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BIOCHEMICAL ALTERATIONS IN WHEAT SEEDLINGS AND SOME WEEDS RELATED TO ALLELOPATHIC POTENTIAL OF SOME MEDICINAL PLANTS

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Abstract

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The present study was conducted to investigate the allelopathic effects of some selected medicinal plants (*Thymus vulgaris, Salvia officnalis* and *Calendula officinalis*) on seeds germination and seedling total biomass of wheat (*Triticum aestivum* L.) and their associated weeds, *Lolium. multiflorum* and *Phalaris paradoxa* by aqueous extracts at (0, 20, 40, 60 mg DW ml^{-1}) concentrations under laboratory conditions. The extracts of *C. officinalis* subterranean parts had ED_{50} values for *L. multiflorum* by (20 mg ml^{-1}) root length, *P. paradoxa* by (15 mg ml^{-1}) dry weight, *T. aestivum* by (23 mg ml^{-1}) root length. *T. vulgaris* vegetative extracts had ED_{50} values for *L. multiflorum* by (30 mg ml^{-1}) and *P. paradoxa* by (30.5 mg ml^{-1}) root length. The change in mineral content increase by *C. officinalis* and *T. vulgaris* than *S. officnalis* aqueous extracts as compared with untreated control. The highest reduction in IAA content of *T. aestivum* seedling achieved from *T. vulgaris* subterranean parts extract by (81.8 %), ABA and GA content from *C. officnalis* vegetative extracts by (78.3 and 421%) at concentration 60 mg ml^{-1} than the untreated control. The addition of C. *officnalis* and *T. vulgaris* vegetative extracts at 20 mg ml^{-1} increased *T. aestivum* seedlings anthocyanin contents by (21.8 and 20.0%) and total proteins by (23.2 and 28.5)%, respectively, than its control. The highest inhibitory effect achieved from *C. officinalis and T. vulgaris* vegetative and subterranean parts extract at 40 mg ml^{-1} reduced tyrosine biosynthesis of *T. aestivum* seedlings by (92.2, 92.2, 86.2 and 89.6%) respectively compared to the control. The obtained results proposed that *C. officinalis* and *T. vulgaris* extracts have herbicidal properties that may provide an alternative to synthetic herbicides for their ability to suppress weed germination and seedling growth in wheat field, but in a limited use especially the highest dose and its prefer to use this extracts in pre emergence stage to prevent weeds emergence and to avoid the adverse effect of allelopathic compounds on wheat seedling and yields.

Key words: allelopathy, mineral content, phytohormones, anthocyanin and amino acids

Introduction

Wheat (*Triticum aestivum* L.) is the most important staple food crop for more than one third of the world population. In Egypt, it can be found in wheat fields, representing the dominant grass weeds, and can be noticed easily in wheat fields, especially in late season, with its long culm and characteristic panicle. Weeds are the plants, which interfere with agricultural operations, compete with crop plants for light, water, nutrients and space, reduce the crop growth, and yield (Rao, 1992) by releasing phytotoxins as leachates, exudates and volatiles and products. As many as 37 species of harmful weeds grow in wheat field in different cropping systems, the most troublesome being *Phalaris minor, Chenopodium album, Convolvulus arvensis, Avena fatua* etc., (Khaliq et al., 2012). *Phalaris* sp., is an annual weed, which can reduce quantitative and qualitative properties of winter crops (Sing et al., 1999). In addition, *Lolium multiflorum* is a vigorously competitive species as such, many attempts to have been made to establish its yield-reducing potential in wheat. The application of herbicides has been a major factor enabling the intensification of agriculture in past decades. Indeed, three million tones of herbicides per year are used in most agricultural systems (Stephenson, 2000). There has been increasing herbicide resistance in weeds and

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widespread concern about adverse environmental effects from herbicide use, (Stephenson, 2000). For this reason, the use of allelopathic plants may provide an alternative to minimize the risk towards agroecosystems by serving in a complementary way with herbicides. Studies have shown a great potential of allelopathy for weeds control in wheat. It is the best alternatives to the synthetic herbicides to control weeds (Bhowmik and Inderjit, 2003; Jabran et al., 2008). These allelopathic compounds can also be used as natural herbicides and other pesticides (Einhelling, 1995). Allelopathy has been broadly defined as the direct or indirect stimulatory or inhibitory influence of one plant on another, through the production of chemical compounds (allelochemicals) that escape into the environment. These compounds can regulate such interactions within and among species in plant communities (Fernandez, et al., 2008). Various parts of same weed have different allelopathic effects on germination and growth of crop (Aziz et al., 2008). Allopathic compound not only reduced germination, but also delayed germination that was affecting seedling greater (Escudero et al., 2000). Allelochemicals may inhibit shoot/root growth and nutrient uptake, (Qasem, 1995), and soluble protein contents, (Rice, 1984). In addition, these compounds exhibit a wide range of mechanisms of action, affect on phytohormone activity (Einhellig, 2002). The present investigation was undertaken to examine allelopathic effect of three dominant medicinal plants species (*Thymus vulgaris, Salvia officnalis* and *Calendula officinalis*) against some weeds (*L. multiflorum and P. Paradoxa*) germination, seedling growth parameters and biochemical aspects of wheat crops (*T. aestivum*).

Materials and Methods

Plant materials: Mature plants of three commonly medicinal plants species: (*Thymus vulgaris, Salvia officnalis and Calendula officinalis*) were collected from North Sinai Egypt. These whole plant samples were collected by hand pulled or by cutting them using a manual cutter at soil level. Plant taxonomist at Desert Research center according to Tâckholm (1974), identified plant specimen. All the plants were gently washed of dust and attached debris using tap water. All the plant samples were ground into powder with a grinder and kept in paper bags under room temperature. Wheat Giza-193 cultivars were obtained from Agriculture Research Center, Cairo Egypt. Little seed (*Phalaris Paradoxa*), ryegrass (*Lolium multiflorum*) seeds were collected from wheat field during 2010 at El Farafra Oasis, Egypt.

