Journal of Applied Biosciences 112: 11005-11015

ISSN 1997-5902

Effects of *Phytolacca dodecandra* Extracts on the Quality Parameters of Stored Maize Grains

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Original submitted in on 6th January 2017. Published online at www.m.elewa.org on 30th April 2017 https://dx.doi.org/10.4314/jab.v112i1.5

ABSTRACT

Objective: subsistence farmers in Mbulu district to control grain pests in stores use Phytolacca dodecandra leaves. The study evaluated the effects of leaf and root extracts of *P. dodecandra* on grain quality: moisture content, insect infestation, seeds germination, colour and odour over 150 days of storage.

Methodology and Results: P. dodecandra plants were collected, identified, processed and extracted using ethanol (95% v/v) and extracts stored at -4 °C for quality tests. Synthetic pesticide (Actellic gold™ dust) at 0.05 g/kg and untreated maize sample were used as positive and negative controls, respectively. No significant increase of moisture content ($p < 0.05$) was observed in treated maize compared to controls. Leaf and root extracts minimized grain damage to <5% and <6%, respectively after 150 days post treatment. Both extracts did not affect odour, though leaf extracts affected grains colour. Both extracts insignificantly reduced seed germination index ($p > 0.05$) to 29%.

Conclusion and Application of findings: P. dodecandra extracts have little impact on the moisture content of the stored maize grains. Germination index of the treated maize was affected neither by the extracts nor by the storage duration up to 150 days. These marginal changes in grains moisture, odour and colour have no significant impact on the local market value of the maize grains. *P. dodecandra* extracts for use as biopesticide are recommended as a sustainable alternative to synthetic pesticides in maize grains storage especially for the subsistence farmers. However, further tests on biosafety and effects of the extracts on the organoleptic contents of the grains prior to consumption are recommended.

INTRODUCTION

The use of botanical pesticides is considered as a substitute to hazardous synthetic pesticides such as pyrethroid and organophosphorus pesticides due to the disturbance in the environment, increasing user cost, pest resurgence, pest resistance to pesticide and toxic residues in food grains. Botanical pesticides are less cost effective, non-toxic to consumers and readily available for storage of large quantities of grain than the use of synthetic insecticides (Arya & Tiwari, 2013). Hence, Subsistence farmers treat grains with plant products and oil, use cultural methods such as open sun drying and storing in barns, earthen pots, jars and airtight containers (Asawalam *et al*., 2006). Botanical pesticides and essential oils from various plant parts (seeds, stem roots, leaves and flower heads) have been used against field and storage pests due to their broad-spectrum pesticide activity. Some plants have been scientifically tested and found to have good pesticide properties (Kamatenesi-Mugisha, 2013). Various botanical grain protectants have been used to protect stored grains and these include *Lippia javanica, Tageta minuta, Lantana camara* (Muzemum *et al*., 2013), *Eucalptus* spp (Shahzadi *et al*., 2010) *Azadirachta indica*, *Nicotiana tabacum*, Lonchocarpus *heptaphyllus*, *Derris elliptica*, *Tephrosia vogelii*, *Annona squamosa*, *Schoenocaulon officinale*, *Chrysanthemum cinerariaefolium, Ryania speciosa*, *Rosmarinus officinale*, *Thymus vulgaris* and *Nicotiana gossei* (EL-Wakeil, 2013). Plant extracts, essential oils and powder from these plants demonstrated contact toxicity (Asawalam *et al*. 2006; Ogendo *et al.,* 2008), repellence (Rosman *et al*., 2007), fumigant toxicity (Lee *et al*. 2003; Rajendran & Muriladharan 2005) and anti-feedant effects (Ogemah 2003; Chebet *et al.,* 2013) when evaluated against several storage insect pests of cereals and legumes. In many parts of the world and Tanzania in particular, the maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae) are serious post-harvest pests which damage on stored grains and pulses may reach 40% particularly where modern storage technologies are not used (Pandey *et al*., 2011). These storage pests affect both farmers and traders. *S. zeamais* larvae are internal grain feeders, which affect ability of seeds to germinate lower market value of grains as well as aesthetic and grain nutritive value (Muzemum *et al*., 2013). Determination of plant efficacy with pesticide activity properties is a key step in the bio-prospecting of new plants based compounds in grains protection. Although the pesticides are meant for grain protection in which they have succeeded at large, some of them have shown to affect the quality of the grains adversely. *P. dodecandra* leaves (Figure 1) are used by subsistence farmers in Mbulu district to control grain pests in stores with minimal loss in quantity and quality. However, the use of this plant species by various societies in Mbulu district as a pesticide has not been scientifically rationalized. Therefore, this study evaluated the effect of treating stored maize grains with *P. dodecandra* leaf and root extracts on grain organoleptic properties (moisture content, grain damage, seed viability, colour and odour).

