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PHYTO-CHEMICALS OF SOME PLANT LEAF POWDER AS ANTI-INSECT AGENTS AGAINST MAIZE WEEVILS *Sitophilus zeamais* (Coleoptera: Curculionidae)

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ABSTRACT

Leaf powder of Purple dead nettle (*Lamium purpureum*), Nut grass (*Cyperus retrorsus*), Wild sage (*Lantana camara*), Sunflower (*Helianthus annuus*), Key lime (*Citrus aurantifolia*) and Queen of the night (*Cestrum nocturnum*) were screened for secondary metabolite constituents and insecticidal activity against Maize weevil (*Sitophilus zeamais*). Phytochemicals screening of the powder revealed the presence of alkaloids, terpenoids, flavonoids, tannins, saponnins, phytosteroids, phenolic compounds, proteins and aminoacids, oil and fats as well as reducing sugars in the plants investigated. The plants powder indicates insecticidal activity in a dose dependent manner, higher doses has stronger effect, all the experimental plants caused significant mortalities ($p \leq 0.05$) of the *S. zeamais*. LD_{50} (g) showed that *L. purpureum* (3.5) was most toxic to *S. zeamais* followed by *L. camara* (3.9) and *C. aurantifolia* (4.7), also *H. annuus* (5.9) and *C. retrorsus* (5.9) were more toxic than *C. nocturnum* (9.3) which was least toxic to adults of *S. zeamais*. Therefore, these phyto-chemical constituents of plants powder have potential to be used as control agents of *S. zeamais* infestations and could be used as replacement or supplements to conventional chemical insecticides which price, availability and technology of applications may be out of reach to poor farmers.

Keywords: Insecticidal activity, Powder, Phyto-chemical, Screening, *Sitophilus zeamais*

INTRODUCTION

Maize weevil *Sitophilus zeamais* is one of the major storage pests of maize; Maize is the third most important cereal crop in the world (FAO, 2002). This insect causes reduced quality and quantity of stored maize in only few months of infestation, consuming up to 15% of the stored maize grain (Bergvinson, 2004). Poor post-harvest storage has accounted for up to 40% lost in cultivated maize (Gerald, 2008). Stored-Product pest management in most part of the world has relied on the use of chemical insecticides; however, chemical control methods are restricted because of the development of pest resistance, health hazards and risk of environmental contamination (Isman, 2006). Therefore, in the current scenario, there is urgent need to develop safer, environmental friendlier and efficient alternatives that have potential to replace synthetic chemical insecticides and convenient to use. Plants powders and their components have shown to possess potential for development as insecticides and they may have advantages over conventional insecticides in terms of low mammalian toxicity, rapid degradation and local availability (Li *et al*., 2001). phyto-chemical compounds such as Alkaloids, Terpenoids, Flavonoids, Tannins, Saponins and Phenolic compounds are reported to possess anti-insects activities (FAO, 1999). The presence of these compounds forms the basis of the insecticidal properties of the plants powder and extracts. These compounds can affect insects in several different ways: they may disrupt major metabolic pathways and caused rapid death (Bell *et al*., 1990) acts as attractants, deterrants, phago-stimulants, anti-feedants or modify ovipositions, retard or accelerate development or interfere with life cycle of the insects in other ways (Bell *et al.*, 1990). Hence in the present study, powder phytochemical constituents of six (6) different plants species were evaluated for anti-insects properties against Maize weevil (*Sitophilus zeamais*).

MATERIALS AND METHODS

Culturing of insects: Maize weevil *S. zeamais* were collected from infested stock of maize grains at Abubakar Rimi Market Kano. *Sitophilus zeamais* was identified as described by Richard (1991). Twenty (20) pairs of *S. zeamais* were used to infest fresh 1000g of maize grains, contained in a label transparent bucket (35cm height and 30cm diameter). The bucket was capped with piece of net 10 mesh/cm which allowed for ventilation but precludes the entry or exit of the insect. The set were maintain under ambient conditions of temperature, relative humidity and photoperiods (32 ± 0.64^0C, 68±3% and 12L: 12D) (Olaifa and Ethan, 1997) in the

Laboratory for two weeks to ensure oviposition. The parent stocks were sieve out and maintained undisturbed until adult emergence. The First Filial (F1) adults emerging over 24hrs period were collected, preserved in another container and used for subsequent experiments (Magaji *et al.,* 2009)

Collection of plant materials and powder preparation: The six (6) plants materials were collected around Sharada phase II industrial area Kano by direct hand picking and identified at herbarium of the Department of Biological Sciences, Bayero university Kano. The plants were washed with clean water and dried under shade at room temperature of about 30^0C for five days. Shade dried Materials of each of the six (6) plants were grinded into fine powder using mortar and pestle as described by Lale (2002). Four different dosages (1g, 2g, 3g, and 4g) of each of the plants powder were prepared using weighing balanced.

