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The Fumigant Toxicity of *Syzygium aromaticum* and *Cymbopogon citratus* Oils on Selected Life Stages of *Tribolium castaneum* (Coleoptera: Tenebrionidae)

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Abstract

In efforts to compliment research on biopesticides, attempts were made to determine the fumigant toxicity of essential oils from *Syzygium aromaticum* and *Cymbopogon citratus* against *Tribolium castaneum* larvae and adults under laboratory conditions. Four dosages (0, 50, 100 and 150µL/L) of each oil were applied in four replications. One-hundred larvae and unsexed adult populations of *T castaneum* were thus subjected to the oils in fumigant bioassays. α and β amylases activities in the fumigated life stages of *T. castaneum* were accessed. Toxicity data was subjected to One-way Analysis of Variance. Also, the relationship between the dosages of the *S. aromaticum* and *C. citratus* oils and the mortality rate among *T. castaneum* was investigated using regression analysis. Mortality rate was directly proportional to the dosages of both oils. The inhibitory potential of the oils, however, was only observed at the highest dose (150µl/L) for the adult stage, where low amylase activity was recorded. On the whole, amylase activity was higher in the insect stages fumigated with *S. aromaticum* oil. Similarly, significant positive linear correlation exists only between the mortality rate and the dosages of *S. aromaticum* oil ($r= 0.994$, $N = 4$, $P = 0.006$). The results gave cues on the action of the essential oils via some stress-related physiological activities in the insect stages that culminated in the killing of the insects.

Keywords: Fumigant toxicity, *Tribolium castaneum*, amylase activity, *Syzygium aromaticum*, *Cymbopogon citratus*, biopesticide.

1. Introduction

In the tropics, stored grains and grain products are significantly affected by pest insects (Jeyansakar *et al.*, 2016). An example of such pests is the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), a polyphagous beetle commonly found in flour, flour products, cereal grains, grain legumes, and even spices. All forms of the insect life cycle can be found in infested stored commodities causing qualitative and quantitative food loss. In developing countries including Nigeria, post-harvest losses due to this insect-pest have been reported to be around 40 % (Pimentel, 1991).

In efforts to abate this occurrence, synthetic insecticides have been extensively and frequently used to control the red flour beetle. However, due to the growing concerns over the associated environmental and consumers' health, the effects on non-target organisms, insecticide resistance, and the high cost of application (Titli *et al.*, 2008; Ajayi *et al.*, 2014), alternative control measures are globally in focus nowadays (Arbes *et al.*, 2003). As such, there is a need to investigate substitutes that will achieve the desired level of efficacy, and more particularly, ones that are benign to the environment.

Already, the approaches of Integrated Pest Management give credence to this idea by adopting control tactics such as organic production to manage pests safely and effectively (Pedigo and Rice, 2009; Beatrice *et al.*, 2016). Similarly, the development of new pesticides from plants has been successfully leveraged on to reduce the pests' populations below the economic injury level (Adedire and Ajayi, 2003; Steven *et al*., 2017). Botanicals have been reported to contain defense compounds such as terpenes, alkaloids and enzyme inhibitors (Tinkeu *et al*., 2004; Schafer and Wink, 2009). These aproteic compounds exert effect on insect gut digestive enzymes, such as amylases (Octavio *et al*., 2002; Mazid *et al*., 2011).

The presence of an inherent rich source of bioactive chemicals make botanicals a promising source of potential alternatives to use as insect-control agents (Isman, 2008; Ballhorn *et al*., 2009; Mazid *et al*., 2011). Specifically, the essential oils of several spices such as the clove oil obtained from *Syzygium aromaticum* ((L.) Merril & Perry) have been found to possess fumigant toxicity to four major stored product pests, including *T. castaneum* (Shaaya *et al*., 1991; Merr and Perry, 2011). Souza *et al.* (2016) evaluated the toxicity of *Citrus aurantium* and *Cymbopogon citratus* on the grain weevil, and found out that the oils presented fumigating properties to be used in

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the management of the pests. Bachrouch *et al*. (2010) reported the potentiality of the control of one-seven-day-old adult *T. castaneum* by plant essential oils. In addition, these essential oils are extracted from botanicals that contain enzyme inhibitors and defending digestive enzymes as a natural defense response against pest attacks (Gerhenzon *et al*., 1991; Grayson, 1998). Tatun *et al*. (2014) reported that plant extracts exhibited inhibitory effects on the activity of amylase enzyme in *T. castaneum*. This characteristic qualifies botanicals as insecticides, as they are able to form complexes with insect digestive enzymes leading to poor nutrient utilization, growth retardation, and ultimately death (Fraga, 1988; Sami, 2014).

