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FUMIGANT TOXICITY OF *MELALEUCA LEUCADENDRON* L. AND *HYPTIS SUAVEOLENS* L. ESSENTIAL OILS AGAINST *PLUTELLA XYLOSTELLA* L. (LEPIDOPTERA: YPONOMETIDAE)

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ABSTRACT

The diamondback moth, *Plutella xylostella* L. (Lepidoptera: Yponomeutidae) is an economically important pests for cruciferous crops throughout the world. The management is commonly done based on repetitive applications of chemicals, resulting in environmental pollution and resistance in pest population.

In the present study, headspace of essential oil (EO) from *Melaleuca leucadendron* and *Hyptis suaveolens* were tested for toxicity and antifeedant effect against larvae of *P. xylostella* L. The two EOs obtained by hydrodistillation of *Melaleuca leucadendron* and *Hyptis suaveolens* were found to be toxic with LD_{50}s of 4.86 and 7.53 $\mu L.L^{-1}$ air and LD_{90}s of 14.47 and 18.22 $\mu L.L^{-1}$ air, respectively. For a given essential oil, LD_{50} values were significantly greater than respective LD_{90} values. Volatiles from both EO also exhibited antifeedant action against 4^{th} instar *P. xylostella* larvae. Long-term effect toxicity against 4^{th} instar larvae of *P. xylostella* has been evaluated. Both vapour phases reduced significantly the rate of emergence of adults. The chemical composition of vapour phase, obtained by headspace (HS) static method, and EO was determined by gas chromatography coupled with mass spectrometry. The main constituents found respectively in *M. leucadendron* EO and HS were 1,8 cineole (50.4%; 48.5%), viridiflorol (16.4%; 0.7%), α-pinene (12.4%; 6.8%) and α-terpineol (8.1%; 14.5%). Those identified in *H.*

ISSN: 2455-6939

Volume: 05, Issue: 06 "November-December 2019"

suaveolens EO and HS were sabinene (29.9%; 20.2%), β-caryophyllene (25.2%; 33.9%), α-pinene (6.6%; 4.0%) and terpinolene (5.5%; 7.3%). The biological effect of *M. leucadendron* and *H. suavolens* EOs on *P. xylostella* and their use in IPM strategy was discussed according to their chemical composition.

Keywords: Chemical composition; Essential oils; Headspace; *Hyptis suaveolens*; *Melaleuca leucadendron*; *Plutella xylostella* vapor phase toxicity.

INTRODUCTION

Plutella xylostella L. (Lepidoptera: Yponometidae), commonly called diamondback moth (DBM), is the most important pest for cruciferous crops throughout the world. The larvae feed on all cruciferous plants, either in fields or in greenhouses (Reddy et al. 2004). On a yearly basis, farmers spend more than US $ 1 billion to control this pest worldwide (Talekar 1992, Chaudhary et al. 2011). Control of this insect relies heavily on the use of synthetic insecticides and fumigants (Zhen et al. 2008). However, irrational use of chemical insecticides brings about serious implications for the environment and constitutes toxicity hazards on non-target organism and on farmers (Isman 2006). Hence, research has focused on identifying environmentally-friendly means to fight this pest. One approach is to extract phytochemical products from plants and use them as pest control agents with no or minimal side effects (Gomah and sahar 2011). Plants defend themselves against phytophageous pest by direct or indirect mechanisms. For direct defence, plants produce antifeedant, repellent, or deterrent compounds against herbivore insects (Karban and Baldwin 1997). For their indirect defence, plants diffuse volatile compounds to attract predators and parasitoids that help reduce crop pest damage (Vet and Dike 1992). These volatiles can then either be used as insecticides of natural origin and sprayed on plant leaves (Bonsignore and Vacante 2011), or the plants themselves can be employed as attractive or repellent plants in the push-pull approaches for crop protection (Marcel and Joop 2000) . During the past 20 years, essential oils (EO) have been widely investigated and tested in order to discover new sources of botanical insecticides and natural antifeedant compounds (Maia and Moore 2011). The use of EO extracted from aromatic plants is now considered as a promising alternative to protect crops, as far as their insecticidal properties towards different pests have been demonstrated (Kimbaris et al.2010, Ilboudo et al. 2010, Yang et al. 2010, Gomah and sahar 2011). Natural products, such as EO, are an excellent alternative to synthetic pesticides as a means to reduce negative impacts to human health and the environment. In previous studies, activities of botanical insecticides against Lepidoptera species have been well documented (Brewer et al. 1995, Zhen et al. 2008, Echereobia et al. 2010, Machial et al. 2010, Vasu et al. 2010). However, toxicity of the vapor phase or emission of volatile molecules contained in EO remains largely unknown.

ISSN: 2455-6939

Volume: 05, Issue: 06 "November-December 2019"

Melaleuca leucadendron L. (Myrtaceae), commonly named cajuput and originated from Southeast Asia was recently introduced in Côte d'Ivoire. In Cuba, Pino et al. (2010) reported that, the volatile compounds emitted by dried leaves of *Melaleuca leucadendron* were used as a mosquito repellent.

