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Research Article

Influence of Female Age on the Reproductive Performance of *Spermophagus niger* Motschulsky and the Insecticidal Activity of *Cymbopogon nardus* (L.) Rendle Essential Oil

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Abstract

Spermophagus niger L. is the main insect pest of stored *Hibiscus sabdariffa* seeds, causing significant damage through seed perforation. This study aimed to enhance our understanding of the biology and management of this pest by evaluating the influence of female age on reproductive performance, and assessing the insecticidal effect of *Cymbopogon nardus* essential oil (EO). The influence of females age was assessed through several reproductive and developmental parameters, including egg size, hatching rate, and development time, among other factors. The insecticidal activity of the EO was evaluated against both adults and freshly laid eggs. Increasing female age negatively affected offspring performance, resulting in smaller eggs, higher larval mortality, extended development time, and reduced adult size at emergence. Chemical characterization of *C. nardus* EO has shown a predominance of monoterpenes, with citronellal (23.61%), geraniol (11.63%), citronellol (9.63%), and D-limonene (1.99%). This EO exhibited significant insecticidal activity against adult *S. niger*, reducing both fertility and egg hatching rates. The median lethal concentration (LC_{50}) and the concentration causing 90% mortality (LC_{90}) values were estimated at 4.29 µL/L (95% CI: 3.96-4.62) and 10.52 µL/L (95% CI: 9.44-11.87), respectively. These results provide valuable insights into the biology of *S. niger* and demonstrate the potential of *C. nardus* EO as an alternative to chemical insecticides for protecting stored *H. sabdariffa* seeds.

Keywords

Hibiscus sabdariffa, *Spermophagus niger*, Control, *Cymbopogon nardus*, Reproductive Performance

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1. Introduction

Hibiscus sabdariffa L. (Malvales: Malvaceae) is a leafy vegetable widely cultivated in savanna and semi-arid regions [1]. It is highly valued for its nutritional, therapeutic, and industrial properties [2, 3]. Its leaves and calyxes are used in the preparation of various food products, including sauces, beverages, and jams [4-6]. Its seeds, which are rich in proteins, lipids, and carbohydrates [7], are processed into a condiment used as a meat substitute, and known in Burkina Faso as *Bi-kalaga* [8, 9].

However, the storage of *H. sabdariffa* seeds is severely threatened by *Spermophagus niger* (Motschulsky, 1866) (Coleoptera: Chrysomelidae), the main insect pest associated with this commodity [10, 11]. The entire life cycle of this insect occurs within the seeds, where the larvae feed on seeds the reserves before adults emerge through characteristic exit holes in the seed coats [12]. In the absence of effective control measures, infestation levels and associated losses may increase up to tenfold after six months of storage [11].

In Burkina Faso, to protect *H. sabdariffa* seeds against *S. niger*, farmers and traders commonly rely on chemical insecticides [13]. This control strategy has many negative effects on human and animal health, as well as harmful impacts on the environment [14, 15]. Furthermore, the excessive use of these chemicals has led to the development of resistance in various insect pests [14] and has negative impacts on non-target organisms, particularly pollinators and other beneficial insects [16].

The ability of *S. niger* to cause damage is closely linked to its population dynamics, which are influenced by factors affecting its morphology, physiology, and adaptation to environment [17, 18]. Among these factors, temperature is considered one of the most important environmental determinants of insect performance, influencing survival, fertility, and development of insects [19]. Previous studies on *S. niger* have shown that its development is optimal at 25 ℃ but is inhibited at 20 ℃ and 39 ℃ [20]. Nevertheless, beyond abiotic factors, intrinsic biological factors such as the age of females at the time of oviposition play a decisive role in reproductive performance. Indeed, in many insects, the age of females influences fertility, egg viability, and the quality of the offspring [21-23]. In *S. niger*, this aspect has not yet been documented.

