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PERSISTENCE OF ACTIVE COMPOUNDS OF ESSENTIAL OILS OF *CLAUSENA ANISATA* (RUTACEAE) AND *PLECTRANTHUS GLANDULOSUS* (LABIATEAE) USED AS INSECTICIDES ON MAIZE GRAINS AND FLOUR

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ABSTRACT

Maize occupies an important place in the resolution of food insecurity problems in the sub-Saharan region of Africa. However due to poor post-harvest technologies, more than 35% of annual crop yield is often lost during storage as a consequence of insect attack. While chemical pesticides constitute an efficient tool for reducing these losses, current excessive usage runs the risk of modifying the quality and safety of foods produced from these grains. The use of essential oils of plant origin for grain storage has been advocated as a non invasive method with limited or no effect on the quality and safety of the grains or their products. This study was carried out as an evidence of bioactivity of two essential oils of *Clausena anisata* (Willd.) Hook and *Plectranthus glandulosus* Hook F. against adults of *Tribolium castaneum* Herbst and *Sitophilus zeamais* Motschulsky, which are two important stored product insect pests in Northern Cameroon. Because of the low persistence of the insecticidal activities of these plants, their essential oils to achieve a complete protection of the stored products must be applied at frequent delays. The present investigation focuses on the occurrence of residues of these oils on treated maize grain and flour. The doses of crude essential oils used to treat adults of *S. zeamais* and *T. castaneum* in a contact and inhalation process were their LD_{99}s. After the evaluation of their insecticidal activity the persistence of each essential oil was observed every 2 days till 14 days. After the disappearance of their insecticidal activities, essential oil was re-extracted and their residual compounds were identified from treated grain and flour. The major compounds of *C. anisata* are, estragole, α-humulene, germacrene D and (E)-nerolidol. In *P. glandulosus* they are: fenchone, α-terpinolene and piperitenone oxide. After 14 days, only 64.24% of compounds of *C. anisata* were recovered on treated flour and 55.16% on grains. Concerning *P. glandulosus*, 48.94% was recovered on grains and 61.23% on flour.

Key woods: Essential oils, persistence, stored products insects

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INTRODUCTION

Regulations for food safety and quality management are world widely enforced by laws to protect consumers. If products are properly treated with insecticides, the side effect residues on consumers and mostly mammals are acceptably low or under control. Because of their persistence many chlorinated pesticides (such as DDT) in spite of their insecticidal efficiency are nowadays prohibited [1]. Aromatic plants locally used as crop protectant by local farmers could be sources of new pesticides. Their essential oils or other extracts are used for crop protection as alternative to hazardous pesticides because of their availability, their insecticidal efficiency, and their biodegradability [2]. To achieve a good control of the pest, the protectant having high biodegradability potential must be applied frequently. This has the consequence of increasing the amount of residue on the treated products. The present work aims to consider the case of essential oils of 2 local aromatic plants which will be used for the control of 2 major pests of cereals grain or flour during storage. Their damages due to insects affect the quality, the quantity, the commercial and agronomic value of the product [1]. The targeted pests *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* Herbst are major pests of stored grains and grain products in the tropics [3]. Coming back to natural products as plant extracts could be an alternative to synthetic insecticides. In the northern part of Cameroon, peasants frequently introduce local aromatic plants in their granaries while filling them up with grain as a means of protecting them against insect attack during storage [4]. Studies of essential oils extracted from these active plants have already shown their potential for pest control and may contribute to alleviate losses recorded during grain storage [5, 6, 7, 8].

Previous investigations have already shown the potential activity of the essential oil of *Clausena anisata* and *Plectranthus glandulosus* that can lead to more than 90% mortality by direct application on adults of *S. zeamais* and *T. castaneum* at a lower concentration [9].

Despite the previous study on bioactivity of *C. anisata* and *P. glandulosus* to various insects, its persistence and residuals components against *T. castaneum* and *S. zeamais* had not been determined. The present research was therefore undertaken to investigate the bioactivity of the essential oil of *C. anisata* and *P. glandulosus* against adults of *T. castaneum* and *S. zeamais*, two important stored product insect pests in grain storage in Northern Cameroon.

MATERIALS AND METHODS

1. Stored product insect pests used

Two pests, *S. zeamais* and *T. castaneum* were used at adult stage and with known age. They were obtained from laboratory permanent rearing kept in the dark in incubators at 28 ± 2.2 °C and 65 ± 5.7% r.h. *S. zeamais* reared on white maize (CMS 8504), while *T. castaneum* was kept on flour of the same maize strain mixed with yeast (10:1, w:w). Adults of the two species of insects used for bio assays were 2 to 4 weeks old. Before being tested, these insects were starved for a 24 h period.

