Journal of Food Security, 2017, Vol. 5, No. 1, 13-18
Available online at http://pubs.sciepub.com/jfs/5/1/3

DOI:10.12691/jfs-5-1-3

Pesticidal Activity of Wild Mushroom *Cantharellus cibarius (FR)* Extracts against *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) in Stored Maize Grains

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Abstract Grain damage due to pest infestation is among the top challenges facing cereals production. Maize grains being among the staple food in different parts of the world is prone to destruction by pests such as vertebrates, fungi and insects who are known to affect maize before harvest and during post harvest storage. *Sitophilus zeamais* is among the potential maize grains infestants. The efforts to control the pest highly depends on the application of synthetic pesticides which are faced by challenges of limited access, fear for toxicity, development of resistance among the pests and environmental pollution**.** This study aimed at assessing the toxicity, anti-feedant and repellence activities of crude methanol extracts of wild mushroom *Cantharellus cibarius* on *Sitophilus zeamais* in stored maize grains towards searching for alternative means of pest control. Assessments were conducted using six levels of concentrations ranging between 0.05 to 0.5 % w/w. Nontreated grains 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 demonstrated high toxicity 21 days after treatment killing 66.7% of the pest. Similarly, a 92.5% reduction in grain damage was observed at 0.5% w/w 21 days after treatment compared to nontreated controls. Furthermore, the extracts indicated pest repellence of 98.3% after 24 hours of exposure. The findings render *C. cibarius* a potential biopesticide for use by subsistence farmers against maize storage pests to support the ongoing Integrated Pests Management strategies. Further studies are recommended on the appropriate frequency and rate of application as well as the maximum duration of protection that can be offered by the extracts.

Keywords: *pesticidal activity, Catharellus cibarius, maize grains, Sitophilus zeamais, grain damage, Tanzania*

Cite This Article: Nelson E. Masota, Joseph Sempombe, Matobola Mihale, Leonia Henry, Veronica Mugoyela, and Fortunatus Sung'hwa, “Pesticidal Activity of Wild Mushroom *Cantharellus cibarius (FR)* Extracts against *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) in Stored Maize Grains.” *Journal of Food Security*, vol. 5, no. 1 (2017): 13-18. doi: 10.12691/jfs-5-1-3.

1. Introduction

Grains damage both pre harvest and post harvest in many parts of the world has become an alarming problem for years. In subsistence farming, cereals are the most affected crops. Like other cereal grains, maize is prone to damages caused by vertebrate pests, insects and fungi. [1,2,3,4,5]. The damage on maize grains can occur before harvesting as well as during post-harvest storage time. Crops’ loss due to pest infestation leads to food insecurity and decreased household’s health and financial wellbeing [6,7]. *Sitophilus zeamais* is among the potential infestant known to damage cereal grains in the field and at storage sites [8,9,10].

Synthetic pesticides have been continuously used by some farmers in attempts towards reductions of grain loss. However, relatively higher costs, and challenges encountered during registration by local regulatory authorities have limited their availability to the subsistence farmers [7,11]. Other concerns of interest which limit the effective application of conventional pesticides are the fear for mammalian toxicity during application and upon consumption of pesticides residues on the preserved food [7,12,13,14]. The development of resistance by the pests and environmental pollution which may cause elimination of other beneficial organisms are as well considered as the limiting factors [15,16,17].

The integrated pest management (IPM) strategies advocate the use of alternatives and more environmentally friendly natural and biological means to combat the pests in order to reduce the use of synthetic pesticides. The benefits associated with the use of botanical pesticides advocate for their use as promising alternatives to synthetic pesticides in pests management [18,19,20,21]. The identification of species with pesticidal potency also sets a base for further studies towards the isolation and development of novel pesticides with improved profiles. So far studies on mushrooms have shown the presence of compounds with antimicrobial, antiviral, antitumor and immune-modulating activities [22,23,24,25]. Insecticidal activity on *Drosophila melanogaster* associated with lectins have been reported [26,27].