Various chemotypes of the EO extracted from *M. leucadendron* were described. The major components of plants grown in Cuba were 1,8 cineole and viridiflorol (Pino et al. 2002, 2010), 1,8 cineole and α-terpineol dominated the specie from Egypt (Farag et al. 2004) and Brazilian specie were characterized by viridiflorol (Silva et al. 2007).

Essential oil of *Hyptis suaveolens* L. (Poit) (Lamiaceae) has also been reported to have insecticidal effect against *Callosobruchus maculatus* (Ilboudo et al. 2010) and *Aedes aegypti* (Cavalcanti et al. 2004). *H. suaveolens* has also different chemotypes with various EO compositions (Cavalcanti et al. 2004, Beena et al. 2008, Grassi et al. 2008, Kossouoh et al. 2010, Moreira et al. 2010). The variability of the chemical composition of *H. suaveolens* EO leads us to justify an investigation on the biological activity of the EO of the species grown in Côte d'Ivoire.

The present work investigated the major chemical constituents of *M. leucadendron* and *H. suaveolens* essential oils vapour phase (HS) and their insecticidal and antifeedant effects on *P. xylostella*.

METHODS AND MATERIALS

Plant material and essential oil extraction

Plants samples were collected in April 2008 in two different geographical areas of Côte d'Ivoire: namely Bingerville (Abidjan), an area with a very humid climate and Yamoussoukro (6°49' N; 5°16' O) with a warmer and less humid climate. Plant samples of *M. leucadendron* were collected from the botanical garden of Bingerville and *H. suaveolens* from savannah areas surrounding Yamoussoukro. After harvesting, the plant material was air-dried at room temperature (28 ± 2°C). EO of each plant was obtained by steam distillation for 3 hours of 500 g of plant material, using a Clevenger type apparatus. The EO obtained was dried over anhydrous magnesium sulphate, and then stored in a tightly closed dark vial at 4°C until use in laboratory assays. EO yields were expressed as weight percentages of dried plant material.

Headspace solid phase microextraction (HS-SPME)

One µL of EO was dropped on a filter paper (Whatman n°1, 4.2 cm diam.). The filter was placed in the bottom of a desiccator (2.5 L) to allow EO volatile compounds (HS) to diffuse in the

ISSN: 2455-6939

Volume: 05, Issue: 06 "November-December 2019"

whole volume. Volatiles from this vapour phase were extracted during 3 hours, using a Mono Trap® disc absorbent (DCC18, GL Sciences Inc., Japan). The absorbed volatile compounds were then extracted from the Mono Trap® with 300 µL of dichloromethane solvent using ultrasonication for 5 min. The extracted solution was then analyzed by gas chromatography (GC) and GC coupled with mass spectrometry (GC-MS).

Chemical analysis

Analyses of EO and of their HS were performed on a GC (Varian, model CP-3380) equipped with a flame ionization detector (FID) and two fused silica capillary columns: HP5 column (5%-phenyl-methyl polysiloxane; 30 m x 0.25 mm i.d. x 0.25 µm film thickness), and Supelcowax-10 column (10% polyethylene glycol; 30 m x 0.25 mm i.d. x 0.25 µm film thickness). The carrier gas N_2 was set at 0.8 mL/min for both columns. The injector temperature was 220°C, the detector temperature was 250°C. The oven temperature programs were 60-200°C at 3°C/min, kept at 200°C during 20 minutes, for HP5 column analysis and 50-200°C at 5°C/min, kept at 200°C during 10 minutes for Supelcowax column analysis.

For both column analyses, 1 µL of EO was injected, with a split ratio 1:100. The linear retention indices (LRI) of every peak were determined relatively to the retention times of a series of *n*-alkanes (C9-C22) analysed in the same GC conditions.

GC-MS analyses were performed using a Hewlett-Packard GC 5890 series II, equipped with a capillary column HP5 (30 m x 0.25 mm, 0.25 µm film thickness) and with a capillary DB-Wax column (30 m x 0.25 mm, 0.25 µm film thickness) interfaced with a quadrupole detector (HP, model 5972). The oven temperature program was 60-200°C at 3°C/min, the injector temperature was 220°C and the MS transfer line temperature set at 250°C. The carrier gas was helium at 0.6 mL/min flow rate. The ionization voltage was 70 eV and the electronmultiplier was 1460 eV. The scan range was 35-300 amu at a scan rate of 2.96 scan/s. The injection was 1µL of EO (10% v/v, in CH_2Cl_2) with a split ratio 1:10 and 1µL of HS solution in a splitless mode.

The identification of the constituents was based on comparison of their relative retention times with either those of authentic samples or with published data in the literature (Adams 2007) and by matching their mass spectra with those obtained from authentic samples and/or the NBS 75K and Wiley 7th NIST 98 EPA/NIH libraries spectra and literature data (Adams 2007). The results are reported in Table 1.

The percentage compositions were obtained from electronic integration measurements from the GC-FID chromatograms on the apolar column without taking into account the relative response factors.

