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OLFACTORY RESPONSES OF *Sitophilus zeamais* L. TO BUSHMINT LEAF POWDER AND METHANOL EXTRACT ON STORED MAIZE

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ABSTRACT

Bushmint (*Hyptis suaveolens* Poit) is a weed with pesticidal properties that have been explored in managing pests of agricultural and medicinal importance. The objective of this study was to evaluate the repellence ability of *Hyptis suaveolens* to prevent infestation of stored maize grain seed. The study involved evaluation of the olfactory responses of adult weevil to maize (*Zea mays* L.) grains treated with 5, 10, 15 and 20% concentrations of leaf powder (LP) and methanol extract (ME) of *Hyptis suaveolens* in the laboratory, with a static air four-chamber olfactometer. Also, the insecticidal effects of the substances on adult mortality and oviposition on maize grains treated with LP and ME were determined in a completely randomised design, with four replicates. The number of weevils in ME chambers decreased with increase in concentration of extracts; while the LP chambers had consistently fewer weevils with numbers, ranging from 2.5 to 3.75. Adult mortality in maize treated with LP was the least and varied between 0.00 (15% concentration) and 2.54% (20% concentration). Maize grains with ME caused significant mortalities that ranged from 65.50% at 15% concentration to 94.92% at 10% concentration. More eggs (3.5 to 5.75) were laid on maize with LP compared with ME treated maize. Generally, ME was richer in phytochemicals than LP. *Hyptis suaveolens* repellence manifested more in the form of LP than as ME; though ME caused more adult mortality and reduced oviposition than LP.

Key Words: Adult mortality, *Hyptis suaveolens, Zea mays*

RESUME

La menthe de brousse (*Hyptis suaveolens* Poit) est une adventice aux propriétés pesticides explorées dans la gestion des ravageurs en agriculture et en médicine. Bien que des données sur son efficacité contre les ravageurs de stocks soient disponibles, sa capacité répulsive contre les insectes de stocks n'a pas été suffisamment documentée. Par conséquent, les réponses olfactives du charançon du maïs en stock traité avec des concentrations de 5, 10, 15 et 20% de poudre de feuilles d'*H. suaveolens* (LP) et de son extrait méthanolique (ME) ont été étudiées au laboratoire à l'aide d'olfactomètre à quatre compartiments à air statique. De même, les effets insecticides des substances sur la mortalité des adultes et la ponte sur les grains de maïs traités avec LP et ME ont été déterminés suivant un dispositif complètement aléatoire à quatre répétitions. Le LP et le ME ont été analysés pour leurs composés phytochimiques. Le nombre de charançons dans les compartiments ME diminuait avec l'augmentation de la concentration d'extrait, tandis que les compartiments LP avaient constamment moins de charançons avec des nombres variant entre 2,5 à 3,75. Le pourcentage de mortalité d'adultes dans le maïs traité avec LP était le plus faible et variait entre 0,00 (concentration 15%) et 2,54% (concentration 20%). Le maïs traité avec ME causait des mortalités significatives allant de 65,50% pour une concentration de 15% à 94,92% pour 10% de concentration. Le nombre le plus élevé d'œufs (3,5 à 5,75) a été pondu sur du maïs traité avec LP, tandis que le nombre le plus faible (1,0) a été enregistré sur celui traité au ME. Des concentrations significativement élevées de composés phytochimiques étaient obtenues dans ME comparativement à LP. Le maïs traité avec LP était hautement répulsif pour *Sitophilus zeamais*, tandis que celui traité avec ME causait la mortalité des adultes et réduisait la ponte.

Mots Clés : Mortalité d'Adulte, *Hyptis suaveolens, Zea mays*

INTRODUCTION

Insect pests threaten food security in west Africa, accounting for 30-40% of yield losses annually in maize (*Zea mays*, L.) (Boxall, 2002; Ojo and Omoloye, 2012; Abraha *et al.*, 2018). Among the insect pests of maize, *Sitophilus zeamais* Motschulsky (maize weevil), is considered as a major pest in storage world over. Infestations by the pest not only cause significant economic losses, but also result in elevated temperatures and moisture conditions that lead to an accelerated growth of moulds, including toxigenic fungal species (Megan *et al.*, 2003). The weevil feeds on the germ of maize grains, thereby reducing the protein content of the grains (Effiong and Sanni, 2009). Adult weevils and larvae feed on maize grains and reduce them to powdery form (Longe, 2016).

