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Original Article

The allelopathic effects of aqueous *Talinum triangulare* (jacq.) willd extracts on the development of *Lactuca sativa* L. seedlings

Efeito alelopático dos extratos aquosos de *Talinum triangulare* (Jacq.) Willd no desenvolvimento de plântulas de *Lactuca sativa* L.

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Abstract

Plants that produce allelopathic compounds against weeds have emerged as a potential solution for the development of ecologically correct bioherbicides. *Talinum triangulare* is noteworthy in this regard, as its phytochemical composition encompasses flavonoids, alkaloids and other metabolites that can be used to develop inhibitory weed growth solutions. *Lactuca sativa* (lettuce) has been widely applied as a bioindicator species for bioherbicides and several chemicals, animal waste, water and soil quality, and atmospheric contamination, among others. In this context, this study aimed to assess the potential allelopathic effect of aqueous *T. triangulare* extracts on the development of *L. sativa* seedlings. A completely randomized design employing a 2x4 factorial scheme (shoot and root extracts) x the concentration of each extract (0, 2.5, 5, 7.5%) was applied, comprising four replications. *Lactuca sativa* seeds were sown on germitest papers soaked with the extracts in a germination chamber at 20°C. Physiological seed evaluations comprising the germination test, where normal and abnormal seedlings are counted on the seventh day after sowing, first normal seedling counts on the fourth day after sowing, and seedling and root length measurements. At the end of the germination test, *L. sativa* seedlings were separated for morphoanatomical characterizations and chlorophyll a fluorescence analyses. The *T. triangulare* extracts significantly influenced *L. sativa* root growth, with shoot extract exposure leading to more abnormal plants and lower root lengths at increasing concentrations and compared to the root extract. Root extract exposure led to evident cellular changes and lower non-photochemical quenching and unregulated dissipation quantum yields at a 5% exposure dose compared to shoot extract exposure. These findings suggest that both aqueous *T. triangulare* root and shoot extracts from 5% exposure doses exhibit high potential as bioherbicides, acting directly on plant structure, anatomy, quality, size and physiology.

Keywords: bioherbicides, water sheet, organic agriculture, chlorophyll *a* fluorescence, allelopathy.

Resumo

Espécies vegetais que possuem substância com efeitos alelopáticos contra plantas daninhas são potenciais soluções para o desenvolvimento de bioherbicidas ecologicamente corretos. Dentre as espécies com potencial de alelopatia, *Talinum triangulare* apresenta em sua composição fitoquímica flavonoides, alcaloides e outros metabólitos que podem ser utilizados para o desenvolvimento de soluções inibitórias do crescimento de plantas daninhas. Já a planta *Lactuca sativa* é bastante utilizada há vários anos como espécie bioindicadora, tanto para bioherbicidas, como para químicos em geral, resíduos animais, qualidade de água e solo, contaminação atmosférica e outros. Nesse cenário, objetivou-se avaliar o potencial efeito alelopático dos extratos aquosos de *T. triangulare* no desenvolvimento de plântulas de *Lactuca. sativa*. O delineamento utilizado foi inteiramente ao acaso, com esquema fatorial 2x4 (extratos de parte aérea e raiz) x concentrações de cada extrato (0; 2,5; 5; 7,5%), com quatro repetições. Para imposição dos tratamentos, as sementes de *Lactuca sativa* foram semeadas sobre os papéis embebidos com os extratos e o material foi acondicionado em câmara de germinação a 20°C. Foram realizadas as avaliações fisiológicas das sementes, sendo elas: teste de geminação (plântulas normais e anormais ao sétimo dia após a semeadura), 1° contagem de plântulas normais ao quarto dia após a semeadura, o comprimento de plântulas e comprimento de raiz. Ao final do teste de germinação foram coletadas plântulas de *L. sativa* para caracterização morfoanatômica e análise de fluorescência da clorofila *a*. Os extratos de *T. triangulare* influenciaram no crescimento de raízes de *L. sativa*, e os extratos aquosos de *T. triangulare* da parte aérea influenciaram em maior valor de plantas anormais, juntamente com menor comprimento de raiz tanto em relação ao aumento da concentração quanto em comparação com o

