East and Central African Journal of Pharmaceutical Sciences
Vol. 20 (2017) 17-26

Pesticidal Activity of Wild Mushroom *Boletus satanas* Lenz Extracts against *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) in Stored Maize Grains

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***Boletus satanas* Lenz (Boletaceae) is a basidiomycete fungus reported to contain monomeric glycoproteins (lectins) which are known to possess insecticidal, larvicidal, ovicidal and anti-nutritional activities. This study was carried out to assess the toxicity, anti-feedant and repellence potential of the crude methanol extract of the mushroom in stored maize grains. Six levels of concentration ranging from 0.05 to 0.5% w/w were used during the assessments. Untreated grains and grains treated with 2% Actellic gold ™ dust (0.05% w/w) were used as negative and positive controls, respectively. The experiments were carried out in a completely randomized design with three replicates made for each treatment level and controls. The 0.5% w/w methanol extract exhibited the highest mean mortality of 68.3% and 94.2% inhibition in F1 progeny. Moreover, 89.7% reduction in grain damage and 98.3% pest repellence were observed. These findings render credence to the use of *B. satanas* as a potential biopesticide by subsistence farmers to preserve grains and corroborate the ongoing IPM strategies.**

Keywords: Pesticidal activity, *Boletus satanas*, maize grains, *Sitophilus zeamais*, grain damage, Tanzania

INTRODUCTION

Maize is one of the staple foods in many communities around the world with about 594 tonnes produced per year [1]. Besides its use for human consumption, maize has also been used for ages as a component in animal feeds and as an industrial raw material for the manufacturing of food products and other commodities.

The practice of maize storage is common among most of its producers and traders. Various modern and local means of maize storage are known [2-4]. Failure to ensure proper storage of grains is associated with losses caused by numerous infesting agents (pests) such as animals, fungi and insects, to mention a few [5-7]. Consequently, this may predispose societies to food insecurity and poor outcomes in the general health and financial status of individuals and families [8-9].

Sitophilus zeamais is a common infestant of maize and other cereal grains and is capable of destroying the grains before harvest as well as during post harvest storage [10-12]. The use of synthetic pesticides in treating maize grains is the most common practice against *S. zeamais* and other grain infestants [13, 14]. However, this approach faces a number of challenges limiting its extensive application especially among subsistence farmers in rural settings. These include relatively high costs and the fear for human toxicity among subsistence farmers [13, 15-16]. The use of synthetic pesticides has also been highly associated with environmental pollution, destruction of unintended biodiversity and development of resistance among the targeted pests [15, 17-20]. These factors call for enhanced efforts in the controlled use of synthetic pesticides and

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advances in seeking alternative means of controlling pests and their damage. Preservation practices such as treatment of grains with powdered plants, smoking and spraying of grains with plant extracts possessing pesticidal activity are reported in some local communities [21, 22]. The potential of some plant powders, extracts and oils to kill, inhibit reproduction, repel and reduce losses in stored grains by common grains infestants have also been studied before [23-29]. The application of effective botanical and biological means in pest control is of great benefit because of their relatively low costs and high safety profiles to both humans and the environment. Insecticidal activities due to secondary metabolite constituents in some mushroom species have been reported against some insects such as *Drosophila melanogaster* and *Spodoptera littoraris* [30, 31]. In our previous studies, the pesticidal potency of the mushrooms *Cantharellus cibarius* and *Amanita muscaria* against *Sitophilus zeamais* was observed [32, 33].

Boletus satanas is a basidiomycete fungus from the family Boletaceae. The mushroom grows in mixed woodlands and is generally regarded as poisonous [34]. Bolesatine, indole alkaloids and hydroxynorvaline derivatives are among compounds which have been isolated from *B. satanas* and the absence of muscarine has been reported in other studies [36-37]. Bolesatine, is a monomeric glycoprotein (lectin) known to be a constituent of *B. satanas* capable of causing serious gastroenteritis, mitogenicity at low doses and protein synthesis inhibition by causing hydrolysis of guanosine triphosphate (GTP) at high doses (IC_{50} =530 nm) [38-39]. Lipid peroxidation induced by bolesatine is linked to its ability to inhibit cell growth in *in vitro* and *in vivo* systems [40]. These observations further led to identification of lectins as being capable of producing immuno-modulating and anti-proliferative activities because of their cytotoxic effects caused by apoptosis. The high stereo-specificity binding of the lectins occurring in a non catalytic manner is caused by a reversible binding with sugars, making them promising anticancer agents [41-42]. Bolesatine is also capable of inducing agglutination of platelets in rats and human erythrocytes *in vitro* [43]. Following its oral absorption, bolesatine is found to be distributed within the gastrointestinal tract, kidneys, liver, thymus, spleen and lung tissues, with about 80% excreted in faeces and urine after 24 hours, without proteolysis [44]. Lectins have been established as key active compounds involved in insecticidal and anti-nutritional activities exhibited by mushrooms and plants in which they exist. These largely affect their survival and fecundity rates [45-49]. Moreover, larvicidal and ovicidal capabilities of lectins have been reported [50-55].

