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Efficacy of Ethanolic Leaf Extract of *Chromolaena odurata* in Controlling *Sitophilus zeamais* in Stored Maize

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Sitophilus zeamais Motschulsky is among the important pests which attack stored maize. It is listed in addition to *Prostephanus truncatus* as the two most damaging species of maize in West Africa. In Ghana about 15% of maize grains harvested is lost to *S. zeamais*. A laboratory study was conducted at the Entomology laboratory of the Faculty of Agriculture of Kwame Nkrumah University of Science and Technology, Kumasi, to determine the efficacy of *Chromolaena odorata* (L) R. M. King and H. Robintson ethanolic leaf extract for *Sitophilus zeamais* control. The bioactivity of these extracts was assessed under average laboratory conditions of 26℃ and relative humidity of 80%. The leaf extract at four dosage levels (0.0, 2.5, 5.0 and 10.0 ml) were mixed with 50 g of disinfested MAMABA maize variety in 750 ml plastic containers and the effect on insect mortality, progeny production and grain damage were assessed. The repellent action of these extracts at 0.0, 5.0, 10.0, 20.0 ml on *Sitophilus zeamais* was also evaluated. The leaf extract showed significant difference between 10.0 and 5.0 ml on one hand and 2.5 and control on the other hand. The 10.0

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ml that recorded the highest mortality could inflict only as low as 8.75% after 7 days. The maize grain treated with the various dosage levels of the leaf extract showed much promise by significantly reducing the number of progeny produced by *S. zeamais* as compared with the control. Grain weight loss in leaf extract treated grains was dose dependent ranging from 3.51% in the highest dose to 11.34% in the control with significant differences. The leaf extracts was not repellent to the weevil.
The correlation between grain weight loss and progeny production was very strongly positively correlated in the leaf extract effect.

Keywords: Storage pest; Sitophilus; maize; leaf extracts.

1. INTRODUCTION

Maize (*Zea mays* L. sp. *mays*), known as corn in some countries, is a major staple crop in Ghana. It is cultivated in all the 10 regions of the country with the Eastern Region being the largest producer In terms of production, maize ranks third only after roots and tubers and plantain [1]. The Ministry of Food and Agriculture [2] reported that, in 2006 1.2 million Mt tons of maize was produced from 793,000 ha.

Maize has three possible uses: as food, as feed for livestock and as raw material for industry [3,4]. As food, the whole grain, either mature or immature, may be consumed in a multitude of ways which vary from region to region or from one ethnic group to the other. It may be processed to give a relatively large number of intermediary products, such as maize grits of different particle size, maize meal, maize flour and flaking grits.

Despite its importance much of the harvest is lost to insect pests during storage. Between 20 – 40 % losses have been attributed to insect pests in the tropics, including Ghana [5,6]. Insect infestation results in weight losses and quality deterioration which constitute a threat to food security especially in developing countries like Ghana [7].

Sitophilus zeamais Motschulsky is one of the most important insect pests that attack stored maize [8,9]. It has been reported that about 15% of maize grains harvested in Ghana is lost to *S. zeamais* [10].

Current control practice for the pest relies on synthetic chemicals [11]. These chemicals are associated with evolution of resistant strains, destruction of natural enemies and non target species, turning innocuous species into pests and contamination of food. The residue of the chemicals on the grain also poses health hazard to consumers [12].

Current research focus in stored products protection includes the development of non-chemical technologies which may eliminate the use of insecticides and have economic and health benefits for applicators, consumers and the environment [13,14,15]. The use of natural methods of protecting harvested crops from insect damage is not only gaining prominence [16] but is also generating positive results [14,17,18].

It is against this background that this study was conducted to find out the efficacy of *Chromolaena odorata* (L) R. M. King and H. Robintson for *S. zeamais* management.

The specific objectives are:

(i) To determine the efficacy of alcoholic leaf extracts of *Chromolaena odorata* in controlling *S. zeamais.*
(ii) To determine the bioactivity of the extract on reproduction of *S. zeamais*
(iii) To determine the repellency effect of the extracts on *S. zeamais* and
(iv) To determine the effects of the extract on the weight loss of stored maize.

2. MATERIALS AND METHODS

2.1 Location

The experiments were conducted at the Entomology laboratory of the Faculty of Agriculture of Kwame Nkrumah University of Science and Technology, Kumasi.

2.2 Experimental Design

The experimental design used was completely randomized design (CRD).