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extrato da raiz. Além disso, o extrato de raiz influenciou em alterações celulares evidentes e menor quenching não fotoquímico e rendimentos quânticos de dissipação não regulada para a dosagem de 5% em comparação com a parte aérea. Dessa forma, sugere-se que o extrato aquoso tanto da raiz quando da parte aérea de *T. triangulare* a partir da dosagem de 5% tem grande potencial para o uso como bioherbicidas atuando diretamente na estrutura, anatomia, qualidade, tamanho, fisiologia das plantas.

Palavras-chave: bioherbicidas, folha d'água, agricultura orgânica, fluorescência da clorofila *a*, alelopatia.

1. Introduction

Weeds are one of the main agricultural productivity constrictions, as they compete with crops for light, water, nutrients, and space (Riemens et al., 2022), and are considered more economically harmful than insects, fungi or other pests, resulting in 50 to 76% yield losses, for certain crops, such as soybean (Gharde et al., 2018). Herbicides are applied in this regard to control weed growth, comprising the main tool for crop yield sustainment, due to their practicality, economy, and efficiency (Kniss, 2017). The indiscriminate use of herbicides, however, has resulted in negative environmental, agricultural ecosystem, and human and animal health impacts, also leading to the establishment of resistant weeds (Bastiaans et al., 2008; Shen et al., 2018). Currently, 253 weed species worldwide are resistant to herbicides (Varah et al., 2020), such as glyphosate (Heap and Duke, 2018).

Allelopathy is a safer and more sustainable alternative in this scenario, employing secondary plant metabolites, termed allelochemicals, as bioherbicides (Hassan et al., 2022; Tigre et al., 2012; Santiago et al., 2017). Due to their easy decomposition in nature and different modes of action, bioherbicides comprise a control alternative in areas where synthetic products cannot be regularly applied. Allelochemicals are usually present in higher concentrations in leaves (Oliveira and Brighenti, 2018), released into the environment by root exudation, leaching, volatilization or plant residue soil decomposition (Blum, 2011; Latif et al., 2017). When released, these compounds reduce the growth and development of other plants by disrupting crucial physiological metabolisms (Hussain et al., 2022), such as respiration, enzyme synthesis, photosynthesis, mineral ion uptake and nucleic acid and protein synthesis, as well as resulting in anatomical seedling changes (Fernandez et al., 2016; Isik et al., 2016).

Among frequently employed bioindicator plant species, *Lactuca sativa* (lettuce) is resistant to a wide pH range, presenting quick and uniform germination and radicle and shoot development (Tigre et al., 2012), and highly sensitive to allelochemicals, which allows for assessments at low allelopathic concentrations (Alves et al., 2004; Coelho et al., 2011; Uhlmann et al., 2018). This species has been frequently used as a bioindicator (Tavares et al., 2019; Izquierdo-Diáz et al., 2023) in bioherbicide (Moura et al., 2018), water quality (Bertan et al., 2020; Guevara et al., 2019), soil quality and/or contamination (Baoune et al., 2019; Silva et al., 2022), atmospheric pollution (Izquierdo-Diáz et al., 2023; Hassan and Basahi, 2013) and metal (Uzu et al., 2010; Guzmán-Morales et al., 2021) evaluations, among others.

Talinum triangulare (Jacq.) Willd is a plant noteworthy for containing a significant number of bioactive compounds in leaves, encompassing flavonoids, alkaloids, tannins and phytosterols (Aja et al., 2010; Thanamool et al., 2013; Reis et al., 2015), comprising potential seed and seedling growth and development inhibitors (Hassan et al., 2022). This species also presents high agronomic and economic potential, presenting phenotypic plasticity and thus, apt for cultivation in different environments (Alexandre et al., 2018). The potential of *T. triangulare* in manipulating habitats in a biological insect control framework has been previously reported (Amoabeng et al., 2020), although its allelopathic potential has not yet been determined.

