Beni-Suef University Journal of Basic and Applied Sciences

RESEARCH

Open Access

Effect of inoculation with arbuscular mycorrhizal fungi on larvicidal activity and phenolic compounds in *Mimosa tenuiflora* cultivated under field conditions

Joao Cleme Ananias de Sousa-Junior[1], Melquisedec de Sousa Oliveira[1*], Carlos Henrique de Araújo Dias[2], Andréia Amariz[1] and Maryluce Albuquerque da Silva Campos[1*]

Abstract

Background *Mimosa tenuiflora* (Willd.) is an essential leguminous tree used in Brazilian northeastern folk medicine, and its extracts have been tested against larvae of *Aedes aegypti*. These tests typically use parts from adult plants collected in natural environments. However, *M. tenuiflora* seedlings can be successfully produced using arbuscular mycorrhizal fungi (AMF) inoculation technology. Previous studies have reported the benefits of inoculation on *M. tenuiflora* growth and the accumulation of secondary metabolites, while data on how inoculation affects the biological activities of extracts remain limited. This work investigated the potential of field inoculation with arbuscular mycorrhizal fungi to enhance *M. tenuiflora* production, focusing on increasing total phenolic levels and improving the larvicidal activity of its extracts against *Ae. aegypti*.

Results Inoculation with *Gigaspora albida* resulted in higher levels of total phenolics, death of larvae, and lower lethal concentration (LC) compared with other treatments. The concentration of phenolics was 249.87 mg. g^{-1}, and the percentage of death after 48 h was 68.33%. At 48 h, the LC_{50} and LC_{90} values were 147 µg. mL^{-1} and 1301.83 µg. mL^{-1} for extracts from plants inoculated with *G. albida*. For the non-inoculated controls, the LC_{50} was 800.67 µg. mL^{-1} and LC_{90} 8194.26 µg. mL^{-1}, while the inoculation with *Claroideoglomus etunicatum* resulted in LC_{50} 1179.16 µg. mL^{-1} and LC_{90} 3050.32 µg. mL^{-1}. No differences were observed in the percentage of larvae mortality between extracts from plants inoculated with *C. etunicatum* and non-inoculated controls. The increased concentration of total phenolics in plants inoculated with *G. albida* might contribute to the observed potent larvicidal activity. Under field conditions, inoculation of *M. tenuiflora* with *G. albida* increases phenolics and larvicidal activity against *Ae. aegypti* L3 larvae, proving more effective than inoculation with *C. etunicatum*.

Conclusions The results herein corroborate mycorrhizal technology for improving biological plant-derived activities, indicating *G. albida* as the best arbuscular mycorrhizal fungus to improve the larvicidal effects of *M. tenuiflora* extracts.

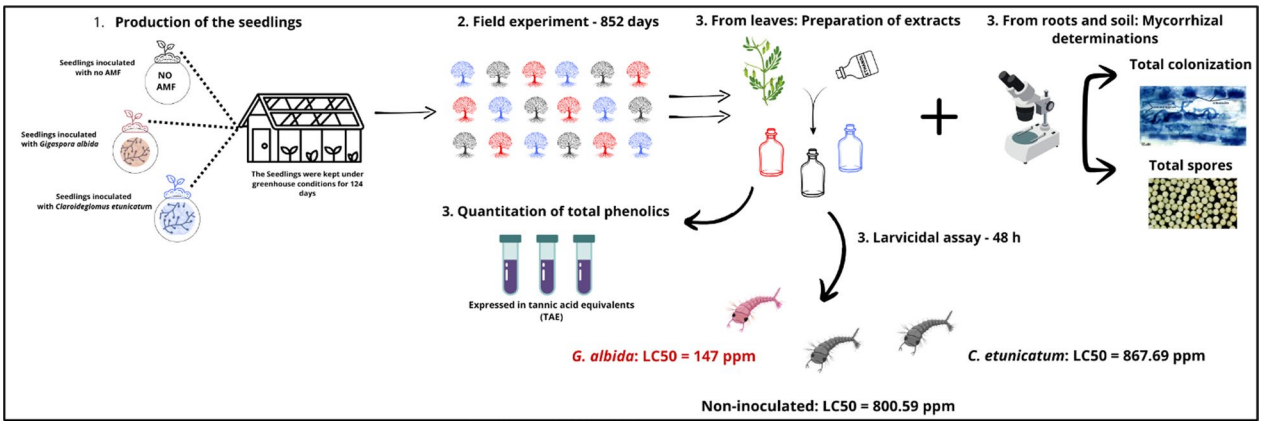
Keywords Microbial biotechnology, Bioprospecting, Fabaceae, Semiarid

*Correspondence:
Melquisedec de Sousa Oliveira
oliveirams@outlook.com
Maryluce Albuquerque da Silva Campos
maryluce.campos@upe.br
Full list of author information is available at the end of the article

Springer Open

© The Author(s) 2025. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

Graphical abstract



1 Background

Caatinga is a semiarid Brazilian biome that harbors many organisms well adapted to harmful conditions such as high temperatures, high UV exposure, and water limitations. Plant species stand out in this biome because they are used as therapeutic resources in folk medicine [1, 2]. Among the roughly 3347 plant species in the *Caatinga*, medicinal plants are of significant interest for bioprospecting and biotechnological applications [1, 3, 4].

Mimosa tenuiflora (Willd.) is a leguminous tree native to the *Caatinga* biome, used as a resource for teas, syrups, and other folk medicines. Ethnopharmacological studies report indications of its leaves and bark for treating diarrhea, wound healing, fever, menstrual colics, bronchitis, and cough [2, 5]. Further investigations showed the presence of phenolic compounds in *M. tenuiflora* tissues, mainly tannins and flavonoids, and these class molecules might be correlated with its biological activities [6–8]. In addition to ethnopharmacology surveys, several authors have reported biological activities such as antimicrobial [9], termiticide [10], anti-inflammatory and antinociceptive [8], and antioxidant [11, 12] from different types of *M. tenuiflora* extracts.

Despite numerous laboratory studies on *Mimosa tenuiflora* extracts, their larvicidal potential has received limited attention. Prospecting for new larvicides is essential, especially in tropical countries, where insects transmit infectious diseases. In South America, dengue is a public health problem affecting millions annually [13]. Dengue is a viral disease transmitted by the mosquito *Aedes aegypti* (L.), which deposits its eggs in water residues, especially during summer and rainy seasons [14, 15]. Different manners to control *Ae. aegypti* might be employed, like larvicides and adulticides. However, resistance has been reported in Brazil [16] and other countries [17, 18]. Thus, the prospect of new larvicidal molecules may contribute to controlling mosquitoes and dengue.

Extracts of *M. tenuiflora* exhibit larvicidal effects; however, there is limited information on their efficacy against *Ae. aegypti*. Aqueous extracts of *M. tenuiflora* lead to the death of *Ae. aegypti*, as reported by Dantas et al. [19]. In addition, in a recent study, Luyen et al. [20] isolated flavonoids from propolis of *M. tenuiflora* and tested them against *Ae. aegypti* and *Aedes albopictus* (Skuse). As a result, larvicidal effects were observed at low concentrations. In contrast, Dos Santos et al. [21] did not find the larvicidal activity of crude extracts against larvae L4.

