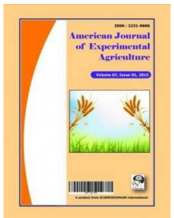

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Synergistic Effects of Wood Ash and Essential Oil on Fecundity, Pupal Eclosion and Adult Mortality of *Callosobruchus maculatus* (Coleoptera: Bruchidae) Cowpea Seed Weevil

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AM and ENN designed the study, wrote the protocol and wrote the first draft of the manuscript. Author AM carried out the experiment. Author CN reviewed the experimental design and all drafts of the manuscript. Author FVV managed the analyses of the dosages and performed complementary statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The use of residual synthetic chemicals, although effective against stored product pests, has led to environmental degradation. However, the risk of target pests developing resistance to insecticides in addition to the high costs of synthetic insecticides has pushed researchers to find alternative control methods. Plant-based insecticides represent a suitable alternative control method because they are less toxic to non-target organisms and biodegradable. The present study investigates the potency of essential oil and wood ashes in the control of the stored product pest

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Callosobruchus maculatus (Coleoptera: Bruchidae).
Study Design: Bioassays were performed in glass jars. Insect rearing, oil extraction and wood ash preparation were done according to an established protocol. For each treatment, a randomized complete block design with four replications was used.
Place and Duration of Study: Laboratory of biology of the Faculty of Science, University of Ngaoundere, Cameroon from February 2013 to February 2014.
Methodology: Wood ash from two different plants and essential oil were used. We evaluated the single effects of our products before combining each wood ash at 0.4, 2, 10, 20 and 40 g/kg with the essential oil of *Lippia adoensis* at the rate of 0.05 mL/kg on different fitness parameters of *C. maculatus*. Data on insect mortality rate and adult emergence were assessed.
Results: In single applications, the susceptibility of *C. maculatus* was significantly (*P* = .05) higher to essential oil than wood ash with LC_{50} values of 2.64 mL/kg and 139.64 g/kg, respectively after the first day exposure. The essential oil was also more effective in reducing egg laying and adult emergence. However, the combination of wood ash and essential oil caused 98.69±1.32% mortality within six days of exposure, compared to 73.53±3.80% for wood ash and 77.44±2.91% for essential oil. The co-toxicity coefficients of the combination of the two substances for mortality (275.9), fecundity (562.61) and fertility (438.92) were higher than 120, suggesting a significant essential oil-ash synergistic interaction.
Conclusion: Essential oil was the most toxic in single applications for all treatments. But when applied in combination, a lowest dose of wood ash (0.8 mL/kg) instead of 40 g/kg was able to kill 98.69±1.32% of adults. Therefore, combining essential oil with wood ash could significantly (*P* = .05) improve on the efficacy of controlling *C. maculatus* in storage facilities. Knowledge gained from this study could be exploited by low income farmers as it could provide a cost effective strategy for crop storage without the use of enormous quantities of wood ash.

Keywords: Callosobruchus maculatus; Vigna unguiculata; essential oil; wood ash; co-toxicity; synergism.

1. INTRODUCTION

Cowpea *Vigna unguiculata* (L.) Walper (Fabaceae: Papillonoidae) is one of the most widely adapted, versatile and nutritious herbaceous legumes with trifoliate leaves, which is drought tolerant and warm weather crop well adapted to the drier regions where other food legumes do not perform well [1]. It is also efficient in fixing nitrogen and enriching the soil [2]. It has been consumed by humans since the earliest practice of agriculture in developing countries of Africa, Asia and Latin America [3]. In West and Central Africa, cowpea constitutes the cheapest source of dietary protein for low-income sector of the population thus helping to alleviate protein malnutrition in human [4]. It is also an important cash crop that makes up part of the export commodities for producing countries. It requires annual rainfall of about 750–1100 mm [5]. In Cameroon, cowpea is cultivated mainly in the northern parts and is grown by nearly 78% of farmers in the Far North, 48% in the North and to a lesser degree in the Adamawa region (1%) [6]. That makes Cowpea the third most important crop in this part of the country after maize, *Zea mays L*., and groundnuts *Arachis hypogea*. The crop is grown in a single cycle but is consumed throughout the year. This situation pushes the farmers to store their crops to spare them from any risk of food shortage during the agricultural off-season [7]. Unfortunately, every year, large quantities of stored cowpea suffer a great damage due to insect attack [8]. The cowpea weevil, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae), is a cosmopolitan field-to-store pest ranked as the principal post-harvest pest of cowpea in the tropics [9]. During larval stages, it causes substantial quantitative and qualitative losses (50-90%) manifested by seed perforation and reductions in weight, market value and germination ability of seeds [2,10]. The insect lays its eggs on the seeds of cowpea, which hatch and produced larvae that bore into the seed cotyledons in which they feed.

