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Toxicity of *Cymbopogon nardus* (Glumales: Poacea) against four stored food products insect pests

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ABSTRACT: Trials on insecticide effects of *Cymbopogon nardus* Linnaeus (Glumales: Poacea) on four stored food products beetles show that the essential oil has a greater insecticidal activity than the saturated ethanol extract. Tests by topical application to adult insects and by contact with filter paper show that the essential oil of *C. nardus* causes a higher mortality than the total ethanol extract in individuals of *Cryptolestes* sp (Coleoptera, Cucujidae), *Palorus subdepressus* Wollaston (Coleoptera: Tenebrionidae), *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrychidae) and *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae). The analysis of the essential oil of *C. nardus* by gas chromatography coupled with mass spectrometry (GC-MS) shows high rates of citronellal (29.2%), citronellol (12.7%), geraniol (29.3%), elemol (5.0%) and limonene (4.1%) compared to the essential oil of *C. citratus* (another insecticide plant) which contains mostly citral (neral (27.3%) + geranial (32.2%)) and myrcene (17.21%). Terpene hydrocarbons (monoterpenes and sesquiterpenes) and oxygen derivatives (aldehydes and alcohols) are the basis of the biocidal activity of *C. nardus*.

***Keywords*:** alcohols, essential oils, insecticide, insect pests, stored food product, terpenes, Cymbopogon nardus.

INTRODUCTION

Losses due to stored food product insect pests are around 10% on average for cereals and legumes, 20% for other stored foods. In some cases, the losses can exceed 50% (Caussanel, 1993).

In Cote d'Ivoire, the two main crops (coffee and cocoa), but also other agricultural staples such as rice, maize and imported wheat are attacked by insects (Agbaka, 1991). To reduce the impact of insects, Ivorian producers use mainly synthetic chemical insecticides. However, because of possible pesticide residues and the risk of emergence of resistant strains of insects, it is recommended to develop alternative methods for protecting stored products (Haubruge & Amichot, 1999). The recommended alternative methods include biological insecticides such as diatomaceous earth (Doumbia, 2014). They also include some plants with biocidal effect. Many scientific studies have revealed particular biocide activity of some plants on insects, fungi and bacteria (Haubruge ., 1989; Lienard, 1992; Baulard, 1999, Jiang, 2010, Nyamador, 2010, Maqbool, 2011; Regnault-Roger, 2012; Gómez-Castillo, 2013; Olivero-Verbel, 2013).

In the North-western rural area of Côte d'Ivoire, local people use the dried leaves of citronella grass *Cymbopogon nardus* to protect maize, beans and millet against pests (Doumbia, unpublished data). Furthermore, that plant is used as beverage by people of the ethnic group in that area who refer to it as "Mahou tea". The essential oil of *C. nardus* is also recognized as a mosquito repellent (Lindsay, 1996, U.S. Environmental Protection Agency, Office of Pesticide Programs, 1996; Fradin, 1998) and is a toxic chemical against the cowpea weevil *Callosobruchus maculatus* F. (Ketoh, 2000). However, according to Luu (2002), for each species of aromatic plants, there are often several "chemotypes" depending on the environment. The chemotypes are the ecotypes of the same plant species characterized by different proportions of chemicals secreted by the plant under the influence of different parameters of the ecosystems such as sunshine, altitude, type of soil, etc. These variations of chemical components in ecotypes' can affect the biological activity of the species according to the considered environment.

To contribute to the knowledge of the insecticidal effects of the Ivorian ecotype of *C. nardus* on four major stored food product beetles in Côte d'Ivoire, experiments on the biocide activity of ethanolic total extract and essential oil of this plant were carried out in the laboratory.

In addition, the chemical composition of that ecotype was determined, thorough an analysis of the oil by gas chromatography coupled with mass spectrometry (GC-MS).