2. Extraction of essential oils of *Clausena anisata* and *Plectranthus glandulosus*

2.1 Extraction from leaves of aromatic plants

Plant materials were collected in the Guinean savannah surrounding the campus of the University of Ngaoundéré in the Adamawa Region of Cameroon, near the point referenced latitude 07°25.11N and longitude 13°22.5E and the altitude 1,036 m. Leaves of two plants were collected and dried without sun light for 2 days and thereafter were cut in pieces with a knife. These leaves cut in pieces were introduced separately in a Clevenger apparatus for a four hours period distillation.

2.2 Extraction from treated flours

Flour treated with essential oils on the day 0 was collected on the day 14 and considered for this study. An amount of 200g of flour was introduced in a Clevenger apparatus for a four hour distillation period. The essential oil obtained was recovered with hexane and the chemical analysis made within a chromatograph and a GS/MS/FID process as that used for the former essential oils obtained from leaves.

The essential oil obtained for each distillation was put in separate flask and kept in a refrigerator at 4°C till use for tests or for analysis.

3- Analysis of chemical composition of essential oils

The GC/FID (Chromatograph Agilent HP-6890) was carried out with HP-5MS column (5% phenyl methyl siloxane) with 30 m length and 250 µm in diameter and 1 µm of thickness. The carrier gas was hydrogen, the oven temperature was programmed from 40 to 230 °C with a rate of 5 °C. min^{-1} with a stay at 230 °C during 5 min. The pressure of the carrier gas was 49.9 KPa and the flux at 74.1 $mL.min^{-1}$. Quantification was carried out by percentage of peak area calculation. The identification of single compounds was performed by comparison of the retention-indices with reference data [10, 11].

4- Insecticidal efficiency of the 2 essential oils

Quantity of each essential oil ranging from 100, 200, 300, 400 µL to 500 µL were dropped and diluted in 10 mL of acetone to formulate the insecticidal solution. For each preparation, 350 µL was pumped and flowed regularly on a disk of filter paper (Whatman n°1) placed in a Petri dish. These concentrations of essential oils were supposed to induce insect mortality ranging from 0 to 100% after 24 h. After application, 25 insects were put on the filter paper within the dish, 4 min later and it was covered. Mortality of insect was noted 24 h after the treatment. For each trial, 4 replications were made. A control with imidacloprid was made. Imidacloprid is the active molecule present in varying proportions in certain insecticides registered in Cameroon and marketed under the name ATTAKAN, and CONFIDOR GAWA. This product is approved for the most part until 2014 [12]. It comes from the Laboratory of Entomology Evolutionary and Environmental of the Faculty of Agronomic Sciences of Gembloux (Belgium), manufactured by Bayer CropScience.

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5 - Persistence of insecticidal activity of the essential oils

The study of the persistence activity of 2 essential oils was made by introduction of insects on treated or flour of maize put in dishes. The mortality was noted 48 hours later. The insecticide that the persistence was to be evaluated was prepared on the day 0 and kept in Petri dishes. On the day 1 and on every 2 days till the day 20, an amount of the prepared insecticide was removed and used to treat 25 insects in new Petri dishes. Four replications were made each time. Dead insects were counted 48 hours after treatment.

Regarding imidacloprid, concentrations ranging from 10 ppm to 80 ppm were prepared in acetone and applications were made as described above in the case of essential oils.

RESULTS

1- Chemical composition of essential oils of *Clausena anisata* and *Plectranthus glandulosus*

The results of the analysis of the chemical composition of the essential oils of *C. anisata* and *P. glandulosus* are represented in the table 1. The only compounds present in the two oils were α- terpinolene and the D-germacrene, but at different proportions. The oil of *C. anisata* was 8 times richer in D-germacrene than the *P. glandulosus* one; often this last contain more than 9 times α-terpinolene than the previous.

Eighteen major compounds with concentration constituting more than 1% were identified in the essential oil of *C. anisata* representing 95.12% of crude oil (Table 1). Sabinene, trans-linalool oxide, estragole, (E)-caryophyllene, β-copaene, α-humulene, D-germacrene and (E)-nerolidol were the major constituents and representing 71.73% of this crude essential oil.

Fifteen compounds with concentration constituting more than 1% were identified in the essential oil of *P. glandulosus*, were β-myrcene, limonene, fenchone, α-terpinolene, and piperitenone oxide representing 74.31% of the compounds of the crude oil (Table 1).