ISSN: 2455-6939

Volume: 05, Issue: 06 "November-December 2019"

Insects rearing

Insect culture of *P. xylostella* was maintained in the laboratory on cabbage plants (*Brassica oleracae* var. KK-cross) grown in plastic pots (20 x 16 x 18.5 cm containing local soil) were place inside oviposition cages. Rearing conditions were 28±2°C and 60±5% r.h. at photoperiod of 12:12 hours L/D. The oviposition cage consisted of transparent cubic Plexiglas's container (30 x 30 x 30 cm), with a fine-mesh net installed on top-side for ventilation. Larvae of *P. xylostella* were reared on the foliage of 5-6 weeks old cabbage plants. In the other hand to synchronize insect rearing, adult of *P. xylostella* were kipped on plants for 21 days. Early 4th instar larvae were used for toxicity test.

Fumigant toxicity assays

Toxicity of the essential oils was tested against the larvae of *P. xylostella* by using fumigation method in a glass desiccator (volume 2.5 liter) (Kouninki et al. 2005). The desiccators were separated in two compartments (1 and 2) by a perforated grid. For each essential oil, a series of sample concentrations was prepared by using a micropipette. A filter paper (Whatman n°1, 4.2 cm diameter) was treated with 2.5 µL, 5 µL, 7.5 µL, 10 µL, 20 µL and 40 µL of oil and placed at the bottom of the desiccator (compartment 2) from which the vapors of oil diffused in the whole chamber. Thus the 6 doses tested varied from 1 to 16 µL/L air. A glass Petri dish (9 cm diameter) containing a cabbage leaf disc on Agar-agar was then deposited in the compartment 1 on the grid. Bioassays conditions were 28±2°C and 60 ± 5% r.h. Twenty 4th instar larvae collected into a small tube were deposited onto each leaf disc in the Petri dish and the desiccator was immediately closed and sealed with wax. One more desiccator was used without EO and served as a control. The desiccators were held at 28 ± 2°C for 24h, after which, the mortality of insects was assessed. Larvae showing no sign of movement when touched with a brush were scored as dead. Each experiment was repeated six times.

After 24 h, the feeding areas were recorded by parchment paper and measured by transparent area measuring device to calculate the antifeedant rate.

After 24 h, the long-term effect of toxicity was determined by observing surviving larvae (after exposure to small doses of 1 µL/L air) maintained on the leaves of cabbage plant without any insecticide in the Petri dish for 6 days. The reduction rate of adult emergence (RE) was calculated relatively to those of the control.

$$\mathbf{RE = ([E - e]/E) \times 100}$$

E = *number of individuals emerged in the control*

ISSN: 2455-6939

Volume: 05, Issue: 06 "November-December 2019"

e = *number of individuals emerged in the test*

Data analysis

The lethal doses (LD) LD_{50} and LD_{90} were calculated using the software biostatistics Win DL version 2.0 CIRAD-CA. The significance of mean differences between essential oils and control was statically compared using an analysis of variance (ANOVA) at 5% probability level with individual pairwise comparison made using Turkey's HSD test through an SPSS version 15.0 software package in Microsoft Windows 7 operating system.

RESULTS

GC-MS analysis of EO and HS

The yields of EO of *M. leucadendron* and *H. suaveolens* were 1.4% (w/w dried material) and 0.4% (w/w dried material), respectively. In details, the major compounds of *M. leucadendron* in the EO and HS were 1,8 cineole (50.4%; 48.5%), α-pinene (12.4%; 6.8%) and α-terpineol (8.1%; 14.5%) respectively. The major compounds of *H. suaveolens* in the EO and HS were sabinene (29.9%; 20.2%), β-carophyllene (225.2%; 33.9%), β-pinene (6.6%; 4.0%), terpinolene (5.5%; 7.3%), and limonene (3.3%; 6.0%) respectively (Table 1).

Fumigant toxicity assays

Headspace from both EO proved toxicity against *P. xylostella* larvae with a LD_{50} of 4.86 $\mu L.L^{-1}$ air for *M. leucadendron* and 7.53 $\mu L.L^{-1}$ air for *H. suaveolens* (**Table 2**). After 24h of contact, the results showed a residual effect of the lower dose tested (1 $\mu L.L^{-1}$ air) of both EO (**Figure 1**). The rates of adult emergence were lower than the control after exposure to HS of both EO.

For antifeedant trials, a significant reduction of leaf consumption was observed when the dose of both EO was applied and exposure done for 24 h (**Table 3**). The antifeedant effect increased with a greater dose.

