

Efficacy of the ethanol extracts of the leaves of some plant species on the mortality of maize weevils, *Sitophilus zeamais* (Curculionidae: Coleoptera) and prevention of grain damage

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ABSTRACT

Sitophilus zeamais (maize weevil) damages maize, a major cereal crop in Ethiopia. A study was conducted to determine the anti-weevil activities of ethanol extracts of leaves of *Juniperus procera*, *Phytolacca dodecandra*, *Ostegia integrifolia*, *Sansevieria trifasciata*, and *Eucalyptus globulus*. Extraction was done using 70% ethanol. Two types of tests (toxicity and efficacy) were conducted using three doses, i.e., 50, 75, and 100 ml/kg of each plant. In the toxicity test, weevil mortality was measured at the 6th, 12th, 24th, 48th, and 72nd hours, then weekly for five weeks. For efficacy test, weevil mortality and grain damage (weight loss, percent holes, and percent germination) were assessed three months after treatment. *J. procera* and *P. dodecandra* were more toxic (96.6% and 96.4% mortality rate, respectively). The 50 and 75 ml/kg doses of *E. globulus*, 75 and 100 ml/kg doses of *J. procera*, of *P. dodecandra*, and of *S. trifasciata* killed 100% of the weevils; in the control mortality was 23.3%. Grain weight loss was high in the control (7.8%) followed by grains treated by 75 ml/kg extracts of *O. integrifolia* (5.9%). Percentage holes was high in the control (70%) followed by 75 ml/kg extracts of *O. integrifolia* (26.7%). Germination was high in grains treated with 50 and 75 ml/kg doses of *J. procera*, *E. globulus*, *S. trifasciata*, and *P. dodecandra* in descending order. The 75 and 100 ml/kg extracts of *J. procera*, *E. globulus*, *S. trifasciata*, and *P. dodecandra* were good candidates in the control of maize weevils with limitations of allelopathy in the 100 ml/kg.

Keywords: *Juniperus procera*, Maize weevil, *Ostegia integrifolia*, *Phytolacca dodecandra*, *Sansevieria trifasciata*, *Sitophilus zeamais*.

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INTRODUCTION

Zea mays (maize or corn) is one of the vital cereal grains widely cultivated and consumed in Africa. Despite its extensive utilization as human and

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animal feed, its productivity is hindered by pest infestation. Annually, more than \$100 billion is lost due to maize grain damage by pests such as weevils (Edelduok *et al.*, 2015). Maize infestation by the larvae and adults of weevil begins in the field and most damage occurs in stores (Khaliq *et al.*, 2014). Damage of maize grains by weevils results in food shortage, increased poverty, low nutritional value, increased malnutrition, reduced grain weight and market values, and reduced germination (Suleiman *et al.*, 2015). Natural pesticides (botanicals), directly extracted from plants, have the potential to replace the otherwise unfriendly synthetic pesticides (Qwarse *et al.*, 2016) and serve as alternatives to synthetic pesticides (Hikal *et al.*, 2017). In Ethiopia, farmers traditionally use non-chemical pest control methods (Gebeyaw Tilahun, 2015). They use local herbs like *Datura stramonium*, *Carissa schimperi*, *Croton macrostachys*, *Phytolacca dodecandra*, *Tagetes minuta*, *Weinia longiflora*, and *Allium sativum* to reduce storage pest infestation by *S. zeamais* (Gebeyaw Tilahun, 2015; Hikal *et al.*, 2017).

