.05$) as analyzed by Duncan test (lower and upper-case letters are used for flavonoids and proline sets, respectively).

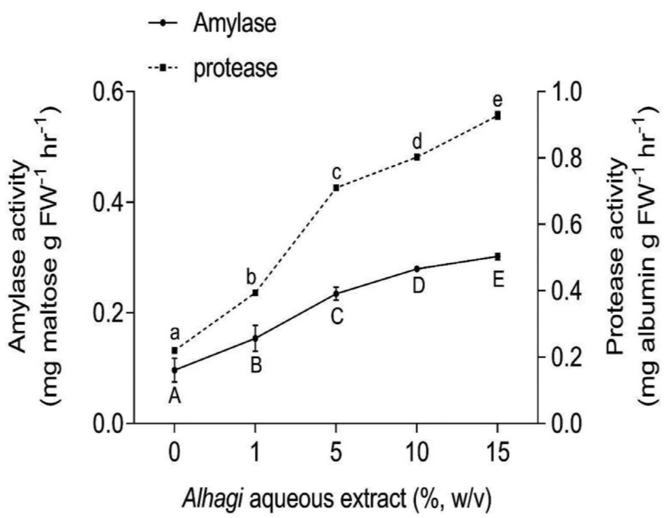

Fig. 7. Phytotoxic effect of different concentrations of *Alhagi* aqueous extract upon amylase and protease activity of 45-day-old pea plants. All the data are the mean of three replicates ($n = 3$), vertical bars show standard error (±SE) and same letters indicate no significant difference ($P \leq 0.05$) as analyzed by Duncan test (lower and upper-case letters are used for amylase and protease sets, respectively).

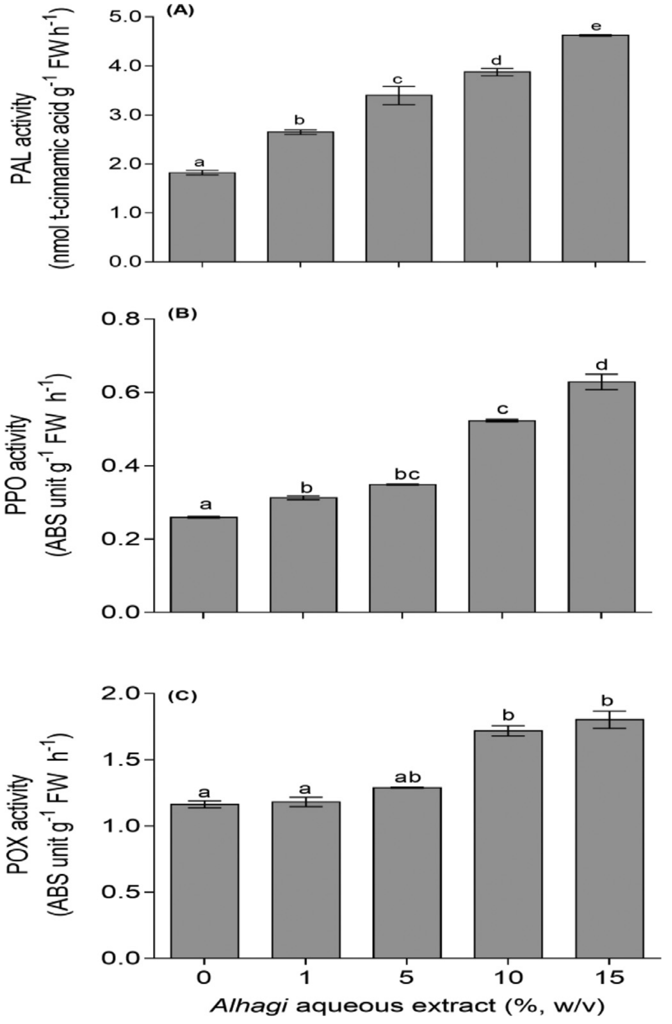

Fig. 8. Phytotoxic effect of different concentrations of *Alhagi* aqueous extract upon the activities of (**A**) PAL, (**B**) PPO and (**C**) POX of 45-day-old pea plants. All the data are the mean of three replicates ($n = 3$), vertical bars show standard error (±SE) and same letters indicate no significant difference ($P \leq 0.05$) as analyzed by Duncan test.

