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Repellency and bioactivity of Caatinga biome plant powders against *Callosobruchus maculatus* (Coleoptera: Chrysomelidae: Bruchinae)

Bruno Adelino de Melo¹, Adrián José Molina-Rugama^{1,2,*}, Khalid Haddi³, Delzuite Teles Leite¹, and Eugênio Eduardo de Oliveira^{3,*}

Abstract

The Caatinga biome represents the 4th-largest area covered by single vegetation in Brazil and contains dry forests rich in aromatic bushes, vines, herbs, and trees. The flora of this ecological region is widely known and employed in folk medicine and has other utilitarian and economic uses; however, its potential for controlling or repelling insects is poorly investigated. In this study, we evaluated the potential use of Caatinga plant species for controlling infestations of *Callosobruchus maculatus* F. (Coleoptera: Chrysomelidae: Bruchinae), the most important insect pest of cowpea, *Vigna unguiculata* (L.) Walp. (Fabales: Fabaceae). Powders of the leaves and stems of 9 plant species, including *Amburana cearensis* A. C. Smith ("cumaru") (Fabales: Fabaceae), *Croton sonderianus* Müll. Arg. ("marmeleiro") (Malpighiales: Euphorbiaceae), *Cleome spinosa* Jacq. ("mussambê") (Capparales: Cleomaceae), *Mimosa tenuiflora* Benth. ("jurema-preta") (Fabales: Fabaceae), *Anadenanthera mac rocarpa* (Benth.) Brenan ("angico-vermelho") (Fabales: Fabaceae), *Aspidosperma pyrifolium* Mart. ("pereiro") (Gentianales: Apocynaceae), *Senna occidentalis* (L.) H.S. Irwin & R.C. Barneby ("manjerioba") (Fabales: Fabaceae), *Hyptis suaveolens* (L.) Poit. ("alfazema-brava") (Lamiales: Lamiaceae), and *Ziziphus joazeiro* Mart. ("juazeiro") (Rosales: Rhamnaceae), were applied on masses of cowpea seeds, and their effects on *C. maculatus* longevity as well as their repellent activities were evaluated. All the leaf and stem powders reduced only the longevity of males and showed strongly repellent activities against females. The preference level of females for untreated beans varied between 73 and 94%, indicating that all the leaf and stem powders can be a part of the integrated management of *C. maculatus* in storage facilities.

Key Words: stored grain pest; bruchid; Vigna unguiculata; plant powder; alternative pest control

Resumo

O bioma Caatinga representa a quarta maior área coberta por um único tipo de vegetação no Brasil. Este bioma se constitui de florestas secas com considerável diversidade de arbustos, ervas, trepadeiras e árvores aromáticas. A flora desta região ecológica é amplamente conhecida e tem sido utilizada para diversos fins utilitários e econômicos, principalmente na medicina popular. No entanto, o potencial destas plantas para controlar ou repelir insetos ainda é pouco investigado. Neste estudo, foi avaliado o uso potencial de espécies de plantas da Caatinga para controlar infestações de Callosobruchus maculatus F. (Coleoptera: Chrysomelidae: Bruchinae), uma das mais importantes pragas no feijão caupi, Vigna unguiculata (L.) Walp. (Fabales: Fabaceae). Pó das folhas e de caules de nove espécies de plantas, incluindo Amburana cearensis A. C. Smith ("cumaru") (Fabales: Fabaceae), Croton sonderianus Müll.Arg. ("marmeleiro") (Euphorbiaceae), Spinosa cleome Jacq. ("Mussambê") (Capparales: Cleomaceae), Mimosa tenuiflora Benth. ("jurema-preta") (Fabales: Fabaceae), Anadenanthera macrocarpa (Benth.) Brenan ("angico-vermelho") (Fabales: Fabaceae), Aspidosperma pyrifolium Mart. ("pereiro") (Gentianales: Apocynaceae), Senna occidentalis (L.) H.S. Irwin & R.C. Barneby ("mangirioba") (Fabales: Fabaceae), Hyptis suaveolens (L.) Poit. ("alfazema-brava") (Lamiales: Lamiaceae) e Ziziphus joazeiro Mart. ("juazeiro") (Rosales: Rhamnaceae), foram aplicados em massas de feijão caupi e seus efeitos sobre a longevidade e repelência de C. maculatus foram avaliados. Todos os pós de folhas e de caules reduziram apenas a longevidade de machos de C. maculatus. Entretanto, estes mesmos pós mostraram alta atividade repelente contra fêmeas destes insetos. O nível de preferência de fêmeas de C. maculatus para grãos não tratados variou entre 73 e 94%, indicando que os pós das folhas e de caules destas plantas podem se constituir importantes ferramentas para o manejo integrado de C. maculatus em unidades de armazenamento.

