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Allelopathic potential of aqueous extracts of passion fruit *Passiflora mucronata* fruit peels on lettuce

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Abstract

Pesticide misuse has led to problems in agriculture, contamination of environment, and human health. However, research into alternative methods of controlling invasive plants is expanding. The allelopathic effect of secondary metabolites from plant parts suggests a potential and sustainable alternative to plant residues for use as bioherbicides. Given the above, the current study aimed to investigate the allelopathic potential of various concentrations of the aqueous extract of fruit peels of genotypes of *Passiflora mucronata* in seed germination and seedling growth of *Lactuca sativa* L. Fruit peels of genotypes of *P. mucronata* were used to obtain an aqueous extract, which was then filtered and diluted at the following concentrations: T1=100; T2=50; T3=25; T4=12.5 mg mL^{-1}; T5= negative control (distilled water) and T6= positive control (glyphosate). Germination was carried out in Petri plates and was conditioned in a biochemical oxygen demand germination chamber at 25 ± 2 °C. Macroscopic evaluation was performed at the germination stage and root and aboveground growth of the seedlings, and the germination velocity index were also evaluated. Cytogenetic analysis was performed, and mitotic index was obtained. The design was entirely randomized, with five repetitions of 25 seeds each. Allelopathic substances found in the bark of *P. mucronata* fruits interfere with seed germination and vigor, as well as initial seedling growth. The germination of *L. sativa seeds* was 100% inhibited by extract concentrations of 50 % and 100 %. The cell cycle of *L. sativa* seeds is inhibited by allelopathic substances present in the bark of *P. mucronata* fruits. It is concluded that aqueous extracts of *Passiflora mucronata* have an allelopathic inhibitory effect on germination and initial growth of *L. sativa*.

Keywords: Bioherbicides; cytogenetics; mutagenicity; passion fruit.
Abbreviations: GVI_germination velocity index; RL_ root length; APL_aerial part length; MI_mitotic index; CA_chromosomal alterations; NA_nuclear alterations.

Introduction

The passion-fruit is a member of the Passifloraceae family, with the genus *Passiflora* being the most economically important. Brazil is considered to be the origin of approximately 150 known species, 87 of which are endemic, making it the genus's center of genetic diversity. It is currently the world's largest producer of passion fruit, with 593,429 tons produced on 41,584 hectares. In recent years, the Northeast region has led Brazilian production, accounting for 64.5 % of total production, followed by the Southeast, South, North, and Midwest regions (Bernacci et al., 2015; Ibge, 2019).

The passion-fruit species *Passiflora mucronata* is native to the Atlantic Forest, and has gained popularity due to its resistance to some diseases, becoming an alternative rootstock under *Passiflora edulis* f. *flavicarpa* (Oliari et al., 2016). It is cold tolerant, with continuous production all year round, as well as having potential for ornamental use, with white flowers that open at night, and chiropterophilic pollination (nectivorous bats) (Meletti et al., 2011).

The passion-fruit has a variety of uses because different parts of the passion-fruit plant such as pulp, seeds, skin, flowers, leaves, and branches have commercial value (Faleiro et al., 2015). In Brazil, the juice industry consumes approximately 40 % of the annual production of passion fruit (Embrapa, 2015). However, according to Andrade Neto et al. (2015), the yield of

raw pulp of passion fruit varies from 34 % to 42 % of the fruit weight, implying that waste generated by the consumption of this product can reach 464,303 tons annually, accounting for approximately 66 % of total production, if peels and seeds are discarded. Given the possibility of reducing the excess of organic waste generated by the passion fruit juice processing industries, studies on passion fruit peel can be conducted to minimize waste and explore the potential of using the peel as an ingredient in new products.

Passifloraceae have been studied for their allelopathic potential in several plant species, including *Lactuca sativa,* and susceptibility to substances released by various species of passion fruit has been observed. The production of allelochemicals is essential for providing plants with self-defense, which can influence the growth and establishment of other coexisting taxa in a given area (Macías et al., 2007; Goldfarb et al., 2009; Teasdale et al., 2012).

