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Alleviation of Allelopathic Effect of *Launae sonchoids* Weed on Wheat Growth by Salicylic Acid

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EXPERIMENT for the study of alleviation of allelopathic effect of *Launae sonchoids* on wheat growth by pretreatment grains with salicylic acid (SA). *Launae sonchoids* was collected from the desert plains at Taif area, Saudia Arabia and sampled in March 2010. The germination and growth were conducted in the greenhouse during April 2010. Diluted extracts of *Launae sonchoids* shoot system was applied to pre-soaked in SA or non soaked wheat grains. The aqueous extract of *Launae sonchoids* at 25% caused an increase in the root and shoots growth and biomass production of the wheat seedlings at pre-soaked in SA or H_2O comparison with control. The concentrated extracts (75 and 100%) were phytotoxic to wheat germination. The percentage declined by pre-soaked wheat grains in 0.05 mM salicylic acid. Betaine levels in seedlings pretreated with salicylic acid significant increase versus untreated seedlings, possibly because such a precursor promotes betaine biosynthesis. This could be responsible for the enhanced growth criteria and preventing the decrease in chlorophyll content in the pretreated seedlings. A relationship between nitrogenous fractions, antioxidant glutathione and *Launae sonchoids* extract tolerance observed in wheat seedlings with or without salicylic acid. Overall, the adverse effects of allelopathic stress could be alleviated by pre-treated with SA.

Keywords: Allelopathic, *Launae sonchoids* aqueous extract, Wheat growth, Salicylic acid, Betaine and non-protein thiols.

Allelopathy is the addition of plant-produced phytotoxins to the plant environment and competitive strategy of plants (Oussama, 2003). Accessions with an allelopathic effect have been found in many crops (Rice, 1984). Out of more than 3000 accessions of oat, several were found with a fluorescent microscope to exude a large amount of an allelochemical, scopoletin (Fay & Duke, 1977). Biodegradable natural plant products rarely contain halogenated atoms and possess structural diversity and complexity; these can act directly as herbicides or may provide lead structures for herbicidal discovery (Bhadoria, 2011). Selection of allelopathic plants is a good and commonly used approach for identification of plants with biologically active natural products (Duke *et al.*, 2000).

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Allelochemicals are released from plant tissue in a variety of ways including emission, exudation, or leaching from above ground parts by rain, dew, fog, etc. (Ben Hammouda *et al*., 2001). Khanh *et al.* (2007) found that inhibitory substances involved in allelopathy are mainly terpenoids, and phenolic substances. Wide arrays of biologically active constituents are produced by plants in the genus *Launae* (Regina & Belz, 2007). Bertholdesson, (2005) showed that volatile oil of *Launae sonchoids* have several biological activities, notably antibacterial, antifungal and antioxidative properties. Monoterpene vapors may cause anatomical and physiological changes in plant seedlings and exposure to volatile terpenes can lead to accumulation of lipid globules, reduction in organelles and disruption of membranes (Monari *et al.*, 2005). Meanwhile the alkaloids gramine and hordenine caused damages to the cell walls, disorganization of organelles, increase cell vacuoles, and the appearance of lipid and globules of root tip cells (Rasmussen *et al.,* 2004).

Salicylic acid is (SA) an endogenous growth regulator of phenolic nature (Hayat *et al.,* 2010). Its role in the defense mechanisms against biotic and a biotic stress has been well documented (Szalai *et al*., 2000, Bosch *et al.*, 2007 and Hussein *et al.,* 2007). SA plays an important role in determining the sensitivity of plants to various a biotic stresses (Dat *et al.*, 1998 and Rao &Davis, 1999), notably at the seedling stage (Borsani *et al*., 2001 and Szepesi *et al.,* 2005). The role of SA in defense mechanism to alleviate stress in plants was studied (Afzal *et al.,* 2006; Hussein *et al.,* 2007 and Javid *et al.,* 2011). The ameliorative effects of SA have been well documented including salt tolerance in many crops such as bean (Azooz, 2009), tomato (Tari *et al*., 2002) and maize (Gunes *et al.,* 2007). Moharekar *et al.* (2006) shows that the salicylic acid (SA), application increased chlorophyll content in both wheat and moong seedlings. Also Noreen & Ashraf (2008) show that ameliorative effect of SA on plant growth under abiotic stress due to its role in nutrient uptake, water relations and stomatal regulation. Meanwhile, Taşgın *et al.* (2005) concluded that exogenous SA can be involved in cold tolerance by regulating apoplastic proteins and antioxidant enzyme activities. It was found that inhibition of catalase, a H_2O_2 scavenging enzyme by SA plays an essential role in the generation of reactive oxygen species (Horváth *et al.*, 2002 and 2007). By increasing H_2O_2 concentration of the tissues, moderate doses of SA may activate the antioxidative mechanisms.

