ISSN 2226-3063 e-ISSN 2227-9555
Modern Phytomorphology **11**: 91–97, 2017

doi: 10.5281/zenodo.1034526

RESEARCH ARTICLE

The influence of alcoholic extract from leaves of *Helianthus annuus* L. on germination and growth of *Sinapis alba* L.

Jakub Oliwa, Katarzyna Możdżeń *, Grzegorz Rut, Andrzej Rzepka

Institute of Biology, Pedagogical University, Podchorążych 2, 30-084 Cracow, Poland;
katarzyna.mozdzen@up.krakow.pl

Received: 19.04.2017 | **Accepted**: 01.08.2017 | **Published**: 21.10.2017

Abstract

The knowledge of plants interactions is used for their better protection and cultivation. The aim of this study was to determine the influence of alcoholic extracts from the leaves of the common sunflower (*Helianthus annuus*) on selected physiological parameters of plants of white mustard (*Sinapis alba* 'Barka'). The seeds germination, the growth, the fresh and dry masses of plants grown from seeds and germinated on the sunflower extracts and plants watered by *H. annuus* extracts during the growth phase were studied. In the growth phase, the fresh masses of all organs were increased on 5% extract, however significantly decreased on 10% extract. The dry mass of *S. alba* was not significantly changed. In the germination phase, much less changes of these parameters were revealed. In general, extracts from *H. annuus* leaves inhibited germination of *S. alba* seeds, but stimulated growth of plants in case of application of 5% concentrations during the germination phase and inhibited their growth in case of application of 10% concentrations during the growth phase.

Keywords: *Helianthus annuus*, *Sinapis alba* 'Barka', mustard, sunflower, allelopathy, growth, morphology, seed germination

Introduction

The progressive degradation of the environment and the need to reduce the use of chemical pesticides caused the interest in the allelopathy phenomenon (Kato-Noguchi *et al.* 2002). The studies on allelopathic effects of different species constitutes the possibility of application of substances of plant origin, which are naturally biodegradable and therefore have a high economic significance (Oliwa *et al.* 2016). Investigations of the number of

allelopathic compounds show that mechanisms of interaction between plants are extremely complex, but nevertheless they are effectively applied in agricultural practice (Lipińska 2006; Afridi & Khan 2014, 2015). Allelopathy refers to secretion of chemical substances by plants, which modify properties of the environment in their immediate surroundings, depending from the intensity of abiotic factors as well as genetic determinants of organisms (Amini *et al.* 2014). The absorption mostly by roots and the transport of allelopathic substances to other plant organs are assisted by regulatory mechanisms at both cellular and tissue levels.

Allelopathic interaction between plants is a result of competition about the environmental resources, revealing by emission of chemical compounds by plants (Kasperczyk & Szewczyk 2007). Allelopathic substances can demonstrate both negative and positive influence on the growth of plants (Ohno & Doolan 2001; Puła *et al.* 2016; Barabasz-Krasny *et al.* 2017).

Helianthus annuus L. (Asteraceae) has a high allelopathic potential. It can actively influence on the growth of the surrounding plants (Leather 1983; Macias *et al.* 2002; Możdżeń *et al.* 2016). In particular, *H. annuus* inhibits germination of such weeds as *Phalaris minor* Retz., *Centaurea* spp., *Erigeron canadensis* L. and *Amaranthus retroflexus* L. (Khalid *et al.* 2002). It was shown that extracts of sunflower reduced seed germination of wild barley (Ashrafi *et al.* 2008). The allelochemicals substances of sunflower also inhibit the germination and growth of *Agropyron repens* (L.) P. Beauv., *Avena fatua* L., *Digitaria ciliaris* (Retz.) Koeler and *Sida spinosa* L. seedlings (Azania *et al.* 2003). The suppressing effect of aqueous extracts from leaves of *H. annuus* was also observed in the case of germination and growth of *Sinapis alba* (Bogatek *et al.* 2006).

Leaves of sunflower are rich source of phenolic compounds, chlorogenic and isochlorogenic acids, and terpenoids, characterised by a wide spectrum of biological activity (Wilson & Rice 1968; Batish *et al.* 2002; Gniazdowska *et al.* 2004). The biochemical analyses of extracts from *H. annuus* leaves also indicated the presence of sesquiterpene lactones exceptionally well soluble in alcohols (Spring *et al.* 1992).

