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Effect of Triton X-100 on the wheat and lettuce growth and contaminant absorption

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Abstract

This study was set to study the effects of surfactants on crops using Triton X-100, one of widely used surfactants for various purposes including agricultural uses, as a target surfactant. The effects of Triton X-100 on the growth of wheat and lettuce were studied and the germination and shoot growth of wheat were not significantly affected by Triton X-100. With lettuce, the increasing Triton X-100 concentrations tend to negatively affect the growth, possibly due to the absorption of Triton X-100 by lettuce. The average lettuce fresh mass was reduced by 31% when Triton X-100 concentration increased from 0 to 240 mg L^{-1}. This may mean that chemicals dissolved or mobilized by Triton X-100 can be absorbed by lettuce. The Cd mobilization was facilitated with Triton X-100, and the absorption of procymidone in soil by lettuce was greater when Triton X-100 was applied (i.e., 0.18 mg kg^{-1}) than when water was applied (i.e., 0.15 mg kg^{-1}), although they were statistically not different (p-value > 0.05). The average lettuce masses in the presence of residual procymidone in soil and Triton X-100 (16 g) were lower than that of the control soils (20 g), although they were statistically not different (p-value > 0.05). The results suggest that surfactants contained in pesticide formulations can potentially affect crop growth and absorption of other contaminants. Therefore, the residual surfactants and active ingredients in pesticide formulations need to be properly managed to protect the environment and to produce crops free of contaminants.

Keywords: Surfactant, Pesticide, Heavy metal, Triton X-100, Crop, Toxicity

Introduction

Agricultural chemicals such as pesticides, insecticides, herbicides, and fungicides are widely used to control crop diseases and promote growth. These chemicals are applied as formulations, and pesticide formulations are mixtures of active ingredients and inert chemicals including various spray adjuvants [1]. Surfactants are one type of spray adjuvants and are used to promote the effectiveness of agricultural chemicals owing to their absorbing, emulsifying, wetting, and spreading properties [2].

When pesticide formulations are applied to crops on agricultural lands, the chemicals constituting the pesticide formulations including active ingredients can enter the soil environment. Once entered, some chemicals can be adsorbed on soil, the others can reach water bodies such as groundwater and rivers [3], and these chemicals can have adverse effects on organisms including microorganisms and fish [3–5]. The chemicals originating from pesticide formulations can also be absorbed by crops, which eventually affect consumers including human. Most studies on the effect of pesticides focus on the entrance of active ingredients of pesticide formulations to the environment, while less attention is paid to the other constituents of pesticide formulations [6]. Previous studies usually investigated the effect of spray adjuvants on the application of active ingredients of pesticide formulations, but their environmental effects were rarely studied [7].

Like the active ingredients of pesticide formulations, surfactants can be absorbed by crops and affect their growth. Surfactants can be toxic to organisms, especially

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to plants [8]. Previous studies reported various effects such as plant growth inhibitions and cellular and membrane damage of plant tissues [8, 9]. Also, when exposed to surfactants, the expression of the plant genes involved in detoxification was affected [10]. Also, when surfactants are present, plants can absorb more chemicals such as minerals, surfactants, and pollutants, which may lead to adverse effects on overall plant growth [11, 12]. In addition, when surfactants are degraded by natural processes such as photolysis in the environmental media, the toxic effects can be increased [13].

This study was set to study the effects of surfactants that may enter and reside in the agricultural environment through pesticide formulation on crops, and Triton X-100 was used as the target surfactant as it is one of widely used surfactants for various purposes including agricultural uses. The effects of Triton X-100 on crop growth were studied using wheat and lettuce as target crops. In order to study the effect of Triton X-100 on fate of contaminants in agricultural soil, cadmium (Cd) and procymidone were used as target contaminants. Cadmium is one of often detected heavy metals in agricultural soils [14], and procymidone is often detected in crops including lettuce and strawberries [15, 16]. The leaching potential of heavy metals by surfactants was studied by running a column test with Cd-contaminated soil, and the pot test was carried out to study the effect of Triton X-100 on absorption of procymidone by lettuce.

