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Evaluation of Genotoxic and Inhibitory Effects of Invasive Weed Wild Poinsettia (*Euphorbia heterophylla* Linn.) in Sunflower

Abstract: Determining the mode of action of allelochemicals is one of the challenging aspects in the allelopathic studies. In this research, the methanolic extracts of wild poinsettia (*Euphorbia heterophylla* Linn.) at different concentrations (0, as untreated control, 8%, 12% and 16%) were tested for genotoxic and inhibitor activity on morphological, biochemical and molecular traits of sunflower (*Helianthus annuus* L.). Seedling emergence was reduced with wild poinsettia leaf extracts in sunflower. Leaf extracts of wild poinsettia decreased chlorophyll *a* and *b* levels and consequently the soluble sugar content was proportional to the increase in the concentrations of the leaf leachates. Some changes occurred in random amplification of polymorphic DNA (RAPD) profiles, profiles of protein bands and total soluble protein of germinated treated seed. These included variation in band intensity, loss of bands and appearance of new bands compared with control. Increased concentration of extracts caused increasing the total soluble protein content, decreasing genome template stability (GTS) value and increasing polymorphism values, the results indicated that they were dose dependent. For instance, the highest and lowest amounts of GTS were observed in 8% and 16%, respectively. In a dendrogram constructed based on genetic similarity coefficients, the treatments were grouped into two main clusters: (a) 8%, 12% and 16% dose clustered together and (b) untreated control grouped alone. Also, we concluded that the basis of interaction between plants, like allelopathy, may be related with genotoxic effects.

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Introduction

Euphorbia heterophylla L., commonly known as fire plant, painted euphorbia, Japanese poinsettia, desert poinsettia, wild poinsettia, fire on the mountain, painted leaf, painted spurge, milkweed and kaliko plant, is an annual broadleaf weed. Wild poinsettia is a recently introduced weed in sunflower (*Helianthus annuus*) and corn (*Zea mays*) farms of Golestan Province, Iran (Savarnjad *et al.*, 2010). It can also pose problems in horticultural crops, vineyards, ornamental plantings and waste areas (Carvalho *et al.*, 2010).

Commonly cited effects of allelopathy include reduced seed germination (Turker *et al.*, 2008) and seedling growth. There is no common mode of action or physiological target site for all allelochemicals (Kato-Noguchi *et al.*, 2002) even though some plant extracts are known to have allelopathic effects on cell division, pollen germination (Turker *et al.*, 2008), nutrient uptake, photosynthesis, specific enzyme function (Han *et al.*, 2008), genomic template stability (GTS) and total soluble protein content of the seedlings (Sunar *et al.*, 2012).

The present research was carried out to study the allelopathic effects of leaf extracts of *E. heterophylla* on morphological, biochemical and molecular traits of sunflower.

Materials and methods

Plant material

The weed plants used in the present study were collected in August 2012 from different fields of Golestan Province, Iran, at the beginning of flowering stage and dried in shadow; the leaves were separated from the stem and all leaves were ground in a grinder to achieve a size of 2 mm in diameter.

Preparation of the methanol extracts (MeOH)

Methanol is a common solvent used for allelochemical analyses because this combination exhibits a good ability to extract both polar and non-polar compounds; therefore, most potential phytotoxins of the plant can be extracted (Fujii *et al.*, 2003). The dried and powdered leaves (500 g) were extracted with 1 L of methanol using a Soxhlet extractor for 72 h at a temperature not exceeding the boiling point of the solvent. The extracts were filtered using filter paper (Whatman No. 1) and then concentrated in vacuo at 40°C using a rotary evaporator. The residues obtained were stored in a freezer at −80°C until further tests.

Treatments of extracts on sunflower seeds

Seeds of sunflower Cultivar DPX were obtained from the Agricultural Research Center, Golestan, Iran. The seeds of sunflower were surface sterilized with 1% sodium hypochlorite for 20 min, then rinsed with distilled water several times. The seeds were soaked in sterile distilled water for 1 h and then 15 seeds were germinated in 25-cm dia Petri dishes on four layers of sterile Whatman No. 1 filter paper. The plant extracts were diluted with deionized water to prepare the concentrations of 8%, 12% and 16%. There were six Petri dishes for each treatment and all Petri dishes were placed in a chamber maintained at 25/16°C day/night temperature (±2°C) and 16/8 light/dark cycle with a photon flux density of approximately 150 μmol photons/m^2/s^1 and relative humidity 78 ± 2% in a complete randomized design. Samples were collected for morphological, biochemical and molecular analysis 20 days after sowing.

Germination was determined by counting the number of germinated seeds at 24-h intervals over a 6-day period and expressed as total percent germination. Germination was deemed to occur only after the radicle had protruded beyond the seed coat by at least 1 mm. Radicle and hypocotyl lengths of sunflower seedlings were measured 6 days after germination. After measuring the radicle and hypocotyl lengths, the dry weights of seedlings were determined by drying the plant material in an oven at 60°C for 24 h prior to weighing.

One-gram samples of sunflower seeds were soaked for 4, 8, 12 and 16 h in wild poinsettia leaf methanol extracts of 8%, 12% and 16%. After an 8-h interval, seeds were taken from the solution, blotted for 2 h between two folds of filter paper, and weighed. The water uptake was calculated by subtracting the original seed weight from the final seed weight. Distilled water was used as the untreated control.

Biochemical analysis

Damage criteria: 1. Hydrogen peroxide (H_2O_2) content. H_2O_2 content was determined using the method given by Velikova *et al.* (2000). 2. Effect on membrane integrity. Loss of membrane integrity (an indicator of cellular damage) was studied in terms of ion (electrolyte) leakage from the leaves of sunflower measuring conductivity of the bathing medium, as per the method of Duke and Kenyon (1993). 3. Determination of lipid peroxidation. Lipid peroxidation was measured in terms of malondialdehyde content (MDA) as described by the method of Heath and Packer (1968).

Non-enzymatic antioxidants: 1. Glutathione (GSH). Reduced GSH and oxidized glutathione (GSSG) levels were determined according to the method of Griffih (1980). 2. Ascorbic acid (AA). The AA concentration was measured using the 2,6-dichlorophenol-indophenol (DCPIP) photometric method of Guri (1983).

Activities of antioxidant enzyme: 1. Superoxide dismutase (SOD) was assayed following the method of Beauchamp and Fridovich (1971), by measuring its ability to inhibit photochemical reduction of nitro blue tetrazolium chloride (NBT). 2. Catalase (CAT) activity was measured as per the method of Cakmak and Marschner (1992). 3. Glutathione reductase (GR) activity was determined spectrophotometrically by monitoring GSSG-dependent oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) at 340 nm as described by Foyer and Halliwell (1976). 4. Ascorbate peroxidase (APX) was assayed by Nakano and Asada (1981). 5. Guaiacol peroxidase (GPX) activity was measured using the method described by Egley *et al.* (1983).

