

Pesticidal Activity of *Phytolacca Dodecandra* Extracts against *Sitophilus zeamais* (Motschulsky) (Curculionidae) and *Tribolium castaneum* (Tenebrionidae) Storage Pests in Maize

Michael Qwarse¹, Matobola J. Mihale^{1,*}, Leonia Henry², Joseph Sempombe³,
Veronica Mugoyela³, Fortunatus Sung'hwa⁴

¹Department of Physical Sciences, Open University of Tanzania (OUT), Dar es Salaam, Tanzania

²Department of Science and Laboratory Technology, Dar es Salaam Institute of Technology, Dar es Salaam, Tanzania

³Department of Medicinal Chemistry, School of Pharmacy, Muhimbili University of Health and Allied Sciences (MUHAS), Dar es Salaam, Tanzania

⁴Department of Chemistry, College of Natural and Applied Sciences, University of Dar es Salaam, Dar es Salaam, Tanzania

*Corresponding author: matobola.mihale@gmail.com

Abstract This study assessed the pesticidal activities of *Phytolacca dodecandra* extracts in controlling *Sitophilus zeamais* and *Tribolium castaneum* pests in stored maize grains. The study was conducted at ambient conditions (25–30°C and 65–70% relative humidity) and designed in a completely randomised design with 8 treatments in triplicate. Contact toxicity, percent mortality, feeding deterrence and grains weight loss were assessed using developed procedures for 21 days and first filial generation progeny was assessed for 42 days of post-pest introduction. Both ethanolic root and leaf extracts of *P. dodecandra* demonstrated significant bioactivities against both *S. zeamais* and *T. castaneum*, with the leaf extract being more active than the root extracts. *P. dodecandra* leaf extracts killed 98% of *S. zeamais* and 99% of *T. castaneum* at concentration of 150 mg/mL on day 3. Similarly, *P. dodecandra* leaf extracts reduced grain damage to 0% and had moderate repellence of 57% and 66% to *S. zeamais* and *T. castaneum*, respectively. Hence, after biosafety studies this plant-based products can be included in the Integrated Pest Management strategies and can be recommended as an alternative remedy in treating maize storage pests in subsistence agriculture. Further isolation and structure elucidation of bioactive ingredients in the plant species are recommended and are underway.

Keywords: Pesticida Activity, *Phytolacca dodecandra*, Mbulu, Tanzania, *Sitophilus zeamais*, *Tribolium castaneum*, maize

Cite This Article: Michael Qwarse, Matobola, J. Mihale, Leonia Henry, Joseph Sempombe, Veronica Mugoyela, and Fortunatus Sung'hwa, "Pesticidal Activity of *Phytolacca Dodecandra* Extracts against *Sitophilus zeamais* (Motschulsky) (Curculionidae) and *Tribolium castaneum* (Tenebrionidae) Storage Pests in Maize." *Journal of Food Security*, vol. 4, no. 6 (2016): 147-155. doi: 10.12691/jfs-4-6-4.

1. Introduction

The agricultural sector in developing countries is heavily facing post-harvest crop losses in quality and quantity caused by storage pests [1]. This contributes to the problem of food shortages and poverty. Storage insects cause qualitative and quantitative pre- and post-harvest losses varying in magnitude from 10% to 100% in tropical countries [2,3]. These losses are mainly caused by *Sitophilus* spp. *Tribolium castaneum*, *Rhyzopertha dominica*, *Callosobruchus chinensis* and *Sitotroga cerealella* in cereals, as well as *Acanthoscelides* spp and *Callosobruchus* spp in legumes [4,5]. More damage in cereal crops is also caused by tenebrionid beetles and *Prostephanus truncatus* in stored cereal crops and cassava [6]. Currently synthetic pesticides are used for controlling storage pests.

However, high costs, low biodegradability, multi resistance to pests and disease causing microorganisms as well as their unavailability in rural areas make the use of synthetic pesticides uneconomical and incompatible in subsistence agriculture. As a result, plants and fungi have been used in pest control [2,7].

Tanzania alone is endowed with a great abundance of flora diversity which is estimated to constitute about 10,000 vascular plant species, of which 25 % are indigenous and among them, about 1,200 species have been reported to occur exclusively in Tanzania [8,9]. Northern part of Tanzania, Mbulu in particular, is well known to have abundance of the natural herbs of which their biological potential is not yet explored [10,11]. A number of botanical plants in the region are known of their insecticidal properties by subsistence farmers, but there is no much scientific knowledge on their bioactivity and toxicity values. Lack of technical resources and

assistance to the majority of subsistence farmers undermine their efforts in diseases and pests management at family level. Consequently, most of bioactive plants used traditionally have not been tested scientifically for their efficacy and toxicity in both human and animals. These cause the loss of crops quality and animal products which may affect the food security and livelihood of many poor communities [2,7].

Phytolacca dodecandra (L' Herit) (synonyms: *P. abyssinica* Hoffin, *Pircunia abyssinica* Moq.) is a perennial herb of the Phytolaccaceae family, which is a climber plant growing rapidly with hanging branches. It has average height of 2 to 3 meters, although it can reach a height of up to 10 meters. The leaves of *P. dodecandra* are pinnate, opposite appearing whorled, ovate with 10-15 cm long, 4-12 cm broad with margins entire and undulating. Flowers of the plant are inflorescences, stand auxiliary, in racemes, rapidly symmetric and hypogynic. The sepals are rounded and fruit are reddish. *P. dodecandra* is distributed in East, West, Central and Southern Africa and some parts of South America and Asia [12,13,14].

