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PHYTOTOXICITY OF DIMETHYL SULFOXIDE IN THE GROWTH TEST

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ФІТОТОКСИЧНІСТЬ ДИМЕТИЛСУЛЬФОКСИДУ У РОСТОВОМУ ТЕСТІ

ABSTRACT

Dimethyl sulfoxide (DMSO) is widely used as an industrial solvent and for a variety of medical purposes. Reports of the toxicity of DMSO to living organisms vary. The plants *Lepidium sativum* L. and *Raphanus raphanistrum* subsp. *sativus* (L.) Domin. are widely used in the biotesting of toxicants. Currently, there is no information on the phytotoxicity of aqueous solutions of DMSO for these test plants, which determined the aim of this study.

Methodology. Phytotoxicity of aqueous DMSO solutions was assessed using a growth test. The following plants were used as test plants: 1) seed radish (*R. raphanistrum* subsp. *sativus*) of the French breakfast variety, 10 seeds of which were placed in a Petri dish on filter paper moistened with distilled water (control) or 0.5 %, 1.0 %, 5.0 %, 10.0 %, 25.0 % or 45.0 % aqueous solution of DMSO (experiment); 2) watercress (*L. sativum*) of the Aphrodite variety, 10 seeds of which were placed in a Petri dish on filter paper moistened with distilled water (control) or a 0.025 %, 0.05 % or 0.1 % aqueous solution DMSO (experiment). The research was carried out in triplicate for 6 days (radish) and 5 days (watercress), during which the energy of seed germination of test plants (%) was determined on the 3rd day, on the 6th day (radish) and on the 5th day (watercress) – seed germination (%) and biometric and morphometric indicators of test plant seedlings (root and aerial part length, mm).

Scientific novelty. In a growth test with *R. raphanistrum* subsp. *sativus* showed extreme phytotoxicity for 5.0-45.0 % aqueous DMSO solutions and no phytotoxicity for 0.025-1.0 % aqueous DMSO solutions in a growth test with *L. sativum* and *R. raphanistrum* subsp. *sativus*.

Conclusions. Aqueous solutions of DMSO in concentrations of 0.025-1.0 % did not show phytotoxic properties according to the growth test with *L. sativum* and *R. raphanistrum* subsp. *sativus*, in contrast to 5.0-45.0 % solutions, which showed extreme phytotoxicity according to the growth test with *R. raphanistrum* subsp. *sativus.* The obtained results indicate that this substance will not inhibit plant growth at concentrations found in wastewater (0.05-0.08 %).

Key words: biotesting, dimethyl sulfoxide, growth test, toxicity, *Lepidium sativum*, *Raphanus raphanistrum* subsp. *sativus*

АНОТАЦІЯ

Диметилсульфоксид (ДМСО) широко використовується як промисловий розчинник і для різноманітних медичних цілей. Повідомлення щодо токсичності ДМСО для живих організмів різняться. У біотестуванні токсикантів широко використовуються рослини *Lepidium sativum* L. та *Raphanus raphanistrum* subsp. *sativus* (L.) Domin. Наразі відомості щодо фітотоксичності водних розчинів ДМСО за цими тест-рослинами відсутні, що й зумовило мету даної роботи.

Методологія. Оцінку фітотоксичності водних розчинів ДМСО здійснювали за ростовим тестом. Як тест-рослини використано: 1) редьку посівну (*R. raphanistrum* subsp. *sativus*) сорту Французький сніданок, насіння якої у кількості 10 штук розміщували у чашці Петрі на фільтрувальному папері, змоченому дистильованою водою (контроль) або 0,5 %, 1,0 %, 5,0 %, 10,0 %, 25,0 % або 45,0 %-ним водним розчином ДМСО (дослід); 2) крес-салат (*L. sativum*) сорту Афродита, насіння якого у кількості 10 штук розміщували у чашці Петрі на фільтрувальному папері, змоченому дистильованою водою (контроль) або 0,025 %, 0,05 % або 0,1 %-ним водним розчином ДМСО (дослід). Дослідження здійснювали у потрійній повторності 6 діб (редька) та 5 діб (крес-салат), протягом яких на 3-ю добу визначали енергію проростання насіння тест-рослин (%), на 6-у добу (редька) та 5-у добу (крес-

салат) – схожість насіння (%) та біометрико-морфометричні показники проростків тест-рослин (довжину коріння та надземної частини, мм).

Наукова новизна. У ростовому тесті з *R. raphanistrum* subsp. *sativus* показано екстремальну фітотоксичність для 5,0-45,0 %-них водних розчинів ДМСО та відсутність фітотоксичності для 0,025-1,0 %-них водних розчинів ДМСО у ростовому тесті з *L. sativum* та *R. raphanistrum* subsp. *sativus*.