2.3 Maize Variety

Exactly twenty kilogrammes of shelled untreated Mamaba maize variety was obtained from a seed grower at Asuoyeboa, a suburb of Kumasi. Basal insect infestations in the maize were disinfested by deep freezing for two weeks [19]. The maize was then air dried under a screen to prevent possible re-infestation by insects. The moisture content of the maize samples was determined before each laboratory experiment.

2.4 Preparation of Botanical Materials

Fresh leaves of *Chromolaena odorata* were collected from the arable farm of the Faculty of Agriculture of Kwame Nkrumah University of Science and Technology, Kumasi. The leaves (chopped up into pieces to facilitate drying) were dried in a well ventilated area at room temperature for 2 weeks. The dried leaves were milled into powder using Christy and Norris Junior ® laboratory mill. Three hundred and fifty grams (350 g) of the leaf powder were soaked in 1,400 ml (1:4 W/V) of ethanol contained in 2,000 ml plastic containers for 96 hours. The leaf extracts were strained using a clean fine muslin cloth. The extracts were stored in plastic containers at room temperature in the insectary as stock solution and used for the study [20].

2.5 Insect Culture

Adult *S. zeamais* used for the study were raised from a stock maintained at the insect laboratory of the Department of Crop and Soil Sciences, Kwame Nkrumah University of Science and Technology, Kumasi. Two hundred unsexed adult *S. zeamais* from the stock were introduced into a plastic container, sealed with a clean fine muslin cloth, holding 1,000 g of the disinfested maize grain. The insects were allowed to oviposit for ten days before they were sieved out and the container sealed again with the cloth to prevent possible escape and/or reinfestation. The F_1 adults that emerged were introduced onto a sample of the test maize and the resulting F_2 emerged weevils were used for the various experiments. The culture was maintained under average temperature of 26℃ and relative humidity of 80%.

2.6 Mortality Test and Progeny Emergence Assessment

Fifty grams of maize grain were introduced into 750 ml plastic containers. Varying volumes or dosages of *C. odorata* leaf extracts (0.0, 2.5, 5, and 10.0 ml) were introduced onto the 50 g maize in the plastic containers and vigorously shaken every 30 minutes for 2 hrs to ensure uniform distribution of the extract over the grain surface. The treated maize was allowed to stand for two hours for the alcohol solvent to evaporate before the introduction of the weevils. Ten pairs of sexed 5-10 days old adults staved for 24 hrs were then introduced into each plastic container. The plastic containers were covered with muslin cloth sandwiched between two wire mesh. Each treatment was replicated four times. The experiment was arranged in a completely randomized design in the laboratory.

The number of dead insects in each plastic container was counted after 1, 2, 3, 4, 5, 6, and 7 days to estimate maize weevil mortality. Insects were certified dead when there was no response to prodding of the abdomen with a sharp pin.

Maize weevil mortality was assessed as:

(Number of dead insects/Total number of insects) x 100

To account for death by natural conditions other than the effect of the plant extracts data on percentage adult weevil mortality was corrected using Abbott's (1925) formula thus:

$$PT = (PO-PC) / (100-PC)$$

Where,

PT = Corrected mortality (%),
PO = Observed mortality (%)
PC = Control mortality (%)

After mortality count on the 7th day, all insects were sieved out of the plastic containers and their contents kept at an undisturbed area in the laboratory for 6 weeks for progeny development from any eggs laid. Emergence count of F_1 generation commenced on the 21st day after infestation and was terminated after 34 days of counting to prevent overlapping of generations.

2.7 Repellency Test

A modified [21] technique for assessing repellents and attractants in stored products as adopted by [22], was used. Plastic bottles measuring 18 cm x 4 cm (height and base diameter respectively) with 2 mm holes created at intervals of 1.5 cm x 1 cm (horizontally and vertically respectively) all round the surface were

used. Two hundred grams of shelled maize treated with varying volumes of *C. odorata* leaf extracts (0.0, 5, 10.0 and 20.0 ml) were put into the plastic bottles. This was placed in a plastic cup measuring 5 cm x 6 cm (height and base diameter respectively) with holes at the base. The setup was placed in a petri dish filled with water.

Ten pairs of unsexed 2 to 3 weeks old adults starved for 24 hours were introduced into the treated maize samples through a long stem funnel. A cage made up of a wooden frame of 21 cm x 18.5 cm x 18.5 cm wide with the sides covered by plastic mesh was inverted over the setup to prevent the escape of insects repelled out of the treated maize in the bottles and also to prevent insects not included in the experiment coming into contact with the set up. Each setup had a control in which untreated maize was used. The *S. zeamais* that moved out of the plastic bottles into the water contained in the Petri dish or on the outside surface of the bottle or on the inside surface of the cage inverted over the setup were deemed repelled away by the extracts and therefore counted. Counting was done at 1, 2, 12 and 24 hours after infestation. Abbot's correction formular was used to eliminate random departures by the insect.

