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Bioassay of Aqueous Extracts from Six Tropical Plants against *Tribolium castaneum* (Coleoptera: Tenebrionidae)

*[1]Ugwu, J. A., [2]Bobadoye, B. O. and [1]Aluko, O. J.

[1]Federal College of Forestry Ibadan, Forestry Research Institute of Nigeria, P.M.B.5087 Jericho Hills, Ibadan, Oyo State, Nigeria
[2]Forestry Research Institute of Nigeria, P.M.B 5047, Jericho Hills, Ibadan, Oyo state, Nigeria
*Corresponding Author's email: dr.amaka2013@gmail.com

Abstract
Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae) is a very important insect pest of stored products causing economic damage to several stored grains. Laboratory bio-assay of aqueous extracts of six plants namely; *Azadirachta indica* (Neem), *Xylopia aethiopica* (Grain of selim), *Cymbopogon citratus* (Lemon grass), *Piper guineense* (African black pepper) Zingiber *officinale* (Ginger) and *Ocimum gratissimum* (African basil) were conducted for their contact and residual toxicity to adult *T. castaneum* under ambient temperatures of 27±2ºC and 80 ±5% relative humidity. Aqueous extracts of the test plants were applied in crude form at1 ml and 2 mls /5adult insects for contact and residual assays respectively. The mortality of adult *T. castaneum* were recorded at 20 minutes intervals for 24hours, data collected were subjected to Analysis of Variance and significant means were separated using Duncan Multiple Range Test (DMRT). The results showed that aqueous extracts of all the plants evaluated had toxic effects on adult *T. castaneum* ranging from 60.0% - 86.6% and 33.4% - 86.6% mortality for contact and residual toxicities, respectively at 24 hours post application. Highest mortality of 86.6% was observed in *A. indica* and *Z. offiinale* extracts for contact and residual toxicities, respectively. The aqueous extracts of plant materials evaluated showed great potentials against *T. castaneum* under laboratory conditions. Hence, their usage should be encouraged as a viable alternate to synthetic insecticide for stored products pest management to mitigate health and environmental hazards associated with synthetic pesticides application.

Keywords: Rust –red flour beetle, aqueous extracts, mortality, plant materials

Introduction
Insect pests of stored grains cause the highest qualitative and quantitative losses to stored- products globally (Fields, 2006). The rust-red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae) is one of the most noxious stored insects pests across the globe (Abdelghany *et al*.. 2010; Green, 2014). It is a cosmopolitan polyphagous pest of flour mills, cereal products, dried stored and processed foods causing significant economic losses (Aboelhadid and Youssef, 2021). The mature beetles and larvae consumes endosperm of the seeds and contaminate flour products which leads to coagulation, stale odor and pinkish coloration of the products as it secretes quinone compounds (Karunakaran *et al*., 2004; Keskin and Ozkaya, 2013). *T. castaneum* cause up to 40% reduction in grain weight (Ajayi and Rahman, 2006; Rees, 2007). They have high rates of reproduction which increases their population speedily (Sabbour, 2014; Saad *et al.,* 2019).

The current and common method of stored insect pest control is application of synthetic insecticides such as phosphine (PH3), organophosphates and pyrethroids (Awan *et al.,* 2012). However, the indiscriminate use of these broad-spectrum insecticides leads to several negative effects such as health hazards, environmental pollution, destruction non target beneficial organisms, development of pest resistance, rapid resurgence of population of target pest and pesticide residues on food (Park *et al.,* 2003). The restriction in exportation of food and horticultural products with pesticides residues above the standard recommended limit has aroused an intensive search for alternative measures for pest management such as the use of botanical pesticides that are acceptable, safer and environmentally friendly (Isman, 2006; Obeng-Ofori, 2007). Moreover, the synthetic pesticides are expensive for resource- poor farmers in developing countries and could cause potential risk to users due to the lack of technical knowledge and skills related to their safe applications (Hodges and Carr, 1999).

