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PESTICIDAL POTENTIALS OF SEED EXTRACTS OF BLACK PEPPER (*Piper nigrum L*) IN THE CONTROL OF MAIZE GRAIN WEEVIL (*Sitophilus zeamais Mots*) IN STORAGE

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ABSTRACT

This study was conducted between November 2009 – May 2010 at the Teaching and Research farm of Imo State University, Owerri, Nigeria. Five rates of ethanolic and powder extracts of black pepper, *Piper nigrum L* were used (0.0, 0.1, 0.2, 0.3, and 0.4) in milliliters and grams as treatments respectively, using Completely Randomized Design (CRD). The parameters determined were mortality rate, grain damage (exit holes), germination percentage, presence of phytochemicals and infrared spectroscopy. Results showed that both ethanolic and powder extracts, killed *Sitophilus zeamais* but the ethanolic extracts were significant, more effective and quicker in action at $P \leq 0.05$. However, it was observed that the bio-efficacy of the extracts increased with increase in the level of concentration. Ethanolic and powder extracts of *P. nigrum L*. at 0.4, 0.3 and 0.2mls/g were significantly ($P \leq 0.05$) more effective in the control of maize grain weevil in storage than 0.1 mls/g within12-48hours and 12-72 intervals respectively. There were no grain damage (exit holes) on treatments with ethanolic extracts but with powder extracts, exit holes were noticed but differed significantly at ($P \leq 0.05$). On seed germination tested 6 months after treatments, 0.2 and 0.4mls gave the highest number of maize grains with radicule and plumule emergence, 80% each while 0.1 and 0.3mls gave 70% each. None of the treatments was significant at ($P \leq 0.05$) except over the control. Phytochemical screening of the plant extract revealed the presence of bio-active compounds comprising alkaloids (50.13 – 8 5.67%), flavonoids (24.56 – 4.02%), Tannins (17.79 – 0.22%), Saponnins (5.51 – 7.52%) and Phenols (2.01 – 0.71) % in decreasing order of percentage. The infrared spectroscopy revealed some functional groups – OH, - COOH radicals and double bond ketones. These compounds have the implication of exerting different toxicities on organisms.
Keywords: P. nigrum, S. zeamais, Maize, Phytochemical, Pesticidal Potentials.
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INTRODUCTION

Maize (*Zea mays L.*) is one of the most important cereal crops in sub-Saharan Africa. It is ranked third after rice and wheat being one of the three most important cereal crops in the world (IITA, 2000). It is a versatile crop that grows across a wide range of agro-ecological zones (IITA, 2000; CGIAR, 2001). In industrialized countries, maize is largely used as livestock feed and a raw material for industrial products, while in low-income countries, it is mainly meant for human consumption (IITA, 2000; CGIAR, 2001). It is an important source of calories for the poor and serves as a good vegetable crop to bridge a "hunger gap" that occurs yearly after the long dry season and a staple food for an estimated 50% of the population in sub Saharan Africa (Oikeh, 2003). About 589 million tonnes of maize were produced worldwide in 2000, on 138 million hectares (IITA, 2000). The United States is the largest maize producer (43% of world production), followed by Asia (25%), Latin America and Caribbean (13%) and Africa (7%) of the world's maize (IITA, 2000). The average yield in 2000 was 4.2 tonnes per hectare. Average yield in the U.S.A. was 8.6 tonnes per hectare, while in sub-saharan Africa it was 1.3 tonnes per hectare (IITA, 2000).