ISSN: 2455-6939

Volume: 05, Issue: 06 "November-December 2019"

Table 1: Chemical composition of essential oil (EO) and headspace (HS) from leaves of *M. leucadendron* and *H. suaveolens*

Constituents			*H. suaveolens*		*M. leucadendron*	
	LRI[a]	LRI[b]	EO	HS	EO	HS
α-Pinene	935	1011	2.1	0.9	12.4	6.8
Benzaldehyde	961	1572	-	-	0.5	3.1
Sabinene	975	1112	29.9	20.2	-	-
β-Pinene	982	1104	6.6	4.0	1.0	3.5
Myrcene	991	1152	0.3	2.1	-	-
Limonene	1030	1157	3.3	6.0	0.5	12
β-Phellandrene	1031	1202	0.3	3.5	-	-
1,8-Cineole	1031	1203	0.3	-	50.4	48.5
γ–Terpinene	1060	1232	0.7	0.7	0.3	0.6
cis-Sabinene hydrate	1068	1396	0.3	1.1	-	-
Terpinolene	1091	1267	5.5	7.3	0.2	0.4
Linalol	1097	1552	-	-	0.2	0.6
trans-Sabinene hydrate	1098	1468	0.2	-	-	-
α-Fenchol	1117	1595	-	-	0.2	0.3
trans-sabinol	1144	-	-	-	0.2	0.4
neo-isopulegol	1149	-	-	-	0.2	1.3
Terpinen-4-ol	1181	1551	1.0	3.2	0.8	2.0
p-Cymen-8-ol	1188	1846	0.1	1.2	-	-
α-Terpineol	1194	1686	0.1	1.1	8.1	14.5
β-Caryophyllene	1428	1576	25.2	33.9	0.6	0.5
Aromadendrene	1458	1615	0.2	0.8	0.2	-
α-Humulene	1469	1641	1.8	1.6	0.1	-
γ-Gurjunene	1486	-	-	-	0.1	0.3
β-Selinene	1499	1724	1.2	1.3	0.4	-
Viridiflorene+α-Selinene	1507	1733	-	-	1.3	0.3
γ-Cadinene	1525	1748	-	-	0.3	0.6
Spathulenol	1595	2107	1.4	-	-	-
Caryophyllene oxide	1603	1955	3.7	1.5	-	-
Viridiflorol	1614	2075	-	-	16.4	0.7
Yield (g/100g)			**0.4**	**-**	**1.4**	**-**
Total identified (%)			**84.2**	**90.4**	**94.4**	**96.3**

The components and the percentages are listed in order of their elution on the apolar column (HP_5)

a = Linear retention indices on HP_5 column (a 5%-phenyl-methylpolysiloxane phase); temperature program 60-200°C at 3°C/mn, then 200°C for 20 min. **b** = Linear retention indices on Supelcowax 10 (polyethylene glycol phase) temperature program 50-200°C at 5°C/min, then 200°C for 10 min. Methods of identification: GC, identification based on co-injection with authentic sample, MS, identification based on comparison of mass spectrum with literature data, LRI, identification based on comparison of retention index with those of published data.

ISSN: 2455-6939

Volume: 05, Issue: 06 "November-December 2019"

Table 2: Vapor toxicity (LD_{50}) in $\mu L.L^{-1}$air of *M. leucadendron* and *H. suaveolens* against 4th instar larvae of *P. xylostella* after 24 hours exposure at room temperature (28±2°C)

Essential oils	n*	Slope ± SE	LD_{50} (µL/L air)	$CI_{95\%}$
M. leucadendron	120	2.71 ± 0.06	4.86	1.76-7.95
H. suaveolens	120	3.57 ± 1.27	7.53	4.44-10.62

*6 experiment with 20 insects

Figure 1: Long-term activity effect of EO vapors from *M. leucadendron* and *H. suaveolens* against 4th instar larvae of *P. xylostella* after 24 hours exposure to small doses of 1 $\mu L.L^{-1}$ air at 28±2°C).

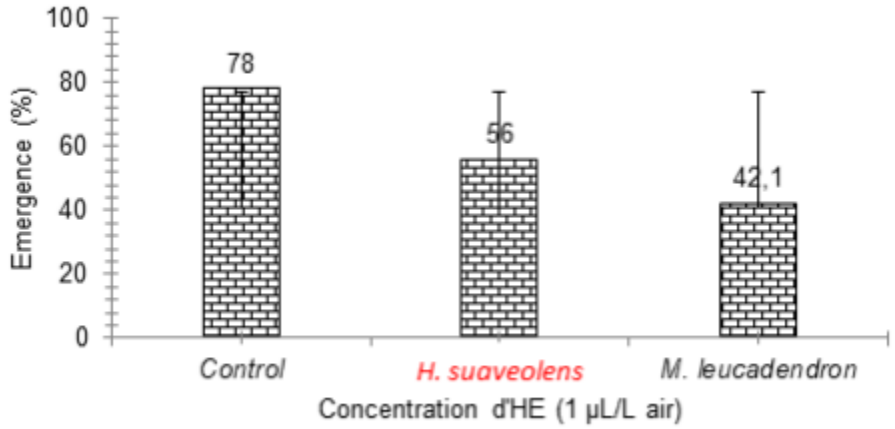

Table 3: Antifeedant effect of vapor phase of EO from *M. leucadendron* and *H. suaveolens* against 4th instar larvae of *P. xylostella* after 24 hours exposure at room temperature (28±2°C)