The current study investigates the acute fumigant toxicity of essential oils of Clove bud (*S. aromaticum*) and Lemon grass (*C. citratus* ((DC.) Staff) against adult and 4th instar larva of *T. castaneum* (Red flour beetle). In addition, the effect of these botanicals on α-amylase and β-amylase activities in these life stages was also explored.

2. Materials and Methods

2.1. Biological Materials

Adults of the American strain of *T. castaneum* used to start the experiments of this study were obtained from laboratory cultures of over one-hundred generations at the Department of Biology, of the Federal University of Technology in Akure Nigeria. The culture was raised on disinfested wheat flour at 30^{o}C ±2^{o}C and 75±5 % relative humidity. The White wheat flour was obtained from a grocery store in Akure metropolis, and was disinfested in Haier Thermocool freezer at 0^{o}F (-18^{o}C) for seventy-two hours. Newly emerged adults and fourth instar larva obtained from the insect culture were used for the bioassays. Oils of *S. aromaticum* clove bud (PCode 100198414), and lemon grass, *C. citratus* (PCode 101507375) were purchased from Sigma Aldrich Co. 3050 Spruce street St Louis, MO 63103 USA. The studies were conducted at the above-mentioned conditions in the laboratory at the Departments of Biology, and Biochemistry of the Federal University of Technology in Akure Nigeria.

2.2. Fumigation Bioassay

The fumigant toxicity bioassay was conducted on *T. castaneum* without flour to simulate surface treatment. This was done according to the methods of Negahban *et al*. (2007) with slight modifications. Air-tight plastic containers, of a one-litre size served as the fumigation chamber. One hundred (fifty pairs) adult insects were placed in each container. Whatman No. 1 (5mm Ɵ) filter paper was glued to the underside of the lid of the containers using a glue gun (AdTech HiTemp Project Pro; Made in Taiwan). Oils at 0, 50, 100 and 150µL/L were applied to the filter paper. The lid with the treatments was used to cover the insects immediately. The seam of each fumigation chamber was taped with Para film wrap to make it gas-proof. Four replicates were set for each dose of each treatment. The same procedure was done for the fourth instar larva. The control was similarly set-up without the application of essential oil. Mortality was observed five days post-treatment.

2.3. Preparation of Samples for Enzymatic Assay

The treated adults and larva were ground separately in a crucible mortar. A 0.5g of each ground insect of the two life stages was weighed into centrifuge tubes, and 500µL of 20mM sodium acetate buffer, pH 4.9 was added. The solution was homogenized at 10,000 rpm for twenty minutes at 4^{o}C. The supernatant was pipetted into Eppendorf tubes and stored in deep freezer at -20^{0}C until used.

2.4. Enzyme Assay

An aliquot of 0.5mL of the insect homogenates was pipetted into test tubes with a blank of 0.5mL reagent grade water in two replicates. The homogenates were incubated at 25^{o}C for three- four minutes to achieve temperature equilibration. Each tube received a 0.5mL starch solution at a fifteen-second interval. The mixture was then incubated at 25^{o}C for three minutes. At a fifteen second-interval, 1mL Dinitrosalicylic Acid (DNSA) color reagent was added to each of the test tubes, and the tubes were incubated in boiling water in a water bath for five minutes. After incubation, the tubes were allowed to cool at room temperature. 10 mL of reagent grade water was added to each tube and agitated. The content of each tube was poured into cuvette and inserted into a spectrophotometer. Absorbance was read at 540_{wv} (A_{540}). Enzyme activity was calculated as follows;

$$\text{Enzyme Activity} = \frac{A}{S \times T \times V}$$

Where; A= Absorbance of enzyme solution, S = Slope of standard graph, T = Time of incubation, V = volume of enzyme used.

