Article

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Phytochemical Profile and Insecticidal Potential of Leaf Essential Oil of *Psidium guajava* Growing in North Central Nigeria

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Abstract. Loss of stored maize grains due to infestation by *Sitophylus zeamais* contributes to food insecurity. This necessitates farmers' use of synthetic chemicals to curtail the pest. Unfortunately, the pesticides are toxic to humans and unsafe for the environment. Interestingly, some plants possess phytochemicals that exhibit insecticidal activity without these drawbacks. This activity is linked to the type of phytochemicals, whose presence depends on environmental conditions that change with the time of collection of plant samples. On this basis, we investigated how the time of harvest affects the phytochemicals and insecticidal activity of *P. guajava* leaf essential oils. To accomplish this, pulverized leaves (500 g) from 7.00 am and 1.00 pm harvests were hydrodistilled individually for three hours using a Clevenger setup. GC–MS technique was used to characterize the oils, while contact toxicity bioassay was used to assess the insecticidal activity of the oils. The yields of essential oils obtained from the leaves were 0.27 ± 0.015 and 0.24 ± 0.018 % (w/w). β-Caryophyllene (14.7 and 18.2 %), α-guaiene (13.7 and 10.6 %), α-selinene (10.9 and 12.9 %), globulol (9.5 and 8.1 %), caryophyllene oxide (7.8 and 7.0 %) and eucalyptol (5.6 and 5.8 %) existed in higher quantities in the GC-MS results. Both oils were active against *S. zeamais* with LT50 of 59.23 and 121.09 hours, and the highest activity was recorded for the afternoon oil harvest. Therefore, the oil from the afternoon harvest can serve as a cheaper and more innocuous substitute to synthetic insecticide for *S. zeamais* management in stored maize.
Keywords: *Psidium; guajava*; β-caryophyllene; insecticidal activity.

Resumen. La pérdida del maíz almacenado por la infestación de *Sitophylus zeamais* contribuye a la inseguridad alimentaria. Lo anterior requiere el uso de substancias sintéticas para detener la peste, las cuales son tóxicas e inseguras para el ambiente. Interesantemente, algunas plantas poseen substancias que muestran actividad insecticida sin las desventajas mencionadas. Esta bioactividad está ligada al tipo de fitoquímicos, cuya presencia depende de las condiciones ambientales que cambian con el tiempo de colecta de las plantas. Sobre esta base, investigamos el efecto del tiempo de cosecha con respecto a la actividad insecticida del aceite esencial de la hoja de la guayaba. Para el logro del objetivo, hojas pulverizadas (500 g) de cosechas de 7:00 am y 1.00 pm fueron hidrodestiladas individualmente por tres horas, usando un equipo Clevenger. Se empleó el análisis por CG-EM para la caracterización de los aceites y se empleó el ensayo de toxicidad por contacto para la valoración de la actividad insecticida. Los rendimientos de los componentes mayoritarios de los aceites esenciales fueron 0.27 ± 0.015 y 0.24 ± 0.018 % (w/w). β-Cariofileno (14.7 y 18.2 %), α-guayeno (13.7 y 10.6 %), α-selineno (10.9 y 12.9 %), globulol (9.5 y 8.1 %), óxido de cariofileno (7.8 and 7.0 %) y eucaliptol (5.6 and 5.8 %). Ambos aceites fueron activos contra *S. zeamais* con LT50 de 59.23 y 121.09 h, y la mayor actividad fue registrada para la cosecha después del mediodía. Por lo anterior, la cosecha

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vespertina puede servir como un substituto barato e inocuo a insecticidas sintéticos contra *S. zeamais* en el manejo de maíz almacenado.

Palabras clave: *Psidium guajava*; β-cariofileno; actividad insecticida.

Introduction

Within sub-Saharan Africa (SSA), maize is crucial in sustaining over 300 million individuals from various cultural and socio-economic backgrounds [1]. Yet, the storage of maize presents a significant challenge due to the potential infestation and harm caused by *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). This particular weevil species, widely distributed and highly destructive, targets maize grains along with other cereals, posing a substantial threat to stored grain integrity [2]. Infesting untreated maize in storage, *S. zeamais* can lead to significant grain weight losses, ranging from 20 % to a staggering 90 %, thereby exacerbating concerns about food security in the region [3,4]. Farmers and grain merchants commonly use synthetic insecticides and fumigants to manage *S. zeamais* in stored maize. However, these chemical solutions have severe repercussions despite their efficacy, adversely affecting human health, livestock, and the environment [5]. Consequently, there's a pressing need to explore and adopt alternative strategies that are both effective and environmentally friendly. One alternative has been using plant extracts such as the extracts from common guava, *Psidium guajava*.

