

The Effects of different liquid extract of Swallow grass (*Cynanchum acutum*) on seed germination, anatomical and morphological structures of wheat (*Triticum aestivum*)

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ABSTRACT: The action of allelochemicals is diverse and affects a various number of biochemical reactions resulting in modifications of different physiological functions. In this study, the allelopathic effects of Leaves extract of Swallow grass during seed germination and seedling stage as anatomic sampling in three concentrations 0, 70, 140 and 210 mL and in two types of aqueous and alcoholic extracts on seeds of wheat and *Sisymbrium irio* were studied. The results showed that type of extract made no difference on the traits. But the Analysis of variance of other treatments showed that different concentrations in both plant extracts decreased germination traits. Anatomical results also showed that all traits except root epidermis affected by different concentrations of extracts. In a way that the extracts reduced the thickness of measured traits.

Keywords: Allelopathy, Anatomical traits, Germination

INTRODUCTION

Due to the growing human population in the world, need for food and supply it as a major problem and a serious concern for humanity today has always been considered one of the most important issues and despite efforts to increase production, constantly is affected by both biotic and abiotic damaging factors (Alsaadawi, 2001). Weeds are one of the biotic factors effective in reducing crop. In recent year, weed management has got much attention in biological topics. The correct weed management is identifying environmental factors that influence their biology (Bilalis et al., 2003). It is known that weeds are the main factors that limit the germination of crops. The chemicals secreted by these plants can be used to control weeds or pesticides used in farmland. Although in most countries, chemical weed control is in progress but reducing the quality of crops, the high cost of weed control, environmental damage and on the other hand increasing weed resistance to herbicides, Show the need for a revision of the methods of controlling weeds (Boz, 2003). So now we need a new herbicide that targets new metabolic status and are safe for the environment. In this regard, studies allelopathic weed extract can be an opportunity for the emergence of a new generation of herbicides natural and inhibited growth. Allelopathic term refers to the interaction of plants by biochemical functions of plants (Grabinski et al., 2008). These biochemical materials are responsible for processes of physiological, morphological and anatomical such as inhibition of

germination, inhibiting cell division, inhibition of respiration and photosynthesis, protein synthesis inhibitors and the activity of other enzymes. Allelopathic is the bioactive molecules by growing plants or debris of plants (Machado, 2007). May be after deformed and entering the environment has a direct or indirect impact affected on the growth and development of the same species or other species. Researchers have shown that allelopathic substances that found in plants reduced total dry matter crops such as wheat, corn, sunflower and soybean (Putnam, 1985; Putnam et al., 1990). Several chemicals such as coumarin, flavonoids, tannins and derivatives of cinnamic and benzoic acids affect various physiological processes and their effects have been demonstrated (Reddy, 2003). The weed plants have been useful and also a good source of biochemistry in the development of natural herbicides and pesticides.

Methods and Materials

Seeds of the Wheat [*aestivum* Merr.] c.v. 'L17' and Iranian *Sisymbrium irio* (*Descurania Sophia*) were surface sterilized in hydrogen peroxide/ethanol solution (10 ml of 30% H₂O₂ and 75 ml of 96% ethanol filled up to 100 ml with sterile distilled water) and rinsed several times with sterile water. The seeds were primed in various concentrations of Swallow grass (*Cynanchum acutum*) extract (0, 70, and 140 mL) in a flask for 8 h. Finally, ten seeds of each group were sown in plastic pots (20 cm diameter) filled with 5 kg autoclaved soil mixture composed of clay, sand and farmyard manure (1:1:1, w/w/w) at the depth of 1-2 cm (total 36 pots). All pots were placed into a growth chamber property of Faculty of Agriculture, Azad University of Saveh, Arak, Iran under controlled conditions (photoperiod: 16 h light and 8 h dark, temperature: 24/20 °C (day/night) and light intensity: 80,000 lux-metal halide source). Seeds were watered every day and watered with full strength of Broughton and Dilworth solution (Broughton and Dilworth, 1971) until seed germinated and leafy plant was achieved. After 20 days of sowing the plants were removed from the pots. The roots were gently washed with water to remove all perlites and vermiculites and then shoot and root were detached and weighted. Stem sample of each treatment was taken and fixed into 70% ethanol for anatomical assay. Stem and leaf sections were cut with a razor blade and placed in distilled water and observed under fluorescence microscopy. Olympus microscope equipped with a digital camera was used.