M. tenuiflora can be cultivated with bioinoculants like arbuscular mycorrhizal fungi (AMF) [22], which are rhizospheric microorganisms that improve plant nutrition and growth [23]. Bonfim [24] inoculated seedlings of *M. tenuiflora* with the AMFs *Claroideoglomus etunicatum* (W.N. Becker & Gerd.) C. Walker & Schüßler and *Gigaspora albida* (N.C. Schenck & G.S. Sm.), registering growth improvements. These results were observed both under greenhouse and field conditions.

In addition, the presence of AMF in the plant root system alters whole plant physiology in many aspects, especially defense responses. As a result, plants inoculated with AMF accumulate significantly more secondary metabolites [25, 26], becoming more resistant to pathogens and plagues [27]. Notwithstanding, extracts from plants inoculated with AMF result in higher activities than control extracts, as Marcolino et al. [28] observed. In such a study, extracts from guava leaves, previously cultivated with AMF, inhibited the growth of resistant *Klebsiella pneumoniae,* effects not observed in the respective controls. Similarly, increases in antioxidant activity

depending on the AMF inoculation were observed in *Cynara cardunculus* (L.). The authors suggested a correlation with phenolics, a variable also improved by the inoculation [29]. Additionally, Giovannetti et al. [30] found that AMF inoculation can increase lycopene levels, resulting in higher antiestrogenic activity. Such results highlight AMF inoculation as a biotechnological strategy for prospecting new molecules and biological activities.

Despite the benefits of AMF on the growth of *M. tenuiflora*, improvement in the production of metabolites was also reported [24]. However, no biological activities with extracts of *M. tenuiflora* inoculated with AMF have been tested. Thus, considering the evidence that AMF technology enhances the synthesis of phenolics and its potential correlation with increased biological activities, this study aimed to evaluate the larvicidal effects of extracts from *M. tenuiflora* inoculated with AMF. Notably, the effects of AMF inoculation to improve larvicidal activity of plant extracts are still under explored.

We evaluated the potential of AMF inoculation to enhance the concentration of phenolic compounds and larvicidal efficacy in foliar extracts of *M. tenuiflora*. Plants were cultivated in a semiarid field environment, and extracts were assayed for activity against *Ae. aegypti* L3 larvae.

2 Methods

2.1 Experimental field of *M. tenuiflora* cultivation

Bonfim [24] previously cultivated seedlings of *M. tenuiflora* under greenhouse conditions in 2018. Seeds of *M. tenuiflora* were surface sterilized with sodium hypochlorite (5 mL. L^{-1}—v/v), and the dormancy breaking was performed by the hot water/cold water method as suggested by Bakke et al. [31]. All seeds were obtained from the natural *Caatinga* area.

Afterward, germination was performed in the sand:vermiculite (1:1) substrate, previously autoclaved (121° C/ 1 h, two times). Plants with two definitive leaves were transplanted to bags of 1,5 L of capacity, containing the substrate composed of sand and vermiculite (1:1) plus vermicompost (50 mL.L^{-1}). AMF inoculation was performed at transplantation by adding soil inoculum (200 glomerospores) of two different fungi: *Claroideoglomus etunicatum* or *Gigaspora albida*. Control plants received mock inoculum (soil without AMF propagules). The AMF was multiplied in a rhizosphere of *Sorghum bicolor* L. and *Zea mays* L., cultivated in the sterile substrate composed of argisol, sand, and vermiculite (1:1:0.5 v/v). Seedlings of *M. tenuiflora* grew under greenhouse for 124 days until transplanting to the field.

In December 2016, the field experiment was set up at the Poço D'água farm, localized in the municipality of Juazeiro, Bahia, Brazil. The area is in the mesoregion of the sub medium São Francisco River Valley. As a semiarid region, the predominant climate is Bsh, based on the Koppen–Geiger classification system (Fig. 1).

Seedlings were transplanted into pits (30 cm × 30 cm × 30 cm) with four meters of space between plants. For the first three months, the plants were drip-irrigated for one hour (flow rate 1.6 L) daily, then rinsed for one hour every other day. After six months, they were rinsed once a week for 1.5 h. Plants were continuously cultivated until the collection of the samples.

2.2 Collection of plant material

Fully developed leaves with no signs of wounds were collected and put in paper bags. Samples of soil and roots from a 20-cm deep layer depth located at the canopy projection were also collected to measure AMF colonization and total spore quantities. The collection of the samples occurred in April 2019, 852 days after transplanting them to fields.

2.3 Assessment of colonization and quantitation of AMF spores

Fine roots were separated from the soil and cleared with potassium hydroxide (100 g. L^{-1}) solution and hydrogen peroxide (30%) overnight. Samples were stained with trypan blue and stored in a lactophenol solution. The total AMF colonization was determined by intersection quadrants described by Giovannetti and Mosse [32]. For total spores quantitation, 50 g of soil samples were submitted to the wet sieving method [33], and the obtained spores were quantified in a stereomicroscope.

2.4 Preparation of extracts and quantification of total phenolics

At 45° C, leaves were oven-dried until constant weight, and dried samples were pulverized in a grinder. In amber flasks, plant metabolites were extracted by maceration with an ethyl alcohol solution (950 mL. L^{-1}). The extraction solution was replaced four times, in intervals of 72 h hours. Then, the final solution was filtered in the qualitative paper, and the solvent was volatilized in the rotated evaporator (low pressure at 50° C). All these procedures resulted in a crude ethanolic extract (CEE), stored at 4 °C prior to larvicidal tests and quantitation of phenolics. Phenolic compounds are the main secondary metabolites of *M. tenuiflora* [34], and therefore, changes in the concentrations of these molecules may affect the larvicidal potency. However, there are currently no reports of these events for *M. tenuiflora*.

Total phenolics were quantified using the Folin–Ciocalteu method described by Georgé et al. [35], using different concentrations of tannic acid as standard (400 µg.

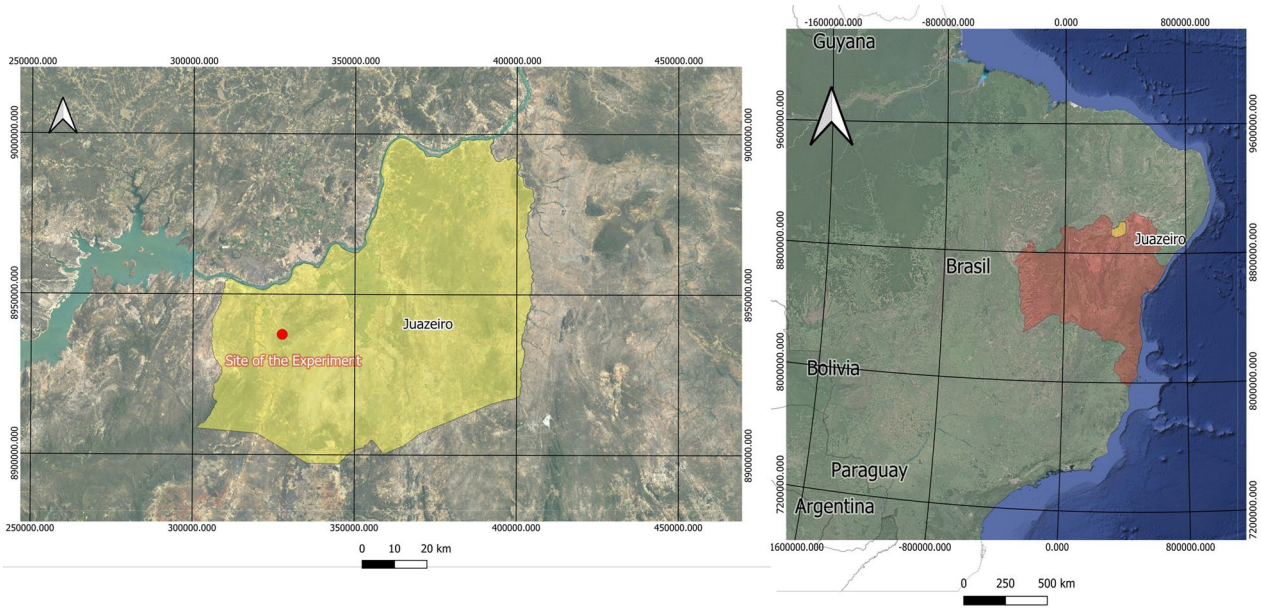


Fig. 1 Localization of the area where the experiment with M. tenuiflora was carried out – 09° 36′ 30.27″S e 040° 34′ 26.70″O