polymerization inhibitor, hydroquinone prevents polymerization of acrylic acid, methyl methacrylate, cyanoacrylate, and other monomers that are susceptible to radical-initiated polymerization Huang and Chou, 2017. Therefore, hydroquinone has a powerful antioxidant properties of and also play pivotal role in allelopathic interactions and showed regulation of biological activities of other plants and weeds (Kamran et al., 2017). In this context, 0.01 mM hydroquinone markedly retard the growth of *Charazeylanica* Willd. (Pandey et al., 2005). Similarly, our results showed that *A. maurorum* aqueous extract negatively affect both growth and biochemical parameters of pea plants. Additionally, sinapyl alcohol (about 25.8% of our phenolic profile; Table 1) is important component of plant metabolic pathways. Different concentration of *A. maurorum* aqueous extract strikingly retarded the growth of pea plants. This may be due to greater quantities of allelochemicals that interfere directly or indirectly with cell division, cell expansion, and growth-regulating substances. Phenolic compounds may either stimulate or inhibit germination and early seedling growth depending on the compound utilized, concentration and the test plant (Reigosa et al., 1999). Our results showed that high percentage of flavonoids in the shoot of pea plants treated with *A. maurorum* aqueous extract has the ability to negatively affect polar auxin transport which play significant role in plant hormones coordination and behavioral processes of plants life cycle (Brown et al., 2001). Decreasing in total carbohydrates reflect another reason for inhibition photosynthetic pigments in treated pea plants. Tejera et al. (2006) and Hussin et al. (2013) reported that the reduction in sugar content under osmotic stress may be lead to decrease of photosynthesis and photo assimilates in the aerial parts of plants. The decrease in insoluble and total carbohydrate contents, photosynthetic pigment contents and all the estimated growth parameters were directly proportional to the applied different concentrations of *A. maurorum* aqueous extract.

It was reported that under allelopathic stress, a delay in nutrient mobilization take place during early stage of seed germination (Mishra, 2015). In this context, it was reported that high concentration of fenugreek extract markedly retard nutrient mobilization by inhibiting the activities of both amylase and protease (Madany and Khalil, 2017) Singh et al. (2013) also reported a marked decrease in the number of leaves in *Parthenium hysterophorus* L. treated with 25% *Cassia occidentalis* L. In addition, this oxidative stress interferes with cell division, cell elongation, and phytohormone induced growth (John and Sarada, 2012; Mishra, 2015). Our results corroborate the findings of Fuad Mondal, et al. (2015) who revealed that number of pods and pod fresh mass decreased significantly when pea plants were under allelopathic stress conditions. In the present work, reduction in total photosynthetic pigments in pea leaves compared to control plants may be due to the accumulation of phenolic aglycone fraction that could interfere with the chlorophylls and carotenoids biosynthesis or their degradation (Delgado-Vargas et al., 2000). Moreover, this reduction in photosynthetic pigments could be due to the ability of *Alhagi* treatments to inhibit the production of phenolic glycosides that could enhance the biosynthesis or retard the degradation of these pigments (Delgado-Vargas et al., 2000). Additionally, the reduction in these pigments could also attributed to the increased leaf resistances to CO_2 transport under both biotic or abiotic stresses (Evans et al., 2009). In addition, the decrease in chlorophyll contents in pea plants treated with *Alhagi* extract was concomitant with a noticeable decrease in proline level. This correlation may support the suggestion that nitrogen may be toward synthesizing proline as osmoregulator instead of chlorophylls (De La Rosa-Ibarra and Maiti, 1995).

Phenolics and flavonoids compound are of great interest to scientist's due to their pivotal role in plants such as pigmentation, growth and reproduction. Moreover, phenolics and flavonoids are considered as secondary ROS-scavenging system in plants protecting them against various environmental disturbances (Fini et al., 2011). Our study revealed that total phenolics significantly decreased through decreasing the levels of phenolic glycoside and increasing level of free aglycone in pea shoots treated with *Alhagi*. This reduction could be attributed to inhibition of phenolics biosynthesis and glycosylation that in turn led to accumulation of phenolic aglycones (phytotoxic). In this context, Al-Wakeel et al. (2007), reported an inhibitory effect on total phenolic compounds in 45-day-old pea plants treated with *Acacia nilotica* leaf residue. On the other hand, an improvement in the levels of flavonoids in the pea shoots was treated with lower and higher rates of *Alhagi* aqueous extract was recorded. This improvement in flavonoids could be due to the implementation of allelochemicals that induce changes in plant secondary metabolism, particularly in the biosynthesis of flavonoids by enhancing the activity of biosynthetic enzymes like phenylalanine ammonia lyase (PAL), a key enzyme in phenylpropanoid pathway, suggesting a shift from sucrose production to defense and repair processes (Cheynier et al., 2013).

