

## Bioactivity of aqueous extracts of *Anadenanthera macrocarpa* to *Meloidogyne incognita* in cotton crop

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### Abstract

The black angico (*Anadenanthera macrocarpa*) contains some phenolic compounds, such as flavonoids and tannins, which are considered as secondary metabolites with antimicrobial properties and can be used in the nematodes management. The experimental design was completely randomized in a factorial (2 x 5) + 1 (leaf and bark x concentrations (20, 40, 60, 80 and 100 g L<sup>-1</sup>) + control water-only), with five replications. The cotton seedlings were inoculated with suspension of 4,400 juveniles and eggs of *M. incognita*. The extracts were applied on soil in a total of 100 mL per pot, in four installments of 25 mL each, in intervals of 15 days. After 60 days of the extracts application, agronomic parameters and parasitism characteristics were evaluated. Plant height and fresh mass of aerial part presented considerable gain with application of leaf extracts. All extracts, regardless of concentration, showed independent suppressive effect to *M. incognita*. The black angico extracts presented potential capacity to control gall nematodes by the presence of metabolite compounds that affect the plants protection. These results reinforce the need of further studies regarding the isolation of chemical compounds with nematicide action.

**Keywords:** Alternative control, *Gossypium hirsutum*, Root-knot nematode, parasitism.

**Abbreviations:** PH\_Plant height; FWAP\_Fresh weight of aerial part; RL\_Redicular length; RV\_Root volume; FWRS\_Fresh weight of radicular system; NG\_Number of galls; JR\_Juveniles in the roots; JS\_juveniles in the soil.

### Introduction

Cotton (*Gossypium hirsutum* L.) represents one of the most important crops in Brazilian agribusiness, with growth of 25.4% of planted area and harvest in 2013/14 compared to previous season (Conab, 2014). However, there are big challenges in the maintenance of crop's technical and economic viability, the presence of phytonematodes that negatively affects plant health and productivity (Ribeiro et al., 2012). In Brazil, the main nematodes that contribute to damage in cotton crop are *Meloidogyne incognita*, *Rotylenchulus reniformis* and *Pratylenchus brachyurus* (Inomoto, 2001). Currently, the cotton-nematode association with species *M. incognita* (Kofoid & White) Chitwood (Mota, 2010) has been observed. This species has greater frequency and economic importance in cotton. The infection of this pathogen occurs on the roots, resulting in epidermal rupture of the xylem and cortical tissue, in response of the development of giant cells and formation of galls (Silva et al., 2011).

In an attempt to reduce the damage caused by nematodes and keeping the biodiversity in the different agro-systems, many forms of management, such as crop rotation, use of resistant genotypes, biological control and chemical products are employed to ensure the production (Santos, 2012). Among these methods, chemical products (nematicides) are

considered as the most important in nematode control, especially for presenting immediate results and avoiding any reduced productivity (Oliveira et al., 2005; Inomoto, 2006). However, the continuous use of nematicides has been causing environmental concerns, since they are mostly chemicals with toxic properties for humans and environment, beside their high costs (Gardiano et al., 2009). Therefore, the need of studies to find alternative control methods it is increasingly noticeable. Some plant extracts may contribute as phytonematode killer to manage damage and dispersion of nematode. They can be used separately or in combination with other traditional management methods.

The use of plant extracts with nematicide or nematostatic represent an economical viable option and with low risk of environmental contamination, due to its biodegradable characteristics (Gardiano et al., 2009). Furthermore, the extracts can come from different parts of plant. Different preparation methods can be used to extract the substances that are toxic to nematodes. The toxic substances are usually plant's secondary metabolism with probable organic nematicide activity (Lopes et al., 2005). A few researches on different plants have already been done, demonstrating nematicide potential of plant extracts. For example, *Chenopodium ambrosioides* L. (Mashela and Nthangeni,

2002); castor bean (*Ricinus communis* L.) (Mello et al., 2006), and *Azadirachta indica* A. Juss (Javed et al., 2008) can produce such as toxic substances. Besides, another plant species exist in the entire northeast region, the *Anadenanthera macrocarpa*, might present nematicide and/or nematostatic potential due to its efficiency in pest control in agronomic crops (Moura et al., 2012) and veterinary, as in ticks *Rhipicephalus (Boophilus) microplus* bovinos (Silva Filho et al., 2013).

