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Allelopathic Influence of Aqueous Extract of *Stachytarpheta cayennensis* (Rich.) Vahl on Seed Germination and Initial Seedling Growth of *Cucumis sativus* L.

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Authors' contributions

This work was carried out in collaboration with all authors. Authors TCBK, OMY and IVS designed the study, wrote the protocol and wrote the first draft of the manuscript. Author ACB performed the statistical analysis. Author MPP performed the study analyzes. Authors MACC and AMR managed the bibliographic searches. All authors read and approved the final manuscript.

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ABSTRACT

Allelopathy studies investigate the positive and negative effects that secondary metabolites of plants, microorganisms or fungi on the development of neighboring individuals. This study aimed to evaluate the allelopathic potential of aqueous extracts of *Stachytarpheta cayennensis* on germination and initial development of *Cucumis sativus* L. seedlings variety. For this, the experiment was performed in the laboratory, using 5 concentrations (0, 5, 10, 20 and 40%) of the extracts, with 4 replicates each. The cucumber seeds were distributed in gerbox boxes lined with germitest paper, totaling 25 seeds per replicate. Subsequently, they were moistened with the

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extracts and kept inside the BOD-type germination chamber, regulated to 26°C and 12 hours brightness, following the completely randomized design for 7 days, and the control, for comparison purposes, was used distilled water. Comparing the zero dose to the other concentrations, the interferences in the GVI (germination velocity index) occurred in a greater proportion in the macerated stem concentrates diluted from 20%. Regarding MGT (mean germination time), the results point to interferences in this variable, in stem and leaf concentrates. Differential behaviors were observed when analyzing root and shoot length of seedlings, where macerated root extracts caused reduction as the doses increased. Extracts of stem showed increase of these variables as the doses increased. In leaf concentrates the result remained stable at shoot length and in smaller proportions regarding the root length of *C. sativus* seedlings. For dry matter, the leaf and stem concentrates increased this variable, while the root extracts had a reducing effect, remaining stable at 20%. The survey of the allelopathic potential of *S. cayennensis* contributes significantly with information of these plants considered as spontaneous and improve studies of the biological properties in the scientific community.

Keywords: Allelopatic effect; aqueous extracts; weed; spontaneous plant.

1. INTRODUCTION

The species *Stachytarpheta cayennensis*, popularly known in Brazil as the purple gervão, rinchão and buttonworm belongs to the Verbenaceae family, which comprises about 100 genera and approximately 2600 plant species [1].

It is part of the plant groups existing in the environment, referred to as spontaneous plants because they occur naturally with spontaneous growth in cultivated areas [2].

In cropping systems, although spontaneous plants are erroneously considered to be harmful, many of them add organic matter into the system, protect the soil surface from erosion, and act on nutrient cycling. In addition to providing the physical and chemical structure of soils; show allelopathic action on certain nematodes and insects [3]. In the present study, it was found that the biological activity in the root zone was similar to that of the roots.

Among the characteristics of the species *S. cayennensis*, is its medicinal use especially by traditional communities where infusions of all its aerial part are used as antipyretics, stomáquicos, in the treatment of chronic liver diseases and for several other purposes. Its roots are used as cicatrizant and as attenuating rheumatic pains. This species has varied chemical composition such as alkaloids, glycosides (verbenaline and verbenine), tannins, saponins, flavonoids, steroids, quinones, phenolic compounds and glycogenic acid [4].

The use of allelopathy in the management and balance of other spontaneous plants contributes not only to the environmental aspect with reduction of pesticides, but also to the production costs of crops [5].

In this sense, investigations into the possibility of using allelopathy in the management of spontaneous plants become important, as there are reports of the existence of plants that release substances that suppress the development of other plants, which could be used to control these species [6].

Experiments in the laboratory have been developed with the objective of analyzing, under optimal conditions of temperature and humidity, the effects of aqueous extracts, both shoot and root, on the germination of seeds of several species, since the allelochemicals may be present in tissues from different parts of the plant [7].