Experimental details

Ten grams of air-dried ground tissue were extracted with 100 ml double distilled deionized water using a rotary shaker for five hours at 25°C. The aqueous extract solutions were made from each sample by dividing it into (vegetative parts and subterranean parts). The mixture was filtered through two layers of cheesecloth to remove debris, and centrifuged for 10 min at 3500 rpm and finally through whatman #4 paper. The filtrate was considered 100 gram dry wt. /liter solution, and diluted to different concentration 0, 20, 40, 60 (mg dry wt. ml^{-1}) using distilled water and kept in the refrigerator at 4^0C until treatments. Seeds were surfaces sterilized using sodium hypochlorite (0.3% v/v) for 10-12 min and washed four times in sterile double-distilled water, and then ten seeds of wheat were put separately in Petri dishes of 9cm diameter containing three layers of Whatman No.1 filter paper. Five mL of the prepared extract was applied to each Petri dish while distilled water was applied to the Petri dishes containing the control treatment. The experiment was run at 25±3°C temperature. The experiment was regularly visited and the extracts were added when needed. The ED_{50} values for each growth parameter were calculated by plotting concentration on a log scale (X) and the response (Y) on probit scale mathematically transformed, the data appeared linear and sign the point in a semi-log graph paper.

Parameters recorded: Growth Parameters after seven days of the experiment, the plants with various treatments were collected to estimate the fresh weights of seedling total biomass, roots and shoot length. The samples were oven dried at 70°C for 72 h. and the dry weights of total biomass were determined.

Biochemical aspect analysis

Measurement of minerals concentration: The concentration and total uptake of micronutrients (Manganese Mn, Zinc Zn, Iron Fe, and Copper Cu) and macronutrients (Potassium K and Sodium Na) in wheat plants and associated weeds (*Lolium. multiflorum* and *Phalaris Paradoxa)* were determined by Atomic Absorption(UNICAM 929 A A spectrometer) sing standard method described by (Cottenie et al., 1982).

Determination of phytohormones: Extraction and estimation of phytohormones were carried out according to the method of Unyayar et al. (1996). Indol 3-acetic acids (IAA), Gibberellic acid (GA), Abscisic acid (ABA) were analyzed. Five gram fresh weight samples were placed in 100 ml methanol: chloroform: 2 N ammonium hydroxide (12:5:3 v/v/v) and homogenized using a Kinematic Polytron Homogenizer. After the addition of 1 µg/100 ml Butylated Hydroxytoluene (BHT), the samples were frozen at -80°C for one week, for further analysis. Then, the extracts were transferred into 250 ml conical flasks and 22.4 ml bi-distilled water was added. To obtain a homogeneous mixture, the conical flasks were shaken three or four times. Thus, with the exception of plant

growth substances, the other organics in methanol were allowed to pass into the chloroform phase. HPLC system equipped with quaternary pump LPG3400SD, a WPS 3000 SL analytical auto sampler, and a DAD-3000 photodiode array detector (HPLC Ultimate 3000Thermo Dionex, Germany) analyzed phytohormones. Samples were run on an analytical column (150× 4.6mm 5µGOLD aQ Hypersil Gold column) using gradient elution. The mobile phase consisted of 0.1% (v/v) acetic acid in water (Soln. A), methanol (Soln. B) using the following linear gradient: 50% to 90% C over 20 min. The flow rate of the mobile phase was 0.70 ml min^{-1} and the injection volume was 20 µl with UV at λ_{max} 245 nm. The extraction, purification and quantitative determination of total IAA, GA3 and ABA were done according to literature methods of Unyayar et al. (1996).

Determination of total Anthocyanins content and total proteins: Fresh samples were homogeneous with 12 mL of 1% (w/v) HCl in methanol for 2 days at 3 to 5°C with continuous shaking. The samples measured at 530 and 657 nm and anthocyanin concentrations calculated by means of (Mancinelli et al., 1975). Protein content was estimated by using Bradford method (1976).

Quantitative determination of total amino acids: Total amino acid composition of wheat seedling was determined by amino acid analyzer apparatus model "INSTRUMENT MODEL: AAA400" using the method of Csomos and Simon-Sarkadi (2002). Acid hydrolysis: A known weight of wheat seeding powder was defeated with soaking in diethylether overnight to remove any fats, pigments and impurities in the samples to be clear. A known weight (0.3 g) of defeat plant material received 10 ml 6 N hydrochloric acid in a sealed tube, and then placed in an oven at 110°C for 24 hours. Hydrolyzates were transferred quantitatively into a porcelain dish and the hydrochloric acid was then evaporated to dryness at 50-60°C on a water bath. Distilled water (5 ml) was added to the hydrolyzate and then evaporated to dryness to remove the excess of hydrochloric acid and finally the residue was dissolved in 10 ml distilled water and filtrate through a 0.45 mm filter. The filtrate was dried under vacuum with a rotary evaporator, then 10 ml of distilled water was added and the samples dried a second time. One ml of 0.2 N sodium citrate buffers at pH 2.2 was added and the samples stored frozen in a sealed vial until separation of amino acids by the amino acid analyzer. Separation of amino acids by amino acid analyzer: Samples of amino acids were injected in amino acid analyzer (AAA400). Each amino acid is separated at specific pH, and then colored by a reagent named Ninhydrin. Ninhydrin (triketohydrindene hydrate) is an oxidating agent, which leads to the oxidative deamination of alpha-amino groups. It is very important for the detection and the qualitative analysis of amino acids. Ninhydrin also reacts with primary amines, however the formation of carbon dioxide is diagnostic for amino acids. Alpha amino acids yield a purple substance that absorbs maximally at 570 NM. Amino acids (Proline) yield a yellow product (absorption maximum 440 nm).

Phytochemical screening: The promise plant parts were analysis by introduced to the following tests: crude fiber contents (Maynard, 1970), total carbohydrate (Herbert et al., 1971), total tannins (Balbaa et al., 1981), total polyphenols (Folin and Denis, 1915), terpenoids (Edeoga et al., 2005), saponins (Hostettmann et al., 1991), and for alkaloids (Woo et al., 1977) Also, testing of flavonoid and phenolic compounds were done according to (Edeoga et al., 2005).

Experimental site and design: Data were statistically analyzed by ANOVA, according to Snedecor and Cochran (1990) and treatment means were compared by LSD test at 5% level of probability. The experimental designs used were randomized with a complete block design and each treatment had three replications and has been repeated independently at twice.

