academicJournals

Vol. 9(27), pp. 1684-1691, 8 July, 2015
DOI: 10.5897/AJMR2015.7574
Article Number: CE3F44254158
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Control of *Sclerotium rolfsii* in peanut by using *Cymbopogon martinii* essential oil

Yrlânia de Lira Guerra[1], Thiago Alves Santos de Oliveira[1], Delson Laranjeira[1], Liziane Maria Lima[2], Péricles de Albuquerque Melo Filho[1] and Roseane Cavalcanti dos Santos[2]*

[1]Plant Pathology, Rural Federal University of Pernambuco, Recife-PE, Brazil.
[2]Biotechnology Laboratory, Embrapa Cotton, Campina Grande-PB, Brazil.

Received 8 May, 2015; Accepted 6 July, 2015

Essential oils of seven species were investigated in order to control peanut plants against white mold (*Sclerotium rolfsii* Sacc.). The assays were carried out by *in vitro* and *in vivo* assays. At first, fungitoxicity and suppression of oxalic acid diffusion (SOAD) bioassays were performed in order to evaluate the mycelial growth of fungus. Then, validation assays were carried out in greenhouse, involving inoculation of fungus in the seeds and further plant treatments with essential oil. Four isolates of *S. rolfsii* were tested in different oil concentrations. *Cymbopogon martinii* oil at 300 ppm inhibited the mycelia growth of *S. rolfsii* in 55% and also the number of sclerotia. In validation assay, we found that a single dose of *C. martinii* oil at 400 ppm reduced the rate of disease in 55%, confirming the *in vitro* assays. The follows traits: number of pods/plant, pod weight and harvest index increased in 57, 54, and 40%, respectively, in all *C. martini* oil treatments. These results demonstrate that *C. martinii* oil at low concentration may serve for new formulations in the treatment and prevention of white mold.

Key words: *Arachis hypogaea,* disease control, toxicity, white mold.

INTRODUCTION

Peanut (*Arachis hypogaea* L.) is one of the world's most important oleaginous, grown widely to attend the edible oil and food markets. More than half of the production area of peanut fall under arid and semi-arid regions, where peanuts are frequently prone to drought stresses (Reddy et al., 2003). In addition, drought conditions influence the growth of weeds, agronomic management and, nature and intensity of pests, including insects, weeds and diseases (Staley et al., 2006).

Diseases caused by fungus are a serious problem to peanut crop. Annually, large amounts of fungicides are sprayed in field in order to control leaf and soil fungus. The indiscriminate use of chemical pesticides has given rise to several problems, such as genetic resistance of pest species, toxic residues in stored products, increasing costs of application, hazards from handling, environmental pollution, and others (Adeyemi, 2010). Genetic resistance to diseases is a main goal in breeding

*Corresponding author. E-mail: roseane.santos@embrapa.br. Tel: + 55 83 31824300.

Table 1. *Sclerotium rolfsii* isolates used in the pathogenicity assay.

Isolate	Code	Access	Local	Host	Lat/Long	Botanic specie
SR5	S.r. 28	LEDP	Cristalina, GO	chickpea	18°10'12"S,47°56'31"W	*Cicer arietinum*
SR14	CMM 2115	CMM	Teresina, PI	cowpea	5°5′20″S,42°48′7″W	*Vigna unguiculata*
SR15	CMM 2930	CMM	Potengi, CE	cowpea	7°5′27″S,40°1′37″W	*V. unguiculata*
SR16	CMM 3051	CMM	Alhandra, PB	cowpea	7°26′20″S,34°54′50″W	*V. unguiculata*

programs, however, depending on the pathogen, the progress is limited due to lack of resistant germplasm.

White mold, caused by *Sclerotium rolfsii* Sacc., is a dangerous pathogen of several crops and is found throughout the major crop areas in many countries, causing yield losses >40% in peanut, bean, garlic, onion, and pepper plant (Fery and Dukes, 2011; Adandonon et al., 2006; Earnshaw et al., 2000). In Brazil, no commercial cultivar of peanut has resistance to white mold, so that management in areas infested with the fungus is often hampered due to limitations to control the disease. Moreover, as inoculum has high persistence in soil, the eradication of pathogen is low efficient and quite expensive (Ozgonen et al., 2010; Punja, 1985). The cost to chemical treatment of the seeds burdens the production system, besides environmental damages caused by pesticide residues. Besides, as resistance development is a real problem faced by the indiscriminate use of synthetic pesticides, it is likely that the protection of plants by biopesticides will be more durable due to various components contained in extracts or essential oils (Koul et al., 2008).