Despite the insecticidal potency of *B. satanas*, there are few or limited reports on its use as a pesticide within communities in which it indigenously exists. This study aimed at determining the pesticidal potency of the mushroom against *Sitophilus zeamais*, a pest of high infectivity on maize grains. Specifically, the study involved the assessment of toxicity, repellence, feeding deterrence and reproductive inhibitory potencies.

MATERIALS AND METHODS

Sample collection and extraction

Samples of fresh *B. satanas* were collected from the southern highlands of Tanzania in Mbeya region in November 2014. They were cut into smaller pieces and air dried under the shade at 22-27°C for three days to reduce moisture content. The samples were then packed into paper bags and transported to the Medicinal Chemistry laboratory at the School of Pharmacy, Muhimbili University of Health and Allied Sciences (MUHAS) for further drying at 40°C for 28 hours in an oven (Köttermann, German). Dried samples were ground into fine powder using an electric laboratory blender (Akita Electronics Co. L.L.C, UAE). Extraction of powdered samples was done by maceration using methanol (99.5% v/v) (Carlo Erba reagents group, German) with periodic agitations for 72 hours to obtain the crude extract. Filtration of the crude extract was done under vacuum using Whatman filter paper (Whatman No. 1 sheets) (GE Healthcare UK Ltd, China). The filtrate was evaporated to dryness using a rotary vacuum evaporator (Bibby Sterilin Ltd, UK) operated at 50 °C and the residue refrigerated at 4 °C prior to further testing.

Insect rearing

Adult *Sitophilus zeamais* were obtained from the maize grains milling station and identified appropriately. The pests were reared in the laboratory on untreated and uninfected maize grains which had been sterilized in an oven at 40°C for four hours [56]. Approximately four hundred (400) unsexed adult *S. zeamais* were placed in a perforated transparent plastic jar (20 cm diameter and 30 cm height) containing one kilogram of maize grains. The top was covered with a fine plastic mesh fastened by elastic bands to allow aeration [24, 25].
The containers were then kept at 25-30°C, 60-70% relative humidity and 12 hours light: 12 hours dark cycle. 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 sieve, 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 the adult insects emerged (25 - 35 days). The newly emerged adults were then removed daily in a similar process and kept in separate jars according to their age for further experiments [24, 26].

Laboratory bioassays

Repellence studies (Choice bioassay)

Repellence of crude methanol extract on adult *Sitophilus zeamais* was carried out using circular plastic containers measuring 45 cm in diameter and 15 cm in height. The bases of the containers were marked into four equal parts with a common centre onto which about 100 mg portions of treated and untreated maize grains were placed in alternation equidistant from the centre [26]. 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 the positive control, Actellic Gold^{TM} 2% dust (0.05% w/w). The containers were arranged in a completely randomized design (CRD). Then, 20 adult *S. zeamais* aged 5 to 10 days were placed at the centre of the containers whose tops were covered with a fine wire mesh to prevent escape.
Recording of the total number of insects which settled on the untreated (N_C) and treated (N_T) grains in each container was carried out 1, 12 and 24 hours post exposure. Percent repellence (PR) was then calculated using equation (1) and interpreted as described elsewhere [27, 28].

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

Feeding deterrence and contact toxicity

Forty untreated maize grains were weighed and put in perforated transparent plastic containers (200 mL). Six crude extract concentrations (0.05, 0.15, 0.25, 0.3, 0.4 and 0.5 %w/w) in 1 mL of methanol were prepared, and thoroughly mixed with the maize grains. The treated maize grains were left in open air under shade for 6 hours to allow for complete evaporation of the solvent. Untreated and methanol treated grains were used as negative controls whereas grains treated with Actellic Gold^{TM} 2% dust (0.05% w/w) served as the positive control. Three replicates were prepared for each concentration and for the controls [21].
Twenty unsexed adult *S. zeamais* aged 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 treatment (DAT). Thereafter, 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 using equation (2) [27,28].

$$\text{Weight loss (\%)} = \frac{(UNd - DNu)x100}{(U(Nd + Nu))} \quad (2)$$

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.

