



ENTOMOTOXIC EFFECTS OF *PLUMBAGO ZEYLANICA* LINNAEUS ON *SITOPHILUS ZEAMAI* MOTSCHULSKY (COLEOPTERA CURCULIONIDAE)

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ABSTRACT

This study investigated the entomotoxic effects of different concentrations of the powder, oil extract and aqueous extract of *Plumbago zeylanica* against *Sitophilus zeamais* in stored grains. Adult *Sitophilus zeamais* was introduced into the container with the treated maize seeds. Weevils were exposed to contact and fumigant toxicity test at 0.5g, 1.0g, 1.5g, 2.0g and 2.5g/50g of maize grains and mortality was assessed at exposure period of 24, 48, 72 and 96 hours post-treatment. Result obtained show that weevils exposed to ethanolic oil extract of *Plumbago zeylanica* showed higher mortality value of (37-100%) at 2.5g concentration and exposure period of 72 hours while that of root powder of *P. zeylanica* showed mortality value of (33.24-100%) at 2.5g concentration and exposure period of 96 hours. Both the powder of *P. zeylanica* and its ethanolic oil extracts significantly reduced adult emergence in treated grains. Furthermore, the powder and oil extract of *P. zeylanica* root bark tested on the liver and kidney of albino rat were found to be non-toxic, since there was no significant difference between the control and the animal treated with the powder and the ethanolic oil extracts. This study showed that the powder and ethanolic oil extract of *P. zeylanica* would provide alternative to synthetic insecticides in the management of *Sitophilus zeamais* infesting maize grains in Nigeria.

KEYWORDS: “*Plumbago zeylanica*”, “*Sitophilus zeamais*”, “Entomotoxic”, “Adult emergence”.

INTRODUCTION

Post harvest pest problems may begin once the crop has attained physiological maturity and is undergoing natural dehydration in the field. For instance, maize weevil, *Sitophilus zeamais* (Motschulsky) is a major economic pest of stored products and found on every continent (Rees, 2004). It is a cosmopolitan pest of sound and wholesome grains, *Sitophilus zeamais* is a serious primary pest of maize but could secondarily be found attacking other crops such as rice, sorghum etc in Nigeria. (Nwana, 1993; NRI, 1996). Since, insects pests infestation has been reported as the major cause of food grain losses in most developing countries during storage (Adedire *et al.*, 2011). The use of conventional synthetic insecticides to control maize weevils and other stored product pests has a lot of attendant problems notably high mammalian toxicity, high level of persistence in the environment, workers safety, insect resistance and health hazards (Adedire and Ajayi, 1996). These have stimulated a search for alternative means of storage-pests control. In view of these, researchers and farmers have diverted 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, 2011), (Isman, 1997; Odeyemi, 1998; Adedire and Lajide, 2003; Arannilewa *et al.*, 2006). Small scale farmers and researchers have often claimed successful use of plant products in insect pest control. Plant materials such as spices, vegetable oils, extracts, powders or inert dust have been reported for their insecticidal efficacy (Keita *et al.*, 2001; Akinkulore *et al.*, 2006; and Adedire *et al.*, 2011). This study is therefore sought to evaluate the insecticidal activity of *Plumbago zeylanica* powder, ethanolic extract and aqueous against adult *Sitophilus zeamais* and also determine the toxic effect of the plant extract and powder on albino rat.

MATERIALS AND METHODS

The plant *Plumbago zeylanica* were collected in Modebiayo camp in Ondo West Local Government area of Ondo State, Nigeria.

Preparation of Plant Materials

Freshly harvested roots of *Plumbago zeylanica* were collected in Modebiayo camp in Ondo West Local Government area of Ondo State. The roots were taken to

the laboratory, for botanical identification and authenticated by Dr Akinneye J. O. of the Department of Biology, Federal University of Technology Akure. The roots were washed thoroughly with water and air-dried in the laboratory for 30 days. The roots bark were then pulverized into fine powder using Binatone Electric Blender (Model 373). The powders were further sieved to pass through 1mm perforation. The fine powders were kept in a separate bottles with screw cap-lid plastic containers and stored at ambient temperature of 28 ± 2 °C and 75±5% rh pending use.

EXTRACTION OF PLANT OIL

The solvent used for the extraction was ethanol. Thereafter, 200g of the pulverized plant root bark were weighed separately into a beaker and packed in a thimble using muslin cloth and extracted with 500ml of ethanol as solvents in a soxhlet extractor. In each case, the extraction was carried out for 3 hours 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 subsequent experiment. (Adegbe *et al*, 2016).

