engineering proceedings

Proceeding Paper

The growth test with Lepidium sativum L. for toxicity evaluation of wet wipes †

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† Presented at the 4th International Electronic Conference on Applied Sciences, 27 October–10 November 2023; Available online: https://asec2023.sciforum.net/.

Abstract: The aim of this study was to investigate the toxicity of wet wipes from manufacturers of different countries using the growth test with garden cress (*Lepidium sativum* L.). The study used 9 variants of wet wipes produced in Ukraine, Turkey and the United Kingdom. Germination energy (the 3rd day), germination and biometric and morphometric characteristics (the 5th day) were determined. The phytotoxic indexes were calculated. It was established that 78% of the tested wet wipes (60% Ukrainian production and 100% foreign) were extreme toxicity. Therefore, the tested wet wipes contain toxic substances (in particular, surfactants), show phytotoxicity and can be a source of environmental pollution.

Keywords: *Lepidium sativum*; surfactants; the growth test; toxicity; wet wipes

Citation: Tkachuk, N.; Zelena, L. Evaluation of the Toxicity of Wet Wipes Based on the Growth Test with *Lepidium sativum* L. *Eng. Proc.* **2023**, *52*, x. https://doi.org/10.3390/xxxxx

Academic Editor(s): Name

Published: date

1. Introduction

Wet wipes (**WW**) are widely used in everyday life and the volume of their market is expected to increase in the future [1–3]. The wet wipes made from non-woven fabric that composed of polyester or viscose fibers. However, the composition of wet wipes also contains various chemical compounds, primarily surfactants, which can negatively affect the environment and human health [4]. It was reported that although the bio-based wet wipes caused the lower toxicity risk than the petroleum-based ones they could have a great impact on the water resources inducing the contamination [5]. That is why it is essential to monitor and control the environmental effects of using wet wipes.

For the practical purpose of determining the toxicity of substances and substrates, biotesting methods are used, in particular with garden cress (*Lepidium sativum* L.) [6,7]. The aim of this study was to investigate the toxicity of wet wipes from manufacturers of different countries using the growth test with garden cress.

2. Materials and Methods

2.1. Materials

The study used 9 variants of wet wipes produced in Ukraine (two manufacturers—**WW1** and **WW2**; **WW3**, **WW4** and **WW5**), Turkey (three manufacturers—**WW6**, **WW7** and **WW8**, respectively) and the United Kingdom of Great Britain and Northern Ireland (one manufacturer—**WW9**) and available in the retail network of Ukraine. We do not mention the names of wet wipes and their manufacturers to prevent accusations of advertising or anti-advertising. Chemical compounds in the composition of wet wipes (according to manufacturer) are:

WW1—demineralized water, glycerin, propylene glycol, benzalkonium chloride, cocamidopropyl betaine, PEG-40 hydrogenated castor oil, PPG-2 methyl ether, ethylparaben, 2-bromo-2-nitropropane-1,3-diol, cetrimonium bromide, extracts of sedum, chamomile, calendula, perfume composition, citric acid. Material of wipes: non-woven fabric (60% polyester, 40% viscose).

WW2—demineralized water, glycerin, propylene glycol, benzalkonium chloride, cocamidopropyl betaine, PEG-40 hydrogenated castor oil, PPG-2 methyl ether, ethylparaben, 2-bromo-2-nitropropane-1,3-diol, cetrimonium bromide, flavor, citric acid. Material of wipes: non-woven fabric.

WW3—water, flavor, citric acid, tocopheryl acetate (vitamin E), aloe vera extract, glycerin, allantoin, cocamidopropyl betaine, polypropylene glycol, phenoxyethanol, polysorbate-20, dehydroacetic acid, benzoic acid, tetrasodium EDTA, cetearyl isononanoate, cetearet-12, cetearete-20, cetearyl alcohol, glyceryl stearate, cetyl palmitate. Material of wipes: not specified.

WW4—water, flavor, citric acid, tocopheryl acetate (vitamin E), sea buckthorn (*Hippophaë rhamnoides*) extract, cranberry (*Vaccinium macrocarpon*) extract, glycerin, allantoin, cocamidopropyl betaine, polypropylene glycol, phenoxyethanol, polysorbate-20, dehydroacetic acid, benzoic acid, tetrasodium EDTA, cetearyl isononanoate, cetearet-12, cetearet-20, cetearyl alcohol, glyceryl stearate, cetyl palmitate. Material of wipes: not specified.

WW5—water, flavor, citric acid, tocopheryl acetate (vitamin E), aloe vera, sea buckthorn (*Hippophaë rhamnoides*) extract, chamomile extract, glycerin, allantoin, cocamidopropyl betaine, polypropylene glycol, phenoxyethanol, polysorbate-20, dehydroacetic acid, benzoic acid, tetrasodium EDTA, cetearyl isononanoate, cetearet-12, cetearet-20, cetearyl alcohol, glyceryl stearate, cetyl palmitate. Material of wipes: not specified.