Several metabolities have been reported as effective biopesticides against various species of phytopathogens, highlighting the essential oils that contain up to 60 distinct chemicals, with more than two main components (Hillen et al., 2012; Abdolahi et al., 2010; Bajpai and Kang, 2010; Bakkali et al., 2008). The toxicity of these oils is more related to phenolic compounds and terpenoids, that have high antimicrobial activity and are found in several plants such as lemon grass (*Cymbopogon* sp.), *Eucalyptus* sp., rosemary (*Rosemarinus sp.*), vetiver (*Vetiveria sp.*), clove (*Eugenia* sp.), thyme (*Thymus* sp.), and others (Melo et al., 2013; Das et al., 2010; Vukovic et al., 2007). In fungus, Chen and Viljoen (2010) report that antimicrobial action of *Cimbopogon* oil involves the passive entry of the oil into the plasma membrane in order to initiate membrane disruption, and after to inhibit the cell growth due to accumulation in the plasma membrane. The bilayer disorder and ion leakage disturb the osmotic balance of the cell through loss of ions. Full inhibition of mycelia growth and spore germination have been demonstrated in *Phakopsora pachyrhizi*, *Colletotrichum gloeosporioides*, *Didymella bryoniae,* *Cladosporium* sp., *Nigrospora* sp. and others (Souza Junior et al., 2009; Mata et al., 2009; Medice et al., 2007; Fiori et al., 2000).

The present work was proposed in order to investigate the antifungal activity of different essential oils to control peanut plants against *S. rolfsii*, based *in vitro* and *in vivo* assays.

MATERIALS AND METHODS

Origin of *S. rolfsii* isolates and disease severity assay

Four *S. rolfsii* isolates (Table 1) were kindly supplied by the Maria Menezes Collection, from Rural Federal University of Pernambuco (UFRPE), Brazil. Previous pathogenicity assays were carried out in greenhouse, using peanut plants, in order to estimating the disease severity, following methodology described in Bastos and Albuquerque (2004).

The isolates were previously grown on autoclaved rice during nine days in Petri dishes and further added to a commercial substrate (Baseplant) at 72 mg.kg^{-1}, in pots (1 L) (Barbosa et al., 2010). Three peanut seeds previously surface-sterilized (hypochlorite solution at 1.5%) were sown in each pot and daily watered. Two earliness-upright cultivars were used in this assay: Senegal 55 437, a Spanish type developed by International Crops Research Institute for the Semi-Arid-Tropics (ICRISAT), and BR 1, a Valencia type developed by Brazilian Company of Agricultural Research (EMBRAPA). Taking in account that the low fertility of substrate could affect the fungus pathogenicity, this assay was repeated in the same conditions with supplementation of 40 g P_2O_5, 15 g KCl and 200 g hummus, added to each kg substrate, based on recommendations in Santos et al. (2006). The experimental design was completely randomized with eight replications.

Plants were monitored daily for 15 days to follow pathogen establishment and development of disease symptoms. The disease severity (DS) was evaluated following the scale described by Fery and Dukes (2002) (that is, 1 = no wilting symptoms, 2 = slight or partial wilting, 3 = general plant wilting, 4 = permanent wilt, and 5 = dead plant). Then, the disease severity index (DSI) was estimated on the basis of this rating scale by adopting the following formula

(Galanihe et al., 2004): $\text{DSI (\%)} = \sum \frac{(P \times Q)}{(M \times N)} \times 100$

Where, P = severity score, Q = number of infected plants showing the same score, M = total number of observed plants, and N = maximum rating scale.

Inhibition bioassays with essential oils *in vitro*

Seven pure essential oils, obtained commercially, were used in this assay: *Cymbopogon martinii* (Roxb.) Stapf var. *motia* Burk (Accession 1), *Cedrus atlantica* Manetti (Accession 2), *Copaifera officinalis* L (Accession 3), *Zingiber officinale* L (Accession 4), *Eucalyptus staigeriana* F. (Muell) (Accession 5),. *Juniperus communis* L. (Accession 6), and. *Ocimum basilicum* L. (Accession 7).

Oils were added separately to potato dextrose agar (PDA) culture

medium at 50°C and poured onto Petri dishes (9 cm diameter). A 0.5 cm-PDA disk containing mycelium from each isolate was deposited in the center of each plate (Melo et al., 2013). The negative control was oil-free. Then, plates were randomized and incubated in a biochemical oxygen demand (BOD) growth chamber at 28°C and 12:12 h photoperiod. The bioassay was completely randomized with seven replications for each concentration. Thereafter, the number of the sclerotia were counted in each treatment every 24 h for 15 days.

Initially, all oils were previously bioassayed at 1500 ppm with *Sclerotium*-isolates in order to evaluating the mycelia growth inhibition. Then, a new screening was performed at low concentrations (300, 400, 500, 600, 700, 800, and 900 ppm) using only the oils that showed initiation of mycelia growth until 1500 ppm. In both bioassays, the experimental design was completely randomized with ten replications.

Suppression of oxalic acid diffusion (SOAD) bioassay

A 523 medium (Kado and Heskett, 1970), supplemented with streptomycin sulfate (150 ppm), penicillin G (150 ppm), and bromophenol blue (150 ppm), was utilized for SOAD bioassay. The pH was adjusted to 4.7 (adapted by Steadman et al., 1994). Essential oils were added to the medium at the lowest inhibiting concentration verified by previous bioassays. A 0.5-cm-diameter disk of the PDA medium with a five-day mycelium was deposited in the center of each plate and incubated in the BOD growth chamber at 28°C for a 12:12 h photoperiod. The negative control was oil-free. The bioassay was completely randomized with five replications. The capacity of each oil to alkalinize the medium was evaluated by restricting the oxalic acid diffusion produced by the pathogen, which was visualized by the formation of a yellow halo of inhibition. The measurements were taken from the diameter of the halo.