Allelochemicals are important ecological tools, as they influence dominance, succession, formation of plant communities and crop productivity and management, and may affect more than one physiological function, such as nutrient absorption. Thus, its knowledge is of great interest in agriculture, including for the production of biological herbicides (Goldfarb et al., 2009; Mauli et al., 2009).

Costalonga and Batitucci (2014) tested allelopathy using *P. edulis* leaves on *Allium cepa* (onion) seeds and observed that this passion fruit species disrupted the cell cycle of onion seeds, resulting in a decrease in the mitotic index (MI) and inhibition of germination. However, positive effects on allelopathy were observed with the use of an aqueous extract of *Passiflora alata* leaf in *L. sativa* seeds, where the extract at lower concentrations induced seed germination (Silva et al., 2019). This disparity in results on allelopathy in Passifloraceae highlights the importance of additional work and research to identify the presence and mode of action of these allelochemicals.

Pesticides disrupt ecosystem balance and, as a result, animal and human life. The effects range from changing the composition of the soil to contaminating water and air, as well as interfering with terrestrial and aquatic living organisms, changing their morphology and function within the ecosystem. As a result, research and the use of alternative methods are required (Jabran et al., 2015; Lopes; Albuquerque, 2018). Allelopathy is the influence of an allelopathic plant on another plant, which can affect germination velocity and percentage, physiology, seedling growth, and plant population, and can benefit or harm the plant that is sensitive to allelopathic substances, as well as genetic factors of neighboring plants (Conti; Franco, 2011; Harun et al., 2014; Teixeira et al., 2018; Siyar et al., 2019; Laizer et al., 2021).

In order to identify allelopathic properties of plant species, called donors, bioassays are performed using receptor species known to be sensitive to the action of allelochemicals, such as *L. sativae*. However, the use of sensitive receptor species can overestimate the allelopathic potential of the donor species, so that the use of spontaneous species or even those that are cultivated in the same area (agroforestry systems and vegetable gardens, for example) present greater similarity to what takes place in a natural environment (Reigosa et al., 2013; Carvalho et al., 2016; Barbosa et al., 2018).

Lettuce (*L. sativa* L.) is one of the most commonly used vegetables for bioassays due to its rapid growth, large number of seeds, and small seeds, all of which contribute to a larger surface area of contact with the substance being tested (Andrade-Vieira et al., 2014). It is an eudicotyledonous plant in the Asteraceae family. The sensitivity of the species, even at low concentrations of allelochemicals, is the main advantage of using lettuce in allelopathic studies. In addition, the species exhibits other characteristics that make it useful, such as rapid germination (in approximately 24 h), insensitive linear growth to pH variations across a wide range, and insensitivity to osmotic potentials (Souza et al., 2005).

In light of these observations, the present study aimed to investigate the allelopathic potential of different concentrations of aqueous extract of fruit peels of *P. mucronata* genotypes on seed germination and growth of *L. sativa* L. seedlings.

Results and discussion

Germination and vigor of L. sativa submitted to treatments

Lactuca sativa seeds were treated with four different concentrations of aqueous extract of bark from *P. mucronata* genotype G1 and two controls, which resulted in a higher percentage of germination, followed by the water control (Table 1). The treatments with different aqueous extract concentrations (100; 50; 25, and 12.5%) did not differ statistically, resulting in a lower mean germination percentage. The seeds with the highest mean GVI were treated with water, and the extract concentrations did not differ significantly from each other.

In terms of root length, the seeds subjected to the water control had the highest mean RL and differed significantly from the other treatments. Glyphosate and the extract's lowest concentration (12.5 %) did not differ statistically ($p \leq 0.05$). Allelochemicals may influence germination; however, these effects are more noticeable with respect to root growth, as other authors have observed (Baličević et al., 2014; Konstantinović et al., 2014).