To overcome the insect pest problem and to extend the maize storage shelf life, several chemical insecticides have been developed and used (Sikirou *et al.*, 2015). Unfortunately, unregulated use of chemical insecticides engenders many problems, including environmental pollution, residual toxicity, death of non-target organisms, risk of user's contamination and promotes pest resistance (Ouko *et al.,* 2017). This led researchers to think of a better alternative, such as biopesticides (Conti *et al.*, 2011, Kumar, 2015; Kamrul *et al.*, 2018). Biopesticides are substances derived from materials such as animals, plants, bacteria and minerals (Kumar, 2015). Farmers reportedly use natural products such as neem leaves, bitter leaves, pepper, ashes, to preserve stored grains (Schneider, 2015). Insecticidal activities of leaf, stem, bark and root from different plants (*Eucalyptus globulus* Labill, *Jacaranda mimisifolia* D. Don, *Matricaria chamomilla* L., *Tagetes minuta* L., *Ocimum tenuiflorum* L., *Moringa oleifera*, *Hyptis spicigera* Lamarck and *Hyptis suaveolens* (L.) Poit (Barbara *et al.*, 2010; Alabi and Adewole, 2017; Sekyere *et al.*, 2018) have been explored for the management of stored products, with mostly promising results.

Originally native to tropical America, Bushmint (*Hyptis suaveolens* Poit) is considered as a weed (Ngozi *et al.,* 2014) with pesticidal properties that have proven effective in managing pests of agricultural importance and for medicine (Conti *et al.,* 2011; Olotuah, 2013). The larvicidal and repellent activity of *H. suaveolens* essential oil against the mosquito *Aedes albopictus* Skuse (Diptera: Culicidae) was investigated by Conti *et al.* (2011). They found that at the highest dosages of 450 and 400 ppm, there were no significant differences on larval mortality between *Hyptis suaveolens* and synthetic insecticide. Olotuah (2013) reported that essential oils from *Hyptis* *suaveolens* using n-hexane, diethyl ether and methanol as solvents repelled *Sitophilus oryzae*, *S. zeamais* and *Callosobruchus maculatus*. In particular, the methanolic extract at 100% concentrations against *Sitophilus zeamais* through contact treatment caused 100% mortality of the insects.

Previous studies have established the insecticidal potency of methanol extract from *Hyptis suaveolens* leaves against stored product and field pests, this suggests that the leaf of *H. suaveolens* has potent insecticidal properties. However, there is dearth of information on the biological activity of the leaf powder, and not extracts, since resource poor farmers in Africa can easily prepare the powder than compounding any extract; this would be a beneficial study to investigate. Therefore, the objective of this study was to evaluate the olfactory responses of *S. zeamais* to *H. suaveolens* leaf powder and methanol extract and their effects against survival of adult weevils.

MATERIALS AND METHODS

Fresh leaves of *H. suaveolens* were collected from the roadside of Abomey-Calavi south of Benin. The leaves were washed with distilled water, chopped into small pieces, and shade-dried at room temperature (26 ± 1 °C) for 21 days. Then, the dried leaves were ground into powder using a laboratory grinder (Bender and Hobein 8042 Zurich) and kept at room temperature of 25-27 °C until use. The ground powder was used as the leaf powder in all experiments.

To prepare the methanol extract from leaves, leaf powder (50 g) prepared above was mixed with 500 ml of methanol for 2 hours, at 40 °C with a magnetic stirrer (IKA C-MAG HS7). The solution was subsequently filtered through Whatmann filter paper, and the resulting filtrate was evaporated off using a Rotavapor (BUCHI RII), at 47 °C to remove the solvents completely. The methanol extracts were stored in falcon tubes at 4 °C.