Validation assay of peanut protection against *S. rolfsii* greenhouse

Based on bioassay results, a validation assay was performed in order to test the effectiveness of the essential oils against *S. rolfsii* in greenhouse. The assay was carried out in conditions adjusted to 77-86% relative humidity and 39–45°C air-temperature.

Although no report of germination inhibition of peanut seeds due to use of essential oils has been found, a preliminary germination test was conducted with 100 peanut seeds using essential oils at 1000 ppm, in growth chamber during seven days. All seeds have normal germination and no occurrence of toxicity was found (data not shown).

Seeds of the cv. BR 1 were sown in pots (5 kg) containing commercial substrate (Baseplant) supplemented with 40 g P_2O_5 + 15 g KCl + 200 g of humus per kg of substrate. *S. rolfsii* was added to substrate at 72 $mg.kg^{-1}$. Three peanut seeds previously surface-sterilized (hypochlorite solution at 1.5%) were sown in each pot and after 15 days, just two plants were remained. Normal watering was maintained throughout trial.

The follows treatments were evaluated: NC, negative control (seed treated with water, oil-free), PC, positive control (seeds previously treated with commercial fungicide, oil-free), ST, seeds previously treated with essential oil at 400 ppm, ST/11- ibid + 11 weekly applications of oil at the same concentration, ST/9- ibid + nine applications of decennial oil at the same concentration, ST/6- ibid + six biweekly applications of oil at the same concentration, and ST/3- ibid + three monthly applications of oil at the same concentration.

In PC-treatment, a fungicide based on Carboxin + Thiram (250 mL/100 kg of seed) was used. To ST-treatment, seeds were kept for 30 min in contact with the oil and then were sown; in the others treatments involving spraying, the oil was mixed in the irrigation water.

The completely randomized design was adopted with five replications. At the harvest, the disease severity was estimated according to the scale described by Fery and Dukes (2002). The traits pod weight, number of pods/plant, and the harvest index index was also estimated. Harvest index (HI) was calculated as total pod yield/total biomass including pod weight at final harvest (Nigam et al., 2005).

Statistical analysis

Data were submitted to analysis of variance using Statistix (version 9.0). The Tukey test ($p < 0.05$) was used for average comparisons. Data from the DSI were previously tested to normality according to Shapiro-Wilk test and further transformed using the function [$\sqrt{(x + 0.5)}$].

RESULTS AND DISCUSSION

Four *S. rolfsii* isolates were bioassayed as to pathogenicity assays in peanut plants, in greenhouse. The first symptoms were verified 48 h after inoculation, which evolved to stem bottleneck and plant wilting with the presence of white mycelium. The disease severity index (DSI) caused by *S. rolfsii* in two peanut cultivars is shown in Table 2. The pathogenicity of isolates was more pronounced in plants grown in fertilized soil. Based on scale reported by Fery and Dukes (2002), the isolate SR5 showed high severity. Therefore, it was chosen for further assays.

The cv. BR 1 showed high sensitivity to *S. rolfsii* isolates (Figure 1). The DSI ranged from 26 to 98% when plants were grown in substrate and from 20 to 76%, in soil with fertilizer supplementation. The relative differences in disease severity due to fertilizer supplementation ranged from 22 to 67%, for BR 1, and 67 to 94%, to Senegal for 55 437, indicating that although BR 1 is more sensitive to the pathogen, fertilization contributed to alleviate the effect of the disease. These results confirm the findings in the literature that the incidence of infection caused by *S. rolfsii* is reduced in well-nourished plants (Basseto et al., 2007; Mascarenhas et al., 2003). Based on pathogenicity assays the isolate *S. rolfsii* - SR5 and the sensitive cv. BR 1 were chosen to further validation assay.

Up till now, no report of tolerance to *S. rolfsii* is Brazilian fields involving peanut commercial cultivar is found. Based on low DSI seen in Senegal 55437 (Table 2), we suggest that it may be a genetic resource with tolerance to white mold and further studies should be encouraged to attest that suggestion. The low severity of disease may be associated with high earliness and short cycle (only 75-80 days), limiting a fast spreading of fungus, especially in reproductive phase (Duarte et al., 2013; Boote and Hammond, 1981).

Mycelia inhibition of *S. rolfsii* with essential oils

These bioassays were carried out with SR5 grown in

Table 2. Disease severity index in peanut cultivars inoculated with *Sclerotium rolfsii* isolates.

