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Original paper

Evaluation of phytochemical composition of *Piper guineense* (Uziza) Seed and its insecticidal potency against common Beans Weevil *(Acanthoscelides Obtectus)*

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ABSTRACT: Insect infestation is a major problem in agriculture, causing significant damage to crops and reducing yields. The use of synthetic insecticides has been the traditional method of controlling insect pests. However, the negative impact of these chemicals on the environment and human health has led to the search for natural alternatives. One such alternative is the use of Uziza seeds, also known as *Piper guineense*, which have been found to possess insecticidal properties. A study was carried out to evaluate the insecticidal potency of *Piper guineense* seeds against the common bean weevil, *Acanthoscelides Obtectus*. The study had two research objectives: (i) to determine the phytochemical composition of the uziza seed with particular emphasis on the piperine contents, and (ii) to evaluate the potency of *Piper guineense* powder in the control of *Acanthoscelides Obtectus*. The *Piper guineense* seeds were collected, dried, and ground into a fine powder. The powder was then extracted using methanol, and the extract was subjected to phytochemical screening to determine the presence of bioactive compounds. The phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, glycosides, steroids, phenols, and piperine in the *Piper guineense* seed extract. The concentration of bioactive compounds was also determined. The insecticidal activity of the extract was tested against *Acanthoscelides Obtectus* using contact toxicity and fumigation methods. The contact toxicity test showed that the extract had a significant insecticidal effect on *Acanthoscelides Obtectus*. The result of the study revealed that with an increase in the concentration of Uziza seed powder, the mortality rate increased from 0 ± 0a to 24 ± 0c when the concentration was increased from 0g to 10g. The results of this study suggest that *Piper guineense* seeds contain bioactive compounds with insecticidal properties that can be used as an effective natural alternative to synthetic insecticides. Further studies are needed to determine the safety and efficacy of *Piper guineense* seed extract as an insecticide in agricultural applications. The use of natural alternatives such as *Piper guineense* seeds can help reduce the negative impact of synthetic insecticides on the environment and human health. In addition, the use of *Piper guineense* seeds can also help reduce the cost of insect control in agriculture. However, it is important to note that further studies are needed to determine the safety and efficacy of *Piper guineense* seed extract as an insecticide in agricultural applications. In conclusion, *Piper guineense* seeds have been found to possess insecticidal properties that can be used as a natural alternative to synthetic insecticides. The use of *Piper guineense* seeds can help reduce the negative impact of synthetic insecticides on the environment and human health while also reducing the cost of insect control in agriculture. However, further studies are needed to determine the safety and efficacy of *Piper guineense* seed extract as an insecticide in agricultural applications.

Keywords: Beans weevil, *Piper guineense,* insecticidal potency

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INTRODUCTION

Plants have the ability to synthesize a wide variety of chemical compounds that can perform important biological functions and as defense against microbial attack (Besong *et al.,* 2016). Of the 265,000 species of flowering plants that have been identified on planet Earth, only 0.5% of them have been studied in details for their chemical composition and medicinal value. In fact, modern scientists only know the chemical composition of

less than 5% of the flora in the rainforest (Besong *et al.,* 2016). The use of various parts of indigenous plants as botanical extracts has become important in pest management in modern days due to the environmental hazards associated with the chemical control measures (Ugwu *et al*., 2021). Using plant materials as biopesticides are preferably safe, ecofriendly, biodegradable and affordable (Ugwu *et al*., 2021).

Natural plant products with bioactivity toward insects include several classes of molecules, for example: flavonoids, alkaloids, phenols, glucosides and other insecticidal substances. These compounds (phytochemicals) have important ecological activities in nature, such as: insecticide, antifeedant, attractant, nematicide, fungicide, repellentand insect growth regulator; acting as a promising source for novel pest control agents or biopesticides (Souto *et al*., 2021).

These phytochemicals present in plants also have several disease prevention activity (Asaduzzaman and Asao, 2018). These plant-derived chemical compounds play important preventive activities mainly anti-inflammatory, antidiabetic, antiaging, antimicrobial, antiparasitic, antidepressant, anticancer, antioxidant, and wound healing. They also have great role in stress tolerance of plants and accumulation of many important bioactive compounds in fruits and vegetables (Asaduzzaman and Asao, 2018).

