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Allelopathic and phytochemical potential of the aqueous extract of *Curcuma longa* L.: contributions to the production of bioherbicides

Potencial alelopático e fitoquímico do extrato aquoso de Curcuma longa L.: contribuições para a produção de bioherbicidas

Leonardo Mendes da Silva *1(ORCID 0000-0001-6510-9005), **Marcela Emiliano Novaes Matilde** 1(ORCID 0009-0000-4085-0956), **Fábio Júnio da Silva** 2(ORCID 0000-0003-2159-5907)

[1]Federal University of Lavras, Lavras, MG, Brazil. *Corresponding author: leonardoifsudestemg@gmail.com
[2]Federal Institute of Southeastern Minas Gerais, Barbacena, MG, Brazil.

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RESUMO

A planta *Curcuma longa* L. é rica em compostos bioativos. No entanto, há poucos estudos disponíveis que investigaram sua atividade alelopática. Neste trabalho, buscamos investigar preliminarmente os principais grupos químicos com atividade alelopática e bioherbicida presentes no extrato aquoso obtido dos rizomas de *C. longa*, bem como seu efeito na germinação e no crescimento inicial de plântulas de alface (*Lactuca sativa* L.) e trigo (*Triticum aestivum* L.). A análise fitoquímica foi realizada por técnicas de precipitação e colorimétricas. Para determinar o potencial alelopático do extrato, foram testadas sete concentrações (1 a 64 g L^{-1}), além de dois grupos controle: água destilada (controle negativo) e glifosato (controle positivo). As sementes permaneceram em contato com as diferentes concentrações por 72 horas. Os parâmetros avaliados foram: porcentagem de germinação (PG), índice de velocidade de germinação (IVG), comprimento da raiz e da parte aérea das plântulas e concentração inibitória média (IC_{50}). Foram identificados quatro compostos com potencial alelopático: alcaloides, esteroides, flavonoides e terpenos. Os resultados do ensaio biológico revelaram que o extrato aquoso exerceu impacto limitado sobre a PG, apresentando efeitos apenas na concentração mais elevada (64 g L^{-1}), ocasionando redução de 20,70% desse parâmetro em ambas as espécies. Além disso, verificou-se que o índice de velocidade de germinação (IVG) foi significativamente influenciado por concentrações superiores a 4 g L^{-1} para ambas as espécies. O comprimento da raiz e da parte aérea das plântulas diminuiu com o aumento das doses. O comprimento da parte aérea foi o parâmetro mais sensível, sendo necessária a IC_{50} de 3,73 e 7,20 g L^{-1} para o trigo e a alface, respectivamente. Os resultados sugerem que o extrato dessa planta apresenta potencial para a produção de bioherbicidas de pós-emergência.

PALAVRAS-CHAVE: alelopatia; controle de plantas daninhas; compostos bioativos; fitotoxicidade.

ABSTRACT

The plant *Curcuma longa* L. is rich in bioactive compounds. However, there are few available studies that have investigated its allelopathic activity. In this work, we sought to preliminarily investigate the main chemical groups with allelopathic and bioherbicide activity present in the aqueous extract obtained from the rhizomes of *C. longa*, as well as its effect on the germination and initial growth of lettuce (*Lactuca sativa* L.) and wheat (*Triticum aestivum* L.) seedlings. Phytochemical analysis was performed using precipitation and colorimetric techniques. To determine the allelopathic potential of the extract, seven concentrations (1 to 64 g L^{-1}) were tested, in addition to two control groups: distilled water (negative control) and glyphosate (positive control). The seeds remained in contact with the different concentrations for 72 hours. The evaluated parameters were: germination percentage (GP), germination speed index (GSI), root and shoot length of the seedlings, and median inhibitory concentration (IC_{50}). Four compounds with allelopathic potential were identified: alkaloids, steroids, flavonoids, and terpenes. The results of the biological assay revealed that the aqueous extract had a limited impact on GP, showing effects only at the highest concentration (64 g L^{-1}), causing a reduction of 20.70% in this parameter in both species. Additionally, it was found that the germination speed index (GSI) was significantly influenced by concentrations higher than 4 g L^{-1} for both species. The length of the root and shoot of the seedlings decreased with increasing doses. Shoot length was the most sensitive parameter, with an IC_{50} of 3.73 and 7.20 g L^{-1} required for wheat and lettuce, respectively. The results suggest that the extract of this plant has potential for the production of post-emergence bioherbicides.

KEYWORDS: allelopathy; weed control; bioactive compounds; phytotoxicity.

INTRODUCTION

Weeds pose significant challenges to agricultural production, imposing considerable constraints. They compete with crops for essential resources such as water, nutrients, space, and light, as well as act as hosts for pests and diseases (NICHOLS et al., 2015; HORVATH et al., 2023). This competition compromises crop yields, resulting in significant production losses. For example, rice crop yields can decrease by 15 to 66%, while corn yields can decline by 8.6 to 51%. In soybeans, yield losses can reach 50 to 76%, and in peanuts, 45 to 70% (GHARDE et al., 2018).

