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Research Article

Phytochemical investigations of *Piper guineense* seed extract and their effects on *Sitophilus zeamais* (Coleoptera: Curculionidae) on stored maize

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Abstract: Three different solvents namely; hexane, ethyl acetate and ethanol were used in partitioning the extracts obtained from *Piper guineense* seeds and solvent extracts were investigated for the presence or absence of secondary metabolites. Extracts were further evaluated on adult maize weevils, *Sitophilus zeamais*. Experiment was carried out under laboratory conditions at 27 ± 2 °C ambient temperatures and 65 ± 5% relative humidity and was arranged in a completely randomised design in four replicates. Parameters assessed, including contact toxicity, repellent effects as well as effect of solvent extracts on the germination of maize seeds. Results obtained showed that the solvent extracts contained all the secondary metabolites tested except flavonoids and steroids. As well, all the solvent extracts were lethal and possessed high repellent action against *S. zeamais*, with ethanol fraction being the most potent. There was no significant difference in the germination of seeds treated with the solvent extracts as well as the ethanol control. The results obtained from this study reveal *P. guineese* as a potential candidate for bio insecticide and could serve as an alternative to synthetic insecticides.

Keywords: *Piper guineense*, secondary metabolites, *Sitophilus zeamais*, contact toxicity

Introduction

Maize, *Zea mays* (L.), is one of the most important cereal crops in the world today. The grains, cobs, stalks, leaves, tassels and silks all have commercial values in most settings, though that of the grain is greatest (Timothy *et al.* 1988; Paliwal, 2000; Akinbuluma and Ewete, 2014). *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) is a serious primary and a major pest of maize in Nigeria and other parts of the world, causing most of the losses incurred in maize annually (Adedire *et al.*, 2012). Declining food production, worsened by huge losses resulting from *S. zeamais* attack during maize storage expose farmers to different magnitudes of food shocks (Nwosu and Nwosu, 2012). *S. zeamais* is capable of penetrating and infesting intact kernels of grain, in which immature stages develop (Lale and Ofuya, 2001) leaving the maize emptied of its nutritional and seed value culminating in outright rejection of the product at the local and international markets (Nwosu *et al.*, 2015). Botanical pesticides are of interest to organic farmers because the chemicals are natural products, hence easily biodegradable

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and are often thought of being safe to handle and use on food products. Some botanicals or their derivatives such as pyrethrum-based products have made an impact in crop protection and this has rekindled hopes for the resurgence of plant-derived pesticides. Plant-derived pesticides are traditionally used and produced by farmers in developing countries where these botanicals are locally readily available and appear to be quite safe and promising (Stoll, 2000). Complex mixtures of lethal and sub-lethal phytochemicals in botanicals often offer multi-factorial selective pressures that retard the development of resistance in pests (Ntonifor *et al*., 2006; Ntonifor, 2011). Several compounds of plant origin exert various physiological and behavioural activities on stored product insects and notable among these plants are various spices and medicinal plants used traditionally for protecting foodstuffs against insects (Echendu, 1991; Ho *et al.,* 1995). Among the plants investigated to date, one showing enormous potential in the control of *Sitophilus zeamais* is member of the family Piperaceae (Dodson *et al*., 2000). Seed powders and oils of *Piper guineense a*re known to adversely affect the biology of maize weevil and also cause mortality (Ivbijaro and Agbaje, 1986; Lajide *et al.,* 1998). The use of its seed oil for the control of insect pests in stored food commodities did not result in health hazards in consumers because *P. guineense* is an edible spice and presently constitutes an integral part of the diets of many Africans and Asians (Lale and Yusuf, 2000). They have also been traditionally used in most parts of the world as pesticide (Awoyinka *et al*., 2006) with high potential for use in insect pest control (Ivbijaro and Agbaje, 1986; Olaifa *et al.,* 1987). However, no recent data has revealed the phytochemical constituents of *P. guineense* seeds extracted using different solvents with a view to isolating and synthesizing its active compounds. The present study determined the secondary metabolites present in *P. guineense* seeds from three different solvents and assessed their insecticidal properties on the maize weevil, *Sitophilus zeamais.*

Materials and Methods

Study locations/experimental conditions

Experiments were conducted at the Entomology Research Laboratory, Department of Crop Protection and Environmental Biology, University of Ibadan, Nigeria under ambient temperature of 27 ± 2 °C, and relative humidity of 65 ± 5%. Phytochemical investigations were carried out in the Organic Chemistry Laboratory, Department of Chemistry, University of Ibadan, Nigeria.

