RESEARCH ARTICLE

Fumigant toxicity of *Cleistopholis patens* (Benth) oil extracts on *Plodia interpunctella* (Hubner) and its toxicological effects in Wistar rats

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Olorunsogbon ST, Akinneye J, Olowu E. Fumigant toxicity of *Cleistopholis patens* (Benth) oil extracts on *Plodia interpunctella* (Hubner) and its toxicological effects in Wistar rats. AGBIR.2023;39(4):627-633.

Background: The efficacy of root and stem barks oil of *Cleistopholis patens* as a fumigant agent on *Plodia interpunctella* infesting maize grains as well as its toxic potential in wistar rats were investigated. Both plant oils were used for fumigant bioassay while only the root oil extract was used for all toxicological studies due to its higher toxicity on *P. interpunctella* when compared to stem oil extract. Both insects and wistar rats were exposed to different concentrations (0.0, 5%, 10%, 15%, 20% and 25%) of the extract. 36 wistar rats were divided into Group A-F and each group received different concentrations of the oil extract except for Group A that received only dimethyl sulphide saline and various toxicological tests were conducted.

Results: Result shows that both stem and root extracts significantly evoked ($p<0.05$) higher larva and adult mortality in treated grains when compared to both controls except at 5% of both oil extracts. Ten percent root oil extract achieved 50% mortality in *Plodia interpunctella* within 48 hrs and 25% achieved 100% mortality in larva and adult of *Plodia interpunctella* within 72 hrs when compared to the amount needed to achieve 50% and 100% mortality in stem oil extract within 48 hrs and 72 hrs. Irrespective of the concentration administered to the animal, there were no significant alteration ($p>0.05$) in the toxicological test using both liver and kidney biochemical parameters.

Conclusion: This study shows that root oil extract of C. *patens* is a very good fumigant poison to *Plodia interpunctella* with no toxic impact in wistar rats.

Key Words: *Cleistopholis patens; Biochemical assay; Fumigant; Plodia interpunctella; Wistar rat*

INTRODUCTION

The Indian meal moth, *Plodia interpuntella* (Hubner), is known to be one of the major economic insect pests of stored product on every continent [1,2] and a secondary storage pest found in farms, food processing plants, households, retails stores and warehouses. The species have gained economic importance because infested products may contain insect fragment and cast larval skin in addition to individuals of each life stage. *Plodia interpunctella* is a cosmopolitan pest that infests a wide range of stored products including maize grains, nuts, beans, processed foods and dried fruits [3]. Infestation of commodity by *Plodia interpuntella* causes 60%-80% postharvest losses of staple food crops in Nigeria leading to major economic losses [4] and in most storage facilities developing countries [5]. Infestation of *Plodia interpunctella* cause direct product loss and indirect economic costs, quality losses and consumer complaints. Indian meal moth has been found to be distributed across the continent of the world, both in temperate and tropical climates of the world including Africa [1]. The larva stage is the most destructive and causes appreciable damage to the infested produce. The newly hatched larvae disperse to find food; first instars can invade food in cans through pinholes of diameter 0.39-0.45 mm [6]. The feeding activity of the larva creates a matted surface on food which contaminates the produce and gives an unpleasant odour. The webbing also contains larval excreta (frass) and exuvial (cast skin) [7-9]. Indian meal moth feeding reduces the weight of the grain. The economic damage caused by Lepidoprean pest on field crops and on stored grain has aggravated the problem of food security and malnutrition in many developing countries [10].

Low temperature storage and heat treatment of storage facilities have potential to control *Plodia interpunctella* [11]. Heat treatment in a feed mill eliminates population of different stored product insects, but the *Plodia interpunctella* population gradually increases after few weeks [12]. Locatelli et al., [13] reported that the larvae stage is vulnerable to low-oxygen conditions using modified atmospheres, but they still survive up to 6 days after such conditions. Amendments of 1996 made to the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) and Food Quality Protection Act (FQRA) as result of food quality control charged the USA Environmental Protection Agency to re-access all currently registered pesticides. Some insecticides such as organophosphate and carbonate have been removed from the post-harvest market, while some others are being considered for removal. In fact, strains of *P. interpunctella* have proved resistance to the organophosphate malathion and several other organophosphates [14-16]. Though the investigation of McGaughey and Johnson [17], and Herrero et al., [18], report have shown that the *Plodia interpunctella* has also developed resistance to the microbial insecticide *Bacillus thuringiensis*. The control of this pest by synthetic insecticide has serious drawbacks [19]. The indiscriminate use of synthetic insecticide has given rise to many serious problems, including genetic resistance by pest species, toxic residues; increasing cost of application; pollution of storage environment and hazard from handling [20]. These have stimulated a search for alternative means of storage-pests control.

In view of these, researchers and farmers have given their attention toward the use of botanical insecticides to control stored product insect pests, because they are eco-friendly, less toxic to humans, easy to use, specific in action and insect pests are not resistance to them Ileke and Oni [4]. Medicinal plants have demonstrated potential as insect control agents [21-23]. Small scale farmers and researchers have often claimed successful use of plant products insect control. Plant materials such as spices, vegetable oils, extracts, powders or inert dust have been reported for their various insecticidal efficacies [5,24,25].

