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Pesticidal Activity of Wild Mushroom *Amanita muscaria (L)* Extracts against *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) in Stored Maize Grains

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Abstract The toxicity, antifeedant activity and repellency of the crude methanol extract of the wild mushroom *Amanita muscaria* on *Sitophilus zeamais* in stored maize grains was determined by assessments, carried out between the extracts concentration of 0.05 and 0.5% w/w. Nontreated and treated grains with 2% Actellic gold ™ 2% dust (0.05% w/w) were used as negative and positive controls, respectively. Three replicates were made for each treatment and experiments were conducted in a completely randomized design. The methanol extract at 0.5% w/w concentration showed highest toxicity 21 days after treatment killing 61.7% of the pest. Interestingly, 68.6% inhibition of F1 progeny was observed at 0.5% w/w 42 days after treatment whereas the reduction in grain damage was up to 86.0% compared to the negative control. The extract demonstrated a pest repellency of up to 96.7% after 24 hours of exposure. The findings were promising for use of *A. muscaria* as a biopesticide for maize grains storage towards supporting the ongoing IPM strategies. The study provides a baseline data that needs to be complimented by doing more research on the active compounds in the mushroom as well as improving the synthetic industry based on the fact that most of the species are endemic.

Keywords: *pesticidal activity, Amanita muscaria, maize grains, Sitophilus zeamais, grain damage, Tanzania*

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1. Introduction

Food insecurity has been a challenge to many communities worldwide over years, leaving a majority with no assurance of adequate and good quality of food when necessary. More than a half of the African countries are reported by FAO to be facing a food deficit [1,2]. The emergence and persistence of this phenomenon are caused by poor control of insestations of vertabrate, insect and fungal pests [3,4,5]. The damage of food grains before harvesting as well as during storage have led to crops' loss with the consequences of decreased household's health, and financial wellbeing [6,7].

Sitophilus zeamais (Motschulsky) [Coleoptera: Curculionidae] is a common and highly destructive pest, capable of infesting different types of grains, during the storage, causing a significant grain loss [8,9,10]. To combat the storage pests, a number of strategies have been employed such as using synthetic pesticides and natural pesticides. Integrated Pest Management (IPM) strategies are also employed to promote the use of natural products over synthetic chemicals as pesticides [11,12,13].

Majority of the smallholder farmers in the sub-Saharan Africa are still facing a number of challenges toward sustainable utilization of synthetic pesticides in the pests management, hence consistently affected by substantial post-harvest grain losses. This is contributed by the relatively high costs involved in the purchasing of the pesticides, poor accessibility of the remote areas, limited knowledge and skills on their use and the fear for toxicity [11,14,15,16]. Moreover, synthetic pesticides are still associated with challenges pertaining to environmental pollution that exposes the nontarget species to negative effects as well as fast development of resistance among the targeted pests [17,18,19].

The use of locally available means in prevention of grain damage by pests is a more affordable practice in many rural based societies. These include the treatment of grains with powdered plants, smoking, and spraying grains with plant extracts to mention the few [13,20]. A number of studies have reported pesticidal potency of dried plant powders, volatile oils as well as different crude

extracts against grains infestants known to cause significant grain losses [21-26].

Amanita muscaria (Amanitaceae) commonly referred as the fly agaric is a well known mushroom for its psychoactive and insecticidal potency. The mushroom is classified as a poisonous mushroom although incidences of human deaths from its ingestion are extremely rare. The mushroom is eaten in parts of Europe, Asia and North America after peeling off of the cuticle and parboiling, which is regarded to lower its toxicity and degrade its psychoactive compounds [27]. Ibotenic acid and muscimol are the constituents known to be associated with the toxicity upon its decarboxylation commonly leading to the formation of muscimol and muscarine, which is the main active principle in *A. muscaria* [31,32]. Ibotenic acid is an example of a known constitutive chemical defense mechanism exhibited by *A. muscaria*. These bioactive compounds have a potential of becoming lead structures towards the development of new drugs or pesticides [39].