When seeds were treated with water, the length of the aerial part (APL) showed the highest average; however, it did not differ statistically among extracts at concentrations of 50, 25, and 12.5 %. The glyphosate positive control had the lowest mean APL, however, it did not differ statistically from that of the treatments at concentrations of 50, 25, and 12.5 % (Table 1).

Seeds of *L. sativa* treated with higher concentrations (100 and 50 %) of aqueous extract of *P. mucronata* fruit peel (genotype G6) did not germinate, indicating allelopathic effects. Seeds subjected to the water control showed a higher percentage of germination, which differed statistically from that of the other treatments (Table 2).

The GVI showed the highest mean in seeds treated with distilled water, which differed significantly from the other treatments (Table 2).

The water control had a longer mean root length (RL) and aerial part length (APL) than those of the other treatments (Table 2). The allelopathic action of aqueous extracts of *E. argentinum, L. divaricata, M. guianensis* and *O. puberula*, may have inhibitory effects, mainly on the primary root and its development (Maraschin et al., 2005).

Table 1. Germination, germination velocity index (GVI), root length (RL), and aerial part length (APL) of *L. sativa*, whose seeds were subjected to different different treatments (100; 50; 25 and 12.5%) of aqueous extract of the G1 genotype of *P. mucronata* and two controls (water and glyphosate).

Treatments (%)	Germination (%)	GVI	RL (mm)	APL (mm)
100	0	0	0	0
50	5	0.219	0	0.3 ab
25	30	1.529	2.2	0.3 ab
12.5	55	4,090	4.2 b	0.3 ab
Water	97 b[(1)]	10.850 a	7.6 a	0.5 a
Glyphosate	98 a	10.640 b	4.0 b	0.3 b

[(1)] Means followed by the same lowercase letter in the columns do not differ by Dunnett's test, at a probability level of 5%.

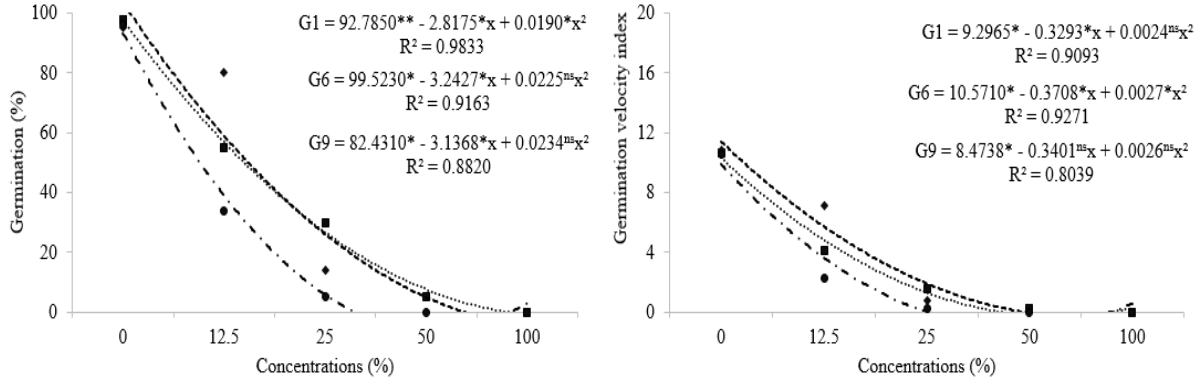

Fig 1. Germination (%)and germination velocity index (GVI) of *L. sativa* seeds treated with aqueous extract of *P. mucronata* genotypes at concentrations of 100; 50; 25 and 12.5% and two controls (water and glyphosate). **, * and ns: significant at 1%, 5% and not significant, respectively. R^2 = coefficient of determination.

Table 2. Germination, germination velocity index (GVI), root length (RL), and aerial part length (APL) of *L. sativa*, whose seeds were subjected to different different treatments (100; 50; 25 and 12.5%) of aqueous extract of the G6 genotype of *P. mucronata* and two controls (water and glyphosate).

