

Full length Research Paper

The bio-efficacy of *Calpurnia aurea* against *Sitophilus zeamais* (Motschulsky) (Coleoptera; Curculionidae) of stored maize under laboratory condition in Ethiopia

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N-hexane (non-polar), chloroform (partial polar), distilled water, methanol, acetone and ethanol (polar) extracts (10 g/100, 20 g/100 and 20 g/100 ml extraction levels) and leaf powder of *Calpurnia aurea* were tested as protectant against maize weevil, *Sitophilus zeamais* on filter paper and in maize grains under laboratory condition. The extracts and powder were applied at rate of 2 and 3 ml on filter paper and at a rate of 5, 10 and 15% (w/w) on admixture bioassay. Parental adult's mortality, F1 progeny emergence, percent protection, percent grain damage and weight loss were measured as efficacy determining parameters. Polar solvent extracts of the *C. aurea* applied at all rates on filter paper from all levels of extraction induced significant ($p < 0.05$) toxicity effect compared to partial and non-polar solvent extracts, as well as the negative controls. Besides, hundred percent adult weevil's mortality was induced by all polar solvent extracts of *C. aurea* applied at rate of 3 ml per filter paper from 30 g/100 ml level of extraction 24 h after treatment application. Furthermore, the powder of *C. aurea* applied at all rates was also induced significantly good degree of protection of maize grains against F1 progeny emergence ($\geq 78\%$), percent grain damage (≤ 4.00) and weight loss (≤ 0.50) by maize weevils in about 2 month's storage period. Therefore, leaf powder and the solvent extracts of *C. aurea* were potent and could be used for managing maize weevils on stored maize under farmer's storage conditions in Ethiopia.

Key words: botanicals, *Calpurnia aurea*, *Sitophilus zeamais*, solvent extracts, leaf powder.

INTRODUCTION

Maize is the major staple food in Africa contributing significantly to the agricultural sector (Tefera et al., 2011). However, during storage, it is heavily attacked by various insect pests, of which the maize weevil, *Sitophilus zeamais* is most economically important. Accordingly,

these insect pests have been reported to be responsible for loss ranging from 30 to 90% in Ethiopia (Getu, 1993) and losses ranging from 40 to 100% in Malawi (Denning et al., 2009).

Thus, they have been recognized as an increasingly

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serious problem in Africa (Markham et al., 1994; Abebe et al., 2009; Tefera et al., 2011), indicating great need for safe grains storage and control interventions. However, control of these insect pests has been heavily relied on the use of synthetic insecticides. This has led to development of resistance strains, environmental and health concerns (Ofuya and Longe, 2009). As a result, the search for the development of safe, affordable and eco-sound alternatives, such as botanicals, has been inspired.

Calpurnia aurea (Ait.) Benth plant may have protective role of stored maize against weevils. It is a small, multi-stemmed tree, 3 to 4 m tall plant. It is widely distributed in Ethiopia. It is widely grown in high land areas (Birhanu and Asale, 2015) and is easily cultivated (Germishuizen and Meyer, 2003). The plant has been commonly used in traditional medicine to treat different medical disorders and parasitic infections, in animals and in humans in Africa including Ethiopia (Watt and Breyer-Brandwyk, 1962). Its leaves and powdered roots are used to destroy lice and to relieve itches and they contain terpenoids, saponins, tannins, flavonoids, steroids, glycosides and alkaloids (Nega et al., 2016).

The objective of the present study was to evaluate the toxicity potency of leaf powder and solvent extracts of the plant against the most economically important storage insect pest of maize and maize weevil (*S. zeamais*) under laboratory conditions.

MATERIALS AND METHODS

The study period

The study was conducted in between 1, October to 30, June of 2016/2017 in the insect science laboratory of zoological science department of Addis Ababa University of Ethiopia.