Treatment	BR1		RD (%)	Senegal 55437		RD (%)
				RD (%)		
	S	S+F		S	S+F	
Control	0dA	0dA	-	0eA	0cA	-
SR5	98aA	76aB	22	24aA	8aB	67
SR14	76bA	36bB	53	14bA	4bB	71
SR15	72bA	24cB	67	4cdA	0.5cB	87
SR16	26cA	20cA	-	8cA	0.5cB	94
Coefficient of variation (%): 14.38						
General average: 5.14						
Standart error: 0.49						
Treatment square mean: 64.54 Freedom degree: 4						
F test: 117.94						

S, Substrate without fertilizer supplementation; S + F, with fertilizer supplementation. RD, relative difference in disease severity based on fertilizer supplementation. Original data transformed by √ (x + 0.5) for statistical analysis. Means with the same letters do not differ statistically by Tukey test ($p < 0.05$). Letters on the line (capitals) represent among-treatment comparisons; letters in the columns (lowercase) represent among-isolate comparisons.

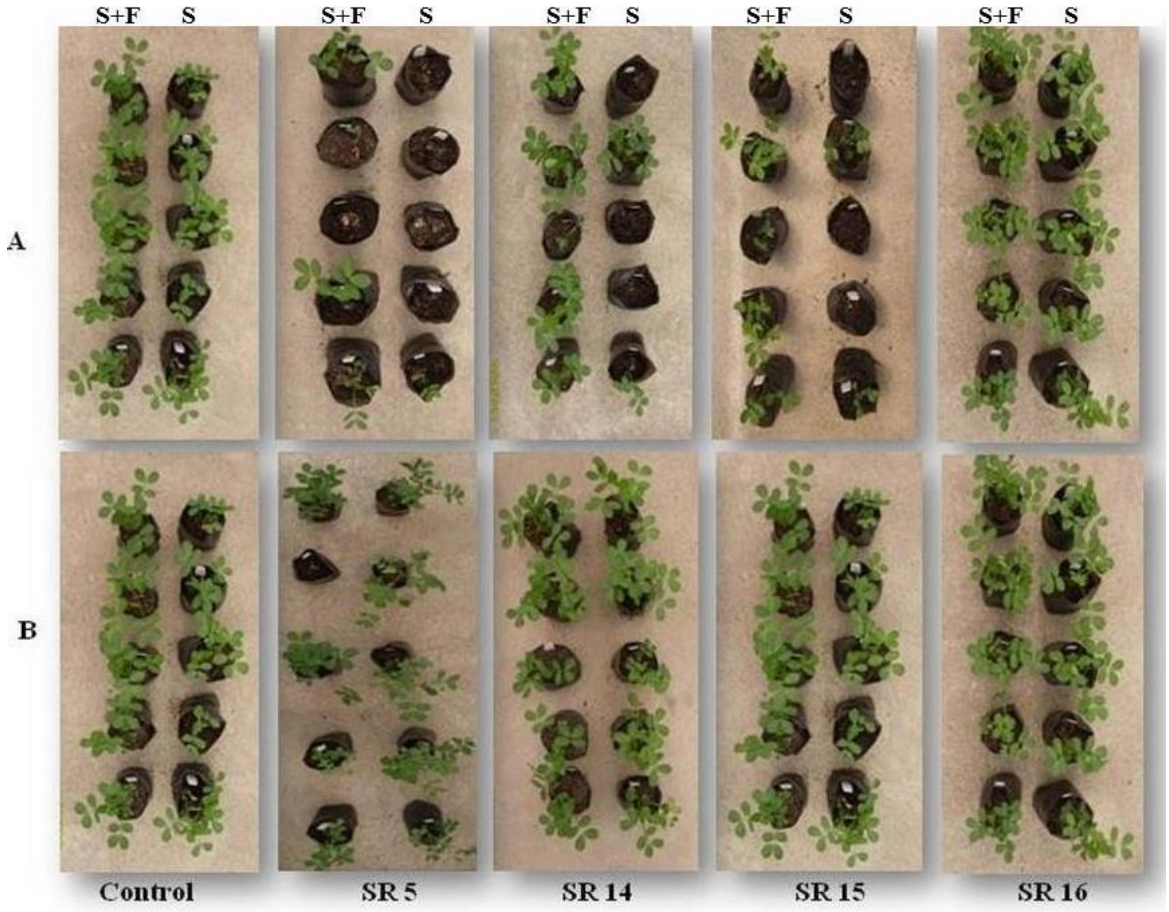

Figure 1. Pathogenicity assays in peanut plants carried out in greenhouse. **A.** BR 1. **B.** Senegal 55 437, S-Substrate, S+F- Substrate + fertilizer.