For this, some plant types that are sensitive to allelochemicals such as *Lactuca sativa* (lettuce), *Solanum lycopersicon* (tomato) and *Cucumis sativus* (cucumber) have been used. These species are considered plants indicative of allelopathic activity because they have sensitivity to secondary metabolites and these are a species-specific characteristic. *C. sativus* is widely used as a test plant because it presents rapid and uniform germination and a degree of sensitivity that allows the expression of results even at low concentrations of allelopathic substances [8].

Understanding the mechanisms of action of various substances is important to understand the interactions between plants [9]. Allelopathy involves interaction between abiotic and biotic stresses, through multiple compounds that may have synergistic relationships that potentiate their actions [5]. Due to the intensive and indiscriminate use of herbicides, allelopathy can be a viable alternative in the management of spontaneous plants, due to its ecological importance and the possibility of providing alternative sources of new chemical structures for the production of agricultural biodefensives [10].

The objective of this work was to study the possible allelopathic influence of aqueous extracts of *S. cayennensis* on seed germination and early development of *C. sativus* seedlings under laboratory conditions.

2. MATERIAL AND METHODS

The present study was conducted from February / 2015 to February / 2017, and conducted at the University of the State of Mato Grosso - UNEMAT, Campus Universitário de Alta Floresta / MT, at the Laboratory of Seed Technology and Weed Science (LaSeM), located at Technological Center of the Southern Amazon (CETAM), Brazil.

Stachytarpheta cayennensis individuals were collected in the urban area of Alta Floresta, located at Latitude 09°52'32 "S and Longitude 56°05'10"W, at the northern end of the State of Mato Grosso, Brazil. The climate is type Am, according to the classification Köppen, tropical rainy season with clear dry season [11].

In order to prepare the extracts, the specimens were collected, and in the laboratory, the material was duly sanitized in running water. Afterwards, the plants were separated into three parts (root, stem and leaves), being packaged separately in kraft paper bags. Subsequently, the materials were dried in a forced air circulation oven for 96 hours at 45°C. After this drying period, they were ground in Willey mill and stored in plastic containers and kept in a refrigerator at 10°C until the moment of their use. *C. sativus* variety was used as test plant, being the seeds free of chemical treatment, acquired in the local commerce and whose germination was previously tested (98%).

For the study of the allelopathic effect with aqueous gervan extracts, the experiment was carried out in a completely randomized design with four replicates, in a 3x5 factorial scheme, and the treatments were obtained by combining 3 parts of the plant (root, stem and leaf) at 5 concentrations (0, 5, 10, 20 and 40 mg mL^{-1}), with dilution in distilled water, with 4 replicates each concentration.

To obtain the aqueous extracts, the maceration procedure was used where the powder of each part of the plant was diluted in distilled water, in the proportion of 1: 25 (p v^{-1}), and the solution obtained was kept under stirring constant for 24 hours on a magnetic stirrer at room temperature and then each solution was subjected to filtering on a quantitative paper filter (JP40 – 25 µm permeability) and immediate use for assay setup.

From the initial solution (40 mg mL^{-1}), dilutions were performed to obtain the other concentrations: 0, 5, 10 and 20 mg mL^{-1}, totaling 5 treatments.

The germination test was carried out in a BOD type germination chamber with 12 hours light/dark photoperiod and constant temperature of 20°C for cucumber, as recommended by Brazil [12]. The tests were carried out in transparent gerbox boxes, lined with two sheets of germitest paper, previously autoclaved at 120°C, for 40 minutes. In each plastic box was added 12.0 mL of each aqueous extract to be tested at the concentrations of 0, 5, 10, 20 and 40 mg mL^{-1}. Subsequently, 25 cucumber seeds were distributed in each plot. All experimental units, represented by gerbox boxes, were arranged inside the BOD.

The percentage of germination was verified every 24 hours, during the period of seven days, and it was necessary to moisten the substrates again, thus generating data for the determination of the germination velocity index (GVI) and the mean germination time (MGT). The root and shoot length, as well as seedling dry matter on the seventh day (end of the evaluation period) were determined as follows:

Percentage of germination - Primary root emission with a length equal to 2 mm was considered as a criterion for germination. The calculations were performed according to the formula below:

$$G(\%) = \left(\frac{N}{A}\right) X100$$

Where:

N = Number of germinated seeds
A = total number of seeds.

