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Influence of the methanol extract and fractions of *Smilax brasiliensis* Sprengel on development *in vitro* of *Nicotiana tabacum* and *Alllium cepa*

Influência do extrato metanólico e das frações de *Smilax brasiliensis* Sprengel no desenvolvimento *in vitro* de *Nicotiana tabacum* e *Allium cepa*

Influencia del extracto de metanol y fracciones de *Smilax brasiliensis* Sprengel en el desarrollo *in vitro* de *Nicotiana tabacum* y *Alllium cepa*

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Abstract
Smilax brasiliensis (Smilacaceae) is a native Brazilian plant found in the Cerrado biome and commonly used in folk medicine. The aim of this study was to evaluate the influence of the methanol extract and fractions from *S. brasiliensis* leaves on development *in vitro* of *Nicotiana tabacum* (tobacco) and *Allium cepa* (onion) seeds. *In vitro* germination protocol of onion seeds was established. Tobacco and onion seeds were placed to germinate on basal medium added of extract or fractions of *S. brasiliensis* leaves and dichlorophenoxyacetic acid (2,4-D), 6-benzylaminopurine (BAP) and gibberellic acid (GA) at different concentrations in the presence of light. The germination ranged from 40 to 100% for tobacco and from 60 to 100% for onion seeds. The results indicated that the extract and fractions promoted higher growth than 2,4-D and GA when analyzed number of nodes, leaves, root and root size for germinated tobacco seeds, and higher growth when analyzed number roots and stem size for onion seeds. Regarding BAP, the extract and fractions inhibited leaf growth, root and root size of tobacco seeds. Further studies are needed to evaluate the possible use of the methanol extract and fractions from *S. brasiliensis* leaves as natural sources of hormones and/or bioherbicides.
Keywords: *In vitro* germination; Auxin; Cytokinin; Gibberellin; Brazilian Cerrado.

Resumo
Smilax brasiliensis é uma planta nativa do Cerrado brasileiro e comumente usada na medicina popular. O objetivo deste estudo foi avaliar a influência do extrato metanólico e das frações das folhas de *S. brasiliensis* no desenvolvimento *in vitro* de sementes de *Nicotiana tabacum* (tabaco) e *Allium cepa* (cebola). Foi estabelecido um protocolo de germinação *in vitro* de sementes de cebola. Sementes de tabaco e cebola foram colocadas para germinar em meio basal adicionado de extrato ou frações das folhas de *S. brasiliensis* e ácido diclorofenoxiacético (2,4-D), 6-benzilaminopurina (BAP) e ácido giberélico (AG) em diferentes concentrações na presença de luz. A porcentagem de germinação variou de 40 a 100% para o tabaco e de 60 a 100% para a cebola. Os resultados indicaram que o extrato e as frações promoveram maior crescimento do que 2,4-D e AG, quando analisados número de nós, folhas, raiz e tamanho da raiz para sementes de tabaco germinadas, e maior crescimento quando analisado número de raiz e tamanho do caule para sementes de cebola. Em relação ao BAP, o extrato e as frações inibiram o crescimento das folhas, raiz e tamanho da raiz das sementes de tabaco germinadas. Mais estudos são necessários para avaliar a possível utilização do extrato metanólico e frações das folhas de *S. brasiliensis* como fontes naturais de hormônios e/ou bioherbicidas.
Palavras-chave: Germinação *in vitro*; Auxina; Citocinina; Giberelina; Cerrado brasileiro.

Resumen
Smilax brasiliensis es una planta originaria del Cerrado brasileño y de uso común en la medicina popular. El objetivo de este estudio fue evaluar la influencia del extracto de metanol y fracciones de hojas de *S. brasiliensis* en el desarrollo *in vitro* de semillas de *Nicotiana tabacum* (tabaco) y *Allium cepa* (cebolla). Se estableció un protocolo de germinación *in vitro* para semillas de cebolla. Se colocaron semillas de tabaco y cebolla para germinar en medio basal al que se le adicionó extracto o fracciones de hojas de *S. brasiliensis* y ácido diclorofenoxiacético (2,4-D), 6-bencilaminopurina (BAP) y ácido giberélico (AG) a diferentes concentraciones en presencia de luz. El porcentaje de germinación varió del 40 al 100% para el tabaco y del 60 al 100% para la cebolla. Se colocaron semillas de tabaco y cebolla para germinar en medio basal al que se le adicionó extracto o fracciones de hojas de *S. brasiliensis* y ácido diclorofenoxiacético (2,4-D), 6-bencilaminopurina (BAP) y ácido giberélico (AG) a diferentes concentraciones en presencia de luz. El porcentaje de germinación varió del 40 al 100% para el tabaco y del 60 al 100% para la cebolla. Los resultados indicaron que el extracto y las fracciones promovieron mayor crecimiento que 2,4-D y AG, al analizar el número de nudos, hojas, tamaño de raíz y raíz para semillas de tabaco germinadas, y mayor crecimiento al analizar número de raíz y tamaño de tallo para cebolla semillas. En relación con BAP, el extracto y las fracciones inhibieron el crecimiento de hojas, raíz y tamaño de raíz de semillas de tabaco germinadas. Se necesitan más estudios para evaluar el posible uso de extracto de metanol y fracciones de hojas de *S. brasiliensis* como fuentes naturales de hormonas y/o bioherbicidas.
Palabras clave: Germinación *in vitro*; Auxina; Citoquinina; Giberelina; Cerrado brasileño.

