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# Journal of Stored Products and Postharvest Research

Table of Contents: Volume 9 Number 3 May 2018

## ARTICLES

- Post-harvest evaluation of selected hybrids to maize weevil *Sitophilus zeamais* resistance** 16  
Khakata S., Nzuve F. M., Chemining'wa G. N., Mwimali M., Karanja J., Harvey J. and Mwololo J. K.
- Efficacy of solvent extracts of *Calpurnia aurea* (Ait.) Benth and *Milletia ferruginea* (Hochest) Baker leaves against maize weevils, *Sitophilus zeamais* (Motsch.) of stored maize in Ethiopia** 27  
Berhanu Hiruy and Emanu Getu

*Full Length Research Paper*

## **Post-harvest evaluation of selected hybrids to maize weevil *Sitophilus zeamais* resistance**

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***Sitophilus zeamais* has been identified as one of the most destructive pests of maize stored in tropical regions. While most maize hybrids are being developed, it is necessary to evaluate their resistance to this pest. This study determined the resistance of selected maize hybrids to infestation by *S. zeamais*. Twenty two hybrids with varying resistance to weevil infestation and two checks DUMA 41-susceptible and MTP0701-resistant were used in a randomized complete block design experiment. Assessment was done at 10, 60 and 120 days of maize storage. Data was collected on percent weevil damage, grain weight loss and number of live and dead weevils. Heritability and correlation of factors were also estimated. Analysis of variance showed significant differences ( $P \leq 0.05$ ) on weight loss. The selection of the resistant genotypes was based on percent weight loss after 60 days. KH631Q and PH4 were selected as the most resistant and moderately resistant hybrids, respectively. The resistant check MTP0701 was also found to maintain resistance to weevil attack. There was a strong positive correlation between weight loss, number of live weevils and percent damage. Moderate heritability estimates of hybrids at 60 days of storage indicated the possibility of their parents to transfer the desirable traits to subsequent generations. Therefore, parents of the resistant hybrids could be utilized in breeding programs for maize weevil resistance and be deployed to farmers for use, respectively.**

**Key words:** Hybrid, maize, post-harvest, resistance, *Sitophilus zeamais*.

### **INTRODUCTION**

Maize plays a major role in peoples' livelihood in the sub-Saharan Africa. It is an important subsistent and cash

crop for a majority of the population in this region (Midega et al., 2016). Several factors including low maize yield

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production and population growth rate have aggravated food insecurity (Cairns et al., 2013; FAO, 2013). Another key constraint which has worsened the situation is losses due to post harvest insect pests. It is estimated that, insect pests result to losses of up to 88% of the total maize produced in a season in sub-Saharan Africa (Ojo and Omoloye, 2012). In spite of several efforts being employed to curb insect pest menace (Midega et al., 2015), challenges contributing to post harvest losses remain a huge hindrance to sufficient food production (Tefera et al., 2011). In addition to feeding, insects further contaminate maize by accumulation of excretory products. Further deterioration is witnessed when insect presence and feeding raise grain temperature and moisture contents resulting to fungal activity (Tefera et al., 2011). In Kenya, more than 75% of local maize production is provided by farmers (Kang'ethe, 2011; Mohajan, 2014). Most breeding work in maize focuses on increased yield, field pests and disease resistance. While these efforts are worth, most of the farmers produce is destroyed after harvesting due to the introduction of improved varieties accompanied by reports of increased susceptibility to storage pests (Fortier et al., 1982; Kossou et al., 1992; Ricker-Gilbert and Jones, 2015). Post-harvest losses to storage insect pests such as the maize weevil *Sitophilus zeamais* have been recognized as an increasingly important problem (Markham et al., 1994; Midega et al., 2016). Maize weevil aggravates shortage of maize food by causing losses of 20 to 90% in Kenya (Giga and Mazarura, 1991; Kumar and Kalita, 2017). This reduces the nutritional value, germination capacity, grain weight and marketability of such grains.

As a remedy to control of these pests, synthetic insecticides have been widely used on stored grains. There is a global concern with respect to environmental hazard, chemical residues on food, insecticides resistance development and associated costs (Cherry et al., 2005; Carvalho, 2017). To this regard, host-plant resistance as a pest control measure has been recognized to be environmentally safe, economically cheaper to farmers and integrates well with other components in pest management initiatives (Chapman, 2000; Carvalho, 2017).

This has directed research to the development of resistant maize varieties. There has been progress in developing maize varieties that have multiple resistances to pests and diseases and improved agronomic performance. Despite the growing effort of developing weevil resistance varieties, little has been done on identifying resistant maize hybrids. Therefore, it was necessary to evaluate hybrids for resistance to maize weevil infestation and identify resistant hybrids.

## MATERIALS AND METHODS

### Source of maize germplasm

Maize grains used in this study were from twenty two hybrids which had been planted at the Kiboko nursery in July 2016. The

genotypes used were provided by the Kenya Agricultural and Livestock Research Institute (KALRO) – Katumani. The hybrids originated from local commercial enterprises within Makueni County.

### Field trial and management

The experimental materials were evaluated at KALRO-Kiboko, a research centre situated in Makueni County. The mean annual rainfall is 530 mm and is spread over two very short rainy seasons. It lies at an altitude of 975 m above sea level and between latitude 2° 25' S and longitude 37° 72' E. Sand-clay type of soil occupies this location. Temperatures are uniformly high with mean maximum value of 35.1°C and the minimum of 14.3°C.

The twenty two hybrids were planted in the Kiboko experimental site. Field sizes were 87.5 × 18 m<sup>2</sup> and 87.5 × 30 m<sup>2</sup>, respectively. Each plot measured 5 × 0.75 m<sup>2</sup>. Fertilizer was applied at a standard rate of 30 kg calcium ammonium nitrate (CAN) and 30 kg Di ammonium phosphate (DAP) per ha. Supplementary irrigation was administered when needed.

The fields were kept free from weeds by hoe weeding. Number of rows per plot was 2 and distance between stations, 0.25 m. Treatments were laid in a randomized complete block design (RCBD) with 4 replicates.

### Grain preparation, insect culture and infestation

At harvest, sieving was done to remove any dirt, dust or broken grains. The mature maize weevil insects used for the evaluation were sourced from CIMMYT/KALRO-Kiboko post-harvest testing laboratory. The insects had been reared on commercial hybrid maize H614 under controlled conditions of 28°C and 70% relative humidity (Tefera et al., 2011). Fifty grams of grains was put in 250 ml capacity no-choice glass jars at room temperature and then thirty unsexed adult insects were introduced into the glass jars (Tefera et al., 2011). Glass jars were then covered with a lid made of wire mesh (1 ml) to allow for adequate ventilation and prevent escape of the weevils (Tefera et al., 2011).

### Categories of samples

At harvest, the maize was arranged into three categories. Each category describes the time when the samples were assessed for insect damage. One category represented materials under storage for 10 days; the second had materials under storage for 60 days while the third, were materials stored for 120 days. Each category consisted of 22 entries replicated 4 times. The experimental set up for these genotypes was done at the same time.

### Experimental design in the screening laboratory

Treatments were laid out in a randomized complete block design and kept on wooden shelves at room temperature in the laboratory. The experiment consisted of 22 germplasms replicated 4 times and put in 3 categories. A total of 264 samples were assessed in this experiment. MTP 0701 (resistant check) and DUMA 4 (susceptible check) to weevil infestation were incorporated in the study. Assessment of the trials was done at 10, 60 and 120 days of storage.

### Data collection and assessment

Data was collected on weight of damaged and undamaged grains,



live and dead weevils. On each assessment date (10, 60 and 120 days), the glass jars were opened, contents separated into grains, insects and dust using 4.7 and 1 mm sieves (Endecott Ltd UK). All maize weevils were separated and removed (by hand) from the maize at the end of these three storage periods. Separation of the damaged and undamaged kernels was done using grain tunneling and holes as the criteria (Tefera et al., 2011). These were counted and the percentage of damaged grain and grain weight loss computed. The percent damage was determined using the converted percent damage method of Baba-Tiertor (1994):

$$\%GD = (WDG \times 100) / WDUD$$

where GD is the damaged grain and WDG is the weight of damaged grain, and WDUD is the weight of damaged and undamaged grains.

