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RESEARCH ARTICLE

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ALLELOPATHIC EFFECT AND OXIDATIVE STRESS INDUCED BY AQUEOUS EXTRACT OF *ACHILLEA SANTOLINA* L. SHOOT ON *TRITICUM AESTIVUM* L. PLANT

ABSTRACT:

The purpose of this study was to investigate the allelopathic effects of *Achillea santolina* L. shoot aqueous extract on dry weight and photosynthetic pigment contents as well as some metabolic and biochemical processes during germination of wheat plant (*Triticum aestivum* L. Sakha 93)**.** To exclude the involvement of osmotic stress in plant reaction to phytotoxic compounds, the study compared the effect of 0.5%, 1%, 2%, 4%, and 8% (w/v) *Achillea* shoot aqueous extracts with distilled water as a control. Most of measured parameters of wheat plant exhibited a great sensitivity to *Achillea* extract. Germination, shoot and root length, dry weight, water content, chlorophyll content, proteins, carbohydrates and proline were significantly inhibited by increasing the concentration of allelochemicals extracted from *Achillea*. *Achillea* extracts enhanced the production of hydrogen peroxide (H_2O_2), electrolyte leakage (EC), and malondialdeyde (MDA) in wheat plant. The activities of antioxidant enzymes (such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR) and glutathione peroxidase (GPX)) beside the contents of non-enzymatic antioxidants as ascorbic acid and glutathione (AsA & GSH) were markedly affected after treatment with *Achillea* extract. The allelopathic effects of *Achillea* on growth alongside with metabolic and biochemical performance of wheat plant is mostly due to the allelochemicals extracted from its shoots.

KEY WORDS:

Phytotoxicity, *Achillea santolina*, Oxidative stress, *Triticum aestivum* L.,Lipid peroxidation, hydrogen peroxide, Electrolyte leakage, Membrane permeability, Antioxidant enzymes,non enzymatic antioxidants.

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INTRODUCTION:

Allelopathy is derived from the Greek words *allelon* "of each other" and *pathos* "to suffer" (Rizvi *et al*., 1992). It therefore translates literally as mutual suffering. It achieved when plants secrete a biochemical materials which prevents other plants from growing near them. These biochemical materials are called allelochemicals that may affect the physiological processes of the plants such as respiration, cell division, water and nutrient uptake, oxidative stress and others. Most plant species, including wild plants, crops and trees are capable of producing such molecules into the environment to inhibit the development of neighbouring plants (Rice, 1984).

Allelopathy plays an important role in the agroecosystems leading to a wide array of interaction between crop–crop, crop–weed and tree–crops (Zahida *et al*., 2006). Generally, these interactions are harmful to the recepient plants but provide a selective benefit to the donor (Adrain *et al*., 2000). Although most plants produce phytotoxic allelochemicals, relatively few have strong allelopathic properties (Xuan *et al.*, 2005; El-Darier and Youssef, 2007; Golisz *et al.*, 2007; Salhi and El-Darier, 2008; El-Darier and Tammam, 2009).

The genus *Achillea* (Family Asteraceae) comprises more than 200 species, most indigenous to Europe and the Middle East (Ahmed *et al*., 1988). Specifically, *A. santolina* L. (Arabic name is Al-Qisum) is considered the only species in genus *Achillea* widely distributed in the northern western desert of Egypt. It grows well in crop fields (barley, wheat, broad bean) under fig and olive trees and on the edges of roads and canals. Field observations during the last few years indicated that the considered species exhibited deleterious effect on the performance and yield of non tillage barley, wheat and broad bean fields in the region. El-Darier and Tammam (2009) in laboratory experiment reported that the aqueous extract of *A. santolina* shoots achieved phytotoxic effect on germination efficiency and metabolite accumulation in barley and broad

bean plants. Production of reactive oxygen species (ROS) and related oxidative stress in general, has been proposed as one of the major mechanisms of action of the phytotoxins (Weir *et al.,* 2004).

These active oxygen species are highly toxic and can damage different cell structures and functions. The oxidative defense systems include several antioxidant enzymes such as super oxide dismutase SOD, EC.1.15.1 for, catalase CAT EC: 1.11.16, ascorbate peroxidase APX EC: 1.11.11, glutathione reductase GR EC: 1.6.4.2 and glutathione peroxidase GPX EC: 1.11.19. In addition to these enzymatic systems, plants also contain non enzymatic antioxidant systems involving substances like β- carotenes, α-topopherol, the reduced form of ascorbate and glutathione. Ascorbate which is oxidized in this process is generated by reduction of dehydroascorbate at the expense of oxidizing glutathione GSSG to reduced glutathione GSH. Subsequently GSH is regenerated by glutathione reductase with consumption of NADPH (Asada, 1994). A relevant defense system is represented by glutathione, which protects many cellular components against oxidative stresses. GSH may also metabolize H_2O_2 by participating in the ascorbate /glutathione cycle (Abdel Nasser, 2000).

However, not much is known about *A. santolina* L. exact mode of action. Also specific details regarding the level and extent of oxidative stress and the induction of anti-oxidative enzyme mechanisms due to *A. santolina* exposure are lacking in wheat plants.

The main objective of the present study was to examine the potential impact of aqueous extract of *A. santolina* shoots on growth, induction of oxidative stress and induction of enzymatic and non-enzymatic antioxidant in wheat plant.

MATERIAL AND METHODS:

1. Infrared spectra:

Preliminary trials were performed to determine the density of extract necessary to produce spectra with a good signal. A known weight of dry plant shoot was analyzed according to Kansiz, *et al*. (1999). For IR analysis the dried material was placed on the infrared microscope stage for spectral acquisition. The spectra were graphed on Perkin Elmer 1430 ratio recording infrared spectrophotometer. The absorbance spectra were between 4000 – 250 cm^{-1} with 10 scans co-added and averaged.

