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Management of *Plodia interpunctella* (hübner) [Lepidoptera: pyralidae] using ethanolic oil extract of *Plumbago zeylanica* (LINN.)

Adeyera, O. J.* and Akinneye, J. O.

Department of Biology, Federal University of Technology, P.M.B. 704 Akure, Ondo State, Nigeria.

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This study investigated the insecticidal effects of ethanolic oil extract of *Plumbago zeylanica* against *Plodia interpunctella* infestation in stored maize grains. Oils from the plant were administered at 5, 10, 15, 20 and 25% concentrations to maize grains inside plastic containers. The treated samples were air-dried for 3 h. The three developmental stages of the moth (egg, larval and adult) were separately introduced into the treated samples, using aspirator. Mortalities were assessed at 24, 48, 72 and 9 6 h post-treatment periods as contact and as fumigant insecticides. The rates at/from 15-25% inhibited egg to hatch and adult emergence as contact and as fumigant insecticides. At 20 to 25% rates, 81 to 100% larval mortalities were achieved within 72 to 96 h post infestation periods as contact and fumigant insecticides. At 20 to 25% application rates of oil extracts as contact and fumigant, the treatments achieved 89 to 100% adult moth mortality within 72 to 96 h post infestation periods. The GC-MS analysis of the ethanolic oil extract shows that *P. zeylanica* has some bioactive compounds that could be co-opted into integrated management of *P. interpunctella* infesting stored products.

Key words: *Plumbago zeylanica*, *Plodia interpunctella*, hatchability, contact and fumigant.

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 all over 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 mankind in the past; but irrespective of these great contributions, their continuous use have triggered a number of ecological, resistance and health-related challenges (Varma and Dubey, 1999); such as the development of resistant pests, resurgence and outbreak of new pests, toxicity to non-target organisms and hazardous effects on the environment, thus endangering the sustainability of the ecosystem (Jeyasankar and Jesudasan, 2001).

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 spent (annually) to manage the side effects of these pesticides on man and environments (USEPA, 2011). These have necessitated the search for eco-friendly and bio-degradable pesticides for crop protection and

*Corresponding author. E-mail: laoluadeyera@yahoo.com.

management. This drive had been greatly encouraged over the last few decades (Prasad et al., 2013). It is expected that the ideal insecticide should control the target pest adequately, rapidly degradable and non-toxic to human and livestock (USEPA, 2011). The use of plant-derived pesticides to make up for various shortfalls identified with synthetic pesticides had been promising over years (Parugrug and Roxas, 2008). There have been reviews on the use of plants' secondary metabolites to control the menace of pests' infestation on stored grains by several authors (Obeng-Ofori, 2007; Tan and Luo, 2011). This study is therefore sought to assess the bioactivity of the oil extract of *Plumbago zeylanica* against the egg, larval and adult stages of the Indian meal moth, *Plodia interpunctella*.

MATERIALS AND METHODS

Sourcing of plant materials

The plant *P. zeylanica* was obtained from a farm Modebiayo Camp, Via Ondo in Ondo East Local Government Area of Ondo State, Nigeria.

Preparation of plant materials

The plant *P. zeylanica* was obtained from a farm Modebiayo Camp, Via Ondo in Ondo East Local Government Area of Ondo State, Nigeria. The required quantity (1000 stands) of the plant *P. zelanica* was completely uprooted from the farm. The plants were brought to the Biological Research Laboratory of the Federal University of Technology, Akure. They were thoroughly washed with water, then the root barks were carefully removed with a 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 mm 2 perforations to obtain labeled samples of fine powders which were kept in separate airtight plastic containers and stored at ambient temperature of 28+2°C and 75 + 5% relative humidity pending use (AOCS, 2001).

Extraction of the plant oil

Two hundred grams of the pulverized plant materials were poured into a muslin cloth of dimension 50 x 50 cm and then transferred into the thimble and extracted with ethanol in a soxhlet apparatus model 77-520 (Hospital Equipment Manufacturing Co. Limited, India). The extraction was carried out for 3-4 h. It was terminated when the solvent in the thimble became clear. Then the thimble was removed from the unit and the solvent recovered by distilling in the soxhlet extractor. The Rotary evaporator was used to separate the solvent from the oil after collection of the resulting extracts from the soxhlet, after which the oil was exposed to air so that traces of the volatile solvent evaporates, leaving the oil extract. The resulting oil was kept in glass bottles and stored at 4°C until required for use (Adegbe et al., 2016).