Studies on the mushroom *C. cibarius* indicated the presence of phenolic and indole compounds, fatty acids, carbohydrates, proteins, free amino acids, sterols, vitamins, enzymes and other elements that render it a nutritious food. The antioxidant, antiviral, antimicrobial and antigenotoxic properties exhibited by *C. cibarius* are probably due to these constituents [24,28,29]. The available data regarding the use of the *C. cibarius* as a biopesticide against *S. zeamais* and other pests is limited. This study aimed at determining the pesticidal activity of crude methanol extracts of an edible wild indigenous mushroom, *C. cibarius*, against *S. zeamais* in stored maize. The findings from this study will help to indicate if *C. cibarius* can be used as a potential biopesticide by subsistence farmers against maize storage pests to support the ongoing Integrated Pests Management strategies

2. Materials and Methods

2.1. Sample Collection and Extraction

Fresh whole bodies of the wild mushroom *C. cibarius* were collected from Mbeya region in Southern highlands of Tanzania during the month of November. The samples were dried under shade at 22-27°C before being packed in paper bags. Then, the samples were transported to the Medicinal Chemistry Laboratory, School of Pharmacy, Muhimbili University of Health and Allied Sciences (MUHAS) for further drying in oven (Köttermann, German) at 40°C for 48 hours. Grinding of the samples to fine powder was carried out using an electric laboratory blender (Akita electronics Co.L.L.C, UAE). Extraction by maceration of powdered samples for 72 hours using methanol (95%v/v) (Carlo Erba reagents group, German) provided a crude extract. The filtrate of the crude extract was obtained through serial filtration with *Whatman* filter papers (Whatman No. 1 sheets) (GE Healthcare UK Ltd, China) under vacuum. The purified extracts were dried *in vacuo* using rotary evaporator (Bibby Sterilin Ltd, UK) operated at 50°C and were then refrigerated at 4°C until further pesticidal activity tests.

2.2. Insects Rearing

The study was conducted in the Medicinal Chemistry laboratory at MUHAS. Insects (*Sitophilus zeamais*) were obtained from the grains milling stations and appropriately identified. Rearing of the insects was done on nontreated and uninfected maize (*Zea mais L.),* grains, after sterilisation in an oven at 40°C for four hours [30].

Maize grains (about one kilogram) were put in a perforated transparent plastic jar (20 cm diameter and 30 cm height). Approximately four hundred unsexed adult *S. zeamais* were placed in the containers and top fine plastic mesh were fastened by elastic bands to allow aeration [31,32].

The pests in the containers were kept at 25-30°C, 60-70 relative humidity (RH) and 12 hours light: 12 hours dark conditions of exposure. The insects were allowed to lay eggs for 14 days, after which all adult insects were removed by gentle sieving. Maize grains were retained by a 3 mm mesh sieves, the insects were collected by a 1 mm mesh sieve and the frass was collected by the holding pan at the bottom. Afterwards the frass and the grains were returned in the containers and kept under similar conditions until when the adult insects emerged (25-35 days). The emerging adults were removed daily by a similar sieving process and kept in separate jars based on their age ready for pesticidal tests [31,33].

2.3. Laboratory Bioassays

2.3.1. Repellence Studies (Choice Bioassay)

Repellence of the crude extracts was carried out using round plastic containers (45 cm diameter and 15 cm height). The bases of the containers were marked into four portions onto which 100 mg portions of treated and nontreated grains were placed in alternation at an equal distance from the centre [33].

Three replicates were made for each level of treatment (0.0, 0.5, 1.0 and 1.5% w/w) of crude methanolic extract and a positive control (Actellic Gold™ 2% dust (0.05% w/w)) (Syngenta, UK). A negative (no choice) control was used where all four portions were composed of nontreated grains was included. The containers were arranged in a completely randomized design (CRD). Then, 20 adult *S. zeamais* (4 - 8 days) were placed at the centre of the containers whose tops were covered with a fine wire mesh to avoid escaping. Recording of the total number of insects which settled on the nontreated (N_C) and treated (N_T) grains in each container was carried out after 1, 12 and 24 hours post exposure. Percent repellence (PR) was then calculated as in equation (1) and interpreted as described in Hassanal and Wekesa et al. [34,35]:

$$PR = \frac{(N_C - N_T)x100}{(N_C + N_T)}. \quad (1)$$

2.3.2. Feeding Deterrence and Contact toxicity Studies

Forty maize grains were weighed before treatments with mushroom methanol crude extracts and put in perforated transparent plastic containers (200 mL). Six levels of treatments (0.05, 0.15, 0.20, 0.25, 0.3, 0.4 and 0.5 %w/w) in methanol (1 mL) were prepared followed by thorough mixing of the resulting solutions with the maize grains. The treated maize grains were left in open air under shed for 6 hours to allow the complete evaporation of the solvent. Nontreated grains were used as negative controls whereas Actellic gold ™ 2% dust (0.05% w/w) treated grains was used as a positive control. Three replicates were made for each level of treatments and for the controls [31].

