Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Original article https://doi.org/10.12980/jclm.5.2017J6-279

Toxicity of lemon grass *Cymbopogon citratus* powder and methanol extract against rice weevil *Sitophilus oryzae* (Coleoptera: Curculionidae)

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ARTICLE INFO

Article history:
Received 6 Dec 2016
Received in revised form 5 Jan 2017
Accepted 20 Jan 2017
Available online 22 Feb 2017

Keywords:
Cymbopogon citratus
Pest management
Sitophilus oryzae
Stored products
Toxicity

ABSTRACT

Objective: To evaluate the toxicity potential of lemon grass [*Cymbopogon citratus* (*C. citratus*)] products against adult rice weevil, *Sitophilus oryzae*.
Methods: Lemon grass (*C. citratus*) leaves were sundried for 7 days, pulverized and sieved using 0.5 mm mesh size to obtain fine powders. About 500 g of the powder were dissolved in 1 000 mL of 90% methanol to produce the extract. The powder and extract were used for the bioassay. The powder was tested at 1.0, 1.5, 2.0 and 2.5 g/10 g rice grains, respectively. The toxic potential of the extract of concentration of 1.0, 1.5, 2.0, and 2.5 mg/mL were evaluated using the filter paper method. The experiment was setup on a completely randomized design using three replicates per treatment.
Results: The results indicated significant difference (F = 7.450; df = 3.15; $P < 0.05$) in mean percentage mortality after 24, 48, 72, and 96 h exposure with the powder compared with the control. Significantly (F = 5.519; df = 3.15; $P < 0.05$) higher percentage adult mortality was also observed in the extract after 24, 48, 72, and 96 h exposure compared with the control. The LC_{50} value of the powder was 4.91 g/10 g of rice while the LT_{50} was 160.51 h. The LC_{50} value of the extract was 2.16 mg/20 mL of methanol with an LT_{50} of 75.10 h. The methanol extract of *C. citratus* showed the highest mortality compared to the powder which was less toxic.
Conclusions: The study showed that *C. citratus* products are promising insecticides and can be used effectively in the management of *Sitophilus oryzae* in storage.

1. Introduction

In Nigeria, *C. citratus* L. (Poaceae) popularly called rice, is an important staple food among other cereal group and a good and cheap source of carbohydrate supplying energy and protein requirements. It is consumed by nearly half of the world's population[1]. It takes about 8 months after planting before harvesting. After harvesting, the harvested crops are often stored for food reserve and to provide income. During storage, insect pests are known to attack the crops. These insect pests are known as stored-product insect. They cause an estimated overall damage of 10%– 20% of stored products[2,3]. The infestation of insect pest of rice is one of the major setbacks for the low yield of rice in Nigeria, which has in turn affected the country's economy. Damaged grains have reduced nutritional values, weight loss and market values[4]. The rice weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae) (*S. oryzae*) is one of the most important stored pests of stored-grain products found commonly in tropical and warm temperate regions of the world[5,6]. The adult weevils tend to multiple very fast in storage, causing very high infestation and a high progeny production rate which usually occur every month[1]. The infestation is one of the major constraints in achieving the yield potentials. Therefore, over the years, it has been the priority of farmers and researchers to reduce the numbers of these pests below economic threshold level.

Synthetic chemical insecticides have been used in the past to store products, since these chemicals are not eco-friendly and thus pose very serious environmental hazards. Rice grains are consumed totally, which limits the acceptability of such toxic

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The journal implements double-blind peer review practiced by specially invited international editorial board members.

synthetic insecticides[7]. Also, the indiscriminate use of chemical pesticides has resulted to problems ranging from genetic resistance of pest species, toxic residues, increasing costs of application, hazards from handling, *etc*. It has been recognized globally that the incessant use of chemical insecticides lead to the development of cross and multi-resistant strains in many important insect species. *S. oryzae* has also been reported to have developed such resistance to synthetic insecticides[8].

The recent trend in farm storage systems in Nigeria and elsewhere is the use of plant-derived materials for controlling insect pests since they are readily biodegradable. Currently, biological scientists are screening plants to examine their botanical insecticidal potential. These botanical insecticides including lemon grass can be used as powders and extracts (crude or purified) to control insect pests[4,9]. Lemon grass, *C. citratus*, is a tropical plant and exists in diverse forms and cultivars[10]. In Nigeria, it is commonly found in the southern region, where it is used as spice, pot herbs and as local medicine[4,11]. Lemon grass including its extracts has been reported to have shown insecticidal potential, thus have been used in insecticidal sprays[4,11].

