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Studies on *Cleistopholis patens* (benth.) [Magnoliales: Annonaceae] powders as fumigant and contact insecticides to *Plodia interpunctella* (Hübner) [Lepidoptera: Pyralidae]

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Post-harvest losses of agricultural produce caused by store grain pest most especially cereals and grains to store pest require developing cheap, ecofriendly and readily available insecticide to combat this threat and achieve the goal of food security in developing countries. This study investigated the effects of *Cleistopholis patens* (leaf and stem bark) on *Plodia interpunctella* infestation in stored maize grains. Powders from the plant were administered at 0.5, 1.0, 1.5, 2.0 and 2.5 g dosages to maize grains containing developmental stages of *P. interpunctella* both as contact and fumigant insecticides. The insecticidal activities were monitored at 24, 48, 72 and 96 h post-treatment periods. As a contact insecticide, *C. patens* was significantly (P<0.05) more effective than as a fumigant against all developmental stages of the pest. The leaf powder was observed to be weakly effective both as contact and fumigant against *P. interpunctella*. At its peak, 69.17% larval mortality was achieved at 2.5 g dosage after 96 h exposure, but 1.5 g dosage of the stem bark achieved 0% egg hatchability and 100% larval and adult mortalities at the same length of exposure. Inferences from these results suggest that the plant has some bioactive constituents which if properly harnessed can be co-opted into integrated management of *P. interpunctella* infesting stored products.

Key words: hatchability, contact, fumigant, bioactivity, Indian meal moth and post- treatment.

INTRODUCTION

Damages to stored grains and their products by insects had been estimated as 5-10% in the temperate countries and 20-30% in the tropical zones (Nakakita, 1998). Grain storage around the world had been relying so heavily on the use of synthetic pesticides, which of course have played a major role in food storage and protection and have tremendously benefited humankind in the past. Aside these great contributions, their continued usage has triggered several ecological, resistance and health-

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related challenges (Verma and Dubey, 1999).

It has been reported that over 2.5 million types of such pesticides are used in the agricultural crop protection annually across the globe and that over \$100 billion was being spent annually to either combat or manage the side effects of these pesticides on man and environments (USEPA, 2011). Hence the search for ecofriendly and biodegradable pesticides for crop protection and management had been greatly encouraged over the last

Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> five decades (Sengottaiyan, 2013). It is expected that the ideal insecticide should control the target pest adequately, rapidly degradable and non-toxic to human and livestock. The use of botanical pesticides to make up for various shortfalls identified with synthetic pesticides had been promising over the years. There have been reviews on the use of plants' secondary metabolites / phytochemicals to control the threat of pests' infestation on stored grains by several authors (Mason et al., 1987; Rosenthal and Berenaum, 1992; Tan and Luo, 2011). This study is therefore designed to assess the bioactivity of the powders from Cleistopholis patens (Benth.) Engl. & Diels against the egg, larval and adult stages of the Indian meal moth, Plodia interpunctella (Hübner, 1813), a world-wide insect pest of stored products and processed commodities.

MATERIALS AND METHODS

Sourcing of plant materials

The required quantity of the plant *C. patens* was completely uprooted from the source in the forest region along Akure-Ondo Road, Akure, Nigeria (located at latitude 7.2571°N and longitude 5.2058°E). The maize grains were bought at Isikan Market, Akure, Nigeria.

Preparation of plant materials

C. patens samples collected from the field were transported in protective bags to the Biology laboratory 2 of the Federal University of Technology, Akure. The plants were thoroughly washed with water, then the root barks were carefully removed with sharp knife and air-dried in the laboratory for 50 days, after which they were pulverized into fine powder using Binatone electric blender (Model 373). The powdery samples were further sieved to pass through 1 perforations to obtain labeled samples of fine powders that mm² were kept in separate airtight plastic containers and stored at ambient temperature of 28±2°C and 75 ± 5% Rh pending use. The maize grains used were first sorted to remove both contaminants and damaged ones. The grains were later disinfected in the oven at 60°C for 4 h and allowed to cool on open laboratory bench for 5-6 h before been stored in plastic containers until usage. Who identified the botanical specimens of C. patens? and in which collections C. patens specimen vouchers were deposited?

