Innovations

Evaluation of the Biocidal Effect of the Seed Extracts of Xylopiaaethiopica (Negro Pepper) and Piperguineensis (Uziza Seed) Against Sitophilusoryzae (Rice Weevil)

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Abstract: *Sitophilus oryzae is a major insect pest that threatens stored grains like rice, leading to significant economic losses. This study investigated the insecticidal potential of seed powders from Xylopiaaethiopica and Piper guineenseagainst adult S. oryzae. Qualitative and quantitative phytochemical analysis was done using standard methods. Seed powders of the plants at different concentrations (10-100 gm/kg) with 0 gm/kg concentration as control were applied in this study. Insect mortality was determined at various time intervals (24 hrs, 48 hrs, 72 hrs, 96 hrs and 120 hrs). Phytochemical evaluation revealed the presence of alkaloids, flavonoids, terpenoids, cardiac glycosides, steroids, saponins, and tannins in varying concentrations. Quantitative phytochemical analysis revealed P. guineense exhibited significantly higher ($p<0.05$) concentrations of alkaloids (3.14±0.67%), flavonoids (3.21±0.46%), terpenoids (5.68±0.81%), cardiac glycosides (5.91±0.32%), and steroids (4.46±0.81%) compared to X. aethiopica. The insecticidal efficacy was evaluated by exposing S. oryzae adults to varying concentrations (0-100 g/kg) of the seed powders over 120 hours. X. aethiopica induced dose-dependent and time-dependent mortality, with the highest concentration (100 g/kg) causing 16.28 mean dead insects at 120 hours. P. guineense demonstrated significantly superior ($p<0.05$) insecticidal activity, achieving 20.00 mean dead insects at 100 g/kg after 120 hours. The combined use of both seed powders exhibited a synergistic effect, with P. guineense contributing more substantially to mortality. Statistical analysis showed a significant difference ($p<0.05$) in the number of insect deaths at the various tested concentrations and time intervals.Statistically, P. guineensis demonstrates a significantly higher contribution to mortality compared to Xylopiaaethiopica. These findings highlight the promising potential of X. aethiopica and P. guineense seed powders as eco-friendly alternatives for controlling S. oryzae*

infestations in stored grains, aligning with sustainable agriculture and integrated pest management practices.

Keywords: *Sitophilus oryzae, Xylopiaaethiopica, Piper guineense,Infestation, Insecticidal.*

1.0 Introduction:

The most important cereal grain is rice, or Oryzasativa (Walp), which provides energy and carbohydrates to over half of the world's population.The harvested crop is commonly stored for various purposes, including food, cash crop, fodder, industrial raw materials, and future planting. However, cereal grains like rice can suffer storage losses ranging from 10 to 20 percent of the total yield, with a major contributor to these losses being stored-product insects [1]

The rice weevil, Sitophilus oryzae, is a significant pest affecting processed cereal products and stored grains in tropical and warm temperate regions worldwide. Due to concerns about the ongoing use of conventional pesticides, researchers are assessing new reduced-risk pesticides for controlling stored-product pests. These concerns encompass pest resistance, environmental persistence, elimination of beneficial organisms, mammalian toxicity, food residues, increased crop production costs, and technical difficulties in application.[2]. There has been a lot of focus on the use of alternative control techniques with low toxicity to humans and the environment to preserve stored-product pests. Numerous spice and herbal plant products, including extracts, distillates, powders, pellets, and essential oils (EO), have been used; however, due to their low toxicity, they may also be effective as fumigants, deterrents, antifeedants, repellents, and toxicants to keep pests associated with stored products out of grain [3].

A significant evergreen medicinal plant found throughout West Africa, Xylopiaaethiopica, is also known as the African pepper or spice tree. Traditional medicine uses mixtures made from this plant's morphological parts to treat a variety of conditions, including stomach-aches, fever, dysentery, skin infections, and candidiasis [4]. Antibacterial and antifungal properties that repel mosquitoes and termites have been reported to be present in extracts derived from Xylopiaaethiopica[5].

The spicy pepper plant Piper guineensis grown in the southern states of Nigeria and has a woody forest. Its leaves and fruits are used in conjunction with other herbs to cure worm infestation, cough, and infantile convulsions. Moreover, reports of insecticidal and antifungal effects have been made on Piperguineensis extracts

[6].The primary objective of this study is to investigate the biocidal effects of seed extracts of X aethiopica and P. guineensis on pests, particularly targeting rice weevils. This research holds significant promise in addressing pest control challenges effectively and sustainably.