WW6—do not contain alcohol and parabens; there are water, phenoxyethanol, perfume, benzoic acid, glycerin, tetrasodium EDTA, cetearyl isononanoate, cocamidopropyl betaine, dehydroacetic acid, cetearet-20, cetearyl alcohol, glyceryl stearate, allantoin, panthenol, cetearet-12, cetyl palmitate, chlorhexidine digluconate, D-limonene. Material of wipes: not specified.

WW7—alcohol-free: deionized water, cetearyl isononanoate, ceteareth-20, cetostearyl, glyceryl stearate, glycerin, ceteareth-12, cetyl palmitate, polysorbate-20, phenoxyethanol, methylparaben, propylparaben, 2-bromo-2-nitropropane-1,3-diol, cocamidopropyl betaine, PEG-7 glyceryl cocoate, EDTA, citric acid, vitamin E, chamomile extract, perfume. Material of wipes: not specified.

WW8—without alcohol and parabens; there are water, C12-15 pareth-12, phenoxyethanol, benzoic acid, dehydroacetic acid, glycerin, perfume, citric acid. Material of wipes: not specified.

WW9—water, polysorbate 20, caprylyl glycol, sodium benzoate, coco-betaine, maleic acid, sodium citrate. Material of wipes: 70% cellulose and 30% plastic (prevents tearing during use).

2.2. The Growth Test with Lepidium sativum

In phytotesting, garden cress seeds were used (producer of Svityaz LLC, Ukraine), which, according to the manufacturer, complies with DSTU 7160-2010. To study the phytotoxicity of wet wipes, a circle with a diameter of 9 cm was cut out of each variant of the wipes, placed in a Petri dish and moistened with distilled water. The filter paper instead of wet wipes was used as a control. 10 seeds of garden cress were planted in each Petri dish for 5 days. The experiment was repeated three times. On the 3rd day, germination energy was determined, on the 5th day—germination and biometric and morphometric characteristics (root and above-ground part length) [6]. The phytotoxic indexes were calculated—the seed germination index (SGI) and the root length index (RLI) [8–10]. The toxicity scale given in the study [9] was used.

2.3. Statistical Analysis

The results were processed statistically using Microsoft Excel 2010: the arithmetic mean, arithmetic mean error and significance of differences (by Student's *t*-test) were calculated.

3. Results and Discussion

Biotesting with garden cress is a sensitive method for the study of toxicants and is widely used in practice [6,7,11–14].

The results of the toxicity study of wet wipes manufactured in Ukraine, Turkey and the United Kingdom are presented in Figures 1–4. The calculated phytotoxicity indices and the interpretation of the bioassay results are given in Table 1.

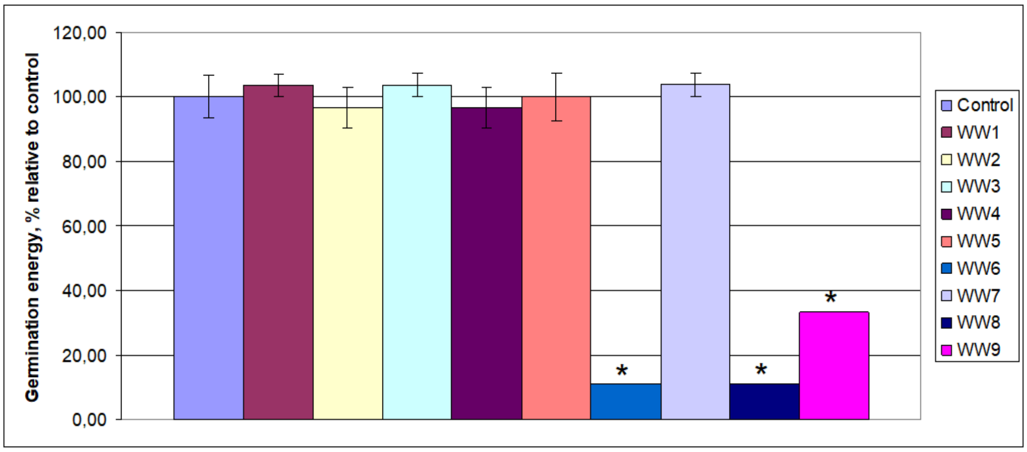

Figure 1. Germination energy of *L. sativum.* * Differences from the control are significant at $p \leq 0.05$.

