



Impact of *Verbesina sphaerocephala* extracts on seed germination and seedling development of tomato and mung bean

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ABSTRACT

Sustainable alternatives to herbicides, agrochemicals, and other synthetic molecules are imperative for the ecological sustainability in the line of United Nations Sustainable Development Goals (SGD) goals. Natural aqueous extracts from different plant species are important sources of biomolecules that can be used to holistically manage crops by improving germination and plant development. The objective of this study was to evaluate the application of two aqueous extract preparations of *Verbesina sphaerocephala* on the germination and development of tomato and mung bean seedlings. Infusion and maceration extracts were prepared using *V. sphaerocephala* leaves at different concentrations (0.25, 0.75, and 1.5 g/l). The effects on seed germination and seedling development were evaluated under nursery conditions. The germination percentage of tomato seeds was significantly reduced following the application of all extracts, which was considered an allelopathic effect. The opposite results were observed in mung bean seeds. Seedling development also differed between species following extract application, although the development of certain agronomic variables, such as stem height, root length, and fresh weight, were favored in both in tomato and mung bean. From the study, it may be concluded that *V. sphaerocephala* extracts delay germination during the first three days of tomato growth, however, they promote optimum seedling development both in tomato and mung bean.

Key words: Seedling vigour, biostimulant, allelopathy, botanical extracts, capitaneja.

INTRODUCTION

The application of chemical pesticides has been found to decrease the nutrient content of agricultural soils by at least 50% (FAO, 4). Thus, sustainable alternatives that minimize the use of chemical pesticides and fertilizers in agriculture, such as botanical extracts that act as biostimulants, must be developed. Botanical extracts are environmentally safe compounds that can improve germination, support crop development, increase yields, and enhance the quality of agricultural products (Fregoni, 6). The benefits of the application of botanical extracts to crops extend beyond plant nutrition and encompass promotive effects like bio-stimulation and protective effects such as phytotoxicity due to the production of secondary metabolites, phytohormonal groups, or other chemical compounds. *Verbesina sphaerocephala* A Gray. (family Asteraceae) is endemic to the states of Jalisco, Michoacán, Nayarit, Guanajuato, and Guerrero in western Mexico.

Commonly, *V. sphaerocephala* is known as *capitaneja*, *vara blanca*, or *palo espino*. This species is not widespread, and researchers have only begun to study the potential of its biomolecules and their agricultural applications as bioremediators (Velasco-Ramírez *et al.*, 18) and growth promoters (Velasco-Ramírez *et al.*, 17). However, *V. sphaerocephala* contains multiple substances that may cause allelopathic effects, which can interfere with the germination and growth of other plants (Elisovetcaia *et al.*, 2).

In recent decades, allelopathic compounds have been proven to be natural alternatives to pesticides and herbicides and have been successfully used to control pests, diseases, and weeds (Panca-Jevera *et al.*, 14). Given that *V. sphaerocephala* has been found to contain potentially allelopathic substances, we propose that aqueous extracts of *V. sphaerocephala* function as growth promoters by inhibiting the presence of weeds and pests. The use of these aqueous extracts, which can be produced alongside crops by the same producers, can minimize the use of synthetic substances, such as herbicides and agrochemicals, in commercially important crops like tomato (*Solanum lycopersicum*) and mung bean (*Vigna radiata*).

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The red tomato is one of the most consumed vegetables worldwide, and Mexico ranks ninth worldwide in production (SIAP, 15). The mung bean is a legume native to Southeast Asia and India (Wintersohle *et al.*, 19). Mung beans contain calcium, iron, magnesium, potassium, and phosphorus, are high in fiber, and lack cholesterol. Despite being a sprout with great nutritional potential, mung beans are not highly consumed in Mexico and can usually only be found in health food stores. Nonetheless, the mung bean is an important experimental model in biostimulants studies and has been used in comparisons of seedling germination and development (Hernández-Herrera *et al.*, 8). This work aimed to evaluate the effects of two aqueous extract preparations (infusion and maceration) of *V. sphaerocephala* on the germination and development of tomato and mung bean seedlings. Additional studies with chemical approaches are necessary to better direct the uses and applications of *Verbesina biomolecules*, as they also functioned as growth promoters.