Therefore, the present study aimed to evaluate the effects of female age on egg size, egg viability, larval survival, and development time. In addition, the insecticidal activity of *Cymbopogon nardus* essential oil against eggs and adults of *S. niger* was assessed. The results obtained should contribute to a better understanding of the biology of this species and provide a scientific basis for the development of strategies for the sustainable management of *H. sabdariffa* seeds.

2. Materials and Methods

2.1. Study Site

The study was conducted from December 2025 to March 2026 at the Laboratory of Fundamental and Applied Entomology (LEFA), Joseph KI-ZERBO University, Ouagadougou, Burkina Faso.

2.2. Host Plant: *Hibiscus sabdariffa*

Seeds of *Hibiscus sabdariffa* (cv. *Altissima* variety) were purchased at the Dassasgho market (Ouagadougou, Burkina Faso). Prior to use, the seeds were cleaned and then stored at -18 ℃ to eliminate any initial infestation.

2.3. Insect: *Spermophagus niger*

The *Spermophagus niger* strain used has been maintained at the Laboratory of Fundamental and Applied Entomology since October 2025. To maintain the strain, 20 pairs of 24-hour-old adults were introduced into Plexiglas containers (15.5 × 11.5 × 4 cm) containing 50 g of healthy sorrel seeds (cv. *Altissima*) and allowed to oviposit for 48 hours. The infested seeds are then transferred to a controlled-environment rearing chamber (32 ± 0.1 ℃, 43 ± 1% RH; L:D = 12:12) until the adults emerge. The adults are then used either for experiments or to maintain the strain using the same procedure.

2.4. Essential Oil Origin

The essential oil of *Cymbopogon nardus* used in this study was obtained from the Institute for Research in Applied Sciences and Technologies (IRSAT, Burkina Faso). According to the supplier, the essential oil was extracted from fresh leaves harvested in the IRSAT botanical garden by steam distillation using a Clevenger-type apparatus. The extracted oil was dissolved in absolute ethanol.

2.5. Chemical Composition of *Cymbopogon nardus* Essential Oil

The chemical composition of the essential oil was determined by gas chromatography–mass spectrometry (GC–MS). A 20 µL aliquot of a 1/5000 diluted sample was transferred into a vial fitted with an insert (VWR, Radnor, PA) and analyzed using a GC–MS system (Trace 1310, Thermo Fisher Scientific) equipped with a 30 m capillary column (0.25 mm i.d.; #36096-1420, Thermo Fisher Scientific). Helium was used as the carrier gas at a constant flow rate of 1 cc/min. Sample injection was performed using an autosampler (TriPlus RSH, Thermo Fisher Scientific). The oven temperature was initially maintained at 45 ℃ for 4 min, then increased to 230 ℃ according to a programmed temperature gradient and held for 6 min, resulting in a total run time of 28.5 min. Chromatographic peaks were manually integrated using the Chromeleon MS Quantitative Processing software (Thermo Fisher Scientific), and compound identification was achieved by comparison with spectra from the online NIST library.

2.6. Effects of Female Age on Developmental Dynamics of *Spermophagus niger*

A male and a female *Spermophagus niger*, both less than 24 hours old, were introduced into to plastic Petri dishes (9 cm × 1.5 cm) containing 20 healthy sorrel seeds of uniform size, with 15 replicates. The adults were transferred daily to a fresh batch of seeds until the females died. The length of the eggs laid was measured daily using binocular loupe LEICA EZ4HD coupled with a desktop computer and LAS EZ software. To prevent larval competition, only seeds bearing a single egg were maintained under controlled conditions (32 ± 0.1 ℃, 43 ± 1% RH; L = 12:12). Six (6) days after oviposition, the number of hatched and unhatched eggs was counted. Hatched eggs were identified by the presence of a reddish spot inside the chorion corresponding to the cephalic capsule of the first-instar larva [12]. Upon adult emergence, insects were counted and removed from the rearing boxes daily for two weeks. The following parameters were then assessed:

1) egg fertility rate (%): the ratio of the number of hatched eggs to the total number of eggs that × 100;
2) larval survival rate (%): the ratio of the number of adults emerged to the number of eggs hatched × 100;
3) development time (T): the time between egg laying and adult emergence, calculated using the formula: (D1*A1 + D2*A2 +…Dn*An) / (number of insects emerged) [24]; D1: day when adults began to emerge (first day); A1: number of adults that emerged on day D1.
4) Adult body size: the size of twenty (20) unsexed adult using binocular loupe LEICA EZ4HD coupled with a desktop computer and LAS EZ software.

