Botânica Aplicada 2

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(Organizador)

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APRESENTAÇÃO

A obra Botânica Aplicada 2 – Inserções Multidisciplinares traz ao leitor diversos temas da área, sendo mais de 28 trabalhos científicos, no qual o leitor poderá desfrutar de pontos da biologia vegetal aplicada abrangentes envolvendo temáticas como de sociedade, conservação do ambiente, produção vegetal, dentre outros.

A obra está seccionada em 4 setores temáticos da botânica: Avaliação da Produção e Desenvolvimento de Plantas; Estudos Taxonômicos de Plantas; Avaliação Botânica para Estudos dos Ambientes; Botânica Aplicada aos Estudos Socioeconômicos do Ambiente, onde os mesmos trarão estudos científicos recentes e inovadores de forma a demonstrar aplicação da biologia vegetal em assuntos como produção de mudas, germinação de plantas, avaliação de áreas degradadas, levantamento florístico para avaliação de ambientes, estudos socioambientais relacionados a botânica, avaliações econômicas de plantas.

A abrangência dos temas nos setores e sua aplicação na preservação, recuperação e avaliação de ambientes é um ponto importante nesta obra proporcionando ao leitor incremento de conhecimento sobre o tema e experiências a serem replicadas. Contudo a obra não se restringe a esta temática, levando o leitor ao conhecimento de temas fisiológicos e de interação entre plantas do nível bioquímico ao fitogeográfico com inúmeras abordagens nos capítulos de espécies pouco conhecidas e estudas no cotidiano do sistema de produção e ambientes naturais proporcionando abertura de novas fronteiras de ideias para suas pesquisas e aprendizado.

Neste sentido ressaltamos a importância desta leitura de forma a incrementar o conhecimento da aplicabilidade da botânica e para o estudo de espécies botânica ainda pouco retratadas tornando sua leitura uma abertura de fronteiras para sua mente. Boa leitura!

André Luiz Oliveira de Francisco

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CAPÍTULO 4

GERMINATION AND SEEDLING DEVELOPMENT OF THE GREEN FERTILIZER *CANAVALIA ENSIFORMIS* (L.) DC. (FABACEAE) UNDER DIFFERENT 2,4-D CONCENTRATIONS

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RESUMO: O 2,4-D (ácido 2,4-diclorofenoxiacético) é um herbicida extremamente tóxico e persistente no ambiente, incluindo corpos d`água. O objetivo deste trabalho foi avaliar a germinação e desenvolvimento inicial de *Canavalia ensiformis* sob diferentes concentrações de 2,4-D, aliando os benefícios da fixação biológica de nitrogênio e capacidade de fitorremediação. As sementes foram desinfestadas e introduzidas em vasos de Leonard modificados, contendo areia e 2,4-D: 0 (controle), 0.35, 2.48 e 4.97 mg/L. A avaliação pós-germinativa foi feita considerando a porcentagem de germinação; comprimento do hipocótilo, epicótilo e da raiz; e, número de folhas. A germinação foi observada a partir do 3º dia. Do 10º ao 17º dia a porcentagem de germinação do controle se manteve em 10%, enquanto nas concentrações de 2.48 e 4.97 mg/L, no 17º, a germinação alcançou 30 e 40%, respectivamente. As plântulas do tratamento controle apresentaram tamanho médio de 17.4 cm, 2 folhas e 8 cm de raiz. Com a adição de 2.48 mg/L de 2,4-D, as plântulas obtiveram o crescimento médio 15.6 cm, 1.33 folhas e 7.1 cm de raiz. O 2,4-D mostrou efeito estimulador da germinação de sementes de *C. ensiformis*. O comprimento das plântulas foi maior no tratamento controle do que sob efeito do 2,4-D. Não se verificou alterações morfológicas no desenvolvimento das raízes sob efeito de 2,4-D. O plantio de *C. ensiformis* é uma alternativa para fitorremediação de 2,4-D. No entanto, estudos precisam ser feitos para verificar a tolerância de *C. ensiformis* a maiores concentrações de 2,4-D e o mecanismo de fitorremediação deste contaminante.

