

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Effect of Hexane and Methanol Extracts of *Piper guineense* (Schum and Thonn) Seeds against the Maize Weevil (*Sitophilus zeamais*) in Stored Maize Grains

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ABSTRACT

Methanol and hexane extract of black pepper *Piper guineense* Schum & Thonn was evaluated for its insecticidal activity against the maize weevil *Sitophilus zeamais* Motschulsky in stored maize grains. The extracts were applied at various concentrations (4000, 2000, 1000, 500, 250 μ l/ml) with ordinary acetone as control. The experimental setup involved placing ten unsexed adults of each insect species into petri dishes treated with each of the extracts as applicable. Also, maize grains weighing twenty grams were treated with the different concentrations of the extracts and the control before being artificially infested with *Sitophilus zeamais* and left for a period of thirty-three days. All of the treatments significantly reduced emergence holes and grain damage compared with the control.

Keywords: Sitophilus zeamais, Hexane extract, Methanol extract, Piper guineense, maize grain

1.0 Introduction

Maize is one of the most widely cultivated cereal crops globally, and it plays a significant role in agriculture and food security (Suleiman *et al.*, 2015; Kamara, *et al.*, 2020; Negbenebor and Nura, 2020). Its importance as a primary source of income for small holder farmers ranks it third among food crops worldwide after rice and wheat (FAO, 2013; Bawa, 2021; Erenstein *et al.*, 2022). Maize serve as a vital source of sustenance for millions of people, and it can be consumed in both fresh and dried forms with moisture content reduced to 14%, making it suitable for extended storage (Paddy *et al.*, 2018). Also, its versatility as a high-yielding crop extends to providing food, feed and raw materials for industrial uses. Maize is a valuable source of biofuel production, such as ethanol, which contributes to renewable energy sources. In terms of output, maize is a major cereal crop (Gariba *et al.*, 2021), with an estimated average national production of 12.74 million metric tonnes cultivated across 5.8 million hectares (USDA, 2023). Africa contributes about 7.5% of the total world production of maize with Nigeria as its leading producer (FAOSTAT, 2022). The genetic plasticity of maize has led to its extensive cultivation in Nigeria, adapting to diverse climates from the wet evergreen climate of the forest zone to the dry ecology of the Sudan savanna (Kamara *et al.*, 2020). The increasing maize demand and its global advance indicate that by the end of 2032, maize will account for the second largest share (34%) of the global cereal trade after wheat (OECD/FAO, 2023).

Biological and environmental variables significantly influence the shelf life of stored maize. Among these variable, grain moisture level and temperature play the biggest part in impacting grain quality, biochemical reactions, dry matter losses, permissible storage durations and overall grain storage management (Singh *et al.*, 2022). Stored maize is prone to infestation by moulds, fungi, mycotoxins and insect pests, such as *Sitophilus* spp and *Tribolium* spp which may be present where maize grain had been previously stored, or by cross-infestation between stores, or by cross infestation between granaries during storage (Kamara *et al.*, 2020). In tropical sub Saharan, the *Sitophilus zeamais* is the most destructive pest of stored maize leading to grain losses ranging from 20 to 90% for stored untreated maize (Muzemu *et al.*, 2013; Suleiman *et al.*, 2022). *Sitophilus zeamais* is associated primarily with maize but capable of developing on all cereal grain and cereal products. These weevils bore holes in grains, consumes a large portion of endosperm, destroy the germ and thus reduce the nutritive value and germination viability of the grain (Pitan and Jallow, 2021).

Peasant farmers often claim successful use of material of plant origin in insect pest control. Such include spices, powders, oil and extracts of plant parts. Plant extracts have been commonly used in insect pest management due to their effectiveness against various insect life stages (Adedire 2001; Nzelu *et al.*, 2020). Their mode of action varies, but with low to moderate dosages, the effect is always repellent or toxic (Rajapaske, 2006). Their main advantage is that they are environmental friendly, target specific, and non-deleterious to non-target organism (Kumar *et al.*, 2021). The wide variety of secondary plant compounds found in *Piper* species are potential leads for novel insecticides while many varieties are used in traditional control of insects that are vectors of disease and damage stored crops. This study was aimed at assessing the effects of *Piper guinense* (West African black pepper) extracts on *Sitophilus zeamais* in stored maize grains.

2.0 Materials and Methods

2.1 Culture of Sitophilus zeamais

Infested maize grains were purchased from Eke-Awka market, Awka. Adult *Sitophilus zeamais* were cultured in the laboratory in plastic jars with the mouths of the jars covered with nylon mesh held in place with rubber bands. Prior to the culturing, the grains were disinfested in the oven for 40°C for 4 hours and kept in the laboratory before use. Thereafter, they were infested with the test insect. The culturing lasted for a period of two months under ambient laboratory temperature and humidity conditions. Freshly emerged adults of *S. zeamais* were then used for the experiments.

