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RESEARCH PAPER

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Allelopathic potential of petal, leaf and seed extracts of sunflower different ecotypes on Zea mays

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Abstract

Allelochemicals have the potential to create friendly-eco products for weed management. The aim of the present investigation was to determine the allelopathic effect of aqueous extract from petal, leaf and seed on physiological parameters of *Zea mays*. Factorial experiments were performed based on a completely randomized block design with three replications. The first factor included two sunflower ecotypes. The second factor was the concentration of the extracts (0, 0.5 g/l). The third factor was the different parts of sunflower (petal, leaf, seed). The results indicated that germination percentage, shoot and root length, peroxidase and catalase activity, chlorophyll a,b, total chlorophyll and carotenoid content of *Zea mays* was Significantly decreased by sunflower ecotypes. Aqueous extract of sunflower petals inhibited physiological paremeters more efficiently in comparison with leaves and seeds. Thus, petal and seed extract of Zardanjan sunflower inhibited physiological paremeters more efficiently in comparison with petal and seed extract of Shahreza sunflower. Therefore, allelopathic potential of these two ecotypes can be consider as a sustainable approach in integrated management systems.

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Introduction

The word allelopathy is derivatived from two separate Greek words: allelon which means "of each other", and pathos means "to suffer" (Rizvi, 1992). It refers to the chemical inhibition of one plant species by another. Allelopathy is biological phenomenon that retains equilibrium among the various plant communities and in natural ecosystems. Some plants release chemicals referred to allelochemicals that often effect the growth, development, survival and reproduction of neighboring plants (Toshiki and Asaduzzaman, 2012). Allelochemicals are frequently, plant secondary metabolites of either acetate or shikimate metabolic pathways. These chemicals include phenolic compounds, long-chain fatty acids, alkaloids, steroids, and derivatives of coumarin, quinines, flavonoids, tannins, terpenes and water soluble organic acids generally with a broad spectrum of activity (Chung et al., 1997).

The toxicity of these compounds is a function of concentration, flux rate, age and physiological stage of the plant, climate and environment. These plant secondary metabolites may be found or extracted from all parts of the plant including roots, rhizomes, stems, leaves, flowers and seeds. They are in vacuoles of cells as glycosides, polymers or crystals so that they do not affect the plant producing them (Chou, 1999; Whittaker and Feeny, 1971). Allelochemicals are released into the environment as leachates, volatilized compounds, exudates and decomposed plant material (Thayaril, 2009).

The mode of action of allelochemicals may be direct or indirect. Direct action includes effects on plant growth and metabolism and indirect effects are within field of alteration of soil confidants, soil nutrition and changes in beneficial and harmful soil microbial populations. Allelochemicals may participate with various important processes such as seed germination, photosynthesis, respiration, water relations, ion uptake and growth, cell ultrastructure and oxidative stress (Rizvi, 1992; Zhao-Hui *et al.*, 2001).

Vyvyan (2002) reported that terpenoids and flavonoids are the most important allelopathic compounds isolated from sunflowers. Allelopathic material from sunflowers can influence antioxidant systems in target plants, causing cellmembrane permeability and cellular injury, reducing the target plants' ability to germinate and causing a gradual loss of seed vigor (Oracz et al., 2007). It seems that the negative effects of sunflower extracts are not due to osmotic potential, but to their toxic effects. Analysis of polyunsaturated fatty acids in target plants' cell membranes has revealed severe damage to membranes and damage to the fat sources stored in the seed (Oracz et al., 2007). finally, isolating chemicals from plants and conducting bioassays is not enough to confirm allelopathic effects.

The present study carried out to evaluate the allelopathic potential of different extracts of sunflowers accumulated of Zardanjan and Shahreza regions on some morphological and physiological parameters of *Zea mays*.

Materails and methods

Plant material and preparation of extracts

Sunflowers (*Helianthus annus* L.) were collected from Isfahan different regions (Zardanjan, Shahreza). Seeds, petals and leaves of sunflowers were collected and air-dried under shade and ground in to fine powder using electric blender. then, 10 gr of each organ powder were extracted with 100 mL distilled water for 72 hours. The mixtures were filtered with whatman filter paper and extracts were stored in the dark kept at 4°C as stock solution for further studies.