2. Sampling and preparation of extract:

Fresh vegetative plants of *A. santolina* L. were collected during late spring 2008 from ten natural agro-fields distributed in the northern western desert of Egypt. The plant material was washed throughly with tap water to get ride of any sand particles then allowed to air dry. Shoots were cut into 1-2 cm pieces and stored at room temperature until use for analysis. To prepare full strength stock, 75 g of dried material were grinding in mortar with phosphate buffer pH7, and then made to a known volume 500ml with distilled water. Plant material was filtered out of the extract with cheese-cloth followed by filtering through Whatman filter paper. Then subsequently treatment concentrations (0.5, 1, 2, 4, and 8 %) were prepared. The extracts were prepared and stored at -5°C. Prior to use in bioassay, the extracts were equilibrated at room temperature for 1 h.

3. Growth experiment:

Grains of wheat (*Triticum aestivum* L.) Sakha 93 were obtained from the Breeding Program of the Agricultural Research Center, Giza, Egypt. Twenty wheat grains were placed in each of four sterile petri-dishs (9 cm in diameter) per treatment, lined with a whatman No.1 filter paper. Four ml of the different concentration levels of *Achillea* extract were added per petri – dish. In another treatment, 4 ml of distilled water were added per petri – dish and considered as a control. Dishes were incubated at 25°C in the dark. The germination percentage and length of plumule and radicle were recorded at the fifth day.

The pot experiment was conducted under laboratory conditions with different treatments of aqueous extract concentrations of *Achillea* shoots. Weighed plastic pots measuring about 500 cm^3 were filled with air dried soils collected from the natural community of *Achillea* and planted with 15 grains of wheat each in three replicates.The pots were placed in a growth chamber maintained at 30/16°C day/night temperature (± 2°C) and 16/8 light/dark with a photon flux density of approximates 150 μ mol photons m^{-2} s^{-1} and relative humidity 78 ± 2%. The pots were irrigated with distilled water (according to the pre-estimated water field capacity). After ten days from sowing, the pots were irrigated with the different treatment concentrations day after day for a period of 10 days. The leaf photosynthetic pigments were determined as described by Moran (1982). The dry matter of roots and shoots was determined after drying in an aerated oven at 80°C to constant weight.

4. Carbohydrate, protein and proline contents:

Carbohydrates content was determined as described by Fales (1951) and Schlegel (1956). Proteins content was determined according to Bradford (1976) using bovine serum albumin as standard. Proline was estimated using acid ninhydrin method described by Bates *et al*. (1973).

5. Oxidative stress:

a- Hydrogen peroxide (H_2O_2) content:

Content of H_20_2 was determined using the method of Velikova *et al*. (2000), in which fresh samples of leaf tissue (100 mg) was extracted with 5 ml of 0.1% trichloroacetic acid (TCA) and centrifuged at 12000 g for 15 minutes. Then 0.5 ml of supernatant was mixed with 0.5 ml of 10 mM phosphate buffer (pH 7.0) and 1 ml of 1 M potassium iodide. The absorbance was determined at 390nm. The amount of H_20_2 could be calculated using the extinction coefficient 0.28 μm^{-1} cm^{-1} and expressed as nmol g^{-1} f. wt.

b- Membrane permeability:

Loss of membrane permeability (an indicator of cellular damage) was studied in term of ion (electrolyte) leakage from the leaves of wheat plants by measuring conductivity of the bathing medium, as described by Duke and Kenyon (1993). Leaf tissue (100 mg) collected from 20-day-old seedlings was dipped in 5 ml of 1 mM MES buffer (2-[N-morpholino]ethanesulfonic acid sodium salt, pH = 6.5) containing 2% sucrose (w/v). A parallel control containing all the materials was also maintained. The conductivity of the bathing medium was measured with a conductivity meter (ECOSCAN CON5; Eutech Instruments Pte. Ltd., Singapore). Leaf samples were then boiled for 15 minutes in order to measure the maximum electrolyte leakage.

c- Determination of lipid peroxidation:

Lipid peroxidation was measured in terms of malondialdehyde content (MDA) as described by the method of Heath and Packer (1968). Leaves (100 mg) were extracted with TCA (0.1 %, w/v) and centrifuged at 10 000 g for 10 minutes. MDA level was used as an index of lipid peroxidation and was expressed as nmol g^{-1} fresh weight. One ml of the supenatant was added to 4 ml of 0.5 % thiobarbituric acid (TBA) disolved in 20 % TCA. The mixture was incubated at 95 °C for 30 minutes followed by quick cooling over ice, then centrifuged at10 000 g for 10 minutes. The absorbance of the supernatant was determined at 532 nm and corrected for non-specific absorbance at 600 nm. MDA amount was determined using the extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as nmol g^{-1} fresh weight.

6. Nonenzymatic antioxidants:

Glutathione (GSH) and total glutathione (GSH+GSSG) were assayed according to Griffth (1980) and described by Abdel Nasser (2000). GSSG was determined from the difference between total glutathione (GSH+GSSG) and glutathione (GSH). Ascorbic acid (AsA) and dehydroascorbic acid (DHA) contents were determined according to the method of Law *et al*. (1983). Total ascorbate was determined through the reduction of DHA to AsA by 0.97 mM dithiothreitol (DTT) and the DHA concentration was determined by estimating the difference between total ascorbate and AsA values. A standard curve covering the range 0 – 25 µmol AsA was used.