2.8 Damage Assessment

Damage assessment was carried out on treated and untreated grains after two months of storage. Samples of 100 grains were taken from each jar and the number of damaged grains (grains with characteristic holes) and undamaged grains counted and weighed. Percentage weight loss was calculated, using [23] method as follows:

$$\% \text{ Weight loss} = [(UaN)-(U+D)] / UaN \times 100$$

Where,

U = Weight of undamaged fraction in the sample
N = Total number of grains in the sample
Ua = Average weight of undamaged grain
D = Weight of damaged fraction in the sample.

2.9 Data Analysis

All percentage with more than 40% range were arcsine transformed (Sine $^{-1}$ ((x+0.5/100). While count data were square root transformed √(x+0.5) [24]. GenStat Release 7.2 Discovery Edition (2007) Computer package was used to analyse variances and least significant differences (LSD) were used to separate means that showed significant differences at Probability level of 5% ($P \leq 0.05$).

3. RESULTS AND DISCUSSION

3.1 Effect of Ethanolic Leaf Extract of *C. odorata* on *S. zeamais* Mortality after 4 Days

Fig. 1 shows the percent mortality of *S. zeamias* after 4 days in maize grains treated with different dosage levels of ethanolic leaf extract of *C. odorata*.

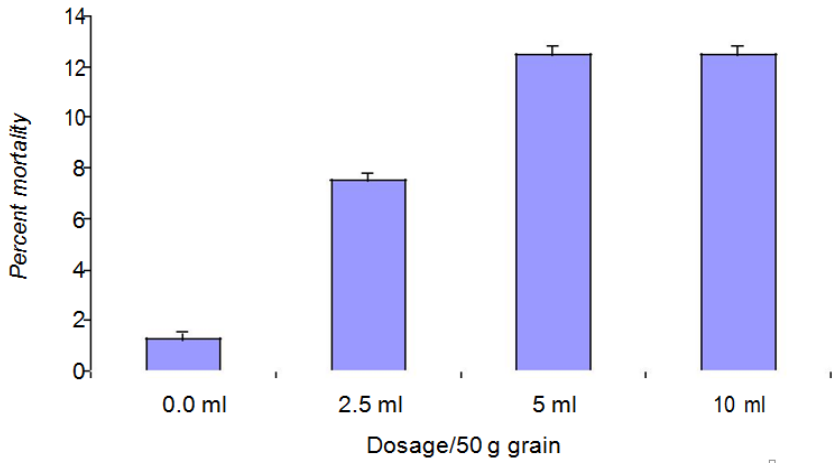

Fig. 1. Mean percent mortality of *S. zeamais* after 4 days in maize grain treated with different dosage levels of *C. odorata* ethanolic leaf extract

There were no significant differences in toxicity between the 5 ml and the 10 ml dosage levels of *C. odorata* ethanolic leaf extract to the insects after 4 days exposure to the treated grain. However, the differences between the 5 ml and 10 ml on one hand and the 2.5 ml and the control on the other hand as shown in Fig. 1 were Significant. The differences between the 2.5 ml and the control were also significant.

3.2 Effect of Ethanolic Leaf Extract of *C. odorata* on *S. zeamais* Mortality after 7 Days

Fig. 2 shows the mean percent mortality of *S. zeamias* after 7 days in maize grains treated with different volumes of ethanolic leaf extract of *C. odorata.*

Similar to the results obtained from 4 days of exposure there were significant ($P < 0.05$) differences in weevil mortality between the different dosage levels of extract after one week exposure The *C odorata* leaf extract at 10 ml and 5 ml dosage levels exhibited significantly greater mortality than the 2.5 ml and the control (untreated grain). Also, the grain treated with 2.5 ml dosage level inflicted significant level of *S. zeamais* mortality ($P < 0.05$) than the control.

3.3 Effect of Ethanolic Extracts of *C. odorata* on *S. zeamais* Mortality

The results showed that the ethanolic leaf extracts that showed some killing power, inflicted as low as 8.75% mortality on *S. zeamais* (Fig. 1). However the extracts cannot be discounted completely as it exerted some degree of mortality on the *S. zeamais.* This perhaps indicated that, a much higher dose may exhibit greater potency. On the other hand, other appropriate alternative solvents (method of extraction) may enhance the efficacy of the leaf extracts as a protectant for maize against the maize weevil [25,26].

