

Original Article

Comparative Assessment of 2,4-D Phytotoxicity on Six Green Manure Plants

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Abstract

Pesticides are widely used in various crops and are associated with environmental impacts. The herbicide 2,4-D, for example, can cause phytotoxicity in different plant species, including leguminous plants that can be used for green manure. In this context, the present research aimed to identify the phytotoxicity of 2,4-D during the initial growth of six terrestrial plant species, including *Cajanus cajan*, *Canavalia ensiformis*, *Crotalaria juncea*, *Dolichos lablab*, *Lupinus albus*, and *Mucuna pruriens*. The studied plants were exposed to different concentrations of 2,4-D (from 0.8 to 4.0 mg kg⁻¹) for 14 days after the germination in control treatments. The endpoints were the germination percentage, the shoot and root lengths, and fresh and dry biomass. Fresh and dry biomass results were interpreted in terms of the Tolerance Index (TI), and the concentrations of 50% inhibition effect (EC₅₀) were calculated for germination and shoot and root length. Four species showed germination above 87.5% for all treatments (EC₅₀ > 4.0 mg kg⁻¹), while this endpoint was below 50% for *C. cajan* and *L. albus* at the highest concentrations. The growth of all species was impaired when they were exposed to 2,4-D, with an increase in herbicide concentrations leading to a decrease in the lengths of shoots and roots. The effect on shoot length was more pronounced for *L. albus*, *M. pruriens*, and *C. cajan* (EC₅₀ < 2.0 mg kg⁻¹). The root length inhibition was more evident for *C. cajan*, *C. juncea*, *L. albus*, and *D. lablab* (EC₅₀ < 1.0 mg kg⁻¹). Swollen roots and yellowing of leaves stood out among the qualitative symptoms of phytotoxicity. Among the tested plants, *M. pruriens* was the most tolerant (TI > 60% in all treatments), standing out as the species with the most significant potential for green manure in crops during the off-season.

Keywords: Biomass; Ecotoxicity; Leguminous; Pesticide; Soil Ecotoxicology.

INTRODUCTION

The expansion of agriculture has been associated with several environmental implications, including the intensive use of pesticides that affect human and environmental health (Rani *et al.*, 2021; Alengebawy *et al.*, 2021; Ogura *et al.*, 2022a). For instance, 2,4-D (2,4-dichlorophenoxyacetic acid) is a widely used herbicide in various crops, including sugarcane, corn, wheat, and soybeans, and it is one of the most consumed pesticides in Brazil (IBAMA, 2019; MAPA, 2022). It is an auxin-simulating herbicide used to effectively control broadleaf weeds, leading to uncontrolled plant growth and disrupted cell division (Pazmiño *et al.*, 2012; Song, 2014;

Ogura *et al.*, 2023). Despite its relatively short half-life (from 7 to 14 days) in soils (Starrett *et al.*, 2000), this herbicide can also affect non-target plants and other terrestrial species (Triques *et al.*, 2021).

The toxic effects of 2,4-D on plants have already been reported, particularly on eudicots due to its mode of action. The herbicide caused a 74% root length inhibition in *Phaseolus vulgaris* at 0.3 mg L⁻¹ (Cenkci *et al.*, 2010), and it substantially decreased the root length of *Raphanus sativus* at 2.35 mg kg⁻¹ (Triques *et al.*, 2021). However, monocotyledons are also susceptible to the impacts of 2,4-D, as the root length inhibition was reported for *Allium cepa* at 2.35 mg kg⁻¹ (Triques *et al.*, 2021), and chromosomal disturbances were detected for *Triticum*

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aestivum at 0.3 mg L⁻¹ (Kumar, 2010) and *A. cepa* from 5 to 20 mg L⁻¹ (Ateeq *et al.*, 2002). Previous studies evidenced the effects of 2,4-D on green manure species, highlighting that root length inhibition was a more sensitive endpoint to assess the phytotoxicity of 2,4-D. For *Dolichos lablab* and *Lupinus albus*, significant decreases in shoot and root lengths were observed at concentrations above 0.06 mg kg⁻¹, while *Canavalia ensiformis* exhibited a reduction in root length starting at 1.00 mg kg⁻¹ (Ogura *et al.*, 2023). The root length of *Crotalaria juncea* was significantly reduced when plants were cultured in a solution containing 2,4-D at 0.2 mg L⁻¹ (Costa *et al.*, 2022).

Green manure plants are relevant for soil cover and sustainable agricultural practices by enhancing soil fertility, suppressing weeds, and cycling nutrients (Cherr *et al.*, 2006; Sandhya Rani *et al.*, 2022). Their extensive root systems also aid in stabilizing soil structure and preventing erosion, and they can be effectively incorporated into crop rotation systems, given their rapid growth, biomass production, and ease of management (Mueller and Thorup-Kristensen, 2001; Ma *et al.*, 2021). In addition, these plants can also potentially uptake soil contaminants, including pesticides (Mendes *et al.*, 2020; Teófilo *et al.*, 2020). Pesticide residues in agricultural soils can impair their development, particularly when considering the potential application during the off-season, within crop rotation systems, or at the edges of fields. The selection of the most tolerant green manure species can improve the application of these plants for soil cover and remediation.

