Fumigation and repellency of essential oils against *Callosobruchus maculatus* (Coleoptera: Chrysomelidae: Bruchinae) in cowpea

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DOI: 10.1590/S0100-204X2017000100002

Pesq. agropec. bras., Brasília, v.52, n.1, p.10-17, jan. 2017

Abstract – The objective of this work was to assess the fumigant and repellent effects of essential oils on adults of *Callosobruchus maculatus* and to identify the chemical composition of two of the tested essential oils. For the fumigation test, the oils of *Schinus terebinthifolius*, *Piper aduncum*, *Syzygium aromaticum*, *Piper hispidinervum*, *Cymbopogon citratus*, *Cinnamomum zeylanicum*, and the eugenol compound were tested at different concentrations on *C. maculatus* adults. For the repellency test, the oils of *S. terebinthifolius*, *P. aduncum*, *P. hispidinervum*, *S. aromaticum*, *Jatropha curcas*, and *Ricinus communis* were evaluated. In the fumigation test, it was observed that *P. aduncum* and eugenol showed the highest and lowest LC₅₀, of 169.50 and 0.28 µL L⁻¹ air, respectively. In the repellency test, the oils of *S. aromaticum* and *P. hispidinervum* were repellent to *C. maculatus*. Gas chromatography coupled to mass spectrometry (GC-MS) analysis of these two oils identified 42 compounds, of which safrole was the main component of *P. hispidinervum* and eugenol of *S. aromaticum*. The essential oils of *S. aromaticum*, *C. zeylanicum*, and the eugenol compound are the most promising to control *C. maculatus*, via fumigation.

Index terms: *Vigna unguiculata*, cowpea weevil, natural insecticides, stored grains.

Introduction

Cowpea [*Vigna unguiculata* (L.) Walp.] is one of the most consumed grains as food, especially in the Northeastern region of Brazil. However, a significant amount of grains and seeds are lost during storage due to stored-grain pests (Almeida et al., 2004), among which the cowpea weevil, *Callosobruchus maculatus* (Fabr.) (Coleoptera: Chrysomelidae: Bruchinae), stands out. This species is a primary pest and internal feeder, which lays eggs that adhere to grain surface and whose infestation starts in the field, before harvest,
intensifying in the stored product, even causing total losses (Medeiros et al., 2007). Fumigant insecticides are the most commonly used against this species on grains and seeds. However, in Brazil, there is only one insecticide currently registered for *C. maculatus* control in postharvest cowpea (Agrofit, 2015), showing the need for alternative chemical control methods using synthetic insecticides.

In general, essential oils can be used as fumigants, as contact and systemic insecticides, and as repellents in stored-grain pest control (Bakkali et al., 2008), opening new perspectives for the management of these pests. Studies on essential oils as pesticides against *C. maculatus* have already been conducted for different plant species. *Eucalyptus staigeriana*, *Foeniculum vulgare*, *Eucalyptus citriodora*, and *Cymbopogon winterianus*, for example, showed insecticidal action from 178.13 to 345.57 ppm, with the last two species presenting potential repellent activity (Gusmão et al., 2013). Brito et al. (2006) evaluated the toxicity of essential oils from *Eucalyptus* spp. and observed reductions in lethal time and lethal concentration 50 (LC₅₀) with the increase in exposure time and doses. The essential oils of plants from the *Citrus* genus and its components have also shown insecticidal activity against this pest (Dutra et al., 2016). Ketoh et al. (2006) found that the essential oil of *Cymbopogon schoenanthus* was effective as a fumigant, with LC₅₀ of 2.7 µL L⁻¹ air.

The essential oils of plants are, therefore, promising for *C. maculatus* control in storage units (Pereira et al., 2008). It should be noted that, although essential oils may contain hundreds of different components, some of them are present in larger quantities. This shows that it is necessary to have a better understanding of these components and to test them separately, in order to determine if they are responsible for the toxic effects of these oils on *C. maculatus*.

The objective of this work was to assess the fumigant and repellent effects of essential oils on adults of *C. maculatus* and to identify the chemical composition of two of the tested essential oils.

**Materials and Methods**

The experiments were conducted at the Agricultural Entomology Laboratory of the Plant Protection Area of Universidade Federal Rural de Pernambuco, in a climatized room with a 12-hour photoperiod.