mL^{-1} until 0.75 µg. mL^{-1}). In brief, 500 µL of CEE were mixed with 2.5 mL of Folin–Ciocalteu reagent (100 mL. L^{-1} v/v) and 2 mL of sodium carbonate (75 g. L^{-1} m/v). The resulting mixture was heated to 90 °C for 15 min and thereafter cooled with cold water. The absorbance was measured at a wavelength of 760 nM. The total phenolics were expressed as tannic acid equivalents (TAEs).

2.5 Larvicidal tests against *Ae. aegypti* larvae

Larvicidal tests were performed using pulps of *Ae. aegypti*, strain Rockefeller. Pulps were submitted to different concentrations of crude extracts, as described by the World Health Organization [36].

After hatching, the larvae were transferred to plastic trays and fed 0.04 g of reptile feed (Alcon®) per day. When the larvae reached the L3 development stage, they were transferred to plastic pots (10 per pot) containing 10 mL of a test solution. Concentration of 1500 µg. mL^{-1}, 1200 µg. mL^{-1}, 800 µg. mL^{-1}, and 500 µg. mL^{-1} 200 µg. mL^{-1} of CEE was tested per pot. Distilled water with 1% DMSO was used as a negative control. All larvae were kept at 25 °C, and survival was observed at 24 and 48 h after extracts were added and the death count was performed. Larvae that did not respond to mechanical stimulation with a clamp were considered dead.

2.6 Experimental design and statistical analysis

Data from secondary metabolites and mycorrhizal parameters were analyzed in a completely randomized design, only testing the effects of the inoculation with AMF on the production of total phenolics and larvicidal properties. For secondary metabolites quantitation, three treatments were tested: i. Control with no previous AMF inoculation in the greenhouse and then cultivated in field conditions; ii. Plants previously inoculated with *C. etunicatum* in the greenhouse and then cultivated in field conditions; iii. Plants previously inoculated with *G. albida* in the greenhouse and then cultivated in field conditions in seven repetitions for each treatment.

The data from larvicidal tests were analyzed in a completely randomized design, with two factors: AMF inoculation and concentrations of CEE. The tests were performed for each AMF inoculation treatment as follows: i. Negative control; ii. 1500 µg. mL^{-1} of CEE; iii. 1200 µg. mL^{-1} of CEE, iv. 800 µg. mL^{-1} of CEE, v. 500 µg. mL^{-1} of CEE, and vi. 200 µg. mL^{-1} of CEE, in triplicate. In summary, all larvicidal tests resulted in 56 experimental units.

Data were submitted for analysis of variance (ANOVA), and the means were compared by the Tukey's test ($p < 0.05$). Finney's probit regression analyses were performed using data on larvae death numbers and concentrations of CEE from inoculated and non-inoculated plants. In addition, the Spearman correlation test was performed to assess whether the production of phenolics was associated with larvicidal activity. All analyses were performed using the software STATISTICA 10 and GraphPad Prism 9.

Table 1 Significance levels for study variables considering isolated effects of AMF inoculation (AMF) or concentrations of crude extracts (CEE) or their interactions (AMF x CEE)

Variable	AMF	CEE	AMF x CEE
Root colonization	*	NS	NS
Density of spores	*	NS	NS
Total phenolics	*	NS	NS
% of larvae death – 24 h	*	**	NS
% of larvae death – 48 h	*	**	NS

*Significance by Tukey's test: * $p < 0.05$; ** $p < 0.01$; NS – not significant

Table 2 The density of spores and percentage of mycorrhizal colonization in *M. tenuiflora* plants inoculated or not with AMF and cultivated in field conditions for 852 days

	Density of spores (50 g^{-1} soil)	Mycorrhizal colonization (%)
Non-inoculated control	40 b	16 a
C. etunicatum	87 ab	13 a
G. albida	99 a	12 a

Means followed by the same letter did not differ by the Tukey's test ($p < 0.05$)

3 Results

In general, inoculation with AMF resulted in high levels of root colonization and spore density. Similarly, AMF improved the concentration of total phenolics and the death rate of larvae; however, responses varied according to the AMF tested. As expected, increases in the concentration of CEE led to progressive death rates. No interaction was observed between tested factors (Table 1).

3.1 Mycorrhizal colonization and spore density

Previous inoculation of seedlings did not increase the colonization rate in adult plants. All plants had similar levels of AMF propagules in the root system, which were also observed even in non-inoculated controls (Table 2). On the other hand, treatments previously inoculated with *G. albida* had significantly higher spore density than the non-inoculated control and *C. etunicatum*, which did not differ (Table 2).

3.2 Concentration of total phenolics

Only the presence of *G. albida* increased the foliar concentration of total phenolics. Inoculation with *C. etunicatum* did not increase total phenolics, showing similar values to non-inoculated controls (Fig. 2). The concentration of total phenolics per gram of CEE was 249.87 mg. g^{-1} in *G. albida* treatments, 191.64 mg. g^{-1} in *C. etunicatum*, and 171.48 mg. g^{-1} in non-inoculated controls.

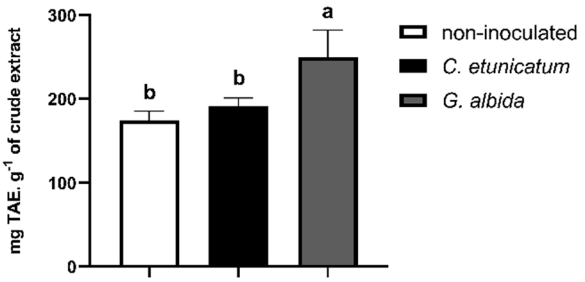


Fig. 2 Foliar concentration of total phenolics (expressed as TAE) in adult plants of M. tenuiflora, inoculated or not with different AMFs, and cultivated for 852 days under field conditions expressed in tannic acid equivalents. Means followed by the same letter did not differ by the Tukey's test ($p < 0.05$)

Table 3 The total number of larvae killed per pot by extracts from *M. tenuiflora* cultivated or not with AMF, along 852 days under field conditions, independently of CEE tested

Inoculation treatment		
	24 h	48 h
Non-inoculated control	2.33 b	3.83 b
C. etunicatum	2.91 b	3.67 b
G. albida	4.66 a	6.83 a

Means followed by the same letter did not differ by the Tukey's test ($p < 0.05$)

3.3 Larvicidal effect against *Ae. aegypti*

Extracts from plants inoculated with *G. albida* resulted in a higher death percentage of larvae, with responses varying according to time (Table 3, Fig. 3). After 24 h, extracts from plants inoculated with *G. albida* killed significantly more larvae than those inoculated with *C. etunicatum* and non-inoculated control. Similar results were observed after 48 h (Table 3). At 24 h, extracts from plants inoculated with *G. albida* killed 46.67% of the larvae, while extracts from plants inoculated with *C. etunicatum* and non-inoculated killed 29.17% and 23.33%, respectively. On the other hand, at 48 h, *G. albida* extracts killed 68.33%, C. *etunicatum* extracts killed 36.67, and non-inoculated control extracts killed 38.33% (Fig. 3).