Results and Discussion

Bioactivity of aqueous extracts on wheat and some associated weeds germination and seedling growth

Effects of different concentrations of (*T. vulgaris, S. officnalis* and C. *officinalis*) extracts on seed germination, seedling growth and seedling dry weight of *T. aestivum, L. multiflorum and P. Paradoxa* were shown in (Tables1-3). Aqueous extracts of *C. officinalis* vegetative extracts added to *L. multiflorum* at 60 mg ml^{-1} decreased seed germination by 68.9% relative to water treated controls (Table 1). Seedling root and shoot length and dry weight was significantly (p=0.05) decreased at a maximum tested concentration of 60 mg ml^{-1} by 77.4%, 90.4% and 50% respectively. Maximum concentrations of 60 mg ml^{-1} were required to be inhabited *P. Paradoxa* growth parameters completely. The aqueous extracts of *C. officinalis* vegetative parts at the 60 mg ml^{-1} concentration were significantly phytotoxic to seedlings of *T. aestivum,* inhibited the root (95.9%), and shoot (99.2%) length as compared with the control. *C. officinalis* vegetative extracts had ED_{50} values for *L. multiflorum by* (40 mg ml^{-1}) germination, (30 mg ml^{-1}) root length, (42.5 mg ml^{-1}) shoot length and (30 mg ml^{-1}) dry weight respectively. The ED_{50} values for *P. Paradoxa* by (38 mg ml^{-1}) germination, (40 mg ml^{-1}) root length, (31 mg ml^{-1}) shoot length respectively. In addition, the ED_{50} values for *T. aestivum* by (25 mg ml^{-1}) root length, (27 mg ml^{-1}) shoot length and (28 mg ml^{-1}) fresh weight respectively. *C. officinalis* subterranean extracts had ED_{50} values for *L. multiflorum* by (58 mg ml^{-1}) germination, (20 mg ml^{-1}) root

length (44 mg ml^{-1}) shoot length and (39 mg ml^{-1}) dry weight respectively. The ED_{50} values for *P. Paradoxa* by (28 mg ml^{-1}) germination, (18.5 mg ml^{-1}) root length, (29 mg ml^{-1}) shoot length (18 mg ml^{-1}) fresh weight and (15 mg ml^{-1}) dry weight respectively. Finally, the ED_{50} values for *T. aestivum* by (38 mg ml^{-1}) germination, (23 mg ml^{-1}) root length, (39 mg ml^{-1}) shoot length and (39 mg ml^{-1}) dry weight respectively.

Aqueous extracts of *T. vulgaris* vegetative extracts at 60 mg ml^{-1} decreased *L. multiflorum* seed germination by 67.9% relative to water treated controls, Seedling root and shoot length and dry weight was significantly (p=0.05) decreased at a minimum tested concentration of 20 mg ml^{-1} by 36%, 24.5% and 66.6% respectively (Table 2). Minimum concentrations of 20 mg ml^{-1} were required to significantly reduce the root length (32.1%) of *P. Paradoxa*. The aqueous extracts of *T. vulgaris* were significantly phytotoxic to seedlings of *T. aestivum* at the 60 mg ml^{-1} concentration, inhibited root (53.6%), and shoot (28.3%) length, germination (46.7%) and fresh weight by (73.9%) as compared with the control. *T. vulgaris* vegetative extracts had ED_{50} values for *L. multiflorum* by (41 mg ml^{-1}) germination, (30 mg ml^{-1}) root length, (44 mg ml^{-1}) shoot length and (30 mg ml^{-1}) dry weight respectively.

Table 1
Effect of *C. officinalis* aqueous extracts on some plant growth parameters

	L. multiflorum					*P. Paradoxa*					*T. aestivum*				
	Concentration (mg ml^{-1})														
	0	20	40	60	LSD 0.05	0	20	40	60	LSD 0.05	0	20	40	60	LSD 0.05
(vegetative parts)															
Germination	9.67	8.00	7.67	3.00	1.89	9.66	8.66	4.66	0.00	1.95	8.33	9.66	10.0	6.33	NS
Shoot length, cm	5.00	4.67	4.33	1.13	0.56	4.23	3.97	2.06	0.00	1.09	13.83	11.16	4.16	0.56	5.32
Root length, cm	6.30	3.33	2.50	0.60	2.22	3.16	1.66	0.16	0.00	2.27	16.66	8.16	3.06	0.13	11.54
Fresh weight, gm	0.45	0.14	0.18	0.05	NS	0.27	0.16	0.03	0.00	NS	2.56	1.27	0.81	0.13	1.34
Dry weight, gm	0.02	0.05	0.03	0.01	0.002	0.05	0.06	0.02	0.00	0.02	0.12	0.02	0.11	0.06	NS
Subterranean parts															
Germination %	9.00	6.00	5.33	4.33	0.98	9.67	5.33	2.33	0.00	2.42	9.67	6.00	4.67	1.67	2.69
Shoot length, cm	6.17	5.50	2.50	1.90	1.17	5.47	3.07	1.77	0.00	2.33	10.50	7.33	2.83	3.83	3.82
Root length, cm	6.17	1.67	0.43	0.17	2.14	3.17	0.50	0.13	0.00	0.25	13.00	4.00	2.17	2.17	4.86
Fresh weight, gm	0.38	0.20	0.18	0.10	NS	0.18	0.03	0.01	0.00	NS	0.95	0.80	0.28	0.14	NS
Dry weight, gm	0.06	0.03	0.04	0.01	0.041	0.05	0.01	0.00	0.00	0.03	0.07	0.17	0.04	0.02	0.04

