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Insecticidal effect of *Cymbopogon schoenanthus* (L.) Spreng essential oil on adults of the rice weevil *Sitophilus oryzae* Linnaeus (Coleoptera: Dryophthoridae) in Togo

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Abstract

Fumigation, most often with phosphine, is commonly used to control pests in stored food and grain. This practice is highly toxic to animal, human, and environmental health. To find alternatives for the safe and healthy storage of grain, the insecticidal effects of the essential oil of *Cymbopogon schoenanthus* (L.), a grass native to Togo, were evaluated on adults of the rice weevil *Sitophilus oryzae* (L.). *In vitro* laboratory biological tests following the method recommended by the Insecticide Resistance Action Committee (IRAC) No. 1 Version 2, coupled with field tests (sorghum storage warehouse), demonstrated a highly significant insecticidal effect on rice weevil adults. *C. schoenanthus* essential oil at 0.5 ml. L^{-1} has an insecticidal effect on adult rice weevils *in vitro* and a repellent/insecticidal effect at 5 ml.L^{-1} diluted in soapy water and sprayed every 3 days for 30 days in farm stores to ensure sorghum quality and quantity. Safety assessment will be important for developing and integrated management strategy using this essential oil against this formidable pest.

Keywords: *Cymbopogon schoenanthus*; *Sitophilus Oryzae*; Essential Oil; Sorghum; Togo

1. Introduction

In rural areas where semi-modern and modern conservation stores do not exist, the use of synthetic chemical pesticides for the preservation of foodstuffs has long been one of the most used methods [1]. However, this practice, often carried out by non-professionals, creates not only environmental problems (air and water pollution) but also for humans (poisoning, cancer, etc.) and develops insect resistance. [2] cited by [3] find that the development of resistance by insect populations, the change in status of secondary pests into primary pests, the effects on non-target organisms and general pollution of the environment with persistent residues caused by synthetic chemical insecticides have led to a drastic change in the philosophy of pest control. Cereals represent for many developing countries, in this case Togo, the bulk of the diet of rural populations, generally on low incomes [4]. Indeed, in the dry Sahelian zone, post-harvest conservation is the only way to ensure the link between the harvest occurring once a year and consumption which is permanent and obligatory. Harvests, generally stored in inadequate conditions, are attacked by insects, rodents and molds [5]. The FAO estimates that post-harvest losses go beyond 60% for cereals and seeds. Sometimes, losses (quality, quantity) can reach 100% of the harvest [6]. Insect pests are responsible for 25 to 40% of loss in 6 months [7]. Many studies in recent years have shown satisfactory results of the antimicrobial, insecticide, insect repellent and acaricide potential of essential oils of some plants [8, 9, 10, 11, 12, 13]. In order to find alternatives to the healthy and safe storage of cereals in the face of the dangers of commonly used synthetic pesticides, the insecticidal effects of the essential oil of *Cymbopogon schoenanthus* (L,) a grass of the Togolese flora, were evaluated on adults of the rice weevil *Sitophilus oryzae* (L.)

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Specifically, this involved carrying out *in vitro* biological tests in the laboratory following the method recommended by [14] coupled with tests in a rural environment in a sorghum storage warehouse in Pana in northern Togo.

2. Materials and methods

2.1. Study site

In vitro tests are carried out at the Agroresources and Environmental Health Research Laboratory of the Higher School of Agronomy of the University of Lomé (LARASE, ESA-UL). Field trials are carried out in Pana, Tône region, in the far north of Togo. These two areas are located in tropical Guinea. Pest identification was carried out at the ESA entomology laboratory coupled with Google images. The site was chosen based on the quantity of sorghum production, conservation practices and the degree of pest infestation.

2.2. Animal material

Adult rice weevils are captured in the sorghum mud warehouse in Pana. A 5 kg sample of grain containing the adult insects in a plastic jar covered with a fine-mesh net is brought back to the laboratory. Samples were taken 10 to 15 minutes before testing.