Висновки. Водні розчини ДМСО у концентраціях 0,025-1,0 % не проявили фітотоксичних властивостей за ростовим тестом з *L. sativum* та *R. raphanistrum* subsp. *sativus* на відміну від 5,0-45,0 %-них розчинів, які проявили екстремальну фітотоксичність за ростовим тестом з *R. raphanistrum* subsp. *sativus*. Отримані результати вказують, що дана речовина не гальмуватиме ріст рослин за концентрацій, виявлених у стічних водах (0,05-0,08 %).

Ключові слова: біотестування, диметилсульфоксид, ростовий тест, токсичність, *Lepidium sativum, Raphanus raphanistrum* subsp. *sativus*

Introduction

Dimethyl sulfoxide (DMSO) is widely used as an industrial solvent and for a variety of medical purposes (Hatton et al., 1994; Makashova et al., 2017; Volkova et al., 2019). DMSO is a commercially produced dipolar aprotic solvent. It is also a naturally occurring substance and is apparently part of the complex sulfur cycle on Earth (Gaylord Chemical Company, L.L.C., n.d.).

DMSO occurs naturally in marine and freshwater environments, soil, rainwater, and the atmosphere, as well as in a variety of beverages and foods (Gaylord Chemical Company, L.L.C., n.d.; Hatton et al., 1994). DMSO has often been detected in industrial wastewater from processes in the manufacture of semiconductors or liquid crystal displays in concentrations 500–800 mg/L (Zhang et al., 2016).

Reports of the toxicity of DMSO to living organisms vary. There are publications that note the low acute and chronic toxicity of DMSO for animals, plants and aquatic organisms, as well as the absence of carcinogenic properties, which led to its use as a neutral solvent in Ames mutagenicity tests (Gaylord Chemical Company, L.L.C., n.d.). However, there are publications that note the toxic properties of DMSO (Gallardo-Villagrán et al., 2022), in particular, phytotoxic ones (Erdman and Hsieh, 1969; Zhang et al., 2016). Thus, it was noted that a DMSO concentration of more than 0.1 % is toxic to plant (Erdman and Hsieh, 1969). *Lepidium sativum* L. (Liwarska-Bizukojc and Urbaniak, 2007; Pavel et al., 2013; Galli et al., 2019; Bożym, 2020; Martínez Barroso and Vaverková, 2020; Radlińska et al., 2020; Tkachuk and Okulovych, 2021; Tkachuk et al., 2022) and *Raphanus raphanistrum* subsp. *sativus* (L.) Domin. plants (Pidkopaylo and Korzh, 2009; Atamaleki et al., 2021; Araniti et al., 2022; Daniel et al., 2022) are widely used in the biotesting of toxicants. Currently, there is no information on the phytotoxicity of aqueous solutions of DMSO for these test plants, which determined the aim of this work.

Materials and methods

Growth test

Phytotoxicity of aqueous DMSO solutions was assessed using a growth test (Tkachuk et al., 2022). The following plants were used as test plants: 1) seed radish (*R. raphanistrum* subsp. *sativus*) of the French breakfast variety, 10 seeds of which were placed in a Petri dish on filter paper moistened with distilled water (control) or 0.5 %, 1 %, 5 %, 10 %, 25 % or 45 % aqueous solution of DMSO (experiment); 2) watercress (*L. sativum*) of the Aphrodite variety, 10 seeds of which were placed in a Petri dish on filter paper moistened with distilled water (control) or 0.025 %, 0.05 % or 0.1 % aqueous solution DMSO (experiment). The research was carried out in triplicate for 6 days (radish) and 5 days (watercress), during which the energy of seed germination of test plants (%) was determined on the 3rd day, on the 6th day (radish) and on the 5th day (watercress) – seed germination (%) and biometric and morphometric indicators of test plant seedlings (root and aerial part length, mm).

Phytotoxic indices

Phytotoxic indexes were calculated – seed germination index (SGI) and root length index (RLI) according to previously given formulas (Bagur-González et al., 2011; Tkachuk and Zelena, 2022).

A toxicity scale was used (Bagur-González et al., 2011):

–0.25 ≤ SGI or RLI < 0 – slight toxicity;
–0.5 ≤ SGI or RLI < –0.25 – moderate toxicity;
–0.75 ≤ SGI or RLI < –0.5 – high toxicity;
–1 ≤ SGI or RLI < –0.75 – extreme toxicity.

Statistical processing of results

When processing the research results, statistical data processing methods were used. The arithmetic mean, the error of the arithmetic mean, the reliability of the differences of the arithmetic means were determined as described (Tkachuk and Zelena, 2023).

Results and Discussion

Phytotest with *R. raphanistrum subsp. sativus*

The results of the study of phytotoxicity of 0.5-45.0 % aqueous solutions of DMSO according to the growth test with *R. raphanistrum* subsp. *sativus* is presented in Fig. 1-5.