The *P. dodecandra* is traditionally used in Ethiopia to control fresh water snails (as molluscicides) and to treat schistosomiasis. The species contains 25% of saponins in berries in which active molluscicide lemmatoxin was isolated [15]. The plant species has been used by subsistence farmers around Lake Victoria [2,7,14] to treat crops in the field and storage. Moreover, the plant powders have been used to produce laundry detergents and to control vectors such as mosquito larvae, housefly, and parasites such as mice [2,16].

The maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae), is one of the most destructive pest which affect stored cereal crops in tropical areas [17], [18]. Weevil adults attack the grains then larvae cryptically feed and develop within grains [19]. Infestation by this weevil begins in the field [20], but most damage occurs during storage. Damaged grains caused by this weevil appear to be reduced in nutritional value, germination, weight, and commercial value [21]. The maize weevil can be conventionally controlled by residual pesticides and fumigation [19]. There has been decision to ban dangerous fumigants like Methyl bromide in developed countries since 2005 and in developing countries the plan was extended to 2015. These provide a wider room for natural pesticides investigation [22,23].

The rust red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae) is a cosmopolitan insect that infest on dried material of animal and plant origin, especially cereal grains and oil seeds. They can only eat damaged or milled grain and are often introduced to a warehouse or production facility through infested flour deliveries [17]. In particular, *T. castaneum* attack stored products, especially food grains and other food products including flour, grinded cereals, nuts and beans [24]. The red flour beetle originated from Indo-Australia. The adult can live up to three years causing enormous grain damage and loss of stored products.

The purpose of this study was to evaluate the biopesticidal activities (contact toxicity, feeding deterrence and repellent effects) of *P. dodecandra* extracts, commonly used traditionally by agro-pastoralists in Mbulu district for pest management. The study intended to scientifically

assess the pesticidal efficacy of crude leaf and root extracts of *P. dodecandra* against *S. zeamais* (Motsch.) and *T. castaneum* storage pests. Hence, the study will form a baseline for further research on the isolation and chemical characterization of the active ingredients against storage pests in the plant species. This will lead to have effective and affordable pesticides which are environmentally safe for use by farmers in subsistence agriculture.

2. Materials and Methods

2.1. Study Area

The study was carried out in Mbulu district, south western part of Tanzania (Figure 1). The area of this district is approximately 7,695 square km (including Lake Eyasi) of which the dry land is approximately 6,700 square km. The altitude of the district 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 and rainfall ranging between 17.3°C and 23.4°C and from 400 mm to 1,100 mm, respectively.

Mbulu district is mainly dominated by hunters and agro-pastoral communities. There are some other socio-economic activities practiced such as afforestation, extensive grazing and smallholder rainfed cultivation as well as mechanized rainfed cultivation with medium to high inputs. The main crops grown in the study area include maize, beans, pigeon pea, sorghum, wheat, vegetable, fruits and coffee [25]. It was reported that, *P. dodecandra* leaves are used by subsistence farmers in Mbulu district to control grain pests in stored grains with minimal loss of quantity and quality [14].

2.2. Sample Collection and Preparation

Phytolacca dodecandra was collected from Mbulu district. Botanical identification of the plant species was done at the University of Dar es Salaam, Department of Botany, where voucher specimen 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 - 2cm long, while leaves were cut into pieces of approximately 1 cm². All samples were then dried at room temperature for another two weeks for further oven dried at 35°C for 48 hours.

2.3. Sample Extraction

Dry samples were grinded into fine powders using a laboratory electric hammer mill. The powdered plant materials were then stored in air-tight 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 [26]. Later, the extracts were filtered, concentrated, and then stored in a refrigerator at -4°C for pesticidal activity tests.

