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Biomonitoring of Allelopathy between *Salix alba* L. and Five *Triticum* Cultivars at Delta Region, Egypt

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ABSTRACT : The present study was conducted to evaluate the allelopathic effects of different concentrations (0, 4, 8, 16, 32 and 64%) of *Salix alba* leaf aqueous extracts (SALAE) as well as crude powder (SALCP) on germination and growth of four *Triticumaestivum*cultivars (Gemmiza 7, Giza 168, Sods 1 and shandaweel 1) and one *Triticum durum* cultivar (Banisuef 1). The effect of SALAE on germination percentage (GP), plumule (PL) and radicle (RL) lengths, as well as fresh and dry weight of wheat seedlings was significant. Similarly, negative allelopathic effect of SALCP on wheat germination and growth was increased with increasing extract and crude powder concentration. Based on the obtained results, Giza 168 was recognized as very resistant wheat cultivar compared to the other cultivars upon applying the aqueous extracts and crud powder of *Salix alba*leaves.
Key words: Phytotoxicity, germination percentage, growth parameters, wheat cultivars.

INTRODUCTION

Allelopathy is the chemical modification of a site tofacilitate better tree growth, and control ecological volume and essential resources (Coder, 1999).This modification is convey through biomolecules known as allelochemicals which released from plant parts by leaching, root exudation, volatilization, residue decomposition and other processes in both natural and agricultural systems (Putnam and Tang, 1986).Nowadays advancement in the research field has been made to study the allelopathic interactions between crops and weeds, crops and crops, crops and trees both stimulatory and inhibitory (El-Darier, 2002 and Hemada*et al*., 2004,Abou-Zeid and EL-Darier, (2014a). Residues and leachate of several crops and weeds have been shown to possess negative impacts on wheat growth and yield. Ben- Hammouda*et al.* (1995)reported that some barley cultivars are phytotoxic to bread wheat (*T. aestivum*).

Salix alba is a species of willow native to Europe and Western and central Asia. Its name is derived from the white tone to the underside of the leaves. It is now cultivated in India, China and Caribbean (Rashmicaphouse.tradeindia.com., 2012).It is a landscaping tree species of family Salicaceae which broadly distributed in the Nile Delta region in Egypt. It extends on the edges of lakes and crop fields to affect the growth and productivity of many crops and vegetables (field observations). Sadiq*et al.* (2014) reported that alkaloids, phenols, tannins, glycosides and steroids were positive while saponins were absent in the tested *S. alba* extract. In the GC-MS 40 phytochemical compounds were identified in the methanolic extract of *S. alba* leaves. The major compounds of the extract were 1, 2 Cyclohexanediol 12.89%, Salicyl Alcohol 7.19%, Stearic acid 12.66%, Linolenic acid 16.17% and Galactose 4,6-O-nonylidene 11.49%.

Wheat is considered the main cereal crop in Egypt. The percentage of production amounted to 53.2% of total consumption and so Egypt imports about 46.8% of its need from wheat yearly (Abd El-Rahman, 2009). Consequently, there is a big gap between wheat production and consumption. Therefore, National Program for Wheat Research developed new wheat cultivars (Gemmiza 7, Giza 168, Sohag 3 and Sakha 93) characterized with its higher yield and persist pests (Anon, 2005).

The present study was carried out to evaluate the probable allelopathic interference of *Salix alba* leaf aqueous extracts (SALAE) on germination and growth of four *Triticumaestivum*cultivars (Gemmiza 7, Giza 168, Sods 1, shandaweel 1) and one *Triticum durum* cultivar (Banisuef 1). Accordingly, one may suggest which of these cultivars is sensitive or tolerant to allelochemicals produced by *Salix alba*.

MATERIALS AND METHODS

Sampling and Preparation of Plant Extract

Leaves of the donor species have been collected from El-Behara governorate, 88 km south of Alexandria city during the vegetative stage in spring. The plant materials were dried in shade then ground in a Wiley Mill to coarse uniform texture and stored in glass jars until use.

Dried powder of leaves of *Salix alba* (50 g for each) were extracted with 300 ml distilled water. The extract was conducted in dark for 24 h at 25^{o}C. The supernatant was taken and centrifuged at 3000 rpm for 15 minutes; this would be considered as a full strength concentration (100%). The extracts were prepared no more than 48 h in advance and were kept in a refrigerator at 5^{o}C until used and the purified extract was adjusted to pH 6.8 with 1M HCl. Series of dilutions were prepared from the stock solution (4, 8, 16, 32 and 64% besides the control) and were tested for their effects on some germination parameters, and seedling growth of the recipient cultivars.