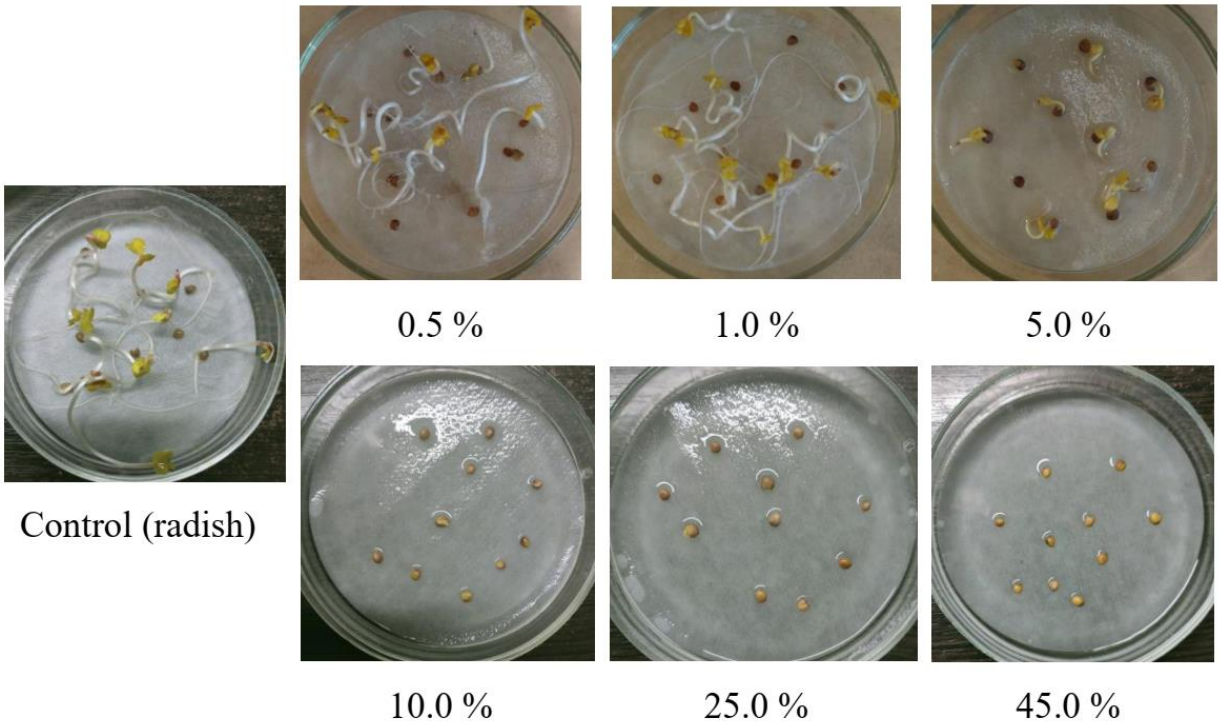

Fig. 1. Radish seedlings (6th day) growing on the tested DMSO solutions

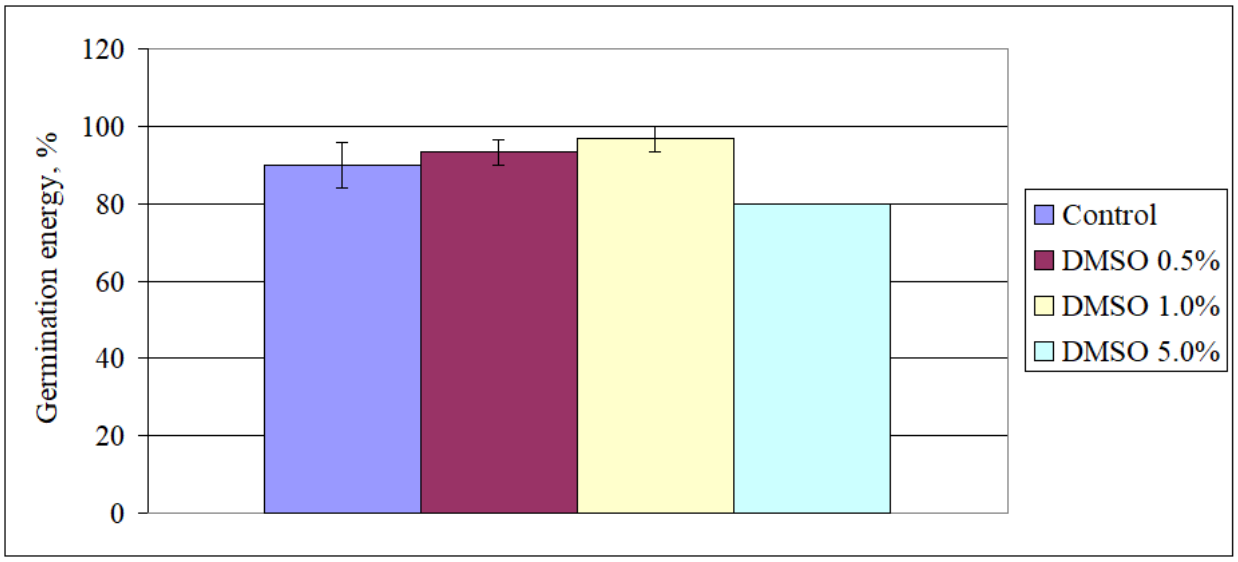

Fig. 2. Germination energy of radish seeds under the influence of different concentrations of DMSO