Germination velocity index (GVI) was performed in conjunction with the germination test, by means of daily counts of the number of germinated seeds and for each sub-sample the value was obtained, according to the formula presented to follow:

$$IVG = \frac{N_1}{D_1} + \frac{N_2}{D_2} + ... \frac{Nn}{Nn}$$

Where:

N1: n = number of seedlings sprouted on day 1, ..., n;
D1: n = days for the occurrence of germination.

Mean germination time (MGT) - Given by the equation below, with the results expressed in days:

$$MGT = \frac{\left(\sum niti\right)}{\sum ni}$$

Where: ni = number of seeds sprouted per day;
ti = time of the evaluation after the beginning of the test;

At the end of the test, all seedlings were removed from each plot and, from them, the seedling length was determined. Subsequently these were packed in a kraft paper bag and dried in a forced circulation oven for 72 hours at 65°C.

Aerial part length and root of seedlings- They were evaluated in conjunction with the germination test, using all normal seedlings of each replicate, measured with ruler graduated in millimeters. The seedling root and shoot lengths, for each sample, were calculated by dividing the total of the measurements by the number of seedlings evaluated, obtaining mean values.

Dry mass (DM) of seedling: Defined as the average mass, expressed in grams, corresponding to the mass of each seedling per repetition, using a drying oven with air circulation, set at 65 ± 3°C for 2 days, weighing in balance with precision 0.001g.

The treatments were submitted to analysis of variance and the means of the qualitative factor (part of the plant) compared by the Tukey test, at a 5% probability level and for the quantitative factor (concentrations) the polynomial regression of the SISVAR computer program [13]. The data collected were transformed into percentages, analyzed and grouped into their respective categories.

3. RESULTS AND DISCUSSION

It was verified according to statistical analysis (Table 1), that interaction between the extract and dose factors occurred for the variables MGT (mean germination time), DM (dry mass), RL (root length) and SL (shoot length). For GVI (germination velocity index) there was a difference between the isolated form factors, both for extract and for dose. Regarding the germination variable, there was no significance for any of the factors tested as well as interaction between them.

According to Martinelli and Silva [14], recent studies show that, although the final percentage of germination may not be significantly affected by the action of allelochemicals, the germination pattern can be modified, verifying differences in the speed and the synchrony of the germination of seeds submitted to such compounds, as was verified in the present work.

In the present study, it was found that the reduction of the germination capacity of the seeds was a major cause of allelopathy. However, other effects, such as seedling development are important indicative values for susceptibility to allelochemicals [6].

In the present research, this fact occurred in all factors analyzed, in which the development of *C. sativus* seedlings was negatively affected by the extracts of *S. cayennensis*.

These results have also been observed in other studies, for example in the work of Belinelo et al. [15], in which the authors observed *Arctium minus* (Hill) variable inhibitory allelopathic activity on root growth, and Oliveira et al. [16], which observed reduced growth of seedlings originated and seeds treated with extracts of *Emilia sonchifolia* (L.).

When analyzing the GVI (germination velocity index), it is observed that, due to the behavior of the concentrates of the tested species, there is a greater interference for this variable in *S. cayennensis* root (Fig. 1A).

As the doses increased, the reduction in the rate of germination of *C. sativus* seeds was higher (Fig. 1B). Similar results were obtained by Borella and Pastorini [17] on the interference of aqueous extracts of roots of *Solanum americanum* on germination and initial growth of radish.

Table 1. Mean square values of the variables germination percentage (G); germination velocity index (GVI); mean germination time (MGT); shoot length (SL); root length (RL) and dry mass (DM) of *Cucumis sativus* grown on substrate moistened with aqueous extract concentrations of different vegetative parts of *Stachytarpheta cayenensis*

	G		GVI		MGT		RL		SL		DM	
Extract (E)	10,4	ns	13,1	**	0,023	**	5949,4	**	9571,8	**	1,3E-06	**
Dose (D)	11,6	ns	21,5	**	0,037	**	58,4	**	656,5	**	1,2E-06	**
(E)x(D)	6,4	ns	1,3	ns	0,002	*	249,4	**	759,5	**	4,6E-07	**
Residue	8,2		0,6		0,0009		45,6		149,1		1,5E-07	
CV(%)	2,91		5,47		0,81		13,76		22,44		9,16	