Although the biological effects of some plant materials against stored product insects have been studied by different authors[12-15], this study seeks to evaluate the toxicity efficacy of lemon grass leaves powder and methanol extract against the rice weevils under laboratory conditions. Understanding the insecticidal potential would be economically viable option to local famers.

2. Materials and methods

2.1. S. oryzae culture

Adults of *S. oryzae* were obtained from infested grains in a local market in Abraka, Delta State in Nigeria. They were identified by Dr. J.E.G. Akeat, Department of Animal and Environmental Biology, Delta State University, Abraka, Nigeria. The identified weevils were cultured on a diet of rice grains in rearing rubber containers with fine mesh gauze covering the opened end to prevent the contamination and escape of weevils. The containers were left undisturbed for seven days to allow for further reproduction. The parent stocks were sieved out and the rice grains containing eggs were left undisturbed until the new adults emerged which were then used for the bioassays. Insect culture was done under laboratory conditions (temperature 28 ˚C and humidity 44%).

2.2. Preparation of plant powder

The research plant was identified as *C. citratus* by Botany Department of Delta State University, Abraka, Nigeria. It was used for the study. The plant was obtained from Ikpoba Okha Local Government Area of Edo State, Nigeria in August 2014. Fresh leaves from the plant were sundried before pulverization using an electric 5.0Hp kitchen grinder and sieved through a 40 holes/mm^2 mesh screen to obtain uniform particle size powders. The resulting powders were placed in an air tight container to prevent active components from evaporating until when needed.

2.3. Preparation of plant extract

The extracts of *C. citratus* were prepared at the Faculty of Science Central laboratory of Delsu, Abraka, Nigeria. Cold extraction method was employed using 90% methanol as the solvent. 1 000 mL of methanol was added to 500 g powder *C. citratus*. The mixture was left to stand on the bench for 24 h and the cover was slightly open to expel oil from the mixture. The mixture was filtered through filter paper and the residues re-extracted for another 24 h before filtration. The filtrate was then extracted using simple distillation method by heating in a water bath to evaporate the solvent (methanol) to obtain the extract. The extract was then kept in air tight flask to prevent the escape of active ingredients and stored in a refrigerator until needed.

2.4. Insecticidal bioassay

2.4.1. Adulticidal effect of C. citratus powders

About 10 g of rice grains were weighed into Petri dishes mixed with 1.0, 1.5, 2.0 and 2.5 g lemon grass powders. The Petri dish contents (treatments and rice grains) were shaken thoroughly to ensure uniform distribution of the botanical powders. Ten unsexed adults *S. oryzae* were then carefully introduced into the contents and exposed to treatments. Control containing untreated rice grains and ten unsexed adults *S. oryzae* were also set up. The treatment was laid out on a completely randomized design on the laboratory bench with 3 replicates per treatment. Mortality counts were recorded 24, 48, 72 and 96 h after setup by carefully sieving out the contents into a white tray while taking census of dead insects to determine LC_{50} and LT_{50}. Insects which did not react or move when probed gently with a needle were considered dead and were discarded while living ones returned to their respective treatments. The data on each assessment day after being summed was considered the cumulative adult weevil's mortality.

2.4.2. Adulticidal effect of C. citratus extracts

The assay of *C. citratus* extracts against adult *S. oryzae* was done according to Ojianwuna and Umoru[4] with some modifications. 20 mg, 30 mg, 40 mg and 50 mg quantities of the extracts were measured and dissolved in 20 mL of 90% methanol in test tubes

and delivered to Petri dishes containing Whatman No. 1 filter paper. The solvent was then allowed to evaporate for 90 min to get the concentrations of the extract as 1.0, 1.5, 2.0, and 2.5 mg/mL, respectively. Ten unsexed adults *S. oryzae* were then introduced into each Petri dish and exposed to treatments. 20 mL of 90% methanol which was also evaporated for 90 min was set up as the control experiment. The Petri dishes were covered and kept in the laboratory. The treatment was established on a completely randomized design in the laboratory with 3 replicates per treatment. Insect mortality was taken 24, 48, 72 and 96 h after setup to determine LC_{50} and LT_{50}. Adult mortality was also determined in percentage.