The bean weevil, *Acanthoscelides Obtectus* (Coleoptera: Chrysomelidae: Bruchinae) is one of the most damaging insects of Phaseolus bean worldwide, particularly in Africa (Hashem *et al*., 2022). The bean weevil is an economically important post-harvest and field pest species of beans and other legume seeds which renders the commodity unsuitable for consumers. The insect pest causes serious damage to dry common beans (Gad, 2019; Hashem *et al*., 2022).

A. obtectus was originally native to Central America and was introduced into Europe at the end of the 19th century from where it subsequently spread around the globe. The beetle now occurs in Europe, Asia, North and South America, Africa, and Australia (Gad, 2019). *A. obtectus* initiates the infestation in the field and continues the infestation during storage. Populations of *A. obtectus* can grow exponentially and destroy stored grains within a few months (Gad *et al.,* 2020). They infest seeds of many grain legumes, both in the field and in storage. Populations of *A. obtectus* are most commonly detected in stores of dried legumes and their life cycle appears well adapted for reproduction in a storage environment (Ahmed *et al.,* 2019).

The weevil has a life span of 21-28 days at the temperature above 30^{o}C (Banga *et al*., 2020). The females lay eggs in clusters under or nearby the seed. The larvae burrow holes into a seed where they live for most of their lives, and rendering seeds unfit for consumption (Ahmed *et al*., 2019). The insect causes damage by reducing the mass and/or volume, reducing the physiological quality and germination capacity of bean seeds. Unlike most of the other bruchids, *A. obtectus*' reproductive cycle is continuous, without imaginal diapause for temperatures between 14-35^{o}C and it attacks the beans in fields as well as stored seeds. This insect completes its entire life cycle within stored dry beans without returning to the field (Ahmed *et al.,* 2019).

A. obtectus proliferation is usually suppressed in large storage facilities by using chemical insecticides. The application of these chemicals has increased concerns over insecticide resistance, human health and environmental contamination (Gad *et al.,* 2020).

The common beans, *Phaseolus vulgaris* originated in tropical America (Mexico, Guatemala, and Peru) and is now widely distributed throughout the world and is grown in all continents except the Antarctic (Tegegne, 2017). The world demand for common beans is highly increasing because of its significance to human nutrition as a source of proteins, complex carbohydrates, vitamins, and minerals. Its importance in reducing blood cholesterol level and combating chronic heart diseases, cancers and diabetics is also gaining recognition (Tegegne, 2017). Common bean (*Phaseolus vulgaris*) is the most consumed legume crop in the world (Mangole *et al*., 2022).

The common bean, *Phaseolus vulgaris* can be infested by the common bean weevil, *Acanthoscelides obtectus*, both in the field and in storage and this renders it unfit for sale and consumption. Insect pest infestation, if unchecked, results to poor yield, damaged seeds, loss in germination capacity, altered sizes and consequent loss in market value (Ojiako *et al.,* 2018).

Piper guineense, commonly known as Uziza, is a plant found in West Africa that has been used for various medicinal and culinary purposes. Recent studies have shown that the seeds of Uziza contain photochemical compounds that have insecticidal properties. This study aims to evaluate the photochemical composition of Uziza seeds and its effectiveness as an insecticide against the common beans weevil, Acanthoscelides Obtectus.

Piper guineense is a herbaceous perennial plant of the family Piperaceae (Nzelu *et al*., 2020).The plant is found in tropical regions of Central and Western Africa where it is cultivated in countries like Nigeria especially in the southern parts (Ojiako *et al*., 2018).Its common English names include West African Black pepper, Benin pepper, Guinea pepper, Ashanti pepper and false cubeb; it is locally known as: “Soro wisa” by the Ghanaians, “masoro” by Hausa speaking tribes, “iyere” by the Yoruba and “uziza” by Igbo speaking tribes of Nigeria (Alagbe *et al*., 2021). The plant is a perennial climber and climbs up to 12m high on trees by means of its adventitious

Figure 1: Dry *Piper guineense* Seeds

rootlets. Its inflorescence is pedicel flower spikes that are about 4- 6cm long. The flowers are greenish-yellow and arranged in a spiral on the spine. The fruit is oval and occurs in clusters. The seeds are reddish brown when ripe but black when dry (Alagbe *et al*., 2021) (Figure 1).

