

*Full Length Research Paper*

# The use of plant extracts in anthracnose control in species of *Heliconia* (*Heliconia psittacorum* cv. Golden Torch and *Heliconia rostrata*)

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Received 10 July, 2018; Accepted 30 October, 2018

The objective of the present study was to assess the anti-fungal activity of medicinal plant extracts on inhibition of *Colletotrichum gloesporioides* mycelial growth, sporulation and germination and on anthracnose control in *Heliconia psittacorum* cv. Golden Torch and *Heliconia rostrata*. Extracts of garlic, lemon grass, cinnamon, lemon balm, eucalyptus, ginger, mint, bitter melon and black pepper were used. The mycelial were assessed *in vitro* by measuring their growth in Petri dishes. Sporulation was assessed by counting spores in a Neubauer chamber. Germination was observed based on germination tube emission. In the *in vitro* tests, the inflorescences were treated with the plant extracts and inoculated with the pathogen 24 h later. The results showed that all the extracts presented anti-fungal activity, in greater or lesser intensity, compared to the control. The garlic extract resulted in the highest mycelial growth inhibition rate. Regarding sporulation, the bitter melon, ginger, mint, popcorn eucalyptus and bitter melon extracts were more efficacious, interfering in spore formation; while the ginger extract most reduced spore germination inhibiting germination tube emission. For the *in vivo* tests, it was observed that all the plant extracts tested were efficient in reducing lesion severity in the inflorescence, showing that the use of plant extracts may be a promising alternative for managing the diseases that affect helconia post-harvest.

**Key words:** Alternative control, fungi, *Colletotrichum gloesporioides*, tropical flowers.

## INTRODUCTION

Floriculture is one of the main segments of agrobusiness in Brazil, although the main consumer of flowers and ornamental plants produced in Brazil is the domestic Brazilian Market itself that accounts for more than 96% of the total (Sebrae, 2015). From 2014 to 2015, the volume

of Brazilian exports was only 21.9 thousand dollars according to the Ministry of Industrial Development and Foreign Trade, values much lower than those presented in 2004 and 2005, which were a total of 12.7 million dollars (Brasil, 2017).

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In 2015 the ornamental plant sector grossed more than R\$ 6 bilhões (Alencar and Galera, 2016), showing its size and importance in the Brazilian economy. In the same period, the planted area was approximately 15.000 hectares, due mainly to a recurrent increase in the area used for this activity in Brazil, as in 2012 and 2013 the area was estimated at about 11.800 and 14.000 ha, respectively (Neves and Pinto, 2015).

There are no recent studies in the literature on the productive chain in the state of Maranhão, Brazil, and the local production is still not very enterprising and focused essentially on supplying São Luís, the state capital. It concentrates on exploiting cut flowers and tropical foliage that include palm trees, bromelias, ferns, miniature roses and crotons (Sebrae, 2015).

The conditions of tropical ornamental plant cultivation, climate and planting density favor disease occurrence. Diseases reduce production and affect flower quality; becoming a bottleneck for quality tropical flower production, because they cause both direct damage, when they infect the inflorescences causing dark spots, and indirect damage, by reducing the photosynthesis area of the plant when they colonise the leaves (Sardinha et al., 2012).

Sardinha et al. (2012), surveyed disease occurrence in tropical flowers on São Luís Island and reported the presence of 16 disease causal agents, especially fungal and nematoid diseases.

Plant diseases are mostly controlled by agricultural chemicals, which results in high costs, environmental and toxicological risks and favors plant pathogen resistance to agricultural chemicals. Society's concern with dependence on toxic agricultural chemicals, that contaminate the environment, has led to the search for alternative control methods that are safe, viable and efficient in plant pathogen fungus control (Silva et al., 2010).

Control alternatives, including the use of biological and antagonistic plant extracts, have been studied, with significant advances for sustainable agriculture (Freitas, 2008).

Considering the potential of tropical flowers as a source of income for small and large producers and the presence of plant pathogens that can affect the yield of this activity, technologies need to be adopted that meet the requirements of these producers, especially in decreasing plant pathogens. Thus the objective of the present study was to assess the anti-fungal activity of crude plant extracts on the pathogen *Colletotrichum gloeosporioides* Penz., causal agent of anthracnose in *Heliconia psitacorum* cv. Golden Torche and *Heliconia rostrata*.

