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Monitoring mortality, seed viability tolerance, toxicological and histopathological effects of powder of *Syzygium aromaticum* dried flower bud used for postharvest control of *Sitophilus zeamais* Motschulsky infestation of maize grains

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Abstract

The insecticidal activities, toxic and histopathological effects of clove, *Syzygium aromaticum* L. were studied. Adult male albino rats weighing 150-60 g maintained in standard cages with free access to food and water were used for this experiment in year 2020. The plant powder was cold macerated in methanol to produce filtrate reconstituted for determination of median lethal dose (LD₅₀) using Bruce's revised up and down procedure. Other assays followed standard methods too. The results showed that the botanical material, host resistance and permethrin are effective in controlling the maize weevil pest, *Sitophilus zeamais* Motschulsky infesting stored maize grains. Varietal resistance had no effect on the mortality of *S. zeamais* adults caused by *S. aromaticum* botanical insecticide at short storage period. Dry flower bud powder of clove, permethrin standard insecticide and resistance status had no effect on seed viability of maize grains when planted. The LD₅₀ for *S. aromaticum* flower bud powder extract was 3000 mg kg⁻¹ and this caused chills, convulsion and eventual death of the rats. The lowest weight gain in group IV rats administered 75% of the powder (2250 mg kg⁻¹) in diet was an index of high powder concentration. The study revealed that 25 (750 mg kg⁻¹) and 50% (1500 mg kg⁻¹) supplementation of *S. aromaticum* did not adversely affect the serum biochemical indices, liver and kidney of the albino rats. The 75% *S. aromaticum* supplementation caused physiological damage in the animals. Therefore, supplementation of < 75% is recommended in the safe use of the botanical insecticide in pest control and herbal therapy.

Keywords: insecticidal activities, clove, median lethal dose, biochemical indices, pest control

Introduction

Clove, *Syzygium aromaticum* L. (Myrtaceae) is native to Maluku islands, Indonesia but it is now cultivated in different parts of the world (Batiha *et al.*, 2020) [2]. The commercial part of the plant is the inflorescence/ flower bud and production of these floral parts commences four years after planting (Cortés-Rojas *et al.*, 2014) [5]. The rush for the dried flower bud of this plant species is on the increase probably because of its multipurpose capacity. From current information, the dried flower bud summarily is highly medicinal, used industrially in perfume production and a powerful spice used as food preservative and recognized for its antimicrobial and antioxidant properties (Batiha *et al.*, 2020) [2]. Recently, Nwosu and Adedire (2019) [19] published the insecticidal activities of powder and extracts of dried flower bud of *S. aromaticum* against the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). The present study will confirm the results of Nwosu and Adedire (2019) [19]. Ultimately, *S. aromaticum* flower bud contains about 18% of essential oil which comprises eugenol, eugenol acetate and β-cariofileno (Jirovetz *et al.*, 2006) [13]. Postharvest phases of maize (*Zea mays* L.) grain is characterised with major challenge posed by *S. zeamais*, the most important storage insect pest of maize (Nwosu, 2018; Ileke *et al.*, 2020) [17, 10]. Maize grain is so important; therefore any shortage due to insect pest attack will affect the world.

Maize grain is staple and consumed by man for survival, it is used industrially to manufacture an array of products and traded locally and internationally for financial credit and economic growth (Nwosu *et al.*, 2015; Ileke *et al.*, 2020) ^[14, 10]. Unequivocally, *S. zeamais* pest causes different degrees of damage to stored maize grains which results to agronomic, food security and commercial problems. The problems emanating from *S. zeamais* and its proclivity to destroy maize have attracted much attention and there is need to provide sustainable solution. Chemical insecticides used conventionally to tackle *S. zeamais* in stored maize are effective but bad consequences undermine their benefits (Nwosu *et al.*, 2016; Ileke *et al.*, 2020) ^[15, 10]. At present, research has focussed on plant materials as alternatives to conventional synthetic insecticides because plant materials are known to pose lower danger to man and his environment. Botanicals are usually available in our environments, are easily formulated into powders without education and training and can easily be removed from grains after pest control process. These are some of the major reasons why there is high emphasis on the need to identify effective plant powders for control of stored-product insect pests. However, many studies test insecticidal potency of plants without extending to possibilities of adverse effects on seed viability and possibilities of toxicological and histopathological effects after pest control process (Golob *et al.*, 1999; Nwosu *et al.*, 2017; Nwosu, 2019) ^[16, 19]. This is a common weakness with the majority of works published on screening of botanical materials for insecticidal activities. Categorically, research is required to clarify effects of botanicals on germination chances of grains after pest control process and also establish their safety in pest control and in the environment. The present study will address these limitations using replicated experiments. This is particularly important for dried flower bud/ inflorescence of *S. aromaticum* because these data are grossly inadequate for this plant material.