Materials and methods

Germination and growth tests using wheat

Triton™ X-100 (laboratory grade) was purchased from Sigma-Aldrich (Munich, Germany). The germination tests with *Triticum aestivum* (wheat) were carried out on a gauze moistened with water containing different concentrations of Triton X-100. The gauze was kept wet by applying the Triton X-100 containing water (0, 200, 400, 600, 800, 1000 mg L^{-1}) regularly. For each condition, ten seeds were used for each sample, and four replicates were prepared. The germination tests with *T. aestivum* (wheat) were also carried out in soil following the ISO 11269-2:2012 method (i.e., determination of the effects of pollutants on soil flora—part 2). The tests were carried out in a temperature-controlled chamber at 24 ± 1.4 °C under the 16 h light and 8 h dark conditions. For each condition, ten seeds were used for each sample, and duplicate samples were prepared. The Triton X-100 containing water (0, 200, 400, 600, 800, 1000 mg L^{-1}) was applied regularly to the soil during the test period to determine the effect of different Triton X-100 concentrations on seed germination and shoot growth.

The wheat growth was also studied using an aquaponics system (waterway length of 2 m; DH-B32, Daesan, South Korea). The wheat seedlings were prepared by germinating seeds and growing the seedlings for 2 weeks in the aquaponics system. The growth medium for leafy green vegetables for the aquaponics system was purchased from Coseal Co., Ltd. (South Korea). The growth medium consists of A medium and B medium, which are mixed at a designated ratio. The growth medium contains 21.5% N, 9.5% P, 43% K, 14% Ca, 0.2% B, 0.07% Mn, 0.007% Zn, 0.35% Fe, and 5.5% Mg. After the wheat seedling preparation, the growth medium with different concentrations of Triton X-100 (i.e., 0, 125, 500, 1000 mg L^{-1}) were prepared and the four seedlings were placed in the aquaponics system at each Triton X-100 concentration to study the growth of seedlings under different concentrations of Triton X-100 for 28 days.

Growth tests with lettuce

Lettuce (*Lactuca sativa*) seedlings prepared for about a month were placed in the aquaponics system to study the effect of different concentrations of Triton X-100 (i.e., 0, 63, 140, 240 mg L^{-1}) on the lettuce mass and chlorophyll and carotenoid concentrations. The lettuce was grown in the same growth medium as for the wheat growth for a week, then exposed to Triton X-100 for 12 days. The temperature was maintained at 20–23 °C during the test.

The chlorophyll and carotenoid concentrations were determined following the procedure described in Su et al. [17]. The lettuce leaves and 80% acetone were mixed and leaves were ground, and the mixture was filtered and brought to a volume of 25 mL with 80% acetone. The absorbance was measured using a UV/Visible spectrometer (UV-1800, Shimadzu, Japan) at 645 nm and 663 nm to determine the chlorophyll concentrations (Su et al. [17]). The total carotenoid was determined by measuring the absorbance at 470 nm [18].

The changes in the Triton X-100 concentrations of the growth medium were also monitored. Before the experiment, the lettuce seedlings were placed in the growth medium for a week, and then different concentrations of Triton X-100 were added to the growth medium to run the test. Four seedlings were used for each test condition (i.e., Triton X-100 concentration). The initially prepared growth medium with Triton X-100 was used over the experimental period without refilling to determine the residual Triton X-100 concentrations. The changes in the residual Triton X-100 concentrations were determined by measuring absorbance at 275 nm using a UV/Visible spectrometer (UV-1800, Shimadzu, Japan).

Pot test

In order to determine the effect of Triton X-100 on the absorption of procymidone by lettuce, pot tests were carried out. The procymidone-contaminated soil was prepared to have the initial concentration of 3.5 ± 0.9 mg kg^{-1} based on the soil procymidone concentration reported in the study by Hwang et al. [15]. Procymidone was not detected in the control soil (i.e., no artificial procymidone contamination). The soils were placed in rectangular pots and lettuce seedlings prepared for about a month were placed in the control and Cd-contaminated soils. The lettuce was grown for a month and watered with water or Triton X-100 containing water (i.e., 50 mg L^{-1}) every 2–3 days. Three lettuce seedlings were placed in each pot and two pots were prepared for each condition.

