



Article

Dicamba Impacts on Aquatic Bioindicators and Non-Target Plants

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Abstract

Use of dicamba, an auxin-mimic herbicide, has increased in recent years. Both the effects of dicamba on non-target plants and the determination of a biological model to determine the dicamba ecotoxicity dynamics are important to monitor the correct and safe use of this herbicide. The objectives of this study were to determine the effects of low doses (simulating herbicide drift) and to determine the acute toxicity of dicamba to aquatic bioindicator species (*Lemna minor*, *Pomacea canaliculate*, *Hyphessobrycon eques*, and *Danio rerio*) and terrestrial non-target plants (*Cucumis sativus*, *Solanum lycopersicum*, and *Lactuca sativa*) in tropical conditions. Measurements of acute toxicity of dicamba at the concentrations that cause 50% of symptoms of injury (LC50) and other biometric variables were performed. Dicamba was virtually non-toxic to all aquatic bioindicator species ($LC_{50} > 118.0 \text{ mg L}^{-1}$), while it was highly toxic to all terrestrial non-target plants ($LC_{50} < 0.5 \text{ mg L}^{-1}$). Severe injury symptoms (70% to 100%) caused by application of low doses of dicamba were found for all non-target terrestrial plants. Severe injury symptoms (70% to 100%) caused by volatilization of dicamba were found only for *S. lycopersicum*. Since *S. lycopersicum* was found as the most sensitive non-target plant, showing high injury symptoms caused by dicamba and significant injury from volatilized dicamba, this species is suitable for environmental monitoring of dicamba applications.

Keywords: environmental dynamics; pesticides; non-target plants; auxin-mimic herbicides; toxicity



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1. Introduction

The increasing growth of the world population poses a challenge to the maintenance of sustainability, since the human population impacts the environment [1]. A growing human population has required increases in food production by increasing cultivated land and more intensive use of agricultural land [2], requiring more pesticides to be used for crop protection. In Brazil, among environmental pollutants, pesticides account for a large part of environmental exposure to contaminants [3], where 800,652 tons of active ingredients were

sold in 2022 [4], especially herbicides (~50%). Herbicides are pesticides generally used to control and manage target plants (weeds), but they can cause injury to non-target plants (e.g., crops, trees, etc.) and natural environments if misused.

Monitoring the effects of herbicides on the environment is crucial for making decisions about how to use them correctly. It helps to determine the effects of acute and chronic ecotoxicity on non-target organisms. This is the first step to determine environmental dynamics. So, tests with bioindicators of contaminant exposure play a fundamental role in the whole environmental assessment process, as they focus on the interaction between contaminants and environmental features, enabling the ecological assessment of the effects arising from exposure [5]. Bioindicator species can be a key in detecting potential toxic effects of chemicals and are important in ecotoxicological studies [6–9].

Bioindicators of exposure to contaminants are an excellent tool to assess the possible environmental effects of herbicides. The need to assess the effects and impacts of herbicides has encouraged the development of a wide range of aquatic and terrestrial ecotoxicological tests [10]. The use of bioindicators of exposure on Brazilian fauna and flora may be a feasible alternative to herbicide monitoring [11]. Among herbicides, dicamba has been used for control of broadleaf weeds in conventional maize, wheat, and pasture crops since the 1960s [12], and more recently in genetically modified crops [13].

Dicamba (3,6-dichloro-o-anisic acid or 2-methoxy-3,6-dichlorobenzoic acid) belongs benzoic acid family of herbicides. It is a synthetic auxin because it mimics indole acetic acid [14]. It is a selective systemic herbicide, absorbed by the leaves and roots, and translocated throughout the plant by symplastic and apoplastic pathways. Dicamba is a moderately volatile compound [15], and by its volatility, it can cause injury to non-target, susceptible plants, especially under high temperatures and low relative humidity conditions. Dicamba has the following physicochemical properties: 6.6 g L⁻¹ of water solubility; 1.25 × 10⁻⁵ mmHg (25 °C) of vapor pressure; 1.83 dissociation constant (pKa); −1.8 of octanol-water partition coefficient (log P); 233.2 mL g⁻¹ sorption coefficient on organic matter basis (Koc); and 1.0 × 10⁻⁴ Pa m³ mol⁻¹ Henry's Law constant [16]. Its half-life in most environments is >180 days [16].

Despite its high efficacy for the control of many weeds, the possible drift effects of dicamba on non-target susceptible crops have raised public concern [17]. The increasing use of dicamba for control of glyphosate-resistant weeds causes even more concern about its effects on non-target, susceptible crops [18–20]. Previous research has addressed the effects of damage, injury, and toxicity of dicamba to non-target plants [21,22], and the volatilization of different dicamba salts [21,23–25]. Low-dose applications of dicamba were reported to injure many non-target crops, e.g., *Glycine max* [24,26], *Phaseolus vulgaris*, *Solanum tuberosum* [27], *Gossipium hirsutum* [28], *Brassica oleracea*, *Capsicum annuum* [29], *Vitis vinifera* [30], *Citrullus lanatus* [31], *Cucumis sativus*, *Cucumis melo*, *Cucurbita sativus*, and *Citrullus lanatus* [32,33]. In general, the studies were performed in non-tropical conditions. Thus, it is desirable to establish bioindicator species for dicamba contamination in both aquatic and terrestrial environments, especially in tropical conditions.

Dicamba applications used to be restricted due to problems of drift to susceptible crops [24], both as droplets and as volatilized herbicide [34]. Several dicamba-based technical herbicides have been recently registered in Brazil, as pointed out in Act No. 39, of 6 July 2020, published in the Brazilian Government Gazette on 9 July 2020. New formulations of dicamba have significantly reduced herbicide volatility compared to old formulations. For example, new diglycolamine salt formulations are less volatile than dimethylamine salt, which was used in the first formulations [34]. Other new formulations with a N,N-Bis-(3-aminopropyl)methylamine salt have lower volatility [35]. Although

new formulations of dicamba are less volatile, herbicide drift to non-target plants was still observed in the first year of their field applications [36].

The availability of dicamba-resistant soybean, in 2016, increased the use of this herbicide, especially in the United States of America. In Brazil, genetically modified dicamba-resistant soybeans were first commercialized in 2021. Despite concerns about potential dicamba drift issues, growers have adopted this technology. Thus, dicamba is a herbicide with great potential for increased use in agriculture in Brazil and worldwide. Thus, research is needed to assess its environmental dynamics in tropical and subtropical conditions. The assessment of toxicity to non-target organisms allows the characterization of the possible effects of doses used in the field in relation to bioindicators of exposure. In addition, the assessment of apparent volatility and the effect of low-dose applications on non-target plants can help determine the possible routes of dicamba application. Furthermore, injury symptom characterization can be used in environmental monitoring programs.

Our objective was to study the impacts of dicamba on aquatic bioindicators and non-target plants in tropical conditions aiming (i) to determine acute toxicity of dicamba to aquatic bioindicators [common duckweed (*L. minor*), snail (*P. canaliculata*), and two fish species, serpa tetra (*H. eques*), and zebrafish (*D. rerio*)]; (ii) to determine acute toxicity of dicamba to terrestrial non-target plants [cucumber (*Cucumis sativus*), tomato (*Solanum lycopersicum*), and lettuce (*Lactuca sativa*)]; and (iii) to determinate the effect of low-dose applications and volatilization (simulated herbicide drift) on terrestrial, non-target plants.