The present study was conducted to asses if the pretreatment wheat grains with low concentrations of SA could ameliorate the adverse allelopathic effects of *Launae sonchoids* on wheat growth. For this purposes growth characteristics, chlorophyll content, betaine, glutathione and nitrogenous fractions were measured.

Materials and Methods

Preparation of extract

Launae sonchoids was collected from the desert plains at Taif area and sampled in March 2010. Whole plants were pulled out of the field at the stage of flowering development. The plants were gently washed with distilled water, dried between two paper towels. The plant materials were chopped into 1 cm long pieces and dried at 50°C for 24 hr. The components were soaked in distilled water for 24 hr at the rate of 5g dry weight per 100 ml distilled water. Extract was filtered through four layers of cheese cloth, Whitman filter paper under vacuum and stored at <5°C. Dilutions were made of the original extracts as, 100, 75, 50, and 25% of the origin extracts.

Culture and treatments

The soil used in pots was sandy soil (85% sand, 10% Silt and 5% clay). Caryopses of wheat (*Triticum aestivum* L. CV, Gomeza 7) washed with running tap water and soaked for 30 sec in 70% ethanol, disinfected with 20% (v/v) sodium hypochlorite for 20 min and rinsed three times with sterile distilled water. Sterilized caryopses were divided two groups one soaked in water and other soaked in 0.05 mM salicylic acid for 6 hr. Twenty caryopses were swon in each pot at relative humidity of 50 ± 4%, day/night temperature 30/20 ±2^{o}C, and light intensity of 350.67 ± 4.16 Lux. in the greenhouse at Botany Department, Zagazig University, during April 2010. The experimental design was a complete randomized design with four replications.

The treatments were

C = Soaked in H_2O, irrigated with distilled H_2O
LA_1= Soaked in H_2O, irrigated with *Launae* extract, 25%
LA_2= Soaked in H_2O,irrigated with *Launae* extract, 50%
LA_3= Soaked in H_2O, irrigated with *Launae* extract, 75%
LA_4= Soaked in H_2O, irrigated with *Launae* conc., extract 100%
SC= Soaked in 0.05 mM salicylic acid (SA), irrigated with distilled H_2O.
SLA_1= Soaked in 0.05 mM salicylic acid, irrigated with *Launae* extract, 25%
SLA_2= Soaked in 0.05 mM salicylic acid, irrigated with *Launae* extract, 50%
SLA_3=Soaked in 0.05 mM salicylic acid, irrigated with *Launae* extract, 75%
SLA_4= Soaked in 0.05 mM salicylic acid, irrigated with *Launae* conc., extract 100%

Growth measurement

Plant growth was estimated after 8 days (from germination) by measuring accumulation of root and shoot weight (after drying the plants material at 70^{o}C for 48-72hr. Water content (WC) and degree of succulence (D. Su.) were measured according to Weatherly & Barrs (1962) and expressed as a percentage according to the following equations:

$$WC (\%) = (FW - DW) / FW \times 100,$$
$$D. Su. = FW / DW.$$

Analyses

Chlorophyll content

Concentration of chlorophyll a & b in green tissues (leaf plus stem) of wheat seedlings at 2, 4, 6 and 8 days were estimated by the equations of Witham *et al*. (1971).

Betaine assay

0.5g FW of wheat seedlings at 2, 4, 6 and 8 days were homogenized with 5ml of methanol and extracts were phase-separated with chloroform and water as described previously (Rhodes *et al.,* 1987). After evaporating the aqueous phase to dryness in an air stream of distilled water and 0.3ml of slurry of Dowex 50 ion exchange resin in the H^+ form (Lerma *et al.,* 1991) were added. Total betaine was determined according to the periodide method (Wall *et al.,* 1960).

Glutathione determination

None-protein thiols were extracted by homogenizing 0.3 gm FW of wheat seedlings at 2, 4, 6 and 8 days in 1.5 ml of 0.1N HCl. After centrifugation at 15000 rpm for 30 min at 4°C, the supernatants were used for analysis. Total glutathione was determined in the homogenates by spectrophotometry at 412 nm (Schupp & Rennenberg, 1988).

Nitrogenous fractions

Total nitrogen, soluble nitrogen; insoluble nitrogen and crude protein were measured in wheat seedlings after, 2, 4, 6 and 8 days from germination according to the conventional micro-Kjeldahl method (Pirie, 1955) and AOAC (1980).