Indeed, the protection of stored grains with plant materials has been an old common practice among peasant farmers in Africa (Hassanali *et al.*, 1990) ^[8]. The safety of most of these botanicals is often overlooked, simply because, most of them have been in use for many years (Odeyemi *et al.*, 2008) ^[20]. Botanicals are not safer than the conventional synthetic insecticides because they are of natural origin (Weinzierl and Henn, 1994) ^[22]. Botanical insecticides are believed to possess characteristics which place them at a higher advantage over conventional synthetic insecticides (Odeyemi *et al.*, 2008) ^[20]. Briefly, these include low mammalian toxicity, less persistence in the environment, selectivity towards target pests and nonphytotoxicity (Isman, 2006) ^[12]. These have led to the belief that botanical insecticides are safer than synthetic chemicals. This, however, is not always the case, Weinzierl and Henn (1994) ^[22] and Odeyemi *et al.* (2008) ^[20] seriously warned. For the avoidance of doubt, nicotine extracted from tobacco is one of the most widely-known botanicals. Although it is effective in pest management, nicotine is highly toxic to mammals and can readily be absorbed through the eyes, skin and mucous membranes (Carr *et al.*, 1991) ^[4]. Rotenone, a polycyclic ketone widely used as a broad-spectrum insecticide extracted from the roots of *Lonchocarpus* spp, *Derris* spp and several other leguminous plant genera, is extremely toxic to aquatic life and also exhibits some level of toxicity to mammals (Carr *et al.*, 1991; Cranshaw, 1992) ^[4, 6]. Therefore, these information justify the extension to toxicological and histopathological evaluations to

reveal the safety of powder of *S. aromaticum* dried flower bud to man and his environment. The specific objectives of this study are to i. determine the effect of powder of *S. aromaticum* dried flower bud on the mortality of adult *S. zeamais* pest, ii. ascertain the effects of the plant powder on the germination of maize grains when planted in the laboratory and iii. evaluate the toxicological and histopathological effects of the botanical in albino rat.

Materials and Methods

Experimental site

The research was carried out at the Department of Animal and Environmental Biology, Imo State University, Owerri, Nigeria. The site was neat and well-ventilated for the work. The site (tropical environment) supports optimum development of maize weevil, *Sitophilus zeamais* Motschulsky. The mean temperature and relative humidity of the site was 30.1 °C and 75.5%, respectively.

Insect culture

The maize weevil, *Sitophilus zeamais* Motschulsky new adults used for the study were obtained from stock culture in the Crop Protection Laboratory of the Department of Crop and Soil Science, University of Port Harcourt, Nigeria. The weevils were bred on a susceptible local maize variety, Mangu. The grains were sorted (to remove any unwanted material), disinfected by cold storage for 7 days at -20 ± 2 °C and air-dried to prevent mould infection (Nwosu and Adedire, 2019) ^[19]. There were four culture-containers (19 cm length; 20 cm diameter) and each (with 1.5 kg maize) was added 100 randomly-picked unsexed adult *S. zeamais*. The containers were covered with muslin net held in place by rubber-band. After 7 days of feeding, mating and egg-laying, all adult *S. zeamais* were disposed and the containers were maintained at 30.1 °C (temperature) and 75.5% (relative humidity) until new adults emerged. From the 3rd generation of insects, adult *S. zeamais* of similar age (1-5 d) were selected for the experiment (Nwosu and Adedire, 2019) ^[19].

Maize varieties

Two maize varieties with known resistance status to the maize weevil infestation were used for the study. These were one improved resistant maize variety, DTSTR-WSYN2 and one local susceptible maize variety, Mangu. The improved maize variety was obtained from International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria whereas the local maize was procured at Ekeukwu Market, Douglas road, Owerri, Imo State, Nigeria. The grains were sorted, disinfested by cold storage and acclimatised to normalise moisture at 12-13% before they were used for the experiment.