	Dose of essential oil (µL/L air)					
	1	2	3	4	8	16
M. leucadendron	+	-	-	-	PS	PS
H. suaveolens	+	-	-	-	PS	PS
Control	+++	+++	+++	+++	+++	+++

+++: high feeding (area consumed including 4-6 mm^2); +: low feeding (area consumed including 1-3 mm^2); - antifeedant (area consumed including 0 mm^2) PS: no survival

ISSN: 2455-6939

Volume: 05, Issue: 06 "November-December 2019"

DISCUSSION

Resultats showed that HS of both EO had an effective insecticidal activity against larva of *P. xylostella*. The two biocides caused 100% mortality at a dose of 8 µL/L air within 24 h of exposure, with the *P. xylostella* larval mortality rate increasing with higher EO dose.

These results are in line with previous records in literature against stored-food coleopteran species. Conti et al. (Conti et al. 2011) reported that essential oil of *H. suaveolens* expressed insecticidal activity against *Sitophilus granarum* L. (Coleoptera: Dryophthoridae). Also, *H. suaveolens* leaf powder had greater insecticidal activity against *Callosobruchus maculatus* (Coleoptera: Bruchudae), compared to *Azadirachta indica*, *A. juss* and *Ocimum gratissimum* L. (Iloba et al. 2006). Insecticidal activities of *H. suaveolens* seed have also been recorded against *P. xylostella* second instar larvae (Kéita et al. 2006). However, our study is the first demonstration of vapor toxicity (HS) of *H. suaveolens* EO specifically against *P. xylostella* larvae.

The chemical composition of *H. suaveolens* EO has been previously reported (Tripathi et al. 2009, Conti et al. 2011), producing similar results to this study, although there were some differences in ratios (relative amounts) of chemicals (Table 1).

The insecticidal activity of HS of *H. suaveolens* EO could be attributed to β-caryophyllene (33.9%), sabinene (20.2%), terpinolene (7.3%) and limonene (6.0%) constituents, that have well-documented insecticidal properties (Kousso et al. 2007, Conti et al. 2011). Nevertheless, some minor components, such as β-phellandrene and terpinen-4-ol could also contribute to activity increasing. The LD_{50} (7.53 µL.L^{-1} air) with the HS of *H. suaveolens*, against *P. xylostella*, in our study corroborates the LD_{50} (10.77 mg/filter paper) reported by Chang-Geun et al. (2007), but is much higher than LC_{50} (1.30 µL.L^{-1}) reported by Ilboudo et al. (2010) against *Callosobruchus maculatus*.

On the other hand, headspace of EO extracted from *M. leucadendron* showed high insecticidal activity. Coitinho et al. (2010) found that EO of *M. leucadendron* induced high insecticidal activity against *Sitophilus zeamais*. Insecticidal and repellent properties of EO of *M. leucadendron* have been reported by Noosidom et al. (2008) against *Aedes aegypti*. These results suggested that EO of *M. leucadendron* could be a good candidate for naturally occurring *P. xylostella* larvae control agents.

The chemical composition of *M. leucadendron* EO and its HS analyzed in this study were similar to the ones previously reported (Sakasegawa et al. 2003, Farag et al. 2004, Kumar et al. 2005, Silva et al. 2007, Noosidum et al. 2008, Pino et al. 2010). Therefore, insecticidal activity observed in this study might be attributed to the major components: 1,8 cineole (50.4%; 48.5%),

ISSN: 2455-6939

Volume: 05, Issue: 06 "November-December 2019"

α-terpineol (8.1%; 14.5%) and α-pinene (12.4%; 6.8%). These are compounds whose insecticidal activity is well known (Pivnick et al. 1994, Asawalam et al. 2008).

Some plant-based compounds have repellent and antifeedant properties (Egigu et al. 2010). A number of research results have also been published on the use of essential oil for controlling insects. For example, Qin et al. (2010) reported the antifeedant effects of essential oil of *Piper sarmentosum* on the larvae and imagoes of *B. longissima*. Our most observations, in the present study, were that the foliar consumption by *P. xylostella* larvae was reduced by both EO of *M. leucadendron* and *H. suaveolens*. These results suggested that HS of *M. leucadendron* and *H. suaveolens* EO could be use for their effective anti-feeding activity to protect cabbages against *P. xylostella* larvae. Previously, antifeedant effects of essential oil of *Piper sarmentosum* on the larvae and imagoes of *P. xylostella* have been reported by Qin et al. (2004).

This study showed the high insecticidal action of headspace of *M. leucadendron* and *H. suaveolens* EO on adult's emergence of *P. xylostella*. Both EO reduced the rate (50%) of emergence of adult insects compared to control at 1 $\mu L.L^{-1}$ air. The effectiveness of both EO against adults of *P. xylostella* was similar that previously observed against *Spodoptera litura* (Hummelbrunner and Isman 2001, Fan et al. 2011). Such materials could become an important part of integrated pest management strategies.

