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Effect of high-temperature extracted plant material fume against southern cowpea weevil (*Callosobruchus chinensis* L.) (Coleoptera: Bruchidae) as a non-chemical novel fumigation technique

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Abstract

Background: Fumes from high-temperature heated plant leaves containing volatile phytochemicals generated from *Lantana camara*, *Cinnamomum zeylanicum*, *Azadirachta indica* and *Ocimum sanctum* were tested for their insecticidal activity against adult southern cowpea weevil (*Callosobruchus chinensis* L.) and their F_1 progeny production/emergence. Volatile phytochemicals containing fume was generated using a flameless dry heat extraction method similar to pyrolysis combustion without air supplement at 180 ± 5 °C. Insect mortalities were assessed up to 72 h after exposure to the different treatments of fumigation by plant-fume and control.

Results: All volatile plant-fume samples contained average of $16.3 \pm 1.5\%$ O_2 and $5.8 \pm 0.5\%$ CO_2 in the test jars. The F_1 progeny emergence was estimated 30 days after treatment. After 36 h of exposure, *L. camara* showed the highest toxicity against *C. chinensis*, followed by *O. sanctum*, *A. indica* and *C. zeylanicum*, with LT_{50} values of 7.3, 9.4, 14.7 and 20.6 h, respectively. The volatile phytochemical containing plant-fume generated by *A. indica* and *C. zeylanicum* produced LT_{99} values that were not significantly different ($P > 0.05$) from each other. The F_1 adult emergence from treated mungbean (*Vigna radiata* L.) samples was significantly inhibited by *L. camara* and *A. indica* volatile plant-fume compared to *C. zeylanicum* fume. However, plant-fume generated from all four plants exhibited effective direct toxicity and F_1 progeny inhibition of more than 86%.

Conclusion: From the study, it can be concluded that volatile plant-fume treatment was highly lethal to *C. chinensis* and significantly reduced F_1 progeny emergence. Therefore, phytochemicals obtained from thermal extraction technique can be used as an alternative technique to chemical fumigation of stored mungbean.

Keywords: *Callosobruchus chinensis*, Cowpea weevil, Fumigation, Mungbean, Phytochemical, Volatile plant-fume

Background

Grain legumes are an important source of dietary protein for low-income earners of many regions in Asia and Africa. About 40% of grain legumes harvested in

Sri Lanka are stored by farmers for personal consumption over a period of 3–6 months [1]. Mungbean (*Vigna radiata* (L.) Wilczek) is the green grain legume most commonly stored in poly-sack bags after harvesting, which similar in many Asian and African countries. This type of on-farm storage results in significant postharvest losses of mungbean due to heavy insect and fungal damage. According to previous study [2], grain damage in Sri

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Lanka could be high as 60% after 6 months of storage in poly-bags.

Stored grain legumes are susceptible to damage by stored product insects such as Southern cowpea weevil (*Callosobruchus chinensis* L.) and cowpea weevil (*Callosobruchus maculatus* F.) (Coleoptera: Bruchidae) throughout the year. Infestation of grain legumes including mungbean starts in the farmers' field [3], and this infestation is carried into storage, resulting in further infestation and deterioration of the stored mungbean. The population of insects develop in the grains at a rapid rate under favourable tropical climatic conditions such as high humidity and temperature [4]. In order to protect mungbean from infestation from beetles, farmers apply hazardous insecticides to their crop soon after harvesting and several times during storage. Phosphine fumigation is the most common technique used for controlling stored product insects in large commercial granaries. However, it is not recommended for farm level application due to safety concerns. Also, the development of resistance to chemical fumigation has become a major problem for control of stored product pest [5].