Psidium guajava L. is a member of the family Myrtaceae and native to southern Mexico, but commonly grown in Europe, South America, Asia, and Africa. In Nigeria, the plant is well known as ‘guaba’ or ‘gilofa’ by the Yorubas, while the Igbos and Hausas refer to it as ‘goba’ and ‘ugwoba’ respectively [6]. It is used in traditional medicine worldwide to treat toothache, diarrhea, wounds, body pain, stomach aches, dysentery, diabetes mellitus, and cholera. Interestingly, extracts from different parts of the plant have been documented to possess antibacterial, anti-inflammatory, antifungal, antidiabetic, antiulcer, and antiviral activities, which justifies why the plant is used traditionally in folk medicine [7-15]. The plant’s extract also displayed insecticidal activity [16,17]. The activities were linked to plant extracts' terpenoids, flavonoids, and steroids [11,13,18,19].

Several workers have analyzed and reported the leaf essential oil of the plant. The analyses revealed the existence of β-caryophyllene and *E*-nerolidol chemotypes in the leaf oils of the plant Indigenous to Nepal and Pakistan [20,21]; limonene in the leaf oil of the Nigerian, Ecuadorian, and Philippino grown *P. guajava* [11,22,23], and viridiflorol in the leaf oil of the plant home-grown in Tunisia [24]. The chemotypic variations of the oils are attributable to the plant ontogeny and environmental conditions at the locations of the plant [21,25-28]. When harvesting the plant materials, there may be variations in the ecological conditions of one plant location. This has affected some essential oils' chemical profiles and biological activities [21,25]. On this basis, we investigated the effect of harvesting time on the phytochemical profile and insecticidal activity of the leaf oil of *P. guajava.*

Experimental

Sample harvest

Fresh leaves (1200 g each) of *P. guajava* were collected from its matured plant at 7 a.m. and 1 p.m. at the University’s Park and Garden, University of Ilorin, Ilorin, Nigeria. The plant was identified at the Plant Biology Departmental Herbarium in the University, and voucher specimens were deposited [UILH/005/0110].

Oil extraction

Samples (500 g each) of the harvested leaves were separately pulverized and hydro-distilled for three hours in a Clevenger set-up in accordance with the British Pharmacopoeia specification [26]. The distilled oils were separately saved in a sealed sample tube and refrigerated until analysis.

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GC-MS characterization of the oils

The oils were characterized using GC (Agilent 190915) coupled with a quadruple focusing mass spectrometer (433 HP-5). The carrier gas was helium, and the gas flow rate was 1.5 ml/min. The GC was filled with a 30 mm x 0.25 mm fused silica capillary column coated with phenylmethyl siloxane at a split ratio of 1:50. The thickness of the film was 0.25 µm. The oven temperature was fixed at 100 °C initially for 5 min and then programmed to 150 °C at a rate of 4 °C/min for 8 min. The temperature was later increased to 250 °C at 20 °C/min. The following were the operating conditions of the MS: Transfer line temperature, 300 °C, ionization potential, 70 eV.

The percentage composition of the oils was computed from the peak areas of each GC chromatogram. The components were identified by comparing the retention indices of the constituents (with the retention times of a series of n-alkanes) and mass spectra with those of authentic samples and with the data from the literature [30,31].

Insecticidal properties of the oils

Insect culture

The culture of maize weevils, *Sitophilus zeamais*, was obtained from heavily infested maize grains purchased from a local market in Ilorin, Kwara State, Nigeria. These were taken to the laboratory and reared on pristine untreated maize in a Kilner jar. The jar's opening was covered with a piece of muslin fabric. The insects were exposed to aeration and prevented from escaping by holding the fabric with a rubber band. The culture was kept on a shelf and maintained under ambient laboratory conditions (28 ± 2 °C, 75 ± 5 % relative humidity; 12 hours photoperiod).