2. Materials and Methods

A commercial formulation of dicamba (Atectra, BASF, 480 g a.i. ha⁻¹, Brazil) was used in this study.

Aquatic standard bioindicators were used in aquatic toxicity tests of dicamba: *L. minor*, *P. canaliculata*, *H. eques*, and *D. rerio*. Terrestrial non-target plants were used as test plants in terrestrial toxicity tests of dicamba: *C. sativus*, *S. lycopersicum*, and *L. sativa*. These species were also used in greenhouse trials to test the impacts of low doses and volatility of dicamba on non-target plants.

Distilled water was used in toxicity tests. In trials on effects of dicamba on terrestrial, non-target plants, a dystrophic Red Latosol soil with the following physicochemical characteristics was used: pH 4.28; 16.97 g dm⁻³ organic matter; 5.97 g dm⁻³ P; 0.73, 3.87, and 3.22 mmol dm⁻³ of K, Ca and Mg, respectively; 13.79, 0.40, 0.14, 1.0, 1.0, 37.84, and 30.84 mg dm⁻³ of S, Zn, B, Mn, Cu, Fe, and H + Al, respectively; 38.67 mmol dm⁻³ of cation exchange capacity (CEC); and 20.3% of base saturation percentage.

2.1. Dicamba Toxicity Tests for Aquatic Bioindicators

Four bioassays were carried out to study the effects of dicamba on four ecotoxicological standard bioindicators, *L. minor*, *P. canaliculata*, *H. eques*, and *D. rerio*, aiming to determine the acute toxicity of dicamba (LC50). Assays were repeated three times for each species. The study was submitted to and approved by the Ethics Committee for the Use of Animals (ECUA), protocol no. 01_24_CEUA_FEB, in accordance with Law no. 11.794, of 8 October 2008, and Decree No. 6.899, of 15 July 2009.

Plants of *L. minor* were acclimated in a bioassay room at 23.0 to 27.0 °C, under constant illumination of 1000 photosynthetically active radiation for three days [37]. After acclimatization, the plants were disinfected with an aqueous solution of sodium hypochlorite (2%) in distilled water. Four plants with three fronds were then selected and transferred to a glass container with a capacity of 100 mL, containing 50 mL of Hoagland's culture medium [38], and then they were acclimated for 24 h. After this period, 50 mL of the culture

medium was added at the concentrations of 0.1, 1.07, 3.4, 11.6, 36.4, and 118.0 mg L⁻¹ of dicamba. A control treatment was also used. A complete randomized design was used with five replicates.

Toxicity to *L. minor* was assessed 3, 5, and 7 days after exposure (DAE) by counting the number of fronds and occurrences of chlorosis and total necrosis of the fronds [37]. For the sensitivity control, a toxicity test was performed with the reference substance sodium chloride (NaCl) with a 99.9% purity content at concentrations of 0.001; 0.01; 0.1; 0.5; 0.8; and 1.0 g L⁻¹ in triplicate, and one control [37]. The concentration causing 50% lethality at 7 days after exposure (LC50; 7d) of NaCl was 0.09 g L⁻¹, with a confidence interval of 95% between 0.09 and 0.13 g L⁻¹.

Hyphessobrycon eques and *D. rerio* fish and *P. canaliculata* snails were acclimatized in a bioassay room at a temperature of 25.0 to 27.0 °C and a photoperiod of 12 h of light, for ten days, in a box (250.0 L), and continuous aeration was provided by air pumps. The fish were fed commercial feed with 28% crude protein [39], and snails were daily fed the aquatic plant *Hydrilla verticillata*. To determine the lethal concentration of 50% at 48 h after exposure (LC50; 48 h) of dicamba, fish weighing 1.0 ± 0.2 g and snails (*P. canaliculata*) weighing between 2.0 and 4.0 g were used.

A control and dicamba concentrations of 0.1, 1.07, 3.4, 11.6, 36.4, and 118.0 mg L⁻¹ were used to determine the concentration range between zero and 100% mortality. A complete randomized design was used with five replicates. Three fish and five snails per replicate were used in a static exposure system.

Mortality was assessed 24 and 48 h after exposure by removing dead organisms from the containers. Temperature (°C), pH, dissolved oxygen (mg L⁻¹), and electrical conductivity of water (µS cm⁻¹) were monitored [39]. For the sensitivity control, a toxicity test was performed with the reference substance potassium chloride (KCl) with 99.9% purity [39]. Dicamba in concentrations of 0.01, 0.10, 0.56, 1.0, 1.56, and 2.44 g L⁻¹ were used for *H. eques* and *D. rerio*, while concentrations of 0.10, 0.25, 0.50, 0.90, 1.20, and 2.0 g L⁻¹ were used for *P. canaliculata*. All the tests were carried out in triplicate, with a control treatment [37]. The dicamba LC50 values at 48h were 1.26 g L⁻¹ for *H. eques* and *D. rerio* and 1.04 g L⁻¹ for *P. canaliculata*.

2.2. Dicamba Toxicity Tests for Terrestrial Non-Target Plants

Bioassays were carried out to study the effects of dicamba on three non-target plants, *C. sativus*, *S. lycopersicum*, and *L. sativa*, used here as potential bioindicators for tropical conditions, with the aim of determining the acute toxicity of dicamba (LC50). The assays were repeated three times for each species.

Seeds of these species were sown in trays in a commercial substrate (Carolina Soil, Brazil), and the plants were grown until they had three true leaves. Then, they were transplanted into containers with 250 g of sand, and kept in a bioassay room at a temperature of 27.0 ± 2.0 °C and a photoperiod of 12 h of light with illumination of 1000 photosynthetic active radiation [40]. After transplantation, irrigation was performed with 50 mL of Hoagland's culture medium [38], and then they were acclimated for 24 h. After this period, 20 mL of the culture medium was added with the concentrations of dicamba described below. A control treatment was also used.

Dicamba concentrations were 0.05, 0.1, 1.07, 3.4, 11.6, 36.4, and 72.8 mg kg⁻¹ for *C. sativus*; 0.01, 0.06, 0.1, 0.7, 1.07, and 3.4 mg kg⁻¹ for *S. lycopersicum*; and 0.06, 0.1, 0.7, 1.07, 3.4, and 11.6 mg kg⁻¹ for *L. sativa*. A control treatment was also used. A complete randomized design was used with five replicates.

Observations of injury symptoms and mortality were carried out during 14 days after application (DAA), following the methodology of Pádúa et al. [41]. Mortality data was

used to estimate the dicamba lethal concentration of 50% at 14 DAA (LC50;14d). At the end of the growing period, the plants were removed from the containers for measurements of shoot and root length (cm).

2.3. Dicamba Low Doses on Non-Target Plants

Three greenhouse trials were carried out to study the effects of applications of low doses of dicamba on three non-target plants, *C. sativus*, *S. lycopersicum*, and *L. sativa*, aiming to simulate the effects of dicamba droplet drift on non-target plants. Greenhouse trials were repeated at least twice for each species.