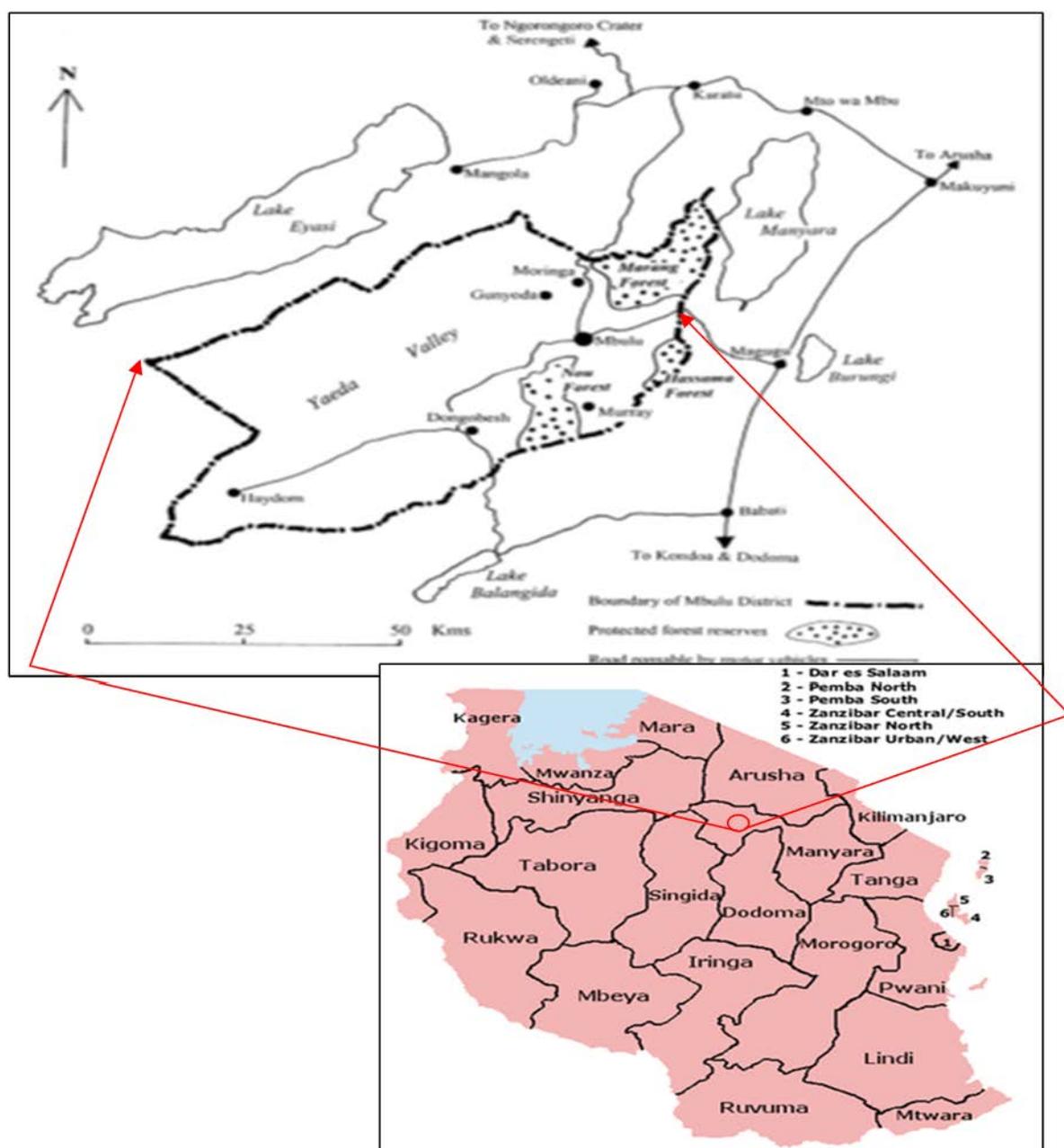


Figure 1. Map of Tanzania showing Mbulu District (Source: Modified from Google Earth)

2.4. Mass Rearing of the Pests

Selected unsexed adult *S. zeamais* and *T. castaneum* species (approximately 250) were introduced into 1-Litre glass jar containing maize grains (500 g) and kept at 25–30°C, 65–70% relative humidity (RH) and 12:12 hours (light: darkness). The jar was covered using plastic stopper reinforced inside with 0.5 mm wire gauze to prevent the insects from chewing through them [6,27,28]. The insects were allowed to lay eggs for 14 days. The beetles and flour frass were separated from the treated grains by repeated gentle sieving through layering of 3 mm and 1 mm mesh sieves, respectively. The grains were retained by the 3 mm sieve, then the beetles were retained by 1 mm sieve and the flour frass were retained in the holding pan. The insects which did not come out during the sieving were forced out by probing with a plastic fibber. The grains and flour frass were then returned into the jar and kept at 65–70% RH until the adults emerged.

2.5. Bioactivity Tests against Storage Pests

The activity of plant extracts against selected storage pests were conducted using contact toxicity, repellence, feeding deterrence and first filial generation (F1) progeny studies as described in the following subsections.

2.5.1. Contact Toxicity and First Filial Generation (F1) Progeny Studies

Maize (40 grains) were weighed into glass jars (250 mL) and admixed with plant extracts at seven different doses (0, 50, 100, 150, 200, 250 and 300 mg/mL) in triplicate. One batch of grains was treated with actellic gold™ 2% dust (0.05% w/w) as positive control. Procedure of assessing contact toxicity was as described in [28] and [29]. Briefly, twenty (20) unsexed adult beetles, *S. zeamais* and *T. castaneum* (5–10 days old) were placed into each experimental jar in a controlled randomised design (CRD) in triplicate. The top of each jar was

covered using plastic stoppers reinforced on the inside with 0.5 mm wire gauze to prevent the insects from chewing through them. The experimental units were kept at 25–30°C and 65–70% R.H. The number of dead (ND) insects in each jar was recorded at 1, 3, 5, 7, 14 and 21 days after treatment (DAT). The adult *S. zeamais* and *T. castaneum* were removed from the grains in the experimental jars at 21 DAT and the grains were returned into the jar and kept for F1 progeny counts. The numbers of the newly emerged adults F1 progeny insects were recorded at 28, 35 and 42 DAT. The percentages reduction in adult emergence or reproduction inhibition rate (IR %) was computed as:

$$IR (\%) = \frac{(C_N - T_N) \times 100}{C_N} \quad (1)$$

where C_N = number of newly emerged adult insects in the un-treated grains and T_N = number of newly emerged adult insects in the treated grains.

