

Determination of the Efficacy of Bitter Leaves (*Vernonia Amygdalina*) Biochar as Bio-Pesticide Against *Sitophilus Zeamais*

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ABSTRACT

Post-harvest storage of grains with chemical pesticides, which are harmful to human health, prompted this experiment. The study, carried out at the Laboratory and Farm of the Department of Agricultural Technology, Federal Polytechnic, Ile-Oluji, Nigeria, used natural means of storing the grains. Biochar from Bitter Leaves (*Vernonia amygdalina*) was applied to stored maize infested with *Sitophilus zeamais* to investigate its effect on the susceptibility index, mortality rate, and germination percentage of the maize. The experiment used a Completely Randomized Design with four Bitter Leaves Biochar (BLB) treatments as 0g, 5g, 10g, and 15g repeated thrice for each of the treatments. Each of the results showed that the control treatment recorded the highest Susceptibility Index (57%), while the lowest value (13%) was recorded at 15 g BLB. In contrast, the control treatment recorded the lowest mortality (12%) and germination percentage (40%). Mortality increased progressively with increasing BLB rate, from 45%, 63%, and 73% at 5, 10, and 15 g BLB, respectively. Similarly, germination percentage increased from 40% in the control to 65%, 73%, and 86% at 5, 10, and 15 g BLB, respectively. The findings show that increasing BLB application rates enhanced the mortality of *Sitophilus zeamais* while improving the germination percentage of stored maize. Bitter Leaf Biochar therefore shows considerable potential as a botanical biopesticide for the management of *Sitophilus zeamais* in stored maize.

Keywords: Bitter leaf Biochar; *Sitophilus zeamais*; Susceptibility Index; Mortality; Germination Percentage; Sustainable Agriculture.

INTRODUCTION

Maize (*Zea mays* L.) is one of the important staple crops globally. As observed by Zhang and Wang (2024), *Zea mays* is consumed locally, and agro allied industries use it for feed mill production and other ingredients for their production. The Food and Agriculture Organization (FAO, 2023) noted that *Zea mays*, are in the same category with wheat and rice, and these grains are produced in their billions globally. Unfortunately, postharvest losses keeps increasing through infestation by insects and pests. Specifically, *Sitophilus zeamais* was known to attack and destroy stored maize grains. This was also substantiated by Molina et al. (2023) and Parmar et al. (2025).

Meanwhile, Phokwe and Mangayi (2023) note that chemical pesticides are widely used to manage insect and pest attacks on stored grain; however, these chemicals can make stored grains harmful to human health when consumed due to their toxicity. These researchers also noted that pests and insects develop resistance to these chemicals with prolonged use, which also causes environmental pollution and depletion.

Growing concerns regarding pesticide residues in food and their potential effects on human health and the environment have consequently increased interest in safer and more sustainable alternatives. Plant-derived biopesticides have received considerable attention as potential alternatives for managing insect pests of stored grains because they are generally biodegradable and may pose fewer risks to humans and the environment (Ayamga et al., 2024; Quintero et al., 2025).

Recent studies have further demonstrated the potential of plant-based biopesticides in suppressing major insect pests associated with stored maize while reducing the adverse effects associated with conventional chemical pesticides (Areej et al., 2024; Zhang & Wang, 2024). *Vernonia amygdalina* (bitter leaf) is a readily available plant with bioactive compounds that may have potential for insect pest management. However, there is limited information on the use of Biochar produced from bitter leaf as a treatment for managing *Sitophilus zeamais* in stored maize.

Therefore, this study investigated the effect of different rates of Bitter Leaf Biochar (BLB) on the mortality of *Sitophilus zeamais* and the germination percentage of stored maize. The experiment delivers an ecologically friendly and potentially biodegradable approach to stored-maize pest management, with the possibility to reduce post-harvest losses, enhance grain protection, and contribute to food security and the sustainable availability of maize for human consumption and raw materials for agro-allied industries.

