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Contact and Fumigant Toxicity of *Uvaria afzelli* (Scott) against *Plodia interpunctella* (Hubner) Infesting Maize Grains in Nigeria

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Abstract

In this study, the insecticidal efficacy of the powder and extract of *Uvaria afzelli* seed is evaluated against different life stages of *Plodia interpunctella* at the ambient temperature of 28 ± 2 ^{o}C and 75 ± 5 % relative humidity. The powder of the plant was tested at the rate of 0.1, 0.2, 0.3 and 0.4g dosages, while the extract was tested at 1, 2, 3 and 4 % concentrations per 20g of maize. The contact and fumigant toxicity of the plant was observed on the mortality and emergence of the adult moth. All the dosages and concentrations of the plant used as treatment through contact toxicity completely inhibited the emergence of the adult moth. Also, all the dosages of plant powder used as treatment achieved 100 % of insect mortality within ninety-six hours of application except the 0.1g dosage which recorded 98.17 % mortality. Within seventy-two hours of exposure, the powder of the plant used as fumigant recorded 100 % mortality of the moth at 0.4g dosage while the extract achieved 100% mortality within twenty-four hours regardless of the concentration used. Also, at 0.1g and above, for both extract and powder, 100 % inhibition of egg hatchability and adult emergence was recorded. The powder and extract of *U. afzelli* are highly toxic against different life stages of *P. interpunctella* when used as contact toxin and fumigant.

Keywords: Hatchability, Emergence, Mortality, *Uvaria afzeli*, Toxicity, Fumigant.

1. Introduction

Maize (*Zea mays* L.) is the third most important crop in the world in terms of growing area and production and grain yield (Shiri *et al*., 2010). It adjusts easily to a wide range of environmental and climatic conditions. Therefore, it is grown both in the tropic and temperate regions of the world. Its availability in stores and on the field has made it a choice of many infesting insects especially the coleopterans and lepidopterans (Gerpacio and Pingali, 2007).

Indian meal moth, *Plodia interpunctella* (Hubner) lepidopterans is one of the primary insect pests infecting maize in the tropics. It has caused an appreciable damage to maize grains both on the field and in stores. Moreover, infestation in the store is more pronounced. The infestation of maize grains by *P. interpunctella* has caused diminution in the quality and market value of the grains (Philips *et al*., 2000; Salami *et al*., 2017). Therefore, the control of this important insect pest of maize has become of major interest among the entomologists and store managers around the world.

For years, the control of this insect pest and other notorious storage pests has relied overwhelmingly on the use of synthetic chemical insecticides that are associated with many dents thwarting their widespread use nowadays. In fact, the governments of many advanced countries including the UK and USA have banned the use of some synthetic chemical insecticides because of their effects on both human and environmental health (Isman, 2006; Oni *et al*., 2016). Therefore, researches have been shifted toward the use of botanical-based insecticides since they were found to have little or no effect on the non-target organisms and the environment (Zibaee, 2011 and Olufemi -Salami *et al*, 2017).

Pepper fruit *Uvaria afzelli* (Scott), known as "*Gbongbose"* among the Yorubas in Nigeria is a medicinal plant which has been used for the treatment of various diseases such as stomach ache, coughing, amenorrhoea, neuralgia etc. (Okepekon, 2004, Odugbemi, 2006). It belongs to the family of *Annonaceae*. The fresh stem and root bark have been shown to be effective in the control of adult moths (*Cleistopholis patens*) reared on paddy rice (Akinneye and Oyeniyi 2016). However, further research has not been done on the entomocidal properties of *U. afzelli* seeds to explore its contact toxicity and fumigant action of its aroma on insects. Hence, the powder and oil of the seeds of *U. afzelli* in the control of insect pests of stored products might be as effective as the bark and the roots. This research, therefore, sought to investigate the contact and fumigant toxicity of the powder and ethanol oil extract of the seeds of *U. afzelli* against *P. interpunctella* infesting maize grains.

