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Research Paper

Assessment of Toxicological Effects of Triclosan on Microbes, Plants, and Genetic Material in Cells

Merry Krisdawati Sipahutar[1]*

[1] *Occupational Safety and Health Study Program, Faculty of Vocation, Balikpapan University, Balikpapan, East Kalimantan, 76114, Indonesia*
***Corresponding author e-mail**: merry.k@uniba-bpn.ac.id

Abstract
Triclosan is an antiseptic ingredient that is commonly found in numerous personal care items that may end up in the environment. Their ecotoxicological profile, however, is still unknown. The current research aims to determine the toxicity of triclosan on *E. coli, Vigna radiata* and *Crotalaria juncea* seeds, and *Allium cepa* roots. The analyses include toxicity assays for microbes, phytotoxicity, and cytogenotoxicity. The results proposed if the triclosan tested (60-150 µM) became exceedingly harmful to the test bacterium, as seen by the decrease in *E. coli* CFU, indicated that triclosan had antibacterial properties and inhibited the test microbe, depending on the concentration of triclosan used. Triclosan at concentrations of 40 and 80 µM, respectively, decreased the sprouting length of *Vigna radiata* and *Crotalaria juncea* by 45-50% and 70-72%, respectively. Thus, the cytogenotoxicity assay using *Allium cepa* revealed that triclosan damages the meristematic cells. Triclosan at 40 µM concentration resulted in a 1.2% aberration index and a 10.4% mitotic index, and 80 µM caused a 1.4% aberration index and an 8.8% mitotic index. All of the findings point to triclosan being potentially hazardous to the biota.

Keywords
Triclosan, Microbial Toxicity, Phytotoxicity, Cytogenotoxicity

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1. INTRODUCTION

The properties of triclosan as an antibacterial, antifungal, and antiviral agent make it widely utilized in a diversity of things for private care, synthetic goods, underware, and toys (Barman et al., 2022; Chen et al., 2023). Triclosan has the IUPAC ID $C_{12}H_7Cl_3O_2$ and the chemical formula 5-chloro-2-(2,4- dichlorophenoxy)phenol. This compound migrates into the environment after being poured into the wastepipe and into the sewage system when used for such a broad range of daily requirements (Nakagawa et al., 2022; Heidler and Halden, 2007). Triclosan is an extremely poisonous substance, but despite this, it is frequently employed in the manufacturing of plastics, textiles, and personal care items. It is persistent and does not break down quickly (Paul et al., 2010). The primary biodegradation of the substance takes weeks to months for complete biodegradation (Balakrishnan and Mohan, 2021).

Typically, 0.1% to 0.3% (w/w) of triclosan exists in products for private care (Lu et al., 2009). At these concentrations, it displays broad-spectrum bacteriostatic action and an excellent human safety profile (Dann and Hontela, 2011). The molecular weight of triclosan is 289.6, it has a pKa value of 8.14, and its logarithm of the octanol-water severance coefficient (log Kow) is 4.8. It is hydrolytically stable, with a solvency of 12 mg/L at 20°C, and a haze pressure of 7×10-4 Pa at 25°C (Baalbaki, 2017).

Around 1500 tons of triclosan are generated universally every year (Sui et al., 2017). Due to the ineffective removal of the compound (72–93%) by traditional systems, a sizable proportion of triclosan is used in the treatment of wastewater and is inevitably released into aquatic areas (Kookana et al., 2011). Consequently, this compound has been discovered in a variety of organisms, including fish, algae, plants, and humans at high consentrations (Oliveira et al., 2009). Additionally, it has been discovered in natural waters and wastewater treatment plant effluents (Fu et al., 2016). Triclosan, for instance, was found in wastewater treatment plant effluent at concentrations ranging from 1-10 µg/L (Adolfsson-Erici et al., 2002; Ying et al., 2007). During wastewater treatment operations, triclosan is diffused into particulate and dissolved organic materials. Biosolid analytes contained triclosan values ranging from 0.33 to 130 mg/kg (Heidler and Halden, 2007). The concentration of triclosan in soil enriched with solid organic matter is calculated at 4.5 mg/kg arid mass (Fuchsman et al., 2010). Antimi-

crobial compounds may be introduced into agricultural soil as a result of biosolids land application. The existence of significant amounts of triclosan in soils may result in the gradual accumulation and hazardous stages of triclosan in the surface-dwelling environment (Fu et al., 2016). Rare studies have revealed the toxicity of triclosan with numerous bioassays.