Assay of lipase activity: Lipase (glycerol ester hydrolase, E.C.3.1.1.3) activity was assayed using a modification of the titrimetric method of Khor *et al.* (1986).

Estimation of chlorophyll: The leaf photosynthetic pigments were determined as described by Moran (1982).

Estimation of sugars: Soluble sugars were extracted following the method adopted by Homme *et al.* (1992) and determined with the anthrone reagent (Whistler *et al.*, 1962).

ABA (abscisic acid): ABA was extracted, separated, identified and quantified by high-performance liquid chromatography using the method described by Shindy and Smith (1975).

Protein analysis: Soluble proteins were determined according to the method described by Bradford (1976). Each experiment was performed at least three times.

Increase rate (IR, %) was calculated by the following formula:

$$\mathrm{IR} = \left(1 - \frac{X}{Y}\right) \times 100$$

Where X and Y are the average values detected in the control and each sample treated, respectively. The identification and characterization of different protein fractions were obtained using one-dimensional SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis). Polyacrylamide slab gel (12.5%) was prepared according to Laemmli (1970). To analyze the banding pattern, molecular weights and band percentage, the destined gels were analyzed by Gel Doc 2000 in the presence of protein molecular weight marker using Gel Proanlyzer Version 3 Media Cyberene Tice Imaging Experts Software.

DNA extraction and RAPD fingerprinting

The roots and first leaves of five to eight seedlings 20 days old were used for genomic DNA extractions and random amplification of polymorphic DNA (RAPD) (Figure 1). Approximately 10–15 mg tissue samples from each plant species were snap frozen in liquid nitrogen in 2 ml Eppendorf tubes. DNA was extracted based on the CTAB (cetyltrimethylammonium bromide) method developed by Saghai-Maroof *et al.* (1984) as described in El-Shazly and El-Mutairi (2006). RAPD-PCR (polymerase chain reaction) fingerprinting was kindly performed at the Agricultural Genetic Engineering Research Institute in Karaj, Iran. For generating DNA profiles, nine 10-mer oligonucleotide DNA primers of arbitrary sequences from the Operon Kit (Operon Technologies, Inc., USA) were

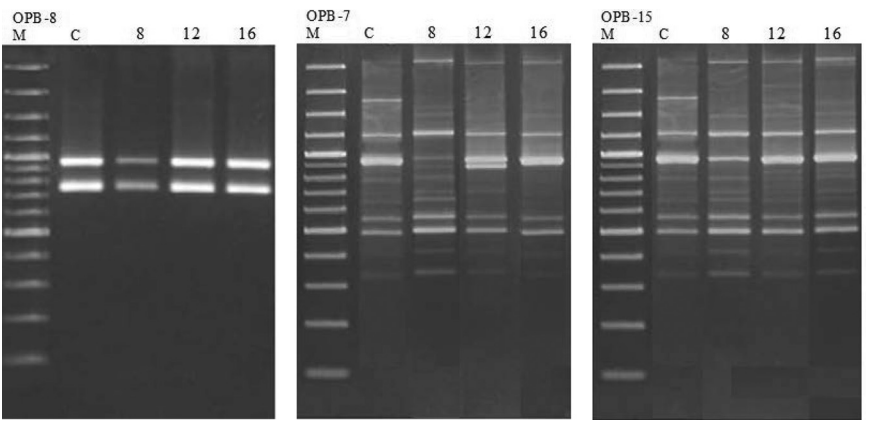

Figure 1: Random amplification of polymorphic DNA (RAPD) profiles of genomic DNA obtained from primers OPB-8, OPB-7, and OPB-15 in sunflower seedling exposed to different wild poinsettia extracts (C: control, 8%, 12%, and 16%). M: GeneRuler 100 bp plus DNA Ladder (100–3,000 bp).

independently used in PCR. Eight of 20 tested primers were able to reveal stable and reproducible RAPD polymorphism in the generated DNA profiles of samples. Sequences (5′ → 3′) from primer 1 to 8 utilized are (OPB-6) TGCCGAGCTG; (OPB-5) AGGGGTCTTG; (OPB-7) GAAACGGGTG; (OPB-8) GTGACGTAGG; (OPB-15) GGGTAACGCC; (OPB-11) CAGCACCCAC; (OPB-14) TCTGTGCTGG; (OPB-18) AGGTGACCGT, respectively. RAPD fingerprinting was performed using each of the used primers in 25-µl reaction volume containing the following reagents: 2.0 µl of dNTPs (deoxynucleotide triphosphates) (2.5 mM), 1.5 µl of $MgCl_2$ (25 mM), 2.5 µl of 109 buffer, 2.0 µl of primer (2.5 mM), 2.0 µl of template DNA (50 ng/µl), 0.3 µl of Taq polymerase (5 U/µl) and 14.7 µl of sterile ddH_2O. Amplification was carried out in a 2400 Perkin Elmer Gene Amp PCR thermocycler using the following setup: one cycle at 94°C for 4 min followed by 40 cycles at 94°C for 1 min; 37°C for 1 min and 72°C for 2 min. The reaction was finally incubated at 72°C for 10 min. The RAPD products were electrophoresed in 1.4% agarose gel in TAE buffer (0.04 M Tris-acetate, 1 mM EDTA, pH 8) at 100 V for 60 min. The gels were stained in 0.2 µg/ml ethidium bromide and photographed using a gel documentation system (Gel Doc BioRad 2000).

All amplifications were repeated twice in order to confirm the reproducible amplification of scored fragments. All amplifications were repeated twice in order to confirm the reproducible amplification of scored fragments. Only reproducible and clear amplification bands were scored for the construction of the data matrix. The marked changes observed in RAPD profiles (disappearance and/or appearance of bands in comparison with untreated control treatments) were evaluated. The robust polymorphic bands were scored as present (1) or absent (0) for each primer. Genetic similarity coefficients (phenetic numerical analysis) among the untreated control and treated seedlings were estimated from Nei's unbiased measure (Nei, 1978) in POPGENE version 1.31. Cluster analysis was performed and a dendrogram generated using the unweighted pair group method with arithmetic means (UPGMA) algorithm of POPGENE. The GTS (%) was calculated for each primer as follows (Sunar *et al.*, 2012):

$$\mathrm{GTS} = \left(1 - \frac{a}{n}\right) \times 100$$

where a is the average number of changes in DNA profiles and n is the number of bands selected in control DNA profiles. Polymorphism in RAPD profiles included disappearance of a normal band and appearance of a new PCR band in comparison to control RAPD profiles (Liu *et al.*, 2007) (Figure 1).