Piper guineense seed is commonly used as a spice and as a medicinal plant in many traditional settings (Ojimelukwe, 2021) and also as food preservative, herbal medicine, and as fragrance in the cosmetic industry (Nwozo *et al*., 2017). *Piperguineense* is used for the treatment of cough, bronchitis, intestinal diseases, and rheumatism. It has also been reported that *P. guineense* stimulates the digestive enzymes, lowers lipid peroxidation, prevents oxidative damage, and inflammation (Nwozo *et al*., 2017).

Solvent extracts of P. guineense leaves and fruits contain different biologically active compounds (Ojimelukwe, 2021).These compounds possess anti-cancer, anti-diabetic and antioxidant properties. It also contains piperine which is responsible for the hotness associated with the Piper species and other antimicrobial phytochemicals (Besong et al. 2016; Ojimelukwe, 2021) Some of the peculiar potential attributes of *P. guineense* include promoting fertility and its anti-inflammatory effects. It is a good antimicrobial agent against food spoilage and pathogenic organisms (Ojimelukwe, 2021).

In some parts of Nigeria, the seeds are consumed by women after child birth, to enhance uterine contraction for the expulsion of placenta and other remains from the womb and also for the weight control (Adebayo *et al*., 2019). It has also been reported that the leaves of *P. guineense* are used traditionally for the treatment of respiratory diseases and correction of female infertility problems, and the seeds used as aphrodisiacagents (Adebayo *et al*., 2019). *P. guineense* seeds have also shown to have analgesic potentials in managing pains and in the treatment of common cold and fever in humans (Salehi *et al*., 2019). Aqueous extract of *P. guineense* was reported to have a positive impact on the male reproductive function because it stimulated the secretions of the testes, epididymis and seminal vesicles (Adebayo *et al*., 2019). The seed oil is commonly used as aromatic in the beverage and pharmaceutical industries. *P. guineense* is also known to have antioxidant, anticonvulsant, antibacterial, anti-inflammatory, fertility, larvicidal and aphrodisiac properties (Nzelu *et al*., 2020).

Piper guineense has been indicated to treat different medical conditions such as boils, bronchitis, catarrh, chest pains, coughs, dyspepsia, impotence, insect repellant, lumbago, rheumatism, uterine fibroid, wounds, stomach discomforts and aches. The fruits are also used as a tonic for easy childbirth and for suppressing tumors (Alagbe *et al.,* 2021) Besong *et al.* (2016) reported that the fruit extract of *P. guineense* is used in China to treat epilepsy. Another preliminary study by Salehi *et al.* (2019) has also reported *P. guineense* usage in the treatment of eczema (*Tinea versicolor*), common cold and fever in humans. Besong *et al.* (2016) also reported that *P. guineense*, which are considered aperitif, carminative and eupeptic, are used in Nigeria to treat respiratory infections, rheumatism and syphilis to relieve flatulence and to treat female infertility and low sperm count in males. On the other hand, the roots are chewed as chewing stick to clean the teeth and the juice swallowed as an aphrodisiac. The analysis carried out by Amadi *et al*. (2019) on the Biochemical effects of *Piper guineense* (African Black Pepper) in female diabetics showed that *P. guineense* is pharmacologically safe in the management of diabetics.

Objective of the study

i) To determine the phytochemical composition of the Piper guineense seed with particular emphasis on the piperine contents of the seed.
(ii) To evaluate the potency of Piper powder in the control of common beans weevil (*Acanthoscelides obtectus*).

MATERIALS AND METHODS

Study area

The analysis and experiment were carried out at Sheda

Science and Technology Complex, Kwali, Abuja. The area lies between latitude of 8o 86' North and longitude 7o 04' East with an average temperature of about 29oC and average humidity of 56%.

Sample collection and preparation

The seeds of "Uziza" (*Piper guineense*) were purchased from the Dutse Alhaji Market, Bwari, Abuja. The seeds of "Uziza" (*P. guineense*) were separated from the stem and sorted to remove debris. The seeds were washed using clean tap water and air dried. They were milled into powder using a blender and stored in air tight containers until required for analysis.