Statistical analysis

The experiment was conducted following a Completely Randomized Design (CRD) with three replications. For all variables, analysis of variance was performed using by software SAS. The means were using LSD and compared at 5%. The significance of differences among treatment means were compared by Duncan's Multiple Range Test (DMRT).

Result and discussion

The analysis of variance showed a significant effect on traits by concentration extract treatment (tab. 1). Comparison of means by Duncan's Multiple Range Test demonstrated that extract treatment decrease root length either shoot length and plant height (Tab. 2). Decrease in root length was parallel with enhancement in extract concentration. The highest root length was observed in control treatment while the lowest one obtained in 210 mL of extract treatment. Similar treatment achieved when shoots length and plant height was measured. The highest and lowest shoot lengths and plant height were obtained in the control treatment and 140 mL of extract treatment. Extract treatment was reduced plant growth and significantly diminished total dry weight. The results showed a downward decrease in total weight because of disincentive effect of extract treatment on plant height (Tab. 2). Allelopathy adversely affects the growth and development of crops, and the results of our study confirm that all growth variables of Wheat drastically decreased with extract treatment. It has been reported that the plants had the reduction in their dry weights because of the proportional increase in toxin substances. This could imply that an ionic effect was being manifested. It is also assumed that in addition to toxic effects of extract, higher concentration of swallow grass extract reduces the water potential in the medium which hinders water absorption and thus reduces plant growth. Weir *et al.*, 2004 also found the adverse effects of allelochemical on shoot and root growth. It has been reported that, decline in plant biomass may be due to excessive accumulation of allelochemical in root zone of wheat, which affects growth rate, and is often associated with a decrease in the nutrient transport activities of plant (Muminovic, 1991). In general, salinity reduces leaf number, leaf area, shoot and root dry weight, leading to low yields (Muminovic, 1991; Islam *et al.*, 2007; Kosobrukhov *et al.*, 2004).

There was no significant difference between control and 70 mL extract treatments on plant height, while increase of extract concentration from 70 to 140 mL dramatically decreased plant height (Table 2). Reduction in growth attributes especially plant height may be due to changes in plant-water relationships under salt stress, which suppress meristem activity as well as cell elongation (Terize *et al.*, 2003).

Microscopic results showed that swallow grass extract induced decrease in cutin synthesis on epidermal cells. Enhancement of swallow grass extract level increased cutin layer thickness in compare with control treatment. Cutin layer is distinguishable by a bright and orange layer on the epidermal cells under florescence microscope (fig. 1). Furthermore, we observed that, in allelopathy stressed plants, number of trichomes was decreased from epidermal root cells. In the other word, increase of salinity level led to more trichomes on epidermal layer in compare with control plants (Data are not shown). There are several reports on increased trichome density under environmental stresses such as drought and salinity (Abernethy et al., 1998; Aguirre-Medina et al., 2002). Allelopathy stress induced structural changes in xylems in roor. In this situation plants, roots vascular cell thickness was much thinner than control treatment; the salinity effect was concentration dependent. Generally, plants grown in alelochemical solution showed higher thickness in cuticle, vascular tissues and vessel than unstressed plant while cortex zone thickness was decreased.

Cell walls are known to become lignified when cell expansion decreases when the cell is under stress and when it differentiates to particular specialized tissues, notably the xylem (Christensen et al., 1998). Alolopathy stress has been associated with a greater deposition of lignin in vascular tissues and/ or xylem development. In bean root vascular tissue, alochemical caused earlier and stronger lignifications, which has been suggested to be a factor that inhibits root growth and, consequently, represents an adaptation mechanism in resisting salinity-imposed stress (Terize et al., 2003).

In conclusion, this study shows that allelopathy stress decreases wheat growth and induces changes in anatomical characteristics such as increment of cutin synthesis on epidermal stem cells and also changes in xylem structure and lignification of them in wheat roots.

Table. 1. Analysis of variance on wheat growth parameters affected by Swallow grass extract

Sources of variation	df	No. of Root	Root length	Shoot length	Plant height	Root dry weight	Shoot dry weight	Total dry weight	Leaf number
Treatment	3	1.20 ns	667.86 *	0.47 **	25.6 **	17.35 **	16.74 **	58.1 **	0.98 ns
Error	8	1.82	157.89	3.12	2.03	4.28	3.56	11.8	0.82
CV (%)		23.32	20.98	9.55	5.45	12.44	8.03	8.58	3.32

*, ** and ns significant at 0.05, 0.01 probability level and no significant, respectively