MATERIALS AND METHODS

This study was carried out between the years 2023 and 2024. Consisted of germinating seeds in germination paper using the taco technique (the seeds were placed on germination paper moistened with all the treatments to be evaluated, and were rolled into a “taco” shape. At the end, the “tacos” were placed inside a polyethylene bag that was placed inside a drying chamber at 29 °C). Leaves (vegetative phase) of wild *V. sphaerocephala* were dried at room temperature (~26 °C) in the laboratory and then pulverized using a blade mill. Subsequently, maceration and infusion extractions were carried out. Extraction by maceration was prepared with 25, 75, and 150 g of leaves (dry weight). The leaves were added to 1 L of distilled water at room temperature and constantly stirred for 15 min. Extraction by infusion was prepared with 25, 75, and 150 g of leaves (dry weight). The leaves were added to 1 L of distilled water (80 °C) and constantly stirred for 15 min. The hot extracts were passed through Whatman No. 40 filter paper and stored at 4 °C. The liquid extracts of *V. sphaerocephala* were designated V.s 0.25 g/l, V.s 0.75 g/l, and V.s 1.5 g/l based on their concentration, and the pH and electrical conductivity (EC, dS m⁻¹) of each extract were measured. The chemical characterization of the aqueous extracts of *V. sphaerocephala* was previously reported by Hernández-Pérez (10) and Velasco-Ramírez *et al.* (17).

Certified seeds of *S. lycopersicum* (indeterminate variety PaiPai) were acquired from Enza Zaden (Enkhuizen, The Netherlands), and certified seeds

of *V. radiata* were acquired from Food to Live (New York, USA). Seeds of both species (95% germination) exhibited uniform size, color, and weight. In July 2023, a germination test was conducted using the standard “between paper method” described by ISTA (2010, 11) to evaluate the effects of the *V. sphaerocephala* extracts. The seeds of both species were germinated on sheets of germination paper that had been previously moistened with distilled water. The seeds were homogenously spaced and organized in a row based on the size of each seed. The seeds were covered with another sheet of germination paper and moistened with 20 mL of V.s 0.25 g/l, V.s 0.75 g/l, V.s 1.5 g/l, or distilled water (control). The sheets were then rolled into a taco shape. The taco sheets were randomly arranged inside a polyethylene bag that was placed inside a plastic tray. At least 10 replicates of 10 seeds were placed between the sterilized and moistened papers and incubated at 25 ± 2 °C in darkness. Seed (n=100) germination was evaluated in each experimental treatment. Prior to germination, the seeds were surface sterilized using a soap solution for 5 min, followed by immersion in a 4% sodium hypochlorite (NaClO) solution for 10 min. After which, the seeds were triple rinsed in sterilize distilled water for 1 min under aseptic conditions. Germination was observed daily over a period of 8 days, according to the methods of the Association of Official Seed Analysts (AOSA, 1). After 8 days of incubation, the germination percentage (over the control) was determined.

Tomato and mung bean seedlings in the rolled taco sheets (30 seeds per treatment; 3 replicas per treatment) were incubated, and germination was measured after 2 weeks. Seedlings were treated with infusion and maceration extracts of *V. sphaerocephala* (0.25, 0.75, and 1.5 g/l) and incubated in a bioclimatic chamber under controlled light (50 μmol photons m⁻² s⁻¹ with white fluorescent tubes), temperature (25 ± 2 °C), humidity (100%), and photoperiod (16-h light /8-h dark) conditions. For each treatment, root and shoot length (cm) were measured with a vernier caliper, and the fresh and dry weights of the seedlings were obtained with an analytical balance after oven drying at 130 °C for 1 h (ISTA, 13). The germination percentage (GP) was calculated with Eq. (1):

$$GP = \frac{\text{Number of germinated seeds at the final count}}{\text{Total number of seeds sets in the bioassay}} \times 100 \quad \text{Eq. (1)}$$

The mean germination time (MGT) was calculated with Eq. (2):

$$MGT = \frac{\sum nd}{\sum n}, \quad \text{Eq. (2)}$$

where n is the number of newly germinated seeds per day, and d is the number of days from the start of the germination test. The germination index (GI) was calculated with Eq. (3):

$$GI = \frac{\text{Number of germinated seeds} \times \text{average root length}}{100} \quad \text{Eq. (3)}$$

The germination rate index (GRI) was calculated with Eq. (4):

$$GRI = \sum \left(\frac{ni}{t} \right), \quad \text{Eq. (4)}$$

where n is the number of seeds germinated, and t is the number of days from sowing to the final day of the experiment. The seedling vigor index (SVI) was calculated with Eq. (5):

$$SVI = \text{Seedling vigor} \times GP, \quad \text{Eq. (5)}$$

where seedling vigor is the root and shoot length in cm, and GP is the germination percentage.

