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Proceeding Paper

Evaluation of the Toxicity of Wet Wipes Based on the Growth Test with *Lepidium sativum* L. †

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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.). This study used nine variants of wet wipes produced in Ukraine, Turkey and the United Kingdom. Germination energy (on the third day), germination, and biometric and morphometric characteristics (on the fifth day) were determined. The phytotoxic indexes were calculated. It was established that 78% of the tested wet wipes (60% from Ukrainian production and 100% from foreign production) possessed 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; growth test; toxicity; wet wipes

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1. Introduction

Wet wipes (WW) are widely used in everyday life, and the volume of them on the market is expected to increase in the future [1–3]. Wet wipes are made from non-woven fabric 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 bio-based wet wipes caused a lower toxicity risk than did petroleum-based ones, they could have a great impact on the water resources, inducing 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

This 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) 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. The chemical compounds in the composition of wet wipes (according to manufacturer) are as follows:

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, and citric acid. The material of the wipes is 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, and citric acid. The material of the wipes is 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, ceteareth-12, ceteareth-20, cetearyl alcohol, glyceryl stearate, and cetyl palmitate. The material of the wipes is 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, ceteareth-12, ceteareth-20, cetearyl alcohol, glyceryl stearate, and cetyl palmitate. The material of the wipes is 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, ceteareth-12, ceteareth-20, cetearyl alcohol, glyceryl stearate, and cetyl palmitate. The material of the wipes is not specified.

WW6 does not contain alcohol and parabens; it contains water, phenoxyethanol, perfume, benzoic acid, glycerin, tetrasodium EDTA, cetearyl isononanoate, cocamidopropyl betaine, dehydroacetic acid, ceteareth-20, cetearyl alcohol, glyceryl stearate, allantoin, panthenol, ceteareth-12, cetyl palmitate, chlorhexidine digluconate, and D-limonene. The material of the wipes is not specified.

WW7 is alcohol-free; it contains 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, and perfume. The material of the wipes is not specified.

WW8 does not contain alcohol and parabens; it contains water, C12-15 pareth-12, phenoxyethanol, benzoic acid, dehydroacetic acid, glycerin, perfume, and citric acid. The material of the wipes is not specified.

WW9—water, polysorbate 20, caprylyl glycol, sodium benzoate, coco-betaine, maleic acid, and sodium citrate. The material of the wipes is composed of 70% cellulose and 30% plastic (which 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. Filter paper instead of wet wipes was used as a control. Ten 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, and on the 5th day, germination and biometric and morphometric characteristics (root and above-ground part length) were determined [6]. The phytotoxic indexes were calculated, including the seed germination index (SGI) and the root length index (RLI) [8–10]. The toxicity scale given in the study in [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.

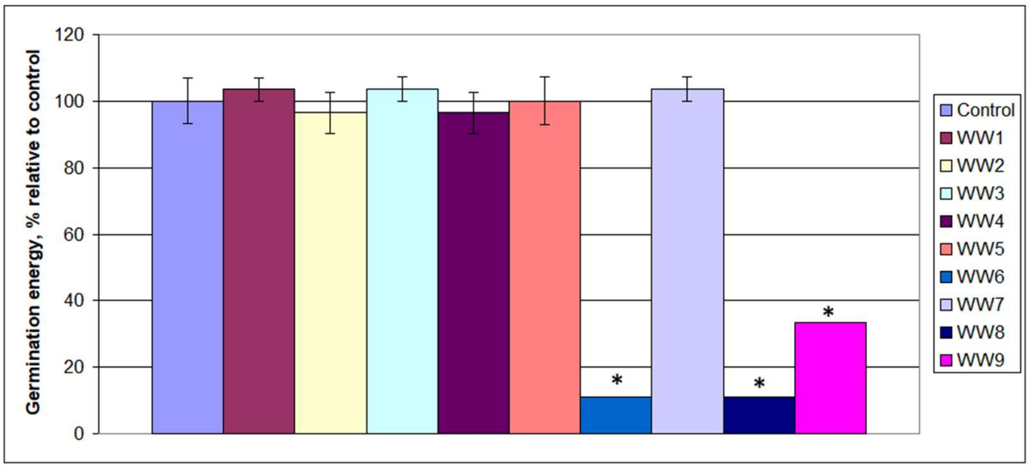

Figure 1. Germination energy of *L. sativum*. * Differences from the control are significant at $p \leq 0.05$.

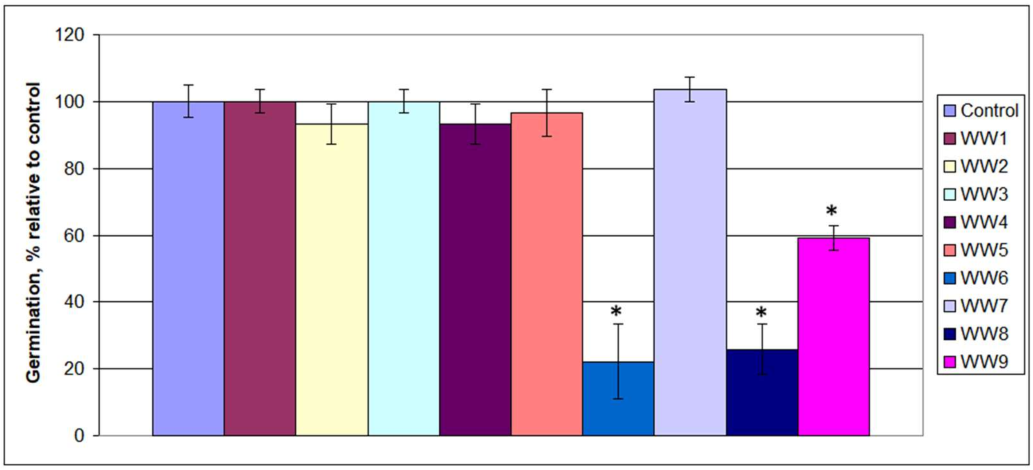

Figure 2. Germination of *L. sativum*. * Differences from the control are significant at $p \leq 0.05$.