Insect pests are important threat to maize production in Africa. Major pests include stem and ear borers, armyworms, cut worms, grain moths, beetles, weevils and virus vectors. A range of pathogens primarily fungi, also damage the plants. About 35% of stored maize grains are lost to insect pests and diseases. If it were possible to exclude these losses with the input of suitable measures, an additional 900,000,000 people would be fed adequately (GTZ, 1979). However, maize grains are usually attacked in storage by a number of insects. The females of these insect pests lay their eggs in or on the grains. In the case of serious infestations up to 90% of the grain may be destroyed within 6 months (Rouanet, 1987). The major storage pest of grains include *Sitophilus zeamais Motschulsky, Tribolium casternum Herbst, Sitophilus oryzae Linnaeus, Sitotroga cereallela Olivier, Trogoderma granarium Everts, Callosobruchus maculatus Fabricius,* and the larger grain borer *Prostephanus truncatus Horn*, which is a recent introduction into Africa from South and Central America (Youdeowei, 1988). Some of these pests actually infest the crops in the field and are subsequently carried into storage where they develop under favourable conditions (Rouanet, 1987, Youdeowei, 1988). For decades, the pest control policy has been dependent upon the use of synthetic pesticides (Dinham, 1993).The use of synthethic insecticides in modern storage practices is viewed as an integral part of the agricultural industry. They are known to have undoubted benefits (Alao, 2008). However, popular grain protection chemicals have been implicated with series of problems including the toxicity of many products to humans and other non- target organisms. The environmental hazards associated with their application and the development of insect resistance has become a major concern in Nigeria and other third world countries, where majority of farmers and pesticide users are not trained in the safe handling and applications of these chemicals (Ahmed and Stoll, 1996; Ohazurike *et al.*, 2003).

Regrettably, these chemicals are still used in stored product pest control even though they are no longer officially permitted in most countries. A need therefore arises to investigate the potency of natural products that are effective, readily available, cheap and non-toxic for protection of stored grains. Plants produce chemicals known as secondary metabolites, which are not directly involved in the process of growth, but act as deterrents to insects. Alkaloids, flavonoids, saponins, tannins, phenols and cyanogenic glycosides as bioactive substances, all fit in this category (Okwu, 2004; 2005*).* Several Nigerian tribes are known to mix a wide range of plants with stored grains to control storage pests. Species used include, *Anona senegalensis Pers, Ocimum americanum L., Nicotiana spp., Luffa aegyptica Mill, Capsicum* *annum, Hyptis spicigera Lam, Afromosia laxiflora Harms, Butyrospermum parkii Kots, Datura stramonium and Ocimum grattissimum* (Giles, 1964). Ivbijaro, 1989 highlighted that oil extracts from the ripe fruits of neem, *Azardirachta indica A. Juss* and brown pepper, *Piper guinense Shum Thorn* added separately to cowpea grains at the rates of 2 and 3 ml kg-1 provided substantial protection from bruchid infestation and damage for 3 months, Oji *et al*., (1991), investigated on the insecticidal activity of dry fruits of *Xylopia aetiopica* and *Piper guinense* on maize weevil with a significant insect mortality in the treated grains from 48-96 hours after treatment. Mueke, (1989), stated that laboratory experiments carried out on the control of *C. maculatus* on cowpea seeds using vegetable oils showed preventive treatment as oil treatment resulted in mortality of adult weevil and eggs on the treated seeds failed to hatch. Umoetuk *et. al.,* (2004) reported the reduction of damage caused to stored cowpea by the bean bruchids using black pepper and alligator pepper for seven months as the treated grains suffered less damage than the untreated ones. Ogendo *et. al*., (2004) reported that plant powders of *Lantana camara* and *Tephrosia vogelli* significantly minimized depression in percentage grain moisture content and had no effect on the percentage germination of maize grains when compared with the control. The current problem of pesticide resistance and detrimental effects on non-target organisms, including humans has revived interest in exploiting pest control potentials of plants.