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2. Materials and Methods

2.1. Insect Culture

The *P. interpunctella* used to establish the culture was obtained from naturally-infested maize grains from the Research Laboratory in the Department of Biology at the Federal University of Technology Akure, Ondo state, Nigeria. The moths larvae were reared in twenty-one plastic containers containing 300g of uninfested maize grains. The culture was maintained by continually replacing the devoured powder and sieving out frass and fragment. The plastic containers were covered with muslin cloth, fastened with rubber band, and placed inside a wire mesh cage of the dimensions 75cm ×50cm ×60cm (L×W×H) with its four strands dipped in water-kerosene mixture contained in a plastic container to prevent the entry of predatory ants into the cage. The culture was maintained at a temperature of 28 ± 2°C and a relative humidity of 75 ± 5% . The whole set up was left inside the breeding cage in the laboratory. The maize variety TZESR-20 obtained from the Agricultural Development Program, Akure, Nigeria was disinfested in the freezer at −2 °C for seventy-two hours. Following that, it was allowed to equilibrate in the laboratory.

2.2. Collection and Preparation of the Plant Materials

The seeds of *U. afzelli* were obtained from a farm at Modebiayo camp, in Ondo East Local Government Area of Ondo State, Nigeria. The seeds were air-dried, pulverized into fine powder using a Binatone Electric Blender (Model 373). The pulverized seeds were sieved with a mesh with a size of $1mm^2$ before being stored in plastic containers with airtight lids for subsequent use.

2.3. Preparation of the Ethanol Oil Extract

Eighty grams of the pulverized seeds were weighed into a muslin cloth and transferred into the thimble and were extracted with ethanol in a Soxhlet apparatus. The extraction was carried out for three hours. The extraction was terminated when the solvent in the thimble became clear. Then, the thimble was removed from the unit, and the solvent was recovered by redistilling using a rotary evaporator. The resulting extract contained both the solvent and the oil. After that, the oil was exposed to air so that the traces of the volatile solvent evaporate, leaving the oil extract. The resulting oil was kept in a plastic container to be used for the subsequent experiment.

2.4. Hatchability Inhibition by Pulverized Seed Powder

Thirty freshly-laid eggs (0-24h old) were placed on 20g of maize grains treated with 0.1, 0.2, 0.3 and 0.4g of the powder of *U. afzelli* seeds inside separate plastic containers (8 cm diameter and 4 cm depth) covered with muslin cloths. A control experiment (20g of maize grain-infested eggs of *P. interpunctella* without any seed powder) was setup under the same environmental conditions with the treatments. All treatments and control experiment were replicated three times. Daily observations were made with a dissecting microscope to determine the number of eggs that hatched. After forty days, the number of adults emerged was determined and the percentage was calculated using the formula:

$$\%\ \text{hatchability} = \frac{\text{number of eggs hatched}}{\text{Total number of eggs introduced}} \times \frac{100}{1}$$

2.5. Contact Toxicity of the U. afzelli Seeds Powder on the 3rd Larvae Instar and Adult P. interpunctella Emergence

The same procedures for the preparation of treatments in the hatchability inhibition of eggs of *P. interpunctella* were repeated for the 3rd instar larvae and 0-24h adults of *P. interpunctella*. Ten 3rd instar larvae and ten adult insects were introduced separately into each of the treatments and control experiment. The numbers of dead larvae and adult insects were counted after 24, 48, 72, and 96 hours following the treatment.

2.6. Contact Toxicity of the U. afzelli Powder on the Mortality of Adult P. interpunctella

Twenty grams of disinfected maize grains were treated with 0.1, 0.2, 0.3 and 0.4g of the *U. afzelli* powder in separate plastic containers (8 diameter and 4 cm depth). The mixtures were agitated gently to homogenize the distribution of powder. Ten pairs of freshly-emerged 0-24 hour old males and females (the sexes were differentiated using size; males are smaller, while females are bigger with a protruding abdomen) were introduced into the treated grains. The container was covered with a perforated lid covered with muslin cloth to provide aeration. The entire sets were kept in the breeding cages, and mortality was recorded every six hours for the duration of twenty-four hours.

2.7. Effects of the U. afzelli Extracts on adult mortality of P. interpunctella

Different concentrations of 1, 2, 3, 4 and 5 % of the oil extract were prepared by adding 0.1, 0.2, 0.3, 0.4 and 0.5 mL of the of seeds (powder) extract into 9.9, 9.8, 9.7, 9.6 and 9.5 mL, of ethanol (solvent) respectively. Twenty grams (20g) of disinfested maize grains were treated with different concentrations of the oil extract of *U. afzelli* seed in a plastic container. The mixtures were agitated gently to homogenize the distribution of the oil extract. Ten pairs of freshly emerged 0-24 hour old male and female of the *P. interpunctella* adults from the stock culture were introduced into the 20g treated maize. The containers were covered with perforated lids to provide aeration. The entire set up was kept in the laboratory, and mortality was recorded every six hours for the duration of twenty-four hours.