As expected, progressive concentrations of CEE led to higher death percentages. The highest rate was observed after the addition of 1500 µg. mL^{-1}, both for 24 h and 48 h (Table 4 and Fig. 4). The lowest larvicidal effect was registered for 200 µg mL^{-1} at 24 h and 48 h. In addition,

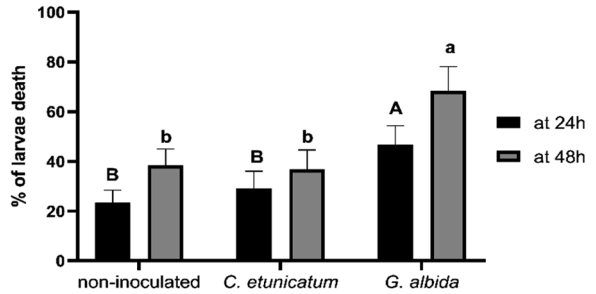


Fig. 3 Percentage of larvae death due to addition of plant extracts, inoculated or not with different AMFs, and cultivated under field conditions for 852 days, independently of tested dose. White bars represent the larvicidal effects 24 h after the addition extract, while black bars represent the larvicidal effect after 48 h. Uppercase letters represent comparison among treatments at 24 h, and lowercase letters at 48 h. Means followed by the same letter did not differ by the Tukey's test ($p < 0.05$)

Table 4 The total number of larvae killed per pot by different CEEs from *M. tenuiflora* cultivated along 852 days under field conditions, independently of mycorrhizal inoculation

CEE treatments		
	24 h	48 h
	0 e	0 c
200 µg. mL^{-1}	1.83 d	3 b
500 µg. mL^{-1}	3 cd	5.5 a
800 µg. mL^{-1}	3.66 bc	5.66 a
1200 µg. mL^{-1}	5 ab	7 a
1500 µg. mL^{-1}	6.33 a	7.5 a

Means followed by the same letter did not differ by Tukey's test ($p < 0.05$)

all larvae survived in negative control (Fig. 4). Spearman's rank correlation analysis revealed a strong positive correlation between the concentration of phenolic compounds and the percentage of larvae mortality at both 24- and 48-h post-exposure (Table 5). This indicates that higher phenolic concentrations were associated with increased larvicidal activity.

Probit regression analysis revealed significantly lower lethal concentrations (LC_{50} and LC_{90}) for extracts from *G. albida*-inoculated plants compared to controls, particularly at 48 h. Specifically, at 24 h, the LC_{50} and LC_{90} were 394.81 and 4643.97, respectively, while at 48 h, these values decreased to 147 and 1301.83 (Fig. 5).

At 24 h, the LC_{50} for the non-inoculated control extracts was 1585.05, while the extracts from *C. etunicatum*-inoculated plants exhibited a lower LC_{50} of 1170.16. Similarly, the LC_{90} at 24 h was 5841.72 for the non-inoculated control and 7271.93 for the *C. etunicatum*-inoculated plants. After 48 h, the LC_{50} for the non-inoculated control decreased to 800.59, and for the *C. etunicatum*-inoculated plants, it increased slightly to 867.69. The LC_{90} at 48 h showed a substantial increase to 8194.26 for the control, whereas it decreased to 3050.32 for the *C. etunicatum*-inoculated plants (Fig. 5).

4 Discussion

4.1 Mycorrhizal colonization and spore density

AMF propagules were found in all plants, even in non-inoculated control, showing the infective capacity of exotic and autochthone fungi to colonize *M. tenuiflora* (Table 2). In natural *Caatinga* areas, autochthone AMF are present and infective, as de Souza et al. [37] reported. The researchers found mycorrhizal colonization ranging from 6.3% to 36.5%. The most common AMF species were *G. albida*, *Gigaspora descipiens*, *C. etunicatum,* and *Rhizoglomus clarum*, while *Acaulospora denticulata*, *Acaulospora tubercutata*, and *Entrophospora infrequens* were found more rarely. In our results, all plants had colonization percentages ranging from 12 to 16%, corroborating previous results [37]. Interestingly, the AMF tested

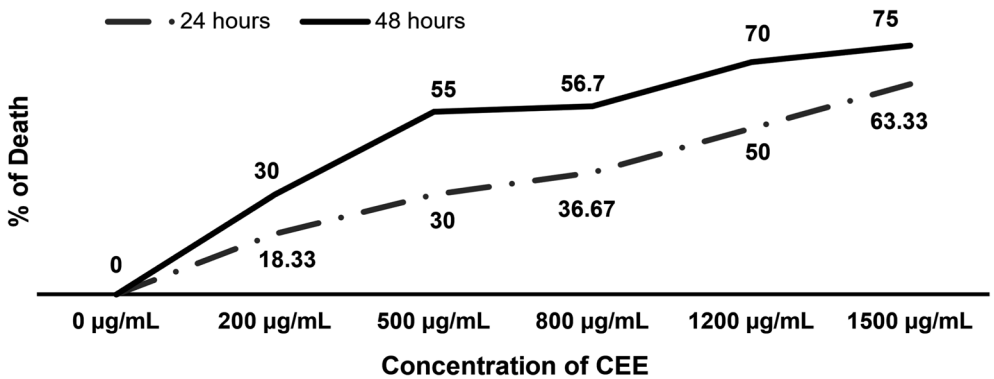


Fig. 4 Percentage of larvae death after the addition different concentrations of crude extracts from M. tenuiflora cultivated 852 days under field conditions, independent of inoculation treatments

Table 5 Correlation between larvae death and concentration of phenolics present in the crude ethanolic extracts from leaves of *M. tenuiflora*

	Concentration of phenolics in CEE	% of larvae death at 48 h	% of larvae death at 24 h
Concentration of phenolics in CEE	—		
% of larvae death at 48 h	0.75***	—	
% of larvae death at 24 h	0.88***	0.87***	—