Table 2. Main effect of salinity on growth parameters and biochemical attributes

Treatments	Root length	Shoot length	Plant height	Root dry weight	Shoot dry weight	Total dry weight
0 mL	20.83 a	36.67 a	59.21 a	4.30 a	9.12 a	13.56 a
70 mL	15.31 b	33.50 b	46.35 b	3.9 ab	8.07 b	12.31 b
140 mL	13.21 bc	24.06 c	39.02 c	3.77 ab	7.78 b	11.59 bc
210 mL	10.18 c	23.14 c	39.0 c	3.56 b	7.6 b	11.31 c

Values within the each column and followed by the same letter are not different at P <0.05 by an ANOVA protected Duncan's Multiple Range Test

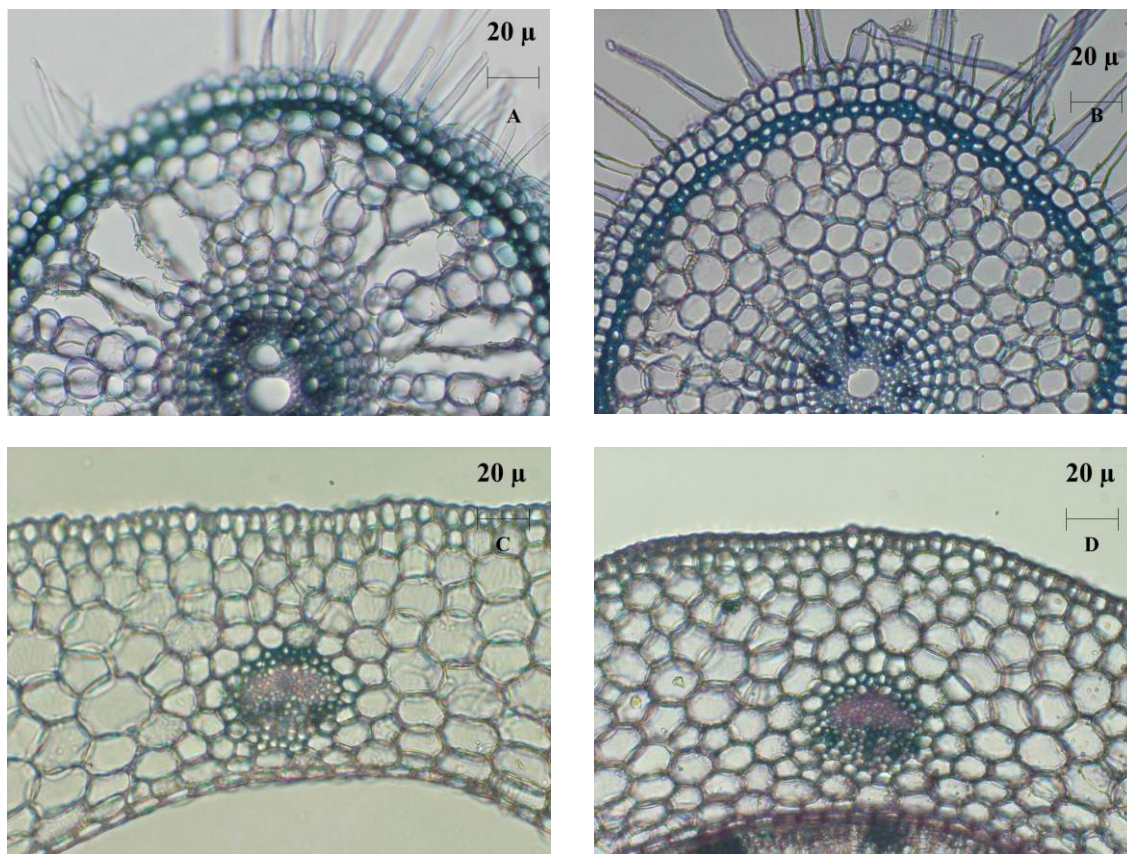


Fig. 1. Microscopic photos of leaf and root sections treated by different concentration of extract.