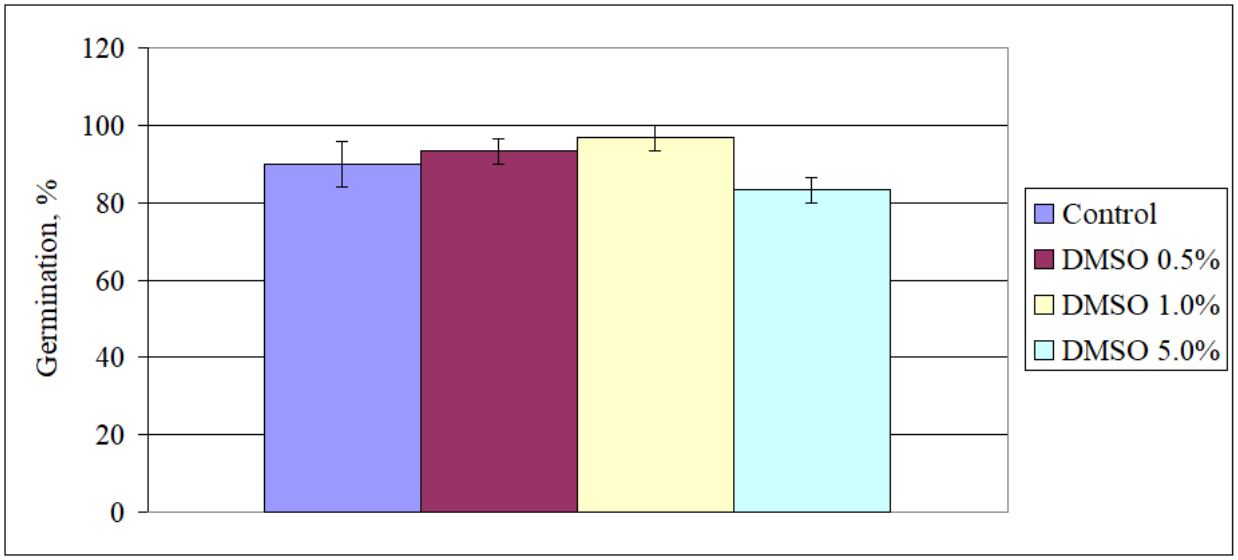

Fig. 3. Germination of radish seeds under the influence of different concentrations of DMSO

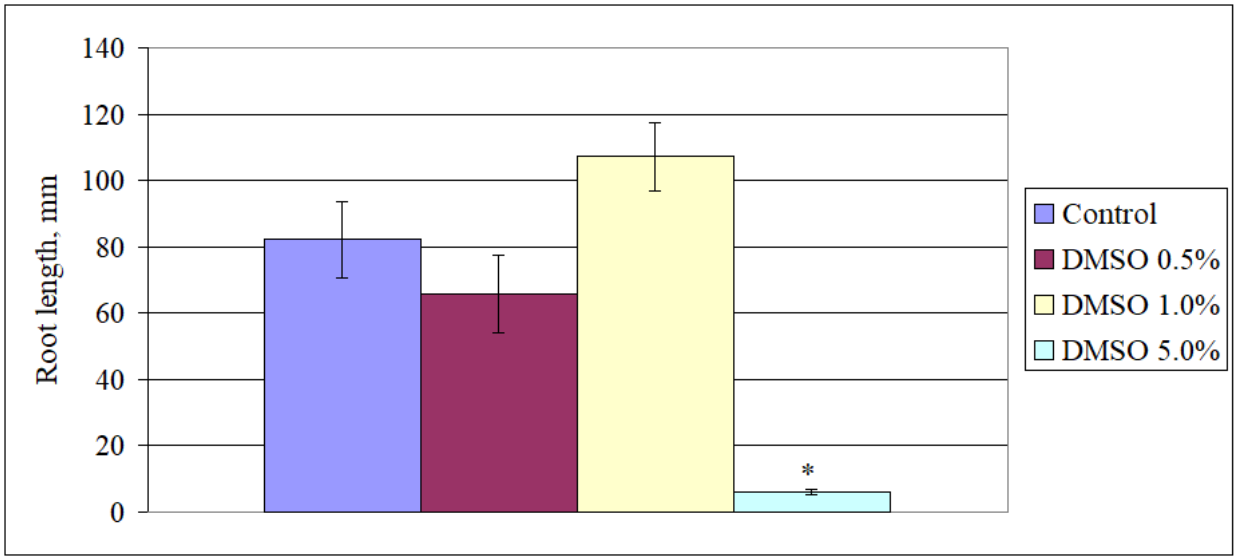

Fig. 4. Root length of radish seedlings under the influence of different concentrations of DMSO
Note: * – the difference is statistically significant compared to the control ($p \leq 0.05$)