The concentrations of procymidone in the soils and lettuce were determined by extracting procymidone and analyzing it with high performance liquid chromatography-diode-array detector (HPLC-DAD; Agilent 1100 series). Briefly, the samples were mixed with acetone and liquid–liquid extraction was used to extract procymidone from the filtrates. The extracts were concentrated further and dissolved in acetone/hexane mixture, which was followed by a cleanup procedure. The sample was then dissolved in acetonitrile for HPLC analysis. The limit of quantification was 0.02 ppm.

Statistical analyses

The linear regression of germination rates was done in GraphPad software (v8.0.1). The One-Way ANOVA and Two-Way ANOVA coupled with the post-hoc test (i.e., Scheffe and LSD) were employed to determine the significant differences between the growth of wheat, lettuce, and duckweed at different Triton X-100 concentrations. One-Way ANOVA, Two-Way ANOVA, and t test for comparison of data were carried out with the SPSS (v21) software and R (v4.0.2).

Results and discussion

Effect of Triton X-100 on the *T. aestivum* germination rates

The wheat seed germination rates (%) on the wet gauze moistened with different Triton X-100 concentrations are shown in Fig. 1. When the germination rates on Day 2 (i.e., when the germination rates of the controls exceed 50%) were compared, the average germination rates decreased with increasing Triton X-100 concentrations. However, only the controls showed statistically significant differences with the Triton X-100-exposed samples (p-value < 0.05), and the different Triton X-100 concentrations did not affect the germination rates significantly (p-value > 0.05; Fig. 1).

Also, the germination duration was longer with the Triton X-100-exposed samples (Fig. 1). For example, the controls took 2 days for near 100% germination, while the Triton X-100-exposed samples took > 5 days. The slopes determined by fitting the data to a linear regression decreased with increasing Triton X-100 concentrations from 0 to 200 mg L^{-1} (i.e., from 332 for the controls to 138 (with the 95% confidence interval of 87–188) for the 200 mg L^{-1} samples), and they were significantly different (p value = 0.0048). However, the slopes were statistically not different for Triton X-100 concentrations from 200 mg L^{-1} to 1000 mg L^{-1} (p value = 0.8158). It can be explained by the weakened germination energy due to exposure to Triton X-100 [19]. In other words, the germination ability (i.e., germination percentage) was not lost by the exposure to Triton X-100, but the time taken for 100% germination became longer. Similarly, the prolonged germination of seeds of lettuce and other crop seeds exposed to contaminated soil was reported. For example, the lettuce seed germination reached the maximum after 7 days when exposed to Triton X-100, while it took 2 days for the controls [19]. Also, the germination duration of tomatoes was longer in the As-contaminated soil samples than the control samples [20]. Considering the effects of time and Triton X-100 on wheat germination, the controls and Triton X-100 exposed samples were significantly different (p value = 0.029, 0.006, 0.000, 0.026, 0.035, respectively, in ascending order of Triton X-100 concentrations from 200 to 1000 mg L^{-1}), while there were no significant differences of growth at different Triton X-100 concentrations.

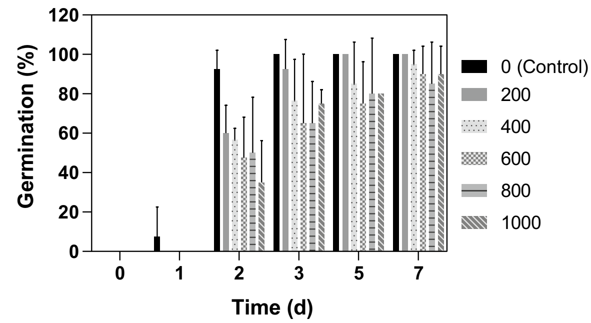

Fig. 1 Germination of *Triticum aestivum* (wheat) seeds on the gauze moistened with different Triton X-100 concentrations (mg L^{-1}). The error bars indicate the average and standard deviations of four replicate samples with each sample containing ten seeds