In this context, this study aimed to evaluate the potential allelopathic effects of *Talinum triangulare* aqueous extracts on *Lactuca sativa* seed germination and seedling development.

2. Material and Methods

2.1. Plant material and extract preparation

Talinum triangulare seeds were sown in polypropylene trays on commercial substrate (vermiculite). The seedlings were transplanted twenty-one days after sowing into 8 L pots filled with soil and placed under a bench in a greenhouse in a shaded environment (50%) for 80 days (reproductive stage). Irrigation was manually applied twice a day, according to the field capacity of the soil-filled pots. Leaves and roots were collected, randomly selected, washed under running water, dried in a circulating air oven at 40°C for 3 days until constant weight and crushed until obtaining a homogeneous powder with the aid of a Wiley-type crusher. The aqueous extracts were obtained by extracting 10 g of the dry material in 90 mL of distilled water at 45°C, 90 rpm for 24 hours in a bench shaker, followed by filtering with two filter paper sheets, adapted from Hassan et al. (2022), resulting in crude aqueous extracts. All analyses were performed by diluting the crude extract. Distilled water was used as the control. The applied experimental design was completely randomized employing a 2 x 4 factorial scheme, in which the first factor corresponds to the plant organ (shoot or root), and the second, to the aqueous extract concentrations (0, 2.5, 5, 7.5%). Each replicate consisted of 25 *L. sativa* seeds, encompassing four replicates.

2.2. Physiological seed evaluations

For the germination test, *L. sativa* seeds were sown on a sheet of blotting paper, in a transparent acrylic box (11 x 11 x 3.5 cm). For the treatments, 2.5 times the weight of the dry extract substrate were applied under the paper, with controls were exposed only to distilled water. The material was then placed in a biochemical oxygen demand germination chamber set at 20°C ± 2°C under a 12-hour photoperiod. The final evaluation took place on the seventh day after sowing, with germinated seed results expressed as percentages and categorized as normal seedlings, with all essential structures developed, and abnormal seedlings, that do not display the potential to continue their development and originate normal plants, even under favorable conditions, according to the Rules for Seed Analysis (RAS) (Brasil, 2009). The first normal seedling count was performed at the same time as the germination test on the fourth day after sowing, also according to the RAS (Brasil, 2009).

2.3. Seedling and root lengths

At the end of the germination test, the total seedling and root lengths of 10 randomly selected seedlings were determined with the aid of a millimeter ruler and the results were expressed as centimeters.

2.4. Morphoanatomical characterizations

Morphoanatomical characterizations were carried out with *L. sativa* seedlings 14 days after the beginning of the germination test. Leaf seedling samples were fixed in Karnovsky's solution (Karnovsky, 1965) for 48 hours, pre-washed in a phosphate buffer, dehydrated in an increasing ethanol series and pre-infiltrated and infiltrated in historesin (Leica, Germany), according to the manufacturer's recommendations. For the structural evaluations, samples were transversely sectioned into 6 µm-thick slices employing a rotating microtome (Model 1508R, Logen scientific, China) and stained with toluidine blue - polychromatic staining (0.05% 0.1 M phosphate buffer, pH 6.8) (O'Brien et al., 1964). Images were photographed under an Olympus microscope (BX61, Tokyo, Japan) coupled to a DP-72 camera using the bright field option.