Black pepper (*Piper nigrum L*.) is one of such plants that possess pesticidal properties, readily available in most rural communities of Nigeria and other West African countries. The plant is under-utilized and neglected but can be improved. Some reports on Black pepper in Nigeria centered on its use in the control of cardiovascular diseases in human beings, herbal medicine and also as antioxidants (Okwu, 2004; Okwu, 2005). Hence *P. nigrum L* has the advantage of providing novel modes of action against insects and can reduce the risk of cross-resistance as well as offering new leads for design of target- specific molecules. Hence, this investigation is designed to add to the existing information by assessing the pesticidal potentials of the seed extracts of *Piper nigrum* as alternative plant-based control measures for subsistence farmers against infestation of stored maize grains.

MATERIALS AND METHODS

2.1 Study Location and structure

The experiments were conducted between November 2006 and May 2007 at the teaching and research farm of Imo State University, Owerri which lies between latitude 5^0 10’ and 6^0 O’ N, longitude 6^0 35’ and 7^0 30’ E. The altitude is 91m above sea level. (Imo State Lands and Survey,1984). A structure measuring 3m x 6m x 4m construction was made using rubber nettings round the sides and top inside a green house. Two tables measuring 1m x 5m were constructed for placing the plastic vials.

2.2 Preparation of plant extracts

Seeds of black pepper were bought from Eke Atta Market in Ikeduru Local Government Area of Imo State, Nigeria. Yellow maize (local variety) was procured from Owerri Main Market in Owerri Municipal Council of Imo State. The seeds were further sun-dried at a temperature of 27 - 30^{o}C for 48 hours, using the method described by Ohazurike *et al*., (2003). One kg of the seed samples of *Piper nigrum* was ground to fine powder using a wooden mortar, manual grinder and finally in an electric blender. The pulverized materials were sieved with 0.4, 0.3, and 0.2mm mesh to obtain 900g each of fine seed powder of *Piper nigrum* L. Using a Soxlet apparatus 150mls of ethanolic extracts were obtained from 600g of ground *P. nigrum L*. The quantity of ethanol used in the extraction was 2.25l, giving a stock concentration of 0.27g/ml. Maize grains used in the experiments were sterilized using one phostoxin tablet on 5kg maize grains for 12 days under a tight jar to kill any latent insect infestation. The sterilized maize was thereafter kept in the farmhouse.

2.3 Rearing of Test Insects: Heavily infested maize grains were screened in the laboratory for confirmation of the insect species. Three hundred (300) unsexed adult *Sitophilus zeamais Motschulsky* insects were reared in 2 litre jars containing 600g of disinfested whole maize grains as described by (Haines, 1991). The top of each rearing jar was covered with nylon mesh fastened tightly with rubber bands, and the insects were allowed a seven-day period for egg- laying, (oviposition). Thereafter, all adults were removed and each jar allowed standing for 25 days during which emerging insects were monitored and kept in separate jars according to their size.

2. 4. Experimental Design

The design used in this experiment was Completely Randomized Design (Steel and Torrie, 1980). There were 6 treatments; each treatment had 5 levels, replicated 4 times. Ethanolic extracts and seed powder of *Piper nigrum* at five levels (0.0, 0.1, 0.2, 0.3 and 0.4) mls and (0.0, 0.1, 0.2, 0.3 and 0.4) g respectively, were measured with graduated disposable syringe and electronic weighing balance. Treatments 0.0mls and 0.0g were the untreated samples. Three small openings were made on each cover of the plastic vial for passage of air so that the weevils will not die of suffocation.

The ethanolic specimens were represented by:-Ethanolic *Piper nigrum*, EPn0= 0.0ml – control, EPn1=0.1ml, EPn =0.2ml, EPn_3=0.3ml, EPn_4=0.4ml. Similarly, the Seed powder specimens were represented by: - Seed powder *Piper nigrum*, $SPoPn_0$ = 0.0g – control, $SPoPn_1$ = 0.1g, $SPoPn_2$ = 0.2g, $SPoPn_3$ = 0.3g, $SPoPn_4$ = 0.4.