2.8. Fumigant Effect of the U. afzelli Seeds' Extracts on the Mortality of Adult P. interpunctella

Different concentrations of 1, 2, 3, 4 and 5 % oil extract were prepared. Whatman 1 filter paper was cut into four equal parts. One part of the paper was dipped into 1 % concentration and then packed in muslin cloth, and suspended using thread. The treated paper strips were introduced by suspension into the plastic containers containing 20g of maize grain and ten pairs of male and female freshly-emerged 0-24 hour-old *P. interpunctella* adults from the stock culture. The lids of the containers were punched and covered with muslin cloth. The same procedures were repeated for 2, 3, 4, and 5 % concentrations. Untreated paper strips dipped in ethanol were used as the control experiment. The set up was

replicated three times. Adult mortality was counted at 24, 48, 72 and 96 hours after application.

The fumigant effect of the plant was also examined on the eggs of Indian meal moth.

2.9. Data analysis

All data on mortality were corrected using Abbott (1925) Formula. Complete randomized bloc design was used for the experiment and data obtained were subjected to analysis of variance (ANOVA), and significantly different treatment means were separated using Tukey's Simultaneous Test ($P < 0.05$). SPSS 16.0 software package was used for all the statistical analysis.

3. Results

3.1. *Contact Toxicity of the U. afzelli Powder and Extract on Egg Hatchability and Adult Emergence of P. interpunctella*

The effect of *U. afzelli* powder and extract of *U. afzelli* on egg hatchability and adult emergence of *P. interpunctella* are shown in Table 1. At all dosages of the *U. afzelli* powder (treatments), hatchability and adult emergence were completely inhibited. The result of the control experiment was completely different from the treatments insomuch that, more than 50 % hatchability and adult emergence were obtained from the control experiment.

The *U. afzelli* ethanol oil extract completely inhibited egg hatchability and the adult emergence of *P. interpunctella* at all concentrations including the control experiment.

Table 1. Effect of *U. afzelli* powder and ethanolic extract on egg hatchability and adult emergence of *P. interpunctella*.

Rate (g/20 maize grain)	Mean % (±S.E) Egg hatch	Mean % (±S.E) Adult emergence
	Powder	
0.0	60.00±0.00^{b}	54.00±2.3^{b}
0.1	0.00±0.00^{a}	0.00±0.00^{a}
0.2	0.00±0.00^{a}	0.00±0.00^{a}
0.3	0.00±0.00^{a}	0.00±0.00^{a}
0.4	0.00±0.00^{a}	0.00±0.00^{a}
	Oil extract	
0.0	72.00±3.2^{b}	58.00±1.5^{b}
0.1	0.00±0.00^{a}	0.00±0.00^{a}
0.2	0.00±0.00^{a}	0.00±0.00^{a}
0.3	0.00±0.00^{a}	0.00±0.00^{a}
0.4	0.00±0.00^{a}	0.00±0.00^{a}

Means followed by the same letter in the same column are not significantly different at P≤0.05

3.2. Contact Toxicity of the U. afzelli Powder on Adult P. interpunctella

The toxicity effect of the *U. afzelli* powder on the adult *P. interpunctella* showed in Table 2 reveals that the toxicity of the seed powder on the adult moth is dosage and time-dependent. It also reveals that a minimum of twelve hours is needed at any dosage greater than 0.1g/20g to achieve a lethal effect on the moth. Moreover, a consistent progressive increase in mortality rate was obtained at 0.2g/20g dosage. Likewise, eighteen hours following treatment, 50 % mortality was achieved in all the dosages above 0.1g/20g. Nonetheless, the 54.17 % mortality recorded in 0.2g/20g dosage eighteen hours following treatment was not significantly different from the 41.67 % mortality recorded at 0.1g/20g dosage at the same post treatment time ($P \leq 0.05$). No significant difference was observed in all the dosages twenty-four hours following treatments. Nevertheless, a complete mortality was obtained in all treatments except in 0.1g/20g where 98.17% mortality was recorded at 24h post treatment

Table 2. Contact toxicity of *U. afzelli* seeds powder on the mortality of adult *P. interpunctella*.