Cletopholis patens (famly: Annonnaceae) is a sun-loving tree about 20-35 m tall found in riverine and swamp forest in largest parts of African countries. They possess roots, stems, barks, leaves, fruits, seeds and flower, which are claimed to have medicinal values in some culture in African countries. *Cleitopholis patens* is commonly called "salt and oil tree" and the local Nigeria name called Apako or Oke (Yourba). It is medicinal plant used for the treatment of headache, malaria, measles and antifertility and infectious diseases caused by *Staphylococcus aureus* [26]. The major phytochemicals in the stem bark are saponins, alkaloids, flavonoids and cardiac glycosides. Its therapeutic impact could be as a result of alkaloids which possess analgesic, antiplasmodic and bacteridal properties. In addition to the phytochemicals present in the stem part extract of C. *patens*, it was also suggested that it

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Received: 01-Jul-2023, Manuscript No. AGBIR-23-108191; **Editor assigned:** 03-Jul-2023, Pre QC No. AGBIR-23-108191 (PQ); **Reviewed:** 17-Jul-2023, QC No. AGBIR-23-108191; **Revised:** 24-Jul-2023, Manuscript No. AGBIR-23-108191 (R); **Published:** 31-Jul-2023, DOI:10.35248/0970-1907.23.39.627-633

has elements such as calcium, magnesium, sodium, potassium, phosphorus, iron, zinc, manganese, copper and cobalt to varying degrees [27,28].

In Africa particularly Nigeria, attention is being to the use of edible plant materials as grain protectant through various laboratory and field research and the tropics is well endowed with these plants' species [21,29]. Some plant parts have been scrutinized for insecticidal activity on stored product pests. Also, oil, powders, or alcoholic extracts from plants leave; stem and roots have been used against pests of stored products [30-32]. Akinneye [33] reported the control of *Ephestia cautella* adult with *Cleistopholis patens* powder and achieved 100% mortality within 72 hours after application. Olawumi [34] also reported the control of *Plodia interpunctella* with *Uvaria afzelli* powder and achieved 100% mortality within 48 hours after application. Numerous insecticides have been used to control *Plodia interpunctella* in stored products, but effectiveness is limited due to the pest resistance [35]. This research work is sought to reveal the fumigant toxicity of *Cleitopholis patens* (Benth) oil extracts on *Plodia interpunctella* (Hubner) (Lepidoptera; Pyralidae) and its toxicological effect on wistar rats and the most potent oil extracts.

MATERIALS AND METHODS

Insect rearing

The *Plodia interpunctella* larvae used to establish the culture was obtained from naturally infested maize grains from School farm, Ondo State, Nigeria. The moths' larvae were reared in 2 litres plastic containers containing 300 g of uninfested grains. The culture was maintained by continually replacing devoured powder and sieving out frass and fragments. The plastic container was covered with muslin cloth, fastened with rubber band, and placed inside wire mesh cage of dimension 75 cm × 50 cm × 60 cm (LWH) with its four strands deep inside dipped in water-kerosene mixture contained in a plastic container to prevent entry of predatory ants into the cages. The culture was maintained at a temperature (28 ± 2°C) and relative humidity (75 ± 55%). The whole setup was left inside the postgraduate research laboratory of the Department of Biology, Federal University of Technology, Akure.

Preparation plant material

The stem barks and root barks of *Cleitopholis patens* were harvested from Otasun farm along Ile Oluji Road, Ondo. These plant parts were brought into the laboratory, washed thoroughly with water, and air-dried in the laboratory for 30 days. Each plant part was pulverized separately into fine powder using Binatone electric blender (Model 373). The powders were further sieved to pass through 1 mm2 perforation. The fine powders were kept in separate airtight plastic containers and stored at ambient temperature of 28 ± 2°C and 75±5% rh.

Extraction of the plant oil

The solvent used for the extraction was ethanol. 300 g of the pulverized plant stem barks and root barks were weighed separately into a beaker and packed in a thimble using muslin cloth and extracted with 500 ml of solvents in a Soxhlet extractor. In each case, the extraction was carried out for 3 hr at 50°C. The extraction was determined when the solvent in the thimble became clear. Then, the thimble was moved from the unit and the solvent recovered by redistilling the content obtained from Soxhlet using rotary evaporator. The resulting extract contained both the solvent and the oil. After which the oil was exposed to air, so that traces of the volatile solvent evaporated, leaving the oil extract. The resulting oil was kept in glass bottles and used for a subsequent experiment.

Insect mortality test

In this research, fumigant toxicity for 24, 48, 72 and 96 hr of *Cleistopholis patens* oil extract were tested on developmental stages of *Plodia interpunctella* to access them as a potential candidate for bioinsecticides. All the experiments were conducted at room temperature of 28 ± 2°C and relative humidity of 75 ± 5%.

Fumigant effect of plant oils on the eggs and larval of Plodia interpunctella

Different concentrations of 5%, 10%, 15%, 20% and 25% oil extract of root barks and stem barks were prepared by dilution of ethanol (solvent). The various concentrations were obtained using graduated syringes. Freshly emerged adults collected from the stock culture were differentiated into male and female using size. The male and female were paired in the ration 1:1 inside glass vessels of dimension 8 cm depth and 3.0 cm diameter. The glass vessels were inverted over Petri dishes lined with filter paper for collection of the eggs. The oil extract of root and stem barks measured with the aid of graduates' syringe at concentration 0.0, 5%, 10%, 15%, 20% and 25% were administered on the filter papers and were allowed to air dried for four hours and placed inside plastic container containing 20 g of maize grains maintained in an airtight condition. Twenty eggs were placed on the treated samples in the plastic container, while 20 eggs were introduced into untreated samples to serve as control. The set up were replicated three times. The eggs were examined daily and the total number of eggs that hatched was recorded. Solvent control treatments were also set up to see the effect of solvent on hatchability and adult emergence. The same procedures were used for larval. Ten larvae were introduced into a plastic container measuring 8 cm depth, 4 cm diameter containing 20 g of maize grains with filter treated at the following concentrations 5%, 10%, 15%, 20% and 25% of oil extract of root and stem barks and replicated thrice. The control experiment i.e., untreated filter paper and solvents treated filter paper were also prepared and replicated three times. Then, larvae mortality was counted at 24, 48, 72 and 96 hr after application.