Insect culture

The maize grains were bought at the Isikan market, Akure, Ondo State, Nigeria. The grains were winnowed and handpicked to remove contaminants and damaged ones. The sorted grains were disinfected in the oven at 600C for 4hours and allowed to cool on the open laboratory bench for 5-6 hours so as to acclimatize them to the laboratory environmental condition of 28+2°C temperature and 75+5% relative humidity. 500 g of the grains was weighed into two Kilner jars (1 L each). Ten newly emerged adults (5 male and 5 female) species of *P.* interpunctella were introduced into each of the jars. The jars were kept in the culturing chamber until the F1 generation emerged (Akinneye et al., 2006).

Insect bioassay

Contact toxicity of the oil extract on egg, larval and adult stages of P. interpunctella

Twenty freshly laid eggs (0-24 h old) were placed on 20g of the grains treated with 0% oil (control). The process was repeated using 5, 10, 15, 20, and 25% of oil extracts of the plant. The treated and the control (untreated) were replicated three times. Daily observations were made to determine the percentage of hatchability and later adult emergence after 40 days. The oil extract from the plant was separately admixed with maize grains at the earlier stated concentrations per 20g of maize grains in a plastic container (8cm diameter and 4cm depth). Ten third instar larvae were introduced into each of the treated and untreated grain samples and were replicated three times. Larvae mortalities were determined after 24, 48, 72 and 96 hours post treatment. The experiments were serially arranged and kept inside a breeding wire mesh cage measuring (75 × 50 × 60 cm) Katunku et al., 2014).

Fumigant toxicity of the oil extracts on egg, larval and adult stages of P. interpunctella

Twenty freshly laid eggs (0-24 h old) were placed on 20 g of grains inside plastic containers (8 cm diameter and 4 cm depth) laid with folded filters treated with 0 (control), 5, 10, 15, 20 and 25% of oil extracts of the plant. The treatments were all in triplicate. Daily observations were made to determine the percentage hatchability and later, adult emergence after 40 days. The same experiment was repeated for larval and adult stages of *P. interpunctella* and mortalities were determined after 24, 48, 72 and 96 hours post treatment (Longe, 2004).

GC-MS analysis of P. zeylanica oil extract

Gas chromatography coupled with mass spectrometry (GC-MS) analysis was used to reveal the profile of compounds contained in the oil extract of *P. zelanica*. One microlitre of the oil extract samples were analyzed using Agilent Technologies. The GC oven temperature was set at 80ºC for 2 min. The temperature increased steadily at 6°C per min to 240ºC and was held for 6 min. The running time for each sample was 36 min. The peak of each chemical compound was expressed based on its retention time and balance (Kadhim and AL-Shammaa, 2014).

Method of data analysis

Data collected were subjected to one-way analysis of variance (ANOVA) using SPSS version 23.0, means were separated using Turkey test.

Table 1. Contact toxicity of root bark oil of *P. zeylanica* to eggs of *P. interpunctella.*

Concentration (%)	Egg hatchability (%)	Adult emergence (%)
5.0	16.67±1.67^{b}	6.67±1.67^{b}
10.0	13.33±1.67^{b}	0.00±0.00^{a}
15.0	0.00±0.00^{a}	0.00±0.00^{a}
20.0	0.00±0.00^{a}	0.00±0.00^{a}
25.0	0.00±0.00^{a}	0.00±0.00^{a}
Solvent	86.67±1.67^{c}	73.33±1.67^{c}

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 root bark oil of *P. zeylanica* to larvae of *P. interpunctella.*

Concentration (%)	24 h	48 h	72 h	96 h
5.0	16.93±1.55^{b}	37.81±3.90^{b}	47.09±4.65^{b}	54.48±2.34^{c}
10.0	25.44±0.44^{bc}	44.91±2.46^{bc}	61.42±0.93^{bc}	70.96±1.27^{d}
15.0	23.60±4.16^{bc}	56.84±2.19^{cd}	75.40±3.50^{c}	88.99±3.29^{e}
20.0	30.44±2.52^{c}	68.95±3.08^{de}	94.91±2.89^{d}	100.00±0.00^{f}
25.0	44.12±2.17^{d}	79.39±2.67^{e}	98.25±1.75^{d}	100.00±0.00^{f}
Control	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Solvent	3.42±2.71^{a}	6.93±1.80^{a}	8.51±4.45^{a}	9.06±1.76^{b}

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

RESULTS

Identification of bio-active compounds in *P. zeylanica* by GC-MS

The results pertaining to GC-MS analysis as shown in Figure 1 led to the identification of number of compounds from the ethanolic extract of *P. zeylanica* plant. The percentage abundance of each of compound is revealed in Table 7 hexadecanoic acid. methyl ester, methyl 10-trans, 12-cis –octadecadienoate, Cis-13-Octadecenoic acid, methyl ethyl esther, methyl stearate, (E)-octadecenoic acid, ethyl ester, Cis- mrthyl 11-eicosenoate, and Bis(2- ethylhexyl) phthalate are the most abundant compounds.