2.5 Treatment Procedure

In the first trial, ethanolic extract of the seed of *Piper nigrum* at five different concentrations of 0.0ml, 0.1ml, 0.2ml, 0.3ml and 0.4ml were applied to 25g of yellow maize in plastic vials. The mixture was thoroughly shaken, exposed to a gentle air blast from an electric fan for a complete evaporation of any ethanol. Five minutes later, ten *Sitophilus zeamais* in the ratio of (6:4) females and males respectively were introduced in each plastic vial and covered.

In the second trial, seed powder of *Piper nigrum* was put into plastic vials containing 25g of yellow maize grains at five different weights 0.0g, 0.1g, 0.2g, 0.3g and 0.4g. The plastic vials were shaken for effective contact of the grains with the powder extracts. Ten *Sitophilus zeamais* in the ratio of (6:4) females and males were introduced in each plastic vial and covered.

2.6 Phytochemical Determination, Screening and Infrared Scanning/Spectroscopy: 10g of grounded *Piper nigrum* was defatted, screened in the laboratory for the presence of phytochemicals – Alkaloids, Tannins, Saponins, Flavonoids and Phenols.

Pure samples measuring 10mls of *Piper nigrum* was analyzed for infrared spectroscopy. This technique was applied to identify the compounds and to investigate the compositions of Black pepper.
Alkaloid determination was done using the method described by Harborne, (1973).
Determination of Total Phenols by Spectrophotometric method was by using the method described by Harborne, (1973).
Tannin Determination was by using the method described by Van Burden & Robinson, (1981).
Saponin Determination was according to the method of Obadoni and Ochuko, (2001).
Flavonoid Determination was by the method described by Bohman and Kocipai (1974).
Standard practices for identification of material by Infrared Absorption Spectroscopy, using the ASTM coded band and chemical classification index was used to classify the phytochemicals (Kuentzel 1951). The choice of spectral range and instrument was dictated by a general consideration of the chemical nature of the samples (Smith, 1965; 1979; Potts, 1962).

2.7 Data collection and statistical analysis

Data were collected based on the following parameters, mortality rate, exit holes, germination percentage, presence of phytochemicals and organic compounds. The data collected were analysed with one-way analysis of variance (ANOVA). Observed differences between treatments were subjected to treatment separation using the Duncan's New Multiple Range Test (Steel and Torrie, 1980).

RESULTS

3.1. Mortality Rate

The effects of different concentrations of ethanolic extracts of *Piper nigrum* on mortality of *Sitophilus zeamais* were summarized in Tables 1a and 1b.

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At 12 hours interval, treatments 0.1, 0.2, 0.3, and 0.4mls of *Piper nigrum* extracts caused deaths of 2, 3, 3, and 3 weevils respectively while no deaths were recorded in the control. There was no significant difference in the mortality caused by treatments 0.2, 0.3, and 0.4mls at $P \leq 0.05$, but differed significantly from 0.1ml and the control (Table 1a).

At 24 hours interval, the various concentrations caused cumulative mortality of 3, 4, 5 and 5 weevils respectively while no deaths were recorded in the control. There were no significant differences among treatments 0.1, 0.2, 0.3 and 0.4mls at $P \leq 0.05$ (Tables 1a and 1b).

At 36 hours interval, the cumulative death records were 4, 5, 6 and 8 weevils respectively while the control produced no deaths. Treatment 0.4ml was significantly higher than all treatment levels applied while there were no significant differences among treatments 0.1, 0.2, and 0.3mls at $P \leq 0.05$ (Tables 1a and 1b).

At 48 hours' interval, the various concentrations of 0.1, 0.2, 0.3, and 0.4mls caused cumulative deaths of 5, 7, 9 and 10 weevils respectively, while no death was recorded in the control. There was no significant difference in the activities of 0.1, 0.2 and 0.4mls of *Piper nigrum*. Similarly, no significant difference existed among treatments 0.2, 0.3 and 0.4 mls but treatment 0.3 mls differed significantly from treatment 0.1ml at $P \leq 0.05$ (Tables 1a & 1b).