MATERIALS AND METHODS

The experiment was conducted at the Demonstration Farm and Pest Laboratory of the Department of Agricultural Technology, Federal Polytechnic, Ile-Oluji, Ondo State, Nigeria. The experimental site is located at approximately latitude 7.236935°N and longitude 4.861769°E.

Fresh leaves of bitter leaf (*Vernonia amygdalina*) were collected from the Demonstration Farm of the Federal Polytechnic, Ile-Oluji, Ondo State, Nigeria. The leaves were initially air-dried to reduce their moisture content from approximately 84% to 16% and subsequently oven-dried at 105°C to approximately 10% moisture content before pyrolysis. One kilogram (1 kg) each of infested and uninfested maize (*Zea mays* L.) was purchased from traders at Odolua Market, Ile-Oluji, Ondo State, Nigeria.

The dried *V. amygdalina* leaves were subjected to pyrolysis at 350°C under limited-oxygen conditions using a locally fabricated Biochar kiln described by Akinfiresoye and Ogidan (2024). The pyrolysis was conducted at the departmental Demonstration Farm. Following pyrolysis, the resulting Bitter Leaf Biochar (BLB) was allowed to cool under ambient conditions. The Biochar was subsequently ground and sieved to obtain a relatively uniform particle size suitable for application to the maize.

Adult *Sitophilus zeamais* were obtained from naturally infested maize. Twenty (20) pairs of adult weevils were introduced into 500 g of clean maize to establish a laboratory culture. The culture was maintained for 40 days under laboratory conditions to allow oviposition, larval development, pupation, and emergence of adult progeny. Newly emerged F₁ adult weevils were collected and used to infest the experimental units.

A Completely randomized Design (CRD) was used for this study. There were four treatments as listed below, and each of the treatment was replicated thrice, with a total of twelve experimental units for the four treatments. Each of the units of the experiment had 20 clean maize grains in a container labelled accordingly.

T₁ (Control): 0 g BLB

T₂: 5 g BLB

T₃: 10 g BLB

T₄: 15 g BLB

The clean maize grain inside the labelled container was thoroughly coated with the specified grams of BLB, while there was no addition of BLB to the control (0 g). Twenty (20) pairs of adult F₁ *Sitophilus zeamais* were now introduced as infestation to each of the experimental units. Muslin cloth was used to cover each of the containers to allow suitable ventilation and prevent the escape of any of the insects.

After 15 days, the adult *Sitophilus zeamais*, dead or alive, were sieved out from the experimental unit and recorded. The experimental units were maintained for another 30 days to give room for emergence of new *Sitophilus zeamais* arising from the oviposition of the initial 15 days. These new emergences were also counted and recorded.

The maize seeds were also examined and separated into damaged and undamaged seeds. The undamaged seeds were subsequently used for germination tests.

Data were collected on the following parameters:

- Susceptibility Index (SI): This was used to determine the susceptibility of stored maize to infestation by *Sitophilus zeamais* under the different BLB treatments.
- Mortality Rate (MR): This was expressed as the percentage of *Sitophilus zeamais* that died following exposure to the different BLB treatments.
- Germination Percentage (GP): This was determined as the percentage of maize seeds that successfully germinated following the treatment and storage period.

These were determined using Equations 1, 2, and 3 according to Akinbuluma (2020)

$$SI = \frac{DS}{MPD} \times 100 \quad \text{equation 1}$$

$$a) \quad MR = \frac{DI}{LI} \% \quad \text{equation 2}$$

$$b) \quad GP = \frac{GS}{TNG} \% \quad \text{equation 3}$$

Where:

DS is the Number of damaged seeds by the weevils

MPD is the Maximum possible damage (total number of seeds)

DI is the Number of dead insects

LI is the Number of live insects introduced

GS is the Number of germinated seeds

TNG is the Total number of grains planted

We analyzed the data descriptively and inferentially using Microsoft Excel (2013 version) and IBM SPSS Statistics. One-way Analysis of Variance (ANOVA) was also performed to establish significant differences among treatments, while Tukey's post hoc test was used to determine which treatments differed significantly at the 5% level of significance ($\alpha \leq 0.05$).