Insect culture

500 g of maize grains was weighed into two kilner jars (1 L each). Ten newly emerged adults 5 males and 5 females *P. interpunctella* were introduced into each of the jars. The jars were kept in the culturing chamber until the F1 generation emerged. Insects were identified using standard entomological keys.

Contact bioassay of *C. patens* against stages of development of *P. interpunctella*

Twenty freshly laid eggs (0-24 h old) were placed on 20 g of maize grains treated with 0.5, 1.0, 1.5, 2.0, 2.5 g and control (untreated) leaf and stem bark powders were separately placed inside plastic container (8 cm diameter and 4 cm depth). Each treatment was

replicated thrice with a corresponding control. Daily observations were made with dissecting microscope to determine the number of hatching eggs from the total number of infested eggs. The experiments were randomly arranged and kept inside a breeding cage with wire mesh cage (75 \times 50 \times 60 cm). After the hatchability period (0-7days) the rearing containers were covered with muslin cloths and held in place with rubber bands; and during 40 days, the number and percentage of adult emerged was determined. The aforementioned procedure was repeated for both the larvae and adult contact bioassay. The same procedure was repeated for the larval and adult experiments; but the container covers were punched with hot iron rod and lined with muslin on the inside to prevent larval and adult from escaping and allow aeration. Ten larvae (third instar) were introduced into each bioassay treated and untreated grains and were replicated three times. The number of dead larvae and adult were counted and percentage mortality was determined.

Fumigant bioassay of *C. patens* against stages of development of *P. interpunctella*

The following dosages; 0.5, 1.0, 1.5, 2.0 and 2.5 g and control (untreated) leaf and stem powders of the plant were separately weighed and sealed in muslin cloth (5cm by 5cm); they were hung on the lid of each of the plastic containers (8 cm depth 4 cm diameter). Twenty freshly laid eggs (0-24 h old) were introduced into each of the plastic containers containing 20 g of maize grains and covered with lid. The plant powder was hung between the lid and the bottom and was made air tight at equal distance. The treated and the control (untreated) were replicated three times. Daily observations were made using dissecting microscope to determine the number of eggs hatched from the total numbers of eggs introduced and the experiment was left inside the insect breeding wire mesh cage pending adult emergence. At the end of 40 days post-treatment period the total number of adults that emerged was determined and percentage mean was calculated. The same procedure was repeated for larvae. The dead larvae were counted and percentage mortality calculated after 24, 48, 72 and 96 h post treatment. The same procedure was repeated for the adult experiments.

The fumigant protocols are the same as the contact protocols, except that leaf and stem powders of the plant were separately weighed and sealed in muslin cloth (5 cm by 5 cm); they were hung on the lid of each of the plastic containers. The plant powder was hung between the lid and the bottom and was made air tight at equal distance. However, it is not clear whether the effect was caused by the release of gases from the powder or by particles from the powder itself. It is suggested to delete this part because *C. patens* was not actually used as a fumigant according to the protocols written in this study. Nevertheless, if the author or authors decide to keep it, then they have to do a one-way analysis of variance to compare Contact vs Fumigant toxicity.

Data analysis

Analysis of data was done using SPSS version 23. Means were separated using Turkey's test.

