



TOXICITY EFFECT OF CYMBOPOGON CITRATUS (LEMON GRASS) POWDER AND METHANOL EXTRACT AGAINST RUST-RED FLOUR BEETLE, TRIBOLIUM CASTANEUM (HERBST) (COLEOPTERA: TENEBRIONIDAE)

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Abstract:

Insect pests have mainly been controlled with synthetic insecticides in the last fifty years and the protection of stored grains from insect damage is currently dependent on synthetic pesticides. Botanicals are a promising source of pest control compounds. The plant kingdom can be a rich source of a variety of chemicals with the potential for development as successful pest control agents. There is, therefore, an urgent need to develop environment friendly alternatives with the potentials to replace the highly toxic chemicals. Hence in the present investigation the toxicity potential of lemon grass (*Cymbopogon citratus*) was evaluated against adult Rust-Red Flour Beetle, *Tribolium castaneum*. The Lemon grass (*C. citratus*) leaves were collected from Kodaikanal hill regions and were shade dried 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 1000 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 cow pea 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. The results indicated significant difference in mean percentage mortality after 24, 48, 72, and 96 h exposure with the powder compared with the control. Significantly higher to 100 % adult mortality was also observed in the extract after 24, 48, 72, and 96 h exposure compared with the control. The LC50 value of the extract was 0.75 mg/20 mL of methanol with an LT50 of 71.10 h. The methanol extract of *C. citratus* showed the highest mortality compared to the powder which was less toxic. The study showed that *C. citratus* products are promising insecticides and can be used effectively in the management of *Tribolium castaneum* in storage of grains and pluses.

Key Words: Toxicity Effect, *C. Citrates* & *Tribolium Castaneum*

1. Introduction:

One of the problems posed to small scale farmers throughout the world is that of insect pest damage to stored-grains and grain products which can be up to 20 - 30 % in the tropical regions (Nakatita, 1998). *Tribolium castaneum* (Coleoptera: Tenebrionidae) is a widespread and destructive secondary pest which attack stored products grains and grain products (Aitken, 1975; Weston and Rattlingourd, 2000). Not only is it capable of breeding throughout the year in warm areas, the insect can live for more than two years, during which the female can produce about 1000 eggs (Pugazhvendon *et al.*, 2009; Shukla *et al.*, 2010).

Stored products of agricultural and animal origin are attacked by more than 600 species of beetle pests, 70 species of moths, and about 355 species of mites causing quantitative and qualitative losses (Rajendran and Sriranjini, 2008) and insect contamination in food commodities is an important quality control problem of concern for food industries. 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 (Rajendran and Muralidharan, 2001 and Phillips and Throne, 2010).

According to the FAO estimate 10 to 25 % of the world's harvested food is destroyed annually by insects and rodent pests (Anonymous (1980). Insect pests cause damage to stored grains and processed products by reducing their dry weight and nutritional value Sinha and Watters, 1985). The confused flour beetle, *Tribolium confusum* and *T. castaneum* is one of the most serious pests of stored cereals and processed cereal products worldwide (Aitken, 1975; Champ and Dyte, 1976 and Hill, 1990).

Control of stored-product insect populations is primarily dependent upon continued applications of insecticides (White and Lees, 1995). Around the world, residual chemical insecticides and fumigation are currently the methods of choice for the control of insects of stored products (Wink, 1993). In spite of its efficacy, their repeated use for several decades has disrupted biological control system by natural enemies and led to outbreaks of insect pests. Their widespread use has led to some serious problems, including the development of insect strains resistant to insecticides (Tang and Eisenbrand, 1992) toxic residues on stored grains, undesirable effects on non target organisms and health hazards to grain handlers.

Increased public concern over the residual toxicity of insecticides applied to stored products, the occurrence of insecticide-resistant insect strains, and the precautions necessary to work with traditional chemical insecticides stress the usage of botanicals to control insects of stored product (Namba, 1993). These problems have highlighted the need for the development of new types of selective insect-control alternatives. Plants may provide potential alternative to currently used insect-control agents because they constitute a rich source of bioactive chemicals (White, 1995). Secondary compounds from plants including alkaloids, terpenoids, phenolics, and flavonoids can affect insects in several ways. They may disrupt major metabolic pathways and cause rapid death, act as attractants, deterrents, phago-stimulants antifeedants or modify oviposition. They may also slow down or accelerate development (Bell, 1986; Houghton, 1996 and Smet *et al.*, 1986).