Target Plant

Corn (*Zea mays*) seeds purchased locally were used as test or target plants in the experiments. Zea mays seeds were provided from pakanbazr Company in Isfahan. Seeds were sterilized with sodium hypochlorite 0.5% and washed with distilled water (3 times). The seeds were placed on sterilized Petri dishes with filter papers of

soaked with extracts (20 seeds per Petri dish).

Bioassay and extraction

The alleolopathic effect of the different extracts (petal, leaf, seed) with concentration 0.5g/l on seed germination was performed with twenty Zea mays seeds of approximately the same size in Petri dishes lined with a double layer of sterile filter paper moistened with 5 ml of each concentration of extract. Distilled water was applied as the control. All treatments were replicated three times and placed in a completely randomized design at 25°C. Seed germination was determined daily; and hypocotyl and radicle lengths were determined 10 days after treatment. Ten-day-old plants were used for measurement of growth parameters(shoot and root length), enzymes activity, and chlorophylls contents. The samples, weighing about 1g, were homogenized with 5 ml of phosphate buffer pH 6.8 (0.1 M). This portion was centrifuged at 4°C for 10 min at 15,000g in a refrigerated centrifuge. The clear supernatant was taken as the enzyme source.

Catalase activity

The activity of catalase was measured in a reaction mixture consisting of a Tris-Glycine buffer (50 mM, pH 7.5), H_2O_2 (10 mM) and enzyme extract. The decomposition of H_2O_2 was followed by the decline in absorbance at 240 nm by a spectrophotometer (Chance and Maehly, 1955).

Peroxidase activity

The peroxidase activity was measured in a reaction mixture consisting of acetate buffer (0.2 mM, pH 4.8), hydrogen peroxide (0.1mM), benzidine (0.04 M) and enzyme extract. Enzyme activity was measured by a spectrophotometer at 530 nm (Koroi, 1989).

Measurement of chlorophyll a, b and carotenoid

Young leaves were homogenized with acetone 80% in a mortar. The absorbance was measured at 663nm, 645nm and 470 nm by a spectrophotometer. Chlorophyll and carotenoid content were calculated according to the method of Lichtenthaler (1994).

Statistical analysis

Experiments followed a randomized complete block design. Three explants per pot and three replications per treatment were tested. Analysis of variance was performed by the General Linear Model procedure (SPSS ver. 16) and differences among treatments were evaluated by Duncan Test ($p \le 0.05$).

Result and discussion

The extract of different parts (petal, leaf, seed) of sunflower plant had a significant effect ($p \le 0.05$) on on germination properties of *Zea mays* seeds (Table 1).

Table 1. Effects of different extracts of sunflower on shoot, root, germination, peroxidase and catalase of *Zea mays in laboratory* (Mean±SD).

Sample	Organ	Concentration (g/l)	Catalase	Peroxidase	Germination (%)	Root (cm)	Shoof (cm)
	Petal	0	0.671±0.02	0.551±0.01	83.33±5.77	6.16±0.28	7.16±0.28
Shahreze		0.5	0.526±0.02	0.306±0.01	76.66±5.77	0.53±0.15	1.83±0.28
	Leaf	0	0.626±0.01	0.393±0.05	93.33±7.63	6.23±0.25	6.26±0.25
		0.5	0.231±0.03	0.231±0.03	85.00±5.00	0.4±0.10	1.66±0.28
	Seed	0	0.689±0.01	0.516±0.03	79.93±6.64	5.76±0.31	6.66±0.35
		0.5	0.609±0.01	0.395±0.02	63.33±5.77	4.26±0.25	4.3±0.26
	Petal	0	0.671±0.02	0.551±0.01	83.33±5.77	6.16±0.28	7.16±0.28
		0.5	0.31±0.01	0.279±0.02	76.66±5.77	1.03±0.35	1.56±0.41
	Leaf	0	0.626±0.01	0.393±0.05	93.33±7.63	6.23±0.25	6.26±0.25
Zardanjan		0.5	0.357±0.03	0.332±0.03	84.33±5.77	0.63±0.11	1.76±0.64
	Seed	0	0.689±0.01	0.516±0.02	79.93±6.64	5.76±0.31	6.66±0.35
		0.5	0.41±0.01	0.379±0.05	53-33±5-77	3.06±0.11	3.13±0.15