F1 Progeny studies

The living *S. zeamais* adults were removed from the containers on 21 days after treatment. Counting and recording of the newly emerged

insects (F1 progeny) was done on 28, 35 and 42 DAT. The reproduction inhibition rate (IR %) was obtained using the equation 3 [24].

$$IR\ (\%) = \frac{(C_N - T_N)x100}{C_N} \quad (3)$$

Where C_N was the number of newly emerged insects in the untreated grains and T_N was the number of newly emerged insects in the treated grains [24]

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 Fishers Least Significance Difference (LSD) testing at 5% significance level. The lethal concentration that can kill 50% of the insects (LD_{50}) and concentration that can repel 75% of insects (RC_{75}) were calculated using Probit Regression analysis.

RESULTS AND DISCUSSION

Contact toxicity

The percentage mortality of adult *S. zeamais* was observed to be significantly ($p<0.05$) associated with an increase in concentration of the extracts and contact duration (Table 1). The highest mean mortality of 68.3% was observed at the concentration of 0.5% w/w of *B. satanas* crude extract 21 days post treatment. A sharp increase in percentage mortality of the pests was observed between the 5^{th} and 7^{th} day of the experiment across all concentrations followed by a relatively gradual increase in the remaining days. Moreover, the differences between the mean percentage mortalities at different treatment concentrations were more significant from day 5 to 21 post treatment using Fishers LSD test ($\alpha = 0.05$).

Using probit regression analysis, the 0.39% w/w concentration was adequate to cause a 50% mortality of the pests (LC_{50}) at 21 DAT, whereas it took about 15.9 days to cause a 50% mortality of the pests (LD_{50}) at 0.5%w/w. Observation has shown that higher mortality rates can be achieved at higher concentrations and longer contact durations. The significant difference ($p< 0.05$) in mean percentage mortality was observed between the negative control and the grains treated with the extract at 0.5% w/w concentration day 3 post treatment. The percentage mortality exhibited by the concentrations in the range 0.15-0.25% w/w on 5 to 21 DAT was also significantly higher ($p< 0.05$) compared with the negative control. However, the positive control, Actellic gold ™ 2% dust, (0.05% w/w) was superior to crude extract treatments over the entire duration of the experiment ($p< 0.05$).

The observed pesticidal activity of the methanol extract of *B. satanas* against *S. zeamais* corroborates the reported insecticidal activities exhibited by mushrooms and plants containing lectins [45-49]. The effect may be attributed to the known insecticidal potential of lectins to induce protein synthesis inhibition hence inducing cell deaths which may affect survival of the exposed insect [41-42]. In another unpublished work, we have demonstrated the pesticidal potency of the mushroom *C. cibarius* and *A. muscaria* against *S. zeamais* in which the mortalities of 66.7% and 61.7%, respectively were 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 [24, 26, 57].

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

Treatment	Concentration (%w/w)	DAT					
		1	3	5	7	14	21
Untreated Control	0.00	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}
	0.05	0.0±0.0^{a}	0.0±0.0^{a}	1.7±1.7^{a}	3.3±1.7^{a}	3.3±1.7^{ab}	3.3±1.7^{ab}
	0.15	0.0±0.0^{a}	1.7±1.7^{a}	3.3±1.7^{ab}	3.3±1.7^{a}	6.7±1.7^{b}	8.3±1.7^{b}
B. satanas	0.25	1.7±1.7^{a}	1.7±1.7^{a}	6.5±1.7^{b}	15.0±2.9^{b}	15.0±2.9^{bc}	15.0±2.9^{bc}
	0.3	0.0±0.0^{a}	0.0±0.0^{a}	8.5±1.7^{bc}	20.0±2.9^{c}	35.0±2.9^{d}	38.3±1.7^{d}
	0.4	0.0±0.0^{a}	0.0±0.0^{a}	10.0±0.0^{c}	40.0±0.0^{d}	58.3±3.3^{e}	61.7±4.4^{e}
	0.5	0.0±0.0^{a}	6.7±3.3^{b}	13.3±1.7^{cd}	40.0±0.0^{d}	65.0±5.0^{e}	68.3±3.3^{e}
Actellic gold^{TM} 2% dust	0.05	100.0±0.0^{b}	100.0±0.0^{c}	100.0±0.0^{e}	100.0±0.0^{e}	100.0±0.0^{f}	100.0±0.0^{f}