It was established that the energy of germination and germination of garden cress seeds when germinated on the tested wet wipes produced in 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 the 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 that for WW1 and WW2. Thus, for WW3–WW5, compared to the control, a significant decrease in the length of garden cress roots, by 3.3 times, 2.7 times and 4.4 times, respectively, was recorded, as was a decrease in 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.

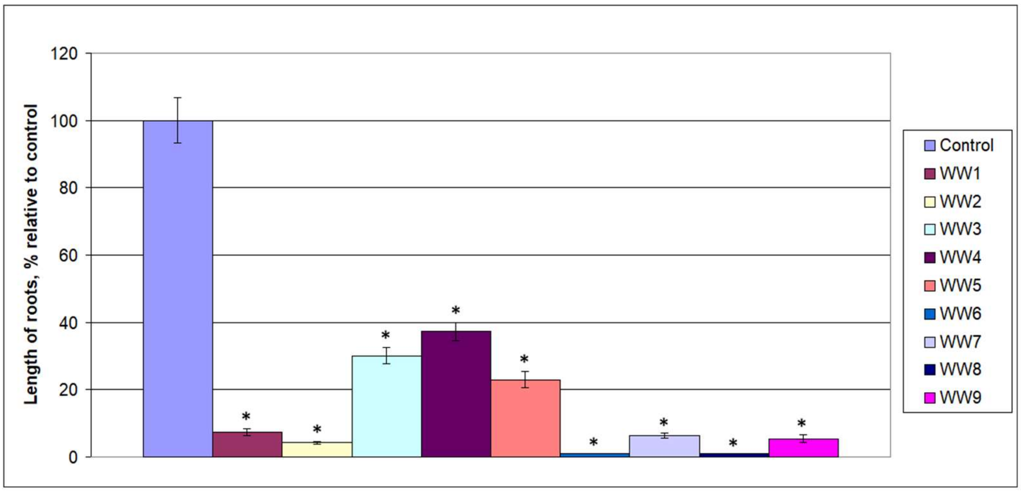

Figure 3. Root length of *L. sativum*. * Differences from the control are significant at $p \leq 0.05$.

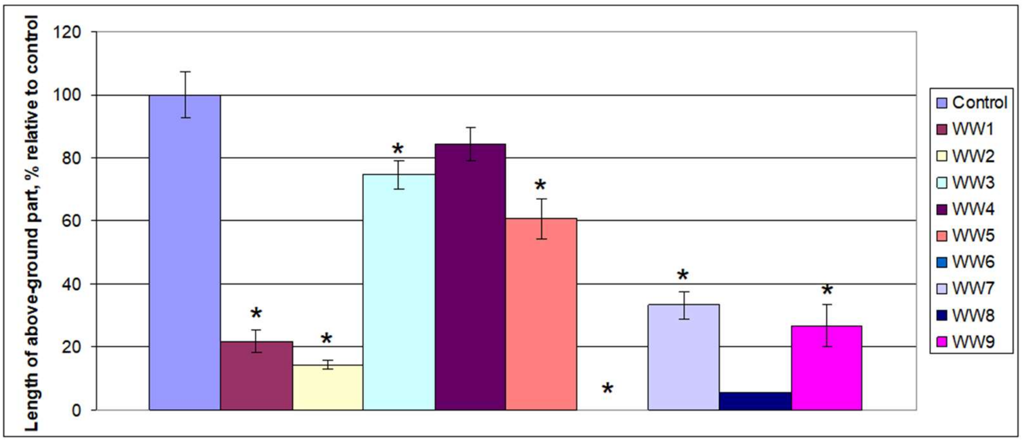

Figure 4. Length of above-ground part of *L. sativum*. * Differences from the control are significant at $p \leq 0.05$.

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
Produced in 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%
Produced in 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%
Produced in the United Kingdom of Great Britain and Northern Ireland				
WW9	−0.408	−0.946	Extreme toxicity	Inhibition of growth more than 90%

This study on germination energy and the 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 that of the control was noted, as a decrease by 9 times (WW6 and WW8) and 3 times (WW9), and so was a decrease in the seed germination index by 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 values in the control was noted in both root length (from 15.9 times to 96.9 times) and the 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 the 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], 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], and of rationalizing the physicochemical interactions between the fabric and the preservatives [15]. Attention should also be paid to the issues of the eco-friendliness of these materials [2,16]. 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% from Ukrainian production and 100% from foreign production) 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 the toxicity and ecological safety of wet wipes, compositions where natural surfactants or biosurfactants prevail are needed.

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