Methods of mineral determination

Mineral contents of Piper guineense seeds were determined by atomic absorption spectrometry, flame photometry and spectrophotometry according to the methods of AOAC (2019).

PHYTOCHEMICAL ANALYSIS

Qualitative analysis

Phytochemical analysis of the dried seed samples of *Piper guineense* were carried out to detect the presence of some secondary metabolites using standard methods described by Manzo *et al*., (2017) and Gul *et al*.,(2017). The phytochemicals that were tested for are alkaloids, saponins, glycosides, phenols, tannins, steroids and flavonoids.

Test for Alkaloids

For the test of alkaloids, the Dradendorff and Mayer reagents were used. For the Dragendorff test: in each tube containing 0.2 ml of crude extract is added 1.5ml hydrochloric acid (2%), then two to three drops of the Dragendorff or the Mayer reagent. The presence of a red or orange precipitate indicates the presence of alkaloids for the Dragendorff test, while for the Mayer test the precipitate characterizing the presence of alkaloids appears whitish (Manzo *et al*., 2017).

Test for Tannins

To detect the presence of tannins, Braymer's test was used as described by Manzo *et al*., (2017). In each tube containing 2 ml of crude extract is 2 ml of distilled water, then two to three drops of 5% Ferric chloride. The formation of brownish green or a dark-blue color indicated the presence of tannins.

Test for glycosides (Kellar–Kilianitest)

The identification of glycosides was performed using the method described by Gul *et al*., (2017). A solution of glacial acetic acid (4.0 ml) with 1 drop of 2.0% $FeCl_3$ mixture was mixed with about 5ml of aqueous extract of sample. Then 1 ml of concentrated H_2SO_4 was added. A brownish green coloration indicated the presence of glycosides.

Test for steroids

Identification of steroids was done by adopting the method of Manzo *et al*., (2017). To 0.5 ml of crude extract is added 2 ml of acetic anhydride, then 2 ml of H_2SO_4. The formation of a purple or violet to blue ring at the interface and indicated the presence of steroids in the extract.

Test for flavonoids

The test for flavonoids was performed by using the method described by Manzo *et al*., (2017). To 1 ml of the crude extract is added eight to ten drops of hydrochloric acid and a pinch of magnesium powder. The mixture is then boiled for ten to15 minutes. A red coloration indicates the presence of flavonoids.

Test for saponins

The test for saponins was performed by using the method of Manzo *et al*., (2017). To characterize the presence of saponins, Foam test is performed. To 5 ml of the crude extract is added 5 ml of distilled water. The tube containing the mixture is then boiled. The formation of a froth indicates the presence of saponins.

Test for phenols

For the test of phenols, Liebermann's test was performed. To 1 ml of the crude extract is added 1ml of sodium nitrite, few drops of diluted sulfuric acid and then 2ml of diluted sodium hydroxide. A deep red or green or blue color indicates the presence of phenols (Manzo *et al*., 2017).

Quantitative determination of phytochemical constituents of Piper guineense seed

The determination of the concentrations of the phytochemical constituents of dried seed sample of *Piper guineense* was performed using standard methods as reported by Ezeonu and Ejikeme (2016) and Adu *et al*. (2020).

Quantitative determination of Alkaloids

Alkaloid determination was performed by the method reported by Ezeonu and Ejikeme (2016).Exactly 200 cm^3 of 10% acetic acid in ethanol was added to powder sample (2.50 g) in a 250 cm^3 beaker and allowed to stand for 4 hours. The extract was concentrated on a water bath to one-quarter of the original volume followed by addition of 15 drops of concentrated ammonium hydroxide dropwise to the extract until the precipitation was complete immediately after filtration. After 3 hours of mixture sedimentation, the supernatant was discarded and the precipitates were washed with 20 cm^3 of 0.1 M of ammonium hydroxide and then filtered using Gem filter paper. Using electronic weighing balance, the residue was dried in an oven and the percentage of alkaloid is expressed mathematically as:

$$\% \text{ Alkaloid} = \frac{\text{Weight of alkaloid X 100}}{\text{Weight of sample}}$$