Four different wheat cultivars (Gemmiza 7, Giza 168, Sods 1 and shandaweel 1) related to *Triticum aestivum* and only one cultivar (Banisuef 1) related to *Triticum durum* were used in the study.

Laboratory Tests

To investigate the allelopathic effect of *Salix alba* leaf extracts on germination of wheat cultivar, wheat seeds were disinfected superficially by 70% ethanol for 1 minute and by 2.5% sodium hypochlorite solution for 3 minutes and then they were washed four times by sterile distilled water.

Germination bioassay

Petri-dish experiment was applied to investigate the potential allelopathic effects of the donor species aqueous extract on germination percentage (GP), inhibition percentage (IP), seed germination index (SGI), energy of germination (GE) and coleoptile (CL) and radicle (RL) lengths of the five cultivars of the recipient species. Twenty grains of the five wheat cultivars were arranged in 9-cm diameter Petri-dishes lined with two discs of Whatman No.1 filter paper under normal laboratory conditions with day temperature ranging from 19-22^{o}C and night temperature from 12-14^{o}C. 10 ml of the respective donor species aqueous extract (4, 8, 16, 32 and 64%) or distilled water as control were added daily to three replicates in a randomized complete block design.

Pot experiment

Pot experiment was performed to test the effect of different concentrations of Salix alba leaves crude powder (SALCP) mixed (w/w) with clay soil (collected from control locations) on some growth parameters, seedling fresh and dry weight as well as pigment content of the recipient species. To achieve this objective, soil samples were collected from the adjacent crop fields away (200 m) from Salix alba tree location, air-dried under shade, sieved to get rid of pebbles and plant debris and stored in paper bags ready for the analysis of some physico-chemical properties.

The soil samples were finally sterilized at (90ºC for 48 h) to remove any microorganisms and weed seeds. Twenty grains of each variety of recipient species and ten of the crop species seeds were sown in plastic pots (16 cm in diameter) with about 1500 g of clay soil thoroughly mixed (w/w) with 8, 16 and 32% of electrically crushed crude powder of the leaves of the donor species. One treatment was run as control with zero percent of crude powder. Treatments were arranged in a randomized complete block design with three replicates. The plants were watered every two days on the average with normal tap water. The amount of water corresponding to average soil–plant evapotranspiration calculated from weight loss over a 24 –hour interval. The experiment was performed under normal laboratory conditions (20±2°C temperature, 70±2% relative humidity, and 14/10 h light/dark photoperiod).

After three weeks, the homogenous seedling were carefully collected from each treatment, washed with tap water to remove the adhering soil particles, and then, by distilled water, gently blotted with filter paper. Seedling length, (shoot and root lengths), fresh weight, leaf area as well as pigment contents were immediately evaluated after harvesting. Other samples were dried at 65^{o}C till constant weight to determine seedling dry weight. Finally, the reduction percentages in the evaluated growth parameters were calculated**.**

RESULTS

Germination Bioassay

Data in Figure 1 showed that almost all wheat cultivars were highly sensitive to allelochemicals extracted from *Salix alba* leaf aqueous extracts (SALAE). Seed germination percentage (GP) was decreased substantially using 32 and 64% extract concentrations. However, there was no significant difference between concentration of 4 and 8%in comparison to the control. Seed germination inhibition (Figure 2) of *S*ods 1 cultivar seeds caused by 64%concentration was the most considerable, so that only 50% of seeds were able to germinate.