Botanical insecticides have been scouted as an attractive alternative to synthetic chemical insecticides for pest management over a long time due to their bio-degradation potential and less degrading to the environment (Isman. 2006). The use of essential oils and other main constituent of plant origins have attracted the researchers to discover an alternatives source of insect control in stored products (Saad *et al.,* 2019). Essential oils from several plant origins are currently being considered for developing plant protection products due to their benefits (Pavela and Benelli, 2016). These plant-derived products are distinguished by their insecticidal, repellent, and antifeedant actions on insects (Hashem *et al.,* 2018; Pavela *et al.,* 2018; Ileke *et al.,* 2020). *Azadirachta indica* (neem) products is one of the botanicals that have been widely proved effective against several stored grain pests such as maize weevils (Ileke and Oni, 2011, grain borers (Malik *et al*., 2012) cowpea beetles (Iqbal *et al*., 2015; Ugwu, 2016; Ahmad *et al.,* 2019) and Marchent grain beetle (*Oryzaephilus mercator*) (Ugwu *et al.*, 2012). It works as feeding deterrent, insect-growth regulator, repellent and sterilant, and may inhibit oviposition (Isman, 2006). The insecticidal potentials of extracts of *C. citratus, P. guineense,* Z. *officinale* and *O. gratissimum* against different insect species have also been documented (Idoko and Adesina, 2012; Ugwu *et al.,* 2012; Hamman *et al.,* 2012; Ugwu, 2016, 2020). However, there is a dearth of information about the insecticidal activity of these plant materials against *T. castanuem.* Hence, this study evaluated the biocidal activity of aqueous extracts *of A. indica*, X. *aethiopica* , *C. citratus, P. guineense,* Z. *officinale* and *O. gratissimum* agains*t T. castaneum* in a laboratory.

Material and Methods

Study site

The study was conducted at Biology and Entomology of Federal College of Forestry (FCF) Ibadan under ambient temperatures (27±2ºC), 80 – 85 % relative humidity and 12: 12 photoperiod. Federal College of Forestry, Ibadan is located within latitude 7° and 9° N, longitude 3° and 58° E of Greenwich Meridian Time (GMT) with annual rainfall of about 1300 to 1500mm and average relative humidity of about 80 to 85% (FRIN, 2014).

Sources and preparation of test plant materials

The fresh leaves of *Cymbopogon citratus* (Lemon grass), *Azadirachta indica* (Neem) and *Ocimum gratissimum* (African basil) were collected at Federal College of Forestry Ibadan while dry seeds of *Piper guineense* (African black pepper*),* fruits of *Xylopia aethiopica* (Grain of salim) *and* rhizome *of Zingiber officinale* were purchased from Dugbe market, Ibadan, Oyo State. The fresh leaves were air dried in a laboratory bench for two weeks and the dried plant samples were further air dried for two days The dried leaves, seeds, and stem of plant samples were pulverized separately into fine powders with the aid of an electric blender (Binatone blender/grinder BLG.450). The botanical and scientific names, families and plant parts used are shown in Table 1.

Extraction of plant materials

Hundred grams of each powder of the test plants were weighed using a sensitive scale into a separate 1liter bottles and then 250 ml of boiled water at 60°C temperature was poured into each bottles containing each powdered sample. The mixtures were agitated at 30 minutes intervals for 12 hours and allowed to stay for 24 hours. The extract solution was then obtained by filtering using a filter paper and the extracts were preserved in air tight glass vials until when used.

Insect culture

Twenty adults of *T. castaneum* were collected from infested wheat grains purchase from local market in Ibadan and introduced into one liter (1L) glass Kilner jar containing whole wheat flour. The beetles were cultured in whole meal wheat flour and the cultures were maintained at 28 ± 5° C and 75- 80 ± 5% relative humidity. The newly emerged a day old and unsexed adults were used for the bioassay.

Contact and Residual bioassay of the plant extracts against adult T. castaneum

The crude aqueous extracts were evaluated for contact toxicity by applying 1ml of each extracts on petri dishes lined with filter paper and containing five newly emerged adult *T. castaneum.* The adult insects were guided with the aid of camel brush to ensure that the abdomen of each insect had contact with the extracts and then the petri dishes were covered with its lid and monitored for mortality. For residual assay, extracts were evaluated by applying 2ml of each extracts on petri dishes lined with filter paper. Petri dishes were left to drain off for 10 minutes before five newly emerged adult *T. castaneum* were introduced into each dish and then covered with its lid. Each experiment was replicated three times in completely randomized design (CRD). The mortality of *T. castaneum* was recorded at 20 minutes intervals for 24 hours for both contact and residual assays.

Data Analysis

Data collected were subjected to analysis of variance (ANOVA) and significant means were separated by Duncan multiple range test (DMRT) using ASSISTAT statistical software 7.