Therefore, this study aimed to assess the phytotoxicity of 2,4-D on six green manure plant species, including *Cajanus cajan* (pigeon pea), *Canavalia ensiformis* (sugar bean pig), *Crotalaria juncea* (sunn hemp), *Dolichos lablab* (lablab bean), *Lupinus albus* (lupine), and *Mucuna pruriens* (velvet bean). In this sense, we evaluated the initial plant growth under five concentrations of 2,4-D, comparing species using the Tolerance Index (TI) and effect concentrations. We expected to discuss potential applications of green manure plants, considering the effects of 2,4-D residue exposure in sugarcane cultivation soils.

METHODS

The soil of study was collected from the Center for Water Resources and Environmental Studies at the University of São Paulo (CRHEA/USP), in Itirapina, Brazil. The site has no history of contamination, and the soil has already been previously used and characterized in the study by Ogura *et al.* (2023). The soil was manually sieved using a metal sieve with a 4 mm opening to remove larger particles, then placed in an oven at 60°C for

24 h to remove fauna and moisture. Classified as Oxisol, the study soil contains 31.9% of clay, 21.7% of silt, 46.4% of sand, 11% of organic matter, a pH of 5.2, a cation exchange capacity (CEC) of 3.52 cmolc kg⁻¹, a density of 1.48 g cm⁻³, and an electrical conductivity of 50.1 μS cm⁻¹.

The seeds of the six plant species were obtained from Pirai Sementes® (Piracicaba, São Paulo, Brazil), including *Cajanus cajan*, *Canavalia ensiformis*, *Crotalaria juncea*, *Dolichos lablab*, *Lupinus albus*, and *Mucuna pruriens*. The tests were adapted from ISO 11269-2 (ISO 2012) and were conducted under controlled conditions of temperature (25 ± 2°C), artificial light (4500 lx), and photoperiod (12h:12h). The studied commercial formulation was DMA®806 BR (Dow AgroSciences Industrial Ltda), with a maximum recommended dosage of 3.5 L ha⁻¹ (equivalent to 4.7 mg kg⁻¹). Five concentrations were established below this dosage, including 0.8, 1.6, 2.4, 3.2, and 4.0 mg kg⁻¹. All results throughout the manuscript were expressed in terms of the nominal concentration of 2,4-D, according to the estimated proportion based on the manufacturer's label.

In each experimental unit (400 mL non-toxic polystyrene plastic pots), 300 g of soil were used, with four replicates for each concentration. Ten seeds were planted in each pot, approximately 2 cm below the soil surface. Due to the size of the *C. ensiformis* and *M. pruriens* seeds, only seven were planted in each pot. The experiments lasted 14 days after germinating at least five seedlings in the control samples; the test was considered valid if the germination rates in the control were above 70%. Seedlings were removed so that five plants remained evenly spaced in the containers to maintain adequate space for plant development. Soil moisture was maintained three times a week at approximately 20% through manual irrigation with distilled water. After the exposure period, plants were removed from the soil, and the measured endpoints were the germination percentage, shoot and root length, and fresh and dry biomass (for shoot and root). Fresh biomass weight was obtained immediately after plant harvesting and the removal of soil particles by rinsing with tap water, followed by distilled water; the excess water was then removed with a paper towel. Dry biomass weight was measured after plant samples were kept in a drying oven at 80°C for 24 h. The difference between the fresh and dry weights indicates the moisture content in the plants.

The germination percentage was calculated from the difference between the number of seeds planted and the number of seedlings developed in each pot, considering the removed seedlings. The effects of phytotoxicity on species exposed to 2,4-D were also determined based on the shoot and root length inhibition percentage. The Tolerance Index (%) was obtained by ratio between the

biomass of a given treatment and the biomass in the control. In this case, the replicates formed a composite sample, so the standard deviation was not calculated. Statistical analyses were performed using the Statistica 7 software. Data normality was checked using the Shapiro-Wilk test, and homogeneity was assessed using the Levene test. The data underwent analysis of variance (ANOVA), followed by Tukey's post-hoc test to consider whether the treatments presented statistically significant differences in plant growth ($p < 0.05$). The Effect concentration (EC_{50}) represents the concentration of a substance at which the growth of plants is inhibited by 50%, considering the endpoints of germination, shoot length, and root length. Estimates were made based on nonlinear regression using the three-parameter logistic curve model, with a 95% confidence interval (lower and upper limits).

The biomass production estimate for the studied species (in $t ha^{-1}$) was calculated proportionally based on data from the seed supplier. The growth of plants subjected to each treatment was compared to the expected biomass in the control group. The expected biomass production provided by the supplier was as follows: 20 to 30 $t ha^{-1}$ for *C. cajan*, 20 to 40 $t ha^{-1}$ for *C. ensiformis*, 40 to 60 $t ha^{-1}$ for *C. juncea*, 15 to 30 $t ha^{-1}$ for *D. lablab*, 20 to 30 $t ha^{-1}$ for *L. albus*, and 40 to 50 $t ha^{-1}$ for *M. pruriens*. Based on these ranges, the biomass yield for each species under the various treatments was proportionally estimated, allowing the assessment of the 2,4-D influence on biomass production relative to the expected baseline.

RESULTS

The average values and standard deviation of germination percentages are presented in Table 1. Four species achieved germination between 87.5% and 100%, and treatments were similar to the control even at the highest doses ($p > 0.05$). However, the germination of *C. cajan* decreased considerably when the plants were exposed to all concentrations of 2,4-D (reductions of approximately 48%, 59%, 66%, 69%, 72%, in order) compared to the control ($p < 0.05$). A significant decrease was observed for *L. albus* at 2.4 $mg kg^{-1}$ and higher concentrations (reductions of approximately 65%, 53%, and 73%, respectively) ($p < 0.05$).

