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Bioactivity of basil (*Ocimum basicilum* L.) on control of the spider mite (*Tetranychus urticae* Koch.) in peanut

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Essential plant oils contain biopesticides that could be used to control many crop pests. *Tetranychus* spp. are mites that cause damage to several crops and are primarily controlled by synthetic pesticides. Literature showed that mites can be controlled with essential oils of plants containing eugenol. In this work, we evaluated the bioactivity of basil (*Ocimum basicilum*) accessions for peanut-spider mites control based on molecular, biochemical and agronomic assays. RNA from four basil accessions, previously chosen by divergence genetic analysis, were used to estimate the expression of eugenol synthase (EGS I) transcripts, by semiquantitative and polymerase chain reaction (qPCR) assays. Chromatography was, thereafter, performed in order to estimate the eugenol concentration. Feeding bioassays were performed using basil leaf extracts in order to estimate oviposition and mortality of spider mites females. Finally, a validation assay was carried out in greenhouse, using peanut plants previously infested with spider mites and weekly sprayed with basil water-extract. One basil accession, OVRS, revealed high phytotoxicity to spider mite females, at 15% water-extract. The mortality rate was 75% and complete inhibition of fecundity was found in BOD assays. In the greenhouse assay, the most severe damage due to mite infestations was found to plant height, number of pods and pod yield, which were reduced to 28, 53 and 52% in non-treated plants (control). Considering that basil is a short-cycle plant, with easy reproduction and management, these results represent an accessible alternative to organic control spider mites in peanut.

Key words: Basil, chromatography, eugenol, eugenol synthase, qPCR, spider mite.

INTRODUCTION

Synthetic pesticides are widely used to control pest crops due to high efficiency to plant protection. Despite this, the indiscriminate use of these products has led to several damages to mammals' health such as neurological, respiratory and reproductive effects, and cancer and to increase pesticide resistance of insect-pests (Gill and

Garg, 2014). Several plants produce a high diversity of secondary metabolites with biopesticide properties, including many nitrogen-free and nitrogen-containing compound. These biopesticides could be an alternative source of plant protectants for farmers that do not have access to synthetic pesticides. These compounds are synthesized in either a tissue-, organ- or developmental-specific way by specific biosynthetic enzymes (Facchini and De Luca, 2008).

Bioactive secondary metabolites and essential oils are considered to be part of the preformed defense system of higher plants (Reichling, 2009). Essential oils are mixtures of different lipophilic and volatile substances and are widely distributed in certain plant families. The biopesticide activity of essential oils against crop pests have been widely reported (Isman, 2006; Lima et al., 2008; Cosimi et al., 2009; Sertkaya et al., 2010; Coitinho et al., 2011). Eugenol (4-hydroxy-3-methoxy-allyl-benzene) is a phenyl propanoid found in many species, such as cinnamon (*Cinnamomum zeylanicum* L.), croton (*Codiaeum variegatum* L.), bay leaf (*Laurus nobilis* L.), basil (*Ocimum basilicum* L.), myrrh (*Commiphora myrrha* Nees), nutmeg (*Myristica fragmas* Houtt), pepper (*Piper nigrum* L.), sassafras (*Ocotea odorifera* Vell.), clove (*Syzygium aromaticum* L.), and others (Ueda-Nakanura et al., 2006; Mora et al., 2010; Wu et al., 2010; Tan et al., 2011). Clove fruits are known for containing high concentration of eugenol, between 80 and 95% (Escobar, 2002; Chaieb et al., 2007). In many aromatic species, however, eugenol is stored in glandular trichomes found in the leaves (Gang et al., 2001). The organic synthesis of eugenol occurs from phenylalanine, followed by eugenol synthase I (EGS I). Its sequence is available at the NCBI gene bank (National Centre for Biotechnology Information, www.ncbi.nlm.nih.gov), from *O. basilicum* (DQ372812.1), *Rosa chinensis* (JQ522949.1) and *Clarkia breweri* (EF467239.1), with an open read frame of 945, 1,151 and 957 bp, respectively (Koeduka et al., 2006; Koeduka et al., 2008; Yan et al., 2012). The expression of *EGS I* is genotype-dependent with higer activity in leaves and seeds (Vieira and Simon, 2000; Lima et al., 2008).

The biopesticide activity of eugenol has been reported in dipterous (*Aedes aegypti* L., Simas et al., 2004), coleopterous (*Sitophilus zeamais* Mots. and *Tribolium castaneum* H.; Huang et al., 2002, *Callosobruchus maculatus* Fabr, Pascual-Villalobos and Ballesta-Acosta, 2003, *Sitophilus oryzae* L. and *Rhyzopertha dominica* Fabr; Ogendo et al., 2008), lepidopterous (*Spodoptera littoralis* Bois., Farag et al., 1994; *Thyrinteina arnobia* Stoll, Soares et al., 2011) and also in pathogens such as *Lasiodiplodia theobromae* Pat., *Colletotrichum musae* Berk. & Curt., *Fusarium proliferatum* Matsushima (Ranasinghe et al., 2002) and *Botrytis cinerea* Pers. (Combrinck et al., 2011). Spider mites (*Tetranychus urticae* Koch, Acari: Tetranychidae) are tetranychids that cause damage to several crops including soybean (*Glycine max* L.), cotton (*Gossypium hirsutum* L.), and peanut (*Arachis hypogaea* L.), among others (Haile and Higley, 2003; Esteves Filho et al., 2010; Boubou et al., 2011).

According to Van Leeuwen et al. (2010) and Khajehali et al. (2011), the short life cycle of the mite associated to high female fertilization and haplo-diploid cytology contribute to a quick evolution of spider mites resistance to synthetic acaricides. The investigation of other means of control, using more agroecological methods may represent an attractive opportunity to control this pest in a way less aggressive to the environment. This work focused on an investigation of basil (*O. basilicum* L.) bioactivity against spider mites of peanut plants (*A. hypogaea*), based on molecular, biochemical and agronomic assays.

MATERIALS AND METHODS

Germplasm and genetic diversity

Nine Brazilian basil accessions were used in this work (Table 1). In order to identify contrasting genotypes, a genetic analysis was performed based on Inter-simple sequence repeat-polymerase chain reaction (ISSR-PCR). Seeds of each accession were seeded in pots containing soil previously fertilized (NPK, 10:10:10, ammonium sulfate, simple superphosphate and potassium chloride) and watered daily, in a greenhouse. After 30 days from emergence, young leaves were collected for DNA extraction (DNA Extraction kit Phytopure, GE Healthcare, U.S.A), following the manufacturer's recommendations. PCR assays were performed in a 25 µl final volume containing 20 ng of each basil DNA, 1.5 µl of $MgCl_2$ (25 mM), 2.5 µl of 10× reaction buffer, 0.5 µl of dNTP mix (10 mM), 0.8 µl of each ISSR primer (10 mM, Table 2) and *0.3* µl *of Taq DNA polymerase (*Fermentas, Ontario, *Canada*, 5 U/µL). The program had one denaturation cycle at 94°C/5 min, followed by 30 cycles of denaturation at 94°C/30s, annealing at 40°C/30 s and extension at 72°C/1 min. A final extension cycle was added at 72°C/5 min. The amplification products were stained with Blue Green Loading Dye (LGC Biotechnology, Cotia, Brazil) and loaded in agarose gel (0.8%). The fragments were photo documented (Bio-Imaging Systems – MiniBis Pro, Uniscience) for further genetic analysis. ISSR markers obtained from the accessions were scored for their presence ‘1’ or absence ‘0’ of bands for each primer. The binary data were used to estimate levels of polymorphism by dividing the polymorphic bands by the total number of scored bands. Pair-wise similarity matrix was generated by Jaccard’s coefficient, by using NTSYS-pc (Rohlf, 1992). A dendrogram was constructed by UPGMA method in order to identify the phenetic representation of accessions. The accuracy of clustering was evaluated by cophenetic correlation coefficient (CCC) and the significance of the groups was tested with 2,000 simulations. The GENES program (2013.0.5) was used for all analysis (Cruz, 2001).