Statistical analysis

Results indicated as mean values±SEM. Differences between control and treated samples were statistically analyzed by COSTAT and MSTATc computer programme and comparative analyses of the means were performed by LSD, least significantly different according to Fisher's test.

Results and Discussion

Effect of Launae sonchoids extracts in the presence or absence of SA on wheat caryopses germination

Launae sonchoids shoot extracts significantly affected germination of wheat caryopses in the presence or absence of SA (Table 1). Results obtained revealed that *L. sonchoids* allelopathy takes the form of heterotoxicity, depressive to wheat. The germination percentage of wheat caryopses after 2 days decreased more than 60 and 42% by *Launae* treatment at concentration of LA_4 and LA_3, respectively. The reduction decreased to 35 and 20% after 8 days, in the caryopses pre-soaked in H_2O. While at pre-soaked in SA the germination in day 8 decreased by 15 and 6% at SLA_4 and SLA_3, respectively. Treatment with LA_1 extract had non-significant effect on the germination of wheat seeds, compared with control in the treated or untreated with SA. In general the negative effects of *L. sonchoids* extract on the germination of wheat caryopses increase with increasing the concentrations of extracts and this inhibition may be alleviated with pre-soaked the caryopses in SA.

TABLE 1. Effect of diluted extracts of *Launae sonchoids* on germination (%) of wheat caryopses in the presence or absence of SA. Values are the means ± SEM of three replicated measurements.

Treatments	Germination %			
	Time (days)			
	2	4	6	8
C	64±5	95±6	100±6	100±6
LA1	60±5	88±5	90±6	92±6
LA2	44±4**	65±5**	80±5*	88±6*
LA3	34±3***	51±4***	74±5**	80±6**
LA4	22±2***	34±3***	46±4***	65±6***
LSD, LA (5%)	1.2	0.65	0.55	0.43
SC	72±5	100±6	100±6	100±6
SLA1	65±5	90±6	95±6	100±6
SLA2	50±4	84±5*	90±6	98±6
SLA3	43±3**	79±5*	85±5	94±6
SLA4	36±3**	65±5**	78±5**	85±6*
LSD, SLA (5%)	1.06	0.95	0.64	0.52

Means within a column followed by different number of stars are significantly different according to Fisher's, *=Significant at P< 0.5 **=Significant at P<0.1 ***=Significant at P< 0.05

There are hundreds of secondary metabolites in the plants kingdom, and many are known to be phytotoxic (Einhellig, 2002). Allelopathic effects of these compounds are often observed to occur early in the life cycle, causing inhibition of germination and/or seedling growth (Halsey, 2004). The compounds exhibit a wide range of action mechanisms, from affects on DNA (alkaloids), photosynthetic and mitochondrial function (quinones), phytohormone activity, ion uptake, and water balance (phenolics). Interpretations of action mechanisms are complicated by the fact that individual compounds can have multiple phytotoxic effects (Einhellig, 2002).

Previous studies demonstrated that salicylic acid plays an important role in determining the sensitivity of plants to various a biotic stresses (Dat *et al.,* 1998 and Rao & Davis, 1999), notably at the seedling stage (Borsani *et al.,* 2001). Lipid peroxidation and membrane permeability, which were increased by stress, were lower in SA treated plants (Horvath *et al.,* 2007). SA treatment was accompanied by a transient increase in the H_2O_2 level. As caryopses treatment with H_2O_2 itself had an alleviating effect on the oxidative damage caused by stress in wheat plants (Wahid *et al.,* 2007), it seems possible that SA may exert its protective effect partially through the transiently increased level of H_2O_2.

Effect of Launae sonchoids extracts in the presence or absence of SA on wheat growth

To determine the allopathic effect of *L. sonchoids* on the growth of wheat seedlings the length and water content (WC) as well as dry weight of roots and shoots was measured either pre-soaked in H_2O or SA (Table 2). The maximum shoot and root lengths were noticed at day 8 SC samples. The reduction in the root and shoot lengths reached to 60 and 75 % by treatment with *Launae sonchoids* LA_3 and LA_4, respectively. The root and shoot length of wheat seedlings were gradually decreased with the increase extract concentration from LA_2 to LA_4 at pre-soaked in H_2O. The length of the seedlings was significantly lower than that of the control, reaching a length more than three folds less at the 8^{th} day of treatment (Table 2) at pre-soaked in H_2O. Kil & Yun (1992) shows that germination percentage and dry weight of wheat plants was slightly increased at lower concentrations of *L. sonchoids* extracts, whereas it was proportionally inhibited at higher concentrations.