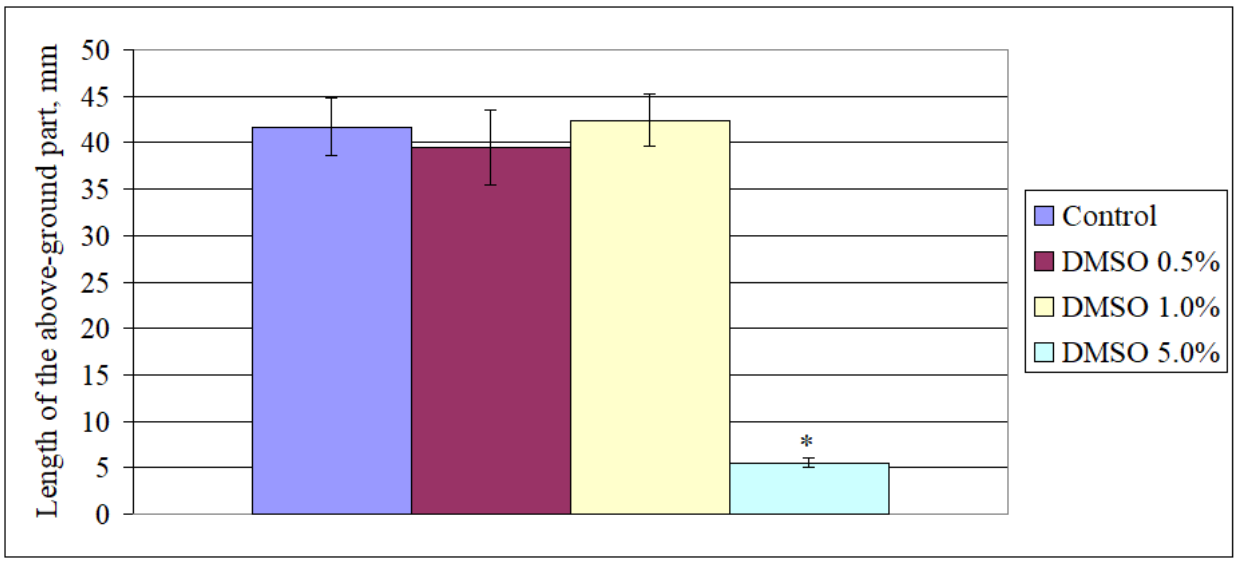

Fig. 5. The length of the above-ground part of radish seedlings under the influence of different concentrations of DMSO
Note: * – the difference is statistically significant compared to the control ($p \leq 0.05$)

It was established that under the influence of DMSO at 0.5 %, 1.0 %, and 5.0 % concentration, germination energy and seed germination were at the same level as the control, no statistically significant difference in these indicators was noted (Fig. 2-3). The length of the roots and the aerial part of the radish seedlings under the influence of 0.5 % and 1.0 % DMSO solutions were at the same level as the control, no statistically significant difference was noted (Fig. 4-5). However, the 5.0 % DMSO solution significantly reduced the length of the roots and the above-ground part of radish seedlings compared to the control – by 14 times and 8 times, respectively (Fig. 4-5). The calculated phytotoxic indices were: SGI -0.1; RLI -0.9. According to the phytotoxic root length index (RLI), a 5.0 % solution of DMSO is extremely toxic.

Also, extremely toxic according to the growth test with *R. raphanistrum* subsp. *sativus* were DMSO solutions with concentrations of 10.0 %, 25.0 % and 45.0 % (Fig. 1). Thus, under the influence of a 45.0 % aqueous solution of DMSO, the seeds of the test plant did not germinate. At a 25.0 % concentration of DMSO, the energy of seed germination was 3.3 %, seed germination was 10.0 %, and the root length of seedlings was 1 mm (110 times less than in the control). At a concentration of 10.0 %, DMSO also affected all tested radish functions: germination energy and seed germination were 10.0 %, root length was 1.3 mm (85 times less than in the control). It was noted that under the action of 10.0-45.0 % concentrations of DMSO solutions, the aerial part of radish seedlings did not develop. Our results confirm the previously obtained results of complete inhibition of seed germination of kidney beans, peas, barley and rye under the influence of DMSO concentrations 10.0 % or more, and an insignificant effect of 5 % concentrations or less (Erdman and Hsieh, 1969).

Phytotest with L. sativum

Since high concentrations of DMSO were found to be extremely toxic and considering that the concentration of DMSO in wastewater is from 0.05 % to 0.08 % (Zhang et al., 2016), the phytotoxicity of 0.025-0.1 % aqueous solutions of DMSO was investigated by growth test with *L. sativum. L. sativum* was chosen as the test plant because it is used for the bioassay of a large number of toxicants. The results of the study of phytotoxicity of 0.025-0.1 % aqueous solutions of DMSO according to the growth test with *L. sativum* are presented in Fig. 6-10.