Insects were raised on grains of the cowpea cultivar BRS Guariba, belonging to the Sempre Verde group, packed in 1-L glass containers that were closed with perforated lids lined with voile fabric, to allow aeration. After confinement for three days in the containers, grains were sieved and insects separated. Then, the grains were returned to the containers and kept in the laboratory until the emergence of adults. The procedure was carried out with successive generations, in order to ensure the amount of adults necessary to perform the experiments.

The grains used in bioassays were first placed in plastic bags and stored in a freezer at -10°C for seven days, to eliminate any external insect infestation and to promote grain moisture balance. After removal from the freezer, all grains were transferred to glass flasks and kept in the laboratory, at room temperature, for ten days, in order to reach hygroscopic equilibrium.

Plant material from different origins and plant parts were used to obtain essential and fixed oils (Table 1). Biological material for essential oil extraction was collected from more than one plant from each species, and a sample per species was analyzed. The essential oils of *S. terebinthifolius*, *P. aduncum*, *S. aromaticum*, *P. hispidinervum*, *C. citratus*, and *C. zeylanicum* were obtained by the hydrodistillation method through a Clevenger-type apparatus. The oil was collected and dried with anhydrous sodium sulfate (Borsato et al., 2013). The fixed oils of *J. curcas* and *R. communis* were obtained by cold pressing. Subsequently, the oils were filtered and treated with emulsifier (Esteves Filho et al., 2013). The eugenol standard synthetic compound (98% purity) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

The fumigation test was conducted in chambers consisting of 2.5-L glass containers, in which *C. maculatus* females with up to 24 hours of age were confined. The following essential oils were tested at different concentrations (µL L⁻¹ air), obtained in preliminary tests: *C. citratus*, 0, 4, 6, 8, 10, 20, and 30; *S. terebinthifolius*, 0, 12, 16, 20, 24, and 28; *S. aromaticum*, 0, 0.1, 0.2, 0.5, 0.6, 0.8, and 1.2; *P. hispidinervum*, 0, 12, 14, 16, 18, 20, 22, and 24; *P. aduncum*, 0, 20, 36, 44, 52, 68, 84, and 100; *C. zeylanicum*, 0.0, 0.04, 0.1, 0.2, 0.6, 0.8, and 1.0; and eugenol, the major component of *S. aromaticum*, 0.0, 0.08, 0.12, 0.16, 0.2, 0.24, 0.28, and 0.32. Fumigation chambers were kept in the laboratory at 26.9±1.4°C, 65.7±2.6% relative humidity, and 12-hour photoperiod.

Pesq. agropec. bras., Brasilia, v.52, n.1, p.10-17, jan. 2017 DOI: 10.1590/S0100-204X2017000100002
The oils were impregnated using an automatic pipettor on filter-paper strips of 5x2 cm, fixed on the lower surface of the container lids.

Insects were placed in glass containers. For full sealing, PVC film and adhesive tape were placed on the edges of the container lids. The mortality rate was assessed 48 hours after the installation of the experiment. Probit analysis was performed using the Polo statistical software (LeOra Software, Berkeley, CA, USA), to calculate LC$_{50}$. Toxicity ratios were obtained individually by the quotient between the LC$_{50}$ of the least toxic essential oil and the LC$_{50}$ of the remaining oils. The oils were tested separately in a completely randomized design, consisting of eight treatments (oils and control) and six replicates.

The repellency test was conducted with the oils of *S. terebinthifolius*, *J. curcas*, *R. communis* (1.0 mL kg$^{-1}$), *P. aduncum*, *S. aromaticum*, and *P. hispidinervum* (0.5 mL kg$^{-1}$), in a climatized room at 26.9±1.04°C, 64.7±2.6% relative humidity, and 12-hour photoperiod. Oil testing was conducted individually in arenas formed by two plastic containers, whose lids were perforated for air circulation. The lids were connected by plastic tubes to a central plastic box (França et al., 2012), and the oils were transferred using an automatic pipette. Subsequently, containers were manually agitated for 1 min. Twenty grams of 'BRS Guariba' cowpea were placed in two different containers: one without any treatment (control) and the other impregnated by the oils in their respective concentrations. *Callosobruchus maculatus* females with up to 24 hours of age were released in the central box. After 48 hours, the insects attracted to each container were counted and discarded, and grains were transferred to other plastic containers. After 12 days, the number of eggs in each grain and the number of emerging adults in each container were counted. The oils were tested separately in a completely randomized design consisting of two treatments: one oil concentration and a control, using ten replicates. The average egg reduction and adult emergence percentage were calculated according to the formula adapted from Obeng-Ofori (1995). Results were subjected to analysis of variance, and means were compared by the t-test (p≤0.05) using the SAS software, version 8.02 (SAS Institute, Cary, NC, USA).