The mechanism of action of plant extracts varies with the type of plant. Extracts can act as repellents (Kareru *et al.*, 2013), as suppressants of reproductive behavior of the pest insects and reducing their fecundity and fertility (Nasiru *et al.*, 2016) or interfering with their life cycle (Kareru *et al.*, 2013). The insecticidal activities of plants are due to their secondary metabolites such as alkaloids, phenolics, tannins, and terpenoids (Perera *et al.*, 2017). Previous reports showed different extracts of *P. dodecandra* (*Phytolaccaceae*) (Qwarse *et al.*, 2016), *S. trifasciata* (*Ruscaceae*) (Potenza *et al.*, 2005), *J. procera* (Karunamoorthi *et al.*, 2014), and *E. globulus* (*Myrtaceae*) (Mandudzi and Edziwa, 2016) have some insecticidal properties against *S. zeamais*. The present study, thus, was aimed at determining the efficacy of *P. dodecandra*, *E. globulus*, *O. integrifolia*, *J. procera*, and *S. trifasciata* leaf extracts on weevil mortality, grain loss due to weevil damage, and seed germination of maize.

MATERIALS AND METHODS

Description of the study area

The study was carried out in Bahir Dar University, Biomedical Research Laboratory, Bahir Dar, Ethiopia. The city of Bahir Dar is located in northwestern Ethiopia at an altitude of 1800 m above sea level, at 11°38' North latitude and 37°15' East longitude. It enjoys a mean annual rainfall is 1419 mm and temperature of 19.6 °C (Mitiku Muluye *et al.*, 2017).

Study Design

Weevils were subjected to three levels of each plant extract (50 mL/kg, 75 mL/kg, and 100 mL/kg) in a completely randomized design (CRD) in the laboratory and each extract level was replicated three times.

Acquisition of grains to be used for rearing test organisms

Some 50 kg of clean maize grain (not treated with chemicals) was purchased from the Bahir Dar market. The grains were disinfected in the oven at 40 °C for 4 h. The disinfected grains were kept in the laboratory in tightly closed plastic beakers before use. Dirt from the maize was removed through winnowing.

Collection and rearing of the maize weevils

Adult weevils were collected from farmers and traders' stores in Bahir Dar and Merawi (35 km southwest from Bahir Dar). The weevils were mixed and released into maize grains reserved for rearing the initial weevil stock. First, they were mass-reared in separate containers (tin cans covered with muslin cloth at the top). They were introduced into the maize seeds and the container was kept at room temperature for reproduction to take place. Unsexed adult weevils were introduced into jars containing uninfested maize grains. The jars were covered with a piece of muslin cloth held in place by a rubber band to prevent the weevils from escaping. The emerging (new generation) weevils (0 to 1-week old) were used for a series of experiments that followed (Edelduok *et al.*, 2015).

Collection, preparation, and extraction of plant materials

Arial parts of plants were collected between October and December 2017 from different places in the Amhara region, Ethiopia. *E. globulus* was collected from the Debre Markos area, *O. integrifolia* from Yejube, *P. dodecandra* from Abay Gorge, near the Ethiopian Grand Renaissance Dam, *J. procera* from Dangila, and *S. trifasciata* from Wollo, near Sirinka. These plants were chosen based on recommendations from local people (informants). The collected plant parts were air-dried under a shade at room temperature in the laboratory, pulverized into powders, and then active ingredients extracted using 70% ethanol (1:5 gm/ml ratio) and placed on a shaker for 21 days (Mandal *et al.*, 1999). After 21 days, the extracts were strained using sterile cotton and filter paper. The filtrates then were stored in sterile brown reagent bottles and placed in a refrigerator at 4 °C until used for experimentation.

Phytochemical analysis

The chemical constituents of the extracts were qualitatively screened for the presence of the phytochemical groups/classes according to the method described before (Jaradat *et al.*, 2015) and the standard technique (Sheela, 2013). The active constituents of the extracts were qualitatively confirmed for the presence of alkaloids, terpenoids, tannins, saponins, phenolics, flavonoids and glycosides. Test for chemical constituents was done as described by Jaradat *et al.* (2015) (phenols), Zohra *et al.* (2012) (tannins), Ayoola *et al.* (2008) (flavonoids), Diouf *et al.* (2016) (saponins), Karthishwaran *et al.*, (2010) (terpenoids), and Zohra *et al.* (2012) and Pandey and Tripathi (2014) (alkaloids).