Fumigant effect of plant oils on the mortality of adult *Plodia interpunctella*

Filter paper measuring 4 × 3.5 cm and 0.5 mm thick was impregnated into the root oil and stem oil at required concentrations of 5%, 10%, 15%, 20% and 25% which were allowed to air dried for 4 hours and then placed inside a plastic container of 20 g of maize. Ten pairs of newly emerged adult *Plodia interpunctella* were introduced into the containers and covered to create airtight condition. Untreated paper was treated with 3.3 ml of ethanol also as solvent control experiment while untreated filter paper was also prepared as the other control. The set up was replicated three times. Adult mortality was counted at 24, 48, 72 and 96 hr after application. At the end of the 96 hours post treatment data on percentage adult mortality was corrected using Abbott (1925) formula, thus

$$Abbottcorrectedmortality = \frac{\%mortality\,\text{int}\,reatment - \%mortalityincontrol}{100 - \%mortalityincontrol \times 100}$$

Where PT=Corrected Adult Mortality

PO=Percentage mortality of treated insects

PC=Percentage mortality on untreated insects

Experimental animal

The animal experiment was approved and done in line with stated rules of ethics committee in animal care of the university (CERAD) with the reference number 241.

Adult male wistar rats weighing 150-160 g used for this experiment were purchased from the breeding colony of the Department of Biochemistry. The rats were maintained at 25°C on 24 hours light/dark cycle with free access to food and water. They were made to acclimate to a new environment for one week prior to the commencement of the experiment.

Animals grouping and extract administration

After a week of acclamation, they were randomly selected and grouped into sizes. Thirty-six wistar rats were randomly grouped into six (group 1-6) of six animals each. The root bark oil extract of *Cleitopholis patens* was administered orally to the rats using a cannular. Group 1 serves as the control group and received 3.8 ml dimethyl sulphide saline, while Group 2, 3, 4, 5 and 6 received 5%, 10%, 15%, 20% and 25% oil extract respectively for 24 hours i.e. a concentration of 5 ml was prepared by diluting 0.5 ml of plant oil extract in 9.5 ml of dimethyl sulphide saline, 10 ml concentration was made by 1.0 ml of plant extract in 9.0 ml of dimethyl sulphide saline. Also, 15 ml, 20 ml and 25 ml concentration were obtained by diluting 1.5 ml, 2.0 ml and 2.5 ml of the plant extract with 8.5 ml, 8.0 ml and 7.5 ml of the dimethyl suplhide saline respectively. Cage sides examination were performed to detect overt signs of toxicity (salivation, lacrimation, convulsion, loss of hair, stress, behavioral abnormalities, and dead rats). After 24 hr, the animals were sacrificed by cervical dislocation.

Biochemical parameter

The procedure described by Yakubu and Akanji was adopted for the preparation of serum. The animals were sacrificed by cervical dislocation and the blood collected by direct heart punctured into EDTA sample bottles

and spinned at 3000 rpm for 20 mins. The serum of both control and treated animals were carefully aspirated with Pasteur pipette into sample bottles for the various biochemical assays which are Alkaline Phosphate (ALP), Aspartate AminoTranferase (AST), Alanine amino Tranferase (ALT), urea, total bilirubin, direct bilirubin, and creatinine were products of Randox Laborites Limited, United Kingdom.

Data analysis

Data were subjected to Analysis of Variance (ANOVA) and treated means were separated using the New Duncan's Multiple Range test. The ANOVA was using SPSS 16.0 software.

RESULTS AND DISCUSSION

Fumigant toxicity of ethanolic oil extracts of *Cleistopholis patens* on egg hatchability and adult emergence of Plodia interpunctellla

The effect of *Cleistopholis patens* ethanolic oil extract on egg hatchability and adult emergence of *Plodia interpunctella* is presented in Table 1. All the concentration of the ethanolic oil extract of the root bark and stem bark of C. *patens* completely inhibited egg hatch and adult emergence of *Plodia interpunctella*. There were not significantly difference (P>0.05) in the percentage egg hatch and percentage adult emergence at all application concentrations of the root bark and stem bark oil extract when compared but differ significantly from untreated and the solvent control.

Fumigant toxicity of ethanoic oil extract of *C. patens* on larvae of *Plodia interpunctella*

Table 2 shows the fumigant effect of oil extract on third instar larvae of *Plodia interpunctella*. There was no mortality observed at 24 hr post treatment period in 5% concentrations of oil extract of the root bark and stem bark. At 10%, 15%, 20% and 25% concentration of the ethanolic oil extract of the root bark caused 20%, 33.3%, 50.0% and 60.0% larvae mortality respectively. At 48 hr post treatment, all concentrations of the root bark oil; 10%-25% concentrations of the stem bark oil caused 53.3%-86.67% larvae mortality and at 72 hr post treatment, all concentration of the root bark oil, and 10%-25% concentration of the stem bark oil evoked 60%-93% larvae mortality. In the treatment, concentration of the root bark oil, after 96 hr, 100% larvae mortality was obtained at 20% and 25% concentration. No concentration of the stem bark oil obtained 100% larvae. So, at 96 hr of post treatment, significant difference (P>0.05) existed between all the treatment used when compared with the untreated control and the solvent treated samples.

Fumigant toxicity of ethanolic oil extract of *C. patens* on adult mortality of Plodia interpunctellla

The fumigant effect of ethanolic oil extract of C. *patens* on adult mortality of *Plodia interpunctella* is presented in Table 3. The 10%-25% of the root bark oil, the 15%-2% of the stem bark oil caused 20%-60% adult mortality after 24 hr post treatment which were significantly different (P>0.05) from untreated and the solvent control. At 48 hr post treatment period, the root bark oil extract of *Cleistopholis patens* caused 50%-80% adult mortality of *Plodia interpunctella* at concentration rates 10%-25% respectively while the stem bark oil caused 50%-70% mortality of adult moth at concentration rates 15%-25%respectively which were significantly different from other concentration used. However, during the 72 hr post exposure period of the root bark oil, 100% adult mortality were obtained at concentration rates 20% and 25%. Whereas, all concentrations of the root bark oil, after 96 hr 100% mortality was obtained except at 5% and 10%. Nevertheless, in the 20% and 25% application concentration rates after 96 hr of exposure of adult moths to *Cleistopholis patens* stem bark ethanolic oil extract mortality of 100% was recorded and so, at 96 hr of post treatment, significant difference (P<0.05) existed between all the untreated and solvent control.