The biological effects of some plant materials against stored product insects have been studied by different authors for several years (Elhag, 2000; Inyang and Emosairue, 2005; Oparaeke and Kuhiep, 2006 and Yankanchi and Gadache, 2010). 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 (Ojianwuna and Umoru, 2010 and Madanat *et al.*, 2016). Lemon grass, *C. citratus*, is a tropical plant and exists in diverse forms and cultivars (Pinto *et al.*, 2015). In India, it is commonly found in the southern and northern region, where it is used as spice, pot herbs and as local medicine (Geetha *et al.*, 2014; Naguib, 2002 and Reena Lawrence *et al.*, 2015). Lemon grass has been used in traditional Indian medicine for a long time to treat fever, rheumatism, headaches, cold and stomach pain. Lemongrass oil has been reported to have various activities like antimicrobial (Inonyea *et al.*, 2001 and Naik *et al.* 2010), anti inflammatory, astringent (Lertsatitthanakorn *et al.*, 2000), carminative (Carbajal *et al.*, 1989), insecticidal (Rabbani *et al.*, 2006), antioxidant and antifungal (Onawunmi, 1989). Lemon grass including its extracts has been reported to have also shown insecticidal potential, thus have been used in insecticidal sprays (Ojianwuna and Umoru, 2010 and Asawalam and Igwe, 2012).

Hence, this study seeks to evaluate the toxicity efficacy of lemon grass leaves powder and methanol extract against the red rust beetle *Tribolium castaneum* under laboratory conditions. Understanding the insecticidal potential would be of great economically viable option to local famers. Therefore, this study was attempted to evaluate the insecticidal activity of lemon grass plant extract.

2. Materials and Methods:

2.1 Collection and Rearing of Insects (*Tribolium Castaneum*):

Adults of *Tribolium castaneum* were obtained from infested grains in a local market in Thanjavur in Tamil Nadu. They were identified by the Department of Zoology, Ayya Nadar College, Sivakasi, Tamilnadu. The identified beetles were cultured on a diet of pea grains in rearing rubber containers with fine mesh gauze covering the opened end to prevent the contamination and escape of beetles. The red flour beetles were maintained in the dark cabinet of an incubator at 28 - 30° C and 60 -70 % relative humidity. The insects were reared in glass containers (0.5 L) containing sterilized grains at 12-13 % moisture content mixed with yeast (10:1, w/w). Insects used in the experiments were about 10 days old adults. The containers were left undisturbed for seven days to allow for further reproduction. Adult beetles were released for egg laying and after 5 days removed from the grains through sieving. The parent stocks were sieved out and the 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 %). The homogenous population was achieved after 28 - 35 days as given by Islam and Talukder (2005).



Figure 1: Showing the food grains infested with *Tribolium castaneum*

2.2 Preparation of Plant Powder:

The plant was collected from Kodaikanal hills, Tamil Nadu, India. The plant was identified as *C. citratus* by the Department of Botany, Ayya Nadar Arts College, Sivakasi, Tamil Nadu. It was used for the study. Fresh leaves from the plant were sundried and pulverized using an electric mixer or blender and sieved

through a 40 holes/mm² mesh screen to obtain uniform particle size powder. The resulting powders were placed in an air tight container to prevent active components from evaporating until when needed.



Figure 2: Lemongrass plant and Lemon grass powder

2.3 Preparation of Plant Extract:

The extracts of *C. citratus* were prepared at the Department of Biotechnology, Centre for Research and Development, PRIST University, Thanjavur, Tamil Nadu, India. Cold extraction method was employed using 90 % methanol as the solvent. 1000 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 cow pea 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 cow pea grains) were shaken thoroughly to ensure uniform distribution of the botanical powders. Ten unsexed adults *Tribolium castaneum* were then carefully introduced into the contents and exposed to treatments. Control containing untreated cow pea grains and ten unsexed adults *Tribolium castaneum* 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 LC₅₀ and LT₅₀. 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 beetle's mortality and delivered to Petri dishes containing Whatman No. 1 filter paper.