Results and Discussion

Results

Contact effects of aqueous extracts of test plant materials

All the plant materials exhibited contact toxicity on the adult *T. castaneum* at varied rates as the time of exposure progressed (Table 2). The contact toxicity began from 60 minutes of exposure on four plant material namely: *A. indica*, *X. aethiopica* , *P. guineense* and *O gratissimum* with *X. aethiopica* recording the highest mortality of 6.7% , followed by *A. indica, P. guineense* and *O. gratissimum* with equal mortality of 3.3%. The contact effect of *Z. officinale* and *C.citratus* on adult *T. castaneum* commenced from 80 minutes insect post exposure with 6.7% and 3.3% mortality respectively.

The contact effect of the plant materials on the adult *T. castaneum* gradually progressed in different proportion until 1440minutes of insect post exposure to the extracts. There were significant (P < 0.01) differences in the rates of adult mortality caused by the different plant extracts at 1440 minutes of exposure. Highest mortality was observed on all the extracts at 1440 minutes post exposure. No mortality was observed on the control at the termination of the experiment.

Residual effects of aqueous extracts of selected plants on mortality of T. castaneum

The residual effects of the test plant materials followed a similar pattern as contact effects by recording higher mortality as the time of exposure progressed. The aqueous extracts of all the plants exhibited residual effects on adult *T. castaneum* at varied proportion as the time of exposure progressed (Table 3). The mortality commenced at 40 minutes post exposure with *Z. officinale* recording the highest mortality of 6.7%, followed by *C. citratus* with 3.3% mortality. *A. indica* and *P.guineense* commenced the residual action from 60 minutes of exposure causing 3.3% and 6.7% adult mortality respectively. *Ocimum gratissimum* was slowest in exhibiting the residual action on the insects by causing 3.3% mortality starting from 100 minutes of insect post exposure. The residual effects of the extracts proceeded gradually until 1440 minutes of insect exposure. The highest rates of mortality were recorded at 1440 minutes of post exposure. There were significant (P<0.05: 0.01) variation on the mortality of adult *T. castaneum* by the different plant extracts at 80, 120 and 1440 minutes of exposure. No mortality was observed on control experiment.

Comparison of contact and residual effects of selected plants on percentage mortality of adult T. castaneum after 24 hours post treatment

The aqueous extracts of all the plant materials showed great potential contact and residual toxicity against adult *T. castaneum* after 24 hours post treatments (Fig. 1). Generally, almost all the test plants showed higher potential for contact actions than residual actions. *A.indica, X aethiopica, P. guineense* and *C. gratissimum* exhibited more contact toxicity than residual effects by causing higher mortality of adult *T. castaneum*. However, only *O. gratissimum* showed a significant difference (p<0.05) on the percentage mortality between contact and residual effects on adult *T. castaneum*. *Zingiber officinale* exhibited more residual effects than contact effects. *Azaditachta indica* with 86.4% mortality recorded highest contact toxicity against adult *T. castaneum* , followed by *O. gratissimum* . For the residual actions, *Z. officinale* recorded highest mortality (86.6%), followed by *A*.indica, *X.aethiopica* and *C. citratus* (66.6%).