This study demonstrated that headspace of EO extracted from leaves of *H. suaveolens* and *M. leucadendron* were highly toxic to *P. xylostella* larvae. Overall, even if it is not easy to determine which are the compounds responsible for the biological activities of the EO used in bioassays, it can be noted that the main differences between the two headspace of EO were that in *H. suaveolens* mono and sesquiterpene hydrocarbons summed 75%, while in *M leucadendron* oxygenated monoterpenes 68.4% reached. Thus, both essential oils constitute promising, alternative pest management tactic against diamondback moth, *P. xylostella* and, an ecologically-sound alternative to synthetic insecticides.

In conclusion, the present study improves the knowledge on composition and the fumigant toxicity of headspace of EO obtained from *H. suaveolens* and *M. leucadendron*. The investigation on their insecticidal activity against *P. xylostella* revealed that headspace of *H. suaveolens* and *M. leucadendron* EO have remarkable toxic effects against *P. xylostella* larvae. The headspace from plant EO or their constituents exhibited fumigant toxicity, a strong antifeedant and long-term effects of toxicity on *P. xylostella* larvae. The fumigant action of two essential oils could be a great advantage for controlling *P. xylostella* larvae population in crops.

Headspace compounds of both essential oils reported in this study are only terpenoids, which we believe to play a role in the feeding and in preventing the emergence of adult of *P. xylostella* as well as in tritrophic interactions.

Actually, pesticides derived from plant essential oils do have several important benefits. Due to their volatile nature, there is a much lower level of risk to the environment than with current synthetic pesticides. Predator, parasitoid and pollinator insect populations will be less impacted because of the minimal residual activity, making essential-oil-based pesticides compatible with integrated pest management programs.

It is also obvious that resistance will develop more slowly to essential-oil-based pesticides owing to the complex mixtures of constituents that characterize many of these oils. Ultimately, it is in developing countries where the source plants are endemic that these pesticides may ultimately have their greatest impact in integrated pest management strategy (Koul et al. 2008).

However, for practical use of these oils as novel fumigants, further investigations should explore the insecticidal properties of EO in integrated pest management strategies namely the association of crops and insecticidal plants in push-pull strategy.

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REFERENCES

Adams, R. P. 2007. Identification of Essential Oil Components by Gas Chromatography/ Mass Spectrometry", 4th Edition. Allured Publishing Corporation, (Ed.). Carol Stream, Il 60188-2787 USA. 804 p.

Asawalam, E. F., S.O. Emosaire, A. Hassanai. 2008. Essential oil of *Ocimum gratissimum* (Labiatae) as *Sitophilus zeamais* (Coleoptera: Curculionidae) protectant. *African Journal of Biotechnology*. 7:3771-3776.

Beena, J., M. Omanakutty, M. Mathew. 2008. Antibacterial effects and chemical composition of the essential oil of *Hyptis suaveolens* Poit leaves. *Journal of Essential Oil-Bearning Plants*. 11:384-390.

Bonsignore, C. P., and V. Vacante. 2011. Influence of botanical pesticides and biological agents on *Orius laevigatus*-Frankliniella occidentalis dynamics under greenhouse conditions. *Journal of Plant Protection Research*. 52:15-23.

Brewer, M. J., T. Meade, J. T. Trumble. 1995. Development of insecticide-resistant and susceptible *Spodoptera exiga* (Lepidoptera: Noctuidae) exposed to furanocoumarins found in celery. *Environment and Entomology*. 24:392-401.

Cavalcanti, E. S. B., M. M. Selene, M. L. Ashley, E. W. P. Santana. 2004. Larvicidal activity of essential of Brazilian plants against *Aedes aegypti* L. Memorias do Instituto Oswaldo Cruz. 99:541-544.

Chang-Geun, Y., K. Min, T. H. Tran, J. Young-su, A. Young-Joon. 2007. Fumigant toxicity of plant essential oils to *Plutella xylostella* (Lepidoptera: Yponeutidae) and *Costesia glomerata* (Hymenoptera: Braconidae). *Journal Asia-Pacific Entomology*. 10:157-163.

Chaudhary, A., P. Sharma, G. Nadda, D. K. Tewary, B. Singh. 2011. Chemical composition and larvicidal activities of the Himalayan cedar, *Cedrus deodara* essential oil and its fractions against the Diamondback moth, *Plutella xylostella*. *Journal of Insect Science*. 11:1-10.

Coitinho, R. L. B. C., J. V. D. Oliveira, J. M. G. C. Gondim, C. A. G. D. Camara. 2010. Persistence of essential oils in stored maize submitted to infestation of maize weevil. *Cienc Rural*.40:1492-1496.

Conti, B., A. Canale, P. L. Cioni, G. Flamini, A. Rifici. 2011. *Hyptis suaveolens* and *Hyptis spicigera* (Lamiaceae) essential oils: qualitative analysis, contact toxicity and repellent activity against *Sitophilus granarius* (L.) (Coleoptera: Dryophthoridae). *Journal of Pest Science*. 84:219-228.

