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Bioefficacy of *Chenopodium ambrosioides* L. (Chenopodiaceae) on adult *Callosobruchus maculatus* F. (Coleoptera: Bruchidae)

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Abstract

The use of synthetic insecticides has become widespread and useful for decades. However, due to not being eco- friendly and development of resistance in these organisms plants natural products essential oil components have shown significant potentials for insect control. Hence, laboratory bioassays were conducted to investigate bio-efficacy of *Chenopodium ambrosioides* L: (Chenopodiaceae) essential oil on adult *Callosobruchus maculatus* F. (Coleoptera: Bruchidae). On the basis of 24 hr and 48 hr median lethal toxicity (L_{50}). Essential oil of *C. ambrosioides* was toxic on *C. maculatus* adults at 0.50 Mg/m^2 and 0.20 Mg /m^2 in 24 hr Lc_{50}. Also 48 hr Lc_{99} values obtained show that essential oil of tested plant was 0.90 Mg/m^2 and 0.18 Mg/ m^2 toxic on adult *C. maculatus*. The study shows that essential oil of *C. ambrosioides* have potentials as fumigants for protecting cowpea from damage of *C. maculatus*

Keywords: Bioefficacy; *Chenopodium ambrosioides*; *Callosobruchus maculatus*; Essential oil; Coleoptera

1. Introduction

It has been estimated that between one quarter and one third of the world stored food products are lost during storage. This may result into quantitative and qualitative losses due to physical, chemical and most importantly biological factors such as pests which may be insects, birds, rodents, and fungi [1, 2,3] The most important among storage pests are insects because apart from their direct damage they create conditions that allow secondary infestation by rot organisms mainly fungi [5].

Damage caused to commodity and stored products include losses in weight, seed viability and nutritive value. Records show that over 600 and 70 species, respectively of beetles and moths attack stored products of agriculture [6,7], causing significant damage to agricultural products intended for human consumptions and animal feeds. Apart from direct losses caused by these insects, indirect losses are also incurred by the contaminants (body parts or exoskeleton, eggs and off–odors) left on produce.

Several methods are used in controlling insect pests of stored foods including physical (smoking, sun-drying, heating), cultural, biological (male insect sterilization, natural enemies, resistant grain varieties), and chemical (synthetic and natural products) methods. The most common and widely used is the chemical method involving mainly the use of synthetic insecticides [4].

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Fumigants and synthetic chemical protectants have played important and beneficial roles in the control of insect pests of stored products and the reduction of insect- borne diseases. Fumigants such as phosphine and methyl bromide are mostly used against stored grain insect pests because of their broad spectrum of activity and penetrating power [7].

Recently, other fumigants such as sulphuryl fluoride, carbonyl sulphide and ethyl formate have also been investigated to control insect pests [8,9]. Although these materials are effective, repeated use for several decades have been reported to have its consequences. These insecticides are often associated with residues that are dangerous to the consumer, the environment even at low doses. Many insecticides are toxic to humans and other animals [10, 11].

The number of confirmed insect species with resistance against synthetic pesticides has continued to rise, apart from risks associated with the use of these chemicals. It has also been estimated that about 2.5 million tons of pesticides are used on stored products yearly which cause billions of naira annually in controlling insect pest leading to high cost of foodstuffs in the markets [12].

Hence, many researchers, farmers, stored managers and homeowners are seeking for less hazardous alternatives to conventional synthetic insecticides. Also, the demand for agriculture stored products worldwide is on the increase. Due to the foregoing reasons, there is a need to search or review the appropriate mechanism that can be used to control the activity of insect pests on stored products.

The ideal insecticide should control target pests adequately and should be target- specific, hence the use of plant materials as traditional protectants of stored products is an age long practices but in a crude way. Recently, extraction of active ingredients from the plant parts such as root, stem and the leaf as protectants is now gaining ground [7]. Botanical insecticides degrade rapidly in sunlight, air and moisture and are readily broken down by detoxification enzymes. This is very important because rapid breakdown means less persistence in the environment and reduced risks to non-target organisms [13, 14, 15].

One of such plant is *Chenopodium ambrosioides*, which naturally has medicinal effects, insecticidal activities and anti-biotic effects [16]. It is widely distributed all over the world especially in the USA where it is listed in National formulary and Pharmacopoenia. In British, it is used as an anthelmintic against roundworms of dogs and for the treatment of cancer [17, 18]. The leaf is used in western Nigeria as mosquito repellant and to treat some diseases [4]. The plant is pungent and resinous accounting for its anti –biotic characteristics.