## MATERIALS AND METHODS

The experiments were carried out in the Plant Pathology Laboratory at the Agronomic Biotechnology Nucleus at the State University of

Maranhão (UEMA), MA, Brazil.

### Plant collection and plant pathogen isolation

Heliconia leaves with disease symptoms were collected during three technical visits to the Vassoural Agrucultural Pole, in the municipality of Paço do Lumiar, on São Luís Island, Maranhão, Brazil. The material collected was taken to the Plant Pathology Laboratory at UEMA for isolation and identification and the isolates were stored.

To isolate the plant pathogens, intermediary fragments of the lesions were cleaned with 70% alcohol, 0.5% sodium hypochlorite solution and washed twice in distilled, sterilised water. The fragments were then plated on Petri dishes containing potato dextrose agar culture medium (PDA) and kept at ambient temperature ( $25\pm 2^\circ\text{C}$ ) for fungal growth. The isolates were identified by observing the morphological aspects under a microscope and when necessary microcultures were used (Menezes and Assis, 2004). After identification, the isolates were placed in the "Prof. Gilson Soares da Silva" plant pathogenic fungus collection, with registration number MGSS114.

### Obtaining the aqueous extract

Extracts used from garlic (*Allium sativum* L.), black pepper (*Piper nigrum* L.) and ginger (*Zingiber officinale* Roscoe), were obtained from retail stores in São Luís; lemon grass (*Cymbopogon citratus* L.), cinnamon (*Cinnamomum zeylanicum* Blume.), lemon balm (*Lippia alba* L.) and mint (*Mentha piperita* L.), were purchased in public markets and eucalyptus (*Eucalyptus globulus* L.); while bitter melon (*Momordica charantia* L.), and neem (*Azadirachta indica* A. Juss.) were collected from the Paulo VI University campus at the State University of Maranhão (UEMA). The garlic and ginger extracts were prepared from bulbs and the other extracts were prepared from fresh leaves.

The proportion used was 200 g/L; the materials were first washed with distilled water and then ground in a blender for 3 min. The extract was filtered three times: using a plastic sieve, then through a glass funnel containing sterile gauze and lastly through nitrocellulose membrane with 0.22  $\mu\text{m}$  diameter pores, attached to a syringe. After filtering, the extracts were placed in a sterile dark recipients and kept refrigerated at  $4^\circ\text{C}$  until the tests.

### Plant extract assessment on *C. gloeosporioides* mycelial growth and sporulation

The plant extracts at 20% concentration were placed in PDA culture medium and poured into a previously autoclaved Petri dishes. Five mm diameter discs containing *C. gloeosporioides* structures were transferred to the centre of the Petri dishes and kept at ambient temperature ( $25\pm 2^\circ\text{C}$ ).

Mycelial growth was assessed by measuring the mycelial growth of the colony, establishing a mean of two measurements taken at two diametrically opposite points, until the control treatment had taken the whole of the Petri dish. The inhibition percentage of the mycelial growth (PIC) was determined from the results, according to Edgington et al. (1971):

$$\text{PIC} = (\text{Control Growth} - \text{Treatment Growth}) / \text{Control Growth} \times 100$$

Sporulation was assessed by evaluating the mycelial growth and 10 ml sterilised distilled water were added to each Petri dish. The colonies were scraped using a Drigalski handle to release the conidia, that were counted using a Neubauer chamber.

### Assessment of plant extracts on *C. gloeosporioides* conidia germination

To assess germination inhibition on *C. gloeosporioides* conidia provided by the plant extracts, a  $4 \times 10^5$  conidia.mL<sup>-1</sup> conidia suspension was prepared using a Neubauer chamber. Twenty microliters of the conidia suspension and 20 µL of each extract to be tested were placed on sterilised glass slides. The control consisted of the conidia suspension with no plant extracts. The slides were placed on Petri dishes containing two layers of moist filter paper and kept in BOD at  $25 \pm 1^\circ\text{C}$  for 9 h (Celoto et al., 2008). At the end of this period, a drop of lactophenol was added to interrupt spore germination. The germination percentage was determined by counting 100 spores under a microscope, separating the germinated from non-germinated spores. A spore is considered germinated when it presents a germination tube bigger or equal to its width.