At 60 hours interval, the various applications of the ethanolic concentrations caused cumulative deaths of 10, 10, 10 and 10 weevils respectively, meaning that all the weevils have died, while no death was recorded in the control. Treatment 0.4ml produced no death. Furthermore, there were significant differences in the activities of 0.1ml, over 0.2ml, 0.3ml, and 0.4mls. The activity of 0.2ml was significant over treatments 0.3 and 0.4mls at ($P \leq 0.05$), (Tables 1a & 1b)

At 72 hours interval, all the weevils have been killed, while the control produced no death (Table 1b). Treatments 0.1, 0.2, and 0.3mls did not produce death of the weevils because all the weevils have died.

Using *P nigrum* seed powder extract on *Sitophilus zeamais* at different time intervals were summarized in (Tables' 2a and 2b).

At 12 hours interval, the application of 0.1, 0.2, 0.3 and 0.4 grammes of seed powder of *P. nigrum* caused deaths of 1, 1, 2 and 2 weevils respectively while the control produced no death. The highest number of deaths after 12 hours were recorded in treatments 0.3 and 0.4 grammes but none of the 4 treatments showed significant difference in activity over each other at $P \leq 0.05$ (Table 2a).

At 24 hours time interval, 0.1, 0.2, 0.3 and 0.4 grammes of seed powder of *Piper nigrum* caused cumulative deaths of 1, 2, 3, and 3 weevils respectively while the control produced no death. There was no significant difference in the activity of the various concentrations at $P \leq 0.05$ (Tables' 2a & 2b).

At 36 hours time interval, 0.1, 0.2, 0.3 and 0.4g of seed powder of *Piper nigrum* caused cumulative deaths of 1, 3, 4 and 5 weevils while the control produced no death. No significant activity of the treatments at $P \leq 0.05$ (Tables 2a & 2b).

At 48 hours interval, 0.1, 0.2, 0.3 and 0.4 grammes caused cumulative deaths of 2, 4, 7 and 8 weevils respectively. Treatments 0.4 and 0.3 grammes recorded the highest number of deaths. None showed significant difference in activity but differed significantly over treatments 0.2 and 0.1 grammes at $P \leq 0.05$ (Tables 2a and 2b).

At 60 hours interval, the 0.1, 0.2, 0.3 and 0.4g caused cumulative deaths of 10, 9, 9 and 10 weevils respectively. Highest numbers of deaths were recorded in treatments 0.1 and 0.2 grammes. At this point, treatment 0.1g differed significantly in pesticidal activity over treatments 0.2, 0.3 and 0.4g. Furthermore, 0.2 grammes differed significantly from treatments 0.3 and 0.4g. No significant activity existed among treatments 0.3 and 0.4 g respectively at $P \leq 0.05$ (Tables 2a and 2b).

At 72 hours interval, 0.2 and 0.3 g caused cumulative deaths of 10 and 10 weevils while no deaths were recorded in treatments 0.1 and 0.4g. There was no significant pesticidal activity among treatments 0.2g and 0.3g at $P \leq 0.05$ (Tables 2a and 2b)

3.2. Grain Damage (exit Holes)

After application of ethanolic extracts of *Piper nigrum*, there were no observable signs of feeding and no exit holes on the maize treated in plastic vials at the end of six months except the control where there was total damage, every grain had several exit holes and all their endosperm eaten up by the weevils. The entire 25 maize grain seeds each in the control was infested (Table 3a).