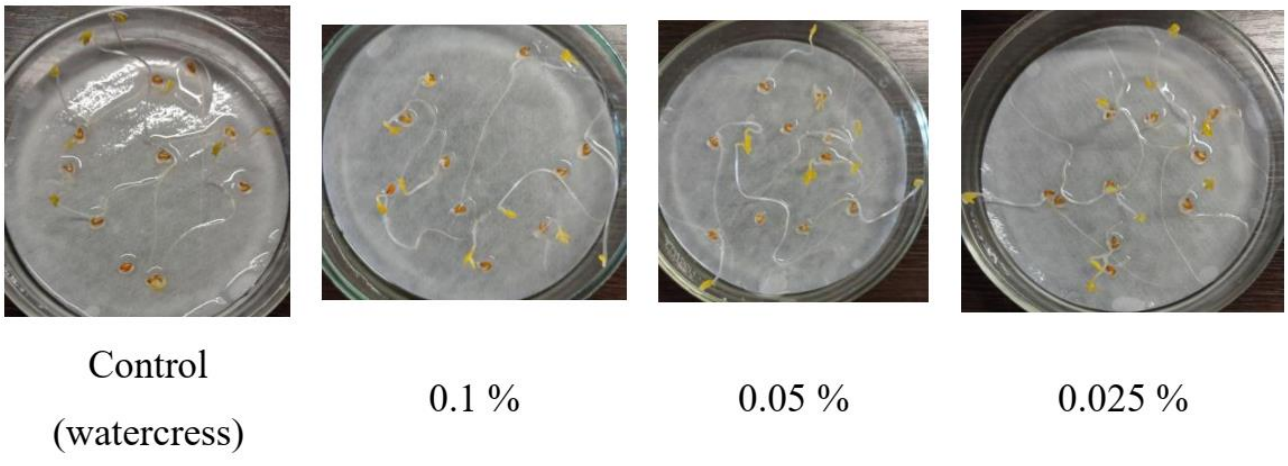

Fig. 6. Watercress seedlings (5th day) growing on the tested DMSO solutions

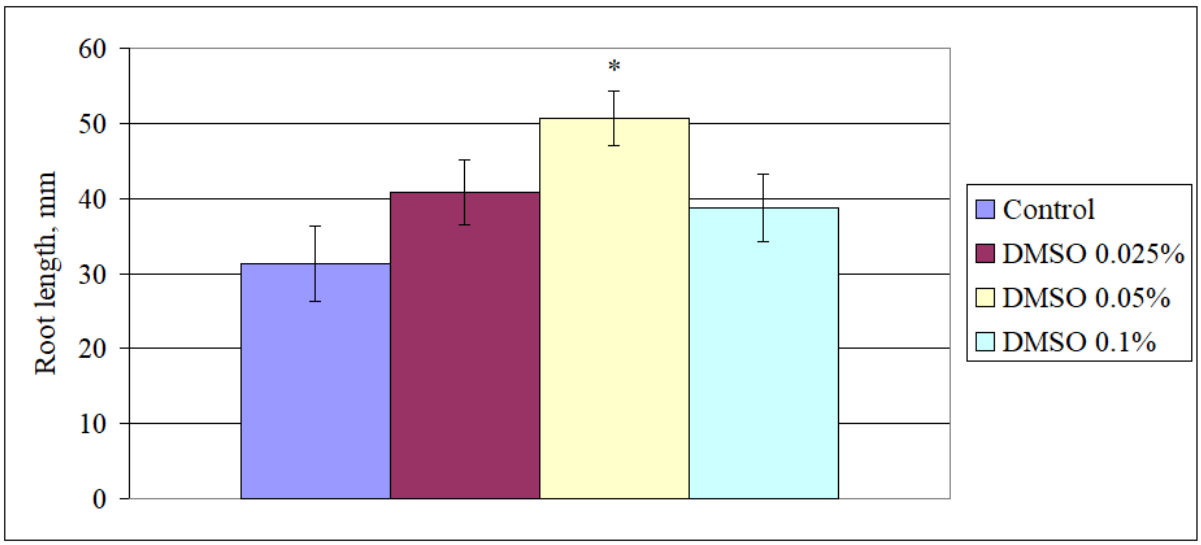

Fig. 7. Root length of watercress seedlings under the influence of different concentrations of DMSO

Note: * – the difference is statistically significant compared to the control ($p \leq 0.05$)