2.5. Data Analysis

To determine acute toxicity, data obtained on the larvae and adults were converted to percentages and Arcsine transformed. The transformed data were subjected to one-way Analysis of Variance (ANOVA). Where significant difference exists, New Duncan's Multiple Range Test (NDMRT) was used to separate the means. The data obtained from biochemical analysis was presented as a mean of two replicates. Regression analysis was used to investigate the relationship between the dosages of the oils and the mortality rate among the *T. castaneum*. All analyses were conducted using the Microsoft excel (2016) and Statistical Package for Social Sciences (SPSS) version 21.

3. Results

3.1. Fumigant Toxicity of S. aromaticum and C. citratus on Larva and Adult T. castaneum

Mortality of larva was dose-dependent (Figure 1). There was no death of larvae in control and fumigation done with 50µL/L of the *S. aromaticum* oil. *C. citratus* was more toxic to larvae than *S. aromaticum*. The highest dose of *S. aromaticum* and *C. citratus* oils (150µL/L) elicited the greatest mortality, 30 % and 41 % of the larvae respectively. (Figure 1). Lower mortality of the beetles was recorded at 100µL/L for *S. aromaticum* (27.5 %) and *C. citratus* (28.5 %) (Figure 2).

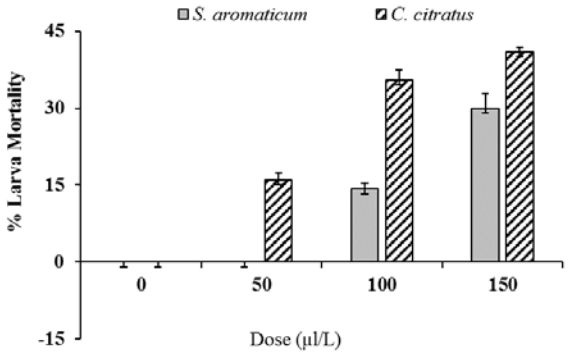

Figure 1 . Percentage mortality of *T. castaneum* larvae fumigated with essential oils of *Syzigium aromaticum*, and *Cymbopogon citratus* (n = 4)

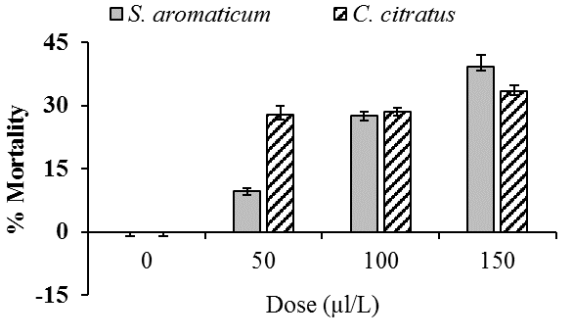

Figure 2. Percentage mortality of adult *T. castaneum* fumigated with essential oils of *Syzigium aromaticum*, and *Cymbopogon citratus* (n = 4)

3.2. Activities of α and β amylase in T. castaneum Larvae and Adult Fumigated with Essential Oils of S. aromaticum and C. citratus

The enzyme activities increased with the increasing of the oil dosage (Figures 3). However, the highest α amylase (50 mol/min/mL) and β amylase (13.64mol/min/mL) activity in the *C. citratus*-fumigated adults, was recorded in the insects fumigated with 100 µL/L (Figure 3 A, B). The α and β - amylase activities were higher in the insect stages fumigated with *S. aromaticum* oils except for β - amylase in larvae at 150 µl/L (Figure 3 C, D).

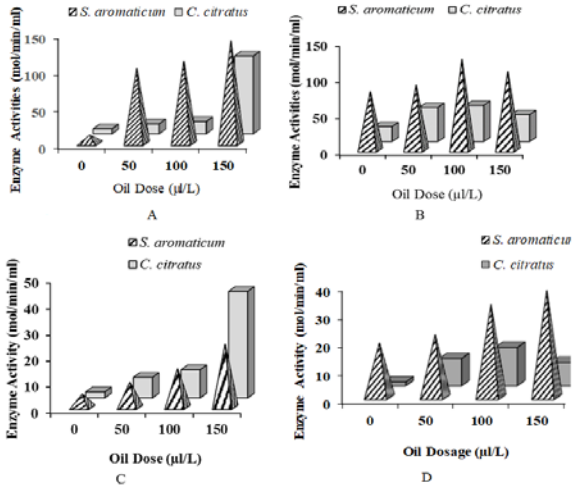

Figure 3. α-amylase ([A] larvae and [B] adult) and β-amylase ([C] larvae and [D] adult) activities in *T. castaneum* fumigated with essential oils of *Syzygium aromaticum*, and *Cymbopogon citratus.*

