Article

Allelopathic effect of *Metopium brownei* and *Viguiera dentata* on *Senna uniflora*

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Abstract

Allelopathy is a biological phenomenon in which chemicals released by one plant species directly influence the growth and development of another; therefore, allelopathic species can be a natural source of herbicides. *Metopium brownei* and *Viguiera dentata* have been shown to have an inhibitory effect on plants and microorganisms. The present work aimed to determine the suppressive effect of different doses of crude extracts of *M. brownei* and *V. dentata* on the *in vitro* germination of a tropical weed (*Senna uniflora*) and *Raphanus sativus*, a species highly sensitive to allelochemicals. It was shown that aqueous extracts of *M. brownei* fruits applied at doses as low as 0.5% suppressed the germination of the weed *S. uniflora* by 100%. The ethanolic extracts of both species demonstrated an inhibitory effect on the germination of *S. uniflora* seeds at concentrations of 8% or higher. On the other hand, aqueous extracts of *V. dentata* flowers were the most effective in inhibiting the germination of *R. sativus* seeds when applied in doses higher than 15%. According to the results obtained in the present work, it is concluded that *V. dentata* has a strong allelopathic effect on *S. uniflora* when used in ethanolic and aqueous extracts, so it can be used as a bioherbicide for the control of the weed in tropical crops.

Keywords: allelopathy, bioherbicides, natural extracts, weeds.

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Introduction

In Mexico, agriculture is an activity of great importance for local, national and international economic development (Macías, 2013). The control of weeds represents one of the main problems to be solved with a view to achieving improvements in agricultural production by reducing costs. In this sense, herbicides that have negative effects on the environment have traditionally been used, causing damages to soil biodiversity and human health (Oliva and Peña, 2004).

A specific example is the use of glyphosate, whose residues such as aminomethylphosphonic acid (AMPA) accumulate in soil and water, causing bioaccumulation in plants, animals and humans, given its high persistence in the environment (Martins-Gomes *et al*., 2022). Likewise, the herbicide 2,4-D has been proven to cause serious health problems in humans, in addition to the ecotoxicological effect it has on aquatic and plant life (Islam *et al*., 2018). Similarly, atrazine has a high toxicity and accumulates in surface and groundwater (Hansen *et al*., 2013).

For its part, paraquat, another widely used herbicide and whose form of action is as a respiration blocker, causes a high rate of intoxication and mortality in mammals and its persistence in the environment is also prolonged (Hernández *et al*., 2008; Rojas, 2018). An alternative to solve this problem is the development of products based on the use of plant species that have been shown to have suppressive effects on the growth and development of weeds, at a lower cost to the environment (Dousseau *et al*., 2008; Celis *et al*., 2009; Oliveira *et al*., 2015).

This inhibitory effect of one plant species on another, mediated by interaction with secondary metabolites, is known as allelopathy; and secondary metabolites that are released into the environment by allelopathic species are known as allelochemicals. An example of these are extracts from the leaves and seeds of *Canavalia ensiformis*, whose bioherbicidal effect has been proven on *Grandifolia ipomoea* and *G. benghalensis* (Mendes and Rezende, 2014). The allelopathic effect in many cases manifests itself in inhibition of germination, plant growth, bud development and root (Sahu and Devkota, 2013).

In tropical crops, *Senna uniflora* (known in some parts of Mexico as 'Cacahuatillo') is a species that is considered a weed in the Yucatán Peninsula and elsewhere in Mexico (Francisco, 2018), whose seeds have the ability to go dormant during times of drought, germinate and grow vigorously in the rainy season (Figueroa and Galeano, 2007). This species has a high presence in tropical crops such as passion fruit (Muraira *et al*., 2016), corn, beans, rice, sugarcane and cotton, coffee and tobacco (Alipi and Flores, 2013), probably due to its allelopathic effect on other weed species (Swati *et al*., 2014), so it easily becomes dominant in the crops in which it is present and may even inhibit the growth of the cultivated species.

According to the above, it is an interesting study model to test the effect of new herbicides, especially those formulated from allelochemicals. On the other hand, *Metopium brownei* and *Viguiera dentata* have a wide distribution in the Yucatán Peninsula, whose allelopathic potential on other plants or microorganisms has been reported in different studies.

Therefore, in this study the aqueous and ethanolic extracts prepared from different organs of both allelopathic species were evaluated to know their effects on the germination of *Senna uniflora*. The above in order to establish the bases that enable the development of a safe and effective bioherbicide product for the control of weeds characteristic of the Yucatán Peninsula.

