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Research Article
Influence of Temperature and Potassium Nitrate (KNO_3) on the Germination/Dormancy of *Tridax procumbens* Linn

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Abstract

Background and Objective: *Tridax procumbens* is a weed with significant medicinal importance in Brazil. This study evaluated the effects of temperature and KNO_3 on *Tridax procumbens* seed germination and dormancy. **Materials and Methods:** Two subsamples of 50 achenes were submitted to treatments using a factorial combination of temperature (25 and 30°C) and moistening the substrate with KNO_3 solution (0% KNO_3, 0.2% KNO_3) in a completely randomized experimental design with five replications. Daily germination counts were made using the primary root protrusion and analyzing the accumulated germination percentage, germination speed and accumulated germination curve. **Results:** Germination percentage increased at the highest temperatures. In contrast, the use of KNO_3 at the same temperature slightly decreased germination speed after 21 days of cultivation. The accumulated germination curve adjusted to the logistic model of all treatments showed an asynchrony in seed germination over time. **Conclusion:** Thus, temperature positively influenced the germination speed index at 21 days, while the presence of 0.2% KNO_3 in the substrate influenced it negatively.

Key words: Coat buttons, germination, medicinal plant, seed, logistic model

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Tridax procumbens is popularly known as coat buttons and belongs to the Asteraceae family. This species is native to Central America, although it has recently spread to South America[1]. It is often found on roadsides, sidewalks, vacant lots and even orchards and pastures in the southeastern and midwestern regions of Brazil[2]. Moreover, this species blooms and bears fruit almost all year round, making it widely invasive and capable of eradicating adjacent species, thus standing out in its habitat[1].

Despite its undesirable reputation, this species has received particular attention in recent years due to its comprehensive composition of secondary metabolites that grant it medicinal properties. *Tridax procumbens* also contains alkaloids, carotenoids, flavonoids (catechins and flavones), saponins and tannins according to its phytochemical characterization[3-6]. In addition, this species contains a variety of chemical constituents, some of which are described for the first time in plants, such as procumbetin, a flavonoid with antioxidant and antibacterial properties isolated from the aerial parts of the plant[5,7] and Oleanolic acid, which was obtained in reasonable quantities and could be used as an antidiabetic agent against alpha-glucosidase[6,8].

Because of the wide range of chemical constituents present in *T. procumbens* leaves as well as research on its use in folk medicine, its extracts have been tested for a variety of biological activities to find a novel source of raw material for medicine, food and beverageproduction[5]. Hence, numerous studies have reported the antioxidant[9], antibacterial[5,10], antifungal[11] and antimalarial efficacy[6,12] and significant anti-inflammatory[13,14], hepatoprotective[15], antidiabetic[16,17], anticoagulant, antiviral and anticancer activities[18,19]. *Tridax procumbens* is also widely used to treat anaemia, colds and inflammations[20,21] and more recently, even as an anti-Parkinson agent[22].

The survival of this species and its medicinal properties are directly connected to its seeds, which reflect its perpetuation and diversity. Despite reports that it can persist indefinitely, *T. procumbens* has initial dormancy and staggered germination, similar to other species considered weeds[1]. This dormancy process can occur during seed formation or due to inadequate germinationconditions[23]. Thus, understanding dormancy mechanisms and assessing the best conditions are critical to manage this species more efficiently and enhance performance with more sustainable production.

Several environmental factors directly influence overcoming seed dormancy in various species, studies on these factors have increased, especially in temperate climate species and pointed the main stimulants for overcoming light dormancy[24-26], such as constant and alternating high temperatures[27,28] and potassium nitrate[29]. Potassium nitrate is a known seed germination stimulant because of its mechanism of action and this compound acts on electron reception and reduces nitrite formation inside the seeds, reoxidizing NADPH and increasing NADP availability to decrease dehydrogenases in the pentose phosphate cycle, thus improving seed dormancy[30,31]. However, species originating from tropical environments, such as *T. procumbens*, need to be better understood to improve their ecological behaviour.