The spore germination inhibition percentage (PIG) was obtained from the results by the following formula:

$$\text{PIG} = (\text{Control growth} - \text{No. of germinated spores the treatment}) / \text{Number of spores germinated in the control} \times 100$$

### *In vivo* assessment of plant extracts for anthracnose control in *H. psittacorum* cv Golden Torch and *H. rostrata*

After collection, the cut helconia were taken to the Plant Pathology Laboratory at the State University of Maranhão and selected with uniform colour and size and no mechanical injury. The flower stems were disinfected by washing with 10% (v/v) sodium hydrochlorite solution for 2 min, then washing in running water. After drying, the stems were submitted to the treatments by pulverisation with the plant extracts at 20% concentration and the addition of Tween 20 (0.02% v/v).

After applying the plant extracts, the plants were kept in a wet chamber for 24 h and then inoculated with *C. gloeosporioides* by placing a disc of pure fungus culture in PDA culture medium on the flower stem lesions and each stem was inoculated at three points. Assessment started three days after inoculation, when the severity was assessed by measuring the lesion size.

The experimental unit consisted of one flower or inflorescence. The assessments were carried out by measuring the mean lesion diameter and establishing the mean of two measurements taken in diametrically opposite positions.

### Statistical analysis

A completely randomised block experimental design was used with 11 treatments and five replications, except for the *C. gloeosporioides* conidia germination assessment, where four replications were used. The data obtained were submitted to analysis of variance and the means compared by the Scott-Knott test at the level of 5%.

## RESULTS AND DISCUSSION

### Effect of plant extracts on *C. gloeosporioides* mycelial growth and sporulation

The result of the analysis of variance indicated differences in the plant extract anti-fungal activity on *C. gloeosporioides* for both mycelial growth and sporulation (Table 1). At the end of the assessments it was observed that the plant extracts tested presented anti-fungal

activity, in greater or lesser intensity, compared to the control, based on the fungus colony diameter.

The garlic extract treatment presented the best result with 50.37% *C. gloeosporioides* mycelial growth inhibition (Table 1), corroborating Venturoso et al. (2011) who assessed the inhibitory effect of plant extracts on plant pathogens and observed that extracts of garlic, clove and cinnamon presented fungitoxic properties and inhibited mycelial growth of the plant pathogens tested (*Aspergillus* species, *Penicillium* species, *Cercospora kikuchii*, *Colletotrichum* species, *Fusarium solan*ie, *Phomopsis* species). Mycelial growth inhibition by natural antimicrobials is due to the presence of bioactive substances found in plants (Chiejina and Ukeh, 2012; Silva et al., 2014).

The potential fungitoxic effect of crude garlic extract on mycelial growth was also observed in fungi that cause anthracnose in strawberry plants (*Colletotrichum acutatum*) (Almeida et al., 2009) and on the causal agent of red rot in sisal (*Aspergillus niger*) (Souza and Soares, 2013). Nascimento et al. (2013) tested aqueous extracts and observed that bitter melon was efficacious in inhibiting mycelial growth of *Cercospora calendulae* at the (10000 mg L<sup>-1</sup>) concentration with 40% inhibition.

Although garlic extract resulted in less mycelial growth compared to the other extracts, when sporulation was observed, the extracts of bitter melon, ginger, mint, neem, eucalyptus, pepper and lemon balm were more efficacious in inhibiting *C. gloeosporioides* spore production (Table 1). However, bitter melon gave the biggest anti-sporulation effect that may have been due to production of bioactive substances such as momordicin, alkaloid, flavonoid, saponin, glycoside, phenol constituents, phenylalanine, arginine, lignan-calceolarioside, and triterpene-momordicine alkaloid zeatin (Martins-Ramos et al., 2010).

When spore formation is more inhibited, the product is more efficient. This is because the spores produce propagules that disseminate and infect the plant.

Several studies have indicated the use of plant extracts for fungal disease control. Simon et al. (2016) studied medicinal plant extracts to control *Diplocarpon rosae* and observed anti-sporulation effect using crude *Equisetum arvense* L. aqueous extract (EBA) and a commercial product based on fermented plant extracts, and further observed protein synthesis in the *D. rosae* mycelium in the treatment with EBAs of *R. officinalis*, *E. arvense*, and *Moringa oleifera*, the commercial plant oil-based product and a citrus matter-based product, respectively. Ferreira et al. (2014) observed an inhibitory of neem seed extract until the third day that reduced sporulation in *C. gloeosporioides* collected from papaya fruits.

### Plant extract effect on *C. gloeosporioides* conidia germination

All the plant extracts tested in the germination experiment

**Table 1.** Effect of different plant extracts on mycelial growth and sporulation of the fungus *Colletotrichum gloeosporioides* after 12 days incubation.

