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Biological Activity of Organic Leaf Extracts against the Maize Weevil, *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae)

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ABSTRACT

Laboratory studies were conducted in order to evaluate the bioactivity of methanolic leaf extracts of four plant species namely Euphorbia balsamifera Aiton, Lawsonia inermis L., Mitracarpus hirtus (L.) DC and Senna obtusifolia L. against Sitophilus zeamais Motsch. Ten 0 to 7 day old weevils were released in 20 g of disinfested sorghum grains treated with methanolic extracts of the botanicals in plastic bottles, at different concentrations of 2.5, 5 and 10% at 30^{o}C and 70% R.H. Adult mortalities of the weevils at 24, 48 and 72 hours after treatment (HAT) were determined. Percentage oviposition deterrence (POD) and adult emergence as well as grain weight losses were also assessed. Results showed that adult mortality of the weevils ranged from 50.00 ± 4.08 to 100.00 ± 0.00% within 72 HAT. POD of the extracts varied between 58.51 ± 2.04 and 94.68 ± 2.68, while no adult emergence was recorded in grains treated with the botanical extracts. Highest (2.96 ± 0.40%) weight losses among the treated grains were observed in 2.5% of S. obtusifolia, while 0.85 ± 0.49% was the least in 10.0% of E. balsamifera. Findings of the study have revealed that the botanical extracts could serve as stored sorghum protectants against S. zeamais infestations.

Keywords: *Adult emergence, Adult mortality, Botanicals, Grain damage, Oviposition deterrence, Sitophilus zeamais*

INTRODUCTION

Sitophilus zeamais infestations to cereals particularly maize and sorghum resulted in serious damage during storage (Wini et al., 2015; Loge, 2016; Shiberu and Negeri, 2017; Suleiman and Rugumamu, 2017). Up to 12.0 to 37.81% weight losses in maize caused by *S. zeamais* were reported (Arannilewa et al., 2006; Ibrahim et al., 2016), while it ranged from 8.30 to 29.33% in sorghum infested for two to four months (Babarinde et al., 2008; Goftishu and Belete, 2014).

Synthetic insecticides have been commonly used for controlling pests in stored products (Baributsa et al., 2017; Nayak and Daglish, 2017; Paudyal et al., 2017). However, the use of chemical insecticides has resulted in problems such as environmental pollution, health hazards, pest resurgence and increase in costs of application arising from the development of resistance to insecticides (Zhi et al., 2012; Cosmas et al., 2018). These problems necessitated a search for alternative, eco-friendly methods for management of insect pests of storage such as the use of plant materials as biopesticides (Suleiman, 2014; Suleiman et al., 2018). Effect of various plant materials has been evaluated against *S. zeamais* in stored grains with recorded success (Adedire et al., 2011; Ajayi, 2013; Suleiman, 2014; Wini et al., 2015).

Solvent extracts of different plant materials have recently been reported to cause high mortality of *S. zeamais* (Ibrahim et al., 2016; Ileke et al., 2016; Suleiman et al., 2018). It was reported that some plant species such *as Olax subscorpioidea* Oliver (Santalales: Olacaceae), *Aframomum melegueta* K. Schum (Zingiberales: Zingiberaceae) *Azadurachta indica* A. Juss (Sapindales: Meliaceea), and *Zingiber officinale* Rosce (Zingiberales: Zingiberaceae) caused adult mortality of *S. zeamais* which ranged from 18.35 to 88.35% within 3 to 45 days after exposure (Oni and Ogungbite, 2015; Tilahun and Daniel, 2016).

Investigations on the level of damage caused to sorghum grains in the store by *S. zeamais* are narrowly reported. However, Babarinde et al. (2008) reported that the weevil caused 8.3%

weight loss of sorghum grains. About 29.33 to 53.30% grain perforations in sorghum due to *S. zeamais* infestations were recorded by Goftishu and Belete (2014) and Suleiman (2014).

This study was carried out to investigate the potentiality of methanolic leaf extracts *of E. balsamifera, L. inermis, M. hirtus and S. obtusifolia* to manage *S. zeamais* infestations in stored sorghum grains.