Contact toxicity of *P. zeylanica* root bark oil on the developmental stages of *P. interpunctella*

The use of the oil extract of *P. zeylanica* in the management of the developmental stages of *P. interpunctella* as contact insecticide yielded positive results. Table 1 shows a progressive reduction in percentage of egg hatchability and adult emergence of the pest. It can be seen that hatchability had been reduced to 0% at 15% oil concentration. Adult emergence also ceases as from 10% concentration. Table 2 reveals the contact effects of the oil extract on the larval stage of pest. The rate of effectiveness increased along the upward concentration gradient. 100% larval mortality was achieved at 20% concentration after 96hrs exposure. Table 3 also showed that 96hrs of contact exposure of the pest to 20%concentration of the botanical achieved 100% adult mortality.

Fumigant toxicity of P. zeylanica root bark oil on the developmental stages of P. interpunctella

The fumigant effects of the oil extract on *P. interpunctella* yielded a similar result to that of contact. Table 4 shows that both the egg hatchability and adult emergence of the pest were completely stopped (0%) at 15% concentration. Tables 5 and 6, reveal that 100% larval and adult mortalities werenot achieved until the concentration was increased to 25% after 96 h exposure. A concentration of 20% yielded the same result when used as contact control.

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

Table 3. Contact toxicity of root bark oil of *P. zeylanica* to adults of *P interpunctella.*

Concentration (%)	24 h	48 h	72 h	96 h
5.0	10.00±0.00^{a}	32.33±1.11^{b}	59.26±3.70^{b}	74.07±3.70^{b}
10.0	26.67±3.33^{b}	57.41±4.90^{c}	77.78±6.42^{c}	88.89±6.42^{bc}
15.0	33.33±3.33^{bc}	74.81±4.12^{cd}	96.30±3.70^{d}	96.30±3.70^{c}
20.0	46.67±3.33^{cd}	89.26±6.43^{d}	96.30±3.70^{d}	100.00±0.00^{c}
25.0	50.00±5.77^{d}	92.59±3.70^{d}	100.00±0.00^{d}	100.00±0.00^{c}
Solvent	3.33±3.33^{a}	2.96±9.10^{a}	7.41±3.70^{a}	7.41±3.70^{a}
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 4. Fumigant toxicity of root bark oil of *P. zeylanica* to eggs of *P. interpunctella.*

Concentration (%)	Egg hatchability (%)	Adult emergence (%)
5.0	36.67±1.67^{b}	13.33±1.67^{b}
10.0	23.33±1.67^{c}	1.67±1.67^{b}
15.0	6.67±1.67^{d}	0.00±0.00^{b}
20.0	0.00±0.00^{d}	0.00±0.00^{b}
25.0	0.00±0.00^{d}	0.00±0.00^{b}
Solvent	78.33±1.67^{a}	63.33±1.67^{a}
Control	85.00±2.89^{a}	55.00±15.33^{a}

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

Table 5. Fumigant toxicity of root bark oil of *P. zeylanica* to larvae of *P. interpunctella.*

Concentration (%)	24 h	48 h	72 h	96 h
5.0	13.77±1.64^{b}	31.64±0.96^{b}	40.06±2.27^{b}	49.12±0.88^{b}
10.0	20.70±0.35^{bc}	43.75±2.53^{bc}	56.34±3.30^{c}	67.25±3.26^{c}
15.0	24.12±1.58^{bc}	47.46±1.44^{cd}	65.50±1.17^{c}	80.02±1.69^{d}
20.0	30.96±2.53^{cd}	59.67±0.94^{d}	81.77±2.01^{d}	87.33±1.56^{d}
25.0	37.98±2.13^{d}	75.48±4.41^{e}	92.79±3.61^{d}	100.00±0.00^{e}
Solvent	1.58±4.48^{a}	6.75±4.39^{a}	5.36±3.04^{a}	1.67±1.67^{a}
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.