Means in columns are significantly different at α=0.05 by Fisher's LSD test

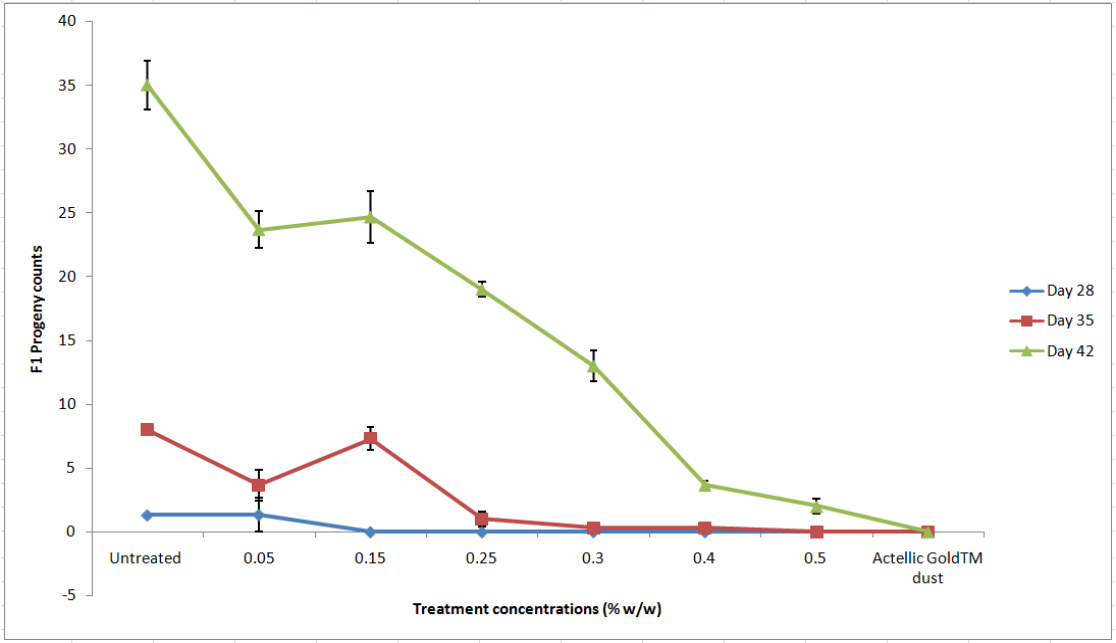

Figure 1: F1 progeny counts (Mean ± SE, n = 3) of *S. zeamais* at varying exposure time and concentrations of *B. satanas* crude methanol extract.

F1 Progeny Studies

A significant dose dependent reduction of the number of newly emerging insects (F1 progeny) was observed (Figure 1). A reduction of 94.2% in F1 progeny was recorded in the grains treated with 0.5% w/w of the extract 42 DAT. The concentration of 0.25% w/w produced a 50% reduction in F1 progeny counts 42 DAT. Higher levels of reduction in F1 progeny were achieved at higher extract concentrations. The observed inhibition in the development of F1 progeny can be associated with the larvicidal and ovicidal potential of lectins reported to be present in *B. satanas* [51-55].

Reduction in grain damage

A significant ($p < 0.05$) dose dependent increase in feeding deterrence was observed, recording a maximum of 89.7% reduction in weight loss of the grains 21 days after treatment (Figure 2). Probit regression analysis indicated that a dose of 0.221% w/w was required to cause a 50% reduction in weight loss over the 21 days duration. The mean percentage weight loss was significantly higher ($p<0.05$) in the untreated grains than the grains treated with the extract. On the other hand, there was no weight loss in the grains treated with the positive control (Actellic Gold™ 2% dust) at 0.05% w/w.

The observed feeding deterrence activity can be attributed by the antifeedant activity of lectins as reported by Pewell *et al.* [58]. The observed trend in reduction of weight loss (Figure 2) suggests that higher protective effects could be achieved with concentration levels higher than 0.5% w/w.

Similar studies reported a reduction in grain damage from 46.2 to 52.2% weight loss when selected plant powders were used to treat stored maize grains against *Prostephanus truncatus* (Coleoptera, Bostrichidae) [24]. The observed reduction in percentage weight loss within the treated as compared to the untreated grains may indicate that the crude extracts possess antifeedant activity.