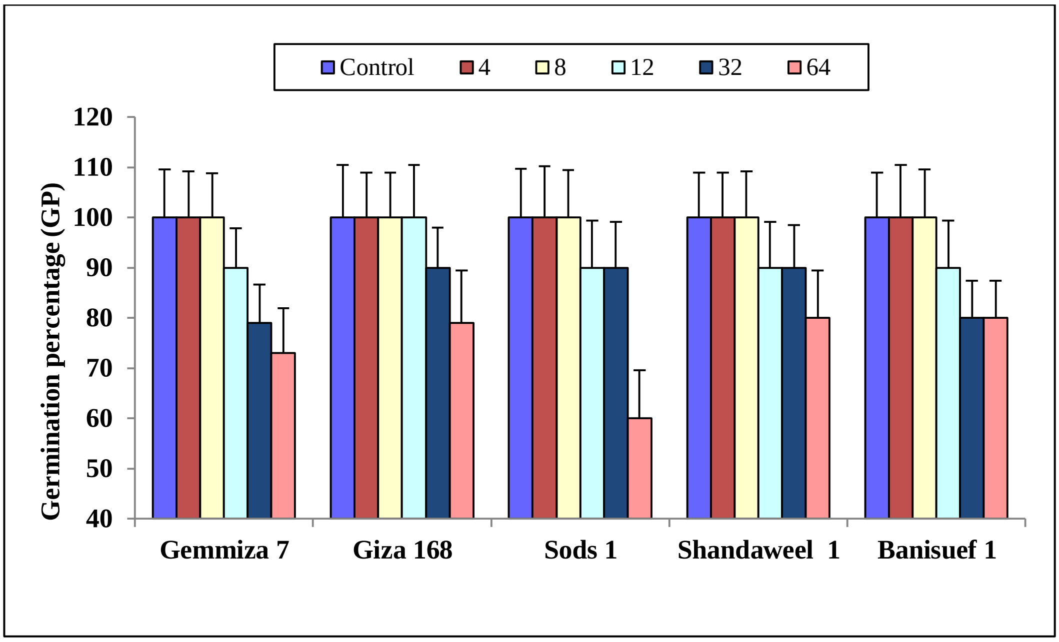

Figure 1. Effect of different concentrations of Salix alba leaf aqueous extracts (SALAE) on germination percentage (GP) of five wheat cultivars seven days after sowing. Bars represent standard deviation.

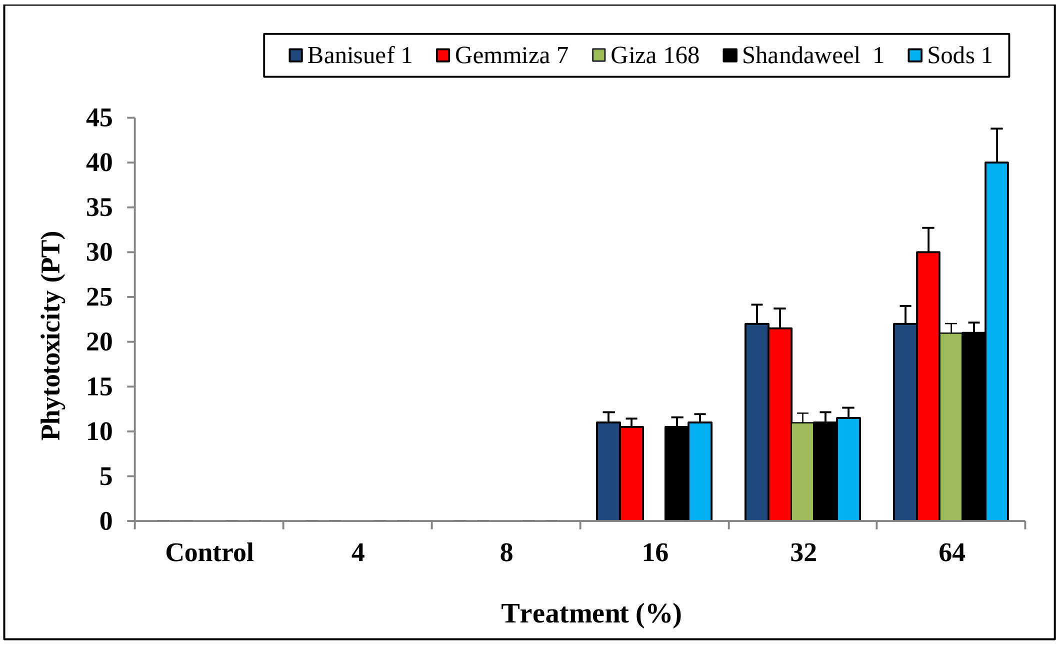

Figure 2.Allelopathic effect of different concentrations of Salix alba leaf aqueous extract (SALAE) on phytotoxicity (PT) of five wheat cultivars seven days after sowing. Bars represent standard deviation.