Table 2
Effect of *T. vulgaris* aqueous extracts on some plant growth parameters

	L. multiflorum					*P. Paradoxa*					*T. aestivum*				
	Concentration (mg ml^{-1})														
	0	20	40	60	LSD 0.05	0	20	40	60	LSD 0.05	0	20	40	60	LSD 0.05
(vegetative parts)															
Germination	8.33	5.33	4.00	2.67	1.57	8.00	7.00	3.33	2.00	2.64	10.00	10.00	6.33	5.33	3.71
Shoot length, cm	6.67	5.03	3.10	2.40	0.82	4.00	3.17	1.67	1.00	1.20	12.33	13.83	10.67	8.83	1.67
Root length, cm	7.33	3.90	2.10	1.30	1.95	4.17	2.83	1.17	0.27	0.54	15.83	17.50	11.50	7.33	4.19
Fresh weight, gm	0.13	0.08	0.08	0.06	NS	0.21	0.12	0.08	0.05	NS	2.53	2.81	2.75	0.66	1.06
Dry weight, gm	0.06	0.02	0.01	0.03	0.02	0.02	0.02	0.03	0.02	NS	0.12	0.13	0.18	0.09	NS
Subterranean parts															
Germination	8.67	6.33	5.50	5.33	2.61	9.00	7.67	7.67	6.33	2.25	10.00	10.00	9.00	7.33	NS
Shoot length, cm	6.50	5.17	4.50	2.77	3.37	4.17	2.83	2.63	1.93	1.26	11.67	14.00	13.67	8.00	2.36
Root length, cm	6.33	5.17	3.83	1.77	2.59	3.77	1.63	1.43	1.20	0.67	13.17	14.00	16.67	9.00	3.49
Fresh weight, gm	0.27	0.20	0.14	0.12	0.12	0.13	0.09	0.09	0.08	0.03	1.57	1.86	2.05	1.71	NS
Dry weight, gm	0.05	0.04	0.03	0.04	NS	0.02	0.01	0.02	0.02	NS	0.20	0.16	0.17	0.09	0.037

The ED_{50} values for *P. Paradoxa by* (40 mg ml^{-1}) germination, (30.5 mg ml^{-1}) root length, (39 mg ml^{-1}) shoot length respectively. *T. vulgaris* subterranean extracts had ED_{50} values for *L. multiflorum* by (45 mg ml^{-1}) root length, (55 mg ml^{-1}) shoot length and (49 mg ml^{-1}) fresh weight, respectively. The ED_{50} values for *P. paradoxa* by (34 mg ml^{-1}) root length, (53 mg ml^{-1}) shoot length respectively. In the presence of *S. officnalis* vegetative extracts at 20 mg ml^{-1} activated *T. aestivuim* significantly (p=0.05) seedling root length, shoot length, fresh and dry weight by 20.6%,9.0%, 29.9% and 108.3% respectively as compared with the control. Minimum inhibition concentration of *S. officnalis* vegetative extracts *was* 60 mg ml^{-1} required to inhabited significantly (p=0.05) *T. aestivum* germination (46.7%), root (77.3%) and shoot (61.7%) length, fresh weight (72.1%) and dry eight (85.3%) relative to water treated controls.

Medicinal plant extracts decreased the germination (%) in all weeds investigated except at *S. officnalis* extract which was low in comparison with the control sample (Table 3). The radical length (cm) was significantly reduced with all the medicinal plant extracts except at *S. officnalis* extract against weeds seedling, which showed no significance in comparison with the control sample. Also, the application of different medicinal plant extracts reduced both the radical and plumule fresh and dry weight (mg) in all the varieties except *S. officnalis* extract against weeds seedling there is no significance different in compared to control. Maximum inhibition was shown by *T. vulgaris* on *T. aestivum, L. multiflorum and P. paradoxa* seed germination, seedling length and seedling fresh and dry weight by using 60 mg ml^{-1} concentration from vegetative parts or subterranean parts. However, the minimum inhibition concentration was 20 mg ml^{-1}. Concerning the germination rate, data of all target species demonstrated a significant degree of suppression and a negative response to the increasing concentration of different medicinal plant extracts. In addition, there were significant differences between the test treatments and control. Suppressive effect was increased with an increase in extract concentration indicating that the effect of plant extracts depends very much on their concentration. Similar observation was found by (Ballester et al., 1982) and (Turk et al., 2003). The germination inhibition may be due to the release of phytotoxins (allelochemicals) from certain specialized organs of donor plants as secondary metabolites (Kobayashi, 2004). Leaching from different parts of various weeds significantly influenced the germination, radical and plumule extension of field crops (Singh et al., 1989). The inhibitory substances present in *T. vulgaris* plants causing allelopathy could be used as a source of potential natural herbicide. Therefore, according to the negative impact of weeds in fields, accurate control in sustainable agriculture systems are essential.

Plants mineral content affected by aqueous extracts allelopathic potential

The data shown in Tables 4, 5 and 6, indicated that mineral content affected by all the aqueous extract of medicinal plants studied. The mineral uptake in all plants reduced by adding the aqueous extract of plants studied regardless of the higher concentration that altered significantly the mineral composition of wheat and some associated weeds seedling. Meanwhile, the change in mineral content Mn, Fe, Cu, Zn, K+ and Na+ increase by *C. officinalis* and *T. vulgaris* than *S. officnalis* aqueous extracts as compared with untreated control. In general: *the* alteration was lower in K^+ and Na+ content of wheat and their weeds seedling regardless of aqueous extracts. In general, increasing level of aqueous extracts concentration caused significant decreasing level in Mn, Zn, Cu, Fe, K+ and Na+ content of wheat and associated weeds seedling. However, the lowest concentration in most treatment increased the mineral levels as compared with untreated control. Iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn) are essential micronutrients for plants and humans (Hao et al., 2007); wheat is the most important dietary source of micronutrients in many developing countries. Increasing the micronutrient concentration of wheat grains has been identified as a way of addressing human micronutrient deficiencies (Pahlavan-Rad and Pessar-

Table 3
Effect of *S. officnalis* (vegetative parts) aqueous extracts on some plants