Treatments (%)	Germination (%)	GVI	RL (mm)	APL (mm)
100	0	0	0	0
50	0	0	0	0
25	14	0.780	1.7	0.3 b
12.50	81	7.153	2.9	0.5 b
Water	98 a[(1)]	11.230 a	7.9 a	0.8 a
Glyphosate	95 b	10.860 b	4.2 b	0.3 b

[(1)] Means followed by the same lowercase letter in the columns do not differ by Dunnett's test, at a probability level of 5%.

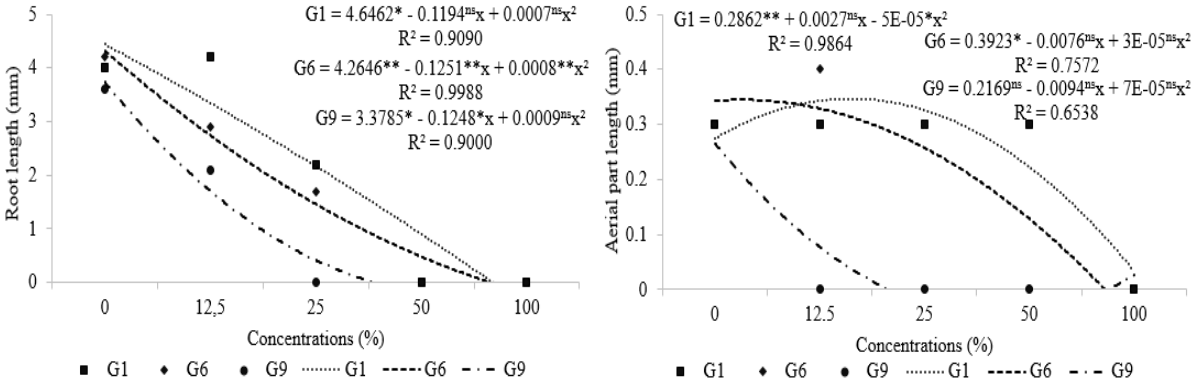

Fig 2. Root length (RL) and aerial part length (APL) of *L. sativa* seeds treated with aqueous extract of *P. mucronata* genotypes at concentrations of 100; 50; 25 and 12.5% and two controls (water and glyphosate). **, * and ns: significant at 1%, 5% and not significant, respectively. R^2 = coefficient of determination.

Table 3. Germination, germination velocity index (GVI), root length (RL), and aerial part length (APL) of *L. sativa*, whose seeds were subjected to different different treatments (100; 50; 25 and 12.5%) of aqueous extract of the G9 genotype of *P. mucronata* and two controls (water and glyphosate).

Treatments (%)	Germination (%)	GVI	RL (mm)	APL (mm)
100	0	0	0	0
50	0	0	0	0
25	5	0.253	0	0.1
12.5	34	2.240	2.1	0
Water	96 a(1)	10.370 b	8.2 a	0.3 a
Glyphosate	96 a	10.543 a	3.6 b	0.2 b

(1) Means followed by the same lowercase letter in the columns do not differ by Dunnett's test, at a probability level of 5%.

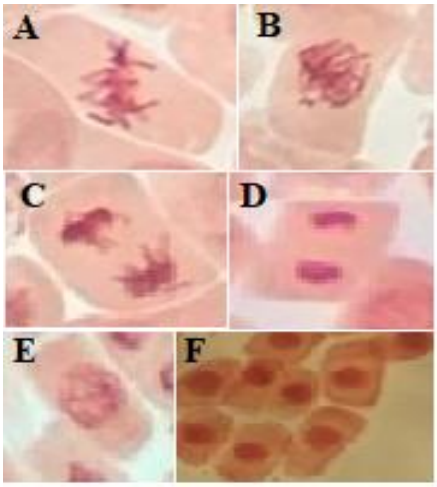

Fig 3. A: Metaphase in meristematic roots of *L. sativa*. B: Adherent chromosome (chromosomal alteration). C: Anaphase mitotic phase. D: Telophase in meristematic roots of *L. sativa*. E: Prophase. F: Condensed Nucleus (chromosomal alteration).