This study aimed to evaluate the potential of leaf and bark extracts from *Anadenanthera macrocarpa* plant on management of *M. incognita* nematode in cotton under greenhouse conditions.

## Results and Discussion

### *Agronomic characteristics of soybean in the soils infested with M. incognita*

The variance analysis showed that there was interaction between the extract sources (ES) and the extract concentrations (EC), with a significant effect on plant height (PH), showing an interdependency between the studied factors. (Table 1). For the other variables, the significant effect was observed singly for the extract sources and concentrations, only on fresh weight of aerial part (FWAP).

The cotton plants presented growth after application of the leaf and bark extracts, with a considerable increase for plant height, where both sources adjusted to the quadratic polynomial regression model in response to the used concentration (Fig 1A). For the bark-based extract, the best concentration with the highest effect to gain plant height was 52.48 g L<sup>-1</sup>, which resulted in an increase of 38.38%, compared to control. For the leaf-based extract, the ideal concentration was 70.72 g L<sup>-1</sup> of extract to achieve an effective height gain of 45.56%, when compared to the control. Monteiro et al. (2005), highlight that the amount of compounds present in plant species might vary considerably depending on seasonal effects, region and especially part of the plant, such as seeds, leaves, bark and fruits.

At the same time, a decrease in plant height was noticed at concentrations higher than the ideal (Fig 1A). This inversion between the increase in the concentration and a reduction in plant growth is possibly directly related to the presence of the main compound of this species, the tannin that is considered as one of the most efficient agents with allelopathic effect. Tannin has capacity to act directly on the cytological characteristics, such as the plant hormones, membranes, germination, mineral absorption, respiration, enzyme activity, etc. (King and Ambika, 2002). Silva et al. (2010) also verified the allelopathic effect of tannin, from ethanolic extracts of *A. macrocarpa*, directly influencing the germination quality for crops such as *Brassica chinensis* (Chinese cabbage) and *Lactuca sativa* (lettuce). However, Ferreira and Áquila (2000), verified that tannin might have an allelopathic action, interfering directly with the plant defense system against several pathogenic agents.

For fresh weight of aerial part, there was a significant difference between the extract sources, in which the leaf-based extract provided increase in this variable 25.6% superior to the bark-based extract (Table 1). Nonetheless, plants showed higher gain in fresh weight of aerial part when treated with extracts with 59.63 g L<sup>-1</sup> concentration, reaching an increase of 48.69%, when compared to plants, which did not receive extract concentrations (Fig 1B).

In higher concentrations the same behavior was observed, in which a decrease in plant height was pointed out. The damaging effects and allelopathic activity of allelochemicals like tannins, cyanogenic glucosides, alkaloids, sesquiterpenes and flavonoids in plant development were studied by Alves and Santos (2002). According to King and Ambika (2002), plants susceptible to allelochemicals might suffer retardation in variable ways such as alterations in plant nutritional characteristics and properties, and influencing the activity of organisms that inhabit the soil. Therefore, the oscillation between the allelopathic and antimicrobial effects observed in this study might be directly connected to tannin concentration exposed to the plants in the soil, with reaction power in different phenological phases.

### *Parasitism characteristics of M. incognita in soybean plants*

Among the parasitism characteristics evaluated, there was significant interaction between the extract sources and concentrations only for juveniles living in the root (JR) (Table 2). However, no significant effect was observed for the extract variations in all the variables when evaluated singly. On the other hand, for the extract sources, there was significant difference for juveniles in the soil.

The leaf and bark extracts negatively influenced the number of juveniles on the root, with exponential reduction on the variable's average due to the extract concentrations (Fig 2A). These results show that the smallest bark extract concentration (20 g L<sup>-1</sup>) was efficient in the reduction of 73.86%, compared to the control (0 g L<sup>-1</sup>), reaching 80.96% in the 40 g L<sup>-1</sup> concentration. At concentrations higher than 20 g L<sup>-1</sup> of leaf extract reduction in juvenile number of roots was observed which was superior to the bark extract, with a reduction percentage of 96.52% in the 60 g L<sup>-1</sup> concentration (Fig 2A). The reduction in parasitic activity of *M. incognita* in this study can be explained by the accentuated presence of tannins (15% to 20%) present in the leave and bark of *A. macrocarpa* (Catharino, 2015). This highlights the antimicrobial action over cellular membrane and metabolism modification (Scalbert, 1991).