Weight loss was determined by the count and weight method of Gwinner et al. (1996):

$$\text{Weight loss}(\%) = (Wu \times Nd) - (Wd \times Nu) \times 100 / Wu \times (Nd + Nu)$$

where Wu is the weight of undamaged grain, Nu is the number of undamaged grain, Wd is the weight of damaged grain, and Nd is the number of damaged grain.

Genotypes were categorized as resistant (1-5%), moderately resistant (5.1-8%), moderately susceptible (8.1-10%), susceptible (10.1-13) and highly susceptible (>13.1%) after 60 days based on percentage weight loss, which was found to be a key trait of discriminating genotypes in relation to resistance (Mwololo et al., 2012; Tefera et al., 2011).

#### Data analysis

The data on percentage weevil damage, grain weight loss, live and dead weevils were subjected to GENSTAT 14th edition software and means separated using Fishers least significance difference at 5% probability level. Heritability was measured based on grain damage. Broad sense heritability was estimated based on Johnson et al. (1955) where by the error mean sum of squares (EMS) was considered as error variance ( $\sigma_e^2$ ). Genotypic variance ( $\sigma_g^2$ ) was derived by subtracting error mean sum of squares (EMS) from the genotypic mean sum of squares (GMS) and divided by the number of replications as given by the formula:

$$\sigma_g^2 = GMS - EMS / r$$

where GMS is the genotype mean sum of squares, EMS is the error mean sum of squares, and r is the number of replications.

Phenotypic variance ( $\sigma_p^2$ ) was derived by adding genotypic variance with error variance as given by the formula:

$$\sigma_p^2 = \sigma_e^2 + \sigma_g^2$$

Broad sense was then calculated as:

$$H^2b = \sigma_g^2 / \sigma_p^2$$

where  $\sigma_p^2 = \text{Phenotypic variance}$  and  $\sigma_g^2 = \text{Genotypic variance}$

Pearson's correlation coefficients were obtained using the GENSTAT 14th edition software. Correlations were computed to establish the interaction between grain weight loss, grain damage, live and dead weevils.

## RESULTS

### Maize weevil damage of hybrids

Genotypes did not differ significantly to weevil damage at 10 days of storage (Table 1). Maize weevil damage on genotypes after 10 days of infestation was negligible. The mean weevil damage was recorded as 0% (Table 3). It was evident that most genotypes had whole maize grains and were not damaged by the weevil.

There were significant differences in hybrids response to maize weevil damage at 60 days and hence performance of hybrids to weevil damage was easily distinguished (Table 1).

The mean weevil damage after 60 days of infestation was at 27%. Maize weevil damage on hybrids ranged from 4 to 48% (Table 3). After 60 days of weevil infestation, MTPO701 (resistant check) was least damaged at 4% followed by KH631Q at 9% (Table 3). The susceptible check was damaged at 29% while the highly damaged hybrid was DK8031 at 48% (Table 3).

Hybrids were not significantly different in reaction to weevil damage at 120 days of storage (Table 1). Mean weevil damage in all hybrids after 120 days of storage was 51% (Table 3). Damage among hybrids varied from 28 to 67%. H513 and WH403 were the highly damaged hybrids after 120 days of weevil attack (Table 3). Damage in these hybrids was 67%. Nevertheless, MTP0701 (Resistant check) and KH631Q were the least damaged hybrids.

The damage in these hybrids was 28 to 31%, respectively (Table 3). It was noted that after 120 days of weevil infestation, 6 hybrids recorded damages of less than 50%.

Based on combined ANOVA analysis, significant differences in weevil damage were only recorded in storage periods. The hybrids and interaction between hybrids and storage periods were not significant (Table 2). Although no damage was recorded after 10 days, the highest damage was at 120 days of storage (Table 3). At 60 days there were significant damages on the hybrids grains but the damage peaked at 120 days. Weevil damage was consistently very low in the resistant check MTPO701 and hybrid KH631Q in all the storage periods (Table 3). They were damaged by 10 and 4%, respectively at 60 days.

### Heritability and variances of weevil resistance on hybrids

Heritability in the broad sense ( $H^2$ ) of weevil resistance was estimated as described earlier by Johnson et al. (1955). Heritability was calculated based on the weevil damage. From the results, heritability was low and varied among storage periods. Broad sense heritability was -4% after 10 days, 19% after 60 days and -0.5% after 120



**Table 1.** Mean sum of squares of resistant parameters at three different storage period among the hybrids.

Source	DF	10 days storage period				60 days storage period				120 days storage period			
		GD (g)	GWL (g)	LMW (count)	DMW (count)	GD (g)	GWL (g)	LMW (count)	DMW (count)	GD (g)	GWL (g)	LMW (count)	DMW (count)
Replication	3	9.44	0.00382	0.5104	0.5694	385.6	578.8	1503.4	12.514	1695.2	2765.8	14620	153.01
Genotype	23	0.00159 <sup>ns</sup>	0.0409*	0.6698*	0.7808*	204.1*	287*	719.4*	7.259 <sup>ns</sup>	472.8 <sup>ns</sup>	761.2 <sup>ns</sup>	6228*	79.74 <sup>ns</sup>
Residual	69	0.00019	0.0183	0.438	0.4535	104.7	142.5	406.8	6.833	481.4	787.3	4131	75.33
CV		0.38	0.32	0.45	0.33	0.27	0.36	0.33	0.41	0.35	0.39	0.32	0.30

Significance level \* $p < 0.05$ . DF: Degree of freedom, GD: grain damage, GWL: grain weight loss, LMW: live maize weevils, DMW: dead maize weevils, ns: not significant.

**Table 2.** Combined mean sum of squares at the 3 storage periods among hybrids.

Source	DF	GD (g)	GWL (g)	LMW (count)	DMW (count)
Replication	3	90.69	34.054	1463.4	793.6
Genotype	29	335.89*	26130.09*	176509.8*	579395.9*
Days of storage	2	124820*	77.047*	2316.5	1127.6*
Genotype x Days of storage	58	222.42*	28.413*	1377.7*	1079
Residual	267	51.51	8.033	668	283.1
CV	-	0.33	0.35	0.35	0.34

Significance level \* $p < 0.05$ . DF: Degree of freedom, GD: grain damage, GWL: grain weight loss, LMW: live maize weevils, DMW: dead maize weevils, ns: not significant.

days. Negative heritability was recorded at 10 and 120 days of storage (Table 4).

### Maize weevil grain weight loss of Hybrids

There was no significant difference in weight loss among hybrids after 10 days of storage. Grain weight loss in all hybrids was considerably low and less than 1% after 10 days (Table 5). The mean grain weight loss of all hybrids was 0%. At this storage period, the hybrids grain weight loss varied from 0 to 0.01. The susceptible check DUMA 41 was not damaged after 10 days of storage (Table 5).

The hybrids showed differences in percentage grain weight loss after 60 days of storage. The mean grain weight loss after 60 days of storage was at 17%. The grain weight loss of hybrids varied from 4 to 27%. According to the criteria of categorizing genotypes (Mwololo et al., 2012; Tadele et al., 2011) 1 genotype was resistant, 1 was moderately resistant, 3 were susceptible and 17 were highly susceptible (Table 5). As expected, the resistant check, MTP0701 had the least damage and hence recorded the least grain weight (Table 5). The susceptible check DUMA 41 recorded a weight loss of 23% and hence was grouped among the highly susceptible genotypes. Among the hybrids, PAN 691 and KH 500-31A

had the most grain weight loss. The weight loss in these hybrids was 27 and 26%, respectively. At this stage about 45% of hybrids had lost more than 20% of the grain weight.

The hybrids did not differ significantly in grain weight loss after 120 days of storage. At this stage, the hybrids had lost more grain weight than after 60 days, and the mean loss was at 39.5%. This storage period recorded the highest grain weight loss which varied from 8.7 to 57.6% (Table 5). The resistant check; MTP0701 had the least weight loss at this storage period. On the contrary, PH3253, DK8031, WH 505 and WH 403 lost the most grain weight at this period. The grain weight loss in these hybrids was above 50% (Table 5).