Palavras Chave: pragas de grãos armazenados; bruquídeo; Vigna unguiculata; pós vegetais; controle alternativo de pragas

The Caatinga biome accounts for about 60% of the northeast Brazilian territory and extends to a small part of the northeastern Minas Gerais State (Sampaio et al. 2002). This area is mainly covered by xeric shrub lands rich in aromatic bushes, vines, herbs, and trees (Almeida et al. 2005) with its native plants presenting utilitarian and economic potential (Albuquerque & Andrade 2002; Lucena et al. 2007, 2008; Ca-

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nuto et al. 2012). Many of the Caatinga plant species are used by native communities as firewood (Ramos et al. 2008), in carpentry (owing to their recognized durability), as seasoning (Canuto et al. 2012), or in folk medicine to treat several diseases (Leal et al. 2000; Albuquerque et al. 2007; Alviano et al. 2008; Cartaxo et al. 2010; Canuto et al. 2012). The great diversity of the Caatinga vegetation is underexploited, and few searches for active biological substances, including those with insecticidal or repellent activity, have been conducted (Almeida et al. 2005; Albuquerque et al. 2007).

Food availability in the Brazilian Caatinga heavily depends on the capacity of farmers (most of them are subsistence producers) to preserve the post-harvest quality of their production. In this region, cereals and beans are grown predominantly by small farmers with little or no technological inputs (Vieira 2004; Ferreira et al. 2013). These farmers have low family income, and they usually keep their production inside their own small storage facilities with high quantitative and qualitative losses, most of them due to insect damage. Natural products from locally available plants with insecticide activity represent a low-cost and sustainable alternative to protect agricultural production. Furthermore, botanical insecticides supposedly pose little threat to the environment or human health compared with synthetic insecticides, and they represent a suitable alternative to controlling mites and insect pests worldwide (Isman 2006; Regnaut-Roger et al. 2012; Kedia et al. 2013).

The cowpea weevil, *Callosobruchus maculatus* F. (Coleoptera: Chrysomelidae: Bruchinae), damages 20–30% of legume seeds in the tropical countries (Kirado & Srivastava 2010) and can cause up to 100% loss when masses of cowpea beans are untreated (Gbaye et al. 2011). Adults mate after emergence and typically live not more than 2 wk depending on ambient temperature. The females deposit eggs on the surface of maturing cowpea pods and seeds. The newly emerged larvae burrow into and feed on a single seed until pupation, and adults do not need to feed (Mitchell 1975; Southgate 1978). Several holes are left in the seed by the emerging adults with severe weight loss facilitating fungal and mycotoxin contamination, which reduces the commercial bean value (Kirado & Srivastava 2010; Kedia et al. 2013).

Insecticidal natural products, such as powders of locally available plants, used by farmers in developing countries in their storage facilities, appear to be safe and promising (Paul et al. 2009; Silva et al. 2013; Tavares et al. 2013, 2014; Fouad et al 2014; Melo et al. 2014). Thus, we evaluated the repellent activity and the effects of powders from 9 Caatinga plant species on *C. maculatus* longevity.