	L. multiflorum					*P. paradoxa*					*T. aestivum*				
	Concentration, mg ml $^{-1}$														
	0	20	40	60	LSD 0.05	0	20	40	60	LSD 0.05	0	20	40	60	LSD 0.05
Germination	9.67	9.67	9.00	7.67	NS	9.67	9.67	10.00	9.00	NS	9.00	10.00	10.00	5.33	2.25
Shoot length, cm	5.43	5.50	6.00	5.17	NS	5.83	5.33	4.83	4.83	NS	11.33	13.67	12.33	4.33	NS
Root length, cm	5.67	5.43	5.17	5.17	NS	3.17	2.50	2.17	1.83	NS	14.67	16.00	15.67	3.33	0.74
Fresh weight, gm	0.27	0.24	0.37	0.25	NS	0.25	0.21	0.22	0.04	NS	2.44	3.15	2.62	0.68	0.27
Dry weight, gm	0.05	0.03	0.05	0.03	NS	0.07	0.07	0.04	0.03	0.02	0.12	0.25	0.20	0.05	0.14

Table 4
Effect of *C. officinalis* aqueous extracts on wheat and associated weeds mineral content (µg/g dry weight)

	L. multiflorum					*P. paradoxa*					*T. aestivum*				
	Concentration, mg ml-1														
	0	20	40	60	LSD 0.05	0	20	40	60	LSD 0.05	0	20	40	60	LSD 0.05
(vegetative parts)															
Mn	6.49	6.27	4.84	0.00	6.00	11.62	8.65	7.39	6.01	5.47	8.09	8.51	7.72	3.04	2.71
Zn	7.04	6.38	5.78	0.00	8.00	8.78	7.79	4.75	1.45	8.45	7.43	7.23	4.79	1.58	2.22
Fe	90.70	102.58	91.96	0.00	14.2	10.43	7.79	5.02	3.23	15.4	69.40	37.13	35.15	23.99	27.5
Cu	4.13	5.06	2.15	0.00	0.12	5.06	5.28	2.18	0.75	NS	2.24	2.90	2.01	1.42	0.29
K	237.05	199.10	201.85	0.00	32.2	328.02	152.46	80.52	23.76	16.7	550.11	560.01	425.37	99.99	45.3
Na	130.90	129.80	61.60	0.00	35.1	180.18	71.28	71.28	68.31	19.4	227.04	225.72	99.66	87.78	37.2
Subterranean parts															
Mn	7.65	9.63	4.28	5.34	NS	7.46	4.29	3.63	2.38	1.6	9.44	8.65	6.04	4.09	3.60
Zn	7.65	9.63	6.66	6.64	10.0	10.89	9.11	6.86	5.87	6.6	8.22	6.17	3.99	2.74	2.99
Fe	95.32	97.90	6.60	6.05	NS	150.15	5.87	1.32	0.53	16.3	23.86	32.97	19.27	12.74	NS
Cu	6.88	6.38	5.39	4.90	0.80	6.40	3.10	1.00	0.65	NS	2.38	2.08	1.02	0.53	1.3
K	218.35	227.26	155.10	154.00	43.5	252.12	237.60	186.12	89.76	18.5	230.01	240.57	93.06	60.06	33.2
Na	116.60	110.00	129.25	116.60	11.9	191.40	205.92	155.76	155.10	42.1	182.16	92.40	48.84	35.64	19.4

Table 5
Effect of *T. vulgaris* aqueous extracts on wheat and associated weeds mineral content (µg/g dry weight)

	L. multiflorum					*P. paradoxa*					*T. aestivum*				
	Concentration, mg ml-1														
	0	20	40	60	LSD 0.05	0	20	40	60	LSD 0.05	0	20	40	60	LSD 0.05
(vegetative parts)															
Mn	5.67	4.40	3.40	2.94	2.3	7.06	6.80	5.37	4.84	2.90	13.20	12.90	11.48	8.15	1.89
Zn	7.21	4.68	6.71	1.24	7.90	11.62	12.47	7.66	6.82	7.59	12.44	11.72	8.71	6.14	0.86
Fe	101.31	32.01	6.00	4.29	10.0	106.79	107.12	92.14	77.28	14.3	66.73	67.39	65.97	12.80	9.0
Cu	4.18	3.08	2.70	1.65	0.18	7.46	7.66	7.06	6.01	0.49	3.96	2.94	2.44	1.95	0.24
K	210.10	160.05	30.25	12.10	63.4	278.52	221.76	186.12	164.34	23.0	302.61	357.72	244.53	237.93	NS
Na	129.25	138.60	104.50	81.40	54.3	118.80	68.64	48.18	23.10	37.1	222.09	218.79	242.88	182.16	15.4
Subterranean parts															
Mn	8.49	7.14	4.36	4.24	6.5	11.22	11.75	10.36	7.66	4.70	19.17	15.21	14.78	7.06	1.60
Zn	8.69	6.93	6.44	6.33	2.31	9.17	8.51	6.14	3.56	11.6	10.30	11.57	7.72	4.21	4.82
Fe	58.69	58.14	58.19	57.37	1.92	120.19	96.36	89.36	67.32	NS	83.36	77.12	65.93	57.98	11.2
Cu	4.62	5.94	4.29	3.85	0.26	5.41	5.48	4.55	3.63	0.11	3.76	3.73	1.55	0.36	0.39
K	210.10	155.10	99.55	72.60	13.4	208.23	186.12	120.12	120.12	28.7	487.08	508.53	425.37	211.53	NS
Na	129.25	95.15	19.25	17.60	19.4	118.80	89.10	89.10	78.54	32.9	175.89	209.55	209.55	132.00	NS

Table 6
Effect of *S. officnalis* aqueous extracts on wheat and associated weeds mineral content (µg/g dry weight)