Sitophilus zeamais culture

Fresh culture of adult *S. zeamais* was established in the laboratory at 27 ± 2 °C, and relative humidity of 65 ± 5% from an initial culture bought from Bodija market, Ibadan. One hundred weevils (1male: 1female) were introduced into 250 g maize grains in each of 8 Kilner jars with mesh lids and arranged on a table whose stands were dipped in plastic bowls containing industrial oil to prevent ants from contaminating the cultures. After two weeks of mating and oviposition, old weevils were removed and jars observed daily. Teneral adults were later removed from the cultures and sexed following the methods of Halstead (1963) using the rostrum as the character for sex differentiation (Akob and Ewete, 2010). Culture was maintained as source of weevils throughout the experiments.

Collection and Preparation of Plant Material

Fresh fruits of *Piper guineense* Schum and Thonn were purchased from a local farm in Kajola, Osun State and Ojoo market in Ibadan, Oyo state. Samples were destalked, washed and air-dried for a period of one week by constantly turning the fruits to prevent fungal growth. Two (2) kilograms of the dry fruits was ground and stored in refrigerator in a well labelled air-tight container till the time for soxhlet extraction.

Extraction and concentration of plant extract

Ground samples were extracted in a Soxhlet apparatus using 95% ethanol at a temperature of 60 °C for a period of 6 hours. Extract obtained was further concentrated on a rotary evaporator

under reduced pressure at a temperature of 38 °C and a speed of 130 rpm. The syrupy crude extract obtained was kept in a refrigerator for subsequent use.

Fractionation of crude extracts

Two hundred millilitre (200 ml) of the crude extract was dissolved in 200 ml 95% ethanol (a polar solvent) and the sample partitioned in 1litre separating funnel. Using equal volume of hexane solvent, the non–polar fraction (hexane fraction) was obtained. This procedure was repeated until all the components soluble in hexane were extracted. Two hundred ml of distilled water was then added to create an interphase between the moderately polar solvent (ethyl acetate) and polar solvent (ethanol). Partitioning was then carried out using equal volume of ethyl acetate to obtain moderately polar components of the sample. Each solvent extract, including the remaining ethanol fraction, was further concentrated separately in a rotary evaporator. One gram (1g) of each of the fractions was dissolved in ten millilitre (10 ml) of 95% ethanol to obtain 100 mg/ml (100,000 ppm) concentration.

Phytochemical screening of crude extract

The phytochemical screening of n-hexane, ethyl acetate and ethanol fractions was carried out using the standard methods as described by Trease and Evans, 1989 (Oloyede, 2005; Akinbuluma *et al.*, 2015). Secondary metabolites namely: alkaloids, carbohydrates, flavonoids, glycosides, phenols, saponins, steroids and tannins, were screened for and presence or absence of any metabolite was recorded for each fraction

Biological activity

Topical application

Ten 1-2 day-old (1 male: 1 female) *Sitophilus zeamais* were placed per petri-dish and each was individually picked and treated by applying 1-2µl drops 100,000 ppm of the partitioned extract on their ventral sides from a micro syringe. The contents of the petri-dishes were provided with 20 grams maize grains (TZPB variety) and the experiment was laid out in a completely randomized design (CRD) with four replications. Insects were observed at 24, 48 and 72 hours with insects considered dead when they did not respond to probing with a seeker. Data on insect mortality were recorded and corrected with Abbott's formula (Abbot, 1925). Data were analyzed and means separated using the least significant difference (LSD) test at $p < 0.05$.

Repellency

Repellency of fractions of *P. guineense* seeds against *S. zeamais* was evaluated using the area preference method described by McDonald *et al.* (1970). The test area consisted of 11.0 cm whatman No. 1 filter papers cut in halves and 200 µl of each of the solvent fraction of *P. guineense* was applied uniformly to half-filter-paper disc with a micro syringe. The other filter paper halves were treated with ethanol (control). The half discs were air-dried for 10 minutes to allow the solvent to evaporate completely. Full discs were re-made by attaching treated halves to untreated halves of the same dimensions with cellotape and each placed in a Petri dish and ten adult weevils released separately at the centre of each filter paper disc in the Petri dish and then covered. The control Petri dish was treated with ethanol only. Treatments were arranged in a completely randomized design (CRD) in four replications. The number of insects present on the control and treated half discs was recorded after 30 minutes exposure and Percent Repellency (PR) for each replicate was estimated as:

$$PR = \frac{(N_c - N_t)}{(N_c + N_t)} \times 100$$

Where (Nt) = number of insects present on the treated half disc and;

(Nc) = number of insects present on the untreated (control) half disc,

A negative PR value was taken as zero and data on percent repellency were analysed.