Seedlings were formed in polystyrene trays and then transplanted into pots when they had three expanded leaves. Plants grew in 1 L pots filled with a mixture of a dystrophic Red Latosol soil and a commercial organic substrate (Carolina Soil, Brazil) in a 1:1, *v/v* ratio. The pots were kept in a greenhouse (± 30.0 °C).

Ten days after transplanting, dicamba was applied at 120.0, 60.0, 30.0, and 15.0 g a.i. ha⁻¹, corresponding to 50%, 25%, 12.5%, and 6.25% of the lowest recommended dose in the field (0.5 L ha⁻¹, representing 240 g a.i. ha⁻¹). A control (no herbicide application) was also used. The herbicide was applied with a CO₂-pressurized backpack sprayer at a constant pressure of 200 kPa, fitted with ultra-thick application tip with air induction (MUG-03) spray bar nozzles (Magnojet, Ibatí, Brazil), with 150 L ha⁻¹ of spray volume at a temperature of 27.0 °C, relative humidity 56.0%, and wind speed of 11 km/h⁻¹. The greenhouse trials were carried out in a completely randomized design with ten replicates.

After application, assessments for injury symptoms (%) were performed, based on the methodology of Pádua et al. [41], at 1, 3, 7, 14, and 21 DAA. At 21 DAA, the shoot and root lengths (cm) and fresh and dry masses (g) of the plants were measured.

2.4. Dicamba Apparent Volatility on Non-Target Plants

Three greenhouse trials were carried out to study the effects of indirect applications of low doses of dicamba in the three non-targeted plants, *C. sativus*, *S. lycopersicum*, and *L. sativa*, aiming to simulate the effects of dicamba vapor drift on non-target plants. The trials were repeated at least twice for each species.

The effect of dicamba vapor drift on the non-target plants was studied by using the methodology published by Ferreira et al. [42]. One kg of dystrophic Red Latosol was placed in plastic trays, and then the soil surface was irrigated with a 5.9 mm water depth (300 mL per tray). After that, the trays were removed from the greenhouse, and herbicide was applied at 13 h after soil irrigation using a CO₂-pressurized backpack sprayer at a constant pressure of 200 kPa, fitted with spray bar nozzles MUG-03 (Magnojet, Ibatí, Brazil), with a 150 L ha⁻¹ of spray volume under environmental conditions of 21.0 °C, 75% relative humidity, and a wind speed of 9.0 km h⁻¹. Dicamba was applied at 45, 90, 180, 360, and 720 g a.i. ha⁻¹, also using a control treatment. Trials were carried out in a completely randomized design with five replicates.

Immediately after dicamba applications, each tray containing the treated soil was placed inside a polypropylene plastic bag and sealed with a PVC hose outlet (0.1 mm thick and 1.1 cm wide). After that, the trays were transported into the greenhouse, and the hoses were used to connect and allow gas exchange between the plastic bag containing treated soil and the other plastic bag containing two pots with non-target plants (Figure 1).

Concomitantly, non-target plants grew in 1 L pots filled with a mixture of dystrophic Red Latosol soil and a commercial organic substrate (Carolina Soil, Brazil) in a 1:1, *v/v* ratio. In a greenhouse (ca. 30.0 °C), pots were kept sealed in a plastic bag for 36 h and connected to the plastic bags where the trays with treated soil were maintained. After that, the plants were removed from the plastic bags and kept outside for assessment.

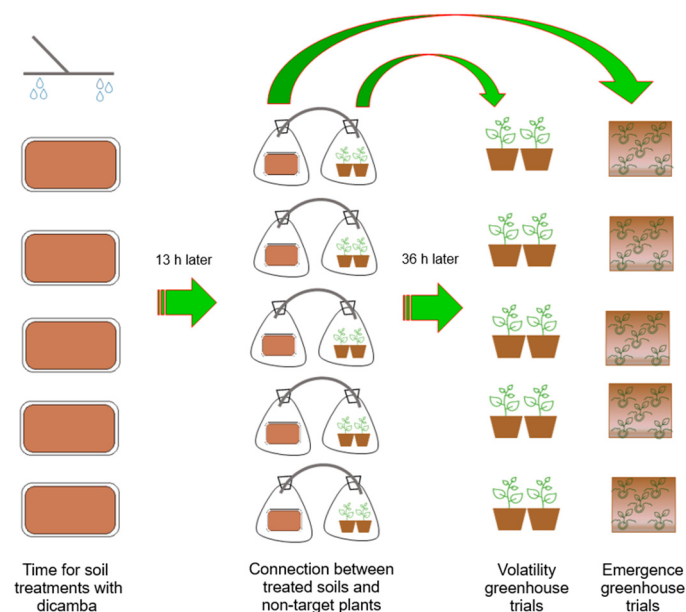


Figure 1. Graphical scheme of the application and assembly of both the volatilization and the emergence greenhouse trials, based on the methodology of Ferreira et al. [42].

Injury symptoms and biometric variables assessments were performed at 14 and 21 DAE, using the control treatment as a comparative pattern. At 28 DAE, the shoot and root length (cm) and the fresh and dry mass (g) were measured.

Additionally, 36 h after dicamba application, the treated soils were used for testing the emergence of *C. sativus* seedlings. From each dose of dicamba, 1.5 kg of soil sample was taken and then placed in plastic containers (300 g per container) for the emergence test. This procedure was performed for each treated soil from bags that were connected to the bags of the three non-target plants. Five containers were used for each soil sample. Five cucumber seeds were sown in each container. Finally, emerging *C. sativus* seedlings were counted at 14 days after sowing (DAS) to test whether dicamba remained in the soil after volatilization and consequently impacted seed germination and seedling emergence.

2.5. Statistical Analysis

Data from dicamba toxicity tests for aquatic and terrestrial organisms were evaluated by linear regression analysis using Trimmed Spearman–Karber software [42] to determine the dicamba concentration that caused 50% lethality (LC50). This toxicity parameter was determined according to the United States Environmental Protection Agency’s classification [43]. Data from greenhouse trials were tested by analysis of variance (ANOVA), and means were compared by Tukey’s test using the software Agroestat [44] to study the effects of low doses and volatilization of dicamba on non-target plants. Statistical significance at the 95% probability level was determined.

3. Results

3.1. Dicamba Toxicity Tests for Aquatic Bioindicators

During the aquatic toxicity bioassays, average water temperature was 23.6 ± 1.2 °C, average electrical conductivity was 246.2 ± 9.1 $\mu\text{S cm}^{-1}$, average dissolved oxygen was 4.3 ± 0.2 mg L^{-1} , and average pH was 7.3 ± 0.2 . No significant change was observed in water quality variables due to the presence of dicamba; i.e., they remained within the parameters recommended by the Organization for Economic Co-Operation and Development [40] for acute toxicity tests.

Mortality of plants of *L. minor* was lower than 17% for all doses of dicamba, while no mortality of either the fish (*H. eques* and *D. rerio*) or the snail (*P. canaliculata*) was observed. The LC50 value at 7d for dicamba was $>118.0 \text{ mg L}^{-1}$ for all the aquatic bioindicator species.