	L. multiflorum					*P. paradoxa*					*T. aestivum*				
	Concentration, mg ml -1														
	0	20	40	60	LSD 0.05	0	20	40	60	LSD 0.05	0	20	40	60	LSD 0.05
(vegetative parts)															
Mn	14.30	8.29	9.27	8.09	NS	7.99	8.05	7.72	3.56	2.92	11.15	11.68	6.83	1.29	1.82
Zn	10.78	12.65	6.90	7.71	8.90	12.94	8.98	7.59	7.93	4.71	12.11	12.51	9.74	3.00	2.34
Fe	63.86	60.89	58.85	2.92	3.70	116.16	92.93	122.76	80.19	2.33	66.92	75.87	51.25	48.02	NS
Cu	6.05	6.00	5.89	5.80	0.51	3.24	2.44	2.44	1.52	0.78	1.98	2.41	0.20	0.07	0.39
K	216.15	163.35	100.65	70.95	16.4	391.38	394.02	252.78	85.14	24.3	390.06	429.99	237.93	188.10	6.5
Na	155.10	161.70	101.20	99.22	10.5	196.81	260.04	224.40	147.84	33.2	242.88	144.54	109.56	35.64	NS

akli, 2009). Allelopathic inhibition of mineral uptake results from alteration of cellular membrane functions in plant roots. Evidence that allelochemicals alter mineral absorption comes from studies showing changes in mineral concentration in plants that were grown in association with other plants, with debris from other plants, with leachates from other plants, or with specific allelochemicals (Nelson, 1985). These allelochemicals can depolarize the electrical potential difference across membranes, a primary driving force for active absorption of mineral ions. Allelochemicals can also decrease the ATP content of cells by inhibiting electron transport and oxidative phosphorylation, which are two functions of mitochondrial membranes. In addition, allelochemicals can alter the permeability of membranes to mineral ions, (Nelson, 1985).

Response of ABA, IAA and GA content to aqueous extracts allelopathic potential

The data shown in Figure 1 indicated that phytohormone affected by all the aqueous extracts, meanwhile the response of ABA, IAA and GA levels to allelopathic potential differed according to plant types and concentrations on wheat seedling we studied. The results revealed that IAA and GA showed a decrease by increasing the concentration of aqueous extract of medicinal plants studied. The reduction response in ABA

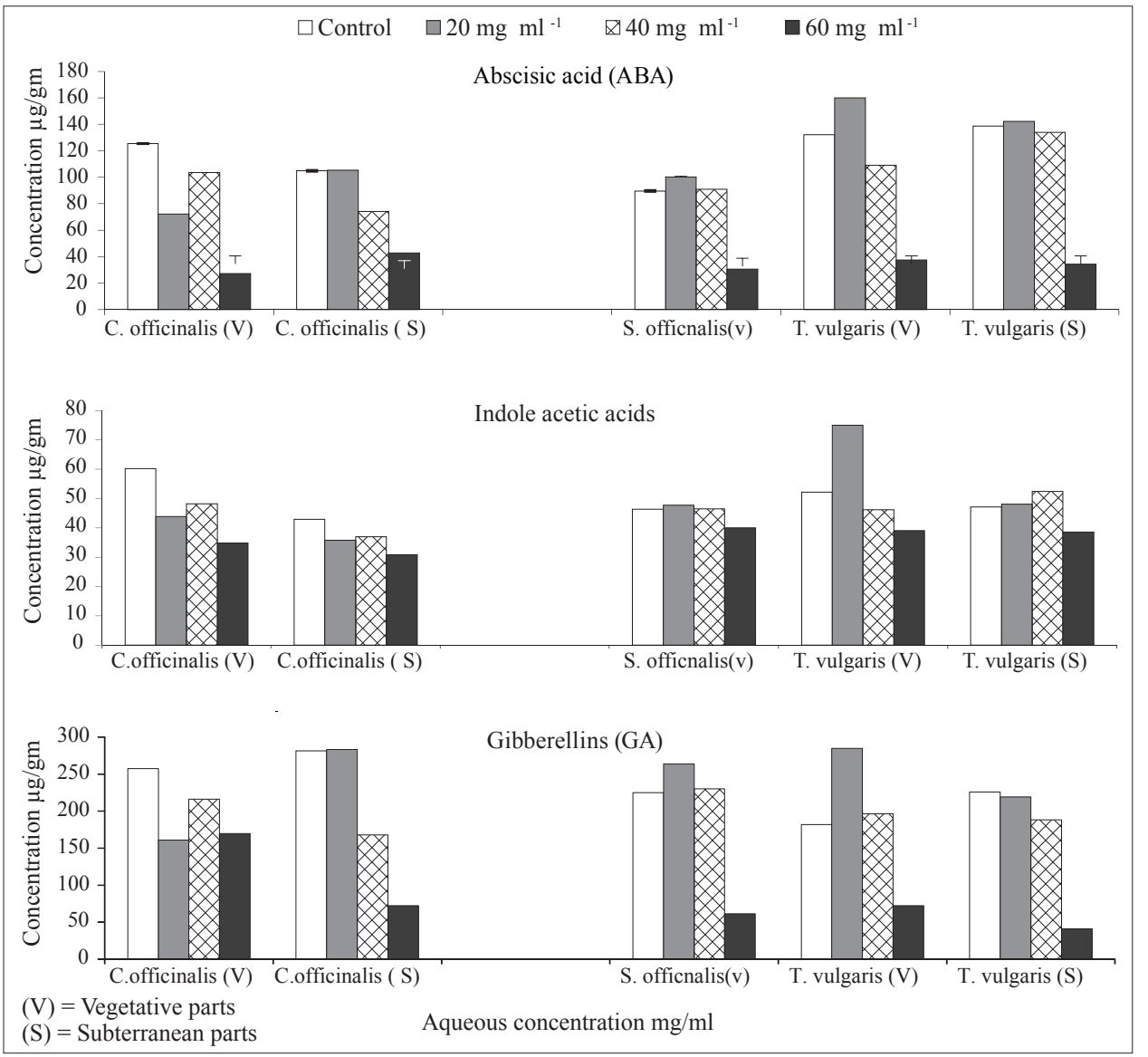

Fig. 1. Response of *T. aestivum* on phytohormones content regardless of aqueous extracts