Determination of Flavonoid

Flavonoid determination was by the method reported by Ezeonu and Ejikeme (2016).50 cm^3 of 80% aqueous methanol added was added to 2.50 g of sample in a 250 cm^3 beaker, covered, and allowed to stand for 24 hours at room temperature. After discarding the supernatant, the residue was reextracted (three times) with the same volume of ethanol. Filter paper was used to filter whole solution of each wood sample. The sample filtrate was later transferred into a crucible and evaporated to dryness over a water bath. The content in the crucible was cooled in a desiccator and weighed until constant weight was obtained. The percentage of flavonoid was calculated as:

$$\% \text{ Flavonoid} = \frac{\text{Weight of flavonoid X 100}}{\text{Weight of sample}}$$

Determination of Saponin

Saponin quantitative determination was carried out using the method reported by Ezeonu and Ejikeme (2016). 100 cm^3 of 20% aqueous ethanol was added to 5 grams of powder sample in a 250 cm^3 conical flask. The mixture was heated over a hot water bath for 4 hours with continuous stirring at a temperature of 55°C. The residue of the mixture was reextracted with another 100 cm^3 of 20% aqueous ethanol after filtration and heated for 4 hours at a constant temperature of 55°C with constant stirring. The combined extract was evaporated to 40 cm^3 over water bath at 90°C. 20 cm^3 of diethyl ether was added to the concentrate in a 250 cm^3 separator funnel and vigorously agitated from which the aqueous layer was recovered while the ether layer was discarded. This purification process was repeated twice. 60 cm^3 of n-butanol was added and extracted twice with 10 cm^3 of 5% sodium chloride. After discarding the sodium chloride layer the remaining solution was heated in a water bath for 30 minutes, after which the solution was transferred into a crucible and was dried in an oven to a constant weight. The saponin content was calculated as a percentage:

$$\% \text{ Saponin} = \frac{\text{Weight of saponin X 100}}{\text{Weight of sample}}$$

Determination of Glycoside

Glycoside quantitative determination methodology used was the method reported by Ezeonu and Ejikeme (2016). 1g of powder sample was weighed into a round bottom flask and about 200 cm^3 of distilled water was added and allowed to stand for 2 hours for autolysis to occur. Full distillation was carried out in a 250 cm^3 conical flask containing 20 cm^3 of 2.5% NaOH (sodium hydroxide) in the sample after adding an antifoaming agent (tannic acid). Cyanogenic glycoside (100 cm^3), 8 cm^3 of 6 M NH_4OH (ammonium hydroxide), and 2 cm^3 of 5% KI (potassium iodide) were added to the distillate(s), mixed, and titrated with 0.02 M $AgNO_3$ (silver nitrate) using a microburette against a black background. Turbidity which was continuous indicates the end point.
Content of cyanogenic glycoside in the sample was calculated as:

$$\text{Glycoside (mg/100g)} = \frac{\text{Titre value (cm}^3\text{) x 1.08 x exract volume}}{\text{Aliquot volume (cm}^3\text{) x weight of sample (g)}}$$

Determination of Tannin

25ml of 80% methanol was added to 0.50g of the pre-treated sample, warmed for 30 min while the solution was filtered through Whatman #1 filter paper, and diluted with 50ml of 80% methanol. 1ml of the extract was mixed with 0.6ml glacial acetic acid, 10ml pyridine (20%) and 2.5 ml Aluminium chloride (10%), and then diluted with 25ml distilled water. The mixture was allowed to stand for 30 min at 25°C. Total tannins was determined by measurement of the absorbance at 420nm. A calibration

standard (0.1- 0.5 mg/L) prepared using tannic acid, was carried out through the same procedure (Adu *et al*., 2020).

Determination of Phenols

Defatting of 2 g of powder sample was carried out for 2 hours in 100 cm^3 of ether using a soxhlet apparatus. The defatted sample was then boiled for 15 minutes with 50 cm^3 of ether for the extraction of the phenolic components. 10 cm^3 of distilled water, 2 cm^3 of 0.1 N ammonium hydroxide solution, and 5 cm^3 of concentrated amyl alcohol were then added to 5 cm^3 of the extract and left to react for 30 minutes for colour development. The optical density was measured at 505 nm. 0.20 g of tannic acid was dissolving in distilled water and diluted to 200 mL mark (1 mg/cm^3) in preparation for phenol standard curve. Varying concentrations (0.2–1.0 mg/cm^3) of the standard tannic acid solution were pipetted into five different test tubes to which 2 cm^3 of NH_3OH, 5 cm^3 of amyl alcohol, and 10 cm^3 of water were added. The solution was made up to 100 cm^3 volume and left to react for 30 minutes for colour development. The optical density was determined at 505 nm (Ezeonu and Ejikeme, 2016).