Material and Methods

INSECT REARING

The original population of C. maculatus was field-collected from small farms in the region of Pombal (Paraíba State, Brazil) and established under laboratory conditions (25 ± 2 °C, 70 ± 5% RH, and12:12 h L:D photoperiod), starting with at least 500 individuals. The identification was based on the traits described previously (Athié & Paula 2002). The population was reared on cowpea bean (Vigna unguiculata [L.] Walp.; Fabales: Fabaceae) grains (free of insecticides) bought from the local market. In order to avoid possible infestations from the field and to reduce any potential insecticide residual effect, the bean grains were kept a temperature of -10 °C for 14 d prior to being offered to C. maculatus. To obtain newly emerged C. maculatus of the same generation, adult insects were released in cowpea bean grain masses that were placed in plastic containers (0.4 L capacity) covered with "organza" cloth. After 5 d of colonization, the adults were removed and the egg-infested grains were maintained under laboratory conditions. The new adults emerged after around 4 wk.

The plant powders used in this study were obtained from the leaves and stems of 9 Caatinga plant species, including Amburana cearensis A. C. Smith ("cumuru-nordestino") (Fabales: Fabaceae), Croton sonderianus Müll.Arg. ("marmeleiro-do-mato") (Malpighiales: Euphorbiaceae), Cleome spinosa Jacq. ("mussambê") (Capparales: Cleomaceae), Mimosa tenuiflora Benth. ("jurema-preta") (Fabales: Fabaceae), Anadenanthera macrocarpa (Benth.) Brenan ("angico-vermelho") (Fabales: Fabaceae), Aspidosperma pyrifolium Mart. ("pereiro") (Gentianales: Apocynaceae), Senna occidentalis (L.) H.S. Irwin & R.C. Barneby ("mangirioba") (Fabales: Fabaceae), Hyptis suaveolens (L.) Poit. ("alfazemabrava") (Lamiales: Lamiaceae), and Ziziphus joazeiro Mart. ("juazeiro") (Rosales: Rhamnaceae) (Table 1), collected in the region of Pombal (Paraíba State, Brazil). We chose only plant species that are used by native communities to treat several diseases, and some of their biological activities have been described (Table 1). During the period between the years of 2009 and 2012, leaves and stems were randomly collected from the adult plants by using pruning scissors. Samples of these plants were compared with material deposited in the herbarium of the Universidade Federal Rural do Semi-Árido (UFERSA, Mossoró-RN, Brazil). All the plant materials were individually wrapped in plastic bags, identified, and brought to the laboratory. Then, these materials were dried by direct exposure to sunlight over a 7 d period, and leaves and stem were separately milled with a manual grinder to powder. The resulting powder was passed through a 25 mesh sieve to obtain a fine dust. The fine dusts were stored individually in glass containers (hermetically closed) that were maintained at a controlled temperature (5 °C) to ensure supply of the material throughout the investigation period.

LONGEVITY BIOASSAYS

The effects of each plant powder on insect longevity were assessed in survival bioassays conducted according to previously described methods (Procópio et al. 2003). Briefly, a pair of newly emerged weevils was confined in a plastic container (100 mL) containing 45 g of untreated (control) or plant powder treated cowpea bean seeds. Each weevil pair in 45 g of bean seeds was an experimental unit. In the treated bean unit, 2 g of the plant powder had been homogeneously distributed among the seeds. Five replicates were used for each plant powder tested, and the male and female insect mortality was monitored daily until the last day of survival. As these insects are excellent fliers, we customized an escape-proof cage that allowed measurements of mortality. This cage had the following dimensions: 40 cm length × 20 cm width × 20 cm height, and its base, back, and front sides were made of wood. Openings of 10 cm diameter were drilled in the back and front sides and were closed with organza cloth. These openings facilitated the insertion and handling of experimental materials. Furthermore, complete and easy viewing and handling of the experimental materials were achieved through the glass used at the top and lateral sides of the cage. The bean seeds were carefully poured onto the plastic trays placed inside the cage. After counting the number of dead insects, all the live insects, bean grains, and plant powders were added back into the experimental units. The insect longevity measurements were subjected to analysis of variance and subsequently to Tukey's test (α = 0.05), when appropriate.