2.5.2. Feeding Deterrence (Grain Damage) Studies

Maize (40 grains) were weighed into glass jars (250 mL) and admixed with plant extracts at seven different dosage (0, 50, 100, 150, 200, 250 and 300 mg/mL) in triplicate. Each set of experiment were kept at 25–30°C and 65–70% RH as described in [27,29] and [30]. Here, twenty (20) unsexed adult insects *S. zeamais* and *T. castaneum* (5–10 days old) were introduced into the treated grains and allowed to feed. After 7 and 21 days, insects were removed and the amount of frass (flour) produced were determined by sieving the samples and weighing the resultant frass (flour). Percentage grain damage was computed using the formula:

$$\text{Weight loss } (\%) = \frac{(UNd - DNu) \times 100}{U(Nd + Nu)} \quad (2)$$

where U = Weight of undamaged grains, D = Weight of damaged grains, Nu = number of undamaged grains and Nd = number of damaged grains.

2.5.3. Repellence Studies

The repellent activity of the plant extracts against *S. zeamais* and *T. castaneum* was assessed using a locally made Y-shape olfactometer. The test sample extracts dissolved in dimethyl sulfoxide (DMSO) were applied onto a filter paper disc (Whatman No. 1, 1.8 cm diameter). DMSO alone was applied on similar filter paper disc as control. The solvent was allowed to evaporate and the treated and control discs were placed in either arms of the olfactometer.

Randomly selected adults (30 pests) of mixed sex and age were introduced into the olfactometer in triplicate. Prior to the introduction of the test material, air suction were applied at the Y junction of the olfactometer by using an aspirator pump to ensure that it did not become saturated with the test materials which are confined on the treated arm.

All bioassays were conducted using seven rates (0, 50, 100, 150, 200, 250 and 300 mg/mL) in triplicate and kept at 25–30°C, 65–70% R.H. Actellic gold™ dust (2%) was used as a positive control. The same procedure used for extracts was applied for the control. All experiments were

performed in a dark room [31]. The assays were left to run for 30 min and after that the number of insects in the control arm (NC) and that in the treated arm (NT) were counted. After each test, the olfactometers were thoroughly cleaned and dried. Percentage repellence (PR) values were computed according to [32]:

$$PR = \frac{(N_C - N_T) \times 100}{(N_C + N_T)} \quad (3)$$

A positive PR value indicated repellence of the insect pests against pesticidal plants, while a negative PR value indicated attracting ability.

2.6. Data Analysis

Data for pesticidal activity were calculated for mean values and analyses using Statistical Package for Social Sciences (IBM SPSS) version 20 where one-way analysis of variance (ANOVA) was used. The resulting data were later presented in form of graphs as described below.

3. Results and Discussion

3.1. Contact Toxicity of *P. dodecandra* Extracts

The leaf extracts of *P. dodecandra* at 150 mg/mL concentration killed 98% of *S. zeamais* and 99% of *T. castaneum* at day 3, respectively (Figure 2: A and B). The same concentration of *P. dodecandra* root extract killed only 5% of *S. zeamais* and 67% of *T. castaneum* at day 3 (Figure 3: A and B). No mortality was recorded from the untreated control maize, whereas maize treated with actellic gold™ dust achieved 100% mortality within one day. Generally, the percentage mortality increased significantly with the increase in concentration and duration of contact with treated maize ($p < 0.05$).

The leaf extracts of *P. dodecandra* shows relative higher toxicity compared to *P. dodecandra* roots against adult *S. zeamais* and *T. castaneum*. There were a significance difference in the toxicity between leaves and roots extract in which the leaf extracts were more active than root extracts. Leaf extract of *P. dodecandra* at concentration range of 150 to 300 mg/mL caused 100% mortality of adult *S. zeamais* and *T. castaneum* on 3 DAT (Figure 2: A and B). On the other hand, root extracts of *P. dodecandra* at concentration range of 250 to 300 mg/mL caused mortality between 80% to 81% of *S. zeamais* and between 80% to 86% mortality of *T. castaneum* on 21 DAT. (Figure 3: A and B). The powders of dry leaves of *P. dodecandra* were reported to be used in treating grain storage insects by subsistence farmers around Lake Victoria basin [2]. Some studies have reported high toxicities of plant crude powders from which eight plant species caused mortality between 58% and 88% of *S. Zeamais* [33]. This toxicity of the plant species supports the current study.

It is evident that *P. dodecandra* leaf extract is a potential protectant against *S. zeamais* and *T. castaneum*. This bioactivity could be associated with the presence of phytochemicals such as saponin, alkaloid, sterol,

triterpenoids, phenol, flavanoid and glycoside [14]. The broad spectrum bioactivity of botanical extract in relation to local availability and simple processing make the pesticidal plant acceptable and cost effective compared to synthetic pesticides in small scale agriculture. Therefore, this plant - based products hold good promises for inclusion in the Integrated Pest Management (IPM) strategies.