As an alternative to fumigation, farmers traditionally use many dried plant materials and their generated smoke after burning the different plant materials to control stored product insects inside the grain storage [6]. In traditional system plant materials is burnt using red-hot coconut shell charcoal. Botanical fumigants have long been known as organic alternatives to synthetic chemical insecticides for pest management in stored products [7]. Although the efficacy and exact mode of action of such methods are not well known, fumigants from plant materials can either be used as protectants or disinfestation methods for stored product pests [8, 9]. This type of smoke or fumes may contain many volatile phytochemicals gasses and other volatile organic compounds (VOCs), which may be released during partial burning/heating or pyrolysis burning of plant materials [10] and some gasses. Practical application of phytochemical extracts (i.e. essential oils or other volatile extracts) into bulk-stored grains may not be an easy task due to their extraction process, nature of volatility and diffusivity. However, fumigation, smoking and fogging are relatively simple methods to apply volatile chemical pesticides in bulk grain storage facilities. Stored product pest management heavily relies on chemical pesticides and chemical fumigation, which cause significant impact on health and environment. Today, considerable amount of grains, nuts, herbs and are produced through organic agriculture, but synthetic pesticides or chemical fumigation must not be applied to preserve stored foods according to the strict regulation of organic produces. Therefore, investigation of traditional knowledge of plant

material derived phytochemical fume/smoke (volatile plant-fume) is important to develop alternative non-chemical, but natural plant material-based fumigation techniques to protect the stored grains. In traditional system-generated smoke may not be an effective and environmentally sound method because smoke may contain a high amount of carbon monoxide (CO), carbon dioxide (CO₂), methane (CH₄), hydrogen (H₂), other gasses and water vapour (H₂O) rather than phytochemicals. Therefore, a modification of traditional knowledge is necessary to develop the novel fumigation method.

The objective of this study was to evaluate the efficacy of organic phytochemical containing volatile plant-fume extracted under high-temperature heating as a new fumigation method for control of *C. chinensis* infested mungbean. High-temperature heating may be similar to the "pyrolysis" of plant materials [6, 7].

Methods

Insect culture

A sample of newly harvested mungbean (variety MI-6) was obtained from the Department of Agriculture, Sri Lanka. Prior to the experiment, grain samples were stored under frozen conditions (−18 °C) for 2–3 weeks to destroy any hidden infestations of insects. Adult *C. chinensis* insects were obtained from a culture maintained on mungbean at the Department of Agricultural Biology, Faculty of Agriculture, University of Peradeniya, Sri Lanka for this study.

Plant-fume generation

Plant materials such as *Lantana camara* (L.), *Ocimum sanctum* (L.) and *Azadirachta indica* (L.) were obtained from Anuradhapura area, North Central province and *Cinnamomum zeylanicum* (L.) was obtained from Southern province of Sri Lanka. Leaves from matured plants of *L. camara* (L.), *C. zeylanicum* (L.), *A. indica* (L.) and areal part of the *O. sanctum* (L.) plants were collected from farmers' fields at the respective area and then brought to the research laboratory of the Department of Food Science and Technology, Faculty of Agriculture, University of Peradeniya, Sri Lanka. Leaves and shoots were separated from the woody branches of the plant material. All the samples were separately air dried at room temperature (32 °C) for about 4 weeks.

Air-dried leaf samples were manually crushed and refrigerated prior to the study. Plant leaves were directly dry heated without air supplement (similar to pyrolysis) in an airtight 500-mL tween-neck round-bottom flask (Fig. 1). The flask containing plant leaf sample was heated using laboratory electric heating mantle unit (AREC, VELP-Scientifica, Italy) at 180 ± 5 °C. Volatile plant-fume generated in this manner was collected from

the top of the flask and sent through a 500-mL gas-wash bottle containing glass-wool and silica gel layers to filter ashes in the fume and reduce the moisture in the fume, respectively. The generated fume was sent into a 2-L glass vacuum desiccator through a copper tube. The desiccator was used as a “test jar” for fumigation bioassays.

The test jar was connected to the gas-wash bottle and the fume was drawn into the jar through a suction pump. Inside the jar, temperature and relative humidity (RH) were monitored using a data logger (EL-USB-2-LCD, Lascar Electronics, Hong Kong). A beaker of 200 mL of saturated sodium chloride (NaCl) solution was kept inside the test jar to maintain the equilibrium relative humidity around 75%. The internal atmosphere of the test jar was agitated with a magnetic stirrer. Oxygen and carbon dioxide concentrations of the inside the test jar were monitored using a gas analyser (Quantek model-902D, Quantek Instruments, Inc, USA) at the input port. The glass-wool layer was replaced after each replicate due to accumulated fine solids partials and tar.