TABLE 2. Effect of diluted extracts of *Launae sonchoids* on root and shoot lengths of 8 days old wheat plants, pre-soaked or none soaked in salicylic acid. Values are the means ± SEM of three replicated measurements.

Treatments	Root length (cm)	Shoot length (cm)
C	4.9±0.4	14.5±0.9
LA1	5.1±0.5	15.2±1
LA2	3.5±0.3*	12.5±0.4*
LA3	2.3±0.2**	8.5±0.6***
LA4	1.6±0.2***	6.7±0.4***
LSD, LA (5%)	0.26	0.92
SC	5.8±0.5	16.8±1.1
SLA1	5.0±0.5	14.2±1
SLA2	4.5±0.4	13.5±0.7
SLA3	4.0±0.3*	12.6±0.7*
SLA4	3.4±0.3*	10.7±0.6*
LSD, SLA (5%)	0.36	1.2

Means within a column followed by different number of stars are significantly different according to Fisher's, *=Significant at P< 0.5 **=Significant at P<0.1 ***= Significant at P< 0.05.

Pre-soaked in SA alleviation the negative effect of the *L. sonchoids* allelopathy on shoot and root lengths.The protective effect of salicylic acid was shown by the greater length of SLA versus LA seedlings (Table 2). On the 8^{th} day, mean SLA_4 lengths were more than twice as much as LA_4 and were accompanied by the appearance of secondary roots in the seedlings. Treatment of wheat plants with 0.05mM SA increased the level of cell division within the apical meristem of seedling roots which increased the in plant growth (Javid *et al.*, 2011).

Water content and degree of succulence were higher in the LA and SLA groups compared with C and SC groups, (Table 3). *L. sonchoids* water extracts increased the water content as well as degree of succulence of the wheat root and shoot either the seeds at pre-soaked in SA or H_2O. Meanwhile at LA groups the increase was more obivious than that of SLA groups. At LA and SLA groups water content and degree of succulence gradually increase in seedling roots with increasing extract concentration. Water content and degree of succulence gradually decrease in the seedling shoots of SLA groups from SLA_1 to SLA_4. The pre-treatment with SA decrease the water content of the seedlings with stress only. Szepesi *et al*., (2005) reported that SA pre-treatments reduced K^+ contents of leaves and increased water potential under stress, so water content and degree of succulence decrease by increasing stress.

TABLE 3. Effect of diluted extracts of *Launae sonchoids* on root and shoot water content (WC) and degree of succulence (D. Su.) of 8 days old wheat plants pre-soaked or none soaked in salicylic acid.

Treatments	Root		Shoot	
	WC %	**D. Su.**	**W.C %**	**D. Su.**
C	46.44	1.88	70.93	3.44
LA1	47.83	1.92*	72.83	3.68
LA2	51.90	2.08	85.90	7.09
LA3	56.73*	2.31	88.89*	9.00*
LA4	58.24*	2.39	88.24*	8.89*
LSD, LA (5%)	0.87	0.08	1.17	0.07
SC	41.83	1.72	71.83	3.52
SLA1	44.44	1.80	74.44	3.91
SLA2	47.12	1.89	74.12	3.86
SLA3	49.97	2.00	72.97	3.7
SLA4	51.37	2.04	70.37	3.38
LSD, SLA (5%)	0.62	0.07	1.02	0.08

Means within a column followed by different number of stars are significantly different according to Fisher's, *=Significant at P< 0.5 **=Significant at P< 0.1 ***=Significant at P< 0.05

Priming treatments significantly affected fresh and dry weight of wheat seedlings (Table 4). Fresh weights of seedlings was drastically decreased due to high concentration of *L. sonchoids* water extract, meanwhile low concentration (LA_1 and SLA_1) improved fresh and dry weight of seedlings as compared with control with or without pre-soaked in SA. The reduction of fresh and dry weight reached to 65% at seedlings treated with LA_4. Meanwhile the reduction reached to about 44% at SLA_4 at the same concentration of *L. sonchoids* extract. Seedlings pre-treatment with SA prevented the decreement in biomass caused by stress. Moreover seedlings pre-treatment with SA (SC) had higher biomass than control seedlings.