Essential oils with the highest and the lowest toxicity, according to LC$_{50}$, were selected and analyzed by gas chromatography coupled to mass spectrometry (GC-MS) on the Agilent 5975C Series GC/MS (Agilent Technologies Inc., Palo Alto, CA, USA), which incorporates a quadrupole system and is equipped with a nonpolar DB-5 column with 60 m x 0.25-mm inner diameter and 0.25-μm film thickness (Agilent J&W GC Columns, Agilent Technologies Inc., Palo Alto, CA, USA) (Brochini & Lago, 2007). A 1-μL solution of known concentration, containing its corresponding essential oil diluted in hexane, was injected at a split ratio of 1:20. In addition, a C9-C34 hydrocarbon pattern-mixture solution, consisting of commercial standards from Sigma-Aldrich (St. Louis, MO, USA), was also injected. GC temperature was set at 60°C for 3 min, and then increased at 2.5°C per min until reaching 240°C, which was held for 10 min. Helium flow was kept at a constant pressure.

| Scientific name                  | Common name     | Part used | Origin                                      |
|----------------------------------|-----------------|-----------|---------------------------------------------|
| *Syzygium aromaticum*            | Clove           | Dried flower buds | Stores in the municipality of Recife, in the state of Pernambuco, Brazil |
| Eugenol (major component of *Syzygium aromaticum*) | Eugenol         | -         | Sigma-Aldrich                               |
| *Schinus terebinthifolius*       | Brazilian pepper tree | Green fruits | Campus of UFRPE$^{(1)}$                     |
| *Cinnamomum zeylanicum*          | Cinnamon        | Leaves    | Campus of UFRPE                             |
| *Cymbopogon citratus*            | Lemon grass     | Leaves    | Campus of UFRPE                             |
| *Ricinus communis*               | Castorbean      | Seeds     | Fazenda Tamanduá, in the state of Paraíba, Brazil |
| *Piper aduncum*                  | Spiked pepper   | Leaves    | Embrapa Acre                                |
| *Piper hispidinervum*            | Long pepper     | Leaves    | Embrapa Acre                                |
| *Jatropha curcas*                | Physic nut      | Leaves    | Fazenda Tamanduá, in the state of Paraíba, Brazil |

$^{(1)}$UFRPE, Universidade Federal Rural de Pernambuco, in the state of Pernambuco, Brazil.
of 100 kPa. MS interface was set to 200°C, and mass spectra were recorded at 70 eV in El mode, with a scanning speed and range of 0.5 s and 20–350 m/z, respectively (Bezerra-Silva et al., 2016). The retention index was calculated for each oil component, which was subsequently confirmed by comparing its respective mass spectra with those available in GC-MS databases – MassFinder 4 (Dr. Hochmuth Scientific Consulting, Hamburg, Germany), NIST08 (National Institute of Standards and Technology, Gaithersburg, MD, USA), and the Wiley Registry of Mass Spectral Data (McLafferty, 2009) – and with those described by Adams (2009). Chromatogram peak areas were integrated and their values were used to determine the respective relative proportions of each compound.

Results and Discussion

The LC50 estimated for the essential oils of P. aduncum, P. hispidinervum, S. terebinthifolius, C. citratus, C. zeylanicum, S. aromaticum, and the eugenol compound varied between 169.50 and 0.28 μL L⁻¹ air (Table 2). The oils of C. zeylanicum, S. aromaticum, and eugenol had the lowest LC50, of 0.88, 0.73, and 0.28 μL L⁻¹ air, respectively. These low LC50 values showed the potential of these essential oils for the control of C. maculatus and the need for studying compounds that may cause mortality. Mahmoudvan et al. (2011) tested the fumigant effect of essential oils of several plant species on stored-grain pests and found that Citrus sinensis var. Hamlin had a good fumigant effect against Tribolium castaneum, Sitophilus granarius, and C. maculatus, with LC50 of 223.48 μL L⁻¹ air. This is a much higher concentration than that found in the present study, especially when compared with that of S. aromaticum, showing the potential of these oils for the control of C. maculatus.