Figure 3 (a,b) represents the reduction percentage (relative to the control) in plumule and radicle length of the 5 tested cultivars. At concentration 4%, the reduction percentage in Gemmiza 7 was 13.7%, however, the same concentration showed a stimulation effect on the other 4 cultivars. The stimulation percentage reached 1.6, 10.7, 48.3 and 48.8% in Giza 168, Shandaweel 1, Sods 1 and Banisuef 1 respectively. The same trend was also observed at concentration 8% except for at this concentration the stimulation percentage was 10.5, 24.4, and 29.3 in Gemmiza 7, Banisuef 1 and Sods 1 respectively. However, at the same concentration, the reduction percentage of both Giza 168 and Shandaweel 1 cultivars reached 6.6 and 21.4 % respectively. Concentration 64% showed the highest reduction percentage in all wheat cultivars especially Gemmiza 7 (95.8) and Banisuef 1(100).

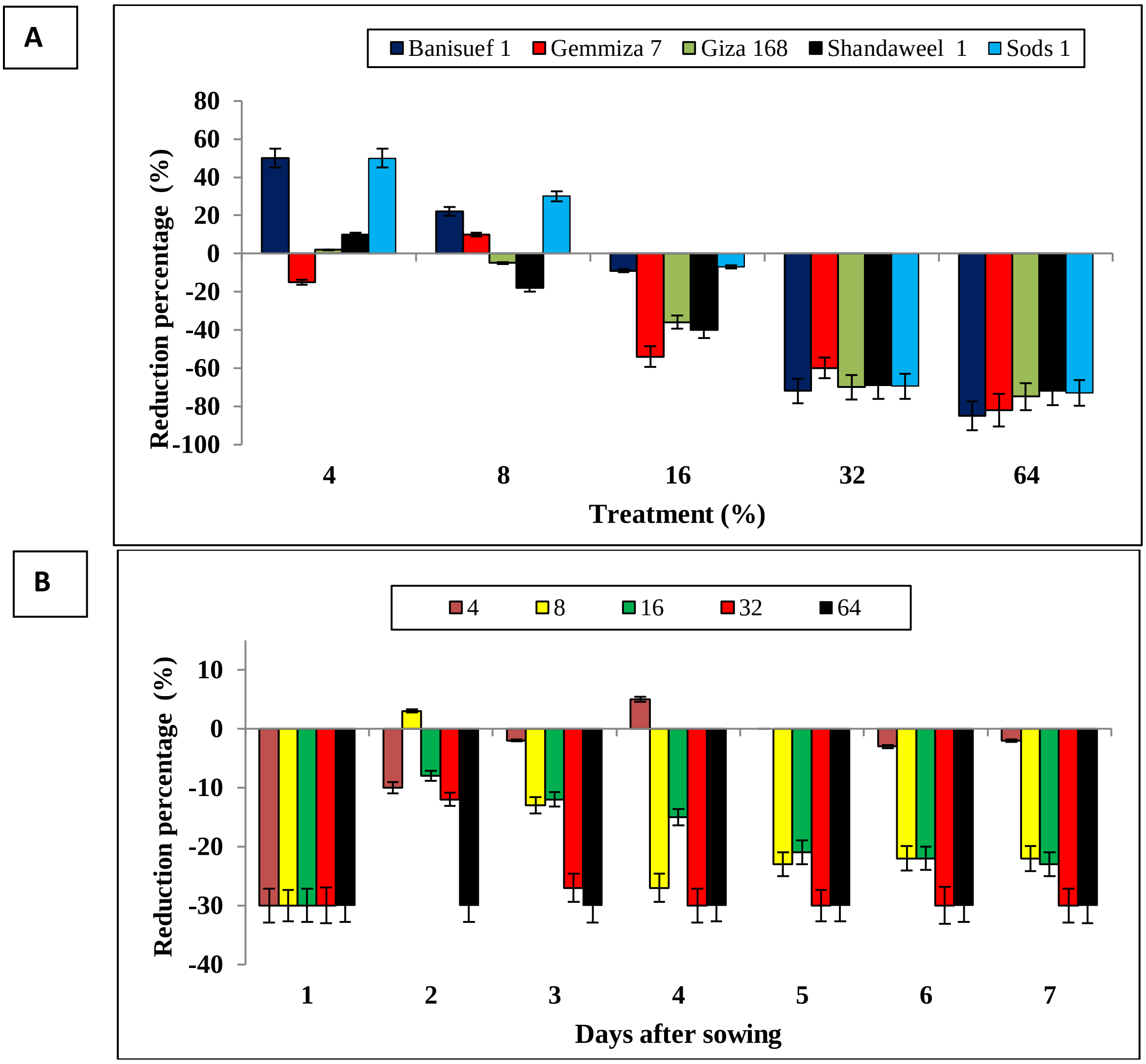

Figure 3. (A & B).Allelopathic effect of different concentrations of Salix alba leaf aqueous extracts (SALAE) on reduction percentage in plumule (PL)(A) and radicle (RL) (B) lengths of five wheat cultivars seven days after sowing. Bars represent standard deviation.

Growth Bioassay

Plumule and radicle lengths

The Allelopathic effect of *Salix alba* leaf crude powder (SALCP) concentration on plumule (PL) and radicle (RL) lengths of the five wheat cultivars is illustrated in Table 1. Obviously, all treatment levels resulted in significant reduction in the two parameters.The most dramatic reduction in PL was observed in Banisuef 1 where the percent reduction was 83%.Compared to control, a gradual decrease in radicle length (RL) of all recipient cultivars was observed along gradual increase in SALCP concentration.