**Table 3.** Maize weevil damage on hybrids for the three storage periods.

Hybrid	Mean weevil damage			Mean
	10 days	60 days	120 days	
DH01	0.0	21.9±4.9	61.8±8.3	27.9
DH04	0.0	36.7±9.2	54.2±9.5	30.3
DK8031	0.1	48.4±4.8	62.1±6.6	36.8
H513	0.0	21.9±3.8	67.3±7.3	29.7
H614D	0.0	29.3±5.6	60.3±6.9	29.9
KH 500-31A	0.0	38.3±1.9	58.4±5.6	32.2
KH 500-33A	0.1	19.5±2.8	46.8±1.4	22.1
KH 600-15A	0.0	42.4±8.1	35.6±1.3	26.0
KH 600-16A	0.0	31.9±5.6	59.6±0.9	30.5
KH 631Q	0.0	9.8±3.2	30.7±6.3	15.8
MTPEH200804	0.0	18.7±2.5	37.6±2.6	16.4
PAN 67	0.1	20.5±5.0	51.7±1.3	24.1
PAN 691	0.0	44.5±7.7	54.3±10.1	32.9
PH 4	0.0	18.5±2.5	45.1±1.3	21.2
PH1	0.0	36.6±8.8	59.0±9.4	31.9
PH3253	0.0	28.1±5.0	61.6±8.8	29.9
SC DUMA 41	0.0	31.2±4.1	51.6±1.2	27.6
SC DUMA 43	0.0	29.5±2.7	58.3±2.1	29.3
SC Simba 61	0.0	28.7±8.7	51.9±2.4	26.9
WH 403	0.1	28.3±4.6	66.7±4.2	31.7
WH 504	0.0	36.5±5.0	58.9±2.1	31.8
WH 505	0.0	18.4±4.9	54.7±9.4	24.4
<b>Checks</b>				
MTP0701R)	0.0	4.1±3.8	28.4±26.1	10.8
DUMA 41(S)	0.0	29.4±1.7	51.6±9.8	27.0
Mean	0.0	26.92	52.84	25.88
P value	-	P ≤ 0.05	P ≤ 0.05	P ≤ 0.05
LSD(Gen × Days)	-	10.1	6.3	20.5
CV (%)	-	28.3	27.9	24.6

R: Resistant, S: susceptible, Gen: genotype, LSD: least significant difference, CV: covariance.

**Table 4.** Heritability and estimated variances in maize hybrids.

Heritability and estimated variances	After 10 days	After 60 days	After 120 days
$V_E$	0.00019	104.7	481.4
$V_G$	-0.0000075	24.85	-2.15
$V_P$	0.0001825	129.55	470.7
HBS (%)	-4.109589041	19.18	-0.46

$V_E$ : environmental variance,  $V_G$ : genotypic variance,  $V_P$ : phenotypic variance, HBS: heritability in broad sense.

From combined ANOVA results, the hybrids did not differ significantly in grain weight loss and in the interaction of hybrids and storage days. Grain weight loss increased gradually after the 60 days of storage and was most observed at 120 days in all hybrids. The least and most grain weight loss was recorded after 10 and 120

days, respectively (Table 5).

#### Number of live weevils in hybrids

After 10 days of storage, hybrids did not show differences

**Table 5.** Maize weevil grain weight loss on hybrids at the three storage periods.

Hybrid	% Grain weight loss			Mean	Remarks
	10 days	60 days	120 days		
MTP0701(R )	0.0	4.5±1.1	8.7±1.3	4.38	Resistant
KH 631Q	0.0	5.0±3.9	18.3±1.4	7.77	Resistant
PH 4	0.0	7.2±1.5	29.2±1.8	12.11	Moderately resistant
DH01	0.0	9.5±0.1	49.8±8.8	19.75	Susceptible
MTPEH200804	0.0	9.7±3.3	13.8±1.2	7.85	Susceptible
WH 505	0.0	9.9±0.9	55.9±1.4	21.94	Susceptible
PAN 67	0.0	12.4±0.1	35.3±1.2	15.9	Highly susceptible
H513	0.0	14.4±0.6	48.5±2.9	20.99	Highly susceptible
SC Simba 61	0.0	15.2±0.5	37.8±1.8	17.68	Highly susceptible
KH 500-33A	0.0	15.8±0.5	34.2±2.8	16.66	Highly susceptible
H614D	0.0	16.2±0.5	47.5±7.1	21.23	Highly susceptible
WH 403	0.0	17.0±1.6	57.6±3.1	24.84	Highly susceptible
KH 600-16A	0.0	18.7±1.8	47.2±5.5	21.99	Highly susceptible
PH3253	0.0	18.8±1.1	55.1±1.6	24.61	Highly susceptible
DK8031	0.0	20.1±1.2	55.9±7.4	25.31	Highly susceptible
DH04	0.0	20.2±1.5	41.5±9.8	20.54	Highly susceptible
SC DUMA 43	0.0	20.9±1.7	41.6±1.9	20.84	Highly susceptible
PH1	0.0	22.2±0.2	44.4±1.5	22.21	Highly susceptible
SC DUMA 41	0.0	22.3±2.6	32.7±1.2	18.33	Highly susceptible
KH 600-15A	0.0	22.7±0.2	17.1±1.4	13.26	Highly susceptible
DUMA 41( S)	0.0	23.3±1.0	36.9±4.5	20.07	Highly susceptible
WH 504	0.0	25.3±0.9	47.5±3.9	24.26	Highly susceptible
KH 500-31A	0.0	26.4±0.6	47.6±2.2	24.67	Highly susceptible
PAN 691	0.0	27.3±3.3	44.7±1.0	23.99	Highly susceptible
Mean	0.00	16.88	39.53	18.80	-
P-value	-	P ≤ 0.05	P ≤ 0.05	P ≤ 0.05	-
LSD (Gen × Days)	-	3.1	8.9	25.80	-
CV (%)	-	24.8	32.4	27.10	-

R: Resistant, S: susceptible, Gen: genotype, LSD: least significant difference, CV: covariance.

in number of live weevils present in the grains. At the start of the experiment, 30 live insects had been introduced in each glass jar containing grains of hybrids. After 10 days of storage the live insects were ranging from 29 to 30 (Table 6). This showed that only one weevil had died in most hybrids.

However, 12 hybrids retained the number of live insects introduced at the start of the experiment.

There were significant differences in number of live weevils among hybrids when hybrids were stored for 60 days. At this storage period, a mean of 33 live weevils was recorded. Nonetheless, the number of live weevils varied from 5 to 64 at this storage period.

The least number of weevils was recorded in resistant check MTP0701. This check had a mean number of five weevils after 60 days of storage. In this check, number of live weevils had reduced by 25. Similarly, in nine hybrids, live weevils had reduced by between 1 and 12 weevils (Table 6). However, in 14 hybrids number of weevils had

increased. DK8031 had the highest number of live weevils. This hybrid had doubled the number of live weevils to 64 at this storage period (Table 6).

Hybrids did not differ significantly in number of live weevils after 120 days of storage (Table 1). At this stage, live weevils ranged from 35 to 181. The mean number of live weevils had tripled after 60 days and was at 99 (Table 6). At this stage, 15 hybrids had at least tripled number of weevils than the introduced. The least number of live weevils were in hybrids, KH 600-15A and SC DUMA 41. The number of live weevils in the two hybrids was at 38 and 52, respectively. The resistant check MTP0701 had the least number of weevils (35 weevils on average). The susceptible check DUMA 41 had 54 weevils at this stage. However, 2 hybrids had lesser number of live weevils than the susceptible check (Table 6).