Cowpea weevil, known as *Callosobruchus maculatus* L. (Coleoptera: Bruchidae) is the most widespread and destructive major insect pest of stored legumes all over the world [19]. Control of this insect population throughout the world has relied principally on the application of synthetic insecticides like organophosphates and fumigants such as methyl bromide and phosphine, which are still the most effective means of protection of stored food and other agricultural commodities from insect infestation [20]. Although effective, such synthetic pesticides cause consequential residual pollution of the environment and toxicity to consumers. Their repeated use for decades has disrupted biological control by natural enemies and has led to the resurgence of stored products insect pests. Many of these stored product insects have developed resistance to commonly used chemicals [21].

These problems have highlighted the need to develop insect control alternatives. Many medicinal plants and spices have been used as pest control agents [22, 23]. Farmers and researchers often claim the successful use of plant materials in insect pest control including ash [24, 25], Vegetable oils [26, 27], Plant extracts [28, 29], and Plant powders [30, 31, 32, 33]. Plant materials should be explored to protect stored products against pest infestation.

This study investigated the bioefficacy of oil extract from *Chenopodium ambrosioides* leaf on adult *Callosobruchus maculatus*.

2. Materials and methods

2.1. Preparation of formulations

The leaves of *Chenopodium ambrosioides* were collected from Apa and Owode towns, Badagry West Local Government Area of Lagos State, Nigeria. The leaves were air dried in the Zoology Laboratory, Lagos State University, Ojo for 72 hours before oil extraction.

2.2. Extraction of oil

Chenopodium ambrosioides leaves were in a 2 litres conical flask with distilled water in the conical flask which was part of the clavenger apparatus held by a retort stand, the whole apparatus was then placed on a 2 litres heating mantle. Attached to the clavenger were two hoses with one running cold water into the clavenger and warm water was passed out through the other hose.

As water in the conical flask was heated by the heating mantle, the vapour which contains oil was condensed back to the lower part of the clavenger which helped in the separation of oil from the water and also the oil from evaporating, making it easier to collect in an amber bottle and kept in a freezer at 4 °C in other to keep the oil from denaturing or evaporating until when needed for bioassays.

2.3. Preparation of *Chenopodium ambrosioides* oil for serial dilution

Six, 1.5 litres of bama bottles were arranged horizontally and labelled; control, 20 % ppm, 40 % ppm, 60 % ppm, 80 % ppm and 100 % ppm respectively. Olive oil (10 ml, 0.8 ml, 0.6 ml, 0.4 ml, 0.2 ml respectively) was measured into the labelled containers and 0 %, 0.2 %, 0.4 %, 0.6 %, 0.8 %, 1.0 % of the oil extracts was added into the olive oil bottles respectively.

Three replicates of a medium-sized cup solution were arranged in front of each dilution, the diluted oil solution was measured into each bottle and twenty adults of *Callosobruchus maculatus* were added into each bottle. The same procedure was used for the other replicates and the control respectively. The mortality rate was checked every 24 hours and 48 hours.

2.4. Test insects

Cowpea weevil-: *Callosobruchus maculatus* was cultured in the Zoology Laboratory of Lagos State University Ojo. The Cowpea (beans) used for the culture was bought at Alaba Market, Ojo, Lagos State. Two sets of Cowpeas were bought from the same market: the clean beans without cowpea weevil and the unclean beans with Cowpea weevils. The insects *C. maculatus* collected from the unclean beans was used to prepare fresh experimental cultures from the original stocks as described by [34] and maintained at 30 ± 1 °C temperature and 70 ± 4 °C relative humidity.

The fumigant effects of *C. ambrosioides* leaf oil was carried out against adult test insects. Bioactivity of *C. ambrosioides* was carried out in air tight Kilner jars (1-1) using the method of [35].

A 7 cm diameter watchman No 1 filter paper was impregnated uniformly with the *C. ambrosioides* oils at 20 % ppm, 40 % ppm, 60 % ppm, 80 % ppm and 100 % ppm concentration and quickly hung down with a thread into the Kilner jars already holding 20 adults of *C. maculatus* and sealed with a cap.

The control insects were left in air tight sealed chambers using olive oil on the filter paper. There were three replicates per treatment.

2.5. Data analysis

Statistical analysis was done using Microsoft excel 2007 version to determine the Lc_{50} and Lc_{99} for the acute toxicity in the essential oil respectively. [36] formula was used to correct for mortality and equation of straight line in calculating Lc_{50} and Lc_{99}

3. Results

At twenty-four hours exposure, total mortality of test insects increased with concentration; 20 % ppm concentration had 9 mortalities of test insects 40 % ppm had 29 mortalities 60 % ppm had 34 mortalities, while each of 80 % ppm and 100 % ppm concentrations had 60 mortalities (table 1).

