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BRIEF COMMUNICATION

Allelopathic effects of sunflower extracts on mustard seed germination and seedling growth

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Abstract

The allelopathic potential of two cultivars of sunflower (*Helianthus annuus* L.) Lech and Ogrodowy was studied. Leaf aqueous extracts of sunflower at 2.5, 5, and 10 % (m/v) concentrations were applied to determine their effect on mustard (*Sinapis alba*) seed germination and seedlings growth under laboratory conditions. Increasing concentration of aqueous extracts of sunflower inhibited seed germination, up to almost complete failure of germination, observed in the presence of 10 % extract from sunflower cv. Ogrodowy. The decrease in germinability was well correlated with increased membrane deterioration, assayed as electrical conductivity and enhanced lipid peroxidation, detected as increased malondialdehyde content.

Additional key words: electrolyte leakage, *Helianthus annuus*, MDA content, *Sinapis alba*.

Allelopathy is defined as a direct or indirect harmful or beneficial effect of one plant on another through the production of chemical compounds released to the environment (Rice 1984). Recently, allelopathy is exploit as a weed control strategy, alternative to the commercial herbicide dominated programs (Bhowmik and Inderjit 2003). Promising results were obtained by selecting allelopathic crop types, using allelopathic companion plants or rotational crops (Weston and Duke 2003). Sunflower (*Helianthus annuus* L.) can actively influence the growth of surrounding plants due to its high allelopathic potential (Azania *et al*. 2003). Macias *et al*. (2002) isolated about 125 natural allelopathic compounds from different cultivars of sunflower, phytotoxic towards many weed species. Although, there are some studies on sunflower allelopathic activities evaluating different plant organs (Irons and Burnside 1982) little is known on their effects in regard to sunflower genotypes.

The allelopathic potential of two sunflower cultivars Ogrodowy (O) and Lech (L) was determined under laboratory conditions. The aim of the presented study was also to establish whether loss of seed germination ability in allelopathy stress is related to membrane deterioration evaluated as malondialdehyde (MDA) content and electrolyte leakage.

Allelopathic extract was prepared from leaves of field grown sunflower (O or L) harvested at the beginning of flowering stage. Air-dried tissue was ground to fine powder and extracted (2.5, 5 or 10 g) for 24 h in 100 cm^3 of distilled water at room temperature. The obtained extract was filtered through filter paper.

Mustard seeds (*Sinapis alba* L.) were germinated in 10 cm Petri dishes (50 seeds per dish) on a filter paper moistened with distilled water (control) at 20 °C in darkness. The influence of sunflower allelochemicals was investigated in the presence of 2.5 %, 5 % or 10 % (m/v) water extract from O or L.

Electrolyte leakage from seeds or young seedlings was measured with a conductivity meter (*OK-102/l*, *Radelkis*, Budapest, Hungary). Seeds (20) or seedlings (5 - 10) were placed in 15 cm^3 distilled water at room temperature in darkness and conductivity in the medium was measured after 2 h. Results are expressed as % of total leakage from seeds or seedlings boiled for 20 min.

Lipid peroxidation in seed and seedlings was determined in terms of malondialdehyde (MDA) content by thiobarbituric (TBA) reaction as described by Heath and Parker (1968).

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Abbreviations: O - sunflower cv. Ogrodowy, L - sunflower cv. Lech
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Control mustard seeds were characterized by a high germination ability (95 - 100 %) after 3 d. Water extracts from sunflower leaves of both L and O had an inhibitory effect on mustard seeds germination (Fig. 1). The degree of inhibition increased with the extract concentration. The effects of allelopathic compounds from O and L were similar at the lowest concentration 2.5 %, and only little impact on mustard seed germination was detected after 7 d treatment. The extract from O at the highest concentration, 10 %, inhibited germination almost completely, while in the presence of 10 % extract from L, 15 % seeds germinated after one week. Sunflower allelochemicals did not affect imbibition, the first phase of germination (data not shown). That indicates that the observed alterations in seeds germination are due to toxicity of sunflower allelopathic compounds not only to water stress. It is in agreement with data presented by Bernat *et al.* (2004). The authors suggested that the lower water availability for seed germination due to binding water by compounds present in extract of sunflower leaves plays a minimal role in reduction of seed germination, so the mode of action of sunflower allelochemicals is mainly to their toxic nature. Inhibition of wild mustard seeds germination in the presence of leaf extracts from four different sunflower cultivars was previously observed by Leather (1983). Macias *et al*. (2000) investigated the effect of several compounds isolated from *Helianthus annuus* on different dicotyledon and monocotyledon species. In general he observed inhibitory effects on germination of dicotyledon species and stimulatory effects on the growth of monocotyledon species. The greatest inhibitory activity was shown on