The *T. aestivum* germination rates on Day 4 (i.e., when the control germination rates exceed 50%) in soil with Triton X-100 application did not show a statistically significant difference (p value = 0.862) between the samples of different Triton X-100 concentrations (Fig. 2). In other words, within the Triton X-100 concentration range used in this study, adverse effects on the germination rates were not observed. When the germination data were

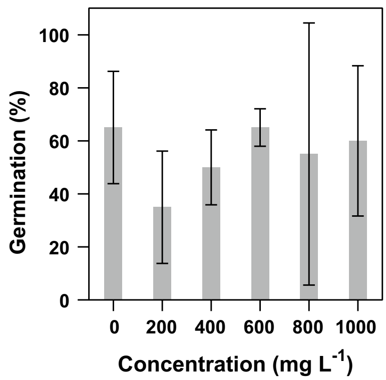

Fig. 2 Effect of applying different concentrations of Triton X-100 solutions (mg L^{-1}) on the germination of *Triticum aestivum* (wheat) seeds in soil on Day 4. The error bars indicate the average and standard deviations of duplicate samples with each sample containing ten seeds

fitted to linear regression, the slopes for different Triton X-100 concentrations were not statistically different (p value = 0.782), suggesting that the germination duration was similar. Overall, the effect of Triton X-100 on the germination rates was not significant when compared to the controls, although the Day 2 germination rates on the wet gauze were higher for the controls than the Triton X-100-exposed samples. When the gauze was moistened with Triton X-100, it could represent a contaminated media (i.e., environmental media with residual Triton X-100). When the soil was watered with Triton X-100 solution, it can represent a contaminant entering the soil environment. Thus, the seeds are expected to have more contact with Triton X-100 on the wet gauze, which, in turn, affects the germination.

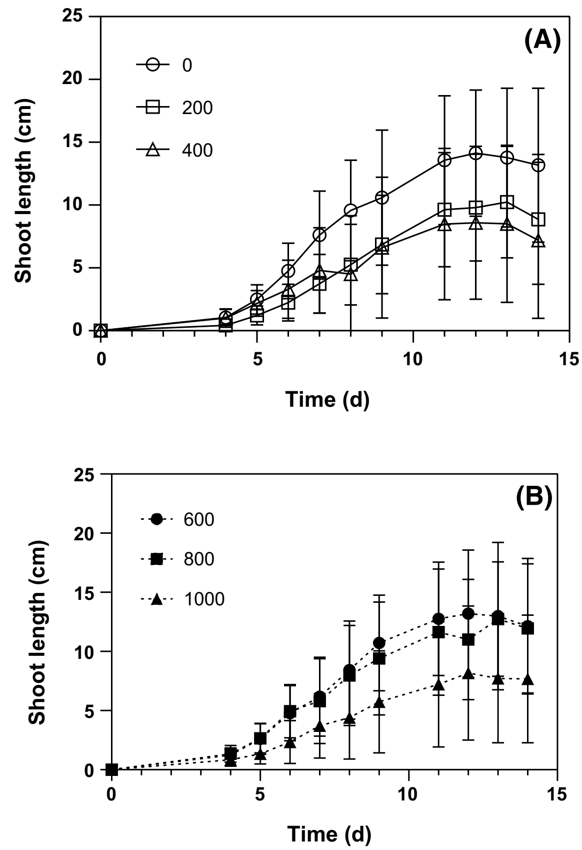

Fig. 3 Effect of applying different concentrations of Triton X-100 containing water (mg L^{-1}) on the shoot length of *Triticum aestivum* (wheat) grown in soil (**a**) effect of Triton X-100 containing water at 0, 200, and 400 mg L^{-1} and (**b**) effect of Triton X-100 containing water at 600, 800, and 1000 mg L^{-1}. The error bars indicate the average and standard deviations of the shoot lengths of all the wheat seedlings germinated and grown (between 7 and 19 seedlings depending on the day and the Triton X-100 concentrations)