2.4.2 Adulticidal Effect of *C. Citratus* Extracts: The assay of *C. citratus* extracts against adult *Tribolium castaneum* was done according to Ojjanwuna and Umoru (2010) with some modifications. 5 mg, 10 mg, 15 mg, 20 mg, 25 mg and 30 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 0.25, 0.50, 0.75, 1.0, 1.25 and 1.50 mg/mL respectively. Twenty five unsexed adults *Tribolium castaneum* 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 LC₅₀. Adult mortality was also determined in percentage.

3. Results:

The percentage mortality of *Tribolium castaneum* exposed to powders of *C. citratus* for a period of 24, 48, 72, and 96 h are shown Table 1. The results showed that higher number of unsexed adult *Tribolium castaneum* mortality on cow pea grains treated with different concentrations of *C. citratus* powder when compared to that of 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 as the concentrations increased from 1.0–2.5 g in 24 – 96 h exposure. The data obtained (Table 1) also revealed that among the various concentrations of *C. citratus* powder the concentration of 2.5 g was most toxic causing 36.00 % mortality, followed by 2.0, 1.5, and 1.0 g with 28.0 %, 25.33 %, and 17.33 % mortality were recorded after 96 h exposure. Percentage probit kill of *Tribolium castaneum* exposed to lemon grass Powder and Methanol Extract (*C. citratus*) at 24 h is shown in fig.3.

The percentage mortality of *Tribolium castaneum* exposed to methanol 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 *Tribolium castaneum* was recorded with methanol extract of *C. citratus* as the concentration increased from 0.25 –1.5 mg/mL while no mortality was recorded in the control treatment. The data indicated that *C. citratus* extracted at a concentration of 1.5 mg/ mL was the most toxic with a percentage mortality of 100 % after shortest exposure of 24 h. Hundred percent mortality was observed after 48 h of exposure for 1.25 mg/mL of *C. citratus* extract and it is dissipated in Table 2. Time-mortality response of *Tribolium castaneum* exposed to lemon grass (*C.citratus*) powder at 1 g and methanol extract 0.25 mg concentration is shown in fig.4. There was however, a significant difference in adult mortality as the concentration increased from 0.25 to 1.5 mg/mL. The median lethal concentration (LC50) of *C. citratus* extract against *Tribolium castaneum* was 0.75 mg/ml of *C. citratus* extract. Even 0.75 mg/ml of methanol extract was able kill the adult beetles more than 50 % of the treated population in 72 h of exposure time.

Table 1: Percentage mortality of red rust beetle *Tribolium castaneum* exposed to powder of lemon grass (*C. citratus*)

Treatments (g)	Mortality (%)				
	24 h	48 h	72 h	96 h	Overall Mean
1.0	5.33 ± 2.31	9.33 ± 2.31	13.33 ± 2.31	17.33 ± 2.31	13.33
1.5	13.33 ± 2.31	18.67 ± 2.31	22.67 ± 2.31	25.33 ± 2.31	20.00
2.0	16.00 ± 4.00	21.33 ± 2.31	22.67 ± 2.31	28.00 ± 4.00	22.00
2.5	25.33±2.31	29.33 ± 2.31	33.33 ± 2.31	36.00 ± 4.00	31.00
Control	2.67 ± 2.31	2.67 ± 2.31	4.00 ± 0.00	5.33 ± 2.31	3.67

Values are means of triplicate observations ± SEM.

Table 2: Percentage mortality of red rust beetle *Tribolium castaneum* exposed to extract of lemon grass (*C. citratus*)

Treatments (mg/ 20 ml of methanol)	Mortality (%)				
	24 h	48 h	72 h	96 h	Overall Mean
0.25	14.67 ±2.31	21.33 ± 2.31	26.67 ± 2.31	33.33 ±2.31	24.00
0.50	22.67 ±2.31	32.00 ± 4.00	41.33 ± 2.31	45.33 ±2.31	35.33
0.75	36.00 ± 4.00	49.33 ±2.31	54.67 ±2.31	61.33 ±2.31	50.33
1.00	53.33 ± 2.31	69.33 ±2.31	77.33 ± 2.31	90.67 ±2.31	72.67
1.25	93.33 ± 2.31	100 ±0.00	100 ±0.00	100 ±0.00	98.33
1.50	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	100.00
Control	1.33 ± 2.31	1.33 ± 2.31	2.67 ±2.31	2.67 ±2.31	2.00