Materials and methods

Samples of leaves, fruits, bark and roots of *M. brownei* trees; as well as of leaves, stems, flowers and roots of shrubs of *V. dentata* were collected. The collections were carried out at the Xamantún experimental ranch, of the Technological Institute of Chiná. The samples were stored in refrigeration until disinfection, using a 5% sodium hypochlorite solution for 10 min. Once this was done, they were rinsed with plenty of sterile water and dehydrated at 55 °C in an industrial oven for three days.

The dehydrated material was crushed and macerated for 72 h with 96% absolute ethanol at room temperature or distilled water (at 4 °C), at a rate of 1:3 v/v (plant material/solvent). Subsequently, the evaluation of the phytotoxicity of the extracts was carried out by soaking Whatman grade 3 filter paper discs with 1 ml of the extracts to be evaluated diluted to obtain concentrations of 0.3, 0.5, 1, 2.5, 8, 15 and 20% (Table 1), similar to the technique reported by Uribe (2008) to evaluate phytotoxic extracts in onions and soybeans.

Table 1. Effect of aqueous and ethanolic extracts of *Metopium brownei and Viguiera dentata* on the germination of *Raphanus sativus* seeds.

Dose	Mb_Leaf	Mb_Fruit	Mb_Bark	Mb_Root	Vd_Leaf	Vd_Flower	Vd_Stem	Vd_Root
				Aqueous extract				
C-	66.6±47.14^{ab}	66.6±47.14^{a}	66.6±47.14^{ab}	66.6±47.14^{ab}	66.6±47.14^{a}	66.6±47.14^{ab}	66.6±47.14^{b}	66.6±47.14^{ab}
C+	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
0.3%	86.6±18.5^{b}	90±14.14^{a}	76.6±14.14^{b}	100±0^{b}	90±8.16^{a}	100±0^{b}	100±0^{b}	80±28.28^{ab}
0.5%	93.3±4.17^{b}	83.3±23.57^{a}	90±23.57^{b}	83.3±23.57^{b}	53.3±38.58^{a}	53.3±41.09^{ab}	93.3±9.42^{b}	86.6±18.85^{b}
1%	93.3±9.42^{b}	90±14.14^{a}	93.3±14.14^{b}	83.3±23.57^{b}	46.6±36.81^{a}	100±0^{b}	100±0^{b}	60±43.2^{ab}
2.5%	90±14.14^{b}	80±28.28^{a}	93.3±28.28^{b}	83.3±23.57^{b}	86.6±9.42^{a}	90±47.14^{ab}	100±0^{b}	100±0^{b}
8%	86.6±18.85^{b}	83.3±23.57^{a}	93.3±23.57^{b}	86.6±12.47^{b}	30±21.6^{a}	33.3±14.14^{ab}	93.3±9.42^{b}	100±0^{b}
15%	86.6±18.85^{b}	70±35.59^{a}	93.3±35.59^{b}	86.6±18.85^{b}	16.6±16.99^{a}	0±0^{a}	76.6±9.42^{b}	83.3±12.47^{ab}
20%	83.3±23.57^{b}	83.3±23.57^{a}	90±23.57^{b}	96.6±4.71^{b}	6.6±9.42^{a}	0±0^{a}	93.3±9.42^{b}	93.3±9.42^{b}
				Ethanolic extract				
C-	66.6±47.14^{abc}	66.6±47.14^{b}	66.6±47.14^{b}	66.6±47.14^{b}	66.6±47.14^{a}	66.6±47.14^{a}	66.6±47.14^{a}	66.6±47.14^{a}
C+	0±0^{a}	0±0a	0±0^{a}	0±0	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
0.3%	90±14.14^{c}	83.3±9.42^{b}	90±8.16^{b}	96.6±4.71^{b}	40±43.2^{a}	46.6±41.06^{a}	46.6±41.09^{a}	60±43.2^{a}
0.5%	83.3±9.42^{bc}	83.3±9.42^{b}	76.6±20.54^{b}	86.6±12.47^{b}	46.6±41.14^{a}	26.6±37.71^{a}	66.6±47.14^{a}	66.6±47.14^{a}
1%	46.6±33.99^{abc}	80±8.16^{b}	83.3±12.47^{b}	76.6±20.54^{b}	33.3±47.14^{a}	36.6±44.96^{a}	10±14.14^{a}	56.6±41.89^{a}
2.5%	13.3±18.85^{ab}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	3.3±4.71^{a}	6.6±9.42^{a}	3.3±4.71^{a}
8%	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
15%	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
20%	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}

Mb= *Metopium brownei*; Vd= *Viguiera dentata*; C-= negative control (water); C+= positive control (pyrocatechol); ^{a,b,c}= different literals in the same column indicate a statistically significant difference (Tukey, $p \leq 0.05$).