Toxicity test against maize weevils

The contact method was used to test the toxicity of the extracts against maize weevil using three different concentrations (50, 75, and 100 ml/kg grain). Twenty grams of maize grains were put into each of 93 plastic vials. Extracts were added and the mixtures were shaken for five minutes to ensure uniform coating of grains. Ten adult *S. zeamais* were introduced into each vial and then covered with the muslin cloth. In the blank control, twenty grams of maize and ten adult insects were introduced. Three replicates were made for each treatment. The weevil mortality was assessed at the 6th, 12th, 24th, 48th, 72nd hours, and weekly up to five weeks after the insects were introduced. The insects were considered dead when they didn't respond to gentle probing with a sharp pin at the abdomen (Ouko *et al.*, 2017). The number of dead and live insects was recorded and percentage mortality was calculated.

Testing the efficacy of the plant extracts on weevil mortality, seed damage, and seed germination

A random sample of 200 g of maize seeds was taken and kept in each of the 93 plastic beakers. The maize samples were treated with 50, 75, and 100 ml/kg of the 70% leaf ethanol extracts of the *P. dodocandra*, *S. trifasciata*, *J. procera*, and *E. globulus*. Each dose was applied in triplicates. Three of the grain samples were not treated by any plant extract and served as controls. Twenty adult weevils were introduced into the treated and control grains and the beakers were covered with a muslin cloth to facilitate proper aeration and to prevent the escape of weevils. Three months after treatment, the weevil mortality and grain damage (weight loss, percent holes, and percent germination) were measured.

Mortality test: Percent weevil mortality was calculated using the formula (Muzemu *et al.*, 2013)

$$\text{Parent weevil mortality} = \frac{ND}{TN} \times 100\%$$

Where ND = **number** of dead weevils and TN = total number of weevils initially introduced into the flask.

Grain damage test: Grain weight loss due to infestation with *S. zeamais* was calculated using the formula used by Melaku Wale and Tesfaye Mengie (2017).

$$\text{Weight loss \%} = \frac{(IDW - FDW)}{IDW} \times 100\%$$

Where IDW = Initial dry weight and FDW = Final dry weight.

Besides, the extent of weevil damage was assessed by counting the number of seeds with holes. Grains with exit-holes were counted and the percentage holes (PH) of the weevils to the grains were calculated using the formula used by Melaku Wale and Tesfaye Mengie (2017).

$$PH = \frac{TNGH}{TNG} \times 100\%$$

Where PH stands for percentage holes, TNGH for the total number of treated grains with holes, TNG for the total number of grains.

Germination test: Surface sterilized maize seeds were treated with all plant extracts at different concentrations (10, 15, and 20 ml). After three months, the germination test was carried out in a completely randomized design by taking 20 seeds from the five treatments of each plant product. A random sample of 20 seeds was taken from each treatment set and placed each in a Petri dish (11 cm diameter) lined with cotton. The Petri dishes were covered to prevent loss of moisture and contamination and placed in an environmental chamber. The temperature was maintained at 23 °C and the dishes exposed to constant light conditions. After one week the number of seeds germinated was counted and recorded. Germination percentage was calculated using the formula given below (Muzemu *et al.*, 2013).

$$\text{Germination percentage \%} = \frac{G1}{G2} \times 100$$

Where $G1$ = total germinated grain, $G2$ = total grain in Petri dish.

Data analysis

Weevil mortality, number of holes per seed, grain loss, and rate of seed germination against the leaves extracts of the test plants were subjected to Analysis of Variance (ANOVA) procedure using the SAS statistical program (SAS, 2003). Means were compared using the Tukey HSD test at $\alpha=0.05$.