Effects of *C. patens* root bark oil extract on liver function of wistar rats

Table 4 presents the effects of C. *patens* root bark oil extract on liver function parameter of albino rats. There was no significant alteration (P>0.05) in the liver biochemical parameters, Aspirate Aminotranferase (AST), Alanine Aminotransferase (ALT) and alkaline phosphatase in rates with 5%, 10%, 15%, 20% and 25% of C. *patens* root bark oil extract in comparison with wistar rats treated with dimethyl sulphide in control.

Effects of *C. patens* root bark oil extract on kidney functions of wistar rats

Table 5 shows the effects of C. *patens* root bark oil extract on kidney function parameter of albino rats. The kidney activities of urea, total bilirubin, and creatinine of the animals treated with 5%, 15%, 20% and 25% C. *patens* root bark oil extract respectively were not significantly different (P>0.05) with treatment in control groups.

TABLE 1

Fumigant toxicity of ethanolic oil extract of *C. patens* on larvae of *P. interpunctella*

Plant oil	Concentration (%)	Mean % mortality after			
		24 hr	48 hr	72 hr	96 hr
Root bark oil	5	0.00 ± 0.00^{a}	46.67 ± 3.33^{b}	60.00 ± 5.77^{b}	63.33 ± 3.33^{b}
	10	20.00 ± 10.00^{b}	53.33 ± 3.33^{c}	70.00 ± 5.77^{e}	80.00 ± 5.77^{c}
	15	33.33 ± 3.33^{c}	66.67 ± 3.33^{d}	83.33 ± 3.33^{d}	90.00 ± 0.00^{d}
	20	50.00 ± 5.77^{d}	86.67 ± 3.33^{e}	93.33 ± 3.33e	100.00 ± 0.00^{e}
	25	60.00 ± 0.00^{c}	86.67 ± 3.33^{e}	93.33 ± 3.33^{e}	100.00 ± 0.00^{e}
	Control 1	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	Control 2	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Stem bark oil	5	0.00 ± 0.00^{a}	36.67 ± 3.33^{b}	56.67 ± 3.33^{b}	60.00 ± 5.77^{b}
	10	6.67 ± 6.67^{b}	43.33 ± 3.33^{c}	60.00 ± 5.77^{c}	70.00 ± 5.77^{c}
	15	23.33 ± 3.33^{c}	56.67 ± 3.33^{d}	73.33 ± 3.33^{d}	80.00 ± 0.00^{d}
	20	36.67 ± 3.33^{d}	76.67 ± 3.33^{c}	83.33 ± 3.33^{e}	90.00 ± 0.00^{e}
	25	40.00 ± 5.77^{c}	76.67 ± 3.33^{c}	86.67 ± 3.33^{e}	90.00 ± 0.00^{e}
	Control 1	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	Control 2	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	33.33 ± 3.33^{a}	3.33 ± 3.33^{a}

Note: a, b, c, d, e means followed by the same letter in the same column are not significantly difference at P>0.05; Control 1 is the maize grain treated with neither extract nor solvent; Control 2 is the grains treated with 3.3 ml of ethanol.

TABLE 2

Fumigant toxicity of ethanolic oil extract on egg hatchability and adult emergence of *Plodia interpunctella*

Plant oil	Conc. (%)	Eggs hatch	% Adults emergence
Root bark	5	0.00 ± 0.00[a]	0.00 ± 0.00[a]
	10	0.00 ± 0.00[a]	0.00 ± 0.00[a]
	15	0.00 ± 0.00[a]	0.00 ± 0.00[a]
	20	0.00 ± 0.00[a]	0.00 ± 0.00[a]
	25	0.00 ± 0.00[a]	0.00 ± 0.00[a]
	Control 1	73.33 ± 3.33[b]	63.33 ± 3.33[b]
	Control 2	69.33 ± 3.33[b]	60.00 ± 0.00[b]
Stem bark	5	0.00 ± 0.00[a]	0.00 ± 0.00[a]
	10	0.00 ± 0.00[a]	0.00 ± 0.00[a]
	15	0.00 ± 0.00[a]	0.00 ± 0.00[a]
	20	0.00 ± 0.00[a]	0.00 ± 0.00[a]
	25	0.00 ± 0.00[a]	0.00 ± 0.00[a]
	Control 1	76.67 ± 3.33[b]	0.00 ± 0.00[a]
	Control 2	70.00 ± 0.00[b]	66.33 ± 3.33[b]

Note: a, b means followed by the same letter in the same column are not significantly difference at P>0.05; Control 1 is the maize grain treated with neither extract nor solvent; Control 2 is the grains treated with 3.3 ml of ethanol.

