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# ALLELOPATHIC POTENTIAL OF AQUEOUS EXTRACTS FROM *Tithonia diversifolia* (Hemsl.) A. Gray

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**ABSTRACT -** *Tithonia diversifolia* (Hemsl.) A. Gray has been recommended as a fertilizer species and its liquid extract as a biofertilizer in organic agroforestry systems. However, studies indicate that plant extracts have inhibitory effects on the germination and growth of several plant species, acting as allelopathic. In this sense, the work aims to evaluate, on a laboratory scale, the biological effect of concentrations of aqueous extract of *T. diversifolia* on the germination and initial development of seeds, using lettuce as an indicator species. Aqueous extracts were produced at concentrations of 5%, 10%, 15%, and 20%, using distilled water as a control. Lettuce seeds were arranged in Gerbox boxes, on filter paper, in the amount of 25 seeds per box, under a constant temperature of 20°C and natural light, for seven days. The percentage of final germination, mean germination time (MGT), germination speed index (GSI), and phytosanitary status of the seed on the seventh day were evaluated. The aqueous extracts of *T. diversifolia* in concentrations between 5% and 20% do not affect the final germination of the seeds, however, they reduce the germination speed; affect seedling size, and induce a higher percentage of abnormalities in germinated seeds. The results suggest that extracts of *T. diversifolia* have allelopathic potential for the germination of species.
**Keywords:** Agroforestry, allelopathy, plant extract, mexican-sunflower.

## *POTENCIAL ALELOPÁTICO DE EXTRATO AQUOSO DE Tithonia diversifolia (Hemsl.) A. Gray*

**RESUMO -** *Tithonia diversifolia* (Hemsl.) A. Gray tem sido recomendada como espécies adubadora e o seu extrato líquido como biofertilizante em sistemas agroflorestais orgânicos. Entretanto, estudos indicam que os extratos da planta apresentam efeitos inibitórios na germinação e crescimento de várias espécies vegetais, atuando como alelopática. Nesse sentido, o trabalho objetiva avaliar em escala laboratorial, o efeito biológico de concentrações de extrato aquoso de *T. diversifolia* sobre a germinação e desenvolvimento inicial de sementes, utilizando alface como espécie indicadora. Os extratos aquosos foram produzidos nas concentrações de 5%, 10%, 15% e 20%, usando água destilada como testemunha. As sementes de alface foram dispostas em caixas Gerbox, sobre papel filtro, na quantidade de 25 sementes por caixa, sob temperatura constante de 20°C e iluminação natural, durante sete dias. Foi avaliado o percentual de germinação final, tempo médio de germinação (TMG) e índice de velocidade de germinação (IVG) e estado fitossanitário da semente no sétimo dia. Os extratos aquosos de *T. diversifolia* nas concentrações entre 5% e 20% não afetam a germinação final das sementes, porém reduzem a velocidade de germinação; afetam o tamanho das plântulas e induzem um maior percentual de anormalidades nas sementes germinadas. Os resultados sugerem que extratos de *T. diversifolia* tem potencial alelopático sobre a germinação de espécies.
**Palavras-chave:** Agroflorestal, alelopatia, extrato vegetal, margaridão.

## INTRODUCTION

Plants have evolved by sharing the same space with numerous other species, competing for resources, and in some cases, producing and releasing into the environment chemicals harmful to other plants (allelochemicals) through allelopathy, thereby influencing the development of the plant community. *Tithonia diversifolia* (Hemsl.) A. Gray (Asteraceae), popularly known as Mexican sunflower or tree marigold, is a shrubby plant native to North America but naturalized in Africa, Australia, and Asia, where it is an aggressive invasive species. (AJAO; MOTEETEE, 2017). The species is a pioneer and thrives in full sun, even in poor and acidic soils, where other species may struggle to establish themselves. (MICCOLIS et al., 2019). Due to its hardiness, the use of *T. diversifolia* as green manure, biofertilizer, cover crop, and nutrient cycler has been recommended in different African countries (ENDRIS et al., 2019), Asia (FAHRURROZI et al., 2019) and Latin America (BOTERO LONDOÑO et al., 2019), in Brazil, it is one of the most widely used plants in Agroforestry Systems. (MATOS et al., 2020).

However, studies indicate that extracts from *T. diversifolia* have inhibitory effects on the germination and growth of various plant species, possibly due to the presence of phytotoxic substances that can act as allelopathic agents. (GARSABALL; NATERA, 2013; TAKIM et al., 2017).