Fumigation bioassay

Fifty mixed sex 1- to 2-day-old adult *C. chinensis* and 250 g of mungbean were enclosed in a cage ($\approx 325 \text{ cm}^3$) of fine steel gauze mesh (#40 mesh) and placed inside the airtight bioassay test jar (2-L vacuum desiccator). Pre-heated flask (180°C) was loaded with approximately 30 g crushed dry leaf samples to generate/release the volatile plant-fume for mungbean fumigation. Prior to the bioassay, the plant sample was generated over 10 min of heating at $180 \pm 5^\circ \text{C}$ (similar to the pyrolysis burning/dry heating) inside the airtight flask (closing D and J in Fig. 1)

and then volatile plant-fume was sent into the bioassay test jar after opening the valves J (Fig. 1) while operating the suction pump (1.5 l/min) by about 2–3 min. The temperature of the fume was brought down to near room temperature ($\approx 38^\circ \text{C}$) passing through the copper tube with a moist cotton wool layer (H in Fig. 1) wrapped around the tube (G in Fig. 1). The cotton wool layer was regularly moistened with flowing ambient water (I in Fig. 1). Accumulated volatile plant-fume inside the airtight test jar was agitated (O in Fig. 1) using a magnetic stirrer after closing valves “I” in the experimental setup. Treated sample jars were maintained at room temperature ($30 \pm 1^\circ \text{C}$) prior to estimation of mortality. Insect mortalities were determined 0, 2, 6, 10, 18, 24, 36, 48 and 72 h after treatments and the samples were discarded after the mortality assessment (as a destructive method). Adults were considered dead if appendages did not move when prodded with an insect pin. Control treatments consisted of heated and filtered air pump through the experimental setup without any fume. All bioassays were repeated in triplicate.

Fecundity

Uninfested mungbean samples (250 g) were enclosed in a fine steel gauze cage similar to previous the bioassays setup. Each mungbean sample was treated/fumigated with the respective volatile plant-fume separately prior to the experiment. The volatile plant-fume-treated sample was stored about 4 h in the bioassay test jar at $30 \pm 1^\circ \text{C}$ to settle down the plant-fume. A group of 50 mixed sex (1–2 days old) *C. chinensis* was released into the