TABLE 3

Fumigant toxicity of ethanolic oil extract of *C. patens* on adult mortality of *P. interpunctella*

Plant oil	Concentration (%)	Mean % mortality after			
		24 hr	48 hr	72 hr	96 hr
Root bark oil	5	0.00 ± 0.00[a]	20.00 ± 0.00[b]	33.33 ± 3.33[b]	53.33 ± 3.33[c]
	10	20.00 ± 10.00[b]	60.00 ± 0.00[c]	76.67 ± 3.33[c]	90.00 ± 5.77[d]
	15	30.00 ± 0.00[c]	66.67 ± 3.33[d]	83.33 ± 3.33[d]	100.00 ± 0.00[e]
	20	56.67 ± 3.33[d]	80.00 ± 0.00[e]	100.00 ± 0.00[e]	100.00 ± 0.00[e]
	25	60.00 ± 5.77[d]	80.00 ± 0.00[e]	100.00 ± 0.00[e]	100.00 ± 0.00e
	Control 1	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	3.33 ± 3.33[a]
	Control 2	0.00 ± 0.00[a]	0.00 ± 0.00[a]	3.33 ± 3.33[a]	10.00 ± 5.77[b]
Stem bark oil	5	0.00 ± 0.00[a]	6.67 ± 3.33[b]	26.67 ± 6.67[b]	46.67 ± 6.66[b]
	10	3.33 ± 3.33[a]	46.67 ± 3.33[c]	63.33 ± 3.33[c]	73.33 ± 3.33[c]
	15	20.00 ± 0.00[b]	53.33 ± 6.67[d]	66.67 ± 3.33[d]	76.67 ± 3.33[c]
	20	36.67 ± 3.33[c]	70.00 ± 0.00[c]	83.33 ± 3.33[e]	100.00 ± 0.00[d]
	25	46.67 ± 3.33[d]	70.00 ± 0.00[c]	86.67 ± 3.33[e]	100.00 ± 0.00[d]
	Control 1	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
	Control 2	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	3.33 ± 3.33[a]

Note: a, b, c, d, e means followed by the same letter in the same column are not significantly difference at P>0.05; Control 1 is the maize grain treated with neither extract nor solvent; Control 2 is the grains treated with 3.3 ml of ethanol.

TABLE 4

Liver function parameters of wistar rats administered with oil extract of *C. patens*

Groups	Concentration of extracts (%)	AST (U/L)	ALT (U/L)	ALP (U/L)
I	0	26.67 ± 0.33[a]	48.67 ± 2.97[a]	101.00 ± 1.00[a]
II	5	25.00 ± 1.52[a]	47.67 ± 3.75[a]	100.00 ± 0.57[a]
III	10	25.00 ± 3.33[a]	48.67 ± 3.70[a]	101.30 ± 2.33[a]
IV	15	26.67 ± 0.33[a]	49.33 ± 3.33[a]	101.00 ± 1.00[a]
V	20	26.67 ± 0.33[a]	48.67 ± 0.33[a]	102.4 ± 0.33[a]
VI	25	26.67 ± 0.33[a]	49.33 ± 0.89[a]	102.7 ± 0.33[a]

Note: Each value is a mean ± standard error of three replicates; a, b Means followed by the same letter along the column are not significantly different (P>0.05) using New Duncan's Multiple Range Test; ALT=Alanine amino transferase; AST=Aspartate amino transferase; ALP=Alkaline phosphatase.

TABLE 5
Kidney function parameters of wistar rats administered oil extract on *C. patens* root bark

Groups	Concentration of extracts (%)	Urea (m/mol)	Total bilirubin (umol/l)	Direct bilirubin (umol/l)	Creatinine (umol/l)
I	0	2.28 ± 0.00[a]	16.33 ± 0.67[a]	335.7 ± 0.88[a]	90.07 ± 0.00[a]
II	5	2.30 ± 0.20[a]	16.33 ± 0.67[a]	335.7 ± 0.88[a]	90.75 ± 0.68[a]
III	10	2.30 ± 0.20[a]	17.00 ± 1.15[a]	336.0 ± 0.00[a]	90.09 ± 13.00[a]
IV	15	2.30 ± 0.20[a]	17.67 ± 0.67[a]	336.3 ± 0.33[a]	90.09 ± 13.00[a]
V	20	2.30 ± 0.20[a]	17.67 ± 0.67[a]	336.7 ± 0.33[a]	90.77 ± 13.10[a]
VI	25	2.33 ± 0.23[a]	17.33 ± 1.45[a]	336.7 ± 0.33[a]	90.09 ± 13.00[a]

Note: Each value is a mean ± standard error of three replicates; a, b Means followed by the same letter along the column are not significantly different (P>0.05) using New Duncan's Multiple Range Test.

Fumigant toxicity of ethanolic oil extract of *C. patens* on egg hatchability and adult emergence, larvae, and adult moths of *P. interpunctella*

The oil extracts of this plant are used in control of stored product coleopteran and lepidoptera because of their relative high efficacy on all developmental stages of insect. The result obtained from this research showed that the ethanolic oil extract of C. *patens* root bark and stem bark at an application rate of 5%, 10%, 15%, 20% and 25% inhibited egg hatch and development to adult stage significantly. This may be as result the inhibition of gaseous exchange between the eggs and the external environment caused by the plant oil which led to inability of the eggs to hatch [36]. On the other hand, the result of this study indicated that the oil extract of the root and stem barks of this plant had obvious effect on post embryonic survival of this effect which resulted in prevention of adult emergence at different concentration as suggested by Annie Bright et al., [37] and Shifa et al., [38].

The ability of the ethanolic oils extract to affect larvae mortality within 96 hr after application can be attributed to fumigant effect of the plant oil on the larvae. This finding agreed with the report of Adedire et al., [21] that Monodara temifolia seed oil was highly toxic to adult and immature stages of C. maculatus thus effectively protecting stored cowpea seed against insect attack. The root bark oil extract of C. *patens* was the most toxic of all the plant parts evaluated against the larval of the moth *P. interpunctella* because 100% mortality was obtained at an application rate of 20%, 25% using ethanol extract, while no rates of C. *patens* stem bark oil extract attained 100% larval mortality but the percentage mortality produced at 96 hrs post treatment was significantly higher than the control. The result of this research was in accordance with the Akinneye et al., [33] in which C. *patens* ethanolic oil was able to cause larval mortality of *Ephestia cautella* within 96 hrs of application.

Plant oils are commonly used in insect control because they are highly effective against virtually all life stages of insects [21,22,39].