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Table 1. Basil accessions used in this work.

Accession	Origin	Lat./Long/Alt.	Leaves		
			Length (cm)	Width (cm)	Color
OCG	Campina Grande (PB)	7°13'51"/35°52'54"/512 m	1.8	1.8	Green
OSJ	Brejo da Cruz (PB)	6°12'32"/37°21'6"/145 m	2.5	1.9	Green
OBA	Salvador (BA)	12°58'13"/38°30'45"/12 m	2.5	1.6	Green
OSP	Guarulhos (SP)	23°27'49"/46º32'1"/769 m	5.2	3.1	Green
ODF	Brazlândia (DF)	15°46'48"/47°55'45"/1130 m	3.3	2.7	Green
OCE	Barbalha (CE)	7°18'20"/39°18'9"/415 m	5.0	3.0	Green
OVRS	Farroupilhas (RS)	29°13'29"/51°21'4"/770 m	8.5	3.6	Green
ORRS	Farroupilhas (RS)	29°13'29"/51°21'4"/770 m	6.5	2.1	Purple
OPE	Vitória de S. Antão (PE)	8°6'50"/35°17'29"/162 m	2.5	2.2	Green

Table 2. ISSR primers from *UBC series,* used to genetic analysis of *Ocimum* accessions.

Primer	Sequence (5'→3')	Primer	Sequence (5'→3')
UBC-813	CTCTCTCTCTCTCTCTT	UBC-853	TCTCTCTCTCTCTCTCRT
UBC-820	GTGTGTGTGTGTGTGTC	UBC-858	TGTGTGTGTGTGTGTGRT
UBC-824	TCTCTCTCTCTCTCTCG	UBC-868	GAAGAAGAAGAAGAAGAA
UBC-827	ACACACACACACACACG	UBC-884	HBHAGAGAGAGAGAGAG
UBC-834	AGAGAGAGAGAGAGAGYT	UBC-892	TAGATCTGATATCTGAATTCCC

Expression of *EGS I* transcripts in basil accessions

Total RNA from leaves of each accession was extracted by using the Invisorb Spin Plant Mini Kit (Invitek, Berlin, Germany). Then, cDNA was synthesized (SuperScript III First-Strand Synthesis SuperMix for qRT-PCR, Invitrogen, Carlsbad, CA, USA) using 1 µg of each RNA. The reverse transcriptase was inactivated at 85°C for 5 min. Then, 2 U of RNase H was added to each sample and reactions were incubated at 37°C for 20 min. All procedures followed the manufacturer's recommendations. To semiquantitative expression of *EGS I*, reactions were performed in a 25 µl final volume containing 2 µl of cDNA (1 µg), 0.04 U *Taq* Polimerase (Fermentas, Ontario, *Canada*), 0.2 mM dNTP set (10 mM), 1.5 µl $MgCl_2$ (25 mM), 1× kit buffer (10×) and 0.8 µl of each forward (ATGGAGGAAAATGGGATGAAAAGC) and reverse (GGCTCTTCTGATCATCCTCTTC) *EGSI*primers (10 mM), designed from eugenol synthase sequence (DQ372812.1), deposited on NCBI. The real time PCR reaction was made as follows: initial denaturation at 95°C/7 min, followed by 35 cycles of denaturation at 94°C/1 min, annealing at 56°C/1 min and extension at 72°C/2 min. A final extension was added at 94°C/5 min. As a constitutive control, a pair of forward (GATRTTGTCATATCTGCACTTGCA) and reverse (GGCTCTTCTGATCATCCTCTTC) *β-actin* primers (10 mM) was used. Both *EGS I* and *β-actin* primers were designed to amplify a fragment of 0.5 kb. The amplicons were analyzed in agarose gel (0.8%) and photodocumented.

The relative expression of *EGS I* transcripts was estimated by qRT-PCR (Eco Real-Time PCR System – Illumina, SD, USA) using Evagreen kit (Biotium Inc., Hayward, CA, USA), according to manufacturer's instructions. The forward (GATRTTGTCATATCTGCACTTGCA) and reverse (G GGCTCTTCTGATCATCCTCTTC) *EGS I* primers were used at 10 mM. A two-step RT-PCR procedure was performed in all experiments. First, 95°C/15 min and 40 cycles of 95°C/20 s, 60°C/20 s and 72°C/20 s were performed. Then, a curve of denaturation (melting curve) was performed after the conclusion of the amplification at 95 and 60°C/15 s, rising 2°C/min until reaching 95°C. Forward (TTGCAGACCGTATGAGCAAG) and reverse (ATCCTCCGATCCAGACACTG) *β-actin* primers (10 mM) were used as a constitutive control. Both *EGS I* and *β -actin* primers were designed to amplify a fragment of 0.2 kb. All reactions were carried out with experimental triplicate and biological duplicate. The threshold cycle (Ct) and PCR efficiency was estimated by Real-time PCR Miner program (Zhao and Fernald, 2005). The analyses of gene expression were performed using the qBASEPlus program (Hellemans et al., 2007). The graphics, Cqs and Melt curves were automatically generated based on the normalization method with a reference gene, ΔΔCq (Livak et al., 2001). The expression pattern was estimated by relative quantification.

Chromatographic analysis of basil leaves

Fresh basil leaves from each accession were dehydrated and extracts were prepared using ethyl ether. Chromatographic analysis was performed with a flame ionization detector (Chromatograph gaseous Shimadzu 2010), equipped with RTX-1 capillary column (0.25 mm × 0.25 µm × 30 m). A volume of 2 µl was injected to each sample, with injector operating in splitless mode, using nitrogen as a carrier gas. The flow rate was maintained at 1 ml/min, with an initial programming of 50°C/5 min, increasing 6°C/min up to 250°C/10 min. The Kováts Index (KI) of samples was estimated by comparing with the retention time of alkane standards (C7 a C24), in the same chromatographic conditions described. The following equation was used: $KI = 100n + 100 [(Rt_x - Rt_n)/(Rt_N - Rt_n)]$ where Rtis the retention time, x is the compound in analysis, n is the carbon number of previous alkane to Trx, and N = n+1. The quantification was performed with injection of eugenol patterns, with dilutions in hexane HPLC at 100, 200, 800 and 1500 ppb. From each pattern sample, 1.0 µl were injected and analyzed in same

conditions of chromatographic parameters established to basil extracts. The retention time of eugenol was 17 min. The concentration of each compound was established in ppb.