RESULTS

Contact bioassay of *C. patens* against stages of development of *P. interpunctella*

The leaf powder of *C. patens* was observed to be weakly

Dosage (g)	Egg hatchability (%)	Length of days for adult emergence	Adult emergence (%)
0.5	80.00±2.89 ^b	27.67±0.33 ^{ab}	68.33±1.67 ^{cd}
1.0	73.33±1.67 ^{ab}	28.00±0.58 ^{ab}	65.00±2.89 ^{bcd}
1.5	68.33±3.33 ^a	30.00±0.58 ^b	61.67±1.67 ^{bc}
2.0	66.67±1.67 ^a	34.33±0.67 ^c	58.33±1.67 ^{ab}
2.5	63.33±1.67 ^a	36.33±0.67 ^c	51.67±1.67 ^a
Control	83.33±1.67 ^b	27.00±0.58 ^a	71.67±1.67 ^d

Table 1. Contact toxicity of leaf powder of C. patens to eggs of P. interpunctella.

Means followed by the same letter(s) within the column are not significantly different (P<0.05) using Tukey's Test.

Table 2. Contact toxicity of leaf powder of C. patens to larvae of P. interpunctella.

Dosage (g)	24 h	48 h	72 h	96 h
0.5	8.33±1.67 ^a	13.68±3.16 ^b	23.43±4.19 ^b	24.84±4.58 ^b
1.0	13.33±1.67 ^a	24.12±1.58 ^c	23.43±2.89 ^b	26.91±1.75 ^b
1.5	13.33±1.67 ^a	36.23±0.61 ^d	47.27±0.10 ^c	44.23±1.70 ^c
2.0	16.67±4.41 ^a	43.16±2.19 ^{de}	54.28±4.72 ^c	55.77±1.70 ^d
2.5	16.67±6.01 ^a	48.25±3.16 ^e	59.96±1.92 ^c	69.17±2.28 ^e
Control	20.00±7.64 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a

Means followed by the same letter(s) within the column are not significantly different (P<0.05) using Tukey's test.

Dosage (g)	24 h	48 h	72 h	96 h
0.5	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
1.0	0.00 ± 0.00^{a}	6.67±3.33 ^{ab}	7.04±3.53 ^a	7.31±3.70 ^a
1.5	3.33±3.33 ^a	6.67±3.33 ^{ab}	10.37±5.79 ^{ab}	10.37±5.79 ^a
2.0	3.33±3.33 ^a	6.67±3.33 ^{ab}	14.54±2.76 ^{ab}	34.72±1.39 ^b
2.5	6.67±3.33 ^a	20.37±5.46 ^b	32.31±9.99 ^b	45.83±5.26 ^b
Control	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00 ± 0.00^{a}

Means followed by the same letter(s) within the column are not significantly different (P<0.05) using Tukey's test.

effective against the spread of *P. interpunctella* when used as contact insecticide. Table 1 shows 63.33% egg hatchability at the highest dosage of 2.5 g powder. The same dosage achieved 69.17 and 45.83% larval and adult mortalities respectively after 96 h post-treatment exposure (Tables 2 and 3).

Unlike the leaf powder, *C. patens* stem powder was observed to be very effective in the control of the developmental stages of *P. interpunctella*. Table 4 reveals the contact effects of the powder on egg hatchability. It shows that hatchability was completely suppressed to 0% at 1.5 g powder dosage as against 63. 33% recorded when the leaf powder was used. Table 5 reveals 100% larval mortality at 2.0 g powder dosage after 72 h post-treatment exposure. Also as contact insecticide, 100% adult mortality was achieved at 1.5 g dosage but after 96 h exposure as reflected in Table 6.

Fumigant bioassay of *C. patens* against stages of development of *P. interpunctella*

The leaf powder was slightly less effective in the control of the developmental stages of the pest when used as fumigant insecticide. Table 4 shows 68.33% egg hatchability at the highest powder dosage of 2.5 g. The same dosage yielded 36.26 and 53.33% larval and adult mortalities after 96 h post-treatment exposure (Tables 7 and 8). Tables 7 to 9 show that the fumigant insecticidal activities of the stem powder was observed to slightly less effective than the contact effect. Table 7 shows 0% hatchability at 2.0 g powder dosage. It was also 0%

Dosage (g)	Egg hatchability (%)	Length of days for adult emergence	Adult emergence (%)
0.5	41.67±6.01 ^b	32.00±0.58 ^b	16.67±1.67 ^b
1.0	10.00±2.89 ^a	0.00±0.00 ^a	0.00±0.00 ^a
1.5	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
2.0	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
2.5	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
Control	83.33±3.33 ^c	24.33±5.17 ^b	71.67±1.67 ^c

Table 4. Contact toxicity of stem bark powder of C. patens to eggs of P. interpunctella.

