CAF2020 Paper No. P-8-3-64

Babarinde SA, Ottun AT, Olaniran OA, Adebayo TA, Oderinde AE, Odewole AF, Alao FO (2021) Acute toxicity of two bio-fumigants against larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae). Pp. 246-252 In: Jayas DS, Jian F (eds) Proceedings of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2020), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Acute toxicity of two bio-fumigants against larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae)

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Abstract

The larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae), is a polyphagous pest of stored products such as dried cassava chips, maize and timbers. It causes a reduction of nutritional value and of product weight, and consequently constitutes a major threat to food security and income generation, if left uncontrolled. Synthetic fumigants, despite their age-long application, have some demerits such as the tendency of a pest to develop resistance against them, the high-cost implications and the concerns for ecological safety. Therefore, it is crucial to search for eco-friendly alternatives. In this research, essential oils (EOs) of *Cymbopogon citratus* and *Thuja plicata* were obtained via distillation for four hours using Clevenger type apparatus. Each EO was separately assayed against *P. truncatus* for fumigant toxicity at 6.66, 13.33, 26.66, and 53.33 µL/L air; and latter subjected to Gas Chromatography Mass Spectrometry. The percentage oil yields from *C. citratus*, *and T. plicata* were 0.263% and 0.393% (v/w), respectively. When each EO was applied at 55.33 µL/L air, *C. citratus* caused significantly higher mortality of 100% against *P. truncatus* at 6 h after treatment (HAT), while *T. plicata* caused 100% mortality at 48 HAT. The predominant compounds identified in *C. citratus* EO were Citral (44.92%), Verbenol (34.87%), and Geraniol (6.51); while *T. plicata* EO was predominated by 3-Carene (30.68%), α-Pinene (24.85%), Caryophyllene (8.60%), Humulene (5.94%) and Cedrol (4.32%). The results indicated the superior bioactivity of *C. citratus* over *T. plicata* as bio-fumigant against *P. truncatus.*

Keywords: *Prostephanus truncatus,* Essential oil, Acute toxicity, *Cymbopogon citratus, Thuja plicata,* GC-MS

Introduction

Prostephanus truncatus (Horn) known as the larger grain borer is a significant pest of dried cassava, maize, and woody plants in the tropics. The insect represents the main destructive storage pest of maize and cassava stock over a short period of time. The adult bores into cassava or maize cobs or grains making neat holes and tunnels by producing a large quantity of dust (Nang'ayo et

al., 2002; Nansen et al., 2004). Losses to *P. truncatus* on field and storage has constituted a major constraint to food security and income generation in sub-Saharan Africa (Abebe et al., 2009; Osipitan et al., 2011).

The use of botanicals to control *P. truncatus* has been in existence in developing countries where they are cheaply available. Several scientists have reported the bio-efficacy of different plants formulation against *P. truncatus* and other insects. *Thuja plicata* is commonly called western cedar, giant cedar. It is a species of *Thuja* an evergreen coniferous tree in the cypress family Cupressaceae native to Western North America. *Cymbopogom citratus* is a perennial plant with long thin leaves, is one of the largely cultivated medicinal plant for its essential oil in part of tropical and subtropical areas of Asia, Africa and America (Chanthal et al., 2012). The leaves usually produced yellow or amber colour aromatic essential oil when squeezed (Adejuwon and Ester, 2007).

Synthetic insecticides have been used against this pest; however, due to several problems associated with the use of synthetic insecticides, it is necessary to consider alternatives to synthetic insecticides that cause no harm to human health and the environment. Since the geographical location of plant has tendency to affect the chemical composition of its essential oil (EO), conducting an empirical study to establish the bioactivity of the EO would not be out of place, even when such studies had been carried out in other geographical locations. To the best of the authors' knowledge, the toxicity of Nigeria-grown *C. citratus* and *T. plicata* against *P. truncatus* has not been studied. Therefore, the research was designed to evaluate the toxicity of the bio-fumigants against *P. truncatus.*

Materials and methods

Insect culture

Prostephanus truncatus adults were obtained from a heavily infested dried cassava chips kept in the Entomology Laboratory of Crop and Environmental Protection, Department, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Nigeria. They were introduced into clean transparent jars that contained uninfested dried cassava chips (cultivar Oko Iyawo), obtained from Araada Market, Ogbomoso and reared according to established methods (Osipitan, 2011). The jars were covered with a muslin cloth and kept in the laboratory, and monitored under a fluctuating temperature (28±2°C) and relative humidity (65±5%).