Feeding bioassays with spider mite females

A *Tetranychus urticae* rearing was maintained in peanut plants (cv. BR 1), grown in greenhouse for further feeding bioassays. Spider mite females were fed on peanut leaves dipped into basil extract at different concentrations. Dehydrated basil leaves were diluted in ethanol (10 g/100 ml ethanol) and stored at RT for 24 h. Then, the extracts were filtered and concentrated on a rotary evaporator at 40°C to remove the solvent. The crude extract of each sample was diluted at 1, 5, 10 and 15%. Young peanut leaves (4 cm × 1.5 cm) were immersed for 10 s in each extract, dried on filter paper and placed in Petri plates (10 cm diameter) containing a polyethylene sponge and filter paper wetted in distilled water. Ten spider mite females were placed on each leaf. The plates were stored in BOD-growth chamber (Mod. BF2 CGFP 275, Biofoco, Brazil) at 25°C ± 1°C, relative humidity of 65 ± 10% and 12:12 h photoperiod. In control treatments, leaf discs were dipped into distilled water (control 1) and ethanol (control 2). The experimental design was completely randomized, with six treatments and four replications. Mortality rate and number of eggs were counted daily for 2 days (Siqueira et al., 2014).

Validation assay to control of peanut-spider mite in greenhouse

Seeds of peanut (cv. BR 1) were sown in pots (5 kg) containing clay loam soil previously fertilized (40 g P_2O_5 + 15 g KCl + 200g of vegetal humus). Two plants were maintained per pot. Fifteen days after emergence, each plant was infested with 20 females of spider mites. Plants were watered daily. Ten days after infestation, plant canopies were weekly pulverized (20 ml/plant) with water-based extract of fresh basil leaves (10%), for 9 weeks, using an atomizer (1 L, mod. 78605/050, Tramontina, Brazil). The experimental design was completely randomized with two treatments and five repetitions. Control plants were sprayed with water (20 ml/plant). In the greenhouse, temperature was 35°C ± 2°C, relative air humidity 58% ± 5% and photoperiod 12:12 h during the assay.

Statistical analysis

For statistical analysis, we used the statistical software's: R v2.14.1 (R Development Core Team, 2011), to verify the homogeneity of variances and normality of error; SISVAR v5.1 (Ferreira, 2007), for analysis of variance; and Scott-Knott to mean comparisons.

RESULTS

Genetic diversity of basil accessions

The polymorphism rate ranged from 50 to 91.6% as shown in Table 3. A rich band pattern was obtained, especially with UCB-813, UCB-824, UCB-868, UCB-834, UCB-853 and UCB-884 primers. Amplicons generated by UCB-813 and UCB-824 are shown in Figure 1. All PCR products were used to generate a similarity matrix for further clustering of accessions, which were represented by dendrogram in Figure 2. Four groups were formed by UPGMA method. Group 1 contained accessions OBA, OSJ, ODF, OCG and OPE, all of them are small green leaves, measuring from 1.8 to 3.3 cm length; groups 2 and 3 had only one accession each, OVRS and ORRS, with extra-large green and purple leaves, measuring 6.5 and 8.5 cm length, respectively; and group 4 had two accessions, OCE and OSP, both phenotypically similar for canopy and leaf traits. A panel with some morphological details of the accessions is shown in Figure 3. Based on clustering results, four accessions were chosen to represent the variability of basil genotypes and further use in molecular and biochemical assays: OVRS, OCG, ORRS and OSP.

Table 3. Polymorphism for the basil accessions generated by ISSR primers.

Primer	NB	NMB	PR (%)
UBC – 813	22	7	68.2
UBC – 824	17	4	76.4
UBC – 827	8	4	50.0
UBC – 834	16	3	81.2
UBC – 853	14	4	71.4
UBC – 858	4	2	50.0
UBC – 868	17	5	70.6
UBC – 884	12	1	91.6
UBC – 892	10	1	90.0

NB-Number of bands, NMB- Number of monomorphic bands, PR-Polymorphism rate.

Expression of *EGS I* transcripts by semiquantitative and qPCR assays

RNA from the four basil accessions selected by genetic analysis were used to estimate the expression of *EGS I* transcripts, based on semiquantitative and qPCR assays. *EGS I* transcripts were differentially expressed in all four basil accessions selected, as high expression in OVRS, median in OSP, and low in OCG and ORRS, based on the β-actin pattern shown in Figure 4A. These results were confirmed in qRT-PCR assays that estimated in 180× the level of *EGS I* transcript, from OVRS leaves shown in Figure 4B.

Quantification of eugenol in basil accessions by gas chromatography

Table 4A shows the standardization of eugenol by mass spectrometry based on retention time at different concentrations, and Table 4B shows eugenol concentrations from basil leaves in basil accessions, ranging from 12,635.6 ppb (OVRS) to 2,781.5 ppb (OCG), and

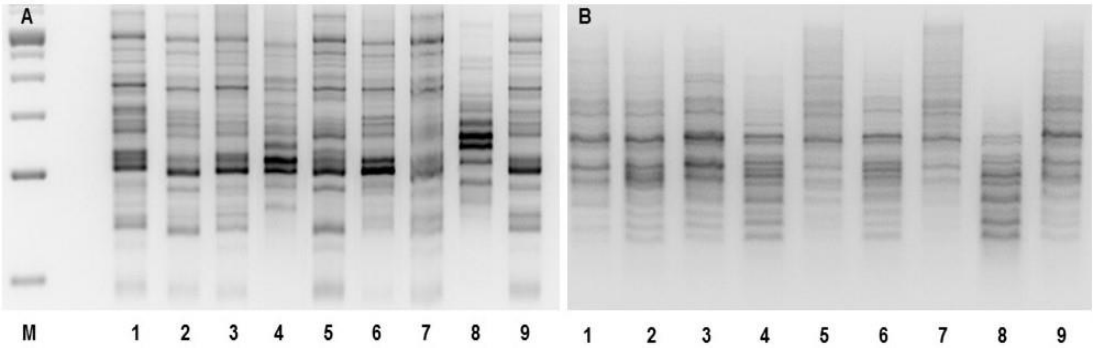

Figure 1. Amplicons generated with UCB 813 and UCB 824 ISSR primers from basil accessions. M- marker 1 kb DNA ladder (Invitrogen, CA, USA), 1- OSP, 2- ODF, 3- OSJ, 4- OCG, 5-OBA, 6- OVRS, 7- OCE, 8- ORRS, 9-OPE.