*** and * significant at 1 and 5% probability respectively by the F. ns test: not significant*

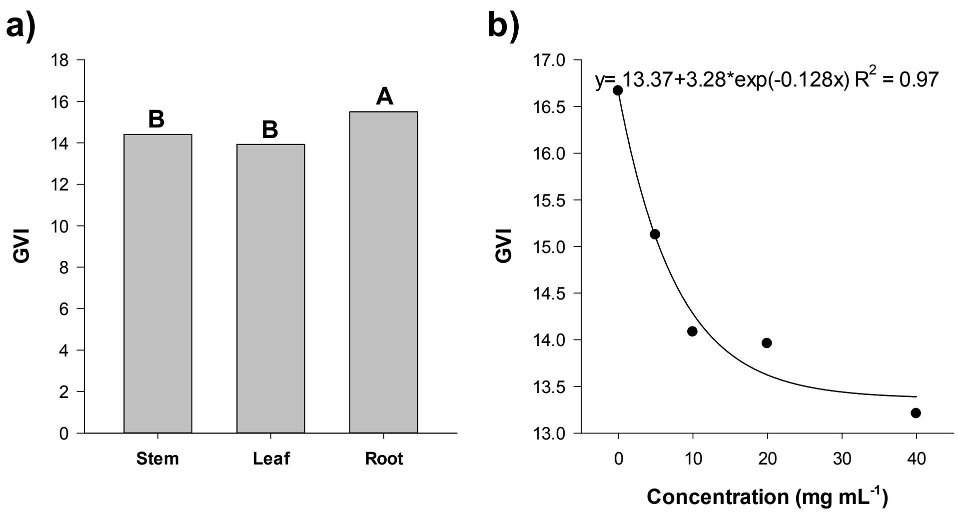

Fig. 1. Seed germination speed index of *Cucumis sativus* as a function of substrate culture moistened with aqueous extracts of root, stem and leaf of *Stachytarpheta cayennensis* (a) and different concentrations of these aqueous extracts (b)

Interferences regarding GVI (germination rate index) were also observed by Castagnara et al. [18], who verified in their experiments that extracts of oats, ryegrass and brachiaria reduced the GVI in cucumber seeds compared to 0% control.

The allelopathic effect may not occur on the final percentage of germination, but on the germination speed or another stage of the process. The interference in the germination speed mainly demonstrates the effect caused by the extracts in this initial process, as happened in the research, in which this interference delayed and compromised the germination [19].

For MGT (mean germination time) lower values are observed for root extract at all concentrations, which is in agreement with the GVI (Table 2). The highest values of time occurred with the use of stem and leaf extracts, which ratifies the velocity data since they are inversely proportional. This is justified by the fact that speed and time are correlated. According to Masum et al. [20], the average germination time is important to estimate the rate of occupation of a species in a given environment by testing the vigor of these species, mainly because it is in contact with the extract and the substances contained in it.

In order to complement the results of the germination tests and to determine parameters related to seed vigor, the Average Germination Time can inform the regularity of the germination distribution during the evaluation period. By means of this variable it is possible to establish a behavior pattern: if they germinate up to a maximum value and then lower or if, after reaching the maximum value, they decline. In general, the germination process was affected by the action of the allelochemicals present in the substrate, causing this variation. There was interaction between the concentrations and the parts of the plant, indicating that in this case, as reported, the concentrations are different depending on the part of the plant, resulting in a more pronounced effect when using leaf and stem in higher concentrations (Table 2).

In phytochemical researches related to the species belonging to the Verbenaceae family, the presence of triterpenes and steroids, iridoids, sugars, flavonoids and phenylpropanoids were indicated in the stem and leaves, and in the roots the presence of iridoids [21]. Under conditions such as allelopathic experiments the plants release the secondary metabolites. Some of these compounds interfere in the initial development of seedlings, which may have occurred in the present work, evidencing the difference between the extracts.

Some hypotheses for the results of the present research are pointed out by Souza et al. [22], where they report that interactions with cyclic hydrocarbon monoterpenes cause changes in the structure and function of membranes, which may impede cell growth and activity. And, normally the presence of flavonoids in the solution may cause a decrease in the osmotic potential generating difficulties in the absorption of solutes through the absorbent hairs, thus causing a reduction in root growth [23]. Thus, the greater presence of flavonoids in leaf and stem extracts may have impaired the development of radicle and affected MGT.