1. Introduction

Allelopathy is defined as a chemical-ecological phenomenon, in which secondary metabolites produced by a plant species are released and interfere with the germination and/or development of other plants in the same environment; this type of interference can be beneficial or harmful (Cheema, Farooq & Wahid, 2013; Soares et al., 2002; Weston & Mathesius, 2013).

Seed germination is a complex process characterized by radicle protrusion through water absorption (Weitbrecht, Muller & Leubner-Metzger, 2011). The germination process can be influenced by physical factors such as water, temperature and light, and by the presence of chemicals that can prevent this process from starting or finishing (Finch-Savage & Leubner-Metzger, 2006; Sirová et al., 2011).

Plants produce signaling molecules known as hormones, responsible for marked effects on development at very small concentrations. Phytohormones or plant hormones are naturally occurring organic compounds, which, at low concentrations, have a deep influence on plant physiology. These are chemical messengers that are produced in small quantities at a specific location and induce responses at other plant locations (Fagan et al., 2015).

Smilax brasiliensis Sprengel (Smilacaceae) is a medicinal species of the Brazilian Cerrado. Previous study showed the allelopathic effect of ethanol extract and fractions from *S. brasiliensis* leaves on growth of hypocotyls and radicles of *Allium cepa* and *Lactuca sativa* (Fonseca et al., 2017). Allelopathic, cytotoxic, genotoxic and antigenotoxic effects were observed in the studies of Amado et al. (2019, 2020a). The phytotoxic effects of the hexane and dichloromethane fractions from the leaves of *S. brasiliensis* was also demonstrated (Barbosa et al., 2021; Fonseca et al., 2019).

This work aimed to evaluate the influence of the extract and fractions *S. brasiliensis* leaves on development *in vitro* of *Nicotiana tabacum* and *Allium cepa* seeds.

2. Methodology

This work is an experimental research, with a quantitative approach. According to Pereira et al. (2018), a quantitative survey, translates opinions and information into numbers to classify and analyze them, using statistical techniques. In this type of research, the relationship between the variables must be formulated and classified to ensure the accuracy of the results, thus avoiding contradictions in the analysis and interpretation process.

2.1 Chemicals

For this study, methanol, petroleum ether, dichloromethane and ethyl acetate (analytical grade) were obtained from Cromato Produtos Químicos® (Brazil). Agar, 2,4-dichlorophenoxyacetic acid (2,4-D), 6-benzilaminopurine (BAP), gibberellic acid (GA), dimethyl sulfoxide and sucrose were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

2.2 Plant material

Leaves of *Smilax brasiliensis* Sprengel were collected in the Brazilian Cerrado in Ijaci, South Minas Gerais State, Brazil (21°13'46"S and 44°55'65"W, average altitude 908 m) in October 2014 (SISBIO n. 24542-5). Fertile samples were collected and the vouchers were identified by Dr. Regina Helena Potsch Andreata, and deposited in the PAMG Herbarium (PAMG 57078) at the Agricultural Research Company of Minas Gerais (EPAMIG). This study has access permission to the components of plant genetic heritage (n. 010455/2014-0/CNPq/CGEN/MMA) and it is registered in the SisGen Platform (Register AEF6C95), according to Brazilian Biodiversity Law (13.123/2015).

2.3 Preparation of *S. brasiliensis* leaves extract and fractions

Petroleum ether and methanol were used as solvents to obtain the extracts from 300 g of dried leaves using a Soxhlet extractor. The extracts were concentrated in a rotary evaporator at 40 °C under reduced pressure to yield petroleum ether (EE, 11.121 g) and methanol (ME, 32.829 g) extracts (Amado et al., 2018).