The shoot and root lengths of plants exposed to 2,4-D are shown in Figure 1. There was a dose-response phytotoxicity as the increase of 2,4-D caused a significant decrease in plant growth. All treatments for *L. albus*, *C. juncea*, *D. lablab*, *C. cajan*, and *M. pruriens* differed statistically from the control ($p < 0.05$). However, for *C. ensiformis*, statistical differences ($p < 0.05$) occurred at

2.4 $mg kg^{-1}$ for shoots and 4.0 $mg kg^{-1}$ for shoots and roots. In most species, the impacts on root length were more evident than those on shoot length, except for *C. ensiformis* and *M. pruriens*. Even though *C. ensiformis* showed no impact up to 3.2 $mg kg^{-1}$, its shoot length showed an inhibition of 98.8% at the highest concentration. On the other hand, other species were more sensitive to 2,4-D. In the case of *C. cajan*, even at the lowest concentration, the roots were significantly affected (86.7% of inhibition, $p < 0.05$).

Table 2 presents the EC_{50} calculated for germination, shoot length, and root length for the six plant species. The EC_{50} for germination was 0.91 [0.21–1.61] $mg kg^{-1}$ for *C. cajan* and 2.64 [2.17–3.09] $mg kg^{-1}$ for *L. albus*. The EC_{50} for the germination for all the other species was calculated to be higher than the maximum tested concentration ($> 4.0 mg kg^{-1}$). The EC_{50} for shoot length was 1.14 [0.90–1.38] $mg kg^{-1}$ for *L. albus*, 1.35 [0.18–2.52] $mg kg^{-1}$ for *M. pruriens*, 1.59 [0.93–2.25] $mg kg^{-1}$ for *C. cajan*, 2.99 [2.77–3.21] $mg kg^{-1}$ for *D. lablab*, and 3.49 [2.85–4.13] $mg kg^{-1}$ for *C. ensiformis*. The EC_{50} for the shoot length of *C. juncea* was above 4.0 $mg kg^{-1}$. On the other hand, the EC_{50} for *C. cajan* (0.001 $mg kg^{-1}$) and *C. juncea* (0.004 $mg kg^{-1}$) were significantly low, and their 95% confidence intervals were not determined. The EC_{50} for root length was 0.31 [0.10–0.51] $mg kg^{-1}$ for *L. albus*, 0.46 [0.33–0.59] $mg kg^{-1}$ for *D. lablab*, and 3.70 [3.04–4.37] $mg kg^{-1}$ for *C. ensiformis*, while the value for *M. pruriens* was above 4.0 $mg kg^{-1}$.

The TI represents the plants' tolerance to the five tested concentrations of 2,4-D compared to the control, considering fresh and dry biomass (Figure 2). In this scenario, *M. pruriens* produced biomass even when exposed to 2,4-D (tolerance higher than 60% in all treatments for both fresh and dry biomass). *C. ensiformis* was tolerant in biomass production when it was exposed to 2,4-D up to 3.2 $mg kg^{-1}$. On the other hand, *C. cajan* and *D. lablab* were considered the least tolerant in terms of biomass production. In our study, both fresh and dry biomass values exhibited a similar pattern across different concentrations of 2,4-D.

The expected total biomass production of the studied species was estimated for different concentrations of 2,4-D based on their TI values and data provided by Pirai Sementes®. According to the manufacturer, *C. juncea* and *M. pruriens* have the most potential for biomass production (up to 60 and 50 $t ha^{-1}$, respectively). However, considering the influence of 2,4-D on biomass production, *M. pruriens* remains the most prolific biomass producer even at the highest concentration (up to 34.87 $t ha^{-1}$). As it was highly sensitive to 2,4-D, *C. cajan* showed the least potential for biomass production (up to only 2.74 $t ha^{-1}$ at the highest concentration).

Table 1. Percentages of germination (%) of the six plant species exposed to five concentrations of 2,4-D

Treatment	0.0 mg kg ⁻¹	0.8 mg kg ⁻¹	1.6 mg kg ⁻¹	2.4 mg kg ⁻¹	3.2 mg kg ⁻¹	4.0 mg kg ⁻¹
<i>C. cajan</i>	72.6 ± 22.2	37.5 ± 5.0 *	30.0 ± 0.0 *	25.0 ± 5.8 *	22.5 ± 5.0 *	20.0 ± 0.0 *
<i>C. ensiformis</i>	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
<i>C. juncea</i>	97.5 ± 5.0	97.5 ± 5.0	92.5 ± 9.6	97.5 ± 5.0	92.5 ± 9.6	87.5 ± 9.6
<i>D. lablab</i>	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
<i>L. albus</i>	100.0 ± 0.0	97.5 ± 5.0	100.0 ± 0.0	35.0 ± 5.8 *	47.5 ± 15.0 *	27.5 ± 5.0 *
<i>M. pruriens</i>	96.5 ± 7.1	100.0 ± 0.0	96.5 ± 7.1	96.5 ± 7.1	100.0 ± 0.0	100.0 ± 0.0