2.0 Materials and Methods

2.1 Study location

This study was conducted in the Biology laboratory at the School of Biological Sciences, Federal University of Technology, Owerri. The laboratory's average temperature ranges from 27°C to 30°C, with a mean relative humidity of 81% to 90%. The Federal University of Technology Owerri is located between latitudes 05° 21' and 05° 42' North and longitudes 07° 48' and 06° 53' East. Owerri is situated in a tropical rainforest zone, receiving an average annual rainfall of 2,250 to 2,800 mm.

2.2 Sample collection and preparation

The study, conducted in the laboratory of the School of Biological Sciences at the Federal University of Technology, Owerri, utilized Sitophilus oryzae obtained from laboratory cultures maintained on untreated rice at 25 ± 1 °C and 65-70% relative humidity. Both male and female adult weevils were used in the experiments. Untreated rice was purchased from Douglas Foodstuff Market, while mature ripe fruits of X. aethiopica and Piper guineensis were also sourced from the same market. The seeds were identified and authenticated by the curator of the Department of Biology's laboratory at the Federal University of Technology, Owerri, where the plant specimens were deposited. The fruits were rinsed with tap water to remove impurities, air-dried in the shade, and then milled into powder using a mechanical blender. The powdered fruits were weighed into several doses, sealed in well-labeled transparent plastic bags, and stored until needed.

2.3 Preparation of the Plant Extract:

Powdered samples of X. aethiopica and Piper guineense were retrieved, and 2000g was measured out. The powdered samples were then macerated in 3.5 liters of distilled water at room temperature for 72 hours with constant stirring. After 72 hours, the mixture was filtered using Mushin cloth. A concentrated extract was obtained by evaporating the filtrate with a rotary evaporator at 45°C.

2.4 Qualitative Analysis of Phytochemicals

The ethanolic extract was qualitatively analyzed for several bioactive elements, including flavonoids, tannins, cardiac glycosides, saponins, steroids, terpenoids, anthraquinones, and alkaloids. Phytochemical screening was conducted on the

extracted material using standard procedures to identify secondary metabolites [7; 8].

2.5 Quantitative Phytochemical Analysis of the Extracts

Phytochemical screening was performed on the extracted material using standard procedures to identify secondary metabolites [7;8;9].

2.6 Biocidal Activity

The rice grains were fumigated with carbon tetrachloride for 48 hours and then aerated for four days to ensure the elimination of any developing larvae or pupae within the grains, following the methods suggested by [10] and [11]. Powders from the fruits of Piper guineensis and Xylopiaaethiopica were mixed with the rice grains at different rates (0, 10, 25, 50, and 100 g/kg). Twenty rice weevils were introduced into each treated batch of rice grains, with three replicates for each setup.

All treatments were arranged using a randomized complete block design. The laboratory conditions were maintained at a room temperature of 27°C and a humidity of 72.5%, as controlled in a laboratory incubator. The mortality of Sitophilus oryzae was determined by daily counts of dead adults for 10 days. After this period, all surviving adults were removed, following the method suggested by [12]. An adult Sitophilus oryzae was considered dead if it did not move when touched with a spatula.

3.0 Results and Discussion

3.1 Results

Qualitative Phytochemical Properties of Some Selected Plants Used as Biocidal Agents against Sitophilusoryzae (Rice Weevil).

Results show (Table 1) that alkaloids, flavonoids, terpenoids, cardiac glycosides, and steroids were found in high amount in P. guineense and in low amounts in X. aethiopica. Tannin was high in X. aethiopica and low in P. guineense, while saponin was high in both plants

Table 1: Qualitative phytochemical analysis of the extracts

Phytochemical	Xylopiaaethiopica	**Piper guineense**
Alkaloids	+	++
Tannins	++	+
Flavonoids	+	++
Terpenoids	+	++
Saponins	++	++
Cardiac glycosides	+	++
Steroids	+	++

Keys: + = low amount; ++ = high amount; - = not present

Quantitative Properties of Phytochemicals of Some Selected Plants Used as Biocidal Agents against Sitophilusoryzae (Rice Weevil).