Plant terpenoids and phenolic acids have been reported to be toxic to insects. For example, *Lantana camara* which has been reported to possess, in major quantities, terpenoids such as caryophyellene and germacrene D exhibited insecticidal activity against *Dactynotus carthamii* [27]. *Chromolaena odorata* has also been reported to possess terpenoids including caryophyellene and germacrene [28] and phelonic acids such as coumarine D in major quantities [29].

According to [30,31] leaf powder *of C. odorata* caused 69% and 64% mortality of *S. zeamais* respectively. In this study, mortality of *S. zeamais* ranged from 3.75% to 8.75% for all the different treatments indicating that the populations of *S. zeamais* were not appreciably controlled at the doses of ethanolic extracts of *C. odorata* tested.

Several reasons could be adduced for the ineffectiveness of ethanolic extracts *C. odorata* to control *S. zeamais* in this study. It has been established that different climatic, soil, and seasonal conditions can affect the type and quantity of the components isolated from extracts. It is probable also that the age of the plant, type of solvent used in extraction and quantity of extracts used among others could have affected the efficacy of the extracts used in this study. [30,31] used *C. odorata* powder which might have contributed to the higher mortality recorded in their studies since the abrasive nature of such materials may be damaging to the insect cuticle leading to dehydration and death (mechanical control), in addition to the insecticidal effect.

3.4 Effect of Ethanolic Leaf Extract of *C. odorata* on *S. zeamais* Progeny Development

The number of progeny produced by *S. zeamais* in untreated grains and grains treated with different dosage levels of ethanolic leaf extract of *C. odorata* are shown in Fig. 3. The first batch of emergence was recorded from 5 ml and 10 ml treated grain lots at 26 days after infestation (DAI) but emergence occurred in all treatments on the 27^{th} DAI. Peak emergence occurred in the 5^{th} week (as depicted by the steepness of the graphs slopes) with the control recording the highest emergence. Emergence then reduced greatly until the 8th week when it stopped.

Significantly greater number of progeny was produced by *S. zeamais* in the untreated grains compared with the grains treated with the highest dosage levels of ethanolic leaf extract. The two highest dosage levels of the ethanolic leaf extract of *C. odorata* significantly reduced the number of progeny produced by the weevil compared to the 2.5 ml and the control.

3.5 Effect of Ethanolic Extracts of *C. odorata* on *S. zeamais* Progeny Development

The results (Fig. 3) obtained from this study demonstrated that the leaf extracts of *C. odorata*

can suppress progeny production (egg production) of *S. zeamais*. Suppression of ovipositional activity could be attributed to the presence of caryophyllene and germacrene D [32]. [32] reported that *Lantana camara* which contains caryophyllene and germacrene D in large quantities exhibited some ovipositional suppression on *Callosobruchus maculates.*

3.6 Effect of Ethanolic Leaf Extract of *C. odorata* Treatment on Grain Weight Loss

Weight loss caused by *S. zeamais* to treated and untreated grains is shown in Fig. 4. Weight loss was dose dependent with significant differences. The weight loss of 11.34% in the control was significantly greater than losses of 5.46% and 3.51% in the 5 ml and 10 ml dosage levels respectively. However treatment at 2.5 ml/ 50 g grain also suffered significant weight loss compared with the grains treated with 10 ml of leaf extract.

3.7 Effect of Ethanolic Extracts of *C. odorata* on Weight Loss of Grains

This study (Fig. 4) indicated that, the leaf extracts significantly protected the stored maize against *S. zeamais* up to two months of storage. The efficacy of the leaf extracts against weevil damage agrees with the findings of [31] that *C. odorata* leaf powder was as effective as Actellic powder in protecting stored maize against *S. zeamais.* Weight loss in stored maize grain was related to the number of insects present [33].