Seed viability

Germination test was conducted using twenty seeds treated with 100,000 ppm of each of the

solvent extracts obtained from *P. guineense* seeds and the ethanol treated control. The seeds were placed on moist filter paper in plastic petri dishes kept in an incubator at 25 °C and the number of germinated seed was counted and recorded and percentage seed viability was calculated as:

$$Viability\ (\%) = \frac{Number\ of\ germinated\ seeds}{Number\ of\ seeds\ sown} \times 100$$

Statistical analysis

All data were analysed using analysis of variance (ANOVA) and treatment means that showed significant differences ($P < 0.05$) were separated using the Least Significant Difference (LSD).

Results

Phytochemical characteristics of ethanol extract

The result of phytochemical screening of partitioned extract of *Piper guineense* presented in Table 1 shows that hexane fraction of the extract contained alkaloids, resins and steroids but gave negative result for flavonoids and phenols. The ethyl acetate fraction contained phenols, resins, saponins, steroids and tannins while ethanol extract of *P. guineense* contained all the metabolites checked for except steroids and flavonoids.

Table 1 Phytochemical characteristics of ethanol extract of *Piper guineense* seeds.

Phytochemicals	n-Hexane	Ethyl acetate	Ethanol
Alkaloids	+	-	+
Flavonoids	_	_	_
Glycosides	_	_	+
Phenols	_	+	+
Resins	+	+	+
Saponins	_	+	+
Steroids	+	+	_
Tannins	_	+	+

+ = present, - = absent.

Contact toxicity

Table 2 shows the effect of topical application of partitioned *P. guineense* seed extract on adult maize weevil 24, 48 and 72 hours post treatment. *S. zeamais* mortality in grains treated with different solvent extracts of *P. guineense* was significantly ($p < 0.05$) different from weevil mortality in the control grains in all the days of trials. Adult mortality increased with the length of exposure. The highest weevil mortality (100%) was recorded in

maize grains treated with ethanol extract at 72 h post treatment, and it was significantly ($p < 0.05$) higher than weevil mortality of 82.50%, obtained in grains treated with ethyl acetate fraction of *P. guineense* extract (Table 2)

Repellency

Table 3 shows the effects of solvent extracts of *P. guineense* seeds on the repellencey of adult *S. zeamais*. Ethanol fraction of *P. guineense* was most repellent (87.50%) followed by n Hexane (70.00%) and ethyl acetate (62.50%). All the solvent extracts were repellent to *S. zeamais*. The percentages of maize grains that germinated after treatment with 100,000 ppm concentration of *P. guineense* solvent extracts are also presented in Table 3. At the end of 7 days germination period, all grains treated with solvent extracts as well as the ethanol-control recorded high viability. The latter had the highest percentage germination of 91.25%; followed by the grains treated with ethyl acetate (88.75%) while grains treated with ethanol had the least percentage germination (86.25%). There was no significant difference in the germination of seeds treated with solvent extracts and the ethanol control (Table 3).

Table 2 Effect of partitioned extract of *Piper guineense* seeds on the mortality of adult *Sitophilus zeamais* over 1-3 days post treatment.

Fractions	Mortality ± SD (%)		
	24 h	48 h	72 h
n-Hexane	77.50 ± 5.00	97.50 ± 5.00	100
Ethyl acetate	62.50 ± 5.00	77.50 ± 5.00	82.50 ± 9.57
Ethanol	87.50 ± 5.00	100	100
Control (EtOH solvent)	0	0	0
LSD	6.67	5.45	7.38

Table 3 Effect of partitioned extract of *Piper guineense* seeds on the repellency of adult *Sitophilus zeamais* and germination of maize seeds.

Fractions	Repellency (%)	Seed viability (%)
n-Hexane	70.00 ± 5.77	87.50 ± 2.87
Ethyl acetate	62.50 ± 5.00	88.75 ± 6.29
Ethanol	87.50 ± 9.57	86.25 ± 4.79
Control (Ethanol solvent)	0	91.25 ± 2.50
LSD	10.43	6.76 (ns)