MATERIALS AND METHODS

2.1. Insect rearing

The choice of test insects (*Cryptolestes* sp, *Rhyzopertha dominica*, *Palorus subdepressus* and *Sitophilus zeamais*) was based on the polyphagous nature of these species which attack a majority of stored commodities in Côte d'Ivoire, including coffee and cocoa (Agbaka, 1991).

Wild strains of *S. zeamais*, *P. subdepresus* and *Cryptolestes* sp were collected from stocks of maize and that of *R. dominica* collected from stocks of sorghum. The different samples of these commodities were collected directly from the market and insects were reared separately on maize. The mass rearing was performed in one-liter glass jars containing foodstuff; each jar was closed by a plastic cover pierced with small holes and covered with a 50 µm wire mesh. The jars were then placed in an air-conditioned room at a temperature of 30 ± 2 °C and a relative humidity of 70 ± 5%. During rearing, the strains were purified by sieving to separate adults from larvae and grains from eggs. The infested grains and the larvae were then introduced in a new rearing jar. The adult insects which newly emerged from the jars were used for the tests. This practice allows to have adult insects of the same generation for the tests.

2.2. Extraction of total ethanolic extract and essential oil of C. nardus

Leaves of *C. nardus* harvested in Laoguié, Sub-prefecture of Agboville (70 km North-East of Abidjan), were dried under in the laboratory during three weeks.

To obtain total ethanolic extract, 50 g of fine powder of dried leaves of *C. nardus* were dissolved during 24 hours in 300 ml of 99.5% ethanol. The solution was then concentrated to 50 ml by evaporation under vacuum at 40°C. In addition, essential oils were extracted from dried leaves by hydro-distillation using a Clevenger-type apparatus during 3 hours.

Total ethanolic extract obtained after concentration on a rotary evaporator, and the essential oil of *C. nardus* were then used in bioassays without dilution.

2.3. Preparation of solutions of plant extracts and the reference product

Ethanol and acetone were chosen as solvents because they have a great dissolving power, a high volatility and are safe chemicals for insects (Shepard, 1958; Busvine, 1971).

For topical application tests, the total ethanolic extracts and the essential oils were applied without dilution. However, the reference product, K-Othrine (Deltamethrin 25 g / l), was diluted in water at 1% as recommended by the manufacturer.

For contact tests on filter paper, the essential oil was diluted in acetone to obtain a 20% oil solution.

2.4. Bioassays

Adult insects of different species and from 1 to 14 days old were removed from the rearing jars one day before the tests. These adults were then placed in 10 x 20 x 7 cm plastic boxes, covered with muslin and containing the grains on which the insects were reared.

2.5. Toxicity test by topical application

Topical application was done on each insect, under the objective of a stereo-microscope magnified 40x, using a micro-syringe mounted on a 50µl microinjector set to deliver a volume of 2µl at a time. During the application, the insect was immobilized, and 2µl of solution or extract of the test substance were applied on its pronotum. The treated insects were gathered in groups of 20 in 90 mm diameter Petri dishes. Control groups of 20 insects were also treated under the same conditions, but either with distilled water (control for essential oil and the reference K-Othrine) or with ethanol (control for saturated ethanolic extract). For each product tested on each insect species, five repetitions were performed. Test boxes were placed in the air-conditioned room.

The first counting of dead adult insects in each treatment and for each species was done after 24 hours and living insects were introduced in 137 cm^3 glass jars with 30 g of foodstuff; the jars were then placed in the air-conditioned room. After 5 days, the jars were checked again and the number of dead insects in each treatment was determined. For a given treatment, the numbers of dead insects in the first and second counting were added up to give a cumulative mortality after 5 days.

2.6. Contact toxicity test on filter paper

The application of a substance or an extract to be tested was done using a micropipette. For each prepared solution, 1 ml was applied to the filter paper (Wahtman N° 1) placed inside a 90 mm diameter Petri dish . After evaporation of the solvent, 20 adults of each insect species were placed on the treated filter paper. Control groups of 20 adults from each insect species were placed on the filter paper treated as described, with the solvent dilution of each extract. The boxes were then placed at a temperature of 30 ± 2°C and a relative humidity of 70 ± 5%. The counting of dead insects was done following the same protocol used in topical application tests.