Pre-treatment with SA regulate the nascent polypeptide-associated complex (NAC) during seeds germination. NAC is a heterodimeric complex that can reversibly bind to eukaryotic ribosome's (Rospert *et al.*, 2002). It is presumed to prevent ribosome-associated nascent polypeptide from inappropriate interaction with proteins in the cytosol (Wiedmann *et al.,* 1994). Yan *et al.* (2005) demonstrated that the NAC was down-regulated in rice (*Oryza sativa*) roots submitted to stress. Also, a proteomic study of sugar beet (*Beta vulgaris*) leaves identified the *α*-chain NAC as being down-regulated in response to stress (Hajheidari *et al.,* 2005). Jumali *et al.* (2011) showed that most genes responding to acute SA treatment are related to stress and signaling pathways which eventually led to cell death. This include genes encoding chaperone, heatshock proteins (HSPs), antioxidants and genes involved in secondary metabolite biosynthesis, such as sinapyl alcohol dehydrogenase (SAD), cinnamyl alcohol dehydrogenase (CAD) and Cytochrome P450 (CYP 450).

TABLE 4. Effect of diluted extracts of *L. sonchoids* on fresh weight (F.W) and dry weight (D.W) of 8 days old plants pre-soaked or none soaked in salicylic acid. Values are the means ± SEM of three replicated measurements.

Treatments	Root (g/plant)		Shoot (g/plant)	
	F.W	D. W	F.W	D. W
C	0.048	0.020	0.079	0.022
LA1	0.051	0.026	0.086	0.028
LA2	0.044	0.011^{*}	0.074	0.021
LA3	0.033^{**}	0.01^{*}	0.063	0.017
LA4	0.022^{***}	0.008^{**}	0.034^{**}	*
LSD, LA (5%)	0.005	0.004	0.008	0.011
SC	0.058	0.023	0.094	**
SLA1	0.061	0.025	0.10	0.002
SLA2	0.048	0.018	0.085	0.037
SLA3	0.043	0.015	0.074	0.042
SLA4	0.032^{**}	0.011^{*}	0.054	0.022
LSD, SLA (5%)	0.006	0.005	0.01	0.020
				0.016
				*
				0.002

Means within a column followed by different number of stars are significantly different according to Fisher's *=Significant at P< 0.5 **=Significant at P< 0.1 ***=Significant at P< 0.05

Effect of Launae sonchoids extracts on chlorophyll contant of wheat seedlings (pre-soaked or none soaked in salicylic acid)

Total chlorophyll was determined in wheat green tissues under the effect of irrigation with *L. sonchoids* extract. In LA_1 and SLA_1 seedlings, total chlorophyll was higher than those of C and SC seedlings, (Table 5). Meanwhile increasing the extract concentration from LA_2 and SLA_2 to LA_4 and SLA_4 were associated with gradually decrease of chlorophyll contents. Yang *et al.,* (2002) showed that allelochemical may reduce chlorophyll accumulation in three ways: The inhibition of chlorophyll biosynthesis, the stimulation of chlorophyll degradation, or both. The present study strongly indicates that chlorophyll biosynthesis of wheat seedlings was effacted by the allelopathic effect of *Launae sonchoids* water extracts.

Seedlings pre-treatment with SA prevented the decrease in chlorophyll content caused by *L. sonchoids* extract stress. Since SA improved the photosynthetic performance of plants under stress conditions (Ananieva *et al.*, 2002), and chlorophyll a fluorescence could give insight into the ability of a plant to tolerate environmental stresses. This can be partially overcome if plants are pre-treated with SA. Since under non-photorespiratory conditions the effective quantum yield of PSII provides useful information concerning photosynthetic performance of C3 plants, these results suggests that SA pre-treatment may improve the gross rate of carbon assimilation during allopathic stress. In the presence of SA, leaves accumulated different compatible osmolytes, such as sugars, sugar alcohol and proline. SA pre-treatment decreased the CAT activity both in the roots and leaves, but the activity of other enzymes associated with the antioxidative defense, superoxide dismutases (SOD), peroxidase (POD), ascorbate peroxidase (APX) and glutathione reductase (GR) exhibited different changes. As a general rule, the activity of these enzymes (CAT, SOD, POD and APX) decreased compared to the control in the leaves of tomato plants (Szepesi *et al.*, 2005).

TABLE 5. Effect of diluted extracts of *Launae sonchoids* on chlorophyll (a+b) content (ug/g F.W) of wheat seedlings pre-soaked or none soaked in salicylic acid. Values are the means ± SEM of three replicated measurements.