was higher than IAA content, while total GA exhibited less response with increasing concentration and allelopathic compounds level. The ABA, IAA, and GA levels did not change significantly when treated with the lower aqueous extracted concentration than the control. However, the highest aqueous leachates concentrations were significantly lower ABA, IAA, and GA levels than the control treatments. *C. officinalis* achieve the highest reduction effect on ABA and GA; however, *T. vulgaris* caused the maximum reduction effect on IAA as compared with its control. The main effect of S. *officnalis* vegetative extracts at 20 and 40 mg ml^{-1} in ABA, IAA, and GA hormones of wheat was activation. However, S. *officnalis* at 40 mg ml^{-1} caused a significant reduction as compared with the control. In this respect, generally most applied concentration caused a significant reduction in ABA, IAA, and GA one week after treatments and the highest relative reduction recorded at 60 mg ml^{-1} in IAA content by *T. vulgaris* subterranean parts extract (81.8 %), ABA and GA content by *C. officnalis* vegetative extracts (78.3 and 421%) than the untreated control. Allelochemicals act upon pathways that are involved in the synthesis and control of plant hormone levels. These could represent an important factor to regulate many metabolic processes that govern plant growth (Olofsdotter1998). In addition, some mechanisms of action of allelochemicals seem to resemble those of synthesis plant hormones (Kruse et al., 2000).Thus, these compounds probably affect inducible hormones of germination such as gibberellin (Rice,1984 and Kruse, et al., 2000) which are necessary for seed germination. The effects of allelopathic compounds on the activity of hormones are considered by experiments done on phenolic growth inhibitors from aqueous extract of weed plants studied, which prove to suppress the activity of IAA and gibberellin (GA), (Kefel and Turetskaya, 1968). In addition, allelochemicals presented in the aqueous extracts of different plant species have been reported to affect different physiological processes through their effects on enzymes responsible for phytohormone synthesis and were found to associate with inhibition of nutrients and ion absorption by affecting plasma membrane permeability (Daizy et al., 2007).

Effect of plants extracts on *T. aestivum* seedling total proteins

The data in Figure 2 revealed that total protein decreased by increasing the concentration of aqueous extracts of medic-

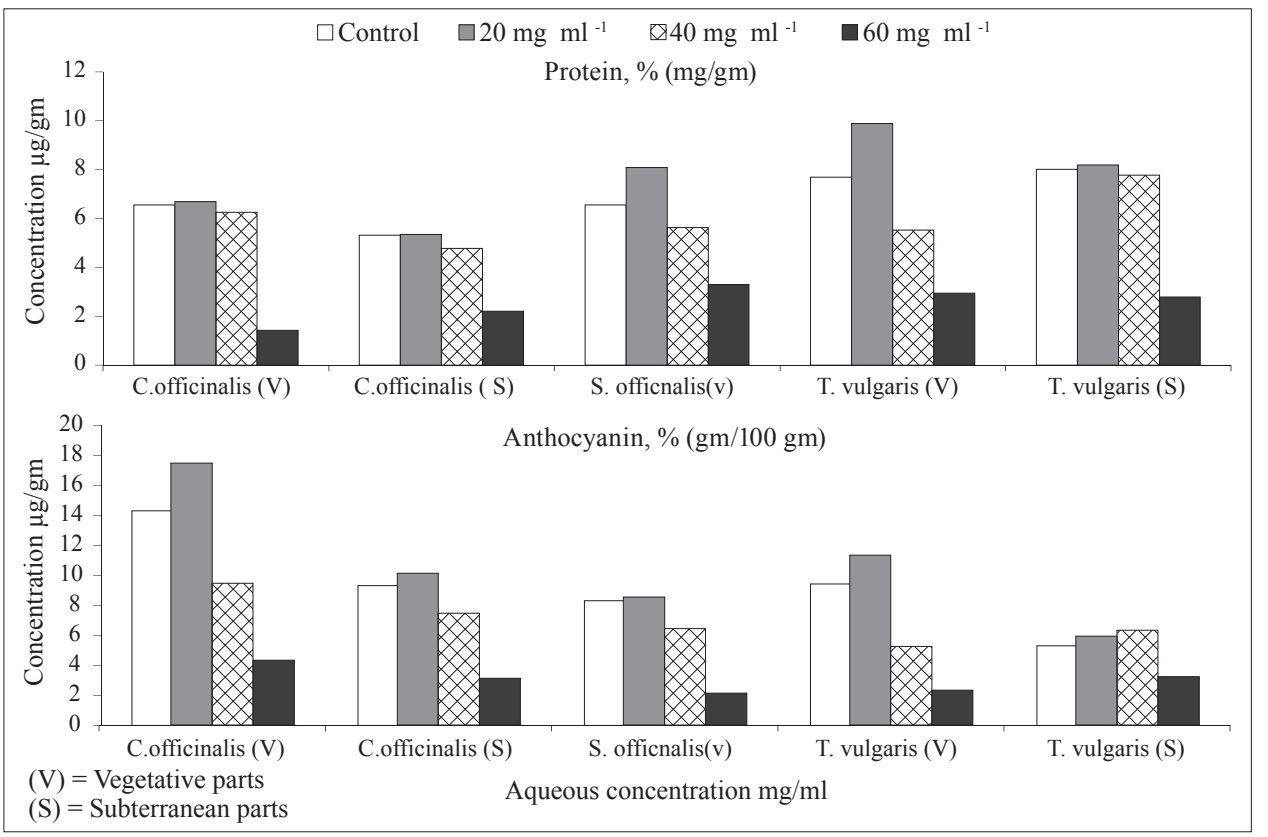

Fig. 2. Response of *T. aestivum* on protein and anthocyanin contents regardless of aqueous extracts

inal plants studied. In the present work the content of proteins were found to be higher in low concentration at 20 mg ml^{-1} for *S. officnalis* and *T. vulgaris* than the highest concentration 40 and 60 mg ml^{-1} for *C. officinalis, T. vulgaris* and *S. officnalis* due to increasing allelopathic compounds amount with increasing aqueous extracts. The addition of all the applied extracts resulted in general activation of protein contents of wheat one week after treatment than the untreated check. The highest activation in protein contents were recorded with *S. officnalis* and *T. vulgaris* vegetative parts extracts at 20 mg ml^{-1} showing 23.2 and 28.5% activation, respectively. However, the highest reductions in protein contents were recorded with *C. officinalis* vegetative parts extract at 60 mg ml^{-1} by 78.1 % activation than the untreated control. The influence of extracts on reduced total protein content was due to the presence of allelochemicals, particularly phenolics and other secondary metabolites like growth regulators, alkaloids, terpenoids and toxins (Rice, 1984). The maximum inhibitory effect was found in *T. vulgaris* followed by *Calendule sp* is due to their high concentration of this phenol content along with other constituents in their compare to control. This phenolic compound might have interference with phosphorylation pathway or inhibiting the activation of Mg and ATPase activity or might be due to decrease synthesis of protein, and nucleic acid (DNA and RNA) or interference in cell division, mineral uptake and biosynthetic processes (Pawar and Chavan, 2004).