The goal of the current study is to clarify the biological danger of triclosan on plant tissue and microorganisms in order to address this issue. *Escherichia coli* (*E. coli*) was used for the assessment of the antimicrobial activity of triclosan. *Vigna radiata* (mung bean) and *Crotalaria juncea* (sunn hemp) seeds were used in phytotoxicity evaluation to determine triclosan uptake because legumes are commonly consumed and are known to be sensitive to contaminants. *Allium cepa* was used for the cytogenotoxicity test. These findings are anticipated to provide additional information on the environmental fatality and latent biological harm of triclosan.

2. EXPERIMENTAL SECTION

2.1 Chemicals and Reagents

Triclosan was supplied by the Tokyo Chemical Industry, Japan. The purity of triclosan was greater than 96%. Acetone was purchased from J.T. Baker (manufactured in the USA). All other compounds were of analytical grade.

2.2 Microbial Toxicity Test

Effect of triclosan on microorganism augmentation was determined by injecting a strain of *E. coli* into a triclosan-conceiving Mueller-Hinton broth and contrasting it to a similar bacterium raised in a triclosan-unrestricted medium (Wen et al., 2015). Briefly, a series of tubes containing 3 mL of untainted Mueller-Hinton broth with or without the supplement of triclosan were prepared (0–150 µM, pH 7.0). Each spout was shot with 1 mL of an *E. coli* cell suspension at a concentration of 2.4×10^9 CFU/mL (CFU, colony forming units) and incubated at a temperature of 35±1°C for 24 hours (Griffin et al., 2000; Kowalska-Krochmal and Dudek-Wicher, 2021).

2.3 Phytotoxicity Test

Considering how sensitive plants are to toxins, the initial toxicity experiment's goal was to ascertain whether legume seedlings were affected by root growth suppression. Germination percentage, seedling survival, and sprouting length are measures of plant growth that have been used to gauge how plants react to different contaminants. *Vigna radiata* and *Crotalaria juncea* were selected for this research because legumes are commonly consumed. Considering the plants' high sensitivity to a toxic substance (triclosan), the goal of such a toxicity experiment was to determine the inhibition of root growth in *Vigna radiata* and *Crotalaria juncea* seeds.

Triclosan with 40 and 80 µM concentrations was extracted with acetone, dried, dispersed to the preliminary quantity in water that had been distilled, and tested for noxiousness. The phytotoxicity of treated samples was evaluated using a bioassay for seed germination (Raj et al., 2014). Five seeds of each *Vigna radiata* and *Crotalaria juncea* were surface-sterilized (Tadashi et al., 2011). In each petri plate, five seeds of either the test solution (triclosan) or deionized water (a control) were deposited on filter paper. The plates were incubated for 72 hours in a growth chamber with 80% humidity, a cycle of 14 hours of light and 10 hours of darkness, and a constant temperature of 25°C. At the end of the exposure period, the level of toxicity was evaluated in relation to seed germination inhibition and sprouting length compared with the control; this reflected non-harmfulness to the seeds and the maximal sprout length.