2- Insecticidal efficiency of the tested essential oils towards *Sitophilus zeamais* and *Tribolium castaneum*

The killing activities of the two essential oils towards the targeted insect pests were evaluated by the calculation of the lethal concentration (LC). A presided LC was determined by the proportion of the experimental population killed. The LC_{50} and LC_{99} was not the same for both essential oils, or for the 2 pests.

In the Table 2, *S. zeamais* was more susceptible than *T. castaneum* with LC_{50} of 267 ppm and, 145 ppm, respectively for *C. anisat*a and *P. glandulosus*. The LC_{99} was at 395 ppm for *C. anisata* and at 246 ppm for *P. glandulosus*. For *T. castaneum*, the LC_{50} concentrations for essential oils, varied from one to the others, and were found at 294 ppm with a slope of 3.41 and at 196 ppm with a slope of 2.67, respectively for *C.*

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anisata and *P. glandulosus*. The LC_{99} of *T. castaneum* were at 433 ppm for *C. anisata* and at 276 ppm for *P. glandulosus*.

With imidacloprid as essential oils, the concentrations obtained vary from one insect to another. They are: LC_{80}= 60 ppm and LC_{99} = 80 ppm for *S. zeamais*, and LC_{80}= 80 ppm and LC_{99}= 100 ppm for *T. castaneum*.

3 - Persistence of insecticidal activity of the tested essential oils

The results from figure 1 show that the mortality rates increasing with time following introduction of insects, and is independent of the type of food. On the maize grains, the mortality observed with essential oils was about 100% of the experimental population of *S. zeamais* after 4 days of exposition (Figure 1).

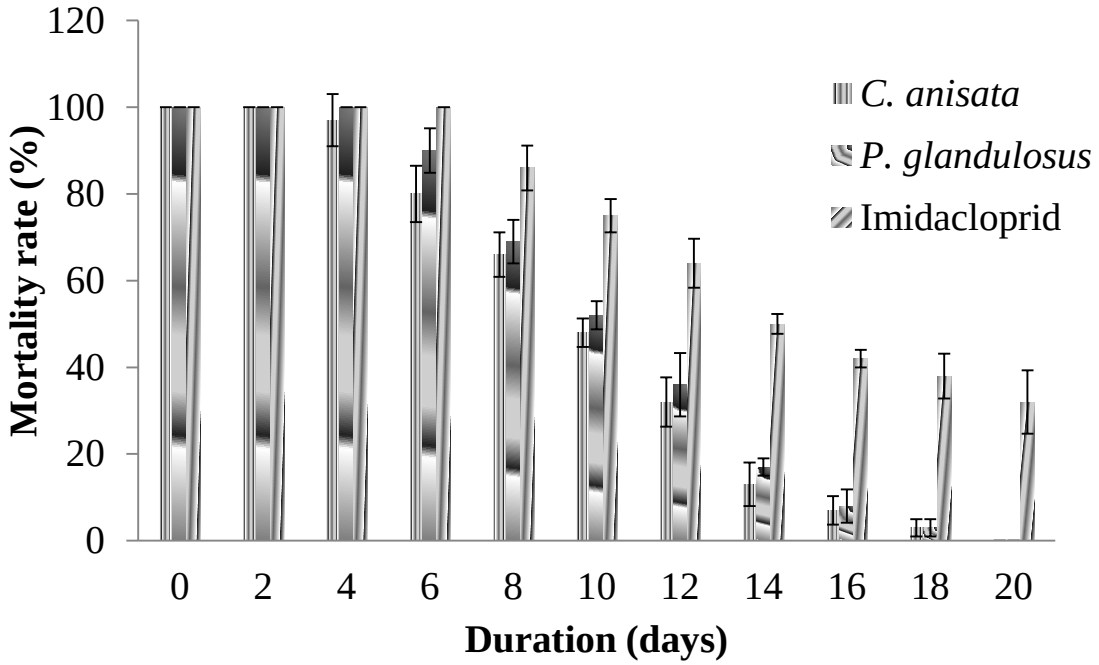

Figure 1: Mortality rate of *Sitophilus zeamais* observed every 2 days during 20 days on the fumigated maize with active matters.

On the corn flour, the mortality rates of *T. castaneum* were 100% at the end of day 6 of exposition (Figure 2). Beyond that time, mortality decreased continually to 80% and 62%, respectively, for *C. anisata* and *P. glandulosus* after 14 days. The analysis of this mortality rates shown a highly significant difference ($p<0.0001$) between the time of introduction of insects for all these essential oils. The Duncan test shows a significantly difference in the level of 5% for the two active matters.