In agriculture, weed control is an extremely important agronomic practice. Due to the shortage of labor in the agricultural sector, there has been a significant increase in the use of synthetic herbicides to control the spread of invasive species around the world. Synthetic herbicides are chemical compounds manufactured in laboratories to control or eliminate unwanted plants. These products are formulated through chemical synthesis processes and exhibit variations in their molecular structures and mechanisms of interaction with biological targets (GUPTA 2018, BRILLAS 2021).

There are two main groups of herbicides: pre-emergence herbicides, which are applied to the soil and inhibit the germination of weeds, and post-emergence herbicides, which are applied to the leaves of plants and suppress their growth or cause their death. However, the indiscriminate and prolonged use of herbicides from both classes has led to the emergence of species with resistant genotypes. In addition, this practice has contributed to the presence of toxic residues in crops, generating ecological imbalances between harmful and beneficial organisms, as well as negative impacts on ecosystems (Aktar et al., 2009; Gandhi et al., 2021; Mehdizadeh et al., 2021).

Currently, bioherbicides have gained increasing recognition as an essential strategy in weed control, emerging as viable alternatives to synthetic herbicides (HASAN et al., 2021; PÉREZ-DE-LUQUE, 2023). Bioherbicides are biological agents, derived from living organisms such as fungi, bacteria, and plants, which are naturally produced during their life cycle and are applied to control weeds (BAILEY 2014). Its first use dates back to the 1980s, and it was initially used in Canada, the United States, Ukraine, and European nations, with the aim of suppressing the germination or growth of unwanted species without causing accumulation or adverse environmental impacts (BAILEY 2014, RADHAKRISHNAN et al., 2016).

Plant extracts have been explored as promising sources for the development of bioherbicides in sustainable agricultural practices. This success can be attributed to the presence of bioactive compounds in the plant extracts, which have specific inhibitory effects on the growth of undesirable plants. It is believed that these compounds interact selectively with target enzymes or specific receptors present in weeds, resulting in the inhibition of weed growth (HOSNI et al., 2013; MAURYA et al., 2022; KOSTINA-BEDNARZ et al., 2023).

Several plant species have the ability to secrete metabolites known as allelochemicals, which include alcohols, fatty acids, phenolic compounds, flavonoids, terpenoids, and steroids. These compounds play important roles in inhibiting the reproduction, growth and development of neighboring vegetation. By releasing these allelochemicals into the environment, plants can suppress the growth of other plants around them, giving themselves a competitive advantage. The exploitation of these allelochemicals as bioherbicides could offer a sustainable and targeted approach to weed control in crops (HUSSAIN 2020, DE SOUZA BARROS et al., 2021, KOSTINA-BEDNARZ et al., 2023).

One of the plant species showing potential for the production of bioherbicides is *Curcuma longa* L., popularly known as turmeric or curcuma. Native to tropical and subtropical regions of the world, it is commonly grown in Asian countries, especially India and China, as well as Brazil. It is an erect perennial plant that stands out for its leafy and lush appearance. Belonging to the Zingiberaceae family, it can reach a height of up to one meter. It has a short stem and bright green, pointed leaves. The funnel-shaped yellow flowers provide a vibrant contrast against the green leaves (AKRAM et al., 2010; LAL, 2012; YADAV & TARUN, 2017; DA SILVA et al., 2023). The plant's rhizomes are oblong, oval, or pear-shaped, and often branched. Three main curcuminoid pigments are identified: predominantly curcumin (60%), followed by demethoxycurcumin (22%), and bisdemethoxycurcumin (18%). In addition, compounds such as zingiberene and turmerones are detected (LORENZI & MATOS 2021).

In view of the above, the aim was to identify the classes of phytochemical constituents with potential allelopathic activity present in the aqueous extract derived from the rhizomes of *C. longa*, as well as to investigate the effects of this extract on the germination and initial development of two model species: lettuce (*Lactuca sativa L.*) and wheat (*Triticum aestivum* L.). It is hoped that the results obtained in this study will contribute to understanding the effects of *C. longa* on seedlings germination and initial seedling development, as well as provide valuable input for the development of new bioherbicides.

MATERIAL AND METHODS

Preparation of solutions

The fresh rhizomes of the *C. longa* plant were collected from the garden of one of the authors of this study, located in the municipality of Ressaquinha, Minas Gerais, Brazil. The material was subjected to a rigorous selection process, carefully washed and sliced into thicknesses of two centimeters using a stainless steel blade. The rhizomes were then subjected to controlled drying in an oven at a constant temperature of 40°C for 120 hours. They were then ground in an industrial grinder and the material obtained was sieved through a 40-mesh sieve, resulting in a fine powder.