Treatment	Mycelial growth (cm)	PIC%	Sporulation
Control	9.00 <sup>a</sup>	-	7.40 <sup>a</sup>
Lemon gras	8.10 <sup>b</sup>	10.37	7.08 <sup>b</sup>
Neem	8.05 <sup>b</sup>	10.66	6.20 <sup>e</sup>
Cinnamon	8.04 <sup>b</sup>	10.83	7.18 <sup>b</sup>
Lemon balm	8.00 <sup>b</sup>	11.78	6.78 <sup>c</sup>
Bitter melon	7.70 <sup>c</sup>	14.89	5.85 <sup>f</sup>
Mint	7.50 <sup>c</sup>	17.20	6.18 <sup>e</sup>
Eucalyptus	7.40 <sup>c</sup>	17.93	6.45 <sup>d</sup>
Ginger	7.20 <sup>c</sup>	20.23	6.13 <sup>e</sup>
Black pepper	7.07 <sup>c</sup>	21.82	6.53 <sup>d</sup>
Garlic	4.50 <sup>d</sup>	50.37	6.90 <sup>c</sup>
CV %	4.91	-	2.24

Means followed by the same letter do not differ significantly by the Scott- Knott grouping test at the level of 5% probability.

**Table 2.** Effect of different plant extracts on spore germination of the fungus *Colletotrichum gloeosporioides* after 12 days incubation.

Treatment	Germinated spores	PIG %
Control	100 <sup>a</sup>	-
Lemon gras	18.72 <sup>g</sup>	81.28
Neem	24.93 <sup>f</sup>	75.07
Cinnamon	74.50 <sup>b</sup>	25.50
Lemon balm	61.70 <sup>c</sup>	38.30
Bitter melon	11.5 <sup>h</sup>	88.5
Mint	17.63 <sup>g</sup>	82.37
Eucalyptus	40.00 <sup>e</sup>	60.00
Ginger	4.68 <sup>i</sup>	95.32
Black pepper	46.4 <sup>d</sup>	53.6
Garlic	8.55 <sup>h</sup>	91.45

Means followed by the same letter do not differ significantly by the Scott- Knott grouping test at the level of 5% probability.

showed potential for spore germination inhibition (Table 2). However, the ginger plant extract presented the highest spore germination inhibition (95.32%), followed by the extracts of garlic (91.45%), bitter melon (88.5%), mint (82.37%) and lemon grass (81.28%). This inhibition was observed by Amadi et al. (2014) when ginger and guava extracts were used on the sporulation and germination of fungus spores stored in melons and was observed that the sporulation and spore germination were inhibited of *Aspergillus flavus*, *A. niger*, *Rhizopus stolonifer* and *Fusarium*.

Only cinnamon and lemon balm, of the plant extracts tested, presented germination inhibition below 50%.

Brito and Nascimento (2015) obtained similar results

when they studied plant extract fungitoxic potential on *Curvularia eragrostidis* and observed that garlic, ginger, neem and citronela, starting at 25% concentration, presented bigger plant toxic effects in the *in vitro* analysis; reducing mycelial growth and sporulation as well as fungus germination. Marcondes et al. (2014) also observed that 20% garlic extract completely inhibited *C. gloeosporioides* conidia germination and reduced the number and germination of *Fusarium moniliforme* conidia.

The fungitoxicity of garlic extracts on fungus spore germination has been reported in several other studies, that it decrease the germination of sexed spores and conidia of a range of fungi pathogenic to plants (Souza

and Soares, 2013; Wilson et al., 1997).

Lorenzi and Matos (2002) reported that aromatic herbs, such as garlic and ginger, have bacteriocide and fungicide action, because they contain allicin, inulin, gingerol and shogaol in their chemical composition that confer high potential to these plants for control of several pathogens. Morais (2004), observed that 20% garlic aqueous extract concentrations inhibited *Fusarium oxysporum* Schlecht., Emend., Snyder., and Hansen. conidia germination.

Oliveira et al. (2008) reported that the use of neem and garlic plant extracts at different concentrations (20, 30 and 40%) may be control alternatives for *Fusarium gutiforme*. Souza et al. (2007) reported that garlic and lemon grass (*C. citratus* Stapf.) extracts inhibited germination of the fungus *Fusarium proliferatum* (Matsush.), but were more efficient at concentrations above 2.5%. According to the authors, these extracts have inhibitory constituents, thus indicating the possibility of using plant extracts to protect the host and/or eradicate the pathogen.