Preparation of plant materials for extraction of essential oil

The bio-fumigants were obtained from fresh leaves of *C. citratus* and air-dried leaves of *T. plicata* via hydro-distillation method for 4 h using Clevenger apparatus. (Shiva Scientific Glass Private Ltd, New Delhi, India). The plants were collected from June to August, 2017. The leaves of *C. citratus* were collected from LAUTECH Teaching and Research Farm, Ogbomoso; while the leaves of *T. plicata* were collected from Ilorin, Kwara State, Nigeria.

Gas chromatography-mass spectrometry analysis

The conditions and the procedures for the Gas Chromatography Mass Spectrometry analysis of the bio-fumigant followed Babarinde et al. *(*2019), using AGILENT (19091S–33HP–5MS) GC (Agilent Technologies, Palo Alto, CA, USA) interfaced with a double focusing mass spectrometer VG Analytical 70–250S) (VG Analytical Ltd, Manchester, UK).

Fumigant toxicity of the bio-fumigants on P. truncatus

To assess the fumigant toxicity of the bio-fumigants on *P. truncatus*, the different doses (5, 10, 20 and 40 µL) of the essential oils corresponding to 6.66, 13.33, 26.66, 53.33µL/L air were separately dissolved in 0.2 mL of acetone and applied to filter papers (Whatman N° 1, ~8 cm²), that was pasted under the bottle cover of 75 mL plastic bottle. The bottle cover was dried in air for 20 min to allow for escape of acetone, and treatment was replicated three times. In the control bottles, 0.2 mL acetone was applied on the filter papers and also replicated three times. Twenty mixed sexed *P. truncatus* adults were placed in the covered plastic bottles. Data were collected at 6, 12, 24, 36, and 48 HAT on the number of dead and alive insects. Percentage of dead adult (PDA) was computed as:

$$PDA = \frac{Number\ of\ dead\ insects}{Total\ number\ of\ assayed\ insects} \times \frac{100}{1}$$

Statistical analysis

The data were subjected to analysis of variance. Significant means were separated using SNK at 5% probability level.

Results and discussion

Yield and chemical composition of studied bio-fumigants

The oil yields from *C. citratus* and *T. plicata* were 0.263 and 0.393% (v/w), respectively. *Cymbopogon citratus* EO was dominated by oxygenated monoterpene with citral (44.92%) and verbenol (34.97%) as the predominant compounds (Table 1); while *T. plicata* was dominated by hydrogenated monoterpene with 3-carene (30.68%) and alpha pinene (24.85%) being the predominant compounds (Table 2).

Table 1. Chemical composition of *Cymbopogon citratus* leaf essential oil

Retention Index [a]	Compound name [b]	Composition (%) [c]	Chemical class
958	Myrcene	0.47	Monoterpene
948	3-Carene	0.06	Monoterpene
1074	p-Menth-8-ene, cis	0.04	Monoterpene
1082	Linalool	1.03	Oxygenated monoterpene
1175	alpha-Cyclocitral	0.09	Oxygenated monoterpene
1131	trans-3(10)-caren-2-ol	0.20	Oxygenated monoterpene
948	Carene, 4,5-epoxy, trans	0.15	Oxygenated monoterpene
1136	cis-Verbenol	0.26	Oxygenated monoterpene
928	5-Isopropyl-1,4-dimethylcyclopentene	0.61	Monoterpene
1206	Carveol	1.13	Oxygenated monoterpene
1196	Isopregol	0.38	Oxygenated monoterpene