Adult toxicity test

Experiments to assess the toxicity of essential oils from the leaves of *P. guajava* harvested in the morning and afternoon to adult *S. zeamais* were prepared in a Completely Randomized Design. Experimental units consisted of lipped Petri dishes into which 10 g of pristine maize grains of a susceptible variety (DMR yellow) were placed. Treatments consisted of each oil type – oils from each harvest. Each oil type (0.1 mL) was applied using a micropipette onto the grains within the respective dishes. The dishes were gently agitated for approximately two minutes to ensure uniform dispersion of the oils. Subsequently, the dishes were uncovered, allowing the oil-coated grains to aerate for five minutes before introducing 10 unsexed adult weevils into each dish. This aeration phase aimed to evaluate the residual effect of the oils. A control treatment was also established, consisting of maize grains placed in Petri dishes without oil application. The experiment was repeated four times for each oil treatment and the control, resulting in a total of 52 experimental units. Weevil mortality data were recorded every 12 hours over three consecutive days to assess the impact of the oils on the weevil population. No synthetic insecticide was included as a positive control since the primary goal of the bioassay was first to confirm whether the essential oils have any toxic effect and establish baseline toxicity for the oils compared to untreated control.

Data analysis

The data for the percentage mortality from the experiment was square root transformed and subjected to a One-Way Analysis of Variance (ANOVA). If there was no significant difference, mean separation was done using Tukey's Honestly Significant Difference (HSD) Test, and the significance level was 5 %. Untransformed means are, however, presented in the result section. Statistical analysis was done using the GenStat Discovery Edition 3 (2007) statistical package.

Results and discussion

Essential oil yields and chemical composition

The quantities of essential oils hydrodistilled from the leaves of *P. guajava* harvested in the morning and afternoon were 0.27 ± 0.015 % and 0.24 ± 0.018 %.

Relative percentages, retention indices, and identities of the compounds identified in the oils are shown in Table 1.

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Table 1. Chemical Composition (%) of Constituents of Essential Oils from Leaves of *P. guajava*.

S/N	Compound	RI	% Composition		MS Data
			7 am	1 pm	
1	Sabinene	897	Tr	Tr	136,121,107,93
2	α-Thujene	902	Tr	–	93,92,91,77
3	β-Pinene	943	0.1	Tr	136,121,93,79
4	α-Pinene	948	0.5	0.4	136,121,103,93
5	α-Phellandrene	969	–	0.1	136,93,91,77
6	cis-β-Ocimene	976	Tr	0.1	136,121,93,79
7	Neo-allo-Ocimene	993	–	Tr	136,121,105,93
8	γ-Terpinene	998	0.1	0.1	136,121,93,77
9	o-Cymene	1042	0.1	0.1	134,119,91,77
10	Eucalyptol	1059	5.6	5.8	154,139,81,43
11	Linalool	1082	Tr	Tr	154,136,93,71
12	Terpinen-4-ol	1137	0.1	–	154,111,93,71
13	α-Terpineol	1143	0.9	0.8	136,121,93,59
14	Butanoic acid, 3-hexenyl ester	1191	–	0.2	101,82,71,67
15	α-Copaene	1221	0.4	0.8	204,189,161,119
16	α-Terpinyl acetate	1333	0.1	0.2	181, 121,93,43
17	δ-Cadinene	1469	0.5	0.6	204,161,134,119
18	α-Selinene	1474	10.9	12.9	204,189,107,93
19	4,4-dimethyltetracyclo [6.3.2.0(2,5).0(1,8)]tridecan-9-ol	1490	11.1	8.9	136,91,79,41
20	α-Guaiene	1492	13.7	10.6	204,189,147,105
21	**β-caryophyllene**	**1494**	**14.7**	**18.2**	204,161,133,93
22	Selina-3,7(11)-diene	1507	1.3	1.7	204,161,122,107
23	Caryophyllene oxide	1509	7.8	7.0	121,93,79,43
24	Globulol	1530	9.5	8.1	204,81,69,43,41
25	α-Caryophyllene	1579	4.7	5.7	204,121,147,93
26	β-Cadin-4-en-10-ol	1580	–	Tr	121,95,43
27	α-Humulene epoxide	1592	0.9	0.7	121,93,79,43

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28	Juniper camphor	1647	2.3	2.0	204,189,81,43
29	Humulane-1,6-dien-3-ol	1757	0.3	1.0	204,161,109,43
	Total (%)		**85.6**	**85.9**	