2.7. Insecticidal Effects of *Cymbopogon nardus* Essential Oil on Fecundity and Mortality of *Spermophagus niger*

Ten (10) pairs of *Spermophagus niger* adults less than 24 hours old were introduced into 1-litre glass jars containing 20 g of healthy seeds of sorrel *Altissima* variety. The essential oil was applied by fumigation at seven concentrations: 2, 4, 6, 8, 10, 12, and 14 µL/L of air. For each treatment, the required volume of essential oil was applied to a Whatman filter paper disc (2 cm in diameter) suspended from the inner side of the jars lid before hermetically sealing the containers. An untreated control was included in the experimental design. Each treatment was replicated four times. After 24 hours of exposure, the insects were removed, and the number of dead individuals and eggs laid were recorded. fecundity was assessed as the mean number of eggs laid per female, whereas the insecticidal activity of the essential oil was evaluated based on the observed mortality rate, calculated using the following formula:

$$\text{Mortality rate (\%)} = (\text{number of dead insects} / \text{total number of exposed insects}) \times 100$$

2.8. Impact of *Cymbopogon nardus* Essential Oil on Egg Hatchability

Forty (40) seeds, each bearing a 24-hour-old egg, were placed in jars identical to those used in the previous experiment. The number of seeds per experimental unit was adjusted to 100 prior to treatment. The eggs were then exposed for 24 hours to the same concentrations of *C. nardus* essential oil as described above. An untreated control was included, and each treatment was replicated four times. After exposure, the seeds were transferred to plastic Petri dishes (9 cm × 1.5 cm) and maintained under rearing conditions. Six (6) days later, the numbers of hatched and unhatched eggs were counted using a binocular loupe LEICA EZ4HD. Average rearing conditions were 26.9 ℃ and 28.85% RH, with a photoperiod of 12 h light / 12 h dark. The egg hatching rate was accessed using the following formula:

$$\text{Hatching rate (\%)} = (\text{number of hatched eggs} / \text{total number of treated eggs}) \times 100$$

2.9. Statistical Analysis

Statistical analyses were performed using R software version 4.2.2. The normality of data distribution and the homogeneity of variances were assessed using the Shapiro–Wilk and Bartlett tests, respectively. The effect of female the age in *Spermophagus niger* on the measured biological parameters (egg size, egg fertility rate, larval survival, development time, size of first-generation adults) was analyzed using one-way analysis of variance (ANOVA).

Similarly, the effect of *Cymbopogon nardus* essential oil on adult mortality, fecundity, and egg fertility were evaluated using the same statistical approach. When the assumptions required for ANOVA were not met, the Kruskal-Wallis test was applied. The lethal concentrations (LC50 and LC90), and their 95% confidence intervals were estimated using Probit analysis. The dose-response relationship between essential oil concentrations and adult mortality was modeled using a generalized linear model with a binomial error distribution and a Probit link function. The model adequacy was assessed based on the regression slope and the confidence intervals of the lethal concentration estimates. Statistical significance was determined at the 5% ($p < 0.05$) for all analyses.

3. Results

3.1. Effect of the Female Age on Egg Length

Egg length decreased significantly with increasing female age ($F = 16.73$; $P = 2.28e-12$). The largest Eggs were laid during the first and second days of oviposition, whereas the smallest eggs were observed on the last day (Figure 1).