Values are means of triplicate observations ± SEM

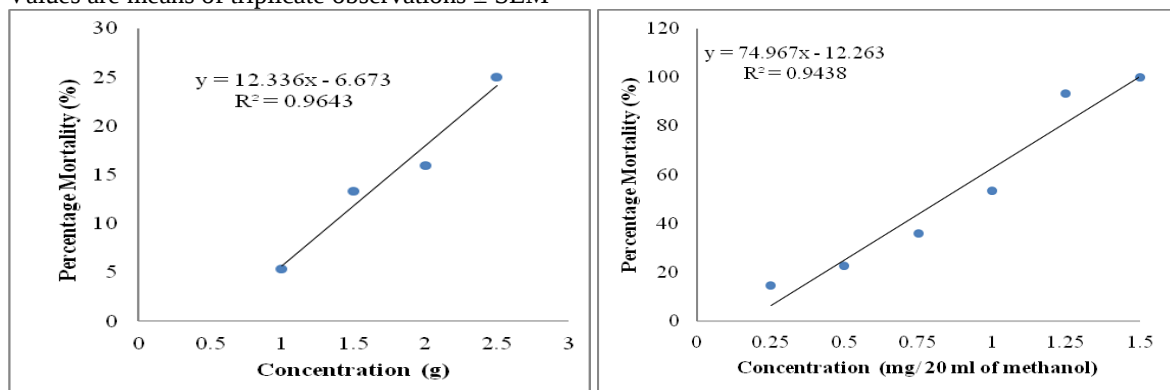


Figure 3: Percentage probit kill of *Tribolium castaneum* exposed to lemon grass Powder and Methanol Extract (*C. citratus*) at 24 h

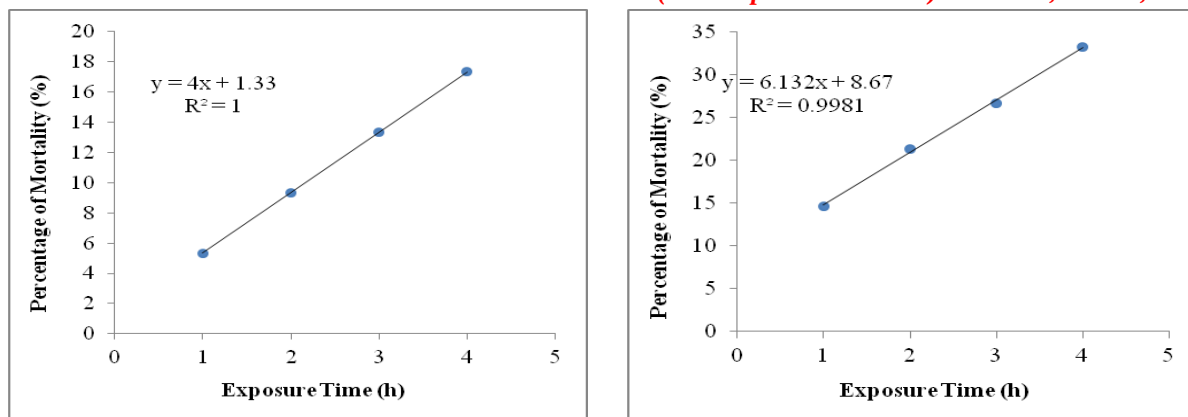


Figure 4: Time-mortality response of *Tribolium castaneum* exposed to lemon grass (*C.citratus*) powder at 1 g and methanol extract 0.25 mg concentration

4. Discussion:

Fang *et al.* (2002) indicated that *Tribolium* species are classified among the least susceptible insect pests of stored-products and often more difficult to kill than other stored-product beetles, though the order of toxicity often varies depending on the particular insecticide. The insecticidal activity of monoterpenes, the major component of essential oils, has been reported against insects of stored-products (García *et al.*, 2005 and Habig *et al.*, 1974).

In this study, the toxicity of *C. citratus* powder and methanol extract was evaluated under laboratory conditions against *Tribolium castaneum* by ascertaining the LC50 for the powder and for the methanol extract. The results of the present study confirms efficacy *C. citratus* powder and methanol extract on the adult mortality of *Tribolium castaneum* 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 (Rees, 2004).