3.2. Toxicity, Low-Dose Applications, and Dicamba Volatility on Cucumber Plants

Dicamba caused light injury symptoms on the *C. sativus* plants in toxicity bioassays (e.g., leaf edge chlorosis, loss of pigmentation, leaf/stem wilting, irregular growth of branches, and irregular leaf development), but it led to a rapid and 100% plant death (Table 1) at 36.4 mg kg^{-1} . Dicamba linearly reduced the full plant length, shoot length, and root length of the *C. sativus* plants at rates of 5.14, 3.60, and $1.54 \text{ cm}(\text{mg kg}^{-1})^{-1}$ (Figure 2A). The linear equation representing the concentration–mortality ratio was $y = 16.739x + 11.94$ ($R^2 = 0.84$), and the calculated LC50 at 14 days was 0.23 mg kg^{-1} .

Table 1. Injury symptoms and mortality caused by dicamba on non-target plant species in terrestrial toxicity tests and low-dose and volatility greenhouse trials.

Non-Target Plants	Cucumber (<i>Cucumis sativus</i>)			Tomato (<i>Solanum lycopersicum</i>)			Lettuce (<i>Lactuca sativa</i>)		
Injury Symptoms	Toxicity	Low Doses	Volatility	Toxicity	Low Doses	Volatility	Toxicity	Low Doses	Volatility
Leaf edge chlorosis	1 (light)	1 (light)	1 (light)	1 (light)	1 (light)	0 (no effect)	0 (no effect)	0 (no effect)	1 (light)
Loss of pigmentation	2 (light)	0 (no effect)	0 (no effect)	2 (light)	0 (no effect)	0 (no effect)	2 (light)	2 (light)	0 (no effect)
Leaf/stem wilting	3 (light)	0 (no effect)	0 (no effect)	3 (light)	0 (no effect)	0 (no effect)	3 (light)	3 (light)	0 (no effect)
Irregular growth of branches and irregular leaf development	4 (light)	4 (light)	0 (no effect)	4 (light)	4 (light)	4 (light)	4 (light)	4 (light)	0 (no effect)
Partial or total necrosis	0 (no effect)	5 (moderate)	0 (no effect)	5 (moderate)	5 (moderate)	0 (no effect)	0 (no effect)	0 (no effect)	0 (no effect)
Leaf wilting	0 (no effect)	6 (moderate)	0 (no effect)	6 (moderate)	6 (moderate)	0 (no effect)	0 (no effect)	0 (no effect)	0 (no effect)
Apical bud necrosis	0 (no effect)	0 (no effect)	0 (no effect)	0 (no effect)	9 (severe)	0 (no effect)	0 (no effect)	9 (severe)	0 (no effect)
Death	10 (severe)	10 (severe)	0 (no effect)	10 (severe)	10 (severe)	0 (no effect)	10 (severe)	10 (severe)	0 (no effect)

Dicamba caused a range of light (e.g., leaf edge chlorosis, irregular growth of branches, and irregular leaf development), moderate (e.g., partial or total necrosis and leaf wilting), and severe (e.g., plant death) injury symptoms on the *C. sativus* plants in the low-dose greenhouse trials (Table 1). Severity of injury increased with time and dicamba dose, ranging from 50.9% (15 g ha^{-1} , 1 DAA) up to 75.4% (120 g ha^{-1} , 21 DAA) (Table 2). Although dicamba did not reduce root length, the lowest dose of the herbicide (15 g ha^{-1}) reduced shoot length by 72%, whole plant fresh mass by 34%, and whole plant dry mass by 37%, while the highest dose (120 g ha^{-1}) reduced shoot length by 87%, fresh mass by 78%, and dry mass by 81% (Table 2).

Dicamba caused only light injury symptoms on the *C. sativus* plants in the greenhouse volatility trials (e.g., leaf edge chlorosis), with no plant death (Table 1). Injury symptoms increased in time, ranging from 1.9% (45 g ha^{-1} , 14 DAE) up to 10.1% (720 g ha^{-1} , 21 DAE), so that the level of injury was also dependent on the dose of dicamba (Table 3). Dicamba volatilization did not significantly reduce shoot and root length and fresh and dry mass compared to the control treatment (Table 3).

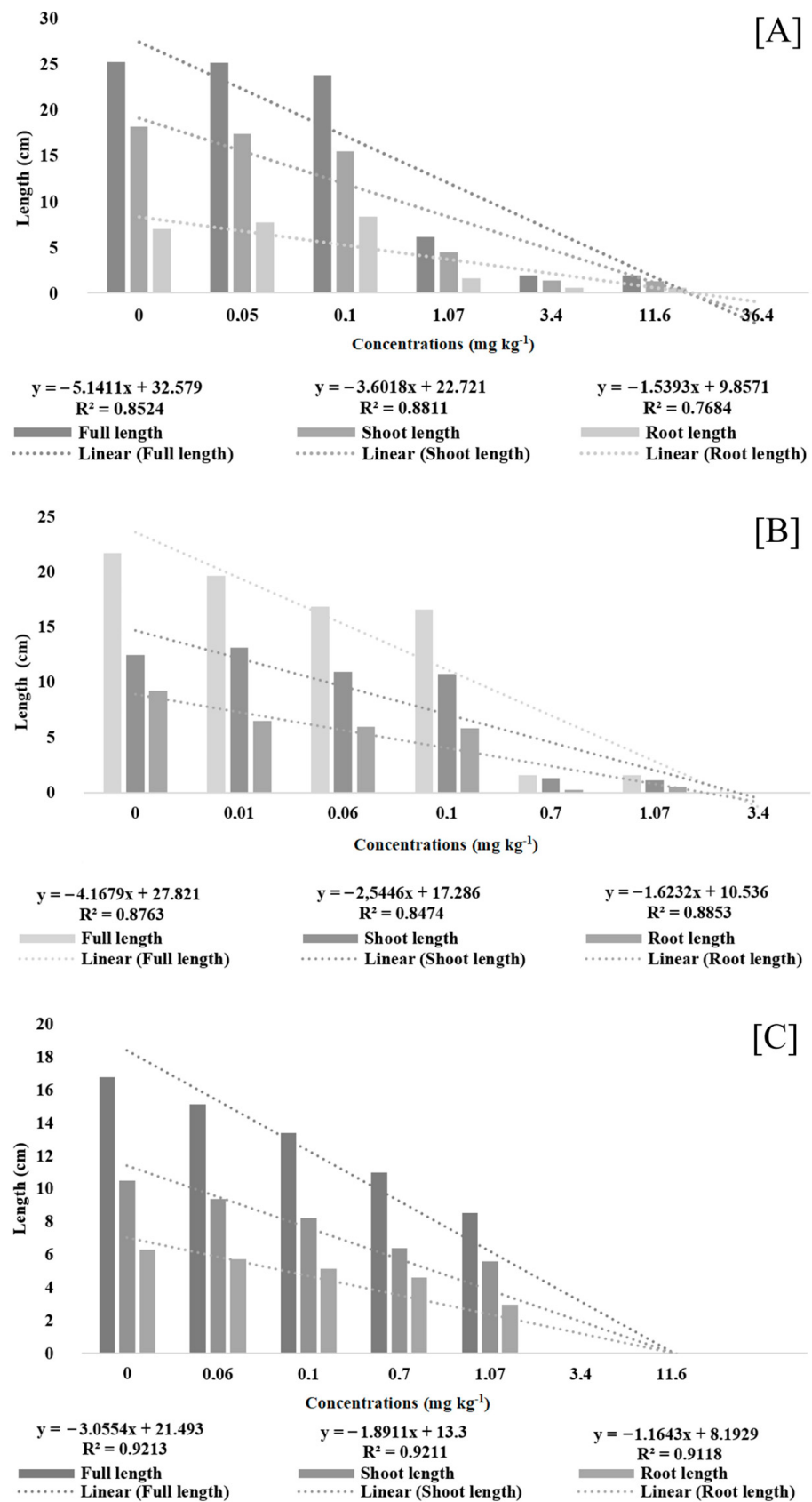


Figure 2. Full length, shoot length, and root length of cucumber (*Cucumis sativus*—A), tomato (*Solanum lycopersicum*—B), and lettuce (*Lactuca sativa*—C) plants at 14 days after exposure to dicamba in toxicity tests.