2.5. Chlorophyll a fluorescence evaluations

Chlorophyll a fluorescence images (640 × 480 pixels) and parameters were obtained using an IMAGING-PAM fluorometer (MAXI version) employing the Imaging Win software (Heinz Walz GmbH, Effeltrich, Germany). To this end, *L. sativa* seedlings were fixed fourteen days after sowing on a support placed 18.5 cm from a loading device camera coupled to a fluorescence device. The first evaluations were performed on dark-adapted seedling presenting fully open reaction centers (all primary acceptors oxidized), with minimal heat loss. Leaf tissues were first exposed to a dim light (0.03 µmol m^{-2} s^{-1}) to determine dark fluorescence (F0). A pulse of saturating light (> 6000 µmol m^{-2} s^{-1}) was the applied for 0.8 s to determine maximum fluorescence (Fm). The maximum PSII quantum yield, Fv/Fm = (Fm-F0)/Fm, was then calculated from these initial measurements. Subsequently, leaf tissues were exposed to actinic light to determined fluorescence (F) values, followed by a saturation pulse to determine maximum fluorescence (Fm'). The PSII effective quantum yield, Y_{II} =(Fm'-F)/Fm' and unregulated dissipation (Y_{NO}) quantum yields (F/Fm), were calculated according to Hendrickson et al. (2004). The non-photochemical extinction coefficient of qN is defined as (Fm-Fm')/(Fm-F0').

2.6. Statistical analyses

The data were subjected to an analysis of variance assessment through the F test ($P< 0.05$), and compared using the Tukey test ($p \leq 0.05$) employing the Variance Analysis System (SISVAR – Version 5.3).

3. Results

3.1. Germination test

No significant *L. sativa* seedling germination differences were noted between the treatment, with control values of 60% and 40% determined at a 7.5% extract concentration (Table 1). Furthermore, no significant differences concerning first normal seedling counts were observed between different aqueous *T. triangulare* shoot and root extract concentrations (Table 1).

Similar results were observed for normal seedling categorizations, with no significant difference between treatments (Table 2). Different shoot extract concentrations led to higher values than the root extracts for abnormal seedlings, although no significant difference between different extract concentrations was noted (Table 2).

3.2. Seedling and root length determinations

Interactions between, total seedling and root lengths were noted. Concerning total seedling lengths, significant differences were observed between the aqueous *T. triangulare* root extracts, increasing with higher concentrations, while no differences were observed between the aqueous shoot extracts (Table 3). Concerning

Table 1. *L. sativa* seedling germination rates and first normal seedling counts following exposure to different aqueous *T. triangulare* shoot and root extract concentrations.

Extract concentrations	% Germination		First normal seedling counts	
	S	R	S	R
0	60 ± 1.4 A	62 ± 5.0 A	12,5 ± 0.7 A	15 ± 0.3 A
2.5	56 ± 3.7 A	59 ± 6.2 A	12,7 ± 1.2 A	14 ± 0.9 A
5	59 ± 2.5 A	59 ± 6.5 A	13 ± 1.2 A	14,7 ± 0.6 A
7.5	40 ± 6.1 A	58 ± 4.3 A	13,7 ± 0.7 A	11,2 ± 0.6 A

Capital letters compare the shoot (S) and root (R) extracts.

Table 2. Normal and abnormal *L. sativa* seedlings following exposure to different aqueous *T. triangulare* shoot and root extract concentrations.

Extract concentrations	Normal seedlings		Abnormal seedlings	
	S	R	S	R
0	60% ± 0.3 A	62% ± 1.2 A	36% ± 0.6 A	28% ± 1.7 A
2.5	56% ± 0.9 A	58,8% ± 1.5 A	38% ± 1.2 A	26% ± 1.2 A
5	58.8% ± 0.6 A	58,8% ± 1.6 A	34.8% ± 1.2 A	24.8% ± 1.3 A
7.5	44.8% ± 0.6 A	58% ± 1.0 A	44. 8% ± 1.5 A	32% ±0.6 A

Capital letters compare the shoot (S) and root (R) extracts.

Table 3. Total *L. sativa* seedling and root lengths submitted to different aqueous *T. triangulare* shoot and root extract concentrations.