The aim of this study was to evaluate the influence of alcoholic extracts from dry leaves of *H. annuus* at 5% and 10% concentrations on plants of *Sinapis alba* L. cultivar 'Barka'. The mustard cultivar 'Barka' is widely grown in Poland and therefore it was selected for the studies. In this study, (1) seeds germination, (2) growth of underground and aboveground organs and (3) their fresh and dry masses were measured for *S. alba* plants watered by *H. annuus* extracts during the germination and growth phases.

Material and methods

The experiments were conducted at the Department of Plant Physiology of Pedagogical University in Kraków (Poland) during September 2016. In the same conditions, the experiments were replicated five times.

Extracts preparation

The leaves of *H. annuus* were collected from cultivated field in south of Poland in August 2016. Alcoholic extracts of allelopathic substances from dried leaves of *H. annuus* of 5% and 10% concentrations were prepared by weighing of plant material (5 g and 10 g were taken, respectively) and soaking by ethanol (95 ml and 90 ml were added, respectively) for 24 hours. The extracts were filtered on a Büchner funnel with Whatman No. 1 filter paper by vacuum pump and placed in a laboratory dryer at 70°C for next 48 hours to evaporate. Then, the extracts were soaked in appropriate amount of distilled water (5% – in 95 ml and 10% – in 90 ml, respectively).

Seeds germination

One hundred seeds of *S. alba* 'Barka' were placed in sterilised 9 cm Petri dishes with 3 layers of Whatman No. 1 filter paper. The dishes were wetted with 6 ml of alcoholic extracts from *H. annuus* leaves and distilled

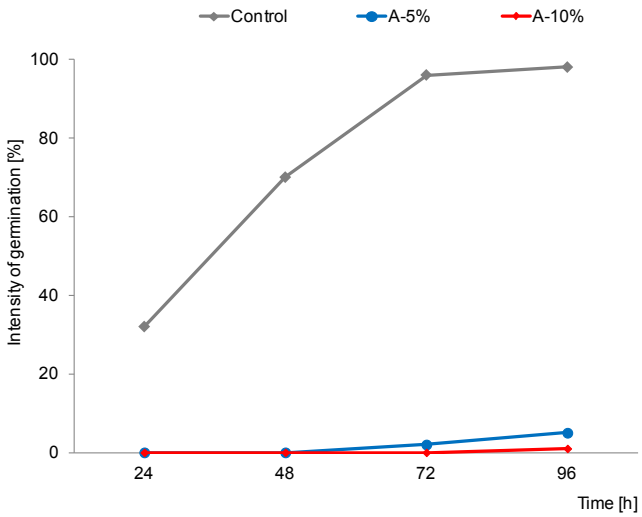

Fig. 1. The germination of *Sinapis alba* 'Barka' seeds on alcoholic extracts from the leaves of *Helianthus annuus* with concentrations of 5% and 10%.

water (control group). The seeds were placed in dark growth chamber at temperature 25 °C. After 24, 48, 72 and 96 hours, the number of germinated seeds was counted. As germinated seeds were considered that seeds, which had at least 2 mm of radical length. The percentage of the germinated seeds was calculated using the formula: germination [%] = (germinated seed × 100) / total seeds sown.

Plant growth conditions

After 72 h of germination, the seedlings of *S. alba* grown on the Petri dishes with *H. annuus* extract were rinsed with distilled water and planted in pots with sand. The first group of plants was watered every 48 h with distilled water and once a week – by Steiner culture medium (Steiner 1961). The second group included plants grown from seedlings germinated on distilled water and they were watered every second day with alcoholic extracts from the leaves of *H. annuus* and once a week during the growth – with culture medium. The control group of plants was watered with distilled water and the Steiner culture medium. Plants were grown in a growth chamber (Angelantoni Lifescience, Italy) with 12/12 h photoperiod, 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light intensity, 25 °C/20 °C alternation, and 60–70% of relative humidity.

Biometric analysis, fresh and dry mass

The lengths of the root, hypocotyl and epicotyl of *S. alba* plants were measured with caliper and average lengths were calculated. The fresh mass and the dry mass (fresh mass dried at 105 °C for 48 h) of plant material were determined.

Statistical analysis

The data were subjected to ANOVA with Statistica 10.0 for Windows. The significance of differences was determined using the Duncan test for homogeneous groups, the mean ± SD of $n = 5$, $p < 0.05$.