2.7. GC-MS analysis of chemical components of essential oils of C. nardus

The essential oils were analyzed by GC-MS using a mass spectrometer coupled to a HP 5989 gas chromatograph HP5890 series II equipped with a split-splitless injector maintained at 250°C. Mass spectra were recorded with electronic impact (70 eV, mass range: 35-350 amu). The optimum conditions were determined as follows. Column: Chrompak CP Sil 57-CB (polyethylene glycol) 48 m x 0.25 mm, df = 0.25µm; temperature program: 40-250°C at 2 C min^{-1} gas Carrier: He (1 ml. min^{-1}).

The molecules were identified based on their retention data by comparing their mass spectra with those of the computerized library Wiley 275 L injection and reference molecules.

For a comparative analysis of chemical components of *C. nardus* with those of the essential oil of *C. citratus* (an insecticidal plant, Ketoh, 2000), the essential oil of *C. citratus* was also analyzed by GC-MS under the same conditions.

2.8. Statistical analysis

Firstly, mortality data were corrected using Abbott's formula.

Then, the comparison of the mortality of insects for the different tests was performed by an analysis of variance ANOVA for each insect species, using the software Minitab 12.

RESULTS AND DISCUSSION

3.1. Toxicity test by topical application

The results show that topical application of the essential oil of *C. nardus* on different insect species leads to a total mortality of treated insects after 24 hours. Total mortality is also observed after 24 hours with K-Othrine, the reference chemical (Table 1).

Table 1. Real mortality after one day and cumulative mortalities after 5 days (mean±standard error) of *S. zeamais*, *R. dominica*, *P. subdepressus* and *Cryptolestes* sp treated by topical application test with saturated ethanolic extract and essential oil of *C. nardus*

Species	Treatments	Tested products					
		RS		EO of *C. nardus*		ESE of *C. nardus*	
		1 day	5 days	1 day	5 days	1 day	5 days
S. zeamais	Test	20.0±0.0	20.0±0.0	20.0 ± 0.0	20.0±0.0	11.4±0.6	12.6±0.2
	Control	0.2±0.2	0.2±0.2	0.0 ± 0.0	0.8±0.4	0.0±0.0	0.8±0.6
	F of Fisher	9801***	9801***	9801***	2633.1***	5.44*	8.1*
R. dominica	Test	20.0±0.0	20.0±0.0	20.0 ± 00	20.0±00	2.2±0.4	3.4±0.5
	Control	0.8±0.4	2.4±0.2	0.0 ± 00	0.4±0.4	0.8±0.4	1.2±0.4
	F of Fisher	2633.1***	5162.7***	9801***	2401***	7*	12.1**
P. subdepressus	Test	20.0±0.0	20.0±0.0	20.0 ± 0.0	20.0±00	1.8±0.6	2.2±0.5
	Control	0.4±0.4	2.8±0.6	0.4 ± 0.4	2.4±0.7	1.4±0.4	1.4±0.4
	F of Fisher	2401***	870.1***	2401***	673.4***	O.3NS	1.6NS
Cryptolestes sp	Test	20.0±0.0	20.0±0.0	20.0 ± 0.0	20.0±0.0	14.2±0.9	17.6±0.7
	Control	0.6±0.2	3.6±0.7	0.2 ± 0.2	2.2±0.4	4.2±0.8	6.2±0.5
	F of Fisher	6272.7***	584***	9801***	2263.1***	72.5***	185.6***

*** = very highly significant difference (P < 0.001); ** = highly significant difference (P < 0.01); * = significant difference (P < 0.05) ; NS = not significant difference; RS = Reference substance (K-Othrine) ; EO = essential oil of *C. nardus* ; ESE = ethanolic saturated extract of *C. nardus*

In addition, the statistical analyzes show clearly that the topical application of the ethanolic saturated extract of *C. nardus* has toxicity effects on *S. zeamais*, *R. dominica* and *Cryptolestes* sp. However, the differences between control and treated insects are less significant compared with the application of the essential oil.