The application of different grammes of seed powder of *Piper nigrum* on number of exit holes by *Sitophilus zeamais*, in plastic vials at the end of six months was as shown in (Table 3b). The application of 0.1, 0.2, 0.3 and 0.4g of seed powder of *Piper nigrum*, revealed exit holes of 4, 3, 3 and 2 respectively. The control treatment, 0.0g with 25 grains had exit holes. Treatment 0.1g recorded the highest number of grains with exit holes, followed by treatments 0.3, 0.2 and 0.4g. Treatment 0.1g differed significantly over treatments 0.3, 0.2 and 0.4g. There was no significant difference in the number of exit holes caused by treatments 0.3 and 0.2 g, they differed significantly in the number of exit holes caused by treatment 0.4 g at $P \leq 0.05$.

Means in each column having the same subscript letters are not significantly different at 5% level of probability using DNMRT.

3.4 Germination percentage

The investigation showed a satisfactory germination percentage in terms of radicule and plumule emergence in maize grains treated with ethanolic extracts of *Piper nigrum* after 6 months of storage (Table 4a). Treatments 0.1, 0.2, 0.3 and 0.4ml produced 70%, 80%, 70% and 80% grains with emerged radicule and plumule respectively. Treatments 0.4 and 0.2mls ranked highest followed by treatments 0.3 and 0.1mls.Treatments 0.0g had no germination. All treatment levels when subjected to statistical analysis at $P\leq0.05$ were the same except the control (Table 4a).

Using seed powder, the application of 0.1, 0.2, 0.3, and 0.4g of *Piper nigrum* yielded 50%, 60%, 70%, and 70% emerged radicule and plumule. Treatments 0.4 and 0.3g produced the highest percentage radicule and plumule emergence (70%) each followed by treatments 0.2g (60%) and 0.1g (50%) respectively. Treatment 0.2g differed significantly from treatment 0.1g on number of seeds that germinated. 0.0g had no germination at $P\leq0.05$ (Table 4b).

3.3. Phytochemicals and their Compositions

The phytochemical constituents of *Piper nigrum* were as shown in (Table 5). The concentration of alkaloids in P. *nigrum was* (50.13 $\pm$ 0.820%). Flavonoids (24.56 $\pm$ 0.820%), tannins (17.79 $\pm$ 0.820%) and phenols (2.01 $\pm$ 0.820%) and saponins (5.51$\pm$ 0.820%)

3.4. Infrared scans of *Piper nigrum.* The infrared analysis of *Piper nigrum* revealed that at 3440 wavelength, which was (broad).The functional group identified was (OH) radical and compound types were alcohol and phenol. At 2920 wavelength, which was (small), the functional group (COOH) radical was identified and the compound type was carboxylic acid (organic acid). At 1680 wavelength, (small) the functional group identified was =C = O which depicted a compound type known as Ketones. (Table 6).

4.0 DISCUSSION

From the result of the experiments, it was observed that black pepper extracts (ethanolic and powder) showed effectiveness in the control of maize weevil, *S. zeamais.* The ethanolic extracts of *P. nigrum* were more effective than the seed powder. This was because the ethanolic extracts and seed powder of *P. nigrum* killed most of the weevils within 12-60 and 12-72 hours interval of exposure, respectively. *Piper nigrum* were potent in the entire parameters tested. Ethanolic and powder extracts showed that their efficacies increased with increase in concentration. Consequently, the higher levels of 0.4ml, 0.4g, 0.3ml, 0.3g were more efficacious than 0.2ml, 0.2g and 0.1ml, 0.1g of ethanolic extracts and powder extracts respectively. 12-60 hours interval was the best time range for the exposure of the insects for the ethanolic extracts. For seed powder of *P. nigrum* the best time range was 12-72 hours. These observations confirmed the works of *Oji et al*., (1991) Ogendo, (2000) and Ohazurike *et al*., (2003) that oil and dust extracts from natural plant products were insecticidal and microbial in action and adequately protected weevils in stored grains within 24-60 hours interval and 48- 60 hours interval for oil and dust respectively.