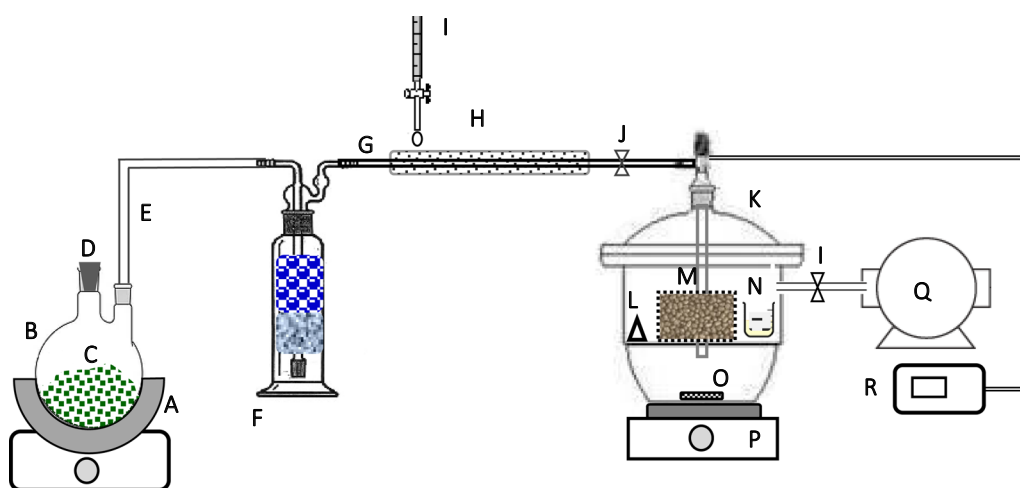


Fig. 1 Laboratory setup of volatile plant-fume generation from dry heat extraction of plant leaves (A) electric heating mantle, (B) tween-neck round-bottom flask, (C) dry plant leaves, (D) stopper, (E) stainless steel tubes, (F) gas-wash bottle with glass-wool and silica gel layers, (G) copper tubes, (H) moist cotton wool layers, (I) flowing ambient water, (J) one-way stop valves, (K) volatile phytochemical fume collecting test glass jar (bioassay jar), (L) Temperature-RH data logger, (M) mungbean sample in steel mesh cage, (N) saturated NaCl, (O) magnet, (P) magnetic stirrer, (Q) suction pump, (R) gas analyser

respective plant-fume-treated mungbean samples to test F_1 progeny production after 30 days. The samples were kept in an aerated glass jars under at ambient conditions (30 ± 2 °C and RH $74 \pm 3\%$). Similarly, control samples of 250 g of mungbean were also treated with air only and then infested with 50 unsexed weevils. All samples were held in a 1-L aerated glass jar and stored under ambient room conditions. The F_1 progeny production/emergence was estimated 30 days after plant-fume-treated and control samples. All samples were kept for 2 days under -10 °C to kill any emerged weevils, which were removed by sieving. The F_1 progeny emergence inhibition percentage (PI%) was calculated by the following simple Eq. (1). All fecundity tests were conducted in triplicate with 3 parental cohorts:

$$PI\% = 1 - \frac{N_T}{N_C} \times 100 \quad (1)$$

where PI% is the progeny emergence inhibition percentage; N_T is the number of insects in treatment and N_C is the number of insects in control sample.

Statistical analysis

All treatments and controls were replicated 3 times. Percentage mortality data of weevils were first corrected using Abbott's corrections. The corrected data were then subjected to Probit analysis by using the following non-linear logistic dose response equation [11]. The SAS-NLIN least square estimation method (SAS 9.1, SAS Institute) was used to fit the experimental data to an Eq. (2). The adjusted regression coefficient (r^2_{adj}), fit standard error (FSE) and F statistic values as goodness-of-fit statistics were used to evaluate the curve fitting to mortality data. The LT_{50} , LT_{99} and F_1 progeny emergence values were analysed using two-way repeated analysis of variance (ANOVA) and the least significant difference (LSD) test was used to separate the means at 95% confidence interval ($P < 0.05$):

$$Y = q + \frac{a}{1 + \left(\frac{t}{b}\right)^c} \quad (2)$$

where Y is the insect mortality (%) at given exposure time, t is the exposure time (h), q is the lowest efficiency of mortality (%) when $t \Rightarrow 0$ assuming $q = 0$, a = highest efficiency of mortality (%) when $t \Rightarrow \infty$ at $a = 100\%$ mortality, b is the model constant of curve transition and c the slope factor of the curves.

Results

Immediately after fumigation treatments average oxygen (O_2) in the test jars was dropped to $16.3 \pm 1.5\%$ and CO_2 content in the test jar was increased to $5.8 \pm 0.5\%$ (Fig. 2).

The temperature inside the test jar was around 37 ± 2 °C. Although high CO_2 and low O_2 contents were detected in the test jar, there was no significant difference ($P > 0.05$) of CO_2 and O_2 contents (%) among the four plant-fume samples. The highest and the lowest CO_2 contents of $6.4 \pm 1.8\%$ and $5.4 \pm 1.1\%$ were observed in *C. zeylanicum* and *O. sanctum* plant-fume-treated jars, respectively. Control samples also showed slightly reduced O_2 content during treatments, but atmospheric O_2 level was regained immediately after treatment. Among the generated plant-fume samples, the lowest O_2 content of $14.2 \pm 1.4\%$ were detected in *C. zeylanicum* fume which was significantly ($P < 0.05$) lower than the O_2 content found in *L. camara* fume.