RESULTS

Phytochemical constituents of the plant extracts

Qualitative phytochemical analysis of secondary metabolites showed that *P. dodecandra* contained alkaloids, terpenoids, tannins, saponins, flavonoids, and phenols. *J. procera* contained all the above except saponins, *E. globulus* contained all the above except flavonoids, and *O. integrifolia* and *S. trifasciata* have all the above except terpenoids (Appendix 1).

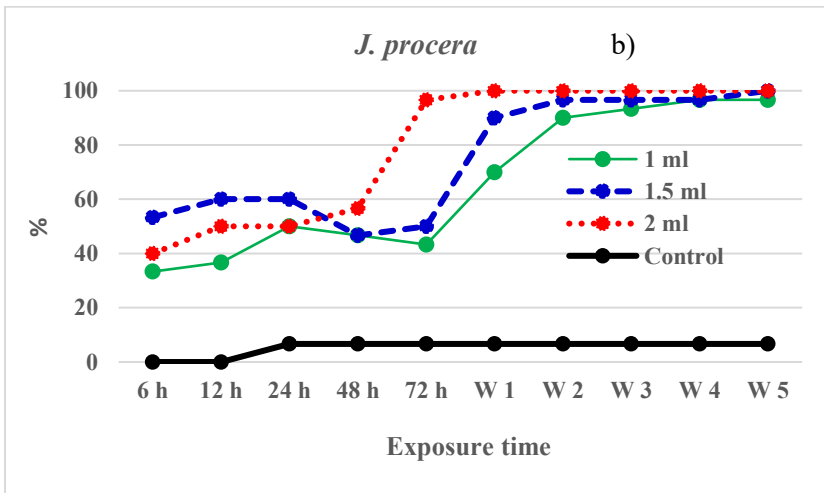
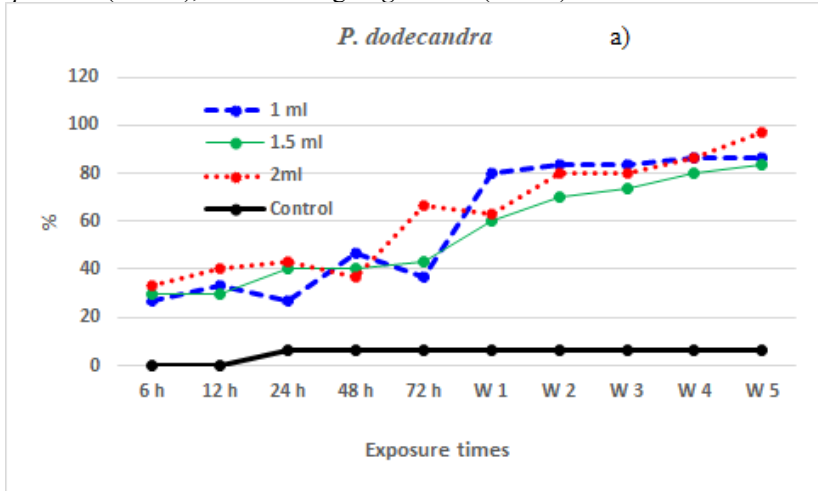
Toxicity test against maize weevils