The solvents were previously removed from the extracts with the use of a rotary evaporator. In the bioassays, seeds of *Senna uniflora* and *Raphanus sativus* were used as recipient species, the latter species because of its susceptibility to allelochemicals (Othman *et al*., 2012; Rahman *et al*., 2022). To do this, the seeds were disinfected with a chlorine solution (10 ml L^{-1}) placing 10 on the discs already soaked with the dilutions of extracts.

The treatments were subjected to experimentation until observing the germination of all the seeds in the negative control bottles (sterile distilled water). Pyrocatechol (35 mg L^{-1}) was used as a positive control. The phytotoxic effect was determined by calculating the percentage of germination obtained in each treatment. Data were analyzed by a one-way Anova and Tukey's test of means (p= 0.05), using the Infostat V. 2017 software.

Results and discussion

Table 1 shows that aqueous and ethanolic extracts of *M. brownei* and *M. brownei* affected the germination of *R. sativus* seeds. This effect had different intensities of the inhibition of seed germination according to the type of extract, concentration of the extract and tissue of the allelopathic plant in question. It was observed that the aqueous extracts of *V. dentata* obtained from leaves, the application of a dose greater than 8% resulted in an inhibition of germination of about 70%.

Likewise, extracts obtained from flowers caused 100% inhibition of germination from a dose of 15%. In general, these extracts were more effective than those obtained from the different organs of *M. brownei*. With respect to ethanolic extracts, those obtained from leaves of *M. brownei* led to a 100% inhibition of the germination of radish seeds when applied in doses from 8%, the most effective being those isolated from fruits, bark and root, applied in doses of 2.5% or higher.

On the other hand, the extracts prepared with leaves of *V. dentata* were the most effective among those prepared with the plant material of this species, inhibiting germination by 100% by applying them also at doses of 2.5% or higher. The extracts obtained from its other organs reached the same effectiveness from the dose of 8%. Therefore, in general it can be established that ethanolic extracts of both species are effective in inhibiting the germination of radish seeds when applied in doses as low as 2.5%. However, the aqueous and ethanolic ones applied at low doses promoted germination (Table 1).

There are reports of several plant extracts that have been evaluated, demonstrating effectiveness similar to that observed in this study. Similarly, studies carried out with aqueous extracts show that they can inhibit or promote plant germination and development, depending on the dose applied. For example, extracts from leaves of 25 Bangladeshi leguminous plants inhibited the germination and growth of radish shoots, confirming their allelopathic activity (Rahman *et al*., 2022), in contrast, another study showed that some doses of extracts from the root and aerial parts of *Deverra tridariata* stimulated the germination and vegetative growth of *Triticum aestivum* L. (Guetat *et al*., 2022).

Similarly, extracts of *Tectona grandis* L. and *Tagetes erecta* L. inhibited the germination of seeds of cucumber (*Cucumis sativus* L.), okra (*Hibiscus esculentus* L.), radish (*Raphanus sativus* L.) and lettuce (*Lactuca sativa* L.), while stimulating the germination of beans (*Phaseolus vulgaris*

L.). Similarly, aqueous extracts of *Calotropis procera* applied in high doses (between 40 and 60%) delayed the germination of seeds of barley (*Hordeum vulgare* L.), wheat (*Triticum aestivum* L.), cucumber (*Cucumis sativus* L.), fenugreek (*Trigonella foenum graecum* L.) and septic weed (*Senna occidentalis* L. Link), while at lower doses (5%), they stimulated the growth of cucumber, septic weed and fenugreek seedlings, even more than the control treatment (Al-Zahrani and Al-Robai, 2007).

Therefore, it is important to consider that allelopathic plant extracts also have active substances capable of promoting faster and more uniform germination (Carrillo-Martínez *et al*., 2018). For example, lactones have been proven to stimulate plant growth and development (Aristizábal *et al*., 2017). On the other hand, the application of secondary metabolites can induce the release of phenolic compounds involved in stress defense physiology (Hernández and González, 2010).