Twenty unsexed adult *S. zeamais* (5 - 10 days) were put in the containers containing treated maize grains and allowed to feed on the grains. The containers were kept in the laboratory at 25–30°C and 65–70% R.H in a CRD. Counting of dead insects was carried out on 1, 3, 5, 7, 14 and 21 days after treatments (DAT). After that, 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 in equation (2).

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

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.

2.4. Data Analysis

The data were analysed using Statistical Package for Social Science (SPSS) version 20. 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. The lethal concentration that can kill 50% of the insects (LD_{50}) and concentration that can repel 50% of insects (RC_{50}) were calculated using Probit Regression analysis.

3. Results and Discussion

3.1. Contact Toxicity

The mean percentage mortality of the insects was observed to be significant at (p< 0.05) associated with the concentration of the treatment and the contact duration (Table 1). The percentage mortality of up to 66.7% was observed after 21 days post treatment of the *C. cibarius* crude methanol extract concentration of 0.5% w/w.

Results showed a significant (P<0.05) increase in the percentage mortality between the 5th and 7th day post treatment for all treatments with crude concentrations ≥ 0.3% w/w. However, there was no a significant increase in the percentage mortality of the insects between the 14th and 21st day post treatment at all concentration levels. Probit regression analysis indicated that the concentration of 0.338 %w/w was required to kill 50% of the insects at 21 days post treatment, whereas the duration required to kill at least 50% of the insects (LT_{50}) was 13.94 days at 0.5% w/w.

A significant difference (P<0.05) in mean percentage mortality was observed between the negative control and treated grains at the highest concentration from day 14 post treatment. However, the crude extracts treatment were inferior (p<0.05) to the positive control (Actellic gold ™ 2% dust (0.05% w/w) over the entire duration of an experiment.

Most of the grains treated with crude extracts exhibited mortality of the pests from the 5th day post treatment onwards; the increase in mortality was sharp from 5th to the 14th day of observation, followed by a relatively smaller increase on the last 7 days of observation. The present linear relationship between the amount of crude extract applied per unit weight of the grains and the percentage mortality of the pests indicates the certainty of attaining an even higher mortality of the pests at upon an increase in the treatment concentrations [33].

The observed decrease in the mortality rate from the 14th day post treatment is suggestive of the possible loss of the killing potency of the crude extracts with time possibly due to environmental factors which may favour degradation of the active constituents [36]. This implies that any attempt to use *C. cibarius* in killing *S. zeamais* already present in the grains should employ high concentrations that will maximize mortality in the first two weeks post treatment.

Other studies have indicated the insecticidal activity of isolated esters and glycerides from *C. cibarius*. However; the activity of the isolates was reported to be lower as compared to that of the crude extracts which is suggestive of a synergistic effect of the compounds present in the crude extract [36]. Indole, sterols, carotenoids and phenolic compounds are among the compounds which have also been isolated from *C. cibarius* associated with the antimicrobial, antiviral and antioxidant activities among others [29]. Percentage mortality of 33% to 93.75% on the genus *Sitophilus* have been reported when other plant powders and crude extracts were tested on maize grains [31,33,37,38].