In the combine ANOVA, significant differences in the number of weevils were recorded between storage

**Table 6.** Number of live weevils in hybrids at the three storage periods.

Hybrid	Number of live weevils			Mean
	10 days	60 days	120 days	
DH01	29.50±0.6	29.00±8.6	124.75±10.5	61.08
DH04	29.50±0.6	35.25±16.7	106.50±1.8	57.08
DK8031	29.50±0.6	63.50±12.6	181.25±2.5	91.42
H513	29.25±1.0	24.75±2.3	101.25±4.3	51.75
H614D	29.00±0.8	35.50±20.6	91.00±7.1	51.83
KH 500-31A	29.00±0.8	49.50±8.0	128.50±3.1	69
KH 500-33A	28.75±0.5	24.25±9.2	84.50±2.4	45.83
KH 600-15A	29.00±0.8	43.25±19.0	38.25±4.2	36.83
KH 600-16A	29.50±0.6	43.25±7.1	107.75±4.9	60.17
KH 631Q	29.50±0.6	19.75±7.3	55.00±16.4	34.75
MTPEH200804	30.00±0.0	18.75±15.9	83.00±18.8	43.92
PAN 67	29.75±0.5	22.25±17.4	94.25±1.2	48.75
PAN 691	29.75±0.5	51.75±5.6	164.00±5.8	81.83
PH 4	28.75±0.5	19.25±14.3	79.50±1.3	42.5
PH1	29.25±1.0	51.25±6.8	103.50±6.4	61.33
PH3253	29.75±0.5	32.00±9.4	176.25±5.4	79.33
SC DUMA 41	29.25±0.5	43.00±22.6	52.75±8.2	41.67
SC DUMA 43	28.50±0.6	40.50±5.3	76.50±0.5	48.5
SC Simba 61	29.25±1.5	32.25±8.4	92.00±6.0	51.17
WH 403	30.00±0.0	36.00±3.0	139.00±7.5	68.33
WH 504	29.75±0.5	34.50±12.8	111.25±5.1	58.5
WH 505	29.75±0.5	23.25±6.0	109.00±4.4	54
<b>Checks</b>				
MTP0701	29.75±0.5	5.00±10.9	35.25±4.4	23.33
DUMA 41	29.75±0.5	21.25±10.1	54.25±5.2	35.08
Mean	29.5	33.3	99.6	54.13
P-value	-	P ≤ 0.05	P ≤ 0.05	P ≤ 0.05
LSD (Gen × Days)	-	15.2	26.1	36.7
CV (%)	-	32.9	36.7	25.2

R: Resistant, S: susceptible, Gen: genotype, LSD: least significant difference, CV: covariance.

periods and hybrids. However, the interaction of the two factors was not significant (Table 2). The live weevils were retained at 10 days but increased after 60 days of storage. However, at 120 days of storage, the live weevils had tripled the introduced number. In all storage periods, the highest number of live weevils were at 120 days of storage and lowest at 10 days (Table 6).

### Number of dead weevils in hybrids

At 10 days of storage, hybrids were significant for number of dead weevils. However, only one weevil had died in 11 hybrids. The rest of the hybrids had live weevils (Table 7).

Hybrids did not show significant differences in number of dead weevils after 60 days (Table 1). At this storage period, dead weevils had increased and were

ranging from 0 to 7 (Table 7). Resistant check MTP0701 and WH504 lacked dead weevils at this stage. The remaining hybrids had at least one or two dead weevils after 60 days of storage (Table 7).

The number of dead weevils was also found to be insignificant among hybrids after 120 days of storage (Table 1). At this stage, dead weevils were at an average of 5 but varied from 1 to 22. At 120 days of storage, the highest numbers of dead weevils were in hybrid PH4.

The check MTP0701 and DUMA 41 had dead weevils averaging at 5 and 6, respectively (Table 7).

In the combined analysis, significant differences in number of dead weevils were only reported in storage periods. The highest number of dead weevils was recorded after 120 days of storage. The number of dead weevils was considerably lower than live weevils at the three storage periods (Tables 6 and 7).

**Table 7.** Number of dead weevils in hybrids at the three storage periods.

Hybrids	Number of dead weevils			
	10 days	60 days	120 days	Mean
DH01	0.50±0.6	0.75±1.0	1.75±1.0	1.00
DH04	0.25±0.5	1.75±1.5	2.25±2.1	1.42
DK8031	0.50±0.6	1.25±1.0	3.75±2.5	1.83
H513	0.75±1.0	0.75±1.0	2.25±1.5	1.25
H614D	1.00±0.8	0.50±1.0	7.25±6.9	2.92
KH 500-31A	1.00±0.8	2.50±1.7	5.75±3.6	3.08
KH 500-33A	1.50±1.0	1.25±1.0	0.75±1.0	1.17
KH 600-15A	1.00±0.8	3.25±4.0	3.75±2.8	2.67
KH 600-16A	0.50±0.6	1.00±0.8	4.00±2.6	1.83
KH 631Q	0.50±0.6	0.75±0.5	2.00±0.8	1.08
MTPEH200804	0.00±0.0	1.50±1.3	1.00±0.8	0.83
PAN 67	0.25±0.5	1.00±1.2	2.75±2.4	1.33
PAN 691	0.25±0.5	2.75±1.7	2.75±1.7	1.92
PH 4	1.25±0.5	6.75±0.5	22.00±7.0	10.0
PH1	0.75±1.0	1.25±2.5	4.50±3.5	2.17
PH3253	0.25±0.5	2.25±1.9	5.50±5.0	2.67
SC DUMA 41	1.00±0.0	1.75±1.7	13.25±7.8	5.33
SC DUMA 43	1.50±0.6	0.75±1.0	3.50±2.1	1.92
SC Simba 61	0.75±1.5	1.00±1.4	5.00±5.0	2.25
WH 403	0.00±0.0	1.00±1.2	5.00±3.8	2.00
WH 504	0.25±0.5	0.25±0.5	3.75±4.2	1.42
WH 505	0.25±0.5	1.00±1.4	7.75±8.0	3.00
<b>Checks</b>				
MTP0701(Resistant)	0.25±0.5	0.25±0.5	6.00±6.8	2.17
DUMA 41( Susceptible)	0.25±0.5	1.25±1.3	5.00±3.4	2.17
Mean	0.6	1.52	5.05	2.39
P-value	-	P ≤ 0.05	P ≤ 0.05	P ≤ 0.05
LSD (Gen × Days)	-	3.2	4.5	7.4
CV (%)	-	32.1	24.4	44.8

R: Resistant, S: susceptible, Gen: genotype, LSD: least significant difference, CV: covariance.

## Correlations

There was a significant correlation among all the factors. In all the factors, numbers of live weevils were strongly correlated to percent of weevil damage. The correlation coefficient in these factors was 0.99. Unlike the inbred lines where weight loss and percent of weevil damage had the most association, correlation coefficient was low in these factors ( $r=0.34$ ) in hybrids (Table 8). Live weevils correlated well with dead weevils giving a coefficient of 0.81 while dead weevils and percent of weevil damage resulting in coefficient of 0.85 (Table 8).

## DISCUSSION

### Maize weevil damage of Hybrids

The present study showed great variation in hybrid

resistance to weevil attack after 60 days of storage and hence hybrids could easily be separated on the basis of resistance at this storage period. Minimum and insignificant damage was witnessed after 10 days whereas maximum damage observed after 120 days of storage. Such variation in maize weevil has been earlier identified to exist in maize genotypes from eastern, southern and western Africa (Gafishi et al., 2012).

This resistance could either be due to physical factors such as grain hardness or antibiosis as a result of biochemical compounds which are toxic to the insects (García-Lara et al., 2004; Siwale et al., 2009; Munyao, 2015). Resistance mechanisms have been classified into three categories: non-preference for oviposition, food or shelter (also called antixenosis), antibiosis referred to the adverse effect of the plant on the biology of the pest, and tolerance or the plant's ability to repair, recover or withstand infestation (Derera et al., 2001; Dhliwayo et al.,

**Table 8.** Correlation coefficients of maize weevil infestation in maize hybrids.

Parameter	Weevil damage (%)	Weight loss (%)	Dead weevils	Live weevils
Weevil damage (%)	1			
Weight loss (%)	0.3358*	1		
Dead weevils	0.8491*	0.287*	1	
Live weevils	0.9938*	0.3214*	0.8136*	1

\*Significant at 5% probability level.

2005). Biochemical properties such as  $\alpha$ -amylase- and protease-inhibitors (Dari et al., 2010) and phenolics (Sori and Keba, 2013), may explain the basis of resistance to weevil infestation in this study.

### Heritability of maize weevil resistance

Estimate of heritability assists breeders to allocate resources necessary to effectively select for desired traits and to achieve maximum genetic gain with little time and resources. Heritability is recommended to be considered in association with genetic advance to predict the effect of selecting superior crops varieties. In this study heritability for weevil resistance in hybrids was low. Dhliwayo et al. (2005) reported that inheritance of weevil resistance is complex and heritability is likely to be small to moderate. Low heritability indicates slow progress in selection for this character. This may explain why resistance to maize weevil resistance is influenced by additive and non-additive gene effects. Also, low heritability levels could be because most of the evaluated hybrids were being developed for other agronomic traits and hence weevil resistance was not considered as a primary factor during selection.