During the study, solutions were prepared using powder obtained from the plant's rhizomes. The material was infused for 10 minutes in 1,000 mL of boiling distilled water, allowing the active substances to be extracted. After this process, the solutions were filtered to obtain filtrates.

To evaluate the allelopathic effects, seven different concentrations obtained from the rhizomes were used, in the proportions of 1, 2, 4, 8, 16, 32, and 64 g L^{-1}. In addition, two control groups were prepared: the first using distilled water as a negative control and the second using the herbicide glyphosate (Biocarb) at a concentration of 10% as a positive control, to compare the response of the extract with that obtained using a commercial herbicide.

Preliminary phytochemical screening

The aqueous extract previously obtained (concentration of 64 g L^{-1}) was stored in a freezer at a temperature of -15°C and then subjected to freeze-drying. The purpose of this procedure was to prepare the extract for phytochemical analysis to identify classes of metabolites with allelopathic potential.

During the phytochemical analysis, several classes of metabolites were investigated in the aqueous extract, including reducing sugars, alkaloids, anthraquinones, coumarins, steroids, flavonoids, phenols, glycosides, saponins, tannins, and terpenes. The methods used for this analysis followed the procedures described in the scientific literature (HARBORNE et al., 1999; MATOS, 2009; SIMÕES et al., 2016) and are detailed in Table 1.

After detecting the chemical classes present in the aqueous extract of *C. longa*, the allelopathic and bioherbicidal potential of these classes was investigated in the scientific literature, with the aim of understanding the mechanisms of action of each compound on seed germination and plant development.

Evaluation of allelopathic potential

Lettuce seeds(*Lactuca sativa var.* Great Lakes TopSeed brand wheat (*Triticum aestivum*) was purchased commercially and used as test material for the bioassay. These species were selected due to their widespread use in evaluating the allelopathic potential of plant extracts, as well as their advantageous characteristics of rapid germination and fast growth. They are also recommended as model organizations by the Organization for Economic Cooperation and Development (OECD 2006).

To evaluate the allelopathic potential of the aqueous extract obtained from *C. longa* rhizomes, an experiment was conducted using a completely randomized design with nine treatments and six replicates. The treatments consisted of seven concentrations of the extract obtained from the rhizomes (1, 2, 4, 8, 16, 32, and 64 g L^{-1}), including two controls (negative control: distilled water; positive control: 10% glyphosate). For each replicate, a Petri dish containing 10 lettuce or wheat seeds was used. The seeds of both species were placed on filter paper moistened with 3 mL of the previously prepared solutions. The plates were sealed with plastic wrap and placed in a germination chamber, where they were kept at a constant temperature of 24 °C without exposure to light.

Table 1. Preliminary phytochemical tests were carried out on the aqueous extract of *C. longa*.

Class evaluated	Test Performed	Observation
Reducing sugars	2 mL of extract + 1 mL of H_20+ $CuSO_4$	Red-orange precipitate
Alkaloids	2 mL of extract + Hager's reagent	Yellow precipitate
Anthraquinones	2 mL of extract + 2 ml of C_6H_6 + 5 mL of NH_3 (10%)	Pink, violet or red color.
Coumarins	2 mL of extract + 3 mL of NaOH (10%) + observation under ultraviolet light (360 nm)	Yellow color
Steroids	2 mL of extract + 2 mL of H_2SO_4 (concentrated)	Brown ring
Flavonoids	2 mL extract + Mg + 2 mL HCl (concentrated)	Orange or red color
Phenols	2 mL of extract + drops of $FeCl_3$	Blue or blue-green color
Glycosides	2 mL of extract + drops of $FeCl_3$ + 1 mL of CH_3COOH	Brownish red ring
Saponins	5 mL of extract + 5 mL of H_2O+ heating	Foam formation
Tannins	2 mL of extract + 2 mL of H_2O+ drops of $FeCl_3$ (1%)	Blue or green color
Terpenes	2 mL of extract + 2 mL of $(CH_3CO)_2O$+ 2 mL of H_2SO_4 (concentrated)	Red color

Checks were conducted every 12 hours to gather data on seed germination, with the goal of determining the germination percentage (GP) and the germination speed index (GSI). After 72 hours, the seedlings were removed from the Petri dishes, and the lengths of the roots and shoots of each seedling were measured using a caliper.

To determine the allelopathic potential of the extracts, the half maximal inhibitory concentration (IC_{50}) was calculated, i.e., the concentration that inhibited the evaluated parameters by 50%. This calculation was carried out using GraphPad Prism 8 software (GraphPad Software Inc.; San Diego, CA, USA). Using these data, it was possible to measure the sensitivity of the species and the evaluated parameters in relation to the *C. longa* extract. The lower the IC_{50} value, the greater the potency, indicating that the species or parameter showed a more significant response to the action of the extract.

Data analysis

The data collected from the analyzed variables, which include germination percentage (PG), germination speed index (GSI), and seedling root and shoot length, were subjected to analysis of variance. Subsequently, the means obtained were compared using the Scott-Knott test, using SISVAR statistical software version 5.6 (FERREIRA 2011).