The obtained toxicity ratios varied from 605.35 to 4.08. The oils of C. zeylanicum, S. aromaticum, and eugenol showed the highest toxicity ratios, of 192.61, 232.19, and 605.35, respectively, whereas P. hispidinervum had the lowest one, of 4.08. However, the concentration-mortality curve of the oil of P. hispidinervum, which had a slope of 8.44, exhibited the greatest inclination, indicating that small variations in the concentrations promoted high mortality responses (Table 2).

In tests with several monoterpenes extracted from plants, eugenol, at a concentration of 5 μL L⁻¹ air, caused 90% mortality in C. maculatus after 24 hours of exposure (Ajayi et al., 2014). The potential of eugenol among other monoterpenes is also confirmed by its toxicity ratio of 605.35. Therefore, eugenol was probably the monoterpene that allowed for a low dose to cause 50% mortality in the population of C. maculatus.

The number of C. maculatus eggs in grains was significantly reduced with all oils used, except for those of R. communis and J. curcas (Table 3). The oils of S. aromaticum and P. aduncum were the ones that reduced oviposition the most, also reducing adult emergence. Although S. terebinthifolius showed a reduction in oviposition of 35.24%, it reduced adult emergence in 60.85% (Table 4).

The number of C. maculatus adults attracted to cowpea grains treated with the essential oils of P. hispidinervum (p<0.0001) and S. terebinthifolius (p = 0.03) was significantly lower when compared with those of the untreated grains, indicating repellent effect (Figure 1).

Even though oviposition reduction has been lower when the oils of P. hispidinervum (44.67%) and S. terebinthifolius (35.24%) have been used, both also significantly reduced oviposition and adult emergence. However, J. curcas and R. communis

Table 2. Lethal concentrations (LC50) and toxicity ratios (TR) of essential oils on Callosobruchus maculatus adults(3).

| Treatment          | N  | Slope (±standard error) | LC50 (μL L⁻¹ air) at CI of 95% | TR50 | χ²  |
|--------------------|-----|-------------------------|---------------------------------|------|-----|
| Piper aduncum      | 400 | 1.01±0.13               | 169.50 (123.14–259.27)          | -    | 5.42|
| Piper hispidinervum| 280 | 8.44±0.94               | 41.46 (39.46–43.39)             | 4.08 | 4.40|
| Schinus terebinthifolius | 200 | 7.59±0.96               | 38.30 (35.28–40.95)             | 4.42 | 1.76|
| Cymbopogon citratus| 240 | 4.16±0.42               | 30.86 (27.54–34.97)             | 4.42 | 1.76|
| Cinnamomum zeylanicum | 240 | 1.57±0.19               | 0.88 (0.67–1.15)                | 192.61 | 2.19|
| Syzygium aromaticum | 240 | 1.97±0.25               | 0.73 (0.46–1.02)                | 232.19 | 5.10|
| Eugenol            | 280 | 3.43±0.45               | 0.28 (0.23–0.32)                | 605.35 | 0.81|

(3) N, number of insects used in the test; CI, confidence interval; and χ², Chi-square.

Pesq. agropec. bras., Brasilia, v.52, n.1, p.10-17, jan. 2017
DOI: 10.1590/S0100-204X2017000100002
Table 3. Effect of essential oils on the oviposition of *Callosobruchus maculatus* on cowpea (*Vigna unguiculata*) grains treated and not treated with essential oils\(^{(1)}\).

| Treatment                  | Concentration (mL kg\(^{-1}\)) | Oviposition (±standard error) | Reduction (%) |
|----------------------------|---------------------------------|------------------------------|---------------|
|                            | Control                         | Oil                          |               |
| *Schinus terebinthifolius* | 1.0                             | 23.60±3.90\*                 | 11.30±3.93    | 35.24         |
| *Syzygium aromaticum*      | 0.5                             | 120.60±13.75\*               | 19.50±11.39   | 74.16         |
| *Ricinus communis*         | 1.0                             | 104.00±27.87                 | 90.00±26.09   | 7.21          |
| *Piper aduncum*            | 0.5                             | 69.50±12.13\*                | 14.20±7.07    | 66.06         |
| *Jatropha curcas*          | 1.0                             | 109.30±14.85                 | 102.60±21.35  | 3.16          |
| *Piper hispidinervum*      | 0.5                             | 82.10±18.01\*                | 31.40±13.16   | 44.67         |

\(^{(1)}\)Fumigation chambers were kept at: 26.9±1.4ºC, 65.7±2.6% relative humidity, and 12-hour photoperiod. *Significant by the t-test, at 5% probability, when compared with the control.