3.2. Feeding Deterrence of *P. dodecandra*

Both leaf and root extracts of *P. dodecandra* exhibited significant feeding deterrence to the test pests ($p < 0.05$). *P. dodecandra* leaf extracts at 150 mg/mL concentration and above deterred *S. zeamais* and *T. castaneum* for damaging maize grains from day 7 to day 21 similar to Actellic gold™ (Figure 4: A and B).

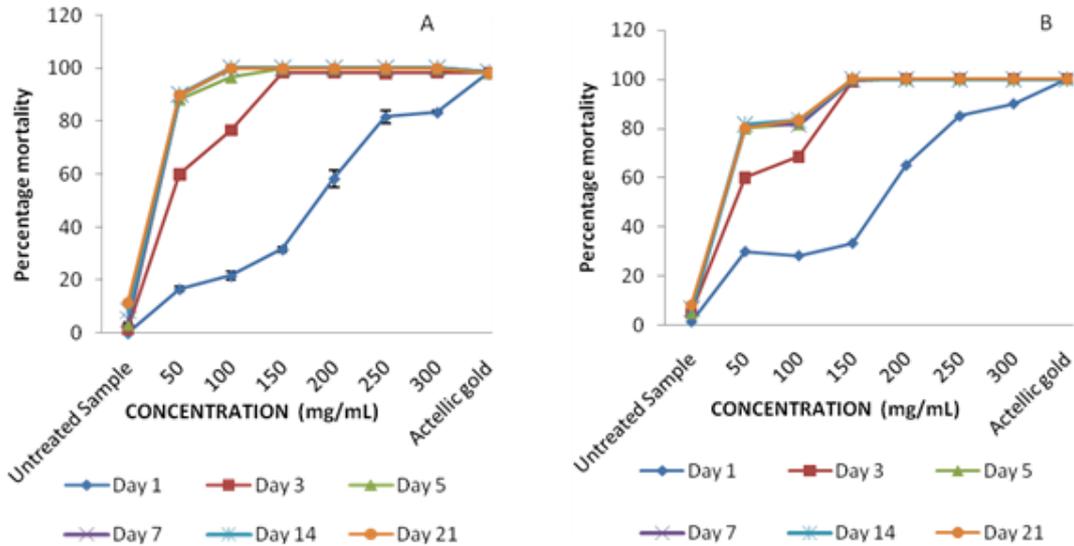


Figure 2. Percent Mortality of *S. zeamais* (A) and *T. castaneum* (B) using *P. dodecandra* Leaf Extracts

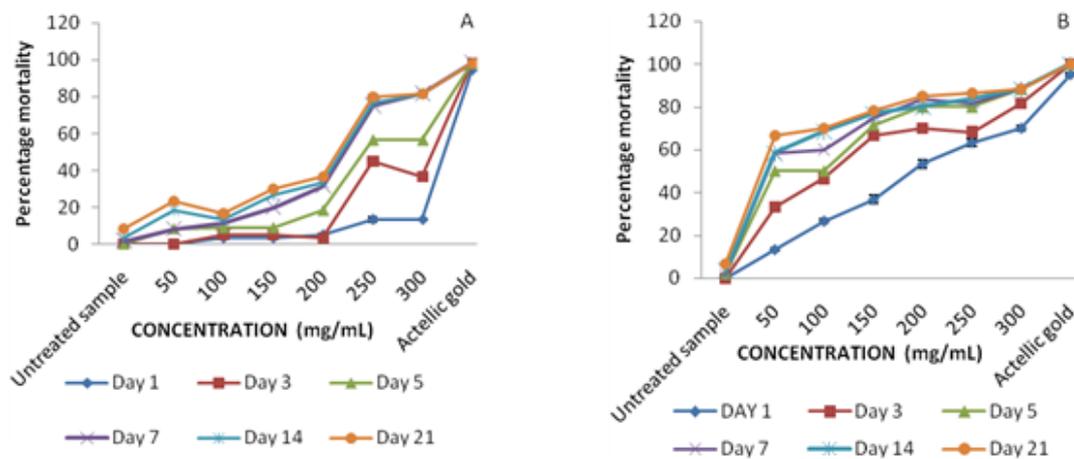


Figure 3. Percent Mortality of *S. zeamais* (A) and *T. castaneum* (B) using *P. dodecandra* Root Extract