Insect rearing. Adults of *S. zeamais* were collected from maize stores in Dantokpa market in the Republic of Benin. Five kilner jars, each of 1 kg capacity, were used for insect production. Each kilner jar contained 500 g of local variety “Gbadé wéwé” of maize grains (white maize), purchased from Comè market, Republic of Benin. The 500 g of maize in each jar, were infested with 50 pairs of newly emerged adults of *S. zeamais,* and allowed to lay eggs for one week. The first generation of emergent adults was used for all experiments.

Olfactometer experiments. The responses of adult maize weevils to odours from powder and methanol extract of different concentrations (5, 10, 15 and 20%) were examined using a Static Air Four-Chamber Olfactometer in the laboratory. The Olfactometer was a cylindrical acrylic glass (4 cm high, 19 cm diameter), divided with vertical plates into four chambers. On the top of the cylinder, a walking arena (1 cm high, 19 cm diameter) was placed consisting of plastic gauze (mesh 0.5 mm) with a rim of plastic (0.9 cm high), and covered with a glass plate according to Gross and Mekonen, (2005). No air flow was generated; this was to simulate a storage system scenario.

Odours emanating from the treated and untreated grains were allowed to diffuse through the gauze, resulting in an odour field in the walking arena above. Both powder and methanol extract from *Hyptis suaveolens* leaf were individually incorporated into 200 g maize grains at 0% (control, untreated) 5, 10, 15 and 20% (w/w). A sample (20 g) of treated maize with either extract or powder at given concentrations, was placed in two opposite chambers.

Two chambers containing untreated maize grains acted as controls. Ten insects were released individually in the centre of the walking arena; and their arrestment times in the four sectors above the arena was recorded every 1 hour, for 4 hours. To avoid bias due to possible orientation preferences of the insects, the position of the Olfactometer was rotated clockwise by 90° at each 1 hour. Each experiment was repeated four times. Protect DP (Pirimiphos-methyl 1.5% + Deltamethrine 0.1%) was included as a control. It was used at 0.05 g 100 g^{-1} of maize grains.

The insecticidal effects of *H. suaveolens* test materials on adult weevil mortality and oviposition were determined in the laboratory using the following procedures.

Leaf powder (LP). Maize grains variety, "Gbadé wéwé", were kept in a deep freezer, at –20 °C for 1 week (Danho *et al.*, 2015) to ensure that any existing insect eggs and larvae were killed before starting the experiment. Ten, 20, 30 and 40 g of leaf powder, corresponding to 5, 10 15 and 20% w/w concentrations, respectively, were added to approximately 200 g of clean undamaged maize (Gbadé wéwé) grains in plastic containers; (each of 300 g capacity) covered with muslin cloth. The grains were mixed with the powder thoroughly for approximately 30 seconds before transferring the treated grains into bioassay containers, according to Ajani (2018). The binary insecticide (Pirimiphos-methyl 1.5% + Deltamethrine 0.1%), with the trade name 'PROTECT'DP, was used as positive control; while the negative control was untreated grains. The effect of methanol on the weevil was also verified. Fifty pairs (25 males: 25 females) from the first generation F1 were introduced in each plastic container containing grains treated with leaf powder and the two controls.

Methanol Extract (ME). The insecticidal activity of *H. suaveolens* was assessed by dissolving 1, 2, 3 and 4 g of methanol leaf extracts, each in 20 ml of methanol; corresponding to 5, 10, 15 and 20% w/v, respectively. Clean undamaged maize grains (200 g) were dipped in 7 ml of each of the different concentrations of leaf extracts; and manually thoroughly mixed for approximately 30 seconds. The mixtures were left for 1 hour to allow methanol to evaporate before infestation; each set up was covered with muslin cloth. Protect DP was used as the positive control, and untreated grains as the negative control.

Fifty pairs (25 males: 25 females) of *Sitophilus zeamais* from the first generation F1 were introduced into each of the plastic cages containing grains treated with the methanol extract, and into the two controls. The experimental variants (LP and ME) were replicated four times in a completely randomised design. Adult mortality was recorded at 2, 4, 7 and 14 days after treatment. The percentage mortality was determined as follow:

Percentage morality =

$$\frac{\text{Number of dead insects}}{\text{Total number of insects}} \times 100$$

Data on percentage mortality were corrected using Abbott's formula (Abbott, 1925)

$$P_t = \frac{P_o - P_c}{100 - P_c} \times 100$$

Where:

P_t = Corrected mortality; P_o = Treated variants mortality; and P_c = Control mortality.