The second stage was 12 days of seed germination and primary root development; the tomato and mung bean seedlings were acclimated to *ex vitro* conditions in the greenhouse nursery. The seedlings were acclimated in 420, 3-inch plastic pots filled with a previously sifted mixture of jal substrate (organic substrate of volcanic origin), coconut powder, and oak leaves in equal parts (33.3%). All pots were irrigated at field capacity. After nine days of acclimation, the first fertilization (soil drench) was conducted by applying 0.5 ml/l of Poliquel Zinc, Arysta Pilatus, and MAP (0.5 g/l). Fertilization was performed three times per week. On the same day as the start of fertilization, the first application of the aqueous extracts of *V. sphaerocephala* was given, and 5 ml/l of extract was applied directly to the soil once a week. The growth parameters of leaf length, root length, and fresh and dry weight were measured after 30 days of growth in both crops. The variables were calculated according to the methods of Hernández-Herrera *et al.* (9).

The experimental design and treatment were similar to those reported by Velasco-Ramírez *et al.* (17). A total of 4 different treatments were employed with 30 replications per crop. The first treatment served as the control, and the plants were grown without botanical extracts. Two factors were randomized for the other three treatments. The first factor was the type of botanical extract (macerated or infusion). The second factor was the concentration (0.25, 0.75, or 1.5 g/l). The experimental units were arranged in a completely randomized two-factorial design; plants received either 5 mL distilled water (control) or 5 mL of botanical extract (experimental treatments). In all cases, normality and homoscedasticity tests were used to evaluate the data. To compare the means of two treatments, a one-way analysis of variance (ANOVA) and the least significant difference (LSD) multiple comparison test ($p = 0.05$)

were used. All statistical analyses were performed in Statgraphics Centurion XV for Windows.

RESULTS AND DISCUSSION

The tomato and mung bean plants differed in their responses to the application of *V. sphaerocephala* extracts in terms of seed emergence (Fig. 1).