Table 1. Percent mortality (mean ± SE, n=3) of adult *S. Zeamais* in grains treated with methanolic crude extracts of *C.cibarius*, negative and positive controls

		DAT					
Treatment	Concentration (%w/w)	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[ab]	10.0±2.9[ab]
C. Cibarius	0.05	0.0±0.0[a]	0.0±0.0[a]	3.3±1.7[a]	3.3±1.7[ab]	8.3±3.3[ab]	10.0±2.9[ab]
	0.15	1.7±0.0[a]	3.3±3.3[a]	6.7±4.4[a]	13.3±1.7[a]	13.3±1.7[ab]	13.3±1.7[ab]
	0.25	0.0±0.0[a]	0.0±0.0[a]	5.0±0.0[a]	5.0±0.0[ab]	11.7±1.7[a]	11.7±1.7[ab]
	0.3	0.0±0.0[a]	0.0±0.0[a]	8.3±1.7[a]	30.0±7.6[a]	46.7±7.3[a]	50.0±5.8[a]
	0.4	0.0±0.0[a]	0.0±0.0[a]	5.0±0.0[a]	35.0±7.6[a]	53.3±6.0[a]	61.7±4.4[a]
	0.5	0.0±0.0[a]	0.0±0.0[a]	8.3±3.3[a]	46.7±9.3[ac]	63.3±6.0[ac]	66.7±4.4[c]
Actellic gold™ 2% dust	0.05	100.0±0.0[b]	100.0±0.0[b]	100.0±0.0[b]	100.0±0.0[d]	100.0±0.0[d]	100.0±0.0[d]

Means in column followed by different letters are significantly different at α=0.05 by Tukey's Studentised range (HSD) test.

3.2. Reduction in Grain Damage

The grain damage studies (feeding deterrence) indicated a significant dose dependent ($p < 0.05$) reduction in the percentage weight loss of the grains 21 days after treatments (Figure 1). The 92.5% reduction in damage caused by *S. zeamais* was observed at the concentration of 0.5% w/w. All treatment concentrations above 0.05 %w/w were significantly superior to the negative controls ($P < 0.05$). The positive control was not superior to the crude extracts in the same aspect at the concentration of 0.4 %w/w and above. Probit regression analysis indicated that a dose of 0.238 %w/w was required to cause a 50% reduction in weight loss over the 21 days duration (Figure 1).

Similar studies reported a reduction in grain damage from 46.2 to 52.2% when selected plant powders were used to treat stored maize grains against *Prostephanus truncatus* (Coleoptera, Bostrichidae) [31]. The observed reduction in percentage weight loss within the treated grains as compared to the nontreated ones may indicate the potential antifeedant activity possessed by the crude extracts. This is supported by the presence of rotenoids among the compounds isolated from *C. cibarius*, which are known for their antifeedant potency [31].

3.3. Repellence Activity

The extent of repellence expressed by the crude extracts on *S. zeamais* was significant ($P<0.05$) and was influenced by the treatment concentrations and the duration of exposure (Table 2). The maximum repellence of 98.3% was observed at the concentration of 0.5 %w/w after 24 hours, taking the percentage repellence expressed by the solvent treated grains (23.3%) as a benchmark.

The positive control (Actellic gold ™ 2% dust) expressed the repellence of 95.0% after 12 hours of exposure and onwards, where the higher killing rate of the positive control did not allow observation of the trend in repellence for more than 12 hours since the pests which remained in the Actellic Gold™ 2% dust treated grains were killed between 1 and 12 hours before migrating to the nontreated grains. Probit regression analysis calculations produced a concentration of 0.223 %w/w which would cause a 75% absolute repellence of *S. zeamais*.

Possession of a repellence activity is an important aspect for an agent to be used as a grains protectant. The repellence potency possessed by the crude methanol extract of *C. cibarius* can be associated with the presence of terpenoids which are characteristic of the insect repellent activity [39]. These compounds are known to exhibit the action by acting on olfactory receptors of the insects in a vapour form [31]. With the observed repellent potency in this species, maize grains treated by the crude extracts and even powders from the wild indigenous mushroom can keep away the pests, thus, rendering the grains safe over a prolonged period of time.