The *A. macrocarpa* wilted leaves are toxic, and even used as a natural defensive system of plant. However, the principles, which provoke the intoxication is not known (Maïke, 2015). Among all the chemical compounds present in *A. macrocarpa*, concentrated tannin inhibits the hatch and the development of plant nematodes, especially the genus *Meloidogyne* of galls (Maistrello et al., 2010).

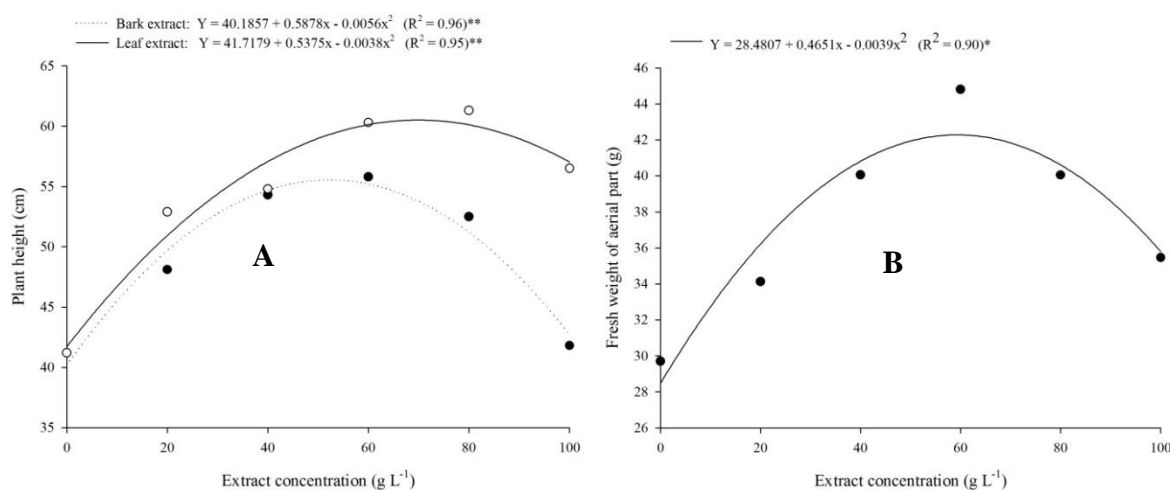
However, there are other plants with great nematicide potential in nematode management in the northeast region. Coimbra et al. (2006), proved the nematicide effect in cassava leaves over the nematode *Scutellonema bradys*, causing the death of 44.0% of the population. This efficiency is due to compounds in the leaves, such as cyanogenic glycoside, especially linamarin, that when hydrolyzed, release cyanide gas, toxic to many microorganisms (Nasu, 2008).

Regarding the concentrations, the smallest number of galls observed in plants was obtained with the extract preparation, using 40 g L<sup>-1</sup>, 77.23% smaller than in plants without extract (0 g L<sup>-1</sup>) (Fig 2B). In this study, the extracts prepared from different plant tissues have demonstrated promising results. Gardiano et al. (2010), observed that *Crotalaria breviflora* and *C. Spectabilis* extracts increased root mass by 38.1% and 56%, respectively, when applied in the nematode infested

**Table 1.** Average values for the agronomic characteristics: plant height (PH), fresh weight of aerial part (FWAP), radicular length (RL), root volume (RV) and fresh weight of radicular system (FWRS) of cotton plants, relating to *A. macrocarpa* aqueous extracts in the management of *M. incognita*.