For decades now, the management of *C. maculatus* has been dominated by chemical control using synthetic Chemicals [11,12]. However, the use of synthetic insecticides in crop protection programmes around the world has resulted in disruption of the environment, pest resurgences, and development of resistance to pesticides, lethal effects to non-target organisms in agroecosystems, toxic residues in food and water bodies, as well as direct toxicity to users [13-15].

This brings to the light the emergency of developing alternative ecologically safer, economical, readily affordable and user-friendly pest control techniques, such as using locally available plants with insecticidal properties [16,17]. It has been reported that resource-poor farmers in Africa employ a range of traditional methods such as the use of sand, dry pepper and botanical extracts [1]. Naturally occurring plant products have been used in the protection of agricultural products against pests for many years in some parts of the world; many authors have reported insecticidal effects of plant products against a broad range of pests. Some of the techniques that can be explored include the use of wood ash and essential oil. In the present circumstance, an approach that would rely on the use of plant products (without involving synthetic pesticides) appears to hold the greatest hope for increased cowpea production in the traditional cereal–dominated cropping system throughout tropical and subtropical countries, including Cameroon.

The present study investigates the effects of combining wood ash from *Lophira lanceolata* and *Hymenocardia acida* with the essential oil of *Lippia adoensis* at the rate of 0.05 mL/kg on mortality, egg-laying and progeny production of *C. maculatus* Fab. (Coleoptera: Bruchidae) with the aim of reducing the enormous quantities of wood ash employed by subsistence farmers in sub-Saharan Africa in their granaries.

2. MATERIALS AND METHODS

2.1 Preparation of Seeds and Insects Rearing

Cowpea seeds of the Mozongo variety were collected from farmers in Lara (Far-North region, Cameroon). They were sun-dried, cleaned and disinfested by keeping in a freezer (-18℃) for 14 days in order to kill any insect pest present. The disinfested seeds were then kept under the experimental conditions for two weeks before use for acclimatization [18]. These seeds were then packed into 1000 mL glass jars and later used for the experiment. The test bruchid, *C. maculatus* were collected from previously infested cowpea seed purchased from Lara (Far-North region, Cameroon). They were reared in white cowpea variety, *Mozongo* at room temperature and relative humidity in the Biology Laboratory of the University of Ngaoundéré for three months (February, March and April 2013) under fluctuation laboratory conditions (T≈22,72±1,06℃, RH≈83,73±1,28%). The glasses were then covered with fine mesh cloth fastened with rubber bands to prevent the contamination and escape of insects. Seven days were allowed for mating and oviposition. The parent stocks were sieved out and the cowpea seeds containing eggs were left undisturbed until the new adults emerge. Only the subsequent F1 progenies of the bruchids, which emerged from the cultures and aged 1-3 days, were used for the experiment.

2.2 Preparation of Test Plant Materials

2.2.1 Wood ash

The plant materials evaluated for insecticidal activity against *C. maculatus* were stems of *L. lanceolata* and *H. acida* which were collected in March 2013 near the campus of the University of Ngaoundéré located in the Adamawa region, Cameroon. The woods were sun-dried until completely moisture lost and then burnt completely to ashes separately in a traditional kitchen. After cooling, the ash was sieved through a 0.4-mm mesh and placed in sealed jar to prevent the absorption of air moisture. Each plant product was labeled and kept at 4℃ until needed for bioassays [19].

2.2.2 Extraction of essential oil

Plant materials used for essential oil extraction were fresh leaves from *L. adoensis*. Plants were collected from Mbe (601masl, latitude 7°82N, longitude13°58E recorded with a GPS Garmin Geko 301) in the reference season Adamawa region. Mbe is located 70km north of Ngaoundéré-Cameroon. The identity of plants was confirmed at the national herbarium of Yaounde (Cameroon), where voucher specimens are deposited. The fresh leaves were used for the extraction of the essential oil by hydrodistillation for 4h using a Clevenger apparatus. The EOs collected were dried over anhydrous sodium sulfate and kept in transparent glass bottle at 4℃ until used for analysis. The essential oil recovered was stored at 4℃ until needed for bioassay.