RESULTS

Phytochemical screening

The chemical components present in the aqueous extract obtained from the rhizomes of *C. longa* were investigated by phytochemical analysis for 11 classes. Six of these were found to be present: reducing sugars, alkaloids, steroids, flavonoids, saponins, and terpenoids (Table 2).

Effects on germination

The aqueous extract obtained from the rhizomes of *C. longa* showed slight effects on the germination percentage (GP) of the species used in the bioassay. At a concentration of 64 g L^{-1}, there was a significant 20.70% reduction in GP for both species (*L. sativa* and *T. aestivum*) compared to the negative control (distilled water) (Figure 1a and Figure 1c). Notably, the results obtained were more promising than those of the herbicide glyphosate 10% (positive control), which did not significantly inhibit the germination percentage for any of the species tested.

Table 2. Class of secondary metabolites identified in the aqueous extract obtained from *C. longa* rhizomes.

Table 2. Classes of secondary metabolites identified in the aqueous extract obtained from the rhizomes of C. longa.

Compounds	Results
Reducing sugars	+
Alkaloids	+
Anthraquinones	-
Coumarins	-
Steroids	+
Phenols	-
Flavonoids	+
Glycosides	-
Saponins	+
Tannins	-
Terpenes	+

+ (Present); - (Absent).

With regard to the germination speed index (GSI), the aqueous extract showed a significant effect on lettuce (*L. sativa*) at a concentration of 4 g L^{-1}, resulting in a 32.97% reduction compared to the negative control (Figure 1b). However, it is important to note that this inhibition was less than that exerted by the positive control (glyphosate), which reduced the GSI by 55.85%. However, at the highest concentrations, the inhibition exerted by the aqueous extract on the lettuce's germination velocity index (GSI) was greater than that observed for the glyphosate herbicide. The concentration of 32 g L^{-1} inhibited the GSI by 62.44%, and the concentration of 64 g L^{-1} inhibited it by 75.95%, compared to the negative control (Figure 1b).

In relation to wheat (*T. aestivum*), the GSI was also affected by the extract at a concentration of 4 g L^{-1}, causing a significant reduction of 13.44% compared to the negative control. This inhibition was statistically similar to that exerted by the herbicide glyphosate, which reduced the GSI by 15.44%. Again, at the highest concentrations of the aqueous extract (32 and 64 g L^{-1}), the greatest inhibitions in GSI were observed, reaching 57.55% and 63.66%, respectively (Figure 1d).

Effects on initial seedling growth

The aqueous extract of *C. longa* rhizomes had phytotoxic effects on lettuce (*L. sativa*) and wheat (*T. aestivum*) seedlings. At a concentration of 2 g L^{-1}, the extract significantly impacted the development of the aerial parts of lettuce seedlings, reducing them by 9.58%.

However, this concentration did not affect root development, and concentrations above 4 g L^{-1} were necessary to observe changes in this parameter. The greatest reductions in root length were observed at concentrations of 32 and 64 g L^{-1}, where root length was reduced by 83.56% and 89.79%, respectively, compared to the negative control (distilled water). The reduction observed in root length at both concentrations was statistically similar to that caused by the herbicide glyphosate (95.59% reduction). It is important to note that at a concentration of 64 g L^{-1}, the aerial parts of the lettuce seedlings did not develop, which was also observed in the positive control group (glyphosate) (Figure 2a).

The length of the aerial part of wheat seedlings (*T. aestivum*) was affected by the lowest concentration evaluated (1 g L^{-1}), causing a reduction of 20.10% compared to the negative control. In turn, root length was significantly affected by concentrations above 2 g L^{-1}, with a 28.43% reduction at this concentration (Figure 2b). Once again, the most significant results were observed at concentrations of 32 and 64 g L^{-1}, with the reduction in the aerial part of the seedlings (86.15%) caused by the 64 g L^{-1} concentration being statistically equivalent to the reduction caused by the glyphosate herbicide (93.62%).