Source/Variation	Agronomic characteristics				
	PH (cm)	FWAP (g)	RL (cm)	RV (cm <sup>3</sup> )	FWRS (g)
ES	462.03**	1079.33**	6.66 <sup>ns</sup>	2.01 <sup>ns</sup>	5.89 <sup>ns</sup>
Bark	48.95 b	33.12 b	31.16 a	12.70 a	11.20 a
Leaf	54.50 a	41.61 a	30.50 a	13.06 a	11.82 a
EC	387.34**	285.09**	27.97 <sup>ns</sup>	37.37 <sup>ns</sup>	6.67 <sup>ns</sup>
(ES) x (EC)	76.12**	77.73 <sup>ns</sup>	33.29 <sup>ns</sup>	11.69 <sup>ns</sup>	10.78 <sup>ns</sup>
CV (%)	8.45	15.7	18.02	32.98	21.41

\*\*significant to 1%; <sup>ns</sup>non significant; ES - Extract source; EC - Extract concentration; CV – Coefficient of variation. Averages followed by the same letter in the column do not differ significantly from one another by the Scott-Knott test in a 5% probability.



**Fig 1.** Mean values for agronomic characteristics: Plant height (A) and fresh weight of aerial part (B) of cotton plants relating to *A. macrocarpa* aqueous extracts' sources and concentrations in the management of *M. incognita*. \*\* significant at 1%; \* significant at 5%.

soil (*C. mucronata*). These authors, applied aqueous extracts of 20 plant species on *M. javanica* in tomato plants and observed that mint extracts (*Mentha spicata*), *Articum lappa*, and castor bean (*Ricinus communis*) can reduce the number of galls about 75.6%, 65.7% and 54.4%, and the number of eggs in 81.7%, 75.9% and 56.6%, respectively. Aqueous extracts of *Azadirachta indica* and *A. Juss.*, in doses of 1.5% and 3.0% applied on the soil reduced the number of galls and eggs of *M. javanica* (Javed et al., 2008). The promising results showing the efficiency of different extract types over various nematode species strengthens the need for expansion of investigation on the use of extracts as a management alternative.

For the number of juveniles in the soil, a significant difference was observed between the extract sources, highlighting the leaf extract, which presented a reduction percentage of 33.52% higher than the bark extract (Table 2). Regarding the leaf and bark extract concentrations, an exponential reduction of juveniles on the soil was observed, in which at 60 g L<sup>-1</sup>, the percentage was 71.47% lower than the control (Fig 2C)

Since application of the extracts in this research was directly applied on the soil, the compounds present in the *A. macrocarpa*'s leaf and bark possibly acted directly by contact on the nematodes, promoting a decrease in the population. Nonetheless, these possible molecules might infiltrate the soil

quickly or slowly, depending on the soil's physical characteristics. However in this study, we noticed that resistance action by the plants was occurred, which induced maintenance efficiency of the extracts against nematodes.