### Maize weevil grain weight loss of hybrids

At 10 days, there was no damage while at 120 days most genotypes had attained maximum damage. However, after 60 days of storage it was easy to identify and categorize genotypes into groups of resistance and susceptibility. Therefore, the hybrids KH631Q and PH4 were identified as resistant and moderately resistant, respectively. It has been reported that resistant varieties offer a sustainable, cost effective and environmental friendly way to reduce damage by *S. zeamais* under storage conditions (Gu et al., 2008). Gafishi et al. (2012) also identifies host plant resistance to storage pests as a component of integrated pest management that is particularly important in developing countries, where maize is stored under often inappropriate conditions due to lack of knowledge or resources.

Therefore, the identified resistant varieties can be used as a source of resistance in breeding programs and

subsequently be adapted by smallholder farmers to diversify the basis of resistance to this pest.

The selected resistant hybrids exhibit genetic factors which confer resistance to maize weevil attack. Within the first storage period (10 days), none of the varieties suffered any significant damage or weight loss. But beyond that, there were increases in weevil numbers, leading to increased weight losses. According to Wangui (2016), despite the shape, size and hardness of the grain, its chemical and nutritional composition are important primarily in resisting insect attack and damage; the length of exposure of the grain to the pest may also affect the level of infestation of maize varieties by *S. zeamais*. This therefore, results in increased grain weight losses. Grain weight losses were generally lower at 10 days of storage with higher losses being at 60% at 120 days. Hossain et al. (2007) reported that grain loss of 12 to 20% is common, but up to 80% has been reported for untreated kernels.

### Number of live weevils in hybrids

The number of live insects remained unchanged after 10 days since new insects had not emerged within this short period. According to Gafishi et al. (2012), complete development for the life cycle of the maize weevil averages 36 days. This could also mean that short storages of studied hybrids up to two weeks are also possible.

However, at 60 days number of maize weevils decreased and increased in some hybrids. The hybrids with highest number of weevils were mostly damaged as it was the case for DK8031. This variety has been found to be susceptible to maize weevil damage and hence a favourable host for maize weevil (Kalunde, 2011). In this study, numbers of live *S. zeamais* varied with the maize hybrid varieties used. Therefore, the shortest developmental times occurred on the varieties which had the largest number of weevils emerging.

On the other hand, the longest developmental times occurred in varieties with the least number of live weevils. The development of an insect is influenced by nature of food the insect is reared on. Generally, more eggs are laid on and development is faster on a more favourable than a non-favourable hosts. Increased maize weevil

emergence is a result of high susceptibility of a genotype on which weevils can feed easily and therefore produce many eggs and progeny. From the study, the number of live weevils is also primarily significant in assessing resistance varieties to maize weevil.

### Number of dead weevils in hybrids

The number of dead weevils was relatively low in hybrids confirming the reports of Abebe et al. (2009) who also found a low mortality rate of maize weevils. However, the rate of mortality of weevils has been revealed not to be a good indicator for weevil resistance among maize varieties.

### Correlations

Four resistance measures were used in this study in order to carefully evaluate the hybrids resistance to maize weevil and to draw confidently accurate conclusions.

The correlations found between the four resistant measures are presented in the results. The results showed that percent damage, percent grain weight loss and number of live weevils are associated with weevil resistance. Therefore, hybrids that were less damaged, had less weight loss and fewer numbers of live insects were considered resistant to maize weevil attack. Also, this implies that increased numbers of weevils results in increased grain damage and hence more grain weight loss. This strong association between the factors has been reported before by Zunjare et al. (2014) and Derera et al. (2010).

### Conclusions

This variation in response to the maize weevil attack among hybrids gives evidence of genetic diversity for breeding that exists in the parents utilized in making the hybrids. This offers a great opportunity to exploit the variability with the aim of reducing post-harvest insect-pest losses through genetic improvement. The selected resistant hybrids should also be used in areas considered to be maize weevil hotspot.

### CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# **Efficacy of solvent extracts of *Calpurnia aurea* (Ait.) Benth and *Milletia ferruginea* (Hochest) Baker leaves against maize weevils, *Sitophilus zeamais* (Motsch.) of stored maize in Ethiopia**

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Distilled water, acetone and ethanol (polar solvent) extracts from 20 g/100 ml and 30 g/100 ml levels of extraction of *Calpurnia aurea* and *Milletia ferruginea* were tested as protectant against maize weevils in maize grains under laboratory condition. They were applied at a rate of 10 and 15% (w/v) in admixture bioassays from both of the aforementioned extraction levels. Parental weevil's mortality, F1 progeny emergence, percent protection, grain damage and weight loss were the parameters measured. All polar solvent extracts of the tested plants applied at a rate of 10 and 15 ml from the two extraction levels induced significant ( $p \leq 0.05$ ) toxicity effect against weevils than solvent treated grains at all dates after treatment. Besides, significantly ( $p \leq 0.05$ ) higher mortality of parental weevils were recorded in all polar solvent extracts (>75%) of the tested botanicals applied at 15 ml dosages from 30 g/100 ml extraction levels than those applied at a rate of 10 ml following 96 h treatment application. Furthermore, all the polar solvent extracts applied at rates of 10 and 15% also induced good degree of protection of maize grains ( $\geq 78\%$ ) against F1 progeny emergence, percent grain damage ( $\leq 1.33$ ) and weight loss ( $\leq 0.28$ ) by maize weevils than negative control in about 2 months storage period (56 days). Consequently, the solvent extracts of *C. aurea* and *M. ferruginea* were potent and therefore, they can be used in management of maize weevils in stored maize under subsistence farmer's storage conditions.

**Key words:** *Calpurnia aurea*, *Milletia ferruginea*, *Sitophilus zeamais*, botanicals, polar solvent extracts, stored maize.

## **INTRODUCTION**

Maize is the major staple food crop in Africa that contributes significantly to the agricultural sector (Tefera et al., 2011). It is also one of the major cereal crops grown for its food, feed, firewood and construction values

(Sori, 2014). Of the cereal crops, it ranks second to tef in area coverage and first in total production nationally (Gemu et al., 2013). But, post-harvest insect pest's grains have been indicted as major problems to food and

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income security of resource poor farmers in sub-Saharan Africa including Ethiopia due to their heavy losses of grains (Tadesse, 1991, 1997; Abebe et al., 2009). The most economically important of these storage insect pests are Coleopterous weevils (Getu and Abate, 1999). Different management strategies such as synthetic insecticides, botanicals and cultural practices have been used to control weevils in particular and storage pests in general, of which synthetic insecticides were the most commonly used farmers in Africa including Ethiopia (Mvumi et al., 1995; Mvumi and Stathers, 2003). However, health and environmental concerns have been associated repeated usage of synthetic pesticides over the years (Ofuya and Longe, 2009). This and other facts mentioned earlier indicates the presence of urgent need for searching cheap, environmentally sound and effective management options such as botanicals for reducing weevils losses in the aforementioned countries.

Birbira, *Milletia ferruginea* (Hochst.) Baker and *Calpurnia aurea* (Ait.) Benth plants may have protective role of stored maize against weevils. The former one is a large shady tree which grows up to a length of 35 m and is endemic to Ethiopia and widely grown at the elevation between 1,000 and 2,500 m above sea level (Jembere, 2002; Getu, 2014). It has been commonly used in traditional medicine. According to MacLachlan (2001), the roots and seeds of this plant are also used as insecticides and pesticides in many parts of the world, and rotenone are responsible for their toxicity. The later one 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 diverse medical conditions and parasitic infestation, in humans and animals in African including Ethiopia (Watt and Breyer-Brandwyk, 1962). Its leaves and powdered roots are used to destroy lice and to relieve itches and they contain tannins, flavonoids, terpenoids, saponins, steroids, glycosides, alkaloids (Nega et al., 2016). Considering that the former plant is reported to contain rotenone and the later one to contain tannins, flavonoids, terpenoids, saponins, steroids, glycosides, and alkaloids, the present study was initiated with the following objectives: (1) to evaluate the toxicity potency of solvent extracts leaves of *C. aurea* and *M. ferruginea* against the most economically important storage insect pest of maize, maize weevil (*Sitophilus zeamais*) under laboratory conditions and (2) to determine the possibility of using these plants for management of insect pests stored maize by poor framers at national level and elsewhere with similar pest problem.

## 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, Addis Ababa University of Ethiopia.

### The test insect's culture

*S. zeamais* adults were collected from maize grains stored in various farmers traditional storage facilities of major maize producing localities Shashogo and Sankura districts of Southern Ethiopia and brought to the Laboratory of Addis Ababa University, Faculty of Life Science, Insect Science Insectary of Zoological Science Department 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 also obtained from farmer's storages of the aforementioned districts. It was the most commonly grown hybrid in the region and considered to be susceptible to insect infestation. 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 13 days. Then, the seeds were kept under laboratory condition until F1 progeny emergence. The F1 progeny, which emerged after 30 days, were sieved out and used for the experiment.