On Grain Damage (exit holes), the ethanolic extracts of *Piper nigrum* did not allow the maize grain weevils to cause any damage (exit holes) on the maize grains at the end of 6 months. The high potency of the ethanolic extracts and powder extracts did not allow the weevils to

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have their normal feeding activity on the maize seeds. This total eradication exerted by the ethanolic extracts and powder extracts reduced the chances of the maize grain weevils feeding and damaging treated maize grains and also the possibility of their acquiring resistance to the seed extracts. This meant that there was deterred feeding resulting to starvation which depicted repellency. The entire control treatments containing 25 grains each were infested and damaged. This result tallied with the observation made by Oji *et al.,* (1991) on extracts of *Piper guinense, which* protected maize grains from having exit holes. Ivbijaro, (1989), Ohazurike *et al.*, (2003), Ogendo *et al.*, (2004), noted that natural plant products protected maize grains from having exit holes. On the other hand it was noted that there was a time lag in the action of powder extracts on the weevils which meant that some seeds were damaged within this period before mortality commenced. Reasons were that the active ingredients of the dusts could be localized or removed as chaff while sieving.

On seed germination, the result showed that the plant extracts (both ethanolic and powder) had no inhibitory effects on seed germination at the end of 6 months. Hence, the extracts did not harm the seed embryo and the viability of the seeds was maintained. There was no germination in the control treatments because the entire seed embryo was eaten up by the weevils meaning that the maize grains were not viable. Umuetok *et al.,* (2004) in their work with alligator pepper and black pepper on reduction of damage caused to stored cowpea revealed that treated cowpea seeds suffered less damage, weight loss, less exit holes and highest percentage germination than other treatments.

Black pepper is rich in phytochemicals such as Alkaloids, Flavonoids, Saponins, Tannins and Phenols. The presence of alkaloids (50$\pm$ 0.820), flavonoids (24.56$\pm$ 0.82), Taninns (17.79$\pm$ 0.820) and Phenols (2.01$\pm$ 0.820) as analysed tallied with Okwu, (2004; 2005) that plants possess phytochemicals in different concentrations that protected plants from external influence.

The result of infrared scan on *Piper nigrum* revealed some functional groups and organic compounds. At 3440 the wave length was broad, at 2920 the wavelength was small, and at 1680 the wavelength was also small. The functional groups were OH, COOH radicals and ketones. The compound types were alcohol and phenol at 3440 wavelength (broad), carboxylic acid (organic acids) at 2920 wavelength (small) and ketones at wavelength 1680 (small). These compounds identified on these wavelengths were compounds that can cause lethal effects on *Sitophilus zeamais Mots* on stored maize grains. These compounds could be poisonous and possess bitter taste properties that can repel or kill pests, which signified that they possess insecticidal properties. This was in line with Okwu, (2004; 2005) that organic compounds like alcohols, carboxylic acids, (organic acids) double bond ketones could exert pesticidal, fungitoxic and antimicrobial activities on pests and diseases.

Finally, there is need for further research studies on these extracts that might in the future improve the results of suppressing the population of *S zeamais* in stored grain.

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APPENDIX

Table 1a. Effects of different concentrations of ethanolic extract of *Piper nigrum* seeds on mortality of *Sitophilus zeamais*.

	Time intervals (Hours)					
Conc. (mls)	**12**	**24**	**36**	**48**	**60**	**72**
0.0	0_c	0_b	0_c	0_d	0_d	-
0.1	2_b	1_a	1_b	1_b	5_a	-
0.2	3_a	1_a	1_b	2_{ab}	3_b	-
0.3	3_a	2_a	1_b	3_{abc}	1_c	-
0.4	$\mathbf{3}_a$	2_a	3_a	2_{ab}	-	-
S.E	0.16	0.34	0.36	o.41	0. 26	

Means in each column having the same subscript letters are not significantly different at 5% level of probability using DNMRT

Table1b. Effects of different concentrations of ethanolic extract of *P. nigrum* seeds on cumulative mortality of *S. zeamais.*

	Time Intervals (Hours)					
Conc. (mls)	12	24	36	48	60	72
0.0	0	0	0	0	0	-
0.1	2	3	4	5	10	-
0.2	3	4	5	7	10	-
0.3	3	5	6	9	10	-
0.4	3	5	8	10	-	-

The result presented in table 1b was obtained by horizontal addition of numbers in each time interval in table 1a gave the sum of the next interval number. The table was not subjected to statistical analysis.