The effect of four different volatile phytochemical containing plant-fume fumigation treatments on adult *C. chinensis* mortality over time is shown in Fig. 3. The results of this study showed that volatile phytochemical

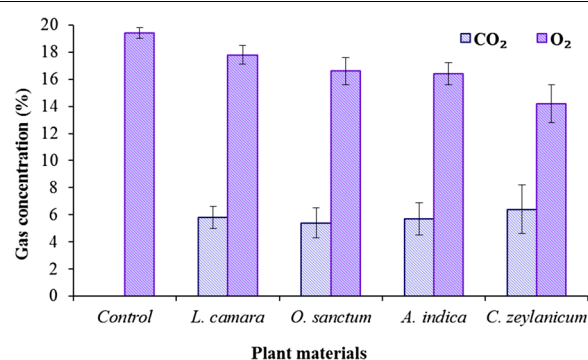


Fig. 2 The percentage content of CO_2 and O_2 yield in the test jar after the high-temperature dry heat extraction of volatiles from four different types of plant leaves (mean \pm SD)

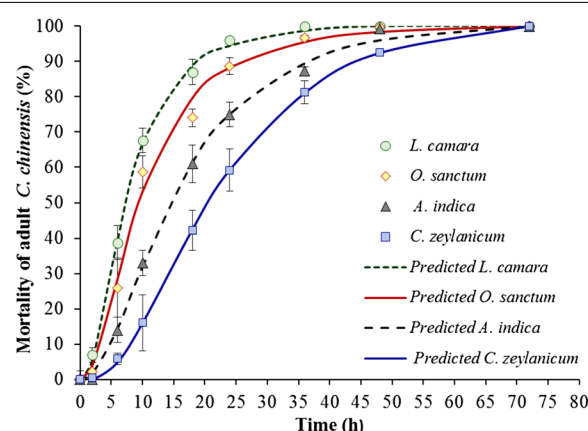


Fig. 3 Predicted and observed mean percentages of *C. chinensis* mortality (mean \pm SD) exposure to volatile plant-fume generated by four different types of plant leaves

contained in plant-fume obtained from all the tested plant leaves was toxic to *C. chinensis*. Among the tested plant-fume, *L. camara* showed highest efficacy followed by *O. sanctum*, *A. indica* and *C. zeylanicum*. Plant-fume of *L. camara* resulted in 100% mortality within 36 h after treatments, while *O. sanctum*, *A. indica* and *C. zeylanicum* treatments showed mortalities of 96.67%, 87.33% and 80.33%, respectively. The plant-fume treatments of *O. sanctum* caused 100% mortality at 48 h after treatment and fume from *A. indica* and *C. zeylanicum* showed 100% mortality at 72 h after fumigation treatment. Weevil mortality was 58% and 68% after 10 h of exposure to fume generated from *L. camara* and *O. sanctum* leaves, respectively. Toxicity of volatile containing plant-fume treatments with *A. indica* was comparatively slow, but >60% mortality was achieved at 18 h after treatment. Volatile plant-fume generated from *C. zeylanicum* took the longest time to attain mortality, taking nearly 24 h to obtain 60% mortality after treatment. The adjusted r^2_{adj} and the F statistics values in the range of 0.98–0.99 and 527.03–1321.34 between the observed and predicted mortality data of *C. chinensis*, respectively. Therefore, logistic dose response Eq. (2) is well fitted to the mortality data of weevils in responds to four different plant-fume fumigation treatments (Table 1).

The ANOVA values LT_{50} and LT_{99} showed that there was significant difference (LT_{50} : $F_{3,11}=36.53$, $P<0.05$; LT_{99} : $F_{3,11}=97.8$, $P<0.05$) in response of *C. chinensis* to the four plant-fume treatments (Table 2). The fume generated by *L. camara* showed the highest toxicity with significantly lower LT_{50} (7.3 ± 0.6 h; $F_{3,11}=36.53$, $P<0.05$) and LT_{99} (37.6 ± 0.8 h; $F_{3,11}=97.8$, $P<0.05$) when compared to the other three plant-fume samples. The highest LT_{50} (20.6 ± 0.7 h; $F_{3,11}=36.53$, $P<0.05$) and LT_{99} (61.2 ± 2.5 h; $F_{3,11}=97.8$, $P<0.05$) was shown by fume of *C. zeylanicum*. According to LT_{50} and LT_{99} values, *L. camara* fume was about 2 and $1.5 \times$ more toxic than *A. indica* and *C. zeylanicum* fume, respectively. There was

no significant difference ($P>0.05$) between LT_{99} values of *A. indica* and *C. zeylanicum*, although *A. indica* LT_{50} values were significantly lower than those of *C. zeylanicum*.