Phyto-chemical Screenings: All the six (6) plant used in this research were screened for the presences of the following commonly found plants secondary metabolites.

1-Alkaloids.6-Saponins
2-Flavonoids.7-Tannins
3-Terpenoids.8-Proteins and Amino acids
4-Oils and Fats.9-Phenolic Compounds
5-Reducing Sugars.10-Phytosterols

1-Alkaloids: Drops (2-3) of Dragendoff's reagent were added to 1ml of the powder solution (Aqueous). An orange red precipitation indicates the presence of alkaloids (Ciulci, 1994).

2-**Flavonoids**: Two milliliter (2ml) of each of powder solution (aq) were dissolved in Sodium hydroxide Solution ($NaOH_{(aq)}$) Yellow solution appeared which disappears on addition of Hydrochloric acids indicates the presence of Flavonoids (Onyeleke and Manga, 2008).

3-**Terpenoids**: Two milliliter (2ml) of Chloroform were mixed with powder Solution (aq) then followed by addition of 3ml of Sulphuric acid ($H_2SO_{4(aq)}$). Formation of reddish brown color indicates the presence of Terpenoids (Abdulwadood *et al.,* 2013).

4-**Oils and Fats:** A small quantity of powder solution was compressed in between two filter papers. Oil stains on the filter paper indicate the presence of oils and fats (Kalita *et al.,* 2011).

5-**Reducing Sugars**: Powder solution (aq) in a test tube was added with few drops of Fehling's A and B solution. The mixture was warmed. Brick red precipitate at the bottom of the test tubes indicates the presence of reducing sugar (Brain and Turner, 1975).

6-**Saponins**: Five milliliters (5mL) of distilled water was added into 1ml of powders solution (aq) in test tubes the mixture was shaken vigorously. A persistent froth that lasted for 15minutes indicates the presence of Saponins (Brain and Turner, 1975).

7-**Tannins**: Two milliliter (2ml) of each plant powders solution (aq) was added with 2-3 drops of 5% ferric chloride ($FeCl_{3(aq)}$) Solution in test tubes. A green-black coloration indicated the presence of Tannins (Ciulci, 1994).

8-**Proteins and Amino Acids**: Each plant powder solutions(aq) were mixed with few ml of $HCl_{(aq)}$ then followed by 2 drops of ninhydrin solutions(10mg of ninhydrin in 200ml of acetone) in a test tubes the mixture was shaken thoroughly, purple coloration indicates the presence of protein and Amino acids.(Kalita *et al*., 2011).

9-**Phenolic Compounds**: Each plants powder solution (aq) in test tubes was added with few drops of neutral 5% Ferric chloride ($FeCl_3$) Solution. A dark green color indicates the presence of Phenolic Compounds. (Kalita *et al.,* 2011).

10-**Phytosterols:** Each plants powder solution (aq) were dissolved in a 2ml of acetic anhydride, then followed by conc.H_2SO_4 (aq) added slowly along the sides of the test tubes. An array of color changes showed the presence of phytosterols (Kalita *et al.,* 2011).

Powder toxicity assay: Method used by Abdullahi *et al.* (2010) were adopted, four different concentrations of all the six (6) plants powders (1g, 2g, 3g and 4g) were admixed with 10g each of preserved maize grains contained in a small transparent plastic containers (4cm height and 6cm diameter) with control treatments having no plants powders.

Ten (10) adults (1-2 days old) of *S. zeamais were* introduced into treated and untreated maize grains and then closed with perforated cap to aid ventilation but preclude the entry or exits of the insect pests. Insects' mortality was scored at 24hrs post-treatment intervals for one week. Insects were considered dead only if they fail three probing blunt test and Abbotts formula (Abbott, 1925) was used to correct observed mortalities where control mortalities exceed 20%.

$$\text{Correct Mortality} = \frac{\%\text{Test Mortality} - \%\text{Control Mortality}}{100 - \%\text{Control Mortality}} \times 100$$

Data Analysis: All data generated from the work were subjected to analysis of variance (ANOVA) using SPSS (version 20) for windows, means were separated ($p \leq 0.05$) using Turkeys tests, while LD_{50} (g) values for powder were also estimated by Probits analysis using same statistical packaged.