Retention Index [a]	Compound name [b]	Composition (%) [c]	Chemical class
1143	alpha-Terpineol	0.17	Oxygenated monoterpene
949	Cyclobutaneethanol, beta-methylene	0.09	Hydrocarbon
1228	Geraniol	7.74	Oxygenated monoterpene
1269	Oxiranemethanol, 3-methyl-3-(4-methyl-3-pentenyl)-	0.13	Oxygenated monoterpene
1136	Verbenol	34.97	Oxygenated monoterpene
1174	Citral	44.92	Oxygenated monoterpene
1224	Epoxy-linalool oxide	1.00	Hydrocarbon
1342	Neric acid	1.23	Acid
1352	Geraniol acetate	0.78	Monoterpene
1030	2,3-Dimethylcyclohexanol	0.70	Alcohol
1430	trans-alpha-Bergamotene	0.08	Sesquiterpene
1449	Tridecanone	1.07	Sesquiterpene
1507	Caryophyllene oxide	0.41	Oxygenated Sesquiterpene
1593	Selina-6-en 4-ol	0.20	Oxygenated Sesquiterpene
1324	(IS,4R)-p-Mentha-2,8-diene, 1-hydroperoxide	0.08	Oxygenated monoterpene
2192	trans-Geranylgeraniol	0.45	Oxygenated diterpene
1211	alpha-Santalene	0.91	Sesquiterpene
1107	Rhodinal	0.55	Oxygenated monoterpene

[a] Kovats retention indices relative to n alkanes on fused silica capillary column Optima® 5MS.
[b] The components are listed in ascending order of retention time.
[c] Percent composition is peak area relative to total peak area obtained from total ion chromatogram peak report.

Table 2. Chemical composition of *Thuja plicata* leaf essential oil

Retention Index [a]	Compound name [b]	Composition (%) [c]	Chemical class
932	2-Bornene	0.08	Monoterpene
903	alpha-Thujene	1.27	Monoterpene
948	alpha-Pinene	24.85	Monoterpene
943	Camphene	1.93	Monoterpene
897	Thujene, 4(10).	1.36`	Monoterpene
943	L-beta-Pinene	1.01	Monoterpene
958	Myrcene	1.41	Monoterpene
948	3-Carene	30.68	Monoterpene
948	2-Carene	0.16	Monoterpene
1042	m-Cymene	0.08	Monoterpene
948	alpha-Pinene	0.59	Monoterpene
1018	d-Limonene	1.96	Monoterpene
976	Ocimene	0.14	Monoterpene

Retention Index [a]	Compound name [b]	Composition (%) [c]	Chemical class
998	Moslene	0.27	Monoterpene
1052	Terpinolene	4.65	Monoterpene
787	Beta-biisocrotyl	0.05	Hydrocarbon
1136	(S)-cis-verbenol	0.02	Oxygenated monoterpene
1125	alpha-Phellandren-8-ol	0.23	Oxygenated monoterpene
1137	1-Terpinen-4-ol	0.31	Oxygenated monoterpene
1197	p-Cymen-8-ol	0.04	Oxygenated monoterpene
1143	alpha-Terpineol	0.02	Oxygenated monoterpene
1117	1-Ethyl-2-methylenecycloheptanol	0.04	Alcohol
1168	4-Isopropylidene-cyclohexanol	0.18	Alcohol
1277	Bornyl acetate	0.16	Oxygenated monoterpene
1032	Terpinolene	0.06	Monoterpene
1152	Bicyclo[3.3.1]non-6-en-3-ol	0.06	Alcohol
1333	alpha-Terpinyl acetate	0.80	Monoterpene
1221	Copane	0,08	Sesquiterpene
1398	beta-Elemene	0.47	Sesquiterpene
1451	Zingiberene	0.05	Sesquiterpene
1458	alpha-Farnesene	0.06	Sesquiterpene
1494	Caryophyllene	8.60	Sesquiterpene
1398	beta-Cedrene	0.70	Sesquiterpene
1416	Thujopsene	0.20	Sesquiterpene
1579	Humulene	5.94	Sesquiterpene
1435	gamma-Muurolene	0.62	Sesquiterpene
1474	alpha-Selinene	0.82	Sesquiterpene
1507	Beta-Chamigrene	0.44	Sesquiterpene
1469	delta-Cadinene	1.14	Sesquiterpene
15.22	alpha-Elemol	0.07	Oxygenated sesquiterpene
1507	Caryophyllene oxide	0.80	Oxygenated sesquiterpene
1539	Ledol	0.45	Oxygenated sesquiterpene
1529	Humulene epoxide II	0.52	Oxygenated sesquiterpene
1543	Cedrol	4.32	Oxygenated sesquiterpene
1580	Cubenol	0.34	Oxygenated sesquiterpene
1580	alpha-Cadinol	0.05	Oxygenated sesquiterpene
1593	Eudesm-4(14)-en-11-ol	0.14	Oxygenated sesquiterpene
1469	Eudesm-4(14)-en-11-diene	0.03	Oxygenated sesquiterpene
1531	Cis-Z-alpha-Bisabolene-epoxide	0.04	Oxygenated sesquiterpene

[a] Kovats retention indices relative to n alkanes on fused silica capillary column Optima® 5MS.
[b] The components are listed in ascending order of retention time.
[c] Percent composition is peak area relative to total peak area obtained from total ion chromatogram peak report.