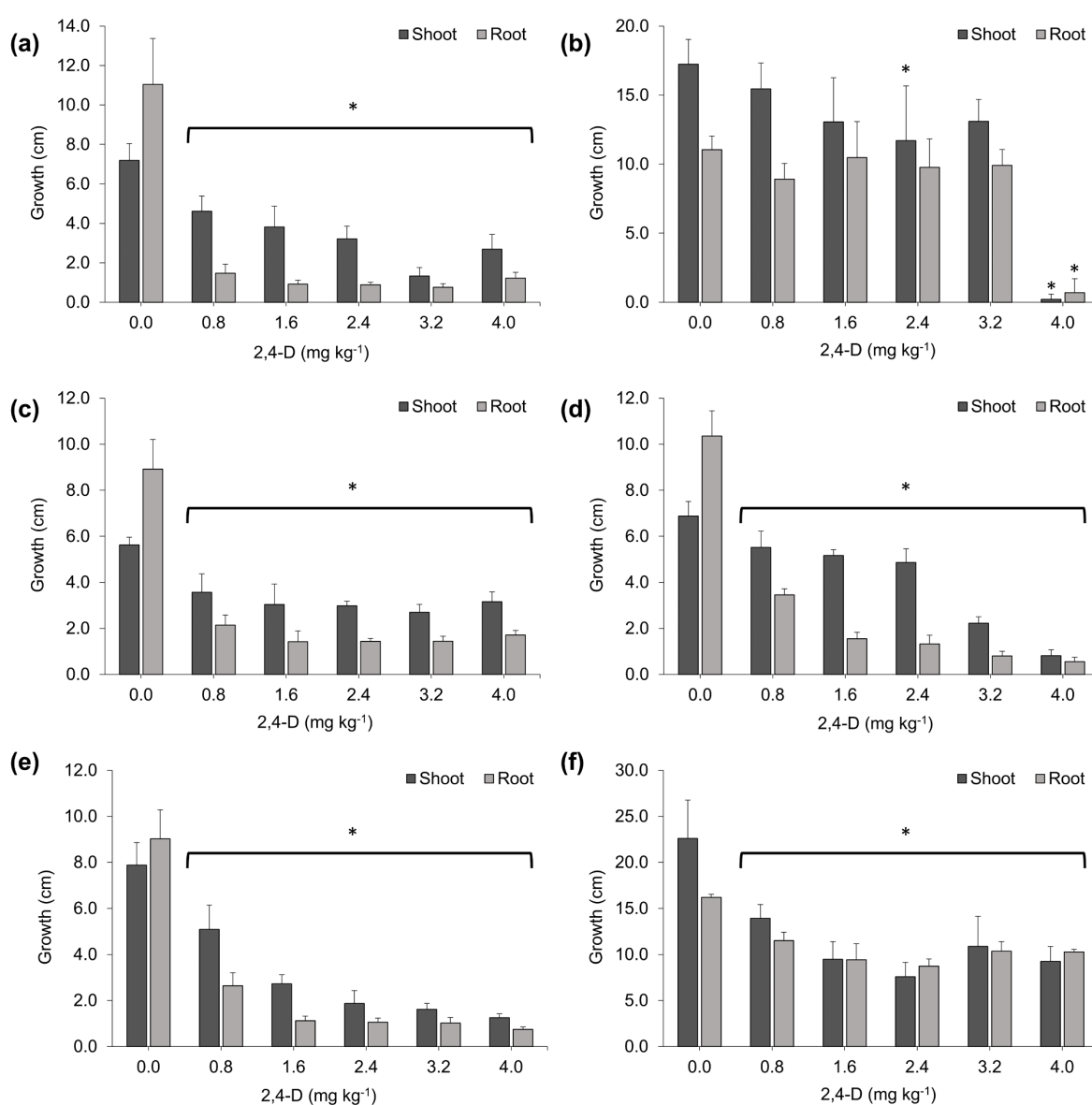
* Treatments that were statistically different from the control ($p < 0.05$).**Figure 1.** Average ± standard deviation for shoot and root length of the plant species exposed to five concentrations of 2,4-D. a) *C. cajan*, b) *C. ensiformis*, c) *C. juncea*, d) *D. lablab*, e) *L. albus*, and f) *M. pruriens*. Asterisks (*) denote treatments significantly different from the control ($p < 0.05$).

Table 2. Effect concentrations (and the 95% confidence intervals) corresponding to 50% inhibition of germination, shoot length, and root length for six plant species exposed to five concentrations of 2,4-D (from 0.8 to 4.0 mg kg⁻¹).

Plant species	Effect Concentrations - EC ₅₀ (mg kg ⁻¹)		
	Germination	Shoot length	Root length
<i>C. cajan</i>	0.91 (0.21–1.61)	1.59 (0.93–2.25)	0.001 (n.d.)
<i>C. ensiformis</i>	> 4.00	3.49 (2.85–4.13)	3.70 (3.04–4.37)
<i>C. juncea</i>	> 4.00	> 4.00	0.004 (n.d.)
<i>D. lablab</i>	> 4.00	2.99 (2.77–3.21)	0.46 (0.33–0.59)
<i>L. albus</i>	2.64 (2.17–3.09)	1.14 (0.90–1.38)	0.31 (0.10–0.51)
<i>M. pruriens</i>	> 4.00	1.35 (0.18–2.52)	> 4.00

n.d. = not determined.

