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TOXIC, ANTIFEEDANT AND REPELLENT ACTIVITY OF AQUEOUS CRUDE EXTRACTS OF *Tephrosia vogelii* HOOK ON THE LARVAL STAGES OF *Helicoverpa armigera* HÜBNER

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Abstract

Laboratory bioassays were conducted to evaluate the bioactivity of aqueous crude extracts of *Tephrosia vogelii* Hook against *Helicoverpa armigera* Hübner larvae. Fresh chickpea leaves, immersed in aqueous crude extracts of *Tephrosia vogelii* at four rates (0, 5, 10 and 20% w/v), were assayed for toxic, antifeedant and repellent effects against 2nd and 3rd instar larvae of *H. armigera* in a completely randomized design (CRD) with 3-5 replicates per treatment. Ordinary water and Dimethoate (Rogor E40) ® at 2% v/v were included as negative and positive controls, respectively. Data on corrected percent mortality, repellence and deterrence coefficient were first homogenized using angular transformations before being subjected to analysis of variance (ANOVA) and means separated by Tukey's HSD test. Results showed that the toxic, antifeedant and repellent effects of crude aqueous extracts of *T.vogelii* against *H. armigera* larvae were significantly ($P<0.0001$) influenced by intra-plant variability, concentration applied, duration (hours) and corresponding factor interactions. At the highest concentration of 20% w/v, the aqueous crude extracts obtained from the leaves (22%) and pods/flowers (av. 11%) of *T.vogelii* were weakly toxic. In the antifeedant bioassay, leaf extracts caused the highest reduction (96%) in weight of larvae followed by pods/flowers (79%) and succulent stems (2.5%), respectively. There were corresponding reductions in larval feeding as the concentration of aqueous crude extracts increased. In the repellence test, except for leaf and pod/flower extracts at 20% w/v and 1 h exposure that produced moderate percent repellence (41.67%) against the larvae, there was a dose- and exposure time-dependent attraction of *H. armigera* larvae to chickpea leaves (food) treated with aqueous extracts of *T. vogelii*. The plant offers hope as a potential cost-effective and environmentally benign antifeedant for *H. armigera* control in chickpea.

Key words: *Tephrosia vogelii*, *Helicoverpa armigera*, toxicity, antifeedant, repellence.

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Introduction

Helicoverpa armigera Hübner (Lepidoptera: Noctuidae) is a highly polyphagous species known to be an important field insect pest of tomato, cotton, pigeon pea, chickpea, sorghum and cowpea. In spite of the use of all available pest control options, the pest still causes annual crop losses estimated at $325 million globally in chickpea (ICRISAT, 1992; Sharma et al., 2005; Prasad and Purohit, 2009). The larva is the only feeding stage in its lifecycle and causes greatest damage in chickpea and tomato. *H. armigera* is particularly difficult to control with insecticides owing to the cryptic nature of the larvae and untimely application of synthetic insecticides (Agboka et al., 2009). Moreover, *H. armigera* damage predisposes chickpea to pre- and post-harvest infestation by the coleopteran pest, Callosobruchus chinensis Linn thereby affecting the quantity and quality of chickpea (ICRISAT, 1992; Sharma et al., 2005). Currently alternative control options include application of pesticides, *Bacillus thuringiensis (*Bt), Helicoverpa nuclear polyhedrosis virus (HNPV), use of resistant cultivars and intercropping, but none provides satisfactory control (Lewis et al., 1997; Ranga- Rao & Shanower, 1999).

Available information indicates that synthetic insecticides are largely incompatible with subsistence agriculture owing to their high costs, health hazards to warm-blooded animals (man and animals), environmental pollution, and inconsistent efficacies. Hence, the age-old botanical pesticides which are locally available, and are broad-spectrum pesticides and are also bio-degradable. They often have low mammalian toxicity and are eco-friendly, eliciting special scientific interest and attention as alternatives to synthetic insecticides (Isman, 2006; Agboka et al., 2009). Botanical insecticides have been successful against a number of field insect pests in Africa (Machocho, 1992). For instance the fish poison bean, *Tephrosia vogelii* Hook (Leguminosae), has recently gained attention with regard to its insect pest control potential. According to Gaskins et al., (1972), leaves of *Tephrosia* species contain at least four compounds that possess insecticidal properties. In their field evaluations, Mugoya and Chinsembu (1995) reported that aqueous fresh-leaf extracts of *T. vogelii* reduced the incidence of maize stemborers. Similarly, several other field studies using aqueous extracts *T. vogelii* extracts showed significant insecticidal and antifeedant effects against American boll worm in cotton, spotted cereal stem borer in maize and insect pests of cowpea (Machocho, 1992; Mathias, 1997; Adebayo et al., 2007). Additionally, preliminary local field studies have shown that crude leaf extracts of *T. vogelii* provides good control against insects feeding on pigeon pea (Minja et al., 2002) whereas its essential oils are effective fumigants of insect pests of stored chickpea grains (Ogendo et al. , 2008) and spotted stalk borer *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) in maize in Zambia (Mugoya & Chinsembu, 1995). The current study evaluated the toxic, antifeedant and repellent activities of aqueous crude extracts of *T. vogelii* against the larval stages of *H. armigera* feeding on chickpea.

Materials and Methods

Mass Rearing of Test Larvae.

Field cultures of *H. armigera* larvae maintained at 26 ± 1°C, relative humidity of 60-70% and 12 hours L: 12 hours D (light: darkness) were used. Test larvae were reared according to Nasreen and Mustafa (2000) on sucrose-based adult diet (1:9) containing honey (10%) and water (90%) in 5 ml plastic vials on cotton wool.

Preparation of Aqueous Extracts of *T. vogelii.*

Separate samples of fresh leaves, succulent stems and pods / flowers of *T. vogelii* were collected in sufficient quantities from EgertonUniversity's Tatton Farm (Field 15) and transported in labeled bags. Thereafter the samples were shade-dried at ambient temperatures (18-28^{o}C) for 2 weeks and further oven dried at 35^{o}C for 48 hours (h) (Ogendo, 2000). Dry samples were ground into fine powders using an electric laboratory hammer mill. The powders were stored in an air tight glass jar in a cool place away from sunlight. Each of the three plant parts was tested at four rates (0, 5, 10 and 20% w/v) using weighed samples of dry crude powders suspended in water for 24 h at room temperature (Garcia-Mateos et al., 2007). Ordinary water and Dimethoate (Rogor E40) ® at 2% v/v were included as negative and positive controls, respectively.