2.2 Collection of Plant Materials

Dried seeds of *Piper guineense* (Schum & Thornn) were procured from Eke Awka market, Awka. This plant material was identified and authenticated at the Botany Laboratory of the Nnamdi Azikiwe University, Awka (NAUH-16). Thereafter, they were dried under room temperature for seven days and pulverized with an electric blender to obtain fine powder.

2.3 Soxhlet extraction from Piper guineense seeds

Both the methanolic and the hexane extraction were conducted using the Soxhlet extractor. In a flat bottomed flask, 250 ml of methanol or hexane was added as applicable and 40g of the milled seeds was placed in the thimble. Hot water bath was used as the source of heat. This process of extraction continued until the colour of the methanol in the extractor became clearer and the solution in the flask darker. This first packet of the milled seeds was removed and another added. The procedure continued until enough extract is gotten. Each extraction of 40g of milled pepper seeds lasted for about 4-5 hours.

2.4 Distillation

A water bath was used to gently heat the distillation flask containing the hexane and methanol extracts. The water bath provided controlled and uniform heating, which is essential for safety and effectively evaporating the solvents without overheating the extracts. After the distillation process (to evaporate the solvents), a thick brownish oil with characteristic piper odour smell was obtained.

2.5 Preparation of Different Concentrations of the Plant Extracts

The serial dilution of the crude extract was prepared to obtain 400%, 200%, 100%, 50% and 25% concentrations thus obtaining 4000µl/ml, 2000µl/ml, 1000µl/ml, 500µl/ml & 250µl/ml per 10 ml respectively with ordinary acetone as the control.

2.6 Residual Application of the Extracts to Determine Lethal Doses

No. 1 Whatmann filter paper measuring 9 cm in diameter was placed in each of the Petri-dishes used for the experiment. The various concentrations of the five levels of the extracts to be used were replicated three times. Control treatment with plane acetone was included. Subsequently, ten unsexed adults of *S. zeamais* were introduced into each of the petri dishes already treated with the hexane and methanolic extracts. Daily mortality counts were taken for seven days. Both moribund and dead insects were counted to determine mortality rates.

2.7 Treatment of Maize Grains

Before applying the various treatments, twenty grams (20g) of the maize grains was weighed into plastic containers for the different levels of treatments including their controls. Each of treatment had three replicates including the control. One milliliter each of the different concentrations of the different extracts was introduced into each of the plastic containers with the food materials accordingly. The controls were maintained with one milliliter of acetone introduced into them. Each treated plastic container was gently shaken to ensure uniform distribution of the test materials. The treatments were then left for twenty four hours to allow for proper distribution of extracts and acetone to evaporate, after which the insect were introduced. Ten unsexed adults of *S. zeamais* were then introduced in each of the different treatment of each oil extract, including the control. Before this time, the lids of the plastic vials were perforated and covered with muslin cloth held in place by a rubber band to secure the mouth of the plastic vials to ensure aeration as well as to avoid entry or exit of insects. The whole set up were then left for thirty three days. At the end of this period, records of number of emergence holes and weight loses were taken.

3.0 Results

3.1Evaluation of mortality caused by P. guineense on S. zeamais

The results obtained in Table 1 demonstrated that both concentration levels and extraction solvents significantly influenced mortality rates (P < 0.05) at 24, 48, 72 hours and 7 days after treatment (DAT). At 24 hours post-treatment, the concentration of 4000 µl/ml (C1) yielded the highest mortality rate of 55.0%, significantly surpassing other concentrations, followed by 2000 µl/ml (C2) at 25.0%, and 1000 µl/ml (C3) at 5.0%. The lowest mortality rates were observed for 500 µl/ml (C4) and 250 µl/ml (C5), both at 0.0%. By 48 hours, the highest mortality was still observed at 4000 µl/ml (60.0%), followed by 2000 µl/ml (35.0%), 1000 µl/ml (15.0%), 250 µl/ml (1.7%), and 500 µl/ml remaining at 0.0%. At 72 hours, 4000 µl/ml resulted in 68.3% mortality, while 2000 µl/ml and 1000 µl/ml showed 43.3% and 20.0%, respectively. Lower rates persisted at 500 µl/ml (0.0%) and 250 µl/ml (1.7%). At 7 DAT, 4000 µl/ml continued to exhibit the highest mortality at 71.7%, followed by 2000 µl/ml at 53.3%, 1000 µl/ml at 23.3%, 500 µl/ml at 5.0%, and 250 µl/ml at 1.7%. The control (C6) recorded mortality of 3.3% at 24, 48 and 72 hours, and 7 DAT. Among the extraction solvents, hexane consistently showed higher mortality rate of 30.0%, while methanol recorded 8.3%. This trend continued at 72 hours, with hexane and methanol showing mortality rates of 32.2% and 13.3%, respectively. By 7 DAT, hexane resulted in a mortality rate of 38.8%, whereas methanol had 14.4%. The least significant difference (LSD) values indicate that these differences were statistically significant (P < 0.05) for all time points. The interaction effect between concentration and extraction solvent was also significant (P < 0.01) at all observed time intervals.