RESULTS

The results found from the investigation carried out on the effect of different rates of Bitter Leaf Biochar (BLB) on the Susceptibility Index (SI), Mortality Rate (MR), and Germination Percentage (GP) of stored *Zea mays* exposed to *Sitophilus zeamais* infestation are as presented in Table 1. These parameters provided an indication of the ability, or otherwise, of BLB to protect stored maize against insect infestation while maintaining the viability of the grains for subsequent germination.

Table 1. Efficacy of Bitter Leaf Biochar as Biopesticide against *Sitophilus zeamais*

TREATMENT	SI (%)	MR (%)	GP (%)
0 g	57	12	40
5 g	27	45	65
10 g	20	63	73
15 g	13	73	86

The results show a clear response of the stored maize to increasing rates of BLB. The control treatment (0 g BLB) recorded the highest Susceptibility Index (57%) and the lowest Mortality Rate (12%) and Germination Percentage (40%). When BLB was applied, the Susceptibility Index progressively decreased from 57% at 0 g to 27%, 20%, and 13% at 5 g, 10 g, and 15 g, respectively. Conversely, Mortality Rate increased progressively from 12% in the control to 45%, 63%, and 73% at 5 g, 10 g, and 15 g BLB, respectively. Similarly, Germination Percentage increased from 40% in the control to 65%, 73%, and 86% at 5 g, 10 g, and 15 g BLB, respectively.

DISCUSSION

As presented in Figure 1. The Susceptibility Index (SI) varied with the rate of Bitter Leaf Biochar (BLB) applied. The control treatment (0g BBL) recorded the highest Susceptibility Index of 57%, while the SI were reduced as the concentration of BLB increases. This showed a progressive reduction in the susceptibility of the stored maize to *Sitophilus zeamais* as the rate of Bitter Leaf Biochar increased.

The relatively high SI recorded in the untreated control indicates that the maize grains were more vulnerable to infestation and damage by *Sitophilus zeamais* in the absence of BBL. However, the marked reduction in SI following BLB application shows that Bitter Leaf Biochar provided a protective effect against the pest. This is consistent with previous research by Musa et al. (2011) showing that *V. amygdalina* has protective activity against *Sitophilus zeamais*. These researchers reported that *V. amygdalina* leaf extracts significantly reduced maize grain damage compared with the untreated control and showed potential for protecting stored maize from maize weevil.

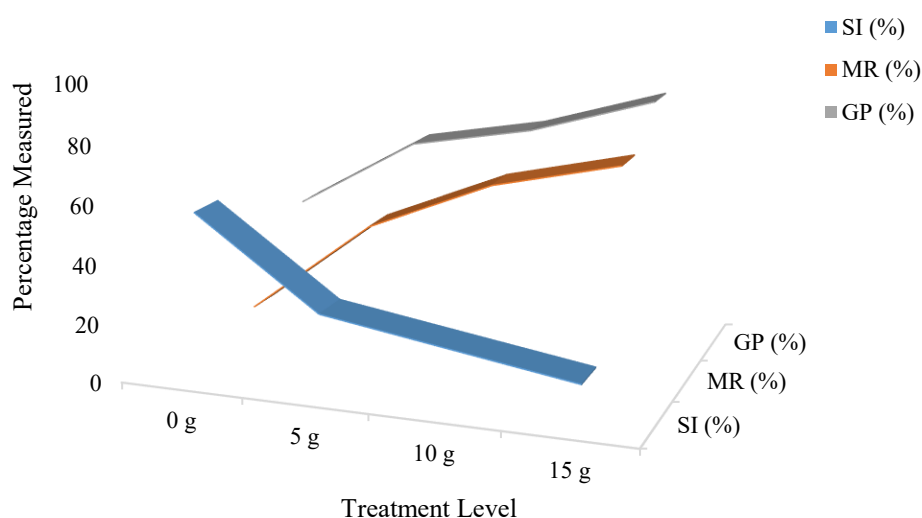


Fig. 1. Effect of Bitter Leaf Biochar on the Susceptibility Index, Mortality Rate, and Germination Percentage of Stored Maize to *Sitophilus zeamais*