Determination of effect of *Syzygium aromaticum* on weevil mortality

The plant material tested for mortality effect on adult *S. zeamais* is clove, *Syzygium aromaticum* L. belonging to the family Myrtaceae. *Syzygium aromaticum* is a popular medicinal material in Nigeria and the powder was generated in the laboratory by pulverizing the dried flower buds and sieving through 1 mm mesh. Therefore, powder of *S. aromaticum* dried flower bud was tested on resistant and moderately-resistant maize at three economically-justified doses 1.0, 1.5 and 2.0% w/w (0.2, 0.3 and 0.4 g/20 g grains respectively). A standard insecticide, permethrin (Rambo: 0.6% dust) and an untreated control were included. Plastic

dishes containing treated or untreated grains were shaken for 1 min (to ensure proper coating of grains) and infested with 20 unsexed 1-5 day-old adult *S. zeamais* before covering with muslin net held in place with rubber-band. After 24 h (in line with 24-hour high-sensitivity test against storage insects recommended by Nwosu (2018) ^[17], post-infestation mortalities were recorded and converted to percentages. The experimental design was randomized complete block design, replicated four times and entire experiment was repeated once to obtain true replicates (2 x 4) and ensure high validity of data.

Determination of effect of *Syzygium aromaticum* on grain viability

After pest control process with 1.0, 1.5 and 2.0% w/w of powder of *S. aromaticum* dried flower bud, seeds of the resistant and moderately-resistant maize were stored in the same experimental conditions for 30 days after which five seeds from each treatment were randomly-selected and planted in the laboratory using standard protocol. The grains were well-spaced on a moistened Whatman filter paper (110 mm diameter) inside a transparent plastic dish (11 cm diameter). Each filter paper in a dish received daily dose of 10 ml of distilled water for a period of 7 days after which the number of germinated grains was counted, recorded and expressed in percentage. Emergence of radicles and primary leaves were used as indices. The experiment was replicated four times and repeated once to obtain true replicates (2 x 4).

Experimental animals

Thirty apparently healthy juvenile male albino rats (age, average of 1.5 months) weighing 150 to 153 g were used. The experiment was conducted in accordance with ethical guidelines of animal experimentation (regulation CEE 86/609). Briefly, the animals were randomly assigned to four groups of six animals per group in standard cages. They had free access to food and water. They were acclimatized under average temperature and relative conditions of 30.1°C and 75.5%, respectively prior to commencement. Experiments on toxicology/ histopathology were replicated six times.

Determination of median lethal dose of *S. aromaticum* dried flower bud

To determine the median lethal dose (LD₅₀) of *S. aromaticum* dried flower bud, the extract was first prepared by cold maceration at room temperature (mean value in our laboratory: 29.23 °C) described in Nwosu *et al* (2017) ^[16]. The extract was reconstituted for oral administration following Saganuwan (2012) ^[21]. Then, the LD₅₀ was estimated using revised up-and-down method described by Bruce (1985) ^[3] and Saganuwan (2012) ^[21]. Five rats were chosen at random, one from each animal group and dosed after an overnight fast. Selection of albino rats from groups was done without replacement. Initial dose of 2000 mg/kg of the extract (0.3 ml in volume) was administered orally to first animal and the rat was observed for toxicity signs for 48 h. The first rat survived, therefore, a dose progression employing logarithm of 3.2 was applied until a toxicity-dose level was attained, causing shivering, delayed reflexes, immobility and death after 96 h. The square root of product of geometric mean of last survived dose and mortality dose was used to estimate the LD₅₀. Sub-lethal doses of *S. aromaticum* dried flower bud powder used for dietary toxicity study were worked out from the result of LD₅₀ adopting Saganuwan (2012) ^[21] standard ratios (25%, 50% and 75%).

Treatment groups for subacute toxicity test with powder of *S. aromaticum* dried flower bud

The diets were worked out following the OECD test guidelines 407 and 451. The albino rats were divided into four groups. Group I rats were used as control and they were fed standard rat diet manufactured by United Africa Company, Nigeria. Furthermore, 25%, 50% and 75% of the plant powder were separately incorporated into the standard rat diet and used to feed Groups II, III and IV rats respectively. The animals in the various groups were allowed to feed for a period of 30 days after which feed consumption data and final body weights were taken.