PALAVRAS-CHAVE: agrotóxico, feijão de porco, herbicida, germinação, leguminosa

ABSTRACT: The herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) is extremely toxic and persistent in the environment, including water bodies. This study aimed to

evaluate the germination and initial development of *Canavalia ensiformis* under different concentrations of 2,4-D to determine its value as both green manure and potential phytoremediate. Seeds were disinfected and introduced into a modified Leonard`s vessel at concentrations of 0 (control), 0.35, 2.48 and 4.97 mg/L 2,4-D. Germination was observed from the 3rd day. From the 10th to the 17th day, the percentage of germination of the controls remained at 10%, while at the concentrations of 2.48 and 4.97 mg/L 2,4-D, it was 30% and 40%, respectively. Post-germination parameters were performed on the 17th day, including percentage of germination, length of hypocotyl, epicotyl and root and number of leaves. Seedlings of control treatment had a mean length (hypocotyl + epicotyl) of 17.4 cm, 8 cm of root and 2 leaves. At the concentration of 2.48 mg/L 2,4-D, the mean length was 15.6 cm, 7.1 cm root and 1.33 leaves. Therefore, 2,4-D showed a stimulative effect on the germination of *C. ensiformis*. Seedling length was higher in the control treatment than other concentrations of 2,4-D. No morphological changes in root development were observed as a result of 2,4-D exposure. Based on these results, *C. ensiformis* appears to represent a novel alternative for phytoremediation of 2,4-D, but further research is needed to determine the tolerance of *C. ensiformis* to higher concentrations of 2,4-D and the mechanisms of phytoremediation of this contaminant.
KEYWORDS: pesticide, jack bean**,** herbicide, germination, leguminous

1 I INTRODUCTION

Green manuring is a sustainable practice in agribusiness because it reduces eutrophication processes and contributes to improving the chemical, physical and biological characteristics of the soil, resulting in better yields for the crops in succession or rotation (QUAN *et al*., 2016; ZHANG *et al*., 2016; TANI *et al*., 2017). Also, the use of green manuring can be applied to the suppression of some weed species by allelopathic effect (MONQUERO *et al*., 2009).

Throughout Brazil, some 2,600 species of legumes can be found (BRAZIL GOVERNMENT, 2013). The plants most used for green manure are *ing*á or ice cream bean (*Inga edulis* Mart), *feijão-caupi*, *feijão-frade* or cowpea (*Vigna unguiculata* (L.) Walp.), *amendoim forrageiro* or peanut forage (*Arachis stenosperma* Krapov & Greg), black velvet bean (*Mucuna aterrima* (Piper & Tracy) Holland), *tremoço-branco* or white lupin (*Lupinus albus* L.), sunn hemp (*Crotalaria juncea* L.) and *feijão-de-porco* or jack bean (*Canavalia ensiformes* L.) (CARVALHO and AMABILE, 2006; AMBROSANO *et al*. 2018; ARAÚJO *et al*., 2018).

According to the National Institute of Amazonian Research (INPA), the use of green manure in Brazil represents about 40 tons of biomass per hectare (BRAZIL GOVERNMENT, 2013). Consequently, the use of green manure can reduce, or eliminate, the need for nitrogenous fertilizers (CARVALHO and AMABILE, 2006), the incidence of weeds by 86.7%, the requirement for irrigation by 23%, as well as lower production costs and more than double the output (SANTOS *et al*., 2018).

Green manuring involves the use of plants that establish symbiotic associations with microorganisms in their rhizosphere. Root nodules form and fix atmospheric nitrogen (N_2) in the soil, consequently producing substantial biomass and accumulating nutrients that serve as a cleaner and safer organic fertilizer (ZHANG *et al*., 2016). In general, legume species present rapid initial development, deep root system and phytomass production sufficient for soil cover. These species have a low rate of decomposition and a carbon to nitrogen ratio favorable to the successful growth of succession crops (CALVO *et al*., 2010; EIRAS and COELHO, 2011). *Canavalia ensiformis* (Fabaceae, Leguminosae) is widely used as a green manure because it presents rapid growth and adaptation to the most diverse edaphoclimatic conditions (CAVALCANTI, 2011; AMBROSANO *et al*., 2018), as well as N_2 fixation in symbiotic association with diazotrophic bacteria (CARVALHO and AMABILE, 2006). In addition, studies of phytoremediation have shown that mycorrhizal colonization of roots of this species is an important resource for its survival in contaminated soils and removal of pollutants (ANDRADE *et al*., 2010).