Contact Toxicity Studies

The contact toxicities of aqueous crude extracts were evaluated according to Brem et al. (2002) with modifications. Ten (10) second larvae of *H. armigera* (NT) were introduced into separate 100 ml sample bottles whose inner walls were rinsed with graded concentrations of aqueous crude extracts of *T. vogelii* as stated above. Separate crude aqueous extracts obtained from leaves, pods/ flowers and succulent stems of *T. vogelii* were applied at four rates (0, 5, 10 and 20% w/v) as treatments. Ordinary water and Dimethoate (Rogor E40) ® at 2%v/v were included as negative and positive controls, respectively. A total of 12 treatments were arranged in a CRD with three replicates per treatment. Fresh chickpea leaves were supplied after every 24 h. The number of dead larvae (ND) was recorded 24, 48 and 72 h post-treatment. The actual percent larvae mortality was computed according to Asawalam et al. (2006) and then corrected for natural mortality using Abbott (1925) formula.

Antifeedant Tests

In order to determine the amount of food consumed, 10 chickpea leaves of the same dimensions were immersed in test *T.vogelii* aqueous extracts and control treatments for 30 minutes. Separately, treated leaves were removed using forceps, placed inside plastic Petri dishes (15 cm in diameter) lined with filter paper (Whatman No. 1) at the base and were then weighed according to Erturk (2006) with some modifications. Ten 2nd and 3rd instar larvae were weighed and introduced into each of the treated diets. Separate aqueous crude extracts obtained from leaves, succulent stems and pods/ flowers of *T. vogelii* were applied at four rates (0, 5, 10 and 20% w/v) as treatments. Ordinary water and Dimethoate (Rogor E40) ® insecticide at 2% v/v were included as negative and positive controls, respectively. A total of 12 treatments were arranged in a CRD with three replicates per treatment. Data on the amount of food consumed and weight of larvae were recorded. The larvae in each experimental unit received fresh treated diet every 24 h. Based on the amount of food consumed, the absolute deterrence coefficient (DC) was calculated using Kielczewski & Nawrot (1979) formula as follows:

$$\text{Deterrence Coefficient (DC)} = \frac{(C-T)}{(C+T)} \times 100 \qquad \text{Equation 1}$$

Where T represented the weight of food consumed by larvae in the experimental unit and C represented the weight of food consumed in the control unit.

Repellence Tests (Choice Bioassay)

Choice bioassay tests using 2nd and 3rd instar larvae were conducted in a circular flat bottomed plastic basin (45cm in diameter by 30 cm high) whose base was divided into four equal portions as described by Ogendo et al. (2004) (Plate 1). Separate aqueous crude extracts obtained from leaves, pods/ flowers and succulent stems of *T. vogelii* were evaluated against *H. armigera* larvae at four rates (0, 5, 10 and 20% w/v). Ordinary water and Dimethoate (Rogor E40) ® at 2%v/v were included as negative and positive controls, respectively. A total of 12 treatments were arranged in a CRD with three replicates per treatment. Alternate treated and untreated 10 chickpea leaves were placed equidistant from the centre of the circular base. This was repeated for all treatments including a no-choice control with untreated chickpea leaves in all four portions. The top of the basin was covered with a nylon mesh to prevent the larvae from escaping. In each treatment, twenty 2nd or 3rd instar larvae were released at the centre of the basin. The number of larvae that settled on the control and treated chickpea leaves was recorded after 1, 12, and 24 h of exposure. Percent repellence (PR) was calculated as described by Ogendo et al. (2003) as follows:

$$PR = 2(C-50) \qquad \text{Equation 2}$$

C is the percent of larvae that settled on the untreated chickpea leaves.

Plate 1

Repellence Test: An Example Showing the Four Portions of the Circular Base with Alternate Treated-Untreated Control Chickpea Leaves

Data Analysis

Data on corrected percent mortality, repellence and deterrence coefficient were first homogenized using angular transformations before being subjected to ANOVA and means separated by Tukey's HSD test (Mead et al., 1994; Ogendo et al., 2008). Data obtained from various concentration-response bioassay (contact toxicity) were further log-transformed before being subjected to probit regression analysis using EPA Probit Analysis Program version 1.4 and LC_{50} values and corresponding 95% fiducial limits obtained from derived regression equations(Finney, 1971). The LC_{50} values in a column were considered significantly different when 95% fiducial limits did not overlap.

Results and Discussion

Contact Toxicity Studies

Results showed that the contact toxicity of aqueous crude extracts of *T.vogelii* against *H. armigera* larvae were significantly ($p<0.0001$) influenced by intra-plant variability, concentration applied, duration (hours) of exposure and corresponding factor interactions.