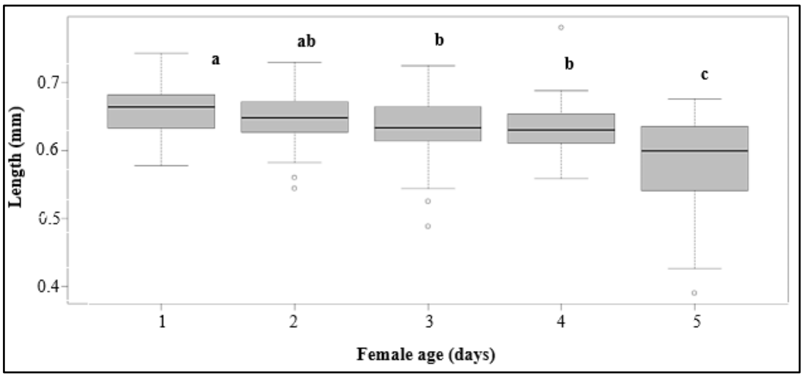

Figure 1. Mean size (mm) of Spermophagus niger eggs by female age. Bars represent means; error bars correspond to standard errors. Different alphabetic letters on the error bars indicate significant differences between means according to Fischer's LSD test at the 5% threshold.

3.2. Effect of Female Age Hatchability, Larval Survival Rate, and Total Developmental Time

Figure 2A shows that the hatchability of eggs laid between the first and fourth days did not differ significantly, but remained significantly higher than that the eggs laid on the fifth day ($F = 2.759$; $P = 0.0343$). larval survival also varied significantly depending on females age ($F = 6.058$; $P = 0.0003$). The survival rates recorded for eggs laid on the first, third, and fourth days were statistically similar and significantly higher than those observed for eggs laid on the fifth day (Figure 2B).

The duration of development ranged from 26.49 days for eggs laid on the first day to 31.30 days for those laid on the fifth day. The shortest developmental times were observed in individuals from eggs laid during the first two days, with no significant difference between them. In contrast, eggs laid on the third and fourth days exhibited intermediate developmental durations, which were significantly longer than those recorded for the first two days but shorter than those observed for the fifth day (Figure 2C).

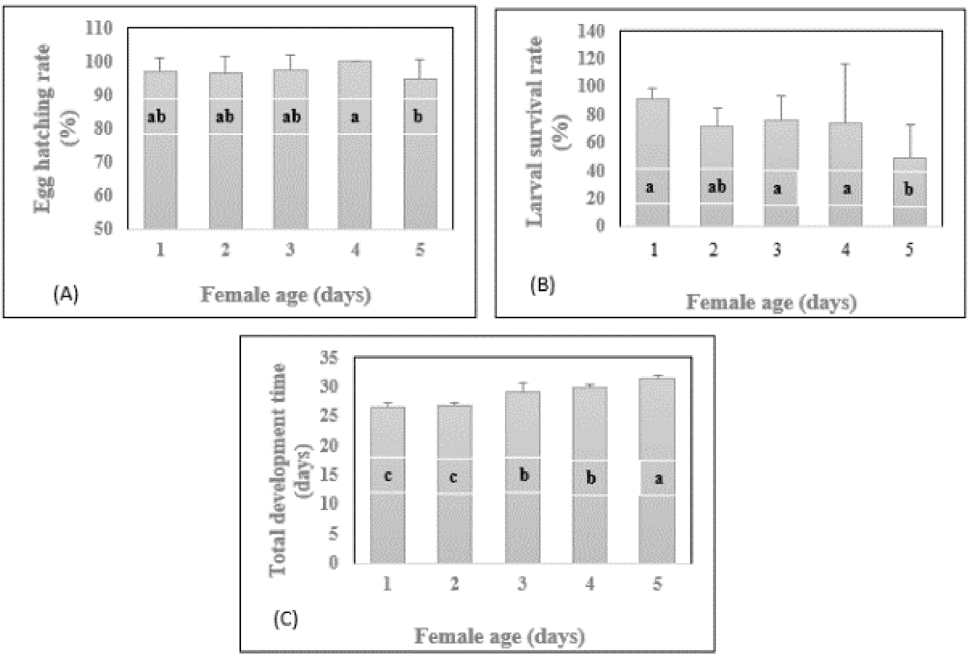

Figure 2. Effect of female age on the reproductive performance of Spermophagus niger (A: mean egg hatchability (%); B: mean larval survival rate (%); C: mean development time (days)). Bars correspond to means, and error bars correspond to standard errors. Different alphabetic letters above the bars indicate significant differences between the means according to Fischer's LSD test at the 5% significant level.