Agrochemicals are widely used for the prevention and control of pests, diseases, weeds and other plant pathogens in order to reduce, or eliminate, yield losses and to maintain product quality standards (DAMALAS and ELEFTHEROHORINOS, 2011). Among agrochemicals, 2,4-D (2,4-dichlorophenoxyacetic acid) is a herbicide that is considered toxic (DOW AGROSCIENCES, 2018a) with short-term to intermediate persistence in Brazilian soils (SILVA *et al*., 2007). Nonetheless, it is widely used throughout the world for the control of broadleaf weeds and other vegetation on rangelands, lawns, golf courses, forests, roadways and parks (AQUINO *et al*., 2007). Application of 2,4-D is also a serious threat to interspersed species adjacent to the agroecosystem since it may reduce auxin concentration and chlorophyll biosynthesis (DE *et al*., 2016). The detrimental effects attributed to the use of 2,4-D have motivated researchers to seek solutions for the remediation of environmental damage caused by its application, particularly soil contamination and subsequent efflux to water bodies.

In this context, some plants have the ability to remove soil contaminants through extraction, accumulation, biotransformation, immobilization and/or degradation, or, in other words, the process of phytoremediation (GERHARDT *et al*., 2017), which may be an alternative method of depolluting soils with a history of 2,4-D application (TRUU *et al*., 2015; WANG *et al*., 2015; PIAIA *et al*., 2017). However, phytoremediation has its limitations as most herbicides are toxic to plants, depending on their composition and dosage (ASSIS *et al*., 2010). Therefore, this study aimed to evaluate the germination and initial development of *C. ensiformis* cultured under different concentrations of 2,4-D. A successful outcome would demonstrate the efficacy of combining the benefits of nitrogen fixation of green manures with those of phytoremediation (SZCZYGLOWSKA *et al*., 2011; FLORIDO *et al*., 2014; MADALÃO *et al*., 2017), thus serving as an alternative means of reducing pollutants in areas exposed to 2,4-D.

2 I MATERIALS AND METHODS

Modified Leonard's vessels (VINCENT, 1970) were used. As a substitute for the traditional use of glass bottles, we used PolyEthylene Terephthalate (PET) bottles (Figure 1) based on the protocol of Santos et al. (2009).

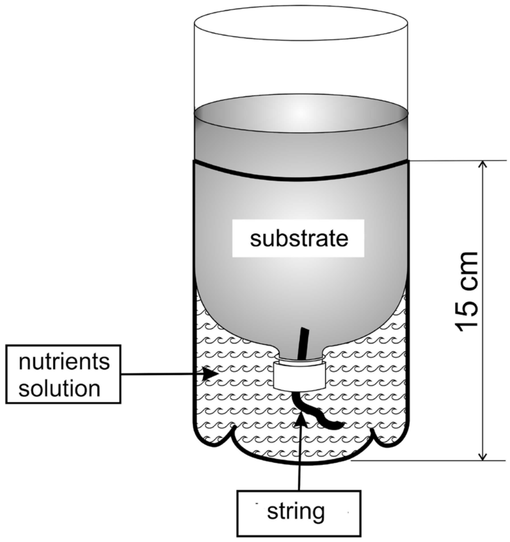

Figure 1. Scheme of modified Leonard`s vessel, using a PET bottle. Substrate: 300 g of washed sand and 300 ml nutrient solution.

The steps of vessel preparation using PET can be seen in Figure 2 A-F. The bottles were cut with scissors at a height of 14-15 cm from the base (Figure 1). For sterilization, bottles were immersed in sodium hypochlorite solution (5%) for 1 hour. Afterwards, the bottles were washed with running water, followed by distilled and autoclaved water, and put in laminar flow to dry (Figure 2 C).