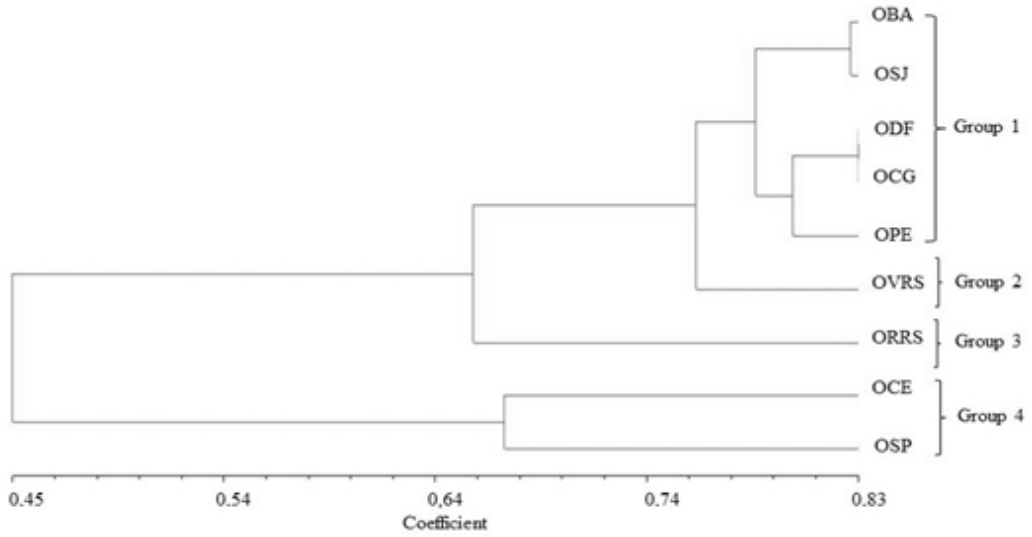

Figure 2. Dendrogram generated by UPGMA method based on ISSR-PCR amplicons from basil accessions. Cophenetic correlation coefficient: 0.87.

confirming that OVRS is different from other basil accessions selected in this study. The chromatograms of basil accessions are shown in Figure 5. No overlap peaks were observed, indicating a clear separation of the different compounds contained in the extracts analyzed. The highest peaks refer to eugenol concentrations in each sample. Only in OGC extract (Figure 5) a complex of compounds was observed, some of them with concentrations higher than eugenol.

Bioactivity of basil extract against spider mites in BOD

Leaf-ethanol extracts of basil accessions were used in feeding bioassays in order to evaluate the effect of eugenol in oviposition and mortality of female spider mites. It was found that the number of eggs and mortality rate of females were concentration-dependent. Data were fitted to a linear regression model shown in Figure 6. The extract obtained from OVRS leaves at 15% showed high phytotoxicity to females, with 75% of mortality rate and complete inhibition of fecundity. In previous bioassays using leaf-water extracts at 15%, the mortality rate of female spider mites were situated in 35% (OCG, OSP, ORRS) and 53% (OVRS). These results indicate that the organic components present in basil leaves were more soluble in ethanol.

Validation of spider mites control with basil leaf extract

Based on previous results obtained in molecular, biochemical and entomological assays, basil water-extract at 10%, from OVRS accession, was chosen to

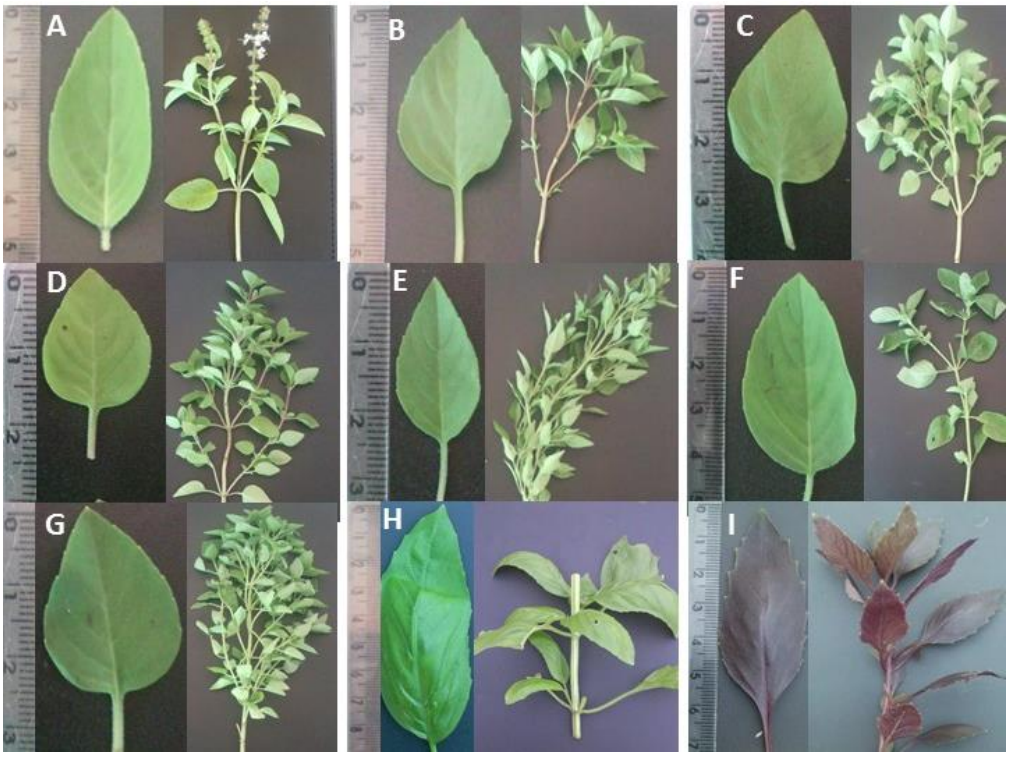

Figure 3. Morphological details of basil accessions used in this work. A- OSP, B- ODF, C- OSJ, D- OCG, E- OBA, F- OCE, G- OPE, H- OVRS, I- ORRS.

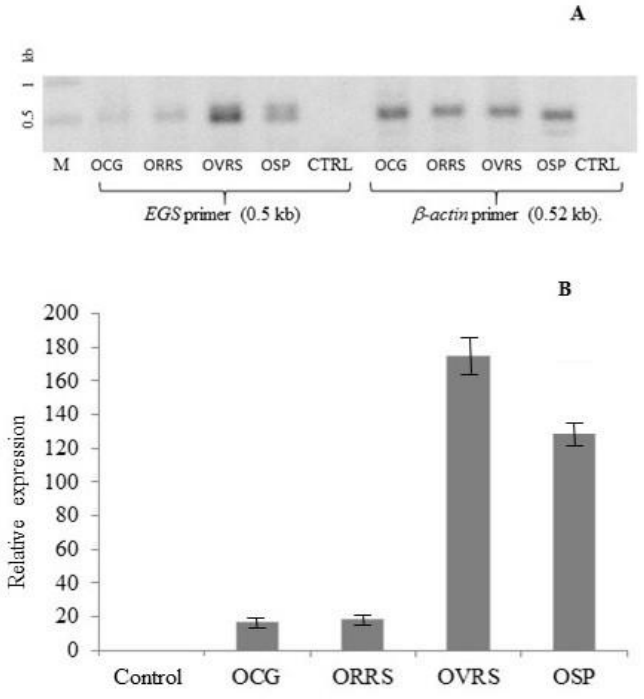

Figure 4. Semiquantitative (A) and relative (B) expression of *EGS I* transcripts in basil accessions. M-1kb DNA ladder (Invitrogen). Graphic generated by Eco Real-Time PCR System software (Illumina) from ΔCq and Melt curve data based on the *β-actin* normalization method.