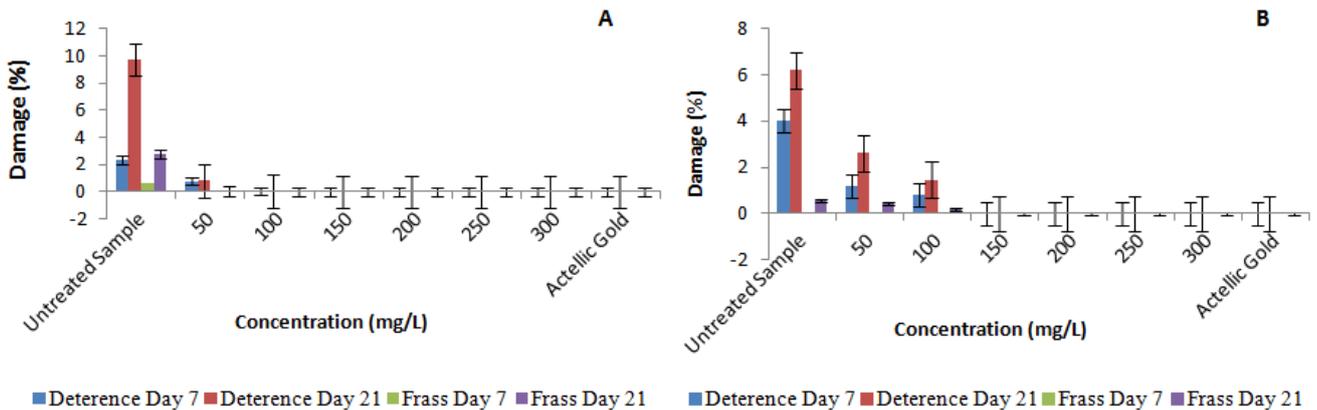


Figure 4. Percent Damage of *S. zeamais* (A) and *T. castaneum* (B) Pests in Maize applied with *P. dodecandra* Leaf Extracts

In the untreated samples, there was up to 10% insect damage by day 21 which was significant ($p < 0.05$). Root extracts of *P. dodecandra* had little feeding deterrence compared to leaf extracts at the concentration of 150 mg/mL (Figure 5: A and B). This shows that feeding deterrence was inversely proportional to grain damage (Figure 5).

The leaf extract of *P. dodecandra* shows relatively higher deterrence (less grain damage) to both *S. zeamais* and *T. castaneum* than the root extract. Furthermore, *S. zeamais* seems to be more destructive than *T. castaneum* though it responded fast to the leaf extract of *P. dodecandra* (Figures 5 and 6). There was high grain damage noted in root extracts than the leaf. However, the grain damage decreased with increasing concentration of both leaf and root extracts. Untreated samples had the highest damage and weight frass percentage in all cases.

The antifeedant property of any plant material depends on the active constituents in the plant such as terpenoids, iridoids, glycosides which may be responsible for observed insecticidal properties [34]. Previous studies reported the presence of anti-feedant compound in *Azadirachita indica*, *Tephrosia vogelli*, and *Lantana camara* [4,6,35,36]. For example, leaves and seeds of *T. vogelli* contain antifeeding deterrents [37,38,39]. The fact that *P. dodecandra* has considerably reduced the damage caused by the adult *S. zeamais* and *T. castaneum*, the observed grains protection

by the crude extracts could partly be attributed to a modification of the physical properties of stored grains that reduce inter-granular air spaces. This discourages insects' penetration, feeding and amount of oxygen available. As a result, the crude extracts succeeded in inhibiting insect feeding and oviposition. As a result, the adult insects mortality recorded in the study may largely be due to starvation. Hence, the antifeedant property of test botanicals may be attributed by their bioactive constituents [4]. These findings are promising for their adoption and rationalization for use in subsistence agriculture.

3.3. Repellence Activities of *P. dodecandra*

P. dodecandra leaf extract demonstrated a moderate repellence ranging from 15.85% at 50 mg/mL to 57% at 300 mg/mL for *S. zeamais* and from 20.99% at 50 mg/mL to 66.67% at 300 mg/mL for *T. castaneum* (Figure 6A). The repellence activity of *P. dodecandra* leaf extract at 150 mg/mL was 75% of the activity of actellic gold™ (standard) against *S. zeamais*. Similarly, the repellence of *T. castaneum* was 88% compared to actellic gold™ (standard). The repellence of the pests against the crude extracts of *P. dodecandra* (Figure 6) concur with the observations indicated by the deterrence experiments (Figure 5).

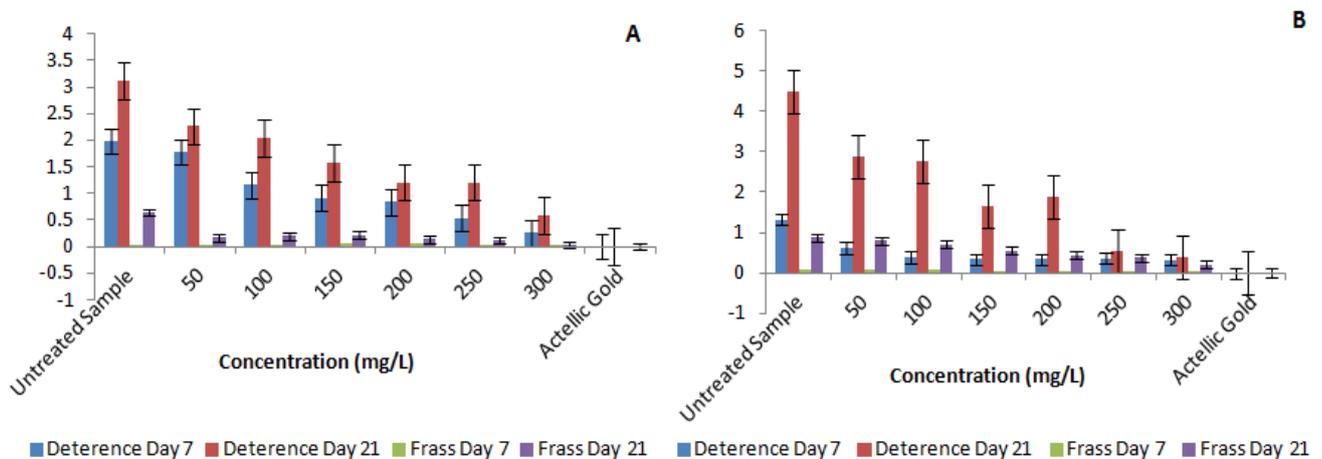


Figure 5. Percent Damage of *S. zeamais* (A) and *T. castaneum* (B) Pests in Maize applied with *P. dodecandra* root extracts