Insecticidal potency of *A. muscaria* has been reported in previous studies, the activity being linked to ibotenic acid. Among the traditional uses of the mushroom include mixing the fruiting bodies with milk or sugar and applying the mixtures to kill house flies [34,35,36,37,38]. Moreover, the activities of the mushroom against some species e.g. genus *Drosophila*, have as well been reported [34,35,36,37,38]. The fruiting bodies of some mushrooms are reported to produce compounds which are either toxic, pungent or bitter which in return are helpful in preventing fungivores from consuming them. This study therefore aimed at determining the pesticidal potential of methanol crude extracts of *A. muscaria* against *S. zeamais* in stored maize grains.

2. Materials and Methods

2.1. Mushroom Sample Collection and Extraction

The fresh whole bodies of wild *A. muscaria* were obtained from Mbeya region in the Southern highlands of Tanzania, where they co-exist with trees from the genus *Pinus*. The collected samples were air-dried under shade at 22 -27 °C for two days and thereafter packed in paper bags and transported to the Medicinal Chemistry laboratory, School of pharmacy, Muhimbili University of Health and Allied Sciences (MUHAS), where they were further dried at 40 °C over a 48 hours in an oven (K ttermann, Germany) coarse powder using an electric laboratory blender (Akita electronics Co.L.L.C, UAE). The powder was extracted by maceration using methanol (95% v/v) (Carlo Erba reagents group, German)for 72 hours. The extract was then filtered under vacuum using *Whatman* filter papers (Whatman No. 1 sheets) (GE Healthcare UK Ltd, China), the filtrate dried *in vacuo* at 50 °C using a rotary evaporator (Bibby Sterilin Ltd, UK) and stored in refrigerator at 4°C for pesticidal activity.

2.2. Rearing of the Test Organisms (*S. zeamais*)

Test insects (*S. zeamais*) were obtained from the milling stations for grains and appropriately identified. Insects' rearing was carried out on nontreated and uninfected maize (*Zea mais L.*) grains, obtained by sterilizing in an oven at 40°C over four hours [21]. About one kilogram of maize grain was placed in a perforated transparent plastic jar measuring 20 cm in diameter and 30 cm in height. Approximately four hundred unsexed adult test insects were placed in the jars and covered with a fine plastic mesh to allow aeration and prevent them from escaping [22,23]. The jars containing maize grains and insects were kept at temperatures between 25-30 ^{o}C, 60-70 relative humidity (RH) and 12 hours light: 12 hours dark conditions for 14 days for the insects to lay eggs. Afterwards all the adult insects were taken out by a 1 mm mesh sieve, collecting the frass by holding a pan at the bottom. Subsequently, the frass and grains were put back in the jars and stored under similar conditions waiting for the emergence of adult insects after 25 to 35 days. The emerging adults were removed using a similar sieving process on daily basis and reserved in separate jars based on their ages ready for pesticidal tests [22,23].

2.3. Experimental Set up

2.3.1. Insect Repellency Tests (Choice Bioassay)

Repellency of the crude methanolic extract was assessed using round circular plastic containers (45 cm diameter, 15 cm high) whose bases were divided into four portions onto which 100 mg fractions of nontreated and treated grains were put in alternation equidistant from the center.

Three replicates were prepared from each level of extract treatment concentrations (0.5, 1.0 and 1.5% w/w) and a positive control, Actellic Gold^{TM} 2% dust (Syngenta, UK) at 0.05% w/w. Moreover, a negative (no choice) control was used where all four portions were composed of nontreated grains was included. The containers were set in a completely randomized design (CRD). Subsequently, 20 adult *S. zeamais* aged 4 to 8 days were placed at the center of the containers whose tops were covered with a fine wire mesh to allow aeration and avoid escaping. The total number of insects settled on the nontreated (N_C) and treated (N_T) grains in each container were recorded after 1, 12 and 24 hours. Percent repellency (PR) was then calculated as in equation (1) and interpreted as described by Hassanal and Weseka et al [2526].