2.4 Cytogenotoxicity Test

Assessment of cytogenotoxicity was performed using *Allium cepa*. Triclosan at 40 and 80 µM concentrations was extracted with acetone, dried, liquified to an early volume in distilled water, and tested for toxicity. As demonstrated by Prasad et al., cytogenotoxicity was tested on meristematic root tip cells of a healthy *Allium cepa* (Prasad et al., 2013). Shortly, healthful *Allium cepa* bulbs were preserved in pure water for 72 hours in a dark place after the outer peel and dehydrated roots were deleted. Every day, the distilled water was changed. After that, the best fresh root tips (±2.5 cm) were chosen for the treatment. For 4 hours, the root tips were subjected to two distinctive concentrations of triclosan (40 and 80 µM). As a control, distilled water was applied to the root tips. Following that, root tips were incubated in glacial acetic acid and 95% ethanol for 90 minutes, steeped in 1 N HCl for 10 minutes, and rinsed in decontaminated water. On a microscope slide, the root tips were stained with 1% aceto-orcein and compressed in 45% acetic acid. Bright field microscopy at 1000× magnification was used to look for chromosomal aberrations and cell dissection in the stained root tips. The mitotic index was determined as the percentage of split cells per total number of cells assessed (1250).

2.5 Statistical Analysis

The result was analyzed by means of a one-way ANOVA with Dunnett's multiple comparisons test. All tests were carried out in triplicate. The mean SD (standard deviation) of the results was displayed.

3. RESULTS AND DISCUSSION

3.1 Antibacterial Activity of Triclosan

Because of its concise phase and wide application in biotechnology and microbiology, the *E. coli* strain is a supreme commencing object for assessing triclosan toxicity. The toxicity of triclosan to *E. coli* was determined by calculating the CFU of *E. coli* in Mueller-Hinton agar. This study revealed that at low triclosan concentrations (5-7.5 µM), the impact of triclosan on bacterial growth was low. Nevertheless, as

triclosan concentration increased (60-150 µM), all of the triclosan tested became extremely dangerous to the test bacterium, as demonstrated by the decreasing number of CFU of *E. coli*. These findings suggest that triclosan may be less toxic to bacteria at low concentrations, but triclosan has much stronger anti-bacterial activity at higher concentrations. The higher the triclosan concentrations, the fewer *E. coli* CFU were present (Figure 1).

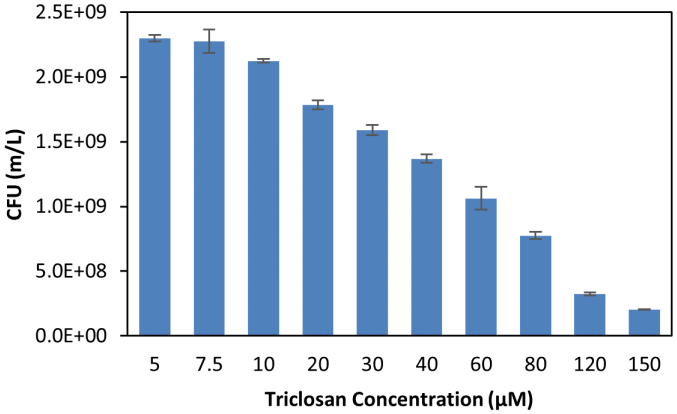

Figure 1. The CFU of *E. coli* Following Exposure to Various Triclosan Doses

Butler et al. discovered that triclosan was toxic to microbial communities in three soils tested (Butler et al., 2012). Despite being consistent with their results, these results could not be matched to theirs due to the several altered experimental conditions used (e.g., triclosan concentrations). In fact, it has been demonstrated that the antibacterial compound triclosan prevents the growth of a diverse group of microorganisms, such as yeasts, algae, and bacteria. It has a long history of use in antiseptics and disinfectants (Chen et al., 2023). Although the exact mechanism is still unknown, it is thought to be connected to their contact with (or possibly infiltration into) the membrane composition of the bacteria. Their integrity of the membrane is compromised by this interaction or penetration, which leads to their amassing on the cell membrane and aggregation (Petkovic et al., 2010).