Figure 1: *Phytolacca dodecandra* plant showing the leaves

MATERIALS AND METHODS

Study Area : The study was carried out in Mbulu district, south west part of Tanzania (Figure 2) with approximately 7,695 square km (including Lake Eyasi), of which 6,700 square km is a dry land.

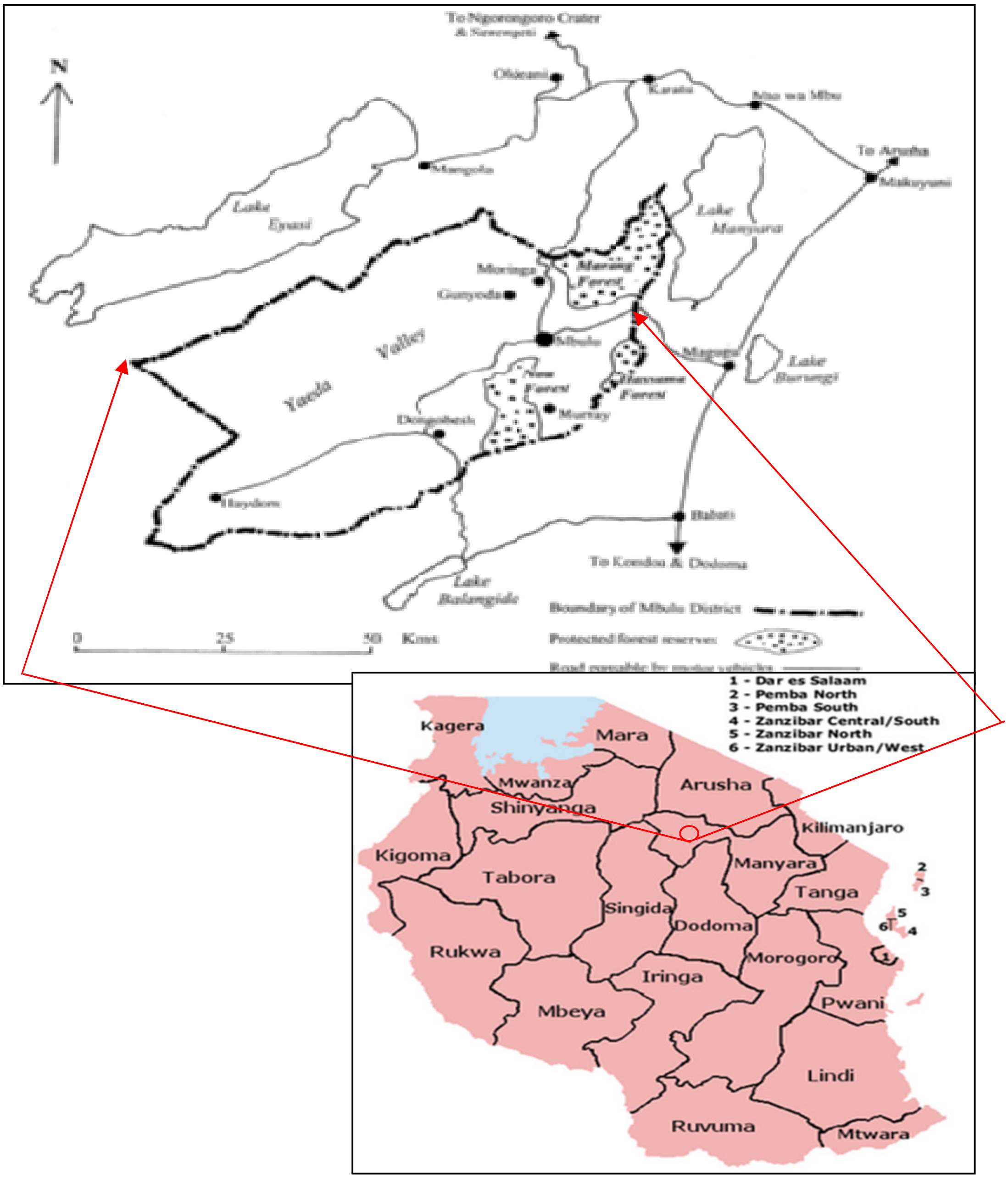

Figure 2: Map of Tanzania showing Mbulu District (Modified from Google Earth)