For each bottle, 300 g of washed sand (Figure 2 A) was used. Sand substrate was autoclaved to asepsis for 1 hour at 121°C. Subsequently, the sand was placed in the vessel compartment (Figure 1, 2 D). In the part designated for nutritive solution (Figure 1, 2 B and D), 300 mL of a solution containing macro- and micronutrients were added, including 10% nitrogen, 9% phosphorus, 28% potassium, 4% magnesium, 6 % sulfur, 0.06 % boron, 0.01 % copper, 0.05% manganese, 0.0075 % molybdenum and 0.02 % zinc in 1000 mL of solution (Hidrogood Horticultura Moderna Ltda). The nutritive solution was transferred to the substrate through a string by capillarity (Figure 1, 2 D). The vessels were covered on the side with waterproof Kraft paper and tied with elastic to protect the nutritive solution from direct sunlight (Figure 2 E).

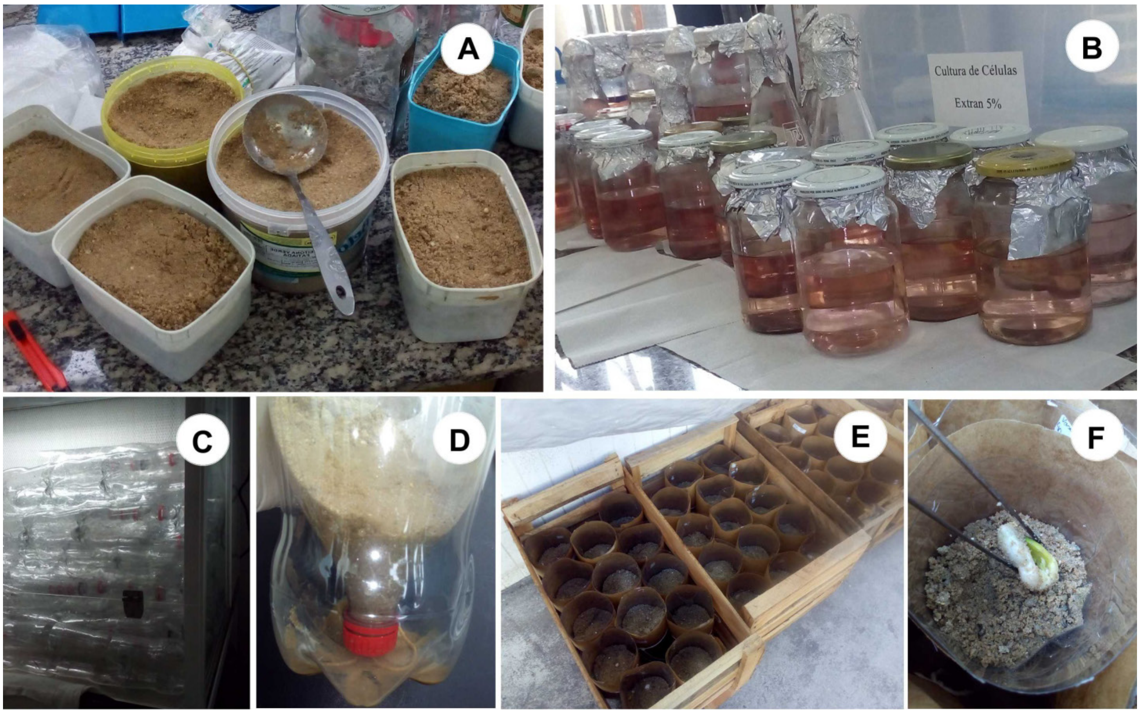

Figure 2. Preparing the experiment with seed germination of Canavalia ensiformis in modified Leonard`s vessel containing sand. **A.** Washed sand used as substrate. **B.** Nutritive solution autoclaved. **C.** PET bottles in the laminar flow after sterilization. **D.** Modified Leonard`s vessel. **E.** Leonard`s vessel maintained under ambient temperature and under plastic roof during month of experimentation, June, 2017. **F.** Seeds of C. ensiformis sown in washed sand after 3 days' growth.