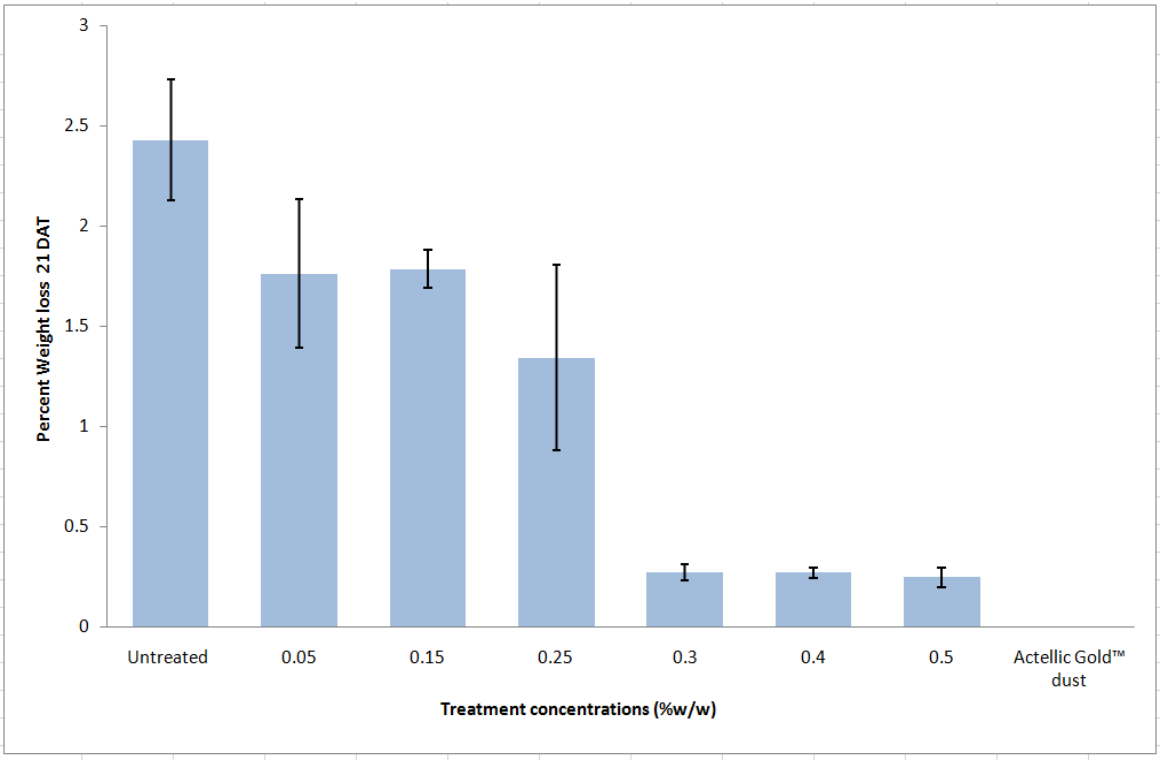

Figure 2: Percent weight loss (Mean ± SE, n = 3) of maize grains at varying exposure time and concentrations of *B. satanas* crude methanol extract 21 DAT

Repellent activity

The percentage repellence of the adult *S. zeamais* was observed to increase as a function of both time and extract concentration. A maximum of 98.3% repellence was recorded at the concentration of 0.5% w/w after 24 hours of exposure (Figure 3). The differences in the mean percentage repellency between the tested extract concentration levels at all time points were significant at $\alpha = 0.05$ using Fishers LSD test. Moreover, 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 untreated grains. Probit regression analysis produced the concentration of 0.177% w/w required to induce 75% repellence after 24 hours of exposure (RC_{75}). This degree of repellence could also be achieved at 0.5%w/w concentration of the extract after 2.83 hours of exposure. The observed repellent profile from this study is suggestive of the presence of volatile constituents capable of reaching the olfactory lobes of the insects and hence inducing noxious stimuli, pushing them away in looking for comfort. Terpenoids and carotenoids have been identified in other members of the genus Boletus and have been associated with the repellent activity in previous studies [59-60].