3.2 Phytotoxicity of Triclosan

Triclosan discharge into the soil not only poses significant concerns for the environment and human health, but it also directly affects soil fertility. As a result, triclosan's phytotoxicity must be evaluated. The phytotoxic activity of triclosan was determined by incubating legume seeds on wet tissue in a petri dish. Phytotoxic activity was detected in seed germinations for 72 hours. This research compared the sprouting length of two legumes, *Vigna radiata* and *Crotalaria juncea*, that had and had not been exposed to triclosan. Figure 2 and Figure 3 show how triclosan affects the sprouting length of *Vigna radiata* and *Crotalaria juncea* at concentrations of 0, 40, and 80 µM. According to our data, the phytotoxic effect of triclosan was the same for both legumes. When seeds were subjected to increasing concentrations of triclosan, sprouting length decreased in comparison to the control. The sprouting length of both *Vigna radiata* and *Crotalaria juncea* decreased by 45-50% and 70-72% when seeds were exposed to triclosan at concentrations of 40 and 80 µM respectively, compared to the control. This result was strengthened by the study of Karnjanapiboonwong et al., which discovered that bean plants (*Phaseolus vulgaris*) cultivated in sand and dirt were poisonous to triclosan (Pullagurala et al., 2018). Our results are compatible with theirs but cannot be equaled because of the varied experimental setups used.

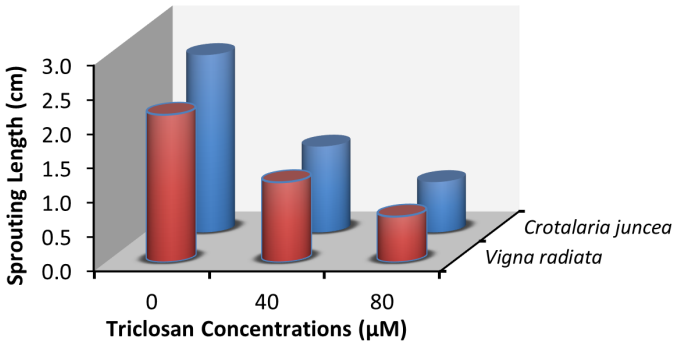

Figure 2. An Evaluation of the Phytotoxicity of Triclosan at Concentrations of 0 (as control), 40, and 80 µM Using *Vigna radiata* and *Crotalaria juncea* Expressed as Sprouting Length

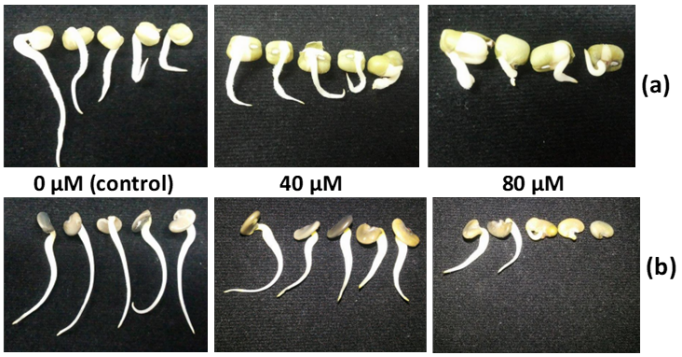

Figure 3. Toxicity Assessment of Triclosan at the Concentrations of 0, 40, and 80 µM on the Sprouting Length of *Vigna radiata* (a) and *Crotalaria juncea* (b)

Since this study focused on the in vitro interactive effect of an increased emission rate of one compound (triclosan), more research on the effects of triclosan in vivo under real environmental conditions is required.

3.3 Cytogenotoxicity

In nature, triclosan and its by products are known to be cytotoxic or carcinogenic (Li, 2021). Based on the results of

Table 1. Chromosomal Aberration and Mitotic Index Examined in Root Tip Cells of *Allium cepa* Treated with Triclosan and Distilled Water

	Concentration of the Compound (µM)	No. of Dividing Cells	Mitotic Index (MI) %	Aberration Index (AI)%	Chromosomal Aberrations			
					ML	MA	AL	CB
Distilled Water (Control)		191[a]	18.2±0.93[a]	0.1±0.03[c]	0	0.63±0.31	0	0
Triclosan	40	108[d]	10.4±0.23[d]	1.2±0.22[a]	3.03±0.86	2.27±0.34	4.35±0.72	2.61±0.41
	80	93[d]	8.8±0.36[d]	1.4±0.11[a]	0	2.99±0.37	2.12±0.34	4.94±0.23