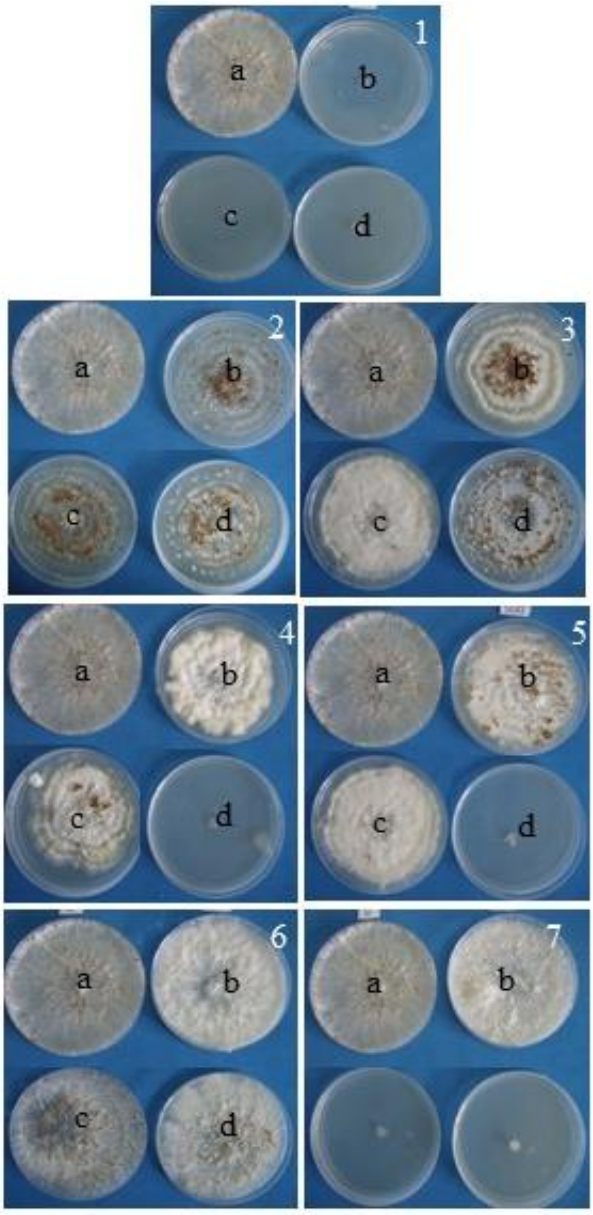

Figure 2. Inhibition of mycelial growth in SR5 *S. rolfsii* grown in (PDA) with several concentrations of essential oils; 1. *Cymbopogon martini*; 2. *Cedrus atlantic;* 3. *Copaifera officinalis*; 4. *Zingiber officinale;* 5. *Eucalyptus staigeriana*; 6. *Juniperus communis*; 7. *Ocimum basilicum*; concentrations were a. control (PDA), b. 500 ppm, c. 1000 ppm, and d. 1500 ppm.

PDA+ essential oils of seven species, at first at 1500 ppm, and then at low concentrations. Only *C. martinii* (Accession 1) inhibited mycelia growth (Figure 2) and sclerotia number (Table 3) in all concentrations. Therefore, oil from Accession 1 was chosen for further assays.

The antimicrobial action of *C. martinii* oil have been reported against several leave and soil pathogens, such as *Alternaria* sp., *Rhizoctonia solani, Aspergillus* sp., *Colletotrichum* sp., *Botrytis cinerea*, and others (Hillen et al., 2012; Stangarlin et al., 2011; Misra et al., 1988).

The biopesticide activity is mainly attributed to citronelal, geraniol and citronelol contents that also exhibit insecticide and nematicide effects (Barros et al., 2009; Hierro et al., 2004; Labinas and Cromo, 2002; Misra et al., 1988). *S. rolfsii* is a soil-born fungus, whose control is quite difficult and expensive. The possibility of control via no-chemical fungicide provides a reasonable perspective of healthy management to several host crops. Some reports have evidenced the control of white mold by using essential oil from *Origanum syriacum* L., *Foeniculum vulgare* Mill. and *Laurus nobilis* L. Mahato et al. (2014) evaluated the sensitivity of *S. rolfsii* towards some fungicides and botanicals and found that the inhibitory effects of different fungicides, essential oils and plant extracts are quite similar, situating at 86 to 95%.

Suppression of oxalic acid diffusion (SOAD)

Although no sclerotia was found at 300 ppm in isolate SP5, SOAD was performed with *C. martinii* oil at 400 ppm, taking in account a reliable safety margin for further recommendation. In this condition, no mycelia or sclerotia were found (Figure 3). The mycelia growth and halo of inhibition were reduced in about 70.5% (Table 4). These data support the bioassay results seen in Figure 2 and Table 3 and provide reliability to oil bioactivity. Inhibition assays by SOAD has been reported as a reliable test, in literature. Oxalic acid is naturally produced by the pathogen during parasitism of the host plant (Deacon, 1997; Kucey et al., 1989). This component combines with calcium, favoring the action of pectinolytic enzymes responsible to plant degradation (Deacon, 1997). According to Almeida et al. (2001), the production of oxalic acid may be one of the major factors contributing to wide host range of *S. rolfsii* and is associated with fast fungus development. The progressive accumulation of oxalic acid by fungus leads to a reduction in pH- growth medium, benefiting the formation of sclerotia (Rollins and Dickman, 2001; Maxwell and Lumsden, 1970).