RI = retention index; MS = mass spectral; Tr = Trace

The Table showed a total of twenty-five and twenty-six compounds representing 85.6 and 85.9 % of the oils from the harvested leaves in the morning and afternoon from their mass spectra. Sesquiterpenoids predominated the oils from the two harvests with a total percentage composition of 78.1 and 78.2 %. Monoterpenoid constituents of the oils from each of the harvests were 7.5 %. β-Caryophyllene (14.7 %) was the sesquiterpenoid with the highest percentage in the oil from morning harvest. Other major sesquiterpenoids in the oil were α-guaiene (13.7 %), 4,4-dimethyltetracyclo [6.3.2.0(2,5).0(1,8) tridecan-9-ol (11.1 %), α-selinene (10.9 %), globulol (9.5 %) caryophyllene oxide (7.8 %), α-caryophyllene (4.7 %), juniper camphor (2.3 %). Selina-3,7(11)-diene (1.3 %), δ-cadinene (0.5 %), and α-humulene epoxide (0.9 %) were found as minor constituents of the oil. Eucalyptol (5.6 %) was the most significant monoterpenoid in the leaf oil from the morning harvest. α-Terpineol (0.9 %) and α-pinene (0.5 %) were found in appreciable quantities in the oil. In comparison, β-pinene (0.1 %), terpinen-4-ol (0.1 %), γ-terpinene (0.1 %), o-cymene (0.1 %), and α-terpineol acetate (0.1 %) were the minor constituents of the oil. Sabinene, α-thujene, cis-β-ocimene, and linalool existed in trace percentage in the oil.

The sesquiterpenoid that constituted the highest percentage in the leaf oil of the afternoon harvest was β-caryophyllene (18.2 %). Other principal sesquiterpenoids in the oil include α-selinene (12.9 %), α-guaiene (10.6 %), 4,4-dimethyltetracyclo [6.3.2.0(2,5).0(1,8) tridecan-9-ol (8.9 %), globulol (8.1 %), caryophyllene oxide (7.0 %), α-caryophyllene (5.7 %) and juniper camphor (2.0 %). Selina-3,7(11)-diene (1.7 %), humulane-1,6-dien-3-ol (1.0 %), α-humulene epoxide (0.7 %), α-copaene (0.8 %) and δ-cadinene (0.6 %) were present in appreciable quantities. β-Cadin-4-en-10-ol existed in trace amounts in the oil. Eucalyptol (5.8 %) was the most abundant monoterpenoid in the oil of the afternoon harvest. While α-terpineol (0.8 %) was detected in appreciable quantity. α-Pinene (0.4 %), α-terpineol acetate (0.1%), α-phellandrene (0.1 %), cis-β-ocimene (0.1 %), γ-terpinene (0.1 %), o-cymene (0.1%) were the monoterpenoids that existed as minor constituents of the oil. Sabinene, β-pinene, neo-allo-ocimene, and linalool occurred in the oil in trace amounts.

The chemotype of the oils was β-caryophyllene since the compound was present in higher quantities than any other phytochemical in the oils of the plant leaves from both times of harvests. Although the compound was the second most abundant compound in the leaf oil of the plant native to southwest Nigeria, limonene was the chemotype of the oil [32]. The chemotypic variations could be attributed to differences in soil conditions, such as pH and temperature, that may influence the activity of the terpene synthases in the plant.

Terpenoid biosynthesis is usually catalyzed by synthases of monoterpenoids and sesquiterpenoids, which are present in highest percentages in plants. The transformation takes place via cationic intermediates in the presence of divalent metal ions. The intermediate cations then undergo a series of rearrangements, such as hydride shifts and cyclizations, until the target terpenoids are formed via dehydrogenation or hydration [31,33]. The abundance of eucalyptol and β-caryophyllene established that the two isoprenoids' synthases mediated all the oils' terpenoids.

Qualitatively, eucalyptol synthase aided the biosynthesis of α-thujene and terpinen-4-ol in the oil of the morning harvest. Still, neither of the compounds was found in the oil of the afternoon harvest. Similarly, 3-hexenyl butanoate, α-phellandrene, neo-allo-ocimene, and β-cadin-4-en-10-ol whose biosynthesis was facilitated by eucalyptol and β-caryophyllene synthases in the oil of the afternoon harvest, were not present in the oil of morning harvest. The non-appearance of some isoprenoids in each of the oils can be ascribed to the inability of the synthases to aid their biosynthesis in the leaves due to unfavorable environmental conditions during the time of harvest at the location of the plant [34].