The data were subjected to one-way analysis of variance, and treatment means separated from the control at $p < 0.05$ or 0.01 applying post hoc Dunnett's test. Statistical analysis was done with SAS system (SAS Institute, 1999).

Results and discussion

Effect on seed germination, seedling growth and rate of water uptake of sunflower

The germination of the control was 98%. Increased concentration of wild poinsettia extracts inhibited germination (Table 1). The degree of inhibition increased with increasing extract concentration. At the highest extract concentration (16%), leaf extracts significantly inhibited seed germination compared with the control. Leaf extract inhibited germination by 13%, 53.4% and 97.8% at the 8%, 12% and 16%, respectively.

All methanol extracts at all concentrations inhibited hypocotyl length compared with the control and the degree of inhibition increased with increasing extract concentration. Hypocotyl development was decreased 90% in the highest concentration (16%) of leaf extract, compared with the control. Adverse effect of leaf extract on radical length and dry weight was similar to that of the hypocotyls (Table 1).

The radical length was more sensitive to all of extracts used in comparison to the other plant parameters measured. Radicle length was relatively more sensitive to allelochemicals than was shoot growth length. These results agree with earlier studies reporting that water extracts of allelopathic plants had more pronounced effects on radicle growth than on hypocotyl growth or shoot growth (Cenkci *et al.*, 2009). Such an outcome might be expected, because it is likely that roots are the first to absorb the allelochemical compounds from the environment (Cenkci *et al.*, 2009).

Although reduced growth of sunflower in the field under wild poinsettia has been attributed to shading and competition for water (Adelusi and Akamo, 2006), our results indicate a possibility of production of phytotoxic natural products by wild poinsettia is a mechanism by which this species may become successful competitors. Similarly, crops yield reduction under wild poinsettia (Jiangbo, 2010) may also be partly explained by the toxic effects of high concentration of phenolics from *Euphorbia* species (Tanveer *et al.*, 2013).

Further, sunflower growth may also be reduced by any toxic effects of phenolic compounds on the nitrogen-fixing symbiotic microorganisms (Carvalho *et al.*, 2010).

According to Ferreira and Borguetti (2004), the allelopathic effect on germination is due to the interference that blocks or delays the progress of metabolic processes during the germination process.

The reduction in the growth of sunflower seedlings in the presence of the different concentrations tested was more drastic than on the germination of seeds.

Table 1: Effects of wild poinsettia methanol extracts on some morphological and biochemical characteristics of sunflower.

Extract concentration (%)	Germination (%)	Hypocotyl length (cm)	Radicle length (cm)	Dry weight per seedling (mg)	Total soluble sugars (mg 100 g^{-1} fr. wt)
0	98 ± 1	5.63 ± 0.3	5.35 ± 0.8	23.4 ± 0.5	200
8	84 ± 1.1*	3.18 ± 0.26**	3 ± 0.7**	20.2 ± 0.9 ns	110**
12	46 ± 8.7**	0.95 ± 0.31**	0.6 ± 0.1**	8 ± 3.1**	78**
16	2 ± 1.1**	0.5 ± 0.17**	0**	3 ± 1.6**	40**
Extract concentration (%)	**ABA (µg 100 g^{-1})**	**Total soluble protein (mg/g)**	**Chl *a* (mg/g fr. wt)**	**Chl *b* (mg/g fr. wt)**	**Carotenoids (mg/g fr. wt)**
0	5	5.15 ± 0.42	0.829 ± 0.12	0.554 ± 0.07	1.251 ± 0.17
8	86**	4.9 ± 0.39*	0.649 ± 0.09*	0.457 ± 0.06 ns	4.156 ± 0.19*
12	100**	4.6 ± 0.33*	0.293 ± 0.04**	0.42 ± 0.05*	12.83 ± 1.78**
16	245**	4.2 ± 0.23*	0.2 ± 0.03	0.337 ± 0.04**	16.8 ± 2.3**
Extract concentration (%)					**Water uptake of sunflower (%), by imbibition period (h)**
	4	8	12	16	20
0	93.8	116.4	118.8	120.6	121.5
8	84.1*	112.4 ns	114 ns	117.4 ns	118.9 ns
12	79.4**	107.1**	110*	113.9*	114.8**
16	73.5**	102.2**	109.5*	112.5**	113.7**
Extract concentration (%)					**Lipase activity of sunflower (% FFAs), by imbibition period (h)**
	4	8	12	16	20
0	1.4	2.26	2.77	3.45	4.92
8	1.35 ns	1.9 ns	2.56 ns	3.28 ns	3.92*
12	1.26 ns	1.7*	2.48 ns	2.96*	3.73**
16	1 ns	1.16**	2.39 ns	2.48*	3.53**

Note: ns: not significant; (*) and (**) represent significant difference over control at $p < 0.05$ and $p < 0.01$, respectively.

According to Ferreira and Aquila (2000), the germination is less sensitive to allelochemicals than the seedling growth, because the allelopathic substances may induce the appearance of abnormal seedlings, for example, with symptoms of necrosis in the root. Maraschin-Silva and Aquila (2005) also indicate that the seeds may suffer loss due to the fact that the germination process uses their own seed reserves.

The results indicate that methanol extracts from wild poinsettia show a phytotoxic influence on sunflower. The degree of inhibition was largely dependent on the concentration of the extracts being tested. At the highest extract concentration (16%), all methanol extracts significantly inhibited seed germination compared with the control (Table 1). This finding is congruent with the results of Han *et al.* (2008) who found that the degree of inhibition increased with increasing extract concentration.

Biochemical analysis

Several enzymes like proteases, lipases and a-amylases play an important role during seed germination. Many enzymatic functions are inhibited by the presence of allelochemicals (Han *et al.*, 2008). Kato-Noguchi and Macías (2005) had reported that the germination of lettuce (*Lactuca sativa* L. cv. Grand Rapids) seeds treated with 6-methoxy-2-benzoxazolinone (MBOA) was positively correlated with the activity of a-amylase. In sunflower seeds, lipases function to mobilize storage triglycerides through hydrolysis to fatty acids during early stages of germination. The fatty acids released by the lipases are channeled into energy-producing pathways, thereby providing energy for the growing embryo and seedling (Staubmann *et al.*, 1999). Lipase activity of sunflower showed inhibitory or tendency with increasing concentration of wild poinsettia methanol extracts (Table 1), which was consistent with that of water uptake. This finding is supported by Moreno *et al.* (2003) who found that grape seed extract inhibited lipase activity in a dose-dependent manner. There may be an indirect association between lower lipase activity and allelopathic inhibition of water uptake. To a large extent, lipase activity is primarily related to water uptake by the seeds (Turk and Tawaha, 2003).