The root bark oil of C. *patens* resulted in higher mortality of *P. interpunctella* compared to the stem bark oil of C. *patens*. This finding conforms to the report of Yalamanchilli and Punukollu [40] who observed that the oil from the leaves of *Curucuma domestica* could effectively protect the cowpea seeds, against *Callosobruchus chinesis* at 2% concentration. The ability of ethanolic oil extracts to effect moth mortality within 72 hrs after application may be attributed to fumigant effect of the plant oil on the adult moth. This finding agreed with the report of Ukeh and Umoetolk [41] that oils extract from the leaves of *Eucalyptus saligna* and *Cupresus sempervirens* against *Sitophilus zeamais* and *Tribolium confusium*, considerably reduce the first filial generation of the pest. The fumigant action of the oil may cause death through respiratory inhibition, inhibition of oxidative phosphorylation and amide metabolism [25]. At 96 hr post treatment, root bark oil of C. *patens* was earliest to attain 100% adult mortality at 15% concentration rate. Whereas the stem barks oil of C. *patens* also attained 100% mortality at 20% concentration within 96 hrs post treatment. The finding coincides with the findings of Ajayi et al., [42] that oil obtained from *Zylopia aethiopica* were the most effective against *Tribolium castaneum* adults. The root bark oil extract of C. *patens* was found to be most effective against adult mortality. This finding agreed with the report of Donald and Ukeh [43] that oil of *Aframomum melegueta* was more effective adult mortality of *Rhizopertha dominica*.

Toxicity of *C. patens* root bark oil extract on liver and kidney functions of wistar rats

Assessment of liver and kidney function is very important in toxicity evaluation of drugs and plants extracts as organs are necessary for the survival of all organisms [44]. Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) are markers of liver damage and can thus be used to assess liver cytolysis with ALT being a more sensitive biomarker of hepatotoxicity than AST [45]. The reduction in the activity of the ALT and AST from the liver without the corresponding increase in the serum enzyme could be due to inhibition of the enzyme activity by components of the extracts [46]. In the present study, the toxicity of ethanolic oil extract of C. *patens* root bark at 5%, 10%, 15%, 20% and 25%/kg body weight did not induce any damage to the liver after 24 hr oral route in albino rats compared with albino rats in the control because the oil extract caused no significant increase in the level of AST and ALT. This agreed with the work of Olorunnisola et al., [47] in which the toxicity of *Anacadium occidentale* observed produced no toxic effect in mice treated with aqueous extracts of *A. occidentale* at doses of 2 g-6 g/kg body weight by oral route.

While Arafa [48] reported no damage in the liver because there is no significant increase in serum level of AST and ALT, this was observed in the experimental studies on the hypolipidemic and haematological properties of aqueous leaf extract of *Cleistopholis patens* in rat. Alkaline phosphatase (ALP) is a plasma membrane marker enzyme and is often employed to assess the integrity of the plasma membrane [46]. The increase in the ALP activity in the serum during both the treatment and recovery periods could be a consequence of leakage of the enzyme from other tissues apart from small intestine and kidney or a reduced rate of clearance of the enzyme from the serum [49]. The observed normal Alkaline Phosphatase (ALP) activities in the liver following the administration of 5%, 10%, 15%, 20% and 25% oil extract of C. *patens* root bark does not hinder the transportation of the required molecules across the plasma membrane when compared with the rat in the control group. This was like the finding of Brown et al., [50] in the activity of *Azadirachta indica* seeds and peel of *Citrus sinesis* on the liver enzymes of albino rats. The oil extract of C. *patens* root bark did not show any significant effect nor caused liver injury, since there is no significant increase in the serum level of ALP compared with the control group. This agreed with the work of Assy et al., [51] observed that there was no change in the serum level of ALP in the anti-hyper lipidemia activity of *T. violacea* rhizome extract. Kidney is to excrete the waste products of metabolism and to regulate the body concentration of water and salt. When there is a compromised of the normal glomerular function, substances normally cleared by the kidney such as urea, creatinine, total bilirubin, and direct bilirubin accumulate in the biological fluid. One of the objectives of this study was to investigate the toxicity of C. *patens* root bark oil by assessment of the urea, creatinine, total bilirubin, and direct bilirubin. Urea is a byproduct from protein breakdown. About 90% of urea products are excreted through the kidney.

Meanwhile, creatinine is a waste product from a muscle creatinine, which is used during muscle contraction. Creatinine is commonly measured as an index of glomerular function. Therefore, damage to the kidney will make the kidney inefficient to excrete both urea and creatinine and causes their accumulation in the serum. The urea, creatinine, total bilirubin of the rats administered with 5%, 10%, 15%, 20% and 25% concentration of the extract of C. *patens* root bark oil compared with the rats in the control group after 24 hrs of oral route, showed that there was no significant change of urea, creatinine, total bilirubin and direct bilirubin which indicate no kidney damage. Similar observations have been made by Bamisaye et al., [44], Doliah et al., [52] in normal rats treated with extract of *Morinda lucida*, caster seed oil and powder *Nigella sativa* for 7 days, 30 days and five weeks

respectively. These results suggested that the evidence of normal AST, ALT, ALP, urea, creatinine, total bilirubin and direct bilirubin level in the serum, the ethanolic oil extract of *C. patens* root bark does not alter the liver and kidney function [53].

CONCLUSION

The research study showed that the root bark oil extract of *Cleistopholis patens* is the most effective for the control of *Plodia interpunctella* on maize grains since they completely inhibited development of *Plodia interpunctella* from eggs to adult stage. The oil extract of *C. patens* root bark was tested on albino rat and are found to be non-toxic since there are no significant differences between the control and the animals treated oil extract of *C. patens* root bark. Therefore, oil extract of *C. patens* root bark could be recommended for use to protect stored maize grains and can also be integrated with other pest management procedures, since the maize grains are safe for consumption even after post-harvest treatment.

Recommendations

Further research could be carried out on characterization of the oil root and stem extracts of *Cleistopholis patens* and tested as fumigant on all stages of *Plodia interpunctella*.