Doses	Total length		Root length	
	S	R	S	R
0	3.6 ± 0.1 Aa	2.9 ± 0.5 Ba	2.0 ± 0.1 Aa	1.6 ± 0.3 ABa
2.5	3.1 ± 0.2 Aa	2.9 ± 0.1 Ba	1.5 ± 0.1 ABa	1.2 ± 0.7 Ba
5	2.9 ± 0.1 Ab	4.4 ± 0.3 Aa	1.4 ± 0.1 ABb	2.0 ± 0.2 ABa
7.5	2.6 ± 0.2 Ab	5.3 ± 0.3 Aa	1.1 ± 0.1 Bb	2.4 ± 0.1 Aa

Lowercase letters between columns compare the shoot (S) and root (R) extracts and capital letters in the same column compare different extract concentrations (0, 2.5, 5, 7.5%) by Tukey's test ($p \leq 0.05$).

the same shoot and root doses, only the 5 and 7.5% aqueous *T. triangulare* root extract doses resulted in different (higher) total seedling and root lengths when compared to the shoot extracts (Table 3).

Root lengths were also significantly influenced by the aqueous *T. triangular* extracts, with decreasing root lengths observed with increasing shoot extract concentrations, while the opposite was observed for the root extracts (Table 3). Concerning the same dose for both extracts, the highest root extract doses increased root length compared to the shoot extracts (Table 3).

3.3. Morphoanatomical characterizations

The *L. sativa* epidermis is unistratified on both sides, with larger epidermal cells present on the abaxial surface compared to the adaxial surface. The mesophyll consists of chlorophyll parenchyma located between the two epidermal surfaces, homogeneously distributed and formed by rounded cells (Figure 1). *Lactuca sativa* leaves displayed altered chlorophyll parenchyma cells, presenting increased cell areas following exposure to the aqueous root extract at 7.5% (Figure 1H).

3.4. Fluorescence chlorophyll a evaluations

Maximum photosystem II quantum yield (*Fv/Fm*) and effective photosystem II quantum yield (Y_{II}) were not significantly different between treatments (Figures 2 and 3). Interactions were observed between the aqueous *T. triangulare* extracts and concentrations for non-photochemical quenching (qN) and quantum yields of unregulated dissipation (Y_{NO}) (Figures 2 and 3).

4. Discussion

Allelochemicals can significantly affect plant performance, in a process termed allelopathy, an interference mechanism in which plants are chemically suppressed through the release of allelochemicals (Meiners et al., 2012). In this regard, allelopathy research has demonstrated high allelochemical sensitivity and selectivity for weed control (Macías et al., 2019). The potential of *T. triangulare* in the manipulation of habitats aiming at biological conservation control has been previously reported (Amoabeng et al., 2020), although it allelochemical potential is still unknown. The effects of aqueous *T. triangulare* root and shoot extracts were, therefore, assessed herein.

A bioherbicidal effect of the aqueous *T. triangulare* shoot extract was noted, increasing abnormal plant germination and affecting their total length. Zhao et al. (2022) reported similar results when assessing the allelopathic effects of sesame extracts on moso bamboo seed germination and seedling growth. Yan et al. (2011) indicated a possible explanation for this phenomenon, reporting that allelochemicals affect plant metabolism and the activity of several enzymes during the seed germination process. This, in turn, alters seedling growth by interrupting several physiological processes, such as photosynthesis, respiration and hormonal balance, among others, with most allelopathic interactions being negative (Soltys et al., 2013).