The ABA concentration of sunflower is shown in Table 1. There is a gradual increase in the ABA concentration in proportional to the increase in the concentration of the leaf extracts. Several reports indicated that allelopathy stress may induce an increase in the ABA accumulation (Noguchi *et al.*, 2002; Khan, 1998).

Treatment with wild poinsettia phytotoxins endures a significant excessive ion leakage in the sunflower seedling as measured by increased conductivity of the bathing medium (MES buffer) (Table 2).

Table 2: Effects of wild poinsettia methanol extract on some indicators of oxidative stress and some non-enzymatic, enzymatic antioxidants mechanisms in sunflower.

Extract concentration (%)	Electrolyte leakage (%)	Malondialdehyde (nmol/g fr. wt)	Hydrogen peroxide (nmol/g fr. wt)	Ascorbate acid (nmol/g fr. wt)	Glutathione (nmol/g fr. wt)
0	53	250	13	3.5	200
8	83*	373*	25**	7.8**	380*
12	89**	700**	35**	9**	500**
16	98**	873**	43**	5.5*	570**
Extract concentration (%)	**Catalase (unit/g fr. wt)**	**Peroxidase (unit/g fr. wt)**	**Ascorbate peroxidase (unit/g fr. wt)**	**Superoxide dismutase (unit/g fr. wt)**	**Glutathione reductase (unit/g fr. wt)**
0	30	20	48	40	8
8	55**	40**	57*	60**	18*
12	66**	52**	73**	71**	30**
16	72**	65**	95**	83**	49**

Note: ns: not significant; (*) and (**) represent significant differences over control at $p < 0.05$ and $p < 0.01$, respectively.

We monitored changes in lipid peroxidation by measuring the thiobarbituric acid-reactive substance in the leaves of sunflower. Increased lipid peroxidation was observed with increasing wild poinsettia concentration in the medium in sunflower (Table 2).

A change in H_2O_2 content is a good indicator of the reactive oxygen species (ROS) scavenging capacity of plants under oxidative stress. In Table 2, sunflower showed significant enhancement in the H_2O_2 content, up to 150% in the presence of 16% methanol leaf extract.

The present study showed a significant excessive ion leakage in sunflower as measured by increased conductivity of the bathing medium (MES buffer). This may indicate that the methanol extract of the allelopathic plant caused stress resulting in disruption of membrane integrity. In addition, a decrease in membrane permeability could be due to peroxidation of polyunsaturated fatty acids in the biomembranes, resulting in the formation of several byproducts including MDA (Darier and Tammam, 2012).

MDA (as an indicator of lipid peroxidation) and electrolyte leakage resulting in loss of membrane integrity are among the key factors that determine cell injury. Generally, various types of environmental stresses (including abiotic, xenobiotic and herbicidal) mediate their impact through oxidative stress caused by generation of ROS (Blokhina *et al.*, 2003). ROS such as singlet oxygen (O_2), superoxide radicals (O_2^-), hydroxyl radical (OH), and H_2O_2 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). In the current work, MDA increased significantly in leaves of the target plant upon treatment with wild poinsettia extract. Increased lipid peroxidation indicates that wild poinsettia extract results in oxidative stress due to generation of ROS species, which causes a loss of cell integrity. Apart from causing peroxidation of membrane lipids, high levels of H_2O_2 have been reported to cause a net reduction in photosynthesis in plants by over 50% (Kaiser, 1979). By channeling most of the photoreductants (electrons produced at photosystem II by the water oxidation complex) for the detoxification of H_2O_2 produced under stress, CO_2 fixation is compromised. This leads to poor growth in sunflower.

Reduced GSH and AA are common antioxidants used by plants to reduce ROS levels in vivo. We investigated the modulations of the levels of these antioxidants in response to varying degrees of phytotoxin-induced oxidative stress (Table 2). In sunflower, AA steadily increased until reaches its maximum at 12% extract concentration level then decreased to a minimum value of about 5.55 at 16% concentration level.

The total GSH (including GSH and its oxidized form) content was significantly higher in stressed conditions when compared to the control (Table 2). GSH content increased significantly by nearly three-fold at 16% concentration of wild poinsettia extract in sunflower.

Several reports indicated that oxidative stress may induce an increase in the GSH accumulation (Darier and Tammam, 2012). GSH plays an important role in the antioxidant defense system of leaves, since it not only participates in the regeneration of ascorbate via dehydroascorbate reductase but also reacts with singlet oxygen and OH^- radicals and protects the protein thiol groups (Asada, 1994). It is used by glutathione S-transferase to detoxify xenobiotics. AA increased in low and at moderate concentrations of wild poinsettia methanol extract in sunflower leaves and then decreased at high concentration; this may be due to participation in reducing H_2O_2 to H_2O by APX. Ascorbate is a ubiquitous soluble antioxidant in photosynthetic organisms and it is also an important soluble antioxidant compound in plant cells (Nakano and Asada, 1981) and the most important reducing substrate for H_2O_2 detoxification. The levels of ascorbate and GSH play an important role in oxidative defense. Tammam (2008) reported that there is a decrease in AA content in cotton cultivars under salt stress.

Increase in CAT activity was recorded in all treatments of wild poinsettia extract concentration with maximum activity at 16% where it reached about 2.4-fold in sunflower (Table 2).

Concerning peroxidase (GPX) activity, the activity increased with increasing wild poinsettia phytotoxin extract (Table 2). Minimum SOD activity was recorded in control of sunflower; SOD exhibited a significant increase in the leaves of seedlings treated with different concentrations of wild poinsettia extract as compared with control (Table 2). Maximum SOD activity was achieved at 16% concentration of wild poinsettia leaf extract where its value was about two-fold in sunflower relative to control.

With regard to APX, a similar trend characterized by a progressive increase in the content with increasing extract concentration level was obtained for sunflower (Table 2). At 16% concentration level the increase in sunflower was about two-fold relative to control. A similar trend was observed with GR activity. It increased in sunflower by six-fold at 16% extract concentration level (Table 2).