Table 1.Variation in plumule(PL) and radicle (RL) lengths (cm) of five wheat cultivars seedlings as affected by different concentrations of *Salix alba* leaf crude powder (SALCP) twenty- one days after sowing.

Treatment (%)	Cultivar									
	Banisuef 1		Gemmiza 7		Giza 168		Shandaweel 1		Sods 1	
	Plumule length	Radicle length	Plumule length	Radicle length	Plumule length	Radicle length	Plumule length	Radicle length	Plumule length	Radicle length
Control	20.3^{c}	8.3^{d}	21.6^{d}	5.7^{d}	22.2^{d}	7.6^{d}	23.1^{d}	6.9^{c}	22.2^{d}	6.9^{c}
8	19.8^{c}	5.2^{c}	20.1^{c}	5.1^{c}	17.3^{c}	5.2^{c}	20.4^{c}	5.4^{b}	21.1^{c}	5.4^{b}
16	12.2^{b}	4.5^{b}	17.8^{b}	4.3^{b}	14.4^{b}	4.3^{b}	18.3^{b}	4.7^{a}	20.3^{b}	4.5^{a}
32	3.4^{a}	3.1^{a}	10.4^{a}	3.2^{a}	8.9^{a}	2.9^{a}	13.6^{a}	4.2^{a}	17.2^{a}	4.1^{a}

Different letters within each column indicate a significant difference at probability level ≤ 0.05 according to ONE WAY-ANOVA.

Seedling dry weight

Compared to control, a significant decrease in seedling dry weight (SDW) of all five cultivars was observed along gradual SALCP concentrations. The control values of the SDW were 0.09 ,0.07,0.06 ,0.06 and

0.09 (g ind.$^{-1}$) in cultivars Banisuef 1, Gemmiza7, Giza 168, Shandaweel 1 and Sods 1 respectively, reduced to 0.05 ,0.04 ,0.02 ,0.04 and0.06 at 32% **(Figure 4).**