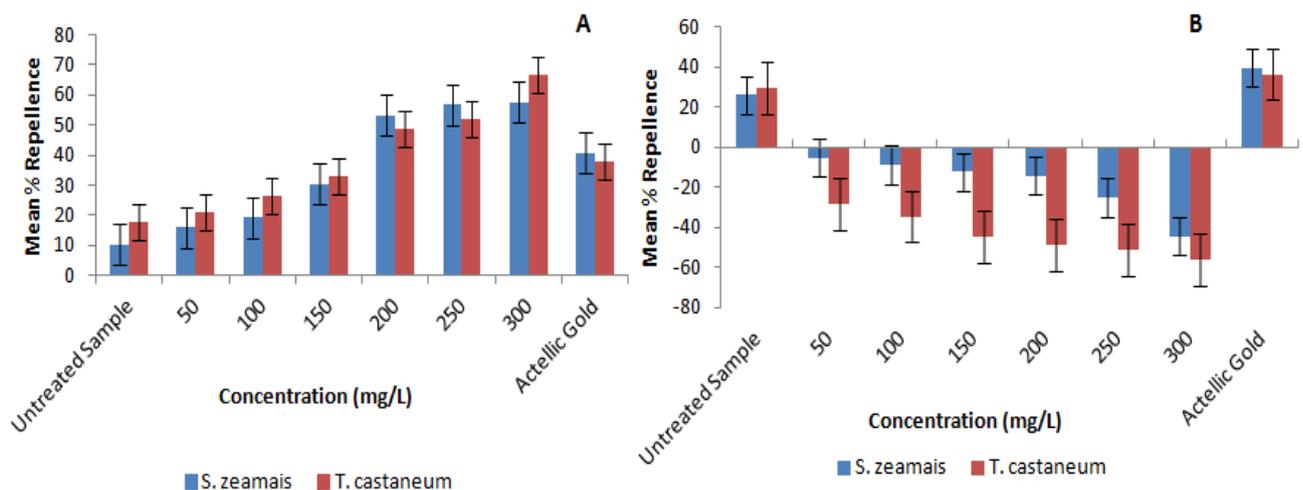


Figure 6. Mean Percent Repellence of Tested Pests against *P. dodecandra* leaf (A) and root (B) extracts

Unlike leaves, *P. dodecandra* root extracts indicated a weak attractance ranging from 5.11% at 50 mg/mL to 44.43% at 300 mg/mL for *S. zeamais* and from 20.99% at 50 mg/mL to 56.18% at 300 mg/mL for *T. castaneum* (Figure 6B). In contrast, actellic gold™ had repellence percent of 40.67% and 37.68% for *S. zeamais* and *T. castaneum*, respectively. The results of the repellence and attractance bioassay show significant difference between the untreated control and treated samples ($p < 0.05$). The percentage repellence (PR) values for all insects' treatment increased significantly with increasing concentration.

It has been reported that the essential oil from plants containing sulphur and saponin compounds contribute to the repellence potency [40]. For example, *Ephestia cautella*, *L. camara* and *T. vogelli* are good repellents to major storage pests [28,41,42]. Similarly, reference [43] reported that neem oil repelled various insects including moths. Leaf extracts of *P. dodecandra* demonstrated significant repellence against *S. zeamais* and *T. castaneum*. The observed repellent activity could partly be attributed to the presence of volatile constituents such as monoterpenes and sesquiterpenes which are well known repellents of phytophagous (biting) insects [14,44,45]. In addition, aromatic compounds from plants have long been used in daily life as insect repellent because they are generally regarded as safe, economically and socially friendly compared to synthetic pesticides [4]. The repellent effect of *P. dodecandra* leaves may have important implications on post harvest protection system, since they facilitate maize weevils to depart from the treated grains [31]. The repellent action of *P. dodecandra* leaves observed is crucial for the traditional poor farming communities in Sub-Saharan Africa whose synthetic pest control is expensive and uneconomical [28].

3.4. First Filial Generation Results

The results of adult F₁ progeny counts for *S. zeamais* and *T. castaneum* were presented in Figures 7: A and B. The *P. dodecandra* leaf and root extracts have reduced F₁

progeny emergence. Crude extract of *P. dodecandra* leaves have shown 100% reduction of F₁ progeny in both *S. zeamais* and *T. castaneum* at 150 mg/mL. The *P. dodecandra* leaf extract showed high reduction of F₁ progeny in *S. zeamais* and *T. castaneum* compared to root extract. Indeed, all *P. dodecandra* botanical treatments indicate significant reduction in F₁ progeny emergence compared to the untreated control. The reduction of F₁ progeny emergence indicated by *P. dodecandra* leaf extract was similar to actellic gold™ (positive control) as indicated in Figure 7A and Figure 7B.