Mbulu district have an altitude, which ranges from 1,110 m to 2,250 m. This difference in altitude contributes to the wide range of climatic conditions with mean annual temperature ranging between 17.3 °C and 23.4 °C. In addition, the mean annual rainfall ranges from 400 mm to 1,100 mm. The district is mainly dominated by hunters and agro-pastoral communities. There are other socio-economic activities, which are practiced including smallholder rainfed cultivation, extensive grazing, afforestation and mechanised rainfed cultivation with medium to high inputs. The main crops grown in the study area includes maize, beans, pigeon pea, sorghum, wheat, vegetable, fruits and coffee (Brornsen *et al*., 2006). *P. dodecandra* leaves are used by small farmers to control grain pests in stores and field crops with minimal loss of quantity and quality in Mbulu district (Qwarse, 2015).

Plant Material Collection and sample preparation: *Phytolacca dodecandra* species were collected from Mbulu district in March 2014. Botanical identification of the species was done by Haji Selemani (taxonomist) at the Herbarium in the Department of Botany, University of Dar es Salaam where voucher specimens were deposited. Plant materials were immediately separated into their component parts (leaves and roots) and placed in the open container in a dark room. The samples were allowed to air dry at room temperature in a dark room with low humidity for about two weeks**.** The samples were subjected to size reduction where the roots were sliced into pieces of 1 – 2 cm long, while leaves were cut into pieces of approximately 1 cm^2. All samples were then dried at room temperature for another two weeks and further oven dried at 35°C for 48 hours. Dry samples were ground into fine powders using a laboratory electric hammer mill. The powdered plant materials were then stored in an airtight glass jars in a cool place ready for extraction. Extraction of the samples was done using cold method (maceration) where plant powders were extracted using ethanol (95% v/v) for 24 hours at room temperature (Omorgbe *et al*., 1996). Later, the extracts were filtered, concentrated and then stored in a refrigerator at -4 °C for pesticide activity tests. Finally, the infested maize grains were collected from local maize market and screened for presence of the intended insect pests in the laboratory.

Mass Rearing of Test Insects: Two hundred (200) unsexed adults *S. zeamais* Motschulsky insects were reared in 5 litres gas jar containing 1kg of disinfested maize grains as described by Haines (1991) and Ogendo *et al*., (2004). The top of rearing jar was covered with nylon mesh fastened tightly with rubber bands. Then the *S. zeamais* insects were allowed to lay eggs (oviposition) for a period of 7 days. Thereafter, all adults were removed and the gas jar was monitored for 25 days where emerging adult insects were taken and kept in separate jars according to their age. The rearing test of *S. zeamais* insects was done in the laboratory at ambient temperature of 25 – 30^{0}C and 38- 69% relative humidity (RH) at 12 hours of light and 12 hours of dark (L12: D12) regime (Ogendo *et al*., 2005).

Disinfesting of Maize grains: Maize grains used in the experiment were disinfested under a gas tight sheet for 10 days using phostoxin tablets (5g PH_3 per 1000 kg of maize) to kill any latent insect infestation according to Haines (1991) and Ogendo *et al*., *(*2004*)*. The disinfested maize grains were kept in the laboratory under ambient conditions. Sub-samples used for grains quality evaluations were further disinfested at 40 °C in an oven for 4 hours (Ogendo *et al*., 2004) and allowed to cool for 2 hours before use.

Grain Quality Evaluations: The extract of *P. dodecandra* plant species was mixed with disinfested maize grains (1 kg). The untreated maize sample was used as negative control. Synthetic insecticide, (actellic gold™ 16 g/kg pirimiphos-methyl + 3 g/kg permethrin), 2% dust at the recommended rate of 0.05% (w/w) was used as comparative positive control (Table 1). The concentrations of *P. dodecandra* extracts used for grain quality evaluations were chosen based on their pesticide activities (contact toxicity, repellence, feeding deterrence) on *S. zeamais* and *T. castaneum* storage pests (Qwarse *et al*., 2015). A total of 20 randomly selected insects per 1kg sample were used as initial infestation.