Similar to adult mortality, all volatile phytochemical plant-fume treatments significantly reduced ($F_{3,11}=30.65$, $P<0.05$) F_1 progeny emergence (Fig. 4) of *C. chinensis* when compared to the control. Control samples had mean F_1 progeny of 1858 ± 354 . The F_1 progeny emergence from *O. sanctum* and *C. zeylanicum* fume treatments (147.7 ± 34.6 and 260.7 ± 51 , respectively) were significantly higher ($F_{3,11}=30.65$, $P<0.05$) than that from *L. camara* and *A. indica* treatments (50 ± 13.6 and 76 ± 11.5 , respectively). Highest and lowest F_1 progeny emergence inhibitions were obtained from plant-fume derived from *L. camara* (98%) and *C. zeylanicum* (87%), respectively (Table 2), but the difference was only 11% between two plant-fume types. In spite of lethality–time relation showing significant differences among the different plant material fumes, F_1 progeny emergence inhibition percentage indicated that all the tested plant material fumes were effectively reduced the F_1 progeny.

Discussion

A large number of phytochemicals extracted from different plant species have already been tested and identified for insect toxicity, as potential fumigants for stored grain legumes [12–14]. Volatile compounds of plant extracts contain many bioactive molecules including monoterpenes, sesquiterpenes and phenyl propanoids, which are the dominant constituents of essential oils. These compounds can act as pesticides, insect repellents, feeding deterrents, antimicrobials and antioxidants [7, 15, 16].

Pyrolysis or high-temperature dry heat extraction is a thermal decomposition method of organic materials at high temperature under a low O_2 environment or in a vacuum, which involves the irreversible change of chemical composition [17]. During the pyrolysis process, plant

Table 1 Estimated model fitting statistics and parameters of the non-linear logistic dose response equation used to estimate the lethal time response of *C. chinensis*

Plant species	Goodness-of-fit statistics			Parameters ($\pm 95\%$ CI) [§]		
	r^2_{adj} *	FSE [†]	F [‡]	a	b	c
<i>Lantana camara</i>	0.99	2.47	950.27	100.68 (± 3.6)	7.35 (± 0.5)	− 2.42 (± 0.41)
<i>Ocimum sanctum</i>	0.99	2.94	844.16	109.2 (± 10.86)	22.24 (± 3.07)	− 2.24 (± 0.46)
<i>Azadirachta indica</i>	0.99	2.30	1321.4	105.25 (± 6.37)	15.43 (± 1.57)	− 2.07 (± 0.3)
<i>Cinnamomum zeylanicum</i>	0.98	3.46	527.03	101.44 (± 6.67)	9.57 (± 1.18)	− 2.104 (± 0.5)

* Adjusted regression coefficient

† Fit standard error

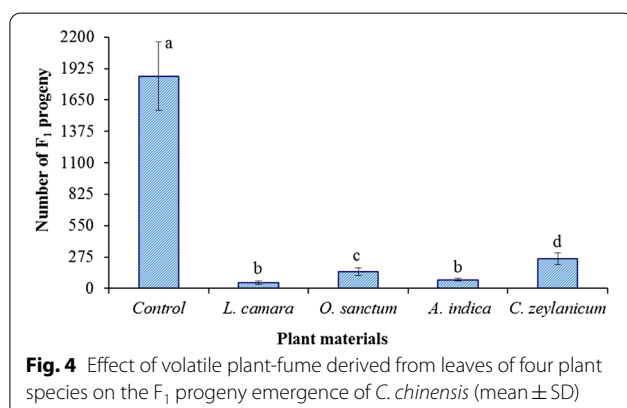
‡ F statistic values

§ 95% confidence interval

Table 2 The lethal time response (\pm SE) and inhibition of F_1 progeny emergence (\pm SE) of *C. chinensis* to plant-fume derived from four different plant leaves