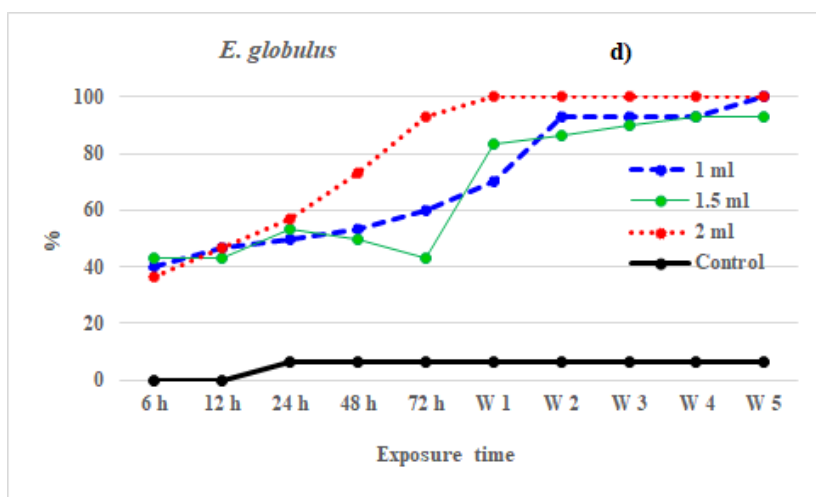
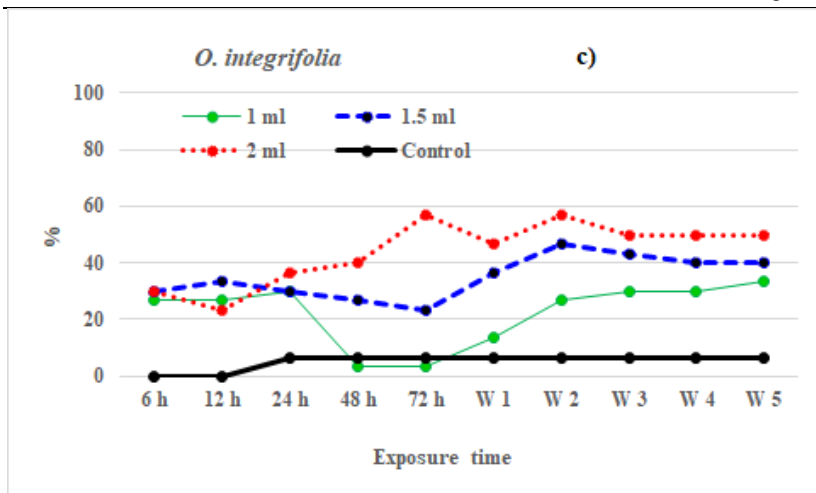
After six and 12 h exposure, *J. procera* extracts showed significantly higher mortality than the other plant extracts (Figure 1b). As exposure time increased, the effectiveness of *J. procera*, *P. dodecandra*, *S. trifasciata*, and *E. globulus* increased. The result of *O. integrifolia* (and the control) was low compared to the other four plant extracts. Based on potency, the six treatments could be grouped into two, i.e., *J. procera*, *P. dodecandra*, *S. trifasciata*, and *E. globulus* (in decreasing order of effectiveness) in one group and *O. integrifolia* and the control in another. The 100 ml/kg dose of the 70% ethanol extract of all plants was most effective. The trend of weevil mortality due to the 70% ethanol extract at 100 ml/kg doses was 100% (*J. procera*, *E. globulus*, and *S. trifasciata*); close to 100% (*P. dodecandra*); and 60% (*O. integrifolia*) (Figures 1a-c).

Weevil mortality and measures of grain damage after extract treatment

Three months after treatment by the 70% extract, the percentages of weevil mortality due to the 50, 75, and 100 ml/kg extracts of *E. globulus*, *J. procera*, *P. dodecandra*, and *S. trifasciata* were significantly higher than that of the 50 and 75 ml/kg extracts of *O. integrifolia* and the control. But mortality due to all doses of all the extracts was significantly higher than that of the control (Table 1). Grain damage was measured indirectly by measuring weight loss, percent holes in grains, and germination three months after extract application. Weight loss in the control grains (7.8%) was significantly higher than grains treated with plant extracts except for the lower doses of *O. integrifolia* (5.9%). Similarly, the percentage of holes in the control group (70%) was significantly higher than grains treated by all the test doses of the extracts. In contrast, significantly low germination was observed in grains treated with 100 ml/kg of *P. dodecandra* (3.3%); 100 ml/kg of *J. procera* (5%); control (13.3%); 50, 70, 100 mg/kg of *O. integrifolia* (15%, 15%, and 16.7% respectively) and the maximum significant germination

was observed in grains treated by the 50 ml/kg *J. procera* (60%), 75 ml/kg *J. procera* (58.3%), and 50 ml/kg *E. globulus* (56.7%).