Discussion

The results of our study showed that aqueous extracts of all the tested plant materials have potential for the management of *T. castaneum* infesting stored products. All the plant materials showed contact and residual effects on adult *T. castaneum*. *A. indica* and *Z. officinale aqueous* extract recorded higher mortality of adult *T. castaneum* for contact and residual effects, respectively. These results corroborate the earlier reports by Ahmed *et al*. (2019) and Epidi and Odili (2009) that higher concentration of *Z. officinale* and *A. indica* powder were effective against *T. castaneum* on whole wheat meal. Similarly, different dosages of Calneem oil derived from neem seed were found toxic and highly repellent to *T. castaneum* (Adarkwah *et al*., 2010). The efficacy of *A. indica* extracts against several insect species have been reported by different researchers (Anikwe, 2013; Malik *et al.,* 2012; Ugwu *et al*., 2012; Ugwu, 2021; Ugwu *et al*., 2022). Aqueous extracts of *A. indica* have been reported to exhibit contact and residual effects against *Phytolyma fusca* (Ugwu, 2021) and *Bactrocera dorsalis* larvae (Ugwu *et al.,* 2022) under laboratory conditions . Anikwe (2013) also reported the high residual actions of six different plant extracts against *Sahlbergella singularis* (brown cocoa mirid) in a laboratory bioassay. Similarly, Ugwu *et al.* (2012) reported that leaf powders of *A. indica* and *Cymbopogon citratus* were potent against *Oryzaphilus mercator* on stored *Irvingia wombolu* kernel. Moreover, *A. indica* to been reported to confer effective control against major pest species of stored grain system (Iqbal *et al*., 2015; Malik *et al*., 2012). Our study further revealed that aqueous extracts *P. guineense*, *X.aethiopica*, *C. citratus* and *O. gratissimum* were potent against *T. castaneum* at both contact and residual assay. These results are in consonance with the report of several other researchers (Mbata and Ekpendu, 1992; Aarthi and Murugan, 2010; Hamman *et al*., 2012; Idoko and Adesina, 2012; Ugwu and Mokwunye, 2019; Aboelhadid and Youssef, 2021). Aboelhadid and Youssef (2021) reported and clove lemongrass extracts had a repellency effect and lethal effect on adult *T. castaneum.* Similarly, Idoko and Adesina (2012) reported that sole application of *P. guineense* powder reduced oviposition, adult emergency and caused adult mortality of cowpea weevils. Likewise, the contact and residual toxicity of *A. indica, P. guineense*, *and Aframomum melegueta* were reported to cause 80 – 100% adult and larvae mortality of *Balanogastris kolae* at various concentrations (Ugwu and Mokwunye, 2019). Mbata and Ekpendu (1992) also reported that application of 0.4 g /5.0 g *P. guineense* seed powder on maize caused 50% mortality of adult *Sitophilus zeamais.* The insecticidal activities *Ocimum gratissimum* leaf extracts had also been earlier reported (Aarthi and Murugan, 2010; Hamman *et al*., 2012). Moreover, Raji and Akinkurolere (2010) reported that ethanol extracts of *X. aethiopica* and *A. occidentale* exhibited high toxicity on the adult *Anopheles gambiae* causing 100% mortality. Eke *et al.* (2018) also reported that the ethanol and aqueous extracts of *X. aethiopica* were effective against *Sitophilus oryzae* on stored rice.

Conclusion

The study established the toxicity by contact and residual actions of aqueous extracts from *A. indica*, *P. guineense*, *C.citratus*, *Z. oficinale*, *X.eathiopica* and *O.*

gratissimum against adult *T. castaneum* under laboratory conditions. *A. indica* extracts exhibited significantly more contact toxicity than the others, whereas, *Z. officinale* showed higher toxicity due to residual effects than the others. These plant materials are readily available, biodegradable, their preparation and application procedures are uncomplicated, thus small holder farmers are encouraged to adopt their use to protect stored products like grains and chips against *T. castaneum* infestation and damage.

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Table 1: List of the plant species and their parts used as botanical insecticides against *Tribolium castaneum*

Scientific name	Common name	Family	Plant part used	Active ingredients	References
Cymbopogon citratus Stapf.	Lemon grass	Poaceae	Leaves	Neral, Gerenial, Linalool, Nerol, Geranyle Acetate	Manzoor *et al*. (2011)
Azadirachta indica. A.juss	Neem	Meliaceae	Leaves	Azadirachtin, nimonol, epoxyazadiradione	Rahman and Talukder (2006) and Upadhyay *et al*. (2011), Mahmoud *et al*. (2011)
Ocimum gratissimum. Linn	African basil	Lamiaceae	Leaves	Estragole, 1,8-cineole, trans- α-bergamotene	Sienkiewicz *et al*. (2013)
Piper guineense Schum and Thonn	African black pepper	Piperaceae	Seed	Piperine, Piperine, chavicine	Shiva Rani *et al*. (2013), Ganesh *et al*.(2014)
Xylopia aethiopica Dunal	Grain of salim	Annonaceae	Seed	β-pinene, γ-terpinene *trans*-pinocarveol and p-cymene	Bakary *et al*. (2003)
Zingiber officinale Roscoe	Ginger	Zingiberaceae	rhizome	Gingerol, b-Phellandrene, Camphene)	Epidi and Odili (2009)

Table 2: Successive contact toxicity of aqueous plant extracts on adult *T. castaneum* (*Time in minutes)*