Results and discussion

Allelopathic interactions usually result in delay or inhibition of the seeds germination and plants growth, which mostly depend from concentration of the active substances contained in substrate (Możdżeń & Oliwa 2015; Oliwa *et al.* 2016). In this study, with increasing of the concentrations of chemical substances in the alcoholic extracts from *H. annuus* leaves, the inhibition of seeds germination of *S. alba* was observed (Fig. 1). After 48 hours of germination on the alcoholic extract from leaves of

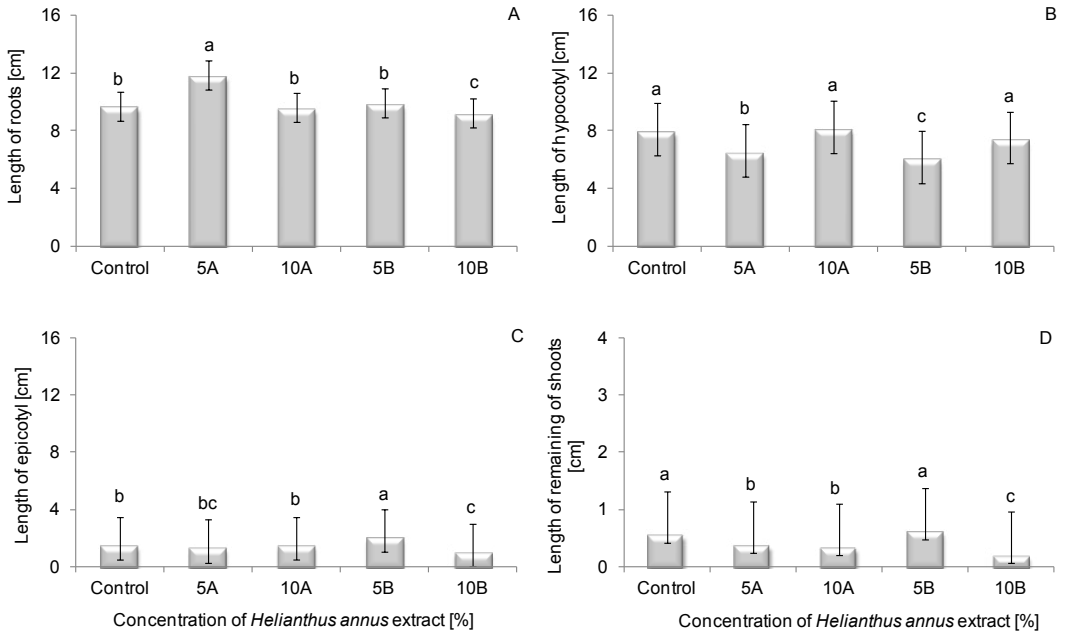

Fig. 2. The length of particular organs of *Sinapis alba* 'Barka' watered with alcoholic extracts from the leaves of *Helianthus annuus* with concentrations of 5% and 10% in germination (**A**) and growth (**B**) phases. Mean values ±SD (n=5); different letters are significantly different according to Duncan's test at $p < 0.05$.

H. annuus no germinated seeds of *S. alba* were observed, while in control group 70% of seeds were germinated. The first seeds germinated on 5% extracts were observed after 72 h. Higher concentration of allelopathic extracts (10%) resulted in complete inhibition of seeds germination up to 96 hours. In such case, after 96 h, only 1% of *S. alba* seeds were germinated. The destabilisation of metabolic pathways in different conditions has both primary and secondary origin, and it has a reflection in the physiological processes associated with growth and development of plants (Możdżeń & Repka 2014). The plant growth inhibition can be a competition between the plants for water, minerals substances, oxygen, and carbon dioxide. Among other factors reducing the plant growth may be the changes of pH and osmotic potential of the soil, and disorder of nitrogen uptake mechanisms (Inderjit & Duke 2003).

In this study, the morphometric analysis revealed that in the germination phase the 5% extract of *H. annuus* increased the root length and decreased the hypocotyl length in *S. alba* plants. Comparing with a control group, the remaining of shoots was inhibited on the 10% extracts. In the growth phase, 10% extracts inhibited the growth of mustard plants (Fig. 2).