Insect Culture

The adult *Sitophilus zeamais* used for this study were obtained from field-infested maize grains from Federal University of Technology, Akure (FUTA) farm, Ondo State, Nigeria. Adult insects were reared in 2 litre plastic containers containing 400g of uninfested maize grains. The culture was maintained by continually replacing devoured powder and sieving out frass and fragments. The plastic container were covered with muslin cloth, fastened with rubber band and placed inside wire mesh cage of dimension 75cm x 50cm x 60cm with its four legs dipped in water-kerosine mixture contained in a plastic container to prevent entry of predatory ants into the cage.

New generation of *S. zeamais* obtained from this stock were reared by infesting 200g clean and uninfested grains with 10 pairs of adult *S. zeamais* to generate the required insect population for the study. The set-up was placed in the Postgraduate Research Laboratory in the Department of Biology, The Federal University of Technology Akure.

Insects Bioassay

Contact toxicity of *Plumbago zeylanica* powders on adult of *Sitophilus zeamais*. Fine powders of *P. zeylanica* root bark, were admixed with maize grains at the rate of 0.5, 1.0, 1.5, 2.0, and 2.5g/ 20g in 250ml plastic containers (8cm diameter and 4cm depth). The container cover was punched with hot rod and lined with muslin on the inside to prevent insect escape and allows for aeration. Ten

pairs of adult *S. zeamais* was introduced into plastic containers containing the treated maize and untreated samples was also infested to serve as control with adult *S. zeamais* and all treatment were replicated three times. Adult mortality at 24, 48, 72 and 96 hours after treatment were counted and recorded. At the end of 96 hours post treatment, data, on percentage adult mortality was corrected using Abbot (1925) formula thus,
Where $P T$ = Corrected Adult mortality
 P_o = Percentage mortality of treated insects
 $P C$ = Percentage mortality on treated insects

Fumigant effect of *P. zeylanica* powders on adult *S. zeamais*

Fine powder of the *P. zeylanica* root bark were sealed in muslin clothes (2cm by 2cm) and hanged on the lid of plastic containers of dimension 8cm depth x 4cm width. Twenty pairs of newly emerged adult were introduced into plastic container containing 20g of maize grains and covered with lid. Plant powder were hanged at equal distance between the lid and the base of the container. Untreated maize grains with adult of *S. zeamais* were also set up as control. The treatments were replicated three times. Adult mortality at 24, 48, 72 and 96 hours after treatment were determined and recorded. At the end of the 96 hours post treatment period, data on percentage adult mortality were corrected using Abbot (1925) formular.

Toxicological investigation of *P. zeylanica* powder feed formulation.

The diets were freshly formulated according to the modified method of Oboh (2005) and were kept air tight containers and stored at 40c until need for use

Animals grouping

After two week's acclimatization, another eighteen albino rats were randomly grouped in six (Group I – VI) of three animal each. Group I was fed with Basal diet, Group II was fed with Basal diet containing 0.5g *P. zeylanica* root bark powder, Group III was fed with Basal diet containing 1.0g *P. zeylanica* root bark powder, Group IV was fed with Basal diet containing 1.5g *P. zeylanica* root bark powder, Group V was fed with Basal diet containing 2.0g *P. zeylanica* and Group VI was fed with Basal diet containing 2.5g *P. zeylanica* root bark powder, the experiment lasted for seven days and cage side examination were perform daily to overt signs of toxicity. Cage side examinations were performed to detect overt signs of toxicity (salvation, lacrimation, convulsion, loss of hair, stress, behavioral abnormalities and dead rats) (Ratnasoriya *et al.*, 2000). After 24 hours the animals were sacrificed by cervical dislocation.

Preparation of Serum

The procedure described by Yakubu *et al.*, (2007) 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 3000rpm for 20mins. The serum was carefully

aspirated with pasture pipette into sample bottles for the various biochemical assays.

Statistical analysis

The data on adult mortality were subjected to one-way analysis of variance (ANOVA) ($P < 0.05$) and treatment means were separated using Duncan's New Multiple Range Test. Contact and fumigant mortality data and dosages of the root bark of *P. zeylanica* were also subjected to probit and log transformation respectively to determine the dosage lethal to 50% and 95% of *S. zeamais* (LD 50 and LD 95). All analyses were determined using SPSS 20.0 software package.