FREE-CHOICE REPELLENCE TEST

The repellent activity of each plant powder was assessed in bioassays conducted in custom-made plastic arenas (35 cm diameter, 12 cm high), according to the modified protocols reported previously (Burkholder & Dicke 1966; Phillips & Burkholder 1981). Six 50 mL plastic

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Table 1. Caatinga plant species collected in the county of Pombal, Paraíba	nty of Pombal, Para	iíba State, Brazil.			
Scientific name	Family	Common name	Biological activity	Isolated compounds	References
Amburana cearensis A. C. Smith	Fabaceae	"cumaru"	anticholinesterase, antinociceptive, antiinflamatory, bronchodilator, antibacterial	courmarin, phenolic glyscosides	(Bravo et al. 1999; Leal et al. 2000; Trevisan & Macedo 2003; Figueredo et al. 2013)
Croton sanderianus Müll. Arg.	Euphorbiaceae "marmeleiro"	"marmeleiro"	antinociceptive, antimicrobial, antifungal	clerodane and cleisthantane type diterpenes; 1,8-cineol	(McChesney et al. 1991; Santos et al. 2005; Fontenelle et al. 2008)
Cleome spinosa Jacq.	Cleomaceae	"musambê"	neuroprotection, cytotoxicity against a number of human cancer cell lines, anti-HIV activity	unsaturated polyprenols, cembranoids, diterpenes	(Faulkner 2001; Hanson 2002; Collins et al. 2004)
<i>Mimosa tenuiflora</i> Willd. Benth.	Fabaceae	"jurema-preta"	anti-inflammatory, antioxidant, antifungal, healing properties	tannins and flavonoids	(Araújo et al. 2008)
Anadenanthera macrocarpa (Benth.) Brenan	Fabaceae	"angico-vermelho"	antibacterial, anti-inflammatory	phenols, flavonoids, free xanthones, leucoantho-cyanidins	(Desmarchelier et al. 1999; Figueredo et al. 2013)
Aspidosperma pyrifolium Mart.	Apocynaceae	"pereiro"	antiplasmodial	monoterpenoid indole alkaloids	(Araújo et al. 2007)
Senna occidentalis (L.) H.S. Irwin & R.C. Barneby	Fabaceae	"mangirioba "	anti-inflammatory, antioxidant, antifungal, healing properties	tannins and flavonoids	(Araújo et al. 2008)
<i>Hyptis suaveolens</i> (L.) Poit.	Lamiaceae	"alfazema-brava"	anti-inflammatory, antioxidant, antifungal, healing properties, anticonvulsant	tannins and flavonoids, β-Caryophyllene, 1,8-cineole	(Akah & Nwambie 1993; Peerzada 1997; Araújo et al. 2008)
Ziziphus joazeiro Mart.	Rhamnaceae	"juazeiro"	antimicrobial, antioxidante, anticholinesterase	saponins	(Alviano et al. 2008; Farias et al. 2013; Ribeiro et al. 2013)

containers were placed at equidistance inside the arena, with 30 g of cowpea bean seeds in each container. Plant powder to be tested (1.5 g per container) was added to alternate containers (3 per arena). To facilitate odor removal, a 5 cm diameter hole was drilled in the center of the arena's lid for the insertion of a 5 cm diameter polyvinyl chloride (PVC) tube 10 cm in height. The external extremity of this tube was covered with organza cloth to prevent escape of the insects. Thirty adult females (aged 1–5 d) were released into the center of the arena, and after 24 h, the total number of insects per container was registered. Five replicates were used for each plant powder tested. In preliminary tests, we found even distribution of insects among containers when all the 6 plastic containers were filled only with untreated cowpea, so there was no indication of a position effect within the arena.