2.3. Plant material and extraction of essential oil

The plant material used is Indian verbena (*C. schoenanthus*), a species adapted to drought conditions and more easily cultivated in all seasons with a significant biomass. It comes from a cultivation plot at the Agronomic Experiment Station of the University of Lomé (SEAL, ESA-UL, Togo) harvested during the flowering phase (June to July 2024) then dried on the laboratory bench at a temperature of 28°C. The extraction of the essential oil was obtained by hydrodistillation of a 50g sample of plant material for 2 hours using a modified Clevenger-type glass device [15]. The oil obtained was placed in the Amber glasses obtained from the CHU Campus of Lomé, cleaned with alcohol and passed through an oven before the introduction of the oil. It is then stored in a refrigerator at 5°C.

2.4. Analysis of the essential oil of *C. schoenanthus* and its major compounds

The analysis and identification of the constituents of the essential oil obtained as well as the determination of its relative centesimal composition were carried out by gas chromatography (GC) equipped with a flame ionization detector (CPGFID) and by gas chromatography coupled with mass spectrometry (GC-MS).[16]

2.5. Biological tests

In the laboratory

Performed according to the method [14], the *in vitro* sensitivity tests were carried out as follows: 25 adult rice weevils were placed in Petri dishes (9cm in diameter) using a fine brush (Ponnet brush) divided into 6 batches of 4 dishes containing 25g of sorghum each. In each batch was introduced cotton soaked in *C. schoenanthus* essential oil at different concentrations (0; 0.2; 0.4; 0.6; 0.8 and 1 ml.L^{-1}). Concentration 0 is the negative control (insect with sorghum). The Petri dishes were then placed in laboratory conditions (room temperature: 28°C; relative humidity 80%) for the different observations.

In the field

1, 2, 3, 4 and 5 ml of essential oil in 1 L of soapy water by volume (locally made potassium soap) in 5 batches are sprayed, respectively in 5 mud stores of dimensions 4m x 2.5m x 3m including 3 stores containing 10 bags of 100 kg of grain sorghum each obtained by hand threshing during the month of December 2024 and 2 containing one ton of sorghum panicle each harvested in the month of October of the same year, repeated every 3 days for a month. These grains contained both weevils and other insects. The stores closed after spraying but had vents and some cracks.

2.6. Determination of the mortality rate

In the laboratory

The mortality rates of insects exposed to the different products at different concentrations were assessed after 24 hours. A binocular magnifying glass was used to count the dead insects. According to [16], an insect is considered dead when it no longer reacts to contact using a slightly heated needle placed on sensitive parts such as antennae. Mortality was calculated and corrected according to the formula of [17]:

$$Mc = \frac{\text{Me} - \text{Mt}}{100 - \text{Mt}} \text{ x } 100$$

Mc = mortalité corrigée en pourcentage
Me = mortalité de l'échantillon testé
Mt = mortalité dans le témoin non traité

In the field

It is assessed by observation. Dead insects are noted as well as those that fled after spraying.

2.7. Analysis of the results

The collected data was entered and processed using Excel 2019 to determine descriptive statistics in terms of percentages and averages. To do this, data from different stores was grouped for overall analysis. The graphs presented in this document were created using the same software.

3. Results

3.1. Analysis of the essential oil

Obtained from dry biomass with a yield of 2.4%, the essential oil of *C. schoenanthus* is colorless and contains 23 compounds representing 98.02% of the identified compounds with piperitone (66.5%) and δ-2-carene (14.96%) as the major compounds. (Table 1)

Table 1 Chemical composition of *C. schoenanthus* essential oil from SEAL (Togo)

Identified compounds	Woodpecker ranges *C. schoenanthus* (%)
Hydrocarbures sesquiterpéniques	*1.69*
β-élémène	0.81
β-caryophyllène	0.09
α-farnésène	0.09
Tr-β-farnésène	0.21
Germaacrene D	0.31
σ-cadinène	0.18
Sesquiterpènes oxygénés	*2.07*
oxyde de caryophyllène	0.2
β-eudesmol	0.76
α-eudesmol+ γ-eudesmol	0.3
α-tglate de citronnellyle	0.81
Monoterpènes oxygénés	*70.69*
Trans hydrate de pinène	1.27
Cis hydrate de pinène	0.53
Terpinéol-4	0.84
α-terpinéol	1.55
Piperitone	66.5
Hydrocarbures monoterpéniques	*23.57*