The test insect's culture

S. zeamais adults were collected from maize stored in various farmers' traditional storage facilities of major maize producing localities Shashogo and Sankura Districts of Southern Ethiopia, and brought to the laboratory insect science stream of zoological science department of Addis Ababa University of Ethiopia. These test insects were cultured at $27\pm 3^{\circ}\text{C}$ and 55 to 70% RH (Jembere et al., 1995; Zewde and Jembere, 2010).

Shone variety of maize grains were obtained from farmer's storages of the survey site. It was the most commonly grown hybrid in the region and considered to be susceptible to insect infestation (from survey finding). The grains were kept at $-20\pm 2^{\circ}\text{C}$ for 2 weeks to kill any infesting insects, cleared of broken kernels and debris and then graded manually according to size, and similar sized grains were selected for the experiment (Gemechu et al., 2013).

Following the methods by Zewde and Jembere (2010), fifteen pairs of the adult of the test insects were placed in 12.1 L glass jars containing 250 g seeds. The jars were then covered with nylon mesh and held in a place with rubber bands to allow ventilation and to prevent the escape of the experimental insects. The parent of the test insects were sieved out after an oviposition time of 14 days. Then, the jars were kept under the aforementioned laboratory

condition until F1 progeny emerged. The F1 progeny, which emerged after 30 days, were sieved out and used for the experiment.

Solvent extract of plant materials

Ground plant material (powder) from the leaves of the test plant was soaked in n-hexane (non-polar), chloroform (partial polar), distilled water, methanol, acetone and ethanol (polar) at the rate of 10 g /100, 20 g /100 and 30 g /100 ml of each solvent (Jembere, 2002). The solution was allowed to stand for 24 h for extraction. After 24 h, the mixtures were filtered with cheese cloth. Then, the filtrates were ready to be used for the different treatments following similar procedures by Zewde and Jembere (2010).

Filter paper bioassay

Following similar procedures of Zewde and Jembere (2010), different levels of each solvent extracts were applied to a filter paper of 9 mm diameter at the rate of 2 and 3 ml per filter paper, and placed in a Petri dish of 10 cm diameter. Variable exposure times were considered, which were based on the nature of the solvent. In case of acetone and methanol, the exposure time was 30 min, while it was 60 min for ethanol (Jembere, 2002). Then, 1 ml of distilled water was added to the entire surface of each treated filter papers, as a carrier of the extracts. Other filter papers were treated with two levels of different solvents as control. After treatment, 5 pairs of 3 to 7 days old unsexed experimental insects were introduced into the treated and control filter papers in the petri dishes. Mortality of the adult insects was counted after 24, 48, 72 and 96 h. When no leg or antennal movements were observed, insects were considered as dead as suggested by Gebre selase and Getu (2009). All treatments of filter paper were arranged in Completely Randomized Design (CRD) in three replications.

Admixture bioassay with botanicals powder

Following the methods by Gebre selase and Getu (2009), 100 g of disinfected maize grains of shone varieties (that were disinfested using the same procedure as indicated in insect culture section) were introduced into 1 L glass jars that were treated differently with the powdered peels of the test plant (that is, 5, 10 and 15 g of the powder) for treatment of powder. Malathion (5%) dust was used as the positive control at a dosage of 0.05 g / 100 g maize grain and untreated grains were served as the negative control. The jar contents were shaken thoroughly for 5 mins to ensure uniform distribution of the treatments with grain surface. After treatment, 20, three to seven-day old experimental insects of unsexed were introduced to the treated and untreated seeds in the glass jars. Then, the jars were covered with nylon mesh and held in place with rubber bands. The treated grains and controls were kept under same experimental condition indicated in insect culture section. All treatments of powder were arranged in CRD in three replications. Mortality observation in the dried powder experiment (the number of dead insects in each jar were sieved and counted) was conducted at 1, 2, 3, 4, 7 and 14 days after treatment application. All live and dead insects were sieved and discarded after 13 days of introduction.

F1 progeny assessment bioassay

The treated grains and controls were also kept until emergence of F1 progeny under same experimental condition indicated in insect capture section after mortality observation. Then the numbers of F1

progeny produced by the experimental insects were counted. Counting was stopped after 56 days from the day of introduction to avoid overlapping of generation (Zewde and Jembere, 2010).