Chromosomal aberration per 1050 cells; Mitotic index (MI) % = (number of dividing cells/total number of cells observed)×100. ML: metaphase lagging chromosome; MA: metaphase aberration; AL: anaphase lagging chromosome; CB: chromosome breaks. Each value represents the mean ± SD of three replicates per treatment. In the same column according to Dunnett's multiple comparison test significant differences at $P < 0.05$ levels over control are indicated by different letters.

this study, triclosan, as an active antimicrobial agent, demonstrated momentous cytotoxicity on *Allium cepa* used in a genotoxicity test. *Allium cepa* assessment is a straightforward, efficient, dependable, and widely recognized method for observing the effects of exposure to probable carcinogens or mutagens on systems. Table 1 shows the mitotic indexes and chromosomal aberrations in, meristematic cells of *Allium cepa* spoiled with triclosan. At 40 µM triclosan concentration, the maximum aberration index (1.2%) was observed, with the lowest percent (10.4%) of mitotic index, and at 80 µM triclosan concentration, the maximum aberration index (1.4%) was observed, with the lowest percent (8.8%) of mitotic index.

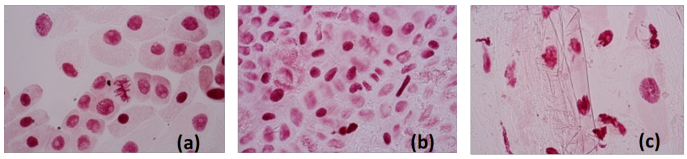

Figure 4. Study of Cytogenotoxicity in *Allium cepa* Meristematic Cells. The Following Conditions were Used: A Control Using Distilled Water (a); Triclosan Treatments at 40 (b) and 80 µM (c)

In contrast to non-toxic distilled water (the control), which showed a greater mitotic index percentage, cells treated with increasing concentrations of triclosan showed a drop in the mitotic index percentage, demonstrating triclosan's toxicity. As concentration rose, the rate of aberration increased. To analyze chromosomal abnormalities, several types of genotoxic damage were investigated, including metaphase lagging chromosomes, metaphase aberrations, anaphase lagging chromosomes, and chromosome breakage. Figure 4(a) shows healthy cell division as seen through microscopic examination of the chromosomes of the control (distilled water) Allium cepa meristematic cells. In both concentrations of 40 and 80 µM, irregular phases were discovered to be the most common anomalies and chromosomal damage (Figures 4(b) and 4(c)). Cells treated with triclosan showed chromosome breakage, abnormal cells, and death cells. The most frequent anomalies were found to be aberrant metaphase, multipolar anaphase, and irregular prophase at concentrations of 40 µM. Chromosome bridges and death were thus observed at 80 µM as a result of insufficient chromosome replication. Similar abnormalities were seen in root cells treated with triclocarban, another antiseptic (Sipahutar and Vangnai, 2017). In cells treated with triclocarban, spindle abnormalities, such as multipolarity, are frequently observed. In cells that have been exposed to the antiseptic chemicals, binucleated cells are present that were most likely produced by multipolar spindles.

4. CONCLUSIONS

We investigated the effects of triclosan on a range of living things, including the bacteria *E. coli*, the legume plants *Vigna radiata* and *Crotalaria juncea*, and the genetic material cells found in the Allium cepa root. According to the latest research, *Allium cepa* roots, *Crotalaria juncea*, and *Vigna radiata* seeds, as well as CFU of *E. coli*, were reliable indicators of the acute toxicity of triclosan. Significant antibacterial action against *E. coli* was present in triclosan. The phytotoxicity of triclosan in bean plants was further demonstrated by the notable reductions in the length of their sprouts. *Allium cepa* root cells used in a cytogenotoxic test showed a wide range of chromosomal aberrations, suggesting that triclosan affects the plant's normal growth. Additional indicators of cytogenotoxic effects in the root meristematic of *Allium cepa* included a reduced Mitotic Index (MI), an increased Aberration Index (AI), other chromosomal abnormalities, and micronuclei. The evaluation of microbiological toxicity, phytotoxicity, and cytogenotoxicity points to the possibility of triclosan having a negative effect on the biota.

5. ACKNOWLEDGEMENT

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