3.2. Contact toxicity test on filter paper

The results of mortality of insects during the contact tests on filter paper show that the 20% acetone solution of essential oil of *C. nardus* is toxic to all tested insect species tested as is the reference product K-Othrine (Table 2).

Table 2. Mortality (mean±standard error) of adults *S. zeamais*, *R. dominica*, *P. subdepressus* and *Cryptolestes* sp one and 5 days after contact with a filter paper impregnated with ethanolic saturated extract and 20% acetone solution of essential oil of *C. nardus*

Species	Treatments	Tested products					
		RS		EO of *C. nardus*		ESE of *C. nardus*	
		1 day	5 days	1 day	5 days	1 day	5 days
S. zeamais	Test	20.0±0.0	20.0±0.0	12.0±1.2	15.4±0.7	0.0±0.0	0.6±0.4
	Control	0.0±0.0	1.4±0.4	0.2±0.2	2±0.3	0.0±0.0	0.0±0.0
	F of Fisher	9801***	2162.3***	96.7***	272.1***	0NS	2.3NS
R. dominica	Test	20.0±0.0	20.0±0.0	6.2±0.7	10.8±1.2	0.0±0.0	0.4±0.3
	Control	0.0±0.0	1.4±0.7	0.0±00	0.2±0.2	0.0±0.0	0.4±0.3
	F of Fisher	9801***	2162.3***	71.2***	71.1***	0NS	0NS
P. subdepressus	Test	20.0±0.0	20.0±0.0	18.8±0.4	20.0±0.0	0.0±0.0	0.8±0.4
	Control	0.0±0.0	0.0±0.0	0.6±0.4	1.4±0.2	0.2±0.2	1.4±0.5
	F of Fisher	9801***	9801***	1104.1***	5766***	1NS	0.9NS
Cryptolestes sp	Test	20.0±0.0	20.0±0.0	20.0±00	20.0±0.0	4.8±0.6	6.6±0.9
	Control	0.4±0.3	0.6±0.3	1.2±0.6	4±0.4	2.6±0.3	5.8±0.7
	F of Fisher	6402.7***	6272.7***	1039***	1280***	12.1**	0.53NS

*** = very highly significant difference (P < 0.001); ** = highly significant difference (P < 0.01) ; * = significant difference (P < 0.05) ; NS = not significant difference; RS = Reference substance (K-Othrine) ; EO = essential oil of *C. nardus* ; ESE = ethanolic saturated extract of *C. nardus*

However, except for *Cryptolestes* sp, saturated ethanolic extract of *C. nardus* shows no toxic effect against the three other species of insects during the contact test on filter paper. From the results of tests by topical application (Table 1) and by contact on filter paper (Table 2), it emerges that the essential oil of *C. nardus* has an effective biocidal activity against beetles studied. The biocidal activity is very clear in the analysis of corrected mortality after 1 day test determined from the Abbott's formula for each product tested according to insect species (Table 3).

Table 3. Corrected mortality rate (in per cent) after 24 h of *S. zeamais*, *R. dominica*, *P. subdepressus* and *Cryptolestes* sp. treated with ethanolic saturated extract and essential oil of *C. nardus*

	Insect tested			
Tested products	*S. zeamais*	*R. dominica*	*P. subdepressus*	*Cryptolestes* sp
RS	100a	100a	100a	100a
EO of *C. nardus*	100a	100a	100a	100a
ESE of *C. nardus*	7b	7b	4b	63c

For each column, the figures having common letter are not statistically different (P < 0.01). RS = Reference substance (K-Othrine); EO = essential oil of *C. nardus*; ESE = ethanolic total extract of *C. nardus*

In this analysis, it appears that for all treated insect species, the essential oil of *C. nardus* shows a level of toxicity similar to that of the reference substance K-Othrine (synthetic pesticides); but saturated ethanolic extract of *C. nardus* does not have a significant biocide activity except on *Cryptolestes* sp which is shown to be very sensitive in terms of mortality observed during contact toxicity test on filter paper (Table 2).