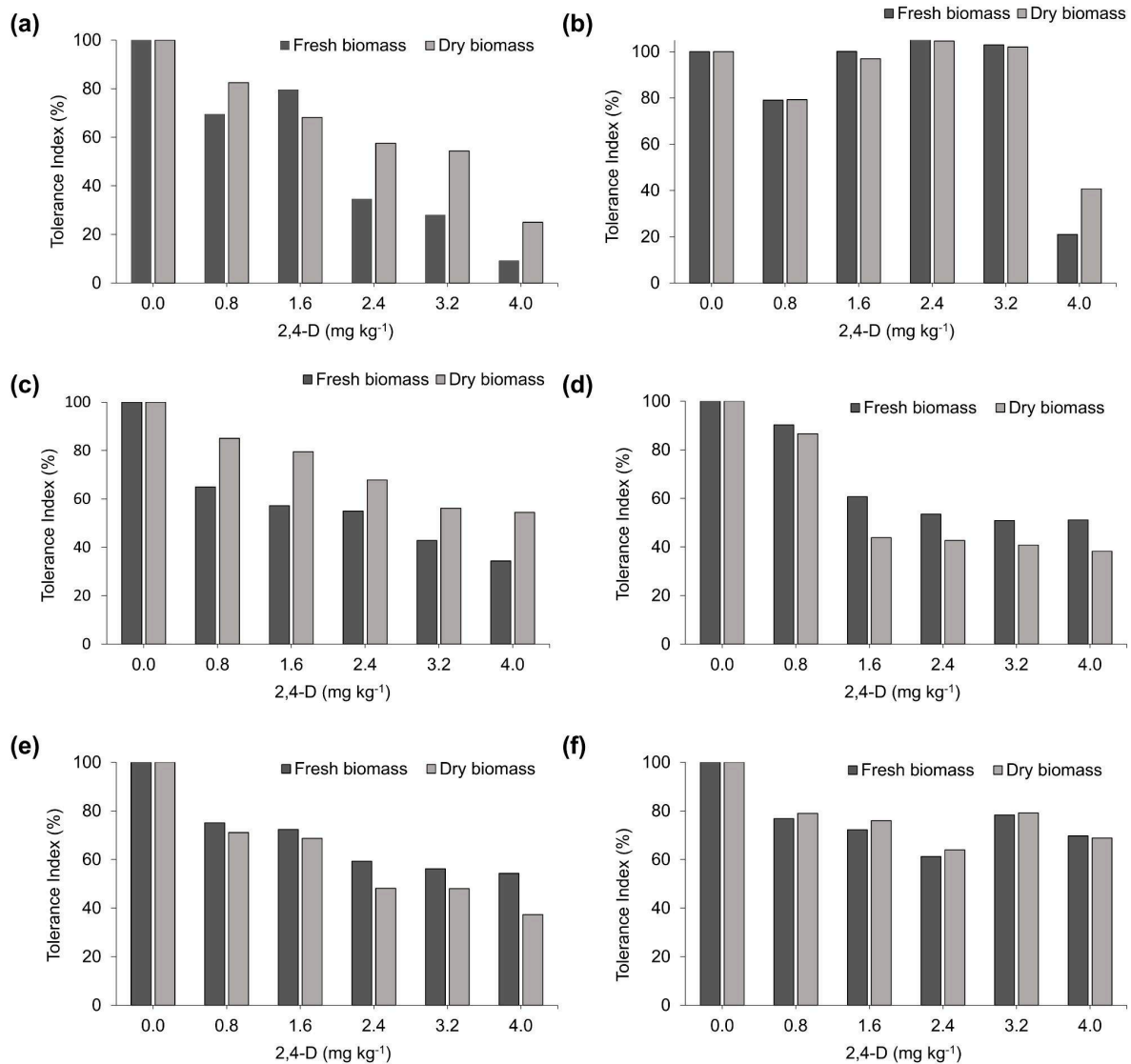
**Figure 2.** Fresh and dry biomass of plant species exposed to five concentrations of 2,4-D. a) *C. cajan*, b) *C. ensiformis*, c) *C. juncea*, d) *D. lablab*, e) *L. albus*, and f) *M. pruriens*.

Table 3. Estimated biomass production for the studied species ($t\ ha^{-1}$), considering the data provided by the manufacturer.

2,4-D	<i>C. cajan</i>	<i>C. ensiformis</i>	<i>C. juncea</i>	<i>D. lablab</i>	<i>L. albus</i>	<i>M. pruriens</i>
0 mg kg ⁻¹	20 – 30	20 – 40	40 – 60	15 – 30	20 – 30	40 – 50
0.8 mg kg ⁻¹	13.9 – 20.85	15.8 – 31.6	25.96 – 38.95	13.54 – 27.07	15.02 – 22.53	30.72 – 38.4
1.6 mg kg ⁻¹	15.91 – 23.87	20.01 – 40.02	22.86 – 34.29	9.09 – 18.2	14.74 – 21.71	28.88 – 36.1
2.4 mg kg ⁻¹	6.91 – 10.36	21.79 – 43.58	22 – 33	8.02 – 16.04	11.86 – 17.78	24.47 – 30.59
3.2 mg kg ⁻¹	5.61 – 8.41	20.59 – 41.18	17.13 – 25.7	7.63 – 15.26	11.23 – 16.84	31.31 – 39.14
4.0 mg kg ⁻¹	1.83 – 2.74	4.19 – 8.39	13.74 – 20.6	7.66 – 15.33	10.85 – 16.28	27.9 – 34.87