This accumulation of phenols has been detected in high quantities in the vacuole and in the cell walls of various species, leading to phenolic oxidation and growth inhibition in woody plants (Jácome and Rojas 2017). Similar phenolization effects were observed in this study in *R. sativus* seeds treated with organic extracts obtained from the plant organs of *M. brownei*, such as that of leaves (1E), bark (1H) and root (1N), applied at 20% (Figure 1). It is possible to attribute the low percentages of germination observed to this phenomenon.

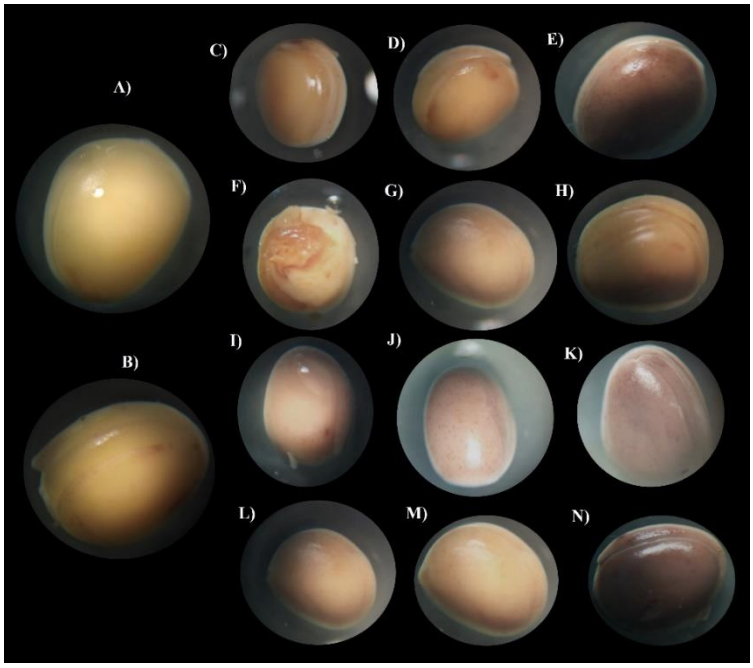

Figure 1. Effect of ethanolic extracts of *Metopium brownei* on *Raphanus sativus seeds*. A) control; B) control+; C) leaf extract at 8%; D) leaf extract at 15%; E) leaf extract at 20%; F) bark extract at 8%; G) bark extract at 15%; H) bark extract at 20%; I) fruit extract at 8%; J) fruit extract at 15%; K) fruit extract at 20%; L) root extract at 8%; M) root extract at 15%; and N) root extract at 20%.

In the study region, *V. dentata* is an herbaceous plant whose biomass is not used and once it has passed its flowering stage, it ends up drying out and being lost in the environments in which it is present. According to the results obtained from bioassays with radish seeds, ethanolic extracts prepared from leaves of this species are postulated as a good bioherbicide alternative, given their high effectiveness and that, in biomass, the leaves exceed the remaining plant organs, which would facilitate the preparation of a product for application in sustainable agriculture.

Based on the above, it was proposed to evaluate the phytotoxicity of extracts on seeds of the weed *S. uniflora*. Table 2 shows that extracts from the leaves of *M. brownei* had the best effects in the inhibition of the germination of this weed. Specifically, the aqueous extracts of its fruits inhibited 100% the germination of *S. uniflora* from the concentration of 0.5 to 20%. In the germination values obtained from the application of aqueous extracts of bark and root, there were no significant differences, although it is worth mentioning that the germination was lower than that obtained in the negative control (water).

Table 2. Effect of aqueous and ethanolic extracts of *Metopium brownei and Viguiera* on the germination of *Senna uniflora* seeds.