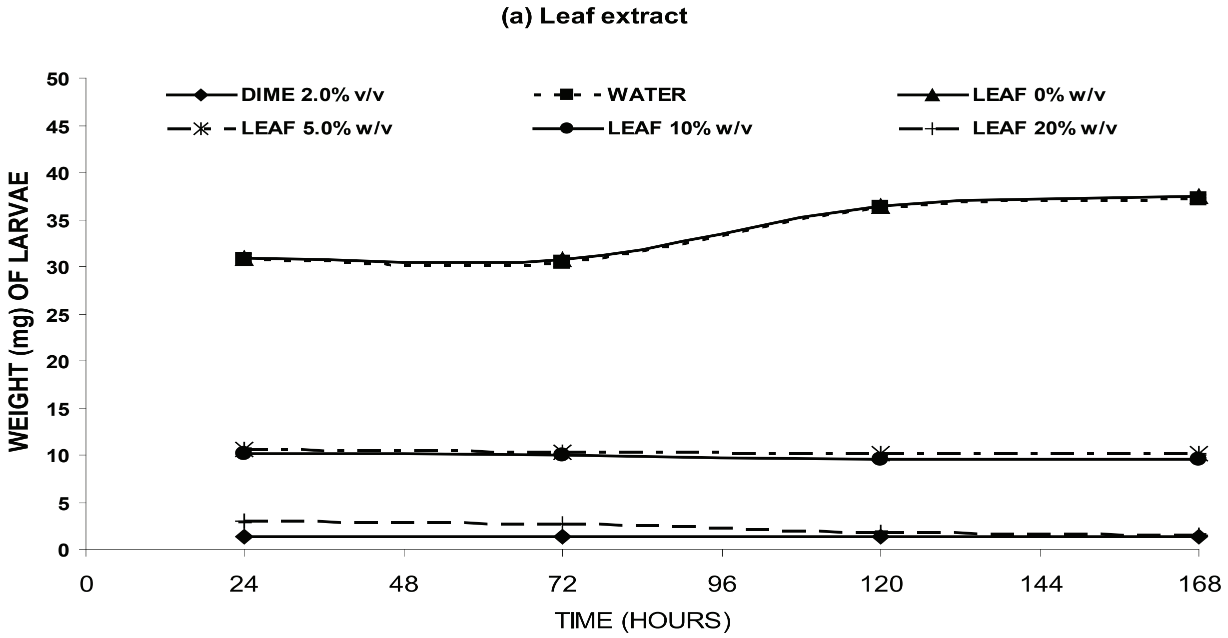
At the highest concentration of 20% w/v and 24 h, the aqueous crude extracts obtained from the leaves and pods/flowers of *T.vogelii* were weakly toxic against *H. armigera* larvae causing 22 and 11% mortality, respectively, whereas the succulent stem extracts were non- toxic (LC_{50} values were insignificant) (Table 1). The positive control, Dimethoate (Rogor E40) ® at 2% v/v, was the most toxic achieving 100% kill within 24 h.

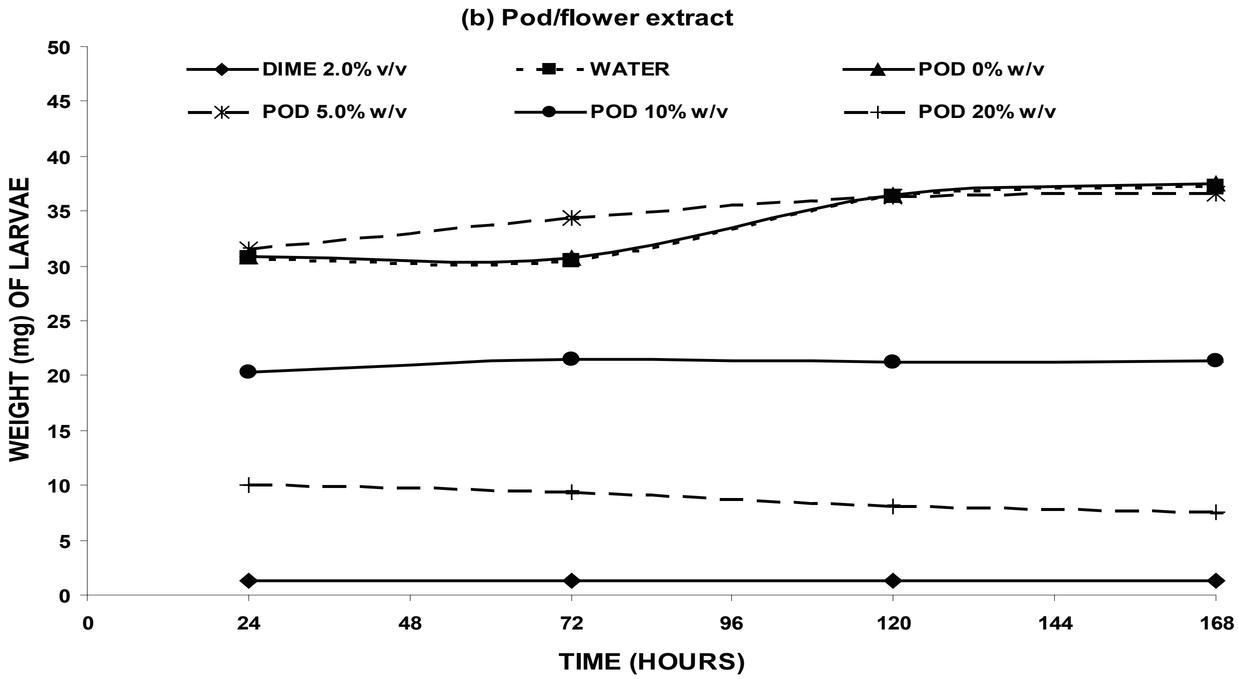
Table 1
Mean Cumulative Percent Mortality of Helicoverpa armigera Larvae Fed on Different Concentrations of Aerial Parts of Tephrosia vogelii

CONTACT TIME (HRS)				
Plant part/Conc (% v/v or w/v).	N	24	48	72
Leaf				
Dimethoate 2.0	10	100.00±0.00	100.00±0.00	100.00±0.00
Ordinary water	10	0.00±0.00	0.00±0.00	0.00±0.00
0.0	10	0.00±0.00	0.00±0.00	0.00±0.00
5.0	10	11.11±0.59	11.11±0.59	11.11±0.59
10.0	10	11.11±0.59	11.11±0.59	11.11±0.59
20.0	10	22.22±1.18	22.22±1.18	22.22±1.18
LC50 Value		136.2	136.2	136.2
95% **FL**		(42.6, 238.5)	(42.6, 238.5)	(42.6, 238.5)
Pods/flower				
Dimethoate 2.0	10	100.00±0.00	100.00±0.00	100.00±0.00
Ordinary water	10	0.00±0.00	0.00±0.00	0.00±0.00
0.0	10	0.00±0.00	0.00±0.00	0.00±0.00
5.0	10	0.00±0.00	0.00±0.00	0.00±0.00
10.0	10	0.00±0.00	0.00±0.00	0.00±0.00
20.0	10	11.11±0.59	11.11±0.59	11.11±0.59
LC50 Value		NS	NS	NS
95% **FL**		-	-	-
Succulent stems				
Dimethoate 2.0	10	100.00±0.00	100.00±0.00	100.00±0.00
Ordinary water	10	0.00±0.00	0.00±0.00	0.00±0.00
0.0	10	0.00±0.00	0.00±0.00	0.00±0.00
5.0	10	0.00±0.00	0.00±0.00	0.00±0.00
10.0	10	0.00±0.00	0.00±0.00	0.00±0.00
20.0	10	0.00±0.00	0.00±0.00	0.00±0.00
LC50Value		NS	NS	NS
95% FL		-	-	-