Collection and pretreatment of blood, liver and kidney

Blood samples were rapidly collected from each rat group by intracardiac puncture under ether anaesthesia and kept in a non-heparinized sample tube (Nwosu *et al.*, 2017) ^[16]. Serum samples from the rats separated 1 h after collection and used to assay biochemical indices. The rats were dissected immediately after blood collection to isolate the liver and kidney. The liver and kidney were preserved in 10% formalin for histopathological studies.

Quantification of serum biochemical indices

The standard methods applied by Nwosu *et al* (2017) ^[16] were used to determine six important serum biochemical indices in rats. Apart from total protein that was determined by the direct Biuret method, in quantification of serum biochemical indices of urea, creatinine, total protein, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase, the absorbance of sample and standard were measured against the reagent blank. Readings were taken at specific wavelength using a spectrophotometer.

Histopathological studies using liver and kidney

The method used by Imafidon and Okunrobo (2012) ^[11] and Nwosu *et al.* (2017) ^[16] was employed during histopathological examinations of the liver and kidney of the albino rats. The samples were fixed for 48 h in 10% formalin-saline and were dehydrated by passing it in a different mixture of ethyl alcohol and water, cleansed in xylene and embedded in liquid paraffin wax for 2 h. A rotary microtome was then used to make 5-6 µm thick sections of the organs. The prepared sections were collected on glass slides, deparaffinized and stained with hematoxylin-eosin prior to mounting in neutral dibutyl phthalate xylene medium. The preparations were examined under a microscope equipped with a digital camera connected to a computer and photographs were taken.

Results and Discussion

Effect of clove powder on mortality of *S. zeamais* adults infesting resistant and moderately-resistant maize grains

Table 1 shows the effect of powder of *S. aromaticum* dried flower bud on mortality of *S. zeamais* adults infesting resistant and moderately-resistant maize grains in storage. There were significant differences in mortality caused by the different treatments ($P < 0.05$). Increasing the rate of dried flower bud powder from 1 to 2% w/w and exposure time 24 to 96 h both increased adult mortality. Ascending from 1 to 2% w/w did not give significant differences ($P > 0.05$) in both resistant and moderately-resistant maize varieties. Mortality caused by the clove powder was noticeable after 24 h and exceeded 50% at all doses tested in resistant and moderately-

resistant maize. In both resistant and moderately-resistant maize, the powder caused minimum 86% and maximum 100% mortality at ≥ 72 h to 96 h of exposure. There were no significant differences in mortalities between resistant and moderately-resistant maize varieties. Mortalities produced by all doses differed significantly from mortality on untreated maize grains. Permethrin insecticide caused a minimum of 79.70% mortality within 24 h, reaching minimum 95% after 48 h, 72 h and 96 h and these differ significantly from mortalities caused by clove at ≤ 48 h of exposure. The high mortality recorded in this study is strongly attributed to effect of active ingredients in clove flower bud. Hassanali *et al.* (1990) [8] noted that potent active ingredients such as eugenol (the principal ingredient) are responsible for insecticidal activities in the plant species. Other constituents include Carvacrol, thymol, and cinnamaldehyde. Differences in

mortality rates across treatment, variety and exposure period revealed that dose and exposure period impacted on *S. zeamais* mortality, but not variety at the storage duration considered. This shows that varietal difference (resistance versus moderate-resistance) had no effect on the mortality of adult *S. zeamais* in maize stored for a short period. The non-significant variety x treatment interaction observed here is supported by Nwosu and Adedire (2019) [19]. We therefore conclude that host resistance, clove dry flower bud and permethrin standard insecticide are effective in the protection of maize grains against infestation and damage by *S. zeamais* in stores. The use of botanicals particularly in combination with resistant varieties will reduce health and ecological problems associated with the use of synthetic insecticides. This is also supported by literature.