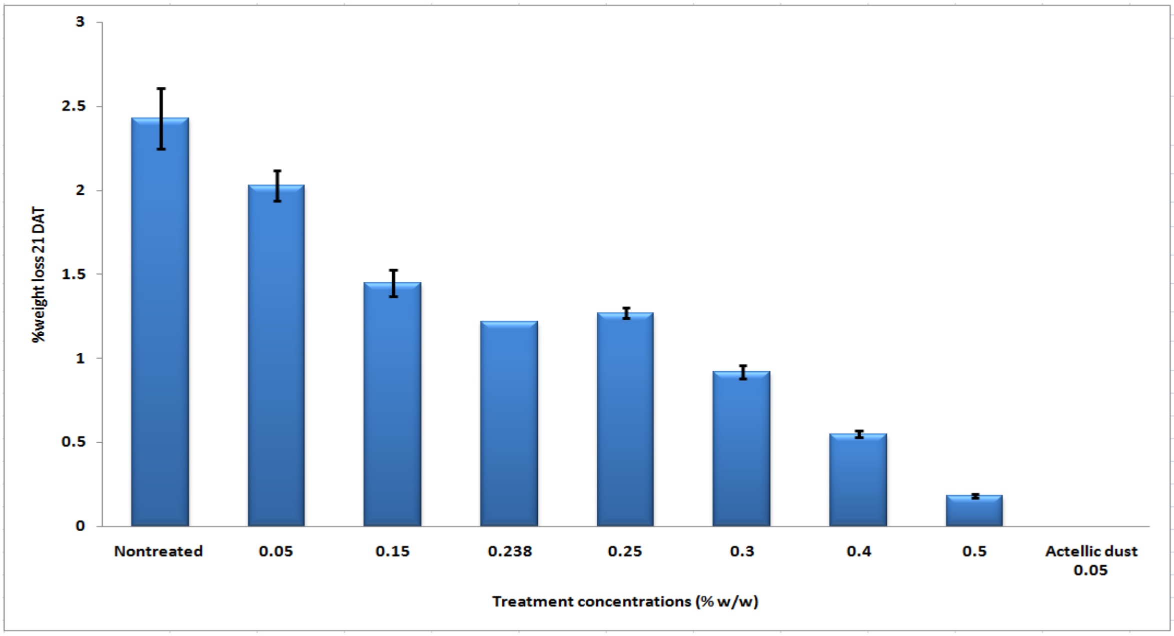

Figure 1. Percentage weight loss of *C. cibarius* treated maize grains 21 DAT

Table 2. Percent repellence (Mean ± SE, n = 3) of adult *S. zeamais* at varying exposure time and concentrations of *C. cibarius* crude extracts

Treatment	Concentration (%w/w)	Exposure Time (h)		
		1	12	24
Nontreated Control	0	1.7 ± 1.7^{a}	3.3 ± 3.3^{a}	8.3 ± 1.7^{a}
C. cibarius extracts	0.15	6.7 ± 3.3^{a}	55.0 ± 2.9^{b}	73.3 ± 4.4^{b}
	0.3	31.7 ± 1.7^{a}	60.0 ± 5.0^{b}	85.1 ± 2.9^{bc}
	0.5	23.7 ± 6.4^{a}	83.3 ± 1.7^{c}	98.3 ± 1.7^{c}
Actellic gold™ 2% dust	0.05	83.3 ± 3.3^{b}	95.0 ± 2.9^{c}	95.0 ± 2.9^{c}

Means in column followed b different letters are significantly different at α=0.05 when analysed by Fisher's LSD test.

4. Conclusion

The findings from this study have indicated the contact toxicity, feeding deterrence and repellence potentials of the crude methanol extracts of wild mushroom *C. cibarius*. This suggests the presence of bioactive compounds; natural or artefact, in the mushroom, which are responsible for observed activities. Being an edible mushroom, where naturally available and where it can be purposefully grown, *C. cibarius* can serve as nutritious food as well as a good biopesticide to the subsistence farmers against the *S. zeamais* storage pests. Further studies are also needed to determine if treatments with a powdered mushroom can demonstrate similar results. Studies to isolate the bioactive compounds and assess their effects on the quality parameters of the treated grains such as viability, moisture, colour and odour over prolonged storage durations are also necessary. Similar studies are recommended in the warm water extracts which is more affordable to the local settings, assuming that warm water extracts could show the activity due to the polarity more or less similar to methanol. This approach can be useful in overcoming existing challenges posed by synthetic pesticides such as availability, affordability and fear for human and environmental toxicity.

Acknowledgements

Authors would like to acknowledge the great support offered by the Tanzanian Commission for Science and Technology (COSTECH) for financial support to project No. CST/SC.327/952/3/2013 extended to this study. 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.

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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