The changes in the values of fresh and dry masses of *S. alba* grown on the alcoholic extracts of *H. annuus* were observed (Tab. 1). The differences of roots mass in plants grown on 5% extracts both during the germination and growth phases were revealed. In growth phase the roots mass was higher than in the germination phase (Tab. 1). This was related also with small decrease in the value of fresh and dry mass of the hypocotyl of *S. alba* watered with *H. annuus* extracts during the growth phase. However in growth phase, higher losses of the fresh and dry masses of hypocotyl were caused by 10% extracts. The fresh mass of epicotyl was decreased in higher concentrations of allelopathic substances both in germination and growth phases, and was

Table 1. The fresh and dry masses of organs of *Sinapis alba* 'Barka' watered with alcoholic extracts from the leaves of *Helianthus annuus* with concentrations of 5% and 10% in germination (**A**) and growth (**B**) phases. Mean values ±SD (n=5); different letters differ significantly within a row by Duncan's test at $p<0.05$.

Organ	Control	Alcoholic extracts of *H. annuus*			
		5%		10%	
		A	B	A	B
		Fresh mass, g			
Root	0.68 ^{c}	0.87 ^{b}	1.12 ^{a}	0.74 ^{c}	0.63 ^{c}
Hypocotyl	0.89 ^{a}	0.93 ^{a}	0.75 ^{b}	0.84 ^{a}	0.57 ^{c}
Epicotyl	0.14 ^{b}	0.13 ^{b}	0.21 ^{a}	0.08 ^{c}	0.04 ^{c}
Blade leaf	0.49 ^{b}	0.49 ^{b}	0.58 ^{a}	0.31 ^{c}	0.12 ^{d}
Whole plant	2.51 ^{b}	2.93 ^{ab}	3.30 ^{a}	2.35 ^{b}	1.59 ^{c}
		Dry mass, g			
Root	0.02 ^{b}	0.02 ^{b}	0.03 ^{a}	0.03 ^{ab}	0.02 ^{ab}
Hypocotyl	0.04 ^{c}	0.07 ^{a}	0.05 ^{b}	0.04 ^{c}	0.04 ^{d}
Epicotyl	0.01 ^{a}	0.01 ^{a}	0.01 ^{a}	0.01 ^{a}	0.01 ^{a}
Blade leaf	0.05 ^{b}	0.05 ^{b}	0.06 ^{a}	0.04 ^{c}	0.02 ^{d}
Whole plant	0.35 ^{b}	0.38 ^{b}	0.47 ^{a}	0.37 ^{b}	0.34 ^{b}

increased only on the lower concentration of allelopathic substances in the growth phase. Similar trend was observed during analysis of these parameters for leaf blades too. The dry mass of *S. alba* epicotyl was not significantly changed in the germination and growth phases, comparing to the control group. The lowest values of leaves mass in the growth phase were in the plants watered by 10 % extracts. In the growth phase, the fresh masses of all organs increased on 5% extract, however on 10% extract they were significantly decreased. In the germination phase, much less changes of these parameters were revealed (Fig. 2; Tab. 1).

In many cases *H. annuus* extracts inhibit or suppress germination and growth of other plants (Leather 1983; Khalid *et al.* 2002; Macias *et al.* 2002; Azania *et al.* 2003; Bogatek *et al.* 2006; Ashrafi *et al.* 2008; Możdżeń *et al.* 2016). But numerous studies also showed that extracts of leaves, stems, roots, flowers and fruits performing toxic effects on many other plant species, although in low concentrations can stimulate selected physiological processes in such plants (Batish *et al.* 2002; Gniazdowska *et al.* 2007; Siegień *et al.* 2008).

There is a positive correlation between the effects of allelopathic substances and plants morphology (Skrzypek *et al.* 2016). The allelopathic substances modify the permeability of cell membranes and disturb the transportation of the mineral substances. They have negative effect on the protein synthesis, inhibit the efficiency of oxidative phosphorylation and photosynthesis, and reduce chlorophyll content (Skrzypek *et al.* 2015, 2016). Many allelopathic substances inhibit the cell division and elongation, and stimulate the oxidation of endogenous and biologically active compounds (Burgos *et al.* 2004).

Conclusions

1. The inhibition of germination of *S. alba* seeds on the 10% alcoholic extracts from the leaves of *H. annuus* was observed;
2. Extracts applied during the germination phase significantly influenced on the plants growth. The growth and the mass of mustard plants were stimulated by 5% concentration of alcoholic extracts of common sunflower;

3. The growth of *S. alba* plants during the growth phase was significantly inhibited by 10% concentration of alcoholic extracts of common sunflower.