Antifeedant Tests

In the antifeedant bioassay, the larval weight, amount of food consumed and feeding deterrence coefficients were significantly (P<0.0001) affected by intra-plant variability, feeding duration (hours), concentration of extract applied and corresponding factor interactions. Except for ordinary water and untreated control treatments, strong dose- and plant part-dependent decrease in larval weights and amount food consumed were recorded (Figures 1-2). At the highest concentration (20% w/v) and 168 h, leaf and pod/flower extracts reduced the larval weights by 75 and 78.7%, respectively (Fig. 1) compared to ordinary water and untreated control. Similar result trends were recorded in which the leaf, pod/flower and stem extracts suppressed the larval food consumption by 89.2, 67.6 and 35.1%, respectively, after 8 days of feeding (Fig. 2). The leaf extracts and synthetic insecticide, Dimethoate (Rogor E40) ® at 2% v/v, equally had the highest antifeedant (reduced by 89.2%) effects on the *H. armigera* larvae. Synthetic insecticide, Dimethoate (Rogor E40)® and *T. vogelii* leaf extracts, after 8 days of feeding, had the highest deterrence coefficients of 90 and 70, respectively, followed by pod/flower (57) and stem (5) extracts in order of decreasing deterrence whereas ordinary water and untreated control treatments had the lowest deterrence values of 1.5-4.3 and 0.0, respectively (Fig. 3).





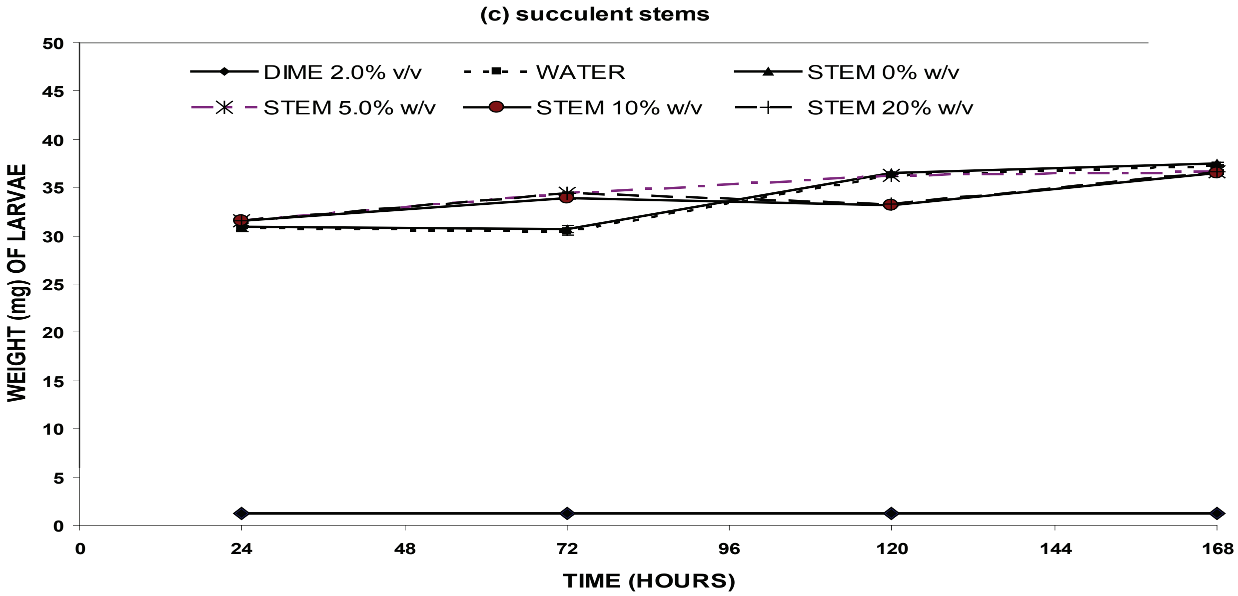
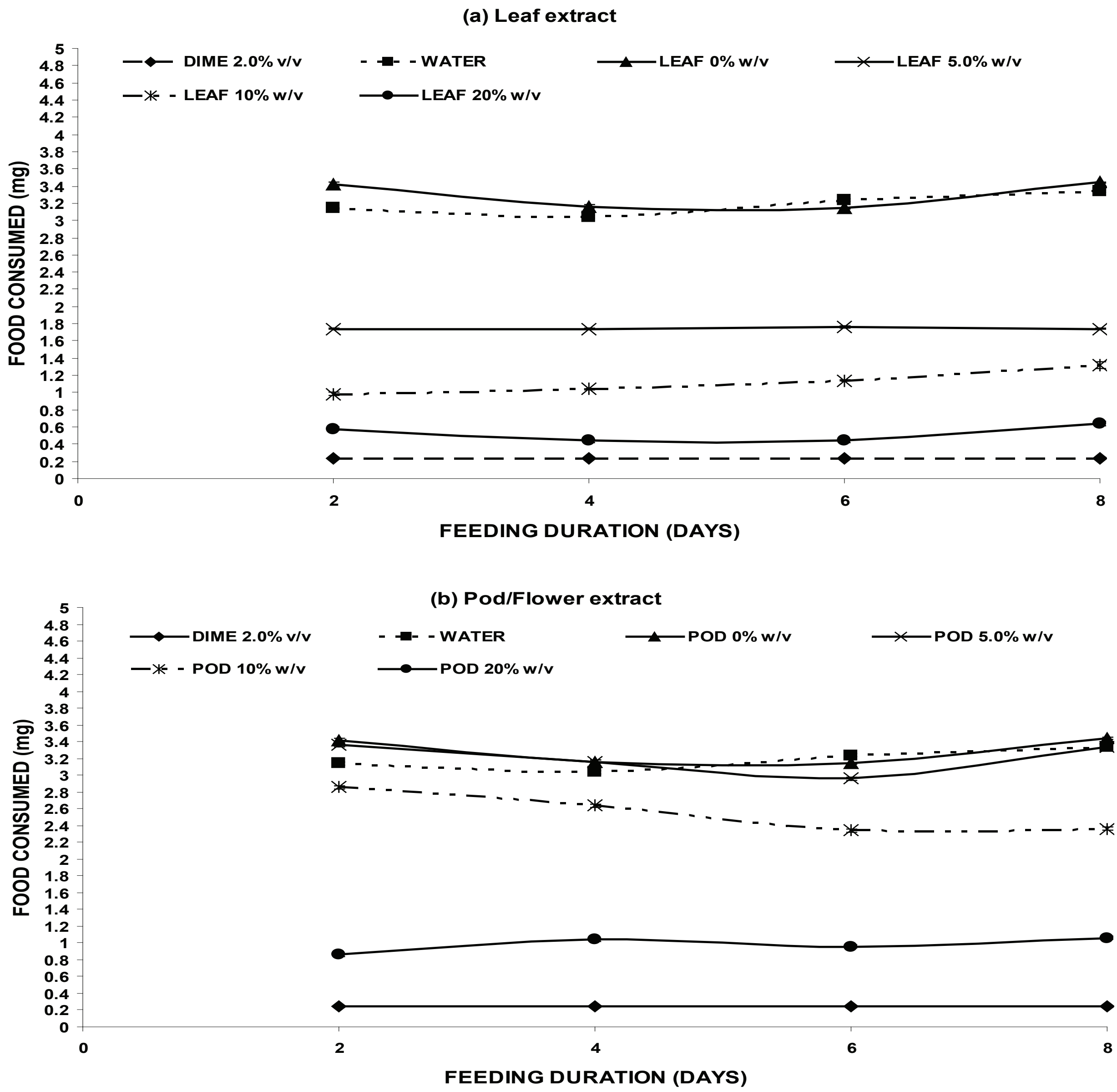