Determination of Piperine concentration of *Piper guineense* seed

Piperine is the most important alkaloid present in the black pepper. A high performance liquid chromatography (HPLC) method proposed by International Organization for Standardization (ISO) was optimized and validated for quantitative determination of piperine in black pepper (Shrestha *et al*., 2020).

Extraction Process

10 g of powdered black pepper seed was extracted with 50 ml glacial acetic acid (maceration for 12 hrs). After 12 hrs, acetic acid extract was gravity filtered. The acetic acid solution was diluted with 50 ml distilled was and extracted with chloroform (50 ml x 3 times) in a separating funnel. The chloroform extract was thoroughly washed with 10% sodium bicarbonate solution followed by 4-5 washing with water. The chloroform extract was filtered through anhydrous sodium sulphate and evaporated to dryness by rotary evaporator.

The obtained residue was redissolved in 3 ml chloroform and precipitation was achieved by adding 30ml of diethyl ether to the chloroform extract. The solution was kept in refrigerator at 4°C for 8 hrs. The creamy white coloured crystals were obtained which was separated by centrifugation. The liquid was then transferred via disposable syringe into an HPLC vial after passing through a syringe membrane filter. The vial was kept into the auto sampler of HPLC for analysis.

Evaluation of the insecticidal potency of *Piper guineense*

Breeding of the bean weevils

The common bean weevil (*Acanthoscelides obtectus)* used for this experiment were obtained from infested beans grains obtained from food stores in Dutse Alhaji Market. They were set in kilner jars covered with muslin cloth, held tightly with rubber bands. The muslin cloth was used to allow adequate aeration of jars, but preventing exit or entry of the beans weevil and other insects.

Experimental bean seeds

The beans seeds used for the experiment were purchased from Dutse Alhaji Market. The seeds were handpicked to ensure that they were free from insect infestation. They were further sterilizedin electric oven at 50°C for 4h to disinfest them thoroughly to avoid future fungal infection in line with Nzelu *et al.* (2020). They were allowed to cool and later sealed in transparent containers.

Test insecticidal material (dry *Piperguineense seeds*)

The test insecticidal material (dry *Piperguineense seeds*) were purchased from Dutse Alhaji Market. The seeds were separated from the stem and sorted to remove debris. The seeds were washed using clean tap water and air dried. They were milled into powder using a blender and stored in air tight containers.

Experiment set-ups

About 100g of healthy looking bean seeds homogenously mixed with the test powder at different doses of 0, 2.5g, 5g, 7.5g and 10g were placed in separate clean transparent bottles covered with muslin cloth. The treated beans were infested with 24 adult *A. obtectus*. The control contained 0% of insecticide (*P. guineense* seed powder) with bean seeds and insects inside (Rugumamu, 2014). The bottles were tightly covered with muslin cloths to allow adequate ventilation. Threereplicates of each treatment dose were set up and observed every 7 days for mortality and possible reproduction of the weevils for a period of 35 days.

Data collection

(i) Number of surviving adult *A. obtectus* were

recorded in periods of 1-7 days, 8-14 days, 15-21 days, 22- 28 days and 29-35 days. At the end of each period, the surviving insects in each treatment were recorded for the determination of effects of the test insecticidal material (*P. guineense* seed powder) to *A. obtectus* as reflected by mortality and reproductive performance at the end of its life cycle in line with the report by Rugumamu (2014).

(ii) The bean seeds were weighed at the end of the experiment with the use of digital weighing scale and recorded against the different doses to determine loss in weight.

(iii) The effects of *Piper guineense* powder on the reproductive capacity of the beans weevils was investigated to observe the emergence of progeny population by counting number of newly emerged insects 21 days after withdrawal of initial insects (Benson *et al*., 2019).