The 1 table also revealed that as concentration increased, percentage corrected mortality also increased. The rates of increase showed that 20 % ppm had the least percentage (15 %) while 80 % ppm and 100 % ppm had the highest (83.33 %).

However, no mortality was recorded in the control set-up during the first 24 hours of exposure.

Table 1 Effects of Essential oil of *Chenopodium ambrosioides* on adult *Callosobruchus maculatus* after 24 hours

Conc.	Mortality in Replicate I	Mortality in Replicate II	Mortality in Replicate III	Total Mortality	Mean Mortality	% Corrected Mortality
Control	0	0	0	0	0	0
20 % ppm	3	2	4	9	0.15	15
40 % ppm	10	9	10	29	0.48	48.33
60 % ppm	12	12	10	34	0.56	56.67
80 % ppm	18	17	18	53	0.88	88.33
100 % ppm	20	20	20	60	1.00	100

At forty- eight hours exposure, total mortality of test insects increased with concentrations, 20 % ppm concentration had 57 mortalities of test insects while 40 % ppm, 60 % ppm, 80 % ppm and 100 % ppm concentrations had 60 mortalities (table 2)

The result also revealed that as concentration increased percentage corrected mortality also increased. The rate of increase showed that 20 % ppm had the least percentage (95%) while 40 % ppm, 60% ppm, 80 % ppm and 100 % ppm had the highest (100%). However, no mortality was recorded in the control set-up during 48 hours of exposure

Table 2 Effects of Essential oil of *Chenopodium ambrosioides* against adult *Callosobruchus maculatus* after 48 hours

Conc.	Mortality in Replicate I	Mortality in Replicate II	Mortality in Replicate III	Total Mortality	Mean Mortality	% Corrected Mortality
Control	0	0	0	0	0	0
20 % ppm	18	20	19	57	0.95	95
40 % ppm	20	20	20	60	1.0	100
60 % ppm	20	20	20	60	1.0	100
80 % ppm	20	20	20	60	1.0	100
100 % ppm	20	20	20	60	1.00	100

The regression line equation and slope of exposure at 24 and 48 hours of adult *C. maculatus* against *C. ambrosioides* showed that at 24 hours 0.50 Mg/ m^2 was needed for 50 % mortality of the test insects. 0.90 Mg/ m^2 was needed for 99 % mortality of adult *C. maculatus*

Consequently, after 48 hours 0.20 Mg/ m^2 was needed to kill 50 % of the test insects while 0.18 Mg/ m^2 were needed for 99 % mortality of adult *C. maculatus* after 48 hours

The implication of this was that 0. 18 Mg/m^2 gave the highest concentration for highest mortality rate of adult *C. maculatus* that is almost all the insect were dead and cannot withstand volatility of the oil extract at that concentration (table 3).

Table 3 Regression line equation and slope of exposure at 24 and 48 hours of adult *C. maculatus* against *C. ambrosioides*

Exposure period	Equation	Lc_{50}	Lc_{99}	Slope
24 hours	Y=20.80x-2144	0.50	0.90	106.67
48 hours	Y=14.7x-31	0.20	0.18	33.3

Figure I below showed the graph of % mortality against concentration (Mg / m^2). This graph shows that mortality increases with the increase in concentration of oil extract. Almost all the insects die at a very low concentration of 0.18 Mg / m^2 at 48 hours to show the bio-efficiency of the essential oil.