Part of the methanol extract (20 g) was dissolved in methanol/water (1:1) and successively extracted with dichloromethane and ethyl acetate, resulting in 1.44, 1.24 and 7.13 g of dichloromethane (DCM), ethyl acetate (AC) and hydromethanol (HM) fractions, respectively (Amado et al., 2020a, b).

2.4 Protocols test for *A. cepa* seed disinfestation

Allium cepa seeds were tested for different methods of disinfestation:

1. Disinfecting with NaOCl (1.0% commercial sodium hypochlorite) for 5 minutes and after washed three times in autoclaved distilled water;
2. Disinfecting with NaOCl (1.0% commercial sodium hypochlorite) for 3 minutes and then washed three times in autoclaved distilled water;
3. Disinfecting with NaOCl (1.0% commercial sodium hypochlorite) for 1 minutes and then washed three times in autoclaved distilled water;
4. Disinfecting with NaOCl (0.5% commercial sodium hypochlorite) for 5 minutes and then washed three times in autoclaved distilled water;
5. Disinfecting with NaOCl (0.5% commercial sodium hypochlorite) for 3 minutes and then washed three times in autoclaved distilled water;
6. Disinfecting with NaOCl (0.5% commercial sodium hypochlorite) for 1 minutes and then washed three times in autoclaved distilled water;
7. Treatment with Captan 5% for 10 minutes;
8. Disinfecting three times with autoclaved distilled water;
9. Without any disinfestation treatment.

The seeds were placed on basal medium MS (Murashige & Skoog, 1962) supplemented with 30 g/L sucrose and solidified with 5 g/L. The pH was adjusted to 5.7 ± 0.1 with 0.1 N NaOH, and the medium was sterilized at 120 °C (1.37×10^5 Pa) for 20 min. The seeds were kept in a growth chamber at 27 ± 1 °C, 16 hours photoperiod and radiation of 45 μmol/m^{2}s for

60 days. The percentages of germinated seeds, oxidation and contamination of the medium and seeds were observed at 7 days intervals.

The parameters analyzed at the end of the test were number root size and stem size.

The experimental design was completely randomized, with ten tubes and each tube containing one seed.

2.5 *In vitro* germination

Fruits containing *N. tabacum* seeds were collected in Santana do Jacaré, Minas Gerais, Brazil (20°50"44.22'S and 45°0"48.87'W) in August 2018. The fruits were dried at room temperature and after drying, were tainted (streaked), separating the seeds, using 30 mesh sieves. After cleaning, the seeds were submitted to germination test.

A. cepa seeds were purchased commercially Red Creole Chata Roxa seeds (Feltrin®, Lot 0003601335026431, Brazil) were used.

N. tabacum seeds were disinfected with 70% alcohol for 1 minute and washed once in autoclaved distilled water. After, the seeds were treated with NaOCl (2% commercial sodium hypochlorite) and a drop of Tween 20 for 30 minutes while stirring. Finally, they were washed six times in autoclaved distilled water.

A. cepa seeds were disinfected with NaOCl (1% commercial sodium hypochlorite) for 1 minute, washed three times in autoclaved distilled water.

N. tabacum and *A. cepa* seeds were placed on basal medium MS (Murashige & Skoog, 1962) supplemented with 30 g/L sucrose and solidified with 5 g/L. The pH was adjusted to 5.7 ± 0.1 with 0.1 N NaOH, and the medium was sterilized at 120 °C (1.37×10^5 Pa) for 20 minutes.

As positive controls were used 1.0 mg/mL 2,4-D (dichlorophenoxyacetic acid), 0.5 mg/mL gibberellic acid (GA), 2 mg/mL BAP (6-benzylaminopurine) and negative control, MS medium supplemented with dimethyl sulfoxide (DMSO).

Methanol extract and dichloromethane, ethyl acetate and hydromethanol fractions were tested at concentrations of 250, 500, 750 and 1000 µg/mL.

After inoculation, *N. tabacum* and *A. cepa* seeds were kept in a growth chamber at 27 ± 1 °C, 16 hours photoperiod and radiation of 45 $\mu mol/m^2 s$ for 60 and 30 days, respectively. The percentages of germinated seeds, oxidation and contamination of the medium and seeds were observed at 7 days intervals.

The parameters analyzed at the end of the test were number of leaves, knots, shoots, root, stem size and root in centimeters for *N. tabacum* seedlings and stem and root size for *A. cepa* seedlings.

The experimental design was completely randomized, with fifteen tubes and each tube containing one seed.