3.3. Relationship between Dosages of S. aromaticum and C. citratus Oils and Mortality Rate in T. castaneum Larvae and Adults

The relationship between the dosages of the *S. aromaticum* and *C. citratus* oil and the mortality rate among the *T. castaneum* larvae and adults is presented in Table 1. Regressing mortality rate in the flour beetle larvae against increasing concentration levels of the *S. aromaticum* oil (r = 0.941, N = 4, *P* = 0.059) and *C. citratus* oil (r = 0.980, N = 4) showed a positive correlation, the correlation was found to be significant (*P* = 0.020) for larvae treated with *C. citratus* oil. Similarly, in the adults, there was a significant positive linear correlation between the mortality rate among the insects and the dosages of *S. aromaticum* oil (r = 0.994, N = 4, *P* = 0.006) and *C. citratus* oil (r = 0.861, N = 4). The correlations were, however, found to be insignificant (*P* = 0.139) for the insect adults treated with *C. citratus* oil.

Table 1. Relationship between dosages of the *S. aromaticum and C. citratus* oils and the mortality rate of the *T. castaneum* larvae and adults.

Essential oil	Correlation coefficient (r)	Regression equation
S. aromaticum (larvae)	0.941	Y = -4.565 + 0.209X
C. citratus (larvae)	0.980	Y = 1.750 + 0.285X
S. aromaticum (adult)	0.994	Y = -1.200 + 0.271X
C. citratus (adult)	0.861	Y = 7.250 + 0.203X

4. Discussion

All doses of the essential oils assayed elicited some level of toxicity to *T. castaneum* larvae and adults more than in the controls. This confirms the presence of bioactive compounds in the oils of these plants (El hag *et al*., 1999; Samarasekera *et al*., 2006; Chaieb *et* al., 2007). The observed low level of toxicity to the life stages of the beetles does not undermine the efficacy of the oils, rather, it suggests that the essential oils may not be an effective fumigant against *T. castaneum* at low doses. Therefore, it may be necessary to increase the dosage to achieve a stronger degree of toxicity. This finding is in agreement with earlier findings on the effects of *C. citratus* oil on several insect pests. The essential oils of *C. citratus* and *S. aromaticum* had been reported toxic to *T. castaneum* in a dose-dependent manner (Verbel *et al*., 2010; Rafeeq *et al*., 2016).

The oil of *C. citratus* was more toxic to *T. castaneum* than that of *S. aromaticum.* The monoterpene hydrocarbons which are characterized by geranial, neral, and myrecene and accounts for about 95 % of the total oil in *C. citratus* have been demonstrated to have high activity against insect pests (Lee *et al*., 2008; Josphat *et al*., 2011). In addition, Mondal and Khalequazzama (2006) investigated contact and fumigant activity of three essential oils, namely *Elettaria cardamomum, Ctenium aromaticum* and *S. aromaticum* against *T. castaneum* larvae and adults and reported that *S. aromaticum* was the least effective as a fumigant.

Starch is a major component of food grains among cellulose and xylose. A number of insects rely on cellulases and amylases for utilizing alpha and beta

polymer of glucose, as a source of carbohydrates. Owing to its exclusive dependency on starch, *T. castaneum* produces a raft of starch-hydrolyzing enzymes such as α and β amylase to help hydrolyze carbohydrates (Chen *et al*., 1992; Sami, 2014). Because the population of *T. castaneum* used in this study were raised on wheat flour, another source of starch, before being fumigated, it is probable that active carbohydrate metabolism had already begun in the insect before being exposed, hence the probable rational for the observed low mortality of insects in this study. The insects' restlessness and erratic movement during fumigation indicate that the oils induced stress in the insects. Increased enzymatic activities in this insects are a physiological response to adapt with abnormal condition.