DISCUSSION

Several factors, such as water availability, temperature, light, and hormonal balance, control plant germination. Silva *et al.* (2018) observed that 2,4-D negatively impacted soybean seed germination, reducing the vigour and physiological quality of the seeds due to hormonal changes. In our study, only *C. cajan* and *L. albus* showed significant reductions ($p < 0.05$) in germination (higher than 48% and 53%, respectively), which may indicate a loss of physiological quality. However, this endpoint was less sensitive than shoot and root length inhibition for most of the tested species. For shoot length, our estimated EC_{50} for *C. ensiformis*, *D. lablab*, and *L. albus* (3.49, 2.99, and 1.14 mg kg⁻¹, respectively) were higher than the values achieved by a previous study with the same natural soil (0.34, 0.34, and 0.68 mg kg⁻¹, respectively) (Ogura *et al.*, 2023). For root length, the EC_{50} in our study for these species (3.70, 0.46, and 0.31 mg kg⁻¹, respectively) were also higher than those estimated by Ogura *et al.* (2023) (0.98, 0.05, and 0.02 mg kg⁻¹, respectively). These results indicate that the effects of exposure to 2,4-D were more evident for roots than shoots for *D. lablab* and *L. albus*, but the same pattern was not observed for *C. ensiformis*.

Since the studied green manure species are eudicots, they can be more susceptible to 2,4-D than monocot species due to its mode of action as an auxin-simulating herbicide (Song, 2014). For example, Triques *et al.* (2021) demonstrated that the eudicot species *Raphanus sativus* is more sensitive to 2,4-D ($EC_{50} = 3.20$ mg kg⁻¹) than the monocot species *Allium cepa* ($EC_{50} = 6.54$ mg kg⁻¹), considering their results for shoot length inhibition in sandy soils. Nonetheless, the effects of 2,4-D on non-target species should not be neglected, especially considering its potential long-term effects on plant development. Irrigation of *P. vulgaris* and *Zea mays* with

water contaminated with 2,4-D at 447 $\mu\text{g kg}^{-1}$ resulted in 45% and 17% inhibition of root length, respectively (Ogura *et al.*, 2022b). Interferences in plant growth may also arise from an auxin overdose in plant tissues, disrupting homeostasis and affecting interactions with other hormones (Grossmann, 2010).

The interaction between soil and pesticides can be dynamic and dependent on several physical and chemical processes, and it can significantly influence phytotoxicity. Due to its high solubility, 2,4-D can exhibit very high mobility in soils, with a soil sorption coefficient (K_{oc}) ranging from 20 to 136 (Kim *et al.*, 2021). It has a relatively short half-life (from 7 to 14 days), but organic matter can contribute to the sorption of 2,4-D in soils, leading to the herbicide being retained for longer periods (Starrett *et al.*, 2000; Prado *et al.*, 2001). Another study indicated an enhancement in 2,4-D toxicity for *R. sativus* when soil moisture was high (80% of WHC), consequently increasing its bioavailability (Triques *et al.*, 2022). The authors estimated EC_{50} from 0.01 to 0.06 mg kg⁻¹ for *R. sativus*, which is a lower value than those obtained for all green manure species (from 1.14 to 4.51 mg kg⁻¹).

In our study, all species suffered some visual symptoms of phytotoxicity, especially in the roots, which showed swelling even at lower concentrations (Figure 3). Root length reduction, irregular growth, and swelling were also identified by a previous study with *C. ensiformis*, *D. lablab*, and *L. albus* (Ogura *et al.*, 2023). Constantin *et al.* (2007) observed symptoms in *Gossypium hirsutum* at 3 days after the application of 2,4-D (from 0.84 to 26.88 g ha⁻¹), including wrinkling of the youngest leaves, purpleness of leaves and stems, and epinasty of cotton petioles. After 14 days, they observed yellowing and necrosis of flower buds, cracks in the stem, and thickening of the main root and base of the stem. Root length inhibition, swelling, and leaf yellowing were

reported in *Plectranthus neochilus* at concentrations above 14.28 kg ha^{-1} , but the latter was not identified in our tested species (Ramborger *et al.*, 2017). Moreover, swelling roots might increase fresh biomass by retaining water, without reflecting an actual increase in plant biomass production.

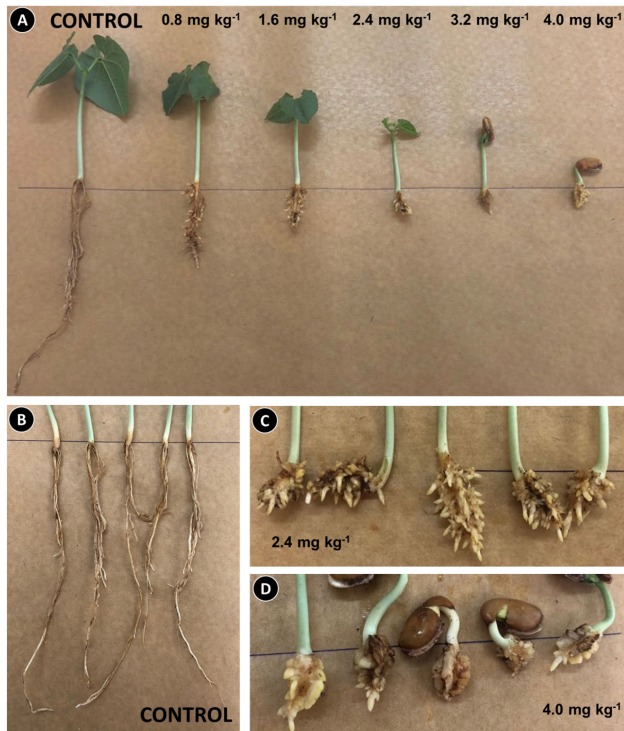


Figure 3. Visual analysis of phytotoxic symptoms on *D. lablab* exposed to five concentrations of 2,4-D compared to the control (a) and zoomed-in views of the roots in control (b) compared to those at 2.4 mg kg^{-1} (c) and 4.0 mg kg^{-1} (d).