References

Afridi R.A., Khan M.A. 2014. Reduced herbicide doses in combination with allelopathic plant extracts suppress weeds in wheat. *Pak. J. Bot.* **46**: 2077–2082.

Afridi R.A., Khan M.A. 2015. Comparative effect of water extract of *Parthenium hysterophorus, Datura alba, Phragmites australis* and *Oryza sativa* on weeds and wheat. *Sains Malays.* **44**: 693–699. https://dx.doi.org/10.17576/jsm-2015-4405-08

Amini S., Azizi M., Joharchi M.R., Shafei M.N., Moradinezhad F., Fujii Y. 2014. Determination of allelopathic potential in some medicinal and wild plant species of Iran by dish pack method. *Theor. Exp. Plant Physiol.* **26**: 3–4. https://dx.doi.org/10.1007/s40626-014-0017-z

Ashrafi Y.Z., Sadeghi S., Mashhadi R.H., Hassan A.M. 2008. Allelopathic effects of sunflower (*Helianthus annuus*) on germination and growth of wild barley (*Hordeum spontaneum*). *J. Agricult. Technol.* **4 (1)**: 219–229.

Azania A.A.P.M., Azania C.A.M., Alves P.L.C.A. Palaniraj R. Kadian H.S., Sati S.C., Rawat L.S., Dahiya D.S., Narwal S.S. 2003. Allelopathic plants. 7. Sunflower (*Helianthus annuus* L.). *Allelopathy J.* **11 (1)**: 1–20.

Barabasz-Krasny B., Możdżeń K., Sołtys-Lelek A., Stachurska-Swakoń A. 2017. The allelopathic potential of *Cirsium oleraceum* (L.) Scop. into the fodder meadow plants. *Not. Bot. Horti Agrobot. Cluj-Napoca* **45 (1)**: 255–261. https://dx.doi.org/10.15835/nbha45110727

Batish D.R., Tung P., Singh H.P., Kohli R.K. 2002. Phytotoxicity of sunflower residues against some summer season crops. *J. Agron. Crop Sci.* **188**: 19–24. https://dx.doi.org/10.1046/j.1439-037x.2002.00526.x

Bogatek R., Gniazdowska A., Zakrzewska W., Oracz K., Gawroński S.W. 2006. Allelopathic effects of sunflower extracts on mustard seed germination and seedling growth. *Biol. Plant.* **50 (1)**: 156–158. https://dx.doi.org/10.1007/s10535-005-0094-6

Burgos N.R., Talbert R.E., Kim K.S., Kuk Y.I. 2004. Growth inhibition and root ultrastructure of cucumber seedlings exposed to allelochemicals from rye (*Secale cereale*). *J. Chem. Ecol.* **30**: 671–689. https://dx.doi.org/10.1023/B:JOEC.0000018637.94002.ba

Gniazdowska A., Oracz K., Bogatek R. 2004. Allelopatia – nowe interpretacje oddziaływań pomiędzy roślinami. *Kosmos – Problemy Nauk Biologicznych* **53 (2/263)**: 207–217. (In Polish)

Gniazdowska A., Oracz K., Bogatek R. 2007. Phytotoxic effects of sunflower (*Helianthus annuus* L.) leaf extracts on germinating mustard (*Sinapis alba* L.) seeds. *Allelopathy J.* **19 (1)**: 215–226.

Inderjit, Duke S.O. 2003. Ecophysiological aspects of allelopathy. *Planta* **217 (4)**: 529–539. https://dx.doi.org/10.10077/s00425-003-1054-z

Kasperczyk M., Szewczyk W. 2007. Dynamika plonowania łąk w zależności od sumy opadów atmosferycznych i rodzaju nawożenia. *Acta Agrophysica* **9 (2)**: 371–378. (In Polish)

Kato-Noguchi H., Ino T., Sata N., Yamamura S. 2002. Isolation and identification of a potent allelopathic substance in rice root exudates. *Physiol. Plant.* **115**: 401–405. https://dx.doi.org/10.1034/j.1399-3054.2002.1150310.x

Khalid S., Ahmad T., Shad R.A. 2002. Use of allelopathy in agriculture. *Asian J. Plant Sci.* **1 (3)**: 292–297.

Leather G.R. 1983. Sunflower (*Helianthus annus*) is allelopathic to weeds. *Weed Sci.* **31 (11)**: 37–41.