Treatments	Time (days)			
	2	**4**	**6**	**8**
C	148±6	212±11	326±16	564±21
LA1	167±6	238±15	390±18*	586±26
LA2	112±4*	176±7**	256±14**	420±16*
LA3	84±3**	151±4***	174±5***	280±6***
LA4	42±2***	94±4***	116±6***	165±8***
LSD, LA (5%)	1.2	0.65	0.55	0.43
SC	164±9	256±16	422±6	629±18
SLA1	193±11	290±16	460±16	656±14
SLA2	184±4	210±11	324±14	512±14
SLA3	134±4*	191±9**	300±11*	470±13**
SLA4	103±3**	114±4***	216±10**	315±9***
LSD, SLA (5%)	1.56	0.95	0.75	0.75

Means within a column followed by different number of stars are significantly different according to Fisher's, *=Significant at P< 0.5 **=Significant at P< 0.1***=Significant at P< 0.05

Pre-treatment of wheat seeds with salicylic acid led to enhanced seedling tolerance to condition of stress during germination , as evidenced by the greater growth of SLA versus LA seedlings evaluated through such parameters as length and biomass of, root and shoot as well as chlorophyll content (Tables 1-4). Szepesi *et al.* (2005) showed that SA substantially improved tomato germination vigor under stress conditions. Sakhabutdinova *et al.* (2003), showed that pre-sowing treatment with SA completely prevented stress induced declines in the concentration of IAA and cytokines in seedlings and reduced accumulation of ABA, which might be a prerequisite for acceleration of growth.

Moharekar *et al.* (2006) reported that salicylic acid, application increased chlorophyll content in both wheat and moong seedlings. Meanwhile Taşgın *et al.* (2005) concluded that exogenous SA can be involved in cold tolerance by regulating apoplastic proteins and antioxidant enzyme activities.

Effect of Launae sonchoids extracts on levels of compatible osmolytes of wheat seedlings (pre-soaked or none soaked in salicylic acid)

Total betaine content reached the maximum level (26 ug /g DW and 18.7 ug /g DW) for SC and C, respectively on the eight day of germination without *L. sonchoids* extract (Table 6). All extracts showed an inhibitory effect on total betaine content of wheat. However, the SLA samples that were pre-soaked in SA showed different degrees of an inhibitory effect.

TABLE 6. Effect of diluted extracts of *Launae sonchoids* on the total betaine levels (ug/g DW) of wheat seedlings pre-soaked or none soaked in salicylic acid. Values are the means ± SEM of three replicated measurements.

Treatments	Time (days)			
	2	4	6	8
C	4.2±.0.3	8.5±0.4	13.2±0.5	18.7±0.8
LA1	3.8±0.3	7.5±0.3	12.5±0.5	17.8±0.7
LA2	3.3±0.2*	7.1±0.3*	10.8±0.5*	15.6±0.7*
LA3	2.5±0.2**	6.5±0.2*	9.5±0.4*	12.4±0.6**
LA4	2±0.2***	5.1±0.2**	8.4±0.4*	10.7±0.6***
LSD, LA (5%)	0.05	0.06	0.08	0.09
SC	4.6±0.1	11.2±0.4*	19.5±1***	26±2***
SLA1	4.1±0.2	10.7±0.4*	18±1***	25.7±2***
SLA2	3.8±0.2	10.1±0.5*	17.8±0.7**	24.8±2**
SLA3	3.5±0.2	9.8±0.3	16.8±0.7**	21.4±2*
SLA4	3.2±0.2*	8.7±0.3	14.4±0.6*	17.9±0.9
LSD, SLA (5%)	0.05	0.07	0.09	0.10

Means within a column followed by different number of stars are significantly different according to Fisher's *=Significant at P< 0.5 **=Significant at P< 0.1 ***=Significant at P< 0.05

Pre-treatment with salicylic acid led to a significant increase in betaine levels in SC and SLA groups versus C and LA seedlings. Such an increase may be attributed to the fact that the addition of this precursor (salicylic acid) promotes betaine formation by stimulating its biosynthesis (Hitz *et al*., 1982). The protective role of betaine against stresses in higher plants, in bacteria and in animals is widely recognized (Rhodes & Hanson, 1993).The significant increase of this osmolytes in plant tissue of pre-treated with SA would help to explain the increase in tolerance to allelopathy. The accumulation recorded in seedlings starting from the fourth day could be responsible for the enhanced growth observed in SLA versus LA seedlings, as well as for preventing the decrease in chlorophyll content.