Validation of control *S. rolfsii* with *C. martinii*

In order to confirm the results obtained in bioassays with *C. martini,* a validation assays was carried out in greenhouse. Plant of negative control (seed treated only with water, oil-free) showed characteristic symptoms of white mold, with DSI of 47% (Table 5). No statistical difference was found among oil treatments, whose DSI average was 21.42%, meaning a reduction in 55%, compared to control treatment. Based on these results we suggest that white mold can be controlled through direct seed treatment in a single dose of *C. martini* oil, minimizing others additional costs of application.

Table 3. Sclerotia number of *S. rolfsii* grown in PDA + *C. martinii* oil.

Treatment	Concentration (ppm)								
	0	300	400	500	600	700	800	900	1000
Accession 3	-	0.11b	0.10b	0.04b	0.50b	0.02b	0.02b	0.01bbb	0.02b
Control	113.5a								
Coefficient of variation (%): 2.82									
General average: 4.24									
Standart error: 0.51									
Treatment square mean: 35.32; Freedom degree: 8									
F test: 23.51									

Means with the same letters do not differ statistically by Tukey test ($p < 0.05$). Original data transformed by $\sqrt{(x + 0.5)}$ for statistical analysis.

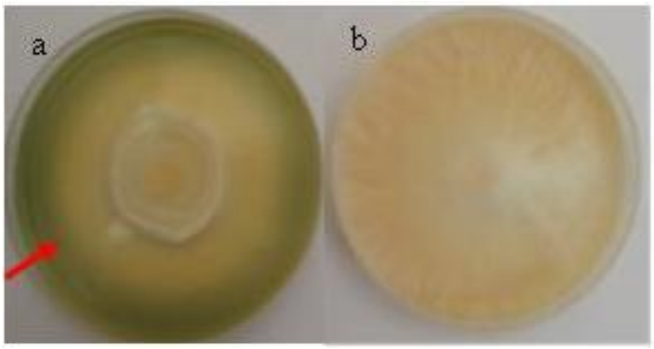

Figure 3. Inhibition of the suppression of oxalic acid diffusion released by *S. rolfsii* in (PDA. **a.** Halo of inhibition with *C. martinii* at 400 ppm (arrow). **b.** Control.

Table 4. Inhibition of the suppression of oxalic acid diffusion in *S. rolfsii* in PDA+ *C. martinii* oil.

Treatment	Mycelia growth (mm)	Halo of inhibition (mm)	Reduction (%)
PDA+ *C. martinii* oil.	26.26a	35.78a	70.5
Control (PDA)	88.75b	1.25b	1.4
Coefficient of variation (%)	4.15	2.64	
General average:	7.28		
Standart error:	1.24		
Treatment square mean:	18.43	Freedom degree: 1	
F test:	201.52		

No statistical differences were found to agronomical traits in fungicide and oil treatments. The control of disease in both treatments allowed gains of 57, 54, and 40% to number of pods, pod weight, and harvest index, respectively. Figure 4 shows a detail of pod production in control and ST treatment. The importance of this result lies in the economic and environmental aspects since *C. martini* oil is cheaper than synthetic fungicides and does not promote environmental damage.

Several studies in literature have highlighted the potential of vegetal essential oils to control plant pathogens. In this study, we confirmed the viability of *C. martinii* oil to control *S. rolfsii*, based on *in vitro* and *in vivo* assays. The trails carried out herein addressed pathogenicity, biochemical (by SOAD) and agronomical assays in order to confirm the effectiveness of *C. martinii* oils against white mold disease. Considering the complexity to control *S. rolfsii* in field, information

Table 5. Disease severity index (DSI) and agronomical traits of BR 1treated with *C. martinii* oil at 400 ppm.

Treatment	DSI (%)	Mature pods/plant		Harvest Index (%)
		Number	Weight (g)	
NC	47.17a	7b	6.7 b	26.53b
PC	13.40c	10a	9.6a	37.93a
ST	21.01b	11a	10.5a	36.91a
ST/11	20.73b	10a	9.5a	35.06a
ST/9	20.80b	10a	9.6a	38.20a
ST/6	21.50b	10a	9.6a	36.84a
ST/3	22.22b	12a	11.4a	37.75a
Coefficient of variation (%)	9.45	12.61	10.78	17.32
General average:	28.12	10.38	10.05	38.46
Standart error:	0.07	0.40	3.51	9.09
Treatment square mean:	345.26	7.49	62.68	172.42
Freedom degree: 6				
F test:	48.86*	4.37*	5.34*	3.89*

Means with the same letters do not differ statistically by Tukey test ($p < 0.05$). NC: negative control (seeds treated with water, oil-free); PC: seeds previously treated with commercial fungicide (positive control, oil-free); ST: seeds previously treated with essential oil; ST/11- seeds previously treated with essential oil + 11 weekly applications of oil at same concentration; ST/9- ibid + 9 applications of decennial oil at the same concentration; ST/6- ibid + 6 biweekly applications of oil at the same concentration; and ST/3- ibid + 3 monthly applications of oil at the same concentration.

Figure 4. Pod production in peanut inoculated with *S. rolfsii*. A. control (seeds treated with water); B. seeds previously treated with essential oil at 400 ppm (ST treatment).

contained in this study provides an alternative to minimize the losses in peanut production and damaging to the environment.