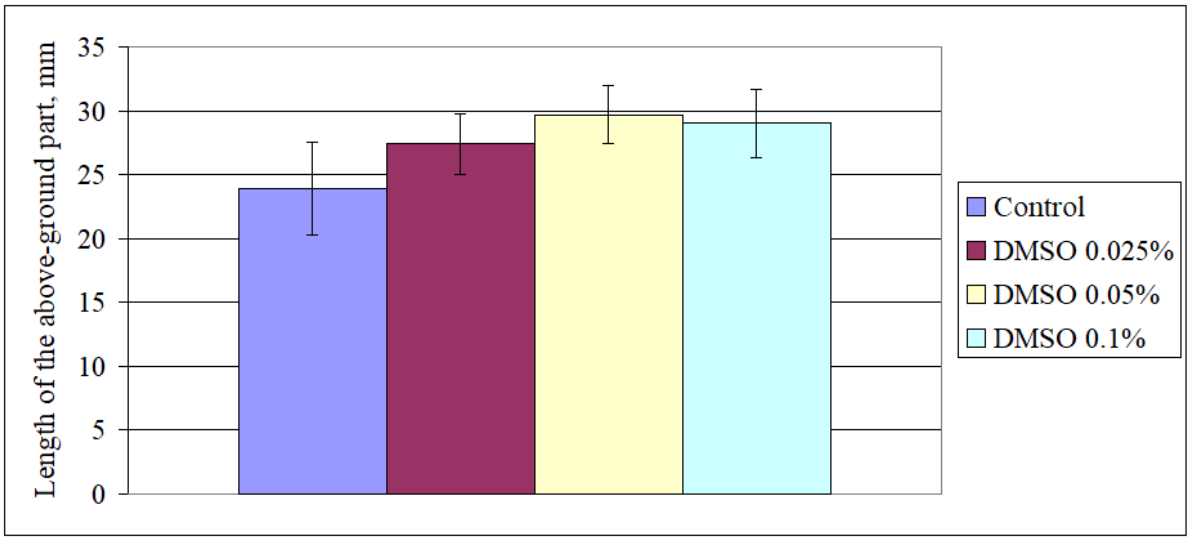

Fig. 8. The length of the above-ground part of watercress seedlings under the influence of different concentrations of DMSO

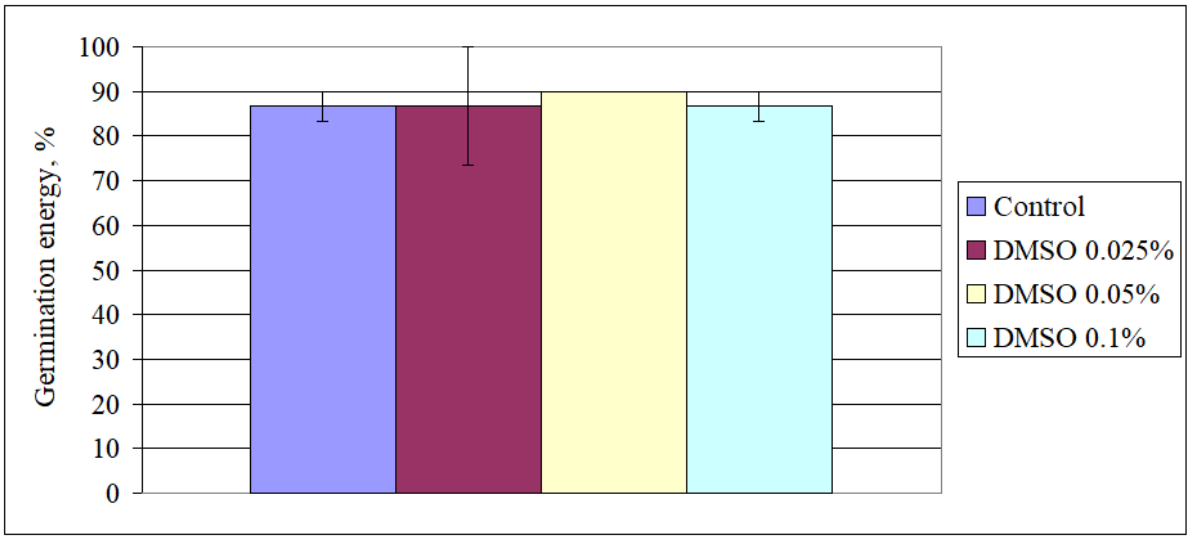

Fig. 9. Germination energy of watercress seeds under the influence of different concentrations of DMSO