Treatments	20	40	60	80	100	120	140	1440
A. indica	0.00	0.00	0.33a	0.33a	0.00a	0.00a	0.33a	3.33a
Z. officinale	0.00	0.00	0.00a	0.67a	0.00a	0.67a	0.00a	2.00ab
X. *aethiopica*	0.00	0.00	0.67a	0.00a	0.00a	0.33a	0.00a	2.67a
C. citratus	0.00	0.00	0.00a	0.33a	0.33a	0.00a	0.33a	2.33ab
P. guineense	0.00	0.00	0.33a	0.33a	0.67a	0.67a	0.00a	1.00bc
O. gratissimum	0.00	0.00	0.33a	0.00a	0.33a	0.00a	0.00a	3.00a
Control	0.00	0.00	0.00a	0.00a	0.00a	0.00a	0.00a	0.00c
Significant level			NS	NS	NS	NS	NS	**

Mean values followed by the same letter along the same columns do not differ statistically. ** Significant at a level of 1% of probability (p<.01)*, Significant at a level of 5% of probability (.01=<p<.05), NS=Not significant (p>=.05)

Table 3: Successive residual toxicity of aqueous plant extracts on adult *T. castaneum* (Time in minutes)

Treatments	20	40	60	80	100	120	140	1440
A. indica	0.00	0.00a	0.033a	0.00b	0.33a	0.00b	1.00a	1.67ab
Z. officinale	0.00	0.67a	0.00a	0.00b	0.00a	1.00a	0.00a	2.67a
X. aethiopica	0.00	0.00a	0.00a	0.67a	0.00a	0.00b	0.67a	2.00ab
C. citratus	0.00	0.33a	0.00a	0.67a	0.67a	0.00a	0.33a	2.00ab
P. guineense	0.00	0.00a	0.67a	0.00b	0.00a	0.33b	0.33a	1.00ab
O. gratissimum	0.00	0.00a	0.00a	0.00b	0.33a	0.00b	0.33a	2.00ab
Control	0.00	0.00a	0.00a	0.00b	0.00a	0.00a	0.00b	0.00b
Significant level		NS	NS	*	NS	**	NS	*

Mean values followed by the same letter along the same columns do not differ statistically. ** Significant at a level of 1% of probability (p<.01)*, Significant at a level of 5% of probability (.01=<p<.05), NS=Not significant (p>=.05)

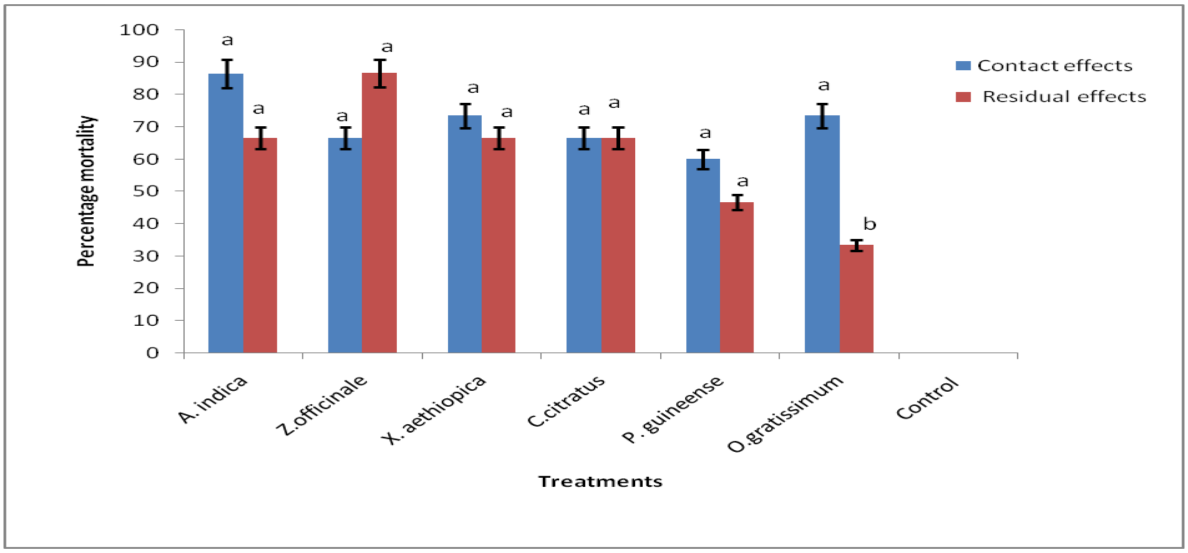

Figure 1: Overall percentage contact and residual effects of selected plant extracts on T. castaneum 24 hours post exposure