Table 4. Concentrations of the standard (A) and calculated eugenol (B) in *Ocimum* accessions by gas chromatography.

Time RT (min)	Area(m)	Height (m)	Eugenol (ppb)
A			
17.216	418.23	157.23	100
17.205	1,617.9	599.5	200
17.204	4,297.03	1,572.2	800
17.195	11,522.33	3,709.66	1,500
B			
Accession	**Time RT (min)**	**Area**	**Conc. (ppb)**
OCG	17.215	21,366.8	2,781.5
ORRS	17.205	24,069.4	3,133.4
OSP	17.212	73,528.3	9,572.0
OVRS	17.199	97,061.6	12,635.6

*RT = Room Temperature.

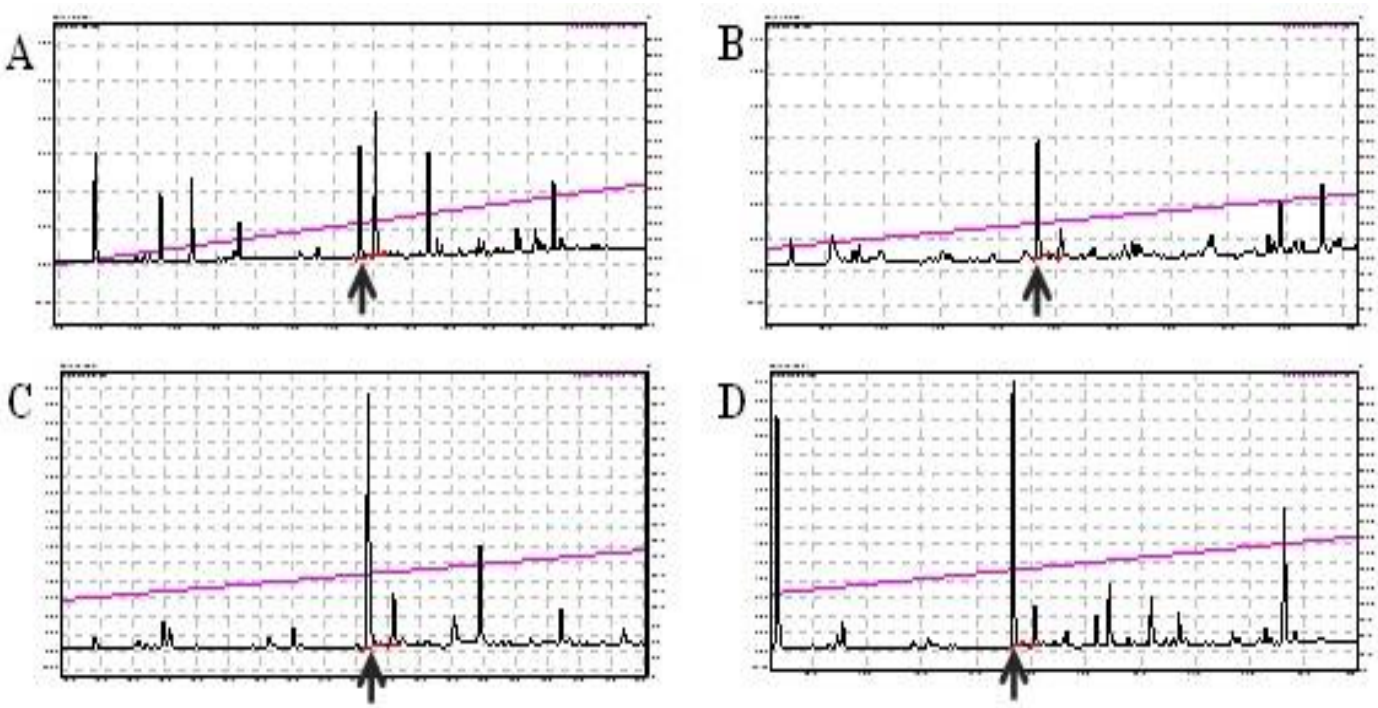

Figure 5. Chromatogram of basil accessions obtained by gas chromatography (GC) with Flame Ionization Detector (DID) for pattern eugenol concentrations. A- OCG, B- ORRS, C- OSP e D- OVRS. Arrows indicate peaks of eugenol.

validate trail against spider mites in peanut plants. It was found that the characteristic symptoms of spider mites in peanut plants were seen soon after one week of infestation. Damages by mites first involved chlorotic areas on leaflets, advancing to plant's death at the end of the life cycle, when high mite populations completely covered the canopy of plants involved in silk webbing. Figure 7 shows the performance of plants in control and treatment (basil-water extract), during three phenological phases, and pod production, at harvest. In general, the spreading of mites on basil-treated plants was slow until the seed formation period, which occurs between 60 and 65 days from planting in early genotypes. In same occasion, control plants were completely infested by mites, showing a severe reduction in photosynthetic area, at 85 days from planting. These plants showed senescence and leaf shedding, followed by wilting and plant death due to high level of infestation. In basil-