In Fig. 2A it is possible to verify that MGT was lower for the root extract. For all the extracts it is noticed an increase of the MGT until the concentration of 10 mg mL^{-1} and from this tends to a stabilization. In the aerial part length of *C. sativus* seedlings, it is observed that according to the increase of the concentrates, differentiated behaviors were observed in the parts (root, stem and leaf) of the species under study. The root concentrate of *S. cayennensis* caused, according to the increase of the doses, reduction in the length of the aerial part of the seedlings. However, when tested the stem concentrations, from 20% occurred an increase in the aerial part of the seedlings. In leaf concentrates a lower effect of the concentrations was observed (Fig. 2B).

In relation to root (RL) and shoot length (SL), the results show that the highest values were observed for leaf extract at concentrations of 5, 10 and 20 mg mL^{-1} (Table 2). At the concentration of 40 mg mL^{-1}, there was no difference between stem and leaf extracts.

Table 2. Deviation of the significant interaction between extracts and concentrations mean germination time (MGT) of seeds and shoot length (SL); root length (RL) and dry mass (DM) of *Cucumis sativus* seedlings at different concentrations of the aqueous extract of different parts *Stachytarpheta cayenensis*

Parts of plants	MGT (days)		RL (mm)		SL (mm)		DM (g $plant^{-1}$)	
5 mg mL^{-1}								
Stem	3,40	b	41,2	b	40,3	b	0,00451	a
Leaf	3,49	a	70,3	a	79,5	a	0,00446	a
Root	3,31	c	33,8	b	31,3	b	0,00444	a
10 mg mL^{-1}								
Stem	3,48	ab	44,2	b	35,9	b	0,00379	b
Leaf	3,55	a	68,2	a	72,9	a	0,00455	a
Root	3,40	b	27,9	c	32,0	b	0,00435	ab
20 mg mL^{-1}								
Stem	3,52	a	42,3	b	46,2	b	0,00358	b
Leaf	3,48	a	66,3	a	73,5	a	0,00390	ab
Root	3,40	b	33,8	b	30,4	b	0,00427	a
40 mg mL^{-1}								
Stem	3,52	a	64,0	a	84,7	a	0,00413	b
Leaf	3,53	a	64,9	a	80,6	a	0,00500	a
Root	3,40	b	24,1	b	19,5	b	0,00413	B

Means followed by equal letters in the column do not differ from each other by Tukey test at 5% significance at each concentration

According to Baličević et al. [8] seedling growth is widely used to evaluate allelopathic effects in laboratory bioassays, perhaps because they are more sensitive to allelochemicals than germination. In this sense, the root extract was shown to be more harmful to the development of seedlings. Thus, the interaction between the factors demonstrates that this species causes significant damages in the process of cell division for the formation of aerial part of the seedlings, especially as the concentrations increase. In addition, the roots of *S. cayennensis*, for this variable, have caused greater negative effects.

For shoot length, root extract caused the inhibitory effect on cucumber seedlings. Conversely, the stem extracts caused a stimulating effect on *C. sativus* seedlings. Barreiro et al. [24] analyzed the allelopathic effect of barbatimão extracts [*Stryphnodendron adstringens* (Mart.) Coville], indicating that the *C. sativus* is sensitive to the action of the allelochemicals during the development of the seedlings. The results pointed out in the research confirm these observations, because the cucumber was sensitive to the action of the tested concentrates. The same authors, also verified that shoot extract of *S. adstringens* was more significant in the development of the seedlings cucumber than in relation to germination.

In this sense, several studies have demonstrated that the increase of the aerial part is not only a consequence of the allocation of reserves in this region, but of what occurs in the whole plant [25]. If stress is maintained for a certain time, self-compensation can be reversed, showing rapid initial growth followed by reduction [26].

For the root length of *C. sativus* seedlings, as the doses increased, different behaviors occurred in relation to the parts tested. The root extracts caused a decrease in seedlings from 10%, however, an increase of this variable occurred in stem extracts from 20%. Leaf extracts also increased, but to a lesser extent (Fig. 2C).