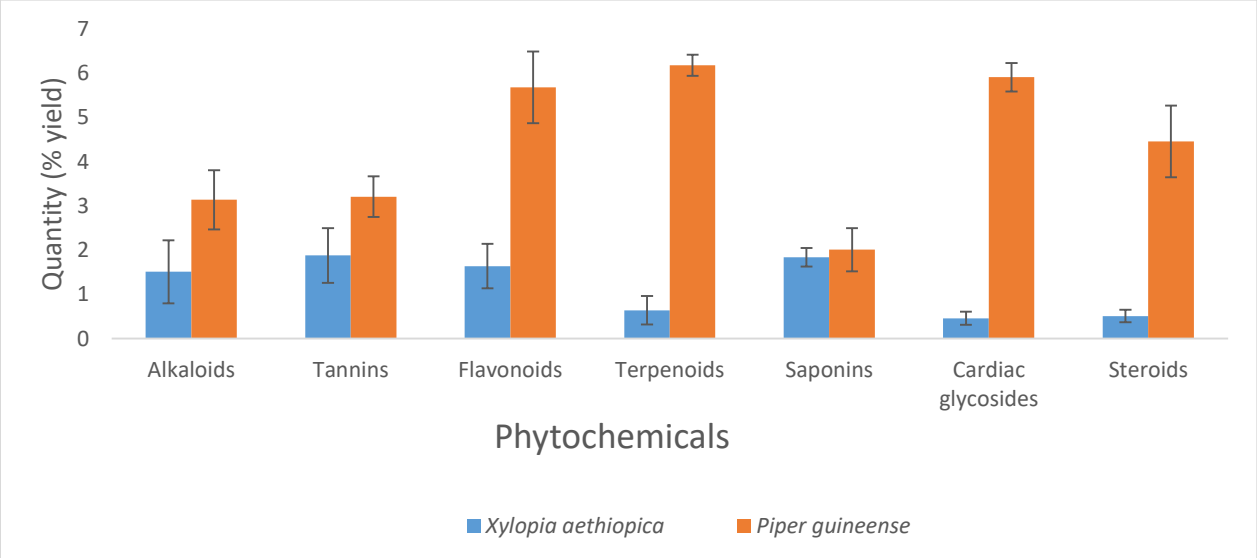

Figure 1: Quantitative Phytochemical Analysis of the Extracts

Figure 1 shows thequantitative phytochemical analysis of X. aethiopicaand P. guineense. The results indicate significant variations ($p<0.05$) in the percentage yield of various phytochemical compounds between Xylopiaaethiopica and Piper guineense with Piper guineensehaving significantly higher phytochemical values when compared with Xylopiaaethiopica.Piper guineense exhibits notably higher levels of alkaloids (3.14±0.67), flavonoids (3.21±,0.46) terpenoids (5.68±,0.81), cardiac glycosides (5.91±,0.32) and steroids (4.46± 0.81) compared to

Xylopiaaethiopica with values of alkaloids (1.51±0.71), flavonoids (1.64±,0.50) terpenoids (0.64±,0.32), cardiac glycosides (0.46±0.15) and steroids (0.51± 0.14).

Effect of Xylopiaaethiopica seed powder on adult Sitophilus oryzae

The effects of X. aethiopicaseed powder at different concentrations concerning time on adult S. oryzae is shown in Table 2. At 24 hours, the number of dead insects ranged from 0 to 3.86, with the highest concentration of 100 gm/kg resulting in the highest mortality.By 48 hours, mortality increased, ranging from 0 to 4.49 dead insects, with higher concentrations leading to more deaths.At 72 hours, mortality continued to rise, ranging from 1 to 11.21 dead insects, with the highest concentration (100 gm/kg) showing the most pronounced impact.After 96 hours, mortality escalated further, ranging from 2.3 to 14.85 dead insects, again with the highest concentration (100 gm/kg) inducing the greatest mortality (14.85).Finally, at 120 hours, mortality peaked, ranging from 2.87 to 16.28 dead insects, with the highest concentration (100 gm/kg) exhibiting the most substantial insecticidal effect (16.28). Statistical analysis showed no significant difference ($p>0.05$) in the number of insect death at the various tested concentration and time interval

Table 2: Effect of Xylopiaaethiopica seed powder on adult Sitophilus oryzae

	Time (hr) /Mean no of dead insects				
Xylopiaaethiopicaconc(gm/kg)	24hr	48 hr	72 hr	96 hr	120 hr
0	0	0	1	2.3	2.87
10	0	1	1.89	2.46	3.12
20	1.24	1.92	4.32	5.12	6.74
50	2.46	3.28	8.46	11.84	13.18
100	3.86	4.49	11.21	14.85	16.28

Effect of P. guineensis seed powder on adult Sitophilus oryzae

The effects of P. guineensis seed powder on adult Sitophilus oryzaeare shown in Table 3. At 24 hours, the mean number of dead insects ranged from 0 to 6.94, with the highest concentration of 100 gm/kg resulting in the highest mortality. At 48 hours, mortality increased, ranging from 0 to 10.84 dead insects, with higher concentrations leading to more effects. At 72 hours, mortality continued to rise,

ranging from 1.18 to 17.12 dead insects, with the highest concentration showing the most pronounced impact. After 96 hours, mortality escalated further, ranging from 4.28 to 18.12 dead insects, again with the highest concentration inducing the greatest mortality. After 120 hours, mortality peaked, ranging from 5.21 to 20 dead insects, with the highest concentration exhibiting the most substantial insecticidal effect.Statistical analysis showed a significant difference ($p<0.05$) in the number of insect deaths at the various tested concentrations and time intervals