7- Antioxidant enzymes:

a) Extraction:

The overall procedure was carried out at 0 to 4 °C. Samples (0.5 g) of leaves and roots, were ground and homogenized in 20 ml of ice-cold extraction buffer (100mM KH_2PO_4/K_2HPO_4 pH7.8), 800 mg polyvinyl polypyrrolidone, 0.5% Triton X-100, 5 mM ascorbic acid). The homogenate was centrifuged at 40,000×*g* (20 min, 4°C). Aliquots of 3 ml of supernatant were passed through a column filled with sephadex G-25 (PD-column-pharmacia-Germany) which had been equilibrated with elution buffer (100 mM KH_2PO_4 / K_2HPO_4, pH 7.0).

According to Asada (1992), the elution buffer for APX contained additionally 1 mM ascorbic acid in order to keep APX enzyme in the active state. The purified extracts were used for the determination of SOD, CAT, APX, and GR.

b) Enzymes assay:

Superoxide Dismutase SOD (EC: 1.15.1.1):

Activity was measured according to the method of Misra and Fridovich (1972). One unit of SOD activity was amount of enzyme activity that inhibited epinephrine formation by 50% .

Ascorbate Peroxidase APX (EC: 1.11.1.11):

Activity was determined as described by Asada (1994). The reaction was initiated by the addition of H_2O_2. The H_2O_2 dependent oxidation of ascorbate was followed by monitoring the decrease in absorbency with an absorption coefficient of 2.8 mM cm^{-1}. One unit of APX was expressed as micromoles ascorbate oxidized per milligram of protein per minute.

Catalase CAT (EC: 1.11.1.6):

Activity was assayed according to Aebi (1983), where decomposition of H_2O_2 is followed spectrophotometrically at 240 nm. One unit of enzyme activity is equal to 1 µmol of H_2O_2 decomposed per min.

Glutathione Reductase GR (EC: 1.6.4.2):

Activity was assayed spectrophoto-metrically according to Smith *et al.* (1988). The reaction was initiated by the addition of GSSG. After the formation of thiobenzoic acid, the absorbance at 412 nm was measured from the linear portion of the curve, usually within 5 min. GR activity was expressed as units per milligram of protein.

Glutathione Peroxidase GPX (EC:1.11.19):

Guaiacol peroxidase (GPX) activity was measured using the method of Egley *et al*. (1983). The reaction mixture (2 ml) consisted of 25 mM phosphate buffer (pH 7.0), 0.05 % guaiacol, 1.0 mM H_2O_2, 0.1 mM EDTA and 0.2 ml of the enzyme extract. Increase in absorbance was measured at 470 nm due to oxidation of guaiacol. The enzyme activity was calculated using an extinction coefficient of 26.6 $mM^{-1}Cm^{-1}$ and expressed as enzyme units g^{-1} fresh weight. One enzyme unit was the amount of enzyme that catalyses oxidation of 1 µM guaiacol min^{-1}.

Statistical analysis:

All data were subjected to standard one-way analysis of variance (ANOVA) using COSTAT 2.0 Statistical Analysis Software manufactured by CoHort Software Company (1986). Comparison of the main effects was performed using the Least Significant Difference (LSD). A significant level of $P<0.05$ were used for all statistical procedures.

RESULTS:

Infrared (IR) spectroscopy of *A. santolina* shoots:

The infrared (IR) spectroscopy as an indicator for the total biochemical composition of *A. santolina* shoot was illustrated in Figure 1. The obtained results recognized the band assignments at the region (4000-250cm^{-1}) which include a number of chemical groups that can account for the chemical differences in *A. santolina*. These chemical groups could be exerted in the soil and have the initiation and/or inhibition effects. The IR spectra gave nearly 15 absorption peaks (Table 1), most of them were found in the frequency regions between 1750 and 1000 cm^{-1} (9 peaks) and between 3500-2000 cm^{-1} (4 peaks). The least two absorption peaks were found at frequency 561 cm^{-1} (one peak) and at 3850 cm^{-1} (one peak).

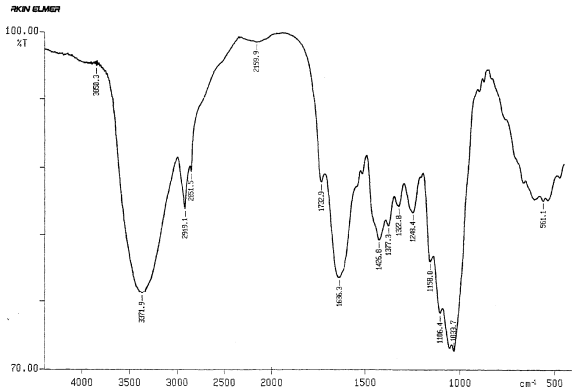


Fig. 1. Infrared spectra of total cell constituents of *Achillea santolina L.* cells.

Table 1. IR spectrophotometer of the standard organic groups of total cell constituents (List of band assignment).

Frequency	Assignment	Comments
4000-3640	vas C-H of methylene groups (Nelson, 1991)	–
3000-2010	vs C-H of methylene groups (Nelson, 1991)	–
2000-1720	V C=O of ester functional groups primarily from lipids and fatty acids (Hedrick *et al*., 1991; Zeroval *et al*., 1995; Williams and Feleming, 1996).	–
~1650	V C=O of amides associated with proteins (Nelson, 1991; Williams and Feleming, 1996)	Usually called the amide 1 band may also contain contribution from C=C stretches of olefinic and aromatic compounds.
~1540	δ N-H of amides associated with proteins (Nelson, 1991; Williams and Feleming, 1996).	Usually called the amide II band may also contain contributions from C=N stretches.
1450-1410	δas CH_3 and Sas CH_2 of proteins (Zeroval *et al*.,1994)	The position of these assignments can vary in the literature.
~1335	δs CH_3 and Ss CH_2 of proteins and vs C-O of COO- groups (Nelson, 1991; Zeroval *et al*., 1994).	The positions of these assignments can vary in the literature.
1240-1160	Vas P=O of the phospho-diester back bone of nucleic acids (DNA and RNA) (Nelson, 1991; Wong *et al*., 1991).	May be due to the presence of phosphorylated proteins and polyphosphate storage products.
1080-1000	Vs P=O of the phosphodiester backbone of nucleic acids (DNA and RNA) (Nelson, 1991 and Wong *et al*., 1991).	May be due to the presence of phosphorylated proteins and polyphosphate storage products.
700-250	V C-O-C of polysaccharides (Wong *et al*., 1991; Zeroval *et al*., 1994).	The predominant polysaccharide in Chlorophyta is starch.