Upon exposure to toxic substances, changes in enzymatic reactions have been reported to be generally developmental and stage-specific in a number of insects including *Tribolium spp* (Mediola-Olaya *et al*., 2000; Bandani and Balvasi, 2006). In these species, a higher enzyme activity is an occurrence commonly recorded in the adults more than in the larvae. Accordingly, the findings in this research, which reveal higher levels of enzyme activity in the adult *T. castanuem* more than in the larvae, appear to be well correlated with the reported findings.

The reduction in α- amylase activity of the adult beetles at the highest dosage of the oils may be precipitated by the defense compounds including inhibitors of digestive enzymes which act on insect enzymes (Franco *et al*., 2002; Jing *et al*., 2005). It is probable that these defense compounds are in proportions high enough to exert effect compared to other levels of dosages administered to *T. castaneum*. More so, since the 150 µL/L oil exhibited a higher proportion of toxicity to the insects relative to the other doses tested, it is most likely that this dose contains high enough amounts of inhibitory compounds that propagated the decline recorded in amylase activity. Moreover, the cytotoxic effects of the essential oils on the epithelial cells of the midgut of *T. castaneum* have been implicated in the reduced α- amylase activity (Tatun *et al*., 2014). While no cytological study was done on *S. aromaticum* and *C. citratus* oils, it could, however, be a probable cause in the decline in amylase activity recorded.

This study shows that the *T. castaneum* larvae at the varied level of doses used had higher enzyme activity than the adults. The chitinous exoskeleton found in the adults compared to the larvae reflects a probable scientific rational for the variation in the mortality rates as this feature may account for the reduction in the quantity of the oil that eventually makes it into the insect body.

5. Conclusion

While validating the potentiality of essential oils in the control of storage pests, it is important to investigate their variety and understand how they exert their effects. The results show that the oil of *C. citratus* was more toxic to *T. castaneum* than to the *S. aromaticum* oil. Generally, insect life stages fumigated with *S. aromaticum* had higher enzyme activity compared to those exposed to *C. citratus*. However, given the generally observed low level of toxicity, there is a need for further research on the essential oils and enzyme activity. The enzyme activity in particular, elicited by exposure to plant oils, needs to be further characterized by gene sequencing, if the industrial application of bio-pesticides is to be fully explored. This sentiment is expressed because, at the corresponding highest toxicity dose, both oils led to a decline in the amylase activity.

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References

Adedire CO and Ajayi, OE. 2003. Potential of sandbox, *Hura crepitans* L. seed oil for protection of cowpea seeds from *Callosobruchus maculatus* F. (Coleoptera: Bruchidae) infestation. *J Plant Dis Protect*, **110(6):**602-610.

Ajayi OE, Appel AG and Fadamiro HY. 2014. Fumigation toxicity of essential oil monoterpenes to *Callosobruchus maculatus* (Coleoptera: Chrysomelidae: Bruchinae). *J Insect Sci*, **2014**: Article ID 917212, 7 pages.

Arbes Jr SJ, Sever M, Archer J, Long EH, Gore JC, Schal C, Walter M, Neubler B, Vaughn B, Mitchell H, Liu E, Collette N, Adler P, Sandel M and Zeldin DC. 2003. Abatement of cockroach allergens (Bla g 1) in low income, urban housing: month 12 continuation results. *J Allergy Clin Immunol* **112**:339-345.

Arbes Jr SJ, Sever M, Mehta J, Gore JC, Schal C, Vaughn B, Mitchell H and Zeldin DC. 2004. Abatement of cockroach allergens (Bla g 1 and Bla g 2) in low income, urban housing: month 12 continuation results. *J Allergy Clin Immunol*, **113**:109-114.

Bachrouch O, Jemâa JM, Talou T, Marzouk B, Abderraba M. 2010. Fumigant toxicity of *Pistacia lentiscus* essential oil against *Tribolium castaneum* and *Lasioderma serricorne*. *Bull Insecto.*, **63(1):**129-135.

Ballhorn DJ, Kautz S, Heil M and Hegeman AD. 2009. Cyanogenesis of wild lima bean (Phaseolus lunatus L.) is an efficient direct defence in nature. *Plant Signal Behav*, **4(8)**:735-745.

Bandani, AR and Balvasi A. 2006. Comparison of α-amylase activity in the larval stages of flour beetles, *Tribolium confusum* (Coleoptera: Tenebrionidae). *Commun Agri Appl Biol Sci*., **71**:537-541.