***($p < 0.01$) – Spearman's rank correlation coefficient

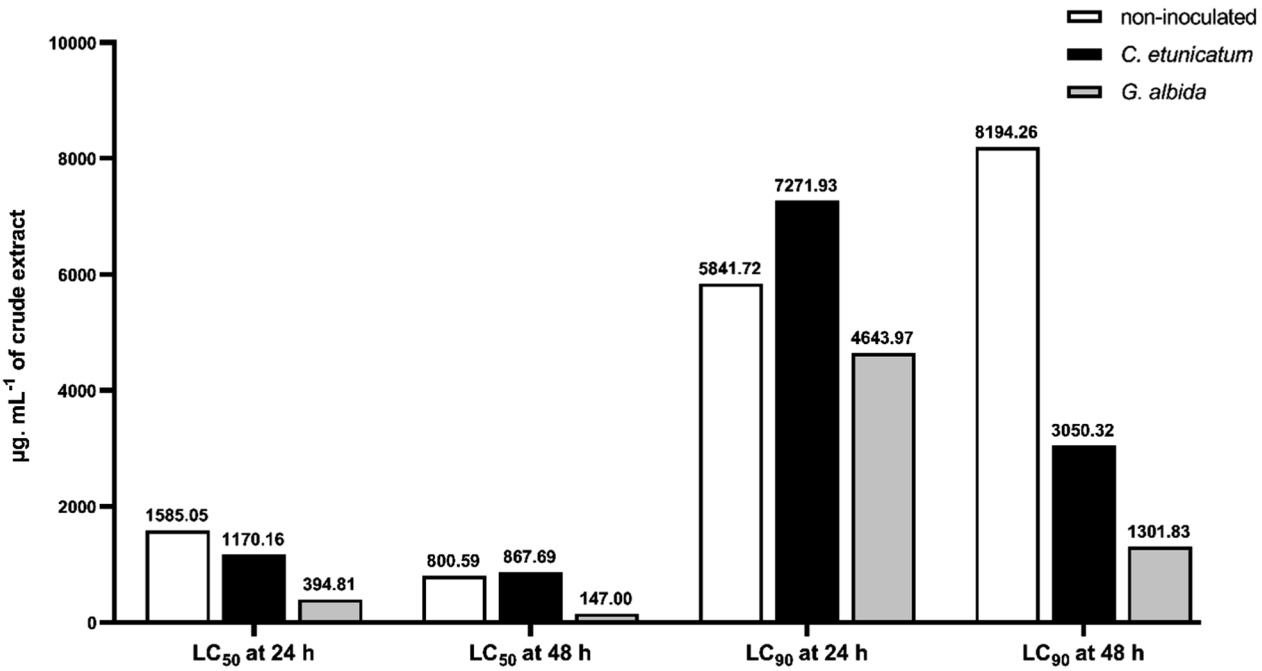


Fig. 5 LC_{50} and LC_{90} against Ae. aegypti larvae due to the addition of crescent concentrations of extracts from M. tenuiflora, inoculated or not with different AMF, and cultivated under field conditions for 842 days. LC 50% and LC 90% were determined by probit regression model. The lower values represent a more potent larvicidal effect

in this study (*G. albida* and *C. etunicatum*) are commonly associated with *M. tenuiflora*, which may explain the similar colonization levels observed in all plants. Bonfim [24] started the field experiment with *M. tenuiflora*, used in our survey, and after 90 days of transplantation, no differences were observed in mycorrhizal colonization, independent of AMF inoculation.

On the other hand, the exotic inoculum of *G. albida* produced more spores than the autochthone AMF, in contrast with data described by Bonfim [24]. It is noteworthy that plant, edaphic, and fungal factors determine the outcomes of mycorrhizal inoculation [38], and that the number of spores may indicate the fitness of the AMF. Additionally, significant compatibility between AMF and the host plant is associated with a high number of spores in native *Caatinga* plants [39]. Thus, considering that *C. etunicatum* and *G. albida* are common AMFs in *M. tenuiflora* rhizosphere, major compatibility probably exists toward *G. albida* despite the similar levels of colonization rate. In addition, AMF spores might be an adequate variable to measure AMF activity over time in the *Caatinga* biome [40].

4.2 Concentration of total phenolics

Despite similar colonization levels among treatments, the inoculation with *G. albida* increased the concentration of total phenolics (Fig. 2). The benefits of AMF inoculation in plants from *Caatinga* are far from only growth variables, and are also related to the synthesis of diverse

metabolites [41]. Our results agree with Bonfim [24], which observed higher concentrations of phenolics in plants inoculated with *G. albida* after 90 days of transplantation. In contrast, after 180 days, no significant differences were observed. Thus, our results confirm the long-term effects of *G. albida* inoculation on total phenolic concentration after 852 days.

A contributing mechanism by which AMF inoculation enhances secondary metabolite production is through the improvement of the host plant's nutritional status. The higher availability of macronutrients leads to increments in biomass yields and biomolecule synthesis, an explanation used by several authors who tested AMF in *Caatinga* plants. Inoculation with AMF increased biomass and phenolic content in *Myracrodroun urundeuva* [25], *Anadenanthera colubrina* [42], and *Inga vera* [43].

On the other hand, the AMF triggers defense responses in plants, resulting in a major concentration of phenolics as a defense compound [44], and these outcomes are not always due to nutritional benefits [45]. The colonization by AMF alters the gene expression in aerial parts, leading to changes in defense-related genes, such as phenylalanine ammonia-lyase and dihydro flavonol-4-reductase, at the transcriptional level [44]. Although we did not determine gene expression, it is possible to speculate that the influence on defense-related gene expression depends on AMF species, as suggested by Feddermann et al. [46], and may explain significant phenolics concentration due to inoculation with *G. albida* and not by inoculation with *C. etunicatum* (Fig. 2).

4.3 Larvicidal effect against *Ae. aegypti*

Due to the biotechnological potential of *Caatinga* biodiversity, many authors have investigated the larvicidal activity of plant extracts from this Brazilian biome. Extracts from native species such as *Amburana cearensis*, *M. urundeuva*, *A. colubrina*, *Caesalpinia ferrea*, and *Dioclea grandiflora* exhibit larvicidal activity against *Ae. aegypti*. Nevertheless, the plant material from all these plants originated from a natural *Caatinga* environment, without prior inoculation with arbuscular mycorrhizal fungi [47]. In relation to *M. tenuiflora*, three publications exist, and all tested extracts from plants without AMF inoculation. Thus, our findings show that for the first time, AMF inoculation increased the potency of extracts against *Ae. aegypti* (Fig. 5).

So, due to these important differences among the source of plant material, comparisons with other studies might be made with parsimony, and must focus on AMF outcomes. The available studies on the larvicidal activity of *M. tenuiflora* have employed varying methodologies for extract standardization, including differences in plant source, concentration, percentage, and molecular characterization [19–21]. Dos Santos et al. [21] did not observe the larvicidal effect of *M. tenuiflora* ethanolic extracts, even testing 500 $\mu g.mL^{-1}$. In contrast, extracts from other plant species and tested at 500 $\mu g.mL^{-1}$ killed *Ae. aegypti* larvae.

Biological activity results depend on the methodology employed to produce the extract, and factors such as plant organs and solvents play crucial roles [48]. While Dos Santos et al. [21] used a mix of leaves, stem bark, and roots, we only used leaves. Thus, it is reasonable to infer qualitative and quantitative differences in the metabolites obtained between the studies. However, the lack of minimal metabolite characterization by [21] makes comparisons with our findings invalid. In another study, twenty percent of foliar aqueous extract of *M. tenuiflora* killed *Ae. aegypti* larvae, representing LC_{50} [19], confirming our larvicidal findings. Unfortunately, the authors did not measure qualitatively or quantitatively any class of secondary metabolites.