2.6 Statistical analysis

The data are presented as the mean ± SE of experiments with ten doses. Statistical differences were determined by analysis of variance (ANOVA) followed by Student's t-test using GraphPad Prism 5.0 software. Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1 Protocols test for *A. cepa* seed disinfestation

Results for the disinfestation protocols tested are shown in Table 1. The seeds without treatment or when disinfected with distilled water only presented 100% of contamination. The best result was observed for seeds disinfected with 1% sodium

hypochlorite for 1 minute, being this protocol chosen for disinfection of seeds during *in vitro* germination test with the extract and fractions, due the better growth and absence of contamination.

Table 1. Protocols test for *A. cepa* seed disinfestation.

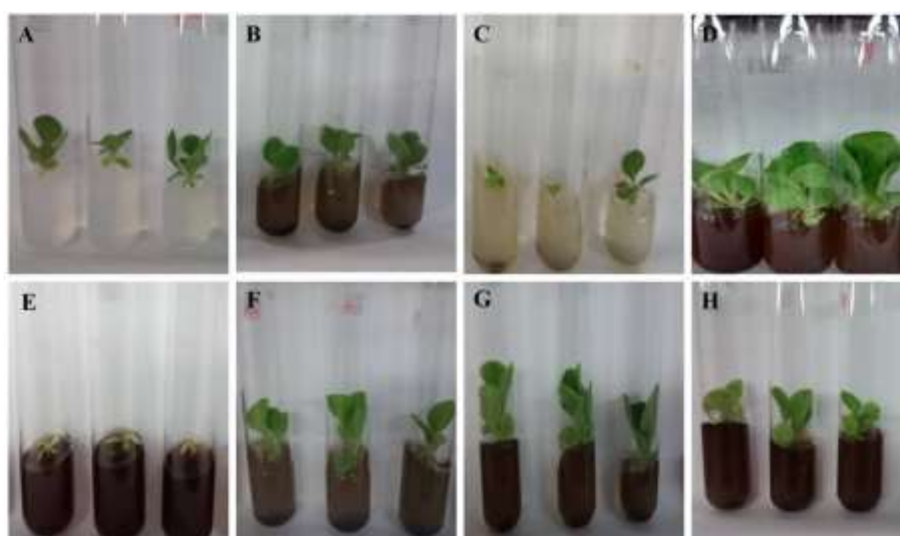
Protocols	Germination (%)	Contamination (%)	Stem size	Root Size
1	60 ± 0.24	10 ± 0.13	1.90 ± 0.78	0.98 ± 0.63
2	70 ± 0.42	20 ± 0.26	3.50 ± 2.31	1.31 ± 0.82
3	70 ± 0.76	0	3.75 ± 1.61	1.88 ± 1.03
4	50 ± 0.42	20 ± 0.26	1.87 ± 0.65	0.89 ± 0.69
5	60 ± 0.77	10 ± 0.13	1.87 ± 0.69	0.99 ± 0.51
6	60 ± 0.40	0	1.84 ± 0.88	0.95 ± 0.51
7	60 ± 0.63	10 ± 0.13	2.68 ± 0.80	8.04 ± 0.75
8	50 ± 0.89	100 ± 1.14	-	-
9	20 ± 0.26	100 ± 0.74	-	-

Results were expressed as mean ± standard error (n = 10). Standard error values were omitted when they are less than 0.099.
Source: Authors.

3.2 *In vitro* germination

The germination percentage varied from 40 to 100% for tobacco seeds and from 60 to 100% for onion seeds (Figure 1 and Figure 2). The percentage of contamination observed shows that onion seeds had greater contamination with percentages ranging from 10 to 60%, while the contamination percentage for tobacco ranged from 10 to 30% (Table 2). There was statistical difference when compared to germination and contamination of the tested samples with the positive control and negative control.

Figure 1. Germination of tobacco seeds treated with methanol extract and fractions of *S. brasiliensis*.



(A) Negative control; (B) ME extract at concentration of 250 µg/mL; (C) DCM fraction at concentration of 500 µg/mL; (D) AC fraction at concentration of 250 µg/mL; (E) AC fraction at concentration of 500 µg/mL; (F) HM fraction at concentration of 250 µg/mL; (G) HM fraction at concentration of 500 µg/mL; (H) HM fraction at concentration of 750 µg/mL. Seedlings after 60 days of germination.
Source: Authors.