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Phytochemical and mineral analyses have revealed the presence of tannins, saponins, flavonoids, and terpenoids (OMOLOLA, 2020), as well as alkaloids and phenols (OLAYINKA et al., 2015).

Knowledge about the effects of these allelochemicals on plant communities enhances the potential use of these substances in controlling spontaneous plants in agroecosystems, allowing for a reduction in the use of agrochemicals and making the systems more sustainable (LIMA et al., 2018). With proper management and understanding of interactions with other species, potentially allelopathic plants can protect crops from spontaneous plants and other pests, thereby minimizing the release of non-degradable synthetic molecules into the environment (HASAN, 2021). In this regard, the search for alternative, environmentally friendly herbicides to control the development of spontaneous species has driven research on allelopathy (REIGOSA et al., 2013).

Although *T. diversifolia* acts as an excellent fertilizer for some (ENDRIS et al., 2019), for others, it is a growth inhibitor for cultivated species (GARSABALL; NATERA, 2013). However, in general, studies only assess quantitative aspects (percentage of germination (%), length (cm) of seedling parts, and fresh/dry mass (g), without considering the qualitative aspects of germination that provide a different perspective on the process. While germination may not be affected, it is crucial to evaluate whether the seedling has sufficient quality to proceed through its development cycle. Therefore, there is a knowledge gap regarding the species, and it cannot be ruled out that incorrect or imprecise information may be disseminated among actors involved in agroecology, potentially causing losses for farmers. Thus, the study aimed to assess, on a laboratory scale, the biological effect of concentrations of aqueous extracts from *T. diversifolia* on the germination and initial development of seeds, using lettuce as an indicator species.

## MATERIALS AND METHODS

Vegetative material from *T. diversifolia* was collected from plants cultivated for approximately 10 years at the Experimental Station Cascata, Embrapa Clima Temperado, Pelotas, Rio Grande do Sul (geographical coordinates 31°37'07.3" S, 52°30'58.6" W., altitude 180 m). Whole plants (aboveground and root parts) were chopped into 15 cm pieces, arranged in trays, and air-dried until a constant weight was achieved. The organic residues initially underwent grinding using an organic waste shredder and subsequently went through an industrial blender, mixing all fractions. Aqueous extracts were produced at concentrations of 5% (50 g of plant material in 1 L of water), 10% (100 g of plant material in 1 L of water), 15% (150 g of plant material in 1 L of water), and 20% (200 g of plant material in 1 L of water), on a dry basis (mass: volume). Distilled water was used as the control treatment. The infusion extracts were kept in 2 L bottles for 24 hours at room temperature, as described by Takim et al. (2017). A fine mesh strainer was used for filtering the extracts at the time of the experiment setup.

Lettuce (*Lactuca sativa* L. cultivar Regina) was seeded in Gerbox containers on filter paper, following the procedure described by Brasil (2009), with 25 seeds per box. The experimental design used was a single-factor, completely randomized design, comprising four concentrations (5%, 10%, 15%, and 20%), in addition to the control treatment, with four replications. The Gerbox containers were kept in a seed germinator (Mangelsdorf model) at a constant temperature of 20°C and natural lighting for seven days. Daily counting of germinated seedlings was conducted from the fourth day onwards (BRASIL, 2009) when all Gerbox containers were moistened with 10 mL of distilled water. The final germination percentage, mean germination time (MGT), germination speed index (GSI), and the phytosanitary status of the seed on the seventh day were evaluated.

Seeds were classified as germinated if they showed a radicle with a minimum length of 2 mm. Subsequently, they were further classified as normal if they emitted an intact radicle or with little defects (Figure 1A) and as abnormal if they emitted radicles that were deformed (Figure 1B), coiled (Figure 1C), or deteriorated (Figure 1D). Seeds that did not germinate were categorized as dead (those in a state of decomposition, those apparently healthy but still without radicle emergence, and those with a solid and rigid consistency) (BRASIL, 2009).