Preliminary simulations of the factors that affect germination in the laboratory may help further elucidate the dormancy mechanisms of this species in its natural environment. Previous studies have shown that *T. procumbens* seeds overcome dormancy when exposed to high temperatures (25-35°C) and photoperiods[32-34]. Dormancy may also be affected by potassium nitrate[35]. Therefore, this study evaluated the influence of constant temperatures and potassium nitrate (KNO_3) on the germinative behaviour of *T. procumbens* under a 12 hrs photoperiod.

MATERIALS AND METHODS

Study area: This study was carried out in the Biochemistry and Seed laboratories of the Federal University of Fronteira Sul (UFFS), Cerro Largo campus (RS), between September and November, of 2020. *Tridax procumbens* achenes were manually collected from various plants in natural populations at the UFFS experimental fields. The achenes were manually dried in the shade before being packed into Kraft bags for the experiments. The samples were separated using a stereomicroscope to exclude any non-viable achenes that did not contain seeds or with malformation, leaving only dark-coloured achenes to show the presence of seeds.

Research protocol: The experiment was performed in BOD germination chambers regulated to provide different temperatures (±0.5°C) and a 12 hrs light/dark cycle using LED lamps. Four tests were carried out at constant temperature: Two at 25°C and two at 30°C. The tests consisted of two treatments using a substrate moistened with distilled water (0% KNO_3) or a 0.2% KNO_3 solution, namely T1, T2, T3 and T4. Germination tests were done in Gearbox boxes with two

subsamples of 50 achenes placed between two sheets of blotting paper for each repetition. The papers were moistened until saturation (2.5 times the mass of the paper), first with distilled water for T1 and KNO_3 solution for T2, both at 25°C. The same procedure was performed for T3 and T4, although at 30°C. Distilled water was added when necessary to maintain the substrate moisture during the tests. The tests were conducted in a completely randomized design and a 2×2 factorial scheme (temperature×moistening) with five replications. The germinated seeds were counted daily for 21 days after the experiment began and seeds with radicle emission visible to the naked eye were considered germinated.

Germination percentage: The accumulated germination was calculated using the daily germination data and considered the germination percentage and Germination Speed Index (GSI)[36]. Germination percentage and GSI were judged based on their averages and presented with the respective standard errors. The germination percentage and GSI data after 21 days were analyzed by analysis of variance (ANOVA) and Tukey's test ($p<0.05$) in the Graph Pad Prism 8.0 program. The GSI was calculated according to the Eq.[36]:

$$GSI = \frac{G1}{D1} + \frac{G2}{D2} + \ldots \frac{Gn}{Dn}$$

where, G1, G2, ..., Gn is the number of germinated seeds observed in the interval of the 1st, 2nd, ..., last count and D1, D2, ..., Dn is the number of days after sowing for the 1st, 2nd, ..., last count.

Statistical analysis: The analyses were performed separately for each temperature. The germination percentage was transformed into arc sine $\sqrt{x/100}$ and the GSI data into $\sqrt{x+0.5}$ for data analysis[34]. Mathematical models were adjusted to the accumulated germination curves with the repetitions of each treatment. This adjustment was made using the curve expert 1.3 software, applying as the model selection criteria model the coefficient of determination (R^2) and the ease of interpreting biological data using the equations. Tukey's test ($p<0.05$) was applied using ANOVA to compare the difference between the tests.

RESULTS AND DISCUSSION

In the study, *Tridax procumbens* seed germination percentage was evaluated for 21 days for each test (Fig. 1). The germination behaviour was not significantly influenced by moistening the substrate with 0.2% KNO_3, not being statistically different from the distilled water treatment (0% KNO_3). The 25°C nitrate test (T2) somewhat decreased compared to the moistened sample (T1). At 30°C, KNO_3 (T4) slightly increased the germination percentage.