Despite the well-known effects of AMF inoculation on biomolecules concentrations [41, 49], information about the outcomes on biological related activities still scarce. Indeed, the correlation between the concentration of molecules from extracts and biological activities is complex. In this context, some inoculated plants show higher secondary metabolite concentrations without changes in biological activity, while others exhibit increased biological activity despite no change in secondary metabolite concentrations. For instance, the inoculation of *Commiphora leptophoelos* seedlings with *G. albida* increased the concentration of total phenolics and total tannins. However, no changes were found in antioxidant activity by the DPPH method [50]. On the contrary, the co-inoculation of *A. longula* and the nematode *Meloigyne enterolobi* did not alter the foliar concentration or content of total phenolics, flavonoids and tannins in guava seedlings; however, it increased antimicrobial activity against a multirresistant *K. pneumoniae* [28].

In our findings, the extracts from *G. albida*-inoculated plants exhibited higher larvicidal activity and concentrations of total phenolics (Table 5; Figs. 2 and 5). In absolute values, inoculation with *G. albida* resulted in 1.3-fold increases in total phenolic content compared to non-inoculated control and 1.43-fold than inoculation with *C. etunicatum*. Considering the LC_{50}, *G. albida* improved 5.44-fold the larvicidal activity relative to non-inoculated controls (Fig. 5). Despite the findings on total phenolics, it is possible that *G. albida* increased the accumulation of specific potent compounds, and to validate such hypothesis, it is necessary complementary experiments. It is interesting to note that AMF inoculation increases the synthesis of particular molecules, which are not detected in respective controls,

as found by [51, 52]. These authors identified specific flavonoids exclusively due to mycorrhizal inoculation in the roots of *Trifolium repens*, and in the roots, and aerial parts of *Lolium multiflorum*.

Isolated polyphenolics have a potent larvicidal effect against *Ae. aegypti*. Six flavonoids obtained from green propolis of *M. tenuiflora* resulted in LC_{50} ranging between 21.11 ppm to 42.98 ppm, 48 h after adding extracts [20]. These findings demonstrate that isolated molecules from *M. tenuiflora* exhibit greater potency compared to crude extracts. Moreover, isolated molecules allow the researchers to investigate the mechanism of larvicidal activity. In the context of larvicidal activity against *Ae. aegypti*, docking studies reveal the involvement of phenolics playing a role as glutathione S-transferase and acetylcholinesterase inhibitors [53, 54] and hemocyte necrotizing [3, 4]

5 Conclusions

Mycorrhizal inoculation alters the concentration of phenolics in adult plants of *M. tenuiflora* and larvicidal activity against *Ae. aegypti*, with responses varying according to fungus. The inoculation with *G. albida* increases the potency of the larvicidal activity of an extract from *M. tenuiflora*. So, we suggest arbuscular mycorrhizal inoculation as an important tool in bioprospecting biological activities and molecules since these microorganisms led to outcomes not observed in uninoculated plants.

However, this study represents the initial step in prospecting for new larvicides, and the bioassays were conducted solely under laboratory conditions. Further studies aimed at better characterizing *G. albida* plant-derived extracts using chromatography, mass spectroscopy, and magnetic resonance will contribute to identifying new molecules and elucidating the mechanisms responsible for enhanced larvicidal activity. In addition, testing these extracts or derived molecules under field conditions, as World Health Organization preconizes [36], is necessary.

Acknowledgements
We wish to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—CAPES for providing a Master's scholarship to Joao Cleme Ananias de Sousa-Junior and a Postdoctoral scholarship to Melquisedec de Sousa Oliveira, and Valdirene Leite Pedone Bonfim for providing the area for the mycorrhizal field experiment.

Author contribution
JCASJ and CHAD performed the larvicidal tests; MSO performed the phytochemical determinations and statistics; MSO and JCASJ wrote the first version of the manuscript; MSO created and edited all figures and tables; MASC and AA supervised all phases and analysis; all authors reviewed the manuscript and contributed to discussions.

Funding
No funding was obtained for this study.

Data availability
No datasets were generated or analyzed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Universidade de Pernambuco, Petrolina, Brazil. [2]Universidade Federal Do Vale Do São Francisco, Petrolina, Brazil.

Received: 4 October 2024 Accepted: 26 March 2025
Published online: 28 April 2025

References

1. Albuquerque UP, Brito AL, Nascimento ALB, Oliveira AFM, Quixabeira CMT, Dias D de Q, Lira EC, Silva FS, Delmondes G de A, Coutinho HDM, Barbosa MO, Landell MF, Alves RRN, Júnior WSF (2020) Medicinal plants and animals of an important seasonal dry forest in Brazil. Ethnobiol. Conserv. 9.
2. de Albuquerque UP, de Medeiros PM, de Almeida ALS, Monteiro JM, de Freitas Lins Neto EM, de Melo JG, dos Santos JP (2007) Medicinal plants of the *Caatinga* (semi-arid) vegetation of NE Brazil: a quantitative approach. J Ethnopharmacol 114:325–354. https://doi.org/10.1016/j.jep.2007.08.017
3. Fernandes DA, Oliveira LHG, Rique HL, de Souza M, F.V. de, Nunes, F. da C., (2020) Insights on the larvicidal mechanism of action of fractions and compounds from aerial parts of *Helicteres velutina* K. Schum against Aedes aegypti L Molecules 25:3015. https://doi.org/10.3390/molecules25133015
4. Fernandes MF, Cardoso D, de Queiroz LP (2020) An updated plant checklist of the Brazilian Caatinga seasonally dry forests and woodlands reveals high species richness and endemism. J Arid Environ 174:104079. https://doi.org/10.1016/j.jaridenv.2019.104079
5. Nunes EN, Ribeiro JE da S, Souza RS, da Cruz DD, de Lucena RFP (2023) *Mimosa tenuiflora* (Willd.) Poir.Fabaceae, in: Farias Paiva de Lucena R, Dias da Cruz D (Eds.), Ethnobotany of the Mountain Regions of Brazil. Springer International Publishing, Cham, pp. 523–530. https://doi.org/10.1007/978-3-030-87251-9_66
6. de Azevêdo TKB, Paes JB, Calegari L, Santana GM (2017) Teor de Taninos Condensados Presente na Casca de Jurema-Preta (*Mimosa tenuiflora*) em Função das Fenofases. Floresta E Ambiente. https://doi.org/10.1590/2179-8087.026613
7. Crepaldi AL, da Bispo ASR, Cruz DCB, Tavechio WLG, Araújo FM, Rocha TVS, Andrade JP, Evangelista-Barreto NS (2022) Triagem fitoquimica, toxicidade e atividade antimicrobiana de diferentes extratos de *Mimosa tenuiflora* sobre cepas de *Aeromonas*. Agrár, Semina Ciênc. https://doi.org/10.5433/1679-0359.2022v43n2p641
8. Cruz MP, Andrade CMF, Silva KO, de Souza EP, Yatsuda R, Marques LM, David JP, David JM, Napimoga MH, Clemente-Napimoga JT (2016) Antinoceptive and anti-inflammatory activities of the ethanolic extract, fractions and flavones isolated from mimosa tenuiflora (Willd.) Poir (Leguminosae). PLoS ONE 11:e0150839. https://doi.org/10.1371/journal.pone.0150839
9. de Souza Araújo E, Pimenta AS, Feijó FMC, Castro RVO, Fasciotti M, Monteiro TVC, de Lima KMG (2018) Antibacterial and antifungal activities of pyroligneous acid from wood of *Eucalyptus urograndis* and *Mimosa tenuiflora*. J Appl Microbiol 124:85–96. https://doi.org/10.1111/jam.13626
10. Cruz CS, Medeiros MB de, Gomes JP, Sousa FC de (2012) Uso de plantas em pó seco com propriedades termiticida sobre a mortalidade de cupins arbóreos. Rev Verde Agroecol E Desenvolv. Sustentável 7.