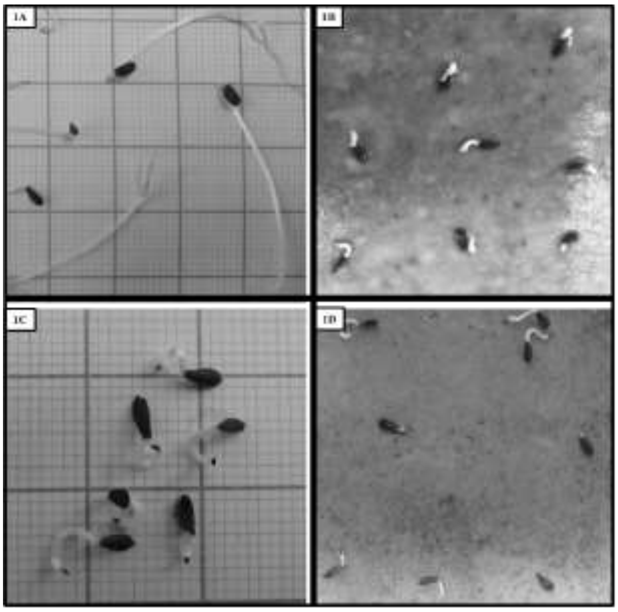

**FIGURE 1 -** Lettuce seeds with intact or slightly defective radicles (A), deformed radicles (B), coiled radicles with oxidized root caps (C), and deteriorated radicles (D).

For statistical analyses, we used R software, version 4.1.0 (R CORE TEAM, 2021). Descriptive statistics, Shapiro-Wilk test for normality of residuals, and Bartlett test for variance homogeneity were applied (SANTANA; RANAL, 2004). Subsequently, Analysis of Variance (ANOVA) or Kruskall-Wallis test for non-parametric data was performed. Post-hoc tests were also conducted using Tukey's test for parametric data and Dunn's test for non-parametric data. All tests were applied with a

5% probability of error. As not all 25 seeds germinated in the control treatment, a simple average of the number of germinated seeds in this treatment was calculated, reaching an average of 23 seeds. This value was used as the baseline (100%) for the analyses.

## RESULTS AND DISCUSSION

Seeds exposed to the highest concentration of the extract (20%) achieved the lowest germination percentage (88%), but the differences were not significant. There was no significant difference in the germination percentage among the tested treatments (Table 1).

**TABLE 1 -** Germination (%), Mean Germination Time (MGT), and Germination Speed Index (GSI) of *Lactuca sativa* L. exposed to different concentrations of aqueous extract from *Tithonia diversifolia* (Hemsl.) A. Gray.

| Extract (%) | Germination (%) | MGT (dias) | GSI (germination/day) |
|---|---|---|---|
| 0 | 100 a* | 2.99±0.37 d | 5.17±0.51 a |
| 5 | 91±2.6 a | 3.43±0.16 cd | 4.50±0.54 ab |
| 10 | 100 a | 3.71±0.29 bc | 4.69±0.12 ab |
| 15 | 89±0.50 a | 4.16±0.21 ab | 4.20±0.15 b |
| 20 | 88±2.38 a | 4.64±0.37 a | 3.89±0.43 b |
| CV(%) | 6.35 | 16.89 | 10.83 |

*Mean (± standard deviation). Different letters in the column indicate a significant difference between them, according to Tukey's test at a 5% probability of error.

The concentration of *T. diversifolia* extract significantly affected the Mean Germination Time (MGT), where seeds required more time to germinate with a higher extract concentration. In contrast, seeds in the control treatment took less than three days for seedling formation, while those exposed to the highest extract concentration (20%) needed an average of five days. Additionally, seeds exposed to higher extract concentrations achieved the lowest Germination Speed Index (GSI), and germination number (days) decreased.

Although the germination percentage did not differ statistically, some germinated seedlings exhibited abnormalities. Seedlings classified as normal represented 91±1.02% in the control treatment, following a decreasing order as the extract concentration increased: 71±4.68% for 5% extract concentration, 56±3.16% for 10% extract concentration, 32±1.86% for 15% extract concentration, and 24±1.38% for seeds exposed to 20% extract concentration. For those seeds that produced abnormal seedlings, the number increased with higher extract concentrations. Moreover, the number of non-germinated seeds was also higher in those exposed to 15% and 20% extracts (Figure 2).

The *T. diversifolia* extract influenced the length of lettuce seedlings, regardless of the concentration used (Figure 3), with the size of the seedlings likely being compromised due to the harmful biological effects of the extracts on lettuce, causing damage to the radicles, such as deformities, necrosis, and coiling. The results demonstrated that the aqueous extract, even without affecting the germination percentage, hindered the growth of germinated seedlings. As allelopathic substances lead to the emergence of abnormal seedlings (SILVEIRA et al., 2021), they reflect on the allelopathic potential of the species and its influence on other plants. The secondary metabolites produced by it (lactones, flavonoids, terpenes) can be explored for the development of natural herbicides from the plant extracts (BRUXEL et al., 2022).