$$PR = \frac{(N_C - N_T)\times 100}{(N_C + N_T)}. \quad (1)$$

2.3.2. Feeding Deterrence and Contact Toxicity Studies

Forty (40) nontreated maize grains, were weighed and placed in perforated transparent plastic containers (200 mL). Six treatment levels (0.05, 0.15, 0.25, 0.3, 0.4 and 0.5 %w/w) in methanol (1 mL) were made and mixed thoroughly with the weighed maize grains. The treated grains were left in open air under shed for 6 hours to ensure complete evaporation of the methanol. Nontreated grains were used as negative controls; whereas, Actellic gold ^{TM} 2% dust (0.05% w/w) was employed as a positive control. Three replicates were made for each treatment level and for the controls [22].

Twenty unsexed adult *S. zeamais* (5-10 days) were placed in the containers with treated maize grains and allowed to feed on the grains. The containers were reserved in the laboratory at 25 – 30°C and 65 - 70% R.H in a CRD. The dead *S. zeamais* were counted on 1, 3, 5, 7, 14 and 21 days after treatments (DAT). Afterwards, the dead insects were removed from the containers, the weights and numbers of undamaged and damaged grains were recorded on the 21st day. The percentage weight loss was obtained as per the equation (2).

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

Where U was the weight of undamaged grains, D was the weight of insect damaged grains; Nu and Nd were the numbers of undamaged and insect-damaged grains, respectively.

2.3.3. F1 Progeny

The living *S. zeamais* adults were taken out of each container on the 21st day after treatments. The newly emerged insects were counted and recorded on 28, 35 and 42 DAT. Thereafter the reproduction inhibition rate (IR%) was obtained as per equation (3).

$$\text{IR } (\%) = \frac{(C_N - T_N) \times 100}{C_N} \quad (3)$$

Where: C_N was the number of newly emerged insects in the nontreated grains and T_N was the number of newly emerged insects in the treated grains [22].

2.4. Data Analysis

Data were analysed using IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp. Mean values of data were subjected to Analysis of Variance (ANOVA) followed by Tukey's Studentised Range (HSD) and Least Significance Difference (LSD) tests at 5% significance level. Probit Regression analysis was used to calculate lethal concentration that can kill 50% of the insects (LC_{50}) and concentration that can repel 50% of insects (RC_{50}).

3. Results and Discussions

3.1. Contact Toxicity

The percentage mortality of the adult *S. zeamais* was significantly associated with a treatment concentration and contact duration ($p<0.05$) (Table 1). The mortality of 61.7% was observed when an *A. muscaria* crude methanol extract of concentration 0.5 %w/w was applied 21 days after treatment. A notable increase in mortality was observed across all treatment levels during the first 14 days post treatments. Moreover, the mortality was increasing with increased concentration of the extract. There was no significant increase in mortality from days 14 to 21 after treatment across all extract treatment levels ($p> 0.05$). Probit regression analysis indicated that a concentration of 0.55 % w/w was required to kill 50% of the insects (LC_{50}), 21 days after treatment.

Mortality rates exhibited by the negative control was significantly ($p<0.05$) lower than those seen in the grains treated by crude extract at the highest concentration (0.5 %w/w) from 7 DAT onwards. However, there was no significant difference in the mean mortalities between the negative control and the rest of the treatment levels. The mortalities in the grains treated with extract were, however, not significantly ($p<0.05$) to the positive control (Actellic gold TM 2% dust (0.05% w/w) over the entire duration of the experiment. Constant

Observation has shown that higher mortality rates of adult *S. zeamais* could be achieved at concentrations higher than the 0.5% w/w reported in this work. Probit regression analysis indicated that the concentration of 1.21% w/w was required to attain 99.0% mortality of adult *S. zeamais* 14 DAT. Moreover, an almost unvarying trend in percentage mortality observed during the last 7 days of the experiment may be due to either changes in the composition of the extract due to environmental factors causing evaporation or degradation of the active compounds within the extract [40]. Adaptation of the insects and hence hiding and feeding in the grains which were bored during the initial days of the experiment is another possible reason.