Table 2. Injury symptoms and biometric variables of non-target plants exposed to low doses of dicamba in greenhouse trials.

Low Doses (g ha ⁻¹)	Injury Symptoms (%) (1 to 21 Days After Application)					Biometric Variables (21 Days After Application)			
	1	3	7	14	21	Shoot Length (cm)	Root Length (cm)	Fresh Mass (g)	Dry Mass (g)
Cucumber (<i>Cucumis sativus</i>)									
0	-	-	-	-	-	99.9 a	24.4 a	60.28 a	9.08 a
15	50.9d	51.9d	54.9d	56.0d	57.0 a	28.3 b	27.0 a	39.97 b	5.72 b
30	51.9c	52.9c	56.1c	57.3ab	62.2 a	20.5 bc	21.6 a	24.69 bc	2.96 bc
60	52.9 b	54.9 b	56.9 b	58.8 ab	72.0 a	13.1 c	17.0 a	15.23 c	1.99 c
120	53.9 a	55.9 a	58.4 a	67.4 a	75.4 a	12.7 c	18.1 a	13.25 c	1.69 c
LSD (5%)	0.38	0.38	0.60	10.38	19.02	12.07	14.12	15.45	2.83
CV	0.60	0.58	0.88	14.39	23.69	27.22	51.39	39.63	50.88
F	166.67 **	333.33 **	85.29 **	3.56 *	2.91 *	150.90 **	1.43 ^{NS}	26.04 **	19.58 **
Tomato (<i>Solanum lycopersicum</i>)									
0	-	-	-	-	-	58.8 a	17.9 a	19.72 a	6.09 a
15	50.9d	51.9d	52.9 c	54.0 c	88.0 ab	25.2 b	18.3 a	14.86 ab	3.07 b
30	52.10 c	53.10 c	54.10 b	65.4 b	66.4 c	20.3 b	16.35 a	10.61 abc	2.35 bc
60	53.10 b	54.10 b	54.10 b	81.0 a	83.80 b	19.9 b	14.2 ab	7.33 bc	1.51 bc
120	54.10 a	54.90 a	56.4 a	81.0 a	96.0 a	8.3 b	4.80 b	2.66 c	0.52 c
LSD (5%)	0.3809	0.3809	0.8301	8.098	10.06	17.58	9.91	9.43	2.29
CV	0.6017	0.5911	1.26	9.54	10.02	52.21	54.49	67.25	66.67
F	187.67 **	168.00 **	45.39 **	38.69 **	22.39 **	19.08 **	5.07 **	7.90 **	13.74 **
Lettuce (<i>Lactuca sativa</i>)									
0	-	-	-	-	-	29.4 a	21.3 a	32.91 a	6.31 a
15	0.0 c	40.5 b	28.10 c	30.00 c	35.00 d	21.5 b	18.45 a	31.78 a	3.94 b
30	0.0 c	41.1 a	39.10 a	49.00 bc	53.00 c	14.0 c	19.3 a	27.15 a	2.91 b
60	20.5 b	34.64 d	37.10 b	69.40 ab	73.00 b	7.5 d	7.9 b	10.28 b	0.95 c
120	21.1 a	35.24 c	39.2 a	77.50 a	100.0 a	0.0 e	0.0 c	0.0 c	0.0 c
LSD (5%)	0.2129	0.2770	0.5039	21.34	13.99	5.40	5.89	7.85	1.37
CV	1.6977	0.6071	1.1661	31.38	17.81	29.36	34.64	30.25	38.28
F	46,275.40 **	2202.27 **	1588.71 **	14.50 **	57.42 **	73.37 **	38.63 **	55.70 **	53.35 **

Different lowercase letters in the column indicate differences between treatments by Tukey's test ($p < 0.05$); LSD = minimum significant difference; CV = coefficient of variation; ^{NS} non-significant at 5% probability; ** significant at 5% probability; * significant at 1% probability.

Table 3. Injury symptoms and biometric variables of non-target plants exposed to apparent volatilization of dicamba in greenhouse trials.

Doses (g ha ⁻¹)	Injury Symptoms (%) (14–21 Days After Exposure)		Biometric Variables (21 Days After Exposure)			
	14	21	Shoot Length (cm)	Root Length (cm)	Fresh Mass (g)	Dry Mass (g)
Cucumber (<i>Cucumis sativus</i>)						
0	-	-	75.8 a	19.0 ab	21.87 ab	3.89 a
45	1.9 e	6.1 e	69.6 a	13.3 b	20.35 b	3.75 a
90	2.9 d	7.1 d	87.1 a	16.1 ab	25.24 ab	4.31 a
180	4.1 c	8.1 c	72.0 a	16.0 ab	24.10 ab	4.38 a
360	5.0 b	9.1 b	72.4 a	20.0 a	29.37 a	4.62 a
720.0	6.1 a	10.1 a	67.60 a	16.7 ab	28.35 a	4.14 a
LSD (5%)	0.35	0.40	26.89	5.71	7.81	1.30
CV	7.07	3.90	27.48	25.65	23.76	23.59
F	345.0 **	250.0 **	1.17 ^{NS}	3.05 *	3.57 **	1.06 ^{NS}
Tomato (<i>Solanum lycopersicum</i>)						
0	-	-	71.8 a	24.8 a	46.62 a	12.09 ab
45	2.0 e	2.9 e	71.0 a	19.4 b	44.96 a	13.62 a
90	30.1 d	70.11 d	73.9 a	19.4 b	38.36 a	10.35 ab
180	30.2 c	72.10 c	70.8 a	19.5 b	35.32 a	8.76 b
360	30.49 c	74.9 b	77.7 a	19.4 b	40.83 a	10.25 ab
720	30.89 a	79.9 a	66.2 a	18.7 b	38.71 a	9.19 b
LSD (5%)	0.035	0.35	12.55	4.94	12.61	4.16
CV	0.11	0.47	13.21	18.52	23.40	29.38
F	2,021,095.00 **	128,648.71 **	1.60 ^{NS}	3.69 **	2.01 ^{NS}	3.40 **

Table 3. Cont.