Oxidative stress could play a role in phytotoxic phenomenon (Weir *et al.*, 2004). This was evident by increased activities of antioxidant enzymes under allelochemical stress. Furthermore, ROS are known to trigger activities and expression of most of the antioxidant enzymes (Apel and Hirt, 2004). Plants possess an enzymatic defense system (SOD, GR, CATs, various peroxidases) for the detoxification of various types of ROS. There are many reports indicated that oxidative stress induced an increase in the responses of enzymatic system-linked ROS-scavenging process

(Apel and Hirt, 2004**)**. SOD is the major scavenger of superoxide ion to form H_2O_2 and O_2 and plays an important role in defense activity against the cellular damage caused by environmental stress (Nakano and Asada, 1981). Increased levels of SOD activity under wild poinsettia indicate an induction of oxidative stress caused by excessive generation of superoxide ion. Ye *et al.* (2006) reported that SOD activity induced an induction of oxidative stress caused by excessive generation of superoxide ion presumably resulting from allelochemicals contained in root exudates of cucumber. Our data are in agreement with other authors for increased activities of SOD and CAT in different plants like cucumber (Macias *et al.*, 2002), tomato (Romero-Romero *et al.*, 2005) and mustard (Oracz *et al.*, 2007) under different allelochemical stress. SOD scavenges the highly reactive free radicals by converting them into H_2O_2. Although H_2O_2 is equally toxic, H_2O_2 is further reduced to H_2O by CAT in the perioxisomes, by APX in the chloroplast and cytosol, and by GPX in the cell wall (Blokhina *et al.*, 2003). These antioxidants cause better survival of plants under stressful condition (Mishra *et al.*, 2006). In this investigation the activities of CAT increased steadily with increasing wild poinsettia extract concentration, the values reached to about 2.7-fold sunflower at 16% extract concentration level. In contrast, there was not to a large extent an increase in the activity of peroxidase (GPX) at lower extract concentrations in sunflower. On the other hand, at 16% extract level sunflower achieved an increase of about 4.2-fold. However, Singh *et al.* (2009) reported that CAT and GPX activities tend to decrease in maize leaves under the influence of high concentration of aqueous leachate of *Nicotiana plumbaginifolia*. Ascorbate peroxidase increased by nearly 2.45-fold in sunflower leaves; GR and APX – the main components of the ascorbate GSH cycle – provide the main defense against oxidative damage in plants (Becana *et al.*, 2000).

Aimed at verifying the genetic effect of the toxic allelochemicals of wild poinsettia, the RAPD analysis was performed on DNA extracted from the seedlings from each replicate treated with leaf extracts in 8%, 12% and 16% treatments. Of the 20 primers tested, only 8 gave specific and stable results. All eight primers produced same RAPD profiles for the untreated seed. On the other hand, RAPD profiles showed substantial differences between untreated control and treated seed with apparent changes (disappearance and/or appearance) in the number and size of amplified DNA fragments for different primers. The changes in RAPD profiles were summarized for sunflower-treated seed in comparison to their controls (Table 3). RAPD profiles generated by OPB-8 were same for all the samples (Table 3, Figure 1). On the other hand, primer OPB-7 produced more informative RAPD profiles for the samples (Table 3, Figure 1). For primer OPB-11, only disappeared bands were observed in RAPD profiles of treatments compared to their controls (Table 3). On the other hand, new RAPD bands appeared mainly for 50- and 75-µl concentrations of leaf extracts especially for primers OPB-18, OPB-15,

Table 3: The number of bands in control and molecular size of (base pair) disappearance (–) and/or appearance (+) of DNA bands for all primers in different concentrations of extracts on sunflower.

Primer		Extract concentration (%)			
		Control	8	12	16
OPB-5	–	3	0	0	0
	+		858	858;1,750	858;1,624;1,750
OPB-6	–	11	2,410;2,559	879;2,559	879;1,045;2,559
	+		731	0	0
OPB-7	–	13	1,850;2,187	668;787;1,850;2,187	668;712;787;1,850;2,187
	+		853;1,664	853	1,077
OPB-8	–	2	0	0	0
	+		0	0	0
OPB-11	–	14	977;1,543;2,412	746;2,412	746;1,543;2,412
	+		0	0	0
OPB-14	–	5	231	2,856	231;2,856
	+		881	404;708;881	708;881
OPB-15	–	13	1,850;2,187	1,850	1,850
	+		0	807;1,646	807;1,646
OPB-18	–	11	945	2,324;2,410	0
	+		1,317	731;1,317	1,317
Total band		72	11(–) 6(+)	12(–) 10(+)	14(–) 9(+)
Polymorphism			23.61	30.6	31.94
Genome stability template			76.39	69.4	68.06

OPB-5 and OPB-14. Increased concentration of extracts caused decreasing GTS value and increasing polymorphism values, we concluded that the polymorphism values and GTS were dose dependent. For instance, the highest amount of GTS was observed in the first (8%) dose and the lowest amount in the third (16%) dose (Table 3). In all cases, polymorphisms were due to the loss and/or gain of the amplified bands in the treated samples in comparison to the control profiles (Table 3). Changes in the RAPD pattern were expressed as decrease in GTS.

Genetic similarity coefficients (phenetic numerical analysis) were constructed to estimate the level of DNA polymorphism in the control and treated seedlings. These coefficients were summarized for the seedlings compared to their controls (Table 4). The estimated genetic similarity coefficients for different concentrations were found to be lower (<1.00) when compared to the control (the genetic similarity value is 1.00 for untreated control treatments). Based on these coefficients, the seedlings treated with 8% showed the nearest distance to controls, but the seedlings treated with 16% indicated the furthest distance (Table 4). In dendrogram constructed using UPGMA, the seedlings exposed to controls, 8%, 12% and 16% treatments showed two main branches for the seedlings (Figure 2). First, the seedlings exposed 8%, 12% and 16% clustered together; second, untreated control grouped alone.

Table 4: Genetic similarity coefficients in the different concentrations of extracts on sunflower.

			Extract concentration (%)	
Genetic similarity	0	8	12	16
	1	0.84	0.80	0.75

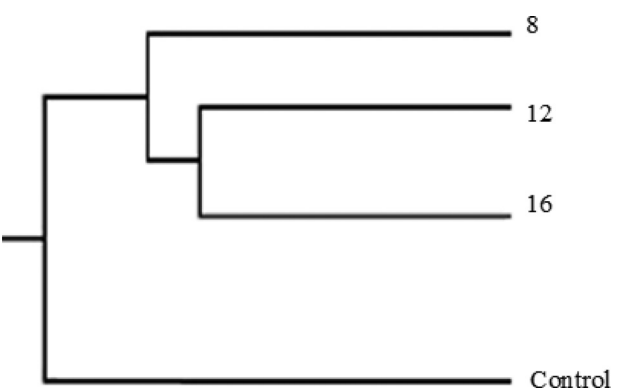

Figure 2: Dendrogram using the UPGMA based on DNA polymorphism among the seedlings exposed to untreated control, 8%, 12%, and 16% concentrations.

Different concentration extracts of wild poinsettia increased significantly the total protein when compared with the control (Table 5). Such an increase was found to be statistically significant ($p < 0.05$). IR% clearly demonstrates the dose-dependent response of wild poinsettia on the levels of total proteins (Table 5). IR values of seedlings treated in different concentrations of extracts were 2.34, 6.35 and 13.82. In addition, on PAGE the separated proteins showed bands ranging from 3 to 69 kDa (Figure 3). There was a little change in profiles

Table 5: Total soluble protein level in different concentrations of extracts on sunflower seedlings.[a]

Concentration (%)	Total soluble protein level (mg/g)	Increase rate (%) (IR)
Control	4.5 ± 0.42	0
8	4.6 ± 0.08	2.34
12	4.8 ± 0.09	6.35
16	5.01 ± 0.4	13.82

Note: [a]The mean difference is significant at the 0.05 level.