2.5. Data analysis

All data obtained from toxicity tests of plant powder and extract were analysed using One-way analysis of variance (ANOVA). Lethal doses (LD_{50} and LD_{90}), lethal concentrations (LC_{50} and LC_{90}) and lethal time values (LT_{50} and LT_{90}) were calculated and also regression analyses were performed.

3. Results

Table 1 shows the percentage mortality of *S. oryzae* exposed to powders of *C. citratus* for a period of 24, 48, 72, and 96 h. The results showed that higher number of unsexed adult *S. oryzae* mortality on rice grains treated with different concentrations of *C. citratus* powder than with the control treatment which had no *C. citratus* powder.There was significant difference in adult mortality (average number of dead adults per replicate) resulting from the treatments ($F = 7.450$, $df = 3.15$; $P < 0.05$) as the concentrations increased from 1.0–2.5 g in 24–96 h exposure. The data obtained (Table 1) also showed that *C. citratus* powder at a concentration of 2.5 g was most toxic causing 43.3% mortality, followed by 2.0, 1.5, and 1.0 g with 30.0%, 23.3%, and 16.7% mortality were recorded after 96 h exposure. The median lethal concentration LC_{50} of *C. citratus* was 4.91 g (Figure 1) with a median lethal time LT_{50} of 160.51 h (Figure 2).

Table 1

Percentage mortality of rice weevil (*S. oryzae*) exposed to powder of lemon grass (*C. citratus*).

Treatments (g)	Mortality (%)				
	24 h	48 h	72 h	96 h	Overall mean
1.0	0.00 ± 0.00	3.30 ± 0.33	10.00 ± 0.58	16.70 ± 0.33	7.5
1.5	0.00 ± 0.00	3.30 ± 0.33	13.30 ± 0.33	23.30 ± 0.88	10.0
2.0	0.00 ± 0.00	10.00 ± 0.58	16.70 ± 0.33	30.00 ± 0.58	14.2
2.5	3.30 ± 0.33	20.00 ± 0.58	33.30 ± 0.33	43.30 ± 0.88	25.0
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.0

Values are means of triplicate observations ± SEM.

The percentage mortality of *S. oryzae* exposed to extracts of *C. citratus* for a period of 24, 48, 72, and 96 h is shown in Table 2. Data obtained showed that significantly higher mortality of unsexed adult *S. oryzae* was recorded with methanol extract of *C. citratus* as the concentration increased from 1.0–2.5 mg/mL while no mortality was recorded in the control treatment. The data indicated that *C. citratus* extracted at a concentration of 2.5 mg/mL was the most toxic with a percentage mortality of 93.3% after 96 h exposure. Mortality was observed after 48 h of exposure for 1.0 mg/mL of *C. citratus* extract (Table 2). There was however, a significant difference ($F = 5.519$, $df = 3.15$; $P < 0.05$) in adult mortality. The median lethal concentration (LC_{50}) of *C. citratus* extract against *S. oryzae* was 2.16 mg/mL (Figure 3) with a median lethal time (LT_{50}) of *S. oryzae* was 75.10 h (Figure 4).

Table 2

Mortality of rice weevil (*S. oryzae*) exposed to extract of lemon grass (*C. citratus*).

Treatments (mg/20 mL methanol)	Mortality (%)				
	24 h	48 h	72 h	96 h	Overall mean
1.0	0.000 ± 0.00	10.000 ± 0.577	23.300 ± 0.667	26.700 ± 0.333	15.0
1.5	6.700 ± 0.667	16.700 ± 0.333	40.000 ± 0.577	53.300 ± 0.333	29.2
2.0	13.300 ± 0.333	40.000 ± 1.528	66.700 ± 0.667	86.700 ± 0.882	51.7
2.5	10.000 ± 0.577	46.700 ± 0.667	73.300 ± 0.333	93.300 ± 0.333	55.8
Control	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.0

Values are means of triplicate observations ± SEM.