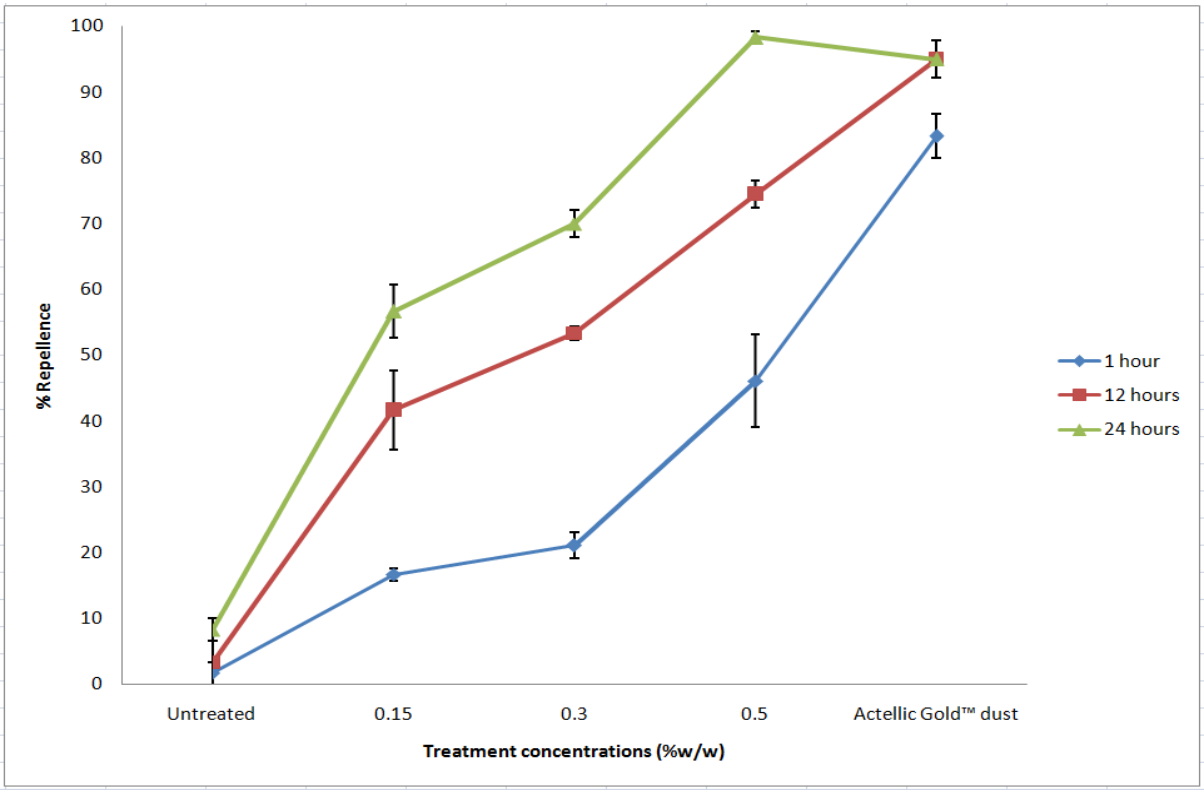

Figure 3: Percent repellence (Mean ± SE, n = 3) of adult *S. zeamais* at varying exposure time and concentrations of *B. satanas* crude methanol extract.

CONCLUSION

The current study has demonstrated the insecticidal, feeding deterrence, reproduction inhibition and repellent potential of the crude methanol extract of the wild mushroom *Boletus satanas*. This may be attributed to the activities of lectins which are known to be among the constituents of the mushroom. The high toxicity of the mushroom endows it with promising pesticidal potency but apparent uncertainty for its application in preservation of consumable grains. However, its application in the storage of grains intended to be used for non-consumption

purposes such as seeds can be advocated. Further studies are needed towards determining if the dried and powdered mushrooms can produce similar results when used in the treatment of stored grains. Studies to demonstrate the possible effects on quality parameters such as seed viability, moisture, colour and odour over prolonged storage duration are needed. In addition, studies using warm water as the extracting solvent (in place of methanol) owing to its ready affordability in local settings are recommended. This can be useful in overcoming existing challenges posed by synthetic pesticides such as availability, affordability and fear for human and environmental toxicity.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the Tanzanian Commission for Science and Technology (COSTECH) for financial support to this study (project No. CST/SC.327/952/3/2013). Our gratitude also goes 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.

REFERENCES

[1] Crop water information: Maize. [Cited 2016 Dec 20]. Available from: http://www.fao.org/nr/water/cropinfo_maize.html

[2] Maize Harvesting and post harvesting handling . [Cited 2017 Jan 03]. Available from : http://teca.fao.org/sites/default/files/technology_files/maize%20harvesting%20and%20post%20harvest%20handling.

[3] J.M. Udoh, K.F. Cardwell and T. Ikotun. J. Stored Prod. Res., 36(2), 2000, 187-201.

[4] Postharvest management and storage of maize. [Cited 2017 Jan 03]. Available from: http://agris.fao.org/agris-search/search.do?recordID=UG9500038

[5] T. Tefera, S. Mugo and P. Likhayo. Afr. J. Agric. Res., 6(10), 2011, 2249–54.

[6] M. Danho, C. Gaspar and E. Haubruge. J Stored Prod. Res., 38, 2002, 259–66.