Doses (g ha ⁻¹)	Injury Symptoms (%) (14–21 Days After Exposure)		Biometric Variables (21 Days After Exposure)			
	14	21	Shoot Length (cm)	Root Length (cm)	Fresh Mass (g)	Dry Mass (g)
Lettuce (<i>Lactuca sativa</i>)						
0	-	-	26.1 a	13.8 a	60.40 a	7.21 ab
45	0.0	6.1 d	25.5 a	13.1 a	55.51 ab	7.02 ab
90	0.0	7.1 c	25.2 a	14.2 a	44.33 b	5.74 b
180	0.0	8.1 b	27.1 a	15.8 a	58.12 ab	8.32 a
360	0.0	9.0 b	22.8 a	15.6 a	44.77 b	6.14 b
720	0.0	10.0 a	25.6 a	13.6 a	54.52 ab	6.78 ab
LSD (5%)	-	0.36	4.62	4.60	14.22	2.01
CV	-	3.50	13.77	24.26	20.32	22.13
F	-	306.50 **	1.67 ^{NS}	1.01 ^{NS}	4.01 **	3.53 **

Different lowercase letters in the column indicate differences between treatments by Tukey's test ($p < 0.05$); LSD = least significant difference; CV = coefficient of variation; ^{NS} non-significant; ** significant at 5% probability; * significant at 1% probability.

3.3. Toxicity, Low-Dose Applications, and Dicamba Volatility on Tomato Plants

Dicamba caused a range of light (e.g., leaf edge chlorosis, loss of pigmentation, leaf/stem wilting, irregular growth of branches, and irregular leaf development), moderate (e.g., partial or total necrosis and leaf wilting), and severe (e.g., plant death) injury symptoms on the *S. lycopersicum* plants (Table 1). Rapid plant death (100%) was observed at 3.4 mg kg⁻¹. On a log scale, dicamba linearly reduced the full length, shoot length, and root length of *S. lycopersicum* plants over a range of application rates from 0 to 3.4 mg kg⁻¹ (Figure 2B). The linear equation representing the concentration–mortality ratio was $y = 22.086x - 17.133$ ($R^2 = 0.90$), and the calculated LC50;14d was 0.1 mg kg⁻¹.

Dicamba caused a range of light (e.g., leaf edge chlorosis, irregular growth of branches, and irregular leaf development), moderate (e.g., partial or total necrosis and leaf wilting), and severe (e.g., apical bud necrosis and plant death) injury symptoms on *S. lycopersicum* plants in low-dose greenhouse trials (Table 1). Injury symptoms increased in time, ranging from 50.9% (15 g ha⁻¹, 1 DAA) up to 56.4% (120 g ha⁻¹, 21 DAA), with the level of injury dependent on the dicamba dose (Table 2). The lowest dose (15 g ha⁻¹) reduced shoot length by 57% and dry mass by 50%, while the half-dose (60 g ha⁻¹) reduced fresh mass by 63%, and the highest dose (120 g ha⁻¹) reduced root length by 73% (Table 2).

Dicamba caused only light injury symptoms on *S. lycopersicum* plants in volatility greenhouse trials (e.g., irregular growth of branches and irregular leaf development), with no plant death (Table 1). Injury symptoms increased in time, ranging from 2.0% (45 g ha⁻¹, 14 DAE) up to 79.9% (720 g ha⁻¹, 21 DAE), with injury level dependent on the dicamba dose (Table 3). Although volatilization of dicamba did not significantly reduce shoot length and fresh mass, volatilization from soils treated with the highest dose (720 g ha⁻¹) reduced root length by 25% and dry mass by 24% compared to the control (Table 3).

3.4. Toxicity, Low-Dose Applications, and Dicamba Volatility on Lettuce Plants

Dicamba caused light injury symptoms on the *L. sativa* plants in toxicity bioassays (e.g., loss of pigmentation, leaf/stem wilting, irregular growth of branches, and irregular leaf development), but it led to a rapid, 100% plant death (Table 1) at 3.4 mg kg⁻¹. On a log scale, dicamba linearly reduced the full length, shoot length, and root length of the *C. sativus* plants a doses from 0 to 11.6 mg kg⁻¹ (Figure 2C). The linear equation representing the concentration–mortality ratio was $y = 17.18x + 0.3533$ ($R^2 = 0.97$), and the calculated LC50;14d was 0.41 mg kg⁻¹.

Dicamba caused light (e.g., loss of pigmentation, leaf/stem wilting, irregular growth of branches, and irregular leaf development) and severe (e.g., apical bud necrosis and plant death) injury symptoms on the *L. sativa* plants in low-dose greenhouse trials (Table 1). Injury symptoms

increased with time (except for 15 g ha⁻¹), ranging from 20.5% (60 g ha⁻¹, 1 DAA) up to 100% (120 g ha⁻¹, 21 DAA) (Table 2). The lowest dose of the herbicide (15 g ha⁻¹) reduced shoot length by 22% and dry mass by 38%, while the half-dose (60 g ha⁻¹) reduced root length by 73% and fresh mass by 53% (Table 2). The highest dose (120 g ha⁻¹) reduced all the biometric variables by 100% (Table 2).

Dicamba caused only light injury symptoms on the *S. lycopersicum* plants in greenhouse volatility trials (e.g., leaf edge chlorosis), with no plant death (Table 1). Injury symptoms were observed only at 21 DAE, ranging from 6.1% (45 g ha⁻¹) up to 10.0% (720 g ha⁻¹, 21 DAE) (Table 3). Although volatilization of dicamba did not significantly reduce shoot and root length, volatilization from soils treated with 90 and 360 g ha⁻¹ reduced fresh mass by 27% and 26%, and dry mass by 20% and 15%, respectively, compared to the control (Table 3).

3.5. Effects of Dicamba-Treated Soils on Cucumber Seedling Emergence

Using herbicide-treated soils from greenhouse volatility trials (as in Figure 1) for emergence tests of *C. sativus* seedlings, differences between both soils and doses were found (Table 4). Seedling emergence was reduced at 90 g ha⁻¹ and higher doses in Soil 1, at 720 g ha⁻¹ in Soil 2 and Soil 3, and also at 90 g ha⁻¹ in Soil 3. Different levels of seedling emergence were observed between soils at 90 g ha⁻¹ and higher doses. In general, average seedling emergence was higher in Soil 2 (79%) than in Soil 1 (44%) and Soil 3 (46%). When using any soil treated with dicamba at 90 g ha⁻¹ and higher doses, seedlings emerged (except for 720 g ha⁻¹ in Soil 3), but the seedling growth and development were impaired.

Table 4. Emergence (%) of cucumber (*Cucumis sativus*) seedlings at 14 days after sowing in dicamba-treated soils.

Doses (g ha ⁻¹)	Soil 1	Soil 2	Soil 3
0	100 Aa	100 Aa	100 Aa
45	67 Aab	71 Aab	100 Aa
90	46 * Bbc	95 * Aab	36 * Bbc
180	29 * Bbc	81 * Aab	50 * ABab
360	38 * Bbc	95 * Aab	45 * Bab
720	25 * ABc	52 * Ab	0 Cc
Average	41 B	79 A	46 B

Obs. Soil 1 originated from the dicamba-treated soil of the volatilization greenhouse trial for *Cucumis sativus*; Soil 2 originated from the dicamba-treated soil of the volatilization greenhouse trial for *Solanum lycopersicum*; Soil 3 originated from the dicamba-treated soil of the volatilization greenhouse trial for *Lactuca sativa*. Different uppercase letters in lines and lowercase letters in columns indicate differences between treatments by Tukey's test ($p < 0.05$); LSD (Doses vs. Soils) = 31.59% and LSD (Soils vs. Doses) = 31.59%, where LSD is the least significant difference; CV = 37.57, where CV is the coefficient of variation; F(DosesxSoils) = 3.49 at 5% probability of errors. * seeds germinated, but seedlings did not grow/develop.