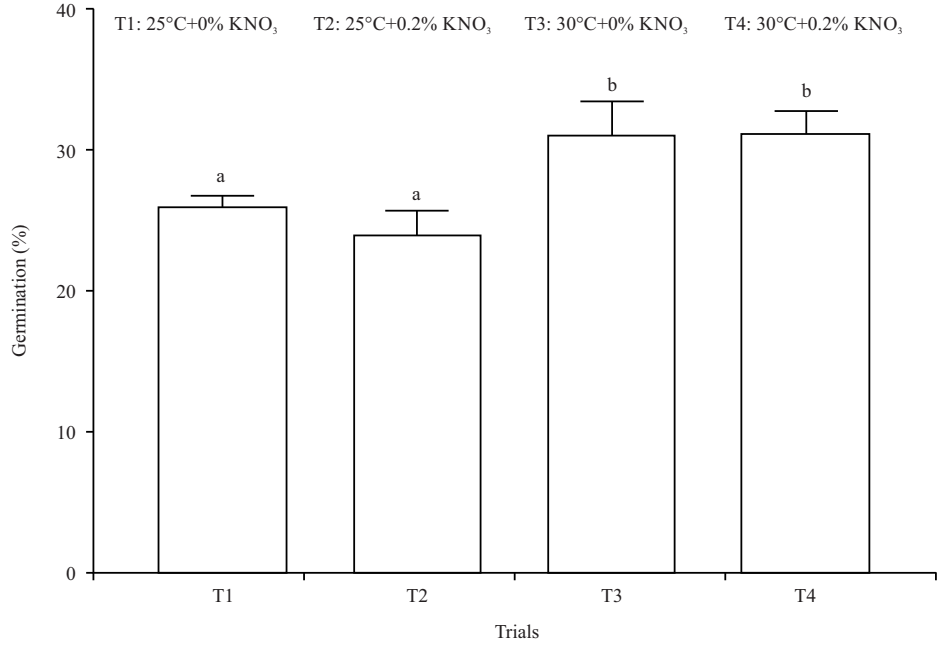

Fig. 1: Germination (%) of *Tridax procumbens* seeds subjected to different temperatures and different moistening conditions
Data were transformed into $\sqrt{x/100}$ sine arc. Different letters in the graph indicate significant differences ($p<0.05$) by Tukey's test

Table 1: Germination speed index (GSI) of *Tridax procumbens* seeds submitted to different temperatures and different moistening conditions at 7, 14 and 21 days

Test	GSI 7 days	CV (%)	GSI 14 days	CV (%)	GSI 21 days	CV (%)
T1	0.84[a]	105.3	1.35[a]	31.63	1.47[a]	21.13
T2	0.91[a]	58.71	1.38[a]	28.94	1.47[a]	18.38
T3	2.04[b]	22.24	2.19[b]	17.91	2.54[b]	15.29
T4	2.13[b]	14.11	2.21[b]	12.41	2.27[c]	11.05

Data were transformed into $\sqrt{x+0.5}$, Different letters in the same column indicate significant differences ($p<0.05$) by Tukey's test. GSI: Germination speed index, CV: Coefficient of variation

Temperature, in turn, was a key influencing factor on germination rate, where the assays at 30°C (T3 and T4) showed the highest germination rates, reaching 31.2 and 31.3%, respectively, being statistically different from the assays at 25°C ($p<0.05$) (Fig. 1). Guimarães *et al.*[32] also observed high germination rates at 25, 30 and 35°C, reaching values over 90%, being faster at 30°C and better distributed in time at 35°C. The authors also noted that germination at extreme temperatures (40°C) was below 6%, with a loss of up to 80% in seed viability, indicating an ideal temperature range between 25-30°C for the highest germination rate.

So, it is noted seed germination is the result of a balance between environmental conditions and the intrinsic characteristics of the seeds, which, when necessary, trigger a sequence of metabolic activities that cause embryonic axis formation and germination[23]. Viable and non-dormant seeds germinate when water and adequate temperature are available[36], in addition to other factors, including the presence of potassium nitrate (KNO_3)[29].