Effect of Triton X-100 on the *T. aestivum* shoot growth

The changes in the shoot length of the germinated wheat are shown in Fig. 3. At all conditions, the average shoot length increased up to Day 11 (Fig. 3). When the shoot lengths on Day 14 were compared, the average shoot lengths tend to show a decreasing trend with increasing Triton X-100 concentration from 0 to 400 mg L^{-1} (Fig. 3A) and the average shoot length increased with further increase in the Triton X-100 concentration to 600 mg L^{-1} (Fig. 3B). Then the average shoot lengths tend to decrease again with further increase in the Triton X-100 concentration to 1000 mg L^{-1} (Fig. 3B). However, on any single day, the differences between the different samples were not statistically significant (p value = 0.576).

The changes in the wheat shoot growth studied using an aquaponics system are shown in Fig. 4. With the controls (i.e., 0 mg L^{-1}), the average shoot lengths increased with time for about 14 days and reached a plateau, while the shoot length of the wheat exposed to the Triton X-100 concentrations of 125 mg L^{-1} continuously increased over the experimental period of 28 days (Fig. 4). But the shoot lengths for the controls and the 125 mg L^{-1} samples did not show a statistically significant difference (p value = 0.336). They could be fitted to a linear regression, and the slope was significantly non-zero indicating the significant shoot growth over 28 days. The shoot lengths at 500 and 1000 mg L^{-1} were statistically different from the controls (p value < 0.05). Also, the shoot lengths of the wheat exposed to 500 and 1000 mg L^{-1} Triton X-100 did not change over the experimental period [i.e., slopes of zero (p value > 0.05)], and this can be related to the leaves turning yellow and withering with time. Similarly, the soybean growth was completely prevented at the Triton X-100 concentrations of 1.0 and 0.1% [21].

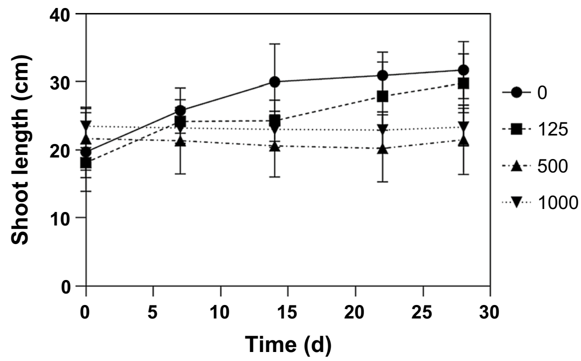

Fig. 4 Changes in the *Triticum aestivum* (wheat) shoot length with time exposed to different Triton X-100 concentrations (mg L^{-1}) in an aquaponics system. The error bars indicate average and standard deviations of the shoot lengths of four wheat seedlings

When the germination of wheat seeds on the wet gauze were considered, the seed germination was negatively affected by Triton X-100, and the shoot growth in the aquaponics system was also negatively affected. Similarly, the morphological traits of wheat and sugar beets were changed when their seeds were treated with Triton X-100 during germination [19, 22].

Effect of Triton X-100 on the growth of lettuce

The lettuce mass increased with time at the Triton X-100 concentrations of 0, 63, and 140 mg L^{-1}, but not at 240 mg L^{-1} (Fig. 5). The controls, the 63 mg L^{-1} samples, and the 140 mg L^{-1} samples were statistically similar (p value > 0.05), although the average values of the 140 mg L^{-1} samples were lower than the others. Up to Day 9, the differences between lettuce masses at different Triton X-100 concentrations were not significant (p value > 0.05), but on Day 12, the lettuce masses of the controls, the 63 mg L^{-1} samples, and the 140 mg L^{-1} samples were statistically greater than that of the 240 mg L^{-1} samples (p value < 0.05). For example, the average lettuce mass (i.e., fresh mass) decreased by about 31% from 35 ± 5 g for the controls to 24 ± 2 g for the 240 mg L^{-1} samples. Furthermore, damages to leaves (i.e., browning of leaves) were observed with the 240 mg L^{-1} samples.

