






Management of *Ephestia cautella* Walker (Lepidoptera: Pyralidae) in Shea (*Vitellaria paradoxa* C.F. Gaertn) Kernel Stocks Using Insecticidal Plant Extracts

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Abstract

Many essential oils (EOs) and powders of aromatic plants with insecticidal properties are increasingly considered alternative insecticides to protect stored products against pests. One of these pests is *Ephestia cautella* Walker, which is a major pest in stored nuts. The larvae of this insect infesting shea kernels cause considerable damage. The aim of this study was to evaluate the effectiveness of neem kernel powder and *Eucalyptus camaldulensis* essential oil in controlling *E. cautella* populations in shea kernel stocks. The study involved applying varying concentrations of neem kernel powder and *E. camaldulensis* EOs to *E. cautella* adults, larvae and eggs. A quantity of 2 g to 15 g of neem kernel powder caused high mortality rates (77.5% to 95%) of larvae by inhibiting their development. However, these same powder quantities proved ineffective in controlling adults. *E. camaldulensis* EOs caused 100% mortality in adults with a concentration of 15 µl/l for 24 hours. By direct contact, this oil caused 100% mortality of larvae at a concentration of 3 µl. It had no significant impact on embryonic development. The difference in toxicity observed between the different stages of the insect suggests a control strategy based on combining both bio-pesticides to optimize their effectiveness in protecting shea kernel stocks.

Keywords

Biological Control, Essential Oils, *Azadirachta indica*,
Eucalyptus camaldulensis

1. Introduction

Burkina Faso is one of West Africa's leading producers of shea kernels, with an estimated annual production potential range from 70,000 to 300,000 tons (Bup *et al.*, 2014) [1]. Bondé *et al.* (2019) [2] reported 506,717.56 tons of fresh fruit, corresponding to 69,067.13 tons of dried shea kernels, which have several uses. Indeed, the butter derived from processing shea kernels is used by local farmers as the main source of fat in the agro-food industries, for making soap and for cosmetic care (Ouédraogo *et al.*, 2013) [3]. However, shea kernels are subject to insect attack during storage. The most significant damage caused by pests is perforation (Kambou *et al.*, 2022) [4]. Faced with the threat of insect pests in stocks, the methods of control are essentially based on the use of synthetic pesticides (Guèye *et al.*, 2011) [5]. Their effectiveness in controlling stock pests is well known. However, the use of synthetic pesticides has several drawbacks such as pollution of food chains and the environment, intoxication of producers and consumers and increased pest resistance to synthetic insecticides (Benhalima *et al.*, 2004) [6]. These insecticides are also a source of ecological disorder (Regnault-Roger, 2002) [7]. The use of bio-pesticides could be an alternative way of reducing the use of synthetic insecticides. The use of plants with insecticidal properties is being promoted worldwide (Guèye *et al.*, 2011) [5]. Indeed, the organs and parts of some plants are used to control pests (Guèye *et al.*, 2011) [5]. Several laboratory tests were carried out with EOs, and the results indicate variable toxicities depending on the EOs used, the pest species present and, above all, the stage of development concerned by the tests (Togola *et al.*, 2014 [8]; Ilboudo *et al.*, 2016 [9]; Aouina and Khelifi, 2018 [10]). The EOs of certain plants act by contact or inhalation, with varying degrees of effectiveness depending on the target insect. Increasingly, the leaves and kernels of neem (*Azadirachta indica*), with their anti-feeding, repellent, toxic and growth-inhibiting properties, have been used extensively to control insects on stored foodstuffs (Facknath, 2006 [11]; Guèye *et al.*, 2011 [5]). Few scientific data exist on the use of insecticidal plants to control *Ephestia cautella* (Walker), the main insect predator of stored shea kernels in Burkina Faso (Kambou *et al.*, 2023) [12]. Therefore, it is necessary to consider a trial to control the activity of *E. cautella* in order to prevent or limit its damage to shea kernel stocks. Hence, this study aimed to evaluate under laboratory conditions the efficiency of insecticidal plant extracts for controlling *E. cautella* populations in shea kernel stocks. This was done by evaluating the insecticidal properties of neem kernel powder on *E.*

cautella adults and larvae, and assessing the susceptibility of *E. cautella* adults, larvae and eggs to *Eucalyptus camaldulensis* EOs.