11. Nascimento MS, Paiva-Souza IO, Fernandes XA, da Moraes SZC, de AraÃojo SS, Shan AYKV, Camargo EA, Santana AEG, AraÃojo BS, Estevam CS (2016) Anti-inflammatory and antioxidant activities of the hydroethanol extract and fractions of the bark of *Mimosa tenuiflora* (Willd.) Poir. Afr J Pharm Pharmacol 10:823–831. https://doi.org/10.5897/AJPP2016.4648
12. de Silva SANM, Barros AB, Souza JMT, Moura AF, de Araújo AR, Mendes MGA, Daboit TC, da Silva DA, Araújo AJ, Marinho Filho JDB (2020) Phytochemical and biological prospection of *Mimosa* genus plants extracts from Brazilian northeast. Phytochem Lett 39:173–181. https://doi.org/10.1016/j.phytol.2020.08.010
13. PAHO, Pan American Health Organization, 2023. As dengue cases increase globally, vector control, community engagement key to prevent spread of the disease [WWW Document]. Dengue Cases Increase Glob. Vector Control Community Engagem. Key Prev. Spread Dis. URL https://www.paho.org/en/news/3-8-2023-dengue-cases-increase-globally-vector-control-community-engagement-key-prevent-spread (accessed 8.1.24).
14. Facchinelli L, Badolo A, McCall PJ (2023) Biology and behaviour of *Aedes aegypti* in the human environment: opportunities for vector control of arbovirus transmission. Viruses 15:636. https://doi.org/10.3390/v15030636
15. Wai KT, Arunachalam N, Tana S, Espino F, Kittayapong P, Abeyewickreme W, Hapangama D, Tyagi BK, Htun PT, Koyadun S, Kroeger A, Sommerfeld J, Petzold M (2012) Estimating dengue vector abundance in the wet and dry season: implications for targeted vector control in urban and peri-urban Asia. Pathog Glob Health 106:436–445. https://doi.org/10.1179/2047773212Y.0000000063
16. de Sá ELR, de Rodovalho CM, de Sousa NPR, de Sá ILR, Bellinato DF, dos Dias LS, da Silva LC, Martins AJ, Lima JBP (2019) Evaluation of insecticide resistance in *Aedes aegypti* populations connected by roads and rivers: the case of Tocantins state in Brazil. Mem Inst Oswaldo Cruz 114:e180318. https://doi.org/10.1590/0074-02760180318
17. WHO, 2023. Report on insecticide resistance in *Aedes* mosquitoes in WHO South-East Asia Region countries.
18. Yang F, Schildhauer S, Billeter SA, Hardstone Yoshimizu M, Payne R, Pakingan MJ, Metzger ME, Liebman KA, Hu R, Kramer V, Padgett KA (2020) Insecticide resistance status of *Aedes aegypti* (Diptera: Culicidae) in california by biochemical assays. J Med Entomol 57:1176–1183. https://doi.org/10.1093/jme/tjaa031
19. Dantas JO, Araújo-Piovezan TG, Santos DP, Alves AEO, Pinheiro SSC, Ribeiro GT (2019) Extracts of potential plants in the control of the *Aedes aegypti* population. Ens E Ciênc Ciênc Biológicas Agrár E Saúde 23:104–108. https://doi.org/10.17921/1415-6938.2019v23n2p104-108
20. Luyen ND, Hao NT, Bastos JK, Ha NX, Hung NH, Gioi DH, Linh NN, Son NT (2024) Flavonoids from *Mimosa tenuiflora* Green Propolis: Chemotaxonomy, Antioxidant, Anti-Inflammation, Anti-Bacteria, Mosquito Larvicidal Activity, and In Silico Approach. Chemistry Select 9:e202305216. https://doi.org/10.1002/slct.202305216
21. dos Santos EA, de Carvalho CM, Costa ALS, Conceição AS, de Moura FBP, Santana AEG (2012) Bioactivity Evaluation of Plant Extracts Used in Indigenous Medicine against the Snail, *Biomphalaria glabrata*, and the Larvae of *Aedes aegypti*. Evid. Based Complement. Altern. Med. ECAM 2012:846583. https://doi.org/10.1155/2012/846583
22. da Silva FA, da Silva FSB (2017) Is the application of arbuscular mycorrhizal fungi an alternative to increase foliar phenolic compounds in seedlings of *Mimosa tenuiflora* (Wild.) Poir., Mimosoideae? Braz J Bot 40:361–365. https://doi.org/10.1007/s40415-016-0320-9
23. Berruti A, Lumini E, Balestrini R, Bianciotto V (2016) Arbuscular Mycorrhizal Fungi as Natural Biofertilizers: Let's Benefit from Past Successes. Front Microbiol. https://doi.org/10.3389/fmicb.2015.01559
24. Bonfim MVLP (2018) Crescimento e produção de compostos bioativos foliares em leguminosas medicinais nativas da Caatinga associadas a fungos micorrízicos arbusculares (doctoral Thesis). Universidade Federal de Pernambuco - UFPE, Recife - PE
25. de Oliveira MS, da Campos MAS, de Albuquerque UP, da Silva FSB (2013) Arbuscular mycorrhizal fungi (AMF) affect biomolecules content in Myracrodruon urundeuva seedlings. Ind Crops Prod 50:244–247. https://doi.org/10.1016/j.indcrop.2013.07.041
26. Oliveira MS, Campos MAS, Silva FSB (2015) Arbuscular mycorrhizal fungi and vermicompost to maximize the production of foliar biomolecules in *Passiflora alata* Curtis seedlings. J Sci Food Agric 95:522–528. https://doi.org/10.1002/jsfa.6767
27. Hafez EE, Abdel-Fattah GM, El-Haddad SA, Rashad YM (2013) Molecular defense response of mycorrhizal bean plants infected with *Rhizoctonia solani*. Ann Microbiol 63:1195–1203. https://doi.org/10.1007/s13213-012-0578-5
28. Marcolino MC, Sousa Júnior JCAD, Dias CHA, Naue CR, Melo FBDS, Campos MADS (2021) Bioprospection: in vitro antimicrobial potential of the leaf extract of mycorrhizal guava infected by *Meloidogyne enterolobii* on *Klebsiella pneumoniae*. An Acad Bras Ciênc. https://doi.org/10.1590/0001-3765202120201559
29. Avio L, Maggini R, Ujvári G, Incrocci L, Giovannetti M, Turrini A (2020) Phenolics content and antioxidant activity in the leaves of two artichoke cultivars are differentially affected by six mycorrhizal symbionts. Sci Hortic 264:109153. https://doi.org/10.1016/j.scienta.2019.109153
30. Giovannetti M, Avio L, Barale R, Ceccarelli N, Cristofani R, Iezzi A, Mignolli F, Picciarelli P, Pinto B, Reali D, Sbrana C, Scarpato R (2012) Nutraceutical value and safety of tomato fruits produced by mycorrhizal plants. Br J Nutr 107:242–251. https://doi.org/10.1017/S000711451100290X
31. Bakke IA, Freire AL de O, Bakke OA, Andrade AP de, Bruno R de LA (2006) Water and sodium chloride effects on *Mimosa tenuiflora* (WILLD.) poiret seed germination. Rev Caatinga 19.
32. Giovannetti M, Mosse B (1980) An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol 84:489–500. https://doi.org/10.1111/j.1469-8137.1980.tb04556.x
33. Gerdemann JW, Nicolson TH (1963) Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. Trans Br Mycol Soc 46:235–244. https://doi.org/10.1016/S0007-1536(63)80079-0
34. Rizwan K, Majeed I, Bilal M, Rasheed T, Shakeel A, Iqbal S (2022) Phytochemistry and diverse pharmacology of genus mimosa: a review. Biomolecules. https://doi.org/10.3390/biom12010083
35. Georgé S, Brat P, Alter P, Amiot MJ (2005) Rapid determination of polyphenols and vitamin C in plant-derived products. J Agric Food Chem 53:1370–1373. https://doi.org/10.1021/jf048396b
36. WHO, 2005. Guidelines for laboratory and field testing of mosquito larvicides. World Health Organization. https://www.who.int/publications/i/item/WHO-CDS-WHOPES-GCDPP-2005.13
37. de Souza TAF, Rodriguez-Echeverría S, de Andrade LA, Freitas H (2016) Arbuscular mycorrhizal fungi in *Mimosa tenuiflora* (Willd.) Poir from Brazilian semi-arid. Braz J Microbiol 47:359–366. https://doi.org/10.1016/j.bjm.2016.01.023
38. Medeiros AS, Scaloppi JC, Damasceno ES, Goto BT, Vieira DCM, Socolowski F, Rodrigues RG, Yano-Melo AM (2023) Arbuscular mycorrhizal fungi communities shaped by host-plant affect the outcome of plant–soil feedback in dryland restoration. J Appl Ecol 60:507–518. https://doi.org/10.1111/1365-2664.14330
39. de Oliveira JRG, de Resende GM, de Melo NF, Yano-Melo AM (2017) Symbiotic compatibility between arbuscular mycorrhizal fungi (autoctone or exotic) and three native species of the Caatinga in different phosphorus levels. Acta Sci Biol Sci 39:59–69
40. Lambais ÉO, de Souza TAF, Késsia P, dos Nascimento GS, Macedo R, de Bakker AP, Lambais GR, Dias BO, da Silva FV (2024) Seasonality and activity of arbuscular mycorrhizal fungi in the rhizosphere of endemic tree species. J Basic Microbiol. https://doi.org/10.1002/jobm.202400354
41. Pedone-Bonfim MVL, Da Silva DKA, Maia LC, Yano-Melo AM (2018) Mycorrhizal benefits on native plants of the *Caatinga*, a Brazilian dry tropical forest. Symbiosis 74:79–88. https://doi.org/10.1007/s13199-017-0510-7
42. Pedone-Bonfim MV, Lins MA, Coelho IR, Santana AS, Silva FS, Maia LC (2013) Mycorrhizal technology and phosphorus in the production of primary and secondary metabolites in cebil (*Anadenanthera colubrina* (Vell.) Brenan) seedlings. J Sci Food Agric 93:1479–1484. https://doi.org/10.1002/jsfa.5919
43. Lima CS, Campos MADS, Da Silva FSB (2015) Mycorrhizal fungi (AMF) increase the content of biomolecules in leaves of Inga vera Willd. seedlings. Symbiosis 65:117–123. https://doi.org/10.1007/s13199-015-0325-3
44. Aseel DG, Rashad YM, Hammad SM (2019) Arbuscular mycorrhizal fungi trigger transcriptional expression of flavonoid and chlorogenic acid biosynthetic pathways genes in tomato against tomato mosaic virus. Sci Rep 9:9692. https://doi.org/10.1038/s41598-019-46281-x