Glutathione content was decreased by *L. sonchoids* extract treatments (Table 7). However, at SLA the glutathione values increased after 8 days compared with C and SC groups. Adaptation to *L. sonchoids* extract involves increases in the antioxidant capacity of the cell to detoxify reactive oxygen species (Bellaire *et al*., 2000). In concordance to Hernadez *et al*. (2000), the glutathione (GSH) content could indicate that this antioxidant soluble compound was involved in the stress tolerance. The increase in glutathione content due to SA treatment enhanced tolerance may be totally or partly due to increased GSH synthesis and/or decrease rates of degradation (Noctor & Foyer, 1998). Exogenous SA treatment leads to increased antioxidant capacity in barley (*Hordeum vulgare*) leaves (Ananieva *et al.*, 2002) and stimulates peroxidase/catalase activities in plant cells (Dixon *et al.*, 1995) because of an enhanced accumulation of hydrogen peroxide under such conditions (Rao *et al.*, 1997). It is known that stress induces the generation of reactive oxygen species in plants (Polle, 1997 and Borsani *et al.*, 2001). Thereby, it is possible that the presently observed induction of such enzymes by SA can provide an explanation for the improvement of *T. aestivum* seed germination under stress.

TABLE 7. Effect of diluted extracts of *Launae sonchoids* on the glutathione content (ug/g DW) of wheat seedlings pre-soaked or none soaked in salicylic acid. Values are the means ± SEM of three replicated measurements.

Treatments				
	2	**4**	**6**	**8**
C	15.2±0.8	19.4±0.9	15.2±0.5	13.7±0.5
LA1	13.8±0.7	17.5±0.7	13.5±0.5	10.8±0.4
LA2	11.3±0.7*	17.1±0.7	10.7±0.5*	8.6±0.3**
LA3	9.5±0.6**	15.5±0.6*	7.5±0.4**	6.4±0.2***
LA4	7.2±0.5***	11.1±0.6**	7.1±0.4**	5.7±0.3***
LSD, LA (5%)	0.08	0.1	0.07	0.06
SC	17.4±1	21.8±2*	18.5±1*	17±2**
SLA1	16.1±0.8	20.1±1.4*	16±1*	18.7±2**
SLA2	15.8±0.7	19.4±1*	14.8±0.7	20.8±2***
SLA3	14.5±0.6	19±1	13.6±0.7	22.4±2***
SLA4	11±0.5*	17.8±0.8	11.4±0.6*	17.3±0.8**
LSD, SLA (5%)	0.13	0.15	0.1	0.1

Means within a column followed by different number of stars are significantly different according to Fisher's, *=Significant at P< 0.5 **=Significant at P< 0.1***=Significant at P< 0.05

Effect of Launae sonchoids extracts on nitrogenous fractions of wheat seedlings (pre-soaked or none soaked in salicylic acid)

It is clear from Table 8 that the values recorded for total nitrogen, soluble nitrogen; insoluble nitrogen and crude protein were decreased by the treatment with *L. sonchoids* extract. The depression induced by the highest level of *L. sonchoids* extract reached to 50% of that control, while the pre-soaked in SA reduced depression to less than 40%.

The pre-soaked with salicylic acid improved all nitrogenous fractions (Table 8). Gunes *et al.* (2007) reported that, salicylic acid acts as endogenous signal molecule responsible for inducing abiotic stress tolerance in plants. They emphasized that exogenous application of SA increased plant growth significantly both in stress and non stress conditions. Plants produce proteins in response to abiotic and biotic stress and many of these proteins are induced by phytohormones such as ABA and salicylic acid (Hoyos & Zhang, 2000). Moreover Gunes *et al.* (2007) demonstrated that exogenously applied SA increased plant growth significantly by improving the uptake of N, Mg, Fe, Mn, and Cu or induced protein biosynthesis. Other processes affected by SA concerned the quality of protein translation, the priming of seed metabolism, the synthesis of antioxidant enzymes, and the mobilization of seed storage proteins. All the observed effects are likely to improve seed vigor (Rajjou *et al.,* 2006).

The mode of action of an allelochemicals can broadly be divided into a direct and an indirect action (Rizvi *et al.*, 1992). Effects through the alternation of soil properties, nutritional status and an altered population or activity of micro-organisms and nematodes represent the indirect action. The direct action involves the biochemical/physiological effects of allelochemicals on various important processes of plant growth and metabolism. Processes influenced by allelochemicals involve mineral uptake. Allelochemicals can alter the rate the ions absorbed by plants. A reduction in both macro- and micronutrients are encountered in the presence of phenolic acids (Bhadoria, 2011). Phytohormones and balanced plant growth hormones [indoleacetic acid (IAA) and gibberellins (GA)] regulate cell enlargement in plants. IAA is present in both active and inactive forms, and is inactivated by IAA- oxidase. IAA- oxidase is inhibited by various allelochemicals (Bhadoria, 2011).