Table 1: Effect of powder of *Syzygium aromaticum* dried flower bud on mortality of *Sitophilus zeamais* Motschulsky adults infesting resistant and moderately-resistant maize grains in storage

Rate (% w/w)	% mortality at indicated hour			
	24	48	72	96
Resistant maize ¹				
1.00	17.10±0.04 ^b	53.00±1.25 ^b	87.00±1.80 ^a	91.00±0.01 ^a
1.50	20.40±0.01 ^b	59.70±4.01 ^b	95.00±0.00 ^a	95.00±0.01 ^a
2.00	24.20±1.05 ^b	65.00±0.03 ^b	100.00±0.00 ^a	100.00±0.00 ^a
Permethrin insecticide	85.00±2.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a
Control	0.00±0.00 ^c	0.00±0.00 ^c	0.00±0.00 ^b	0.00±0.00 ^b
Moderately-resistant maize ²				
1.00	16.80±2.30 ^b	53.80±0.01 ^b	86.40±0.50 ^a	90.00±0.50 ^a
1.50	18.10±0.05 ^b	66.80±0.64 ^b	93.00±0.02 ^a	95.00±1.00 ^a
2.00	22.50±2.10 ^b	64.00±0.01 ^b	95.00±0.01 ^a	95.00±0.00 ^a
Permethrin insecticide	79.70±0.01 ^a	95.00±0.00 ^a	95.00±0.00 ^a	95.00±0.50 ^a
Control	0.00±0.00 ^c	0.00±0.00 ^c	0.00±0.00 ^b	0.00±0.00 ^b

Maize varieties: ¹DTSTR-WSYN2; ²Mangu.

Data are means \pm SEM of four replications.

Mean values in a column with same letter are not significantly different by HSD ($\alpha = 0.05$).

Effect of clove powder and permethrin insecticide on the viability of stored resistant and moderately-resistant maize grains

Table 2 presents the effect of powder of *S. aromaticum* dried flower bud and permethrin standard insecticide on the viability of stored resistant and moderately-resistant maize. All the grains germinated (100%) in both treated and untreated maize irrespective of resistance status. Thus, the

present study has revealed that resistance status (whether resistant, moderately-resistant or susceptible) of a maize variety has no effect on seed viability. The study also revealed that clove dry flower bud powder and permethrin standard insecticide will not affect the viability of maize grains after storage with the materials. This study has provided missing information on the influence of botanical insecticides on seed viability.

Table 2: Effect of powder of *Syzygium aromaticum* dried flower bud and permethrin standard insecticide on the viability of stored resistant and moderately-resistant grains in the laboratory

Application rate (% w/w)	No. of germinated seeds/dish	% germinated seeds/dish
Resistant maize ¹		
1.00	5.00±0.00	100.00±0.00
1.50	5.00±0.00	100.00±0.00
2.00	5.00±0.00	100.00±0.00
Permethrin insecticide	5.00±0.00	100.00±0.00
Control	5.00±0.00	100.00±0.00
Moderately-resistant maize ²		
1.00	5.00±0.00	100.00±0.00
1.50	5.00±0.00	100.00±0.00
2.00	5.00±0.00	100.00±0.00
Permethrin insecticide	5.00±0.00	100.00±0.00
Control	5.00±0.00	100.00±0.00

No. of seeds planted/dish = 5

Data are means \pm SEM of four replications.

Means in each column are the same.

Feed intakes of albino rats used for sub-acute toxicity evaluation

Table 3 presents the feed intakes of albino rats used for sub-acute toxicity test of powder of *S. aromaticum* dried flower bud. There were no significant differences ($P > 0.05$) in the amount of feed consumed by the animals. However, the albino rats fed standard rat diet plus 75% powder of *S.*

aromaticum dried flower bud consumed the highest quantity of feed. Whereas, the rats fed standard rat diet plus 50% powder of *S. aromaticum* dried flower bud had the lowest feed intake. The albino rats fed well during the period of assessment and consumed about 10% feed of their body weights. This analysis is supported by Saganuwan (2012) [21].

Table 3: Feed intakes of albino rats used for sub-acute toxicity test of powder of *Syzygium aromaticum* dried flower bud

Treatment	Feed intake (g/rat/day)
Standard rat diet (control)	15.03±0.01 ^a
Standard rat diet + 25% powder of <i>S. aromaticum</i> dried flower bud	15.01±0.55 ^a
Standard rat diet + 50% powder of <i>S. aromaticum</i> dried flower bud	14.80±0.00 ^a
Standard rat diet + 75% powder of <i>S. aromaticum</i> dried flower bud	16.01 ±0.05 ^a

Data are means ± SEM of six replications.