Oviposition was assessed seven days after infestation, by counting the number of eggs laid on a sample of 100 grains taken from each variant plastic container. *Sitophilus zeamais* females deposited single eggs in holes bored into the grain, and sealed with a gelatinous egg plug which stained cherry red with acid fuchsin (Danho *et al.*, 2015).

The method used to stain the egg plugs was adapted from that of Holloway (1985). It involved soaking grains for 1 to 2 minutes in warm water (25-30 °C); followed by immersion for 1 to 2 minutes in an acid fuchsin solution (0.5 g L^{-1} of water). The maize grains were finally rinsed with distilled water to eliminate excess staining solution, and then dried on filter paper for 60 minutes at room temperature. The egg plugs appeared cherry red in colour, when observed under a stereo-microscope.

Phytochemical analysis of test materials. The quantity of saponins was determined using Obadoni and Ochuko (2001) method. Tannin content was determined by Folin - Ciocalteu method. Total flavonoid content was measured by the aluminium chloride colorimetric assay (Obadoni and Ochuko, 2001). Hager's test was conducted to determine the alkaloids in *H. suaveolens* (Harborne, 1973). The presence of phenols was determined by Ferric Chloride's test; density was measured at 760 nm against a blank. The total phenolic contents were calculated on the basis of the calibration curve of Gallic acid and expressed as Gallic Acid equivalents (GAE), in milligrammes per gramme of the sample (Singleton *et al.*, 1999).

Statistical analysis. Statistical analysis was carried out using R version 3.5.0 software (R core Team, 2019). Responses of *S. zeamais* in the static air four-chamber olfactometer to LP and ME odours were analysed using Student's t-test at 0.05 level of significance. Data on adult mortality and number of *S. zeamais* eggs were analysed using Analysis of variance (ANOVA) and means were separated using Student Newman Keuls (SNK) test at 0.05 level of significance.

RESULTS

Responses of maize adult weevils to treatments. The number of adult weevils in the untreated compartments of the olfactometer was significantly higher ($P<0.05$) than that in treated compartments with Protect DP and LP (Table 1). However, in the ME set up, the number of *S. zeamais* in treated compartment decreased with the increasing bio-extract concentrations; from 6.5 (5% ME) to 2.5 (20% ME), and increased in untreated compartment from 3.5 (5 ME) to 7.5 (20 ME) (Table 1).

Adult weevil mortality. There was no significant ($P>0.05$) effect of concentration of leaf powder on mortality of *S. zeamais*. Adult mortality was very low on grains treated with the different concentrations of leaf powder (Fig. 1). Mortalities recorded on maize treated with methanol extracts were remarkably high ($P< 0.05$) for all concentrations; and varied between 60.50 and 100%, especially at 7 days after treatment for 5 to 15% concentrations and reached 98.50 at 20% concentrations, 2 days after exposure (Fig. 1). Grains treated with Protect DP resulted in high mortality of *S. zeamais,* ranging from 98.50% (2 days after

TABLE 1. Number of *Sitophilus zeamais* in chambers with odours of *Hyptis suaveolens* leaf powder, methanol extract, Protect and untreated chambers

Variant	Treated chamber	Untreated chamber	P values
Protect DP	3.25 ± 1.89	6.75 ± 1.89	0.040*
5LP	2.5 ± 1.29	7.5 ± 1.29	0.000*
10LP	2.75 ± 0.96	7.25 ± 0.96	0.000*
15LP	2.5 ± 0.58	7.5 ± 0.58	0.000*
20LP	3.75 ± 1.26	6.25 ± 1.26	0.030*
5ME	6.5 ± 1.29	3.5 ± 1.29	0.020*
10ME	4.25 ± 0.50	5.75 ± 0.50	0.010*
15ME	3.75 ± 0.96	6.25 ± 0.96	0.010*
20ME	2.5 ± 1.29	7.5 ± 1.29	0.000*