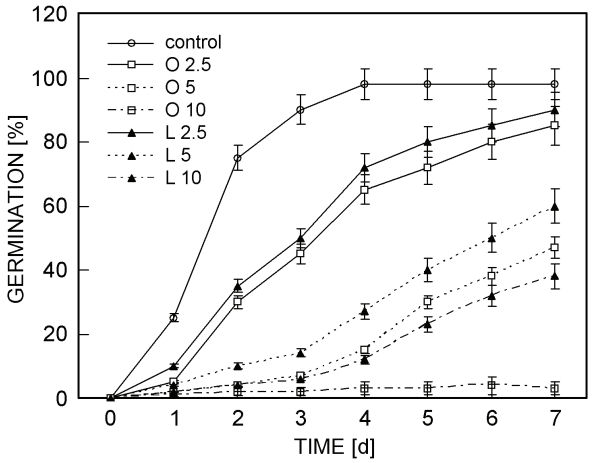

Fig 1. Germination of mustard seeds in the presence of various concentrations [%] of aqueous extracts from leaves of O or L sunflower. Each reported value represents the means ± SD of 4 independent experiments.

lettuce germination, and the effect was dependent on the dilution of allelochemicals (Macias *et al*. 2000).

Electrical conductivity of allelopathy stressed seeds and seedlings increased progressively with the extract concentration and duration of the experiment. It increased rapidly after 7 d of experiment with 10 % extract, reaching value 52 % for seeds treated with L extract and 27 % for seeds germinated in the presence of O allelochemicals (Table 1). These observations are in agreement with data obtained on cucumber cotyledons treated with dehydrozaluzanin C (Galindo *et al*. 1999). Increased electrolyte leakage reflected a loss in membrane integrity. This phenomenon may indicate an inability to maintain coherent membranes, resulting in losses in germinability. Allelochemicals from sunflower leaves caused an increase in lipid peroxidation determined as the increase in MDA concentration (Table 1). Two tested sunflower cultivars affected lipid peroxidation of mustard seed in the same way, but the highest level of malondialdehyde (MDA) was observed in mustard seeds germinated in the presence of 10 % extract of sunflower leaves. It supports the idea that loss of seed germinability may be associated with membrane lipid peroxidation.

Sunflower leaf extracts of both L and O modified mustard seedling growth exhibited as reduction in radicle and hypocotyl length (data not shown), similarly as it was observed on cress, lettuce, timothy and ryegrass in the presence of leaf extract of *Pueraraia thumbergiana* or capsaicin (Kato-Naguchi 2003/4, Kato-Naguchi and Tanaka 2003/4). Besides inhibiting radicle and hypocotyls elongation other morphological abnormalities occurred in the presence of water extracts from both cultivars of sunflower. Roots of treated plants were thicker with a brownish color as compared to control. Comparable observations were done on bean and bottle gourd plants cultured in the presence of aqueous leachate of *Sicyos deppei* leaves (Cruz-Ortega *et al*. 1998).

Sunflower extracts also influenced fresh mass of mustard seedlings (Table 1). Fresh masses of allelopathy stressed seedlings were significantly lower than in the control independently of sunflower cultivar used as allelochemicals source, but the effect was more pronounced for the extracts at higher 5 and 10 % concentrations.