Mean values in a column with same letter are not significantly different by HSD ($\alpha = 0.05$).

Assessment of body weight of albino rats

Table 4 shows the extent of changes in body weight of albino rats fed standard diet and standard diet plus powder of *S. aromaticum* dried flower bud. There were no significant differences ($P > 0.05$) in the initial weights of the rats in the different treatment groups. The final weights of the various animal groups fed different amount of clove powder did not also differ significantly ($P > 0.05$). However, at the end of the experiment, percent weight gain was significantly lowest in the albino rats fed standard rat diet plus 75% powder of *S. aromaticum* dried flower. The body weight gains in rats fed standard basal diet (control) and in rats fed standard basal diet

plus clove dry flower bud powder suggests that basal diet and up to 75% supplementation of clove flower bud powder will not affect body weight of rats adversely. It is now obvious why human beings use the material as tea, spice and herbal therapy without losing body weight. The significantly lowest body weight gains in rats fed basal diet plus 75% of the plant material can be attributed to effect of high powder concentration. Ileke *et al.* (2014) [9] associated weight loss in albino rats to treatment with petroleum ether extract and powder of *Nigella sativa*. In our own study, no loss in body weight was recorded, eliminating the possibility of powder toxicity.

Table 4: Change in body weight of albino rats fed standard diet and standard diet + powder of *S. aromaticum* dried flower bud

Treatment	Initial weight (g)	Final weight (g)	% weight gain (g)
Standard rat diet (control)	153.00±0.00 ^a	160.05±0.02 ^a	7.95±0.91 ^a
Standard rat diet + 25% powder of <i>S. aromaticum</i> dried flower bud	151.05±0.02 ^a	158.95±0.05 ^a	7.90±0.45 ^a
Standard rat diet + 50% powder of <i>S. aromaticum</i> dried flower bud	150.34±0.25 ^a	157.84±1.7 ^a	7.50±0.01 ^a
Standard rat diet + 75% powder of <i>S. aromaticum</i> dried flower bud	151.01 ±0.01 ^a	153.11±5.00 ^a	2.10±0.01 ^b

Data are means ± SEM of six replications.

Mean values in a column with same letter are not significantly different by HSD ($\alpha = 0.05$).

Acute toxicity of clove inflorescence powder

The median lethal dose (LD₅₀) of methanolic extract of *S. aromaticum* inflorescence powder was 3000 mg kg⁻¹ of albino rat. This shows that the LD₅₀ value of clove dry flower bud powder is moderately high. This indicates that the extract of the plant material has a moderately high safety margin. The assertion here is supported by Nwosu *et al.* (2017) [16].

Effect of powder of clove dried flower bud on some serum biochemical indices of albino rat

Table 5 shows effect of powder of *S. aromaticum* dried flower bud on some serum biochemical indices of albino rats. Significant differences ($P < 0.05$) occurred in the serum biochemical indices of the albino rats considered. The albino rats fed standard rat diet plus 75% powder of *S. aromaticum* dried flower bud consistently had the highest serum content of

urea, creatinine, total protein, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase. Associations between serum biochemical indices and liver and kidney functions of albino rats have been reported (Ileke *et al.*, 2014; Nwosu *et al.*, 2017) [9, 16]. Some of the serum biochemical indices are necessary marker enzymes for probing damage to organs such as liver and kidney. The observation that the animals fed standard rat diet plus 75% powder of *S. aromaticum* dried flower bud consistently had the highest serum content of urea, creatinine, total protein, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase is an indication that 75% of the botanical material may have caused the rats physiological problems. The study revealed that clove dry flower bud powder supplementation of ≤ 75 % did not cause injury to liver and kidney of the animal.

Table 5: Effect of powder of *Syzygium aromaticum* dried flower bud on some serum biochemical indices of albino rats