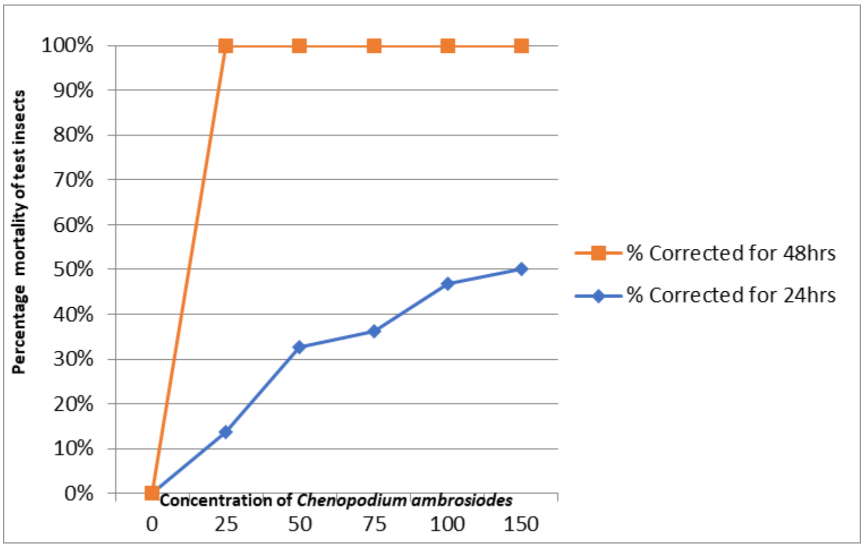

Figure 1 The effect of oil extract from *C. ambrosioides* against adult *C. maculatus* for 24 h and 48 h

Figure 2 below is a graphical presentation of mortality in replicate I - III within 24 hours. The graph shows that as concentration increases mortality of *C. maculatus* also increases.

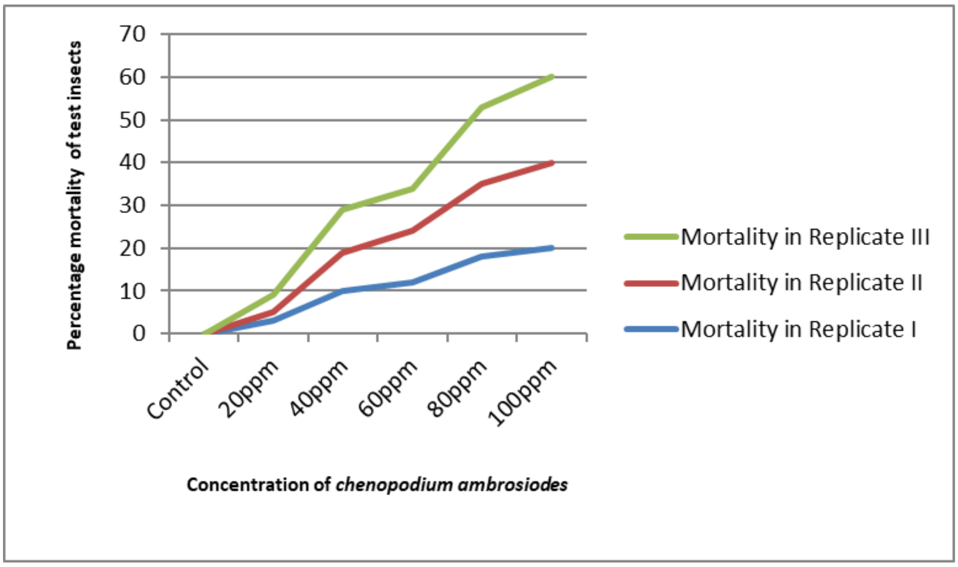

Figure 2 A graphical presentation of mortality in replicate I-III

Figure 3 present the percentage mortality for 24 hours. As concentration increases percentage mortality increases. Only small quantity of the essential oil is needed to kill 50 % of *C. maculatus.*

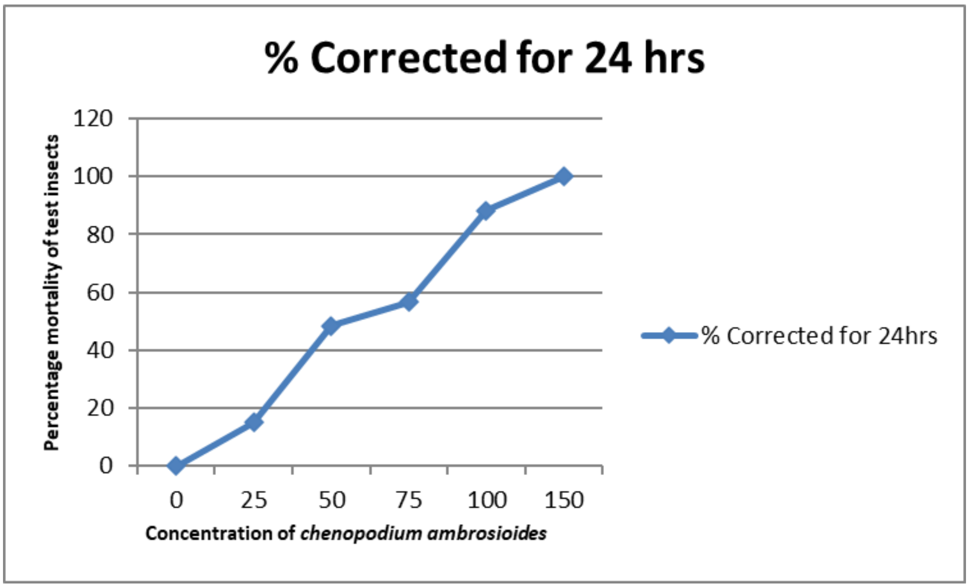

Figure 3 A graphical presentation of percentage mortality within 24 hours

Figure 4 showed the percentage mortality for 48 hours. As concentration increases percentage mortality increases. Only small quantity of the essential oil is needed to kill 50 % of *C. maculatus* i.e. 0.20 Mg / m^2 and very small quantity 0. 18 Mg / m^2 was needed to bring about 99 % mortality within 48 hrs.