Physiological *L. sativa* assessments evaluated through chlorophyll a fluorescence determinations revealed that 5 and 7.5% aqueous *T. triangulare* root extract resulted

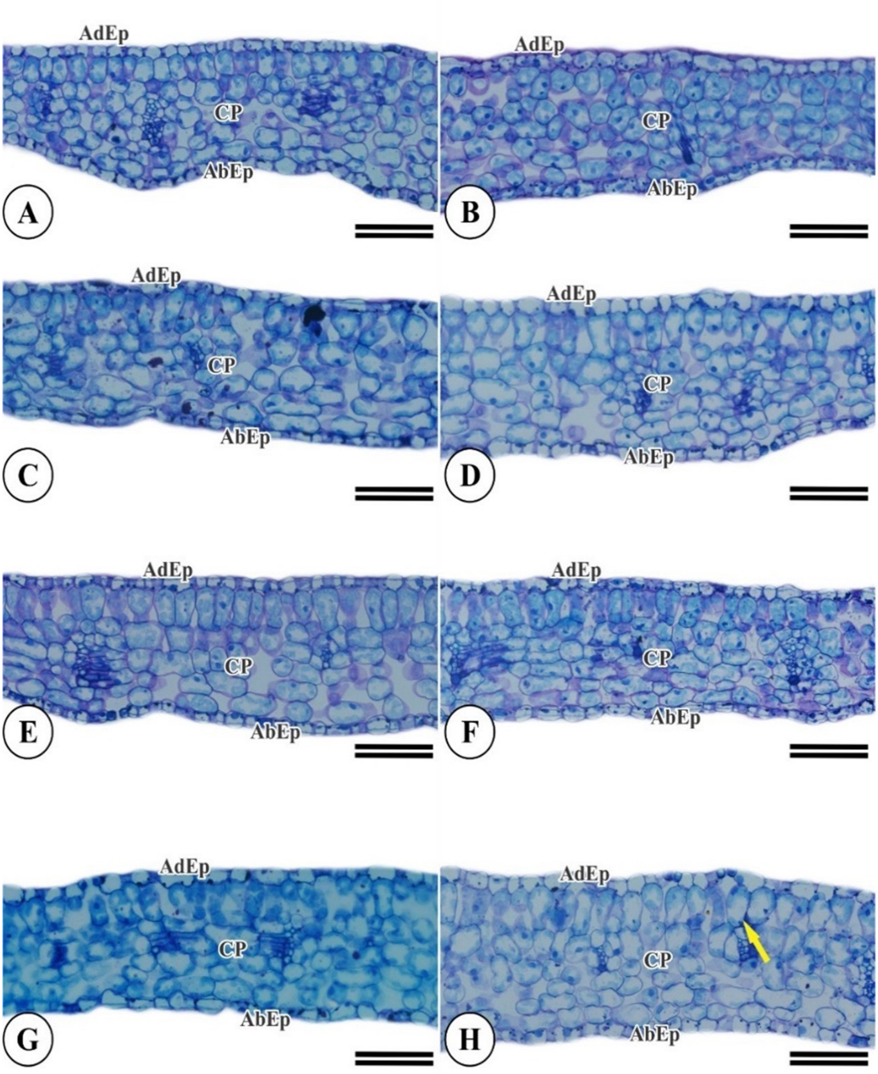

Figure 1. Leaf anatomy of *Lactuca sativa* seedlings after 14 days of sowing on germitest paper moistened with aqueous *Talinum triangulare* shoot and root extracts at different concentrations. (A-B) control (0 mg L^{-1}), (C-D) 2.5%, (E-F) 5%, (G-H) 7.5%. (EpAd) adaxial epidermis. (EpAb) abaxial epidermis. (CP) chlorophyll parenchyma. Left column - shoot extracts. Right column - root extracts. Yellow arrows indicate cellular changes. Scale bar 100 µm.