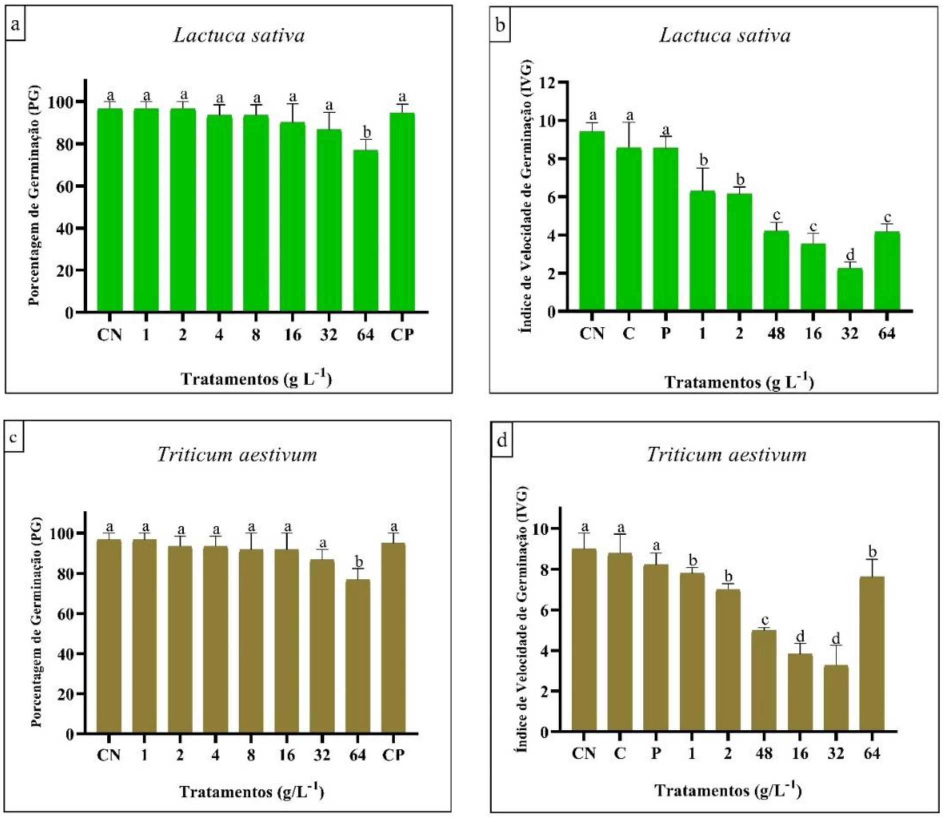

Figure 1. Germination percentage (GP) and germination speed index (GSI) of lettuce (*L. sativa*) and wheat (*T. aestivum*) seeds exposed to different concentrations (1 to 64 g L^{-1}) of the aqueous extract obtained from the rhizomes of *C. longa* and the controls (CN = distilled water and CP = glyphosate). Data are expressed as means ± standard deviation. Averages followed by the same letters do not differ statistically (Scott-Knott test 5%).

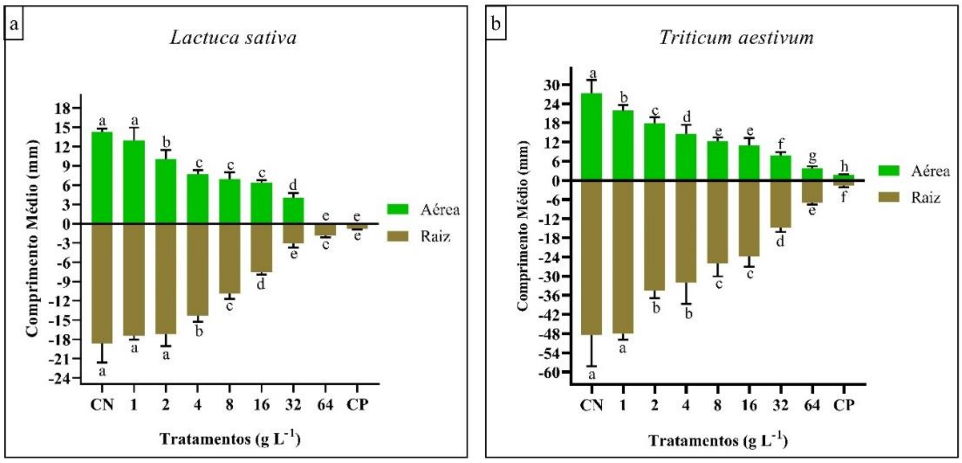

Figure 2. Average length (mm) of lettuce (*L. sativa*) and wheat (*T. aestivum*) seedlings exposed to different concentrations (1-64 g L^{-1}) of the aqueous extract obtained from *C. longa* rhizomes and the controls (CN = distilled water and CP = glyphosate). Data are expressed as means ± standard deviation. Averages followed by the same letters do not differ statistically (Scott-Knott test 5%).

Inhibition data

In this study, the tested concentrations were unable to inhibit germination by 50%. Consequently, it was not possible to determine the half maximal inhibitory concentration (IC50) for this variable.

However, the extract had a significant effect on the germination speed index (GSI) of the plants used in the allelopathic test. Among these, *L. sativa* proved to be particularly sensitive, requiring a concentration of 7.12 g L^{-1} to reduce this index by 50% (Figure 3a). In relation to seedling root length, the species *T. aestivum* showed the greatest sensitivity, with an IC50 of 6.97 g L^{-1} (Figure 3d). Finally, in terms of shoot length, *T. aestivum* also stood out, requiring a concentration of 3.73 g L^{-1} to cause a 50% reduction in this parameter (Figure 3f).