Data analysis

The data collected was subjected to analysis of variance (ANOVA) using SPSS software package and the level of significance at $p< 0.05$.

RESULTS AND DISCUSSION

Phytochemical analysis of *Piper guineense* seed

Qualitative analysis

The qualitative determination of the phytochemicals present in *Piper guineense* seed revealed the presence of Alkaloids, Tannins, Flavonoids, Saponins, Glycosides, Steroids, Phenols and Piperine as presented in (Table 1).

Quantitative determination of phytochemical constituents of Piper guineense seed

The concentrations of the phytochemical constituents of dried seed samples of *Piperguineense* is presented in (Table 2).

The insecticidal potency of *Piper guineense* seeds

Part of this study was to determine the insecticidal potency of *Piper guineense* seed powder against common bean weevil *Acanthoscelides obtectus.* Table 3 shows the mean number of adult mortality and the number of adult emergence. Table 6 shows the means of the loss in total weight of bean seeds after insect infestation and treatment. The phytochemical composition of *Piper guineense* is shown in (Tables 1 and 2). It can be deduced that *P. guineense* seeds are a good source of alkanoids, flavonoids, tannins, phenols and glycosides needed for healthy living. Phytochemicals are not vitamins or minerals but are bioactive compound found in plant foods that work with nutrient and dietary fibers to protect against disease (Besong *et al*., 2016). Alkaloids are one of the most efficient therapeutically significant bioactive substances in plants (Nwankwo *et al*., 2016). Alkaloids are natural antibacterial compounds potent against a panel of gram –positive and gram-negative bacteria including significant human pathogens (Mgbeahuruike *et al*., 2018).

Flavonoids possess antioxidant, anti-inflammatory, anti-tumor,anti-allergic and antiplatelet properties; and have cholesterol lowering ability (Isikhuemen *et al*., 2020); hence *Piper guineense* could have anticancer and anti-ulcer activity and protection against the different levels of carcinogenesis. *Piper guineense* seeds contain tannins as also reported by Besong *et al*. (2016). Plants that contain tannins as their primary component are astringent; they are beneficial or the management of diarrhea and dysentery (Isikhuemen *et al*., 2020). The level of saponins in *Piper guineense* shown in this study is very low. Saponins at low levels are said to be safe and non –toxic (Nwankwo *et al*., 2016). This implies that *P. guineense* seeds are safe for human consumption. High saponin levels have been associated with gastroenteritis, manifested by diarrhea and dysentery (Nwankwo *et al*., 2016). *Piperguineense* also contains cardiac glycosides as reported by Besong *et al*. (2016). Cardiac glycosides are useful in the management of diseases associated with the heart (Besong *et al*. 2016). *Piper guineense* seeds contain high level of Piperine, which is an insecticidal active ingredient (Ojiako *et al.,* 2018).

The potency of *Piper guineense* seed powder as insecticidal material is shown in Table 5. The result from this study indicates that common bean weevil *Acanthoscelides obtectus* thrived in the absence of the insecticidal material till the end of its life cycle. The mortality of the weevils increased with increasing concentration of the *Piper guineense* seed powder as also reported by Benson *et al.,* (2019). This is due to the pungent principal component, piperine as reported by Salehi *et al*., (2019). Ukpai *et al.* (2017) also reported high content of flavonoid in *P. guineense* seed and suggested that the bioactivity and mortality of maize weevil *Sitophilus zeamais* Motschulsky in maize stored with the botanical can be attributed to the presence of alkaloids, flavonoids and phenols. The highest mortality was recorded at 5g, 7.5g and 10g treatment from 1-7 days. The pungency of the piper seed increased with increase in the treatment concentration. The common bean treatment with *P. guineense* powder suppressed the emergence of the progeny population as also

Table 1: Result for the qualitative analysis of *Piper guineense* seed.

Phytochemicals	Composition level
Alkaloids	+++
flavonoids	++
Tannins	++
Saponins	+
Glycosides	+++
Steroids	++
Phenols	++
Piperine	+++

KEY: Highly present = +++, moderately present = ++, lightly present = +

Table 2: Quantitative phytochemical analysis of Piper guineense seed.