Effect of plant extracts on *T. aestivum* anthocyanins pigments content

In the presence of *T. vulgaris* and *C. officinalis*, anthocyanins pigments increased significantly at low concentrations 20 mg ml^{-1} of aqueous extract of medicinal plants studied (Figure 2). However, high concentrations 40 and 60 mg ml^{-1} of these extracts reduced anthocyanine content significantly different from the control plants. The addition of all the applied extracts at 20 mg ml^{-1} resulted in the activation of anthocyanin contents of wheat, while C. *officnalis* and *T. vulgaris* vegetative extracts which recorded the highest activation by 21.8 and 20.0%, respectively than the control one week after treatment. However, the highest reductions in anthocyanin contents were recorded with *T. vulgaris* subterranean parts extract at 60 mg ml^{-1} by 75.3% than the untreated control. Different studies have shown that many biochemical and physiological processes affect allelochemicals, including phenolic compounds, such as pigments content (Ahrabi et al., 2011). Allelochemicals are synthesized in certain specialized organs of donor plants as secondary metabolites (Kobayashi, 2004). In addition, some studies show that environmental stresses on plant such as salinity, drought stress, heavy metal and secondary metabolites, cause increase in the low molecular weight, carotenoids and anthocyanins, in plants and algae (An et al., 1998).

Evaluation of plants extracts allelopathic activity on *T. aestivum* amino acid percentage

The data revealed that the free amino acid percentage decreased by all aqueous extract of medicinal plants at 40 mg ml^{-1} as compared to the control (Table 7). The decreased in free amino acid percentage by all aqueous extracts of medicinal plants due to the release of allelochemicals that reduced free amino acid percentage in wheat plant. Generally extracts of most treatments decreased serine, alanine, valine and Lysine amino acids, however methionine, arginine and ammonia content increased by all aqueous extracts than the control. In this respect, *C. officinalis* aqueous extracts prom-

Table 7
Effect of medicine plants extracts at 40 mg ml^{-1} on *T. aestivum* amino acid percentage by g 100 g^{-1}

Amino acid Percent % / Weeds extract	Arg	Amm	Lys	His	Phe	Tyr	Leu	Ile	Met	Val	Ala	Gly	Pro	Glu	Ser	Thr	Asp
Control	1.34	1.55	0.59	0.73	0.14	1.16	1.06	0.45	0.02	0.94	1.12	1.11	0.02	1.58	0.50	0.26	1.46
C. officinalis (vegetative parts)	1.79	2.04	0.28	0.64	0.09	0.09	0.74	0.30	0.06	0.67	0.72	0.87	0.006	1.74	0.32	0.17	1.47
C. officinalis (Subterranean parts)	1.59	1.43	0.22	0.46	0.05	0.09	0.59	0.26	0.038	0.53	0.56	0.71	0.034	1.66	0.34	0.13	0.69
S. officnalis (vegetative parts)	2.64	3.01	0.41	0.77	0.13	0.11	0.83	0.41	0.07	0.77	0.90	1.23	0.02	1.34	0.42	0.21	1.74
T. vulgaris (vegetative parts)	0.93	1.89	0.56	0.80	0.11	0.16	1.10	0.50	0.04	0.87	1.10	1.35	0.01	1.58	0.48	0.33	2.19
T. vulgaris (Subterranean parts)	1.05	1.49	0.43	0.69	0.18	0.12	0.90	0.36	0.07	0.72	0.87	1.11	0.02	2.12	0.42	0.27	1.23

Table 8
Phytochemical screening of *T. vulgaris* parts by gm/100gm dry weight

	Flavonoids	Terpenoids	Total polyphenols	Total tannins	Total carbohydrate	Crude fiber
Vegetative parts	0.63	0.37	5.40	3.20	21.61	16.15
Subterranean parts	0.32	0.52	3.00	2.20	12.41	19.90

ised by inhibited threonine amino acids than other treatments, while *T. vulgaris* reduced arginine amino acid than their control and than other treatments. The highest inhibitory effect achieved from *C. officinalis* vegetative and subterranean parts*, T. vulgaris* vegetative and subterranean parts and *S. officnalis* vegetative parts aqueous extracts at 40 mg ml^{-1} reduced tyrosine biosynthesis of *T. aestivum* seedling by 92.2, 92.2, 86.2 and 89.6 and 90.5% compared to the control. This result was in harmony with (Alagesaboopathi 2010) who suggests that allelopathic inhibition is complex and can involve the interactions of different classes of chemical like flavonoids, alkaloids, steroids, terpenoids, phenolic compounds and amino acids.

As for example to previous allelopathic plants, the phytochemical tests implemented on *T. vulgaris* (vegetative and subterranean parts) to quantify crude fiber contents, total carbohydrate total, tannins total, polyphenols, terpenoids, saponins, alkaloids (non detected), flavonoid compounds by gm/100 gm dry weight. Data in (Table 8) indicated that divers of chemical compounds were found that might be explaining the multifaceted affects of the extracts on weeds and wheat crops due to allelopathic constituents.

Conclusions

Natural plant extracts from *C. officinalis, T. vulgaris* and *S. officnalis* have herbicidal properties that may provide an alternative to synthetic herbicides for their ability to suppress weeds germination and seedling biomass. However, the results of the instant study revealed that *T. vulgaris* and *C. officnalis* are the stronger and potential candidates to be used for weed control in wheat. These allelochemicals may manipulate shoot/root growth, fresh/dry weight, nutrient uptake, synthesis of protein and they influence the synthesis of amino acids. It is likely that the use of high doses of plant extracts with allelopathic activity would decrease wheat biochemical aspects (minerals content, proteins, anthocyanine and amino acids under laboratory conditions. However, natural conditions are more complicated, hence, the field experiments are necessary before any conclusions are made on allelopathic effect of these medicinal species. So, there is a limited use on wheat seedling especially the highest extracts concentration and its prefer to use this extracts in pre emergence stage for weeds to prevent weeds emergence and ovoid the adverse effect of allelopathic compounds on wheat seedling and yields.

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