Table 1: Treatments Used for the Grains Quality Evaluation

Treatments	Description	Dosage
T1	Untreated grain sample (negative control)	0 mg/mL
T2	Actellic gold™ 2% dust (positive control)	0.05 w/w
T3	*P. dodecandra* leaf with extract in ethanol	150 mg/mL
T4	*P. dodecandra* root with extract in ethanol	300 mg/mL

A total of 4 treatments in triplicate were arranged in a completely randomised design (CRD) on one-metre high wooden benches in the laboratory. All treatments were kept at temperature of 25-30 °C, 38-69% RH and at L12: D12 regime. The baseline parameters used for quality evaluations is given in Table 2.

Table 2: Baseline Parameters for Grains Quality

Quality parameter	Value (± S.E, where applicable)
Grains moisture contents (%)	14.7 ±0.14
Grain damage (%)	00 ± 00
Seed viability (%)	86 ± 0.30
Grains odour	Odourless
Grains colour	natural white with few yellow gains

A cylindrical grain sampler (25 mm in diameter) was used to take sub-samples (250 g) from each replicate after 0, 30, 60, 90, 120 and 150 days of grains storage according to Haines (1991) and Ogendo *et al*., (2004). The sub-samples were used to determine the grain quality parameters: moisture content (%), insect damage (%), seed viability index (%), grain colour and odour as described below.

Grain Moisture Content: A weighed sub-sample of about 100 grams of untreated maize grains was put in a capacitance grain moisture meter (LDS-1G, China). Direct temperature-corrected moisture content (%) readings were recorded for each grain sub-sample in triplicate.

Grains Damage: The grain sub-samples were assessed for damage arising from insect infestations after 0, 30, 60, 90,120 and 150 days of storage. Each sub-sample was separated into undamaged and insect-damaged grains. The number of grains in each category was counted, weighed and the percentage weight loss (percent grains damage) of maize grains in storage was computed according to the Musundire *et al*., (2015). as follows:

$$\%\text{Weight loss} = \frac{(\text{UNd} - \text{DNu})\text{x}100}{(\text{U}(\text{Nd} + \text{Nu}))}$$

Where: U = weight of undamaged grains, D = weight of insect damaged grain, Nu = number of undamaged grains and Nd = number of insect damaged grains.

Seed Viability Index: The effect of extract treatments, storage duration and their interactions on seeds viability were expressed as the percent germination as investigated over a 150-day grains storage period. Unbiased sub-samples of 100 undamaged grains were obtained according to the methods described in Haines (1991) and Ogendo *et al*., (2004). The sub-samples (100-grains) were germinated on moistened cotton wool in Petri dishes arranged in a CRD in triplicate. The experiment was maintained under laboratory conditions as described above. The number of emerged seedlings from each Petri dish were counted and recorded after 7 days. The percentage germination was computed according to Musundire *et al*., (2015) as follows:

$$\text{Viability index (\%)} = \frac{\text{NGx}100}{\text{TG}}$$

Where: NG = number of seeds that germinated and TG = total number (=100) of test seeds placed in each Petri dish.

Grain Colour and Odour: The change in colour and odour of untreated and treated maize grains were assessed on a monthly basis for five months consecutively. Grain sub–samples were assessed for change in colour and odour by modification of the method described by Musundire *et al*., (2015). The assessment method was done by using 1-5 scoring scale which define separately each of the two parameters. However, scores at day zero (0) represent the values just before grain treatment. Scoring for change in grain colour was done according to the following scale: 1 = No detectable change i.e. natural colour (white/gray) with a few yellow grains, 2 = Slight change (≤5%) from natural colour to light brown, 3 = Moderate change (>5 to 30%) from natural colour to brown, 4 = Great change (>30 to 50%) from natural to dark brown, and 5 = Highly significant change (>50%) making grain unacceptable for human consumption. Scoring for change in odour was done as follows: 1 = Grain is odourless, 2 = Grain has little offensive odour, 3 = Grain has moderately offensive odour, 4 = Grain has offensive odour that is acceptable for human consumption and 5 = Grain has very offensive odour making grains unacceptable for human consumption. To obtain unbiased scores, each grain sample was coded and presented in a well-lit and ventilated room for assessment. A panel of six independent assessors record the change in maize grains colour and odour. The assessors were allowed into the assessment room once at a time in rotation to ensure their records were independent from each other. The procedure was repeated on a monthly basis for five month and the same six panellists were retained over the entire assessment period. Blank scoring sheets were used for the different assessment dates to ensure that the previous data do not bias subsequent scores tests (Musundire *et al*., 2015).