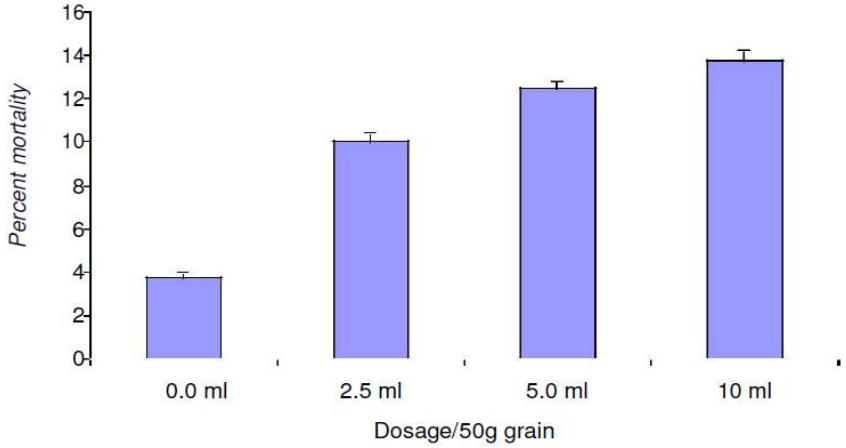

Fig. 2. Percent mean mortality of *S. zeamais* after 7 days in maize grain treated with different dosage levels of ethanolic *C. odorata* leaf extract

The error bars represent standard error of means

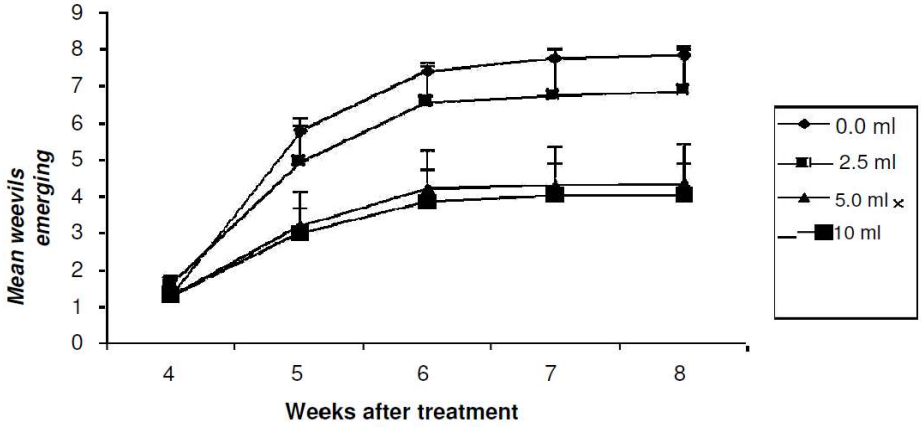

Fig. 3. Mean number of *S. zeamais* that emerged in maize grain treated with different dosage levels of ethanolic *C. odorata* leaf extract for 8 weeks of storage

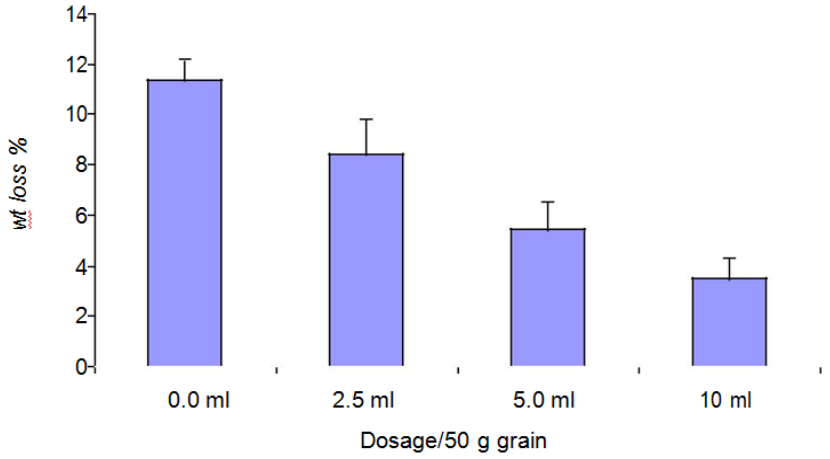

Fig. 4. Percent weight loss caused by *S. zeamais* damage of maize grains treated with different dosage levels of ethanolic leaf extract of *C. odorata* after 8 weeks of storage

Although, the mode of action of these plant materials are not yet fully known, these extracts negatively affected oviposition rate, fertility of eggs or larval growth and development of hatched eggs or a combination of two or all of these factors [34] resulting in fewer number of insects in the treated grains.

3.8 Correlation between Grain Weight Loss and Progeny Production in Stored Maize Treated with Ethanolic *C. odorata* Leaf Extract

Fig. 5 shows the correlation between grain weight loss and progeny production in stored maize treated with *C. odorata* leaf extract.