For treatments carried out with the control, imidacloprid, mortality rates were stable at 100% until the sixth first day for *S. zeamais* and eighth first day for *T. castaneum*. Beyond these times, these mortality rates fall gradually to 38 and 32% for *S. zeamais*, and 42 and 30% for *T. castaneum*, respectively at 18 and 20 days.

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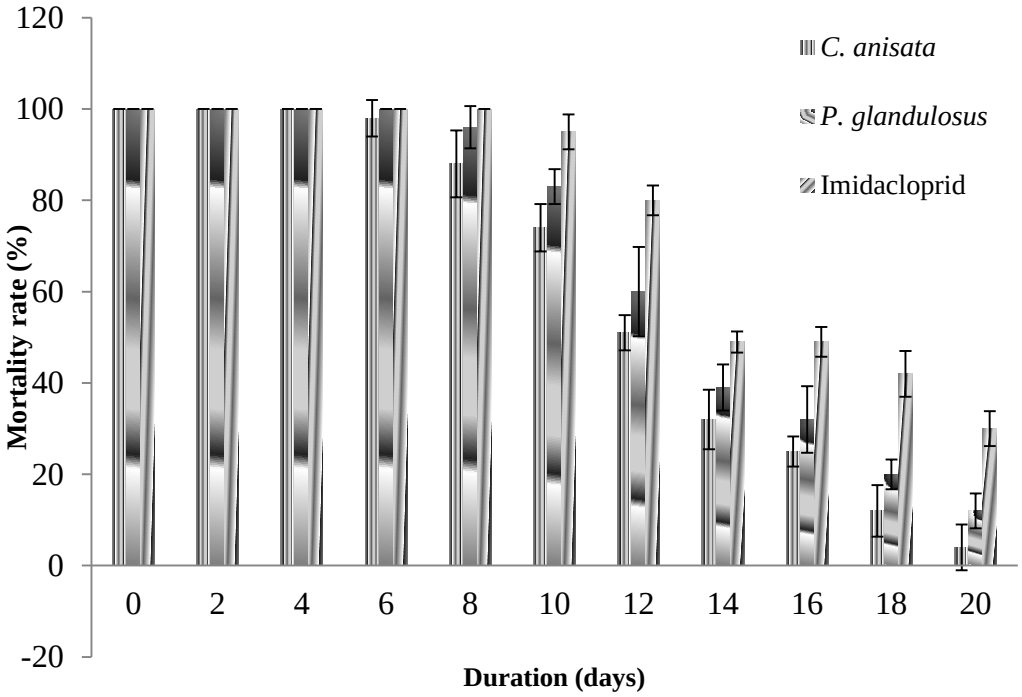

Figure 2: Mortality rate of *Tribolium castaneum* observed every 2 days during 20 days on the treated flour with active matters.

4- Residues of the essential oils used on flour 14 days after application

It is evident from the table 1 that compounds of essential oils decreased after 14 days of exposition to the food. The concentration of active ingredients in the essential oil of *C. anisata* goes from 95.12% for the raw oil to 64.42 and 55.16%, respectively, for residual oil extracted on the corn flour and grains. The cis-p-menth-2 did not exist in the residual oil extracted from the grains. Concentration of active ingredients of *P. glandulosus* oil decreased from 93.82% to 61.23 and, 48.94% for residual oil on the corn flour and grains, respectively. This residual *P. glandulosus* oil no longer contained camphor (Table 1).

DISCUSSION

The plant species which evolve in different ecological and geographical conditions can present some variations in the concentration and the quality of their secondary metabolites [13]. The resource in water, the availability of nutriments and conditions of lighting can influence mechanisms of synthesis of essential oils. The relation exists between the chemical composition of an essential oil and the geographical variations [14]. The composition of the oil of *C. anisata* studied was similar to the one found by [15], but at a different concentrations.

The oil of *P. glandulosus* presents similar composition to that found by [16] which showed that *Plectranthus* genus is also a rich source of piperitone and piperitinone derivatives. The essential oil of *P. incanus* from India, has been found to contain 35.7% of cis-piperitone and 45% of piperitenone oxide [17], while the *P. defoliatus* from Burundi, contained mainly piperitenone oxide (88-53%) [18]. Essential oil of *P. glandulosus* differs from the two species, with a higher percentage of fenchone (29.81%), terpinolene (29.28%) and a lower percentage of piperitenone oxide (11.08%). This difference could be due to ecological conditions of the different regions and plants.