RESULTS

Contact toxicity of *P. zeylanica* root bark powder on adult mortality of *S. zeamais*

The contact toxicity of *P. zeylanica* powder on mortality of adult *S. zeamais* is presented in Table 1. There was no mortality at 24hr post treatment using 0.5g of the root bark powder, while 1.0- 2.5g of the root bark caused over

15.0% adult mortality at 48hr post treatment, all rates of the root bark caused 15-73% adult mortality of the weevils. The root bark at 2.5g was the earliest to cause 100% mortality at 96hr post treatment. The mortality obtained at 2.0g and 2.5g of the root bark were not significantly different ($P > 0.05$) at 96hr post treatment.

Fumigant toxicity of *P. zeylanica* root powder on adult mortality of *S. zeamais*

The fumigant toxicity of *P. zeylanica* root bark powder on mortality of adult *S. zeamais* is presented in Table 2. There was no mortality at 0.5g rate of the root bark powder, at 24hr post treatment while 1.0 - 2.5g of the root bark caused over 18.0% adult mortality. At 48hr post treatment, all rates of the root bark caused 16-63% adult mortality of the weevils. The root bark at 2.5g was able to cause above 90% mortality at 72hr and 93% at 96hr post treatment. The mortality obtained at 2.0g and 2.5g of the root bark were not significantly different ($P > 0.05$) at 96h post treatment.

Table 1: Contact toxicity of *P. zeylanica* powder on Adult mortality of *S. zeamais*(M ± SE).

Rate (g/20g)	Mean %Adult Mortality After			
Maize (grain)	24 Hrs	48 Hrs	72 Hrs	96 Hrs
Control	0.00±0.00 a	0.00±0.00 a	0.00±0.00 a	0.00±0.00 a
0.5		0.00±0.00 a	15.00±2.62 b	24.22±1.7 b
1		20.00±2.17 b	33.00±7.28 c	45.23±5.8 c
1.5		32.33±1.67 c	48.23±5.67 d	65.55±4.33 d
2		34.23±1.77 c	62.45±6.21 e	83.31±6.7 e
2.5		47.23±2.77 d	73.45±7.34 f	90.00±0.00 f
				100.00±0.00 d

Means followed by same alphabet are not significantly different at $P < 0.05$ using New Duncans Multiple Range Text.

Table 2: Fumigant toxicity of *P. zeylanica* powder on Adult mortality of *S. Zeamais* (M ± SE).

Rate (g/20g Maize grain)	Mean %Adult Mortality After			
	24hrs	48hrs	72 Hrs	96hrs
Control	0.00±0.00 a	0.00±0.00 a	0.00±0.00 a	0.00±0.00 a
0.5	0.00±0.00 a	16.00±2.62 b	22.12±1.7 b	43.24±6.19 b
1	18.00±2.17 b	32.05±7.28 c	42.23±3.8 c	61.23±4.71 c
1.5	32.33±1.67 c	45.28±5.67 d	55.55±4.33 d	77.42±3.34 d
2	38.23±1.77 c	8.45±6.01 e	82.31±6.7 e	87.26±4.55 d
2.5	57.03±2.77 d	63.25±7.34 f	90.30±0.00 f	93.20±0.00 d

Means followed by same alphabet are not significantly different at $P > 0.05$ using New Duncans Multiple Range Text..

Effects of *P. zeylanica*'s root bark powders on liver biochemical parameters of albino rats

Table 3 revealed the effects *P. zeylanica*'s root bark powders on liver biochemical parameter of albino rats, which showed no significant different ($P > 0.05$) in serum level of both aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphate (ALP) for the rats in group I, II, III, IV and V when compared with the control rats (Group 1).

Effect of *P. zeylanica*'s root bark on kidney biochemical parameter of albino rats

The effects of *P. zeylanica*'s root bark powders parameters of albino rats were presented on Table 4. The kidney activities of urea, total bilirubin, direct bilirubin, creatinine of the animals fed with basal diet (Group 1), and animals fed with basal diet containing 0.5g 1.0g, 1.5g, 2.0g and 2.5g of *P. zeylanica*'s root bark powders were not significantly different ($P > 0.05$).

Table 3: Biochemical parameters of Liver of Albino rats fed with *P. zeylanica*'s root bark powder.