α-thujène	1.29
α-pinène	0.39
β-pinène	0.18
δ-2-carène	14.96
α-phellandrène	0.46
α-terpinène	1.62
Limonène γ-terpinène	2.21 2.46
Total identifié (%)	98.02

3.2. Variation of the mortality rate according to the concentrations as a function of time *in vitro*

Figures 1a, 1b, 1c and 1d describe the evolution of weevil mortality over time.

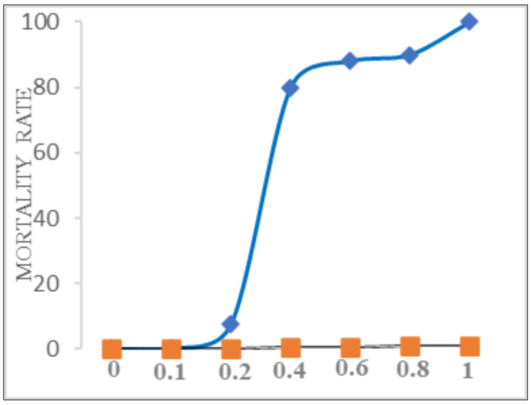

Figure 1a Variation in mortality rate after 1 hour of exposure

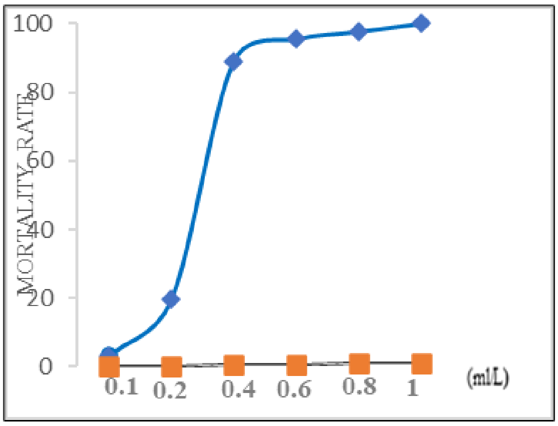

Figure 1b Variation in mortality rate after 3 hours of exposure

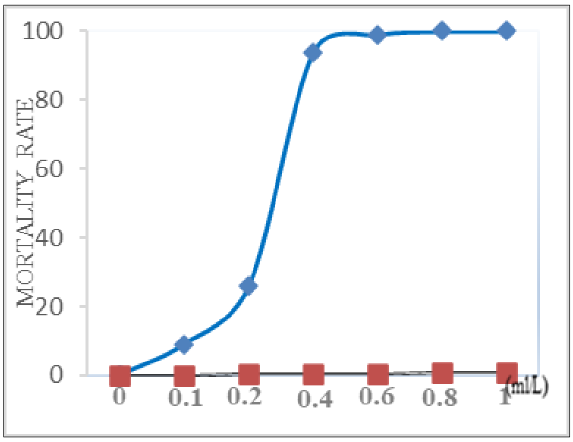

Figure 1c Variation in mortality rate after 6 hours of exposure

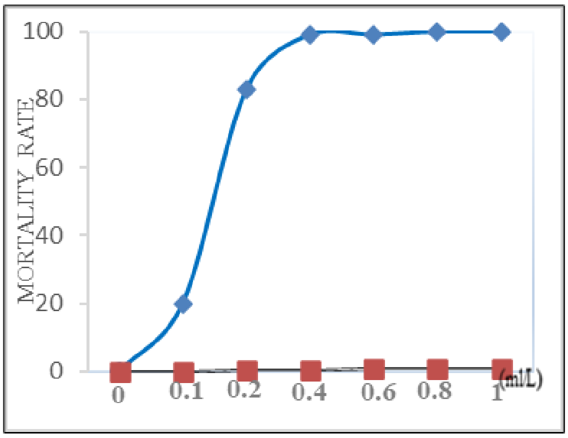

Figure 1d Variation in mortality rate after 24 hours of exposure

The statistical analysis of the results obtained after 1h, 3h, 6h and 24h of exposure to the essential oil and the discrimination of the means using the Duncan test at the 5% threshold made it possible to distinguish 3 homogeneous groups (table 2).