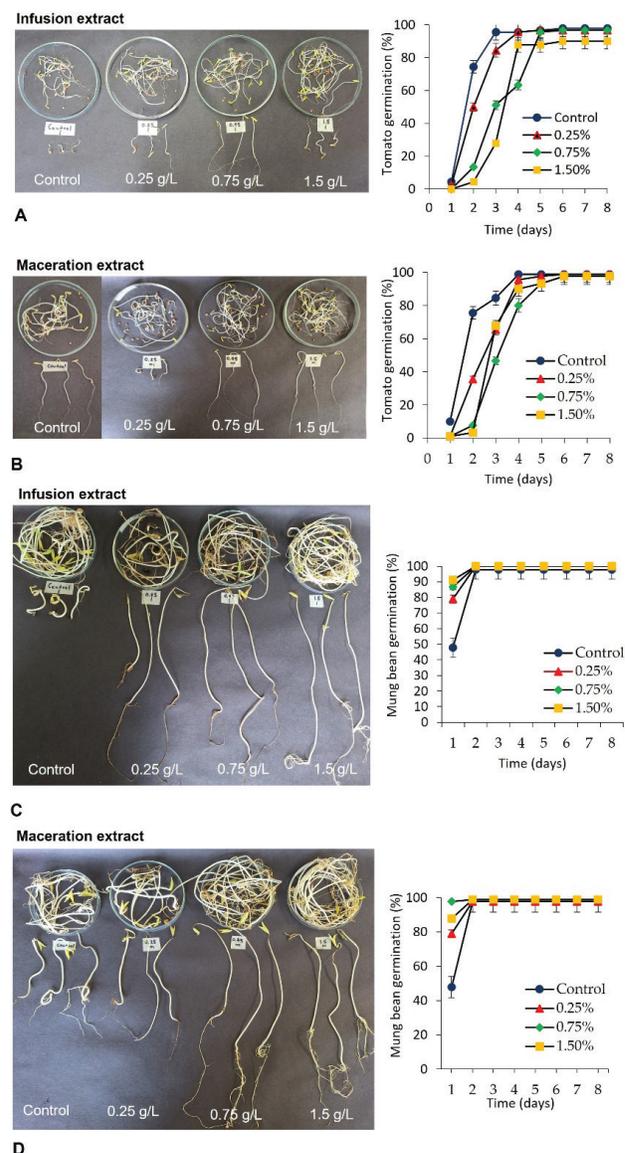


Fig. 1. Effects of *Verbessina sphaerocephala* infusion and maceration extracts at concentrations of 0.25 g/l, 0.75 g/l, and 1.50 g/l on the germination (%) of (A) tomato seeds treated with the infusion extracts, (B) tomato seeds treated with the maceration extracts, (C) mung bean seeds treated with the infusion extract, and (D) mung bean seeds treated with the maceration extract. Values are averages ($n = 100$ seeds); bars are the standard error.

The effect of the *V. sphaerocephala* infusion and maceration extracts at all concentrations delayed tomato germination during the first 3 days following the application of the extract, and GP decreased with the application of the infusion extract (1.50 g/l) (Fig. 1A, B). In contrast, mung bean germination occurred in all treatments one day later. The effect of the *V. sphaerocephala* infusion and maceration extracts at all concentrations (0.25, 0.75, and 1.50 g/l) increased in GP compared to the control (Fig. 1C, D). Both high-concentration treatments (0.75 and 1.5 g/l) increased GP (Fig. 1B, C). Tomato seeds treated with the botanical infusion and maceration extracts at all concentrations decreased GP, exhibited the longest average delay in MGT (5.80 days), were the last to germinate, and exhibited the greatest variation in germination time. The highest GI was recorded following the application of the maceration extract (1.5 g/l); the GI values of the other treatments ranged from 3.68 to 4.20. The GRI did not respond positively to extract application (53.75), as the highest value was obtained in the control (74.25). Only SVI improved with the application of the infusion extract (0.75 g/l; 2546.52) and maceration extract (1.5 g/l; 2228.16) (Table 1). In contrast, mung bean seeds treated with *V. sphaerocephala* infusion and maceration extracts showed higher germination rates associated with lower MGT and increased SVI (Table 2). The infusion and maceration extracts resulted in high GP (up to 89 and 90%,

respectively), low MGT (4.5 days), GI values of 5.29 (1.5 g/l), and high GRI (85.88 [0.25 g/l] to 89.00 [0.25 g/l]) and improved SVI (2143.65 [1.5 g/l] and 2263.48 [0.25 g/l]) compared to the control. In the greenhouse experiment, application of the infusion and maceration extracts significantly enhanced the growth of tomato plants ($p \leq 0.05$). The soil drench application of the infusion extract was more effective in increasing plant height than the maceration extract (Fig. 2A, B). The interaction between treatment and extract concentration demonstrates that plants treated with the infusion extract at 0.25, 0.75, and 1.5 g/l exhibited an increase in shoot length (15, 14.7, and 14.4 cm, respectively). Application of the infusion extract (0.75 and 1.50 g/l) resulted in an increase in root length (14.5 and 12 cm, respectively). In contrast, plants treated with the maceration extract only displayed an increase in shoot length (<14.9 cm) with the concentration of 1.5 g/l (Fig. 2A, Fig. 2B, and Fig. 3). In addition, positive effects on fresh weight (3.6 and 3.5 g, respectively) were observed following application with the *V. sphaerocephala* infusion extract (0.25 g/l) and maceration extract (0.75 g/l) (Fig. 2C). The dry weight of tomato plants was unaffected by treatment with the infusion extract; however, treatment with the maceration extract (0.25 g/l) had a positive effect on dry weight (0.297 g) compared to the control (0.257 g) (Fig. 2D). In the greenhouse experiment, the soil drench application of the infusion and maceration extracts significantly

Table 1. Effects of *Verbesina sphaerocephala* treatments on the germination percentage (GP), mean germination time (MGT), germination index (GI), germination rate index (GRI), and seed vigor index (SVI) of tomato.