2.3 Toxicity Tests with Single Dosages

2.3.1 Bioassay with wood ash

Each wood ash was separately applied to 50 g of cowpea seeds in 1000 mL glass jars at 0.4, 2, 10, 20 and 40 g/kg. There was untreated check which did not contain any plant material. Four replications were maintained for each dose. The jars containing cowpea were gently shaken for about 2 minutes to ensure thorough admixture of cowpea seeds and wood ash. The ash was allowed to settle down for about 15 seconds before ten pairs of adult *C. maculatus* of mixed sex aged one to three days old were added to each jar. The jars were covered with fine mesh cloths fastened with metal lid, labeled and left at room temperature and relative humidity. Treated jars and untreated controls were laid out in a completely randomized design. Adult mortality was recorded 1, 2, 4 and 6 days after treatment. Insects that did not move after being touched with a brush were considered dead [20].

2.3.2 Bioassay of *Lippia adoensis* essential oil

Five dosages (2.5, 5, 10, 20 and 40 µL) of essential oil diluted in 1 mL of hexane were introduced separately in 900 mL glass jars containing each 50 g of cowpea (corresponding to concentrations 0.05, 0.1, 0.2, 0.4 and 0.8 mL/kg). Each jar was shaken manually for 5 minutes for the product to adhere uniformly to grains. Control groups were treated only with 1 mL hexane. These jars were kept open for 20 minutes at ambient laboratory conditions (T ≈ 21.79±2, 49 to 23.69±2.24℃, RH ≈ 34.90 to 83.73±1.28%) for the solvent to evaporate completely [21,22]. Twenty weevils aged between 1 to 3 days of mixed sex were added to each jar. The jar was sealed with thin cloth and closed with perforated lid. The experimental design was a randomized complete block with four replications. The determination of the living and dead weevils was done after 1, 2, 4, and 6 days of exposure. Were considered dead, insects that did not move after being touched with a brush [20].

2.3.3 Toxicity tests with combined doses

In 900 mL glass jars containing 50g of cowpea, we introduced a single volume of 2.5 µL (corresponding to 0.05 mL/kg) of essential oil *L. adoensis* diluted in 1 mL of hexane. The jars were shaken manually for 5 minutes to enable the essential oil to adhere to the seeds. The jars were left open for 20 minutes for the solvent to evaporate completely [21,22]. After evaporation, the masses of 0.02, 0.1, 0.5, 1 and 2 g of wood ash of *H. acida* (corresponding to respective dosages 0.4, 2, 10, 20 and 40 g/kg) were added. Then the jars were shaken manually for 2 minutes. Then, batches of 20 adult *C. maculatus* of indeterminate sex and aged 1 to 3 days were introduced. Control groups were treated only with hexane. The experimental design was a randomized complete block with four replications. The same experiment was carried out for the wood ash of *L. lanceolata*. The number of dead and the living insects were determined 1, 2, 4 and 6 days post- exposure [23]. Were considered dead, insects that did not move after being touched with a brush [20].

2.4 Fecundity and Fertility Tests

The fecundity of *C. maculatus* was evaluated through the number of eggs laid by the females and the fertility through the number of adult emergence. The same procedure described above for the mortality test has been applied as well as for the control (seeds exposed to 100% hexane). Fifty grams were introduced in each treated container and provided as oviposition sites for 10 males and 10 females of *C. maculatus* (aged 1-3 days) [24]. The experimental design was a randomized complete block with four replications. After the oviposition period, data on eggs laid and adult emergence were all monitored and recorded.

2.5 Data Analysis

Data on % cumulative mortality, % reduction in eggs laying and % reduction of adult emergence were arcsine-transformed and the number of F_1 progeny produced was log-transformed. The transformed data were subjected to the ANOVA procedure using the Statistical Analysis System [25,26]. Tukey's Studentized Range HSD test ($P = 0.05$) was applied for mean separation. Probit analysis [27,26] was applied to determine lethal dosages causing 50% (LC_{50}) mortality of *C. maculatus* at 1, 2, 4 and 6 days after treatment application. Abbott's formula [28] was used to correct mortality before probit analysis and ANOVA. OriginPro 8.5.1 was used to draw graphs and curve.

The dose-mortality response was analysed by probit analysis [27,29] using the maximum likelihood estimation. The co-toxicity coefficient per wood ash-essential oil mixture was used to determine their responses: A co-toxicity coefficient of less than 80 is considered as antagonistic, between 80 and 120 as additive, and higher than 120 as synergistic [30].

Toxicity index (TI) of A=100

Toxicity index (TI) of B= (LC_{50} of A/ LC_{50} of B) x 100

Actual TI of M = (LC_{50} of A/ LC_{50} of M) x 100Theoretical TI of M = TI of A x % of A in M + TI of B x % of B in M

Co-toxicity coefficient = (Actual TI of M/ Theoretical TI of M) x 100

If one component of the mixture alone (for example B) causes low mortality at all doses (< 20%), then the Co-toxicity coefficient of the mixture is calculated by the formula: Co-toxicity coefficient = LC_{50} of A alone / LC_{50} of A in the mixture x 100 and was computed.