Table 4. Germination and germination velocity index (GVI) of *L. sativa* seeds treated with extracts of the three genotypes of *P. mucronata* at concentrations of 100; 50; 25 and 12.5% and two controls (water and glyphosate).

Treatments	Germination (%)			GVI		
	G1	G6	G9	G1	G6	G9
100	0	0	0	0	0	0
50	5	0	0	0.219	0	0
25	30	14	5	1.529	0.780	0.253
12.5	55	81 b	34	4.090	7.153 b	2.240
Water	97 a(1)	98 a	96 a	10.850 a	11.230 a	10.370 a
Glyphosate	98 a	95 a	96 a	10.643 a	10.867 a	10.543 a

(1) Means followed by the same lowercase letter in the columns do not differ by Dunnett's test, at a probability level of 5%.

Table 5. Mitotic index (MI), chromosomal alterations (CA) and nuclear alterations (NA) of *L. sativa* meristematic cells treated with the aqueous extract of the G1 genotype the *P. mucronata*.

Treatments (%)	MI	CA	NA
100	0	0	0
50	0	0	0
25	5.94 ab(1)	0.16 b	0.38
12.5	10.46 a	0.50 ab	0.30
Water	8.40 a	0.60 a	0.20 b
Glyphosate	4.24 b	0.20 b	0.66

(1)Means followed by the same lowercase letter in the columns do not differ by Dunnett's test, at a probability level of 5%.

Table 6. Mitotic index (MI), chromosomal alterations (CA) and nuclear alterations (NA) of *L. sativa* meristematic cells treated with the aqueous extract of the G6 genotype the *P. mucronata*.

Treatments (%)	MI	CA	NA
100	0	0	0
50	0	0	0
25	0.06 b[(1)]	0 b	9.60 a
12.5	10.24 a	0.34 b	1.40 ab
Water	13.42 a	0.86 a	1.18 b
Glyphosate	3.96 b	0.20 b	1.52 ab

[(1)] Means followed by the same lowercase letter in the columns do not differ by Dunnett's test, at a probability level of 5%.

Table 7. Mitotic index (MI), chromosomal alterations (CA) and nuclear alterations (NA) of *L. sativa* meristematic cells treated with the aqueous extract of the G9 genotype the *P. mucronata*.

Treatments (%)	MI	CA	NA
100	0	0	0
50	0	0	0
25	0,02	0	1.32 a
12.5	4.93 b[(1)]	0.32 b	0,30 ab
Water	13.67 a	0.90 a	0.18 b
Glyphosate	6.20 b	0.86 ab	0.44 ab

[(1)] Means followed by the same lowercase letter in the columns do not differ by Dunnett's test, at a probability level of 5%.

Seeds subjected to water and glyphosate controls (Table 3) showed higher germination percentages and did not differ statistically. Germination on the other hand, was inhibited by treatments with aqueous extract concentrations of 100 % and 50 %. *P. edulis* has been investigated for its inhibitory substances (generically called allelochemicals), the primary function of which is to guarantee seed dormancy and ensure that seeds do not germinate inside the fruit, but which also exhibit considerable allelopathic action on the germination of other species (Coelho et al., 2011). However, it can be observed that the aqueous extract of *P. mucronata* fruit peels showed allelopathic behavior.

Lactuca sativa seeds showed a higher mean germination velocity index (GVI) when subjected to the glyphosate control (10.543). However, the root and shoot length showed higher means in the negative control (water), statistically differing from the other treatments. Seeds subjected to concentrations of 100 % and 50 % aqueous extract did not show average germination or vigor (Table 3).

Treatments with concentrations of the aqueous extract of *P. mucronata* fruit peel (Tables 1, 2, and 3) inhibited root and aerial part growth of *L. sativa*, which corroborated the findings of Freitas et al. (2016), who tested the extract of *P. alata* in lettuce seeds and found a reduction in root and APL.