MATERIALS AND METHODS

Mass Rearing of *S. zeamais*

One hundred adults of *S. zeamais* were obtained from infested sorghum grains and then introduced into two 500 ml rearing bottles containing 250 g disinfested grains of a local sorghum variety, "Farar Kaura". The bottles were covered with muslin cloth and secured with rubber bands. They were then kept in an incubator for oviposition at 30°C and 70% R.H. for seven days, after which the parents were removed. The bottles were maintained in the incubator under the same condition for adult emergence. Progeny of 0 – 7 days old were sieved and used for the experiments.

Preparation of the Extracts

Fresh leaves of *E. balsamifera, L. inermis, M. hirtus* and *S. obtusifolia* were shade-dried and pulverized into powder. Fifty grams of each of the plant powders was soaked in 200 ml of methanol in conical flasks. Mouths of the conical flasks were properly corked and kept for 48 hours. The mixture was separated using muslin cloth and filtered with Whatman No.1 filter papers following Suleiman et al. (2018). The filtrate was concentrated by evaporating excess solvent using rotary evaporator at 3 to 6 rpm for 8 hours. The resulting extract was stored in a refrigerator at 4°C (Abou-Elnaga, 2015).

Determination of Adult Mortality of *S. zeamais* in Treated Sorghum Grains

Crude extracts were diluted with methanol to make different concentrations of 2.5, 5 and 10%. Forty grams of sorghum grains were weighed and put into 250 ml plastic bottles. The grains were impregnated with 2 ml of the methanolic extracts at the three concentrations, while the same amount of the solvent only was added to the grains and served as the control. The grain mass was mixed thoroughly with the aid of glass rod and air-dried. Ten weevils were then released into each of the bottles and covered by using muslin cloth which was secured with rubber bands. The set-ups were inspected daily and dead weevils in each treatment were removed and recorded at 24, 48 and 72 hours after treatment (HAT).

Percent adult mortality was assessed as:

$$\text{Mortality (\%)} = \left(\frac{\text{Number of Dead Weevils}}{\text{Total Number of Weevils}}\right) \text{X } 100$$

Determination of Oviposition Deterrence of Methanolic Extracts on *S. zeamais*

The same set-up for mortality test was maintained to study the number of eggs deposited by female S. zeamais 14 days after their introduction in the treated and untreated sorghum. Twenty grains were randomly taken from sorghum treated with the extracts at varying concentrations of 2.5, 5 and 10% as well as the untreated ones. The grain samples were viewed under photo micrographic microscope after they had been stained with acid fuchsin for identification of egg plugs. Presence of cherry red egg plugs indicated the existence of eggs. The plugs were counted and their numbers were recorded accordingly.

Percentage of oviposition deterrence (POD) was calculated by the following formula as given by Vanmathi et al. (2012):

$$\text{POD} = \frac{E_C - E_t}{E_C} \text{ x } 100$$

Where:

POD = Percentage of oviposition deterrence;

E_c = Number of eggs laid in control grains; and

E_t = Number of eggs laid in treated grains.

Examination of Adult Emergence of *S. zeamais* in Treated Sorghum Grains

Grains were inspected daily after examination of egg plugs for adult emergence of the weevils. The emerging progenies from each bottle were removed daily, counted and recorded for 49 days after which observations stopped to avoid overlapping of generations.

Assessment of Grain Weight Loss (WL) in Treated Sorghum Infested by *S. zeamais*

Twenty grains from the extract treatments were sampled after 49 days of emergence of F_1 to assess weight losses according to FAO (1985) as follows:

$$\text{\% Weight Loss (WL)} = \frac{[\text{UaN} - (\text{U} + \text{D})]}{\text{UaN}} \text{ X } 100$$

Where:

U = weight of undamaged fraction in the sample;

N = total number of grains in the sample;

Ua = average weight of one undamaged grain;

D = weight of damaged fraction in the sample.