in lower plant effects in comparison to shoot extracts. Under stress conditions, the unregulated dissipation of energy (Y_{NO}) is employed by plants to avoid photosynthetic machinery damage (Hendrickson et al., 2004). Non-photochemical quenching (qN) refers to the maximum fluorescence level (Fm), in which photochemical efficiency is at its peak, and thermal dissipation, at a minimum (Maxwell and Johnson, 2000). Decreases in both qN and Y_{NO} were observed when *L. sativa* seedlings were exposed to the aqueous *T. triangulare* root extract, which may indicate potential photosynthetic machinery damage. Additionally, chlorophyll parenchyma *L. sativa* leaf alterations of increased cell areas were observed following exposure to the aqueous *T. triangulare* root extract at 7.5%. Allelochemical species, such as phenolics, are among the most common classes of exuded compounds (Hagan et al., 2013). Zhao et al. (2022), for example, reported an increase in phenolic compounds when exposing moso bamboo seedlings to sesame extracts, also noting increased cell membrane lipid peroxidation and consequent damage. The phenolic compound increase may, therefore, comprise the main reasons for the inhibitory effects and anatomy alterations observed herein in *L. sativa* seedlings.

Knowledge on the ideal concentration of allelochemicals is required to obtain promising results. Herein, aqueous *T. triangulare* root and shoot extracts were assessed by applying a dose-response curve and evaluating *L. sativa* seedling germination, anatomy and physiology, and the findings indicate that highest aqueous *T. triangulare* root and shoot extract doses (5 and 7.5%) display significant

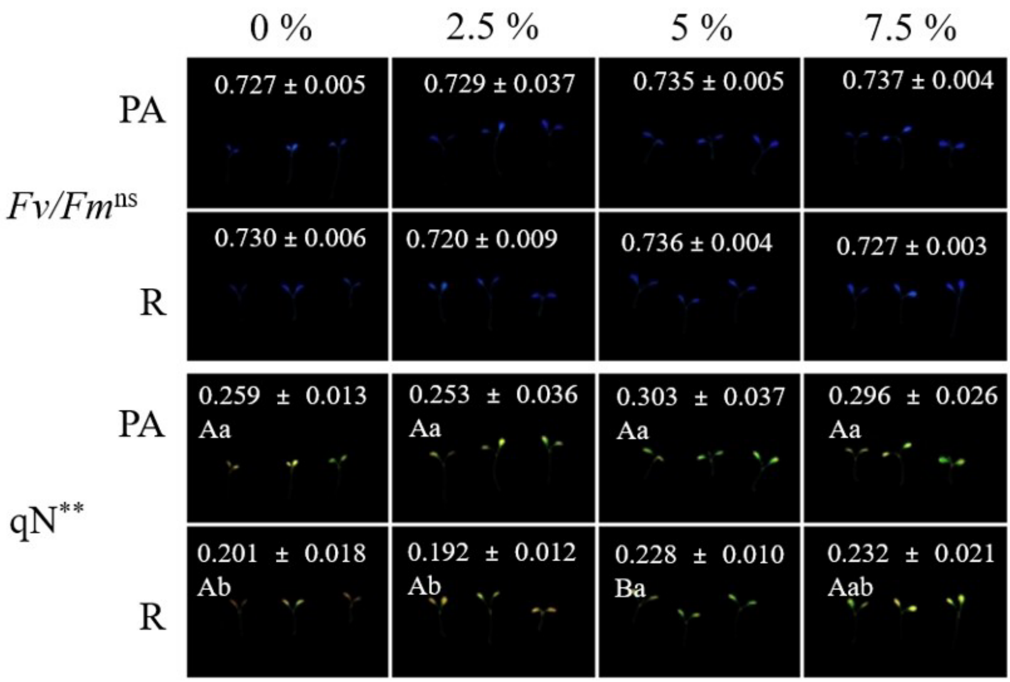

Figure 2. Maximum photosystem II quantum yield (*Fv/Fm*) and non-photochemical photosystem II quenching (qN) of *Lactuca sativa* seedlings after 14 days of exposure to different aqueous *Talinum triangulare* shoot and root extract concentrations. Uppercase letters compare the shoot (S) and root (R) extract and lowercase letters, different extract concentrations (0, 2.5, 5, 7.5%). Asterisks indicate significant differences at 1% (**) probabilities by Tukey's test ($p \leq 0.05$) (NS) indicates non-significance.