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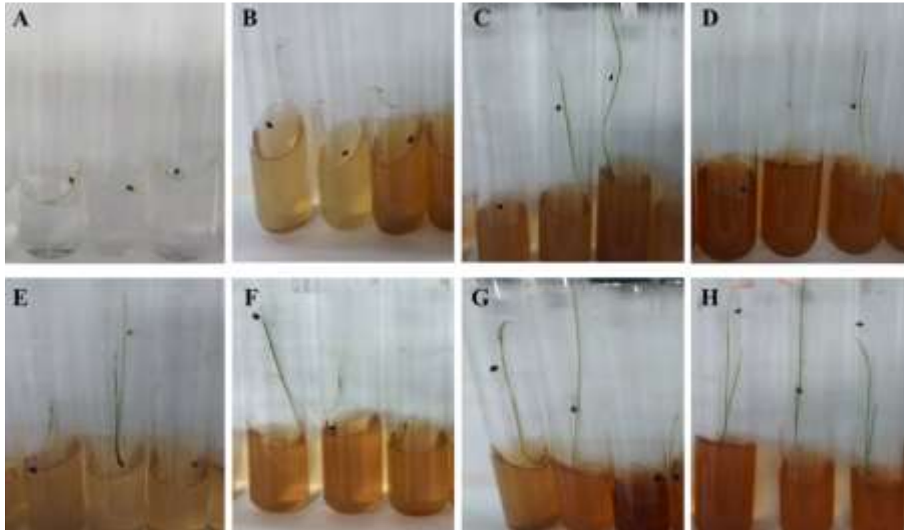
The Figure 1 shows the seedlings of *N. tabacum* after seed germination. The greatest growth can be observed in (G), when they were treated with the HM fraction at concentration of 500 µg/mL. Meanwhile, the smallest growth was observed in (E), when they were treated with the AC fraction at the same concentration.

Figure 2. Germination of onion seeds treated with methanol extract and fractions of *S. brasiliensis*.



(A) Negative control; (B) ME extract at concentration of 250 µg/mL; (C) ME extract at concentration of 500 µg/mL; (D) ME extract at concentration of 1000 µg/mL; (E) AC fraction at concentration of 250 µg/mL; (F) AC fraction at concentration of 500 µg/mL; (G) HM fraction at concentration of 500 µg/mL; (H) HM fraction at concentration of 750 µg/mL. Seedlings after 30 days of germination.
Source: Authors.

Figure 2 shows the germination of *A. cepa* seeds, in which there was only the growth of the aerial part and root, without leaf growth. As can be seen, the negative control stimulated small aerial part growth. The best results were observed when the seeds were treated with the extract and the fractions.

Table 2. Germination and contamination percentage of *N. tabacum* (tobacco) and *A. cepa* (onion) seeds.

Samples	Treatments (µg/mL)	Tobacco		Onion	
		Germination (%)	**Contamination (%)**	**Germination (%)**	**Contamination (%)**
2,4 – D	1000	100 ± 1.12	10 ± 0.35	80 ± 0.68	50 ± 0.79
BAP	2000	100 ± 1.12	20 ± 0.71	100 ± 0.95	20 ± 0.32
GA	500	100 ± 1.12	10 ± 0.35	60 ± 0.95	10 ± 0.16
NC	0	100 ± 1.12	0	80 ± 0.26	10 ± 0.16
ME	250	100 ± 1.12	0	100 ± 1.58	40 ± 0.63
ME	500	100 ± 0.99	10 ± 0.35	100 ± 0.95	30 ± 0.47
ME	750	100 ± 0.99	0	100 ± 0.91	30 ± 0.47
ME	1000	70 ± 0.46	10 ± 0.35	100 ± 1.05	20 ± 0.32
DCM	250	100 ± 0.89	0	100 ± 1.38	30 ± 0.47
DCM	500	100 ± 0.81	0	100 ± 0.95	10 ± 0.16
DCM	750	40 ± 0.24	0	90 ± 0.91	30 ± 0.47
DCM	1000	70 ± 0.46	0	100 ± 1.38	10 ± 0.16
AC	250	100 ± 1.12	0	100 ± 1.17	20 ± 0.32
AC	500	100 ± 0.65	30 ± 1.06	80 ± 0.73	30 ± 0.47
AC	750	100 ± 1.12	20 ± 0.71	100 ± 0.98	10 ± 0.16
AC	1000	100 ± 1.12	0	80 ± 0.73	30 ± 0.47
HM	250	100 ± 1.12	20 ± 0.71	100 ± 1.38	10 ± 0.16
HM	500	100 ± 1.12	0	70 ± 1.11	30 ± 0.47
HM	750	100 ± 1.12	0	100 ± 1.05	20 ± 0.32
HM	1000	100 ± 0.81	10 ± 0.35	90 ± 0.83	60 ± 0.95

Results were expressed as mean ± standard error (n = 15). Standard error values were omitted when they are less than 0.099.
There was no statistical difference in relation to 2,4-D, GA, BAP and negative control.
2,4 - D: 2,4-dichlorophenoxyacetic acid, BAP: 6-benzylaminopurine, GA: gibberellic acid, NC: negative control, ME: methanol extract, DCM: dichloromethane fraction, AC: ethyl acetate fraction, HM: hydromethanol fraction.
Source: Authors.