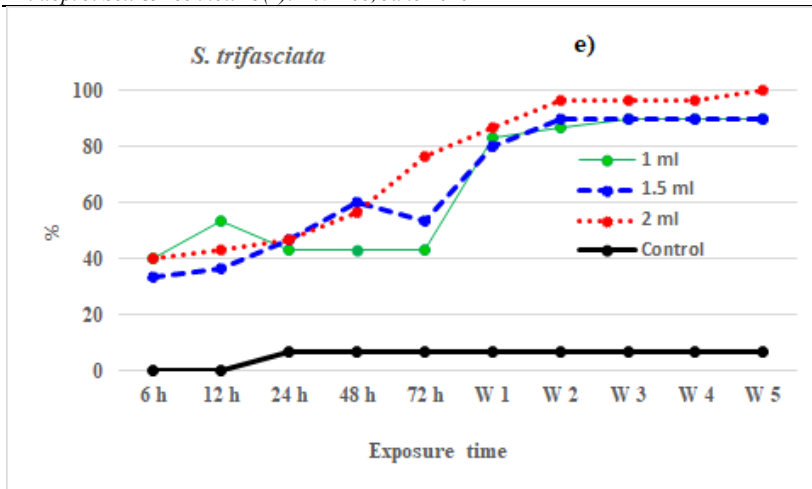


Figure 1a-e. Effect of the different doses of the plant extracts against maize weevil. (Note: h = hour; W = week)

DISCUSSION

In the current study, leaf extracts of the five plant species had alkaloids, tannins, and phenols. Similar findings were reported before (Anbu *et al.*, 2009; Teponno *et al.*, 2016). In a previous study, preliminary phytochemical screening of the leaf extracts of *S. trifasciata* had alkaloids, flavonoids, saponins, glycosides, terpenoids, tannins, proteins, and carbohydrates (Anbu *et al.*, 2009). In contrast, Dagnew Bitew (2015) failed to find tannins on extracts of *J. procera*, a condition that might happen as a result of differences in the method of extraction or the species lacks this metabolite.

Terpenoids were detected in the ethanolic extract of leaves and roots of *P. dodecandra*, *E. globulus*, *J. procera* (Qwarse *et al.*, 2016). The presence of terpenoids revealed that the plants can act mainly as antifeedant, growth disruptors, and toxicants towards insects (Adeniyi *et al.*, 2010). But the absence of these phytochemicals in the extracts of *S. trifasciata*, and *O. integrifolia* may be the cause of marked reduction of their insecticidal properties. Saponins were found in all the ethanolic extracts of the leaves of *P. dodecandra*, *E. globulus*, *O. integrifolia*, and *S. trifasciata* but not in *J. procera*. In the present study, flavonoids were detected in the extracts of *P. dodecandra*, *O. integrifolia*, *J. procera*, and *S. trifasciata* but not in the extracts of *E. globulus* which may be responsible for their insecticidal properties (Adeniyi *et al.*, 2010).

The initial bio-potential of plant extracts was mainly based on concentration and exposure period (Figure 1a-e). All plants tested were not effective during the first 48 hrs. This may be because botanicals do not have immediate knockdown effects as do synthetic pesticides, but the poisoned insects will stop feeding almost immediately (<https://hgic.clemson.edu/factsheet/less-toxic-insecticides/>). However, after 72 hours, the rate of mortality increased sharply. Maximum weevil mortality was recorded for the leaf extracts of *J. procera*, *P. dodecandra*, *S. trifasciata*, and *E. globulus*, but was less for *O. integrifolia*. This may be due to the presence of terpenoids in the former plant extracts. Terpenoids in plants have antifeedant and growth disruptor properties towards insects (Adeniyi *et al.*, 2010).