Beatrice N, Dingha JO, Appel AG and Jackai LEN. 2016. Integrated pest management of the German cockroach (Blattodae) in manufactured homes in rural North Carolina. *Fla Entomol*, **99 (4):**587-592.

Castle S, Palumbo J, Merten J, Cowden C and Prabhaker N. 2017. Effects of foliar and systemic insecticides on whitefly transmission and incidence of Cucurbit yellow stunting disorder virus. *Pest Manag Sci*, **73(7):**1462-1472.

Chaieb K, Hajlaoui H, Zmantar T, Kahla-Nakbi AB, Rouabhia M, Mahdouani K and Bakhrouf A. 2007. The chemical composition and biological activity of clove essential oil, *Eugenia caryophyllata* (*Syzigium aromaticum* L. Myrtaceae): A Short Review. *Phytother Res*, **21**:501–506.

Chen MS, Feng G, Zen KC, Richardson M, Valdes-Rodriguez S, Reeck GR and Kramer KJ. 1992. Alpha amylase from three species of stored grain Coleopteran and their inhibition by wheat and corn proteinaceous inhibitor. *Insectbiochem Mol Bio*, **22**:261-268.

El Hag EA, El Nadi AH, Zaiton AA. 1999. Toxic and growth retarding effects of three plant extracts on Culex pipiens larvae (Diptera: Culicidae). *Phytother Res*, **13**:388–392.

Fraga BM. 1988. Natural sesquiterpenoids. *Nat Prod Rep*, **5**:497-521.

Franco OL, Rigden DJ, Melo FR and Grossi-de-Sa MF. 2002. Plant α-amylase inhibitors and their interaction with insect α-amylase Structure, function and potential for crop protection. *Eur J Biochem*, **269**:397-412.

Gershenzon J and Croteau R. 1991. Terpenoids. *In*: Rosenthal GA, Berenbaum MR (Eds.), **Herbivores their Interaction with Secondary Plant Metabolites, Vol I: The Chemical Participants**, 2nd Ed., Academic press, San Diego, pp.165-219.

Graham Jp, Corella BV, Avitia DR and Gurian P. 2005. The in-home environment and household health: a cross-sectional study of informal urban settlements in northern Mexico. *Int J Environ Res Public Health*, **2**:394-402.

Grayson DH. 1998. Monoterpenoids. *Nat Prod Rep*, **5**:497-521.

Isman MB. 2008. Perspective botanical insecticides: for richer, for poorer. *Pest Manag Sci*, **64**:8-11.

Jeyasankar A, Chennaiyan V and Chinnamani T. 2016. Evaluation of five essential plant oils as a source of repellent and larvicidal activities against larvae of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *J Entomol***, 13**:98-103.

Jing L, Fang Y, Ying X, Wenxing H, Meng X, Syed M, Syed N and Fang C. 2005. Toxic impact of ingested Jatropherol-1 on selected enzymatic activities and the ultrastructure of midgut cells in silkworm *Bombyx mori* L. *J Appl Entomol*, **129**:98-104.

Josphat CM, Isabel NW, Jesca LN and Anderson MK. 2011. Chemical composition of *Cymbopogon citratus* essential oil and its effect on mycotoxigenic *Aspergillus species*. *Afr J Food Sci*, **5(3) :**138-142.

Lawless J. 1995. **The Illustrated Encyclopedia of Essential Oils: The Complete Guide to the Use of Oils in Aromatherapy and Herbalism.** Element Books Ltd, Shaftesbury, UK, pp 256.

Lee Y, Kim J, Shin S, Lee S and Park I. 2008. Antifungal activity of Myrtaceae essential oils and their components against three phytopathogenic fungi. *Flavour Fragr J*, **23(1):**23-28.

Mazid M, Khan TA and Mohammad F. 2011. Role of secondary metabolites in defense mechanisms of plants. *BLM* , **3(2):**232-249.

Mendiola-Olaya E, Valencia-Jimenez A, Valdes-Rodriguez S, Delano-Frier J and Blanco-Labra A. 2000. Digestive amylase from the larger grain borer, *Prostephanus truncatus* Horn. *Comp Biochem Physiol*,l **126**:425–433.