Plant species	LT ₅₀ (\pm SE) (h)	LT ₉₉ (\pm SE) (h)	Percent progeny inhibition (\pm SE%)
<i>Lantana camara</i>	7.30 (\pm 0.57) ^a	37.60 (\pm 0.83) ^a	98.37 (\pm 0.45) ^a
<i>Ocimum sanctum</i>	9.41 (\pm 0.46) ^b	52.87 (\pm 1.1) ^b	93.13 (\pm 1.1) ^b
<i>Azadirachta indica</i>	14.74 (\pm 0.42) ^c	58.41 (\pm 2.1) ^c	97.00 (\pm 0.41) ^a
<i>Cinnamomum zeylanicum</i>	20.60 (\pm 0.70) ^d	61.23 (\pm 2.52) ^c	87.04 (\pm 1.6) ^c

Means in a column followed by the same letters are not significantly different ($P > 0.05$) by LSD



materials may produce various volatile vapours including some gases, moisture, oils, liquids, tar and char. High-temperature extraction of plant leaves also produces many volatile phytochemicals, which may be subject to thermal degradation or isomerization [18], known as derived phytochemicals. But many volatiles such as essential oils may easily escape into the fume without much thermal decomposition. Previous study by Havilah et al. [19] found that *L. camara* produces some volatiles during pyrolysis. High yield of azadirachtin has also been obtained by using pyrolysis of *A. indica* leaves [20].

In this study *L. camara* fume was shown to exhibit maximum toxicity with the lowest LT₅₀ and LT₉₉ values compared to the other volatile plant-fume tested. However, longer exposure of test insects to the less toxic phytochemical plant-fume gradually increased mortality. The highest LT₅₀ and LT₉₉ values were produced by *C. zeylanicum* fume followed by *A. indica* and *O. sanctum*. Extract of *L. camara* has been shown to be insecticidal against all developmental stages of storage pests and suppress their emergence from treated grains due to the presence of glycoalkaloids, lantoniside, linaroside and carminic acid [21, 22]. Previously studies of Zoubiri and Baaliouamer [9] and Mofunanya and Nta [23] have reported that leaf extract of *L. camara* caused 100% mortality on

Callosobruchus spp. and *Sitophilus* spp. within 24–96 h after treatment. The leaf oil of *C. zeylanicum* and *Ocimum* spp. has eugenol as a major component, which has high potential to act as a contact insecticide and inhibit progeny production of stored product insects [24–26]. Previous study of plant-smoke treatments of *L. camara*, *O. sanctum* and *A. indica* to control *Sitophilus oryzae* showed more or less similar LT₅₀ values to the results of this study [6]. This indicates that volatile phytochemical containing plant-fume derived from the same plant species exert similar toxicity, irrespective of insect species. This may be because of release of volatile phytochemicals or formation of other toxic volatile gases during pyrolysis of plant materials. Leiner et al. [10] reported that during high-temperature pyrolysis, phytochemicals such as linalool (synthesised from isoprenoid) may form toxic thermal isomerization molecules of cis- and trans-pinane-2-ol. Selvaraj et al. [27] confirmed that the smoke of *Adhatoda vasica*, *A. indica* and *O. sanctum* produced by burning in hot charcoal was toxic to adult mosquitoes and also reduced mosquito activity for 6–8 h.

It was shown that the percentage of F_1 progeny emergence in the fume treatments was significantly different from that of the controls. In the current study, lowest F_1 progeny emergence was recorded from *L. camara* and *A. indica* volatile phytochemical plant-fume-treated samples, but comparatively high progeny was recorded from the *C. zeylanicum* fume-treated samples. The plant-fume-treated mungbeans may hinder mating and oviposition through the mortality of weevils and/or act as oviposition deterrents on females and affect the egg-laying behaviour and hatchability of laid eggs, due to the phytochemical composition of the plant material fume [23–26]. Some toxic VOCs or gases in the fume may also prevent neonatal larvae from development inside the eggs laid on the surface of mungbean seeds. Compared to the other fume treatments, *C. zeylanicum* and *O. sanctum* volatile fumes were less effective as oviposition deterrents or as toxicants. Apparently, this may be due to

either steady evaporation of effective volatile chemicals into the surrounding air space after volatile fumigation treatments or weevils may simply be less susceptible to these volatile fumes. It is possible that some of the volatile phytochemicals may dissolve in H₂O vapour, but according to Weidenhamer et al. [28] unsaturated solutions of monoterpenes in water vapour may also act as potent biological inhibitors.