The results are in agreement with the previously observed insecticidal potency of *A. muscaria* on house flies and some species of the genus Drosophila reported to be caused by the constitutive secondary metabolite iboteic acid [34,35,36,37,38]. In another unpublished work, we have demonstrated the pesticidal potency of the mushroom *C. cibarius* against *S. zeamais*, in which the mortality of 66.7% was attained at the concentration of 0.5% w/w 21 DAT. Percentage mortality of 33% to 93.75% on the genus *Sitophilus* have also been reported when other plant powders and crude extracts were tested on maize grains [22,24,41].

Table 1. Percent mortality (mean ± SE, n=3) of adult *S. zeamais* in grains treated with methanol crude extracts of *A. muscaria*

Treatment	Concentration (%w/w)	DAT					
		1	3	5	7	14	21
Nontreated Control	0	0.0±0.0^{a}	0.0±0.0^{a}	1.7±1.7^{a}	5.0±0.0^{a}	8.3±3.3^{a}	10.0±2.9^{a}
A. muscaria	0.05	0.0±0.0^{a}	1.7±1.7^{a}	1.7±1.7^{a}	3.3±1.7^{a}	6.7±4.4^{a}	6.7±4.4^{a}
	0.15	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{ab}	6.7±4.4^{a}	8.3±4.4^{a}
	0.25	3.3±3.3^{a}	5.0±5.0^{a}	6.7±4.4^{a}	8.3±4.4^{a}	8.3±4.4^{a}	8.3±4.4^{a}
	0.3	0.0±0.0^{a}	1.7±1.7^{a}	5.0±2.9^{a}	8.3±1.7^{a}	11.7±1.7^{a}	11.7±1.7^{a}
	0.4	0.0±0.0^{a}	0.0±0.0^{a}	5.5±4.9^{a}	10.0±2.9^{ac}	13.3±1.7^{a}	13.3±1.7^{a}
	0.5	0.0±0.0^{a}	1.7±1.7^{a}	10.0±2.9^{a}	23.3±4.4^{d}	60.0±7.6^{b}	61.7±9.3^{b}
Actellic	0.05	100.0±0.0^{b}	100.0±0.0^{b}	100.0±0.0^{b}	100.0±0.0^{e}	100.0±0.0^{c}	100.0±0.0^{c}

Means in column followed by different letters are significantly different at α=0.05 by LSD test.