The different mortality rates between the essential oils would be due to the qualitative and quantitative specific composition of the aromatic plants from which they were extracted [19]. Other investigators have shown the anti-insect activities for these and other sources of the essential oil [6, 20, 21, 22]. One investigator suggested a synergy between all compounds [21]. The difference of efficiency observed within the different species is due to the genetic diversity within the population, which can even vary from a population to another within the same species [22]. The loss of activity of essential oils could be due to the reduction of their quantity and the quality on food [23].

The duration of insecticidal activity of the studied essential oils depends on the insect and the nature of treated product. These insecticide activities decreased quickly, because their compounds are vegetal molecules belonging to groups of monoterpenes, diterpenes, sesquiterpenes, which are volatile from their photolability (loss of molecular structure due to interaction with light)[2]. Furthermore, fast deterioration of monoterpenes hydrocarbons, such as the sabinene; 1,8 cineole, and α-pinene; as well as the alcoholic compounds, are due to a high speed of the oxidization of these essential oils [19].

Previously researchers [5, 24] have shown that the essential oils of *Ecalyptus calmadulensis, E. citiodora, Lippia rugosa* and *Ocimum gratissimum* formulated with kaolin had a persistence of 10 days and 6 days for essential oils of *Hyptis spicigera* and *L. rugosa,* respectively, diluted in acetone. This weak persistence of essential oils suggests that the efficient use of these oils requires substrata that prolong in a substantial manner of their activity to improve the frequency of the successive treatments.

CONCLUSION

The present study examined persistence of active compounds of essential oils of *Clausena anisata* (Rutaceae) and *Plectranthus glandulosus* (Labiateae) for use as insecticides on maize grains and flour in northern Cameroon. This study showed that persistence of essential oils was 10 days when applied to maize and 12 days when applied to flour. At the end of this time, the concentration of active chemicals of these essential oils were still present, but reduced to one half. Local farmers can exploit this result for treatment of their products to reduce pest losses.

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Table 1: Chemical composition identified by GC of the essential oil extracted from fresh leaves *Clausena anisata* and *Plectrantus glandulosus* from Cameroon and from treated maize flour 14 days after application

		C. anisata		*P. glandulosus*	
RT	Compounds	Crude oil	After 14days	Crude oil	After 14days
851	1-hexanol			1.23	0.56
943	α-pinene			1.06	0.87
977	sabinene	4.91	1.51		
991	β-myrcene			5.13	2.32
1008	δ-3-carene			1.1	0.51
1027	limonene			2.7	0.93
1076	trans-linaloooxide	4.25	3.76		
1089	fenchone			29.81	27.55
1090	α-terpinolene	2.94	1.92	28.29	14.67
1091	cis linalooloxide	1.08	0.86		
1100	linalool	1.21	1.18		
1127	cis-p-menth-2-en-1-ol	1.73	0.34		
1142	camphor			1.34	
1146	terpinene-4-ol			2.51	1.33
1179	ρ-cymene-8-ol			2.8	0.87
1193	estragole	23.68	20.04		
1201	methyl salicylate	2.12	1.85		
1234	Z-ocimenone	2.11	2.03		
1243	E-ocimenone	2.08	1.34		
1247	cis-piperitoneoxide			2.82	0.88
1292	thymol	6.07	3.06		
1315	piperitenone			1.23	0.27
1348	Δ-elemene	2.07	0.85		
1353	piperitenoneoxide			11.08	8.86
1389	α-copaene	1.11	0.64		
1399	isopulegone-4-methyl			1.11	0.98
1438	E-caryophyllene	4.68	1.61		
1445	β-copaene	4.57	1.34		
1473	α-humulene	9.78	8.24		
1499	germacrene D	10.61	5.42	1.61	0.63
1571	E-nerolidol	10.12	8.43		
	Total	**95.12**	**64.42**	**93.82**	**61.23**

RT = retention time; Compounds considered in this analysis have their rate above 1%.

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Table 2: Insecticidal efficiency through calculation of lethal concentration of essential oils of *Clausena anisata* and *Plectrantus glandulosus* used against *Sitophilus zeamais* and *Tribolium castaneum*.

		Imidacloprid	*C. anisata*	*P. glandulosus*
S. zeamais	LC_{50}	60	267	145
	LC_{99}	80	395	246
	IC	40 < IC < 100	245 < IC < 427	127 < IC < 298
	slope	1,88	2.32	2.07
T. castaneum	LC_{50}	80	294	196
	LC_{99}	100	433	276
	IC	60 < IC < 120	269 < IC < 458	163 < IC < 309
	slope	2,10	3.41	2.67

IC : Confidence Interval.

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