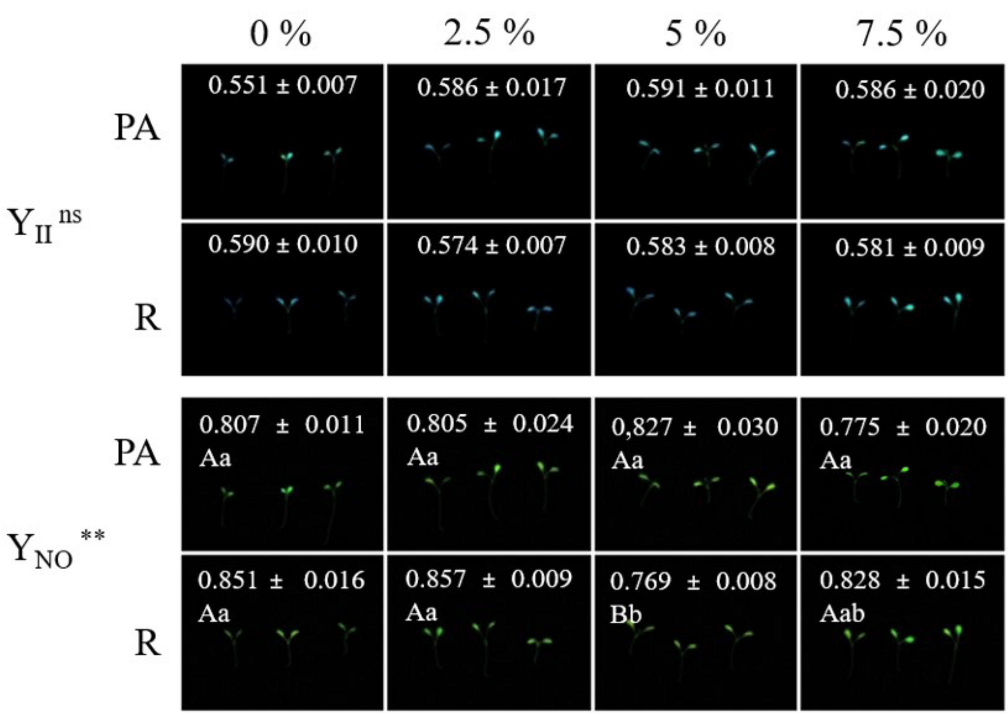

Figure 3. Effective photosystem II quantum yield (Y_{II}) and unregulated photosystem II dissipation quantum yield (Y_{NO}) of *Lactuca sativa* seedlings after 14 days of exposure to different aqueous *Talinum triangulare* shoot and root extract concentrations. Uppercase letters compare the shoot (S) and root (R) extract and lowercase letters, different concentrations (0, 2.5, 5, 7.5%). Asterisks indicate significant differences at 1% (**) probabilities by Tukey's test ($p \leq 0.05$) (NS) indicates non-significance.

potential as bioherbicides. Dai et al. (2022) applied different aqueous *Flaveria bidentis* leaf extract concentrations to the seeds of three different plants, observing increasing inhibitory effects with increasing extract concentrations.

T. triangulare was, therefore, confirmed herein as a potential source for the development of preventive bioherbicides against weeds, providing scientific evidence for the research and development of environmentally friendly herbicides in the future.

5. Conclusion

Exposure of *L. sativa* seedlings to the aqueous *T. triangulare* shoot extract investigated herein resulted in a higher number of abnormal seedlings with smaller root lengths in comparison with the aqueous *T. triangulare* root extract with increasing shoot extract concentrations. In turn, the aqueous *T. triangulare* root extract led to evident cellular *L. sativa* seedling changes, as well as lower non-photochemical quenching and unregulated dissipation quantum yields when applied at 5% compared to the shoot extract. Thus, both aqueous *T. triangulare* root and shoot extracts applied at 5% and above exhibit significant bioherbicidal potential, acting directly on the structure, anatomy, quality, size, and physiology of *L. sativa* seedlings.

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