Table 3: Effect of P. guineensis seed powder on adult Sitophilus oryzae

	Time (hr) /Mean no of dead insects				
P. guineensisconc (gm/kg)	24hr	48 hr	72 hr	96 hr	120 hr
0	0	0	1.18	4.28	5.21
10	2.12	3	6.28	7.14	10.61
20	3.64	6.89	8.48	14.86	14.6
50	5.88	10.14	12.84	17.61	18.46
100	6.94	10.84	17.12	18.12	20

Combined Mortality Effects of Xylopiaaethiopicaand P. guineensis on Adult Sitophilus oryzae

The Table below shows the combined mortality effects of Xylopiaaethiopica and P.guineensis on adult Sitophilus oryzae observed at different time intervals for various concentrations (gm/kg) of the plants. At 24 hours, the mean number of dead insects ranged from 0 to 7, with the highest concentration of 100 gm/kg resulting in the highest mortality. By 48 hours, mortality increased, ranging from 1.17 to 7.64 dead insects, with higher concentrations leading to more significant effects. At 72 hours, mortality continued to rise, ranging from 2 to 15.12 dead insects, with the highest concentration showing the most pronounced impact. After 96 hours, mortality escalated further, ranging from 3.12 to 18.27 dead insects, again with the highest concentration inducing the greatest mortality. At 120 hours, mortality peaked, ranging from 4 to 19 dead insects, with the highest concentration exhibiting the most substantial insecticidal effect.Statistical analysis showed a significant difference ($p<0.05$) in the number of insect deaths at the various tested concentrations and time intervals.Statistically, P. guineensis demonstrates a significantly higher contribution to mortality compared to Xylopiaaethiopica.

Table 4: Combined mortality effects on adult Sitophilus oryzae

	Time (hr) /Mean no of dead insects				
combined mortality (gm/kg)	24hr	48 hr	72 hr	96 hr	120 hr
0	0	1.17	2	3.12	4
10	1.86	2.81	3.71	6.47	7.84
20	3.41	4.32	6.18	11.86	13.19
50	6.12	6.81	8.42	13.47	16.92
100	7	7.64	15.12	18.27	19

Discussion

Plants are well-known sources of a wide variety of bioactive chemical substances that act as biochemical and physiological agents. A notable feature of plants is their rich phytochemical composition .

The phytochemical evaluation conducted on Piper guineense and Xylopiaaethiopica revealed the presence of various phytochemical constituents, including flavonoids, alkaloids, terpenoids, cardiac glycosides, steroids, saponins, and tannins, in varying concentrations (Table 1). The findings for Xylopiaaethiopica are consistent with a previous report by Yusuf et al. [14], which also identified the presence of these phytochemicals in the plant. Similarly, the detection of these phytochemical compounds in Piper guineense aligns with the findings reported by [13]

The presence of various phytochemical constituents, including flavonoids, alkaloids, terpenoids, cardiac glycosides, steroids, saponins, and tannins, in Piper guineense and Xylopiaaethiopica, as revealed by the phytochemical evaluation (Table 4.1), could potentially contribute to their biocidal effects observed on the number of dead insects.

Ukaomaet al. (2024) [15] reported that many of these phytochemical compounds are known to possess insecticidal, pesticidal, or insect-repellent properties. This could explain the observed increase in the mean number of dead insects with higher concentrations and longer exposure times to the plant extracts.

The varying concentrations of these phytochemicals in Piper guineense and Xylopiaaethiopica could contribute to their observed biocidal effects on insects. The higher concentrations of certain compounds, such as terpenoids and cardiac glycosides in Piper guineense, may explain its more potent insecticidal activity compared to Xylopiaaethiopica, as evidenced by the higher mean number of dead insects at higher concentrations and longer exposure times.