Conidia germination inhibition and deformities in the germination tubes due to plant extract action have been reported. Bonaldo et al. (2004) assessed autoclaved *Corymbia citriodora* aqueous extract on *Colletotrichum lagenarium* (Pass.) Ellis and Halst. conidia germination and observed that at concentrations of over 5% fresh leaves (p/v), there was 90% germination inhibition and when 25% plant extracts were used the inhibition was 100%.

Deformity has also been reported concerning the germination tube morphology of the germinated conidia, an effect accentuated by increase in concentration. Souza et al. (2007) observed decrease in *F. proliferatum* spore germination due to increased concentration in garlic and lemon grass plant extracts; at 10% concentration there was 90 and 81% inhibition, for the two extracts, respectively. Bonaldo et al. (2007) assessed *C. citriodora* essential oil at different doses (5 to 60 µL), and reported 100% inhibition in apressorium germination and formation in *Colletotrichum sublineolum* for all the doses tested.

The variation in the results obtained when using plant extracts for plant disease control was probably due to the variable quantity and chemical composition of the plant extracts (Silva, 2006).

#### **Assessment of plant extract action on anthracnose control in *H. psitacorum* cv Golden Torche and *H. rostrata***

The results of the *in vivo* test with *C. gloesporioides* in *H. psitacorum* cv. Golden torch showed that all the plant extracts tested were efficient in reducing lesion severity on the inflorescences. The garlic, ginger and eucalyptus extracts gave the best results, significantly reducing

lesion diameter (Figure 1).

The first symptoms started to appear three days after inoculation, the final assessment was made seven days after the first symptoms were manifested and was characterised as circular, dark brown necrotic lesions around the inoculation location (Figure 2).

Disease control using plant extracts has been shown in other pathogen systems. Cinnamon essential oil sprayed on papaya plants maintained low lesion percentage on leaves for up to 14 days after inoculation with *Corynespora cassiicola*; but when it was applied after the start of infection, it could not control the disease (Bitu et al., 2016).

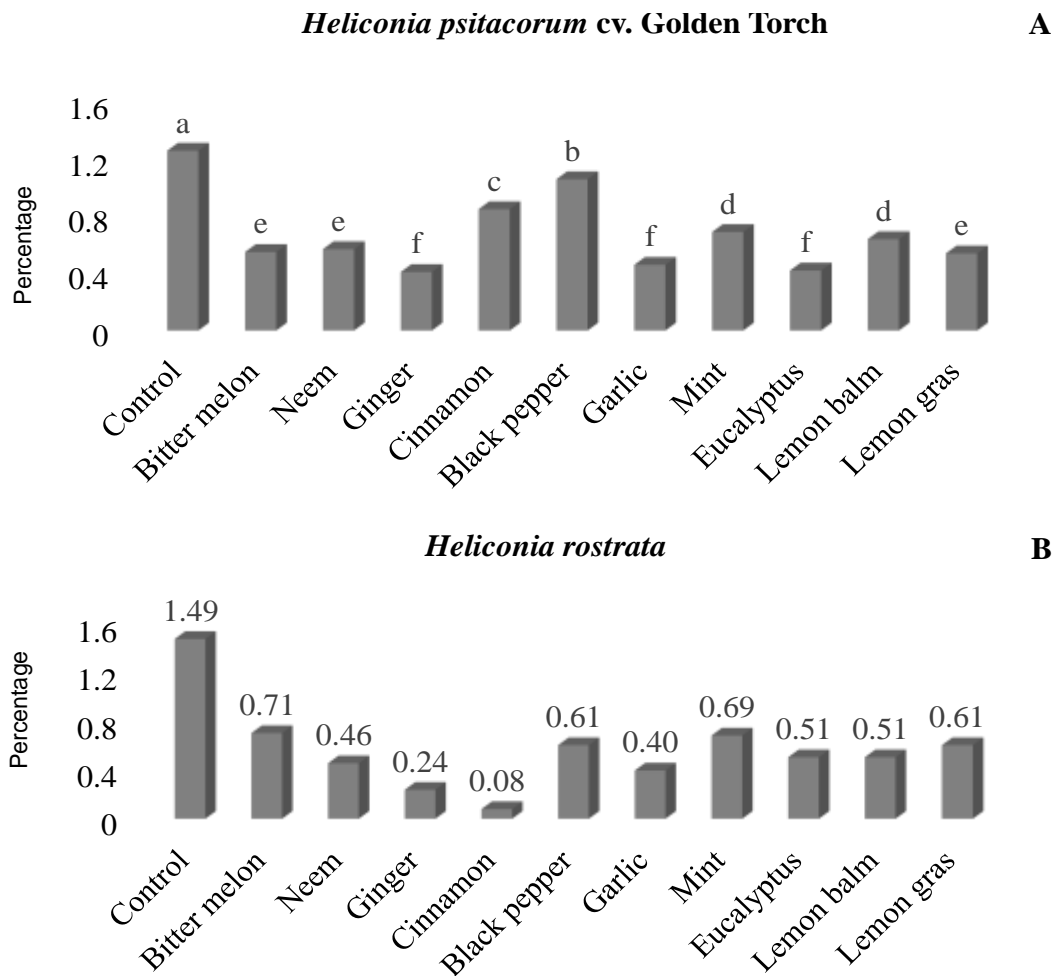
Eucalyptus and citronella essential oils reduced severity of lemon grass rust, but the efficiency of treatments with essential oils was directly related to the environmental conditions and the characteristics of the pathogen system involved (Lorenzetti et al., 2012).