The seeds used were obtained from Futuro Fértil (S. T. Irajá Agrícola Ltda.) located in the State Supply Centers (Ceasa-RJ). These seeds belonged to the 2015 harvest, lot 08/2015, presenting purity of 98% and germination rate of 75%. For deinfestation, seeds were soaked in 70% alcohol for 2 minutes, followed by commercial sodium hypochlorite from 2% to 2.5% for 3 minutes. Seeds were washed with distilled and authoclaved water 3 times between steps. Seed manipulation was done in a laminar flow.

The seeds were introduced into modified Leonard's vessels (Figure 2 F), containing sand as substrate and nutrient solution. Different concentrations of 2,4-D were tested: 0 (control); 0.35; 2.48 and 4.97 mg/L with 2 seeds per vessel, totaling 5 pots for each concentration (n = 20).

The concentrations of 2,4-D used in this work corresponded, respectively, to the application of Dow AgroSciences DMA 806BR herbicide with active ingredient 2,4-D in the concentration of 0.2, 1.4 and 2.8 L/ha (liters per hectare). According to the herbicide leaflet, dosage depends on the crop for which the herbicide is intended, ranging from 0.3 to 3.5 L/ha, but for most crops, the recommended application values range from 1 to 2 L/ha of the commercial product (DOW AGROSCIENCES, 2018b).

The seeds were observed every two days for 17 days. Germination was established from radicle protrusion. Germination and seedling development were observed punctually on the 3rd, 10th and 17th days. The germination percentage was evaluated, and after germination at 17 days, the following developmental parameters were measured: hypocotyl and epicotyl length (shoot length), leaf number and root length.

The experiment was carried out in June 2017 on the campus of the Fundação Centro Universitário Estadual da Zona Oeste (UEZO) of the West Zone, RJ (22°53’58.1”S, 43°34’45.1”W), under natural light and temperature conditions. The vessels were protected by a transparent plastic roof.

3 I RESULTS AND DISCUSSION

3.1 Germination

Germination was evaluated for 17 days, as well as the initial post-germination development of the seedlings. Seed germination of C. ensiformis started on the 3rd day after introduction (Figure 3 A, B). After 10 days, the control treatment and treatment with 0.35 mg/L of 2,4-D resulted in seeds reaching a total percentage of 10% (Table 1). At concentrations of 2.48 and 4.97 mg/L of 2,4-D, the percentage of germination increased from the 10th to the 17th day by 10% and 20%, respectively. At the end of 17 days, germination increased by 30% and 40%, respectively (Table 1). Thus, germination was found to improve after introducing certain concentrations of 2,4-D, indicating that it may play an overall positive role in seed germination of C. ensiformis.

2,4-D (mg/L)	Germination (%)*		
	3rd day	10th day	17th day
control	10	10	10
0.355	0	10	10
2.485	10	20	30
4.975	10	40	40

Table 1. Germination of *Canavalia ensiformis* during 17 days in modified Leonard`s vessels under different 2,4-D concentrations, n= 20. The results were evaluated cumulatively.

Total germination for each day. No significant diferences according Fisher`s exact test, p<0.05.

2,4-D has a physiological effect on the regulation of plant growth. It mimics the hormone auxin which plays an essential role in stimulating the growth of plant cells by interacting with endogenous hormones, such as ethylene, gibberellic acid, abscisic acid and even auxinic acids (ZIMDAHL, 2015). Breaking seed dormancy under the effect of 2,4-D has already been reported (BRADY & MCCOURT, 2003). According to Wang et al. (2016), 2,4-D can cause germination by stimulating mitosis or the cellular endocycle of seeds in studies with Arabidopsis thaliana. Studies in the literature have reported on seed dormancy of sword bean or feijão-espada (Canavalia gladiata) and other species of Canavalia genus, owing to the hardness of the integument (PONCE et al., 2017). However, as shown by the results in Table 1, it is possible to observe the occurrence of dormancy breakage or the stimulation of germination because higher concentrations of 2,4-D correlate with greater percentages of germination.