The emergence of filial generation progeny decreased as concentration of *P. dodecandra* increased. Effective botanicals to various insect pests at different dosages have been reported by [1,46]. There was no significant difference in F₁ progeny counts in maize grains treated with crude root extract and that of crude leaf extract ($P > 0.05$) for both *S. zeamais* and *T. castaneum*. The results have shown a clear dose-dependent reduction in the adults *S. zeamais* and *T. castaneum* F₁ progeny count by *P. dodecandra* leaf extract at concentrations between 150 to 300 mg/mL and at concentration of 300mg/mL for root extract. The leaf extracts with high concentration reduced F₁ progeny count by 100% (similar to actellic gold™ dust at 0.02% w/w), while root extracts reduced F₁ progeny count by 93.3% compared to the untreated control (Figure 7).

The reduction of F₁ progeny emergence in the treated grains might be due to increased adult mortality, ovicidal and larvicidal properties of the tested *P. dodecandra* leaf and root extracts. This is in line with the findings observed in [29] that dry leaves of plants inhibited oviposition and subsequent reduction of progeny production of pests. The botanical pesticide probably weakens adult pests by making them lay fewer eggs than normal leading to reduced hatchability of larvae and hence reduced final metamorphosis to adults. The findings reported in this study are similar to those reported by [4]. In that study, maize grains treated with *L. camara*, *T. vegolii* and *A. indica* powder produced 71.6%, 69.7% and 85.6% of F₁ progeny reduction, respectively.

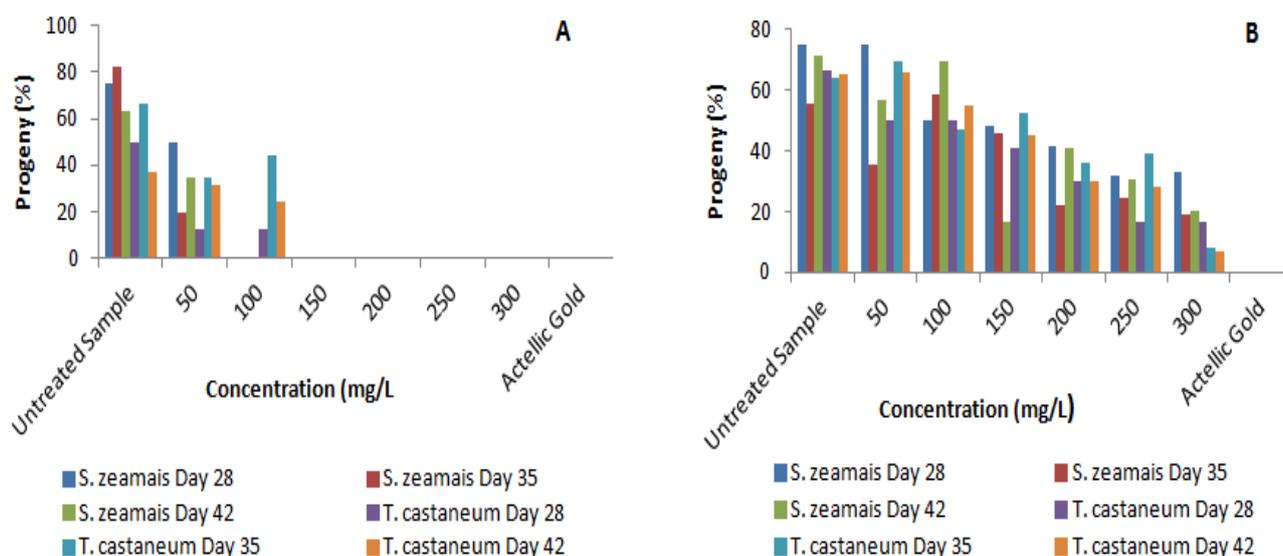


Figure 7. First Filial Generations of *S. zeamais* and *T. castaneum* Pests in Maize applied with *P. dodecandra* leaf (A) and root (B) Extracts

4. Conclusion and Recommendations

The findings have shown that *P. dodecandra* leaf extracts are potential protectants of stored agricultural products against the larger grain borer, *S. zeamais* (Motsch.) and *T. castaneum* pests. The broad-spectrum bioactivity of these botanical extracts coupled with their local availability and simple processing method makes them more acceptable and cost-effective alternatives to synthetic pesticides in smallholder agriculture. Hence, these plant-based products hold good promise for inclusion in IPM strategies especially where the emphasis is on ecological preservation and food safety.

It is recommended that biosafety studies should be carried out to confirm whether maize treated with the botanical pesticide can be safely consumed before promoted to farmers. Apparently, depletion of natural forests has called for isolation of important bioactive natural products and identification of their structures. This will later help in making the appropriate naturally based synthetic pesticides.

Competing Interests

The authors declare that they have no competing interests.

List of Abbreviations

CRD = completely randomised design;
 IPM = integrated pest management;
 OUT = Open University of Tanzania;
 ITM = Institute for Traditional Medicines;
 F1 = first filial generation;
 DAT = days after treatment;
 DMSO = dimethyl sulfoxide;
 SPSS = Statistical Package for Social Sciences;
 ANOVA = analysis of variance

Acknowledgements

The authors would like to thank the Tanzania Commission for Science and Technology (COSTECH) for financial support to project No. CST/SC.327/952/3/2013. Authors are also 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 used in this study.

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