LP = leaf powder; ME = methanol extract, * = significant at $P < 0.05$

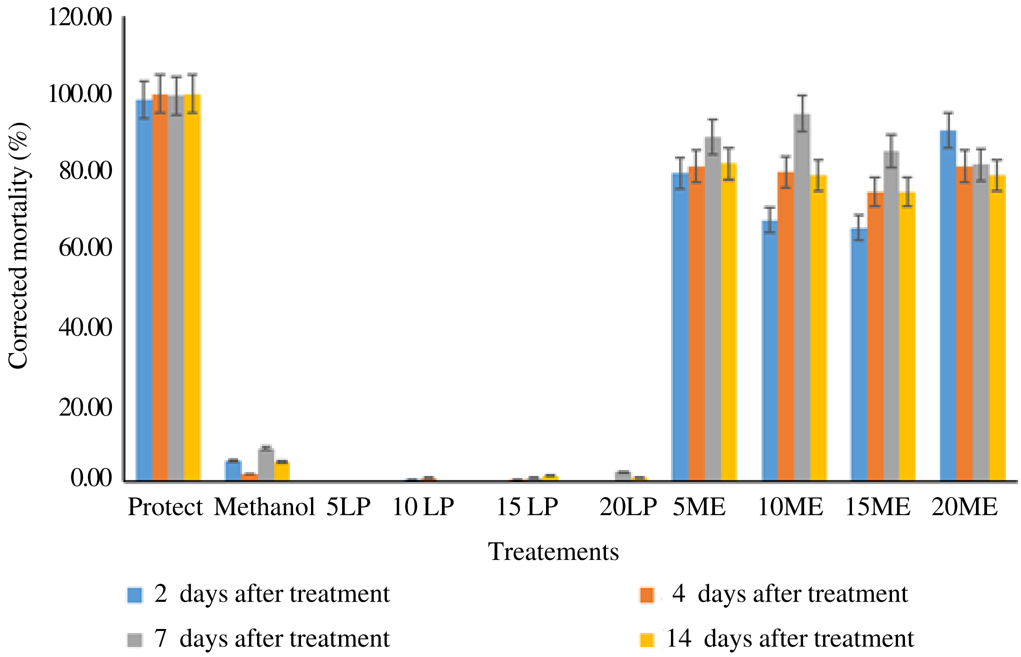

Figure 1. Adult mortality (%) on maize treated with leaf powder, methanolic extract of *Hyptis suaveolens* and Protect DP.

treatment) to 100% (14 days after treatment) (Fig. 1).

Oviposition of adult weevils. The number of *S. zeamais* eggs laid on maize treated with *H. suaveolens* leaf powder, methanol extract and Protect DP are presented in Figure 2. The number of eggs laid on maize grains treated with different concentrations of leaf powder were significantly higher than that laid on maize coated with different concentrations of methanol extract ($P<0.05$). No eggs were laid on maize grains coated with 10 and 20% methanol extracts and the effect was not significantly different from 5% methanol extract (1.00). Few eggs (0.75) were laid on grains treated with Protect DP. There was no significant difference ($P>0.05$) between the number of eggs laid on untreated grains (3.5) and treated grains with *H. suaveolens* leaf powder (Fig. 2).

Phytochemical constituents of the extracts. *Hyptis suaveolens* leaf contained saponins, tannins, alkaloids, flavonoids and total phenols as phytochemicals (Table 2). Phytochemicals were very high when extracted with methanol but very low in leaf powder with highest concentration of saponins in leaf powder and methanol extract and lowest concentration of Tannins.

DISCUSSION

From our result, it was found out that the number of eggs laid on maize grains treated with different concentrations of leaf powder were higher than that laid on maize coated with different concentrations of methanol extract. This can be explained by the fact that Hyptis methanol extract adhered more to the grains than Hyptis powder, which easily rubs off the grain and then facilitate the oviposition of maize weevils.