3.2 Evaluation of post-germination development of *C. ensiformis*

Initial development of seedlings is a predictor of their development in the field as both green fertilizer and phytoremediator. More specifically, morphological and physiological features at this stage provide the indicia necessary to evaluate whether plants will or won't be healthy later on and, hence, good or bad candidates as 2,4-D phytoremediators.

Figure 3 A-G shows the stages of germination up to the 17th day of initial development. The growth of the control seedlings was higher than the seedlings subject to the effect of 2,4-D (Table 2, Figure 3 F-G). After 17 days, the post-germination evaluation indicated a lower number of leaves (1.6) compared to control treatment (2.0) and a reduced value of epicotyl growth in seedlings, the seeds of which were treated with 4.97 mg/L of 2,4-D (Table 2). This conclusion was based on the resultant lower mean seedling length (hypocotyl + epicotyl = 11.7 cm) compared to control seedlings (17.4 cm, Figure 3 E) (Table 2). Only the hypocotyl length of the plants, the seeds of which were treated with 0.35 mg/L of 2,4-D, showed a greater mean length than the control (Table 2). Epicotyl growth did not appear at this concentration, and no leaf expansion occurred (Figure 3 F). The seedlings cultured under concentrations of 2.48 mg/L of 2,4-D (Figure 3 G, right) and 4.97 mg/L 2,4-D (Figure 3 C) showed two expanded leaves above the cotyledon. No change in rooting of C. ensiformis was noted (Table 2, Figure 3 D). The growth of the plantlets was continuous for 30 days (Figure 3 D). Among the evaluated parameters, negative results were observed only for the length of the seedlings, but without significant differences. Therefore, these concentrations did not show toxicity to seedlings, and exposure of this species to concentrations of 2.48 and 4.97 mg/L of 2,4-D resulted in the development of stalk, roots and leaves.

	Seedling development parameters			
2,4-D (mg/L)	Hypocotyl (cm)	Epicotyl (cm)	Leaves (number)	Root (cm)
Control	11.0	6.4	2.0	8.0
0.355	15.0	*	*	5.5
2.485	10.0	5.6	1.3	7.1
4.975	9.1	2.6	1.6	7.9

Table 2. Post-germination development of seedlings of *Canavalia ensiformis* at 17 days under different 2,4-D concentrations, n= 10.

*without development responses. No statistical differences among data using ANOVA test, $p<0.05$.

Pacheco et al. (2007) evaluated the development of millet plants (Pennisetum americanum) for 15 days after application of 2,4-D. The higher doses of 2,4-D at 670 and 1,005 g/ha at any application time caused less growth and biomass production. Our results of initial seedling development under 2,4-D resulted in shorter hypocotyl and epicotyl lengths.

According to Cavalcanti (2011), the height of C. ensiformis using sand as a substrate resulted in shorter plant length after 30 days when compared to sand combined with other substrates. We also used sand as substrate, and after 17 days, our results showed that height reached from 11.7 to 17.4 cm. This suggests that other substrates may be used and subsequently evaluated for their contribution to better seedling development of C. ensiformis after germination

It is worth mentioning that C. ensiformis is a plant that presents tolerance and growth in different environments, and its phytoremediative capacity for some contaminants has been reported, such as diesel oil (BALLIANA et al., 2017), lead (Pb) (ALMEIDA et al., 2008), copper (Cu) (SANTANA et al., 2018), and cadmium (Cd) (FRANCATO ZANCHETA et al., 2015). This species has also shown efficient phytoremediation of herbicides, including trifloxysulfuron sodium (PIRES et al., 2003), atrazine (ARTHUR et al., 2000), imazethapyr and imazapic (SOUTO et al., 2015). 2,4-D may exhibit phytotoxic responses in sensitive plants through more than one pathway in plant cells (ISLAM et al., in press). However, our results corroborate the effective use C. ensiformis in phytoremediation processes, as well as 2,4-D, since it can germinate and develop in soil with the presence of this substance in the concentration range with which it is commonly applied in the field.