J. procera and *P. dodecandra* had the highest mortality rate within five weeks of exposure at all concentrations. The effectiveness of *J. procera* against insects has also been done by other researchers. For example, it was highly effective against mosquito larvae (*An. arabiensis*) (Karunamoorthi *et al.*, 2014). *P. dodecandra* was the second-best performer next to *J. procera* against maize weevils. *E. globulus* was also effective against snails, mosquito larvae, houseflies, and rodent pesticides such as rats (Qwarse *et al.*, 2016).

The next effective plant species was *S. trifasciata*. Similarly, it was effective against *S. zeamais* in previous work (Potenza *et al.*, 2005) followed by *E. globulus*. According to Mandudzi and Edziwa (2016), *E. globulus* had a repellent effect on the olfactory and gustatory system of *S. zeamais*. The order of mortality of the weevils was: *J. procera* > *P. dodecandra* > *S. trifasciata* > *E. globulus* > *O. integrifolia*. However, there was some difference in mortality between the doses of each plant extract. The 2 ml of each extract was the most effective of all. Ojebode *et al.* (2016) reported similar results in which 2% extract resulted in complete mortality. Toxicity has increased with increasing dose and time of exposure. *O. integrifolia* had little effect on weevil mortality when compared to the other plant extracts. The untreated grains had the lowest weevil mortality as compared with the extract-treated grains.

There was an increase in mortality with an increase in extract dose from 75 and 100 ml/kg grain. In another study, the effectiveness of *P. dodecandra* was also justified, whereby its application at a rate of 150 mg/ml concentration killed 98% of *S. zeamais* (Qwarse *et al.*, 2016). The presence of holes indicates that weevils have fed on grains. As a whole, 70% of the untreated and less than 1% of the *J. procera*, *P. dodecandra*, *S. trifasciata*, and *E. globulus* treated maize grains had holes at the end of the 3rd month. However, in grains treated with *O. integrifolia* extract, the percentage of

holes was 25.0%, 26.7%, and 6.7% for the doses of 50, 75, and 100 ml/kg grain, respectively. This implies that the extracts of *J. procera*, *P. dodecandra*, *S. trifasciata*, and *E. globulus* had a protective role against weevil infestation.

Grain weight loss in seeds treated with *J. procera*, *P. dodecandra*, *S. trifasciata*, and *E. globulus* extracts was significantly lower than grains treated with *O. integrifolia* and the control. Grain damage or weight loss recorded in the treated maize seeds with the four plant extracts was generally low. This result agrees with previous reports (Melaku Wale and Tesfaye Mengie, 2017). The development of weevils in the untreated grain was not hindered and they had a high rate of feeding (Cosmas *et al.*, 2012).

The percentage of seed germination in grains treated with *O. integrifolia* extract was significantly lower than seeds treated with *E. globulus* (50 and 75 ml/kg), *J. procera* (50 and 75 ml/kg), *P. dodecandra* (50 and 75 ml/kg), and *S. trifasciata* (50, 75, and 100 ml/kg) and was comparable to the control group. However, germination of seeds treated by the highest dose (100 ml/kg) of *E. globulus*, *J. procera*, and *P. dodecandra* was very low indicating the allelopathic effects of these extracts at the highest doses. Generally, germination of the seeds treated with the plant extracts was relatively low because of their allelopathic secondary metabolites like phenols. For example, *Eucalyptus* contains phenols (active allelochemicals) (Ziaebrahim *et al.*, 2007).

CONCLUSION

The evidence gathered from the current study confirmed that the leaf extracts of *J. procera*, *P. dodecandra*, *S. trifasciata*, and *E. globulus* protected weevil attack of maize grains and reduced grain damage. Therefore, these plants could be used as candidate anti-weevils in botanical preparations. In contrast, compared to the other four plant species, *O. integrifolia* did not protect seeds from the weevil attack, and worse, it reduced seed germination.