Table 4. Effect of essential oils on reducing adult emergence of *Callosobruchus maculatus* in cowpea (*Vigna unguiculata*) grains, treated and not treated with essential oils\(^{(1)}\).

| Treatment                  | Concentration (mL kg\(^{-1}\)) | Emerged insects (±standard error) | Reduction (%) |
|----------------------------|---------------------------------|----------------------------------|---------------|
|                            | Control                         | Oil                              |               |
| *Schinus terebinthifolius* | 1.0                             | 18.90±4.33\*                    | 4.60±2.07     | 60.85         |
| *Syzygium aromaticum*      | 0.5                             | 111.60±14.60\*                  | 14.60±9.55    | 76.86         |
| *Ricinus communis*         | 1.0                             | 61.70±12.70                     | 66.00±15.35   | -3.67         |
| *Piper aduncum*            | 0.5                             | 55.90±11.32                     | 10.40±5.68    | 68.63         |
| *Jatropha curcas*          | 1.0                             | 84.40±12.46                     | 63.00±15.41   | 14.52         |
| *Piper hispidinervum*      | 0.5                             | 61.00±11.88\*                   | 23.50±10.20   | 44.38         |

\(^{(1)}\)Fumigation chambers were kept at: 26.9±1.4ºC, 65.7±2.6% relative humidity, and 12-hour photoperiod. *Significant by the t-test, at 5% probability, when compared with the control.

**Figure 1.** Number of *Callosobruchus maculatus* adults attracted to cowpea (*Vigna unguiculata*) grains treated and not treated with essential oils. The fumigation chambers were kept at: 26.9±1.04ºC, 64.7±2.6% relative humidity, and 12-hour photoperiod. \(^{\text{ns}}\)Nonsignificant. *Significant by the t-test, at 5% probability, when compared with the control.
did not cause repellency in adults or reduced oviposition and emergence (Tables 3 and 4). The essential oil of *P. aduncum* has not shown repellent effect on *C. maculatus* (*p* = 0.49), despite causing 100% mortality when applied to *C. maculatus* in the concentration of 50 μL per 20 g (Pereira et al., 2008), which contrasts with the results of the present study, in which *P. aduncum* was the least toxic oil.

GC-MS analysis of the essential oils with higher repellent effect showed 31 compounds in the essential oil of *P. hispidinervum* and 11 in that of *S. aromaticum*, totaling 98.33 and 99.22% identification of essential oils, respectively (Table 5). The following four compounds were common among these oils: α-copaene, E-caryophyllene, α-humulene, and δ-cadinene. However, 27 compounds were