3.2 Evaluation of emergence holes in maize grains treated with P. guineense

The different concentration levels significantly affected the number of emergence holes (P < 0.05) in Table 2. At the highest concentration of 4000 µl/ml (C1), no emergence holes were observed (0.00), demonstrating the most effective inhibition of *S. zeamais* emergence. As the concentration decreased, the number of emergence holes increased, with 2000 µl/ml (C2) showing 0.50 emergence holes, and 1000 µl/ml (C3) showing 0.83 emergence holes. At lower concentrations, the number of emergence holes increased more noticeably, with 500 µl/ml (C4) resulting in 3.00 emergence holes and 250 µl/ml (C5), resulting in 4.67 emergence holes. The control group (C6), which received ordinary acetone, exhibited the highest number of emergence holes at 7.67. Regarding the extraction solvents, the study showed that there was no significant difference (NS) between hexane and methanol in terms of their effect on the number of emergence holes (P > 0.05). Hexane-treated samples had an average of 2.50 emergence holes, while methanol-treated samples had a slightly higher average of 3.06 emergence holes. The effect of interaction between concentration and extraction solvent was also not significant.

3.3 Evaluation of total weight loss in maize grains treated with P.guineense

The results in Table 3 demonstrated that concentration levels significantly influenced the percentage weight loss (P < 0.05). At the highest concentration of 4000 µl/ml (C1), the percentage weight loss was the lowest at 0.01%, indicating the most effective protection of the grains from *S. zeamais* damage. As the concentration decreased, the percentage weight loss increased: 2000 µl/ml (C2) resulted in 0.11% weight loss, 1000 µl/ml (C3) had 0.39% weight loss, 500 µl/ml (C4) showed 0.55% weight loss, and 250 µl/ml (C5) exhibited a higher percentage weight loss at 2.12%, apart from the control. The control group (C6), which received ordinary acetone treatment, experienced the highest percentage weight loss at 13.01%. This clear inverse relationship between concentration solvents, the study showed that there was no significant difference between hexane and methanol in terms of their effect on percentage weight loss (P > 0.05). Hexane-treated samples had an average percentage weight loss of 2.42%, while methanol-treated samples had a slightly higher average percentage weight loss of 2.98%. Despite hexane showing a marginally better performance, the lack of significant difference suggests that both solvents were similarly effective in reducing the percentage weight loss when used to extract *Piper guineense*.

	Mortality			
Treatment	24hrs	48hrs	72hrs	7DAT
Concentration (Conc.)				
C1 - 4000 µl/ml	55.00	60.0	68.3	71.7
C2 - 2000 µl/ml	25.00	35.0	43.3	53.3
C3 - 1000 µl/ml	5.00	15.0	20.0	23.3
C4 - 500 µl/ml	0.00	0.0	0.0	5.0
C5 - 250 µl/ml	0.00	1.7	1.7	1.7

C6 – acetone	3.33	3.3	3.3	3.3
LSD	6.88	8.88	11.23	13.62
Extraction Solvent (ES)				
Hexane	23.89	30.0	32.2	38.8
Methanol	5.56	8.3	13.3	14.4
LSD	3.97	5.13	6.49	7.86
Interaction				
Conc X ES	**	**	**	**

LSD= Least significant difference, ** = 0.01 level of significance

Table 2: Effect of concentration and extraction solvents on emergence holes in maize grains treated with P. guineense

Treatment	Emergence Holes	
Concentration (Conc.)		
$C1 - 4000 \ \mu l/ml$	0.00	
$C2-2000\ \mu l/ml$	0.50	
$C3 - 1000 \ \mu l/ml$	0.83	
$C4-500\ \mu l/ml$	3.00	
$C5 - 250 \ \mu l/ml$	4.67	
C6 – acetone	7.67	7
LSD (0.05)	1.31	13
Extraction Solvent (ES)		
Hexane	2.50	
Methanol	3.06	
LSD (0.05)	NS	
Interaction		
Conc. X ES	NS	

LSD= Least significant difference, ** = 0.01 level of significance

Table 3: Effect of concentration and extraction solvents of total weight loss in maize grains treated with P.guineense

Treatment	% Weight Loss
Concentration (Conc.)	
$C1-4000 \ \mu l/ml$	0.01
$C2-2000\;\mu l/ml$	0.11
$C3 - 1000 \ \mu l/ml$	0.39
$C4-500\ \mu l/ml$	0.55
$C5-250\ \mu l/ml$	2.12
C6 – acetone	13.01
LSD (0.05)	3.497
Extraction Solvent	
Hexane	2.42