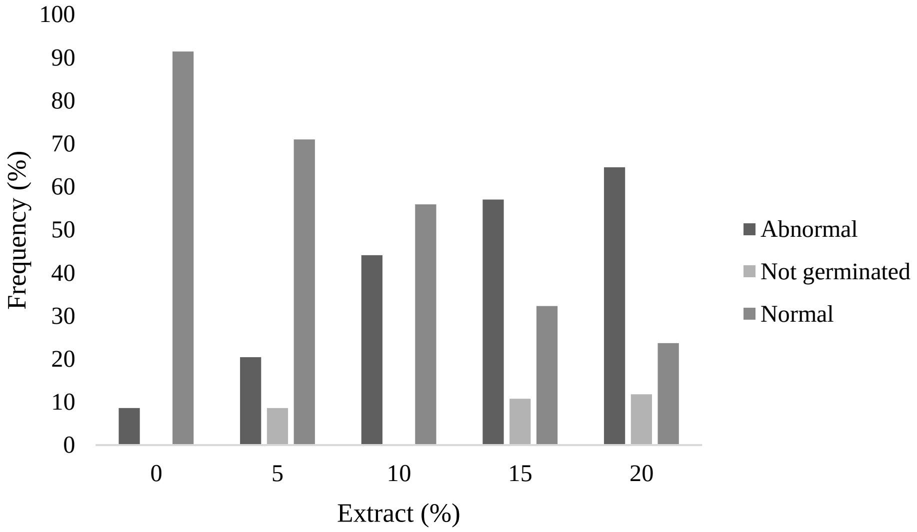


**FIGURE 2 -** Percentage of normal, abnormal, and non-germinated lettuce seedlings exposed to concentrations of aqueous extract from *Tithonia diversifolia* (Hemsl.) A. Gray on the seventh day.

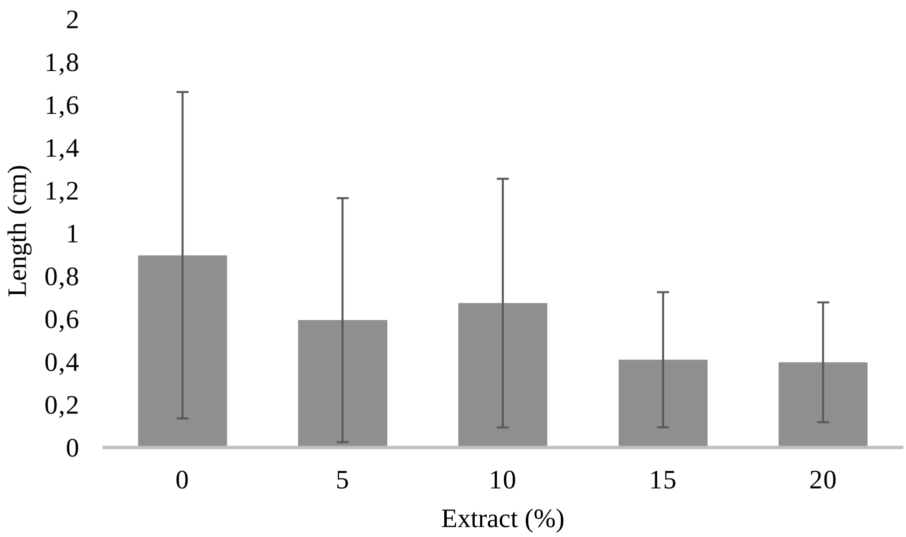


**FIGURE 3 -** Average length of the radicle (root) + hypocotyl (above-ground part) of lettuce exposed to concentrations of aqueous extract from *Tithonia diversifolia* (Hemsl.) A. Gray. *Statistical difference by Dunn's test ($p<0.05$).

When evaluating the same concentrations of *T. diversifolia* leaf extract on lettuce, Garsaball and Natera (2013) concluded that the higher the extract concentration, the lower the germination and growth. However, the methodology used by the authors differed from the method followed in the present study, as they used only the leaves of the plant for extract production and allowed the extract to rest for 48 hours. They performed two daily irrigations with the extracts on the seeds and concluded the experiment at 14 days.