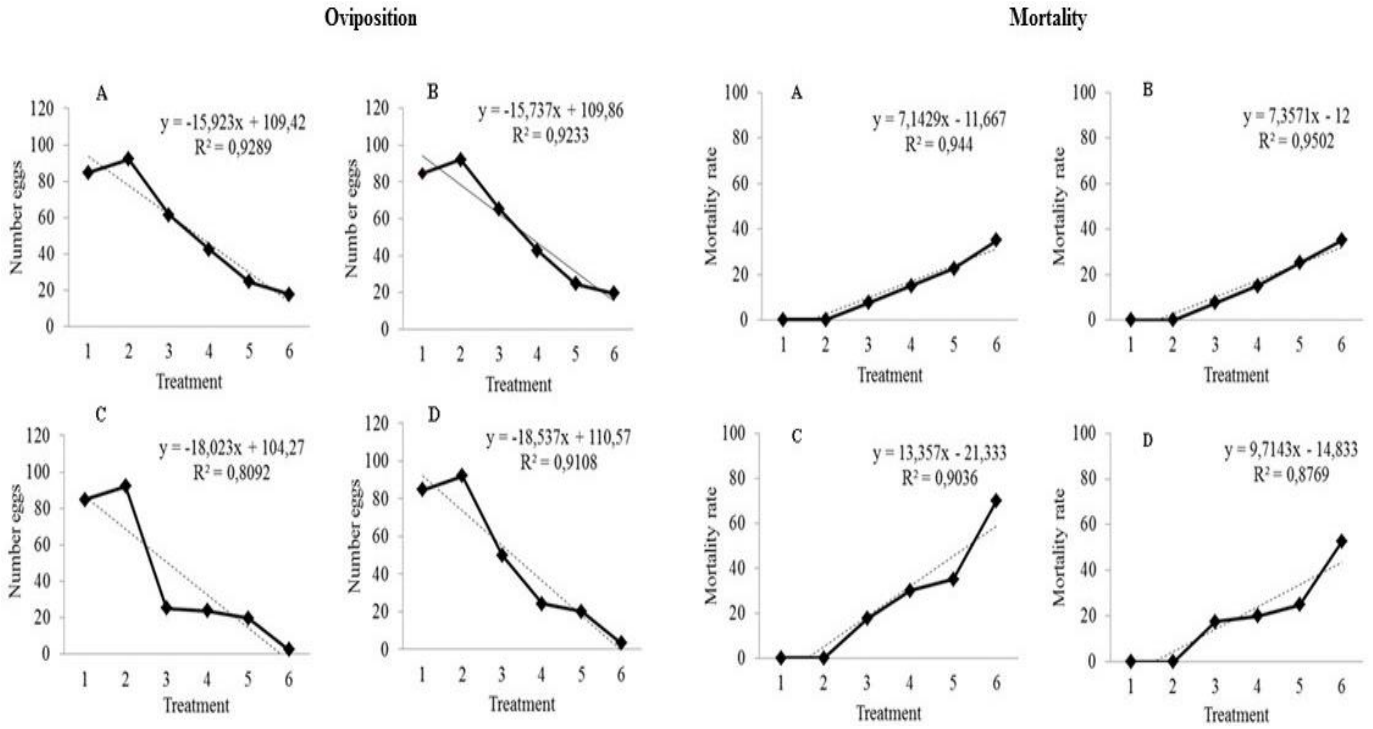

Figure 6. Number of eggs (left) and mortality rate (right) of female spider mites fed on peanut leaves dipped in leaf-ethanol extracts of basil for 48 h. A- OCG, B- ORRS, C- OVRS; D- OSP. Treatments: 1- control 1 (water), 2- control 2 (ethanol), 3 to 6-basil ethanol extract at 1, 5, 10 and 15%. The goodness of fit ($P < 0.001$) is found to each accession.

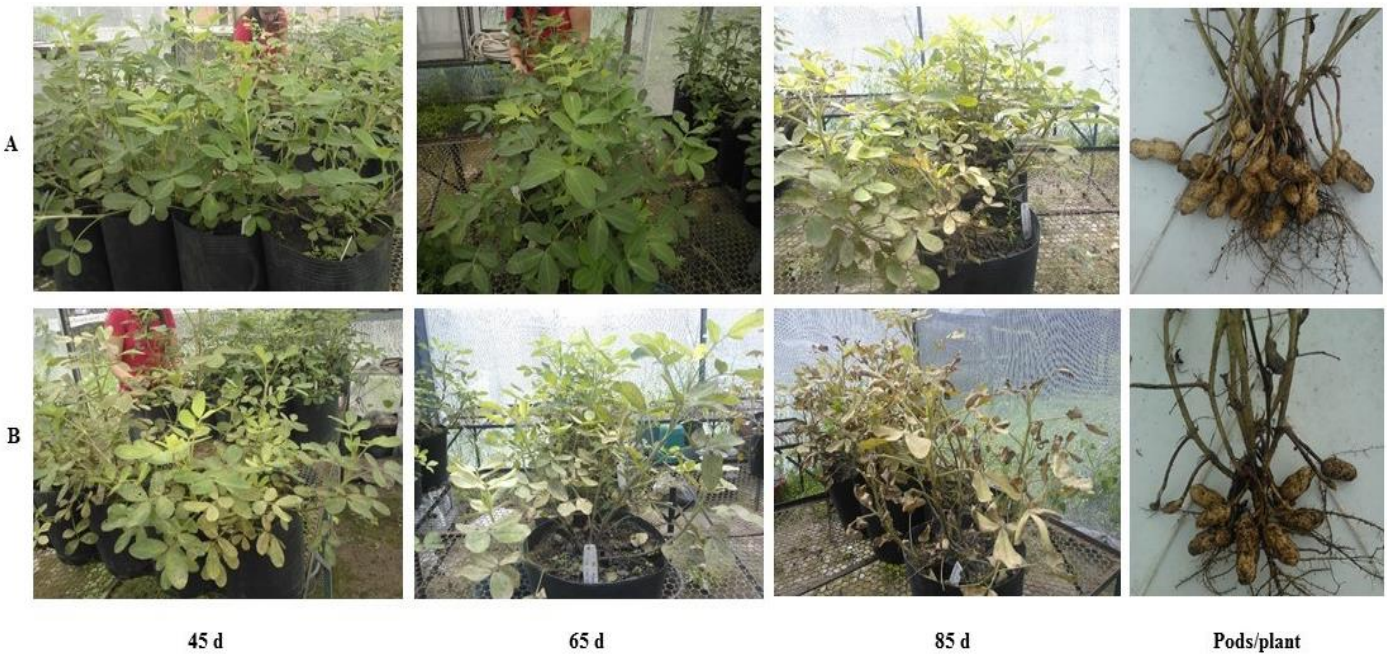

Figure 7. Performance of peanut plants infested with spider mite in greenhouse. A. Plants treated with basil-water extract at 10%; B. Plants treated with water (control).

treated plants, the spreading of mites was increased just from 80 to 85 d, but it did not commit pod production in these plants because maturation of pods was already established. The pod and seed weights, number of

Table 5. Growth and production data of peanut plants treated with basil water extract, in a greenhouse.

Treatment	HMA (cm)	B (dae)	NP	100 PW (g)	100SW (g)	S/P	PL (mm)	PWP (g)
Basil extract	39[a]	22[b]	19[a]	86[a]	45[a]	3.3[a]	3.6[a]	16.5[a]
Control	28[b]	25[a]	10[b]	84[a]	45[a]	3.4[a]	3.4[a]	8.5[b]
Mean	33	23	14	85	45	3.3	3.5	12.3
CV (%)	11.5	12.1	9.1	8.2	6.5	8.6	7.2	9.7

HMA- Height of main axis at 85d, B- blooming, NP- number of pods/plant, 100PW- 100 pods weight, 100SW- 100 seeds weight, S/P- number of seeds/pod, PL- pod length, PWP- pod weight/plant. Means with same letter are not statically different by Scott-Knott test ($p \leq 0.05$). Control treatment was water. CV- coeficiente of variation.

seeds/pod and pod length were not influenced by the spider mite infestation, in both control and basil-extract treatments (Table 5). The blooming was slightly delayed in control plants. The most severe damage due to mite infestations was found to plant height, number of pods and pod yield, which were reduced to 28, 53 and 52% in non-treated plants (control).

DISCUSSION

Spider mites are hosted by several commercial crops including soybean, peanuts, bean, corn, cotton and others, causing damage up to 70% at the commercial production of crops, depending on period of infestation (Haile and Higley, 2003; Oliveira and Moreira, 2009; Esteves Filho et al., 2010; Boubou et al., 2011). The spreading is fast, destroying photosynthetic area of plants, leading to dwarfism, shedding of leaves and fruits, and further losses in production. Despite the genetic improvements and tolerance of the new cultivars to spider mites, pesticides are still necessary as a control method. Therefore, the use of synthetic pesticides is still the main means of control, in spite of potential damage to humans and environment (Copping and Duke, 2007). Studies have shown that spider mites have a great ability to develop resistance to pesticides (Herron and Rophail, 2003; Khajehali et al., 2011). Considering the risks that it presents to agriculture, other strategies must be encouraged, so that the control could be more accessible and secure (Alabouvette et al., 2006). The literature offers a large number of articles reporting the biopesticide potential of extracts and essential oils from several plant species. The adoption of these products could contribute to reduction of management costs, and also environmental risks and dependency of synthetic pesticides (Mazzonetto and Vendramim, 2003; Lima et al., 2008).