REFERENCES

1. Rees D. Insects of stored grain: a pocket reference. CSIRO publishing; 2007.
2. Mohandass S, Arthur FH, Zhu KY, et al. Biology and management of *Plodia interpunctella* (Lepidoptera: Pyralidae) in stored products. J Stored Prod Res. 2007; 43(3):302-311.
3. Silhacek D, Murphy C, Arbogast RT. Behavior and movements of Indian meal moths (*Plodia interpunctella* Hübner) during commodity infestation. J Stored Prod Res. 2003; 39(2):171-184.
4. Ileke KD, Oni MO. Toxicity of some plant powders to maize weevil, *Sitophilus zeamais* (motschulsky)[Coleoptera: Curculiondae] on stored wheat grains (*Triticum aestivum*). Afr J Agric Res. 2011; 6(13):3043-3048.
5. Adedire CO, Obembe OM, Akinkurolere RO, et al. Response of *Callosobruchus maculatus* (Coleoptera: Chrysomelidae: Bruchinae) to extracts of cashew kernels. J Plant Dis Prot. 2011; 118:75-79.
6. Tsuji H. Ability of first instar larvae of the Indian-meal moth, *Plodia interpunctella* Hubner, to reach their food. Med Entomol Zool. 2000; 51(4):283-287.
7. Almasi R. The effect of nutrition on fertility and number of generations of Indian meal moth *Plodia interpunctella* Hbn. Lepidotera: Pryralidae), infesting cocoa beans in storage. 1984:25.
8. Fasulo TR, Knox MA. Indianmeal Moth, *Plodia interpunctella* (Hubner). 2015.
9. Ramanujam B, Rangeshwaran R, Sivakmar G, et al. Management of insect pests by microorganisms. Proc Indian National Sci Acad. 2014; 80(2):455-471.
10. Grieshop MJ. Evaluation of three species of *Trichogramma* egg parasitoids for the biological control of the Indianmeal moth in retail stores and warehouses. Kansas State University; 2005.
11. Fields PG. The control of stored-product insects and mites with extreme temperatures. J Stored Prod Res. 1992; 28(2):89-118.
12. Sauer JA, Shelton MD. High-temperature controlled atmosphere for post-harvest control of indian meal moth (Lepidoptera: Pyralidae) on preserved flowers. J Econ Entomol. 2002; 95(5):1074-1078.
13. Olorunsogbon ST, Akinneye J, Olowu E. Fumigant Toxicity of *Cleistopholis patens* (Benth) Oil extracts on *Plodia interpunctella* (Hubner) and Its Toxicological Effects in Wistar Rats.
14. Attia FI. Insecticide resistance in *Plodia interpunctella* (Hubner)(Lepidoptera: Pyralidae) in New South Wales, Australia. Aust J Entomol. 1977; 16(2):149-152.
15. Attia FI. Insecticide resistance in pyralid moths of grain and stored products. Gen Appl Entomol: The J Ent Soci of New South Wales. 1981; 13:3-8.
16. Arthur FH, Phillips TW, Hui YH, et al. Stored-product insect pest management and control. In Food plant sanitation. 2003; 341-358.
17. McGaughey WH, Johnson DE. Indianmeal moth (Lepidoptera: Pyralidae) resistance to different strains and mixtures of *Bacillus thuringiensis*. J Econ Entomol. 1992; 85(5):1594-1600.
18. Herrero S, Oppert B, Ferré J. Different mechanisms of resistance to *Bacillus thuringiensis* toxins in the Indianmeal moth. Appl Environ Microbi. 2001; 67(3):1085-1089.
19. Sharaby A. Anti-insect properties of the essential oil of lemon grass, *Cymbopogen citratus* against the lesser cotton leafworm *Spodoptera exigua* (Hbn). Int J Trop Insect Sci. 1988; 9:77-80.
20. Leelaja BC, Rajashekar Y, Reddy PV, et al. Enhanced fumigant toxicity of allyl acetate to stored-product beetles in the presence of carbon dioxide. J Stored Prod Res. 2007; 43(1):45-48.
21. Adedire CO, Lajide L. Ability of extract of ten tropical plant species to protect maize grains against infestation by the maize weevil *Sitophilus zeamais* during storage. Nigerian J Exp Biol. 2003; 4(2):175-179.
22. Arannilewa ST, Ekrakene T, Akinneye JO. Laboratory evaluation of four medicinal plants as protectants against the maize weevil, *Sitophilus zeamais* (Mots). Afr J Biotechnol. 2006; 5(21).
23. Ashamo MO, Odeyemi OO, Ogungbite OC. Protection of cowpea, *Vigna unguiculata* L.(Walp.) with *Newbouldia laevis* (Seem.) extracts against infestation by *Callosobruchus maculatus* (Fabricius). Arch Phytopathol Plant Prot. 2013; 46(11):1295-1306.
24. Akinkurolere RO, Adedire CO, Odeyemi OO. Laboratory evaluation of the toxic properties of forest anchomanes, *Anchomanes difformis* against pulse beetle *Callosobruchus maculatus* (Coleoptera: Bruchidae). Insect Sci. 2006; 13(1):25-29.
25. Onaolapo Akinneye J, Ayobami Oyeniyi E. Insecticidal efficacy of *Cleistopholis patens* (Benth) against *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae) infesting rice grains in Nigeria. J Crop Prot. 2016; 5(1):1-0.
26. Adonu Cyril C, Ugwu OP, Esimone Co O, et al. Phytochemical analyses of the menthanol, hot water and n-hexane extracts of the aerial parts of *Cassytha filiformis* (Linn) and leaves of *cleistopholis patens*. Res. J. Pharm. Biol. Chem. Sci. 2013; 4:1143-1149.
27. Akinmoladun AC, Ibukun EO, Afor E, et al. Chemical constituents and antioxidant activity of *Alstonia boonei*. Afr J Biotechnol. 2007; 6(10).
28. Amole OO, Ilori OO. Antimicrobial activity of the aqueous and ethanolic extracts of the stem bark of *Alstonia boonei*. Int J Phytopharm. 2010; 1(2):119-123.
29. Akinkurolere RO, Boyer S, Chen H, et al. Parasitism and host-location preference in *Habrobracon hebetor* (Hymenoptera: Braconidae): role of refuge, choice, and host instar. J Econ Entomol. 2009; 102(2):610-615.
30. Akinkurolere RO. Assessment of the insecticidal properties of *Anchomanes difformis* (P. Beauv.) powder on five beetles of stored produce. J Entomol. 2007; 4(1):51-55.
31. El-Salam A, Ahmed ME. Fumigant toxicity of seven essential oils against the cowpea weevil, *Callosobruchus maculatus* (F.) and the rice weevil, *Sitophilus oryzae* (L.). Egypt Acad J Biol Sci F Toxicol Pest Control. 2010; 2(1):1-6.
32. Derbalah A, Ahmed S. Oil and powder of spearmint as an alternative to Sitophilus oryzae chemical control of wheat grains. J Plant Prot Res. 2011.
33. Akinneye JO. Aspects of Biology and Control of Tropical Warehouse Moth, *E. cautella* (Walker)(Lepidotera: Pyralidae) in Cocoa Beans.
34. Olawumi FK. Some aspects of biology and control of *Plodia interpunctella*. 2014.
35. Arthur FH. Grain protectants: current status and prospects for the future. J Stored Prod Res. 1996; 32(4):293-302.
36. Akinneye JO. Biology and control of the yam moth, Euzopheroides vapidella (Mann) Lepidotera: Pyralidae). Federal University of Technology, Akure, M. Tech.
37. Bright AA, Babu A, Ignacimuthu S, et al. Efficacy of crude extracts of *Andrographis paniculata* nees. on *Callosobruchus chinensis* L. during post-harvest storage of cowpea.
38. Vanmathi JS, Padmalatha C, Sing AJ, et al. Efficacy of selected plant extracts on the oviposition deterrent and adult emergence activity of *Callosobruchus maculatus* F (Bruchidae; Coeleoptera). Glob J Sci Front Res. 2010; 10(8):2-6.