The germination percentage and GVI of *L. sativa* seeds (Table 4), did not differ significantly from each other when compared to the positive and negative controls of the genotypes G1, G6, and G9; however, they did differ from those of the other treatments.

Based on the data presented thus far, it is possible to conclude that the concentrations of 100, 50, 25, and 12.5 % of aqueous extract of *P. mucronata* fruit peels interfere with germination, in the GVI, and in the root and aerial part development of *L. sativa* seeds.

The percentage of germination and GVI (Figure 1) decreased as the concentration of aqueous extracts of *P. mucronata* increased, suggesting an allelopathic action that negatively interferes with the germination and vigor of *L. sativa* seeds.

The relationship between the concentrations of aqueous extracts of *P. mucronata* fruit peels and the length of the root and aerial part of *L. sativa* in the length of the aerial part (Figure 2) revealed that the highest concentrations had the lowest values of part growth; however, when the concentrations were lower, there was an increase in length, suggesting a beneficial interference, as observed by Silva et al. (2019). However, in the case of root length, this behavior was not observed, with a decrease as concentrations increased.

Cytogenetic analysis

The MI (Table 5) had higher averages in the (water) negative control and at the lowest concentration of the extract (12.5 %). However, the treatment at a concentration of 25 % did not differ statistically from the treatments (the two controls (water and glyphosate) and of concentration of the extract and 12.5%). The treatments with aqueous extract with lower mean MI suggested an allelopathic action, resulting in a lower percentage of germination (Costalonga and Batitucci, 2014), which was consistent with the data from the previous tables. The decrease in MI may indicate changes resulting from chemical action in the growth and development of exposed organisms (Leme et al., 2009; Silva et al., 2013).

The mean number of CA was higher in the water control; however, it did not differ statistically from that of the treatment with a 12.5 % extract concentration.

The NA had the highest mean in the glyphosate control (Table 5), which differed significantly from that of the other treatments.

The treatments with higher concentrations (100 % and 50 %) showed no MI, CA and NA of meristematic cells.

The MI showed significantly higher averages in the water control and at the lowest concentration of the extract (12.5 %), compared to those of the other treatments (Table 6). This behavior points to an allelopathic action that inhibits germination. Normal mitotic processes are essential for plant growth (Teerarak et al., 2010). The occurrence of the blockage of mitotic division processes and nucleus death prevents the start of prophase and cell division, resulting in a decrease in the MI, suggesting that reduced root growth is related to a reduction in MI (Andrade et al., 2010).

The mean number of CA was higher in the water control than in the other treatments.

The NA had a higher mean with the 25 % concentration treatment; however, it did not differ significantly from that of the 12.5 % concentration treatment or the glyphosate control (Table 6).

Higher concentrations (100 and 50%) were not analyzed because they did not show MI, CA and NA of meristematic cells.

The MI (Table 7) had a higher mean in the water control than with the other treatments. Lower mean MI values suggest an allelopathic action as a result of *P. mucronata* extract treatment and the glyphosate control.

The mean number of CA was higher in the water control, but it did not differ statistically from that of the glyphosate treatment.

The treatment with 25 % extract had the highest NA; however, there was no difference between the treatment with 12.5 % extract and the glyphosate control (Table 7).

A higher frequency of some mitotic division phases (A, C, D, and E) and the most common chromosomal alterations (B and F) (Figure 3) were detected in *L. sativa* meristematic roots, where cells with adherent chromosomes were observed in all treatments. However, when compared to water and glyphosate, the adherent chromosomes found at both concentrations of aqueous extract are the result of toxic effects. The action of toxic agents on cell cycle proteins can result in chromosome adherence (Amin, 2011).