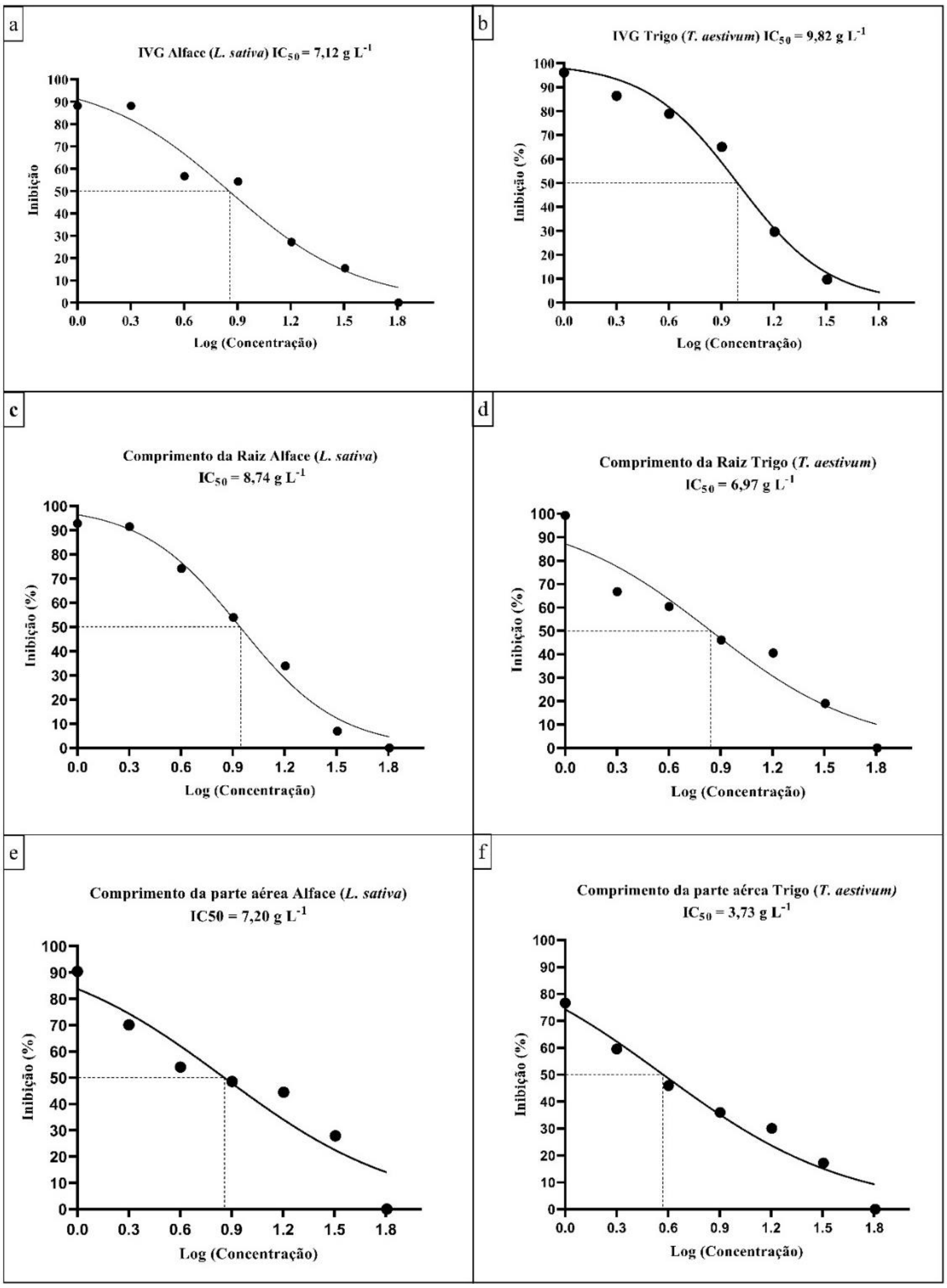

Figure 3. Average inhibitory concentration (IC50) of the aqueous extract obtained from the rhizomes of *C. longa* against the evaluated parameters: germination speed index (GSI), root length, and shoot length of lettuce and wheat seedlings.

DISCUSSION

The choice of water as the extracting solvent was based on previous studies showing its effectiveness in extracting compounds from *C. longa* rhizomes. These studies reported contents of 12.22% in water, 9.20% in alcohol, and 7.31% in ether (m/m) (CHANDEL et al., 2011). UTHAYARASA et al. (2010) performed sequential extractions using solvents of different polarities on the rhizomes of *C. longa*, identifying specific compounds in each extract. None of the evaluated classes (alkaloids, flavonoids, cardiotonic glycosides, saponins, tannins and terpenoids) were found in the hexane extract. The dichloromethane and ethyl acetate extracts tested positive for alkaloids, cardiac glycosides and terpenoids. The ethanolic extract revealed the presence of tannins, alkaloids, flavonoids, and terpenoids. The hydroalcoholic extract of *C. longa* rhizomes contained alkaloid compounds, reducing sugars, glycosides, tannins, resins, saponins, sterols, and fixed oils (GOVIND 2011).