3. RESULTS AND DISCUSSION

3.1 Toxicity Tests

3.1.1 Single products

The results show that the adult mortality rate was dose dependent and increased with the increasing concentrations (Fig. 1). Indeed, the lowest concentration (0.05 mL/kg) of *L. adoensis* essential oil induced 41.22±1.47% adult mortality in *C. maculatus* within 6th days of exposure; while at the highest concentration (0.8 mL/kg), 77.44±2.9% adult mortality of *C. maculatus* was obtained (more details including the response on days exposure are provided in Table 1s of the supplementary materials of this article). *L. adoensis* essential oil was the most effective in inducing the adult mortality in single treatments (Fig. 1). However, we surprisingly recorded a non-significant ($P > .05$) death in two control treatments that may be occasioned by the higher relatively humidity of the laboratory (RH ≈ 83.73±1.28%). (Table 1s. of supplementary materials).

3.1.2 Combined products

All the tested wood ashes products increased the adult mortality of *C. maculatus* at a significant level (P< .0001) when combined to a unique dose of essential oil 0.05 mL/kg compared to the controls (Fig. 1 and Table 1s). Regression analysis of data indicated significant correlation between percentage mortality and period of exposure in all treatments (P= .05) (Table 1s of supplementary materials). The mortality was positively dose-dependent (Fig. 1). When applied alone for 48 h, the two wood ashes were less effective against *C. maculatus* than the *L. adoensis* essential oil (Table 1). Overall, *C. maculatus* adults were more sensitive to the *L. adoensis* essential oil than to the wood ash (Fig. 1). The results we obtained in the present experiment have shown that *L. lanceolata* was more effective than *H. acida* against *C. maculatus* even though their mixture with the *L. adoensis* essential oil was the most effective. The sixth day LC_{50} values clearly demonstrate that wood ash from *L. lanceolata* (2.36 g/kg) was more toxic to *C. maculatus* than wood ash form *H. acida* (7.76 g/kg).

The toxicity of an admixture of *L. adoensis* essential oil with wood ash from *L. lanceolata* and *H. acida* were greater compared to their separate use (Fig. 1). The lowest LC_{50} values (LC_{50}=6.52 and LC_{50}=5.37, for the first and second mixture respectively), the combination increased the susceptibility of *C. maculatus* in comparison to the treatments alone. Moreover, these combinations showed synergistic action with co-toxicity coefficient values higher than 120 (Table 1).

3.2 Progeny Production

3.2.1 Effect of insecticidal products on the egg-laying inhibition of *Callosobruchus maculatus*

Fig. 2 revealed that the highest number of eggs laid was recorded on seeds treated with 0.4 g/kg compared to those treated with 20 g/kg but was not significantly different (P>0.05). Control treatment has the highest number of eggs laid on the cowpea seeds and was significantly different from other treatments (P<0.05).

In spite of the early death of *C. maculatus* adults (3-6 days post-infestation), no concentration of the essential oil completely prevented the females from laying eggs (Fig. 2). The number of eggs laid was inversely proportional to the tested concentrations (Fig. 2 and Table 2s of supplementary materials for more details).

Thus, with the lowest concentration (0.08 mL/kg), the median number of eggs laid by the female was 26.00±0.41% compared to the control

(50.75±0.85%). A ratio of the inhibition average of 48.69±1.64% was recorded compared to the control (0.00±0.00). At the highest concentration (0.4ml/kg), this median number of eggs laid decreased and reached 8.00±0.91% with an inhibition ratio of the laying 84.21±1.88% compared to the control (Table 2s of supplementary materials) The response of the essential oil at four different concentrations regarding eggs laying was found to be statistically significant because it has reduced significantly the oviposition of *C. maculatus* with the lowest LC50 value (Table 2).

3.2.2 Effect on pupal eclosion of adults *Callosobruchus maculatus*

We recorded the pupal success (number of adults that emerged) from all the treatments were significantly (P = .05) different (Fig. 3). 31% of adult emerged from the treatment with 0.4 g/kg of wood ash from *H. acida* while less number of adults emerged from seed treated with 20 g/kg. However, we recorded the highest number of adult emergence in the control. The inhibition rate is inversely proportional to increasing concentrations (Fig. 3).