GROUP	AST (U/L)	ALT (U/L)	ALP (U/L)
I	21.67 1.33 a	66.00 0.00 a	20.50 1.50 a
II	22.67 2.03 a	67.00 11.00 a	19.17 1.59 a
III	21.67 2.96 a	66.00 0.00 a	18.00 2.31 a
IV	21.33 1.20 a	67.00 11.00 a	19.67 0.67 a
V	22.67 2.03 a	67.00 11.00 a	20.33 1.33 a
VI	22.67 2.03 a	66.00 0.00 a	20.83 0.16 a

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

Table 4: Biochemical parameters of kidney of Albino rats fed with *P. zeylanica*'s root bark powder.

Groups	Urea (mol/l)	TOTAL Bilirubin (mol/l)	Direct Bilirubin (mol/l)	Creatinine (mol/l)
I	8.06 0.31 a	20.97 1.23 a	271.00 2.87 a	120.1 30.00 a
II	8.24 0.32 a	21.00 0.60 a	264.00 2.00 a	120.1 30.00 a
III	8.14 0.09 a	22.22 1.05 a	270.00 3.79 a	121.2 30.00 a
IV	8.79 0.23 a	20.97 1.23 a	268.30 2.33 a	120.1 30.20 a
V	7.70 0.32 a	20.37 1.85 a	266.70 0.67 a	121.1 30.0 a
VI	8.41 0.09 a	20.98 1.85 a	266.00 0.00 a	120.1 30.1 a

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

Keys

Group I – normal control rats, fed with basal diet (18% corned starch, 18% rice grain, 50% skinned milk, 4% minerals, vitamin primates and 10% vegetable oil);

Group II – rats fed with basal diet with 0.5g *P. zeylanica*'s root bark powder;

Group III – rats fed diet supplemented with 1.0g *P. zeylanica*'s root bark powder;

Group IV – rats fed diet supplemented with 1.5g *P. zeylanica*'s root bark powder;

Group V – rats fed diet supplemented with 2.0g *P. zeylanica*'s root bark powder;

Group VI – rats fed diet supplemented with 2.5g *P. zeylanica*'s root bark powder

DISCUSSION

A lot of problems associated with the usage of fumigants and contact synthetic chemicals in the control of stored product pests have highlighted the need for other control measures that are believed to be safer and cheaper. Consequently, the use of botanicals has been advocated as an alternative to most synthetic insecticides in the control of stored product pests (Ofuya and Lale, 2001). But, the efficacy of botanicals on target species varies as it depends on several factors such as part of plant from which they have been extracted, plant species, mode of application among others. Present study investigates the potential of root powder of *P. zeylanica* as a contact and fumigant insecticide for management of *S. zeamais* infesting maize grains. Considerable reduction observed in the numbers of adult emerged in *S. zeamais* exposed to both contact and fumigant toxicity test of *P. zeylanica* powders and ethanolic oil extract especially at the highest experimental dosage and exposure time, shows that this plant possesses insecticidal properties against *S. zeamais*. This is in agreement with Ashamo and

Akinawonu (2012). Where insecticidal efficacy of different plant materials against *S. zeamais* has been reported. The high contact toxicity of root of this plant may be due to physical abrasion of the wings and cuticle of *S. zeamais* by this plant material. This could have resulted in loss of body fluid, dehydration and high mortality of *S. zeamais* observed in this study. Higher mortality was also observed in weevils exposed to fumigant test when compared to those exposed to contact test this is because of the pungent smell released by the plant part. This further suggests *P. zeylanica* may be better used as a fumigant against *S. zeamais* due to the fact that the product did not taint the produce or give it another colour. Although it may also be used as a contact insecticide as there were no significant differences when compared. The high fumigant toxicity of this plant against the insect may be ascribed to its high volatility of the active ingredients contained in the plant which kills the insect by diffuse in through the spiracles. This might have led to the blockage of the weevil spiracles, suffocation and high mortality observed in insects exposed to fumigant test.