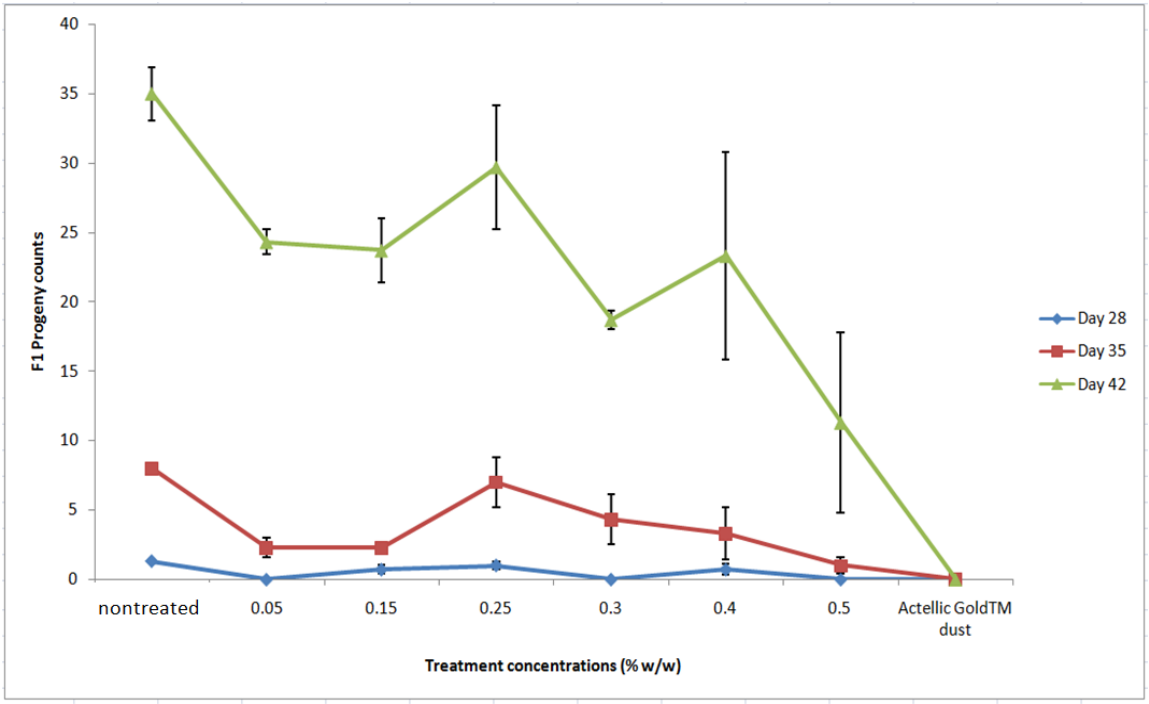

Figure 1. F1 progeny counts (Mean ± SE, n = 3) of *S. zeamais* at varying exposure time and concentrations of *A. muscaria* crude methanolic extract

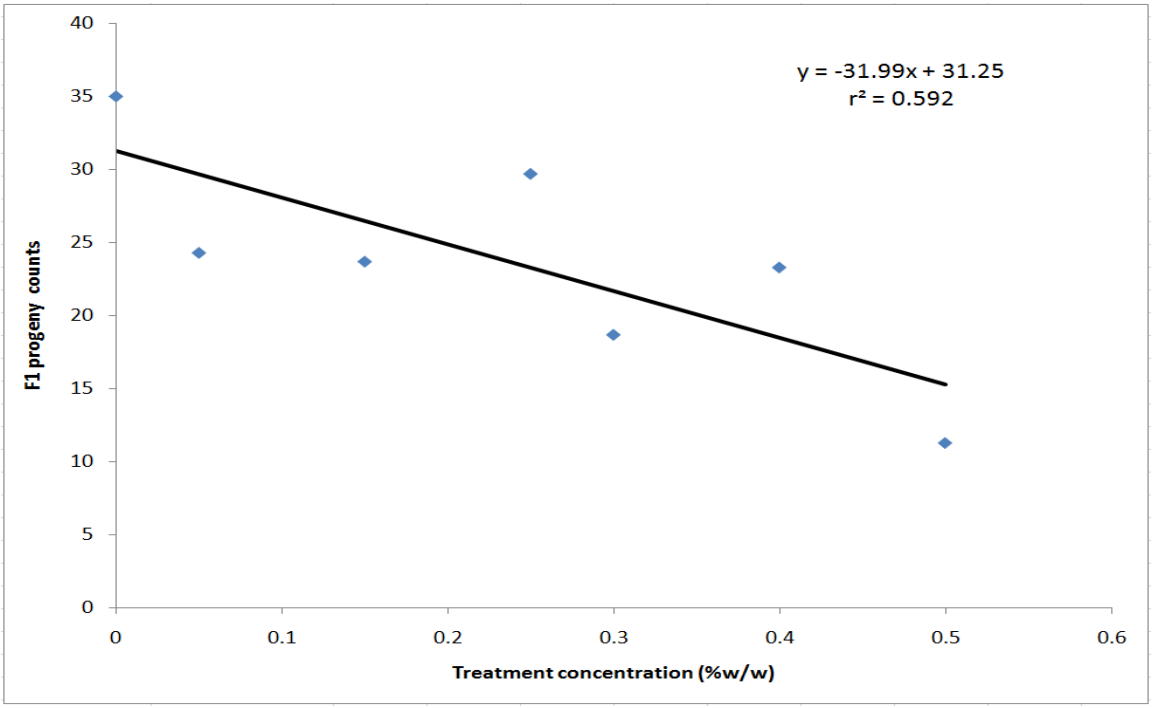

Figure 2. A scatter plot for regression and correlation of F1 progeny counts and *A. muscaria* methanol extracts treatment concentration