Means followed by the same letter(s) within the column are not significantly different (P<0.05) using Tukey's Test.

Table 5. Contact toxicity of stem bark powder of C. patens to larvae of P. interpunctella.

Dosage (g)	24 h	48 h	72 h	96 h
0.5	18.33±1.67 ^b	42.37±4.33 ^b	52.73±3.04 ^b	57.25±2.18 ^b
1.0	28.33±1.67 ^c	54.12±3.67 ^{bc}	69.35±5.78 ^{bc}	73.73±4.62 ^c
1.5	31.67±1.67 ^c	60.96±2.11 ^b	78.53±5.72 ^c	82.94±6.20 ^c
2.0	56.67±3.33 ^d	86.58±4.33 ^c	100.00±0.00 ^d	100.00±0.00 ^d
2.5	63.33±1.67 ^d	93.33±4.41°	98.25±1.75 ^d	100.00±0.00 ^d
Control	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a

Means followed by the same letter(s) within the column are not significantly different (P<0.05) using Tukey's Test.

Table 6. Contact toxicity of stem bark powder of C. patens to adult of P. interpunctella.

Dosage (g)	24 h	48 h	72 h	96 h
0.5	10.00±0.00 ^a	34.44±2.94 ^b	55.19±2.89 ^b	66.39±2.17 ^b
1.0	26.67±3.33 ^b	48.15±1.85 [°]	69.72±5.28 ^c	88.80±0.72 ^c
1.5	43.33±3.33 ^c	82.59±3.76 ^d	88.80±0.72 ^d	100.00±0.00 ^d
2.0	46.67±3.33 ^c	86.30±3.16 ^d	100.00±0.00 ^d	100.00±0.00 ^d
2.5	63.33±3.33 ^d	92.96±3.53 ^d	100.00±0.00 ^d	100.00±0.00 ^d
Control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00±0.00 ^a	0.00±0.00 ^a

Means followed by the same letter(s) within the column are not significantly different (P<0.05) using Tukey's Test.

under contact treatment but at 1.5 g dosage (Tables 10 to 12).

DISCUSSION

Food security for the increasing world population, most especially in the countries where pest control management is not of major concern had been and still being significantly challenged over years (Olotuah, 2014). Grain storage across the globe had been relying so heavily on the use of synthetic pesticides against insect infestation, the use of which have triggered a number of ecologica, health-related and pest resistance problems (Verma and Derbey, 1999). Works on organic pesticides to make up for these short falls had been promising. Several botanical products have been discovered as

potent in the control of storage pest infestation (Ofuya and Dawodu, 2002; Adedire and Ajayi, 2003; Tan and Luo, 2011). The results of this study have shown that the botanical powders of various compositions from C. patens are toxic to egg, larval and adult stages of P. interpunctella in stored products, most especially maize grains. This is in agreement with Akinneye et al. (2006) that showed the efficacy of root bark, stem bark and leaf powders of C. patens at varied compositions both as contact and fumigant insecticides in the control of egg and adult emergence stages of some Coleopteran and Lepidopteran storage pests. This result reveals a significant contact effect as compared with the fumigant effects of the powders on the moth pest. A concentration of 1.5 g leaf powder at all levels affected 100% egg, larval and adult mortalities after 72 h exposure when used as contact insecticides. The fumigant effect only