Table 3 shows the number of nodes, shoots, leaves, root, stem size and root size for germinated tobacco seeds. The results indicated that the tested samples of *S. brasiliensis* that presented statistical difference in relation to 2,4-D and BAP, when evaluated number of nodes, leaves, root and root size, presented higher averages than the tested control.

Table 3. Number of nodes, shoots, leaves, root, stem size and root size of germinated *N. tabacum* seeds.

Samples	Treatments (µg/mL)	N° nodes	N° Shoots	N° Leaves	N° Root	Stem Size	Root Size
2,4 – D	1000	0.50 ± 0.41	0.70 ± 1.22	2.10 ± 3.33	0.60 ± 1.09	0.15 ± 0.22	0.14 ± 0.27
BAP	2000	1.60 ± 0.70	0.40 ± 0.52	3.10 ± 0.99	1.00	0.18 ± 0.10	0.25 ± 0.28
GA	500	3.53 ± 1.09	1.20 ± 0.91	7.50 ± 2.03	7.20 ± 5.49	1.16 ± 0.61	3.95 ± 1.65
NC	0	4.90 ± 0.99	0.90 ± 0.27	10.20 ± 1.75	6.80 ± 4.03	0.63 ± 0.07	4.16 ± 1.63
ME	250	3.54 ± 0.83[a]	1.60 ± 1.07	7.60 ± 0.98[acd]	5.40 ± 4.14[ac]	0.30 ± 0.14	2.43 ± 1.37[c]
ME	500	4.20 ± 1.07[ac]	1.30 ± 1.21	7.70 ± 1.81[acd]	6.80 ± 2.89[ac]	0.37 ± 0.15	0.97 ± 0.51[d]
ME	750	3.80 ± 0.97[c]	0.60 ± 0.51	7.20 ± 1.33[acd]	4.80 ± 1.55[abc]	0.25 ± 0.07	0.66 ± 0.36[d]
ME	1000	2.00 ± 1.07[d]	0.60 ± 0.38	4.20 ± 1.82[bd]	2.50 ± 1.72[bd]	0.18 ± 0.08	0.21 ± 0.23[bd]
DCM	250	3.10 ± 1.51[a]	0.50 ± 0.44	5.90 ± 2.32[acd]	4.10 ± 4.54[abcd]	0.19 ± 0.12	0.83 ± 0.76[bd]
DCM	500	4.00 ± 0.86[ac]	0.80 ± 0.44	8.00 ± 1.28[acd]	5.30 ± 2.79[ac]	0.21 ± 0.06	0.66 ± 0.25[bd]
DCM	750	0.80 ± 0.82[bd]	0.40	1.70 ± 1.26[bd]	0.40[bd]	0.06 ± 0.06	0.06 ± 0.06[bd]
DCM	1000	1.50 ± 0.90[d]	0.20 ± 0.49	3.10 ± 1.62[bd]	1.40 ± 0.82[bd]	0.13 ± 0.07	0.19 ± 0.18[bd]
AC	250	4.10 ± 1.08[ac]	1.70 ± 1.00	9.20 ± 2.63[ac]	12.90 ± 8.27[abcd]	0.67 ± 0.21	3.78 ± 1.95[ac]
AC	500	2.40 ± 0.70[d]	0.70 ± 0.48	5.20 ± 1.47[abd]	4.30 ± 2.83[abcd]	0.33 ± 0.08	0.32 ± 0.23[bd]
AC	750	1.70 ± 0.89[d]	1.00 ± 0.29	4.00 ± 1.31[bd]	2.70 ± 2.47[bd]	0.29 ± 0.13	0.21 ± 0.09[bd]
AC	1000	1.60 ± 0.64[d]	0.50 ± 0.51	3.50 ± 0.83[bd]	1.70 ± 1.12[bd]	0.21 ± 0.12	0.17 ± 0.06[bd]
HM	250	4.60 ± 0.67[ac]	1.20 ± 0.40	9.20 ± 0.65[ac]	8.80 ± 4.41[ac]	0.65 ± 0.21	4.36 ± 0.72[ac]
HM	500	4.50 ± 0.63[ac]	0.90 ± 0.27	9.30 ± 1.03[ac]	11.70 ± 6.75[abcd]	0.46 ± 0.13	5.47 ± 1.79[ac]
HM	750	3.90 ± 0.84[ac]	1.00 ± 0.26	8.40 ± 1.91[ac]	7.50 ± 5.40[ac]	0.33 ± 0.11	4.86 ± 1.54[ac]
HM	1000	2.80 ± 0.80[a]	0.90 ± 0.39	6.30 ± 1.40[acd]	2.90 ± 1.05[abd]	0.22 ± 0.09	2.47 ± 1.55[ac]