Vas: asymmetric stretch; Vs: symmetric stretch; δas: asymmetric deformation (bend). δs: symmetric deformation (bend).

Germination percentage and Length of Radicle and Plumule:

The different concentration levels of *Achillea* shoot extract significantly ($P< 0.05$) suppressed the different germination paramaters of wheat plant after five days of germination (Table 2). For example, it was obvious that 8% of the extract achieved the maximum inhibition percentage of about 36 of control. Furthermore, radicle length were decreased from 3.7 to 0.7 cm, the corresponding figuers for plumule were from1.5 to 0.5 cm.

Table 2. Allelopathic effect of different concentrations of aqueous extract of *Achillea santolina* L. shoot on germination percentage and radicle and plumule length (cm) of *Triticum aestivum* L. (after 5 days).

Concentration (%)	Germination (%)	Radicle length (cm)	Plumule length (cm)
Control	100^{d}	3.70^{e}	1.50^{d}
0.5	71^{c}	2.06^{d}	1.22^{c}
1	48^{c}	1.62^{c}	0.91^{b}
2	42^{b}	1.25^{b}	0.81^{b}
4	40^{b}	0.90^{a}	0.74^{b}
8	36^{a}	0.70^{a}	0.51^{a}

Different letters indicate a significant difference at the 0.05 level of probability evaluated by ANOVA test.

Growth parameters:

Generally, shoot and root lengthes were reduced to a ratio of about 57 and 72%, respectively at the maximum concentration level (8%) compared to control (Table 3). The results also showed that values of root and shoot dry weight decreased to about 22 and 17%, respectively as the extract level increased (8%) which coincided with the decrease in root: shoot ratio. Data also indicated that the shoot and root water content were reduced dramaticly with the increase in extract concentration. With respect to the pigment content (Table 4), the values of Chl a, Chl b and the total pigments, as well as total carotenoids were significantly ($P<0.05$) decreased with increasing *Achellia* extract concentration level. However, at the same level of extract the Chl a/b ratio were significantly decreased indicating that chlorophyll a was more affected than chlorophyll b.

Table 3. Allelopathic effect of different concentrations of aqueous extract of *Achillea santolina* L. shoot on Length (cm), dry matter (g) and water content (g) of *Triticum aestivum* L. plant shoot and root (20 day-old)

Treatment	Length			Dry matter		Water content	
	Shoot	Root	R/S	Root	Shoot	Shoot	Root
Control	18.9 [a]	13.8 [a]	0.73	0.59 [d]	0.71[c]	88.3[a]	86.3 [b]
0.5	17.1 [a]	12.0 [a]	0.70	0.41[c]	0.66 [c]	87.1[a]	85.5 [b]
1.0	15.2 [a]	10.3 [a]	0.67	0.33b[c]	0.50 [b]	86.6[a]	81.3 [b]
2.0	13.8 [a]	8.8 [a]	0.63	0.27ab[c]	0.33 [b]	79.1[a]	77.3 [b]
4.0	10.2 [a]	5.7 [a]	0.55	0.21a [b]	0.17 [a]	77.2[a]	72.1[b]
8.0	8.1 [a]	3.8 [a]	0.47	0.13[a]	0.12 [a]	75.3[a]	56.5[a]

Table 4. Allelopathic effect of different concentrations of aqueous extract of *Achillea santolina* L. shoot on pigment content (mg g^{-1} dry weight) of *Triticum aestivum* L. plant (20 day-old). Different letters indicate a significant difference at the 0.05 level of probability as evaluated by ANOVA test

Concentration (%)	Chl "a"	Chl "b"	Chl "a/b"	Carotenoids	Total Pigments
Control	11.2[f]	6.7[e]	1.67[a]	3.6[c]	21.5[f]
0.5	10.3[e]	6.5[e]	1.58[a]	4.8[d]	20.8[e]
1	9.5[d]	5.6[d]	1.69[a]	3.7[c]	19.0[d]
2	8.2[c]	4.6[c]	1.78[a]	3.1[b]	15.7[c]
4	3.9[b]	2.4[b]	2.78[b]	2.8[a]	7.8[b]
8	3.0[a]	1.7 [a]	1.76 [b]	2.0	6.7 [a]

Different letters indicate a significant difference at the 0.05 level of probability as evaluated by ANOVA test.

Carbohydrate, protein and proline contents:

The soluble carbohydrate and proline content (Table 5) exhibited a significant increase ($P<0.05$) with a parallel decrease in the content of total carbohydrates in wheat plant with the progressing increase in *Achillea* aqueous extract. On the other hand, protein content (soluble and insoluble) in both shoot or root of wheat plant decreased gradually by increasing the concentration of *Achillea* aqueous extract except at 0.5% concentration level where an increase in shoot soluble protein was achieved.