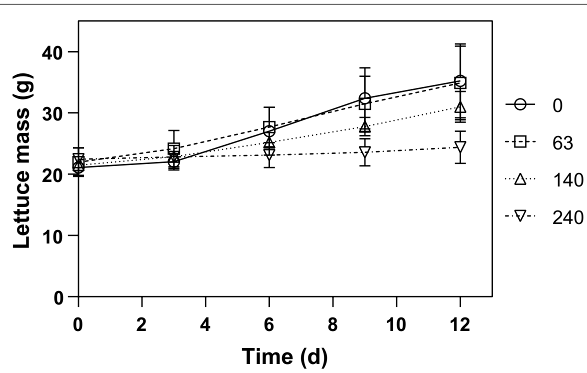

Fig. 5 Changes in the mass (g) of *Lactuca sativa* (lettuce) grown in an aquaponics system under different Triton X-100 concentrations (mg L^{-1}). The error bars indicate average and standard deviations of the masses of four lettuce seedlings

The chlorophyll and carotenoid concentrations of the lettuce affected by different concentrations of Triton X-100 are shown in Fig. 6. The chlorophyll-a concentrations can be used to indicate the general health status of the plant since photosynthesis is one of the important processes [23]. The average chlorophyll-a concentrations increased with increasing Triton X-100 concentrations from 0 (i.e., 1.06 mg L^{-1}) to 240 mg L^{-1} (2.15 mg L^{-1}). The controls and 63 mg L^{-1} samples did not show a statistically significant difference (p value = 0.291), and the 63 mg L^{-1} samples were not statistically different from the 140 mg L^{-1} samples and 240 mg L^{-1} samples (p value > 0.05). However, the chlorophyll-a concentrations of the 140 mg L^{-1} samples and 240 mg L^{-1} samples were statistically higher than that of the controls (p value < 0.05) (Fig. 6). Overall, the exposure to Triton X-100 did not result in different chlorophyll-a concentrations.

The average chlorophyll-b concentrations and the average carotenoid concentrations also showed similar trends as the chlorophyll-a concentrations. The average chlorophyll-b and carotenoid concentrations increased with increasing Triton X-100 concentrations from 0 to 63 mg L^{-1}, but no further increase was observed with further increase in the Triton X-100 concentrations.

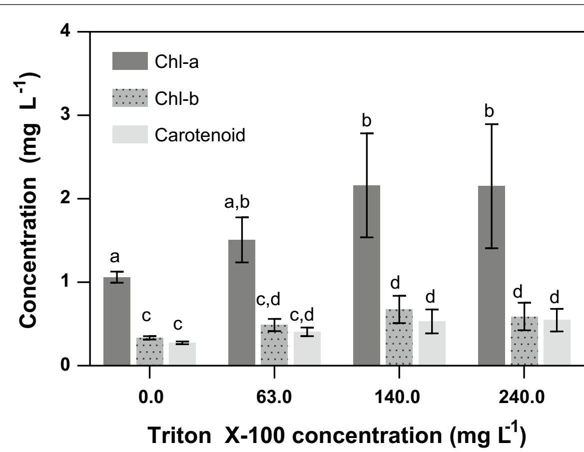

Fig. 6 Effect of Triton X-100 on the chlorophyll and carotenoid concentrations of *Lactuca sativa* (lettuce). The small letters indicate the significance differences at the confidence level of 0.05. The error bars indicate average and standard deviations of the chlorophyll and carotenoid concentrations of four lettuce seedlings

The chlorophyll-b and carotenoid concentrations of the controls showed statistically significant differences with the chlorophyll-b and carotenoid concentrations of the 140 mg L^{-1} samples and 240 mg L^{-1} samples, but not with the 63 mg L^{-1} samples. Also, the chlorophyll-b and carotenoid concentrations of the Triton X-100-exposed samples were statistically similar (p value > 0.05). Overall, there were no statistically significant effects of Triton X-100 on the chlorophyll-b and carotenoid concentrations.