Damage and weight loss assessment

Two days after the last F1 count of 56 days, samples of 100 g were taken randomly from each jar and the number of damaged (grains with characteristic hole) and undamaged grains were counted and weighed. Grain damages were conveyed as a percentage of the entire number of grains in each of the aforementioned three replicates. Percentage weight losses were calculated by count and weight method following the methods by FAO (1985), Boxall (1986), Haile et al. (2003) and Haile (2006) as follows:

$$\% \text{ Loss in weight} = \frac{\text{UNd} - \text{DNu}}{\text{U} (\text{Nd} + \text{Nu})} \times 100$$

Where:

U = weight of undamaged grain, D = weight of damaged grain, Nd = number of damaged grain and Nu = number of undamaged grain.

As adopted by Gebre selase and Getu (2009), percent protection or inhibition in F1 progeny emergence (% IR) was also calculated using the following formula:

% IR = $(\text{Cn} - \text{Tn}) \times 100 / \text{Cn}$, where Cn is the number of newly emerged insects in the untreated (control) jar and Tn is the number of insects in the treated jar.

Data analysis

The data's collected for this study were managed by the Microsoft Excel package 2013 and analyzed using the Statistical Program for Social Sciences (SPSS) version 16. To observe the effects of botanicals treatments against weevil's adults' mortality and F1 progeny emergence, as well as grain damage and weight loss of maize grains at a particular time, appropriate statistical methods, Univariate analysis (for the former one) and one-way analysis of variance (ANOVA) (for the rest of parameters measured) were used. Standard errors (\pm se) were given following means in tables and in the form of error bars in figures. Correlation between the treatments and the efficacy measuring parameters were determined using Pearson's correlation of SPSS program of version 16.

RESULTS

Filter paper bioassay

Percentage adult weevil's mortality was increased ($p < 0.05$) significantly with increased dosage (concentration), extraction level, polarity and exposure time after treatment for the tested botanical in general. Polar solvent extracts (distilled water, methanol, ethanol and acetone extracts) of the *Calpurnia aurea* applied at all rates (2 and 3 ml) from all of the three levels of extraction (10 g/100, 20 g/100 and 20 g/100 ml) induced significant ($p < 0.05$) toxicity effect compared to non-polar and partial solvent extracts, as well as the negative controls

(solvents alone) (Figure 1). Significantly, ($p < 0.05$) higher mean percentage mortality of *S. zeamais* (≥ 60 and $\geq 80\%$) was occurred in all polar solvent extracts (distilled water, methanol, ethanol and acetone extracts) of the tested plant applied at a rate of 2 and 3 ml per filter paper from the higher level of extraction (20 and 30 g/100 ml), respectively 24 h after treatment. Besides, hundred percent adult weevil's mortality was induced by all polar solvent extracts of *C. aurea* applied at rate of 3 ml per filter paper from 30 g/100 ml level of extraction 24 h after treatment application (Figure 1).