Data Analysis: Data collected were subjected to analysis of variance (ANOVA) statistical analysis. The data presentation in form of graphs was done using Microsoft office excel 2007.

RESULTS AND DISCUSSION

Moisture Content of Treated Grains: The effects of botanical pesticides on the percent grain moisture content over a given duration of storage are presented in Figure 2. Generally, there was a slight increase in moisture content of maize treated with botanicals as compared to controls. This increase was significant ($p < 0.01$). This indicated that the botanical extracts have little impact on the moisture content of the stored maize grains. The results showed that the moisture content of treated and control maize grains were significantly influenced by duration of storage as indicated in Figure 3. Ogendo *et al.,* (2004) reported that percentage moisture content of maize grains treated with leaf and root ethanol extracts followed a more or less similar trend. Storage products are usually hygroscopic and as such, they absorb moisture from the surroundings. As there was change in temperature of the environment, the moisture holding capacity of the air increases three times (Ogendo *et al*., 2004). When stored products release moisture to the surroundings during low humidity, moisture content of grains is depressed.

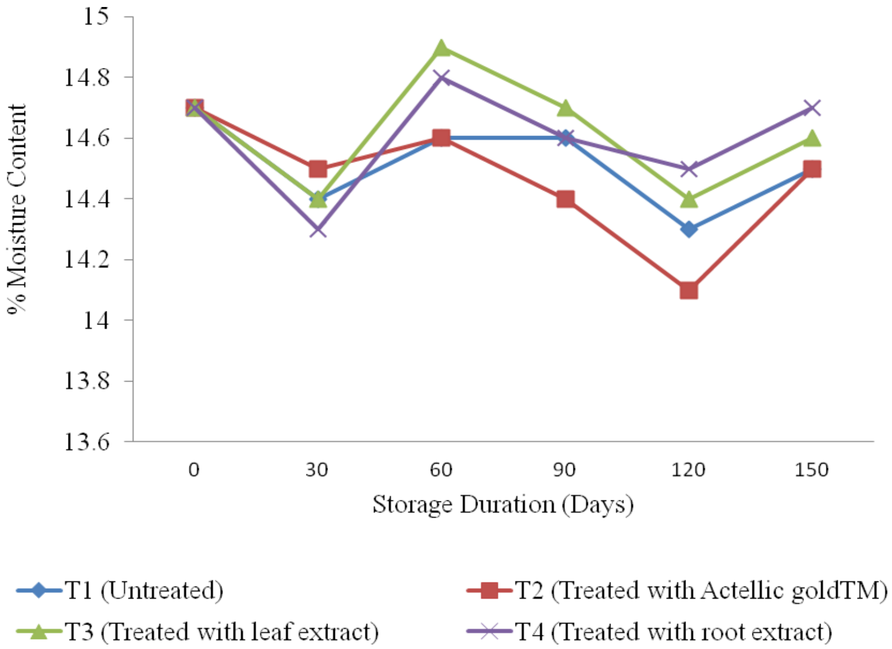

Figure 3: Effect of the Biopesticide on Moisture Content in Stored Maize grains

When the relative humidity and air temperature vary, the maize grains moisture content is likely to vary (Ogendo, *at el*., 2004). This is justifiable particularly in the coastal tropics where ambient humidity and temperature tend to change throughout depending on the season in the year.

Seed Viability of Treated Grains: Percent germination did not significantly ($p > 0.05$) decrease with storage duration for the untreated maize grains, treated maize grains with actellic gold™ and with *P. dodecandra, a* botanical pesticide (Figure 4). Germination decreased rapidly from 81% at day 30 to around 29% at day 90 for both botanical treatments, and later stabilised at around viability index of 29% after 120 days. The decrease of percent germination of seeds treated with *P. dodecandra* leaf and root extracts dropped from 86% on day zero to 29% on day 90 as compared to 73% of the actellic gold™ standard. However, this decrease was not significant ($p > 0.05$). In addition, there were no significant effects of treatment and storage duration by treatment interaction on the percentage germination. Correlation analysis results show that seed viability was weakly correlated with the grain moisture content, botanical treatments and storage duration. Maize grains treated with leaf extracts had the lowest germination viability after 90 days.