Previous study reported lower chlorophyll contents of leaves grown in crude oil-contaminated soils than that of the controls [24]. Thus, the reduction in the chlorophyll content can be used to indicate environmental contamination [24]. However, increasing soil mercury concentrations stimulated the synthesis of chlorophyll at the early stages of wheat growth [25]. The chlorophyll content increased with increasing soil mercury concentration after 14 days-exposure; however, with longer exposure to mercury, the chlorophyll content decreased with increasing soil mercury concentration [25].

The residual Triton X-100 concentrations in the growth medium were continuously reduced over the 12 days-cultivation period (Fig. 7). The reduction can be largely attributed to the absorption by lettuce. The reduction in the Triton X-100 concentrations was fitted to the first-order reaction, and the first-order rate constants were −0.563 (95% confidence interval of −1.59 to 0.466), −0.264 (95% confidence interval of −0.554 to 0.0260), and −0.164 (95% confidence interval of −0.319 to 0.00940)/day for the 60, 140, and 240 mg L^{-1} samples, respectively. The first-order rate constants were statistically different (p value = 0.028). It suggests that the

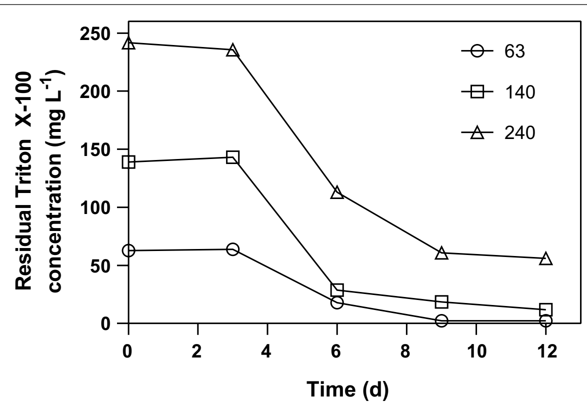

Fig. 7 Changes in the residual Triton X-100 concentrations in the growth medium containing different Triton X-100 concentrations (mg L^{-1}) during the 12 days-lettuce cultivation period. The data are from single measurement. The figure was prepared from single measurement

lettuce absorbed Triton X-100 more slowly in the presence of higher Triton X-100 concentrations. It can also be related to the increase in the lettuce mass over the 12 d-cultivation period. The total increase in the lettuce masses was greater in the 60 mg L^{-1} samples than the other samples. The changes in the lettuce masses in the 140 and 240 mg L^{-1} samples were 74% and 15% of what was observed in the 60 mg L^{-1} sample, respectively. In other words, the absorption of Triton X-100 by lettuce was less at higher Triton X-100 concentrations, because the lettuce growth was less at higher Triton X-100 concentrations. But it is clear that lettuce absorbs Triton X-100 and it implies that the absorption of other chemicals such as pesticides can be affected by the presence of Triton X-100. Surfactants are often used to increase the mobility of contaminants in soils for remediation, thus, the contaminants with increased mobility have greater chances of being absorbed by plants [26, 27].

Effect of Triton X-100 on absorption of procymidone in soil by lettuce

In order to study the effects of Triton X-100 on the mobility of other contaminants such as pesticides, the absorption of procymidone by lettuce was studied. Figure 8 shows the procymidone concentrations in the lettuce grown in the control soils and the procymidone-contaminated soils and the masses of lettuce. The average procymidone concentration was higher when Trion X-100-containing water was used to water lettuce (i.e., 0.18 mg kg^{-1}) than when water was used (i.e., 0.15 mg kg^{-1}) (Fig. 8A). However, they were not statistically different (p value > 0.05). The masses of lettuce tend to be affected by both procymidone in soil and Triton X-100 in water; although the differences were not statistically significant (Fig. 8B). The average lettuce masses were lower when Triton X-100-containing water was applied regardless of the presence of procymidone in soil (Fig. 8B). However, they were not statistically different. Interestingly, the average lettuce mass was higher in the procymidone-contaminated soil (16 ± 2 g) than the control soil (13 ± 6 g) when Triton X-100-containing water was applied, although they were not statistically different (p value > 0.05) (Fig. 8B). Overall, the effects of Triton X-100 on the absorption of procymidone in soil by lettuce and on the lettuce mass were not significant.