Sunflower petal extract from Shahreza inhibited *Zea mays* seed germination more efficiently in comparison with sunflower petal and leaf extract from Zardanjan (P<0.05) (Figure 1). Germination percentage against petal of Shahreza ecotype was 50%, and against Zardanjan ecotype was 74.44%.

In addition, sunflower petal and leaf extract application from Shahreza ecotype cause sharply decreases in shoot and root length in contract to sunflower petal and leaf extract from Zardanjan ecotype (P<0.05) (Figure 2, Table 1).

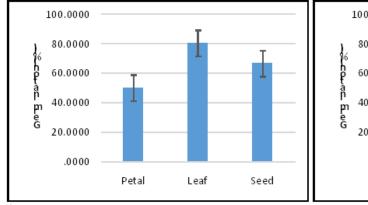
Table 2. Effects of different extracts of sunflower on total chlorophyll, chlorophyll a, chlorophyll b and carotenoid of Zea mays in laboratory (Mean±SD).

		Concentration	Chlorophyll	a Chlorophyll	b Total Chlorophyll (mg/g)	Carotenoid (mg/g)
Sample	Organ	(g/l)	(mg/g)	(mg/g)		
	Petal	0	18.12±0.12	9.85±1.39	27.90±1.39	3.89±0.62
		0.5	7.94±0.21	7.07±0.54	15.04±0.36	0.78±0.21
	Leaf	0	18.12±0.12	9.85±1.39	27.91±1.39	3.89±0.62
Shahreza		0.5	7.48±0.14	8.91±0.26	5.91±0.07	14.82±0.31
	Seed	0	17.73±0.15	8.38±0.54	26.12±0.39	4.42±0.31
		0.5	3.98±0.16	5.62±0.11	12.18±1.22	2.01±0.09
Zardanjan	Petal	0	18.12±0.12	9.85±1.39	27.90±1.39	3.89±0.62
		0.5	3.81±0.09	3.62±0.06	7.53±0.25	0.72±0.01
	Leaf	0	18.12±0.12	9.85±1.39	27.91±1.39	3.89±0.62
		0.5	7.48±0.21	6.49±0.18	13.97±0.02	1.53±0.07
	Seed	0	17.73±0.15	8.38±0.54	26.12±0.39	4.42±0.31
		0.5	3.98±0.06	3.81±0.06	7.79±0.02	0.67±0.04

Also, sunflower seed extract application from Zardanjan ecotype cause decreases in germination, shoot and root length in contract to sunflower seed extract from Shahreza ecotype (P<0.05) (Figure 1,2).

The extract of different parts (petal, leaf, seed) of sunflower plant had a significant effect (p≤0.05)

on peroxidase and catalase activity (Figure 3). Seed extract was more effective than leaf and petal extract. However, Sunflower extracts application from Zardanjan ecotype cause decreases in peroxidase and catalase activity in contract to sunflower extracts from Shahreza ecotype (P<0.05) (Figure 3, Table 1).



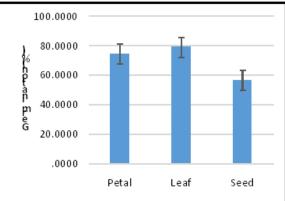


Fig. 1. Interactive effects of sunflower aqueous extract (different parts) on germination percentage *Zea mays* (left figure: Shahreza, right figure: Zardanjan).

The extract of different parts (petal, leaf, seed) of sunflower plant (Shahreza and Zardanjan), had a greater allelopathic effects, so that these plants caused a significant decrease ($p \le 0.05$) in total chlorophyll,

chlorophyll a, chlorophyll b and carotenoid of *Zea mays* in laboratory assay (Table 3).