4. Discussion

4.1. Dicamba Toxicity for Aquatic Bioindicators and Terrestrial Non-Target Plants

The acute aquatic toxicity results (Table 1 and Figure 2) corroborated the results of Tunić et al. [45] and Sanford et al. [46]. In addition, *L. minor* was less susceptible to dicamba than they found it was to 2,4-D [45,46], another auxin-mimic herbicide. Since both the dicamba and 2,4-D herbicides have the same mode of action [45,46], this difference may be due to differences in chemical formulations, uptake and metabolism, activity at the molecular targets (auxin receptors), or/and experimental conditions. Acute toxicity of dicamba for *L. minor* was similar to *Lemna gibba* and *L. minor* exposed to simazine (LC50 = 276.1 mg L⁻¹), and to *Lemna paucicostata* exposed to atrazine

($LC_{50} = 342.2 \text{ mg L}^{-1}$) [47]. These herbicides have different modes of action from dicamba, but the results are similar for the same plant species. So, it indicates that plants of the same genus can show similar responses to herbicides with different modes of action. The LC_{50} values of dicamba for *H. eques* and *D. rerio* were equivalent to dicamba ($LC_{50} = 2264.98$) and 2,4-D (919.81 mg L^{-1}) on *Cnesterodon decemmaculatus* [48], and also to dicamba ($LC_{50} = 285.0 \text{ mg L}^{-1}$) for *D. rerio* embryos [49]. The *P. canaliculata* snails were more susceptible to dicamba than imazapyr [50]. These organisms were not susceptible to auxin-mimic herbicides (e.g., dicamba and 2,4-D), but they were susceptible to ALS inhibitor herbicides (e.g., imazapyr).

The acute terrestrial toxicity results (Table 1 and Figure 2) revealed that *S. lycopersicum* was more susceptible to dicamba than *C. sativus* and *L. sativa* (Figure 1 and Table 2). Dicamba was more toxic to *S. lycopersicum* than 2,4-D, reducing root biomass by 60% [51]. Dicamba was also more toxic to *R. sativus* than clomazone, reducing shoot length by 32% [52]. In addition, dicamba was more toxic to *S. lycopersicum* than sulfentrazone ($LC_{50} = 1.40 \text{ mg kg}^{-1}$) was to *Raphanus sativus* [53], which was classified as moderately toxic [43]. Dicamba reduced the fresh and dry mass of *S. lycopersicum* plants by 100%, being more toxic than sulfentrazone was to *R. sativus* plants [53]. Other studies indicate that *C. sativus* and *L. sativa* were potential bioindicators for herbicide toxicity, such as sulfentrazone [53], bentazon, and atrazine [52]. However, *S. lycopersicum* was more susceptible to dicamba than those plant species. This high level of response of *S. lycopersicum* to dicamba indicates that this species could be a highly sensitive bioindicator for dicamba in tropical, terrestrial environments.

The results of the aquatic and terrestrial toxicity assays, using four aquatic ecotoxicological standard bioindicators and three non-target plants, revealed that the lethal concentration by 50% of dicamba was $>118.0 \text{ mg L}^{-1}$ for all the aquatic bioindicators, while it was $<0.5 \text{ mg L}^{-1}$ for all the non-target plants. The aquatic acute toxicity results indicate that dicamba is not a significantly toxic pesticide in aquatic habitats [43] and has low potential for environmental harm to aquatic ecosystems. Conversely, our terrestrial acute toxicity results indicate that dicamba has high potential for injuring susceptible terrestrial non-target plant species [43], with implications for potential disruption of terrestrial ecosystems.

4.2. Low-Dose Applications of Dicamba on Non-Target Plants

Dose–response experiments using low doses of herbicides have been used to simulate the effects of herbicide droplet drift on non-target plants [29,30,54–58]. The occurrence of herbicide drift outside the targeted spray area has occurred since the first year of use of dicamba in resistant crops in the USA [59], promoting a series of reports of injuries to susceptible plant species [60]. The main reasons for herbicide drift are incorrect spraying methods, due to the use of inappropriate spray nozzles, inappropriate height of the spray bar, or incorrect application speed [61]. Spray drift to non-target areas occurs mainly with the use of small droplet size (e.g., $<100 \mu\text{m}$) [62] under inappropriate wind, temperature, and relative humidity conditions [60]. Herbicide drift usually occurs under conditions of high temperature, low relative humidity, and/or wind speed above 3.73 mph [57]. Thus, applicators must take care to use correct application equipment and methods under appropriate environmental conditions in order to avoid spray drift problems on non-target, susceptible plants.

The results of low-dose applications of dicamba on non-target *C. sativus*, *S. lycopersicum*, and *L. sativa* (Tables 1 and 2) corroborated the findings previously reported on the high toxicity of this herbicide to terrestrial, susceptible plants. Dicamba was found to severely injure *L. sativa* and *G. max* plants at doses from 28.0 g ha^{-1} [55], and *Olea europaea* [57] and *Fragaria* $\times$ *ananassa* [58] have also been reported to be highly sensitive. We observed a range of injury symptoms, e.g., leaf edge chlorosis, loss of pigmentation, leaf/stem wilting,

irregular growth of branches and irregular leaf development, partial or total necrosis, leaf wilting, apical bud necrosis, and death (Table 1). Some of these symptoms were previously found in *S. lycopersicum* exposed to 2,4-D [51], also an auxin mimic herbicide, and these symptoms have also been reported for other plant species. These injury symptoms are characteristic of the auxin-mimic herbicides [63].

Growth, as measured by shoot length, root length, fresh mass, and dry mass, was reduced due to exposure to simulated dicamba drift, the magnitude of the effect depending on the plant species (Table 2). Dicamba drift reduced shoot length of *L. sativa* plants by 65% [56], as well as plant height and shoot length of *G. max* plants by 50% and 65%, respectively [55]. Biometric variables were also found to be reduced by 2,4-D and/or dicamba on *Beta vulgaris*, *C. sativus*, *S. lycopersicum* [64], *Citrullus lanatus*, *Phaseolus vulgaris* [32], *Solanum tuberosum* [28], etc. Auxin-mimic herbicides cause growth anomalies in susceptible broadleaf species [65]. Branch stem epinasty and leaf cupping are the most characteristic and common symptoms, often leading to plant death [57]. Effects on the vegetative performance of the susceptible plants and the injury symptoms during low-dose exposure indicate the occurrence of changes in plant metabolism after exposure to dicamba.