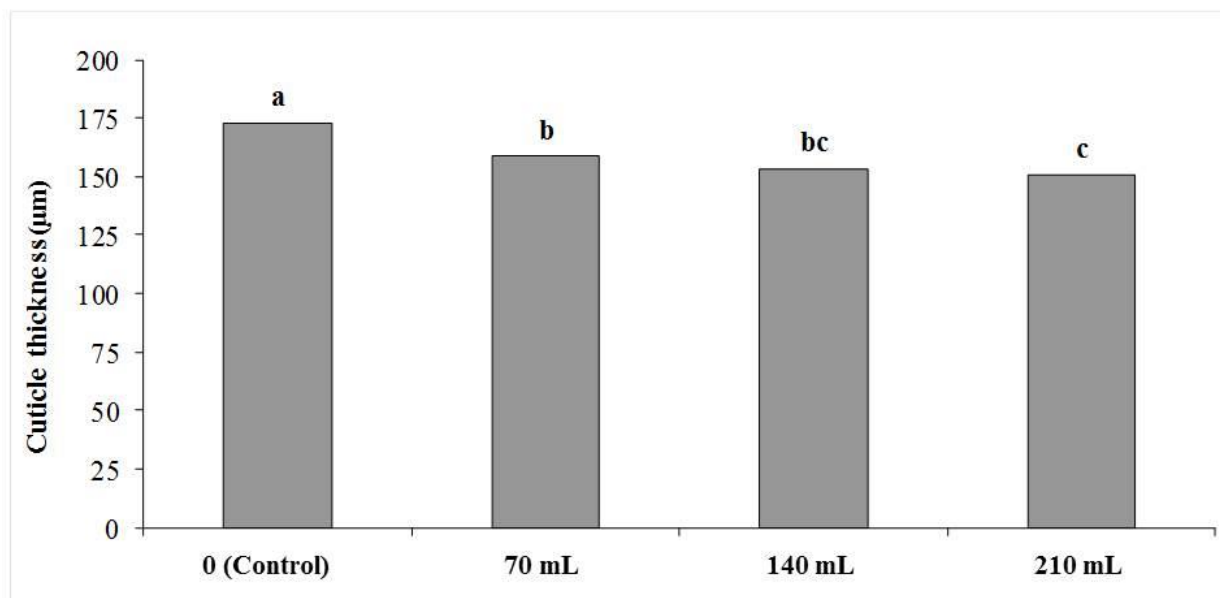


Fig 2. Change in root Cuticle thickness affected by different extract concentration. Within each column followed by the same letter are not significantly differences ($p < 0.05$).

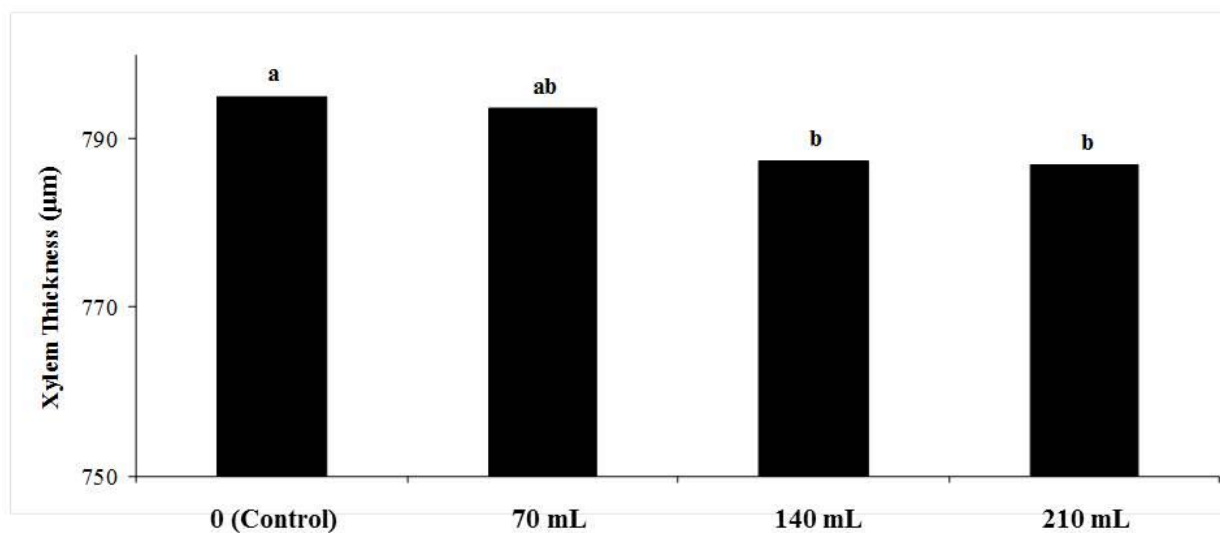


Fig 3. Change in root Xylem thickness affected by different extract concentration. Within each column followed by the same letter are not significantly differences ($p < 0.05$).