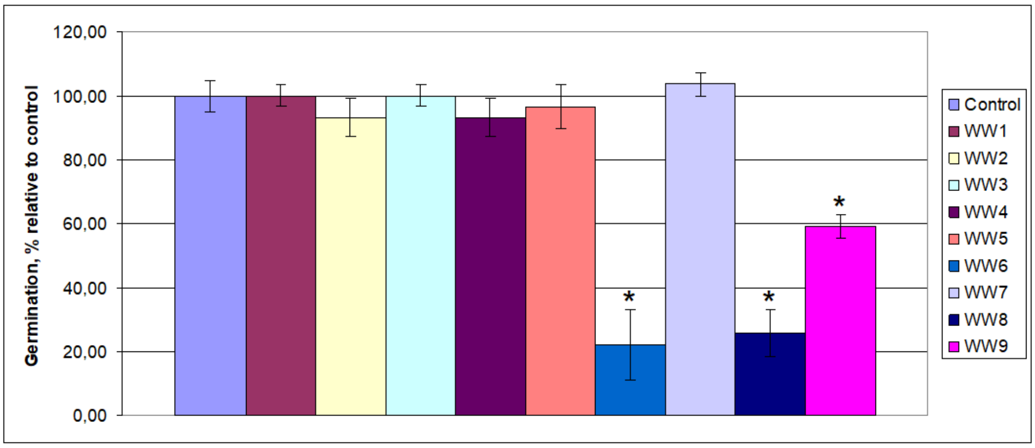

Figure 2. Germination of *L. sativum*. * Differences from the control are significant at $p \leq 0.05$.

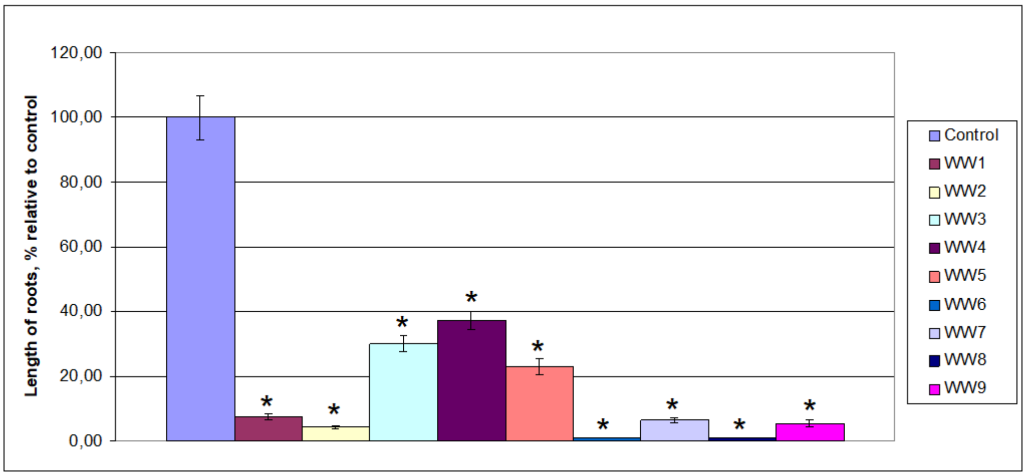

Figure 3. Root length of *L. sativum*. * Differences from the control are significant at $p \leq 0.05$.

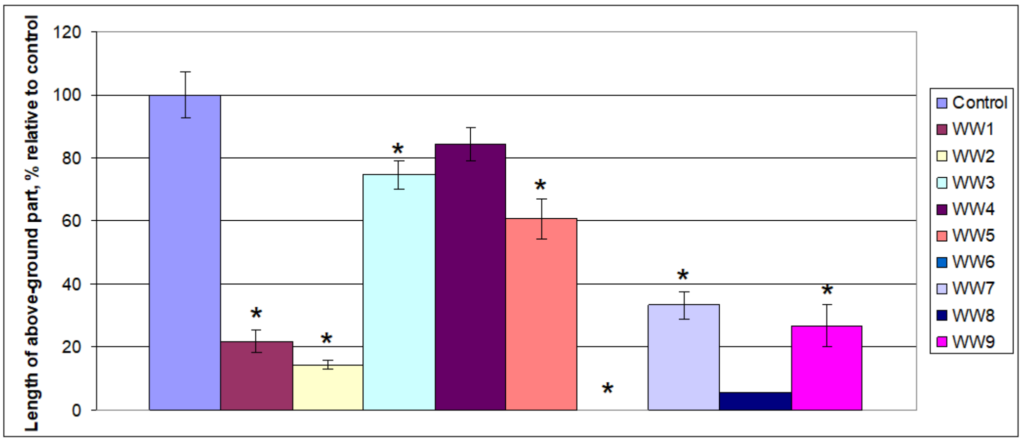

Figure 4. Length of above-ground part of *L. sativum*. * Differences from the control are significant at $p \leq 0.05$.

Table 1. Interpretation of the bioassay data.