Table 1. Linear regression of adult mortality 1, 2, 4 and 6 days after treatment

Products	**n**	**Slope ± SE**	R^2	**LC_{50} (50% FL) (g/kg and mL/kg)**	**Co-toxicity coefficient relative to LC_{50}**	χ^2
				1 day		
H. acida	5	0.36±0.10	0.73	4359(415.90-1255.072)		0.14^{ns}
L. lanceolata	5	0.56±0.10	0.72	139.64 (60.52-668.27)		0.04^{ns}
L. adoensis	5	0.88±0.15	0.96	2.64 (1.05-36.47)		0.28^{ns}
H. acida+ L. adoensis	5	0.76±0.10	0.96	36.58 (23.48-68.98)	**275.9**	3.35^{ns}
L. lanceolata+ L. adoensis	5	0.80±0.09	0.96	12.68 (9.02-18.82)	**255.0**	1.75^{ns}
				2 day		
H. acida	5	0.47±0.10	0.15	392.55 (113.61-6443)		0.34^{ns}
L. lanceolata	5	0.47±0.09	0.95	45.17 (22.49-54.91)		0.58^{ns}
L. adoensis	5	0.96±0.14	0.94	2.63 (1.04-35.70)	**205,88**	0.25^{ns}
H. acida+ L. adoensis	5	0.76±0.90	0.94	5.37 (3.72-7.65159)	**573,32**	0.60^{ns}
L. lanceolata+ L. adoensis	5	0.90±0.13	0.90	6.52 (2.89-14.75)	**160.00**	6.53*
				4 day		
H. acida	5	0.64±0.09	0.90	15.16 (9.89-26.14)		1.88^{ns}
L. lanceolata	5	0.40±0.08	0.94	16.28 (8.38-45.58)		0.73^{ns}
L. adoensis	5	1.14±0.14	0.94	0.54 (0.34-1.28)		0.56^{ns}
H. acida+ L. adoensis	5	1.13±0.20	0.96	2.40 (0.56-5.87)	**146.00**	13.18**
L. lanceolata+ L. adoensis	5	1.00±0.09	0.93	1.61 (1.11-2.20)	**234.00**	3.60*
				6 day		
H. acida	5	0.60±0.08	0.97	7.76 (4.98-12.53)		5.47*
L. lanceolata	5	0.61±0.08	0.97	2.36 (1.33-3.68)		1.91^{ns}
L. adoensis	5	1.26±0.14	0.98	0.09 (0.06-0.13)		0.03^{ns}
H. acida+ L. adoensis	5	1.13±0.21	0.97	0.86 (0.10-2.21)	**209.00**	12.43^{ns}
L. lanceolata+ L. adoensis	5	1.23±0.11	0.95	1.05 (0.75-1.40)	**5198**	5.07*

*ns: not significant (P >. 05) ; *: significantly different (P< .01) ; ***: highly significant (P< .001) ; FL : Fiducial Limits; n: number of dosages; LC_{50}: Lethal concentration killing 50% of adults C. maculates*

Table 2. Linear regression of F1 progeny production (T≈22.92±1.07℃, RH ≈ 83.63±1.28%)

Products	**n**	**Slope ± SE**	R^2	**LC_{50} (50% FL) (g/kg and mL/kg)**	**Co-toxicity coefficient**	χ^2
H. acida	5	0.50±0.10	0.99	3.11 (1.63-5.65)		1.1570^{ns}
L. lanceolata	5	0.46±0.10	0.99	3.78 (1.92-7.62)		0.9601^{ns}
L. adoensis	5	1.09±0.20	0.99	**0.06 (0.03-0.08)**		0.7965^{ns}
H. acida+ L. adoensis	5	0.66±0.10	0.99	0.71 (0.32-1.21)	**438.92**	0.9448^{ns}
L. lanceolata+ L. adoensis	5	0.44±0.10	1.00	0.35 (0.05-0.85)	**1082.19**	1.0781^{ns}

*ns: not significant (P = .05); *: significantly different (P< .01) ; ***: highly significant (P< .001); FL : Fiducial Limits; n: number of dosages; LC_{50}: Lethal concentration inhibiting 50% of adult emergence*