### Description of the tested botanicals and their preparation

Plant materials (that is leaves) used for the study were collected from natural habitats of Hadiya zone, Southern Ethiopia and the identities of the plants were confirmed into *C. aurea* and *M. ferruginea* species at the National Herbarium of Life Science Faculty of Addis Ababa University.

### Admixture bioassay with botanicals leaves solvent extracts

Disinfested shone variety of maize grains (100 g) were placed in 1 L glass jars and treated with the water, acetone and ethanol extracts of the test botanicals at a rate of 10 and 15 ml from the two extraction levels; 20 g/100 ml and 30 g/100 ml following similar procedures by Zewde and Jembere (2010). The jar contents were shaken thoroughly for 5 min to ensure uniform distribution of the solution over grain surface. Then, the treated grains were kept for 24 h for acetone extracts and 36 h for ethanol extracts to allow complete evaporation of solvents before conducting of bioassay based on their property. Malathion 5% dust at the recommended dose rate of 0.05 g per 100 g of maize grains (positive control) and solvent treated grains (negative control) served as comparison and control tests respectively (Arannilewa et al., 2006). Then after, 20, three to seven day old unsexed experimental insects were introduced to the treated and untreated grains in each of the glass jars. The jars were covered with nylon mesh and held in place with rubber bands. Then, treated grains and controls were then kept under same experimental condition indicated in insect capture section. All treatments of solvent extracts were arranged in Completely Randomized Design (CRD) in three replications. Mortality was evaluated 24, 48, 72 and 96 h after the beginning of exposure following similar procedures by Gebreselassie and Getu (2009) and Zewde and Jembere (2010). All live insects were also 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. Then the numbers of F1 progeny produced by the experimental insects were counted. Counting were stopped after 56 days from the day of introduction to avoid overlapping of generation following similar procedures Gebreselassie and Getu (2009) and Zewde and Jembere (2010).

### Damage and weight loss assessment assay

Two days after the last F1 count of 56 days, samples of 30 grains were taken randomly from each jar and the number of damaged (grains with characteristic hole) and undamaged grains were counted and weighed. Damaged grains were expressed as a percentage of the total number of seeds in each replicate. Percentage weight losses were calculated by count and weight method following similar procedures earlier researchers (FAO, 1985; Gebreselassie and Getu, 2009; Zewde and Jembere, 2010) as follow:

$$\text{Loss in weight (\%)} = \left[ \frac{\text{UNd} - \text{Dnu}}{\text{U} (\text{Nd} + \text{Nu})} \right] \times 100$$

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

Following similar procedures by Gebreselassie and Getu (2009) also percent protection or inhibition in F1 progeny emergence (% IR) was calculated using the following formula:

$$\text{IR (\%)} = \left( \frac{\text{Cn} - \text{Tn}}{\text{Cn}} \right) \times 100$$

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

Data on parental adult mortality, F1 progeny emergence, and grain damage and weight loss were managed with the Microsoft Excel version 2013 and then were subjected to analysis of variance (ANOVA) of SPSS Version 16. Data of the former one was analyzed using appropriate statistical method, Univariate analysis, but data of the later ones were analyzed by one-way ANOVA. Significant differences between means of different treatments and time of exposure were separated using Tukey's studentized (HSD) test at 5% confidence interval. Difference among means were stated significant when  $p < 0.05$  and highly significant when  $p < 0.01$ . Standard errors ( $\pm$ SE) are given the following means in Tables and as T-shaped beams in figures. Correlation between the treatments and the efficacy measuring parameters like weight loss and others were determined using Pearson's correlation of SPSS program of version 16.

## RESULTS

### *C. aurea* and *M. ferruginea* leaf solvent extracts on the mortality of maize weevil

All polar solvent extracts (Distilled water, acetone and ethanol) the *C. aurea* and *M. ferruginea* applied at the rates of 10 and 15 ml of the two tested levels of

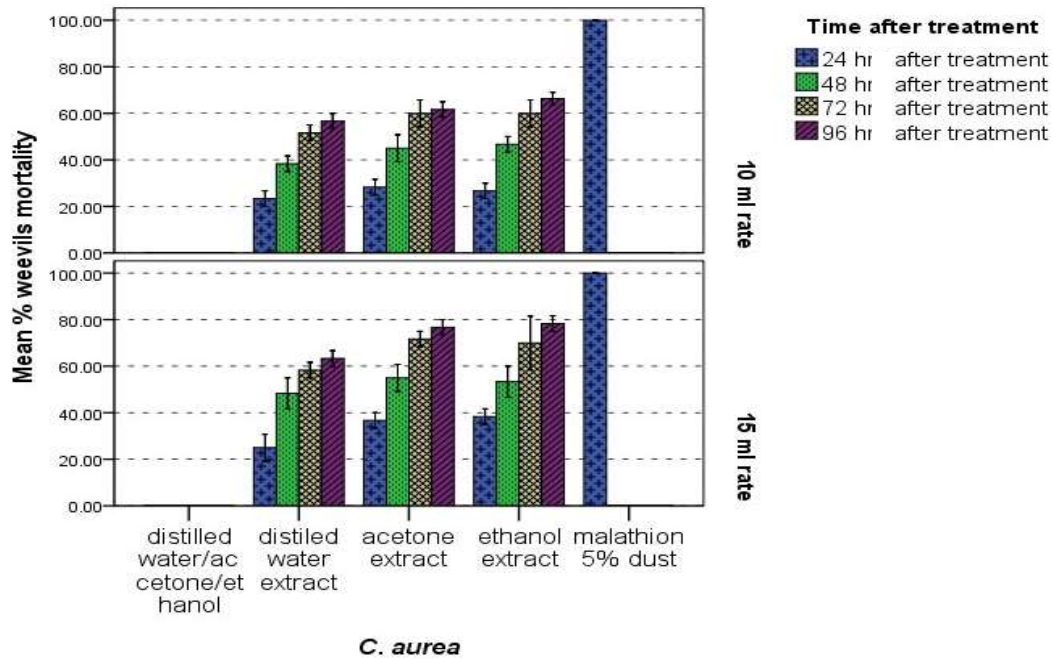
extraction (20 g/100 ml and 30 g/100 ml) caused significantly ( $p < 0.05$ ) higher mortality of maize weevils at all dates after treatment than the negative control. Besides, their efficacy was increased with increased dosage, extraction level and exposure time after treatment application. Polar solvent extracts of the tested plants leaves applied at rates of 10 and 15 ml from the aforementioned two levels of extraction also induced significantly ( $p < 0.05$ ) higher toxicity effect against weevils following 72 ( $\geq 55\%$ ) and 96 h ( $\geq 60\%$ ) post treatment exposure than prior to them (Figures 1 and 2).

The efficacy of the tested plants leaves solvent extracts in weevil's mortality was also varied ( $p < 0.05$ ) significantly with the type of solvent used for extraction; the highest being occurred in ethanol extracts, followed by in acetone and in distilled water extracts in general. Significantly ( $p < 0.05$ ) higher mortality ( $>75\%$ ) of parental *S. zeamais* adults were also recorded in all polar extracts of the tested botanicals applied at 15 ml dosages from 30 g/100 ml extraction levels than those applied at a rate of 10 ml following 96 h treatment application, the maximum of which occurred in ethanol extract ( $>80\%$ ), followed by in acetone and in distilled water extracts (between 75 and 80%) in general (Figures 1 and 2).