Tomato	Germination (%)		Mean germination Time (MGT; days)		Germination Index (GI)		Germination Rate Index (GRI)		Vigor Index (SVI)	
	Infusion	Maceration	Infusion	Maceration	Infusion	Maceration	Infusion	Maceration	Infusion	Maceration
Control	88	89	5.09	5.09	4.20	4.23	74.25	74.75	2053.34	2076.68
V.s 0.25 g/l	87	89	5.25	5.43	4.06	4.02	69.63	66.63	2291.40	2167.89
V.s 0.75 g/l	87	88	5.73	5.74	3.68	3.75	57.75	58.75	2546.52	2175.88
V.s 1.5 g/l	81	88	5.80	6.45	3.47	4.43	53.75	61.75	2152.42	2228.16

Table 2. Effects of *Verbesina sphaerocephala* treatments on the germination percentage (GP), mean germination time (MGT), germination index (GI), germination rate index (GRI), and seed vigor index (SVI) of mung bean.

Mung bean	Germination (%)		Mean germination Time (MGT; days)		Germination Index (GI)		Germination Rate Index (GRI)		Vigor Index (SVI)	
	Infusion	Maceration	Infusion	Maceration	Infusion	Maceration	Infusion	Maceration	Infusion	Maceration
Control	88	88	4.74	4.74	4.34	4.34	82.38	82.38	2097.92	2097.92
V.s 0.25 g/l	90	88	4.59	4.59	4.47	4.38	87.63	85.88	2223.48	2263.48
V.s 0.75 g/l	90	89	4.56	4.50	4.48	4.45	88.50	88.88	2215.18	2207.62
V.s 1.5 g/l	90	89	4.54	5.42	4.49	5.29	89.00	87.75	2143.65	2080.67

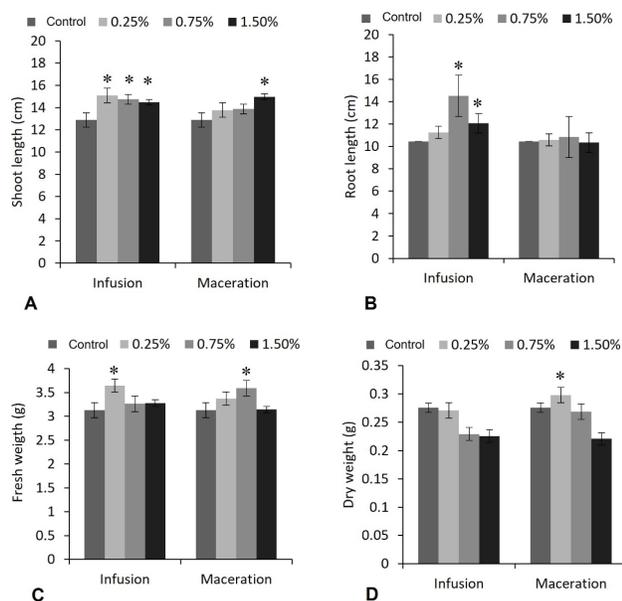


Fig. 2. Effects of *Verbesina sphaerocephala* infusion and maceration extracts applied *via* soil drench to tomato plants on (A) shoot length, (B) root length, (C) fresh weight, and (D) dry weight at concentrations of 0.25, 0.75, and 1.50 g/l. Distilled water was applied to control plants. Columns denoted by an asterisk are significantly different ($p \leq 0.05$). Values are averages ($n = 90$ plants); bars are the standard error.

enhanced the area part of mung bean seedlings ($p \leq 0.05$). The interaction between treatment and concentration demonstrates that mung bean plants treated with the infusion extract (0.25, 0.75, and 1.5 g/l) displayed an increase in shoot length (14.7, 13.7, and 13.6 cm, respectively), whereas plants treated with the maceration extract (0.25 and 0.75 g/l) displayed an increase in shoot length (14.6 and 14.2 cm, respectively) (Fig. 4A). Mung bean root

length was unaffected by treatment with the infusion or maceration extracts (Fig. 4B and Fig. 5).