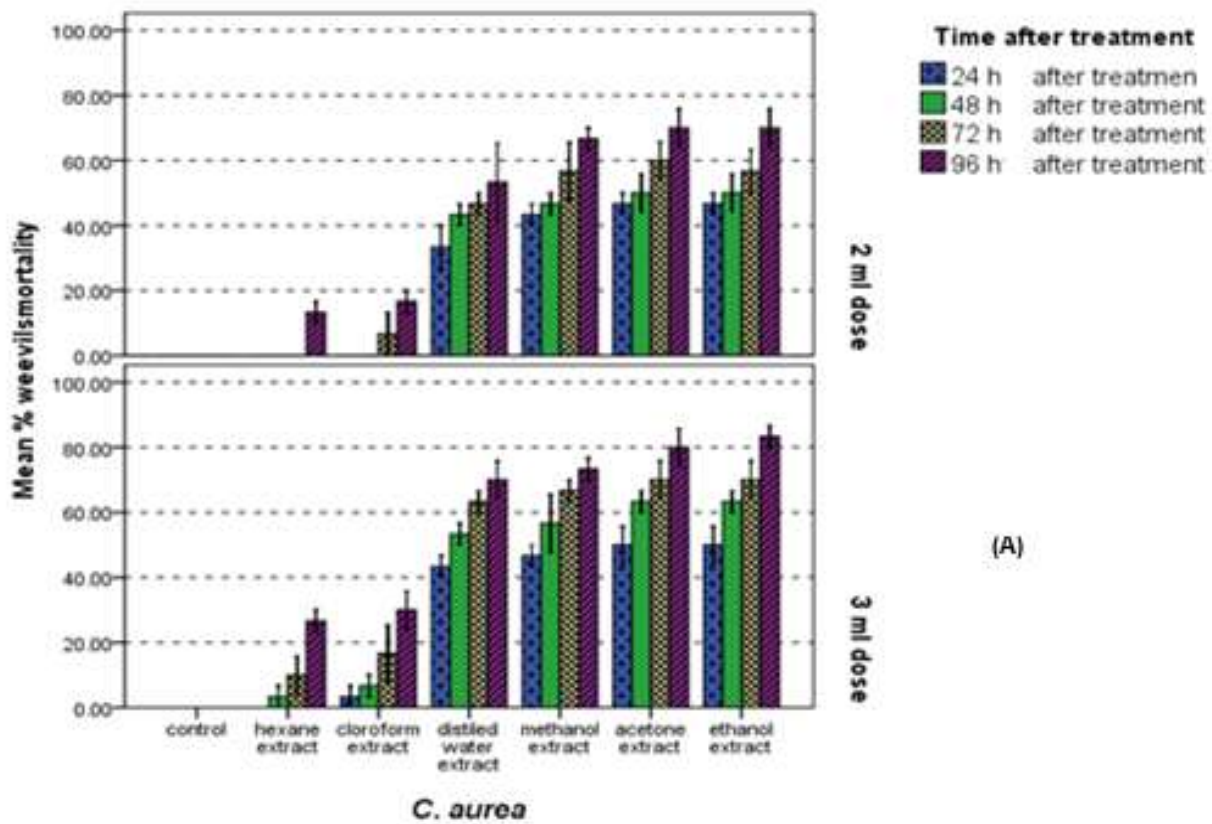
Admixture bioassay

The percentage adult weevil's mortality was increased significantly ($p < 0.05$) with increased dosage (concentration) and exposure time after treatment for the tested botanical in general in all bioassays tested. The tested botanical had significant ($p < 0.05$) effect on percent mortality of adult weevils in comparison to the untreated grain (negative control). However, significant percentages weevil's mortality were not induced at 5 and 10% rates of the tested botanical prior to four days of post treatment exposure (Figure 2).