Rate (g/20g maize grain)	Mean% mortality after			
	6h	12h	18h	24h
0.0	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
0.1	0.00±0.00^{a}	0.00±0.00^{a}	41.67±2.64^{b}	98.17±0.7^{b}
0.2	0.00±0.00^{a}	23.33±2.01^{b}	54.17±3.33^{b}	100±0.00^{b}
0.3	0.00±0.00^{a}	26.67±2.1^{b}	70.80±3.00^{c}	100±0.00^{b}
0.4	0.00±0.00^{a}	76.70±2.11^{c}	95.83±2.00^{d}	100±0.00^{b}

Means followed by the same letter in the same column are not significantly different at $P \leq 0.05$

3.3. Contact Toxicity of the U. afzelli Extract on Adult P. interpunctella

The effect of the extract of *U. afzelli* on the mortality of adult *P. interpunctella reported* in Table 3 reveals that the effectiveness of the extract is concentration and time-dependent. Twenty-four hours following treatment, the mortality rate increases with concentration; this explains why the highest mortality of 53.00 % after twenty-four hours of treatment was recorded at the 4 % concentration treatment. Moreover, forty-eight hours following treatment, all the concentrations used achieved over 50 % mortality of the moth except the 1 % concentration which achieved 33.33 % mortality ($P \leq 0.05$).

Table 3. Effect of extract of *U. afzelli* on adult mortality of *P. interpunctella*.

Rate (%)	Mean % mortality after			
	24h	48h	72h	96h
1	0.00±0.00^{a}	33.30±3.33^{b}	77.00±3.0^{b}	83.01±3.3^{b}
2	0.00±0.00^{a}	80.00±0.00^{c}	83.33±3.3^{b}	100±0.00^{c}
3	33.33±3.33^{b}	86.70±6.67^{c}	96.67±3.0^{c}	100±0.00^{c}
4	53.00±3.33^{c}	96.67±3.30^{c}	100±0.00^{c}	100±0.00^{c}
**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}

Means followed by the same letter in the same column are not significantly different at $P \leq 0.05$

**Control 1 is the maize grains treated with 2ml of ethanol

**Control 2 is the maize grains treated with neither extract nor solvent

3.4. Fumigant Toxicity of the U. afzelli Seeds' Powder on Adult Mortality of P. interpunctella

More than 50 % mortality of adult *P. interpunctella* was recorded at the 0.4g/20g dosage twenty-four hours

after treatment through fumigant action. The fumigant action proved to be dosage-dependent as shown in Table 4. The fumigant effect of 0.1g/20g dosage is very consistent. All the dosages at seventy-two hours following treatment produced adult mortality greater than 50 % through fumigant action. Complete mortality of adult *P. interpunctella* through fumigant action was achieved at all dosages greater than 0.1g/20g over a post-treatment period of seventy-two hours (Table 4).

Table 4. Fumigant toxicity of *U. afzelli* seeds powder on adult mortality of *P. interpunctella.*

Rate (g/20 maize grains)	Mean % mortality after			
	24h	48h	72h	96h
0.0	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
0.1	0.00±0.00^{a}	33.30±3.33^{b}	77.00±3.0^{b}	83.01±3.3^{b}
0.2	0.00±0.00^{a}	80.00±0.00^{c}	83.33±3.3^{b}	100±0.00^{c}
0.3	33.33±3.33^{b}	86.70±6.67^{c}	96.67±3.0^{c}	100±0.00^{c}
0.4	53.00±3.33^{c}	96.67±3.30^{c}	100±0.00^{c}	100±0.00^{c}

Means followed by the same letter in the same column are not significantly different at $P \leq 0.05$

3.5. Fumigant Toxicity of the Ethanol Oil Extract of U. afzelli on Adult Mortality of P. interpunctella

The fumigant effect of the *U. afzelli* ethanol oil extract on the mortality of adult *P. interpunctella* shown in Table 5 shows that the fumigant action of the oil extract irrespective of the concentration elicited more than 50 % and 100 % *P. interpunctella* mortality at eighteen and twenty-four hours post treatments.