Table 5. Allelopathic effect of different concentrations of aqueous extract of *Achillea santolina* L. shoot on protein ,carbohydrates and Proline fractions (mg g-1 dry weight) of shoot and root of *Triticum aestivum* L. plant (20 day-old)

Concentration (%)	Organ	Protein		Carbohydrate		Proline content
		SP	TP	SC	TC	
Control	Shoot	48.81[c]	95.07[f]	119.76[a]	274.55[f]	81[a]
	Root	43.94[f]	79.31[e]	21.80[a]	175.48[f]	52[a]
0.5	Shoot	51.68[f]	84.50[e]	121.78[b]	261.59[e]	89[ab]
	Root	35.23[e]	65.91[d]	41.24[b]	124.99[e]	41[a]
1	Shoot	47.40[e]	82.22[d]	133.78[c]	183.37[d]	95[ab]
	Root	31.96[d]	57.76[c]	64.76[c]	85.35[d]	62[b]
2	Shoot	41.93[d]	72.64[c]	152.96[d]	191.013[c]	98[b]
	Root	27.14[c]	48.55[b]	73.50[d]	70.75[c]	72[b]
4	Shoot	39.16[b]	67.26[b]	182.03[e]	162.41[b]	140[c]
	Root	18.73[b]	85.42[f]	92.15[e]	30.79[b]	81[b]
8	Shoot	25.01[a]	61.19[a]	192.39[f]	131.37[a]	190[d]
	Root	17.73[a]	27.76[a]	121.71[f]	19.91[a]	99[c]

Different letters within the same organ indicate a significant difference at the 0.05 level of probability as evaluated by ANOVA test.

Electrolyte leakage and lipid peroxidation:

Data of the present study indicated that *Achillea* extract exhibited a significant excessive ion leakage as measured by increased electric conductivity (Fig. 2). Such increase was associated with a significant increase in H_2O_2 and MDA with the increase in extract concentration level until their maximum values (7.6 and 22, respectively) at 8% extract.

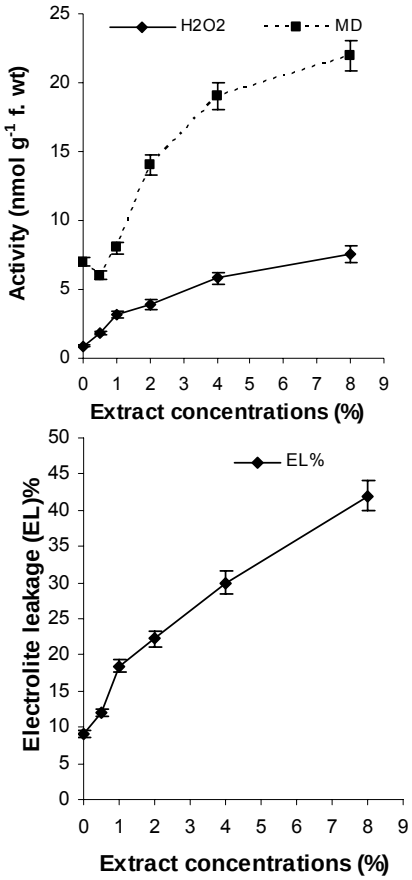


Fig. 2. Effect of different concentration levels of Achillea santolina aqueous extract on electrolyte leakage (EL), lipid peroxidation (MD) and H2O2 accumulation on Triticum aestivum L. (Data are the mean of three independent replicates ± standard errors)

Non-enzymatic antioxidants:

Results presented in table 6 show that the total ascorbate (AsA+DHA) was significantly increased in leaves of wheat plant exposed to different concentration levels of *A. Santolina.* Likewise, the level of AsA was strikingly decreased to a low level particularly at 8% concentration level on the contrary to the trend of DHA and consequentely AsA/DHA ratio was decreased.Furthermore, the acumulation of glutathione (GSH) was enhanced on the payments of GSSG. Consequently, the ratio of GSH/GSSG was increased.

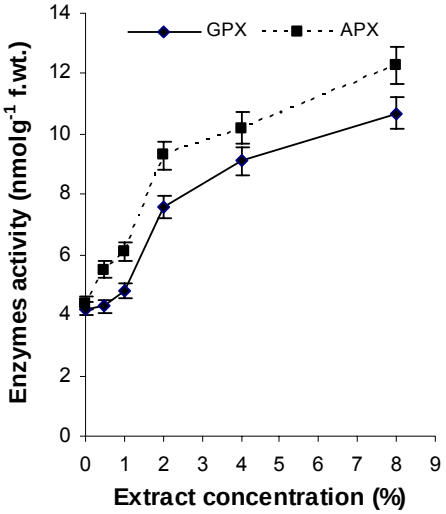
Table 6. Allelopathic effect of different concentrations of aqueous extract of *Achillea santolina* L. shoot on endogenous levels of reduced ascorbate (DHA), oxidized ascorbate (AsA), reduced glutathione (GSH) and oxidized glutathione (GSSG) µmol g^{-1} of *Triticum aestivum* L. plant (20 day-old)

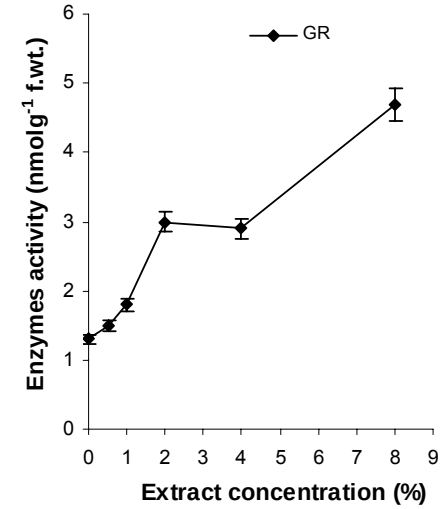
Concentration (%)	GSH	GSSG	GSH+ GSSG	GSH/ GSSG	AsA	DHA	AsA+ DHA	AsA/ DHA
Control	52[a]	39[b]	91	1.33	58[c]	112[a]	170	0.51
0.5	63[b]	31[ab]	94	2.03	43[b]	129[a]	172	0.33
1	75[c]	28[ab]	103	2.67	37[b]	159[b]	196	0.23
2	87[d]	20[ab]	107	4.35	21[a]	179b[c]	200	0.11
4	113[e]	15[a]	128	7.53	13[a]	191[c]	204	0.06
8	122[e]	10[a]	132	12.2	9[a]	203[c]	212	0.04