Dosage (g)	Egg hatchability (%)	Length of days for adult emergence	Adult emergence (%)
0.5	86.67±1.67 ^d	27.33±0.33 ^{ab}	73.33±1.67 ^b
1.0	81.67±1.67 ^{cd}	28.33±0.33 ^{ab}	65.00±2.89 ^{ab}
1.5	76.67±1.67 ^{bc}	29.33±0.67 ^b	63.33±1.67 ^{ab}
2.0	73.33±1.67 ^{ab}	32.00±0.58 ^c	60.00±2.89 ^a
2.5	68.33±1.67 ^a	34.00±0.58 ^c	60.00±2.89 ^a
Control	88.33±1.67 ^d	26.67±0.33 ^a	75.00±2.89 ^b

Table 7. Fumigant toxicity of leaf powder of C. patens to eggs of P. interpunctella.

Means followed by the same letter(s) within the column are not significantly different (P<0.05) using Tukey's Test.

Table 8. Fumigant toxicity of leaf powder of C. patens to larvae of P. interpunctella.

Dosage (g)	24 h	48 h	72 h	96 h
0.5	0.00±0.00 ^a	0.00±0.00 ^a	3.33±3.33 ^{ab}	3.51±3.51 ^a
1.0	1.67±1.67 ^a	10.00±2.89 ^b	13.68±3.16 ^{bc}	13.68±3.16 ^{ab}
1.5	5.00±0.00 ^{ab}	18.33±1.67 ^{bc}	17.19±1.40 ^c	17.19±1.40 ^{bc}
2.0	8.33±1.67 ^{bc}	25.00±2.89 ^{cd}	25.88±3.07 ^{cd}	27.29±0.49 ^{cd}
2.5	11.67±1.67 ^c	28.33±1.67 ^d	32.63±3.99 ^d	36.26±2.92 ^d
Control	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a

Means followed by the same letter(s) within the column are not significantly different (P<0.05) using Tukey's test.

Table 9. Fumigant toxicity of leaf powder of C. patens to adult of P. interpunctella.

Dosage (g)	24 h	48 h	72 h	96 h
0.5	0.00 ± 0.00^{a}	3.33±3.33 ^a	3.33±3.33 ^a	10.37±5.79 ^a
1.0	0.00±0.00 ^a	3.33±3.33 ^a	10.37±0.37 ^{ab}	21.11±5.48 ^{ab}
1.5	3.33±3.33 ^{ab}	13.33±3.33 ^a	13.33±6.67 ^{ab}	21.11±5.48 ^{ab}
2.0	10.00±0.00 ^{bc}	16.67±3.33 ^a	27.78±4.01 ^b	38.89±5.56 ^{bc}
2.5	16.67±3.33 ^c	43.33±6.67 ^b	51.48±4.55 [°]	53.33±4.63 ^c
Control	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a

Means followed by the same letter(s) within the column are not significantly different (P<0.05) using Tukey's Test.

Table 10. Fumigant toxicity of stem bark powder of C. patens to eggs of P. interpunctella.

Dosage (g)	Egg hatchability (%)	Length of days for adult emergence	Adult emergence (%)
0.5	73.33±1.67d	29.67±0.33 ^b	43.33±4.41 [°]
1.0	48.33±4.41 [°]	31.67±0.88 ^b	18.33±1.67 ^b
1.5	26.67±4.41 ^b	22.00±11.00 ^b	3.33±1.67 ^a
2.0	0.00 ± 0.00^{a}	0.00±0.00 ^a	0.00 ± 0.00^{a}
2.5	0.00±0.00 ^a	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Control	86.67±1.67 ^e	27.33±0.33 ^b	75.00±2.99 ^d

Means followed by the same letter(s) within the column are not significantly different (P<0.05) using Tukey's test.

achieved 23.37, 21.67 and 75.56% egg, larval and adult mortalities respectively at the same concentration. The fumigant effect of 2.0 g concentration achieved 100%

larval and adult mortalities after 96 h exposure.