3.3. Effect of the Female Age on the Size of First-Generation Offspring

The size of the emerged adults varied significantly depending on the age of the breeding female (F = 3.539; P = 0.0091). no significant differences in adults' size were observed among individuals originating from eggs laid during the first four days of oviposition. However, adults emerging from eggs laid on the first day were significantly larger (3.59 ±0.27 mm) than those emerging from eggs laid on the fifth day (3.33 ± 0.34 mm) (Figure 3).

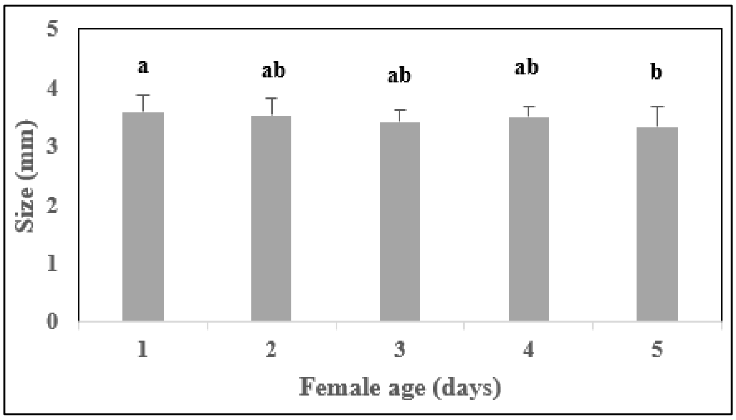

***Figure 3**. Effect of female age on the mean size (mm) of emerged Spermophagus niger adults. Bars correspond to means, and error bars correspond to standard errors. Different alphabetic letters above the bars indicate significant differences between the means according to Fischer's LSD test at the 5% significant level.*

3.4. Chemical Composition of *Cymbopogon nardus* Essential Oil

The identified compounds accounted for approximately 96% of the total essential composition. Monoterpenoids were the predominant constituents, representing about 62% of the total composition. Among these, citronellal, geraniol, and citronellol were the most abundant compounds, occurring at proportions of 23.61%, 11.63%, and 9.63%, respectively. Sesquiterpenoids, accounted for nearly 32% of the total composition and were mainly represented by β-elemol (11.2%) and cadina-1(6), 4-diene (4.09%) (Table 1).

***Table 1**. Chemical composition of Cymbopogon nardus essential oil tested against adults and fresh eggs of Spermophagus niger.*

Number of Peaks	Retention time	Compounds	%
		Monoterpenoid	61.83
70	8.66	ß-Myrcene	1.89
75	8.99	Pseudolimonene	0.13
79	9.44	D-Limonene	1.99
80	9.56	trans- ß-Ocimene	0.81
83	9.76	ß-Ocimene	1.19
91	10.51	Isoterpinolene	0.18
94	10.74	Linalool	0.74
104	11.66	Citronellal	23.61
105	11.82	(-)-Neoisopulegol	1.19
108	12.17	Isopulegol	0.13
114	12.88	Citronellol	9.63