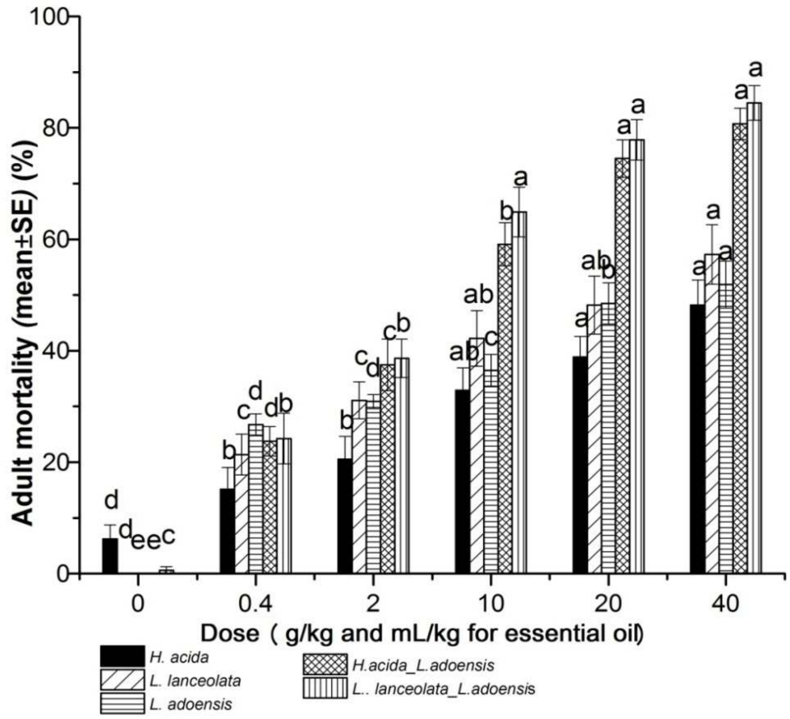

Fig. 1. Adult mortality of *Callosobruchus maculatus* exposed to five dosages of wood ash and essential oil

NB: Means with the same letter are not significantly different after comparison with Tukey's Test at (P< .05)

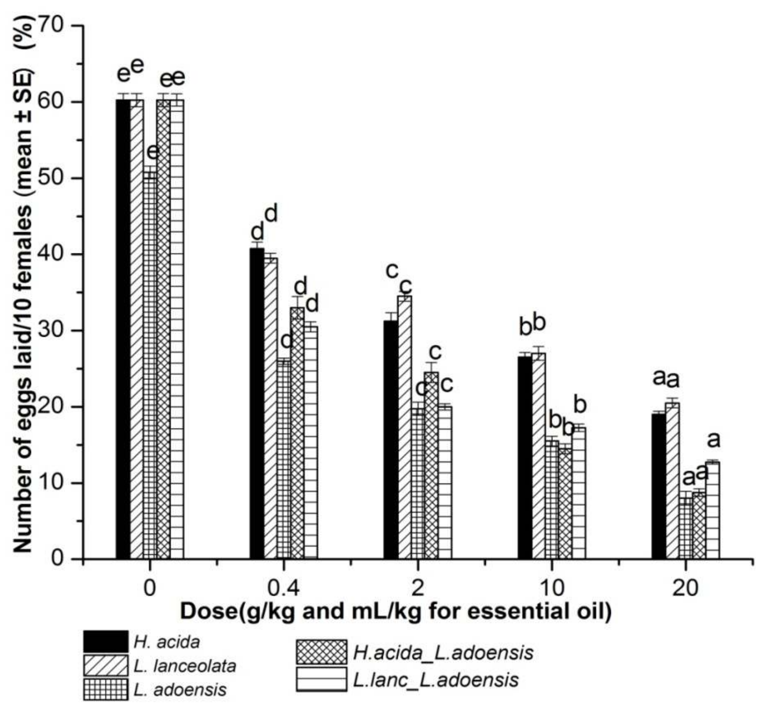

Fig. 2. Mean number of eggs laid by females exposed to five dosages of wood ash and essential oil

NB: Means with the same letter are not significantly different after comparison with Tukey's Test at (P< .05)