Merr L and Perry LM. 2011. *Syzygium aromaticum* (L.). Germplasm Resources Information Network (GRIN), ITIS report, Beltsville, Maryland.

Mondal M and Khalequazzama M. 2006. Toxicity of essential oils against red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *J Bio-sci*, **14**:43-48.

Negahban, M., S. Moharramipour and F. Sefidkon, 2007. Fumigant toxicity of essential oil from *Artemisia sieberi*Besser against three stored-product insects. *J Stored Prod Res*, **43**:123-128.

Nujira Tatun N, Vajarasathira B, Tungjitwitayakul J and Sakurai S. 2014. Inhibitory effects of plant extracts on growth, development and α-amylase activity in the red flour beetle Tribolium castaneum (Coleoptera: Tenebrionidae). *Eur J Entomol*, **111(2):**181–188.

Octavio LF, Daniel JR, Francislete RM and Maria FG. 2002. Plant alpha-amylase inhibitors and their interaction with insect alpha amylases structure, function and potential for crop protection. *Eur J Biochem*, **269**:397-412.

Pedigo LP and Rice ME. 2009. **Entomology and Pest Management**, 6th Ed. Prentice Hall, Upper Saddle River, New Jersey.

Pimentel D. 1991. World resources and food losses to pests. *In*: Gorham JR. (Ed), **Ecology and Management of Food Industry Pests.** (FDA Technical Bulletin 4), ISBN 0935584455, p 5-11.

Rafeeq KUMA, Umadevi D, Sajitha N and Gokuldas M. 2016. Repellency of essential oil extracted from *Cymbopogon citratus* against the nuisance household pests, *Luprops tristis* FAB. (Coeleoptera: Tenebrionidae). *Int J Recent Sci Res*, **7(4):**10309-10313.

Samarasekera R, Kalhari KS and Weerasinghe IS. 2006. Insecticidal activity of essential oils of *Ceylon cinnamomum* and *Cymbopogon* species against *Musca domestica*. *J. Essent. Oil Res*, **18(3):**352-354.

Sami AJ. 2014. *Azadirachta indica* derived compounds as inhibitors of digestive alpha-amylase in insect pests: Potential bio-pesticides in insect pest management. *Eur J Exp Biol*, **4(1):** 259-264.

Schafer H and Wink M. 2009. Medicinally important secondary metabolites in recombinant microorganisms or plants: progress in alkaloid biosynthesis. *Biotechnol J*, **4(12):**1684-1703.

Sever M, Arbes Jr SJ, Gore JC, Santangelo RG, Vaughn B, Mitchel H, Schal C and Zeldin DC. 2007. Cockroach allergen reduction by extermination alone in low-income, urban homes: a randomized control. *J Allergy Clin Immunol*, **120**:894-855.

Shaaya, E, Ravid U, Paster N, Juven B, Zisman U and Pissarev V. 1991. Fumigant toxicity of essential oils against four major stored-product insects. *J Chem Ecol*, **17(3):**499-504.

Souza VN, Oliveira CF, Matos CH and Florentino DK, 2016. Fumigation toxicity of essential oils against *Rhyzopertha dominica* (F.) in stored maize. *Rev Caatinga*, **29(2):**435–440.

Tatun, N, Vajarasathira B, Tungjitwitayakul J and Sakurai S. 2014. Inhibitory effects of plant extracts on growth, development and α-amylase activity in the red flour beetle *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Eur J Entomol*, **111 (2):** 181-188.

Tinkeu, L. Goudoum SN, Ngassoum A, Mapongmetsem MB, Kounninki PM and Hance T. 2004. Persistence of the insecticidal activity of five essential oils on the maize weevil *Sitophilus zeamais* (Motsch.) (coleopteran: Curculionidae). *Commun Agric Appl Biol Scien*, **69**:145-147.

Titli M, Josipovi-Jeli Z and Punda A. 2008. Headache caused by pesticides- a review of the literature. *Acta Med Croatica*, **62**:233-236.

Verbel OJ, Luz SN and Stashenko EE. 2010. Biaoctivity against *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) of *Cymbopogon citratus* and *Eucalyptus citriodora* essential oils grown in Colombia. *Pest Manag Sci*, **6**:66.