Conflict of interests

The authors did not declare any conflict of interest.

ACKNOWLEDGEMENTS

The National Scientific and Technological Development (CNPq) is acknowledged for the financial support and grants.

REFERENCES

Abdolahi A, Hassani A, Ghosta Y, Javadi T, Meshkatalsadat MH (2010). Essential oils as control agents of postharvest *Alternaria* & *Penicillium* rots on tomato fruits. J. Food Saf. 30:341-352.

Adandonon A, Aveling TAS, Labuschagne N, Tamo M (2006). Biocontrol agents in combination with *Moringa oleifera* extract for integrated control of *Sclerotium*-caused cowpea damping-off and stem rot. Eur. J. Plant Pathol. 115:409-418.

Adeyemi MMH (2010). The potential of secondary metabolites in plant material as deterents against insect pests: A review. Afr. J. Pure Appl. Chem. 4:243-246.

Almeida AMR, Abdelnoor RV, Calvo ES, Tessnman D, Yorinori JT (2001). Genotypic diversity among brazilian isolates of *Sclerotium rolfsii*. J. Phytopathol. 149:493-502.

Bajpai VK, Kang SC (2010). Antifungal activity of leaf essential oil and extract of *Metasequoia glyptostroboides* Miki ex Hu. J. Am. Oil. Chem. Soc. 87:327-336.

Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008). Biological effects of essential oil: a review. Food Chem. Toxicol. 46(2):446-475.

Barbosa RNT, Halfeld-Vieira BA, Nechet KL, Souza GR (2010). Método para inoculação de *Sclerotium rolfsii* em tomateiro. Rev. Agro@mbiente 4:49-52.

Barros LA, Yamanaka AR, Silva LE, Vanzeler MLA, Braum DT, Bonaldo J, (2009). In vitro larvicidal activity of geraniol and citronellal against *Contracaecum* sp (Nematoda: Anisakidae). Braz. J. Med. Biol. Res. 42:918-920.

Basseto M A, Ceresini PC, Valerio Filho WV (2007). Severidade da mela da soja causada por *Rhizoctonia solani* AG-1 IA em função de doses de potássio. Summa. Phytopathol. 33:56-62.

Bastos CN, Albuquerque SB (2004). Efeito do óleo de *Piper aduncum* no controle em pós-colheita de *Colletotricum musae* em banana. Fitopatol. Bras. 29:555-557.

Boote KJ, Hammond LC (1981). Effect of drought on vegetative and reproductive development of peanut. Proc. Am. Peanut Res. Educ. Soc. 13:86.

Chen W, Viljoen AM (2010). Geraniol — A review of a commercially important fragrance material. S. Afr. J. Bot. 76:643–651.

Das K, Tiwari RKS, Shrivastava DK (2010). Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. J. Med. Plants Res. 4:104-111.

Deacon JW (1997). Modern mycology. (3rd ed.) Blackwell Science, Cambridge.

Duarte EAA, Melo Filho PA, Santos RC (2013). Características agronômicas e índice de colheita de diferentes genótipos de amendoim submetidos a estresse hídrico. Ver. Bras. Eng. Agríc. Ambient. 17(8):843–847.

Earnshaw DM, Mcdonald MR, Boland GJ (2000). Interactions among isolates and mycelial compatibility groups of *Sclerotium cepivorum* and cultivars of onion (*Allium cepa*). Can. J. Plant Pathol. 22:387-391.

Fery RL, Dukes PD (2002). Southern blight (*Sclerotium rolfsii* Sacc.) of cowpea: yield-loss estimates and sources of resistance. Crop Prot. 21:403-408.

Fery RL, Dukes PD (2011). Southern Blight (*Sclerotium rolf*sii Sacc.) of cowpea: Genetic characterization of two sources of resistance. Int. J. Agron. ID 652404:1-6.

Fiori ACG, Schwan-Estrada KRF, Stangarlin JR, Vida, JB, Scapim CA, Cruz MES, Pascholati SF (2000). Antifungal activity of leaf extracts and essential oils of some medicinal plants against *Didymella bryoniae*. J. Phytopathol. 148:483-487.

Galanihe LD, Priyantha MGDL, Yapa DR, Bandara HMS, Ranasinghe JADAR (2004). Insect pest and disease incidences of exotic hybrids chilli pepper varieties grown in the low country dry zone of Sri Lanka. Ann. Sri Lanka 6:99-106.

Hierro I, Valero A, Perez P, Gonzalez P, Cabo MM, Montilla MP, Navarro MC (2004). Action of different monoterpenic compounds against Anisakis simplex s.l. L3 larvae. Phytomedicine 11:77–82.

Hillen T, Schwan-Estrada KRF, Mesquini RM, Cruz MES, Stangarlin JR, Nozaki M (2012). Atividade antimicrobiana de óleos essenciais no controle de alguns fitopatógenos fúngicos *in vitro* e no tratamento de sementes. Rev. Bras. Plant. Med. 14:439-445.