Number of Peaks	Retention time	Compounds	%
115	13.07	Neral	0.30
116	13.26	Geraniol	11.63
119	13.53	α-Citral	1.27
123	13.89	Thymol	0.11
132	14.73	Citronellol acetate	2.94
133	14.83	Eugenol	0.41
135	15.14	Geranyl acetate	2.80
147	16.22	Copaen-4-α-ol	0.19
149	16.40	α-Himachalene	0.23
150	16.47	Bicyclosesquiphellandrene	0.12
208	21.95	Neodihydrocarveol	0.34
		Sesquiterpenoids	31.87
138	15.42	Elemene	3.78
142	15.9	Caryophyllene	0.17
151	16.61	Muurolene	0.52
152	16.73	Germacrene D	0.36
154	16.93	a-Muurolene	1.52
156	17.19	Cadina-1(6), 4-diene	4.09
158	17.44	(-)-α-cadinene	0.40
159	17.57	β-Elemol	11.20
162	17.98	Germacrene-4-ol	0.15
166	18.32	(-)-Globulol	0.38
170	18.58	epi-γ-Eudesmol	0.13
171	18.67	epi-γ-Eudesmol	1.66
172	18.77	α-Cadinol	7.2
181	19.58	Germacra-4(15), 5, 10 (14)-trien-1α-ol	0.14
204	21.41	Diepicedrene-1-oxide	0.17
		others	2.18
7	3.80	3-Methyl-3-pentano	0.33
32	5.58	3-Methylcyclopentanol	0.51
62	7.97	2-Nitrohexane	0.61
248	25.14	Docosane	0.21
276	26.86	Heneicosane	0.52
Total identified		95.88	

Compounds less than 0.1% of the essential oil have not been listed.

3.5. Effect of *Cymbopogon nardus* Essential Oil on Adult of *Spermophagus niger*

Adult mortality in *Spermophagus niger* increased with increasing concentration of *Cymbopogon nardus* essential oil after 24 hours of exposure. It is nearly zero in the control group (1%), and then increased slightly at 2 µL/L before rising sharply at 4 µL/L (~57%), reaching approximately 96% at 14 µL/L. This dose-dependent response highlights the insecticidal activity of the essential oil (Figure 4).

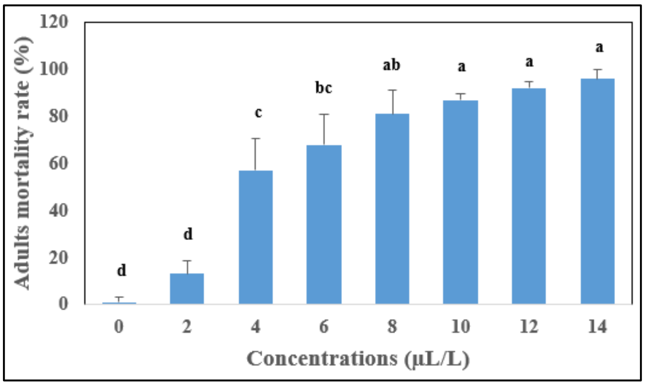

Figure 4. *Effect of Cymbopogon nardus essential oil on adult Spermophagus niger after 24 hours of exposure in 1-liter glass jars. Bars correspond to means, and error bars correspond to standard errors. Different alphabetic letters above the bars indicate significant differences between the means according to Fischer's LSD test at the 5% significant level.*

Probit analysis confirmed a strong dose-response relationship, with a regression slope of 3.29. The lethal concentrations LC50 and LC90 values were 4.29 µL/L (95% CI: 3.96-4.62) and 10.52 µL/L (95% CI: 9.44-11.87), respectively. These results demonstrate the increasing efficacy of *C. nardus* essential oil against *S. niger* as increasing with increasing dose (Table 2).

Table 2. *Toxicity parameters of Cymbopogon nardus essential oil against adult Spermophagus niger after 24 hours of exposure in 1-liter glass jars.*

Parameters	Value (µL/L of air)	CI 95%
Slope	3.29	
LC50	4.29	3.96-4.62
LC90	10.52	9.44-11.87

LC50 = Lethal Concentration; CI = Confidence Interval

3.6. Effect of *Cymbopogon nardus* Essential oil on Fertility and Hatch Rate of *Spermophagus niger*

Treatment with *Cymbopogon nardus* essential oil resulted in a significant reduction in the number of eggs laid by female *Spermophagus niger* at all tested concentrations compared with the untreated control (60.2) ($F = 10.54$; $P = 8.46 \times 10^{-7}$) (Figure 5A). However, no significant differences in fecundity were observed among the different essential oil concentrations tested. Egg hatchability in the untreated control was approximately 94%, which was significantly higher than those observed for treated eggs, regardless of the concentration applied (Figure 5B).