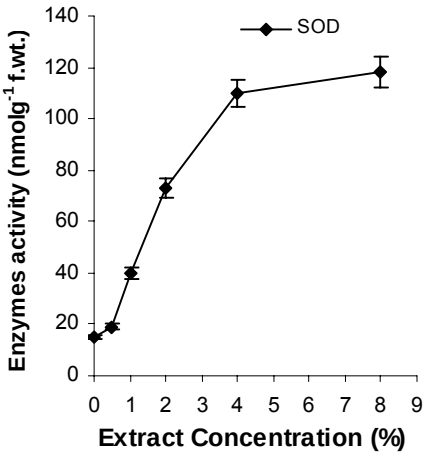
Different letters indicate a significant difference at the 0.05 level of probability as evaluated by ANOVA test.

Enzymatic antioxidants:

Activity of antioxidant enzymes (SOD, CAT, APX, GR, and GPX) were studied in this work showed a differend trend in response to *Achillea* extract except catalase (CAT) enzyme. For example, the activities of SOD, APX, GR, and GPX were icreased steadely with increasing *Achillea* extract concentrations reaching values amounted to 7.8, 2.3, 2.6, and 2 fold respectively with respect to their innitial values (Fig. 3). While, CAT enzyme exhibit another trend as it decrease with increasing the *Achillea* extract concentration (Fig. 3).







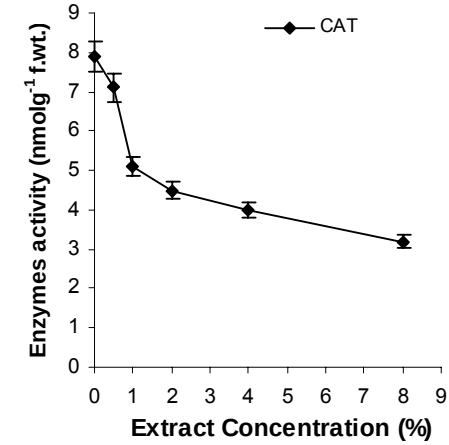


Fig. 3. Effect of different concentration levels of Achillea santolina aqueous extract on the activity of ascorbate peroxidase (APX) , glutathione peroxidase (GPX), glutathione reductase (GR), superoxide dismutase (SOD) and catalase (CAT), in the leaves of Triticum aestivum L. (Data are the mean of three independent replicates ± standard errors).

DISCUSSION:

Allelopathy plays an important role in the agroecosystems leading to a wide array of interaction between crop –crop, crop –weed and tree –crop (Zahida *et al.*, 2006). Generally, these interactions are harmful to

the recepient plants but provide a selective benefit to the donor (Adrain *et al*., 2000). Although most plants produce phytotoxic allelochemicals, relatively few have strong allelopathic properties (Xuan *et al.*, 2005).

Phytochemical screening of *Achillea santolina* shoots showed that it contains about 8 mg/g, 29.70 mg/g and 0.3% of total flavonoids, total phenolics and essential oil respectively also it contain a moderate content of sterols and glycosides while the content of tannins was low (El Darier and Tamman, 2009).

The infrared analysis for *Achillea santolina* shoots indicated that they contained some of the important organic groups which acted as initiator for the production of allelochemicals and/or a precursor of such compounds as also indicated by Aline Meda *et al.* (2005). The IR spectra gave nearly 15 absorption peaks (Table 1 & Fig. 1), most of them were found in the frequency regions between 1750 and 1000cm^{-1} (9 peaks) and between 3500-2000 cm^{-1} (4 peaks) which represent the Vas and VS C-H of methylene groups (Nelson, 1991). The least absorption peaks were found at frequency 561cm^{-1} (one peak) which represents VC-O-C of polysaccharides (Wong *et al*., 1991; Zeroual *et al*., 1994) and at 3850 cm-1 (one peak) which represents Vas VS C-H of methylene groups (Nelson, 1991). Owing to the fact that the major constituents of phytochemicals in *Achillea santolina* are the phenolics and the flavonoids compounds, we speculate that the allelopathic effect of this plant could be presented within the frequency regions between 1750 and 1000 cm^{-1} and between 3500 and 2000 cm^{-1}.

In this connection, Palá *et al*. (2001), Verpoorte *et al*. (2002), and Teixeira da Silva (2004) reported that several products (acetylenes, essential oils, flavonoids, sesquiterpenes) obtained from *Achillea santolina* have been investigated for their biological activities. Naturally-occurring sesquiter-penes, for example, serve a function in allelopathy, fungal pheromones, phytoalexins, phytotoxins, allomones, juvenile hormones, picrotoxins and essential oils.