Other than CO₂ and volatile chemicals, many other gases escape during the heating similar to pyrolysis of plant materials such as H₂O, CO, CH₄, H₂, etc. [17, 29]. However, the type of gaseous form during pyrolysis is dependent on the temperature and residence time of the materials. During pyrolysis at temperatures of around 150–200 °C, the probable gasses formed are volatile phytochemicals, CO₂, H₂O and/or CO. Despite differences in the plant-fume-generated samples, all four plant-fumes produced more or less similar CO₂ during pyrolysis. However, some differences of O₂ content in the range of 14–18% were found among the generated plant-fume samples could be due to the burning of plant materials under pyrolysis condition. Other than CO₂, other gases may also derive and escapes into the test jar during heating. Therefore, resultant test insect mortality may not only depend on the VOCs, but also on the collective effect of different gases in the plant-fume. The drop of O₂ content <5% was most critical factor for the death of bruchids than rise of CO₂ content in the intergranular atmosphere stored grains [2]. In this study, we did not observe very low O₂ (>25%) and very high CO₂ (<6%) contents in the plant-fume inside the test jar. Therefore, rather than toxic gases, volatile phytochemical contained in the fume may have influenced significantly on the mortality and fecundity of *C. chinensis*. However, due to limited residence time and low pyrolysis temperature, formation of toxic gasses such as CO may be restricted during the heating process. Tihay and Gillard [29] found pyrolysis of 3 plant species (leaves and twigs) at the 200–600 °C produced significantly lower amounts of CO and CH₄ than CO₂ and H₂O. Therefore, the effect of other gases may be lower than phytochemical effect of plant-fume, but further investigations are needed to identify the active compounds of test volatile phytochemical plant-fume and other gasses.

Conclusion

Dry heat extraction similar to pyrolysis burning of plant materials and plant-fume derived from dry leaves of *L. camara*, *C. zeylanicum*, *A. indica* and *O. sanctum* contained toxic volatile phytochemicals. Volatile plant-fume treatment was highly lethal to *C. chinensis* and significantly reduced F₁ progeny emergence. Comparatively,

highest and the lowest volatile phytochemical plant-fume was derived from the leaves of *L. camara* and *C. zeylanicum*, respectively. Therefore, plant materials-derived phytochemical containing plant-fume can be used as an alternative but traditional technique to grain fumigation. However, further research is needed to find out the active compounds of the volatile phytochemical in plant-fume and to develop an appropriate fumigation technology.

Abbreviations

RH: Relative humidity (%); PI%: Progeny emergence inhibition percentage; N_T: Number of insects in treatment; N_C: Number of insects in control sample; Y: Insect mortality (%); t: Exposure time (h); q: Lowest efficiency of mortality; b: Model constant of curve transition; c: Slope factor of the curves; LT₅₀: Lethal time for 50%; LT₉₉: Lethal time of 99%; ANOVA: Analysis of variance; SD: Standard deviation; SE: Standard error; LSD: Fisher's least significant difference test; P: Probability %; r²_{adj}: Adjusted regression coefficient; F: F-statistics; FSE: Fit standard error; CI: 95% confidence interval.

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Authors' contributions

The author has contributed in the conceptualization and designing of the experiment, statistical analysis and manuscript preparation. All technical assistants support to carry out the laboratory studies. The author read and approved the final manuscript.

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Availability of data and materials

All available data are shown in the figures and tables.

Ethics approval and consent to participate

Not applicable.

Consent for publication

The author give their personal consent for publication.

Competing interests

The author declares that he has no competing interests.

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