The *Ocimum* genus is known for its aromatic and pesticide properties. The species are herbaceous or bushes, with an appreciable aroma in leaves and stems due to the components of essential oils, such as eugenol, methyl-chavicol, methyl-eugenol, saffron, geranial, thymol, linalool, among others (Blank et al., 2005). Eugenol has been reported as a main oil component. Basil is a short cycle plant, spread by seed or branches, and commercially used in culinary, cosmetics and as insect repellents. Several articles have demonstrated the role of extract and essential oil from basil to insect and pathogen controls, most of them focusing on assays to control temperature and photoperiod using BOD growth chamber. In the present work, molecular, biochemical and agronomic approaches were adopted in order to validate the use of basil to control spider mites in peanut plants (Pasay et al., 2010). Molecular analysis focused on *EGS I*, a precursor enzyme of eugenol biosynthesis.

Semiquantitative and relative expressions of *EGS I* transcripts revealed genotype-dependence response in basil accessions evaluated. In chromatography assays, eugenol was confirmed as the main oil component, with different peaks between accessions, confirming the RT-PCR findings. Ethanol-extract from four contrasting basil accessions, identified by genetic analysis, was used in feeding bioassays using spider mite females. Extract obtained from OVRS at 15% inhibited completely the reproduction of the spider mite eggs while promoting a mortality rate at 75%. This result is promising, however, considering the ability of spider mites to acquire tolerance to pesticides (Van Leeuwen et al., 2010; Khajehali et al., 2011), a validation trial was carried out in order to certify the results in natural condition. Peanut plants were previously infested with spider mites and further sprayed weekly with water-basil (OVRS) extract at 10%, during 9 weeks. Basil extract contained the mites spreading, maintaining the pod production in satisfactory level. It is possible that the population of spider mites in peanut plants from 85 d were minor, if we had used a higher concentration of basil-extract than that adopted in this work. However, as spreading of mites happened from 7th spraying, at full pod maturity phase of cv BR 1, it is suggested that basil-extract at 10% is an adequate concentration to earliness peanut cultivar (Vasconcelos et al., 2015; Santos et al., 2010). The result presented here has great potential use of basil leaf extract in mite control. The next step of the research is confirmation that eugenol is the main oil component essential through the isolation and identification of this active compound responsible for the control of insecticide.

Conflict of Interests

The authors have not declared any conflict of interests.

Abbreviation

PCR, Polymerase chain reaction; **ISSR,** inter simple sequence repeat; ***UBC,*** *University of British Columbia;* **UPGMA,** unweighted pair group method to obtain an arithmetic mean; **NCBI,** *National Center for Biotechnology Information;* **RT,** room temperature; **BOD,** biochemical oxygen demand; **DAE,** days after emergence.

REFERENCES

Alabouvette C, Olivain C, Steinberg C (2006). Biological control of plant diseases: the European situation. Eur. J. Plant Pathol. 114: 329-341.

Blank AF, Silva PA, Arrigoni-Blank MF, Silva-Mann R, Barreto MCV (2005). Influência da adubação orgânica e mineral no cultivo de manjericão cv. Genovese. Rev. Cienc. Agron. 36:175-180.

Boubou A, Migeon A, Roderick GK, Navajas M (2011). Recent emergence and worldwide spread of the red tomato spider mite, *Tetranychus evansi*: genetic variation and multiple cryptic invasions. Biol. Invasion 13:81-92.

Chaieb K, Hajlaoui H, Zmantar T, Nakbi, KAB, Rouabhia M, Mahdouani K, Bakhrouf A (2007). The chemical composition and biological activity of clove essential oil, Eugenia caryophyllata (*Syzigium aromaticum* L. Myrtaceae): a short review. Phytother. Res. 21:501-506.

Coitinho RLBC, Oliveira JV, Gondim Junior MGC, Câmara CAG (2011). Fumigation and toxicity, contact and ingestion of essential oils in *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) Ciênc. Agrotec. 35:172-178.

Combrinck S, Regnier T, Kamatou GPP (2011). *In vitro* activity of eighteen essential oils and some major components against common postharvest fungal pathogens of fruit. Ind. Crops Prod. 33:344-349.

Copping LG, Duke SO (2007). Natural products that have been used commercially as crop protection agents. Pest Manage. Sci. 63:524-554.

Cosimi S, Rossi E, Cioni PL, Canale A (2009). Bioactivity and qualitative analysis of some essential oils from Mediterranean plants against stored-product pests: evaluation of repellency against *Sitophilus zeamais* Matschulsky, *Cryptolestes ferrugineus* Stephens and *Tenebrio molitor* (L.). J. Stored Prod. Res. 45:125-132.

Cruz CD (2001). Genes program: computational applicative in genetics and statistics: Windows version. Viçosa: Eds. UFV. P. 648.

Escobar RG (2002). Eugenol: propriedades farmacológicas y toxicológicas, Ventajas y desventajas de su uso. Rev. Cubana Estomatol. 39:135-142.

Esteves Filho AB, Oliveira JV, Torres JB, Gondim Junior MGC (2010). Comparative biology and behavior of *Tetranychus urticae* Koch (Acari: Tetranychidae) and *Phytoseiulus macropilis* (Banks) (Acari: Phytoseiidae) in cotton Bollgard and no transgenic isoline. Neotrop. Entomol. 39(4):338-344.

Facchini PJ, De Luca V (2008). Opium poppy and Madagascar periwinkle: model non-model systems to investigate alkaloid biosynthesis in plants. Plant J. 54:763-784.

Farag AM, Boese CJ, Woodward DF, Bergman HL (1994). Physiological changes and tissue metals accumulation in rainbow trout exposed to foodborne and waterborne metals. Environ. Toxicol. Chem. 13:2021-2029.

Ferreira DF (2007). Programa Sisvar versão 5.1. Department of Exact Sciences, Federal University of Lavras, Minas Gerais, Brazil.

Gang DR, Wang J, Dudareva N, Nam KH, Simon J, Lewinsohn E, Pichersky E (2001). An investigation of the storage and biosynthesis of phenylpropenes in sweet basil (*Ocimum basilicum* L.). Plant Physiol. 125:539-555.

Gill KH, Garg H (2014). Pesticides: environmental impacts and management strategies. In. Larramendy ML and Soloneski S (Eds.), InTech. Pesticides toxics aspects. pp. 187-230.

Haile FJ, Higley LG (2003). Changes in soybean gas-exchange after moisture stress and spider mite injury. Environ. Entomol. 32:433-440.

Hellemans J, Mortier G, De Paepe A, Speleman F, Vandesompele J (2007). qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. Genome Biol. 8:119-126.

Herron GA, Rophail J (2003). First detection of chlorfenapyr resistance in two-spotted spider mite (Acari: Tetranychidae) from nectarines in an Australia orchard. Exp. Appl. Acarol. 31(2):131-134.