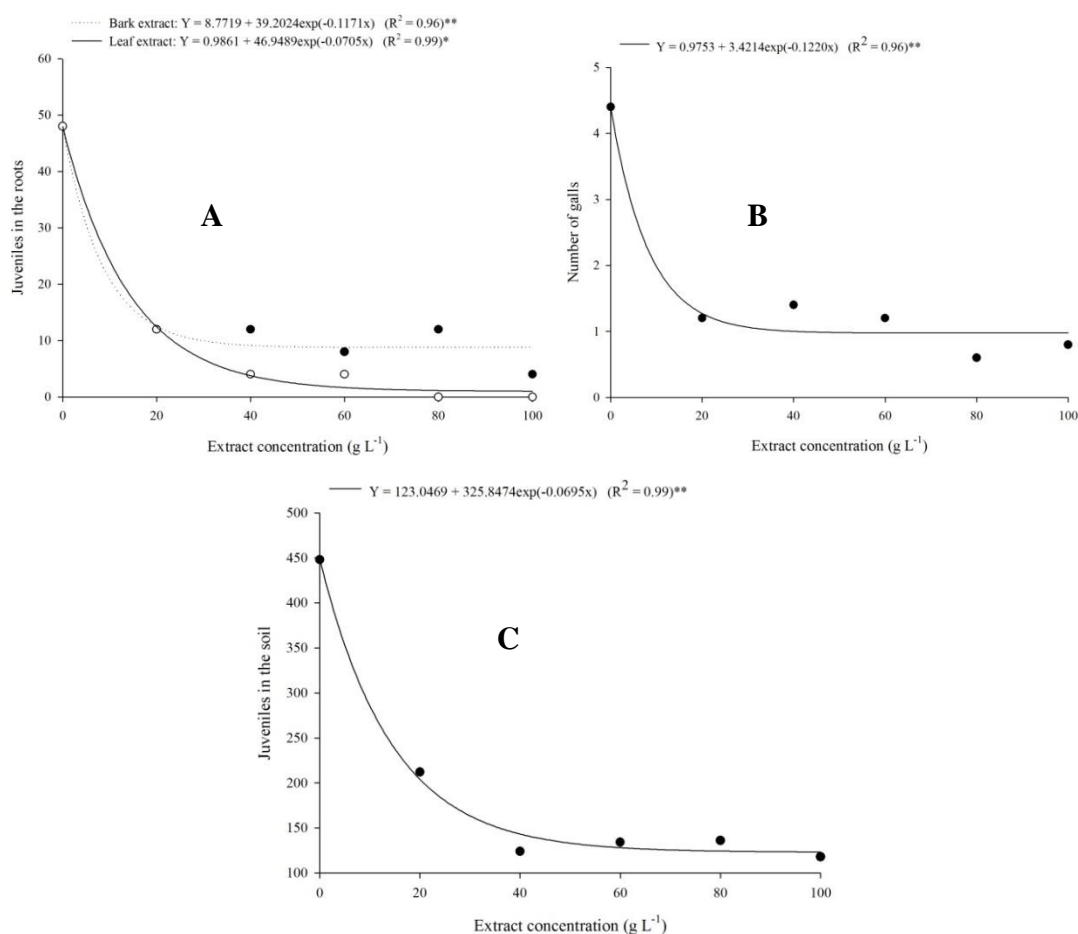
The nematicide potential of extracts mainly depends on the chemical composition of plant material. Other factors such as concentration and application method may give divergent results. Franzener et al. (2007), verified the nematicide effect of aqueous *Tagetes patula* extracts starts at 0.005g mL<sup>-1</sup>, when applied directly on the soil, resulting in a reduction of 61.5% of *M. incognita* juveniles in tomato plant roots. Moreira et al. (2013) observed a great variation in the number of *M. incognita* galls reduction, due to the application method of eugenol extract, with reductions 67.60% higher when applied directly on the soil rather than leaf pulverization, regardless the dosage of the product.

In general, a positive action of the extracts with nematicide behavior is observed, thanks to the presence of metabolite compounds, which enable plant protection. Besides this potential, it reinforces the appeal to society for environmental care, predisposing another option in the plant nematode's pathogenic neutralization. To do so, the realization of new studies in the field is necessary to complement information on the employed extracts, as well as further development of other possible compounds which mitigate the parasitic activity of the nematodes.

**Table 2.** Average values to the parasitism characteristics: number of galls (NG), juveniles in the roots (JR) and juveniles in the soil (JS) of *M. incognita* related to *A. macrocarpa* aqueous extracts.

Source/Variation	Parasitism characteristics		
	NG (unit.root <sup>-1</sup> )	JR (unit.root <sup>-1</sup> )	JS [unit.(100 cm <sup>-3</sup> de soil) <sup>-1</sup> ]
ES	2.40ns	326.66ns	92826.66*
Bark	1.40 a	16.00 a	234.66 a
Leaf	1.80 a	11.33 a	156.00 b
EC	18.68**	2934.66**	164938.66**
(ES) x (EC)	1.12ns	54.66*	8042.66ns
CV (%)	28.9	32.50	34.04

\*significant to 5%; \*\* significant to 1%; <sup>ns</sup>non significant; ES - Extract source; EC - Extract concentration; CV – Coefficient of variation. Averages followed by the same letter in the column do not differ significantly from one another by the Scott-Knott test in a 5% probability.



**Fig 2.** Mean values for parasitism characteristics: Juveniles in the roots (A), number of galls (B) and juveniles in the soils (C) of *M. incognita* relating to the *A. macrocarpa* aqueous extracts' sources and concentrations. \*\* significant at 1%; \* significant at 5%.

## Materials and Methods

### Location and conduction of experiment

The experiment was conducted in a greenhouse in the Laboratório de Fitopatologia, in the Universidade Federal do Piauí, Campus Prof<sup>a</sup>. Cinobelina Elvas, in Bom Jesus-PI, from August to December, 2014. Cotton seedlings were multiplied in expanded polystyrene trays with 128 cells, with a soil-sand-manure based substrate in the proportion of 3:2:1, respectively, sterilized in autoclave at 120°C and pressure of 1.05 Kgcm<sup>-2</sup>, for two hours.