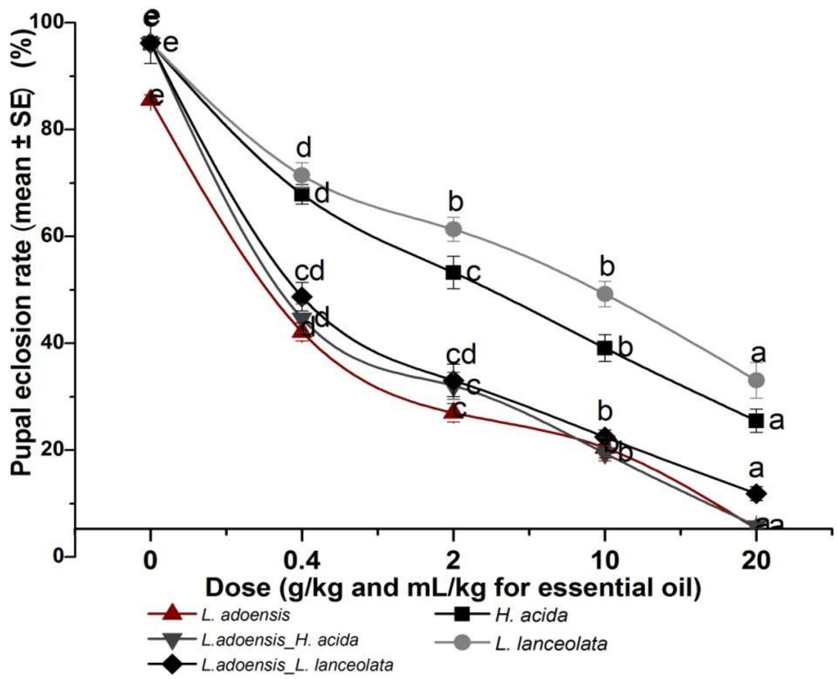

Fig. 3. Mean pupal eclosion of eggs exposed to five dosages of wood ash and essential oil
NB: Means with the same letter are not significantly different after comparison with Tukey's Test at (P = .05)

Out of the 26.00±0.41% of eggs which were laid at the lowest concentration, only 18.25±0.85% hatched whereas at the highest concentration (8.00±0.91%), the progeny production was totally suppressed (Table 3s of supplementary materials). The present study revealed also that the adult emergence decreased with increasing doses. The response of the essential oil at four different concentrations regarding eggs laying was found to be statistically not significant (Table 3). The *L. adoensis* essential oil was the most effective (Fig. 3). The LC_{50} was 0.05 ml/kg (Table 3).

4. DISCUSSION

In this experiment, our results have shown that wood ash from *Lo. lanceolata*, *H. acida* and essential oil from *L. adoensis* had detrimental effect on cowpea weevils for the parameters measured. This suggests that our plant products possess some toxic components which could significantly inhibit egg-laying and adult emergence and cause noticeable mortality of the weevil *C. maculatus* thereby impacting negatively the weevils more than the controls and could therefore have greatest potential as stored grain legume protectants.

On the basis of the LC_{50} values, *C. maculatus* was more susceptible to the essential oil (LC_{50}=2.64) than wood ash (LC_{50}=139.64). Egg laying by female adults of *C. maculatus* was much significantly reduced when exposed to essential oil compared to wood ash, and similar effects were recorded for subsequent egg hatching and larval survival. This is due to the fact that essential oil could diffuse inside the grains whereas wood ash can not.

The insecticidal effect of essential oil tested was translated in the adults of *C. maculatus* by intoxication. This could have blocked the transmission of the nerve impulse by inhibition of the hydrolysis of acetylcholine through mechanisms that have not been fully identified. Ryan and Byrne [31] suggested that the toxic effect of essential oil may be attributed to reversible competitive inhibition of acetylcholinesterase by occupation of the hydrophobic site of the enzyme's active centre.

The steepness of slope in probit mortality regression indicates that there is a large increase in the mortality of insects with a relatively small increase in the concentration of toxicants [32,33]. That was true for the contact toxicity of *C. maculatus* within the experimental conditions of the present study.

Table 3. Linear regression of eggs laying ability of females *Callosobruchus maculates* (T≈22.92±1.07℃, RH ≈ 83.63±1.28%)

Products	n	Slope values ± SE	R^2	LC_{50} (50% FL) (g/kg)	Co-toxicity coefficient	χ^2
H. acida	4	0.61±0.10	0.97	5.22 (3.23-9.09)		2.84*
L. lanceolata	4	0.70±0.10	0.96	2.39 (1.48-3.64)		1.57*
L. adoensis	4	1.91±0.55	1.00	0.05 (--)		9.14*
H. acida+ *L. adoensis*	4	0.87±0.27	0.99	0.34 (--)	**1337.22**	10.58***
L. lanceolata+ *L. adoensis*	4	0.73±0.11	0.99	0.37 (0.14-0.67)	**562.61**	2.48*

** (P< .05): significant; * (P< .001): slightly significant; ***: highly significant; FL: Fudicial limits; n: number of dosages; LC_{50}: Lethal concentration inhibiting 50% of eggs laid, (--): computing Fudicial limits cannot be determined*

It was suggested that persistence of the insecticidal activity of essential oil depends on its chemical composition [34]. The essential oil with a high content of hydrogenated compounds are the most susceptible to oxidation and lose their activity quicker than those containing mainly oxygenated compounds [35]. Therefore, the *L. adoensis* essential oil could have a high persistency probably because of oxygenated monoterpenes and its biodegradability. This hypothesis is being verified in the ongoing experiment in our laboratory where we are trying to identify the chemical constituents of the essential oil and their effects on some fitness parameters of *C. maculatus*.