The present work was carried out to investigate any possible inhibitory effects of *Achillea santolina* aqueous extract on wheat plant. The result obtained in table 2 showed that the allelopathic treatment at different concentrations (0.5, 1, 2, 4, and 8%) had an inhibitory effect on germination percent, and plumule and radicle lengths, indicating that aqueous extract of *Achillea santolina* contained some allelochemicals compounds that have an inhibitory effect (Rice, 1974; Qasem, 2002). Zzet and Yusuf (2004) stated that, plant may directly affecting growth of another plant either positively or negatively through exuding some chemical substances which play an important part in the inhibition of growth. Khan *et al.* (2005) and Daizy *et al.* (2007) have reported that allelochemicals presented in the aqueous extracts of different plant species have an effect on the different physiological processes through their effects on the activities of some enzymes responsible for phytohormone synthesis which were found to associate with inhibition of nutrients and ion absorption by affecting plasma membrane permeability. Similar results were also obtained by Salhi and El-Darier (2008) on the effect of some medicinal plant extract on the growth of some weed species.

A great differences in response of wheat plant to the aqueous extract of *Achillea santolina* shoots was shown in some physiological and biochemical processes. For example, it was found that *Achillea santolina* extract was significantly suppressed the dry mass accumulation at all concentrations used particularly at high concentration (Table 3). The reduction in growth parameters measured in this study such as shoot height and root length and subsequently decrease in the R/S ratio could be attributed to the effect of *Achillea santolina* on cell division in one hand and on cell enlargment due to the decrease in water content on the other hand

In this connection, El-Darier (2002) reported that the allelopathic effects of *Eucalyptus* aqueous extract on growth of broad bean and maize plants were mainly due to inhibition of some interacting physiological processes such as nutrient uptake which is positively correlated to the dry matter production and such suppresive effect was more obvious in dicotyledonous (broad bean) than in monocotyledonous (maize) plants.

Chlorophyll and carotenoids are the central part of the energy manifestation of evergreen plant systems and therefore, any significant alterations in their levels is likely to cause a marked effect on the entire metabloism of the plant (Prasad *et al*., 2004). The result in table 4 showed that,the photosynthetic pigments decreased significantly by increasing the treatment concentrations amounted to the decrease in chlorophyll a, b and carotenoids (73, 74, and 44%, respectively). Several modes of actions have been suggested, including direct inhibition of PSII components and ion uptake, interruption of dark respiration and ATP synthesis and ROS-mediated allelopathic mechanisms (Inderjit and Duke, 2003). Contrarley, carotenoids increased significantly in broad bean and barley plants under the effect of allelochemicals extracted from *Achillea* shoot (Hu *et al.,* 2000)

The data in table 5 showed that, soluble, total protein and total carbohydrates content decreased with increasing extract concentration while proline and soluble carbohydrates accumulated (shoot > root)

upon subjecting wheat plant to different concentration levels of *Achillea* aqueous extract which may act as osmoprotectants. This accumulation might indicate a potential osmoregulation of proline and soluble carbohydrates which in turn exert a positive role in the alleviation of the imposed allelochemical stress. Ramon *et al*. (2003) reported that exposing plants to any stress lead to a series of reactions which generates numerous free radicals which may be reflected by altered levels of major anions and accumulation of proline. Proline is supposed to participate in the reconstruction of chlorophyll, activates the Krebs cycle and costitutes an energy source, it is also an important part of structural proteins and enzymes and participates in repair processes (Alia *et al*., 1993).

The present study showed a significant excessive ion leakage as measured by increased electric conductivity (Fig. 2), which may indicate that the aqueous extract of the allelopathic plant caused stress resulting in disruption of membrane integrity. Membrane disruption by mono-terpenoids is one of the mechanisms responsible for cell death, as suggested by Harrewijn *et al.* (2001). In addition, a decrease in membrane permeability observed could be due to lipid peroxidation of plasma membrane (Fig. 2) (Maness *et al*., 1999). Generally, various types of environmental stresses mediate their impact through oxidative stress caused by generation of reactive oxygen species ROS (Sminrnoff, 1995 and1998; Blokhina *et al*., 2003). This result was in consistence with those obtained by Scrivanti *et al*. (2003) and Zunino and Zygadlo (2004) who reported that monoterpenes enhance lipid peroxidation. Increased lipid peroxidation indicates that *A. santotiana* extract results in oxidative stress due to generation of ROS species, this, causes a loss of cell integrity. ROS such as singlet oxygen, superoxide radicles and hydroxyl radicle (OH), hydrogen peroxide are highly reactive and toxic molecules that can cause oxidative damage to membranes, DNA, proteins, photosynthetic pigments and lipids (Apel and Hirt, 2004). Recently, ROS generation and related oxidative stress has been proposed as one of the modes of action of plant growth inhibition by allelochemicals (Weir *et al*., 2004). Bais *et al*. (2003) reported that catechin, a putative phytotoxin, inhibit plant growth due to severe oxidative burst in root tips, resulting in cell death.

Lipid peroxidation was determined as malondialdehyde level. The cellular level of MDA represents a balance of oxidative stress. Thus it can be regarded as a sink for oxidative radicle.In the current work (Fig. 2) MDA increased significantly in leaves of the examined plant species upon treatment with *A. santolina* aqueous extract.Also increased levels of H_2O_2 enhance lipid peroxidation and oxidative stress levels in the target tissues indicating that *A. santolina* can generate the production of a powerful oxidation. In this connection, Canals *et al*. (2005) reported that the allelopathic effects on other plant species have been recognized as an important survival strategy.

However, very little is known about the action of allelochemicals phytotoxins in inducing ROS mediated oxidative damage. To explore whether *A. santoliana* extract induces a similar response, some enzymatic and non enzymatic antioxidant mechanisms linked with oxidative stress were assessed in leaves of wheat plant.