In the present study it was found that methanol extract of very lower concentration was able to give more 50 % mortality of the adult beetle. This study also found a proportional increase in mortality of *Tribolium castaneum* 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 cow peas, there was corresponding increase in the mortality of the beetle. The result also indicated that as the extract concentrations increased, there was also significant increase in the percentage mortality of the beetle. The findings 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 (Naguib, 2002 and Selim, 2011). It was found that there was lower percentage of mortality of the beetles in the powder within the first 24 h of exposure with concentrations of 1.0 g, whereas in concentrations of 1.5, 2.0 and 2.5 g the mortality rate was in the increasing order, which suggests that the efficacy of *C. citratus* lie at higher concentration.

This possible explain the highest mortality observed in the powder was 36.0 % 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, but there was higher percentage of mortality were noticed in methanol extract of *C. citratus*. Similar studies have observed that essential oils of *Pelargonium graveolens* and *C. citratus* resulted to adult mortality in *Sitophilus zeamais* (Devaraj and Srilatha 1993 and Kabera *et al.*, 2011). Also, Dike and Mbah (1992) had reported the efficacy of lemon grass extracts in the control of *Callosobruchus maculatus* on stored cowpea.

The mortality of *Tribolium castaneum* was noticed after 48 h exposure in 1.0 g concentration of powder, with percentage mortality of only 9.33 %, whereas it was 18.37 %, 21.33 % and 29.33 % for 1.5 g, 2.0 g and 2.5 g respectively. When exposed with the methanol extract, there was lowest mortality after 24 h of exposure in 0.25 mg/mL and 100 % mortality in 1.5 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 Ali and Mohammed (2013) who also observed that the percentage mortality of red beetle *Tribolium confusum* increased as the exposure periods increased when treated with some selected plant extracts.

In the present investigation the highest percentage mortality with the lemon grass methanolic extract was 100 % after 96 h of exposure at 1.5 mg/ml concentration. Asawalam and Igwe (2012) 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.

Similar findings are attributed in the studies on toxicity of some crude plant extracts against *T. confusum* investigated by several authors could depend on several factors among which are the chemical

composition of the crude plant extract and insect susceptibility. Also Khoshsould and Khayamy (2008) refers to the insecticidal effects of ethanolic extract from *Verbascum cheiranthifolium* Boiss against two insect pest species of stored-products and indicated that the mortality of the exposed insects increased with the increase of the exposure interval and dose rate. It was clear from their results that the botanical extract on insects was effective. Another study made by Iram *et al.* (2013) indicated that the ethanol extract was found to be remarkably more potent than the powder form of same plant. Thus our present findings corroborated with the above investigators.

The palatability and nutritional value of the cow pea treated with the lemon grass 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 of red beetle *Tribolium castaneum*. For example, this study identified the median lethal concentration (LC50) for the extract. The LC50 occurred at a high concentration and long hour of exposure indicating that the plant powder might not be very effective for the control of *Tribolium castaneum*. The lethal concentration (LC50) of the extract was 0.75 mg with a median lethal time (LT50) of 71.2 h which could be attributed to the fact that enough content of the volatile compounds and other secondary metabolites are present in the plant sample. Further study has to be made to confirm what would be the possible compounds responsible for the toxicity activity against *Tribolium castaneum*.

The study found that both lemon grass powder and methanol extract showed adult mortality against *Tribolium castaneum* and mortality 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. The ecological friendly and biodegradable nature of plant products as pesticides, the use of lemon grass could be well encouraged. Also, for enhanced efficacy, the use of lemon grass – methanol extract should be preferred.

5. Conclusion:

The findings of the present investigations indicate that the selected plant might be useful as store insect control agents for commercial use. Both the plant powder and methanol extract of the tested plant extracts were effective to some degree in reducing the number of *T. castaneum*. The results of the present study indicated that by considering the mean mortality as a main index and concentration, the *methanol extract* proved to be the most effective material against the flour beetle *T. castaneum*. A study has to be taken in detail to improve the effectiveness of botanical derivatives as insecticides that might benefit the agricultural sectors of developing countries, as these substances are not only of low cost, but also have less environmental impact in terms of insecticidal hazard.

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