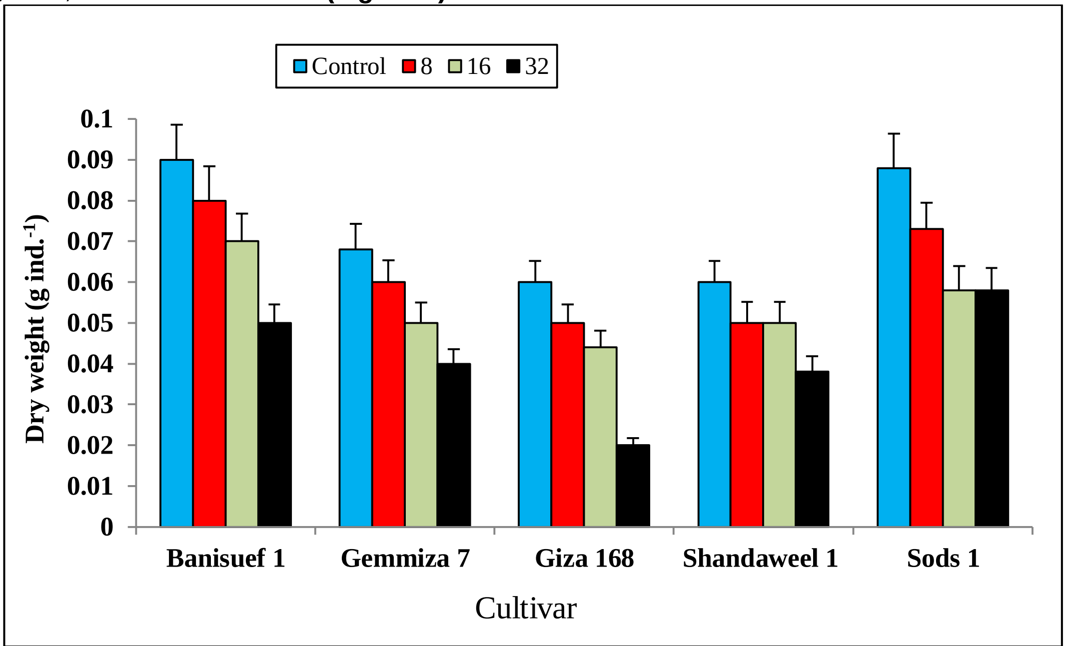

Figure 4.Variation in dry weight (g ind^{-1}) of five wheat cultivars seedlings as affected by different concentrations of *Salix alba* leaf crude powder (SACP) twenty- one days after sowing.

Photosynthetic pigments

Table 2.Variation in the mean concentration of different pigment fractions, Chl "a/b" and Total pigments of five wheat cultivars seedlings as affected by different concentrations of *Salix alba*leaf crude powder (SACP)twenty one days after sowing.

Treatment (%)	Cultivar Banisuef 1		Gemmiza 7		Giza 168		Shandaweel 1		Sods 1	
	Chl a/b	Total pigments	Chl a/b	Total pigments	Chla/b	Total pigments	Chl a/b	Total pigments	Chl a/b	Total pigments
Control	1.95^{b}	3.67^{c}	1.78^{a}	5.29^{c}	1.67^{a}	4.42^{d}	2.17^{b}	5.25^{c}	2.32^{a}	5.28^{c}
8	3.70^{d}	2.53^{a}	3.80^{b}	2.88^{b}	4.86^{b}	3.02^{c}	1.02^{a}	4.80^{b}	2.43^{c}	3.29^{b}
16	2.65^{c}	2.50^{a}	14.5^{c}	2.27^{a}	12.80^{c}	2.54^{b}	10.7^{c}	2.58^{a}	6.92^{d}	3.10^{b}
32	0.86^{a}	2.82^{b}	1.23^{a}	2.51^{a}	12.43^{c}	1.35^{a}	2.21^{b}	2.82^{a}	2.52^{b}	2.45^{a}

Data in Table 2 indicated that the photosynthetic pigment contents of all five cultivars were significantly decreased with increasing SALCP concentrations. The reduction in total photosynthetic pigments in leaves of Banisuef 1, Gemmiza7, Giza 168, Shandaweel 1 and Sods 1 at 32% SALCP was 23, 52.5, 69.4,46 and 53% respectively. The reduction of total photosynthetic pigments of Giza 168 (being the most affected cultivar), can be ascribed to the decrease in Chl. b content. For example, at the highest SALCP concentration the decrease in Chl. a content of Giza 168 was 54% compared to 93% reduction in Chl. b content of the same cultivar.On the other hand, the carotenoids content at the highest SALCP level has increased in cultivars Banisuef 1, Shandaweel 1 and sids1. The percent increase was (48.7%),(53.3%)and (20%) respectively. While decreased in the other 2 cultivars Gemmiza (72%) and Giza168 (71%).

DISCUSSION

In the present study, a series of different concentrations (4, 8, 16, 32 and 64% besides the control) of Salix alba leaf aqueous extract (SALAE) were applied to investigate their allelopathic effects on germination percentage (GP), plumule (PL) and radicle (RL) lengths, seed germination index (SGI) and energy of germination (GE) of five recipient cultivars (Banisuef 1, Gemmiza 7, Giza 168, Shandaweel 1 and Sods 1) of Triticum aestivum L.. For all cultivars, GP, Pl, RL, SGI and GE were significantly affected by applying the different concentrations of SALAE. At the maximum concentration (64%) of SALAE, 40% of cultivar Sods 1 grains were completely inhibited. With respect to the PL, Banisuef 1 (100%) and Gemmiza 7 (95.8%) cultivar could be recognized as very susceptible wheat cultivars to the extracts. At 64% extract concentration radicle growth of Banisuef 1 cultivar was completely inhibited and the highest reduction percentage of RL was attained in all wheat cultivars especially Shandaweel 1 (90.8%) and Banisuef 1(100%).