Fumigant toxicity of two bio-fumigants on Prostephanus truncatus

Of the two bio-fumigants that were assayed against *P. truncatus*, the toxicity of *C. citratus* was superior to that of *T. plicata;* and the toxicity was generally dose and exposure duration dependent. Application of *C. citratus* at 13.33 and 26.66 µL/L air evoked significant mortality at 36 and 48 h after treatment (HAT), whereas in *T. plicata*, significant mortality was observed when 26.66 µL/L air at 48 HAT. Application of *C. citratus* EO at 53.33 µL/L air evoked 100.0% mortality at 6 HAT, whereas 100.0% mortality was observed in *T. plicata* at 48 HAT (Tables 3 and 4).

Table 3. Fumigant toxicity of *Cymbopogon citratus* essential oil against *Prostephanus truncatus*

	Percentage mortality at Hours After Treatment				
Treatment (µL/L air)	6	12	24	36	48
Acetone	0.00±0.0a	0.00±0.0a	1.67±1.7a	1.67±1.7a	10.00±2.9a
6.66	0.00±0.0a	0.00±0.0a	6.67±3.3a,b	13.33±1.7a	21.67±1.7a
13.33	0.00±0.0a	0.00±0.0a	15.00±2.9b	15.00±2.9b	23.33±6.0a
26.66	0.00±0.0a	0.00±0.0a	25.00±5.0c	25.00±5.0b	58.33±10.1b
53.33	100.00±0.0b	100.00±0.0b	100.00±0.0d	100.00±0c.0	100.00±0.0c
ANOVA	df=4,14	df=4,14	df=4,14	df=4,14	df=4,14
Result	F=2931	F=2931	F=264.538	F=200.107	F=45.120
	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001

Means (±SE) followed by the same letter of alphabet within the column are not significantly different using SNK 5% significance level.

Table 4. Fumigant toxicity of *Thuja plicata* essential oil against *Prostephanus truncates*

	Percentage mortality at Hours After Treatment				
Treatment (µL/L air)	6	12	24	36	48
Acetone	0.00±0.0a	0.00±0.0a	1.67±1.7a	1.67±1.7a	10.00±2.9a
6.66	0.00±0.0a	0.00±0.0a	5.00±2.9a	5.00±2.9a	20.00±2.9a
13.33	0.00±0.0a	0.00±0.0a	5.00±2.9a	10.00±5.8a	25.00±5.0a
26.66	0.00±0.0a	0.00±0.0a	13.33±3.3a	28.33±6.0b	46.67±7.3b
53.33	16.67±1.7b	26.67±1.7b	50.00±2.9b	83.33±7.3c	100.00±0.0c
ANOVA	df=4,14	df=4,14	df=4,14	df=4,14	df=4,14
Result	F=100.00	F=256.000	F=51.607	F=42.948	F=68.412
	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001

Means (±SE) followed by the same letter of alphabet within the column are not significantly different using SNK 5% significance level.

The toxicity of the bio-fumigants agrees with previous authors (Buxton et al., 2014; Gariba et al., 2021) who showed various levels of bioactivity of plant products against adult *P. truncatus.* The principles of fumigation include inhalation of the EOs by insects via the spiracles. Consequently,

specific compounds had different mechanisms of action which include neurotoxicity and modulation of some enzymatic reactions. Essential oils are preferred to other botanical formulations because they are effective at low doses without detrimental effects on non-target organisms (Babarinde et al., 2015). Although, the scope of the present study did not include evaluation of the specific EO compounds, the major compounds identified in the bio-fumigants have been reported to be insecticidal. In conclusion, the study showed the potentials of the two bio-fumigants for use in the control of *P. truncatus* under simulated hermetic storage devices.

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