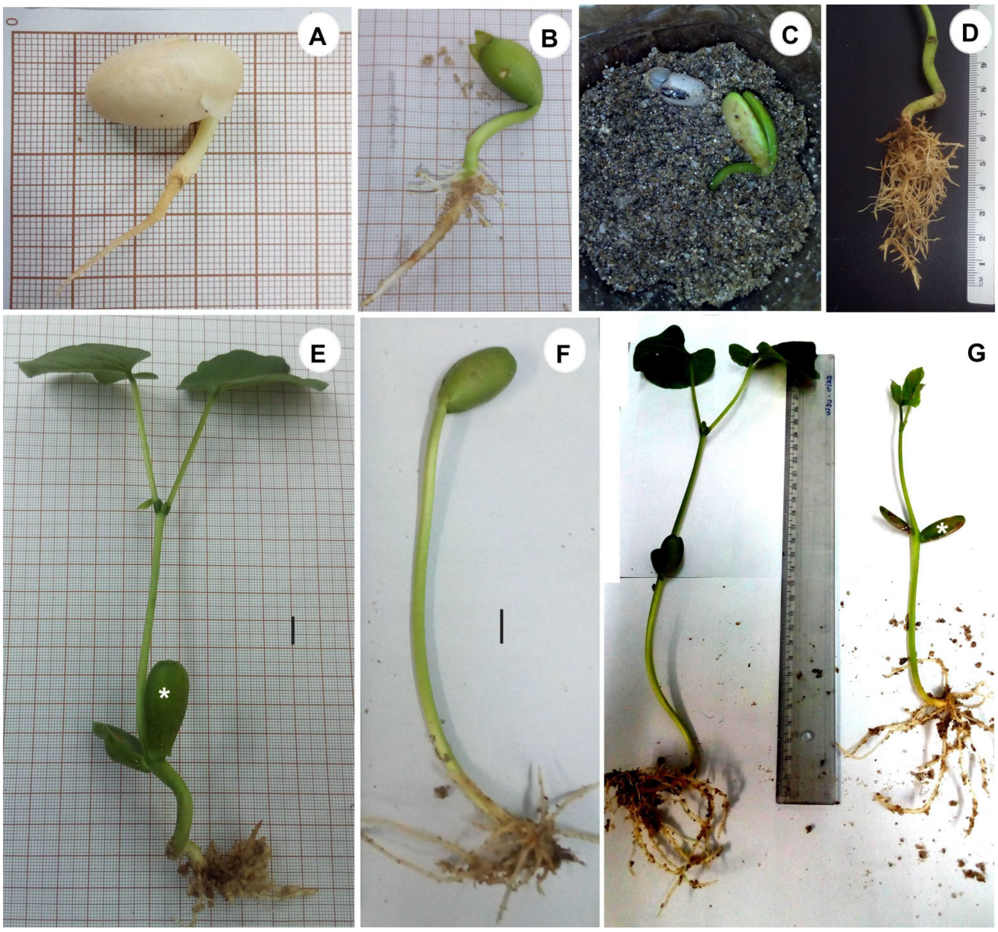

Figure 3. Germination and seedling of Canavalia ensiformis in modified Leonard`s vessel containing washed sand as substrate. **A.** Third day of seed germination in control treatment showing radicle protrusion. **B**. Fourth day of germination control. **C.** Seedlings at 10 days under treatment with 4.97 mg/L 2,4-D. **D.** Roots of seedlings under control after 30 days. **E.** Seedling development at 17 days under control treatment (Bar= 1.0 cm). **F.** Seedlings at 17 days under 0.35 mg/L of 2,4-D with no

leaves and no growth of epicotyl (Bar= 1.0 cm). **G.** Comparison of seedlings at 17 days grown under control (left) and 2.48 mg/L of 2,4-D (right). *indicates cotyledons.

4 I CONCLUSION

The germination of *C. ensiformis* seeds occurred in all concentrations of 2,4-D, but the higher concentration of 2,4-D resulted in a greater stimulus for the physiological process of germination. The evaluation of post-germinative development paramaters showed that the plantlets did not present negative responses to 2,4-D concentrations compared with control, except the length of epicotyl and hypocotyl at the higher concentration. Further research is needed to evaluate the application of the green manure *C. ensiformis* as a potential plant for 2,4-D phytoremediation in areas contaminated with this herbicide.

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