Echereobia, C. O., C. S. Okerere, K. C. Emeaso. 2010. Determination of repellence potential of some aqueous plant extracts against okra flea beetles *Podagrica uniforma*. *Journal Biopesticides*. 3:505-507.

Egigu, M. C., M. A. Ibrahim, A. Yahya, J. K. Holopainen. 2010. Yeheb (*Cordeauxia edulis*) extract deters feeding and oviposition of *Plutella xylostella* and attracts its natural enemy. *Biocontrol*. 55:613-624.

Fan, L. S., R. Muhamad, D. Omar, M. Rahmani. 2011. Insecticidal properties of *Piper nigrum* fruit extracts and essential oils against *Spodoptera litura*. *International Journal Agriculture Biology*. 13:517-522.

Farag, R. S., A. S. Shalaby, G. A. El-Baroty, N. A. Ibrahim, M. A. Ali, E. M. Hassan. 2004. Chemical and biological evaluation of the essential oils of different Melaleuca species. *Phytotherapy Research*. 18:30-35.

Gomah, E. N., I. A. I Sahar. 2011. Chemical composition and the insecticidal activity of certain plants applied as powders and essential oil against two stored-products coleopteran beetles. *Journal of Pest Science*. 84:393-402.

Grassi, P., M. J. Nunez, T. S. U. Reyes, C. Franz. 2008. Chemical variation in the essential oil composition of *Hyptis suaveolens* (L.) Poit (Lamiaceae). *Natural Products Communications*. 3:1137-1140.

Hummelbrunner, L. A., and M. B. Isman. 2001. Acute, sublethal, antifeedant and synergistic effects of monoterpenoid essential oil compounds on the Tobacco cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae)″ *Journal Agricultural and Food Science*. 49:715-720.

Ilboudo, Z., L. C. B. Dabire, R. C. H Nebie, I. O. Dicko, S. Dugravot, A. M. Cortesero, A. Sanon. 2010. Biological activity and persistence of four essential oils towards the main pest of stored cowpeas, *Callosobruchus maculatus* F. (Coleoptera: Bruchidae). *Journal Stored Products Research*. 42:124-128.

Iloba, B. N., and T. Ekrakene. 2006. Comparative assessment on insecticidal effect of *Azadirachta indica, Hyptis suaveolens* and *Ocimum gratissimum* on *Sitophilus zeamais* and *Callosobruchus maculates*. *Journal of Biology Science*. 6:626-630.

Isman, M. B. 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annual Review Entomology*. 5:45-66.

Karban R., I.T. Baldwin. 1997. Induce responses to herbivory, University of Chicago, Press, Chigago, IL, 1997.

Keita, S. M., S. B. A. Umoetock, J. G. Smith. 2006. The insecticidal activity of petroleum ether extract of *Hyptis suaveolens* Poit (Labiatae) seeds on *Plutella xylostella* (L) (Lepidoptera: Yponomeutidae). *Agricultural Journal*.1:11-13.

Kimbaris, A. C., D. P. Papachristos, A. Michaelakis, A. F. Martinou, M. G. Polissiou. 2010. Toxicity of plant essential oil vapours to aphid pests and their coccinellid predators. *Biocontrol Science and Technology*. 4:411-422.

Kossouoh, C., M. Moudachirou, V. Adjakidje, J-C. Chalchat, G. Figueredo. 2010. A comparative study of the chemical composition of the leaves and fruits deriving the Essential Oil of *Hyptis suaveolens* (L.) Poit from Benin. *Journal of Essential Oil Research*. 22:507-509.

Koul, O., S. Walia, G. S. Dhaliwal. 2008. Essential oils as green pesticides: potential and constraints. *Biopesticide International*. 4:63-84.

Kouninki, H., E. Haubruge, F. E. Noudjou, G. Lognay, F. Malaisse, M. B. Ngassoum, A. Goudoum, P. M. Mapongmetsem, L. S. Ngamo, T. Hance. 2005. Potential use of essential oils from Cameroon applied as fumigant or contact insecticides against *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae). Communications in *Agricultural Applied Biological Science*. 70:787-792.

Kousso, D. K., P. Atachi, T. E. Zannou, S. Bougourou. 2007. Evaluation de l'activité insecticide de deux plantes *Hyptis suaveolens* (L.) et *Khaya senegalensis* (A. Juss) sur les insectes ravageurs du niébé (*Vigna unguiculata* L.). *Science Naturelle*. 4:17-26.

Kumar, A., S. Tandon, A. Yadav. 2005. Chemical composition of essential oil from fresh leaves of *Melaleuca leucadendron* L from north India. *Journal of Essential Oil-Bearing Plants*.8:19-22.

Machial, C. M., I. Shikano, M. Smirle, R. Bradbury, M. B. Isman. 2010. Evaluation of the toxicity of 17 essential oils against *Choristoneura rosaceana* (Lepidoptera: Tortricidae) and *Trichoplusia ni* (Lepidoptera: Noctuidae). *Pest Management Science*. 66:1116-1121.

Maia, M. F., S. J. Moore. 2011. Plant-based insect repellents: a review of their efficacy, development and testing. *Malaria Journal*. 10:1-11.