Results were expressed as mean ± standard error (n = 15). Standard error values were omitted when they are less than 0.099.
[a]Statistical difference in relation to 2,4-D ($p < 0,05$).
[b]Statistical difference in relation to GA ($p < 0,05$).
[c]Statistical difference in relation to BAP ($p < 0,05$).
[d]Statistical difference in relation to negative control ($p < 0,05$).
2,4 - D: 2,4-dichlorophenoxyacetic acid, BAP: 6-benzylaminopurine, GA: gibberellic acid, NC: negative control, ME: methanol extract, DCM: dichloromethane fraction, AC: ethyl acetate fraction, HM: hydromethanol fraction.
Source: Authors.

The results indicated that the growth of seeds was inhibited by gibberellic acid, and the seeds treated with the extract and the fractions also had lower average number of leaves, root and root size (Table 3).

Regarding the negative control, the results observed for the extract and fractions showed lower mean values for number of nodes, shoots, leaves, root and root size (Table 3).

For the onion seed test, the results demonstrated that the methanol extract and the fractions were statistically different from the 2,4-D, GA, BAP and negative control only for stem size (Table 4). The results indicated highest average values for the extract and fractions in relation to the positive and negative controls.

Table 4. Number of root, stem size and root size of germinated *A. cepa* seeds.

Samples	**Treatments (µg/mL)**	**N° Root**	**Stem Size**	**Root Size**
2,4 - D	1000	0.80 ± 0.79	1.21 ± 1.33	0.18 ± 0.19
BAP	2000	0.60 ± 0.52	2.73 ± 2.45	0.14 ± 0.12
GA	500	1.00	1.89 ± 1.94	1.40 ± 1.94
NC	0	1.50 ± 1.43	2.47 ± 2.03	1.15 ± 1.56
ME	250	1.00	2.80 ± 1.72[a]	0.60 ± 0.76
ME	500	1.00	3.56 ± 2.13[ab]	0.74 ± 0.85
ME	750	1.00	2.93 ± 2.10[a]	0.24 ± 0.29
ME	1000	1.00	3.93 ± 0.74[abd]	0.36 ± 0.30
DCM	250	1.00	2.74 ± 1.84[a]	0.45 ± 0.46
DCM	500	1.00	2.77 ± 1.69[a]	0.36 ± 0.31
DCM	750	0.90 ± 0.32	1.43 ± 1.27	0.32 ± 0.34
DCM	1000	1.00	1.22 ± 1.41[c]	0.25 ± 0.35
AC	250	1.40 ± 0.84	3.31 ± 2.08[ab]	0.89 ± 1.08
AC	500	1.30 ± 0.82	3.13 ± 2.31[a]	1.13 ± 1.66
AC	750	2.10 ± 1.10	3.75 ± 1.38[abc]	0.81 ± 0.73
AC	1000	0.90 ± 0.57	2.40 ± 2.07	0.19 ± 0.14
HM	250	2.00 ± 0.94[c]	3.30 ± 1.94[ab]	0.77 ± 0.58
HM	500	1.40 ± 1.58	2.61 ± 2.29[a]	0.85 ± 1.08
HM	750	1.80 ± 0.79	5.20 ± 2.14[abcd]	1.11 ± 0.62
HM	1000	2.10 ± 1.52[c]	3.80 ± 2.04[ab]	0.97 ± 0.81

Results were expressed as mean ± standard error (n = 15). Standard error values were omitted when they are less than 0.099.
[a]Statistical difference in relation to 2,4-D ($p < 0,05$).
[b]Statistical difference in relation to GA ($p < 0,05$).
[c]Statistical difference in relation to BAP ($p < 0,05$).
[d]Statistical difference in relation to negative control ($p < 0,05$).
2,4 - D: 2,4-dichlorophenoxyacetic acid, BAP: 6-benzylaminopurine, GA: gibberellic acid, NC: negative control, ME: methanol extract, DCM: dichloromethane fraction, AC: ethyl acetate fraction, HM: hydromethanol fraction.
Source: Authors.