Proline accumulation in pea plants increased significantly with treatment of *A. maurorum* aqueous extract over the control plant. Barakat and Nazeir (2016) reported significant increase in proline contents of the test species under the effect of aqueous extract of *Calotropis procera* as compared with control plants. Proline is the most common stress marker and its accumulation induces leniency and protection against both biotic and abiotic stresses (Al-Wakeel et al., 2013). Moreover, proline protects proteins from denaturation and enzyme protection in plant metabolism (Singh and Rao, 2003). Moreover, proline can act as a reservoir of nitrogen and carbon sources (Fukutoku and Yamada, 1984) and/or radical scavenger that protects cells against oxidative damage (Hong et al., 2000). Proteins considered as vital precursor for active development processes occurring during germination and growth. Our results revealed a reduction in total protein levels especially insoluble protein that are associated with increased activity of protease enzyme in pea shoots. This response may shed the light upon the ability of phenolic phytochemicals to interfere with the activity and function of hydrolytic enzymes, the enhancement in protease enzyme activity indicates its role in facing the stress induced by *Alhagi* aqueous extract, which in turn lead to great breakdown of proteins in which required to afford precursors for active development processes occurring during germination and growth. In accordance with these results, Madany and Saleh (2015) reported that protease activity was markedly improved upon treatment with *Euphorbia helioscopia* water extract, which in turn cause a significant reduction in protein levels.

The activities of antioxidant enzymes showed a noticeable enhancement in pea shoots under treatments with *Alhagi* extract. The stimulation of phenylalanine ammonia lyase (PAL), the key enzyme in phenylpropanoid pathway, could explain the accumulation of flavonoids in pea shoots (Schusterand Retey, 1995). Moreover, Cheynier et al. (2013) stated that improving activity of some antioxidant enzymes including PAL directed the plant metabolism toward protection and resistance instead of synthesis of sugars. Therefore, it considered another reason in a significant decrease in total carbohydrates in pea plants under effects of *Alhagi* water extract (w/v,%) as compared to control. In this respect, Madany and Saleh (2015) reported that activity of PAL significantly increased in wheat and pea seedlings under all *Euphorbia helioscopia* treatments. Moreover, fenugreek extract significantly enhance the activity of PAL in both faba bean and maize seedlings (Madany and Khalil 2017).

The enhanced production of reactive oxygen species (ROS) would accelerate the ROS scavenging enzymes, especially polyphenol oxidase (PPO) and peroxidase (POX). Antioxidative enzymes, such as peroxidases (POX) and polyphenol oxidase (PPO) are the most important components in the ROS scavenging system (Lee et al., 2002). It has been found a close relationship between the enhanced activities of polyphenol oxidase, peroxidase and the concentration of phenolic substances (Saiedian et al., 2007). Under stress condition a number of antioxidative enzymes increased massively to prevent the oxidative damage caused by ROS (Singh et al., 2009b). In the present study, a significantly enhancement peroxidase activity was recorded. POX are therefore probably the most important enzymes in the detoxification of H_2O_2 despite the increase of POX activity works to protect the plant from H_2O_2. At the same time have a negative effect on growth rate of 45-day-old pea plants treated with different concentrations of *A. maurorum* aqueous extract and is further evidence in causes of the slow growth rate of the treated peas plant. Lin and Kao (2000) reported that high levels of POX activity in condition of saline stress are correlated with a more reduced growth of plants and it appeared that POX activity caused the reduction in the growth of aerial parts of plant, rather than in protecting tissues against the accumulation of H_2O_2 because of interfering with photosynthetic activity in chloroplasts, and thereby reduces plant growth (Takeda et al., 1995). Devi and Prasad (1992) demonstrated that ferulic acid (1 mM) stimulation POX activity. Also, Soares et al. (2011) showed that POX activities were significantly increased by approximately 43% under effect of the L-3,4-dihydroxy-phenylalanine (L-DOPA) concentration, when compared to control plant. The phenolic compounds under stress conditions are stored in crop plants, the oxidation and breaking of these poisonous constituents happen through the activity of polyphenol oxidase (PPO). Phenolic compounds are supposed to be of significant important to increase plant resistance. Polyphenol oxidase (PPO) has been described to increase the biosynthesis of these phenolic compounds (Vaughn and Duke, 1984) By raising the activity of PPO, the oxidation of phenolic compounds will be enhanced. Our results showed that for increasing flavonoids and both antioxidant enzymes (PAL and POX). In which, PAL is considered the first rate-limiting enzyme of this phenylpropanoid pathway and POX the last enzyme that leads to lignin synthesis.

5. Conclusions

We concluded that allelopathic effects of the aqueous extract of the *A. maurorum* shoots inhibited the growth of pea (*Pisum sativum*) seedling. This inhibition was concentration-dependent. The phytochemical analyses revealed the presence of several phenolic compounds with a high percent in phenolic profile like hydroquinone and sinapyl alcohol. They were considered as inhibitory compounds for plant growth that had a clear and effective effect on many harmful field weeds. Therefore, these natural products could be used as “eco-friendly” bio-herbicides for weed control.

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Declaration of interests

None.

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