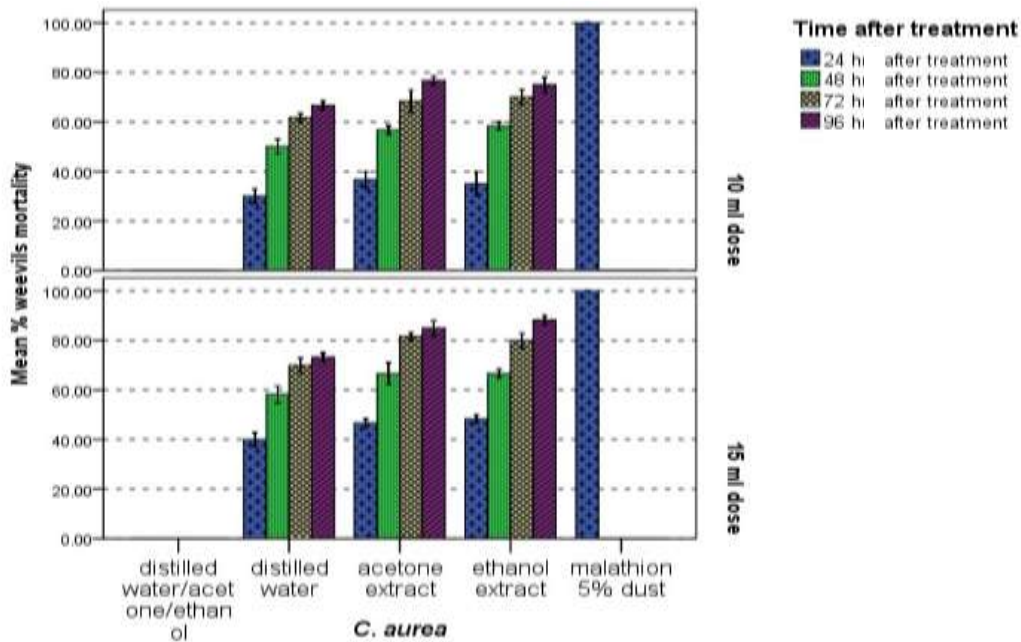
### Damage and mean percentage weight loss

The number of F1 progeny produced, percentage grain damage and weight loss caused by *S. zeamais* in all treatments of botanicals solvent extracts were significantly ( $p < 0.05$ ) lower as compared to negative control (each solvent treated grains). All polar solvent extracts treatments of the two tested botanicals applied at all rates (10 and 15% of solvent extracts) induced more than 73% inhibition in F1 progeny production and significantly ( $p < 0.05$ ) higher reduction in grain damage ( $\leq 4.33$ ) and weight loss ( $\leq 0.58$ ) of maize grain by *S. zeamais* than negative control. However, 100% F1 progeny production inhibition, as well as no grain damage and weight loss of maize were observed in polar solvent extract treatments of the tested botanicals applied at a rate of 10% from 30 g/100 ml extraction levels and in those applied at 15% from both 20 g/100 ml and 30 g/100 ml extraction levels likewise that of the positive control (Tables 1 and 2).

The correlations among the treatments solvent extracts of the tested plants leaves applied at different dosage and the efficacy parameters measured were found to be highly significant. The correlations between the various treatments of solvent extracts of the tested plants leaves and the various parameters measured (the number of F1 progeny emerged, percentage grain damage and weight loss) were negative. However, they were strongly positive between F1 progeny produced and percent grain damage and weight loss of all treatments of the tested plants solvent extracts (Tables 3 and 4).



a)



b)

**Figure 1.** Mean% mortality (mean ± SE) of maize weevil adult exposed in grains treated with *C. aurea* leaf solvent extracts applied at a rate of: (a) 20 g/100 ml and (b) 30 g/100 ml.

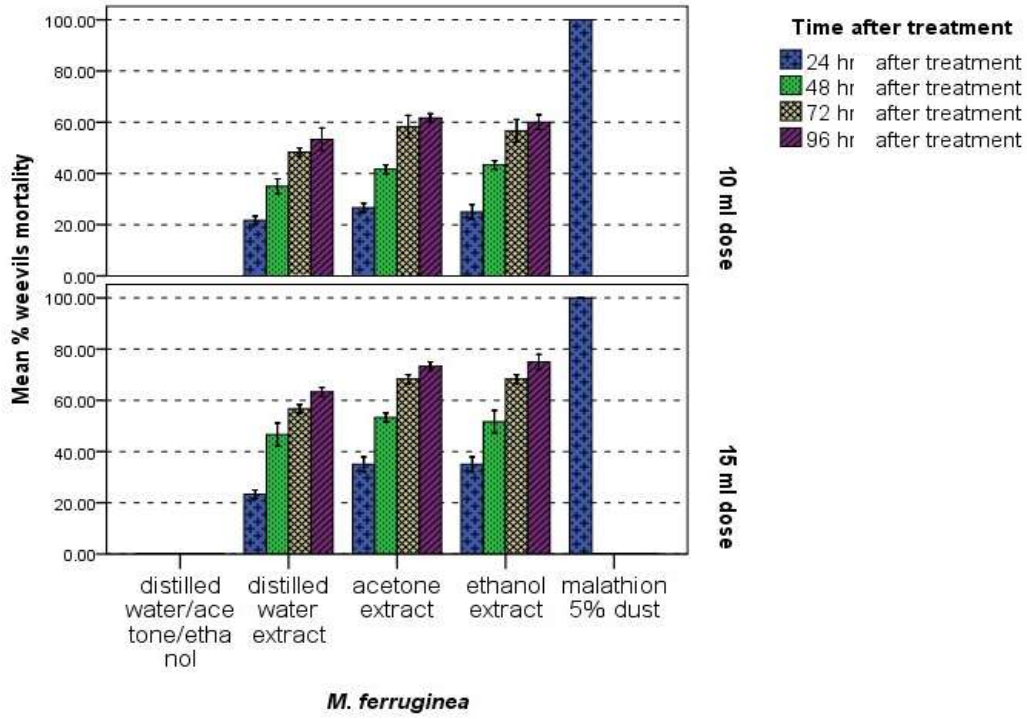
**DISCUSSION**

The current study revealed that the percentage of adult weevil’s mortality was increased ( $p \leq 0.001$ ) significantly with increased dosage (concentration), extraction level, and exposure time after treatment for both tested botanicals in general in the bioassay tested. This result is in line with the findings of Gebreselassie and Getu

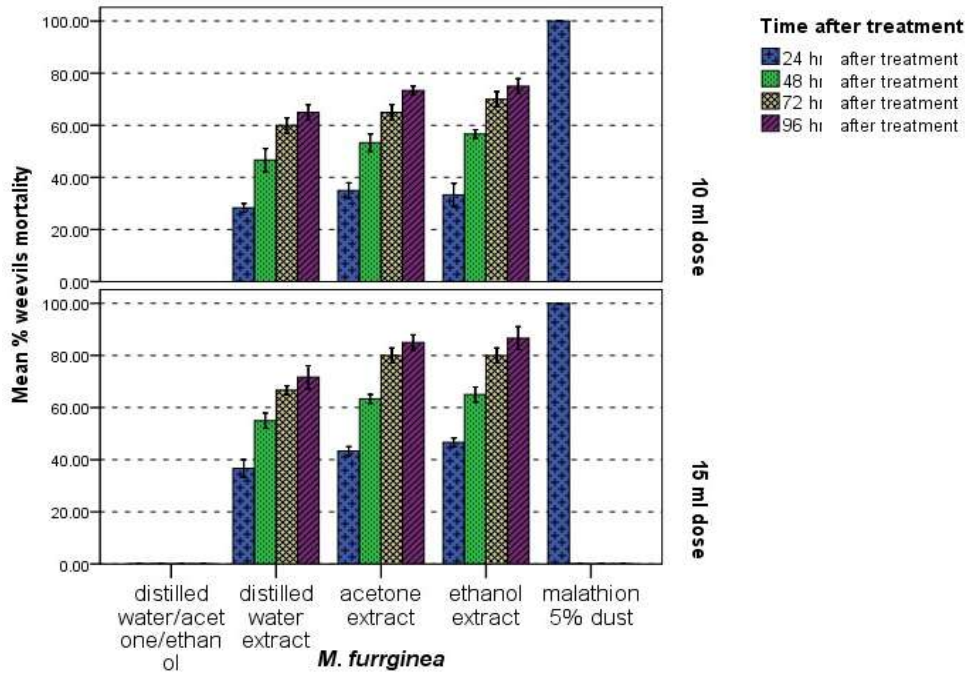
(2009), Zewde and Jembere (2010), Qwarse (2015) and Gebre-Egziabiher (2016) in which mortality effect of botanicals were indicated to be dose and exposure time dependent.