Sunflower extract interaction was significant for all the

measured traits unless chlorophyll a from Shahreza ecotype (Table 3). Leaf extract of Shahreza as well as leaf extract of Zardanjan were able to reduce total chlorophyll content. Interestingly, petal, leaf and seed extracts of Shahreza and Zardanjan could decrease

significantly total chlorophyll content. Additionally, sunflower extract of Shahreza as well as sunflower extract of Zardanjan were able to reduce total chlorophyll, chlorophyll a, chlorophyll b and carotenoid of *Zea mays* (Figure 4).

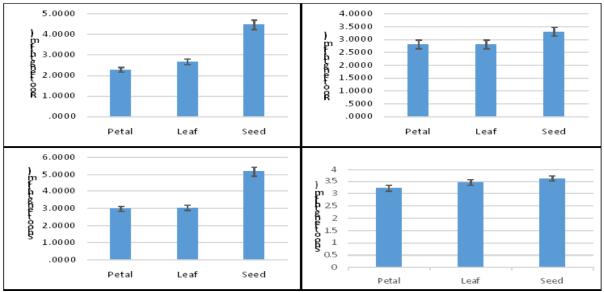


Fig. 2. Interactive effects of sunflower aqueous extract(different parts) on shoot and root length of *Zea mays* (left figure: Shahreza, right figure: Zardanjan).

The results evidently demonstrated that sunflower different extracts significantly inhibited and decreased seed germination, seedling elongation, peroxidase and catalase activity, chlorophyll a,b and carotenoid content. High inhibition of sunflower different extracts were obtained whenever petal

Zardanjan sunflower and seed extracts of applied as allelochemical in comparison with petal and seed extracts of Shahreza sunflower. The present study confirmed sunflower results allelopathic inhibition impact some of on physiological parameters of weeds.

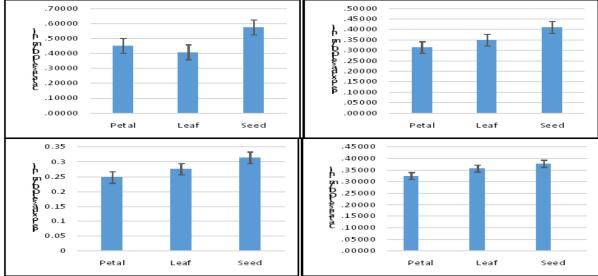


Fig. 3. Interactive effects of sunflower aqueous extract(different parts) on peroxidase and catalase activity of Zea mays (left figure: Shahreza, right figure: Zardanjan).

Allelochemicals stunted the growth of different parts of the plants including branches, leaves and plant height (Wu *et al.*, 1998).

Allelopathic effects not only decrease germination, but also delay it which can bring about various effects on the competition of the plants. In fact, bigger seedlings can have a competitive advantage under adverse environmental conditions like low soil moisture or nutrient limitation (Escudero *et al.*, 2000).

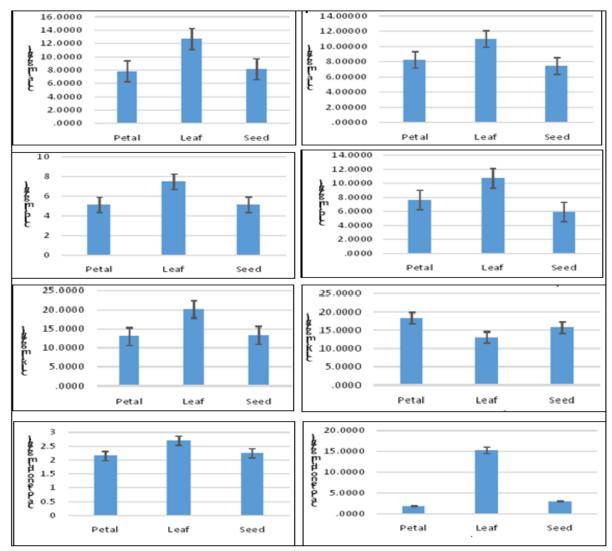


Fig. 4. Interactive effects of sunflower aqueous extract (different parts) on total chlorophyll, chlorophyll a, chlorophyll b and carotenoid content of *Zea mays* (left figure: Shahreza, right figure: Zardanjan).