Experimental Design and Statistical Analysis

All treatments were arranged in a completely randomized design (CRD) and replicated four times. Data generated were tested for normality using Shapiro-Wilk and Jacque-Bera normality tests (Sokal and Rohlf, 1995; Le (2003). Data from adult mortality, POD, and grain WL were subjected to two-way ANOVA using GraphPad Prism (version 7.03). Significantly different means were separated by Bonferroni's multiple comparisons tests (Le, 2003). All analyses were performed as statistically significant $p < 0.05$.

Results

Adult Mortality of *S. zeamais* in Sorghum Grains Treated with Methanolic Leaf Extracts

Methanolic leaf extracts of the selected plant species applied at different concentrations exhibited varying percentage mortalities of *S. zeamais* within 24, 48 and 72 HAT. The highest mortality was recorded in grains treated *with E. balsamifera*, while the least was in those treated with *S. obtusifolia.* No mortality was observed in untreated sorghum during the study period.

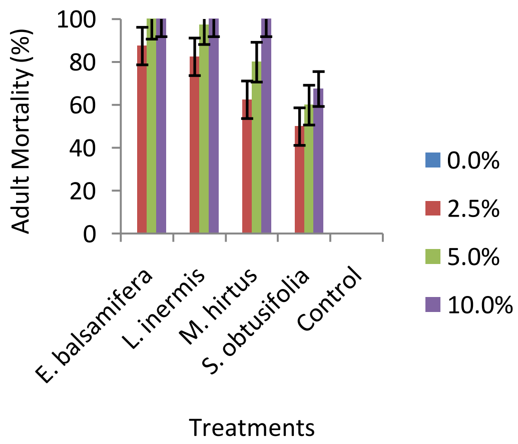

Figure1. *Percentage adult mortality of S. zeamais in treated sorghum grains at 72 HAT*

The mortalities differed significantly among the botanicals ($F_{4, 45}$ = 614.60, $p < 0.0001$) as well as within the varying concentrations of the botanicals ($F_{2, 45}$ = 48.65, $p < 0.0001$) at the end of 72 HAT. Bonferroni's multiple comparisons showed that the mortalities caused by both *E. balsamifera* and *L. inermis* were the same and higher than *M. hirtus* and *S. obtusifolia* at all the three concentrations.

Oviposition Deterrence of Methanolic Botanical Extracts against *S. zeamais*

The numbers of egg plugs of *S. zeamais* in grains treated with methanolic leaf extract of the test plants were decreased with increase in the concentrations. The highest POD (94.68 ± 2.68) was recorded in grains treated with *E. balsamifera* at 10%, while the least (58.51 ± 2.04%) was in *S. obtusifolia* at 2.5% (Figure 2). The POD of *L. inermis* and *M. hirtus* ranged from 68.09 ± 2.75 to 75.53 ± 5.32 and 64.89 ± 7.65 to 81.91 ± 4.72, respectively, while it was zero in the control.

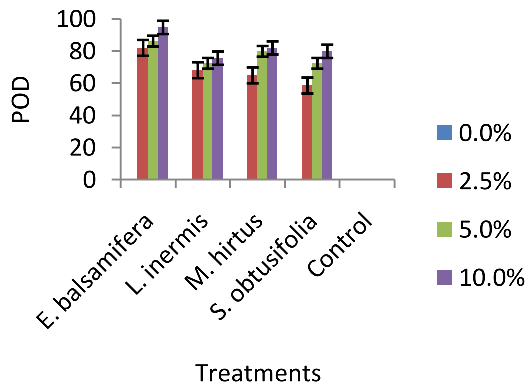

Figure2. *Percentage oviposition deterrence (POD) of methanolic extracts of some botanicals on S. zeamais*

Two-way ANOVA showed that there was a highly significant difference in POD among the botanicals ($F_{4, 45}$ = 237.90, $p < 0.0001$). Similarly, a highly significant difference ($F_{2, 45}$ = 11.48, $p < 0.0001$) existed in POD among the varying concentrations. Multiple comparisons test showed that at 2.5%, the mean POD of *E. balsamifera* was greater than the other three botanicals. At 5%, *E. balsamifera* had statistically the same POD as M. hirtus and was higher than that of *L. inermis* and *S. obtusifolia.* The comparisons test further revealed that the POD of *E. balsamifera* was significantly higher than those from *L. inermis, M. hirtus* and *S. obtusifolia* at 10%.