3.2. F1 Progeny Studies

An increase in the number of F1 progeny *S. zeamais* was observed across all concentration levels on 28, 35 and 42 DAT. Generally, no concentration inhibited the emergence of the F1 progeny counts by 100% despite the observed dose related variations. However, a 68.6% reduction in F1 progeny counts at the concentration of 0.5% w/w 42 DAT was observed (Figure 1).

Regression analysis showed a coefficient of determination (r^2) of 0.592 indicating that an increase in treatment concentrations of the extract could determine the decrease in the F1 progeny by only 59.2%. However, the correlation coefficient (r^2) was found to be 0.769, suggesting a strong positive correlation between the increase in extract's treatment concentration and the decrease in F1 progeny counts (Figure 2).

Moreover, there was a significant difference ($p<0.05$) between the mean F1 progeny counts of the negative and positive controls to that of the grains treated with extract at 0.3 – 0.5 %w/w. The observed reduction in F1 progeny may be correlated with the mortality of the adult *S. zeamais* which lessened the number of pests before oviposition. Apart from the anti-oviposition or ovicidal potentials;

lavicidal potential of the crude extract may be another factor in play where the aqueous extract of *A. muscaria* have been reported to cause larval deaths of up to 88.4% [42]. Compounds closely related to ibotenic acid such as isoxazoles are as well reported to exhibit a potential lavicidal profile [43]. The results are suggestive of the potency of the extract in inhibiting the multiplication of *S. zeamais* in treated grains. The use of higher concentrations could ensure a total inhibition in the F1 progeny.

3.3. Reduction in Grain Damage

A decrease in grain damage (% weight loss) was significantly ($p<0.05$) proportional to the extract's concentration and the duration of exposure (Figure 3). A maximum of 86.0% reduction in weight loss was observed at the extract concentration of 0.5% w/w 21 DAT, in which the percentage weight loss was 0.34% as compared to 2.43% in the nontreated maize grains. Moreover, no loss in weight of the grains was observed in the grains treated with the positive control.

The differences in the mean weight losses of the grain at treatment levels 0.0 to 0.15% w/w were significant ($P<0.05$) compared to those of the grains treated with 0.5% w/w extract as well as the positive control. Probit regression analysis showed that an extract concentration of 0.72 %w/w was required to cause a 99% reduction in weight loss of the treated grains at 21 DAT.

The results are implicative of the antifeedant potency of the crude methanol extract of *A. muscaria* on *S. zeamais*. This is in line with the previous reports on the antifeedant potency of *A. muscaria* against the insects *S. littoraris* and *D. melanogaster* as well as the animal *D. virginiana*, associated with muscimol, a known constituent of *A. muscaria* [44,45].

Other studies in which plant powders were used to treat stored maize grains against the pest *P. trancatus*, reported a reduction in grain damage in a range of 46.2 to 52.2% [22].

3.4 Repellency Studies

Repellency of the adult *S. zeamais* by the grains treated with *A. muscaria* extract was observed to be influenced by both the extract's concentration and the duration of exposure (Figure 4).

The mean percentage repellency of each concentration level was significantly ($P<0.05$) different from other tested concentration levels. A maximum repellency of 96.7% was achieved at 0.5% w/w after 24 hours. Moreover, there was no a significant $p<0.05$) difference in the percentage repellency between the grains treated with extract at 0.5 %w/w and the positive control, 24 hours after exposure. The concentration of 0.35 %w/w after 24 hours of exposure and the time duration of 13.6 hours at the extract concentration of 0.5 %w/w were calculated to be required for ensuring 75% repellency (RC_{75}) of adult *S. zeamais* using probit regression analysis.