RESULTS

Phyto-chemical constituents of plants powder

The phyto-chemical compounds were identified by preliminary phyto-chemical screening and the results obtained were presented in Table 1.

Table 1: Phyto-chemical constituents of powder of the 6 experimental plants.

Test	*L.purpureum*	*C.retrorsus*	*L.camara*	*H.annuus*	*C.aurantifolia*	*C.nocturnum*
Alkaloids	+	-	+	-	+	-
Flavonoids.	-	-	+	-	+	-
Terpenoids.	+	+	+	-	+	-
Saponnin.	+	-	+	+	+	+
Tannin.	+	+	+	+	+	+
PC	+	+	+	-	+	+
Phytosterols.	-	-	+	+	+	-
PAA.	+	-	-	+	-	-
OAF	+	-	+	+	+	-
RS	-	-	+	+	+	-

***Key** +=Presence, - =Absence, PC=Phenolic Compounds, PAA=Protein and Amino Acids, OAF=Oils and Fats, RS=Reducing Sugars,

Alkaloid was found in powder of *L. purpureum, L. camara* and *C. aurantifolia,* also Flavonoids was detected in the powder of *L. camara*and*C. aurantifolia* only, Similarly Tannins was identified in powder of all the six (6) plants, the same with Saponins which is only absent in powder of *C. retrorsus*.

Phenolic compound was not found in the powder of *H. annuus* but found in all other plants powder screened, Phytosterol was detected only in powder of *L. camara*, *H. annuus*and *C. aurantifolia*, but protein and Amino acids were found in powder of *L. purpureum* and *H. annuus*respectively. Oils and Fats were found in powder of *L. purpureum, L. camara, H. annuus*and *C. aurantifolia*. While reducing sugars were found in powder and of *H. annuus, C. aurantifolia* and powder of *L. camara*.

Powder Toxicity

For all the plants powder used, the percentage mortality increased with increased in dosage levels of the plants powders for *S. zeamais,* the effects indicate by different levels of dosage of plants powder used on the percentage mortality of adults *S. zeamais* were presented in Table 2.

Table 2: Percentage mortality (%) of Adult Insects Treated with Powder of experimental plants at 96hrs Post exposure

	Powder Amount (g) in 10g of Maize (*S. zeimais*)				
Plant Used	**Control**	**1.00**	**2.00**	**3.00**	**4.00**
L.purpureum	10.1±1.2b.	57.6±0.5a.	57.6±0.5a.	91.1 ±0.8a.	100.0±0.0a.
C.restrorsus	11.2±1.1b.	32.4±1.0c.	51.7±0.5b.	61.0±0.4c.	83.4±0.6b.
L.camara	16.6±1.2a.	56.1±0.5a.	61. ±0.4a.	63.2±0.4c.	100.0±00a.
H.annuus	19.1±1.0a.	43.2±0.7b.	52.1±0.5b.	62.1±0.4c	74.2±0.6c.
C.aurantifolia	11.2±1.1b.	45.6±0.7b.	50.0±0.5b.	71.4±0.6b.	100.0±0.0a.
C.nocturnum	10.1±1.2b.	36.1±1.0c.	42.1±0.7c.	52.1±0.5d.	57.3±0.5d.

Each value is the mean *(*±SE) of three replicate. Means in each column by same alphabet(s) are not significantly different ($p \leq 0.05$) by Turkey's tests.

While the results of Probits analysis indicating LD_{50} (g) of the experimental plants used against *S. zeamais,* were presented in Table 3.