Many plant extracts are known to have ovicidal, repellent, antifeedant and insecticidal activities against various insect species resulting in the reduction of F_1 progeny production of stored product pests [36].

The mortality caused by wood ash at varying degree compared to the controls could be attributed to stomach poison since the weevils feed directly on the grains [37]. Also, during insects crawling over the grains, the chemical constituents of ash could lodge between cuticular segments and increase water loss through abrasion of the cuticle. Similar observation was made by Ebeling et al. [38]. The higher beetle mortality in cowpea treated with ash powders was recorded with *Lo. lanceolata* which is more effective than *H. acida* against *C. maculatus*. This may be due to their difference in proportion of chemical compounds to abrasive action. The slope and LC_{50} values of *Lo. lanceolata* are higher than that of *H. acida*. Badea et al. [39] revealed that ash powder contained silica, iron, and calcium and sometimes traces of metals, phosphorus and nitrogen which could be responsible of insecticidal effects. According to Jean Wini Goundougou et al. [40], the wood ash from *H. acida* is mainly constituted of Calcium, Magnesium, Potassium, Sodium, Zinc, Iron and Phosphorus which on the basis of their effectiveness in this experiment, could be less potent than that of *L. lanceolata*. However, further research need to be done to determine the chemical compounds of *L. lanceolata* wood ash before confirming this assumption.

Meanwhile, synergy studies aims at determining the scientific reasons about the better activity of plant derivatives compound and essential oils when compared to single treatments. Thus, the combination of wood ash and essential oil caused 98.69±1.32% mortality to *C. maculatus* adults within six days of exposure, compared to 73.53±3.80% and 77.44±2.91% for single wood ash and essential oil treatments respectively. However, for subsequent pupal eclosion and larval survival tests, the essential oil was reported to be the most efficient. The co-toxicity coefficients of the combination of the two substances for mortality (275.9), fecundity (562.61) and fertility (438.92) were all higher than 120, suggesting a significant essential oil-wood ash synergistic interaction. The present results corroborates the findings of Khalequzzaman and Sadia Nazneen Rumu [41] who reported the synergistic action of three essential oils with pirimiphos-methyl against *C. maculatus*. It was thought reasonable that the essential oil act as synergist with wood ash to enhance its effectiveness. The review of literature, however, revealed that no information is available on the combined action of essential oil and wood ash for the control of *C. maculatus* and other stored grain pests. Katamssadan et al. [42] studied the insecticidal potency of *A. indica* powders and recorded a significant dose-dependent mortality to *C. maculatus* and *S. zeamais,* as well as a complete suppression of progeny production and grain damage. Slightly similar results have been reported by Jean Wini Goundoungou et al. [39] when they applied wood ashes, leaf powder and diatomaceous earth on the development of immature stages of *S. zeamais.* To our knowledge, this experiment is the first studying the combined effects of wood ash from *L. lanceolata* and *H. acida,* two plants highly used for firewood in Cameroon and the essential oil from *L. adoensis* highly used in traditional medicine. This study is therefore a contribution to the promotion and development of local plants as grain protectants against stored pest infestations.

5. CONCLUSION

The present study shows that wood ashes from *H. acida* and *L. lanceolata* and the essential oil of *L. adoensis* could be of value in the reduction of the infestation of cowpea by *C. maculatus,* especially among the subsistence farmers of Africa, who store small quantities of grains in traditional granaries. The essential oil prevented oviposition and inhibited progeny production of *C. maculatus* at relatively low concentrations (0.05 mL/kg). We also found that the increasing mortality was dose dependent. *L. adoensis* essential oil was the most effective in all the single treatments (mortality, fecundity and fertility) with the steepest slope and the lowest LC_{50} due to its ability to diffuse inside the grains whereas the admixed treatments were effective for the mortality test. Both combinations (0.05 mL/kg essential oil at varied concentrations of wood ashes) revealed synergistic effects in all fitness parameters studied. Thus, combining wood ash with essential oil would go a long way in reducing the large quantities of the former employed wood ash in traditional granaries, with a better protection of stored grains against the attacks of *C. maculatus* and probably against other insect pests. We can therefore promote wood ash not only as protectant, but also a support which may fix the volatile components of the essential oil. These would lead to a healthier environment and alleviate food insecurity.

6. RECOMMENDATIONS

- Further work should be done to identify and isolate active compounds contained in *L. adoensis* essential oil and plant powders to determine the efficacy and methods of formulations. This may involve chemists, biochemists and environmental scientists.
- These botanical powders should be incorporated into grain protection practice of resource-poor farmer.
- In addition, there is the need to investigate the shelf life of the powders to find out if repeated application is needed after a given period.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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