The observed repellent activity have as well been indicated in previous studies, where *A. muscaria* was reported to exhibit repellency against *S. littoraris* and *D. melanogaster* [44]. Muscimol, a secondary metabolite in *A. muscaria* was indicated to possess a repellent activity in *D. virginiana [45]*. Repellent activity is highly associated with the volatility of the noxious agents and hence capable of reaching the olfactory lobes of the insects before making contacts with the source. Possession of a repellent activity is an essential aspect for an agent to be used as grains protectant, whereby it provides assurance on keeping the treated grains safe from being approached by infestants. This property, in combination with the contact toxicity and feeding deterrence together creates a better pesticidal profile of an organic pesticide.

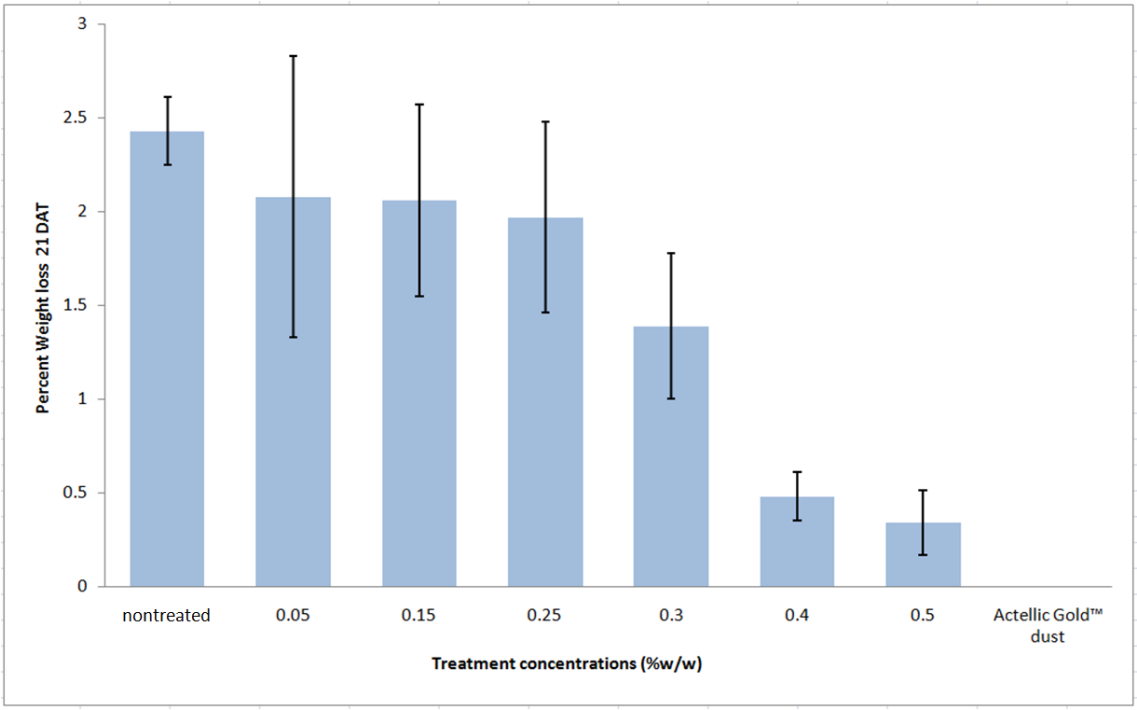

Figure 3. Percent weight loss (Mean ± SE, n = 3) of maize grains at varying exposure time and concentrations of *A. muscaria* crude methanolic extract 21 DAT