Previous study showed that a surfactant increased the mobility of metribuzin in soil [28]. Thus, surfactants can be used to remove residual pesticides in soil by solubilizing sorbed pesticides [29]. The increased mobility of pesticides in the presence of surfactants can lead to increased absorption by plants in soil; however, our results show that the absorption in the presence of Triton X-100 was not significantly different from that in

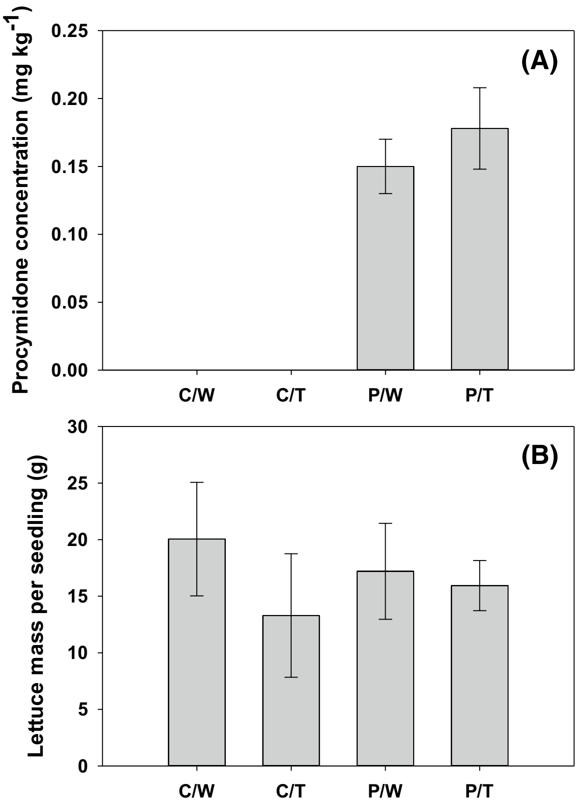

Fig. 8 Effect of Triton X-100 on **A** the absorption of procymidone by lettuce and **B** the lettuce mass per seedling. C/W: control soil/water, C/T: control soil/Triton X-100-containing water, P/W: procymidone-contaminated soil/water, P/T: procymidone-contaminated soil/Triton X-100-containing water

the absence of Triton X-100 (i.e., watered with water). Yet, the higher average procymidone concentration in lettuce in the presence of Triton X-100 and the lower average lettuce masses in the presence of residual procymidone in soil and Triton X-100 compared to the control soils suggest that both the surfactants and the active ingredients of pesticide formulation need to be properly managed.

In summary, residual Triton X-100 in environment can adversely affect the plant growth. The effect of Triton X-100 on the wheat seed germination on the wet gauze was significant when compared to the controls; and the growth of wheat, lettuce, and duckweed was adversely affected by Triton X-100. The wheat seed germination rates on the wet gauze had significant differences; however, the wheat seed germination rates in soil did not show significant differences between the controls and Triton X-100-exposed samples. High concentrations of Triton X-100 in an aquaponics system showed adverse effects on wheat growth. Also, Triton X-100 adversely affected the growth of lettuce and duckweed such as lower masses and shorter root lengths. Triton X-100 was absorbed by lettuce suggesting that chemicals dissolved in Triton X-100 or mobilized by Triton X-100 can be absorbed by lettuce at the same time. The absorption of procymidone in soil by lettuce was greater when Triton X-100 was applied than when water was applied, although they were not statistically different. Overall, residual surfactants can potentially affect plant growth and absorption of other contaminants such as heavy metals and pesticides in soil. Therefore, the residual surfactants and active ingredients in pesticide formulations need to be properly managed to protect the environment and to enhance the production of crops that are free of contaminants.

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Authors' contributions
SS: Methodology, formal analysis, visualization, writing—review and editing, EHJ: conceptualization, formal analysis, writing—original draft, writing—review and editing, visualization, supervision, project administration, funding acquisition. HSP: investigation, methodology. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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