Table 2 Discrimination of mortality means at the Duncan 5% threshold on the sensitivity of *S. oryzae* after 1 hour of exposure to C. sheathes essential oil

Concentration (ml.L^{-1})	**Mean**	**Homogeneous group**
0,2	2	a
0,4	12	a
0,6	53	b
0,8	69	b
1	98	c

3.3. Variation of mortality rates according to the duration of exposure for each concentration

The mortality rate increases with exposure time regardless of the concentration used.

This rate does not exceed 50% mortality at concentrations between 0 and 0.4 ml/L.

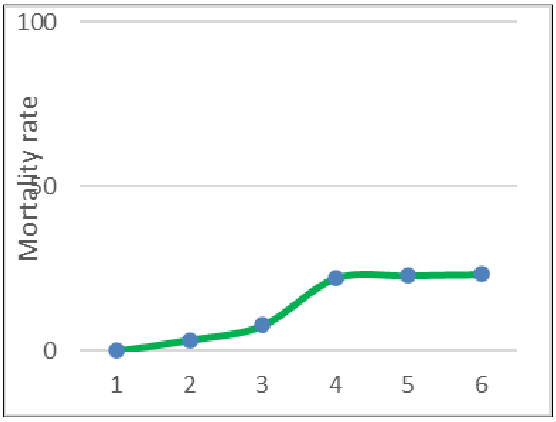

Figure 2a Variation of mortality rate in f(t) and concentration (0.2- and 0.4-ml. L^{-1})

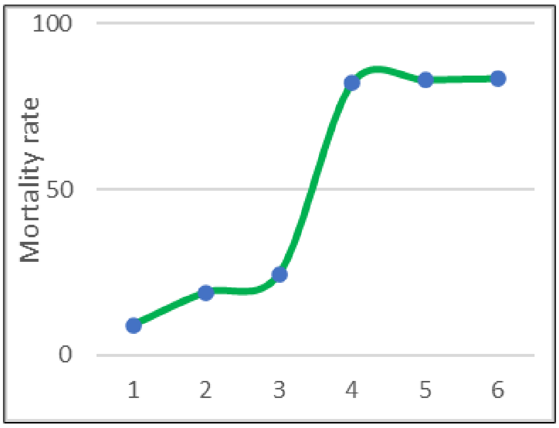

Figure 2b variation of mortality rate in f(t) and concentration (0.6 ml. L^{-1})

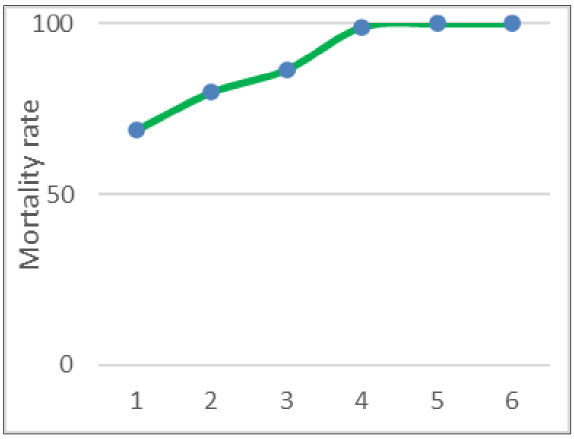

Figure 2c Variation of mortality rate in f(t) and concentration (0.8 ml. L^{-1})