[7] T. Tefera, G. Demissie, S. Mugo and Y. Beyene. Crop Prot., 46, 2013, 94–9.

[8] C. Hadley, M.B. Mulder and E. Fitzherbert. Public Heal. Nutr., 10 (6), 2007, 544–51.

[9] A.V.F .Ngowi, T.J. Mbise, A.S.M. Ijani, L. London and O.C. Ajayi. Crop Prot., 26(11), 2007, 1617–24.

[10] R. Suleiman, K.A. Rosentrater and C.J. Bern. J. Stored Prod. Res., 64, 2015, 97–102.

[11] K.E. Ileleji, D.E. Maier and C.P. Woloshuk. J. Stored Prod. Res., 43(4), 2007, 480–8.

[12] J. Derera, K.V. Pixley, D.P. Giga and I. Makanda. J. Stored Prod. Res. 59, 2014, 24–35.

[13] N. Stadlinger, A.J. Mmochi, S. Dobo, E.Gyllbäck and L. Kumblad. Environ. Dev. Sustain., 13(3), 2011, 641–56.

[14] Chemical Control techniques. [Cited 2017 Jan 03]. Available from: http://www.fao.org/docrep/t1838e/T1838E1g.htm.

[15] J.L. Lu and K. Cosca. Int. J. Environ. Stud., 68(2), 2011, 197–208.

[16] E.E. Lekei, A.V. Ngowi and L. London. BMC Pub. Health., 14(1), 2014, 389.

[17] M.E. DeLorenzo, G.I. Scott and P.E. Ross. Environ. Toxicol. Chem., 20(1), 2001, 84–98.

[18] L.J. Zettler and G.W. Cuperus. J. Econ. Entomol., 83(5), 1990, 1677–81.

[19] G.P. Opit, T.W. Phillips, M.J. Aikins and M.M. Hasan. J. Econ. Entomol., 105(4), 2012, 1107–14.

[20] P.C .Lopes, É. Sucena, M.E. Santos and S. Magalhães. PLoS One, 3(11), 2008, e3741.

[21] D.M. Firake, D. Lytan, D.P. Thubru, G.T. Behere, P.D. Firake, N.S.T. Azad. Ind. J. Hill Farm., 26(1), 2013, 58–61.

[22] T. Abate, A. van Huis and J.K. Ampofo, Annu. Rev. Entomol., 45, 2000, 631 -59.

[23] A.J. Bekele, D.H.A. Obeng-Ofori. Int. J. Biomed. Sci., 42(2), 1996, 139–42.

[24] F. Chebet, A.L. Deng, J.O. Ogendo, A.W. Kamau and P.K. Bett. Plant. Protect. Sci., 49(1), 2013, 34–43.

[25] A. Ebadollahi and M. Mahboubi. Chilean J. Agr. Res., 71(3), 2011, 406-412.

[26] J.O. Ogendo, S.R. Belmain, A.L. Deng. Insect. Sci. Appl., 23(2), 2003, 127–35.

[27] A. Hassanali, W. Lwande, N. Ole-Sitayo, L. Moreka and A.C. Nokoe. Discov. Innov., 2, 1990, 91–5.

[28] I. Wekesa, L.A. Onek, A.L. Deng, A. Hasanali and J.O. Othira. J. Stored Prod. Postharvest. Res. 2(6), 2011, 113–9.

[29] L.S. Mulungu, M.T. Kubala, G.G. Mhamphi, R. Misangu, and M.W. Mwatawala. Int. Res. J. Plant Sci., 1, 2010, 150-4.

[30] K.S. Mier, N.D. Mier, M. Wang, L. Chavant and D. Fournier. Acta Botanica Yunnanica. 20(2), 1998, 193-196.

[31] S. Camazine, J. Chem. Ecol. 9, 1983, 1473.

[32] N.E. Masota, J. Sempombe, M. Mihale, L. Henry, V. Mugoyela and F. Sung'hwa. JFS., 5(1), 2017, 13-8.

[33] N.E. Masota, J. Sempombe, M. Mihale, L. Henry, V. Mugoyela and F. Sung'hwa. JFS., 5(2), 2017, 26-32.

[34] *Boletus satanas* . [Cited 2017 Jan 03]. Available from: http://eol.org/pages/163492/details.

[35] P. Matzinger, P. Catalfomo, and C.H. Eugster. Helv. Chim. Act., 55(5), 1972, 1478-1490.