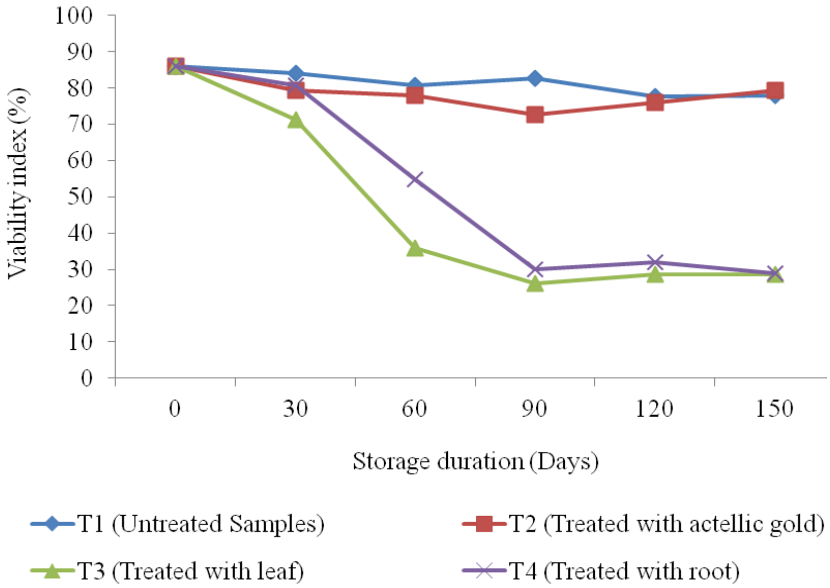

Figure 4: Change in Viability of Maize Grains Treated with *P. dodecandra* Extracts during Storage

The *P. dodecandra* extracts showed significant ability to affect the germination of other plants due to chemicals that can alter the physiological and biochemical processes occurring inside the seeds (Maryam, 2010). For example, the percentage germination decreased from 90% of control to 50% when the crop seeds were treated with neem oil extract for 4 hours at 0.08 mg/mL due to botanical compounds (Rath *et al*., 2013). Studies indicated that decrease in percentage germination of seeds in various crops after treatment with botanical extracts is attributed to phytotoxins, which are common in many plant species (Babu *et al.,* 2014; Mendez & Mannuel, 2014; Mishra, 2014). However, some extracts have no effects on seed germination (Ogendo *et al.,* 2004; Musundire *et al.,* 2015) similar to our observations.

Grains Damage during Storage: The level of grains damage was higher in untreated (negative control) maize grains than those treated with actellic gold™ and *P. dodecandra* extracts (Figure 5).

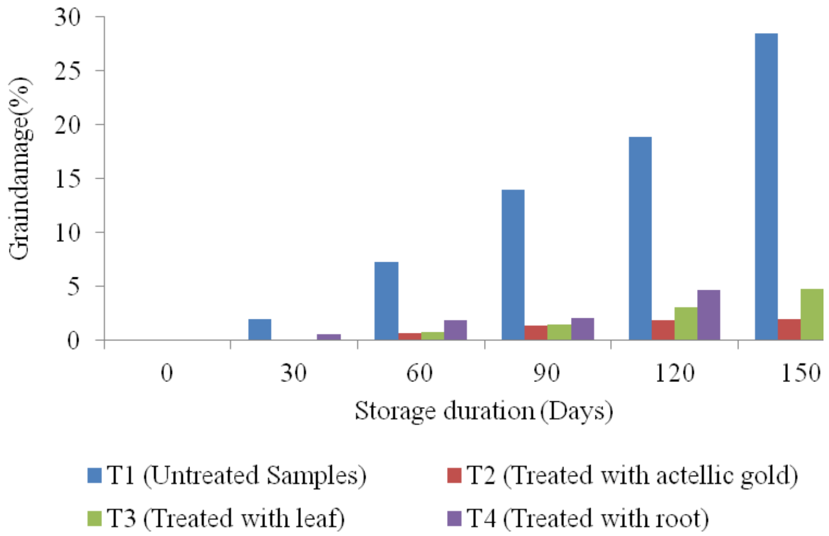

Figure 5: Effect of *P. dodecandra* Extract on Percent Grains Damage on Stored Maize Grains