The results of the *in vitro* test with *C. gloesporioides* in *H. rostrata* showed that all the extracts tested were efficient in reducing the lesion severity on the inflorescences (Figure 1), but the cinnamon and ginger extracts gave the best results, significantly reducing the lesion diameter.

Similar results were reported by Itako et al. (2008) who assessed the protective effect of root aqueous extracts (EBAs) of the medicinal plants, *Achillea millefolium* (yarrow), *Artemisia camphorata* (camphor), *C. citratus* (lemon grass) and *Rosmarinus officinalis* (rosemary) against *Alternaria solani* in tomato plants in a greenhouse. A significant reduction was observed in the number of lesions compared to the control and the extracts had a systemic effect.

Other examples have been reported, such as control of brown spot (*Bipolaris sorokiniana*) in wheat, using camphor aqueous extract (*Artemisia camphorata*) (Franzener et al., 2003), tomato plant oidium (*Oidium lycopersici*) by *Azadirachta indica* emulsion oil (Carneiro, 2003), of anthracnose (*C. lagenarium*) in cucumber by *C. citriodora* extract (Bonaldo et al., 2004) and white mold (*Sclerotinia sclerotiorum*) in lettuce by *Z. officinale* (Rodrigues et al., 2007); indicating that plant extracts and vegetable oils are promising alternatives for use in plant disease control.

Plant extracts produce biologically active substances, that influence the metabolism of a determined organism and they act by contact or systemically triggering metabolic pathways. According to Schwan-Estrada and Stangarlin (2005) root plant extracts and/or essential oil actions include direct anti-fungal action, physiological alterations in the plant, or by inducing enzymes related to the pathogenesis, phytoalexins, and leaf lignification. Therefore, fractioning the metabolites of these plants and determining the biological activity of these molecules in relation to the elicitory or antimicrobial activity may contribute to greater understanding that reinforces their possible use as an alternative method for plant disease



**Figure 1.** Effect of the plant extracts on reducing the severity of leaf spots caused by *C. gloesporioides* in inflorescences of *Heliconia psitacorum* cv. Golden Torch (A) and *Heliconia rostrata* (B), 10 days after inoculation. Means followed by the same letter do not differ significantly by the Scott- Knott grouping test at the level of 5% probability.



**Figure 2.** Inflorescences of *Heliconia psitacorum* cv. Golden Torche (A) and *Heliconia rostrata* (B) with anthracnose symptoms.

control (Schwan-Estrada et al., 2000).

## Conclusion

All the extracts presented anti-fungal activity, in greater or lesser intensity, compared to the control. The garlic extract resulted in the highest mycelial growth inhibition rate. The bitter melon, ginger, mint, popcorn eucalyptus and bitter melon extracts were more efficacious, interfering in spore formation; while the ginger extract most reduced spore germination inhibiting germination tube emission. It was observed that all the plant extracts tested were efficient in reducing lesion severity in the inflorescence, showing that the use of plant extracts may be a promising alternative for managing the diseases that affect helconia post-harvest.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## ACKNOWLEDGEMENTS

The authors appreciate the Maranhão Foundation for Support for Research and Scientific Development – FAPEMA for their support.

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