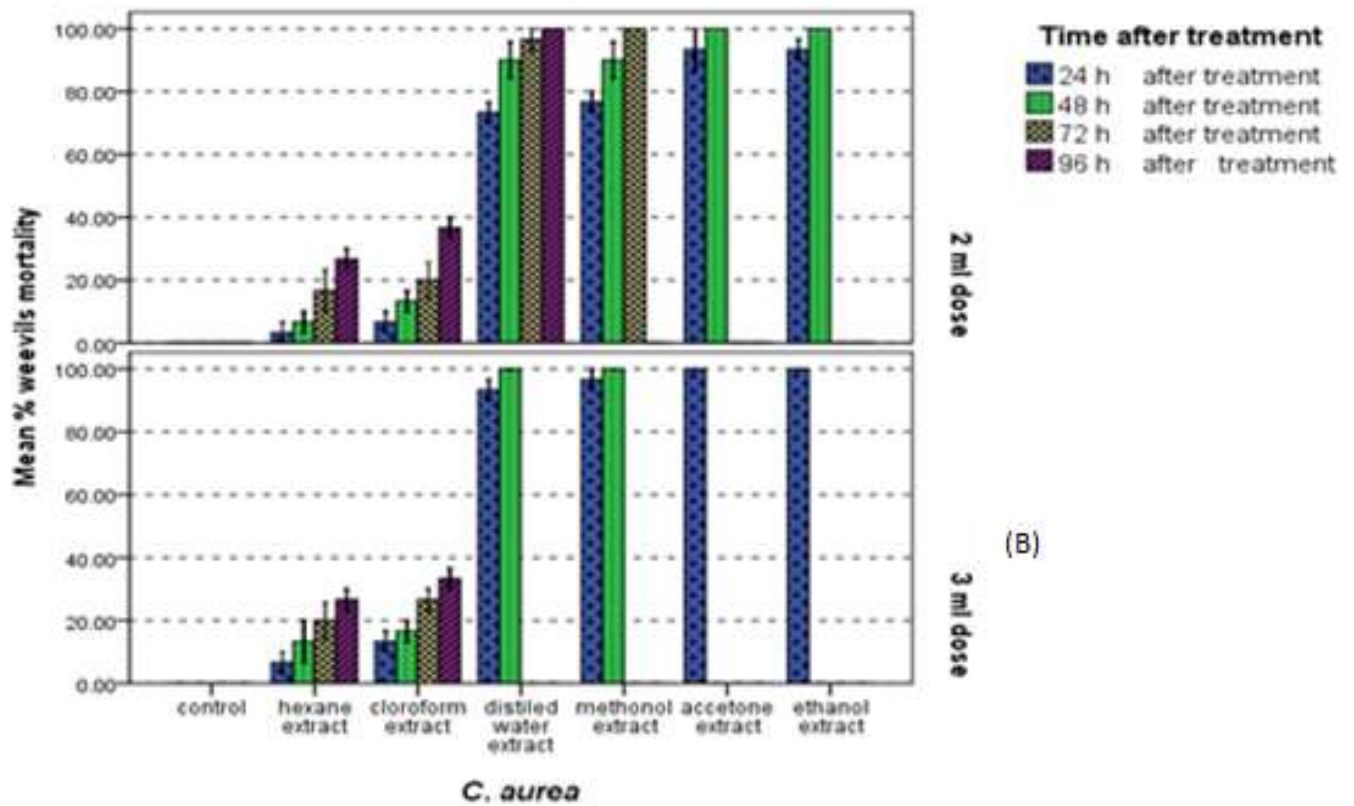
Significantly, ($p < 0.05$) high weevil's mortality ($\geq 50\%$) was caused by all treatments of the tested botanical applied at all dosages, following four days treatments post exposure, as compared to 1 to 3 days after treatment and untreated grains. Besides, the weevil's mortalities were significantly ($p < 0.05$) higher ($\geq 83\%$) in all treatments of the tested botanical applied at 5% dose and it became 100% in all treatments of the tested botanical applied at rates of 10 and 15%, 7 days after treatment application. Furthermore, the magnitude of mortality became 100% in all treatments of applied at all rates of the test botanical, following 14 days of treatment application similar to that of the positive control or Malathion 5% dust applied at recommended dose (Figure 2).

The number of F1 adult progeny produced, percentage grain damage and weight loss caused by *S. zeamais* in all treatments of botanical leaf powder were significantly ($p < 0.05$) lower compared to negative control (untreated cheek). Almost all leaf powders treatments of the tested botanical applied at higher dosage (10 and 15%) induced more than 78% protection in F1 progeny production by *S. zeamais* respectively as compared to negative control. Similarly, almost all leaf treatments of the tested botanical caused significantly ($p < 0.05$) higher reduction in grain damage (≤ 4.00) and weight loss (≤ 0.50) of maize grain as compared to the negative control.