Kado CJ, Heskett MG (1970). Selective media for isolation of *Agrobacterium, Corynebacterium, Erwinia, Pseudomonas* and *Xanthomonas*. Phytopathology 60:969-976.

Koul O, Walia S, Dhaliwal GS (2008). Essential oils as green pesticides: potential and constraints. Biopestic. Int. 4:63-84.

Kucey RMN, Janzen HH, Legget ME (1989). Microbially mediated increases in plant available phosphorus. Adv. Agron. 42:199-228.

Labinas MA, Crocomo WB (2002). Effect of java grass (*Cymbopogon winteranus)* essential oil on fall armyworm *Spodoptera frugiperda* (J. E. Smith, 1979) (Lepidoptera, Noctuidae). Acta Sci. 24(5):1401-1405.

Mahato A, Mondal B, Dhakre DS, Khatua DC (2014). *In vitro* sensitivity of *Sclerotium rolfsii* towards some fungicides and botanicals. Scholars Acad. J. Biosci. 2(7):467-471.

Mascarenhas HAA, Tanaka RT, Wutke EB, Braga NR, Miranda MAC (2003). Potássio para soja. Agron. 55:20-21.

Mata MF, Araújo E, Nascimento LC; Souza AEF, Viana S (2009). Incidência e controle alternativo de patógenos em sementes de mandacaru (*Cereus jamacaru* DC, Cactaceae) R. Bras. Bioci. 7(4):327-334.

Maxwell DP, Lumsden RD (1970). Oxalic acid production by *Sclerotinia sclerotiorum* in infected bean and in culture. Phytopathology 60:1395-1398.

Medice R, Alves E, Assis RT, Magno Júnior, Lopes EAGL (2007) Óleos essenciais no controle da ferrugem asiática da soja *Phakopsora pachyrhizi* Syd. & P. Syd. Cien. Agrotec. 31:83-90.

Melo RMCA, Melo Filho PA, Câmara MPS, Lima WG, Santos RC (2013). Preventive control of cotton ramulosis using clove oil at low concentration. Int. J. Agric. Sci. Res. 2:69-66.

Misra N, Batra S, Mishra D (1988). Antifungal efficacy of essential oil of *Cymbopogon martinii* (lemon grass) against *Aspergillus*. Int. J. Crude Drug Res. 26:73-76.

Nigam SN, Chandra S, Rupa Sridevi K, Manoha Bhukta A, Reddy GS, Nageswara Rao RC, Wright GC, Reddy PV, Deshmukh MP, Mathur RK, Basu MS, Vasundhara S, Vindhiya Varman P, Nagda AK (2005). Efficiency of physiological trait-based and empirical selection approaches for drought tolerance in groundnut. Ann. Appl. Biol. 146:433-439.

Ozgonen H, Akgul DS, Erkilic A (2010). The effects of arbuscular mycorrhizal fungi on yield and stem rot caused by *Sclerotium rolfsii* Sacc. in peanut. Afr. J. Agric. Res. 5:128-132.

Punja ZK (1985). The biology, ecology and control of *Sclerotium rolfsii*. Annu. Rev. Phytopathol. 23:97-127.

Reddy TY, Reddy VR, Anbumozhi V (2003). Physiological responses of groundnut (*Arachis hypogea* L.) to drought stress and its amelioration: A critical review. Plant Growth Regul. 41:75–88.

Rollins JA, Dickman MB (2001). pH signaling in *Sclerotinia sclerotiorum*: identification of a pacC/RIM1 homolog. Appl. Environ. Microbiol. 67:75-81.

Santos RC, Rego GM, Santos CAF, Melo Filho PA, Silva APG, Gondim TMS, Suassuna TF (2006). Recomendações técnicas para o cultivo do amendoim em pequenas propriedades agrícolas do Nordeste brasileiro. Embrapa Algodão-Circular Técnica: Campina Grande. 102:1-7.

Souza Junior IT, Sales NLP, Martins ER (2009). Efeito fungitóxico de óleos essenciais sobre *Colletotrichum gloeosporioides*, isolado do maracujazeiro amarelo. Biotemas 22(3):77-83.

Staley JT, Mortimer SR, Mestres GJ, Morecroft MD, Brown VK , Taylor ME (2006). Drought stress differentially affects leaf-mining species. Ecol. Entomol. 31:460-469.

Stangarlin JR, Kuhn OJ, Assi L, Schwan-Estrada KRF (2011). Control of plant diseases using extracts from medicinal plants and fungi. In: Méndez-Vilas A (ed) Science against microbial pathogens: communicating current research and technological advances. Formatex 3(1):1033-1042.

Steadman JR, Marcinkowska J, Rutledge S (1994). A semi-selective medium for isolation of *Sclerotinia sclerotiorum*. Can. J. Plant Pathol. 16:68-70.

Vukovic N, Milosevic T, Sukdolak S, Solujic S (2007). Antimicrobial activities of essential oil and methanol extract of *Teucrium montanum*. Evid. Based Complement. Alternat. Med. 4(S1):17-20.