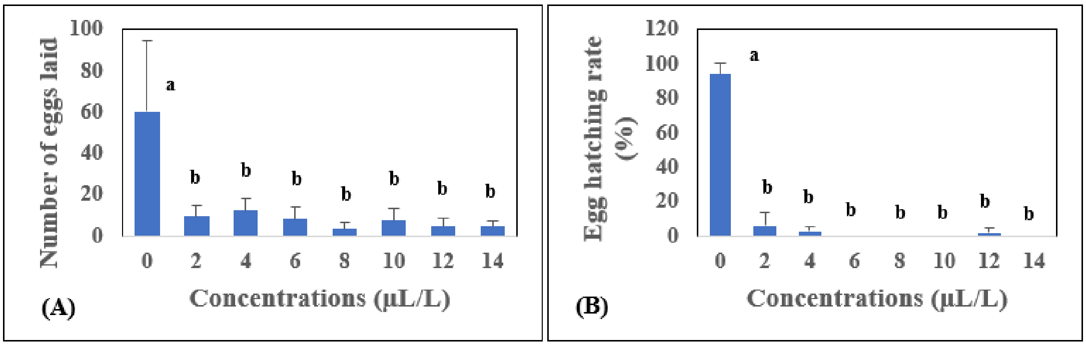

Figure 5. *Effect of Cymbopogon nardus essential oil on the number of eggs laid (A) and the hatching rate (B) in Spermophagus niger after 24 hours of exposure in 1-liter glass jars. Bars correspond to means, and error bars correspond to standard errors. Different alphabetic letters above the bars indicate significant differences between the means according to Fischer's LSD test at the 5% significant level.*

4. Discussion

In many insect species, female age influences offspring quality [25]. Offspring produced by older females generally exhibit higher mortality rates, longer developmental times, and smaller adult sizes than those produced by younger females [25]. Furthermore, several studies have shown that older females often lay smaller eggs [26, 27]. In *Callosobruchus maculatus* (Coleoptera: Chrysomelidae), for example, larvae originating from eggs laid by older females are less likely to reach adulthood and require more time to complete their development [21, 22, 28, 29]. The results of the present study are consistent with this general trend. In *Spermophagus niger*, offspring performance declined with increasing female age. In fact, eggs laid on the fifth day were significantly smaller than those laid during the first few days of oviposition. This reduction in egg size was associated with increased larval mortality, a lengthening developmental time, and the emergence of smaller adults. The observed decrease in egg size may explain these results. It could reflect a gradual depletion of maternal resources, leading to the production of smaller and potentially lower-quality eggs toward the end of the oviposition period, as previously reported in others insect species [30, 31]. According to Fox [32], females with access to food resources lay larger eggs than those subjected to nutritional stress. Similarly, maternal nutrition has been shown to increase egg size in many insect species [33-36]. In *S. niger*, emerged adults did not feed after emergence, which could support the hypothesis of a gradual depletion of resources in females. Analysis of the chemical composition of *Cymbopogon nardus* essential oil revealed a predominance of oxygenated monoterpenes (61.83%), mainly represented by citronellal (23.61%), geraniol (11.63%), and citronellol (9.63%), as well as limonene and other terpenic derivatives present in lower proportions. This composition is generally consistent with previous reports on *C. nardus* essential oil, in which these compounds are commonly identified as the major constituents [37-39]. However, the relatively low proportions of citronellal and geraniol observed in the present study differ from some values reported in the literature [37, 39]. These variations could be related to the existence of different ecotypes of the plant. Indeed, the chemical composition of essential oils is influenced by various intrinsic and extrinsic factors, including climatic conditions, geographic origin, phenological stage, plant age, and the ecological and soil characteristics of the growing environment [40, 41]. These compositional variations allow for the distinction of several chemotypes, which are generally defined according to the predominant compound [42, 43]. Based on the present results, the *C. nardus* essential oil studied could be classified as a citronellal chemotype. This finding is similar to the results of studies conducted in Togo by [44, 45], and [46], who likewise reported citronellal as the dominant constituent of *C. nardus* essential oils of this species.