Methanol	2.98	
LSD (0.05)	NS	
Interaction		
Conc. X ES	NS	
LSD= Least significant difference, $** = 0.01$ level of significance		

4.0 Discussion

The mortality rates of *Sitophilus zeamais*, varied significantly depending on both the concentration of *Piper guineense* extract and the extraction solvent used. This could be attributed to *Piper guineense* extract containing bioactive compounds such as alkaloids and terpenoids known for their insecticidal properties (Talukder, 2006). Hexane was more effective at extracting the insecticidal compounds from *Piper guineense* extract compared to methanol and highlights hexane's superior ability to extract insecticidal compounds from *Piper guineense* extract. Akinbuluma *et al.* (2017) recorded similar results, noting that the hexane fraction of *Piper guineense* recorded higher mortality than ethyl acetate and ethanol. Souza *et al.* (2010) also reported that the hexane extract of *Tabebuia heptaphyla* showed similar mortality effect rates on *S. zeamais* which were higher than those of ethyl acetate on the fifth and tenth day post-treatment. They attributed the observed mortality effect to the presence of secondary metabolites in the hexane fractions. Asawalam *et al.* (2006) also recorded that hexane extract of *Piper guineense* caused almost 100% mortality *Sitophilus zeamais* at 7 days after treatment.

The emergence holes in maize grains treated with *Piper guineense* extract varied significantly depending on the concentration of the extract used. The control (acetone) had the highest number of emergence holes, which was significantly different from the treated samples, showing the increasing progression of *S. zeamais* infestation in the absence of *Piper guineense* (ordinary acetone) treatment. This aligns with findings from Asawalam *et al.* (2007) and Ileke *et al.* (2020), who reported that *Piper guineense* completely protected cowpea seeds and maize grains from damage by *Callosobruchus maculatus* and *Sitophilus zeamais*, respectively. Asawalam *et al.* (2007) recorded that weevil perforation was highest in the control compared to treated maize grains, indicating positive protectant ability. Ileke *et al.* (2020) also found that beetle perforation index was zero in cowpea seeds treated with seed powder at a rate of 2g and 3g/20g of cowpea seeds, while the untreated control recorded more than a 50% perforation index.

The effect of *Piper guineense* extract on the total weight loss in maize grains infested with *Sitophilus zeamais* varied significantly with concentration, although the choice of extraction solvent did not exhibit a statistically significant effect. This minimal weight loss can be attributed not only to the high levels of bioactive compounds but also to the potential disruption of insect feeding behaviour and physiological processes. *Piper guineense* may interfere with the digestive enzymes of *S. zeamais*, making it difficult for the insects to process the grain effectively, leading to reduced damage (Isman, 2006). Furthermore, *Piper guineense* has been reported to have fumigant properties that can enhance its effectiveness in a stored grain environment by creating a hostile atmosphere for the pests, further reducing weight loss (Don-Pedro, 1989). Asawalam and Arukwe (2004) revealed that *P. guineense* gave some protection against weight loss to the maize seed quality them as effective maize protectants. In a similar experiment carried out by Rosulu *et al.*, (2022), they reported that three grams of *C. annuum* powder reduced to the barest minimum the number of eggs laid or hatched by *Callosobruchus maculatus* preventing adult emergence and survival at 38 days after infestation. The effect of interaction between concentration and extraction solvent was also not significant, indicating that the combined effect did not produce a synergistic impact, possibly because the individual contributions of concentration and extraction solvents were either too similar or too small to result in a noticeable difference when combined. This phenomenon has been observed in studies such as Cedergreen (2014), where mixture toxicity did not always lead to significant combined effects, and Knudsen and Barker (1997), where the efficacy of formulations was not significantly impacted by the interaction of different factors.

4.1 Conclusion

The methane and hexane extracts of *Piper guineense* caused mortality to *Sitophilus zeamais* and demonstrated efficacy in reducing emergence holes and reducing weight loss in maize grains. Higher concentrations of *Piper guineense* extract generally resulted in increased mortality rates over time, while the control exhibited the lowest mortality and protectant ability on maize grains. Further research is recommended to conduct quality assessment and safety evaluation of *Piper guineense* extracts to ensure their safety for consumers and environmental impact.

Acknowledgements

The authors wish to thank Mr. Benjamin Chidi Umeanor and all the staff of the Department of Parasitology and Entomology, Nnamdi Azikiwe University for their technical support in the entire project and laboratory analysis. The authors are also deeply grateful to Mr. Chigaemezu Kennedy Onyewuchi of the Biochemistry Laboratory, Nnamdi Azikiwe University for providing the special facilities and resources that were needed for the completion of this study.

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