While the phytochemical evaluation revealed the presence of various phytochemical constituents, including alkaloids, flavonoids, and saponins, in both Xylopiaaethiopica and Piper guineense, there were notable differences in the relative compositions of these compounds compared to the findings reported by [13]. Specifically, the extracts of X. aethiopicaanalyzed in this study exhibited higher relative compositions of alkaloids (1.51%), flavonoids (1.64%), and saponins (1.84%) than those reported by Evuenet al. (2022) [13], which were 1.49%, 0.22%, and 0.18%, respectively. These observed differences in the relative compositions of phytochemical constituents between the two studies could be attributed to several factors such as variations in the analytical techniques or methodologies employed for phytochemical evaluation can lead to differences in the quantification of various compounds, the stage of plant growth and the timing of harvesting can influence the biosynthesis and accumulation of phytochemicals, resulting in variations in their compositions, environmental factors, such as temperature, rainfall, and soil conditions, can affect the metabolic processes and phytochemical production in plants, leading to differences in their phytochemical profiles. Similarly, the findings on the phytochemical components of P. guineense align with previous reports by other authors [16; 17]. Echo et al. (2012) [16] reported that the alkaloid composition in P. guineense was 1.67%, which is lower than the 3.14% found in this study. Additionally, this study observed a tannin composition of 3.21% in P. guineense, contrasting with the 0.30% reported by [16].

The efficacy of Xylopiaaethiopica and Piper guineense seed powders on Sitophilus oryzae mortality was investigated in various studies [18; 19]. Xylopiaaethiopica and Piper guineense seed powders at rates of 100gm kg^-1 were found to be effective in controlling Sitophilus oryzae [18]. Additionally, Piper guineense seed powder, either alone or mixed with Zingiberofficinale, demonstrated high efficacy against Sitophilus zeamais, with mortality rates reaching up to 96.66% at 96 hours after treatment [20]. Furthermore, Eugenia aromatica and Piper guineense powders were effective against Sitophilus oryzae, with E. aromatica inducing complete weevil mortality after 72 hours at a higher dose compared to P. guineense[21]. These findings collectively suggest that Piper guineense seed powder exhibits significant

potential in controlling Sitophilus species infestations. The results of this study provide a scientific rationale for the use of P. guineense in post-harvest protection.

Xylopiaaethiopica, commonly known as the Ethiopian pepper or the Negro pepper, is a plant species that has shown promising potential as a natural insecticide and pest control agent, particularly against Sitophilus oryzae, a significant pest that threatens stored rice. The efficacy of Xylopiaaethiopica in controlling Sitophilus oryzae can be attributed to its diverse phytochemical composition. These phytochemicals are known to possess insecticidal, insect-repellent, and antifeedant properties, contributing to the plant's effectiveness against pests [22]. The synergistic effects of the phytochemicals present in the ethanol extract may enhance its insecticidal activity, making it a recommended method for pest control in stored rice.

Ekeh et al. (2018) [19] reported that X. aethiopica has a pungent smell that acts as an insect repellent and possibly causes suffocation and death of the weevils. The insecticidal properties of the plant's extract are likely due to its constituent components, pungent smell, and mode of action. These factors may explain its effectiveness in controlling S. oryzae and other insects.

In a study conducted by [23], the efficacy of X. aethiopica seed powder in controlling S. oryzaewas evaluated [19]. The researchers observed that the powder, when applied at varying concentrations, led to increased mortality rates of S. oryzae weevils. The powder exhibited antifeedant properties, causing a progressive increase in weevil mortality with higher concentrations and longer exposure periods. Additionally, the study found that X. aethiopica seed powder was effective in reducing the damage caused by weevils to stored grains. These findings align with the findings of this study.

In addition to its insecticidal properties, Xylopiaaethiopica has been found to possess antioxidant properties. Antioxidants play a crucial role in counteracting the detrimental effects of various environmental stressors, including pesticides like glyphosate [24]. The presence of antioxidant compounds in Xylopiaaethiopica could potentially mitigate the harmful effects of synthetic pesticides, making it a valuable complementary or alternative solution for pest management [24].

The use of Xylopiaaethiopica seed powder as a natural insecticide offers several advantages over conventional synthetic pesticides. It is environmentally friendly, biodegradable, and less likely to contribute to the development of resistance in pest populations. Additionally, the powder form facilitates easy application and storage, making it a convenient option for smallholder farmers and households [25]

Furthermore, the utilization of Xylopiaaethiopica as a pest control agent aligns with the principles of sustainable agriculture and integrated pest management (IPM) strategies. IPM approaches aim to minimize the reliance on synthetic pesticides by incorporating various cultural, biological, and natural methods to manage pest populations while reducing environmental impacts [22]. Xylopiaaethiopica seed powder presents a viable and environmentally friendly alternative for managing Sitophilus oryzae infestations in stored rice. Its diverse phytochemical composition, including insecticidal compounds and antioxidant properties, contributes to its effectiveness as a natural pest control agent.

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