Positive effects on fresh weight were observed following the application of the infusion extract (0.25 and 0.75 g/l; 5.4 and 5.5 g, respectively) and maceration extract (0.75 g/l; 5.6 and 5.8 g, respectively) (Fig. 4C). The fresh weight of mung bean plants increased following the application of the infusion extract (0.75g/l) (Fig. 4C) and maceration

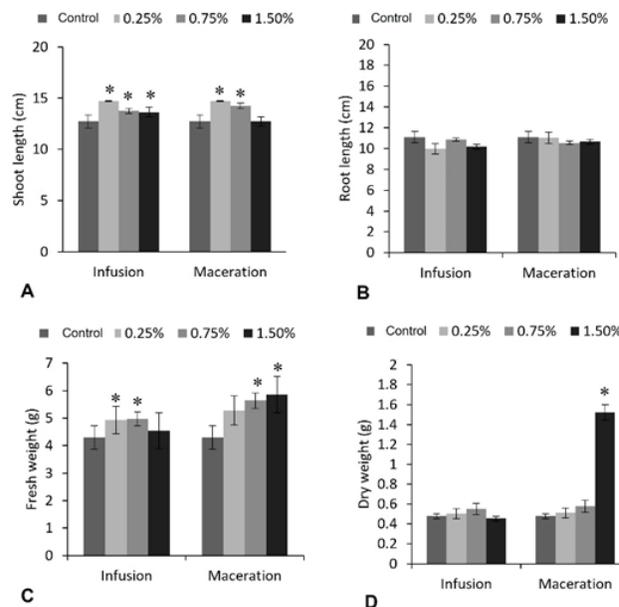


Fig. 4. Effects of the *Verbesina sphaerocephala* infusion and maceration extracts applied *via* soil drench to mung bean (A) shoot length, (B) root length, (C) fresh weight, and (D) dry weight at concentrations of 0.25, 0.75, and 1.50 g/l. Distilled water was applied to control plants. Columns denoted by an asterisk are significantly different ($p \leq 0.05$). Values are averages ($n = 90$ plants); bars are the standard error.

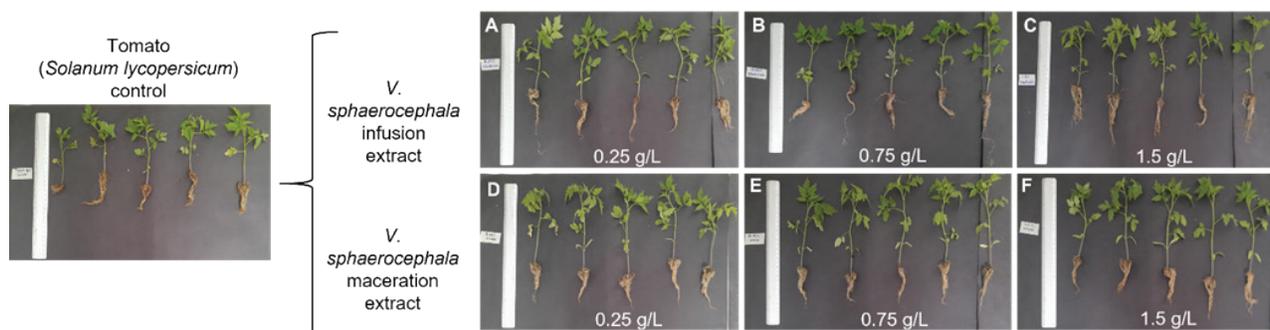


Fig. 3. Differences in seedling development between treatments in the nursery. Tomato seedlings treated with *Verbesina sphaerocephala* infusion extracts at concentrations of 0.25 g/l (A), 0.75 g/l (B), and 1.5 g/l (C), and tomato seedlings treated with *V. sphaerocephala* maceration extracts at concentrations of 0.25 g/l (D), 0.75 g/l (E), and 1.5 g/l (F).



Fig. 5. Differences in seedling development between treatments. Mung bean seedlings treated with *Verbena sphaerocephala* infusion extracts at concentrations of 0.25 g/l (A), 0.75 g/l (B), and 1.5 g/l (C), and mung bean seedlings treated with *V. sphaerocephala* maceration extracts at concentrations of 0.25 g/l (D), 0.75 g/l (E), and 1.5 g/l (F).