2. Materials and Methods

2.1. Study Conditions

This study was carried out at the Laboratory of Fundamental and Applied Entomology of Joseph KI-ZERBO University under ambient laboratory conditions. During the experiment, the mean temperature was $28.33 \pm 5.03^\circ\text{C}$ and the mean relative humidity was $41 \pm 7.59\%$.

2.2. Biological Materials

E. cautella individuals come from the mass rearing of strains isolated from naturally infested shea kernels in Ziro province (Kambou *et al.* 2022) [4]. *Azadirachta indica* kernel powder and *Eucalyptus camaldulensis* EOs were used as botanical material. *A. indica* almond powder was obtained from shelled seeds, then ground using a mortar and pestle. *A. indica* almond powder was stored in a hermetically sealed glass jar and refrigerated at 4°C . *E. camaldulensis* EO tested was obtained from “Institut de Recherche en Sciences Appliquées et Technologies (IRSAT)” in Ouagadougou, Burkina Faso, and kept refrigerated at 4°C .

2.3. Methods

2.3.1. Toxicity Test of *Azadirachta indica* Kernel Powder on Adults and Larvae of *E. cautella*

Four (04) quantities, namely 2 g, 5 g, 10 g and 15 g of neem kernel powder, were tested on insects. The choice of these doses was made after a preliminary study in the laboratory.

For the adulticidal tests, ten (10) pairs of *E. cautella* less than 24 h old were introduced into 1500 ml plastic cans in contact with 30 healthy shea kernels (i.e., around 100 g) coated with a dose of powder. The containers were closed with mosquito nets for 72 h. Insects that could move when pressure was applied to the abdomen with flexible forceps were considered dead and removed from the boxes on a daily basis. For each powder dose, four (04) replicates were performed. An untreated control was also performed using four (04) replicates.

For larvicidal tests with neem kernel powder, twenty (20) late-stage *E. cautella* larvae were introduced into plastic boxes containing 30 healthy shea kernels coated with each of the doses of neem powder previously tested on adults (2 g, 5 g, 10 g and 15 g). Each box was covered with a piece of mosquito net and placed under rearing conditions. Larvae were monitored until emergence to estimate the mortality rate.

2.3.2. Assessment of the Toxicity of *E. camaldulensis* Essential Oil on Adults, Larvae and Eggs of *E. cautella*

Four (04) increasing doses corresponding to volumes of *E. camaldulensis* essential

oil (2.5 μ l; 5 μ l, 10 μ l, 15 μ l) were tested on adults aged 0 - 48 hours. The EOs charge was applied with a micropipette to absorbent cotton, which was then placed in the presence of 10 pairs of insects and 10 shea kernels (weighing around 30 g) in 1-liter glass jars. As the active ingredient acts by fumigation, the jars were hermetically closed. Four repetitions were made for each dose, with a control trial without EOs. Insects were kept in jars in the presence or absence of EO for 24 h, after that dead insects were counted.

For larvicidal tests with *E. camaldulensis* EOs, the test consisted of direct contact by topical application of oil charge on the larva's thorax. Five (05) doses (0.2 μ l, 0.5 μ l, 1 μ l, 2 μ l, 3 μ l) were tested, and a negative control was the larva without treatment. Each test was repeated 4 times. Treated larvae were placed in jars containing 10 healthy almonds for 24 h. At the end of 24 h, living and dead larvae were counted.

Eggs were tested for their susceptibility to *E. camaldulensis* EOs using four (04) doses: 10 μ l, 15 μ l, 20 μ l and 30 μ l. Thirty (30) eggs, no more than 24 h old, were isolated from almond clutches and placed in boxes in the presence of each dose of EOs applied to cotton for 24 h. The eggs were then transferred to new boxes containing 20 g of shea kernel powder and incubated on benches. The number of hatched and unhatched eggs (aborted eggs) was identified five (05) days after exposure using a Leica Wild M3Z binocular loupe. Hatched eggs were recognizable by their translucent appearance. Four replicates per dose and a control without essential oil, were set up.