These results corroborate with those observed in the shoot development of C. sativus plants, showing a negative effect on the interaction between concentration and parts of the weed, with a higher expression of malformation and reduction in development as the concentration was increased and especially also when the extract was removed from the roots.

The behavior observed in the present study corroborates with some literature that chemical substances such as phenolic compounds, coumarins, terpenoids, flavonoids, alkaloids, glycosides, tannins and quinones, which are found as secondary metabolites in plants, may trigger beneficial or harmful effects on plants. Malicious effects are identified as potential inhibitors of cell division and growth [19]. This occurred in the present research, because changes in structures of the species under study were verified.

The *S. cayennesis* species has flavonoids among the secondary compounds, an indication for such anatomical changes observed in the experiment, in which there was an increase in the structures measured in the test species submitted to the extracts.

In addition, it was possible to verify thickening of the radicle, especially in the base of the seedling and the reducing effect of the radicles that had their growth reduced due to these inhibitors.

There is also the possibility that the observed allelopathic activity is due to the synergism of different allelochemicals present in this species. In this case, the synergistic effect is one of the main factors responsible for the high potential for inhibition. This is confirmed by the fact that as the extract fraction is fractionated, there is a decrease in the inhibition power due to the separation of the allelopathic compounds [27].

Macerated stem extracts at 20% and 40% evidenced a stimulating effect on cucumber root growth (Fig. 2C). As the concentration of extract increased, there was a marked increase in the length of the *C. sativus* roots. In the extract macerated root, an inhibition occurred as the dose increased, since the same extraction procedure was used, but in different parts of the studied species, different results occurred.

This stimulus observed in the part of the plant, under the effect of the concentration of the extract, has been portrayed in bibliographies that define it as a stimulating effect from the biological point of view, as a strategy of the organism for optimal allocation of its resources. Thus, this increase in the length of the plant part is due to the self-compensation of the plants under conditions of exposure to momentary stresses. This is due to the allocation of reserve sources, which were destined to the growth and development of the plant [28].

In allelopathic studies, bioactivity generally tends to exhibit a dose-response pattern; the inhibition observed is dependent on the concentration of available allelochemicals, whereby the compounds tend to act as inhibitors at higher concentrations and this activity tends to decrease with the dilution [5].

The results have effects of losses, but also of stimuli and, similarly to the present study, [29] state that the secondary compounds of plants can be continuously synthesized and degraded in the cells, with specific purposes, that can among others, promote the accumulation of substances, causing a stimulatory action, until a certain concentration.

For the dry mass of *C. sativus* seedlings, differences between the root, stem and leaf parts occurred at doses 10, 20 and 40 mg mL^{-1}, and the lowest masses were observed for the stem extract, which did not differ only from the root extract at concentrations of 10 and 40 mg mL^{-1} and leaf at the concentration of 20 mg mL^{-1} (Table 2).

In studies of allelopathic potential, according to Luz et al. [30], the variations in the intensities of the observed effects are commonly related to the concentrations of the extracts applied, as well as to the tissue and the recipient species and this justifies the different responses of the extracts on the variables analyzed.

The dry mass of cucumber seedlings from the 10% concentrates increased when submitted to leaf and stem extracts in comparison to the other concentrations tested. In concentrates from 5% reduction occurred and stabilized from 20% (Fig. 2D).