Dose	Mb_Leaf	Mb_Fruit	Mb_Bark	Mb_Root	Vd_Leaf	Vd_Flower	Vd_Stem	Vd_Root
				Aqueous extract				
C-	67±33.99^{a}	67±33.99^{b}	67±33.99^{a}	67±33.99^{a}	67±35.59 ^{a}	67±35.59^{a}	67±35.59^{a}	67±35.59^{a}
C+	0±0^{a}	0±0^{a}	0 ±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0 ^{a}
0.3%	20±28.28^{a}	90±14.14^{b}	10±14.14^{a}	30±42.42^{a}	40±43.2^{a}	43±41.89^{a}	60±43.2^{a}	16±23.57^{a}
0.5 %	13±18.85^{a}	0±0^{a}	13±18.85^{a}	33±47.14^{a}	40±44.96^{a}	53±41.09^{a}	13±18.85^{a}	23±32.99^{a}
1%	16±23.57^{a}	0±0^{a}	13±18.85^{a}	13±18.85^{a}	0±0^{a}	37±44.96^{a}	20±28.28^{a}	40±43.2^{a}
2.5%	20±28.28^{a}	0±0^{a}	23±32.99^{a}	23±32.99^{a}	23±32.99^{a}	73±42.42^{a}	3±4.71^{a}	10±14.14^{a}
8%	7±9.42^{a}	0±0^{a}	26±37.71^{a}	26±37.71^{a}	0±0^{a}	52±41.09^{a}	3±4.71^{a}	3±4.71^{a}
15%	20±28.28^{a}	0±0^{a}	20±28.28^{a}	33±47.14^{a}	13±18.85^{a}	58±43.2^{a}	26±37.71^{a}	20±28.28^{a}
20%	20±28.28^{a}	0±0^{a}	20±28.28 ^{a}	23±32.99^{a}	0±0^{a}	41±44.96^{a}	43±41.89^{a}	33±47.14^{a}
				Ethanolic extract				
C-	67±33.99^{b}	67±33.99 ^{a}	67±33.99^{b}	67±33.99 ^{b}	67±35.59^{b}	67±35.59^{a}	67±35.59^{a}	67±35.59^{a}
C+	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
0.3%	16.67±23.57^{ab}	16.67±23.57^{a}	6.67±9.42^{a}	43.33±41.89^{a}	13.33±18.85^{a}	63.33±41.89^{a}	63.33±41.89^{a}	43.33±41.89^{a}
0.5%	16.67±23.57^{ab}	23.33±33.99^{a}	10±14.14	33.33±47.14^{a}	10±14.14^{a}	30±43.2^{a}	30±41.89^{a}	33.33±47.14^{a}
1%	0±0^{a}	23.33±33.99^{a}	13.33±18.85^{a}	30±18.85^{a}	6.67±9.42^{a}	53.33±43.2^{a}	53.33±47.14^{a}	30±42.42^{a}
2.5	0±0^{a}	10±14.14^{a}	0±0^{a}	0±0^{a}	10±14.42^{a}	20±28.28^{a}	28±28.28^{a}	10±14.14^{a}
8%	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	6.67±9.42^{a}	0±0^{a}	0±0^{a}	0±0^{a}
15%	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	3.334.71^{a}	0±0^{a}	0±0^{a}	0±0^{a}
20%	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}

Mb= *Metopium brownei*; Vd= *Viguiera dentata*; C-= negative control (water); C+= positive control (pyrocatechol). ^{a,b,c}Different literals in the same column indicate a statistically significant difference (Tukey, $p \leq 0.05$).

The extracts from *M. brownei* leaves had a greater effect on germination inhibition. Specifically, aqueous extracts of fruits inhibited 100% from the concentration of 0.5%. In the germination values obtained from the application of aqueous extracts of bark and root, there were no significant differences.

With the aqueous extracts of *V. dentata* obtained from leaves, the lowest percentage of germination was obtained when compared with the extracts obtained from its other organs and in general, they were less effective than the aqueous extracts of *M. brownei*. With respect to ethanolic extracts, those obtained from leaves of *M. brownei* led to an inhibition of 100% of the germination of *S. uniflora* from the concentration of 1%. Similarly, ethanolic extracts of *V. dentata* were more efficient in controlling the germination of the grass than aqueous extracts obtained from the same species.

In this way, doses above 8% led to 100% inhibition, especially when using extracts prepared with roots, stems and flowers of *V. dentata*. It is important to note that, although high percentages of germination were observed with some treatments, in all cases these remained below the values observed in the negative control (water). Thus, although in some cases the suppressive effect was mild, all the extracts evaluated had a negative effect on the germination of *S. uniflora*.

The extracts of various species evaluated in other studies demonstrate a lower effectiveness than that observed with the extracts evaluated in this study. For example, aqueous extracts of *Azadirachta indica* A. Juss, *Murraya koenigii* (Linn.) *Spreng* and *Paederia foetida* Linn used at doses of 10% on *Vigna radiata* (L.) *Wilczek* demonstrated a low suppressive efficiency, observing a germination of 73.3%, when applied at a rate of 5% there was a germination of 80% and when applied in doses of 1% there was a germination of 86.7%.