Quantitatively, β-pinene, α-pinene, α-terpineol, 4,4-dimethyltetracyclo [6.3.2.0(2,5).0(1,8) tridecan-9-ol, caryophyllene oxide, globulol, α-humulene epoxide, and juniper camphor were found in higher percentages in the oil of the morning harvest than the oil from the afternoon harvest. On the other hand, cis-β-ocimene, eucalyptol, α-copaene, α-terpineol acetate, α-selinene, δ-cadinene, β-caryophyllene, selina-

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3,7(11)-diene, α-caryophyllene, and humulane-1,6-dien-3-ol were of more significant quantities in the oil of the afternoon harvest than the oil of the morning harvest. The presence of some compounds in lower amounts in each of the oils may be due to the earlier termination of their formations from their precursor intermediates in the plant leaves as dictated by environmental conditions at harvest [34]. The proposed biogenesis of the terpenoids is shown in Reaction Schemes 1 and 2 (*Supplementary file*) [35-37].

Insecticidal activity

The efficacy of plant essential oils for pest control, particularly against stored products, has been attributed to their constituents, notably monoterpenoids [38]. These constituents include α-pinene caryophyllene oxide, eucalyptol, β-caryophyllene, α-selinene, α-caryophyllene, globulol, δ-cadinene, and α-guaiene The compounds can work individually or synergistically with physiological effects that range from insecticidal, ovicidal, and repellency on insect pests in storage [39-41].

The toxicity of the volatile oils from fresh leaves of *P. guajava* harvested in the morning and afternoon increased with time of exposure, eventually causing significant mortality of 47.50 % and 52.50 %, respectively, 72 hours after setup (Table 2). This attests to the residual potency of the oils, considering that a significant portion of essential oil was allowed to vaporize after grain treatment but before introducing the insects.

Table 2. Mean percentage mortality of *S. zeamais* exposed to the leaf essential oils of *Psidium guajava* from morning and afternoon harvests.

Treatments	**Exposure period (Hours)**					
	12	24	36	48	60	72
LF 7 am	15.00a	20.00a	25.00a	27.50a	35.00a	47.50ab
LF 1 pm	32.50a	35.00a	45.00a	47.50a	50.00a	52.50ab
Control	0.00a	0.00a	0.00a	0.00a	0.00a	0.00b

PS: Values in the same column followed by the same letter(s) are not significantly different at P = 0.05
LF 7 am = oil from fresh leaves of *P. guajava* harvested at 7 am
LF 1 pm = oil from fresh leaves of *P. guajava* harvested at 1 pm

The 50 % lethal time of the oils was 59.23 and 121.09 hours for the oils of the leaves harvested in the morning and afternoon, respectively, as presented in Table 3.

Table 3. Relationship between probit of kill and Log exposure time (hours) of essential oils used at a fixed dose (0.1mL/10g seeds) as insecticides against adult maize weevil.

Oil Sample	**Regression Equation**	***R²**	****LT₅₀**
LF 7 AM	Probit of Kill = 1.1275(Log Exposure Time) + 2.6513	0.8730	121.09
LF 1 PM	Probit of Kill = 0.7139(Log Exposure Time) + 3.7346	0.9282	59.23

*R^2 measures how well the regression equation fits or explains the observed mortality data.
R^2 ranges from 0 (No fit) to 1.0 (Perfect fit).
**LT_{50} is the time (in hours) required for a fixed dose (0.1mL) of the oil that caused 50 percent mortality of the test insect population.

While no significant differences ($P > 0.05$) were observed between the oils regarding the number of weevils killed at each period, oil from the leaves harvested in the afternoon had a shorter lethal time that was half of the deadly time of the other oil. This implies that the oil acted faster and caused roughly the same number

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of weevil deaths in half the time as oil from the morning harvest. This difference in activity may be due to the qualitative and quantitative variation of the compounds in the oils. Nevertheless, both oils performed significantly better ($p < 0.05$) than the control treatment, where no weevil mortality was recorded at all exposure periods. Regarding their mode of action, it has been reported that terpenic compounds can penetrate insects through their spiracles during respiration and quickly interfere with normal physiological functions [39-42]. However, the swift efficacy of essential oils and their elements against specific pests hints at a neurotoxic mechanism of action. It was established that essential oil constituents interfere with the neuromodulator octopamine and the GABA-gated chloride channels [43,44]. This interference with key neural components suggests a neurological impact on the pests, potentially affecting their normal functioning and contributing to their demise.

Conclusions

The harvest time affected the phytochemical profiles of leaf essential oils from 7 am to 1 pm harvests. As a consequence, the insecticidal activity of the oils was also affected since the activity depends on their phytochemical profiles, which in turn depend on the environmental factors at the plant location at the time of harvest. Irrespective of the time of collection, both oils from morning and afternoon harvests showed toxicity against *S. zeamais*. However, the oil from the afternoon harvest was more active. The oil (afternoon harvest) can be a suitable replacement for synthetic insecticides.

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