4. Discussion

For *in vitro* germination to become a reliable source of aseptic material, disinfection methods must be effective, providing the absence of pathological agents.

The most commonly used germicidal substances in seed disinfestation protocols are ethanol and sodium hypochlorite (Quisen & Angelo, 2008). The most used sodium hypochlorite concentrations in disinfestation protocols range from 0.5% to 2.0% active chlorine, with exposure times ranging from a few seconds to minutes (Torres et al., 2000). The high exposure to hypochlorite, as well as ethanol, ends up causing the opposite effect, promoting tissue oxidation and tissue death, which was not observed in this study, since the exposure time and concentration were not detrimental onion seed germination (Smith, 2000).

The onion is an excellent organism for bioassays because once rehydrated, it goes into germination process, undergoing rapid physiological changes and becomes highly sensitive, expressing any external changes to which it is submitted

(Costa & Menk, 2000; Souza et al., 2005). Tobacco, in turn, offers numerous advantages as a model plant for several studies, due to its short life cycle and large number of seeds per capsule (Brasileiro, 1998).

With the discovery of the effects of plant regulators on plants and the benefits promoted by these substances, many compounds and combinations of these products have been researched.

These natural substances can be applied directly to plants (leaves, fruits, seeds), causing changes in vital and structural processes in order to increase production, improve quality and facilitate harvesting (Castro & Melotto, 1989).

The parameters analyzed in this study indicated that the extract and fractions tested promoted higher growth and development of tobacco and onion seeds when compared to 2,4-D. According to Cordoba (1976), auxins would be involved in the germination process because it indirectly controls the transport of gibberellin from the embryonic axis to endosperm cells. Auxin is a growth-related hormone because it promotes increased cell stretching rates. They also have the ability to promote the elongation of coleoptile, stem segments, and in the presence of cytokinins promote cell division in callus cultures, formation of adventitious roots in leaves or cut stems (Taiz & Zeiger, 2013).

Cytokinins, on the other hand, can stimulate or inhibit a variety of physiological, metabolic, biochemical processes in the context of development. They are responsible for stimulating the process of cell division or cytokinesis and cell differentiation, especially in the formation of stem buds (Kerbauy, 2008). When comparing the results obtained for BAP and the results obtained for the extract and fractions, it was observed that the best results were expressed for BAP, with the averages of the samples of *S. brasiliensis* being lower.

The results observed and compared with gibberellin show better results for the extract and fractions. Gibberellic acid accelerates seed germination and thus ensures uniformity in germination. When seeds begin germination (triggered by water absorption), the embryo releases gibberellins, which diffuse into the aleurone cells and then stimulate the synthesis of hydrolytic enzymes, which digest the nutrient reserves of the starch endosperm (Taiz & Zeiger, 1991). Gibberellins play an important role in breaking seed dormancy or latency and are of fundamental importance in breaking down endosperm reserve substances from seeds. These responsible for stem elongation, also acting in the transition from the juvenile to the adult stage of the plant, consequently causing the induction of flowering (Taiz & Zeiger, 2013).

The main compounds identified by Amado et al. (2020b) in the methanol extract and dichloromethane, ethyl acetate and hydromethanol fractions of *S. brasiliensis* were glycosylated and non-glycosylated flavonoids, especially quercetin, and phenylpropanoids, such as chlorogenic acid. According to Shirley (1998) and Gould and Lister (2006) flavonoids have significant effects in seeds germination. In the plants, flavonoids act as internal physiological messengers and for this function flavonoids are required in small amount (Samanta, Das & Das, 2011). The flavonoids are secondary metabolites present in most plant seeds and play important roles in the protection of the seeds against pathogen and herbivores and act in seeds dormancy and maturation (Shirley, 1998).

No studies on the influence of *S. brasiliensis* on *in vitro* seed germination were found.

5. Conclusion

In order to improve crop yield, it is suggested that bioactive compounds found in *S. brasiliensis* leaf may in future be used as plant hormones and/or natural herbicides, since the results indicated growth promotion and inhibition of the analyzed parameters in relation to the tested positive controls.

The results of this work suggest that future studies aim to identify which bioactive compounds present in the extract and in the fractions may have the effect of plant hormones.

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