The mortality rate of *Sitophilus zeamais* increased gradually as the concentration of BLB increased. The untreated control (0 g BLB) recorded the lowest mortality rate of 12%, while mortality increased to 45%, 63%, and 73% at 5, 10, and 15 g BLB, respectively. The result shows that application of BLB enhanced the mortality of *Sitophilus zeamais*, with the highest mortality recorded at the 15 g application rate.

The increase in the mortality rate due to the increase in the concentration of BLB may be associated with the insecticidal or insect-repellent properties of compounds originally present in bitter leaf and retained or transformed during Biochar production, as observed by Asawalam & Hassanali (2006). These researchers discovered that *Vernonia amygdalina* contains several biologically active phytochemicals and other secondary metabolites that are toxic to insects when inhaled. The porous structure of Biochar may also contribute to its physical interaction with insects and its ability to retain biologically active compounds on its surface. This finding also agrees with Musa et al. (2011), who reported that *Vernonia amygdalina* leaf extract caused substantial mortality of adult *Sitophilus zeamais*, with mortality reaching 88.9% at a 3% application rate after three days. Likewise Akinlosoye et al. (2025) in their study discovered that Rice Husk Biochar significantly affects the mortality rate of *Sitophilus zeamais*. Their study reported mortality rates of 74%, 79%, and 84% at 5, 10 and 15 g rice husk Biochar, respectively, compared with much lower mortality in the control. They concluded that mortality increased progressively with increasing Biochar concentration. The similarity between their findings and the present result is particularly important because both studies demonstrate the potential of Biochar as a plant-derived material for managing maize weevil during storage.

The germination percentage of the stored maize increased progressively with increasing rates of BLB when compared to the control with 0 g of BLB. The application of 5 g, 10 g and 15 g BLB resulted in germination percentages of 65%, 73% and 86%, respectively. The result therefore indicates that increasing the rate of BLB was associated with improved germination performance of the stored maize. The low germination percentage observed in the control may be attributed to the greater level of infestation and feeding damage caused by *Sitophilus zeamais* in the absence of BLB. Maize weevil infestation can damage the embryo and other vital portions of the grain, thereby reducing seed viability and subsequent germination. Contrarily, the higher germination percentages recorded in the grains treated with BLB may be associated with the protective effect of the Biochar, which reduced insect infestation and consequently minimized damage to the grains. In their observation, Akinlosoye et al. (2025) also observed that germination increased with increasing Biochar application and attributed this progress to the clampdown of *Sitophilus zeamais* attack on the stored maize.

Analysis of Variance (ANOVA)

The result was further subjected to one-way ANOVA to determine whether the different rates of the Bitter Leaf Biochar (BLB) really produce significant differences in the measured parameters. The ANOVA test shown in Table 2 revealed significant differences among the BLB treatments for all the parameters assessed.

Table 2: Analysis of variance of the effect of Bitter Leaf Biochar on susceptibility index, mortality rate, and germination percentage

Parameter	F-value	df	p-value
Susceptible Index (%)	43.889	3, 8	<0.001
Mortality Rate (%)	58.815	3, 8	<0.001
Germination Percentage (%)	19.161	3, 8	0.001

Susceptible Index differed significantly among treatments, $F(3, 8) = 43.889$, $p < 0.001$. Likewise, mortality rate showed a highly significant treatment effect, $F(3, 8) = 58.815$, $p < 0.001$, and, Germination percentage was also significantly influenced by BLB treatment, $F(3, 8) = 19.161$, $p = 0.001$. These results indicate that the application rate of BLB significantly affected the susceptibility index, mortality, and germination percentage.