2.4. Data Analysis

The tests were performed after a preliminary check of the data distribution using the Shapiro-Wilk normality test. Where ANOVAs were significant, the means were compared using the Student-Newman-Keuls test for a probability threshold of 5%. Lethal concentrations of essential oil were determined using the probit-log model of Finney (1971) [13]. The analysis resulted in LC_{50} and LC_{95} values expressed in μ l/l, with their confidence limits and slope values. If the Chi-2 values were insignificant at 5%, the goodness-of-fit test model was valid. Data on mortality caused by different concentrations of bio-insecticides were analyzed using R software.

3. Results

3.1. Toxicity of Neem Kernel Powder on *E. cautella* Adults and Larvae

3.1.1. Susceptibility of *E. cautella* Adults to Neem Kernel Powder

The highest mortality rate was 20% with the highest dose of 15 g (Figure 1). The analysis showed that there was no significant difference in mortality ($P = 0.322$) between the doses tested and the control (D0).

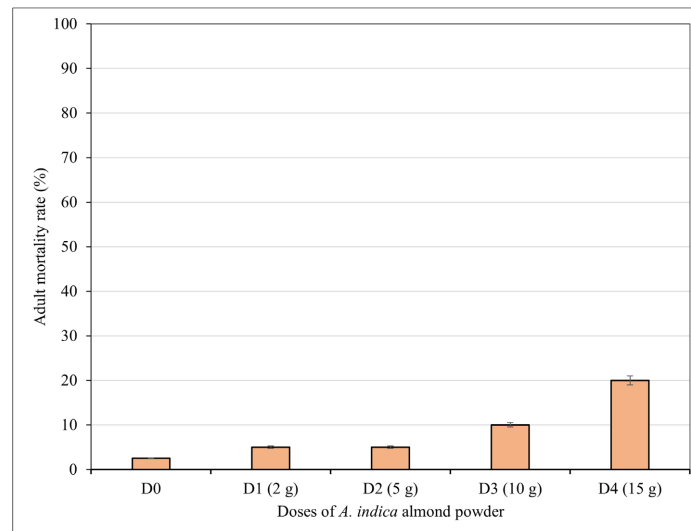


Figure 1. Evolution of mortality of *E. cautella* adult exposed to neem kernel powder for 72 hours.

3.1.2. Susceptibility of *E. cautella* Larvae to Neem Kernel Powder

Powdered neem kernels produced very high mortality rates, ranging from 77.5% to 95% for doses of 2 g and 15 g, respectively (Figure 2). Compared with the different doses, 10% mortality was recorded in the control group. Mortality rates generated by the neem kernel powder were significantly higher ($P < 0.001$) than those of the negative control (larva without treatment). However, among the different doses tested, larval mortality rates did not differ significantly.

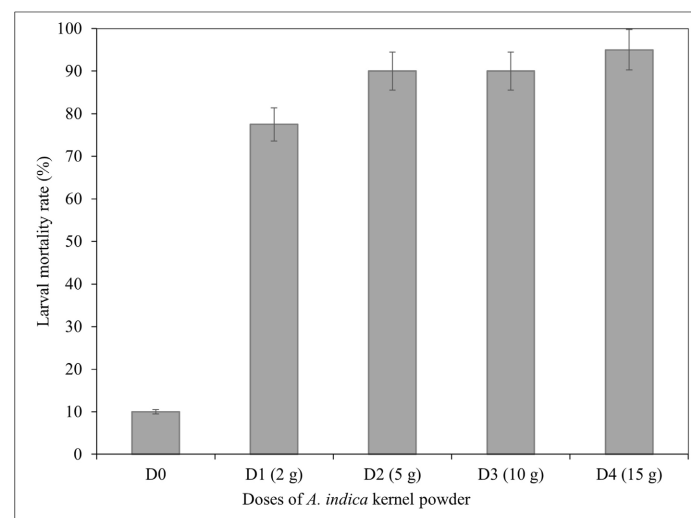


Figure 2. Evolution of late instar larval mortality of *E. cautella* exposed to neem kernel powder.