Huang Y, Ho SH, Lee HC, Yap YL (2002). Insecticidal properties of eugenol, isoeugenol and methyleugenol and their effects on nutrition of *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae) and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). J. Stored Prod. Res. 38:403-412.

Isman MB (2006). Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulatory world. Annu. Rev. Entomol. 51:45-66.

Khajehali J, Van Nieuwenhuyse P, Demaeght P, Tirry L, Van Leeuwen T (2011). Acaricide resistance and resistance mechanisms in *Tetranychus urticae* populations from rose greenhouses in the Netherlands. Pest Manage. Sci. 67:1424-1433.

Koeduka T, Fridman E, Gang DR, Vassão DG, Jackson BL, Kish CM, Orlova I, Spassova SM, Lewis NG, Noel JP (2006). Eugenol and isoeugenol, characteristic aromatic constituents of spices, are biosynthesized via reduction of a coniferyl alcohol ester. Proc. Natl. Acad. Sci. 103:10128-10133.

Koeduka T, Louie GV, Orlova I, Kish, CM, Ibdah M, Wilkerson CG, Bowman ME, Baiga TJ, Noel JP, Dudareva N (2008). The multiple phenylpropene synthases in both *Clarkia breweri*and *Petunia hybrida* represent two distinct protein lineages. Plant J. 54:362-374.

Lima RK, Cardoso MG, Moraes JC, Vieira SS, Melo BA, Filgueiras CC (2008). Composition of the essential oils from the Japanese star anise *Licium verum* L. and lemon grass *Cymbopogon citratus* (DC.) Stapf: evaluation of their repellent effects on *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae). Bioassay 3:81-85.

Livak KJ, Schmittgen, TD (2001). Analysis of relative gene expression data using real time quantitative PCR Method. Methods 25(4):402-408.

Mazzonetto F, Vendramim JD (2003). Vegetal Powder effect on *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae) in Stored Beans. Neotrop. Entomol. 32(1):145-149.

Mora A, Baby P, Roddaz M, Parra M, Brusset S, Hermoza W, Espurt N (2010). Tectonic history of the Andes and sub-Andean zones: implications for the development of the Amazon drainage basin. In. Hoorn F.P., Wesselingh C. (Eds.), Amazonia: Landscape and Species Evolution – A Look into the Past. Oxford. Pp. 38-60.

Ogendo JO, Kostyukovsky M, Ravid U, Matasyoh JC, Deng AL, Omolo EO, Kariuki ST, Shaaya E (2008). Bioactivity of *Ocimum gratissimum* oil and two constituents against five insect pests attacking stored food products. J. Stored Prod. Res. 44:328-334.

Pasay C, Mounsey K, Stevenson G, Davis R, Arlian L, Morgan M, Vyszenski-Moher D, Andrews K, McCarthy J (2010). Acaricidal activity of eugenol-based compounds against scabies mites. PLoS One. 5(8):e12079.

Pascual-Villalobos MJ, Ballesta-Acosta MC (2003). Chemical variation in an *Ocimum basilicum* germplasm collection and activity of the essential oils on *Callosobruchus maculatus*. Biochem. Syst. Ecol. 31(7):673-679.

R Development Core Team (2011) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Ranasinghe L, Jayawardena B, Abeywickrama K (2002). Fungicidal activity of essential oils of *Cinnamomum zeylanicum* (L.) and *Syzygium aromaticum* (L.) Merr et L.M.Perry against crown rot and anthracnose pathogens isolated from banana. Lett. Appl. Microbiol. 35(3):208-211.

Reichling J (2009). Plant microbe interactions and secondary

metabolites with antibacterial, antifungal and antiviral properties. Annu. Plant Rev. 39:214-347.
Rohlf JF (1992). NTSYS-pc: Numerical taxonomy and multivariate analysis system: version 1.70. New York. Applied Biostatistics.
Santos RC, Rêgo GM, Silva APG, Vasconcelos JOL, Coutinho JLB, Melo Filho PA (2010). Productivity advanced peanut lines under rainfed conditions in Northeast Brazil. Ver. Bras. Eng. Agr. Amb. 14(6):589-593.
Sertkaya E, Kaya K, Soylu S (2010). Acaricidal activities of the essential oils from several medicinal plants against the carmine spider mite (*Tetranychus cinnabarinus* Boisd.) (Acarina: Tetranychidae). Ind. Crops Prod. 31:107-112.
Simas NK, Lima EC, Conceição SR, Kuster RM, Filho AMO (2004). Natural products for control of dengue transmission – larvicidal activity of *Myrozylon balsamum* (red oil) and terpenoids and phenylpropanoids. Quim. Nova 27:46-49.
Siqueira FFS, Oliveira JV, Ferraz CS, Oliveira CRF, Matos CHC (2014). Acaricide activity of aqueous extracts of caatinga plants on the cassava green mite. Caatinga 27(4):109-116.
Soares CSA, Silva M, Costa MB, Bezerra CES (2011). Insecticidal action of essential oils on the defoliator caterpillar *Thyrinteina arnobia* (Stoll) (Lepidoptera: Geometridae). *G*reen Mag. Agro. Ecol. Sustain. Dev. 6:2.
Tan KH, Tokushima I, Ono H, Nishida R (2011). Comparison of phenylpropanoid volatiles in male rectal pheromone gland after methyl eugenol consumption, and molecular phylogenetic relationship of four global pest fruit fly species - *Bactrocera invadens*, B. *dorsalis*, B. *correcta* and B. *zonata*. Chemoecology 21:25-33.
Ueda-Nakamura T, Mendonça-Filho RR, Morgado-Díaz JA, Maza PK, Filho BPD, Cortez DAG, Alvino DS, Rosa M, Do SS, Lopes AHCS, Alviano CS, Nakamura CV (2006). Antileishmanial activity of Eugenol-rich essential oil from *Ocimum gratissimum*. Parasitol. Int. 55:99-105.
Van Leeuwen T, Vontas J, Tsagkarakou A, Dermauw W, Tirry L (2010). Acaricide resistance mechanisms in the two-spotted spider mite *Tetranychus urticae* and other important Acari: a review. Insect Biochem. Mol. Biol. 40:563-572.
Vasconcelos FMT, Vasconcelos RA, Luz LN, Cabral NT, Oliveira Júnior JOL, Santiago AD, Sagrillo E, Farias FJC, Melo Filho PA, Santos RC (2015). Adaptability and stability of genotypes erect grown peanuts in the Northeast and Midwest. Cienc. Rural 45(8):1375-1380.
Vieira RF, Simon JE (2000). Chemical characterization of basil (*Ocimum* ssp.) found in the markets and used in traditional medicine in Brazil. Econ. Bot. 54:207-216.
Wu G, Feng X, Stein L (2010). A human functional protein interaction network and its application to cancer data analysis. Genome Biol. 11:52-53.
Yan H, Wang H, Zhang H, Tang K (2012). Cloning and Expression Analysis of Eugenol Synthase Gene RcEGS1 in *Rosa chinensis* 'Pallida'. Acta Hortic. Sin. 39(7):1387.
Zhao S, Fernald RD (2005). Comprehensive algorithm for quantitative real-time polymerase chain reaction. J. Comput. Biol. 12:1047-1064.