Ascorbic acid AsA and glutathione GSH in the oxidized and reduced formes are among the most important non enzymatic cellular anti oxidant deffence compound (Noctor *et al*., 1998; Abdel nasser, 2000). The ratio of the reduced/oxidized formes is considered an important indicator of the redox state of the cell and the degree of oxidative stress (Asada, 1994). To function as antioxidant AsA must be maintained in the reduced form (DHA) in the presence of dehydroascorbic acid reductase (DHAAR) enzyme in addition to GR enzyme which catalyses the reduction of GSSG to GSH (Zhang and Kirkham, 1996; Hatata and Abdel-Aal, 2008).

In this study the result, given in table 6 shows that the content of AsA was markedely decreased with the increase of *A. santolina* extract concentration. This decrease in the reduced form of ascorbic acid was corresponding to the increase in the level of oxidized form particularly at high concentration of *A. santolina* extract. On the other hand, the reverse was true for the change in the content of GSH and GSSG indicating that *A. santolina* extract can be added to the list of stressors on the basis of the markedly evoked GSH/GSSG ratio with increased the concentration of *A. santolina* extract.However, the structural similarity between phytochellating and GSH suggests that GSH might be involved in the synthesis of phytochellating or other proteins which could detoxify the *A. santolina* effect.

Furthermore, it was found that the presence of biologically active flavonoids in leaf extract of *Achillea santolina* stimulates the production of superoxide dismutase and catalase which inactivate active forms of oxygen, preventing superoxidation of lipids and damage to the cells (Bader *et al*., 2003). Therefore, in this study, the activities of some antioxidant enzymes such as SOD, CAT, APX, GR, and GPX have been studied in the leaves of wheat plant treated with *A. santolina* extract. Figure 3 shows that *A. santolina* extract significantly affected the total activities of these enzymes for example, *A. santolina* extract particularly at high

concentration (8%) was found to stimulate SOD activity by about 7.8 fold compared to control. The result also showed that activities of APX, GR and GPX enzymes displayed a similar pattern as SOD (Fig. 3) indicating that the *A. santolina* caused an oxidative stress (Yu *et al*., 2003; Ye *et al*., 2006). At the same time, high level of *A. santolina* decreased the catalase CAT activities, propably due to the compensation for the increase of the APX activity. In this connection, Krizek *et al.* (1993) reported that a reduction in catalase activity and increase in APX activity in leaves of cucumber seedlings upon exposure to stress. Furthermore, APX and GR activities were found to be correlated with the change in GSH/GSSG ratio, suggestig that both enzymes play a role in the regulation of oxidative reduction status (Abdel Nasser, 2000).

CONCLUSION:

In conclusion, the present study proved that allelochemicals extracted from *A. santolina* suppressed the germination parameters, shoot and root length, accumulation of dry weight, chlorophyll, carotenoids and protein. Conversely, it increased the accumulation of soluble protein and proline. Additionally, the extract experienced an increase in the generation and accumulation of ROS in leaves which accompanied by enhanced lipid peroxidation, accumulation of hydrogen peroxied contents increased levels of scavenging system including non-enzymztic and enzymatic systems of wheat. However, further studies on allelochemicals uptake, compartment-alization and detoxification is necessary to elucidate the mechanism involved in their specific recoginition ability.

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التأثير الأ ليلوباثى و الإجهاد التأكسدى الناجم بمستخلص المجموع الخضرى لنبات البعيثران على نبات القمح

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تهدف هذه الدراسة إلى بحث التأاثير الأليلوباثى للمستخلص المائى للمجموع الخضرى لنبات البعيثران على الوزن الجاف والمحتوى الصبغى بجانب بعض الخطوات الأيضية والبيوكيميائية أثناء فترة الانبات لنبات القمح (*riticum aestivum*). وقد اوضحت الملاحظات الحقلية ان كثافة وقوة نبات القمح تتاثر بدرجات متفاوتة بوجود نبات البعيثران, ولمنع تاثير الاجهاد الاسموزى فى تفاعل النبات مع مركبات السمية النباتية فقد تم دراسة تاثير تركيزات مختلفة للمستخلص المائى لنبات البعيثران (0.5, 1، 2، 4، 8%) مع استخدام الماء المقطر فى التجربة القياسية. وقد اوضحت النتائج ان معظم العوامل التى تم قياسها فى نبات القمح قد تاثرت كثيرا بالمستخلص المائى للمجموع الخضرى لنبات البعيثران, كالانبات وطول كل من السيقان والجذور والوزن الجاف والمحتوى المائى والمحتوى الكلوروفيلى والبروتينات والكربوهيدرات والبرولين. وقد كان التاثير المثبط الناتج يزداد بزيادة تركيز المستخلص المائى لنبات البعيثران. و من جهة اخرى فان المستخلص المائى لنبات البعيثران قد ساعد على زيادة انتاج التسرب الإلكترونى (EC) و كذلك المالوندى الدهايد (MD) فى نبات القمح كما أوضحت النتائج ان أنشطة الأنزيمات المضادة للاكسدة مثل السوبر اكسيد دزميوتيز superoxide dismutase, الكاتاليز catalase , الاسكوربيت بيراوكسيديز ascorbate peroxidase, جلوتاثايون ريداكتيز glutathione reductase ، و جلوتاثايون بيراوكسيديز glutathione peroxidase وكذلك محتوى نبات القمح من المضادات الغير انزيمية مثل حمض الأسكوربيك Glutathione, Ascorbic acid والجلوتاثيون (GSH,AsA) التى تاثرت تاثرا كبيرا بعد معاملة نبات القمح بالمستخلص المائى لنبات البعيثران. ويستخلص من النتائج التى تم الحصول عليها ان تاثيرات المستخلص المائى لنبات البعيثران على نمو وأداء النشاط اللأيضى والبيوكيمائى لنبات القمح ناتج أساسا من وجود المواد الأليلوكيميائية بالمستخلص المائى.

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