3.3. Chemical components of C. nardus and C. citratus

The GC-MS analysis revealed that essential oils of *C. nardus* and *C. citratus* are composed mainly of monoterpenes, sesquiterpenes and alcohols. In terms of quantity, the oil of *C. nardus* is characterized by a high rate of citronellal (29.2%), citronellol (12.7%), geraniol (29.3%), elemol (5.0%) and limonene (4.1%) (Table 4), while the oil of *C. citratus* is composed mainly of citral (neral (27.3%) + geranial (32.2%)) and myrcene (17.21%) (Table 4).

Table 4. Chemical composition and average percentage of the constituents of the essential oil of *C. nardus* analyzed by GC-MS

Names of chemical compounds	$t'_{r\,(1)}$	Area %
Myrcene	0.520	1.55
Limonene	0.568	4.08
(Z) beta-ocimene	0.644	0.18
Citronellal	1.000	29.23
Linalool	1.074	1.09
Calarene	1.137	0.50
(Z,E)-Alpha-farnesene/ beta-elemene	1.156	0.23
Germacrene D	1.163	1.02
Citronellyl acetate	1.246	0.77
Neral	1.268	0.96
Beta-cubebene	1.321	0.59
Geranial	1.336	1.05
Alpha-Muurolene	1.341	0.25
Geranyl acetate	1.370	0.68
Delta-cadinene	1.384	1.16
Citronellol	1.426	12.68
Geraniol	1.521	29.29
Elemol	1.772	4.95
Eugenol	1.851	0.85

* in bold type = majority of chemicals

(1) Relative retention times (reference citronellal)

Table 5. Chemical composition and average percentage of the constituents of the essential oil of *C. citratus* analyzed by GC-MS

Names of chemical compounds	$t'_{r\,(1)}$	Area %
Myrcene	0.527	17.61
(E) beta-ocimene	0.628	0.22
(Z) beta-ocimene	0.654	0.34
6-Methyl-5-heptene-2-one	0.777	1.83
Allo ocimene	0.842	0.20
3-(4-Methyl-3-pentenyl)-Furan	0.906	0.45
Citronellal	1.000	1.96
Linalool	1.093	3.43
M = 152 (Not identified)	1.134	3.55
(Z.E)-Alpha-farnesene	1.176	0.23
2-Undecanone	1.181	0.37
Neral	1.303	27.27
Geranial	1.374	32.02
Citronellol	1.454	0.20
2-Tridecanone	1.473	0.29
Geraniol	1.546	5.03
Elémol	1.805	0.32

* in bold type = majority of chemicals

[1] Relative retention times (reference citronellal)

4. Discussion

To evaluate biocide activities of chemical compounds, toxicity tests by topical application and by contact filter paper are widely used (Haubruge, 1989; Doumbia, 1998).

The experiments carried out using topical application highlighted a highly significant (P <0.001) toxic effect of the essential oil of *C. nardus* on four insect species of commodities that were treated (Table 1). Like for saturated ethanolic extract of C*. nardus*, toxic action was also observed during the test by topical application, but at a lower level compared with that of the oil. In addition, saturated ethanolic extract did not show any toxic action on *P. subdepressus*. Low toxic activity of saturated ethanolic extract is very well observed for contact test on filter paper. However, the essential oil still showed a toxic effect on all insect species in the contact tests on filter paper (Table 2).

Results in this study indicate therefore that the Ivorian ecotype of *C. nardus* contains biocide substances which kill all the species of insect pests that underwent the various tests. In addition, it appears that the biocide active substances are more powerful in the essential oil than in the saturated ethanolic extract of *C. nardus*. These findings on the low biocide activity of the saturated ethanolic extract confirm those of Buledi and Gakuru (1995) who observed that extracts from the powder of leaves of *C. nardus* and *C. citratus* were less toxic to the bean weevil *Acanthocelides obtectus* Say. The biocide activity of the essential oil of *C. nardus* is also reported on bacteria, insects, and on human and plants pathogenic fungi (Pattniak ., 1997; Oussou, 2001; Koba ., 2003; Bankole and Joda, 2004; Jiang, 2010; Regnault-Roger, 2012).