A binomial test (P < 0.01) was used to evaluate the significance of differences between the percentages of females that moved to untreated and powder treated bean seeds. The percentage of repellency was calculated as proposed by Mazzonetto & Vendramin (2003): $RI = (2 \times T) \div (T + C) \times 100$, where RI = repellency index, C = number of insects in the untreated container, and T = number of insects in the treated container. The RI values ranged between 0 and 2, which denoted the following: RI = 1, neutral activity; RI > 1, attraction; and RI < 1, repellency. As a safety margin for this classification, the standard deviation (SD) of each treatment was added/subtracted from the value of 1 (indicative of neutrality). The repellency index results were subjected to analysis of variance, and the averages were compared by using the Scott–Knott groupment analysis test (Scott & Knott 1974) at a probability level of 0.05.

Results

LONGEVITY BIOASSAYS

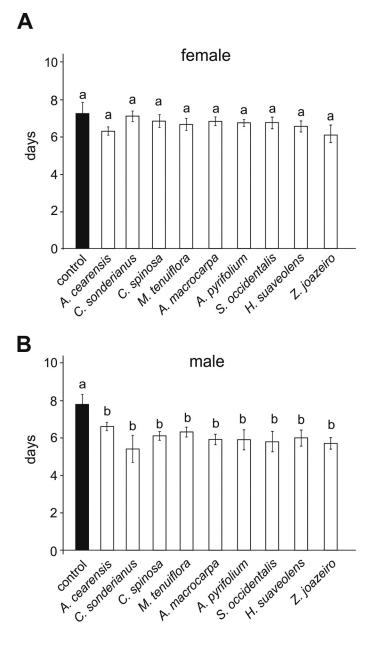
There were no significant differences ($F_{8,76} = 1.99$; P > 0.05) among the longevities of females exposed to leaf or stem powders of each plant tested, which allowed us to pool these longevity data and compare them with the longevity of females on untreated bean masses (Fig. 1). In general, the average longevity of females treated with plant powders was 7.4 ± 1.01 d and did not differ significantly ($F_{1,76} = 0.86$; P > 0.05) from that of the control females (7.8 ± 1.09 d; Fig. 1A). Likewise, the males showed similar longevities ($F_{8,76} = 0.82$; P > 0.05) when exposed to leaf or stem powders of each plant tested. However, the average longevity of males was significantly reduced ($F_{1,76} = 8.15$; P <0.01) from 7.8 ± 1.79 d (control males) to 6.06 ± 1.25 d (males that lived on plant powder–treated beans) (Fig. 1B).

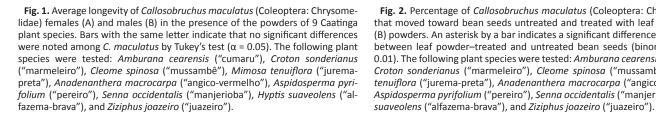
REPELLENT ACTIVITIES

All of the Caatinga plant powders were strongly repellent to females. The percentages of the females that preferred untreated beans ranged from 77% to 94% and were significantly greater (P < 0.01, binomial test) than those of females that preferred the leaf powder– treated beans (Fig. 2A). Similar results were obtained in the repellency bioassays with stem powders, where females significantly preferred (P< 0.01, binomial test) untreated bean seeds (Fig. 2B).

Although all the plant powders significantly repelled females, the leaf powders of *A. pyrifolium, S. occidentalis, H. suaveolens,* and *Z. joazeiro* exhibited greater repellency levels (Table 2). With regard to the stem powders, the plant species *C. sonderianus, C. spinosa, H. suaveolens,* and *Z. joazeiro* presented greater repellency levels. Furthermore, the leaf and stem powders of *A. pyrifolium* and *S. occidentalis* showed differential repellent activities (Table 2), with the leaf powders presenting greater repellency levels.