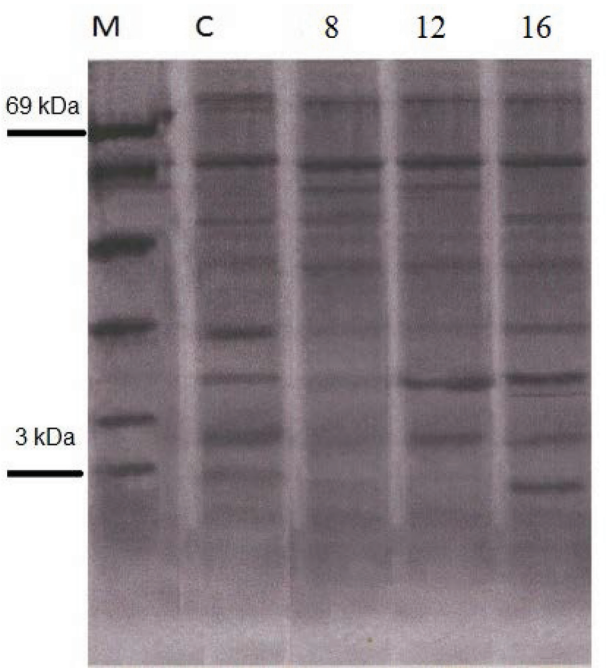

Figure 3: Comparison of total soluble protein level in sunflower seedlings exposed to different wild poinsettia extracts (M: Marker, C: control, 8% 12%, and 16%).

but the accumulation of the proteins and the intensity of the bands have showed variability. Changes in protein band profiles may cause point mutations, genetic and chromosomal rearrangements, deletion, insertions and methylation in DNA. Also, increase in total protein levels can be suggested as one of the mechanisms for their genotoxic effects resulting in cell death.

In "genetic-ecotoxicology" or "eco-genotoxicology," the effective evaluation and proper environmental monitoring of potentially genotoxic allelochemicals will be improved with development of sensitive and selective methods to detect toxicant-induced alterations in the genomes of a wide range of flora (Cenkci *et al.*, 2009). RAPD technique has been successfully utilized to detect various types of DNA damage and mutation in plants induced by allelochemicals (Bozari *et al.*, 2012; Sunar *et al.*, 2012). In the present study, we have evaluated the potential of the RAPD assay to measure toxic allelochemical-induced DNA effects in the sunflower seedlings. This study is the first report on genotoxic effect of allelochemical wild poinsettia extracts in sunflower. Some recent studies have reported that phenolic compounds from several allelopathic plants and heavy metal caused accumulation of ROS (H_2O_2 and O_2) in some plants (Mutlu *et al.*, 2011; Darier and Tammam, 2012; Cenkci *et al.*, 2009). Oxidative stress occurs when the production of ROS exceeds the plant's natural antioxidant defense mechanisms, causing damage to macromolecules such as DNA, proteins and lipids. Changes in the RAPD pattern and decreases in GTS may be due to oxidative damage caused by the allelochemical. According to the RAPD results, DNA damage induced by treated seedlings was reflected by changes in RAPD profiles like loss of bands, appearance of new bands and variation in band intensity. In our study, the number of lost bands was found higher than that of extra bands. The lost bands in sunflower seedlings exposed to toxic allelochemicals were determined generally as PCR products with high molecular weight. It is suggested that the DNA damage may be serious in the majority cells in the roots and leaves of sunflower seedlings exposed to toxic chemicals. The disappearance of normal bands (band loss) may be related to the events such as DNA damage (e.g. single- and double-strand breaks, modified bases, abasic sites, oxidized bases, bulky adducts, DNA–protein cross-links), point mutations and/or complex chromosomal rearrangements induced by genotoxins (Bozari *et al.*, 2012; Sunar *et al.*, 2012). When Taq DNA polymerase encounters a DNA adduct, there are a number of possible outcomes, including blockage, bypass and the possible dissociation of the enzyme/adduct complex which will cause the loss of bands (Cenkci *et al.*, 2009). Appearance of new PCR products (extra bands) was also detected in RAPD profiles. New PCR amplification products may reveal a change in some oligonucleotide priming sites due to mutations [new annealing event(s)], large deletions (bringing to pre-existing annealing site closer) and/or homologous recombination

(juxtaposing two sequences that match the sequences of primer) (Atienzar *et al.*, 1999). Atienzar *et al.* (2000) reported that mutations can only be responsible for the appearance of new bands if they occur at same locus in a sufficient number of cells (a minimum of 10% of mutations may be required to get new PCR product visible in agarose gel) to be amplified by PCR. The new bands could be attributed to mutations while the disappeared bands could be attributed to DNA damage (Atienzar and Jha, 2006).

Conclusion

Wild poinsettia has harmful effects on crops, including reduced seed germination and emergence of sunflower. Additionally, the extract experienced an increase in the generation and accumulation of ROS in leaves, leading to increased lipid peroxidation and H_2O_2 contents. Increased levels of scavenging system included a non-enzymatic system as GSH and the enzymatic system of sunflower. Increased levels of scavenging enzymes indicate their induction as a secondary defense mechanism in response to allelopathy stress present in methanol extract. Genotoxicity judgments can be elicited qualitatively and quantitatively via the comparison of the control and treated groups. The diagnostic analysis (considering band intensity differences or disappearance and/or appearance of RAPD bands) and the phonetic numerical analysis (providing information on the overall genetic diversity of populations; i.e. genetic similarity analysis) are important parameters in RAPD method (Wolf *et al.*, 2004). Based on the phenetic numerical analysis, the dendrogram construction based on UPGMA analysis of RAPD data is one of the most effective methods in numerical computation and it can show the relationships of every sample (Zhiyi and Haowen, 2004). In this sense, the phenetic approach differs from the genetic approach in that RAPD profiles are not considered as genotypes. In the present study, the estimated genetic similarity coefficients for 8%, 12% and 16% concentrations were found to be lower from control treatment (the genetic similarity value is “1.00” for untreated control treatments). These lower values show that DNA damage was increased with an increase in concentrations of allelochemicals. The possible mechanism of the allelopathy may be related to this genotoxic effect. Additional studies are being conducted to test this hypothesis.

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References

Adelusi A.A. and Akamo, O.A., 2006. The combined effects of (*Euphorbia heterophylla* Linn.) and nitrogen fertilizer on the reproductive yield and competitive ability of *Macrotyloma geocarpa* (Harms) Marechal and Beaudat. International Journal of Botany 2(3): 69–73.