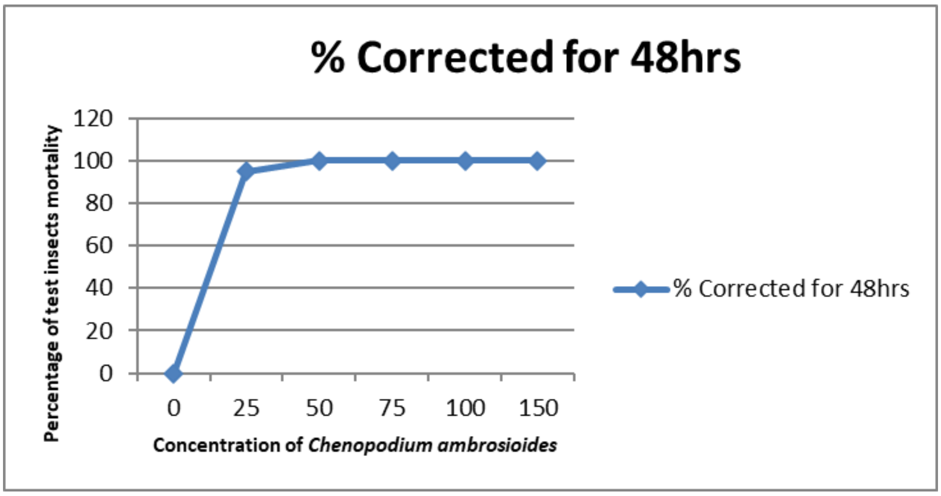

Figure 4 A graphical presentation of percentage mortality within 48 hours

4. Discussion

The results demonstrated the bioactivity of *Chenopodium ambrosioides* leaf oil extract against adult *Callosobruchus maculatus. C. ambrosiodes* showed some appreciable bioactivity properties required as fumigant against adult *C. maculatus*. These findings justify the use of the leaf by farmers in Malawi, Benin Republic to protect cowpea [37, 38] and people of Badagry- Nigeria who use it as a protection against mosquito bites by hanging it on the door post of their house[39]. -

The study revealed that oil extracts from *C. ambrosioides* have repellent effects on adult *C. maculatus*, resulting in mortality based on concentration and contact time interval. It was observed that test insects introduced into glass jar (air tight) containing the leaf extracts recorded 100 % mortality rate due to their exposure to high concentration of the extracts over a long time

This work complements earlier reports of [16, 39, 37] on toxicity of *C. ambrosioides* (Chenopodiaceae) against storage insects. This result indicates that *C. ambrosioides* oil extracts have potential for grain protection which can be harnessed as an alternative to synthetic insecticides.

5. Conclusion

The result indicates that *C. ambrosioides* can be used as fumigant against *C. maculatus*. It was clear from the study that *C. ambrosioides* leaf oil extract could be an alternative biological method for insect pest control.

The result confirmed the medicinal use of the leave *C. ambrosioides* by traditional community in Asia and Africa in controlling different diseases and to ward off mosquitoes by hanging the leaf at the entrance of their doors.

Therefore, the use of the plant products may help to develop new insecticides and serve as control in small concentration against insect pests especially storage pest of agricultural products such as *C. maculatus.*

Recommendation

Based on the findings of this study, it is recommended that *C. ambrosioides* plants can be used as potential plants in controlling and safe guarding economic crops such as cowpea plants

Conservation should be encouraged so as to prevent *C. ambrosioides* plant so that it would not be extinct but available for use.

Government should also encourage research and give adequate support in terms of finance, awareness and publicity to landmarks made by researchers so that they can be encouraged.

There is a need for further work to investigate its environmental effect and toxicity on non- target organism

Research on *C. ambrosioides* should also be expanded to investigate how the oil extracts of the plants can be added to extract from other botanic plant with some fragrance to form essential oils which can be used as fumigant and protectant insecticides on a commercial basis.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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