Ikeda *et al.*[34] reported that *T. procumbens* germination is stimulated by light and should be ideally maintained at 25°C, although the authors also observed that alternating light and temperature (15°C in the dark and 35°C in the light) stimulated germination and was influenced by KNO_3. According to another study[1], *T. procumbens* seeds in the field present initial dormancy and staggered germination. In some species, seed dormancy is caused by a physical blockage due to a resistant and impermeable integument that inhibits seed imbibition or embryo oxygenation by preventing aqueous transit and gas exchange[37]. In general, laboratory tests have shown that, under favourable temperature and in the presence of light, bluegrass seeds have faster and more uniform germination (i.e., dormancy breakage is anticipated)[1,32,34].

The germination rates found here were lower than those described by the authors mentioned above and this difference may have occurred because of different seed sowing management. In their trials, Ikeda *et al.*[34] cut the pappus for the final part of the seed to increase contact with the substrate. To calculate the germination rate, the authors considered germinated seeds that had a germinative structure visible to the naked eye, unlike the present study, which considered the seed that presented radicle emission to be germinated.

Guimarães *et al.*[32] observed germination rates of up to 81% at 30°C using 3-6 days old seeds. The ages of the seeds employed here were unknown and collected two months before the trials. Therefore, it can be affirmed that the difference in seed quality is likely associated with the edapho climatic conditions during seed growth[38]. Nevertheless, this infeasibility may also be linked to selecting the achenes of each assay because the parameters used do not effectively reveal whether the seeds are viable or not, this can be discerned only after the germination period.

In addition to the factors evaluated, the origin of the seeds can also influence *T. procumbens* germination percentages, which may explain the differences reported herein. Current results corroborate[39], who obtained a maximum germination percentage of just 32% at 25°C in Nigeria, not finding significant differences between this temperature and 30 and 35°C.

The GSI was determined for 7, 14 and 21 days in all trials. This parameter is important because it reflects the germination behaviour during all treatment days and not just the final result. For *T. procumbens*, the highest GSI values were achieved considering 21 days of treatment, indicating that higher germination speeds are achieved several days after beginning the experiment since, in general, primary root protrusion begins after 4 days of contact with the substrate. This can be observed, for example, by comparing GSI7 with GSI21 in all trials in Table 1.

Tests performed at 30°C (T3 and T4) resulted in a higher GSI rate, suggesting that temperature affects germination speed (Table 1). This effect was also reported by Guimarães *et al.*[32] and Ikeda *et al.*[34], who reached a GSI21 of 4.9%. In addition, KNO_3 significantly affected GSI 21 (Table 1, column 3), surprisingly reducing germination speed. This was not expected because potassium nitrate in the substrate has been reported as an additional factor