| Compound | *P. hispidinervum* | *S. aromaticum* |
|----------|-------------------|-----------------|
| α-pinene | 932 (0.43%) | - |
| β-citronellene | 942 (0.06%) | 947 (0.02%) |
| Camphene | 946 (0.18%) | - |
| Myrcene | 988 (0.11%) | - |
| α-phellandrene | 1,002 (0.67%) | - |
| δ-3-carene | 1,008 (0.14%) | - |
| α-terpinene | 1,014 (0.19%) | - |
| O-cymene | 1,022 (0.06%) | - |
| Limonene | 1,024 (0.06%) | 1,027 (0.06%) |
| Sylvestrene | 1,025 (0.27%) | - |
| (Z)-β-oicimene | 1,032 (0.06%) | - |
| 2-heptyl acetate | 1,038 (0.60%) | - |
| (E)-β-oicimene | 1,044 (1.78%) | - |
| γ-terpinene | 1,054 (0.18%) | - |
| Terpinolene | 1,086 (5.71%) | - |
| Allo-oicimene | 1,128 (0.05%) | - |
| Benzyl acetate | 1,157 (0.01%) | - |
| P-cymen-8-ol | 1,179 (0.34%) | - |
| Safrole | 1,285 (82.07%) | - |
| δ-elemene | 1,335 (0.07%) | - |
| Eugenol | 1,356 (80.49%) | - |
| α-copaene | 1,374 (0.09%) | 1,377 (0.07%) |
| β-elemene | 1,389 (0.06%) | - |
| Methyl eugenol | 1,403 (0.06%) | - |
| α-gurjunene | 1,409 (0.03%) | - |
| E-caryophyllene | 1,417 (0.59%) | 1,422 (8.18%) |
| Aromadendrene | 1,439 (0.03%) | - |
| α-humulene | 1,452 (0.08%) | 1,457 (0.75%) |
| 9-epi-(E)-caryophyllene | 1,464 (0.14%) | - |
| Germacrene D | 1,484 (0.19%) | - |
| Bicyclogermacrene | 1,500 (3.16%) | - |
| γ-Cadinene | 1,513 (0.06%) | - |
| Eugenol acetate | 1,521 (9.53%) | - |
| δ-cadinene | 1,522 (0.16%) | 1,527 (0.03%) |
| Spathulenol | 1,577 (0.66%) | - |
| Caryophyllene oxide | 1,582 (0.07%) | 1,586 (0.07%) |
| Guaiol | 1,600 (0.04%) | - |
| Dillapiole | 1,620 (0.15%) | - |

**Table 5.** Components of the essential oils of *Piper hispidinervum* and *Syzygium aromaticum*.

1 Components are listed in order of elution in the DB-5 nonpolar column. 2 RI, retention index of Kratz according to the literature (Adams, 2009). 3 RI, retention index calculated through time retention in comparison with n-alkane series. -, undetected. %, relative percentage of the component found in the essential oil.
exclusive components of *P. hispidinervum* and 7 of *S. aromaticum*. Safrole (82.07\%) was identified as the major component of *P. hispidinervum*, and eugenol (80.49\%) of *S. aromaticum* (Table 2), in which it showed a chromatographic profile that was already described in previous studies (Sauter et al., 2011; Fayemiwo et al., 2014). Eugenol and safrole are described in the literature as bioactive for *Tribolium castaneum* and *Sitophilus zeamais*, important stored-grain pests (Coitinho et al., 2010).

When tested individually, the major component of *S. aromaticum*, eugenol, caused the highest fumigant effect on *C. maculatus*. Therefore, it has a lower lethal concentration in comparison with the compound mixture from the essential oil of *S. aromaticum* (Table 3). Some physiological effects of essential oils and their components suggest neurotoxic action in insects. Essential oils can affect octopaminergic target sites, reinforcing the hypothesis that they are strongly insect-specific, as is the octopamine neurotransmitter, having the ability to mimic octopamine action at low concentrations (Kostyukovsky et al., 2002). Linalool, a monoterpane, showed action on the nervous system, affecting ion transport and acetylcholinesterase release in insects (Re et al., 2000).

The essential oils of *S. aromaticum*, *C. zeylanicum*, and of the eugenol compound are the most promising, via fumigation, for the management of *C. maculatus* in stored cowpea, because they are more toxic at lower concentrations, showing both a greater efficiency against this plague, which has a high biotic potential, and a probable economic viability due to the use of lower concentrations. The oils of *P. hispidinervum* and *S. terebinthifolius* are the most promising for repellency, because, besides repelling adults, they reduce oviposition and adult emergence. These results provide subsidies for the management of *C. maculatus*, since the oils with fumigant and repellent effects can be used in the curative and preventive control of this pest, respectively.

**Conclusions**

1. The essential oils of *Syzygium aromaticum* and *Cinnamomum zeylanicum* are more toxic at lower concentrations and, therefore, the most promising, via fumigation, for *Callosobruchus maculatus* control in stored cowpea (*Vigna unguiculata*).

2. The essential oils of *Schinus terebinthifolius* and *Piper hispidinervum* are repellent to *C. maculatus* in cowpea.

3. The essential oils of *S. aromaticum* and *P. aduncum* are more effective in reducing oviposition and adult emergency of *C. maculatus* in cowpea.

4. Safrole is the major component of *P. hispidinervum*, and eugenol of *S. aromaticum*.

**Acknowledgments**

To Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, process No. 306288/2011-7), for the research productivity grant.

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Received on October 30, 2015 and accepted on July 28, 2016