Marcel, D., and J. A. V. L. Joop. 2000. Multitrophic effects of herbivore-induced plant volatiles in an evolutionary context. *Entomology Experiment Applied*. 97:237-249.

Moreira, A. C. P., L. E. Oliveira, P. A. Wanderly, E. S. Carmo, E. L. De Souza. 2010. Chemical composition and antifungal activity of *Hyptis suaveolens* L. Poit leaves essential oil against *Aspergillus* species. *Brazilian Journal of Microbiology*. 41:28-33.

Noosidum, A., A. Prabaripai, T. Chareonviriyaphap. 2008. Excito-repellency properties of essential oils from *Melaleuca leucadendron* L., *Litsiea cubeba* (Lour.) Persoon, and *Listsea salicifolia* (Nees) on *Aedes aegypti* (L.) mosquitoes. *Journal of vector Ecology*. 33:305-312.

Picket, J. A., T. J. A. Bruce, K. Chamberlain, A. Hassanali, Z. Khan, M. C. Matthes, J. A. Napier, L. E. Smart, L. J. Wadhams, C. M. Woodcock. 2006. Plant volatiles yielding new ways to exploit plant defence″, In M. Dike and W. Takken (eds) Chemical Ecology: From Gene to Ecosystem. Springer, Wageningen, the Netherlands. 11:161-173.

Pino, A. J., L. E. Regalado, L. J. Rodriguez, D. M. Fernandez. 2010. Phytochemical analysis and in vitro free-radical-scavenging activities of the essential oils from leaf and fruit of *Melaleuca leucadendron* L. *Chemistry and Biodiversity*. 7:2281-2288.

Pino, J., A. Bello, A. Urquiola, J. Agüero, R. Marbot. 2002. Chemical composition of Cajuput oil (*Melaleuca leucadendron*) from Cuba. *Journal of Essential Oils Research*. 14:10-11.

Pivnick, K. A., B. J. Jarvis, G. P. Slater. 1994. Identification of olfactory cues used in host-plant finding by Diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae). *Journal of Chemical and Ecology*. 20:1407-1427.

Qin, W., S. Huang, C. Li, S. Chen, Z. Peng. 2010. Biological activity of the essential oil from the leaves of *Piper sarmentosum* Roxb (Piperaceae) and its chemical constituents on *Brontispa longissima* Gestro (Coleoptera: Hispidae). *Pesticide Biochemistry and Physiology*. 96:132-139.

Qin, W. Q., M.X. Zhang, B. Ling, Z. Q. Peng. 2004. Influence of three volatiles from tropical rank grasses on behavior of *Plutella xylostella*. *Journal South China Agric. Univ*. 25:39-42.

Reddy, G. V. P., E. Tabone, M. T. Smith. 2004. Mediation of host selection and oviposition behavior in the diamondback moth *Plutella xylostella* and its predator *Chrysoperla carnea* by chemical cues from cole crops. *Biology Control*. 29:270-277.

Sakasegawa, M., K. Hori, M. Yatagai. 2003. Composition and antitermite activities of essential oils from two melaleuca species. *Journal Wood Science*. 49:181-187.

Silva, C. J., L. C. A. Barbosa, C. R. A. Malta, A. L. Pinheiro, F. M. D. Ismail. 2007. Comparative study of the essential oils of seven Melaleuca (Myrtaceae) species grown in Brazil. *Flavour Flagrance Journal*. 22:474-478.

Talekar, N. S. 1992. Management of Diamondback Moth and other crucifer pests: Proceedings of the second International Workshop. Shanhua, Taiwan: Asian Vegetable Research and Development Center. 630 p.

Tripathi, A. K., and U. Shikha. 2009. Repellent and insecticidal activities of *Hyptis suaveolens* (Lamiaceae) leaf essential oil against four stored-grain coleopteran pests″. *International Journal Tropical Insect Science*. 29:219-228.

Vasu, D., H. W. Wayne, R. B. Sarah, C. Eric, G. D. Jeannie, K. J. Dhara, L. Bill, E. Dennis. 2010. Essential oil composition of nine Apiaceae species from western United States that attract the female Indra Swallowtail butterfly (*Papilio indra*). *Biochemistry System and Ecology*. 38:538-547.

Vet, L., and M. Dike. 1992. Ecology of infochemical use by natural enemies in a tritrophic context. *Annual Review Entomology*. 37:141-172.

Yang, N. W., A-L. Li, F. H. Wan, W. X. Liu, D. Johnson. 2010. Effects of plant essential oils on immature and adult sweetpotato whitefly, *Bemisia tabaci* biotype B. *Crop Protection*. 29:1200-1207.

Zhen, H., C. Z. Fu, X. Di, M. Afzal, H. M. Bashir, A. Shaukat, F. Shoaib. 2008. Antifeedant activities of secondary metabolites from *Ajuga nipponensis* against *Plutella xylostella*. *Pakistan Journal of Botanic*.40:1983-1992.