Figure 1

Mean Weights (Mean± SE; N=4) of H. armigera Larvae Fed on Aqueous Extracts Obtained from (a) Leaves, (b) Pods/Flowers and (c) Succulent Stems of Tephrosia vogelii Hook



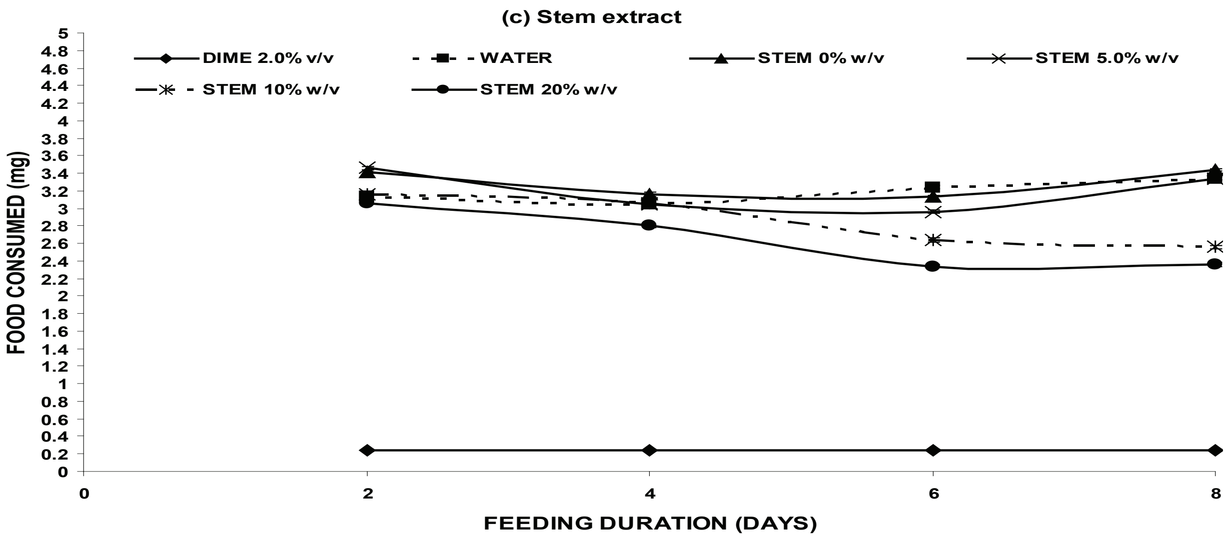
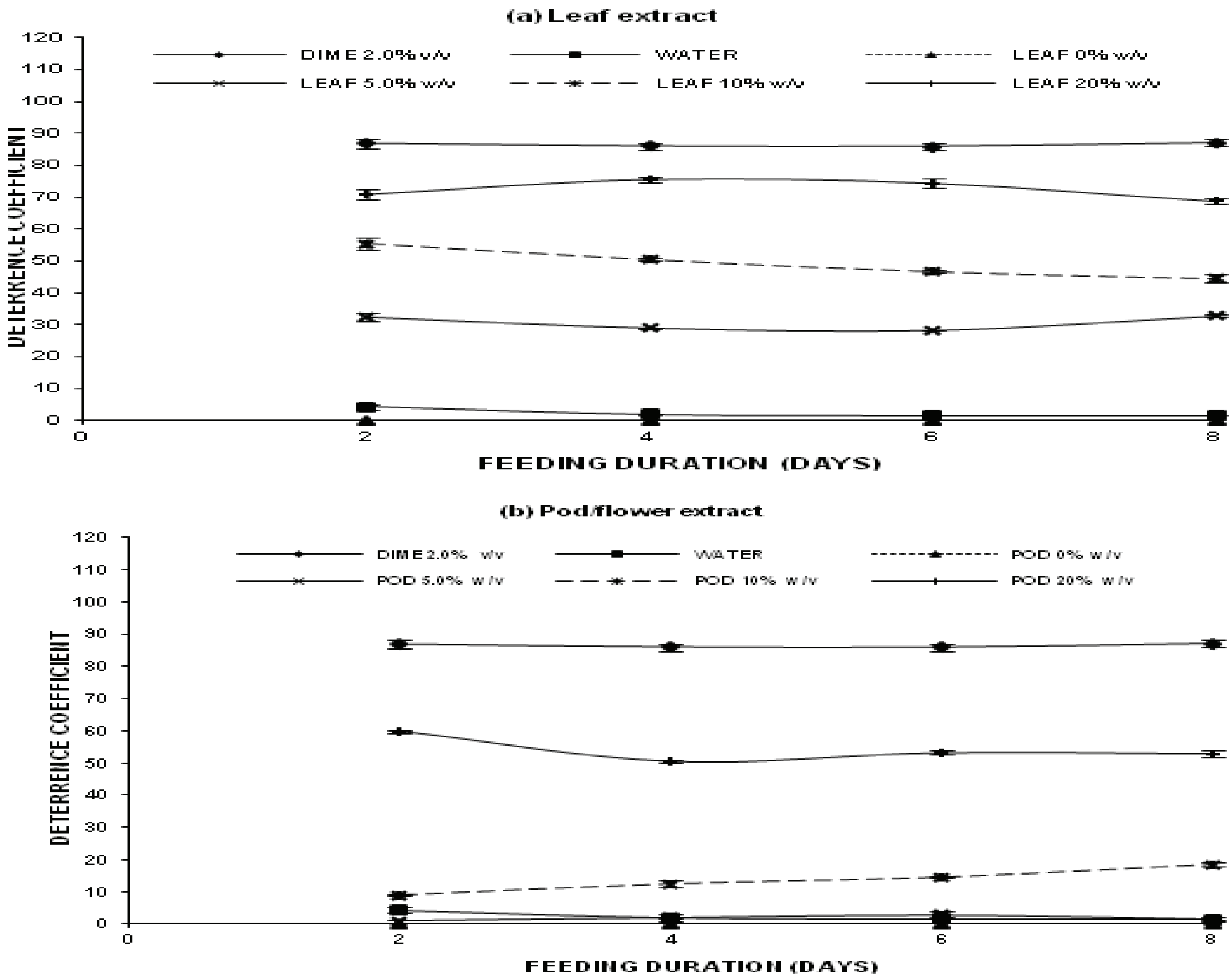