A series of processes involving enzymatic activation and expression of certain genes is involved in the mode of action of auxin-mimic herbicide [60]. These herbicides mimic the effects of indole acetic acid, causing great alterations in gene expression [66], and consequently trigger a wide range of distinct responses in plant metabolism [67]. Auxin-mimic herbicides promote the synthesis of ethylene and abscisic acid by activating the transcription of responsive genes encoding enzymes for their synthesis [68,69]. Excessive ethylene and abscisic acid concentrations trigger a series of biochemical and physiological events (e.g., stomatal closure and senescence) [70], stimulating meristematic cell abnormal growth, blocking phloem vascular tissue, and depriving the plant of essential nutrients [71]. As a result, uncontrolled growth of some cells occurs, leading to epinasty, leaf cupping, and other irregular tissue responses [65]. At high doses, auxin-mimic herbicides also inhibit carbon assimilation and photochemical reactions [65], preventing electron transport to Photosystem II, decreasing carotene and chlorophyll concentrations, and increasing the xanthophyll/carotene ratio, causing plant senescence [72]. The range of metabolic and physiological changes caused by dicamba can consequently lead to the death of susceptible plants.

4.3. Effects of Apparent Volatility of Dicamba and Dicamba-Treated Soils on Non-Target Plants

The results of apparent volatility of dicamba on non-target plants (Tables 1 and 3) showed that dicamba vapor drift can cause low (*C. sativus* and *L. sativa*) and moderate-high (*S. lycopersicum*) levels of injury on susceptible terrestrial plants. The method for testing dicamba apparent volatility used in this study was previously performed to simulate the effects of the vapor drift of mixtures of herbicides and adjuvants on soybean plants [42]. It was based on methodology recommendations by Ouse et al. [73] with adaptations described by Ferreira et al. [42]. This methodology successfully indicated dicamba volatilization on susceptible soybean plants, and the authors concluded that it is feasible for evaluating dicamba volatilization [42] on other non-target plants as well. Volatilization is one of the main challenges of using auxin-mimic herbicides [34] (e.g., dicamba). The volatilization is most influenced by vapor pressure of the compound. Acidic dicamba is moderately volatile [15], and cases of dicamba injuries in susceptible plants have been reported [21].

The volatility of dicamba depends on several factors, including the amount applied, temperature, humidity, formulation, and the surface on which it was deposited [74]. Since the volatility is dependent on pH [34], changes in the pH of the herbicide solution modify the proportion of ionized molecules. Thus, the increase in pH reduces the proportion of

nonionized molecules that are more prone to volatilization [34]. In addition, the type of salt and formulation influence volatilization of dicamba. As reported for 2,4-D, the ester formulation is more volatile than the amine formulation of dicamba [34]. The literature indicates that the diglycolamine and N,N-bis-(3-aminopropyl)methylamine dicamba salts are less susceptible to volatilization than the dimethylamine dicamba salt [18,24,26,34]. Finally, relative humidity seems to play an important role in the distance at which a plant is injured by dicamba vapor drift [24].

Dicamba volatilization caused more injuries on *S. lycopersicum* than *C. sativus* and *L. sativa* plants, and significant reductions in the root length of *S. lycopersicum* were observed (Table 3). This result corroborated the observations in both the toxicity assays and low-dose trials, showing *S. lycopersicum* as a potential bioindicator for tropical, terrestrial environments. Studies on the effects of volatilized herbicides on non-target plants are scarce. Injury by 71% was observed in both *S. lycopersicum* (in this study) and susceptible *Glycine max* used as a bioindicator by Zaccaro-Gruener [75]. As susceptible *G. max* is highly susceptible to dicamba [76], our results indicate that *S. lycopersicum* also shows high susceptibility to dicamba, since they are both broadleaf species.

Dicamba has a relatively low affinity for soil particles due to its low organic carbon partition coefficient [76]. Since it is present in soil moisture after spraying, dicamba can volatilize from the soil [34] and can also be absorbed by the plant root system [77]. In this study, although dicamba volatilized from dicamba-treated soils, the herbicide remained in the soil, affecting both seedling emergence (e.g., Soil 3, 720 g ha⁻¹) and especially seedling growth and development (all soils, >90 g ha⁻¹) of *C. sativus* (Table 4). We have found no previous studies testing the effects of dicamba-treated soils on seed germination and seedling emergence of non-target plants, even though dicamba is sometimes used as a pre-emergence application for weed control [34,77,78]. It is not surprising that both germination and emergence of non-target plants are affected, even if herbicide volatilization from dicamba-treated soil has occurred.

5. Conclusions

The acute toxicity pattern of dicamba for aquatic and terrestrial organisms was determined. In addition to the symptoms of damage, the toxicity of dicamba on test plants in toxicity, low-dose, and volatility studies allows us to understand the environmental dynamics of this herbicide. Herbicide application conditions can expose organisms to different effects of low doses, volatilization, and acute and chronic effects. Thus, establishing the effects of droplet or vapor drift, as in this study, can help to establish the effects of the ecological impacts of dicamba, as demonstrated by plant–herbivore interactions (*Abutilon theophrasti*) and the significant increase in the abundance of the silverleaf whitefly (*Bermisia tabaci*), which feeds on phloem, in plants exposed to dicamba at drift rates of 0.5% and 1.0% of the field dose (5.6 g ha⁻¹) [79].

Dicamba was found to be virtually non-toxic to the aquatic bioindicator organisms, but it was highly toxic to terrestrial plants. *Solanum lycopersicum* was the most susceptible plant species. Low doses applied directly to all the plant species caused severe injuries, especially to *S. lycopersicum*. In addition, *S. lycopersicum* showed the highest sensitivity among the study species to dicamba volatilization. Therefore, our results confirm with different methods and conditions (in this case, under tropical conditions) from those previously published that *S. lycopersicum* is a sensitive bioindicator species for environmental monitoring of off-target dicamba movement after its application.

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Abbreviations

The following abbreviations are used in this manuscript:

LC50	Lethal concentration 50%
EC50	Effective concentration 50%
DMA	Dimethylamine salt
DGA	Diglycolamine salt
BAPMA	N,N-Bis-(3-aminopropyl)methylamine
CTNBio	The national biosafety technical committee
BASF	Badische Anilin- und Sodafabrik
pKa	Electrolytic dissociation constant
K _{ow} or P	Partition coefficient
L _{pg} Pow	Partition coefficient
K _{oc}	Sorption coefficient on organic matter basis
pH	Hydrogenionic potential
P	Phosphorus
K	Potassium
Ca	Calcium
Mg	Magnesium
CTC	Cation exchange capacity
V%	Saturation percentage
S	Phosphate
Zn	Zinc
B	Boron
Mn	Manganese
Cu	Copper
Fe	Iron
H + Al	Hydrogen + aluminum
°C	Degrees celsius
NaCl	Sodium chloride
mL	Milliliter

mg	Milligrams
L	Liter
g	Grams
ECUA	Ethics Committee for the Use of Animals
KCl	Potassium chloride
μS	Microsecond
cm	Centimeter
Kg	Kilo
DAA	Days after application
CRD	Completely randomized design
CO ₂	Carbon dioxide
km	Kilometer
PVC	Addition polymer polyvinyl chloride
DAE	Days after exposure
DAS	Days after sowing
ANOVA	Analysis of variance
ha	Hectare
LSD	Minimum significant difference
CV	Coefficient of variation

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