Silva *et al.* (2017) exposed *Lactuca sativa* to various pesticides, including 2,4-D (from 0 to 900 g ha^{-1}), and the biomass reduction was greater than 60% in all treatments, after 10 days of application. Duarte Júnior and Coelho (2008) studied *C. ensiformis*, *C. juncea*, and *M. aterrima* in a sugarcane cultivation area where the soil was fertilized and not contaminated. Under these conditions, *C. juncea* exhibited the highest biomass production, as evaluated by dry biomass. However, in our study, *M. pruriens* outperformed *C. juncea* in terms of tolerance to 2,4-D, resulting in higher biomass production. Therefore, *M. pruriens* may be a better alternative for green manure in cases where soils are contaminated with pesticide residues.

CONCLUSION

Although all six studied species have the potential for application in green manure, some were more adversely affected by the phytotoxic effects caused

by 2,4-D. Therefore, selecting the most tolerant species, such as *C. ensiformis* and *M. pruriens*, for application in sugarcane cultivation is the most advantageous option for improving soil quality and consequently, crop productivity. However, some limitations of our research include the lack of quantification of the studied pesticide in both soil and plants, which could provide insights into its transport and bioaccumulation. Scenarios of co-contamination with other pesticides or contaminants could also enhance the toxicity of 2,4-D. Additionally, our research was conducted under controlled laboratory conditions, which may not fully reflect field applications and natural environmental variations. To address these limitations, future studies could also extend the duration of experiments to evaluate the variation in phytotoxicity effects over time.

AUTHOR CONTRIBUTIONS

LMP: Investigation, Writing – Original Draft; **ELGE:** Supervision, Resources, Writing – Reviewing and Editing; **APO:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – Original draft preparation, Reviewing and Editing, Supervision.

REFERENCES

- Alengebawy A, Abdelkhalek ST, Qureshi SR, Wang MQ (2021) Heavy Metals and Pesticides Toxicity in Agricultural Soil and Plants: Ecological Risks and Human Health Implications. *Toxics* 2021, Vol 9, Page 42 9:42. <https://doi.org/10.3390/TOXICS9030042>
- Ateeq B, Abul Farah M, Niamat Ali M, Ahmad W (2002) Clastogenicity of pentachlorophenol, 2,4-D and butachlor evaluated by *Allium root* tip test. *Mutat Res Toxicol Environ Mutagen* 514:105–113. [https://doi.org/10.1016/S1383-5718\(01\)00327-8](https://doi.org/10.1016/S1383-5718(01)00327-8)
- Keneci S, Yildiz M, Cigerci IH, et al (2010) Evaluation of 2,4-D and Dicamba genotoxicity in bean seedlings using comet and RAPD assays. *Ecotoxicol Environ Saf* 73:1558–1564. <https://doi.org/10.1016/j.ecoenv.2010.07.033>
- Cherr CM, Scholberg JMS, McSorley R (2006) Green Manure Approaches to Crop Production: A Synthesis. *Agron J* 98:302–319. <https://doi.org/10.2134/AGRONJ2005.0035>
- Constantin J, De Oliveira RS, Fagliari JR, et al (2007) Efeito de subdoses de 2,4-D na produtividade do algodão e suscetibilidade da cultura em função de seu estágio de desenvolvimento. *Eng Agrícola* 27:24–29. <https://doi.org/10.1590/S0100-69162007000200004>
- Costa D dos S, Silva CCA da, Direito ICN, Victório CP (2022) In vitro development of green manures:

- phytotoxicity and remediation of 2,4-D. *Brazilian J Dev* 8:40551–40568. <https://doi.org/10.34117/BJDV8N5-507>
- Duarte Júnior JB, Coelho FC (2008) Adubos verdes e seus efeitos no rendimento da cana-de-açúcar em sistema de plantio direto. *Bragantia* 67:723–732. <https://doi.org/10.1590/S0006-87052008000300022>
- Grossmann K (2010) Auxin herbicides: current status of mechanism and mode of action. *Pest Manag Sci* 66:113–120. <https://doi.org/10.1002/PS.1860>
- IBAMA (2019) Instituto Brasileiro do Meio Ambiente dos Recursos Naturais Renováveis Boletins 2009–2018 Vendas Ingredientes Ativos
- ISO (2012) International Organization and Standardization. Soil quality - Determination of the effects of pollutants on soil flora - Part 2: Effects of contaminated soil on the emergence and early growth of higher plants, ISO 11269-2.
- Kim S, Chen J, Cheng T, et al (2021) PubChem in 2021: New data content and improved web interfaces. *Nucleic Acids Res* 49:D1388–D1395. <https://doi.org/10.1093/nar/gkaa971>
- Kumar S (2010) Effect of 2,4-D and Isoproturon on chromosomal disturbances during mitotic division in root tip cells of *Triticum aestivum* L. *Cytol Genet* 44:79–87. <https://doi.org/10.3103/S0095452710020027>
- Ma D, Yin L, Ju W, et al (2021) Meta-analysis of green manure effects on soil properties and crop yield in northern China. *F Crop Res* 266:108146. <https://doi.org/10.1016/J.FCR.2021.108146>
- MAPA (2022) Ministério da Agricultura, Pecuária e Abastecimento. AGROFIT. http://agrofit.agricultura.gov.br/agrofit_cons/principa1_agrofit_cons. Accessed 22 Jun 2022
- Mendes KF, Maset BA, Mielke KC, et al (2020) Phytoremediation of quinclorac and tebuthiuron-polluted soil by green manure plants. *Int J Phytoremediation* 1–8. <https://doi.org/10.1080/15226514.2020.1825329>
- Mueller T, Thorup-Kristensen K (2001) N-Fixation of Selected Green Manure Plants in an Organic Crop Rotation. *Biol Agric Hortic* 18:345–363. <https://doi.org/10.1080/01448765.2001.9754897>
- Ogura AP, da Silva AC, Castro GB, et al (2022a) An overview of the sugarcane expansion in the state of São Paulo (Brazil) over the last two decades and its environmental impacts. *Sustain Prod Consum* 32:66–75. <https://doi.org/10.1016/J.SPC.2022.04.010>
- Ogura AP, Lima JZ, Silva LCM da, et al (2023) Phytotoxicity of 2,4-D and fipronil mixtures to three green manure species. *J Environ Sci Heal Part B* 58:1–11. <https://doi.org/10.1080/03601234.2023.2178789>
- Ogura AP, Moreira RA, Silva LCM, et al (2022b) Irrigation with water contaminated by sugarcane pesticides and vinasse can inhibit seed germination and crops initial growth. *Arch Environ Contam Toxicol* 1:12. <https://doi.org/10.1007/s00244-022-00914-x>
- Pazmiño DM, Romero-Puertas MC, Sandalio LM (2012) Insights into the toxicity mechanism of and cell response to the herbicide 2,4-D in plants. *Plant Signal Behav* 7:425–427. <https://doi.org/10.4161/psb.19124>
- Prado AGS, Vieira EM, Rezende MO de O (2001) Monitoring of the harmful concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) in soils with and without organic matter. *J Braz Chem Soc* 12:485–488. <https://doi.org/10.1590/S0103-50532001000400007>
- Ramborger BP, Ortis Gularte CA, Rodrigues DT, et al (2017) The phytoremediation potential of *Plectranthus neochilus* on 2,4-dichlorophenoxyacetic acid and the role of antioxidant capacity in herbicide tolerance. *Chemosphere* 188:231–240. <https://doi.org/10.1016/j.chemosphere.2017.08.164>
- Rani L, Thapa K, Kanojia N, et al (2021) An extensive review on the consequences of chemical pesticides on human health and environment. Elsevier Ltd
- Sandhya Rani Y, Jamuna P, Triveni U, et al (2022) Effect of in situ incorporation of legume green manure crops on nutrient bioavailability, productivity and uptake of maize. *J Plant Nutr* 45:1004–1016. <https://doi.org/10.1080/01904167.2021.2005802>
- Silva DRO da, Silva EDN da, Aguiar ACM de, et al (2018) Drift of 2,4-D and dicamba applied to soybean at vegetative and reproductive growth stage. *Ciência Rural* 48:8. <https://doi.org/10.1590/0103-8478CR20180179>
- Silva PV Da, Nicolle G de, Ribeiro NM, et al (2017) Efeitos fitotóxicos sobre a cultura do alface ocasionados pela deriva simulada de herbicidas utilizados no milho. *Rev Ensaios Pioneiros* 1:1–13. <https://doi.org/10.24933/REP.V111.21>
- Song Y (2014) Insight into the mode of action of 2,4-dichlorophenoxyacetic acid (2,4-D) as an herbicide. *J Integr Plant Biol* 56:106–113. <https://doi.org/10.1111/jipb.12131>
- Starrett SK, Christians NE, Austin T Al (2000) Movement of herbicides under two irrigation regimes applied to turfgrass. *Adv Environ Res* 4:169–176. [https://doi.org/10.1016/S1093-0191\(00\)00020-4](https://doi.org/10.1016/S1093-0191(00)00020-4)
- Teófilo TM da S, Mendes KF, Fernandes BCC, et al (2020) Phytoextraction of diuron, hexazinone, and sulfometuron-methyl from the soil by green manure species. *Chemosphere* 256:127059. <https://doi.org/10.1016/j.chemosphere.2020.127059>
- Triques MC, Oliveira D, Goulart BV, et al (2021)

Assessing single effects of sugarcane pesticides
fipronil and 2,4-D on plants and soil organisms.
Ecotoxicol Environ Saf 208:111622
<https://doi.org/10.1016/j.ecoenv.2020.111622>

Triques MC, Ribeiro F, Oliveira D de, et al (2022) The
ecotoxicity of sugarcane pesticides to non-target soil
organisms as a function of soil properties and
moisture conditions. *Int J Environ Res* 16:1–17.
<https://doi.org/10.1007/S41742-022-00433-6>