Emergence of Adult *S. zeamais* in Treated Sorghum Grains

There was no emergence of adult *S. zeamais* in sorghum grains treated with methanolic extracts of the selected botanicals. However, the number of emerged weevils in untreated grains was 165.80 ± 4.13.

Weight Losses(WL) of Sorghum Grains Treated with Methanolic Leaf Extracts

Sorghum grains treated with methanolic extracts of *E. balsamifera* lost 0.85 ± 0.49 to 2.25 ± 0.43% of their weight to *S. zeamais*. The WL in *L. inermis*, *M. hirtus* and *S. obtusifolia* treatments ranged from 1.34 ± 0.45 to 2.25 ± 0.46%, 1.78 ± 0.06 to 2.57 ± 0.41% and 1.74 ± 0.08 to 2.96 ± 0.40%, respectively.

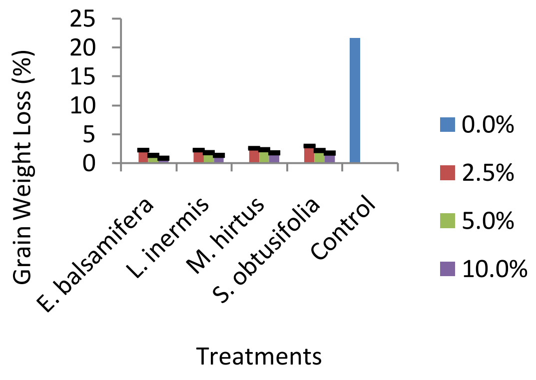

Figure3. *Percentage weight loss in treated sorghum grains caused by S. zeamais*

There was a highly significant difference in WL among the botanicals ($F_{4, 45}$ = 395.00, p < 0.0001). Multiple comparisons test showed that the difference existed between WL of grains treated with each of the botanicals and that in untreated ones only. Further, No significant difference was recorded in grain WL among the varying concentrations ($F_{2, 45}$ = 1.609, p = 0.2114). Bonferroni's multiple comparisons test revealed that WL in grains treated with *E. balsamifera* at 2.5, 5 and 10% was respectively lower than in the rest of the botanicals, though statistically the same.

Discussion

Effect of Methanolic Leaf Extracts on Adult Mortality of *S. zeamais*

Application of methanolic leaf extracts of all the botanicals offered a highly significant effect on adult mortality of *S. zeamais* in sorghum grains within 72 hours. *E. balsamifera* proved to be the most effective botanical followed by *L. inermis*. The total mortality of the weevils recorded in higher concentrations of *E. balsamifera*, *L. inermis* and *M. hirtus* is in line with findings of Ileke *et al*. (2016) that total mortality of adult *S. zeamais* was achieved in 20 g maize grains treated with 2.0% methanolic extracts of *M. fragrans* and *A. melegueta* at 96 hours after treatment.

Methanolic extracts of the selected botanicals were found to be effective by resulting in high mortality of adults of *S. zeamais* in sorghum grains at all concentrations applied. This might be attributed due to presence of secondary metabolites such as the steroids, phenolic compounds, tannins terpenoids, flavonoids, alkaloids, saponins and glycosides with wide range of biological activity reported to have great impact on insecticidal activities (Doughari *et al*., 2008; Biswas *et al*., 2016; Hikal *et al*., 2017). Similarly, Ileke et al. (2016) reported the presence of alkaloids, flavonoids, saponins and tannins, in the powders and methanolic extracts of *M. fragrans* and *A. melegueta* and concluded their insecticidal activity against *S. zeamais*.