Besides, 100% F1 progeny production inhibition, as well as no grain damage and weight loss of maize were observed in powder treatments of the tested botanical applied at a rate of 15% likewise that of the positive control (Malathion 5% dust). However, significantly higher ($p < 0.05$) F1 emergence of *S. zeamais*, grain damage



(A)



(B)

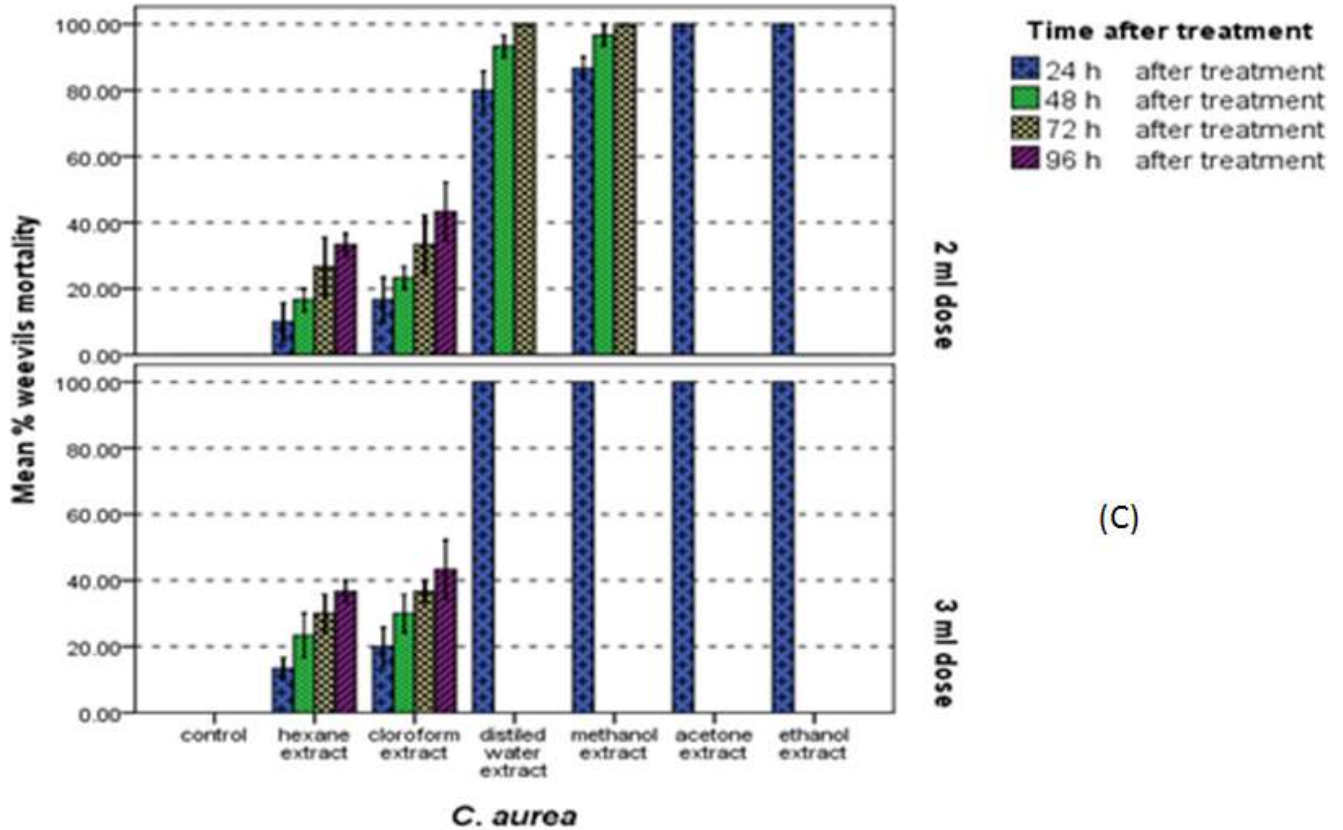


Figure 1. Mean % mortality (mean \pm SE) of *S. zeamias* due to solvent extracts of *C. aurea* extracted at the rate of: 10 g / 100, 20 g / 100 and 30 g / 100 ml and applied at the dose of 2 and 3 ml after different post treatment exposure. Source: The different colors and markings at different days after treatment indicate means that are significantly different at $p > 0.05$.