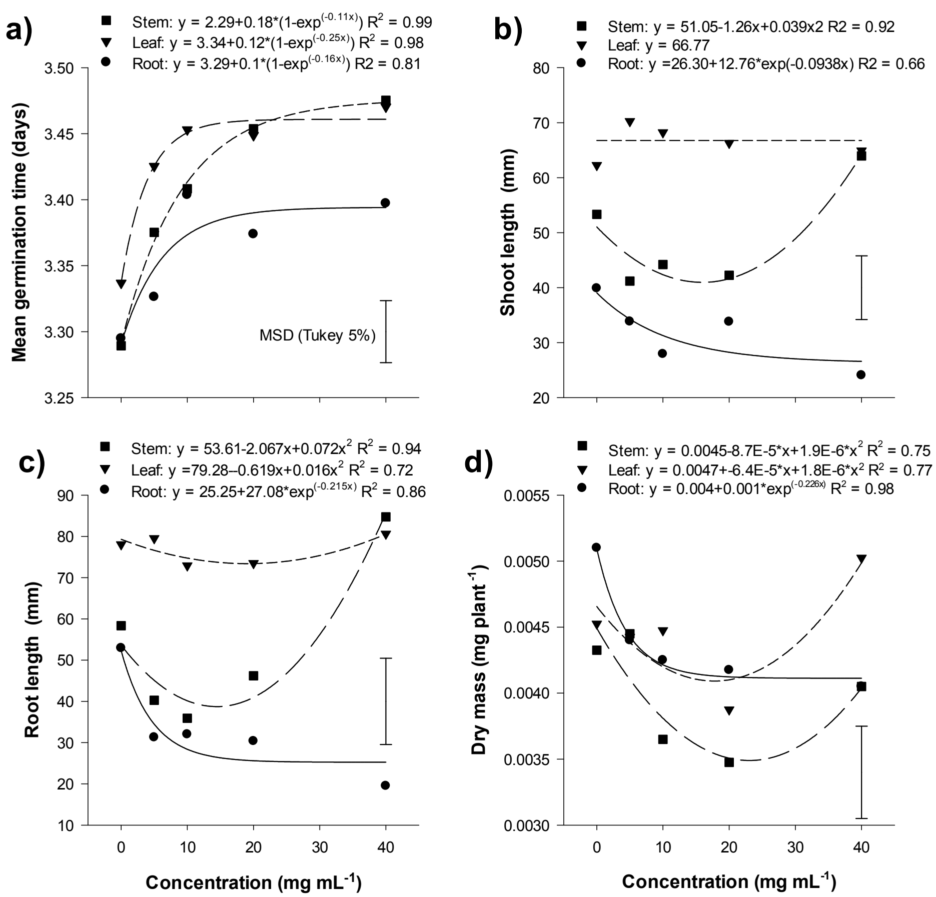

Fig. 2. Mean germination time (a) of seeds, (b) shoot length, (c) root length and (d) dry mass of *Cucumis sativus* seedlings as a function of different concentrations of aqueous extracts obtained from root, stem and leaf of *Stachytarpheta cayennensis*

When comparing the different types of extracts obtained from plant parts of *S. cayennensis* in each concentration, it is observed that the dry mass of *C. sativus* when tested the macerated extracts interfered with the dry mass gain of the seedlings. This effect on seedlings is probably due to the plants use of the seed nutritional reserve. Aqueous extracts are mixtures which may contain substances of various classes, and which have complex effects on the plant tested yet not fully elucidated [6].

The results evidenced that macerated extracts of stem and leaf of *S. cayennensis* caused an increase in dry mass of cucumber seedlings from the dose of 20 mg mL^{-1}.

These results are in agreement with studies that affirm the diversity of mechanisms of the allelochemicals, among them the growth compromise and the dry matter gain of the seedlings. These, however, act on the activity of phytormons that act in the division and / or stretching of cells, in the synthesis of nucleic acid and proteins, in the amount of oxygen that reaches the embryo, in the permeability of membranes and inhibition of photosynthesis [31]. These affirmations corroborate with the results mainly when the cell elongation was mentioned possibly due to the action of the allelochemicals present in the extracts of this species.

In addition, typically allelopathic inhibition results from the combined action of groups of allelochemicals that collectively interfere with various physiological processes [5]. However, when isolated or in minor proportions, these secondary compounds may act as growth promoters in some plant species [31]. Thus, given the response in the expression of these allelochemicals, it would be possible to use them both for the development of natural herbicides and for the production of stimulants for the growth of some plants.

4. CONCLUSION

In the conditions of the present study it can be concluded that the macerated aqueous extracts of *Stachytarpheta cayennensis* present allelopathic effect in *Cucumis sativus*. The greatest effects are provided when tested for macerated root concentrates at 20 and 40 mg mL^{-1}.

From the identification of the allelopathic results obtained in the execution of the research, it is possible to provide another tool in this approach of spontaneous species, serving as important information in the sense of raising the characteristics of these plants contributing significantly to the management of weeds through this biological mechanism that is less aggressive to the environment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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