The significant ANOVA results indicate that these observed differences were statistically significant; nevertheless, the specific treatment pairs responsible for the differences was established using a post-hoc test.

Tukey HSD Post Hoc Test

The result of the post hoc test are presented in Table 3. The superscript letters shows the significance grouping of the means of the treatment. Means bearing the same superscript letter are not significantly different, whereas means bearing different superscript letters differ significantly at the 5 % probability level ($p \leq 0.05$). Means with two superscripts, such as ^{ab} or ^{bc}, are statistically comparable with means carrying either of the corresponding letters.

Table 3: Tukey HSD Post Hoc Comparison of Mean Susceptible Index of Bitter Leaf Biochar Treatments

Bitter Leaf Biochar (g)	Mean Susceptible Index (%)	Mean Mortality Rate (%)	Mean Germination (%)
0	56.67 ^c	11.67 ^a	40.00 ^a
5	26.67 ^b	45.00 ^b	65.00 ^b
10	20.00 ^{ab}	63.33 ^c	73.33 ^{b,c}
15	13.33 ^a	73.33 ^c	86.67 ^c

Note: Means with different superscript letters differ significantly at $p < 0.05$, while means sharing the same letter are not significantly different.

For the Susceptibility Index, the control treatment (0 g BLB) recorded the highest mean value of 56.67% (^c), which was significantly higher than the values recorded at 5 g (26.67%^b), 10 g (20.00%^{ab}), and 15 g (13.33%^a) BLB. The 10 g treatment was statistically comparable with both the 5 g and 15 g treatments, as indicated by its combined superscripts (^{ab}). The progressive decrease in susceptibility index with increasing BLB application suggests that BLB reduced the susceptibility of maize to *S. zeamais* infestation.

There was a contrary trend for the Mortality Rate as it increased with increasing BLB application. The control recorded the lowest mortality (11.67%^a), which was significantly lower than the mortality recorded at 5 g (45.00%^b), 10 g (63.33%^c), and 15 g (73.33%^c) BLB. However, the 10 g and 15 g treatments were not significantly different from each other because they shared the same superscript (^c). This shows that although mortality increased numerically from 63.33% at 10 g to 73.33 % at 15 g, the difference was not statistically significant.

A related increasing trend was observed in Germination Percentage. The control recorded the lowest germination percentage (40.00%^a), which differed significantly from the 5 g (65.00%^b), 10 g (73.33%^{bc}), and 15 g (86.67%^c) treatments. The 5 g and 10 g treatments were not significantly different, as they shared the superscript b, while the 10 g and 15 g treatments were also statistically comparable because they shared the superscript c. However, germination at 15 g BLB was significantly higher than at 5 g BLB.

The Tukey HSD results demonstrate that increasing BLB application generally reduced the susceptibility of maize to *S. zeamais*, increased weevil mortality, and improved maize germination. It was discovered that BLB of 15 g gives the lowest SI and the highest values for MR and GP, making it the most effective.

CONCLUSION

It is seen from this research that Bitter Leaf Biochar (BLB) can be used as a biopesticide against *Sitophilus zeamais* in stored maize. The higher the concentration of BLB, the higher is its effectiveness on the Susceptibility Index (SI), the Mortality Rate (MR), and the Germination Percentage (GP) of the stored maize.

It is recommended that BLB be considered as an alternative to synthetic pesticides, being readily and naturally available. It is environmentally friendly and not toxic to human health when consumed. Further study is recommended to determine the maximum concentration of Bitter Leaf Biochar needed for optimal performance, and also the on-the-field experiment should be carried out with the farmers who have stored maize.

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