The result showed that there was no significant effect ($P < 0.05$) of storage duration and treatment duration on the level of grains damage of the stored grains. The percent of grains damage increased as duration of storage increased. This can be explained probably by the volatility or biodegradability of bioactive constituents over time. However, there was still a considerable reduction in grains damage (5%) for the 5 months of storage. Unlike the treated grains, the untreated maize grains suffered high percent grains damage compared to grains treated with actellic gold™ 2% dust and the botanical treatments (Figure 5). As indicated in Figure 5, the present study is in agreement with a study done by Ahmed *et al*., (2014) who observed that *Callosobruchus maculates* seeds treated with extracts of neem seeds for six month reduced insect damage of the grains from about 95% to around 23%. However, a similar study by Ogendo *et al*., (2004) reported that maize grains treated with *Lantana camara* and *Tephrosia vogelii* for five months had insect damage percent of up to 0.7%. The relatively high insect damage observed in this study could be due to high temperature and humidity that always prevails in coastal areas. This result are in agreement with Musundire *et al*., (2015) who reported that stored maize grains treated with plant powders of *Tegetes minuta* can reduce grains damage.

Effect of Botanical Treatment on Colour and Odour Change in Maize Grains during Storage : Figure 6 shows the mean evaluation scores of colour for treated samples with *P. dodecandra* extracts as compared to those treated with actellic gold™ 2% dust and untreated maize grains. The result shows that there was no significant difference ($p > 0.05$) of the effect of storage duration, quality assessors, botanical treatments and storage interaction on the colour of the stored maize grains. The Wilcoxon scores (rank sum) increased with increased storage duration. The effect is higher in *P. dodecandra* leaf extract treatment compared to root extract treatment. This could be due to chlorophyll materials available in the leaves and eliminating the chlorophyll from the leaf extract might reduce this effect.

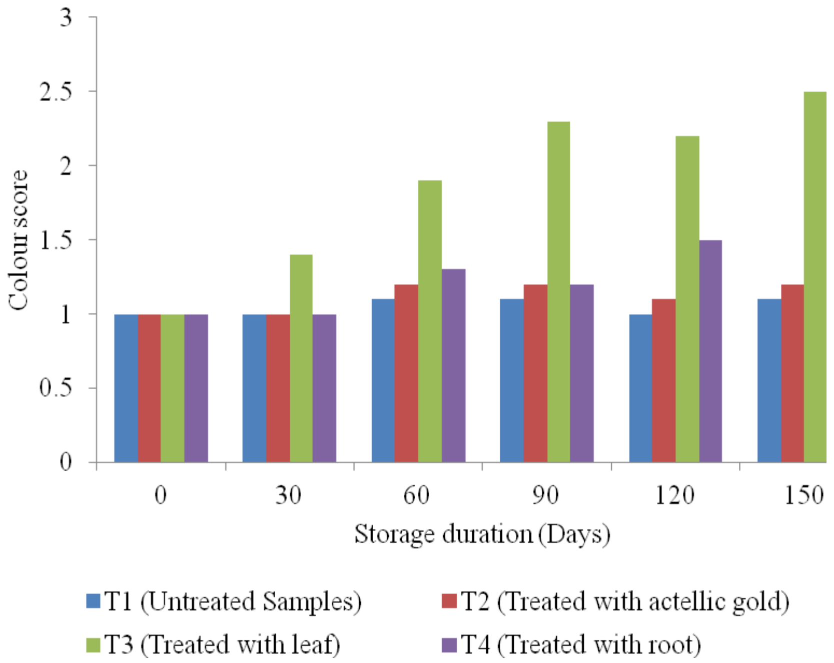

Figure 6: Effect of *P. dodecandra* Extracts on Colour of Stored Maize Grains

The results of change in odour during storage of untreated maize grains, those treated with actellic gold™ 2% dust and those treated with ethanol extract of P. *dodecandra* leaf and root are presented in Figure 7. The result shows that there was significant difference ($p < 0.05$) of effects of storage duration, quality assessors, botanical treatments and storage interaction on the odour of the stored maize grains. The Wilcoxon scores (rank sums) increased at 30 days of storage for botanical treatment and decreased to 120 days of storage. The effect was higher in *P. dodecandra* leaf extract treatment compared to root treatment.