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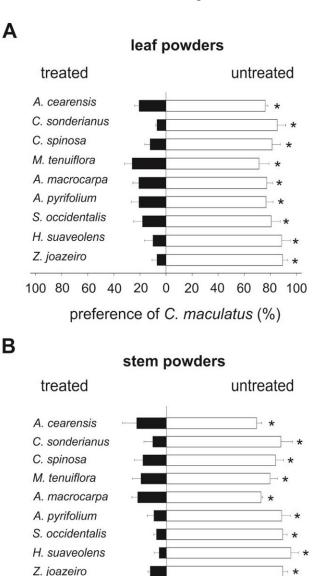


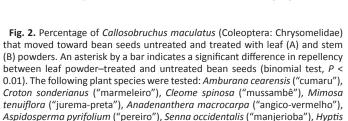


Discussion

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Despite its great territorial expanse and significant biodiversity, the Caatinga biome is still an underexploited source of molecules with insecticidal/repellent activities. Most studies with plant products from this ecological region have focused on extracts or essential oils to control insect disease vectors (Lima et al. 2006; Farias et al. 2010; Souza et al. 2011; Santos et al. 2012; Barbosa et al. 2014). Few studies investigated





0

preference of C. maculatus (%)

20

40 60 80 100

20

100 80 60

40

the potential of Caatinga plant powders as commodity protectants, and they normally evaluated only mortality effects (Souza & Trovão 2009; Cruz et al. 2013). Here, we evaluated the insecticidal and repellent activities of 9 Caatinga plant species (A. cearensis, C. sonderianus, C. spinosa, M. tenuiflora, A. macrocarpa, A. pyrifolium, S. occidentalis, H. suaveolens, and Z. joazeiro) against the cowpea weevil, C. maculatus. Leaf and stem powders from these plants had major insecticidal effects on males and repelled the females, demonstrating their potential for use in the integrated management of C. maculatus in storage facilities.

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 Table 2. The repellency index (RI) obtained for each plant powder tested against

 Callosobruchus maculatus (Coleoptera: Chrysomelidae).

	Repellency index [®]	
Plant species	Leaves	Stems
Amburana cearensis	0.46 ± 0.05 Aa	0.42 ± 0.04 Aa
Croton sonderianus	0.22 ± 0.10 Ba	0.15 ± 0.03 Ba
Cleome spinosa	0.35 ± 0.08 Aa	0.26 ± 0.06 Ba
Mimosa tenuiflora	0.39 ± 0.07 Aa	0.54 ± 0.07 Aa
Anadenanthera macrocarpa	0.46 ± 0.05 Aa	0.42 ± 0.06 Aa
Aspidosperma pyrifolium	0.20 ± 0.06 Bb	0.43 ± 0.07 Aa
Senna occidentalis	0.16 ± 0.03 Bb	0.36 ± 0.08 Aa
Hyptis suaveolens	0.11 ± 0.04 Ba	0.20 ± 0.07 Ba
Ziziphus joazeiro	0.25 ± 0.02 Ba	0.14 ± 0.04 Ba

^aMeans followed by the same lowercase letter in a row or the same capital letter in a column are not significantly different based on the Scott–Knott groupment analysis test at P < 0.05.