Table 5. Fumigant toxicity of extract of *U. afzelli* on adult mortality of *P. interpunctella.*

Concentration (%)	Mean% mortality after			
	6h	12h	18h	24h
0	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
1	16.67±3.3^{b}	25.00±2.9^{b}	72.00±4.4^{b}	100±0.00^{b}
2	20.00±0.0^{b}	26.67±3.3^{b}	85.00±2.9^{b}	100±0.00^{b}
3	20.00±0.0^{b}	37.00±6.7^{b}	88.00±3.0^{b}	100±0.00^{b}
4	25.00±0.56^{c}	60.00±5.8^{c}	93.00±3.2^{c}	100±0.00^{b}

Means followed by the same letter in the same column are not significantly different at $P \leq 0.05$

4. Discussion

The insecticidal activity of the *U. afzelli* seeds extract against eggs hatchability and 3rd larva instar development to adult investigated in this study shows that the seeds' extract is very efficient in inhibiting the development of eggs to larvae and 3rd larva instar to adult. At all rates of application, the powder completely inhibited the egg hatching and the development to adult. The systematic toxicity of the seed powder might be traced to the ingestion of the powder into the alimentary system of the insect thereby causing some unpleasant situation in the feeding and digestion of the insects.

The powder of the seeds of *U. afzelli* inhibited hatchability. This may be attributed to the suffocation caused by the powder to the developing embryos. The air surrounding the eggs may also be polluted because of the sharp aromatic nature of the seeds. Concurrently, the extract inhibited egg hatching and development to adult stage from the 3rd larva instar. This result can be better explained since the ethanol oil extract used for the treatment of the maize grains has a better penetrating ability because of the size of the solutes. Similar extracts from the *Annona senegallensis* root and bark were effective against the egg hatching of *Callosobruchus maculatus* (Aku *et al*., 1998). *Cleisthopholis patens* oil extract has also been reported to have inhibited the egg hatch and development of *E. cautella.* Moreover, a significantly low number of adult's *C. maculatus* were reported to have emerged from the seeds treated with the extracts of African nutmeg seeds [*Monodora myristica* (Gaertn.) Dunal] (Akinneye, 2003; Okosun and Adedire, 2010).

The toxic effect of the powder and rate of application of *U. afzelli* on the mortality of adult *P. interpunctella* depend on the mass of the powder and exposure periods. The injuriousness of the powder was pronounced within a short range of time on adult *P. interpunctella*. The adaptation of the adult moth to survive with the toxicity posed by the powder proved useless. The efficacy of this powder to control adult *P. interpunctella* at a very low concentration is time-dependent. The phytochemical property of the seeds powder might have resulted in the cytoplasmic coagulation of the insect (Bhaduri *et al.* 1990). The results agreed with the findings of Lajide *et al.* (2003) who reported that *U. afzelli, Eugenia aromatic* and *Aframomum melegueta* powder were toxic to *C. maculatus* at all level of treatment within twenty-four hours of application. Akinneye *et al.* (2009) also reported the efficacy of plant powder in the control of *Ephestia cautella* with *Cleisthopholis patens.* Additionally, Ashamo and Akinneye (2004) reported that a minute quantity of *Eugenia aromatica* powder caused greater than 43.3 % mortality of the yam moth *Euzopherodes vapidella*.

The protective ability of the *U. afzelli* seed powder to the grains might be a result of the systematic blockage of the spiracles and diffusion of the aromatic property of the plant into the insect respiratory system thereby causing suffocation. This can be interrelated with the sharp increase in mortality of the insects as the powder mass increased. Similar findings have been reported by Adedire *et al.*, (2011); Ileke and Olotuah, (2012).

The ability of the extract to cause 100 % adult moth mortality in all the concentrations at a period twenty-four hours of exposure may be attributed to the fumigant effect of the plant oil on the adult moth since there was a strong correlation between the death rate of the contact toxicity and fumigant action of the oil. The fumigant action of the oil might have caused death through respiratory inhibition, inhibition of oxidative phosphorylation, and amide metabolism (Ashamo, 2000).

Based on the results obtained from this research, it can be deduced that the *U. afzelli* powder ethanol oil extract used directly or in the form of fumigant can protect maize grain from infestation by *P. interpunctella* during storage.

The powder and extract of *U. afzelli* were good candidates for the control of *P. interpunctella* on maize grains since they completely inhibited the development of

P. interpunctella from egg to adult. Also, the powder and the ethanol oil extract after twenty-four hours of treatment, resulted into an adult mortality of 100 %. Therefore, both the powder and the ethanol oil extract of *U. afzelli* seeds are recommended for use to protect stored maize grains, and can also be integrated with other pest management procedures, since the plant powder and oil were edible, locally available, and medicinal. It is also recommended that the *U. afzelli* seeds could be produced and packaged on a large scale as botanical insecticides.

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