These results indicated that S. alba leaves release allelopathic substances which accumulate in bioactive concentrations and adversely affect seed germination and seedling growth of the studied wheat cultivars. Batish et al. (2007) reported that nettle-leaved goosefoot (Chenopodiu mmurale) roots and their exudates exerted allelopathic effects on wheat by releasing water-soluble phenolic acids as putative allelochemicals in soil. Earlier, inhibition of wheat (Triticum aestivum) radicle growth was positively associated (r = 0.66) with concentrations of total phenolics contained in sorghum (Sorghum bicolor) plant parts (Ben-Hammouda et al., 1995). The potential allelopathic effects of different concentrations of Zygophyllum album (donor species) aqueous extract (2.5, 5, 7.5 and 10%) on germination percentage of Bromus tectorum (weed) and Triticum aestivum (crop species) under laboratory conditions showed to have the greatest inhibitory effect on the recipient species in mixed culture compared to that of pure culture (Salhi et al., 2011b). Additionally, a gradual reduction was obtained by Tanveer et al. (2010) on the effect of Euphorbia helioscopia aqueous extract on SGI of Triticum aestivum, Cicer arietinum, and Lens culinaris. The considerable inhibition of seed germination in the present study may be due to the inhibitory effect of allelochemicals such as water soluble saponins, hormones, enzymes and polyphenols found in S. alba leaves which could affect growth directly or by altering the mobilization of storage compounds during germination (Cheikh-Rouhou et al., 2007). Daizy et al., 2007 and Zzet and Yusuf (2004) stated that, 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. They also found that the allelochemicals were associated with inhibition of nutrients and ion absorption by affecting plasma membrane permeability. Alam and Islam (2002) suggested that the inhibition percentage (IP) of crop plants may be due to the disturbance in the activities of peroxidase, alpha-amylase and acid phosphates. Bioassays of germination, radicle, hypocotyl and coleoptile growth are used to test the allelopathic potential of crop species (Ben-Hammoudaetal., 2001).The elongation of the hypocotyl or coleoptiles can be used in conjunction with GP. Growth bioassays are often more sensitive than germination bioassays (Bhowmik and Doll, 1984). Fuentes et al. (2004) observed that seed germination has been regarded as a less sensitive method than plumule and radicle length when used as a bioassay for the evaluation of phytotoxicity.

Increasing S. alba crude powder (SACP) concentration in the present study caused a dramatic decrease in seedling length (SL), plumule length (PL), radicle length (RL), seedling fresh (SFW) and dry (SDW) weights of all tested cultivars. The results of the present study also showed that there was an inverse proportional relationship between increasing the severity of different percentages of SACP on one hand and content of leaves of chlorophyll a and b, carotenoids and total pigment content on the other hand. The results of the present study are matched with that obtained by khalil and Ismael (2010) on the effect of water stress on pigment content of Lupinus termis leaves. Our results were explained on the concept of the effect of SACP on the water uptake by the recipient species which may be the essential factor for tissue drought. The result were fortified by those of Flexas et al., (2000), El-Tayeb and Hassanien (2000), Misra and Srivastava (2000), Wang and Li (2001), Sawhney and Singh (2002). Such reduction in the photosynthetic pigment content in response to SACP stress was attributed to the ultra-structural deformation of plastids including the protein membranes forming the thylakoids which in turn causes untying of photo system 2 which capture photons, so its efficiency declined, thus causing declines in electron transfer, ATP and NADPH production and eventually CO_2 fixation processes (Maslenkova and Toncheva, 1997 and Zhang et al., 2006).

The study concluded that the more resistant wheat cultivar in the present study is Giza 168. It is recommended to cultivate it in the locations close to Salix trees. Furthermore, extension of wheat farming most probably will increase allelopathic induced damage of Salix alba to wheat. Therefore, in Egypt strategies for extension in wheat cultivation in new vast areas should include studies on the allelopathic actions of trees especially those which are used as windbreaks, or for shelters (Salix alba trees should be keep away from the system).

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