extract (1.5 g/l, 5.8 g); the maceration extracts also increased the dry weight of plants (1.6 g) compared to the control (0.4 g) (Fig. 4C, D). The chemical properties of *V. sphaerocephala* extracts (secondary metabolites) have been previously reported in crops such as tomato (*S. lycopersicum*) (Hernández-Pérez *et al.*, 10), strawberry (*Fragaria × ananassa*) (Velasco-Ramírez *et al.*, 17), and cucumber (*Cucumis sativus*) (Velasco-Ramírez *et al.*, 18). These secondary metabolites enhance the growth and development of the plants; however, the results of this study indicate that tomato germination was inhibited in the first days following extract application, especially at high doses. This was not observed following the application of *V. sphaerocephala* extracts to mung bean seeds. This difference could be due to the morphological characteristics of tomato and mung bean seeds. The tomato endosperm contains all the nutrients necessary for the initial development of the embryo; the testa, or seed coat, is made up of a hard and impermeable tissue covered with hairs that surrounds the embryo of the endosperm. Tomato seed germination is divided into three stages, the first of which lasts approximately 12 h. During this phase, the seed rapidly absorbs water. Therefore, adding the treatment of the aqueous extract of *V. sphaerocephala* seems to absorb it rapidly and to have caused an allelopathic effect due to the secondary metabolites it contains. Allelopathy is an ecological chemical phenomenon in which secondary metabolites produced by a plant species are released and interfere with the germination of other plants. The secondary metabolites in *V. sphaerocephala* act as mediators (allelochemicals) and inhibited nutrient-mobilizing enzymes (e.g., amylase and maltase) during the early stages of seed germination, preventing the endosperm from

breaking down. Espejo *et al.* (3) demonstrated that as the concentration of an aqueous extract of *Helianthus annuus* increased, the germination percentage of *Setaria unguolata* seeds decreased due to allelopathic inhibition. Similarly, González *et al.* (7) reported that botanical extracts of *H. annuus* inhibited tomato growth. In a recent study, Velasco-Ramírez *et al.* (17) reported that *V. sphaerocephala* extracts inhibited tomato germination.

Although previous studies have reported an inhibition of tomato germination during the initial stage due to the allelopathic effects of some botanical extracts, the subsequent stages of germination were not affected. This is because the initial germination stage is followed by a resting period of approximately 40 h during which no anatomical or metabolic changes are observed. Following this period of rest, the tomato seed begins to absorb water once more and transitions into the growth stage, which is associated with the emergence of the radicle (Nuez, 13). However, mung beans are higher in volume than tomato seeds; consequently, mung bean embryos have a greater amount reserve (Faria *et al.*, 5). As a result, application of the *V. sphaerocephala* infusion and maceration extracts did not physiologically inhibit the germination process of mung bean seeds. The extracts of *V. sphaerocephala* significantly inhibited the germination of tomato seeds; however, seedling development was supported by the application of the extracts, which acted as plant biostimulants due to one of the functions that the enzyme tannase performs in the shikimic acid pathway. Shikimic acid is known as the starting point compound for the synthesis of a vast number of natural substances, such as gallic acid (precursor of tannins) and quinic acid (precursor of secondary metabolites such as those with antimicrobial properties) (Singh *et al.*,

16) and its presence likely aided the development of tomato and mung bean seedlings. Although the root length of tomato and mung bean seedlings was not significantly affected by the application of the *V. sphaerocephala* infusion or maceration, seedling development was supported at a physiological level due to the observed enhancement in leaf length. Similarly, no noticeable effects were observed in the root surface, and a higher number of root hairs was observed. Nutrients and aqueous extracts that pass through the Caspary bands into the xylem through a symplastic route are absorbed by the stem, generating turgor in the seedlings (Liu *et al.*, 12).

V. sphaerocephala extracts exhibited dual effects *i.e.*, suppressing tomato seed germination likely due to allelopathic compounds, while enhancing seedling growth in both tomato and mung bean, particularly in later stages. Mung bean showed overall positive responses, suggesting species-specific sensitivity. These findings highlight the potential of *V. sphaerocephala* extracts as selective weed inhibitors and natural growth promoters. Future research should focus on isolating active compounds, understanding their mechanisms, and evaluating their application in sustainable crop and weed management.

AUTHORS' CONTRIBUTION

Conceptualization of research (APVR); Designing of the experiments (RMHH & ARHP); Contribution of experimental materials (SFVR, ACRA & AVR); Execution of field/lab experiments and data collection (ARHP & APVR); Analysis of data and interpretation (ARHP & RMHH); Preparation of the manuscript (APVR & SFVR).

DECLARATION

The authors declare that they do not have any conflict of interest.

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