3.2. Toxicity of *E. camaldulensis* Essential Oil on *E. cautella* Adults, Larvae and Eggs

3.2.1. Susceptibility of *E. cautella* Adults to *E. camaldulensis* Essential Oil

Mortality increased with dose, giving a dose-dependent effect. Mortality with all

doses was significantly higher than the control ($P < 0.001$). However, for doses below $7.5 \mu\text{l}$, mortality rates did not differ significantly from those of the negative control. The highest concentration ($D5 = 15 \mu\text{l/l}$) resulted in total adult mortality (**Figure 3**).

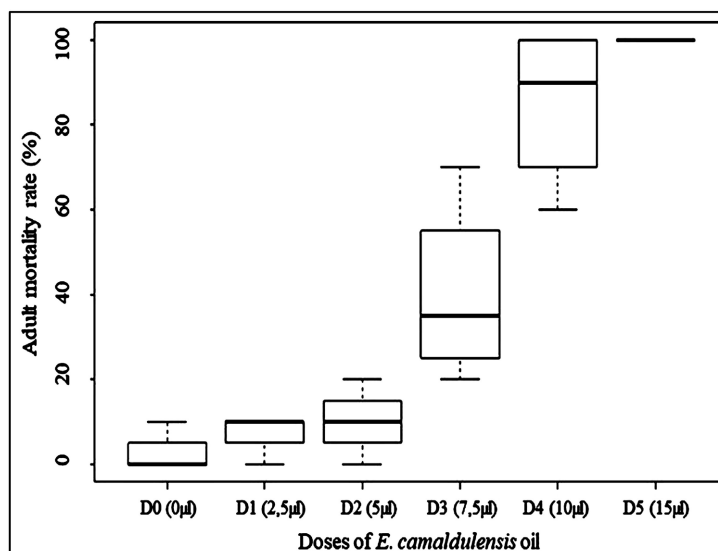


Figure 3. Evolution of *E. cautella* adults mortality exposed to *E. camaldulensis* essential oil for 24 hours.

3.2.2. Susceptibility of *E. cautella* Larvae to *E. camaldulensis* Essential Oil

Direct contact of *E. camaldulensis* EO with *E. cautella* larvae caused dose-dependent mortality (**Figure 4**). All doses produced significantly higher mortality than the control ($P < 0.001$). The lowest oil dose tested ($D1 = 0.2 \mu\text{l}$) resulted in 40% mortality, while the highest dose ($D5 = 3 \mu\text{l}$) caused total larval mortality.

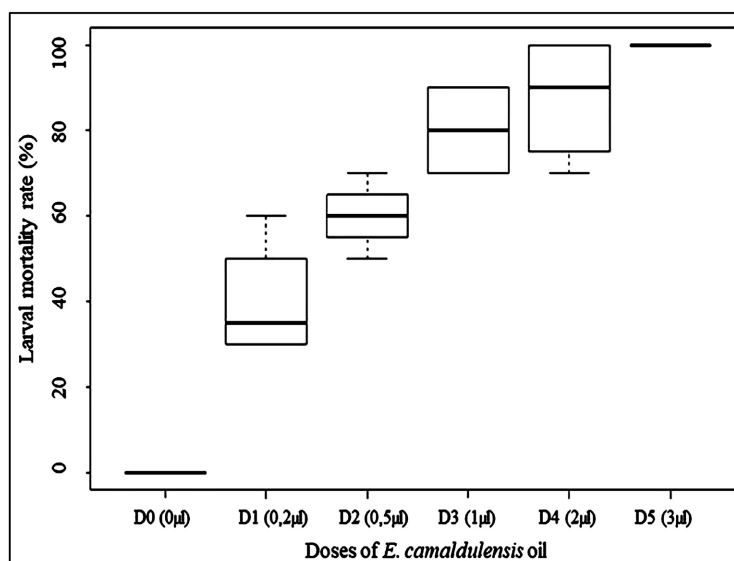


Figure 4. Evolution of late instar *E. cautella* larvae -mortality exposed to *E. camaldulensis* essential oil for 24 hours.