Materials and Methods

Plant materials

The experiment was conducted at the Cytogenetics Laboratory and in the Seed Analysis Laboratory at the campus of the Center for Agricultural Sciences and Engineering of the Federal University of Espírito Santo (CCAE-UFES), in the municipality of Alegre-ES. Fruit peels from three genotypes (G1, G6, and G9) of passion fruit (*P. mucronata),* cultivated in an espalier, were used in the experimental area of CCAE-UFES, Alegre-ES, with latitude 20º 45'S, longitude 41º 30 ''W, and altitude of 250 m. The seeds of *Lactuca sativa* (Crespa-Isla ® cultivar) used in the experiment were purchased at the local market.

Treatments performed

The peels of *P. mucronata* genotypes (G1, G6 and G9)were dried in an oven at 60 °C for 48 h and then ground manually in a porcelain crucible. The extract obtained peels of *Passiflora mucronata* genotypes was filtered in filter paper and diluted in water to get at the following concentrations: T1=100, T2=50, T3=25, T4=12.5 mg mL^{-1}, T5= negative control (distilled water), and T6= positive control (glyphosate). Throughout the study, distilled water and glyphosate were used as negative and positive controls, respectively.

Macroscopic variables analyzed

For macroscopic assays, the germination percentage, germination velocity index (GVI), root length (RL), and aerial part length (APL), comparing the aqueous extracts of barks of *P. mucronata* genotypes with the negative control (water) and the positive control (glyphosate).

Germination was carried out in Petri dishes lined with autoclaved filter paper and moistened with 2.5 mL of each solution, or the controls (as described above). Five repetitions with 25 seeds were used for each treatment. The plates were sealed with film paper and conditioned in a biochemical oxygen demand (BOD) germination chamber at 25 ± 2 °C under constant light. Macroscopic analysis was performed during the germination phase and root and aerial growth of the seedlings.

The germination velocity index (GVI) was counted after eight; 16; 24; 32; 40 and 48 h of exposure to the treatments. Root and aerial growth were evaluated after 48 h and 120 h of exposure to the treatments, respectively.

Microscopic analysis

For microscopic evaluation, after root measurement (48 h), 10 seedling roots from each Petri dish were collected, fixed in ethanol: acetic acid (3:1), and stored at - 4 ºC for 24 h. Subsequently, the rootlets were washed with distilled water three consecutive times for ten minutes (for a total of 30 min of washing), before being subjected to acid hydrolysis in 5N HCl for 18 min to make the slides. The root tips were sectioned and stained with 2 % acetic orcein (Andrade-Vieira et al., 2014), after which the coverslips were placed, and the material was gently macerated with the aid of a round-tipped pen. For cytogenetic analysis, 5000 meristematic cells were analyzed per treatment, and the different phases of mitotic division and possible chromosomal and nuclear alterations were observed and quantified.

The mitotic index (MI) was obtained from the quotient of the number of cells in division (prophase, metaphase, anaphase, and telophase) by the total number of cells analyzed in each treatment, while the frequencies of chromosomal (CA) and nuclear (NA) changes were calculated by the quotient of the number of changes (chromosomal and nuclear, respectively) by the total number of cells analyzed (Andrade-Vieira et al., 2014).

Statistical analysis

The design used was entirely randomized, in a factorial scheme 3 × 4 + 2 controls (genotypes of passion fruits (G1, G6, and G9) x concentrations (100; 50; 25 and 12.5 mg mL^{-1}) + negative control and positive control. The data were submitted to the test of normality of residuals, without data transformation, and the analysis of variance; when the F value was significant at the 5 % level, Dunnett's test was used to compare the means. For the effect of extracts, the data were submitted to regression analysis and for the adjustment of equations ($\hat{Y}$=) the significance of betas ($p \leq 0.05$) was used as a criterion. R software was used for statistical analyses (R Core Team, 2021).

Conclusion

Allelopathic substances found in the fruit peel of *P. mucronata* disrupt the physiological cycle of *L. sativa* seeds, interfering with germination, vigor, and seed development. The bark extracts at concentrations of 50 and 100 % inhibited the germination of *L. sativa* seeds. Allelopathic substances present in the skin of the fruit of *P. mucronata* inhibit the cell cycle of *L. sativa* seeds.

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