### Inoculation of cotton nematode

The cotton seedlings were transplanted thirteen<sup>th</sup> day after the emergence, where two seedlings per polypropylene vase were kept, with a maximum capacity of 5 dm<sup>3</sup>. The thinning was held ten days after the transplanting, keeping one single plant as the experimental unit. Fourth day after the transplanting, the substrate surface was inoculated with 4,400 juvenile nematodes and nematode eggs, from the species *M. incognita* with the help of a pipette. A 10 mL of inoculum suspension, distributed in three 3.0 cm deep holes, with a 2.0 cm distance from one another and from the cotton hypocotyl.

Previously, the inoculum was obtained from a *M. incognita* soy field population, located in the city of Bom Jesus-PI. The nematode and egg extractions obtained in roots were carried by the Hussey and Barker technique (1973), modified by Boneti and Ferraz (1981). These were isolated and inoculated in tomato plants (*Solanum lycopersicum* L.) cv. "Santa Clara" cultivated in vases and kept in a greenhouse, for 55 days, for proliferation. The prior identification of the species were carried out with temporary slides in formalin, looked through the optical microscope, confronting the characteristics found with those from literature (Handoo and Golden, 1989).

#### **Preparation and application of plant extracts**

After 72 hours of the substrate's infestation with the nematodes, 100 mL of the extracts were applied in each vase, where the application was divided in four portions of 25 mL each, in a 15 days interval.

The plant material (leafs and barks) of *A. macrocarpa* were used because the compounds are distributed distinctly in the plant. The plant species was collected in Bom Jesus-PI, processed in the Phytopathology laboratory through dehydration in room temperature for 10 days, pulverized in mechanic mill, being reduced to powder and stored in a 1000 mL beaker until the preparation of the fractioned aqueous extract.

The extracts were only prepared 24 hours before the treatments application. The leaf and bark powder, in the concentrations of 0, 20, 40, 60, 80 and 100 g L<sup>-1</sup> were submitted to cold extraction, through the adaptation of the methodology used by Ferris and Zheng (1999), where 10 g of the powder (leaf and bark) were mixed separately, for each 100 mL of distilled water, resting for 24 hours. The resulting extractive solution was filtered with gauze and subsequently applied via soil.

The trial design was entirely randomized, in a factorial scheme 2 x 5 + 1, constituted by two extract sources (leaf and bark) of *A. macrocarpa* in five concentrations (0, 20, 40, 60, 80, 100 g L<sup>-1</sup>) plus a control – water, with five repetitions.

#### **Variables evaluated**

The evaluations were performed 60 days after the extracts application by determining agronomic characteristics: Plant height (PH) and radicular length (RL) with a graduated ruler, fresh weight of aerial parts (FWAP), fresh weight in the radicular system (FWRS), obtained with a semi-analytic scale and; root volume (RV), calculated by the difference of water dislocated in a test tube after the roots immersion. A test tube with 1000 mL volume was used and a standard volume of 800 mL was considered.

For the parasite characteristics we measured: number of galls in the roots, estimate number of juveniles in the soil for each treatment, extracted in 100 cm<sup>3</sup> of soil per centrifugation and flotation (Jenkins, 1964) and estimate number of juveniles in the roots (Coolen and D'herde, 1972).

#### **Statistical analysis**

The data were submitted to variance analysis in the statistic software Assisat version 7.7 beta (Silva, 2014). For the diagnostic of the significant effects between the extract sources and concentrations, a "F" test was performed, and the averages of the significant parameters compared by the Scott-Knott test in a 5% probability. The quantitative data (extract concentration) were adjusted by regression equations, with the software SigmaPlot 10.0.

#### **Conclusion**

The fresh weight of aerial part had a greater gain with the plant's leaf extract at the concentration of 59.63 g L<sup>-1</sup>, reaching an increase of 48.69%, compared to control. The best concentrations of leaf and bark extracts on plant height were observed in the concentrations of 52.48 g L<sup>-1</sup> and 70.72 g L<sup>-1</sup>, respectively. The extracts from the bark and the leaf, regardless the concentration, were efficient in the management of the nematode galls (*Meloidogyne incognita*) in cotton plants.

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