being significantly challenged over the years (Olotuah, 2014). This calls for effective grain storage and safe pest management methodologies. Grain storage across the globe had been relying so heavily on the use of synthetic pesticides against insect infestation, the use of which has triggered a number of ecological, health-related and pest resistance problems (Varma and Derbey, 1999). Works on organic pesticides to overcome the challenges associated with synthetic pesticides had been yielding positive results. 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). Akinyemi et al. (2005) also reported on the effectiveness of the use of plant extracts in the management of stored products insects and described the approach as an ancient practice. The result of this study shows that the oil extracts of various compositions from *P. zeylanica* is toxic to egg, larval and adult stages of *P. interpunctella* in stored products, most especially maize grains. This is in agreement with the

Table 6. Fumigant toxicity of root bark of *P. zeylanica* to adults of *P. interpunctella.*

Concentration (%)	24 h	48 h	72 h	96 h
5.0	6.67±3.33^{ab}	24.81±2.59^{b}	42.96±1.48^{b}	64.44±2.22^{b}
10.0	20.00±0.00^{bc}	50.00±3.21^{c}	71.48±3.29^{c}	82.22±3.39^{c}
15.0	26.67±3.33^{cd}	60.74±3.23^{cd}	82.22±3.39^{cd}	85.56±3.90^{cd}
20.0	36.67±3.33^{de}	68.15±5.19^{d}	89.63±5.79^{de}	96.67±3.33^{de}
25.0	43.33±3.33^{e}	85.56±3.90^{e}	100.00±0.00^{e}	100.00±0.00^{e}
Solvent	3.33±3.33^{a}	3.33±3.33^{a}	3.33±3.33a	3.33±3.33^{a}
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 7. Compounds with highest percentage abundance in oil extract of *P. zeylinica*

SN	Name of compounds	Percentage of total	Retention time (Min.)	CAS No
001	Hexadecanoic acid.Methyl ester	16.294	16.830	000112-39-0
002	Methyl 10- trans, 12- cis - octadecadienoate	4.341	18.918	1000336-44-2
003	Cis -13-Octadecenoic acid, Methyl ethyl esther	3.484	19.028	1000333-58-36
004	Methyl stearate	3.813	19.283	000112-61-8
005	(E)- Octadecenoic acid, ethyl ester	4.422	19.758	00611-18-7
006	Cis- Mrthyl 11- eicosenoate	4.389	21.260	002390-09-2
007	Bis(2- ethylhexyl) phthalate	3.257	24.324	000117-81-7