The inability of the eggs to hatch may be because powder inhibits gaseous exchange between the eggs and

Dosage (g)	24 h	48 h	72 h	96 h
0.5	6.67±1.67 ^{ab}	20.00±2.89 ^b	29.21±2.89 ^b	75.90±24.10 ^{ab}
1.0	11.67±1.67 ^b	26.67±3.33 ^b	39.65±1.53 ^c	43.57±2.49 ^{bc}
1.5	16.67±1.67 ^b	45.00±2.89 ^c	60.35±1.53 ^d	72.81±2.72 ^{bc}
2.0	31.67±4.41 [°]	73.33±3.33 ^d	96.58±1.71 ^e	100.00±0.00 ^c
2.5	43.33±1.67 ^d	95.00±2.89 ^e	100.00±0.00 ^e	100.00±0.00 ^c
Control	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a

Table 11. Fumigant toxicity of stem bark powder of C. patens to larvae of P. interpunctella.

Means followed by the same letter(s) within the column are not significantly different (P<0.05) using Tukey's test.

Table 12. Fumigant toxicity of stem bark powder of C. patens to adult of P. interpunctella.

Dosage (g)	24 h	48 h	72 h	96 h
0.5	3.33±3.33 ^a	26.67±3.33 ^b	45.93±7.73 ^b	51.85±3.70 ^b
1.0	16.67±3.33 ^{ab}	43.33±6.67 ^{bc}	60.74±3.23 ^b	74.07±3.70 ^c
1.5	23.33±3.33 ^b	63.33±3.33 ^c	88.89±6.42 ^c	96.30±3.70 ^d
2.0	33.33±3.33 ^{bc}	86.67±3.33 ^d	96.30±3.70 ^c	100.00±0.00 ^d
2.5	50.00±5.77 ^c	90.00±5.77 ^d	100.00±0.00 ^c	100.00±0.00 ^d
Control	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a

Means followed by the same letter(s) within the column are not significantly different (P<0.05) using Tukey's test.

their external environment (Akinneye, 2003). The relatively high mortality rate of *C. patens* to the pest may be attributed to the chemical composition of the powder just as reported by Ketunku et al. (2014) that saponin found in Eugenia aromatic (L.)[Bail] affected the respiratory system of certain storage insects thereby preventing their spread. It may also be attributed to the odour or characteristic bitterness associated with the leaf powder.

This corroborates the findings of Lale and Abdurahman (1990) that mortality of storage insects could be associated with pungent odour produced by plant powders against them. The finding is also in line with the report of Akinneye (2003) that C.patens inhibits egg hathchability and development of adult stages of Ephestia cautella (Walker)[Lepidoptera; Pyralidae]. Ashamo and Ogungbite (2014) also discovered that E.aromatica prevented the emergence of some adult storage moths even at 2% concentration. This result is also in agreement with the work of Adedire and Laiide (2001), and Longe (2004) that *E.aromatica* powder has significant contact and fumigant actions on Calosobruchus maculatus (F.) (Coleoptera: Bruchidae). The progressive reduction in percentage adult emergence with increasing concentration and exposure period could suggest the death of the pests at larval stage due to their inability to fully cast off their exoskeleton which remains a link to the posterior parts of their abdomen just as reported by Oigiangbe et al. (2010). This result also tallies with the findings of Adedire and Lajide (2001) that spulverized powder of *Piper umbrellatum* (Linn.)[Piperaceae] seed and *E. aromatica* were toxic to *C. maculatus*, producing 100% mortality at 24 h post treatment across all concentrations. This study had revealed positive contact and fumigant effects of *Cleitopholis* patens powders on the *P. interpunctella* across the concentration gradient and exposure period. The findings suggest that the botanical product could serve as an alternative to synthetic chemicals against *P. interpunctella*.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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