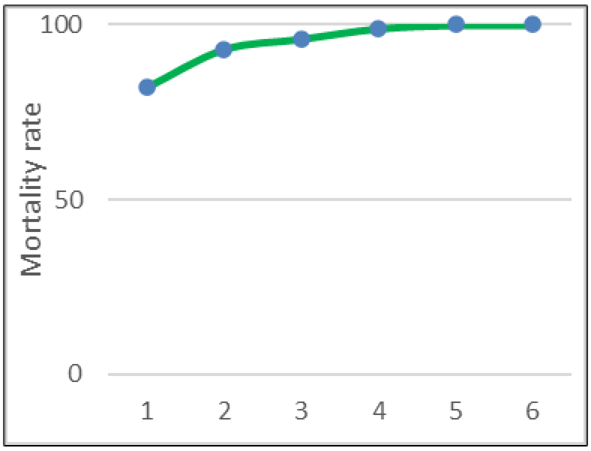

Figure 2d Variation of mortality rate in f(t) and concentration (1 $ml.L^{-1}$)

3.4. Effect of *C. schoenanthus* essential oil in a rural environment

The observation is the same in the five (5) stores. Criteria such as presence, distribution, mortality were evaluated.

Table 3 Pest reaction (*S. oryzae*) in stores

Concentration ($ml.L^{-1}$)	Store containing sorghum grain	Store containing sorghum panicle
1	++	++
2	++	++
3	+	-+
4	-	-
5	-	-

++ abundant presence, +moderate presence, - for absence

We noticed the presence of pests at concentrations of 1 and 2$ml.L^{-1}$ (Figure 3).

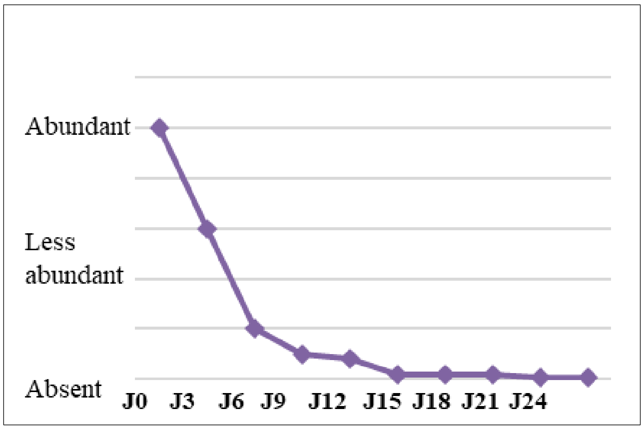

Figure 3 Insect distribution according to days at 1-2 $ml.L^{-1b}$

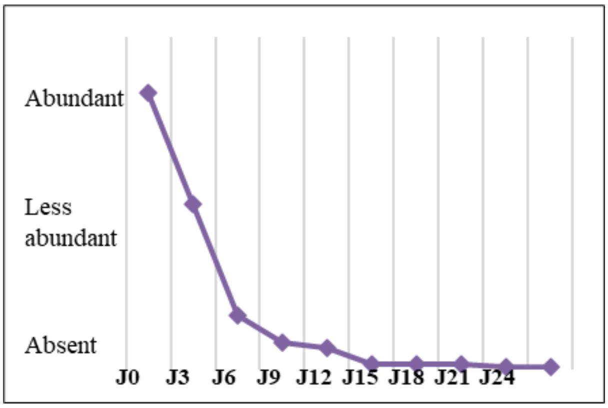

Figure 4 Insect distribution according to days at 3 ml.L^{-1}

The insects fled (the flying ones) and returned 24 hours after spraying at a concentration of 3 ml.L^{-1} (Figure 4) during the 2 weeks after the start of the tests. They were absent on day 21.

With 4 and 5 ml.L^{-1}, their distribution decreased. Six hours after spraying on day 1, we observed a few dead insects and noted movement inside the bags and panicle piles. After 5 sprays, that is, on the evening of day 15, the noise in the bags decreased, and we noticed the presence of weevil corpses observed at the bottom of the panicle piles. Figure 5 illustrates their chronological distribution.