The number of weevils in leaf powder compartments were consistently lower across all the concentrations compared with the untreated (control) compartments. This means that the weevils preferred the untreated compartments containing clean grains, to

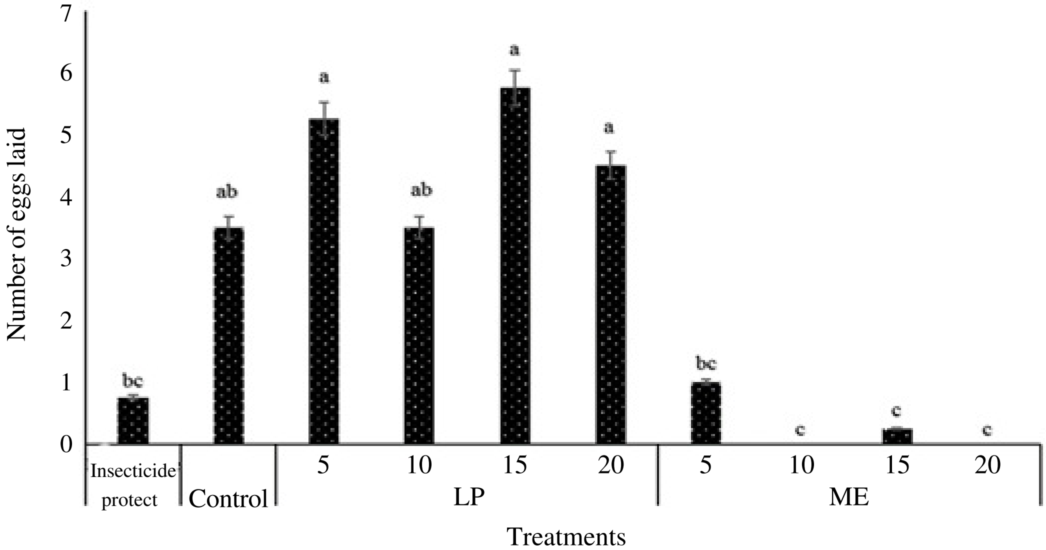

Figure 2. Number of eggs laid by *Sitophilus zeamais* on maize grains treated with *Hyptis suaveolens* leaf powder, methanolic extract and Protect DP.

TABLE 2. Quantitative phytochemical screening of *Hyptis suaveolens* leaf powder and methanol extract

Phytochemical	*Hyptis suaveolens* leaf powder	*Hyptis suaveolens* methanol extract
Saponin (%)	8.09±0.00	14.38±0.00
Tannin (mg g^{-1})	0.35±0.001	1.63±0.005
Alkaloid (%)	2.56±0.00	10.35±0.02
Flavonoid (mg g^{-1})	0.59±0.003	4.75±0.012
Total phenol (mg g^{-1})	1.14±0.004	5.53±0.005

compartments exuding leaf powder odours. This suggests that the leaf powder contain bioactive compounds as was reported by Noudogbessi *et al* (2013) and Adou *et al.* (2019) who stated that several volatile organic compounds (Sabinene (7.3-31.3%), eucalyptol (14.0-24.6%), β-caryophyllene (6.9-12.7%), 1,8-cineole (11.5%), β-phellandrene (10.2%), terpinolene (8.7-9.6%), fenchone (4.1-8.1%), p-mentha-2 (7), 8-diene (7.9%), bicyclogermacrène (4.7-7.5%), β-pinene (4.9-7.4%), (Z)-β-ocimene (6.9%) and terpinen-4-ol (5.4-5.9%) were found in essential oil extracted from *H. suaveolens* leaves. These compounds in *H. suaveolens* leaf according to Noudogbessi *et al.* (2013) and Adjou *et al.* (2019) have pesticidal properties.

Our result show that the number of adult weevils reduced as the concentration of leaf powder increased. The explanation of this result is that the leaf powder exude volatile compounds that repelled the weevils from the treated compartments. This is a first report that demonstrates that volatile compounds emanating from the leaf powder of *Hyptis suaveolens* repelled *Sitophilus zeamais*. Hence, small holder farmers could easily apply the plant powder on stored maize grain to forestall weevil infestation.