The present study also revealed that all polar solvent extracts (distilled water, ethanol and acetone extracts) of the tested botanicals leaves at tested rates (10 and 15 ml) from tested extraction levels induced significant toxic





a)



b)

**Figure 2.** Mean% mortality (mean  $\pm$  SE) of maize weevil adult exposed in grains treated with *M. ferruginea* leaf solvent extracts applied at a rate of: (a) 20 g/100 ml and (b) 30 g/100 ml. *C. aurea* and *M. ferruginea* Leave's Solvent Extracts on F1 Progeny, % Protection, % Grain.

effect against *S. zeamais* than negative control. This suggests the presence of more polar solvent soluble phytochemicals in leaves of *C. aurea* and *M. ferruginea* which are responsible higher weevil's mortality and as

most of them probably might be polar since like dissolves like. 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

**Table 1.** Mean number of F1 progeny produced, percent protection, grain damage and weight loss caused by *S. zeamais* on maize grains treated with *C. aurea* solvent extracts.

Treatment	Level of extraction	Dosage (ml/100 g)	Mean number of F1 progeny	Percent protection	Grain damage (%)	Weight loss (%)
Ethanol	20	10	1.67±1.67 <sup>ab</sup>	94.72	1±0.588 <sup>b</sup>	0.13±0.01 <sup>a</sup>
		15	0.00±0.00 <sup>a</sup>	100.00	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
	30	10	0.00±0.00 <sup>a</sup>	100.00	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
		15	0.00±0.00 <sup>a</sup>	100.00	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
Acetone	20	10	1.67±1.67 <sup>ab</sup>	94.72	1±0.588 <sup>b</sup>	0.13±0.01 <sup>a</sup>
		15	0.00±0.00 <sup>a</sup>	100.00	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
	30	10	0.00±0.00 <sup>a</sup>	100.00	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
		15	0.00±0.00 <sup>a</sup>	100.00	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
Distilled water	20	10	3.33±1.67 <sup>b</sup>	89.58	1.33±0.67 <sup>b</sup>	0.28±0.03 <sup>b</sup>
		15	0.00±0.00 <sup>a</sup>	100.00	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
	30	10	0.00±0.00 <sup>a</sup>	100.00	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
		15	0.00±0.00 <sup>a</sup>	100.00	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
Ethanol/Acetone/Distilled water	0	15	31.67±1.67 <sup>c</sup>	0.00	11.67±0.34 <sup>c</sup>	5.30±0.14 <sup>c</sup>
Malathion 5% dust	0	0.05	0.00 <sup>a</sup>	100	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>

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

**Table 2.** Mean number of F1 progeny produced, percent protection, grain damage and weight loss caused by *S. zeamais* on maize grains treated with *M. ferruginea* solvent extracts.

Treatment	Level of extraction (g/100 ml)	Dosage (ml/100 g)	Mean number of F1 progeny	Protection (%)	Grain damage (%)	Weight loss (%)
Ethanol	20	10	3.33±1.67 <sup>b</sup>	89.49	1.67±0.88 <sup>ab</sup>	0.13±0.01 <sup>a</sup>
		15	0.00±0.00	100.00	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
	30	10	1.67±1.67 <sup>ab</sup>	94.73	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
		15	0.00±0.00 <sup>a</sup>	100.00	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
Acetone	20	10	3.33±1.67 <sup>b</sup>	89.49	2.33±1.2 <sup>b</sup>	0.23±0.03 <sup>ab</sup>
		15	0.00±0.00 <sup>a</sup>	10.000	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
	30	10	3.33±1.67 <sup>b</sup>	89.49	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
		15	0.00±0.00 <sup>a</sup>	100.00	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
Distilled water	20	10	6.67±1.67 <sup>d</sup>	78.94	2.67±1.33 <sup>b</sup>	0.42±0.01 <sup>b</sup>
		15	3.33±1.67 <sup>b</sup>	89.49	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
	30	10	5.00±2.89 <sup>c</sup>	84.21	0.00±0.00 <sup>a</sup>	0.32±0.01 <sup>ab</sup>
		15	0.00±0.00 <sup>a</sup>	100.00	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
Ethanol/Acetone/Distilled water	0	15	31.67±1.67 <sup>e</sup>	0.00	11.67±0.34 <sup>c</sup>	5.30±0.14 <sup>c</sup>
Malathion 5% dust	0	0.05	0.00 <sup>a</sup>	100	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>

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



**Table 3.** Correlation among efficacy determining parameters of Ca solvent extracts.

Efficacy parameter	CA S. ext dose	F1 ext 1	F1 ext 2	GD ext 1	GD ext 2	WL ext 1	WL ext 2
CA S. ext dose	1						
F1 ext 1	-0.380*	1					
F1 ext 2	0.000	-	1				
GD ext 1	-0.073	0.652**	-	1			
GD ext 2	0.000	-	0.966**	-	1		
WL ext 1	-0.371*	1.000**	-	0.638**	-	1	
WL ext 2	0.000	-	0.973**	-	0.998**	-	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, WL: weight loss, ext 1: extraction level of 20 g/100 ml and ext 2 = extraction level of 30 g/100 ml.

**Table 4.** Correlation among efficacy determining parameters of mf solvent extracts

Efficacy parameter	MF S. ext dose	F1 ext 1	F1 ext 2	GDs ext 1	GD ext 2	WL ext 1	WL ext 2
MF S. ext dose	1						
F1 ext 1	-0.380*	1					
F1 ext 2	0.000	-	1				
GD ext 1	-0.098	0.480**	-	1			
GD ext 2	0.000	-	0.966**	-	1		
WL ext 1	-0.033	0.655**	-	0.735**	-	1	
WL ext 2	0.000	-	0.973**	-	0.998**	-	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. MF: *Milletia ferruginea*, GD: grain damage, WL: weight loss, ext 1: extraction level of 20 g/100 ml and ext 2: extraction level of 30 g/100 ml.

polar in nature after he studied castor bean plant against ectoparasites of animals. Jembere et al. (2005) also indicated that high *Zabrotes subfasciatus* mortality was caused by *M. ferruginea* water extract that probably might be due to the presence high water soluble chemicals in the seeds of it. 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 polar solvent extract treatments (distilled water, ethanol and acetone extracts) of the two tested botanicals also caused significantly higher mortality of adult *S. zeamais* than negative control at all dates after treatment. They also induced significantly higher inhibition in F1 progeny emergence, as well as significantly higher reduction in grain damage and weight loss than negative control in about 2 months storage period. This higher efficacy the crude extracts may be attributed due to either the toxic or repellent effects of phytochemicals in the tested plants or starvation and interference with respiration due to suffocation of maize

weevils. This result thus, suggests the potency of both the solvent extracts of the tested plants in protecting maize grains against weevils. Toxicity caused by crude extracts of the two botanicals tested against maize weevils in the current study was also in accordance to the result of pervious researchers (Kasa and Tadese, 1996; Jembere et al., 2005; Zewde and Jembere 2010; Gebreselassie and Getu, 2009; Getu, 2014).

In the present study, the efficacy of the tested botanicals in weevil's mortality also varied ( $P \leq 0.05$ ) significantly with the type of solvent used for extraction; the highest being occurred in ethanol extracts, followed by in acetone and in distilled water extracts. This result also agrees with finding of Jembere (2002) in which the water extracts of *M. ferruginea* was indicated to be the 3rd effective, 3 days after post treatment exposure, following acetone and ethanol extracts against maize weevils. In similar manner, Fredrick (2012) also indicated that the deleterious effects of the plant extracts against the maize weevil varied with the type of solvent extract applied.

The aforementioned highest efficacy in ethanol might also be probably due to its broad solubility properties of organic compounds of the tested botanicals. In accordance with this, it was also shown that ethanol is an accepted solvent for contact application because of its broad solubility properties and low toxicity, among the

various solvents (water, ethanol, acetone, petroleum ether and others) that have been used in the preparation of plants extracts for testing their toxicity to insect pests (Amoh, 2010). Similarly, Koffi et al. (2010) also showed that ethanol extract were found to be more effective than aqueous extracts of the same plant in general, as a result of higher solubility of organic compounds in ethanol.

## Conclusion

In conclusion, all the tested polar solvent extracts (distilled water, ethanol and acetone extracts) of *C. aurea* and *M. ferruginea* were potent in protecting maize grains against maize weevils attack at a rate of 10 and 15%. This in turn confirmed the presence of possibility to exploit the potential of *C. aurea* and *M. ferruginea* in the management of *S. zeamais* under substance farmer's storage conditions. Thus, solvent extracts, particularly water extracts of *C. aurea* and *M. ferruginea* which is cheap relatively can be used for management of maize weevils in 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 the outcomes this study.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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