Figure 2
Mean Weight (Mg) of Food Consumed (Mean±SE; N= 5) by H. armigera Larvae as Influenced by Feeding Duration (Days) and Concentration of Aqueous Extracts Obtained from (a) Leaves, (b) Pods/Flowers and (c) Succulent Stems of Tephrosia vogelii Hook.

Repellence Tests

Repellent activity of aqueous crude extracts of *T.vogelii* against *H. armigera* larvae was significantly (p<0.0001) influenced by intra-plant variability, concentration of extract applied and exposure time. Except for leaf and pod/flower extracts at 20% w/v and 1 h exposure time with moderate repellence (PR value: 40%), a strong dose-dependent attraction of *H. armigera* larvae was observed (Fig. 4). The number of larvae that visited the chickpea leaves treated with *T. vogelii* extracts was higher compared to the negative control, an indication of attraction. The number of larvae that were attracted by the untreated chickpea leaves (food) increased with exposure time (Fig. 4).



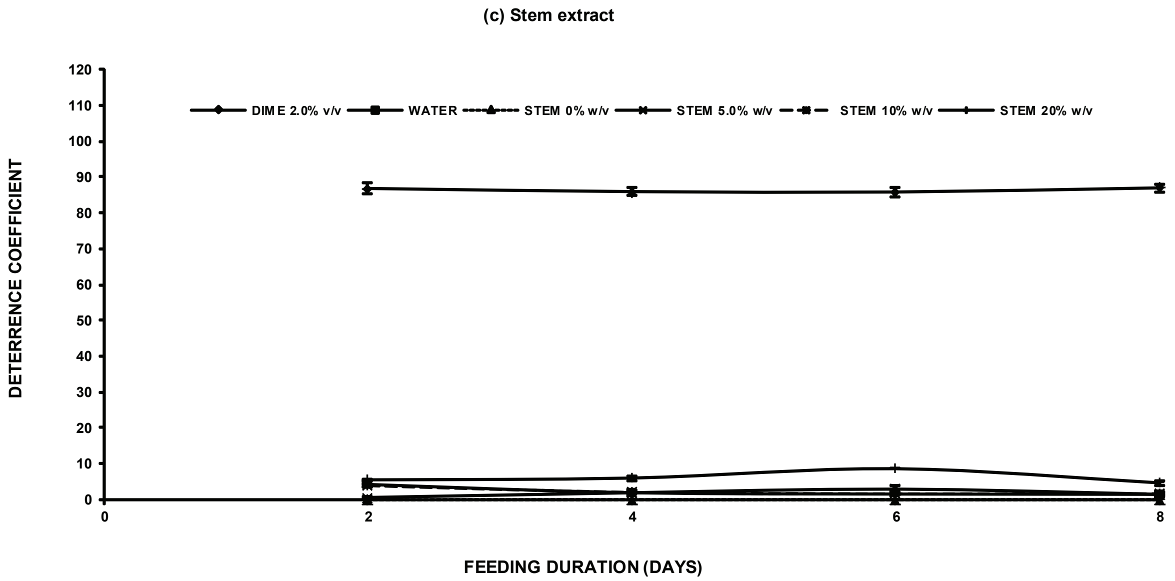
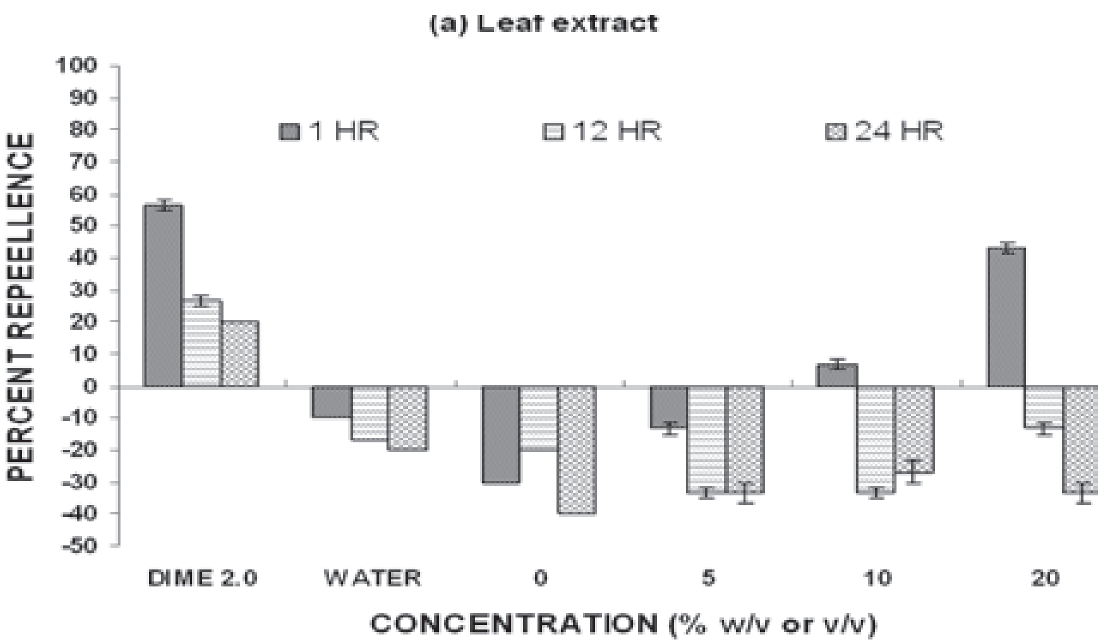
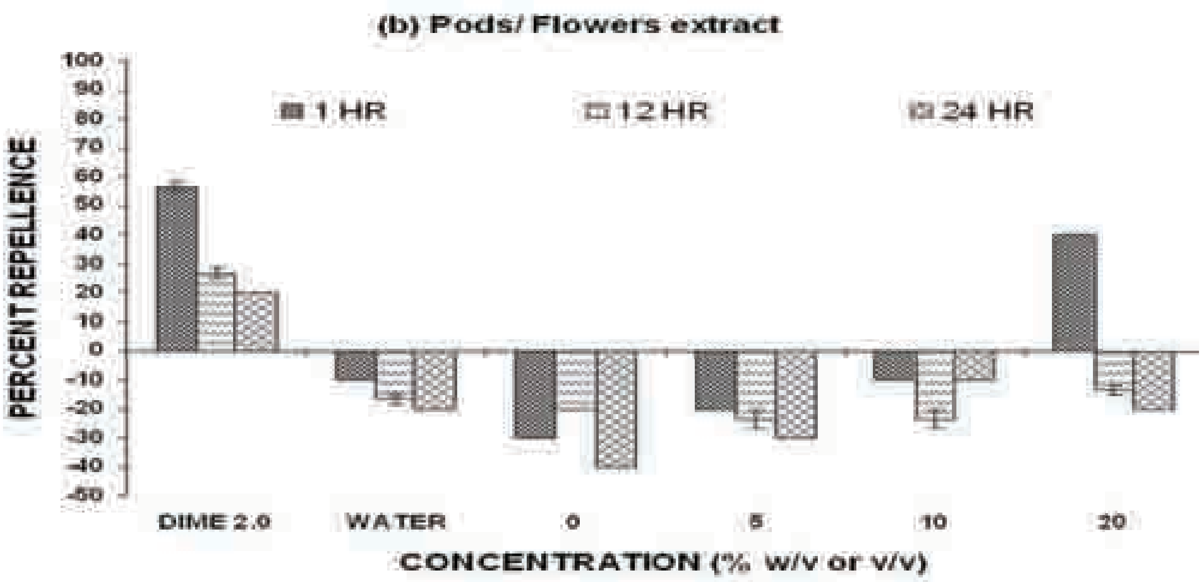


Figure 3
Deterrence Coefficient (Mean± SE; N= 5) Against H. armigera Larvae as Influenced by Feeding Duration (Days) and Concentration of Aqueous Extracts Obtained from (a) Leaves, (b) Pods/Flowers and (c) Succulent Stems of Tephrosia vogelii Hook.





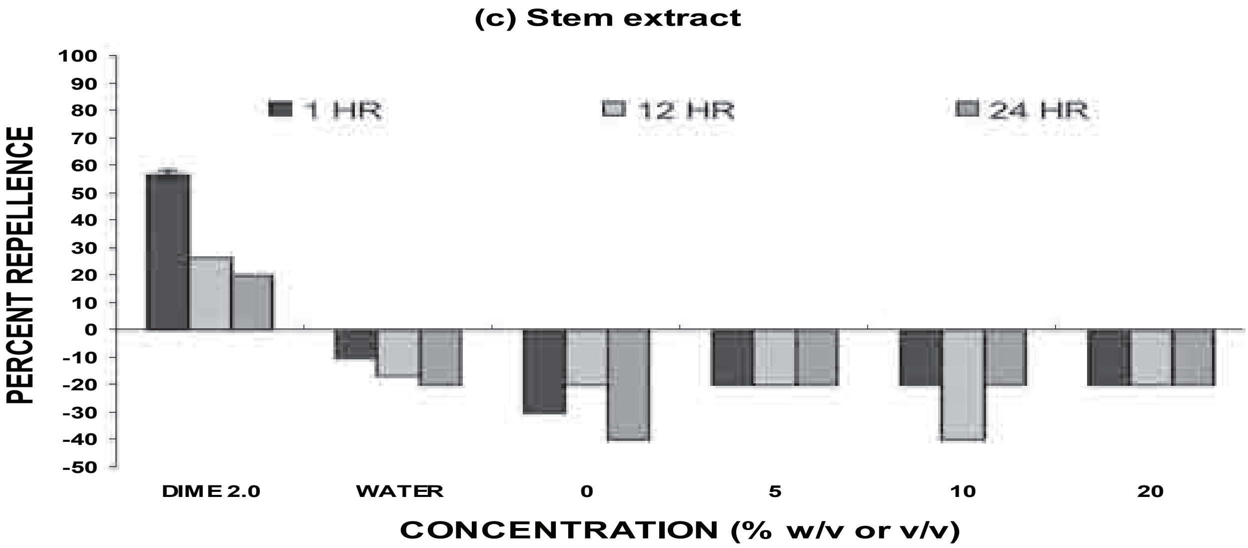


Figure 4
Percent Repellence (Mean± SE; N=4) of H. armigera Larvae to Aqueous Extracts Obtained From (a) Leaves, (b) Pods/Flowers and (c) Succulent Stems of Tephrosia vogelii Hook in a Choice Bioassay