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Table 2a: Effects of seed powder of *Piper nigrum* seeds on mortality of *Sitophilus zeamais*

	Time interval (Hours)					
Powder extracts (g	12	24	36	48	60	72
0.0	0_b	0_b	0_b	0_c	0_d	0_b
0.1	1_a	0a	0a	1_b	8_a	-
0.2	1_a	1_a	1_a	1_b	5_b	1_a
0.3	2_a	1_a	1_a	3_a	2_c	1_a
0.4	2_a	1_a	2_a	3_a	2_c	-
SE	0.57	0.37	0.45	0.40	).42	0.47

Means in each column having the same subscript letters were not significantly different at 5% level of probability using DNMRT.

Table 2b: Effects of seed powder of *Piper* ni*grum* seeds on cumulative mortality of *Sitophilus zeamais.*

	Time Interval (Hours)					
Powder extracts (g)	12	24	36	48	60	72
0.0	0	0	0	0	0	0
0.1	1	1	1	2	10	-
0.2	1	2	3	4	9	-
0.3	2	3	4	7	9	-
0.4	2	3	5	8	10	-

The result presented in table 1b was obtained by horrizontal addition of numbers in each time interval in table 1a, which gave the sum of the next interval number. The table was not subjected to statistical analysis.

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Table 3:. Effects of ethanolic extract of *Piper nigrum* seed extract, on the development of exit holes by *Sitophilus zeamais* Motschulsky at the end of six months.

Con C. (mls)	Number of grains with exit holes
0.0	25(all infested)
0.1	0
0.2	0
0.3	0
0.4	0
S.E	0

Table 3b: Effects of seed powder of *Piper nigrum* on the development of exit holes by *Sitophilus zeamais* Motschulsky after six months storage.

Con (grams)	*No of grains with exit holes*
0.0	25(all infested)
0.1	4_a
0.2	3_b
0.3	3_b
0.4	2_c
S.E	0.34

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Table 4a: Effects of different concentrations of ethanolic extracts of *Piper nigrum* on germination percentage of maize grains after six months of storage

Conc. (mls	**Germination percentage**
0.0	(0%) 0^{b}
1	(70%) 7^{a}
2	(80%) 8^{a}
3	(70%) 7^{a}
4	(80%) 8^{a}
S.E	0.40

Table 4b: Effects of seed powder of *Piper nigrum* on germination percentage of maize grains after six months of storage

Conc. In gms	**Germination percentage**
i	
0.0	(0%) 0^{d}
0.1	(50%) 5^{c}
0.2	(60%) 6^{b}
0.3	(70%) 7^{a}
0.4	(70%) 7^{a}
S.E	0.46

Means in each column having the same subscript letters were not significantly different at 5% level of probability using DNMRT.

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Table 5: Phytochemical constituents of the seeds of *Piper nigrum* on dry weight basis expressed as mg$^{100-1}$g

Phytochemicals	*Piper nigrum*
Alkaloids	50.13 ±0.820
Flavonoids	24.56 ±0.820
Saponins	5.51 ±0.820
Tannins	17.79 ± 0.820
Phenols	2.01 +0.820

Results are mean of three determinations by dry wt. basis ± standard deviation

Table 6: Infrared analysis of *Piper nigrum*

Wave lengths		
Infrared (cm^{-1})	Functional group	Compound type
3440W (broad)	-OH	Alcohol, phenol
2920(small)	-COOH	Carboxylic acid
1680(small)	-C=O	Ketones