39. Don-Pedro KN. Insecticidal activity of some vegetable oils against *Dermestes maculatus* Degeer (Coleoptera: Dermestidae) on dried fish. J Stored Prod Res. 1989; 25(2):81-86.

40. Yalamanchill TS, Punukollu DT. Activity of essential oils from *Curcuma domestica* leaf against *Callosobruchus chinesis*. J Stored Prod Res. 2000; 37:207-209.

41. Ukeh DA, Umoetok SB. Effects of host and non-hosts plant volatiles on the behaviour of the lesser grain borer, *Rhyzopertha dominica* (Fab.). J Entomol. 2007; 4(6):435-443.

42. Ajayi FA, Olonisakin A. Bio-activity of three essential oils extracted from edible seeds on the rust-red flour beetle, *Tribolium castaneum* (Herbst.) infesting stored pearl millet. Trakia J Sci. 2011; 9(1).

43. Ukeh DA. Bioactivities of essential oils of *Aframomum melegueta* and *Zingiber officinale* both (Zingiberaceae) against *Rhyzopertha dominica* (Fabricius). J Entomol. 2008; 5(3):193-199.

44. Bamisaye FA, Odutuga AA, Minari JB, et al. Evaluation of hypoglycemic and toxicological effects of leaf extracts of *Morinda lucida* in hyperglycemic albino rats. Int Res J Biochem Bioinforma. 2013; 3(2):37-43.

45. Adebayo AH, Abolaji AO, Opata TK, et al. Effects of ethanolic leaf extract of Chrysophyllum albidum G. on biochemical and haematological parameters of albino Wistar rats. Afr J Biotechnol. 2010; 9(14):2145-2150.

46. Akanji MA, Olagoke OA, Oloyede OB. Effect of chronic consumption of metabisulphite on the integrity of the rat kidney cellular system. Toxicology. 1993; 81(3):173-179.

47. Olorunnisola DS, Amao IS, Ehigie DO, et al. Antihyperglycemic and hypolipidemic effect of ethanolic extract of Chrysophyllum albidum seed cotyledon in alloxan induced diabetic rats. Res J Appl Sci. 2008; 3(2):123-127.

48. Arafa HM. Curcumin attenuates diet-induced hypercholesterolemia in rats. Med Sci Monit: International Medical Journal of Experimental and Clinical Research. 2005; 11(7):BR228-234.

49. Ribarova F, Atanassova M, Marinova D, et al. Total phenolics and flavonoids in Bulgarian fruits and vegetables. J U Chem Metal. 2005; 40(3):255-260.

50. Brown AS, Bakker-Arkema RG, Yellen L, et al. Treating patients with documented atherosclerosis to National Cholesterol Education Program-recommended low-density-lipoprotein cholesterol goals with atorvastatin, fluvastatin, lovastatin and simvastatin. J Am Coll Cardiol. 1998; 32(3):665-672.

51. Assy N, Kaita K, Mymin D, et al. Fatty infiltration of liver in hyperlipidemic patients. Dig Dis Sci. 2000; 45:1929-1934.

52. Dollah MA, Parhizkar S, Izwan M. Effect of *Nigella sativa* on the kidney function in rats. Avicenna J Phytomed. 2013; 3(2):152.

53. Ashamo MO. Development of the yam moth, *Dasyses rugosella* Stainton (Lepidoptera: Tineidae) in different species of yam/Entwicklung der Yamsmotte, *Dasyses rugosella* Stainton (Lepidoptera: Tineidae) an verschiedenen Arten von Yam. J Plant Dis Prot. 2005; 398-404.