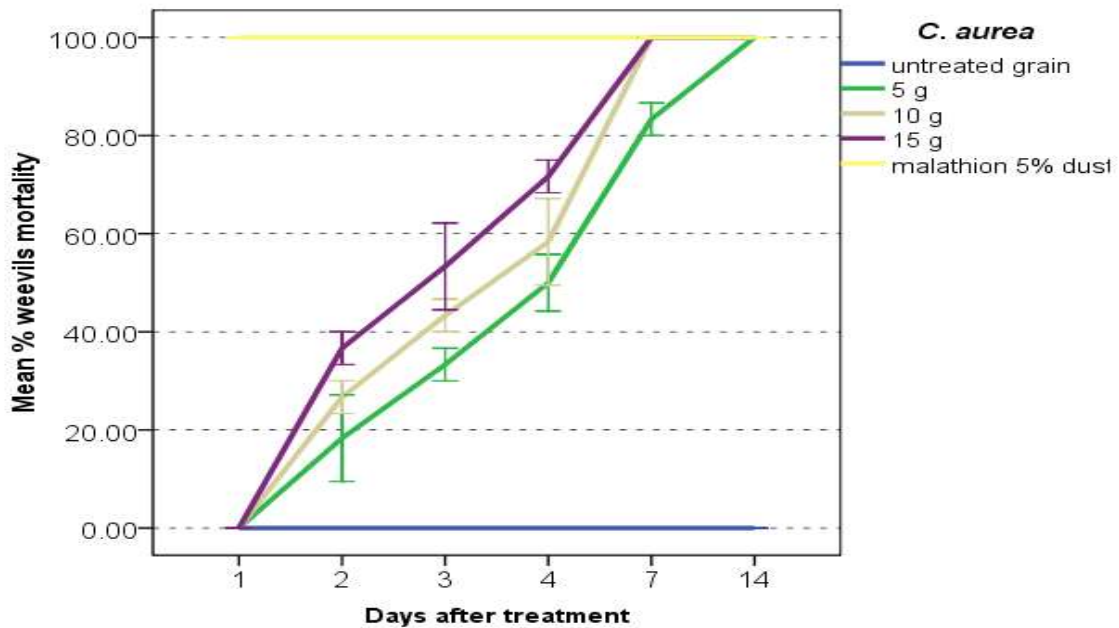


Figure 2. Mean % mortality (mean \pm SE) of maize weevil adult exposed in grains treated with *C. aurea*.

Table 1. Mean number of f1 progeny produced, percent protection, grain damage and weight loss caused by *S. zeamais* on maize grains treated with *Calpurnia aurea* leaf powder.

Treatments	Dosage (g/100 g)	Mean number of F1 progeny	Percentage protection	Mean % grain damage	Mean % weight loss
Dosage	5	6.67±4.41 ^c	78.94	4.00±0.58 ^b	0.50±0.01 ^b
-	10	3.33±1.67 ^b	89.49	1.00±1.00 ^{ab}	0.22±0.01 ^{ab}
-	15	0.00±0.00 ^a	100	0.00±0.00 ^a	0.00±0.00 ^a
Malathion 5%	0.05	0.00±0.00 ^a	100	0.00±0.00 ^a	0.00±0.00 ^a
Control (untreated)	0	31.67±1.67 ^d	0.00	11.67 ^c	5.30±0.17 ^c

Means followed by the same letter in a row are not statistically significantly different at $p < 0.05$.

Table 2. Correlation among efficacy determining parameters of CA leaf powder.

Efficacy determining parameters	Rate	F1 CA	% GDCA	% WLCA
Rate	1	-	-	-
F1CA	-0.943**	1	-	-
% GD CA	-0.920**	0.972**	1	-
% WL CA	-0.866**	0.920**	0.964**	1

Correlation coefficients with two asterisks (**) represent highly significant association at p values < 0.01 (2-tailed), with hyphen (-) represent no association and those without asterisk are non-significant. CA = *Calpurnia aurea*, GD = grain damage and WL= weight loss

and weight loss of maize grains occurred in powder treatments applied at lower dose (5%) when compared to those subjected to higher dosages (10 and 15%) (Table 1).