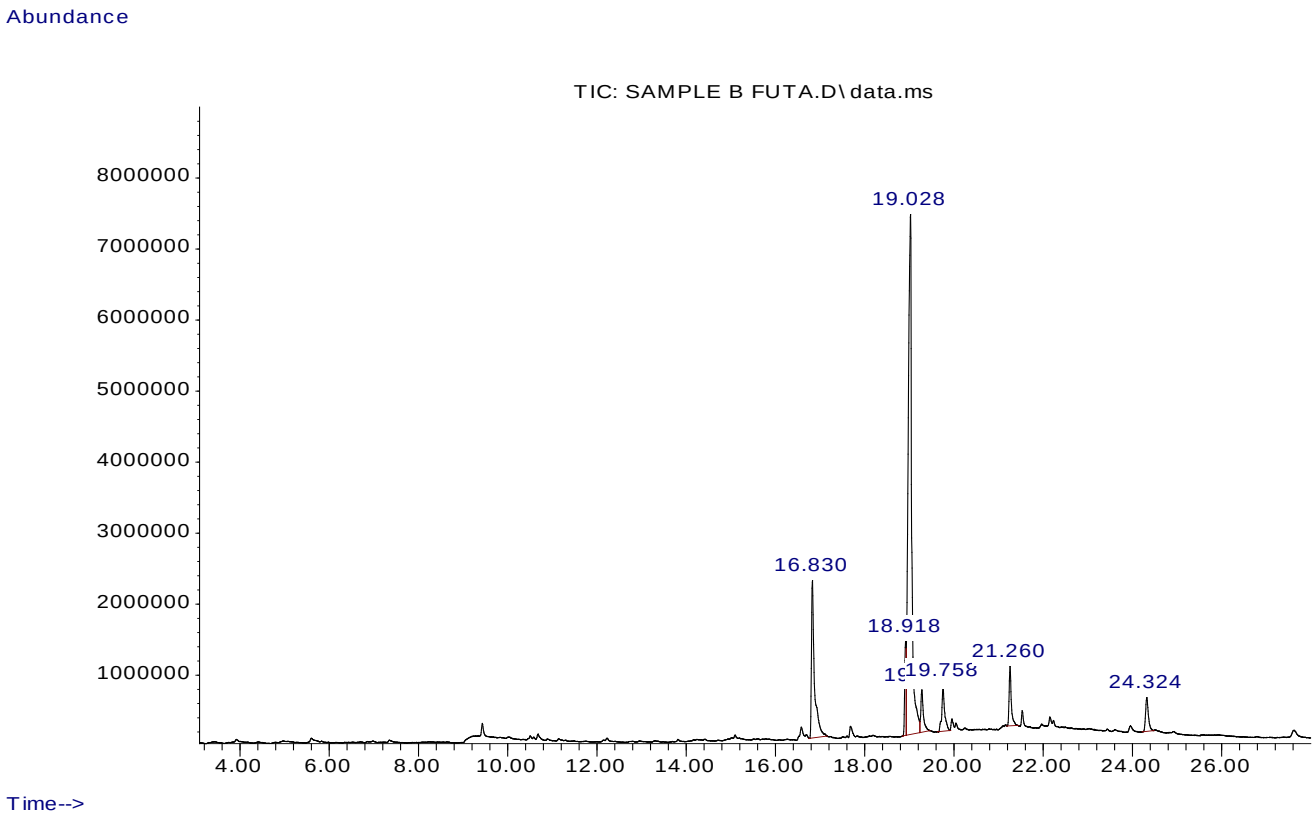

Figure 1. Chromatogram of ethanolic oil extract of *Plumbago zeylanica* root bark.

findings of Akinneye et al. (2006) that revealed the efficacy of root bark, stem bark and leaf powders of *Cleithopholis 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 showed a significant contact effect as compared with the fumigant effects of the oil extracts on the pest. A concentration of 15% of the ethanolic oil extract achieved 0% egg hatchability, which is significantly different ($P < 0.05$) from the result obtained under fumigant control. The hatchability was not completely prevented until the concentration was increased to 20%. 100% larval and adult mortalities were achieved at concentration 20% after 96 hours exposures when used as contact insecticides. The concentration had to be increased by 25% before the same result could be achieved under the fumigant methodology. The inability of the eggs to hatch may be because such botanicals have a way of inhibiting gaseous exchange between the eggs and their external environment (Akinneye, 2003). The relatively high mortality rate recorded as a result of the use of *P. zeylanica* to the pest may be attributed to the chemical composition of the plant, just as reported by Ketunku et al. (2013) that Saponin found in *E. aromatica* affected the respiratory system of certain storage insects thereby prevented their spread. It may also be attributed to the odour or characteristic bitterness associated with the root bark of the plant. This corroborates the findings of Lale and Abdurahman (1990) that mortality of storage insects could be associated with the pungent odour produced by plant powders against them. The finding is also in line with the report of Akinneye (2003) that *C. paten* inhibits egg hatchability and development of adult stages of *Ephestia cautella*. Ashamo and Ogungbite (2014) also discovered that *E. aromatica* prevented the emergence of some adult storage moths even at a concentration 2%. This result is also in agreement with the work of Adedire and Lajide (2001); and Longe (2004) that *E. aromatica* powder has significant contact and fumigant actions on *Calosobruchus maculatus*. 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). Furthermore, the larvicidal activities obtained in this study are also in agreement with the reports of Sosan et al. (2001) that larvicidal activities of essential oils of *Ocinum gratissium*, *Cymbopogon citratus* and *Ageratum conyzoides* against *Aedes aegypti*, achieving 100% mortality at concentrations of 120, 200, and 300 ppm respectively. Das et al. (2007) reported that the LC_{90} values of methanol and ethanol extract from *Aristolochia saccata* roots, *Annona squamosa* leaves and *Gymnopetelus cochinnensis* fruits' pericarp against *Aedes albopictus* and *Curlex quinquefasciatus* larvae ranged from 31.80-155 ppm. The potency of the ethanolic oil extract of *P. zeylinica* against the different stages of *P. interpuntella* can be attributed to the abundance of esters/fatty acids in its biochemical breakdown. The potency of botanical fatty acids have been extensively research for more than three decades (Su, 1976; Su et al., 1972; Messina and Renwick 1983; Abdallah et al., 1986) reported the insecticides potency against weevil species.

Conclusion

This study revealed the positive contact and fumigant effects of *P. zeylanica* oil extract on the *P. interpunctella* across the concentration gradient and exposure period. It was more effective when used as contact compared with fumigant control. The findings suggest that the botanical product could serve as an alternative to synthetic chemicals being used against *P. interpunctella* infestation.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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