Treatment	Urea (mg/dL)	Creatinine (mg/dL)	Total protein (g/dL)	AST* (U/L)	ALT** (U/L)	ALP*** (U/L)
Control (Standard rat diet)	13.01±2.55 ^b	0.31±0.02 ^b	4.00±0.00 ^a	18.05±0.05 ^b	11.01±0.01 ^c	40.78±10.00 ^{cd}
Standard rat diet + 25% powder of <i>S. aromaticum</i> dried flower bud	14.17±0.42 ^b	0.35±0.10 ^b	4.27±0.35 ^a	17.39±0.01 ^b	11.03±0.02 ^c	45.24±1.50 ^{bc}
Standard rat diet + 50% powder of <i>S. aromaticum</i> dried flower bud	15.00±0.00 ^b	0.42±0.01 ^b	4.50±0.55 ^a	20.20±2.05 ^b	30.75±3.70 ^b	62.00±0.00 ^{ab}
Standard rat diet + 75% powder of <i>S. aromaticum</i> dried flower bud	52.70±10.00 ^a	1.27±0.03 ^a	4.60±0.01 ^a	45.11±2.00 ^a	64.08±4.50 ^a	70.33±2.00 ^a

* Aspartate aminotransferase; ** Alanine aminotransferase; *** Alkaline phosphatase

Data are means ± SEM of six replications.

Mean values in a column with same letter(s) are not significantly different by HSD ($\alpha = 0.05$).

Effect of powder of clove dried flower bud on the histopathology of albino rat liver and kidney

Figure 1 shows normal liver structure with intact muscles and sinusoids in rats fed standard rat diet (control), standard rat diet plus 25% powder of *S. aromaticum* dried flower bud and standard rat diet plus 50% powder of *S. aromaticum* dried flower bud. Figure 2 showed haemosiderin granules in the liver sinusoids of rats fed standard rat diet plus 75% powder of *S. aromaticum* dried flower bud. This was a significant observation. Figure 3 showed normal kidney tissues in rats fed standard rat diet (control) and in rats fed standard rat diet plus 25% powder of *S. aromaticum* dried flower bud. Figure 4 showed haemorrhage and vein congestion in the kidney of albino rats fed standard rat diet plus 50% powder of *S. aromaticum* dried flower bud and standard rat diet plus 75% powder of *S. aromaticum* dried flower bud. The histopathological investigations of the liver and kidney of the albino rats revealed physiological disorders at dry flower bud supplementation of 75%. The health problems caused by high powder concentration of botanical agree with findings of Akparie (2004) [1], Ileke *et al.* (2014) [9] and Nwosu *et al.* (2017) [16].

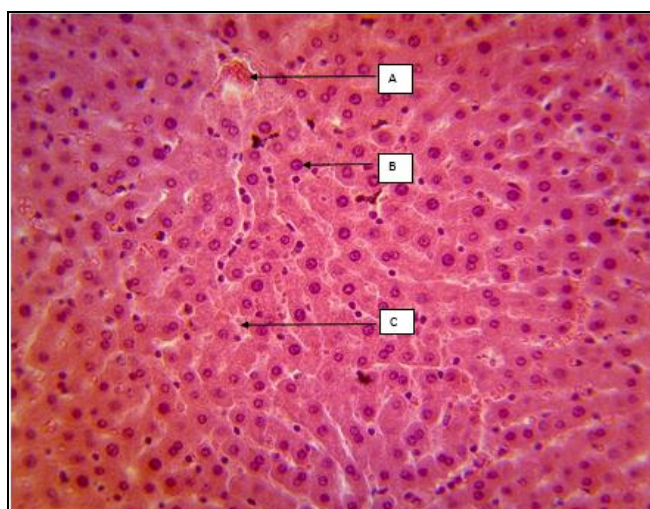


Fig 1: Normal liver structure with intact muscles and sinusoids in rats fed standard rat diet (control), standard rat diet + 25% powder of *S. aromaticum* dried flower bud and standard rat diet + 50% powder of *S. aromaticum* dried flower bud. Arrows A = central vein, B = hepatocytes and C = sinusoid.

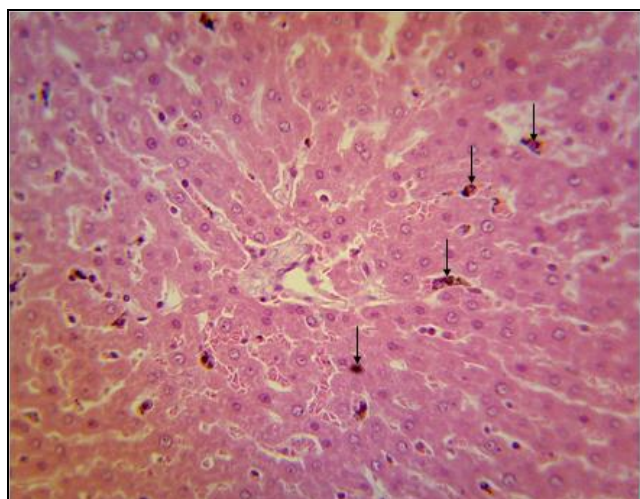


Fig 2: Arrows show haemosiderin granules in the liver sinusoids of rats fed standard rat diet + 75% powder of *S. aromaticum* dried flower bud.