The correlations among the treatments of powder of the tested plant leaves applied at different dosage and the efficacy parameters measured were found to be highly significant. The correlations between the various treatments of powder of the tested plant leaves applied at different rates and the various parameters measured (the number of F1 progeny emerged, percentage grain damage and weight loss) were negative. However, the correlations between F1 progeny produced, and percent grain damage and weight loss of all treatments of the tested botanical powder were strongly positive. Besides, the correlations were strongly positive between the percentage grain damage and weight loss of all of the treatments of the tested botanical powder applied at different rates (Table 2).

DISCUSSION

The current study revealed that the percentage adult weevil's mortality was increased ($p \leq 0.001$) significantly with increased dosage (concentration) and exposure time after treatment for the tested botanical in general in bioassays tested. This result is in line with findings of pervious researchers (Gebre selase and Getu, 2009; Zewde and Jembere, 2010; Gebre-Egziabihir, 2016) in

which mortality effect of botanicals were indicated to be dose and exposure time dependent.

The present study also revealed that polar solvent extracts (distilled water, ethanol and acetone extracts) of the tested botanical leaves at tested rates (2 and 3 ml in filter paper) from all extraction level induced significant toxic effect against *S. zeamais* as compared with negative control. This suggests the presence of more polar solvent soluble phytochemicals in leaves of *C. aurea* which are responsible for higher weevil's mortality. It also suggests the active phytochemicals of these botanicals were highly soluble in all of polar solvents (water, ethanol and acetone) and as most of them probably might be polar.

Similarly, Amante (2016) also suggested that the active ingredients in the leaf extract of the plant reside in the polar fractions indicating that the active principles are polar in nature after he studied castor bean plant against ectoparasites of animals. Jembere et al. (2005) also indicated that high *Z. subfasciatus* mortality was caused by *M. ferruginea* water extract that probably might be due to the presence of highly water-soluble chemicals in the seeds of *M. ferruginea*.

Getu (2014) also indicated that the polar solvent extracts (acetone and water) of *M. ferruginea* seeds caused significantly high toxicity to *Z. subfasciatus* 48 h after treatment. Furthermore, Blum and Bekele (2002) also reported that *C. aurea* has been used as a natural pesticide to improve grain storage. It was also indicated that *C. aurea* possess potent activities (louscidal and

acaricidal effects) against ectoparasites of animals (Amante, 2016).

In the current study, all leaf powder treatments of the tested botanical also caused significant mortality of adult *S. zeamais* than negative control at all dates. Besides, all leaf powder treatments of the tested botanical induced higher mortality (100%) following 7 and 14 days of treatment application. These higher efficacies of leaf powder than negative control may be attributed to either the toxic effects of phytochemicals in the tested plant or starvation and interference with respiration due to suffocation of maize weevils. These results thus, suggest the potency of the leaf powder in protecting maize grains against weevils for the tested plants. Toxicity caused by crude extract of the tested botanical in the current study was also in accordance to the result of previous researchers (Jembere et al., 2005; Zewde and Jembere, 2010; Gebre selase and Getu, 2009; Getu, 2014; Bulito et al., 2017).

Conclusion

The leaf powder and the polar solvent extracts of *C. aurea* were potent in protecting maize against maize weevils attack at all rates (at 2 and 3 ml in filter paper and 5, 10 and 15% for powder admixture bioassays). This in turn confirmed the presence of possibility to exploit the potential of *C. aurea* in the management of *S. zeamais* under subsistence farmer's storage condition. Thus, the powder extracts of *C. aurea* at 5% and above could be used for managing maize weevils on stored maize under subsistence farmer's storage conditions in Ethiopia and elsewhere with similar pest problems. However, their effect on human being, natural enemies and cost effectiveness in farmer's storage conditions need further study before wide implementation of outcomes this study.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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