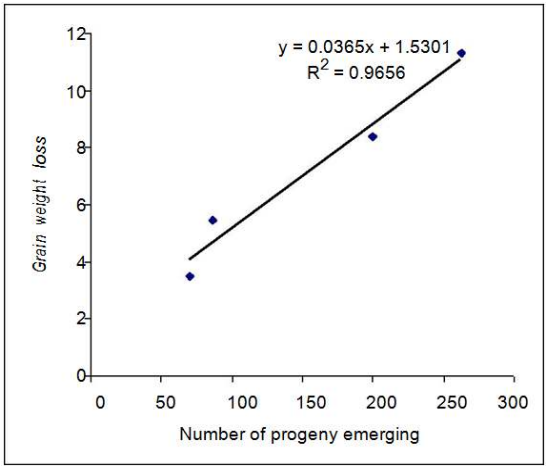

Fig. 5. Correlation between grain weight loss and progeny produced in stored maize treated with *C. odorata* leaf extract

The results of this study showed a very strong positive correlation between grain weight loss and weevil progeny produced. Ninety-six percent (96%) of the variation in grain weight loss was related to the variation in weevil progeny produced.

3.9 Repellence Effect of Ethanolic Leaf Extract of *C. odorata* on *S. zeamais*

Fig. 6 represents cumulative mean repellence of *S. zeamais* on maize grain treated with different dosage levels of *C. odorata* ethanolic leaf extract for 24 hours period. Analysis of variance indicated there were no significant differences between the responses to the four dosage levels tested.

3.10 Repellence Effect of Ethanolic Leaf Extracts of *C. odorata* on *S. zeamais*

The ethanolic leaf extracts of *C. odorata* did not significantly repel the weevil relative to the control (Fig. 6). Caryophyllene and germacrene-D are major constituents in *Lantana camara* and the essential oils have also been reported to repel bees, mosquitoes and cattle flies [35]. The results of this study seek to suggest that *C. odorata* extracts do not repel *S. zeamais*. Pharmacophagous sequestration of pyrrolizidine alkaloids (PAs) in *C. odorata* by *Zonocerus variegatus* was suggested by [36]. Furthermore chemoecological studies by [37] established that pyrrolizidine alkaloids such as rinderine and intermedine (PAs) served as attractants to the African grasshopper, *Z. variegatus*. This study however could not conclude whether the

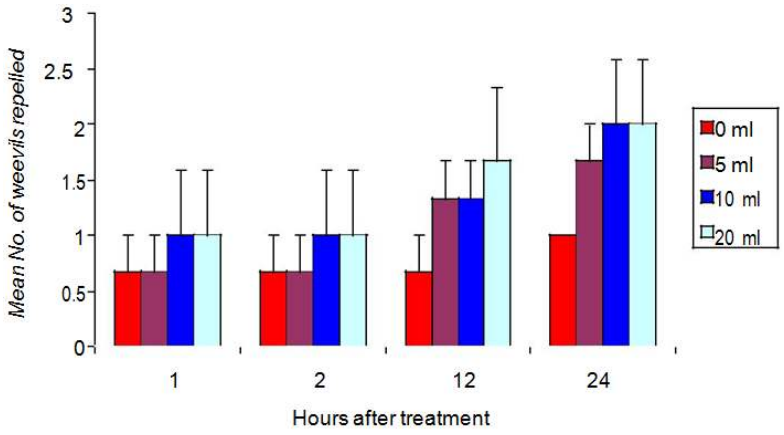

Fig. 6. Mean cumulative repellence of *S. zeamais* in maize grain treated with different dosage levels of ethanolic *C. odorata* leaf extract for 24 hours period

non-repellence of S. *zeamais* by the *C. odorata* extracts was the result of the attractant activity of the PAs as in the case of *Z. variegatus* or otherwise.

4. CONCLUSIONS AND RECOMMENDATIONS

4.1 Conclusions

This study has shown that:

1. The ethanolic leaf extract effected a better control of the weevil; however the percentage of weevil survival (> 91%). in the highest dosage i.e. 10 ml was still very high and capable of inflicting economic damage.
2. Leaf extracts did not repel the weevil significantly.
3. The leaf extracts reduced emergence of the weevil and also reduced grain weight loss.
4. The leaf extracts might be of practical use to farmers if it is used to reduce weevil multiplication (reproduction) thus reducing number of insects feeding and hence weight loss of grain.

4.2 Recommendations

Dried leaves of *C. odorata* were used for the study. This might have resulted in the loss of volatile oils. It will therefore be necessary to test freshly harvested *C. odorata* materials. Further studies should also be conducted with higher dosages of the extract.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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