Further research found that the aqueous extract tested positive for flavonoids and alkaloids, but did not contain glycosides, reducing sugars, tannins, or saponins (CHAKRABORTY & SENGUPTA 2012). Our findings corroborate these results, since we also identified the presence of alkaloids and flavonoids in the aqueous extract of *C. longa*. However, in contrast to the aforementioned study, we observed the presence of reducing sugars and saponins in the aqueous extract (Table 2). These discrepancies may be related to the specific genotype of the plant, its age, time and place of collection, and other factors that influence the production of secondary metabolites.

Of the six classes of compounds identified in the aqueous extract (Table 2), four have been the subject of studies investigating their allelopathic potential and potential use as bioherbicides. These classes include alkaloids, steroids, flavonoids, and terpenoids (PIRES & OLIVEIRA, 2001).

Alkaloids are nitrogen-containing compounds widely found in various sources, including plants, fungi, bacteria, and animals (CUSHNIE et al., 2014). In seed plants, especially in roots, fruits, and stems, the presence of alkaloids is well documented, and around 31,000 alkaloid compounds have already been identified (SHI et al., 2014). Due to their highly bioactive properties, alkaloids play a prominent role in allelopathy. Studies have shown that these substances can induce oxidative stress in seedlings, generating reactive oxygen species and affecting the metabolism and physiology of target plants (OGUNSUSI et al., 2018). In addition, alkaloids cause damage to exposed seedlings, leading to necrosis (Lederer et al., 2004). Other alkaloid compounds have been identified as inhibitors of photosystem II, interfering with photosynthetic processes (Dayan et al., 2015).

Certain alkaloids also have cytotoxic properties that are associated with their direct interaction with vital cell components such as DNA, RNA, and enzymes, including telomerases, polymerases, and topoisomerases (SAJITHA et al., 2018; FILIPPIN et al., 2018).

Steroids play essential roles in plants, with diverse functions. Phytosterols, for example, are fundamental components of plant cell membranes, regulating their fluidity and permeability, thus ensuring the integrity and proper functioning of the membranes. Another group of steroids present in plants is steroidal alkaloids, which have insect-repellent properties and can act as chemical barriers against pathogens, contributing to plant defense against harmful organisms (SIMÕES et al., 2016). Studies on the allelopathic potential of sterols have shown that they can affect the quantum efficiency of photosystem II reaction centers in leaves, impacting the growth and development of target plants. In addition, steroids can affect cell membranes, inhibiting steps in the mitosis process and impairing proper cell membrane formation after cell division (MACÍAS et al., 2019).

Flavonoids are compounds with diverse biological functions, including protection against ultraviolet (UV) radiation and phytopathogens, signaling during nodulation, regulation of male fertility, auxin transport, and floral coloration to attract pollinators. In addition, they contribute to the display of colors in plants, helping to protect leaf cells against photooxidative damage and enhancing the efficiency of nutrient recovery during the senescence phase (FALCONE FERREYRA et al., 2012; SIMÕES et al., 2016; NABAVI et al., 2020). With regard to allelopathy, flavonoids can affect auxin degradation through IAA oxidases and peroxidases, potentially impacting root growth in target species. Studies have shown that combinations of flavonoids can inhibit root growth, reduce the frequency of cell division in the meristematic regions of the root, and suppress the formation of root hairs and statoliths in the cells of the root apex (WESTON & MATHESIUS 2013).

Terpenoids play essential roles in inter- and intraspecific plant interactions, acting as communication and defense agents in response to biotic and abiotic stresses (THIMMAPPA et al., 2014; SILVA et al., 2016). Studies have investigated the ecological characteristics of terpenoids and their allelopathic effects on plants, revealing that they can cause inhibition, promotion, or autotoxic effects on seed germination and seedling growth (CIMMINO et al., 2014; ALWATTAR et al., 2023). The toxicity of terpenoids can be associated with mechanisms such as inhibition of ATP formation, disruption of hormonal activity, alkylation of nucleophiles, complexation with proteins, binding with free sterols, and inhibition of respiration (BACHHETI et al., 2020).

Despite chemical analysis showing the presence of compounds with allelopathic activities in the aqueous extract obtained from *C. longa* rhizomes, few studies in the literature have evaluated this potential and the application of this plant for bioherbicide production. This study found that the extract had a limited effect on the germination percentage of lettuce and wheat seeds. To achieve a significant reduction in this parameter in both species, exposure to a concentration of 64 g L^{-1} was necessary. However, it is important to note that this concentration resulted in greater inhibition than that caused by glyphosate, a post-emergence herbicide (as shown in Figures 1a and 1c). Studies have shown that seed germination is less sensitive to allelopathic compounds compared to other processes, such as root and shoot growth (SHAHRAJABIAN et al., 2019; WEI et al., 2020; WANG et al., 2022). This may be related to the structure of the seeds, such as the integument, pericarp, caryopsis, and seed coat, which have selective permeability and protect the seeds from stressors (BEWLEY et al., 2012; TAIZ & ZEIGER, 2017), such as allelopathic compounds.