Also from our study, the number of weevils decreased with increase in concentrations of methanol extract but not as obvious as in the leaf powder. Hence, the methanol extract exuded low concentrations of odours that was lower compared with the leaf powder. The interesting aspect of these results is the low quantities of leaf powder needed to repel the weevil, hence a smallholder farmer would need a small quantities of leaf powder to protect maize grains against weevil infestation.

The leaf powder of *H. suaveolens* did not result in mortality of adult maize weevil. Although the powder repelled the weevils in the olfactory experiment, it did not possess the ability to cause death of the insects. The maize grains had smooth surfaces that did not permit the powder of *Hyptis suaveolens* to stick to them. Therefore, the adults could lay eggs, hence the higher number of eggs recorded on grains treated with the leaf powder.

In the case of the methanol extract, the high adult mortality on treated maize grains right from the second day of treatment application, also suggests that the methanol extract contained lethal compounds that killed the insects possibly through contact and feeding. The marked death of adult weevils from the second day after treatment, implies that the number of eggs laid at 7 days after treatment will be considerably reduced. Many of the adults that should lay eggs by one week after treatment had died earlier. Indeed, there were no eggs laid on maize grains treated with 10 and 20% methanol extract.

Similar results were reported by several researchers who noted that repellent and toxicity of extracts from leaf and seed of *H. suaveolens* (Musa *et al.,* 2009; Pavunraj *et al*, 2014). Methanol extract from seed and leaf of *H. suaveolens* enhanced khapra beetle (*Trogoderma granarium* Everts) adult mortality, reduced its oviposition and suppressed larval and adult emergence from stored groundnuts (Musa *et al.,* 2009). Pavunraj *et al* (2014) reported antifeedant and insecticidal activity of ethyl acetate leaf extract of *H. suaveolens* against some lepidopteran pests.

Both the leaf powder and methanol extract contained saponin, tannin, alkaloid, flavonoid and total phenol. However, leaf powder was poorer in these substances that its methanol extract counterpart. Thus, the high concentrations of the phytochemicals in methanol extract were responsible for the adult mortality.

From previous reports, there are variations in the phytochemicals obtainable from *Hyptis suaveolens*; although this is not peculiar to Bushmint, but to all plants generally (Akob and Ewete, 2007; Ukeh *et al.,* 2009; Noudogbessi *et al*., 2013). Endogenous (genetic, development, etc.), botanical source, edaphic and climatic factors could alter the chemical composition of plants (Akob and Ewete, 2007; Ukeh *et al.,* 2009; Noudogbessi *et al*., 2013). Hence there is the need to always conduct phytochemical screening of plant materials when they are explored for insecticidal properties.

It is clearly evident from the laboratory experiments in this study that the modes of action of the leaf powder is repellence against weevils and that of methanol extract was contact toxicity and antifeedant against the weevils. *Hyptis suaveolens* leaf powder and methanol extract could be put into multiple uses based on the intent of the user or applicator. Leaf powder of *H. suaveolens* when applied to newly stored maize grains will reduce rapid colonisation of grains by the weevils. On the other hand, if the produce had been infested with weevils, application of methanol extract will rapidly decimate pest population on the stored grains as quickly as a synthetic insecticide would also act. Consequently, there will be reduction in number of eggs laid by surviving weevils and subsequent F1 generation. This is a novel approach in the protection of stored maize from weevil attack and damage.

Further studies should be carried out to determine the volatile organic compounds (odours) in the leaf powder and the most potent compounds could be synthesized and formulated into dusts that farmers and house wives can easily apply to stored grains to ward off weevils infestation.

CONCLUSION

Hyptis suaveolens is a potential biopesticide which could be used as preventive and curative substance against *Sitophilus zeamais* infestation on stored maize as established from the results of this study. *H. suaveolens* methanol extract is also highly effective as an insecticide, while the leaf powder is efficacious as a repellent against *S. zeamais*. Both Leaf Powder and Methanol extracts contain saponin, tannin, alkaloid, flavonoid and phenol as phytochemicals; the former is richer than the latter. However, the specific causes of repellence in Leaf Powder, and phyto-toxicity in methanol extract and the bioactive compounds of *H. suaveolens* need further elucidation.

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