Discussions

The results obtained from this study showed that the three solvent fractions of *P. guineense* seeds evoked high mortality of adult *S. zeamais* in treated maize grains. The hexane and ethanol fraction are more effective than the ethyl acetate fraction. The significantly high mortality of *S. zeamais* recorded on grains treated with extracts from these fractions may be linked to its high volatility and concentration of the bioactive components in the fractions. Adedire *et al.* (2012) reported 100% mortality of *S. zeamais* with ethanol extract of cashew kernel, which was significantly higher than those obtained with other solvent extracts. Only the ethanol fraction from this study caused 100% mortality at 48 and 72 hrs post treatment. Souza *et al.* (2010) reported that the hexanic and ethanolic extract of *Tabebuia heptaphyla* showed similar mortality rates of *S. zeamais* which were higher than those of ethyl acetate on the fifth and tenth day post treatment. They attributed mortality effect observed to the presence of secondary metabolites in the potent fractions. Incidentally, the phytochemicals from the three fractions are reported in this study. The classes of compounds present from the phytochemical investigations might be responsible for curative activities against several pests and pathogens and therefore could justify the ethno botanical uses of the plant for treatment of wide range of illnesses and as pesticides. For instance, Augusti and Cherian (2008), reported that glycosides, saponins, flavonoids and alkaloids have hypoglycaemic and anti-inflammatory activities. The secondary metabolites present in the extract in this study might be responsible for its insecticidal properties. Results from the present investigation showed that *P. guineense* seed extract is rich in phytochemicals, even though the phytochemical screening of the different fractions revealed some differences in their constituents. Phytochemical screening serves as the initial step in predicting the types of potential active compounds from plants. Partitioned *Piper guineense* extracts in this study were repellent to *S. zeamais*. *P. guineense* seed products have equally demonstrated their repellent, insecticidal and ovicidal potencies (Ntonifor and Monah, 2001; Tchoumbougnang *et al*., 2009) as well as behaviour modifying capacities of the maize weevil, *S. zeamais* (Awasalam and Emosairue, 2006; Awasalam *et al*., 2007). *Sitophilus zeamais* adults were significantly repelled by odours from *P. guineense* (Ukeh *et al*., 2010). A laboratory assessment of the repellent and anti-feedant properties of aqueous extracts of *P. guineense* against the banana weevil, *Cosmopolitus sordidus* Germar revealed potent repellent and feeding deterrent activities of the extracts (Inyang and Emosairue, 2005). These reports agree with the observation from the current study. Viability test of grains in this study also revealed no difference in the percentage germination in treated maize grains compared with the untreated. This is index of the fact *P. guineense* extract does not have adverse effect on grain germination. This agrees with the report of Adedire *et al.* (2005) which gave no significant differences in viability of seeds pre-treated with 0.5% and 2.0% of four plant extract concentrations and the control. In summary, results obtained from this study confirmed that *Piper guineense* extracts, especially ethanol fraction, was highly effective in controlling the population of *S. zeamais* in treated grains. *Piper guineense* could serve as an alternative to synthetic insecticides for use by resource-poor farmers who store small quantities of grains for their consumption, sale and planting. Further step should include characterization and identification of the active constituents of the plant.

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بررسی ترکیبات شیمیایی عصاره بذر فلفل *Piper guineense* و تأثیر آن روی شپشه ذرت *Sitophilus zeamais* (Coleoptera: Curculionidae) در بذور ذرت انباری

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چکیده: سه حلال مختلف شامل هگزان، اتیل استات و اتانول برای استخراج و بررسی وجود و یا عدم وجود ترکیبات ثانویه گیاهی از بذور فلفل *Piper guineense* مورد استفاده قرار گرفت. سپس عصاره‌ها روی حشرات کامل شپشه ذرت *Sitophilus zeamais* ارزیابی شدند. این مطالعه در شرایط آزمایشگاهی در دمای ۲ ± ۲۷ درجه سلسیوس و رطوبت نسبی ۵ ± ۶۵ درصد در قالب طرح کاملاً تصادفی در چهار تکرار انجام گرفت. پارامترهای ارزیابی شده شامل اثرات عصاره‌ها روی سمیّت تماسی، دورکنندگی و تأثیر بر جوانه‌زنی بذور ذرت بودند. نتایج به‌دست آمده حاکی از وجود انواع ترکیبات ثانویه به‌جز ترکیبات فلاونوئیدی و استروئیدی بودند. هم‌چنین تمام عصاره‌ها دارای خاصیت کشنده و دورکننده بودند با این تفاوت که عصاره اتانولی بیش‌ترین تأثیر را داشت. این درحالی است که عصاره‌ها همانند اتانول به‌عنوان شاهد تأثیری بر جوانه‌زنی بذور نداشتند. نتایج این مطالعه نشان می‌دهد که عصاره بذر فلفل می‌تواند به‌عنوان حشره‌کش زیستی کاندید مناسبی به‌جای سموم شیمیایی محسوب شود.

واژگان کلیدی: فلفل *Piper guineense*، متابولیت‌های ثانویه، شپشه ذرت، سمیت تماسی