[36] Y.J. Lee, B.S. Hwang, J.G. Song, D.W. Kim, E. Woo, I.K. Lee and B.S. Yun. Kor. J. Mycol., 43(1), 2015, 68-70.

[37] Z.A. Mahmood. Natural Products, Springer-Verlag, Berlin Heidelberg, 2013, pp 523-52.

[38] R. Ennamany, J.P. Lavergne, J.P. Reboud, G. Dirheimer, and E.E. Creppy. Toxicol., 100(1), 1995, 51-55.

[39] O. Kretz, L. Barbieri, E.E. Creppy, and G. Dirheimer. Toxicol., 73(3), 1992, 297-304.

[40] R. Ennamany, S. Marzetto, D. Saboureau, and E.E. Creppy,. Cell Bio. Toxicol., 11(6), 1995, 347-354.

[41] G. Konska. Int. J. Med. Mushroom., 8(1). 2006, 19-30.

[42] R.S. Singh, H.P. Kaur, and J. Kanwar. Curr. Protein Pept. Sci., 17, 2016, 797-807.

[43] C. Gachet, R. Ennamany, O. Kretz, P. Ohlmann, C. Krause, E.E. Creppy, G. Dirheimer and J.P. Cazenave. Hum. & Exp. Toxicol., 15(1), 1996, 26-29.

[44] O. Kretz, E.E. Creppy and G. Dirheimer. Xenobiotica. 21(1), 1991, 65-73.

[45] H. Wang, T.B. Ng and V.E. Ooi. Mycol. Res., 102(08), 1998, 897-906.

[46] M. Wang, V, Triguéros, L. Paquereau, L. Chavant, and D. Fournier. J. Econ. Entomol., 95(3), 2002, 603-607.

[47] R. Singh, I.M. Tiwari, H.M. Jagadeesh, R. Kansal, R.N. Gupta, K.R. Koundal and R. Saini. Ind. J. Biotech., 11(2), 2012, 134-41.

[48] A.M. Gatehouse, K.S. Powell, W.J. Peumans, E.J. Van Damme, J.A. Gatehouse. Insecticidal properties of plant lectins: their potential in plant protection. In Lectins: Biomedical Perspectives. Taylor & Francis, London, 1995, pp 35-58.

[49] J. Pohleven, J. Brzin, L. Vrabec, A. Leonardi, A. Čokl, B. Štrukelj, J. Kos and J. Sabotič. Appl. Microb. Biotech. 91(4), 2011, 1141-8.

[50] C.H. Eisemann, R.A. Donaldson, R.D. Pearson, L.C. Cadogan, T. Vuocolo and R.L. Tellam. Entomol. Experim. 72(1), 1994, 1-10.

[51] R.A. Sá, N.D. de Lima Santos, C.S. da Silva, T.H. Napoleão, F.S. Gomes, B.S. Cavada, L.C. Coelho, D.M. Navarro, L.W. Bieber and P.M. Paiva. Toxicol. & Pharmacol., 149(3), 2009, 300-6.

[52] R.E. Down, A.M. Gatehouse, W.D. Hamilton and J.A. Gatehouse. J. Insect Physiol., 42(11), 1996, 1035-45.

[53] K. Singh, M. Kaur, P.J. Rup and J.J. Singh. Env. Bio., 30(4), 2009, 509-514.

[54] V. Trigueros, A. Lougarre, D. Ali-Ahmed, Y. Rahbé, J. Guillot, L. Chavant, D. Fournier and L. Paquereau. Biochim. Biophys. Acta-Gen. Sub., 1621(3), 2003, 292-8.

[55] A. Sadeghi, E.J. Van Damme, W.J. Peumans and G. Smagghe. Phytochem., 67(18), 2006, 2078-84.

[56] T. Matsumoto, W. Trueb, R. Gwinner and C.H. Eugster. Helv. Chim. Acta, 52, 1969, 716-720

[57] W. Auamcharoen, A. Chandrapatya, A. Kijjoa and Y. Kainoh. Pak. J. Zool., 44 (1), 2012, 227-32.

[58] K.S. Powell, A.M. Gatehouse, V.A. Hilder and J.A. Gatehouse. Entomol. Exp. Appl., 75(1), 1995, 51-9.

[59] B. Muszynska, K. Sulkowska-Ziaja, H. Ekiert. Acta Sci. Pol. Hort. Cult., 12, 2013, 1107-16.

[60] D.E. Zavastin, A. Bujor, C. Tuchiluș, C.G. Mircea, S.P. Gherman, A.C. Aprotosoaie and A. Miron. J. Plant Dev., 23, 2016, 87-95.