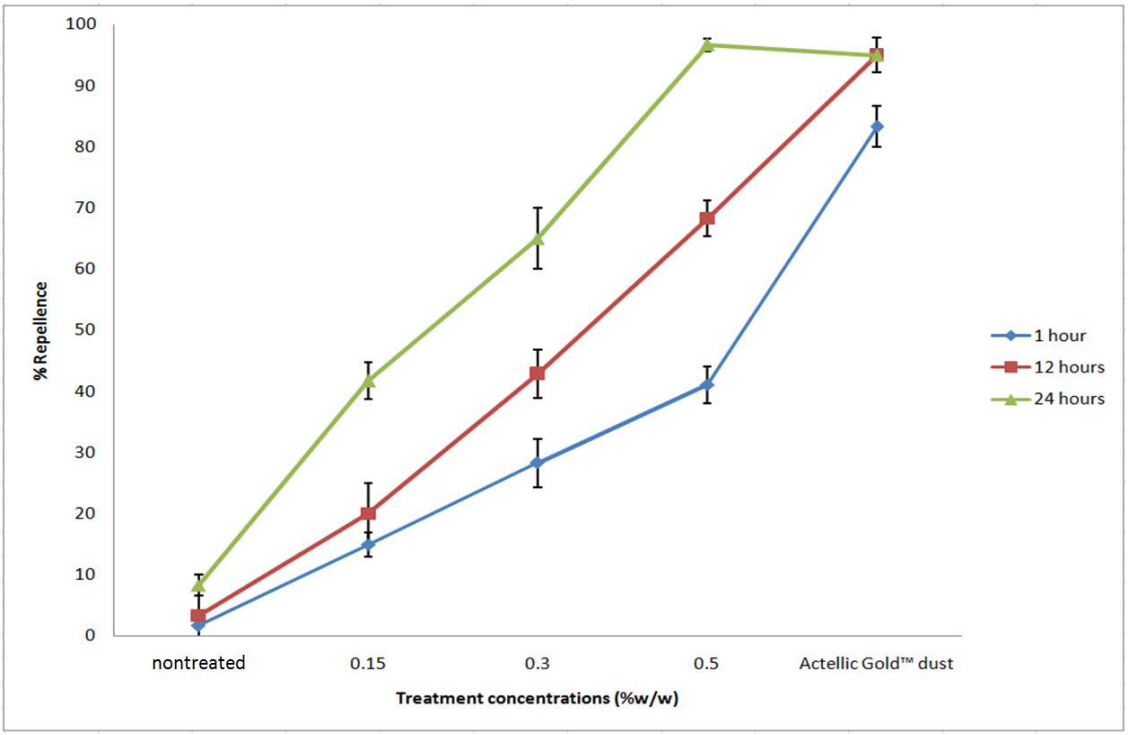

Figure 4. Percent repellency (Mean ± SE, n = 3) of adult *S. zeamais* at varying exposure time and concentrations of *A. muscaria* crude methanolic extract

4. Conclusion

The crude methanol extract of *A. muscaria* demonstrated promising results in terms of contact toxicity, feeding deterrence, reproduction inhibition and repellency potentials against the pest *S. zeamais*. The toxicity could be attributed to the known constituents of *A. muscaria* which are reported to enhance the insecticidal activities of the mushroom. However, based on the demonstrated potencies, the mushroom can be used as a biopesticide by the subsistence farmers against *S. zeamais* storage pests in grains intended for being used as seeds in the forthcoming seasons. The use of *A. muscaria* in storing grains for consumption is discouraged as the toxicity of the plant to human is still uncertain. Further studies are recommended to assess the effect of *A. muscaria* extracts treatment on the quality parameters of the treated grains such as viability, moisture, colour and odour over prolonged storage durations. Moreover, similar studies are recommended for water extracts which is more affordable to the local settings, assuming that water extracts could show the activity due to extraction of polar compounds compared to methanol extract. This approach can be useful in overcoming existing challenges posed by synthetic pesticides such as availability, affordability and fear for human and environmental toxicity.

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Statement of Competing Interests

The authors have no competing interests.

List of Abbreviations

MUHAS:	Muhimbili University of Health and Allied Sciences
DAT:	Days after Treatments
IR:	Inhibition Rate
COSTECH:	Tanzanian Commission for Science and Technology

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