Discussion

Our results have demonstrated that aqueous extracts obtained from the aerial parts of *T. vogelii* had weak to no contact toxicity against the larval stages of *H. armigera.* This makes the use of *T. vogelii* extracts as a toxicant against H. armigera in Chickpea less likely. However these findings are in contrast with those of Simmonds et al. (1990), Machocho (1992) and Morris (1999) who reported the presence of compounds in *Tephrosia* species with strong insecticidal and feeding deterrent effects against insect pests of different field crops. Additionally, recent local laboratory studies showed that *Tephrosia vogelii* essential oils produced strong contact (3-83% kill) and fumigant toxicities against four coleopteran pests of stored cereal and legume grains including insects feeding on pigeon pea and chickpea (Minja et al., 2002; Ogendo et al. , 2008). The significantly low contact toxicity of *T. vogelii* extracts could be explained bymorphological and physiological variations between *H. armigera* larvae compared to the other insects known to be more susceptible.

The results of antifeedant have shown that aqueous extracts obtained from leaf and pods/flowers of *T. vogelii* caused a dose-dependent reduction of the weight of larvae and amount of food consumed which are clear antifeedant indicators. The converse was true for resultant deterrence coefficients (DC) in which a dose-dependent increase in DC values as the amount of food consumed and subsequent larval weights decreased. These findings concur with past studies in which *T. vogelii* leaves and seeds have been reported as a source of rotenoids, including rotenone, tephrosin, and deguelin, known to possess strong feeding deterrent activity (Arnason et al., 1987; Adebayo et al., 2007; Ogendo, 2008). Hence, the antifeedant activity principle in the aqueous extracts obtained from aerial parts of *T. vogelii* could partially be attributed to the presence of known chemical constituents.

In the choice bioassay studies, dose- and exposure time-dependent attractions (negative PR values) of *H. armigera* larvae to chickpea leaves (food) treated with aqueous extracts of *T. vogelii* were observed. The differential repellence (PR values) responses could be attributed to intra-plant variations in the amounts of chemical compounds / principles associated with repellent activity against insects. These results are in contrast with previous local studies in which *T. vogelii* powders, at 10% w/w, were strongly repellent (PR value: 87.5% after 24 h exposure) against adult S. zeamais (Ogendo et al., 2003). Hence, *T. vogelii* has low potential as *H. armigera* larvae repellent.

Conclusion

It can be concluded from the findings of this study that aqueous extracts obtained from aerial parts of *T. vogelii* Hook cannot be relied upon as toxicants and repellents of *H. armigera* larvae. However, these plant extracts have great antifeedant potential as manifested in depressed feeding and growth (weight) of *H. armigera* larvae. This is welcome scientific hope for rationalized use of these extracts in the control of *H. armigera* in chickpea. It further fits well with current regional policies which advocates for reduction in dependence on synthetic pesticides and development of locally available, biodegradable, cost-effective and environmentally benign pest management technologies. Further in-depth bioactivity (growth inhibition, blend effects), biosafety and product formulation studies of *T. vogelii* materials and its constituents against *H. armigera* and other major insect pests of chickpea are recommended. If followed to a logical conclusion, the results of such studies will contribute to improved national, regional and global food security and livelihoods of farming communities.

Acknowledgement

The authors are grateful to the Botanical Pesticides Project, Egerton University, funded by the Inter-University

Council of East Africa through the Lake Victoria Research Initiative (VicRes). The support by the host institution, EgertonUniversity (Kenya) and the Department of Crops, Horticulture and Soils' staff are hereby acknowledged.

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EVALUATION OF SWEET POTATO PROCESSED PRODUCTS

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Abstract

A study was carried out to assess the taste, texture, acceptance and demand for cookies, mandazi, cakes and bread made from varying proportions of sweet potato (SP) to wheat flour with the objective of adding value to sweet potatoes and reducing their bulkiness. Sweet potato flour was made from freshly dug sweet potato tubers. The tubers were cleaned in fresh water, peeled and placed in a basin of water. They were then grated with a kitchen grater and left in water to avoid oxidation. The grates were removed from water and sun - dried for two to three days. Dried grated sweet potatoes were ground into flour which was used in the study. Sweet potato flour was mixed into three ratios (25:75, 50:50, 75:25) with wheat flour. Each of these ratios was incorporated into a standard recipe for bread, cakes and cookies and baked in an oven at 180^0C for one hour, 160^0C for 40 minutes and 180^0C for 15 minutes, respectively. Mandazi balls were deep fried in oil. The products were presented to a panel of randomly selected respondents for evaluation on a 5-point scale as follows: taste (1 = very poor, 2 = poor, 3 = average, 4 = good, 5 = very good) and texture (1 = very coarse, 2 = mildly coarse, 3 = average, 4 = soft, 5 =very soft). Consumer preference was determined in the form of a questionnaire. Respondents were also asked to determine possible market price of the various products. In general, the test products were accepted by the respondents at varying degrees. Cookies with SP flour ratios of up to 50% had highest preference rating. Increasing SP flour to 75% produced coarse cookies. Mandazi, on the other hand, were preferred at SP flour ratio of 75%, which was also the most expensive to make because of the amount of oil used. Preference for cakes and bread increased with decreases in SP flour. The most profitable sweet potato products according to the economic analysis were cakes and cookies. Demand for the products was also influenced by the availability of similar products made from wheat flour. Chi-square test of goodness of fit showed that acceptance of the products was influenced by taste and texture as a result of the various proportion of sweet potato flour to wheat.

Key words: Sweet potato, sweet potato (SP) flour, processing, product, market.