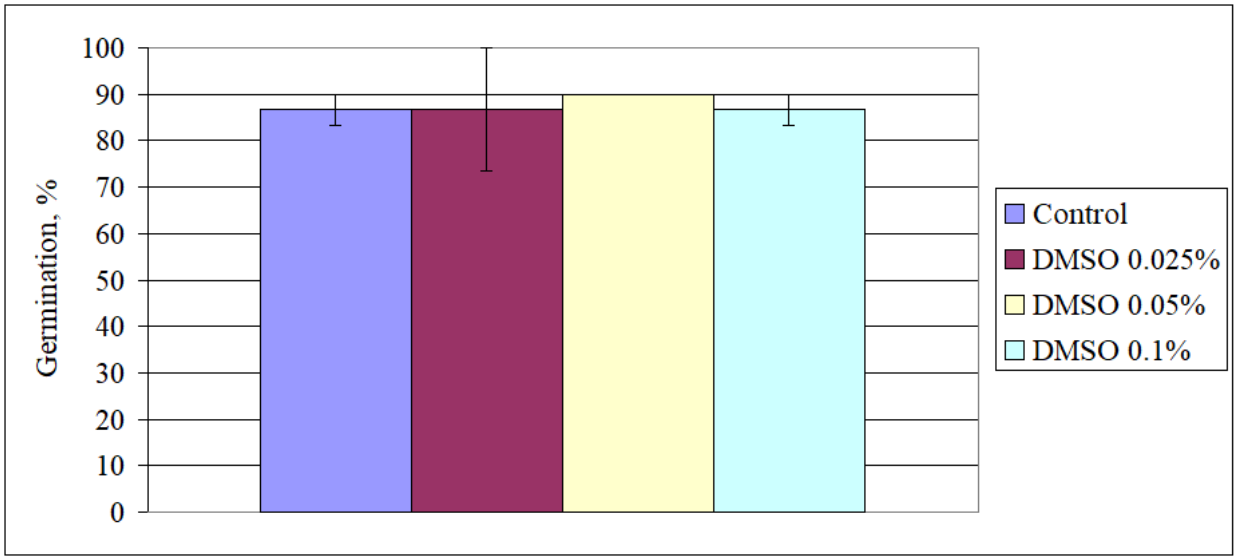

Fig. 10. Germination of watercress seeds under the influence of different concentrations of DMSO

It was established that the changes in indicators of the studied test functions of watercress under the influence of 0.025-0.1 % aqueous solutions of DMSO are statistically insignificant and are within the control limits (Fig. 6-10), except for the root length under the influence of a 0.05 % DMSO solution (Fig. 7). In the latter case, a significant increase in the indicator (by 1.6 times) was recorded compared to the control (Fig. 7).

The calculated phytotoxic indices are shown in Table 1.

Table 1

Phytotoxic indices of the effect of aqueous solutions of DMSO on watercress

Research option	SGI	RLI	Interpretation of the results of phytotest	Comments
Control (distilled water)	0.00	0.00	No toxicity	No inhibition of growth
DMSO 0.025 %	0.00	0.30	No toxicity	No inhibition of growth
DMSO 0.05 %	0.04	0.62	No toxicity	No inhibition of growth
DMSO 0.1 %	0.00	0.24	No toxicity	No inhibition of growth

Note: SGI – seed germination index; RLI – root length index

The obtained results indicate the absence of phytotoxic properties of DMSO aqueous solutions at concentrations of 0.025-0.1 % for watercress.

In general, reports on the toxicity of DMSO to living organisms vary. Thus, it is noted that DMSO has low acute and chronic toxicity for animals, plants and aquatic organisms, without carcinogenic properties (it is used as a neutral solvent in the Ames tests for mutagenicity) (Gaylord Chemical Company, L.L.C., n.d.). It does not show teratogenic properties in mice, rats or rabbits (Gaylord Chemical Company, L.L.C., n.d.). Because of this, DMSO is used as a solvent or co-solvent in pesticides that are applied to the emergence of crops or to the formation of edible parts of food plants (Gaylord Chemical Company, L.L.C., n.d.). There are also reports that the use of clay minerals and silica with additives of DMSO and plant extracts in the complex treatment of patients with hemophilia reduces the level of endointoxication and increases the procoagulant activity of blood, and in general – accelerates the recovery of the patient's body after a hemorrhagic complication (Kovzun et al., 2008).

However, there are bioassay results with various test organisms that indicate the toxicity of DMSO. Thus, in a study of the effect of DMSO on human fibroblast-like synoviocytes in

rheumatoid arthritis, which included five different samples collected from the joints (fingers, hands and pelvis) of five women with rheumatoid arthritis, induction of caspase-3 and PARP-1 cleavage was shown (two phenomena related to the mechanism of cell death) at high concentrations (>5 %) of DMSO (Gallardo-Villagrán et al., 2022). Even at a 0.5 % concentration of DMSO, analyzes of the antiproliferative test demonstrated strong toxicity after 24 hours of exposure (≈25 % cell death). Therefore, to ensure a minimal effect of DMSO on human fibroblast-like synoviocytes in rheumatoid arthritis, the concentration of DMSO to be considered safe must be less than 0.05 % (Gallardo-Villagrán et al., 2022).

Exposure to DMSO (0.56-13.54 mM) caused phytotoxicity for biomass growth and root viability of rice seedlings (Zhang et al., 2016). DMSO induced oxidative stress in rice seedlings, leading to H_2O_2 accumulation in roots.

It is obvious that the reaction of living organisms to the influence of DMSO solutions depends on the studied species and the chosen concentration. Therefore, biotesting research should be comprehensive and include particular model organisms as well as organisms at different levels of organization.

Conclusions

Aqueous solutions of DMSO in concentrations of 0.025-1.0 % did not show phytotoxic properties according to the growth test with *L. sativum* and *R. raphanistrum* subsp. *sativus*, in contrast to 5.0-45.0 % solutions, which showed extreme phytotoxicity according to the growth test with *R. raphanistrum* subsp. *sativus.* The obtained results indicate that this substance will not inhibit plant growth at concentrations found in wastewater (0.05-0.08 %).

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Набір даних доступний за запитом до авторів / Dataset available on request from the authors.

Заява інституційної ревізійної ради / Institutional Review Board Statement

Не застосовується / Not applicable.

Заява про інформовану згоду / Informed Consent Statement

Не застосовується / Not applicable.

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