45. Stratton CA, Ray S, Bradley BA, Kaye JP, Ali JG, Murrell EG (2022) Nutrition vs association: plant defenses are altered by arbuscular mycorrhizal fungi association not by nutritional provisioning alone. BMC Plant Biol 22:400. https://doi.org/10.1186/s12870-022-03795-3
46. Feddermann N, Finlay R, Boller T, Elfstrand M (2010) Functional diversity in arbuscular mycorrhiza – the role of gene expression, phosphorous nutrition and symbiotic efficiency. Fungal Ecol 3:1–8. https://doi.org/10.1016/j.funeco.2009.07.003
47. Barbosa PBBM, De Oliveira JM, Chagas JM, Rabelo LMA, De Medeiros GF, Giodani RB, Da Silva EA, Uchôa AF, Ximenes DFDFMM (2014) Evaluation of seed extracts from plants found in the Caatinga biome for the control of *Aedes aegypti*. Parasitol Res 113:3565–3580. https://doi.org/10.1007/s00436-014-4022-6
48. Sultana B, Anwar F, Ashraf M (2009) Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Molecules 14:2167–2180. https://doi.org/10.3390/molecules14062167
49. Zhao Y, Cartabia A, Lalaymia I, Declerck S (2022) Arbuscular mycorrhizal fungi and production of secondary metabolites in medicinal plants. Mycorrhiza 32:221–256. https://doi.org/10.1007/s00572-022-01079-0
50. Lima CS, Santos HRS, de Albuquerque UP, da Silva FSB (2017) Mycorrhizal symbiosis increase the level of total foliar phenols and tannins in *Commiphora leptophloeos* (Mart.) J.B. Gillett seedlings Ind Crops Prod 104:28–32. https://doi.org/10.1016/j.indcrop.2017.04.020
51. Ponce MA, Bompadre MJ, Scervino JM, Ocampo JA, Chaneton EJ, Godeas AM (2009) Flavonoids, benzoic acids and cinnamic acids isolated from shoots and roots of Italian rye grass (*Lolium multiflorum* Lam.) with and without endophyte association and arbuscular mycorrhizal fungus. Biochem Syst Ecol 37:245–253. https://doi.org/10.1016/j.bse.2009.03.010
52. Ponce MA, Scervino JM, Erra-Balsells R, Ocampo JA, Godeas AM (2004) Flavonoids from shoots and roots of *Trifolium repens* (white clover) grown in presence or absence of the arbuscular mycorrhizal fungus *Glomus intraradices*. Phytochemistry 65:1925–1930. https://doi.org/10.1016/j.phytochem.2004.06.005
53. Bezerra França S, Barros C, De Lima L, Da Silva R, Cunha C, Santos Anunciação D, Ferreira Da Silva-Júnior E, De Sá E, Barreto Barros M, Da Paz J, Lima D (2021) Larvicidal activity and in silico studies of cinnamic acid derivatives against *Aedes aegypti* (Diptera: Culicidae). Bioorg Med Chem 44:116299. https://doi.org/10.1016/j.bmc.2021.116299
54. Inaba K, Ebihara K, Senda M, Yoshino R, Sakuma C, Koiwai K, Takaya D, Watanabe C, Watanabe A, Kawashima Y, Fukuzawa K, Imamura R, Kojima H, Okabe T, Uemura N, Kasai S, Kanuka H, Nishimura T, Watanabe K, Inoue H, Fujikawa Y, Honma T, Hirokawa T, Senda T, Niwa R (2022) Molecular action of larvicidal flavonoids on ecdysteroidogenic glutathione S-transferase Noppera-bo in *Aedes aegypti*. BMC Biol 20:43. https://doi.org/10.1186/s12915-022-01233-2

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.