AKTER et al. (2018) revealed results that were inconsistent with the findings of this study. They investigated the effect of the methanolic extract derived from the rhizomes of *C. longa* on the germination of lettuce (*L. sativa*), radish (*Raphanus sativus* L.), watercress (*Lepidium sativum* L.), and prickly pear (*Bidens pilosa* L.), observing significant inhibition of this parameter at concentrations of 0.5, 1, and 2 mg mL^{-1}. Based on these results, the authors discussed the potential of using the *C. longa* plant to produce bioherbicides. The discrepancy between this study and that of Akter et al. (2018) may be associated with the extraction method used to obtain the active ingredients from the rhizomes. In this study, the aqueous extract was chosen due to its simplicity, ease of preparation, and absence of toxic solvents. However, this choice may have affected the concentration and availability of the active compounds present in the extract.

Unlike the germination results observed, the aqueous extract of *C. longa* rhizomes showed significant interference with the initial development of the seedlings, both in the roots and the aerial parts, and this inhibition was dose-dependent. Some concentrations of the extract showed inhibitory effects statistically similar to those caused by the 10% glyphosate herbicide (Figures 2a and 2b). These results can be directly attributed to the presence of chemical compounds identified in the aqueous extract (Table 2), such as alkaloids, steroids, flavonoids, and terpenoids. These substances, as previously discussed, have allelopathic potential.

C. longa has four specific curcuminoids in its rhizomes: tetrahydrobisdemethoxycurcumin, bisdemethoxycurcumin, demethoxycurcumin and curcumin, which possibly influenced the inhibition of lettuce and wheat seedling growth observed in this study (Figure 2). In the study conducted by Yano et al. (2000), two of these compounds (bisdemethoxycurcumin and curcumin) were identified at low levels in the aqueous extract of the rhizomes. In the research conducted by AKTER et al. (2018), the methanolic extract of the rhizomes revealed the presence of all the curcuminoids mentioned. These compounds have been shown to inhibit the growth of prickly pear (*B. pilosa*) seedlings. The author also pointed out that the presence of methoxyl groups in these curcuminoids can enhance their allelopathic activity.

In addition to these compounds, *C. longa* rhizomes contain three important sesquiterpenes: ar-turmerone, β-turmerone and α-turmerone, which have been documented in the literature for their influence on weed growth after emergence (IBÁÑEZ & BLÁZQUEZ 2019). Studies have also shown that the aqueous extract obtained from the rhizomes of *C. longa* has cytotoxic effects on *Allium* cepa L., reducing cell division in the meristematic zones and the length of the exposed roots (DA SILVA et al., 2023). Therefore, it is likely that the presence of these chemical constituents in the extract, combined with their associated cytotoxic activity, suppressed the growth of *L. sativa* and *T. aestivum* seedlings.

In the allelopathic bioassays conducted, the half maximal inhibitory concentration (IC50) was determined for each parameter evaluated. Among the parameters analyzed, the length of the aerial part of the seedlings proved to be the most sensitive, requiring lower concentrations of the extract to achieve 50% inhibition of this parameter for both species (Figure 3). This finding has substantial relevance in the context of herbicide application in agricultural environments, since the aerial parts of plants represent the main route of entry for herbicides (DAYAN 2019), making it a critical point for efficient weed control. In addition, choosing a lower concentration of the extract to achieve the same inhibitory effect results in a reduction in the amount of product required, making it more economically viable and aligned with environmentally sustainable practices.

Although the pharmacological activities of *C. longa* are widely recognized, there is a significant lack of studies related to its allelopathic and bioherbicidal activity. The results of this study present promising prospects for the development of new molecules with phytotoxic potential. However, field studies are needed to take into account abiotic and biotic factors. In addition, other species, including weeds, should be used in biological tests. In addition, it is crucial to conduct toxicity tests to determine whether the extract has accumulative potential and is toxic to soil microfauna or non-target organisms such as insects.

CONCLUSION

The aqueous extract obtained from the rhizomes of *C. longa* revealed the presence of four important classes of allelochemicals: alkaloids, steroids, flavonoids and terpenoids.

In the biological test, the aqueous extract had a limited effect on the germination percentage of lettuce and wheat seedlings, requiring high concentrations to significantly inhibit this parameter. However, it showed promising effects on the growth of wheat and lettuce seedlings, even at the lowest concentrations tested. Among the parameters used to assess allelopathy, greater sensitivity was observed in the aerial parts of the seedlings of both species.

The results demonstrate the potential of *C. longa* as a source of phytotoxic substances for the development of bioherbicides. This, in turn, could lead to a reduction in the use of synthetic herbicides, ultimately contributing to more sustainable weed management.

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