Similar to the lack of insecticide activity against C. maculatus females observed here for all the plant powders, root powder of M. tenuiflora showed very small insecticidal activity against termites (Isoptera) (Cruz et al. 2013), and powders of A. macrocarpa did not show any insecticidal activity against the maize weevil, Sitophilus zeamais Motschulsky (Coleoptera: Curculionidae) (Souza & Trovão 2009). Furthermore, powders from other medicinal plants (thyme, Thymus vulgaris L. [Lamiales: Lamiaceae]; lavender cotton, Santolina chamaeyceparissus L., and stinking bean trefoil, Anagyris foetida L. [Fabales: Fabaceae]) neither affected the longevity of southern cowpea weevil, Callosobruchus chinensis L. (Coleoptera: Chysomelidae) males nor females (Righi-Assia et al. 2010). These differential insecticidal activities of plant powders might have resulted from multiple factors involving the way they work and the resistance mechanisms of the insects. Plant powders can control insects by eroding the cuticle layer and causing dehydration (Kedia et al. 2013); blocking the spiracles and causing asphyxiation (Denloye 2010); or impairing physiological processes by penetrating the insect body via the respiratory or alimentary system (Ofuya & Dawodu 2002). Plant powders of S. occidentalis caused significant mortality in C. maculatus (Adesina et al. 2011), and insecticidal properties of A. pyrifolium (Torres et al. 2006), C. sonderianus (Morais et al. 2006; Lima et al. 2006, 2013), A. cearensis (Farias et al. 2010; Souza et al. 2011), and Z. joazeiro (Souza et al. 2011) have been documented in different insect species. The repellent activities of Caatinga plant powders need further study although the repellency of many other plant powders against stored pests has been reported (Elhag 2000; Kéita et al. 2001; Mazzonetto & Vendramin 2003; Silva-Aguayo et al. 2005; Sanon et al. 2006; Kabir & Muhammad 2010).

The present study extends knowledge on *Caatinga* plants for use as stored product protectants because it demonstrates that the leaf and stem powders of 9 Caatinga plants show strong repellent activities against C. maculatus females. Leaf powders of A. pyrifolium and S. occidentalis repelled C. maculatus more efficiently than the stem powders of these plants, suggesting that these plants possess different active constituents or that they have the same constituents but with different concentrations in various plant parts (Ravi Kiran et al. 2006; Autran et al. 2009). Such differential activities of the powders of leaves and stems of other plants such as neem, Azadirachta indica A. Juss. (Sapindales: Meliaceae) (leaf and stem bark powders), have been described, with the leaf powder showing higher repellent activities against C. maculatus than the stem powder (Kabir & Muhammad 2010). The striking repellency results obtained here for M. tenuiflora and A. macrocarpa powders are noteworthy, because these plant products had been previously reported to have no (Souza & Trovão 2009; Santos et al. 2012)

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Furthermore, *H. suaveolens* plant products demonstrated noticeable repellent activity against *C. maculatus* females, as demonstrated with other insect species (Sanon et al. 2006; Ilboudo et al. 2010; Benelli et al. 2012). However, products from this plant species can cause detrimental effects on natural enemies in storage environments (Sanon et al. 2011), thus requiring caution when used as grain protectants. In addition, other plant species of the *Cleome* genus showed repellent actions against ticks (Parasitiformes) and insects (Ndungu et al. 1995; Nyalala & Grout 2007), but the present study is the first to report on the insecticidal/repellent potential against *C. spinosa*.

The application of plant materials with insecticidal or repellent properties to stored grains is a common traditional method in rural areas around the world (Regnault-Roger et al. 2012; Kedia et al. 2013). Tropical ecosystems (such as the Caatinga biome) are particularly rich in plants that are used by local communities to treat diseases, thus indicating the potential to discover new compounds (Albuquerque et al. 2007, 2008). Further investigations exploring the toxicological aspects of the major constituents or identifying the principal volatiles produced by the Caatinga plants tested here will provide new insights on how these plants exhibit their insecticidal/repellent activities.

Our findings not only extend the knowledge on the Caatinga plants but also provide information about plants that can be used to protect cowpeas against *C. maculatus* infestations. All the plants tested are readily available in the Caatinga Region, and these anti-insect materials are affordable to low-income farmers who are normally constrained to sell their production early after harvest or, even worse, have their stored bean seeds (normally saved on the farm from the previous harvest) prone to infestation by stored product pests.

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