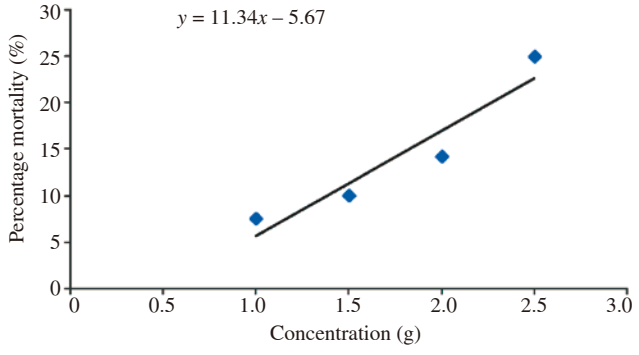

Figure 1. Percentage probit kill of rice weevil (*S. oryzae*) exposed to lemon grass (*C. citratus*) powder.
Regression equation inclusive $LC_{50} = 4.91$ g.

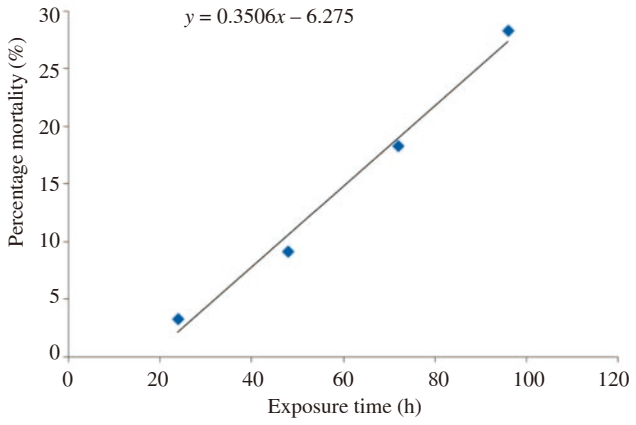

Figure 2. Time-mortality response of rice weevil (*S. oryzae*) exposed to lemon grass (*C. citratus*) powder.
Regression equation inclusive: $LT_{50} = 160.51$ h.

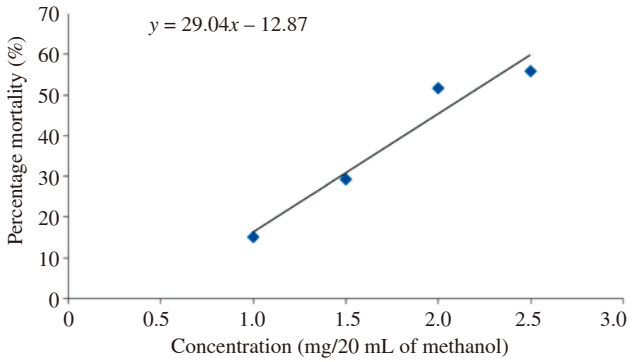

Figure 3. Percentage probit kill of rice weevil (*S. oryzae*) exposed to lemon grass (*C. citratus*) extract.
Regression equation inclusive LC_{50} = 2.16 mg/mL.

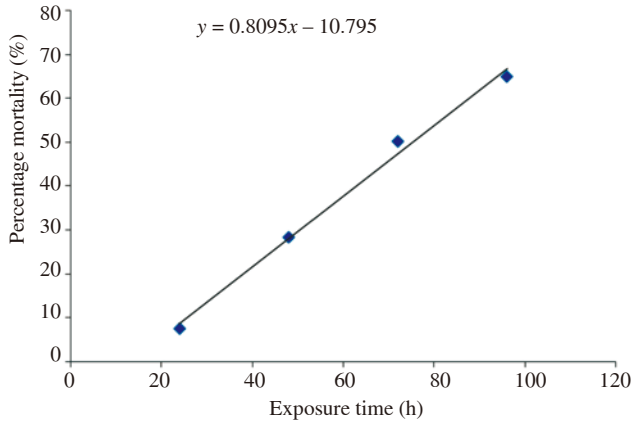

Figure 4. Time-mortality response of *S. oryzae* exposed to *C. citratus* extract.
Regression equation inclusive LT_{50} =75.10 h.