The toxicity of the essential oil of *C. nardus* on bacteria, fungi and insects is attributed to the active chemicals such us terpenes present in this oil (Ketoh, 2000; Oussou, 2001; Jiang*,* 2010). Chemical analysis of the essential oils of *C. nardus* and *C. citratus* shows that these particular two oils are qualitatively and quantitatively very different (Tables 4 and 5). Indeed, the essential oil of the Ivorian ecotype of *C. nardus* is mainly composed of limonene (4.1%), citronellal (29.2%), citronellol (12.7%), geraniol (29.3%) and elemol (5.0%) (Table 4). The essential oil of *C. citrates,* another insecticide plant, is mostly composed of myrcene (17.6%), neral (27.3%) and geranial (32.0%) (Table 5). The comparison of the chemical composition of the Ivorian ecotype of *C. nardus* with Ethiopian and Zimbabwean ecotypes of this species (ALNAP Database, 2001) suggests also quantitative differences between their major components. In fact, among the majority of chemicals, citronellal, which represents 29.23% of the essential oil of the Ivorian ecotype of *C. nardus,* is about 35% in Zimbabwean and Ethiopian ecotypes (ALNAP Database, 2001). Similarly, citronellyl acetate and geranyl acetate are in low proportions (respectively 0.77% and 0.68%) in the Ivorian ecotype, compared to Ethiopian and Zimbabwean ecotypes in which these components represent on average 5% and 6.5% respectively. On the other hand, the Ivorian ecotype is rich in geraniol (29.3%) and elemol (5.0%) compared to ecotypes in Ethiopia and Zimbabwe where these components account respectively for 24.9% and traces. Chemical analysis by GC-MS has also revealed the existence of quantitative differences in chemical constituents between the major Ivorian ecotype of *C. nardus* and the Ethiopian and Zimbabwean.

Despite these quantitative and qualitative differences with regard to chemical components between ecotypes of *C. nardus* and *C. citratus*, all essential oils extracted from these plants have bactericidal (Pattniak, 1997; Oussou, 2001; Koba, 2003), fungicide (Oussou, 2001; Bankole and Joda, 2004) and insecticidal activities (Jiang*,* 2010).

Works on some chemical components of essential oils have revealed the most active substances in terms of biocide activity. According to Pattniak . (1997), geraniol and linalool identified in the essential oil of *C. nardus* (Table 4) are potent bactericides. Furthermore, geraniol and elemol, two major chemical components of *C. nardus* (Table 4), are recognized respectively as inhibitor of hydrolases (sucrase, lactase, alkaline phosphatase) (Carnesecchi, 2001) and anti-cholinesterase (Miyazawa, 1998). It is therefore highly possible that geraniol, due to its high concentration in the essential oil of *C. nardus,* act at two levels, by inhibiting insect hydrocarbons, or as an accelerator of intracellular transfer of other biocides (Carnesecchi ., 2001). Elemol, meanwhile, has a well-known anti-cholinesterase activity (Miyazawa ., 1998; Regnault-Roger, 2012).

These combined actions, probably reinforced by other components of the essential oil of *C. nardus,* may explain the toxic action of the essential oil of *C. nardus* on stored food product insect pests. In the present experiments, the tested insects tetanized for a while and died. This could be actually caused by inhibition of acetylcholinesterase at nerve synapses.

The essential oil of the Ivorian ecotype of *C. nardus*, although qualitatively and quantitatively different from the Ethiopian and Zimbabwean ecotypes in terms of chemical constitution, has a biocide action on dangerous pests of stored coffee and cocoa in Cote d'Ivoire. However, further studies on doses extension in rural environment, on persistence and stability are needed before recommendations for any widespread use by stakeholders.

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