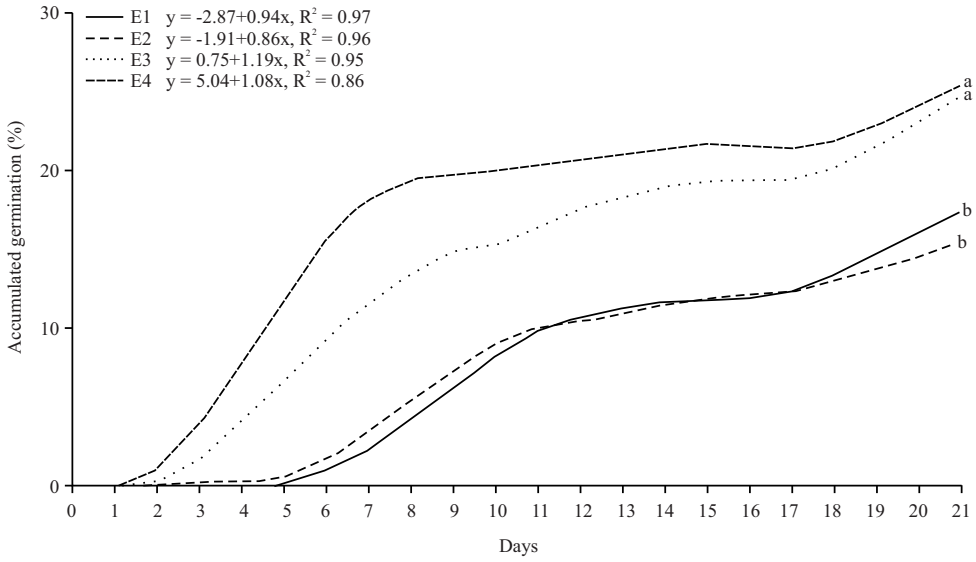

Fig. 2: Accumulated germination percentage for *Tridax procumbens* seeds submitted to different temperatures and different moistening conditions

Different letters in the curves indicate significant differences ($p<0.05$) by Tukey's test

in overcoming seed dormancy[29,31]. Ikeda *et al.*[34] found that KNO_3 stimulated germination and increased the GSI 21 of seeds grown under alternated temperature conditions.

The accumulated germination percentage for each treatment was estimated and plotted in curves. The best adjustment of the curves for the logistic model was obtained:

$$y = a+bx$$

where, y is the accumulated germination percentage, x is the germination time in days and a and b the coefficients. The logistic model was adjusted with the coefficients of determination (R2) ranging from 0.86-0.97 in Fig. 2. Once again, it was verified that the only influential factor in the accumulated germination rate was temperature. Germination occurred rapidly in T3 and T4, with the first seeds germinated on the second day, while in T1 and T2, the process started on the 6th day. This was also true when analyzing GSI 7 (Table 1). The days with the highest number of germinated seeds during the cultivation period were those after a period when the substrate was drier, followed by manual moistening when this was observed. There is no constant temperature and humidity in the field, although high values of both factors on the soil surface during the hottest periods of the day can partly account for the distribution of weather emergencies. Additionally, if seeds remain for long periods in environments that do not favour germination, secondary dormancy may be induced[23]. This mechanism may contribute to the disuniformity of the process, which is a weed survival strategy, enabling its emergencies to differ in time and space.

It is also noteworthy that the plants in T1 and T2 (25°C), several days after germination and emission of cotyledons, presented fragile roots and died rapidly in Fig. 3a. Nevertheless, the seeds germinated at 30°C (T3 and T4) remained largely intact until the end of the trials and their roots continued to grow with no signs of rotting in Fig. 3b. One of the most significant factors in seed germination is temperature. According to Xia *et al.*[27] the temperature may be essential for the germination process because it influences both the percentage and speed of germination. Moreover, it can interfere with the speed of water absorption and biochemical reactions and even influence all stages of plant growth[39] as observed here.

Although high germination rates were not compared to other experiments, many of the conditions observed in nature for the plant were noted and without human interference (e.g., cutting the pappus) as well as the strategy that the plant may have adopted, which seems to germinate on scales after going through a substrate drying cycle→excess heat→moistening. Further research with varying temperatures and alternating light methods and more significant contact with the substrate is necessary (e.g., non-tillage in vermiculite) as these conditions are closer to the reality of *T. procumbens* seeds in nature.

Fig. 3(a-b): *Tridax procumbens* seeds germinated at (a) 25°C and (b) 30°C
Scale bar: 2 mm

CONCLUSION

This study found that temperature affected the germination percentage of *Tridax procumbens* seeds, with germination rates being higher for seeds grown at 30°C. Temperature positively influenced the germination speed index at 21 days was while the presence of 0.2% KNO_3 in the substrate influenced it negatively.

SIGNIFICANCE STATEMENT

This study deals with the germination and development of an important medicinal plant, which is often considered invasive. Thus, understanding the mechanisms of germination and dormancy and what the best conditions to overcome them are is important to develop more effective management of this species, to enhance the results from an ecological point of view and integrated with more sustainable production. This will serve as a basis for future studies with this plant. Both studies seek to discover new medicinal properties and studies that seek its control as an invasive species.

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