Contact toxicity of *P. zeylanica* on adult mortality of *S. zeamais*

The contact toxicity of *P. zeylanica* depends on concentration, and exposure periods. Mortality varied with rate of application and exposure periods. At 24hrs post treatment, the 0.5g rate of the root bark showed no adult mortality of the insect. This findings was similar to observation of Akinneye (2011) in the control of *E. Cautella* with *C. patens* powder. The root bark powder at the rate of 2.0g and 2.5g concentration was effective against *S. zeamais*, producing 100% mortality within 96 hrs post treatment. This observation also tallies with the findings of Adedire and Lajide (2001) that the pulverized powder of *P. umbrellatum* seed and Eugenia. aromatic

were toxic to *C. maculatus* producing 100% mortality at 24hr post treatment across and concentrations. The toxic effect of the root of *P. zeylanica* in this study could be attributed to their pungency which evoked suffocating action of the insect. The powder may also bind to the enzyme, cholinesterase thus preventing the removal and resultant accumulation of acetylcholine, the neurotransmitter, restlessness convulsion and paralysis may occur resulting in death of insect (Ashamo, 2000).

5.2 Fumigant toxicity of *P. zeylanica* Powders on adult mortality of *S. zeamais*.

The fumigant toxicity of *P. zeylanica* root bark powders on adult mortality of *S. zeamais* observed at 96 hrs post treatment with different dosage level revealed the insecticidal potency of *P. zeylanica* root bark. The percentages mortality varied with rate of application and exposure periods. There was no mortality at 24hrs post treatment, at 0.5g rate of root bark. At 48hrs post treatment, all rates of the root bark, i.e 1.0-2.5g rate caused greater than 18% adult mortality of the insect. This findings is similar to the findings of Ashamo and Akinneye (2004) that *Eugenia aromatic* caused greater than 43.3% mortality of *E. vapidella*, at 0.05g/15g of yam. The powder of Plant material acted mainly through fumigant modes of action. This powder may cause death through respiratory inhibition, inhibition of oxidative phosphorylation and amine metabolism (Ashamo 2000).

Toxicity of *P. zeylanica* root bark powder on liver and kidney biochemical parameters of albino rats

The measurement of the activities of various enzymes in tissues and body fluids play a significant and well known aid in disease investigation and diagnosis (Yakubu *et al.*, 2003; Bamisaye *et al.*, 2013). The sub-acute toxicity study conducted where the albino rats feed with basal diet containing 0.5g, 1.0g, 1.5g, 2.0g and 2.5g of *P. zeylanica* root bark powder for 7 seven days, the albino rats does not produce any behavioral changes during 7 days observation. Indicators of liver damages AST, ALT, ALP were not negatively affected although the powder caused an insignificant increase in the level of AST, ALP and ALP compared with the control. Similar observations have made by Awais *et al.*, (2008) in normal rats with the methanolic extract of *Tulbaghia violace* rhizomes in wistar rats. The plant powder did not show any significant effect on marker of kidney function such as urea, creatinine, total bilirubin and direct bilirubin. The results of the present study showed that the addition of 0.5, 1.0, 1.5, 2.0 and 2.5g of the *P. zeylanica* root bark to the basal diet for 7 days did not change the biochemical parameters of kidney function. Since it showed that no significant change in the serum level of urea, creatinine, total bilirubin and direct bilirubin of animals fed with 0.5, 1.0, 1.5, 2.0 and 2.5g groups compared with the control (basal diet) group. Therefore, the level of serum urea, creatinine, total bilirubin and direct bilirubin indicate no kidney damage. Similar observation have been made by Bamisaye *et al.*, (2013) and Dollah *et al.*, (2012) in normal rats treated with the

extract of *Morinda lucida* castor seed oil and powder of *Nigella sativa* for 7 days, 30 days and five weeks respectively. These result suggested that the plant powders does not alter the liver and kidney biochemical function and also suggest that consumption of *P. zeylanica* root bark powder by human beings to protect maize grains against *S. zeamais* will not cause toxicity effect on the liver and kidney function.

CONCLUSION

In this study, the root bark powder and ethanol oil extract of *P. zeylanica* were found to be the most effective for the control of *Sitophilus zeamais* on maize grains since they completely inhibited development of *S. zeamais* from eggs to adult stage. The powder and ethanol oil extract were able to achieve 100% adult mortality after 72hours post-treatment. Furthermore, the powder and oil extract of *P. zeylanica* root bark tested on the liver and kidney of albino rat and were found to be non-toxic, since there was no significant difference between the control and the animal treated with the powder and the ethanolic oil extracts.

Therefore, both the powder and the oil extract of *P. zeylanica* root bark could be recommended for use to protect stored maize grains and can also be integrated with other pest management procedure. Additionally, the maize grains are safe for consumption after post-harvest treatment since the root bark and oil were found to be non-toxic to mammals.

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