Lipińska H. 2006. Allelopatyczne działanie wybranych gatunków traw na kiełkowanie nasion *Phleum pratense* w zależności od ich zagęszczenia. *Acta Agrobot.* **59 (2)**: 17–28. (In Polish)

Macias F.A., Lopez A., Verela R.M., Torres A., Molinillo J.M.G. 2002. Bioactive terpenoids from sunflower leaves cv. *Peredovic*. *Phytochemistry* **61**: 687–692. https://dx.doi.org/10.1016/S0031-9422(02)00370-9

Możdżeń K., Oliwa J. 2015. The morphological changes of *Phaseolus vulgaris* L. exposed to the aqueous extracts of the leaves of *Juglans regia* L. *Mod. Phytomorphol.* **7**: 81–86.

Możdżeń K., Oliwa J., Migdałek G., Radlińska J., Rut G., Rzepka A. 2016. Morphological and physiological changes in *Sinapis alba* cv. Barka treated with aqueous extracts from the roots of *Helianthus annuus* L. In: Grzesiak M., Rzepka A., Hura T., Grzesiak S. (eds), Plant functioning under environmental stress: 47–56. The F. Górski Institute of Plant Physiology PAS, Cracow.

Możdżeń K., Repka P. 2014. Allelopathic influence of aqueous extracts from the leaves *Morus alba* L. on seed germination and seedling growth of *Cucumis sativus* L. and *Sinapis alba* L. *Mod. Phytomorphol.* **5**: 93–99. https://dx.doi.org/10.5281/zenodo.161010

Ohno T., Doolan K.L. 2001. Effects of red clover decomposition on phytotoxicity to wild mustard seedling growth. *Appl. Soil Ecol.* **16**: 187–191. https://dx.doi.org/10.1016/S0929-1393(00)00113-X

Oliwa J., Możdżeń K., Skoczowski A. 2016. Allelopatyczne oddziaływanie wodnych ekstraktów z kłosówki wełnistej (*Holcus lanatus* L.) na kiełkowanie i początkowy wzrost życicy wielokwiatowej (*Lolium multiflorum* Lam. cv Mitos). In: Synowiec A., Parkitna K. (red.), Wiedza i technologia motorem gospodarki: nauki przyrodnicze, ścisłe i weterynaryjne: 123–135. Omnibuss, Częstochowa. (In Polish)

Puła J., Barabasz-Krasny B., Możdżeń K., Sołtys-Lelek A., Lepiarczyk A. 2016. Effect of aqueous extracts of sticky willy (*Galium aparine* L.) on the growth of seedlings of selected maize varieties (*Zea mays* L.). *Not. Bot. Horti Agrobot. Cluj-Napoca* **44 (2)**: 518–524. https://dx.doi.org/10.15835/nbha44210475

Siegień I., Trocka A., Bosa K., Bogatek R., Gniazdowska A. 2008. Potencjał allelopatyczny słonecznika. *Postępy Nauk Rolniczych* **336**: 55–71 (In Polish)

Skrzypek E., Repka P., Stachurska-Swakoń A., Barabasz-Krasny B., Możdżeń K. 2015. Allelopathic effect of aqueous extracts from the leaves of peppermint (*Mentha × piperita* L.) on selected physiological processes of common sunflower (*Helianthus annuus* L.). *Not. Bot. Horti Agrobot. Cluj-Napoca* **43 (2)**: 335–342. https://dx.doi.org/10.15835/nbha43210034

Skrzypek E., Repka P., Stachurska-Swakoń A., Barabasz-Krasny B., Możdżeń K. 2016. Seedlings growth of common sunflower under influence of peppermint extract. *Mod. Phytomorphol.* **9**: 69–73. https://dx.doi.org/10.5281/zenodo.159695

Spring O., Ulrich R., Macias F.A. 1992. Sesquiterpens from noncapitate glandular trichomes of *Helianthus annus. Phytochem.* **31**: 1541–1544. https://dx.doi.org/10.1016/0031-9422(92)83102-5

Steiner A.A. 1961. A universal method for preparing nutrient solutions of a certain desired composition. *Plant Soil* **15 (2)**: 134–154. https://dx.doi.org/10.1007/BF01347224

Wilson R.E., Rice E.L. 1968. Allelopathy as expressed by *Helianthus annuus* and its role in old field succession. *Bull. Torrey Bot. Club* **95 (5)**: 432–448. https://dx.doi.org/10.2307/2483475