Research Option	SGI	RLI	Interpretation of the Results of Phytotest	Comments
Control	**0.000**	**0.000**	**No toxicity**	**No inhibition of growth**
Production of Ukraine				
WW1	0.001	−0.927	Extreme toxicity	Inhibition of growth more than 90%
WW2	−0.068	−0.958	Extreme toxicity	Inhibition of growth more than 90%
WW3	−0.001	−0.699	High toxicity	Inhibition of growth more than 60%
WW4	−0.068	−0.627	High toxicity	Inhibition of growth more than 60%
WW5	−0.034	−0.770	Extreme toxicity	Inhibition of growth more than 75%
Production of Turkey				
WW6	−0.778	−0.990	Extreme toxicity	Inhibition of growth more than 90%
WW7	0.037	−0.937	Extreme toxicity	Inhibition of growth more than 90%
WW8	−0.741	−0.990	Extreme toxicity	Inhibition of growth more than 90%
Production of the United Kingdom of Great Britain and Northern Ireland				
WW9	−0.408	−0.946	Extreme toxicity	Inhibition of growth more than 90%

It was established that the energy of germination and germination of garden cress seeds when germinated on the tested wet wipes produced of Ukraine (**WW1–WW5)** were at the control level (Figures 1 and 2). However, according to biometric and morphometric characteristics, the tested wet wipes reliably showed different degrees of phytotoxicity in the growth test with garden cress (Figures 3 and 4). Thus, the highest phytotoxicity in this group was established for wet wipes **WW1** and **WW2** (manufacturer 1). Compared to the control, a decrease in root length (by 13.6 and 23.7 times, respectively) and above-ground part (by 4.6 and 6.9 times, respectively) was recorded for them. For wet wipes **WW3–WW5** (manufacturer 2), lower phytotoxicity was reliably established than for **WW1** and **WW2**. Thus, for **WW3–WW5**, compared to the control, a significant decrease in the length of garden cress roots was recorded by 3.3 times, 2.7 times and 4.4 times, respectively, and the above-ground part by 1.3 times, 1.2 times and 1.6 times, respectively. According to the calculated toxicity indices (Table 1), wet wipes **WW1**, **WW2** and **WW5** are extremely toxic, and **WW3** and **WW4** are highly toxic.

A study of germination energy and germination of garden cress seeds under the influence of wet wipes produced in Turkey and the United Kingdom showed that only **WW7** did not show a negative effect on these characteristics (Figures 1 and 2). For the other studied variants of wet wipes, a significant decrease in the germination energy index compared to the control was noted: 9 times (**WW6** and **WW8**) and 3 times (**WW9**), as well as the seed germination index: 4.5 times (**WW6**), 3.9 times (**WW8**) and 1.7 times (**WW9**). For all studied variants of wet wipes manufactured in Turkey and the United Kingdom, a reliable significant decrease compared to the control was noted in both root length (from 15.9 times to 96.9 times) and above-ground part (from 18 times to 3.4 times) (Figures 3 and 4). Under the influence of **WW6** on garden cress, the absence of the above-ground part of the test plants was observed (Figure 4). According to the calculated toxicity indices (Table 1), wet wipes **WW6–WW9** are extremely toxic.

The obtained results of phytotoxicity of the examined wet wipes are consistent with the chemical compositions used in their manufacture. Since most of the compounds in the composition of wet wipes are synthetic surfactants, the toxicity of which is known [4], therefore, high or extreme toxicity is manifested in wipes impregnated with these compounds. Currently, the need to review the compositions of wet wipes is considered from the angle of protection against microbial spoilage [14], rationalize the physicochemical interactions between the fabric and the preservatives [15]. Attention should also be paid to the issues of eco-friendliness of these materials [2]. The use of natural surfactants or biosurfactants in the production of wet wipes can solve the problem of their toxicity and environmental safety.

4. Conclusions

It was established that 78% of the tested wet wipes (60% Ukrainian production and 100% foreign) were extreme toxicity. Therefore, the tested wet wipes contain toxic substances (in particular, surfactants), show phytotoxicity and can be a source of environmental pollution. To solve the problem of toxicity and ecological safety of wet wipes, compositions where natural surfactants or biosurfactants will prevailing in their composition are needed.

Supplementary Materials: The conference presentation related to this paper can be downloaded as PDF using the link: https://sciforum.

Author Contributions: Conceptualization, N.T. and L.Z.; Methodology, N.T.; Validation, N.T.; Formal Analysis, N.T.; Investigation, N.T.; Resources, N.T.; Writing—Original Draft Preparation, N.T. and L.Z.; Writing—Review & Editing, N.T. and L.Z.; Visualization, N.T.; Supervision—N.T.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data used in this research are available upon request.

Conflicts of Interest: The authors declare no conflict of interest.

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