On the other hand, Rodríguez-Cala and González-Oliva (2017) concluded that *T. diversifolia* extract did not show a strong allelopathic effect on the germination and initial development of lettuce. The methodology used by these authors also differed from the one carried out in the present study, where they used an aqueous leaf leachate, simulating a field condition where rainwater reached lettuce plants after coming into contact with *T. diversifolia* leaves. Apart from the methodology, the difference in results could be related to the chemical composition of *T. diversifolia*, which may vary depending on the plant part used (leaves, stems, roots, or flowers) (MEJÍA-DÍAZ et al., 2017; OLUWAFEMI, 2013), or with the environmental fluctuations that the species may have faced during its development (temperature, water variations, soil quality, luminosity, plant size, or seasonality) (SAMPAIO et al., 2016). These variables, combined with the effects of biota and the physicochemical structure of the soil, can interfere not only with the production of metabolites but also with the chemical structure and the degree of activity of substances released by plants in their environment (REIGOSA et al., 2013).

The significant increase in MGT is an interesting result when considering the use of the extract as an organic herbicide. Working with cowpea (*Vignia ungulata* L.), Ajayi et al. (2017) found that *T. diversifolia* extracts stimulated plant growth from germination to maturity while reducing the density of spontaneous plants such as *Bidens pilosa* and *Brachiaria brizantha*. In this case, the slower development of spontaneous plants provided an advantage to cowpea, probably because the extract was toxic to spontaneous plants, inhibiting germination and delaying root and aerial development through allelopathic effects of chlorogenic acid present in the species. Chlorogenic acid is an important phenolic acid in lignin synthesis and, together with cellulose, is responsible for maintaining the rigidity of woody plants (REIS et al., 2015). The higher the GSI, the more vigorous the seed lot, and the loss of vigor in a plant can be caused by modifications related to membrane permeability, DNA transcription, and translation, secondary messenger functioning, oxygen sequestration respiration, enzyme and receptor conformation, or a combination of these factors (ALTIZANI JÚNIOR, 2020).

If the results of the present study were reproduced with the same effect on spontaneous species, and they, like lettuce, showed a reduction in vigor, it could mean fewer seeds germinating each day and consequently, fewer spontaneous plants competing with target species before their establishment. This result becomes interesting, possibly indicating less aggressiveness in the dominance of crops by spontaneous plants.

In allelopathy studies, it is common not to observe allelopathic effects on germination but rather on plant growth parameters (GARSABALL; NATERA, 2013). Therefore, it is crucial to evaluate not only the germination of seeds subjected to the test but also the quality of germination, as it can interfere with the establishment of seedlings in the field and their ability to compete for available resources. According to Ferreira and Áquila (2000), evaluating the normality of seedlings is very important because the germination process is less sensitive to allelochemicals than seedling growth. Thus, discrete experimental quantification (germinated or not germinated) is not sufficient, as even with germination, abnormal seedlings may appear, with radicle necrosis being one of the most common symptoms. The results found point exactly in this direction, as at the end of the experiment, the germination percentage did not show a significant difference, but when observing the quality of seedlings, it became evident that the extract had a biological effect on lettuce.

Although laboratory studies must show that the plant produces bioactive compounds and only suggest that, under natural conditions, these compounds may have some kind of effect on the growth of neighboring species (REIGOSA et al., 2013), it is interesting to pay attention to the behavior of species cohabiting with *T. diversifolia* in agroforests. If not properly managed, it can have deleterious effects on the regeneration and restoration of native vegetation or the development of cultivated agroforestry species (MICCOLIS et al., 2019).

Resistance or tolerance to allelochemicals often depends on the sensitivity of the species, as observed for lettuce, a highly sensitive species and therefore widely used in laboratory bioassays (ALTIZANI JÚNIOR, 2020). Therefore, for future studies, it is recommended to test other species commonly used in agroforests. Additionally, there is a need to evaluate extracts produced from different parts of the plant individually to identify which part of the *T. diversifolia* plant concentrates allelochemical substances, enabling more effective isolation of these compounds.

Given these results and considering the use of *T. diversifolia* as a potential natural herbicide for organic agricultural production systems, the importance of future studies identifying the plant components where allelochemicals are concentrated is emphasized. Further studies assessing the effects of the extract on other plant species are also crucial.

## CONCLUSION

The aqueous extracts of *T. diversifolia* at concentrations between 5% and 20% do not affect the final seed germination; however, they reduce the germination speed, influence the size of seedlings, and induce a higher percentage of abnormalities in germinated seeds. These results suggest that *T. diversifolia* extracts have allelopathic potential for the germination of species.

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