Apel, K. and Hirt, H., 2004. Reactive oxygen species: Metabolism, oxidative stress and signal transduction. Annual Review of Plant Biology 55: 373–399.

Asada, K., 1994. Production and action of active oxygen species in photosynthetic tissues. *In*: Foyer, C.H., Mullineaux, P.M. (eds) *Causes of photooxidative stress and amelioration of defense system in plants*, CRC Press, Boca Raton, Ann Arbor, MI, London, and Tokyo, pp. 77–100.

Atienzar, F.A., Conradi, M., Evenden, A.J., Jha, A.N. and Depledge, M.H., 1999. Qualitative assessment of genotoxicity using random amplified polymorphic DNA: Comparison of genomic template stability with key fitness parameters in Daphnia magna exposed to benzo[a]pyrene. Environmental Toxicology and Chemisty 18: 2275–2282.

Atienzar, F.A., Cordi, B., Donkin, M.E., Evenden, A.J., Jha A.N., and Depledge, M.H., 2000. Comparison of ultraviolet-induced genotoxicity detected by random amplified polymorphic DNA with chlorophyll fluorescence and growth in a marine macroalgae, Palmaria palmate. Aquatic Toxicology 50: 1–12.

Atienzar, F.A. and Jha, A.N., 2006. The random amplified polymorphic DNA (RAPD) assay and related techniques applied to genotoxicity and carcinogenesis studies: A critical review. Mutation Research 613: 76–102.

Becana, M., Dalton, D.A., Moran, J.F., Iturbe-Ormaetxe, I., Matamoros, M.A. and Rubio, M.C., 2000. Reactive oxygen species and antioxidants in legume nodules. Physiologia Plantarum 109: 372–381.

Blokhina, O., Virolainen, E. and Fagerstedt, K.V., 2003. Antioxidants, oxidative damage and oxygen deprivation stress: A review. Annals of Botany 91: 179–194.

Bradford, M.M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Annals of Biochemistry 72: 248–254.

Beauchamp, C. and Fridovich, I., 1971. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry 44: 276–286.

Bozari, S., Agar, G., Aksakal, O., Erturk, F.A. and Yanmis, D., 2012. Determination of chemical composition and genotoxic effects of essential oil obtained from *Nepeta nuda* on *Zea mays* seedlings. Toxicology and Industrial Health 29(4): 339–348.

Cakmak, I. and Marschner, H., 1992. Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase and glutathione reductase in bean leaves. Plant Physiology 98: 1222–1227.

Carvalho, L.B., Bianco, S. and Guzzo, C.D., 2010. Interference of *Euphorbia heterophylla* in the growth and macronutrient accumulation of soybean. Planta Daninha 28(1): 33–39.

Cenkci, S., Yıldız, M., Cig˘erci, I.H., Konuk, M. and Bozdag, A., 2009. Toxic chemicals-induced genotoxicity detected by random amplified polymorphic DNA (RAPD) in bean (*Phaseolus vulgaris* L.) seedlings. Chemosphere 76: 900–906.

Darier, S.M. and Tammam, A.A., 2012. Potentially phytotoxic effect of aqueous extract of *Achillea santolina* induced oxidative stress on *Vicia faba* and *Hordeum vulgare*. Romanian Journal of Biology – Plant Biology 57(1): 3–26.

Duke, S.O. and Kenyon, W.H., 1993. Peroxidizing activity determined by cellular leakage. *In*: Böger, P. and Sandmann, G. (eds) *Target assays for modern herbicides and related phytotoxic compounds*, Lewis Publ., Boca Raton, FL, pp. 61–66.

Egley, G.H., Paul, R.N., Vaughn, K.C. and Duke, S.O., 1983. Role of peroxidase in the development of water impermeable seed coats in *Sidas pinosa* L. Planta 157: 232.

El-Shazly, H.H. and El-Mutairi, Z., 2006. Genetic relationships of some barley cultivars, based on morphological criteria and RAPD finger-printing. International Journal of Botany 2: 252–260.

Ferreira, A.G. and Aquila, M.E.A., 2000. Alelopatia: uma area emergente da ecofisiologia. Revista Brasileira de Fisiologia Vegetal 12: 175–204.

Ferreira, A.G. and Borguetti, F., 2004. *Germinacao: do basicoaoaplicado*, 323 p. Porto Alegre: Artmed.

Foyer, C.H. and Halliwell, B., 1976. Presence of glutathione and glutathione reductase in chloroplasts: A proposed role in ascorbic acid metabolism. Planta 133: 21–25.

Fujii, Y., Parvez, S.S., Parvez, M.M., Ohmae, Y. and Iida, O., 2003. Screening of 239 medicinal plant species for allelopathic activity using the sandwich method. Weed Biology and Management 3: 233–241.

Griffih, O.W., 1980. Determination of glutathione and glutathione disulphide using glutathione reductase and 2- vinyl pyridine. Analytical Biochemistry 106: 207–212.

Guri, A., 1983. Variation in glutathione and ascorbic acid content among selected cultivars of *Phaseolus vulgaris* prior to and after exposure to ozone. Canadian Journal of Plant Science 63: 732–737.

Han, C.M., Pan, K.W., Wu, N., Wang, J.C. and Li, W., 2008. Allelopathic effect of ginger on seed germination and seedling growth of soybean and chive. Scientia Horticulturae 116: 330–336.

Heath, R.L. and Packer, L., 1968. Photoperoxidation in isolated chloroplasm kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics 125: 189–198.

Homme, P.M., Gonalez, B. and Billard, S., 1992. Carbohydrate content, fructan and sucrose enzyme activities in roots, stubble and leaves of rye grass (*Lolium perenue* L.) as affected by source and sink modification after cutting. Plant Physiology 140: 282–291.

Jiangbo, H. E., 2010. New compounds from *Euphorbia helioscopia* and absolute configuration determination by computational methods. Bulletin of the Korean Chemical Society 31(8): 2211–2214.

Kaiser, W.M., 1979. Reversible inhibition of the Calvin cycle and activation of oxidative pentose phosphate cycle in isolated intact chloroplast by hydrogen peroxide. Planta 145: 377–382.

Kato-Noguchi, H., Ino, T., Sata, N. and Yamamura, S., 2002. Isolation and identification of a potent allelopathic substance in rice root exudates. Physiologia Plantarum 115: 401–405.

Kato-Noguchi, H. and Macías, F.A., 2005. Effects of 6-methoxy-2-benzoxazolinoneon the germination and a-amylase activity in lettuce seeds. Journal of Plant Physiology 162: 1304–1307.

Khan, M., 1998. Allelopathic potential of dry fruits of *Washington filfera* L. Linden inhibitor of germination and growth. Pakistan Journal of Botany 30: 2–11.