The loss of germination speed may result from the slowdown of living processes of the plants due to the loss of seeds respiration caused by chemicals, too (Rezaee *et al.*, 2008).

The allelopathic properties of sunflowers are well-recognized; their effects on many weeds and crops have been documented. Macias *et al.* (2002) isolated 125 natural allelopathic

compounds that are phytotoxic towards many plants from different sunflower cultivars. Sunflower extracts completely inhibited seed germination of *Sinapis alba* L. (Bogatek *et al.*, 2006. Kupidlowska *et al.*, 2006), although sunflower phytotoxins did not affect seed viability (Kupidlowska *et al.*, 2006). Anjum and Bajwa (2005) studied the effects of bioactive annuionone from aqueous extracts of sunflower leaves on growth

of five weeds including *Chenopodiun album* L., *Coronopis didymus* L., *Medicago polymorpha* L., *Rumex dentatus* L. and *Phalaris minor*; they reported this extract can be used as a natural herbicides.

Terpenoids and flavonoids are the most important allelopathic compounds isolated from sunflowers (Vyvyan, 2002). Flavonoids change the infiltration of mitochondrial and chloroplast membrane and as a result, the rate of electron transport and photophosphorylation is changed. (Kefeli *et al.*, 2003).

Some studies demonstrated that Sunflower residues decreased growth of different weeds such as *Cyamopsis tetragonoloba*, *Pennisetum americanum* and *S. biocolor* (Batish *et al.*, 2002). They concluded that due to decomposing tissue of sunflower by soil microorganisms, some allelochemicals such as phenolics were released and inhibited the growth of those weeds (Batish *et al.*, 2002).

Jamil *et al.* (2009) reported that sunflower water extract can be used for controlling wild oats (*Avena fatua*) and canary grass (*Phalaris minor*).

Allelopathic material from sunflowers can influence the antioxidant systems in target plants, causing cell-membrane permeability and cellular damage, reducing the target plants' ability to germinate and causing a gradual loss of seed vigor (Oracz et al., 2007). It seems that the negative effects of sunflower extracts are not due to osmotic potential, but to their toxic effects. Analysis of polyunsaturated fatty acids in target plants' cell membranes has revealed severe damage to membranes and damage to the fat sources stored in the seed (Oracz et al., 2007). Ultimately, isolating chemicals from plants and conducting bioassays is not enough to confirm allelopathic effects.

The present study was, therefore, carried out to

evaluate the herbicidal potential of sunflower petal, leaf and seed extracts against *Zea mays*.

The changed activity of antioxidants and antioxidant enzymes is perhaps a secondary effect of many allelochemicals. It seems that the receiving plant increases the activities of these enzymes in an attempt to counteract the harmful effects of ROS generated either by the various oxidative states of allelochemicals themselves or by a plant signaling cascade that is induced by the allelochemical. (Rocio *et al.*, 2007).

The germination Zea mays can be related to the change of the activities of enzymes which influences the translocation of stored assimilates germination (Jamil et al., Allelopathic chemicals act through affecting germination or seedling growth, the former by inhibiting cell division and the latter by inhibiting cell elongation. Known as one of the most essential components of allelopathic compounds, phenols can inhibit the division of root cells (Bhowmik and Dol, 1982). Consequently, it is very likely that the loss of germination percentage in the current study was associated with these facts. Results from this experiment showed that allelopathic chemicals of sunflower can potentially serve as an alternative herbicide against plants.

Our research also suggests that using sunflower allelopathy to control weeds, such as *Amaramthus reteroflexus*, *Chenopodium album*. However, more experimentation in the allelopathic effects of sunflowers for weed control is needed in real greenhouse and field conditions.

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