On the other hand, the extracts of *Paederia foetida*, a germination of 76.7% was obtained with the concentration of 10%, while a high percentage of germination, of 93.3%, was obtained with the concentration of 5% and with the concentration of 1% there was a germination of 96.7% (Kakati and Baruah, 2013). Some authors emphasize that the inhibitory activity of allelopathic extracts depends on the concentrations of extract used; the species of donor and recipient plants also influence.

It is even possible to obtain a positive effect on plant germination and growth, similar to the behavior observed in some of the highest concentrations evaluated in this work. For instance, in a study in which aqueous extracts of *Ruta graveolens*, *Baccharis alnifolia* and *Caesalpinia spinosa* were evaluated in the germination of *Chenopodium album*, *Amaranthus hybridus* and *Brassica rapa* subsp., it was found that those obtained from *R. graveolens* roots stimulated the germination of *B. rapa* (Calderón, 2018).

Similarly, aqueous extracts isolated from *V. dentata* flowers in doses of 2.5% appear to be promoting the germination of *S. uniflora*. Figure 2 shows the effects produced by ethanolic extracts of *M. brownei*. In general, necrosis of the seeds is observed, verifying the lethal effect of all treatments from dilution at 8%. A similar effect is observed in seed treated with pyrocatechol (2B).

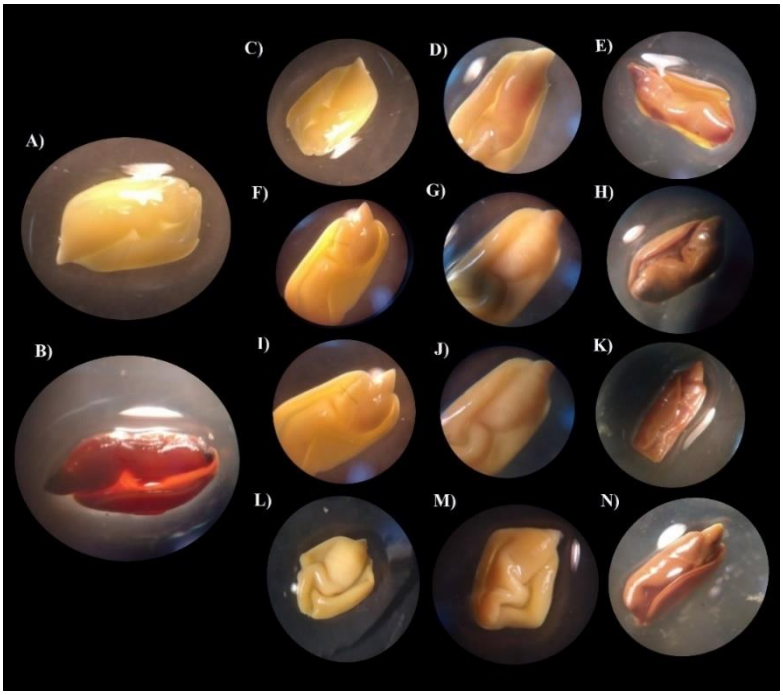

Figure 2. Seeds of *Senna uniflora* after being subjected to different doses of *M. brownei*. A) control-; B) control+; C) leaf extract at 8%; D) leaf extract at 15%; E) leaf extract at 20%; F) flower extract at 8%; G) flower extract at 15%; H) flower extract at 20%; I) bark extract at 8%; J) bark extract at 15%; K) bark extract at 20%; L) root extract at 8%; M) root extract at 15%; and N) root extract at 20%.

Finally, Figure 3 shows the effects produced by the ethanolic extracts of *M. brownei* on the initial development of *Senna uniflora* seedlings. In general, points of necrosis on the leaves and stem, chlorosis and root inhibition are observed.

Figure 3. Seeds of *Senna uniflora* after being subjected to different doses of ethanolic extracts of *M. brownei*. A) Phenolized seed, product of treatment with leaf extract at 1%; B) seedlings derived from treatment with fruit extract at 1%; C) seedlings derived from treatment with fruit extract at 2.5%; and D) phenolized seedling, derived from treatment with bark extract at 1%.

Conclusions

The aqueous and ethanolic extracts of *Metopium brownei* and *Viguiera dentata* have an allelopathic effect responsible for inhibiting the germination of seeds of the tropical weed *Senna uniflora* and *Raphanus sativus*. The species *M. brownei* has a higher inhibitory effect when fruits are used to obtain extracts, while *V. dentata* was more efficient when leaves were used to obtain extracts. Both species have potential to be used as bioherbicides in tropical crops.

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