Khor, H.T., Tan, N.H. and Chua, C.L., 1986. Lipase-catalyzed hydrolysis of palm oil. Journal of the American Chemical Society 63: 538–540.

Laemmli, U.K., 1970. Cleavage of structural proteins during assembly of head of bacteriophage-T4. Nature 227(5259): 680–685.

Liu, W., Yang, Y.S. and Zhou, Q., 2007. Impact assessment of cadmium contamination on rice (*Oryza sativa* L.) seedlings at molecular and population levels using multiple biomarkers. Chemosphere 67(6): 1155–1163.

Macias, F.A., Verela, R.M., Torres, A., Galindo, J.L.G. and Molinillo J.M.G., 2002. Allelochemicals from sunflower: Chemistry, bioactivity and application. *In*: Inderjit Mallik, U. (ed.) *Chemical ecology of plants: Allelopathy in aquatic and terrestrial ecosystem*, Birkhauser Verlag, Basel, pp. 73–87.

Maraschin-Silva, F. and Aquila, M.E.A., 2005. Potencial alelopatico de *Dodonaea viscosa* (L.) Jacq. Iheringia – SerieBotanica, Porto Alegre 60: 91–98.

Mishra, S., Srivastava, S., Tripathi, R.D., Govindrajan, R., Kuriakose, S.V. and Prasad, M.N.V., 2006. Phytochelatin synthesis and response of antioxidants during cadmium stress in *Bacopa monnieri* L. Plant Physiology Biochemistry 44: 25–37.

Moran, R., 1982. Formulae for determination of chlorophyllous pigments extracted with N-N Dimethyl formamide. Plant Physiology 69: 1376–1381.

Moreno, D.A., Ilic, N., Poulev, A., Brasaemle, D.L., Fried, S.K. and Raskin, I., 2003. Inhibitory effects of grape seed extract on lipases. Nutrition 19: 876–879.

Mutlu, S., Atici, O., Esim, N. and Mete, E., 2011. Essential oils of catmint (*Nepetameyeri*) induce oxidative stress in early seedlings of various weed species. Acta Physiologiae Plantarum 33: 943–951.

Nakano, Y. and Asada, K., 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant and Cell Physiology 22: 867–880.

Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583–590.

Noguchi, H.K., Tanaka, Y., Murakami, T., Yamamura, S. and Fujihara, S., 2002. Isolation and identification of an allelopathic substance from *Citrus junos*. Phytoch 61: 849–853.

Oracz, K., Bailly, C., Gniazdowska, A., Come, D., Corbineau, F. and Bogatek, R., 2007. Induction of oxidative stress by sunflower phytotoxins in germinating mustard seeds. Journal of Chemical Ecology 33: 251–264.

Romero-Romero, T., Sanchez-Nieto, S., Sanjuan-Badillo, A., Anaya, A.L. and Cruz-Ortega, R., 2005. Comparative effects of allelochemical and water stress in roots of *Lycopersicon esculentum* Mill Plant. Solanaceae. Plant Science 168: 1059–1066.

Saghai-Maroof, M.A., Soliman, K.M., Jorgensen, R.A. and Allard, R.W., 1984. Ribosomal DNA spacer- length polymorphism in barley. Mendelian inheritance, chromosomal location, and population dynamics. Proceedings of the National Academy of Sciences USA 81: 8014–8018.

SAS Institute., 1999. SAS Online Doc. v. 8, SAS Inst, Cary, NC.

Savarnjad, A., YounesAbadi, M. and Habibian, L., 2010. Introduction of new invasive weeds in soybean fields in Golestan province. Weed flora in soybean fields in Golestan province. Research Department of Plant Protection, Agriculture and Natural Resources, Golestan Province. http://www.golestan-tarvij.ir/chakadalborz.com/images/File/alaf. (last accessed 12.07.2014).

Shindy, W.W. and Smith, O., 1975. Identification of plant hormones from cotton ovules. Plant Physiology 55: 550–554.

Singh, A., Singh, D. and Singh, N.B., 2009. Allelochemical stress produced by aqueous leachate of Nicotiana plumb aginifolia Viv. Plant Growth Regulation 58: 163–171.

Staubmann, R., Ncube, I., Gubitz, G.M., Steiner, W. and Read, J.S., 1999. Esterase and lipase activity in *Jatropha curcas* L. seeds. Journal of Biotechnology 75: 117–126.

Sunar, S., Yildirim, N., Aksakal, O. and Agar, G., 2012. Determination of the genotoxic effects of *Convolvulus arvensis* extracts on corn (*Zea mays* L.) seeds. Toxicology and Industrial Health 29(5): 449–459.

Tammam, A.A., 2008. Increased antioxidants activity under the combined effects of salinity and indole 3-butyric acid and the mechanism of salt tolerance in cotton cultivars. Advances of Food Science 30: 78–88.

Tanveer, A., Khaliq, A., Javaid, M.M., Chaudhry, M.N. and Awan, I., 2013. Implications of weeds of genus euphorbia for crop production: a review. Planta Daninha 31(3): 723–731.

Turk, M.A. and Tawaha, A.M., 2003. Allelopathic effect of black mustard (*Brassica nigra* L.) on germination and growth of wild oat (*Avena fatua* L.). Crop Protection 22: 673–677.

Turker, M., Battal, P., Agar, G., Gulluce, M., Sahin, M.F. and Erez, E., 2008. Allelopathic effects of plants extracts on physiological and cytological processes during maize seed germination. Alleopathy Journal 21: 273–286.

Velikova, V., Yordanov, I. and Ereva, A., 2000. Oxidative stress and some systems acid rain-treated bean plants. Plant Science 151: 59–66.

Weir, T.L., Park, S.W. and Vivanco, J.M., 2004. Biochemical and physiological mediated by allelochemicals. Current Opinion in Plant Biology 7: 472–479.

Whistler, R.L., Wolform, M.L., BeMiller, J.N. and Shafizadeh, F., 1962. Anthrone colorimetric method. *In*: Scott, T.A., Melvin, E.H. (eds) *Methods in carbohydrate chemistry*, vol 1. Academic Press, New York and London, p. 384.

Wolf, H.D., Blust, R. and Backeljau, T, 2004. The use of RAPD in ecotoxicology. Mutation Research 566: 249–262.

Ye, S.F., Zhou, Y.H., Sun, Y., Zou, L.Y. and Yu, J.Q, 2006. Cinnamic acid causes oxidative stress in cucumber roots, and promotes incidence of Fusarium wilt. Environmental and Experimental Botany 56: 255–262.

Zhiyi, R. and Haowen, Y, 2004. A method for genotoxicity detection using random amplified polymorphism DNA with Daniorerio. Ecotoxicology and Environmental Safety 58: 96–103.