Dry mass of control mustard seeds and seedling decreased during culture period (Table 1). Dry mass of mustard seedlings, which were exposed to low and medium concentration of allelopathy extract, was higher in comparison to the control. In the presence of the highest, 10 % concentration of sunflower extracts obtained from L or O almost no seed germination was detected, so dry mass of seed and seedlings reminded at a constant level during the culture period. Sunflower extract at the highest concentration, 10 %, inhibited growth processes in germinated seeds but did not influence seed viability determined by tetrazolium test (data not shown). During the first five days of germination in the presence of 10 % extract from O almost all seeds remained viable. A longer treatment was lethal and all seeds were dead after two weeks.

Strong inhibition of seed germination and seedling growth in the presence of sunflower allelopathics were

Table 1. Influence of various concentrations of aqueous sunflower leaf extracts (O or L) on mustard seedling growth (fresh and dry mass), electrolyte leakage and MDA concentration after 1 and 7 d of culture. Means ± SD of 3 - 5 replicates.

	Time [d]	Control	L [%] 2.5	5	10	O [%] 2.5	5	10
Fresh mass of seeds	1	22.0 ± 2.0	20.0 ± 1.0	19.0 ± 1.0	15.0 ± 1.0	21.0 ± 1.0	19.0 ± 1.0	15.0 ± 1.0
or seedlings [mg]	7	69.0 ± 5.0	55.0 ± 5.0	23.0 ± 3.0	15.0 ± 3.0	73.0 ± 11	48.0 ± 5.0	16.0 ± 2.0
Dry mass of seeds	1	9.0 ± 1.0	11.0 ± 2.0	9.0 ± 1.0	7.0 ± 1.0	11.0 ± 2.0	9.0 ± 1.0	6.0 ± 1.0
or seedlings [mg]	7	6.0 ± 1.0	11.0 ± 2.0	10.0 ± 2.0	7.0 ± 2.0	9.0 ± 1.0	10.0 ± 2.0	7 ± 1.0
Electrolyte leakage	1	8.5 ± 1.0	19.1 ± 2.3	19.4 ± 2.0	20.2 ± 2.7	16.2 ± 1.0	17.3 ± 2.5	22.1 ± 4.0
[% total]	7	9.1 ± 1.0	19.5 ± 2.1	20.3 ± 3.0	52.5 ± 9.9	18.1 ± 3.0	22.3 ± 4.0	27.0 ± 5.0
MDA	1	7.3 ± 1.5	7.2 ± 2.0	7.0 ± 2.4	6.1 ± 1.4	6.1 ± 1.4	6.2 ± 1.6	6.0 ± 1.8
[pmol g^{-1} (d.m.)]	7	7.5 ± 1.5	9.3 ± 2.3	11.1 ± 3.3	18.2 ± 3.0	7.5 ± 1.7	12.0 ± 3.0	16.2 ± 4.5

obtained also during field experiments. Sunflower residues remaining in the field drastically reduced growth of *Cyamopsis tetragonoloba*, *Sorghum vulgare*, *Pennisetum americanum* and *Zea mays* (Batish *et al*. 2002). Significant inhibition in growth of mustard plants due to the presence in the soil both sunflower roots exudates and/or tiny not possible to collect roots and root hairs was detected by Ciarka *et al.* 2004. It was suggested that the reduced growth and yield of crops can be attributed to the release of phytotoxic phenolics from decomposing sunflower residues, since the soil collected from sunflower fields was rich in phenolics (Batish *et al*. 2002). Phenolics compounds released during red clover decomposition were phytotoxic to wild mustard seedlings and the bioassay with aqueous red clover extract shoved a linear reduction in mustard root growth with increasing extract concentration (Ohno *et al*. 2000).

Leaf extract from two different cultivars of sunflower O and L exhibited allelopathic activity against mustard plants. The results demonstrated that sunflower O is characterized by the higher allelopathic potential than L and inhibition in seed germination in due to enhanced membrane deterioration.

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