3.2.3. Susceptibility of *E. cautella* Eggs to *E. camaldulensis* Essential Oil

High hatching rates were recorded regardless of the oil dose tested (Figure 5). Hatching rates ranged from 93.33% to 98.33% with 15 µl and 30 µl doses respectively. They did not differ significantly from the control group ($P = 0.773$). Thus, the EO of this plant did not significantly impact the embryonic development of *E. cautella*.

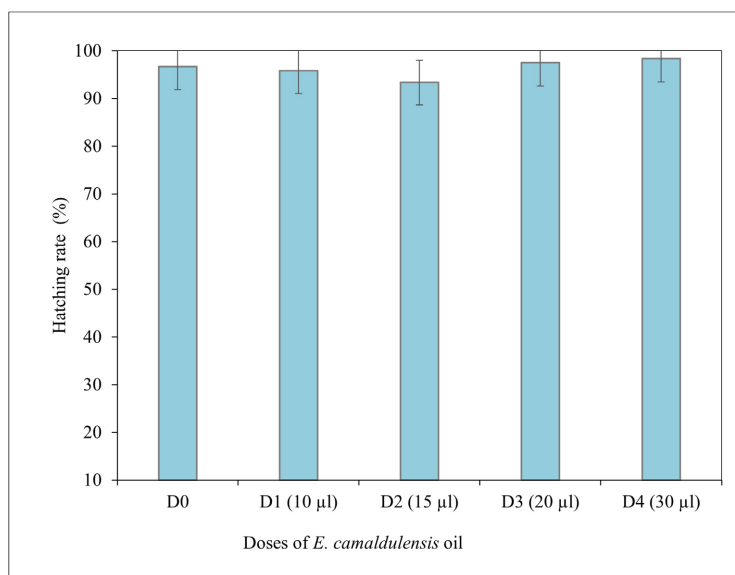


Figure 5. Evolution of *E. cautella* eggs hatching rate of exposed to different doses of *E. camaldulensis* essential oil for 24 hours.

3.3. Assessment of Lethal Doses of *E. camaldulensis* Essential Oil on *E. cautella* Adults and Larvae

The LC_{50} and LC_{95} of *E. camaldulensis* EOs obtained against *E. cautella* adults by inhalation were 7.22 µl/l and 15.13 µl/l, respectively (Table 1). For larvae tested by direct contact with *E. camaldulensis* EOs, the LC_{50} and LC_{95} are 0.32 µl/l and 2.92 µl/l, respectively. The low slope values showed that the insect populations used were heterogeneous. The chi square value (0.99) was insignificant at the 5% level for the larval test, showing a good fit for the analytical model. However, it was significant for the test with adults ($p < 0.05$).

Table 1. LC_{50} and LC_{95} of *E. camaldulensis* essential oils against adults and last instar larvae of *E. cautella*.

Parameters	Adults	Larvae
Slope	5.12	1.69
LC_{50} (µl/l)	7.22	0.32
(CL 50%)	(6.04 - 8.48)	(0.19 - 0.43)
LC_{95} (µl/l)	15.13	2.92
(CL 95%)	(11.99 - 24.20)	(1.90 - 6.13)
Chi square (X^2)	0.01	0.99

LC: lethal concentration; CL: confident limits.

4. Discussion

Neem kernel powder used as a coating on shea kernels was less effective in controlling *E. cautella* adults after three days of exposure. The low toxicity observed in adults could be explained by the fact that adults did not feed on treated shea kernels. However, previous work has shown that neem kernel powder possessed insecticidal properties characterized by a decrease in female fecundity and a reduction in the number of insects emerging in the first generation (Koussoubé, 2018) [14]. The energy-intensive egg-laying activity of the females could explain the low mortality rate recorded in the control. In fact, the dead adults were all females. *E. cautella* larvae showed greater susceptibility to neem kernel powder. Indeed, this powder caused high mortality among the larvae. As a result, it is effective in controlling larvae associated with shea kernel stocks, with dose-dependent toxicity. This toxicity would be due to the high concentration of azadirachtin in neem seeds compared with other parts of the plant (Isman, 2006 [15]; Singh *et al.*, 2010 [16]). Azadirachtin acts as a growth regulator by blocking the insect's endocrine system, resulting in the slowing or total arrest of morphogenetic development and physiological disorders followed by behavioral disorders in the insect (Isman, 2006 [15]; Petit, 2008 [17]). Therefore, this chemical compound inhibited the development of the late larval instar of *E. cautella*. On the other hand, long larval and pupal development times may impact emergence rates. This could explain the mortalities recorded in the control.