Insecticide tests have shown that the essential oil of *C. nardus* exhibits insecticidal activity against *S. niger*. This activity resulted in a significant reduction in female fertility, as reflected by a decrease in the number of eggs laid. A significant decrease in the egg hatching rate and high adult mortality were also observed. This adulticidal toxicity was further confirmed by the LC_{50} and LC_{90} values, estimated at 4.29 µL/L of and 10.52 µL/L of air, respectively. This insecticidal activity of the essential oil may be attributed to its high content of oxygenated monoterpenes, particularly the acyclic monoterpenes geraniol and citronellol, as well as the monocyclic monoterpene D-limonene. These compounds are widely recognized for their insecticidal properties against numerous agricultural and stored-product insect pests. Their modes of action primarily involve disruption of the nervous system and interference with metabolic pathways essential for insect survival and development.

Geraniol, one of the major components of *C. nardus* essential oil, is known for its neurotoxic activity through the inhibition of acetylcholinesterase, a key enzyme involved in nerve impulses transmission. This activity has been demonstrated in several insect pest's species, including *C. maculatus*, *Rhyzopertha dominica*, *Sitophilus oryzae*, and *Sitophilus zeamais* [47]. Furthermore, [48] reported that geraniol significantly reduced the viability of *Spodoptera frugiperda* eggs, even at low concentrations, by inducing morphological alterations in both the eggs and the chorion structure. Citronellol may also contributes substantially to the biological activity of the essential oil through its repellent, toxic, and growth- inhibitory effects. According to [49], this compound acts primarily by disrupting the insect nervous system, altering the integrity cell membrane integrity, and interfering with respiratory processes. Furthermore, the presence of D-limonene may enhance overall insecticidal activity of the oil. Indeed, this monoterpene hydrocarbon exhibits toxic, strong repellent properties, and ovicidal activity effects against several insect pests such as *C. maculatus*. All of these mechanisms could explain the reduced fertility, lower egg hatching rates, and high mortality observed in *S. niger* following exposure to *C. nardus* essential oil.

5. Conclusion

The results of this study show that the age of *Spermophagus niger* females influences the performance of their offspring. As females age, there is a gradual decrease in maternal investment in the eggs, which results in increased larval mortality, a longer development period, and smaller adult size at emergence. The Analysis of the chemical composition of *Cymbopogon nardus* essential oil revealed a predominance of monoterpenes, with geraniol, citronellol, and D-limonene as the major constituents. Biological tests showed that this essential oil possesses strong insecticidal activity against *S. niger* adults, while significantly reducing female fertility and egg hatching rates. Overall, these results improve our understanding of the biology of *S. niger* and highlight the potential of *C. nardus* essential oil as a promising botanical alternative to chemical insecticides for the protection of stored *Hibiscus sabdariffa* seeds.

Abbreviations

LC50	Lethal Concentration 50%

LC90	Lethal Concentration 90%
CI	Confidence Interval
GC–MS	Gas Chromatography–Mass Spectrometry
ANOVA	Analysis of Variance

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Author Contributions

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Edouard Drabo: Conceptualization, Data curation, Methodology

Koi Wenceslas Kam: Writing – review & editing

Mouniratou Rahmat Ouedraogo: Data curation

Antoine Waongo: Supervision, Validation

Antoine Sanon: Supervision, Validation

Zakaria Ilboudo: Supervision, Validation

Conflicts of Interest

The authors declare no conflicts of interest.

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