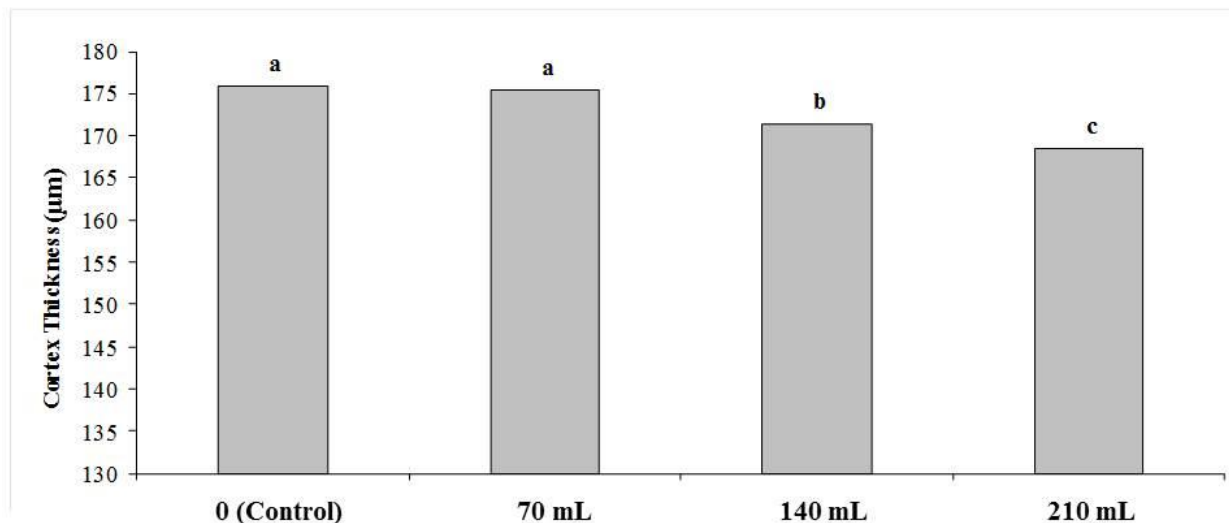


Fig 4. Change in root Cortex thickness affected by different extract concentration. Within each column followed by the same letter are not significantly differences ($p < 0.05$).

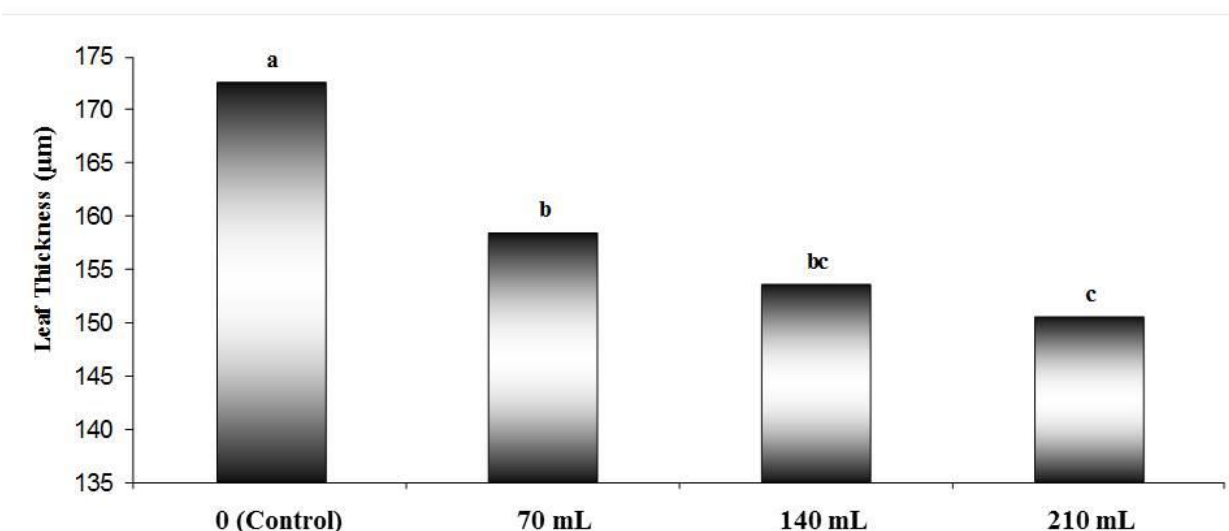


Fig 5. Change in Leaf thickness affected by different extract concentration. Within each column followed by the same letter are not significantly differences ($p < 0.05$).

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