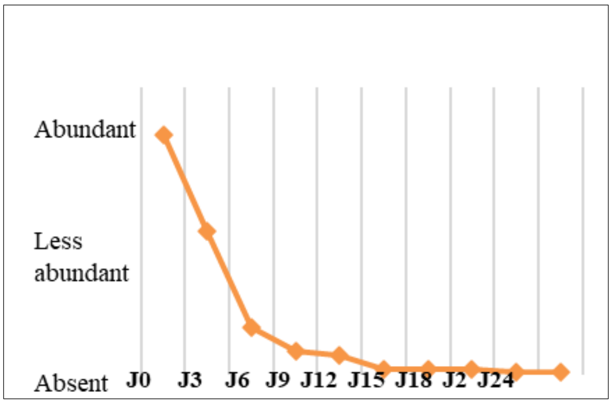

Figure 5 Insect distribution according to days at 4-5 ml.L^{-1}

4. Discussion

The chemical composition of the essential oil of *C. schoenanthus* used is close to that found previously by other Togolese authors [16, 18, 19, 20], but is different from that described in Burkina Faso by [21] with lower proportions of piperitone (42%) and δ-2-carene (8.2%). Its chemical composition is also different from that found in Tunisia by [22] with limonene (10.5–27.3%), β-phellandrene (8.2–16.3%), σ-terpinene (4.3–21.2%) and α-terpineol (6.8–11.0%) as the major compounds as well as that found by [23] in Brazil whose main compounds are geraniol (62.5%), geranial (12.5%) and neral (8.2%). These differences in chemical composition may arise from the plant organ, the extraction and analysis process, harvest and post-harvest treatments and the harvest period [23].

The results obtained with the biological tests show that there is on the one hand a relationship between the mortality rate and the concentration and on the other hand the mortality rate and the duration of exposure. [25] showed that the toxic or repellent effects of an essential oil would depend on several factors including its concentration, its chemical composition and the level of sensitivity of the target insects, thus corroborating with our results. Indeed, *S. oryzae* is a beetle whose cuticle protects it. This explains a high concentration compared with what [16] found on aphids. It proved effective with an insecticidal activity at concentrations higher than 0.5 ml.L^{-1} in a controlled environment (laboratory) and 5ml.L^{-1} in a peasant environment. Our results confirm the hypothesis put forward by [27] according to which essential oils would act directly on the cuticle of insects and mites, especially those with soft bodies. The larvae of the rice weevil live inside the grains. Only adults are visible. A low concentration would not reach them, since they take refuge inside the grains once the danger is felt. This also explains the high concentration obtained in a rural environment, not to mention other factors such as ventilation and the presence of other pests that serve to leave cracks capable of sheltering them. [27] showed that the essence of *C. schoenanthus* has repellent effects of nearly 69.6% on adults of *A. gossipi*, placing it in class 3. These conclusions corroborate with our results in a rural environment which showed repellent effects a few minutes after spraying. The work of [28] also showed a significant repellent effect of essential oils extracted from *Syzygium aromaticum, Eucalyptus smithii* and *Pimenta racemosa* on the insect *Callosobruchus maculatus* after half an hour of exposure. From our results, we conclude that the dose effect is co-related with repellency. Indeed, the higher the concentration, the more repellent the substance.

5. Conclusion

In order to contribute to the valorization of the essential oil of Indian verbena (*C. schoenanthus*) on the one hand and to find alternatives in the healthy and safe conservation of cereals on the other hand, series of tests were carried out on the rice weevil *S. oryzae*. It appears that the essential oil at 0.5 $ml.L^{-1}$ has an insecticidal effect on the adults of the rice weevil and a repellent/insecticide effect at 5 $ml.L^{-1}$ in stores in rural areas diluted in soapy water and sprayed every 3 days for 30 days to guarantee the quality and quantity of sorghum throughout the period of its conservation (6-9 months in rural areas). It has proven to be both insect repellent and insecticide. Our work is continuing in this direction in order to carry out tests on other beetles that infest granaries in northern Togo.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

We, Yendouboame SOAMPA and Kansame BONI, authors, declare that we have no conflict of interest with competing products to those mentioned related to this subject.

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