Table 3: LD_{50} (g) of experimental Plants powder against *S. zeamais*

Plant Used	**LD_{50}(g)**	**df**	**Chi^2**
L. purpureum	3.51	5	0.374
C. restrorsus	9.33	5	0.307
L. camara	3.90	5	0.846
H. annuus	5.88	5	0.022
C. aurantifolia	4.71	5	0.167
C.nocturnum	5.88	5	0.022

Out of the six (6) plant species screened, *L.camara* and *C .aurantifolia* possess the highest number of phytochemical compounds nine (9), *L. camara* has eight (8), *L. purpureum* seven (7) compounds are found, *H. annuus* that has six (6) phytochemical compounds in its powder. The least number of phytochemical compounds (3) was found in powder of *C. retrorsus and C. nocturnum*. Hence insecticidal activities indicated by *L. purpureum, C. retrorsus, L. camara, H. annuus, C. aurantifolia* and *C. nocturnum* could be attributed to the compositions of these compounds in their powder.

LD_{50} (g) estimated showed that *L. purpureum* (3.5) was most toxic to *S. zeamais*, followed by *L. camara* (3.9) and *C .aurantifolia* (4.7), also *H. annuus* (5.9) and *C. nocturnum* (5.9) were more toxic than *C. retrosus* (9.3) which has least toxic effect to adults of *S. zeamais* (Table 3).

DISCUSSION

The results of analysis of variance (ANOVA) indicates that all levels of dosages of plants powder used have significant powder toxicity against *S. zeamais*, Hence the results of post-experimental comparisons (Turkey's Tests) Showed that, *S. zeamais* was susceptible to all plants powder treatment with highest mortality (100%) observed in *L. camara, C. aurantifolia* and *L. purpureum* treatments at dosage level 4.0g 96hrs post-treatments intervals (Table 2), however, all the plants powder treatments at all dosage levels used indicate significant powder toxicity to *S. zeamais*, when compared with controls ($p \leq 0.05$) (Table 2).

The mortality caused by the phyto-chemical constituents of plants powder could be attributed to several mechanisms and the plants powder could have resulted to death of the insects due to contact poisoning, interference with acetylcholine receptors (Rattan, 2010), ingestions of the powder constituents which may in turn interfere with metabolic activities of the insects causing rapid death (Bell *et al.,* 1990) blockage of spiracles or interference with cuticular development of the insect pest as a results of direct contact between the insects and plants powder (Abdullahi *et al*., 2010). The differences in anti-insects properties of the experimental plants powder could be due to the differences in compositions of the active compounds or phyto-chemicals in their powders.

These results were in agreement with many other works on the use of plant powders against stored products insects' pests. For example Ajayi (2013) reported that powder and extracts of *Delonix regia* seeds were effective in controlling Maize weevils *Sitophilus zeamais.* Results of findings of Awoke *et al*, (2014) showed that leaves powders of *Melia azedarach, Mentha piperita* and *Schinus molle* were effective in controlling *S. zeamais*. Olaifa and Erhun (1997); Fasakin and Aberejo (2002) Observed that *Piper guineese* spice powder prevent oviposition on *Callosobruchus maculatus* and *Dermestes maculates* respectively, and therefore, reducing the longevity of the insects. Similarly, Abdullahi *et al*., (2010) reported that Citrus peel powder was effective in suppressing the survival of *T. castaneum* when applied at 4, 6 and 8g respectively. Furthermore, Akinkurolere (2012) reported that plants powders of *Tetrapleura tetraptera, Monodora myristica* and *Momordica charantia* were found to be effective in controlling Cowpea weevils' *C. maculatus.* Bernard and Daniel (2013) reported that Basil plant powder (*Ocimun basilicum*) were only effective for short durations as protectants against Maize weevils *S. zeamais*. Popoola (2013) also reported those powders and whole forms of *Allium sativum, Allium cepa*and *Capsicum annum* to cause significant mortality and reduction in F1 adults' emergence of *Oryzaephilus dutinamensis* infesting Date fruits. Similarly, Aswalam and Onu (2014) recorded the effectiveness of plants powder prepared from different parts of *A. sativum, Zingiber officinale, Curcuma longa, Ficusexa sperata* and *Garcinia kola* in killing and Controlling of *Trogoderma granarium* in stored groundnut.

CONCLUSION

The phytochemicals or secondary metabolites such as alkaloids, terpenoids, flavonoids, tannins, saponnins and phenolic compounds present in the powder of *L. purpureum, C. retrorsus, L. camara, H. annuus, C. aurantifolia* and *C. nocturnum* were responsible for the anti-insects properties indicated by their powders, hence these compounds can be developed as a replacement or supplement to most widely used synthetic pesticides which; price, availability and technology of applications may be out of reach to poor farmers, as well as its effect to the environment, man and livestock and also the development of resistance by the insect pests.

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