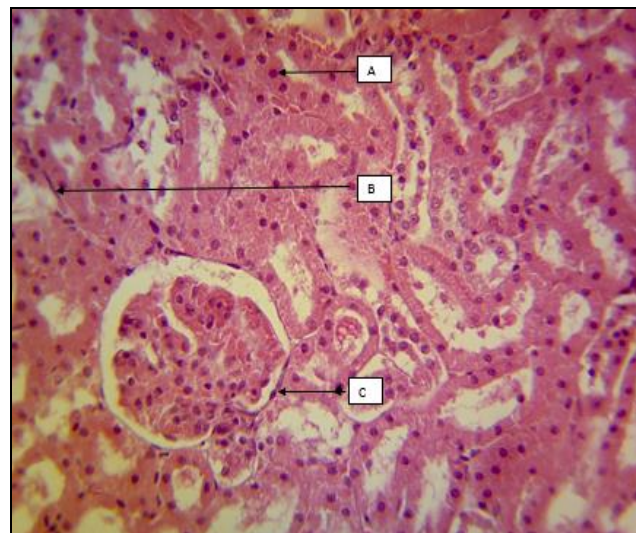


Fig 3: Normal kidney tissues in rats fed standard rat diet (control) and in rats fed standard rat diet + 25% powder of *S. aromaticum* dried flower bud. Arrows A = papillary ducts B = connective tissue stroma and C = glomerular capsule.

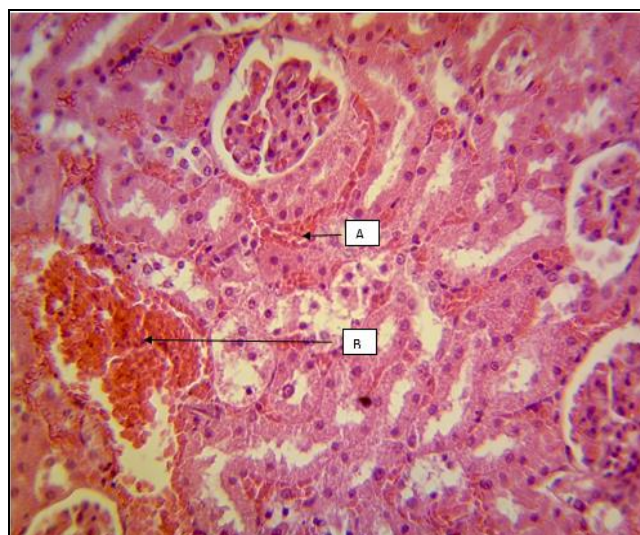


Fig 4: Haemorrhage (arrowed A) and vein congestion (arrowed B) in the kidney of albino rats fed standard rat diet + 50% powder of *S. aromaticum* dried flower bud and standard rat diet + 75% powder of *S. aromaticum* dried flower bud.

Conclusion

The insecticidal activity, toxicological and histopathological effects of dry flower bud powder of clove, *Syzygium aromaticum* L. were investigated in the laboratory. The study found that the botanical material, host resistance and permethrin are effective in controlling the maize weevil pest, *Sitophilus zeamais* Motschulsky infesting and damaging stored maize grains. Furthermore, varietal resistance difference (resistance and moderate-resistance) had no effect on the mortality of *S. zeamais* adults caused by *S. aromaticum* botanical insecticide. It was found that dry flower bud powder of clove, permethrin standard insecticide and resistance status had no effect on seed viability of maize grains when planted in the laboratory. The value of the median lethal dose indicates that the botanical insecticide has moderately high-safety margin. The toxicological and histopathological examinations revealed that high concentration (75% supplementation) of dry flower bud powder of clove caused the albino rats physiological disorders and therefore its use as

food, medicine and in pest control should be limited to < 75% supplementation (< 2250 mg kg⁻¹) in terms of mg/kg body weight.

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