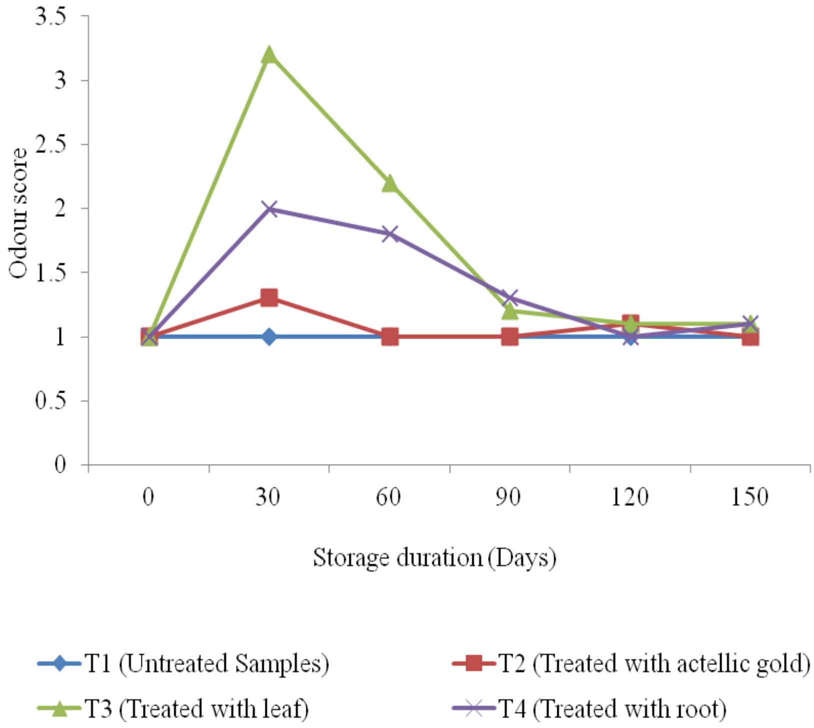

Figure 7: Mean Evaluation Scores of Maize Grains odour after application of *P. Dodecandra extracts*

The Wilcoxon scores for colour increased as duration of storage increased while in odour parameter scores increased from zero days to 30 days then decreased to 90 days and became constant until 150 days of storage for the maize grains treated with *P. dodecandra* extracts. The untreated maize grains and actellic gold™ treated maize grains had slight changes of score during storage period. This implies that the concentration decreased with time, possibly due to the volatility of the bioactive compounds (Ogendo *et al.,* 2004). The evaluation results indicated that admixing maize grains with botanicals treatment started to show slightly changes in grains colour and odour over the five month of storage similar to studies done by Ogendo, (2000) and Musundire *et al.,* (2015). This could indicate that the constituents from the botanical plant powders were not absorbed by the grains (Ogungbite *et al*., 2014). The deterioration of colour in maize grains is probably due to relatively high moisture, high relative humidity, storage period and respiration process in grains, which favour the growth of fungi such as *Aspergillus favus*, *Penicilliun* spp and *Fusarium* spp that produce mycotoxins (Suleiman *et al*., 2013). The average value for colour change was below 2.5 indicating a slight change that is tolerable in the local market. Similarly, the odour change indicated a slight offensive odour after 30 days that faded away as storage time increased.

CONCLUSION

The findings from this study have shown that *P. dodecandra* leaf extracts reduces grain damage and have shown promising results as a natural pesticide in storing maize grains. In addition, botanical treatment of this plant causes marginal changes in grains moisture, odour and colour with no significant impact on the local market value of treated maize grains. However, the botanical treatment reduces seeds germination of the maize grains. Further tests are recommended to investigate the penetration of such botanical compounds into stored maize grains and their impacts on the organoleptic content of the grains. More studies on the biosafety and levels of botanical residues on the treated grains and their potential adverse effects should be done before the extracts are used to treat grains for consumption.

ACKNOWLEDGEMENTS
This work was sponsored by the Tanzania Commission for Science and Technology (COSTECH) through project No. CST/SC.327/952/3/2013 to which the authors are very indebted. The authors are grateful to the Departments of Medicinal Chemistry and Microbiology, School of Pharmacy, Muhimbili University of Health and Allied Science (MUHAS) for providing the laboratory facilities to carry out this work.

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