E. camaldulensis EOs acts by fumigating exposed insects. It caused a dose-dependent mortality rate in adult *E. cautella*. With 15 µl/l, a 100% mortality in adults was recorded. LC₅₀ and LC₉₅ values were low for adults, attesting to the efficacy of *E. camaldulensis* EOs on *E. cautella* adults for stock protection. This efficacy was proven by Togola *et al.* (2014) [8], who showed that impregnating jute bags and treating the bags with EOs of *E. camaldulensis* and *Cymbopogon citratus* is an effective method of protecting stored rice against *Sitophilus oryzae* and *Sitotroga cerealella*. The insecticidal effect of *Eucalyptus saligna* has also been reported on *Callosobruchus maculatus* in cowpea seeds protection trials (Tapondjou *et al.*, 2003) [18]. The insecticidal activity of EOs of the genus *Eucalyptus* is linked to its main components (1, 8-cineole or eucalyptol, α -pinene and d-limonene) which proportions vary from one species to another (Erau, 2019) [19]. In general, the chemical compounds accountable for actions on stock pests are mainly monoterpenes (1 - 8 cineole, eugenol, camphor) with insecticidal effects (Kouninki *et al.*, 2007 [20]; Noudogbessi *et al.*, 2009 [21]) or alkaloids with inhibitory effects on pest larval development (Ngamo *et al.*, 2001) [22]. However, the toxic and repellent effects of these plant materials are based on several factors, including their chemical composition and the level of susceptibility of the insects (Guèye *et al.*, 2011) [5].

Larvae exposed to *E. camaldulensis* EOs showed direct contact susceptibility and dose-dependent mortality rates. All larvae died at doses of 3 µl/larva. Therefore, *E. camaldulensis* EOs is highly effective on *E. cautella* larvae by direct

contact.

E. cautella eggs 24 h old are less sensitive to the chemical compounds of *E. camaldulensis* EOs. After exposing eggs to increasing concentrations of oil for 24 h, hatching rates recorded were not significantly different from the control. However, marked ovicidal activity has been reported on *C. maculatus* and on *Anopheles gambiae* eggs with EOs of *Ocimum americanum*, *Lippia multiflora*, *Hyptis suaveolens* and *Hyptis spicigera* (Wangrawa *et al.*, 2015 [23]; Ilboudo *et al.*, 2016 [9]). The observed tolerance of *E. cautella* eggs may be due to the low penetrating power of the EO. According to Ilboudo *et al.* (2016) [9], the ovicidal activity of EOs is based on their penetrating power or direct toxicity. Similarly, the ovicidal activity of oils can vary according to the age of the eggs and the method of use. Papachristos and Stamopoulos (2002) [24] demonstrated the toxicity of *Lavandula hybrida*, *Rosmarinus officinalis* and *Eucalyptus globulus* on *Acanthoscelide obtectus* eggs, with a difference in sensitivity significantly correlated with age. The authors found that sensitivity was highest after three days, probably due to greater permeability of the chorion or vitelline membrane, which facilitates the diffusion of volatile compounds.

5. Conclusion

The results obtained using neem kernel powder show that it could be used to control *E. cautella* larvae. The insecticidal effect of this powder is low in toxicity to adults. *E. camaldulensis* essential oil, tested for its contact and fumigant activity, is highly effective in controlling *E. cautella* adults, with mortality rates varying according to concentration. It inhibits larvae by direct contact. The difference in toxicity observed between the different developmental stages of the insect calls for integrated control of the two bio-pesticides to optimize their efficacy. Thus, the use of these insecticidal plants could be an interesting alternative to chemical control.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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