4. Discussion

In this study, the toxicity of *C. citratus* powder and methanol extract was evaluated under laboratory conditions against *S. oryzae* by ascertaining the LC_{50} for the powder, the LC_{50} for the methanol extract and the LT_{50} for both the powder and the extract. The results of the present study confirm the efficacy *C. citratus* powder and methanol extract on adult mortality of *S. oryzae* at different concentrations for powder, different concentrations for extract and at different time of exposure for powder and extract. These findings reaffirm the reported potential of plants and plant products in stored insect pest management[16].

This study also found a proportional increase in mortality *S. oryzae* and concentration of *C. citratus*. For example, results showed that as the concentration of the powder increased from 1.0 to 2.5 g/10 g of rice grains, there was corresponding increase in the mortality of the weevil. The result also indicated that as the extract concentrations increased, there was also significant increase ($P < 0.05$) in the percentage mortality of the weevil. The finding of the present study is in agreement with those of other workers who had previously reported that plant powders and extracts including those of lemon grass are toxic at different concentrations[17,18]. We found no mortality in the powder within the first 24 h of exposure with concentrations of 1.0, 1.5, and 2.0 g, which suggests that the efficacy of *C. citratus* lie at higher concentration. This possible explain the highest mortality observed in the powder was 43.3% after 96 h exposure in 2.5 g. The results of this study showed that *C. citratus* leaves powder and methanol extract caused adult mortality. Similar studies have observed that essential oils of *Pelargonium graveolens* and *C. citratus* resulted to adult mortality in *Sitophilus zeamais*[19,20]. Also, Dike and Mbah[21] had reported the efficacy of lemon grass extracts in the control of *Callosobruchus maculatus* on stored cowpea.

The mortality of *S. oryzae* was noticed after 48 h exposure in 1.0, 1.5, and 2.0 g with percentage mortality of only 3.3%, 3.3% and 10.0%. With the extract, there was no mortality after 24 h of exposure in 1.0 mg/mL treatment. However, as the exposure period increased, the mortality was found to increase at considerable levels as both with the powder and extract. These findings were in agreement with the work of Saljoqi *et al*.[22], who also observed that the percentage mortality of rice weevil, *S. oryzae* treated with *C. citratus* increased as the exposure periods increased. The highest percentage mortality with the lemon grass extract was 93.3% after 96 h of exposure. Asawalam and Igwe[11] also found that 35 days after treatment of methanol extract of *C. citratus* against *Trogoderma granarium*, the percentage mortality recorded was 27.51%. The difference with this present study might be attributed to difference in pest species, concentration of the treatment, methodology of research, commodity variations, laboratory and environmental differences.

This study is limited by not studying the mode of action of the plant products against *S. oryzae*. However, Oparaeke and Kuhiep[14], as well as Ofuya and Dawodu[23] attributed efficacy of *C. citratus* powder to respiratory impairment orchestra by blocking of spiracles. Further study would need to confirm and examine this. Secondly, the palatability and nutritional value of the rice treated with lemon grass the powder may have been affected. Additional studies are needed to examine the impact. Despite these limitations, this study adds strength to community based pest control seeking to employ lemon grass *C. citratus* powder and methanol extract against rice weevil *S. oryzae*. For example, this study identified the median lethal concentration (LC_{50}) for the powder and the extract and the median lethal time (LT_{50}) for both treatments. The results from this study showed that the median lethal concentration LC_{50} of the powder was 4.91 g, with a median lethal time LT_{50} of 160.51 h. The LC_{50} occurred at a high concentration and long hour of exposure indicating that the plant powder might not be very effective for the control of rice weevil. The lethal concentration (LC_{50}) of the extract was 2.16 g with a median lethal time (LT_{50}) of 75.10 h; this can be attributed to the fact that enough content of the volatile compounds has been inhaled.

The study found that both lemon grass powder and methanol

extract showed adult mortality against rice weevils and mortality of increases with concentration. Also, mortality was higher for methanolic extract compared to lemon grass powder. This implies that the potential of lemon grass powder could be improved by dissolving it in methanol. Given the ecological friendly and biodegradable nature of plant products as pesticides, the use of lemon grass should be well encouraged. Also, for enhanced efficacy, the use of lemon grass – methanol extract should be preferred.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The laboratory assistance received from Mr. Ogbonnaya Emmanuel and Mr. Obanemenya Emmanuel is highly appreciated.

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