Phytochemicals	Concentration (%)
Alkaloids	2.067 ± 0.123
flavonoids	0.243 ± 0.015
Tannins	0.283 ± 0.012
Saponins	0.033 ± 0.006
Glycosides	1.833 ± 0.025
Steroids	0.190 ± 0.010
Phenols	0.237 ± 0.015
Piperine	4.811 ± 0.003

*Values are means of triplicate determination ± S. D

Table 3: Effect of *Piper guineense* seed on the mortality and reproductive capacity of Common Bean Weevil *Acanthoscelides obtectus.*

P. guineense **seed powder treatments(g**	**Mortality in weevils**		**with time in days**			**Number of Adult emergence**
	1-7	**8-14**	**15-21**	**22-28**	**29-35**	
0	0 ± 0 [a]	0 ± 0 [a]	3 ± 0.82 [a]	3.67±0.47 [b]	17.33±1.25 [a]	18±1.25[a]
2.5	6 ± 1.41[b]	7.33±1.25 [b]	10.67±1.25 [b]	0 ± 0[c]	0 ± 0 [b]	5±1.62[b]
5	24 ± 0 [c]	0 ± 0 [a]	0 ± 0 [c]	0 ± 0[c]	0 ± 0 [b]	0±0[c]
7.5	24 ± 0 [c]	0 ± 0 [a]	0 ± 0 [c]	0 ± 0[c]	0 ± 0 [b]	0±0[c]
10	24 ± 0 [c]	0 ± 0 [a]	0 ± 0 [c]	0 ± 0[c]	0 ± 0 [b]	0±0[c]
Means	15.6±10.46	1.47±2.93	2.73±4.13	0.73±1.47	3.47±6.93	4.6±6.97
CV	0.67	1.99	1.51	2.01	2.00	1.52

Values are means of triplicate determination ± S. D
Means followed by same alphabet in the same column are not significantly difference at 5% probability level from ANOVA

recorded in (Table 4). The control recorded the highest number of adult emergence (18 adults) followed by 2.5g treatment (5 adults) after 21days of withdrawing the initial insects. 5g, 7.5g and 10g treatments recorded no emergency. The increasing concentration of *P. guineense* adversely affected adult emergence due to the consequent mortalities that prevented the weevils from laying eggs. This result also agrees with the evaluation reported by Benson *et al.* (2019) on the control of cowpea weevils using *Piper guineense.* According to the report, the number of adult emergence reduced with increasing concentration of *P. guineense.* The highest number of adult emergence was observed on the control untreated seeds while the lowest number of adult emergence was recorded from the highest treatment concentration (1.0 g piper/20 g seed).

Table 4 shows the loss in weight of bean seeds after treatment. The control bean seeds recorded the highest loss in weight of seeds (9.093g). This is due to the absence of the insecticidal material which encouraged the weevils to thrive and cause damage to bean seeds thereby reducing the mass of the seeds.

Table 4: Loss in weight of Bean seeds.

Seed powder treatments (g)	Loss in weight of bean seeds (g)
0	9.093 ± 0.088[a]
2.5	2.133 ± 0.116[b]
5	0.005 ± 0.001[c]
7.5	0.005 ± 0.001[c]
10	0.004 ± 0.001[c]
Total mean	2.248 ± 3.520
CV	1.566

Values are means of triplicate determination ± S. D
Means followed by same alphabet are not significantly difference at 5% probability level from ANOVA

Conclusion

It is also important to understand the potential benefits of using plant-derived materials such as *Piperguineense* as insecticides. These materials are often considered to be more environmentally friendly than traditional chemical insecticides, as they are derived from natural sources and are less likely to harm non-target organisms. Additionally, plant-derived insecticides may be less likely to contribute to the development of insecticide resistance, which can be a major problem with chemical insecticides. One of the key advantages of using plant-derived materials such as *Piper guineense* as insecticides is that they can be relatively easy to produce and apply; and are relatively inexpensive, which can make them an attractive option for farmers and other growers who are looking for effective pest control solutions.

One of the most important output or finding from this study is the quantitative determination of the phytochemicals in the *Piper guineense* seed, particularly the confirmation of the piperine as the insecticidal component of *P. guineense* seeds.

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