Oviposition Deterrence of Methanolic Leaf Extracts against *S. zeamais*

The botanical treatments had negative effects on deposition of eggs by *S. zeamais* in sorghum grains as reflected by a relatively fewer number of egg plugs. The methanolic extracts of the selected botanicals have also shown oviposition deterrence against *S. zeamais* in sorghum grains. Similar results were reported by Ileke (2014) in which the number of eggs laid by *S. zeamais* was reduced from 36.25 ± 2.27 in the control to 8.00 ± 0.91 in aqueous stem bark extracts of *A. boonei* applied at 0.4 ml / 20 g maize grains.

The effectiveness of the botanicals in reducing egg deposition by S. zeamais concurs with Kosar and Srivastava (2016) who reported oviposition deterrence of aqueous and ethanolic extracts of *Euphorbia hirta* against C. maculatus. Additionally, 52.90 was reported by Chudasama et al. (2015) as POD of aqueous extracts of *L. inermis* against *C. maculatus*.

The oviposition deterrence of the botanicals concurs with earlier findings, that *E. balsamifera* and *L. inermis* leaf powders resulted in early mortality of *C. maculatus* thus interfering with their ability to commence a fresh cycle of oviposition (Vanmathi et al., 2012; Suleiman and Suleiman, 2014). These effects could also be linked to respiratory impairment of the weevil's body (Ileke, 2014).

Effect of Methanolic Leaf Extracts on Adult Emergence of *S. zeamais*

Complete suppression of adult emergence of *S. zeamais* by methanolic extracts of the test botanicals is in accordance with Rivera et al. (2014) that no emergence of adult *S. zeamais* was recorded in maize treated with Peumus boldus foliage powder at 1.0% w/w. Similarly, Ileke (2014) reported a complete inhibition of adult emergence of *S. zeamais* exposed to aqueous extracts of *A. boonei* applied at 0.4 ml/20 g maize. The suppression activity in adult emergence of *L. inermis* was also recorded against *C. maculatus* where 45.76% inhibition was reported when applied at 5% concentration (Chudasama et al., 2015). The inhibition activity of the botanicals in adult emergence of *S. zeamais* might be attributed to their toxic effects to the few eggs deposited. This is in agreement with Chudasama et al. (2015) who reported that toxic substances present in the extracts may enter into the egg through chorion and suppressed further embryonic development.

Effect of Methanolic Leaf Extracts in Reducing Grain Weight Losses of Sorghum Infested by *S. zeamais*

Grain weight loss is an important evaluating factor which indicates the level of grain damage caused by insect pests. There was a drastic reduction in weight losses of sorghum treated with *E. balsamifera, L. inermis, M. hirtus and S. obtusifolia*. The plant materials showed strong ability in the protection of sorghum from damage by *S. zeamais*. This concurs with Yeshaneh (2015) that there was a reduction in weight losses in sorghum grains treated with *Tagitus minuta, Datura stramonium and Carissa schimperi* applied at 5.0% against *S. oryzae*.

Findings of this study revealed that the weight losses in sorghum grains were related to the number of *S. zeamais*. This means that as most of the weevils had died shortly after their introduction and no adult emergence was recorded in the treated grains, feeding activities were reduced and hence, less weight losses were recorded. The present outcome agrees with Asawalam and Hassanali (2006) that no progeny and grain weight loss were recorded in maize grains treated with 0.30 % essential oil of *Vernonia amygdalina* Delile. In addition to their toxicity, chemical constituents of the botanicals might have inhibited feeding on the treated sorghum grains by the weevils, hence, protecting the grains from damage (Oboho et al., 2016).

CONCLUSION

It was found that application of methanolic extracts of the selected botanicals resulted in high adult mortality of *S. zeamais* even at the lower concentration of 2.5% after 72 hours of treatment with *E. balsamifera* as the most effective followed by *L. inermis* and therefore could be used to reduce *S. zeamais* infestations in sorghum grains. Methanolic extracts of the botanicals were highly effective as anti-oviposition and adult emergence inhibition agents against *S. zeamais*. It was revealed that the botanicals conferred protection to sorghum grain against *S. zeamais*. All the botanicals reduced grain damage at all the concentrations and *E. balsamifera* was found to excel, followed by *L. inermis*. These botanicals could be used as alternatives to chemical insecticides in reducing *S. zeamais* infestations in stored sorghum.

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