ACKNOWLEDGMENTS

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Table 1. Effect of different plants extracts against maize weevils (mortality and damage).

Dose (ml/kg)	Weevil mortality (%)	Measures of grain damage (%)		
		Weight loss	Holes	Germination
Control				
0	23.3 ± 5.24 ^f	7.8 ± 0.4 ^a	70.0 ± 3.3 ^a	13.3 ± 2.7 ^{e-g}
<i>E. globulus</i>				
50	100.0 ± 5.24 ^a	1.5 ± 0.4 ^g	0.0 ± 3.3 ^e	56.7 ± 2.7 ^{ab}
75	100.0 ± 5.24 ^a	1.5 ± 0.4 ^g	0.0 ± 3.3 ^e	45.0 ± 2.7 ^{bc}
100	98.3 ± 5.24 ^a	1.9 ± 0.4 ^g	0.0 ± 3.3 ^e	25.0 ± 2.7 ^{de}
<i>J. procera</i>				
50	98.3 ± 5.24 ^a	1.8 ± 0.4 ^g	1.7 ± 3.3 ^e	60.0 ± 2.7 ^{ab}
75	100.0 ± 5.24 ^a	1.0 ± 0.4 ^g	0.0 ± 3.3 ^e	58.3 ± 2.7 ^{ab}
100	100.0 ± 5.24 ^a	1.3 ± 0.4 ^g	0.0 ± 3.3 ^e	5.0 ± 2.7 ^{fg}
<i>O. integrifolia</i>				
50	66.7 ± 5.24 ^{b-d}	4.3 ± 0.4 ^{b-c}	25.0 ± 3.3 ^{b-d}	15.0 ± 2.7 ^{e-g}
75	45.0 ± 5.24 ^{d-f}	5.9 ± 0.4 ^{ab}	26.7 ± 3.3 ^{bc}	15.0 ± 2.7 ^{e-g}
100	80.0 ± 5.24 ^{a-c}	4.3 ± 0.4 ^{b-e}	6.7 ± 3.3 ^{de}	16.7 ± 2.7 ^{e-g}
<i>P. dodecandra</i>				
50	96.7 ± 5.24 ^a	1.6 ± 0.4 ^g	0.0 ± 3.3 ^e	50.0 ± 2.7 ^{a-c}
75	100.0 ± 5.24 ^a	1.3 ± 0.4 ^g	0.0 ± 3.3 ^e	46.7 ± 2.7 ^{bc}
100	100.0 ± 5.24 ^a	1.7 ± 0.4 ^g	0.0 ± 3.3 ^e	3.3 ± 2.7 ^g
<i>S. trifasciata</i>				
50	93.3 ± 5.24 ^{ab}	2.1 ± 0.4 ^{e-g}	0.0 ± 3.3 ^e	51.7 ± 2.7 ^{a-c}
75	100.0 ± 5.24 ^a	1.6 ± 0.4 ^g	0.0 ± 3.3 ^e	50.0 ± 2.7 ^{a-c}
100	100.0 ± 5.24 ^a	1.6 ± 0.4 ^g	0.0 ± 3.3 ^e	26.7 ± 2.7 ^{de}

Note: Similar letters in the column indicate not significant difference ($P=0.05$).

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Appendix 1. Phytochemical composition of the plant extracts.

Plant extract	Secondary metabolites					
	Alkaloids	Terpenoids	Tannins	Saponins	Flavonoids	Phenols
<i>J. procera</i>	+	+	+	-	+	+
<i>P. dodecandra</i>	+	+	+	+	+	+
<i>E. globulus</i>	+	+	+	+	-	+
<i>O. integrifolia</i>	+	-	+	+	+	+
<i>S. trifasciata</i>	+	-	+	+	+	+

Note: The qualitative results are expressed as (+) for the presence and (-) for the absence of phytochemicals.