Once the mechanism of SA action is better understood, new opportunities for agricultural biotechnology may become evident. Alongside unraveling the SA mode of action, other aspects such as uptake, transport and stability of SA as well as the development of SA analogues with high activity, should continue to be explored. It is only with this combined knowledge that unique mechanisms of stress resistance can lead to implementations, with predictable effects of SA application in the field, allowing for the full potential of SA to be harnessed in the future. Plants produce proteins in response to abiotic and biotic stress and many of these proteins are induced by phytohormones such as ABA (Jin & Chen, 2000) and salicylic acid (Hoyos & Zhang, 2000). Salicylic acid is an endogenous growth regulator of phenolic nature, which influence a range of diverse processes in plants, including seed germination (Cutt & Klessig, 1992), stomatal closure (Larque-Saaveda, 1979); ion uptake and transport (Harper & Balke, 1981), membrane permeability (Barkosky & Einhellig, 1993), photosynthetic and growth rate (Khan *et al*., 2003).

SA treatment caused accumulation of both ABA and IAA in wheat seedlings under salinity (Javid *et al.,* 2011), however, the SA treatment did not influence on cytokinin content. Thus, protective SA action includes the development of antistress programs and acceleration of normalization of growth processes after removal stress factors (Sakhabutdinova *et al.,* 2003). The results obtained strongly argue that SA could be a very promising compound for alleviation of the allelopathic stress sensitivity of wheat due to mitigate the damaging effects of various stress factors in the plant.

Conclusions

Our results indicate that salicylic acid alleviat the allopathic effect of *Launae sonchoids* on wheat plant growth. The aqueous extract of *L. sonchoids* decreases the growth parameter of wheat. The pre-soaked wheat seeds in 0.05 mM salicylic (SA) increase the tolerance to allelopathy of *L. sonchoids*. Betaine levels, chlorophyll, glutathione and nitrogenous fractions contents increased in the pretreated seedlings with salicylic acid as scavators. A relationship between nitrogenous fractions, antioxidant glutathione and *Launae sonchoids* extract tolerance observed in wheat seedlings with or without salicylic acid. It was concluded that SA could be used as a potential growth regulator to improve allelopathic tolerance in wheat plant.

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تخفيف التاثير الألوباثي لحشيشة نبات اللونيا على نمو نباتات القمح باستخدام حمض السالسليك

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تمت هذه الدراسة بغرض معالجة التاثير الضار لحشيشة نبات اللونيا على انبات حبوب و نمو نبات القمح عن طريق المعاملة باستخدام حمض السالسليك قبل الزراعة. تم تجميع نبات اللونيا من السهول الصحراوية من محافظة الطائف بالمملكة العربية السعودية في شهر ابرايل ٢٠١٠م وتم تحضير مستخلص مائي للنبات بالطرق العلمية المعروفة وعمل تخفيفات من المستخلص المائي تبدأ من ٢٥٪ إلى ١٠٠٪ . قسمت حبوب القمح إلى مجموعتين احدهما نقعت في محلول مخفف من حمض السالسليك والأخرى نقعت في الماء وتم زراعة البذور في تربة رملية ثم الري بالتركيزات المختلفة للمستخلص المائي لنبات اللونيه.

أظهرت النتائج أن الري بالمستخلص المخفف ٢٥٪ لنبات اللونيه ادى إلى زيادة نسبة انبات و نمو بادرات القمح متمثلا في طول الجذر والساق والكتلة الحية سواء كانت الحبوب قد نقعت في حمض السالسيلك أو الماء مقارنة بالعينات القياسية (تروى بالماء). أما الري بالتركيزات العالية من ٧٥٪ إلى ١٠٠٪ أحدثت تاثيرا مثبط لأنبات الحبوب ونمو البادرات وادي نقع الحبوب في المحلول المخفف لحمض السالسليك إلى تقليل التاثير الضار لهذه التركيزات. نقع الحبوب في المحلول المخفف لحمض السالسليك يزيد بعض المركبات الأيضية التي تلعب دورا في مقاومة النبات للأجهاد مثل البيتان لتاثير حمض السالسليك على تنشيط مناشئه. اوضحت النتائج أن هناك علاقة بين تحمل نبات القمح للتاثير الضار الناتج عن الري بالمسخلص المائي لنبات اللونيه ومكونات النبات من المركبات النيتروجنية والجلاتاثيون مضاد الأكسدة، واستنتج من هذه الدراسة أن المعاملة بحمض السالسليك تخفف من التاثير الألوباثي لنبات اللونيا على انبات حبوب القمح ونمو البادرات.