Susceptibility of Selected Legumes in Northern Nigeria to *Callosobruchus maculatus* (F.) Infestation

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ARTICLE HISTORY
Received Date: 20th April, 2025
Accepted Date: 8th August, 2025

http: www.njphr.nspri.gov.ng
ISSN: 2630-7022

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CONFLICT OF INTEREST: None
ETHICAL APPROVAL: Not Applicable

This is a publication of the Nigerian Stored Products Research Institute (NSPRI)

OPEN ACCESS

Abstract

This study assesses the susceptibility of 31 legume varieties to Callosobruchus maculatus, a significant pest of these globally important, protein-rich staple crops, which are crucial for food security in sub-Saharan Africa. Field surveys and market visits across Northern Nigeria yielded 31 cowpea varieties, which were subsequently collected and evaluated for their susceptibility to Callosobruchus maculatus infestation. Physical attributes, including seed length, width, thickness, weight, density, volume, sphericity, porosity, surface area, and moisture content, were measured. The samples were then artificially infested with C. maculatus to assess resistance. Data analysis, including hierarchical data analysis, dendrogram clustering, histograms, and correlation analysis, was employed to reveal patterns and groupings of cowpea varieties based on physical traits. Distinct groupings of cowpea varieties emerged from the study, with smaller seed dimensions forming a cluster that included pigeon pea, Azuki beans, Achi Shiru, Farin Akidi, and Bakin Akidi. Another cluster, representing medium to large-seeded varieties, comprised 573-1-1, Kidney Beans, Shamjir Brown, and Zebra Beans. Overall, Farin Akidi exhibited the highest susceptibility to C. maculatus, followed by Agwugwu and Zebra Beans. In contrast, Lima Black, Lima Silver, and Kaki beans demonstrated the most substantial resistance. The study found negative correlations between pest resistance and seed traits, such as thickness, diameter, surface area, and weight, indicating their role in resistance. These findings provide valuable insights for breeders to develop more resilient cowpea varieties, thereby enhancing storage, adaptability, and genetic diversity, which supports global food security.

Keywords: *Callosobruchus maculatus*, Cluster Analysis, Legume Resistance, Pest Susceptibility, Physical Seed Traits, Seed Morphology

Introduction

Cowpea (*Vigna unguiculata* L. Walp), a widely cultivated legume, plays a crucial role in food security, income generation, and agricultural sustainability across the semi-arid tropics of Africa, Asia, Southern Europe, and the Americas. Nigeria is the world's leading producer, contributing over 2 million metric tons annually, which accounts for approximately 58% of global production (Omoigui et al., 2020). Despite its significance, cowpea productivity faces major threats from *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae), commonly known as the cowpea weevil. This pest infests stored cowpea seeds, resulting in severe postharvest losses and economic damage. Understanding the susceptibility and resistance of different cowpea varieties to *C. maculatus* is essential for developing effective pest management strategies (Ayodeji et al., 2014; Kpoviessi et al., 2020; Omoigui et al., 2020; Uzoma et al., 2022).

This study aimed to assess the susceptibility of various cowpea varieties to *C. maculatus* infestation and analyse the relationship between seed physical traits and pest resistance. By identifying the most and least vulnerable varieties, the research provides critical insights for selecting and cultivating pest-resistant cowpea lines. Enhancing cowpea resistance through varietal selection can significantly improve storage efficiency, reduce

How to cite:
Anugwom, U. D., Joel, J., Awotunde, D. R., Abubakar, A., Adamson, T. O., Bello, I. A., Ahmad, S., Ibrahim, B., Dayo, O., & Olaleye, O. O. (2025). Susceptibility of Selected Legumes in Northern Nigeria to *Callosobruchus maculatus* (F.) Infestation. *Nigerian Journal of Post-Harvest Research*, 3(5), 70-78.

postharvest losses, and contribute to global food security (Mogbo et al., 2014; Abdulrasak & Abiola, 2017; Audu et al., 2019; Josephine et al., 2022).

Several seed physical traits, such as length, width, thickness, weight, bulk density, true density, geometric mean diameter, volume, sphericity, porosity, and surface area, have been reported to influence bruchid infestation (Kpoviessi et al., 2020). Understanding these characteristics is crucial for identifying resistance-associated traits and guiding breeding efforts toward more pest-resilient varieties (Shams et al., 2012; Mogbo et al., 2014; Dieter et al., 2017; Josephine et al., 2022). Previous studies have also highlighted the usefulness of statistical and clustering approaches in revealing relationships between seed traits and resistance levels, thereby providing a basis for classifying cowpea varieties according to their vulnerability (Mbah & Silas, 2007; Shams et al., 2012; Roman et al., 2021; Dante & Rovetti, 2023).

By situating cowpea resistance within this broader context, the present research contributes to breeding programs, improved agronomic practices, and sustainable pest management strategies that can reduce economic losses and strengthen agricultural resilience.

Materials and Methods

The research was conducted in the Entomology Laboratory of the Nigerian Stored Products Research Institute in Kano from June to December 2023.

Source and preparation of legumes used

Thirty-one (31) legumes namely; Pigeon pea, Azuki beans, *Achi Shiru, Farin Akidi*, *Bakin Akidi*, Kidney beans, *Shamjir Cream*, *Shamjir Brown*, Iron beans, Honey Black, Sampea-7, *Gutun Naran, Oloyin*, *Waken Tanga*, Honey Gold, 573-2-1, 573-1-1, 277-2, Zebra beans, 216, *Bwala Gurum*, *Ire-Ire*, *Agwugwu*, *Kaki Beans*, *Dan-Yinusa*, *Dimpled Brown Crowther*, *Dogon Naran*, *Black* and *White Capsule*, Butter beans, Lima Silver and Lima Black, were sourced from markets in 10 states in Northern Nigeria: Kano (Dawanau Grain Market, IITA), Kogi (Dekina market and Ukwo market, Ankpa L.G.A), Zamfara (Nasarawar Burkullu Market), Kaduna (Samaru market, Zaria), Bauchi (Bogoro Market), Jigawa (Shuwarin Market, Kiyawa L.G.A.), Adamawa (Michika market and Madagali market), Gombe (Kaltungo market), Taraba (Gembu market), and Plateau (Ampang West Market and Mangu Market). After sourcing, the cowpea samples were sorted to obtain wholesome seeds, cold-shocked according to variety, and organised into seed lots of 200 seeds per variety for subsequent evaluation.

Study insect

Adult beetles (*Callosobruchus maculatus* F.) used in this study were collected from naturally infested cowpea seeds stored in facilities across Kano State, Nigeria. To maintain a healthy and uniform population for experiments, the insects were reared in the Entomology Laboratory at a 12% moisture content, 27 ± 2 °C temperature, 65 ± 5% relative humidity, and a 12-hour light/12-hour dark cycle.

Determination of physical characteristics

The physical properties of legume seeds were assessed using various methods. Seed dimensions (length, width, and thickness) were measured using a digital vernier calliper, and mass was determined with a digital balance. The square mean diameter (Ds) was calculated, according to Heidarbeigi et al. (2009).

$$D_s = (LW + WT + TL)^{1/2}$$

The degree of sphericity was determined using standard equations according to Mohsenin (2007):

$$\emptyset = \frac{(LWT)^{1/3}}{L}$$

Where $\emptyset$ the sphericity index in %, L is is the length of the seed in mm, W is the width of the seed in mm, and T is the thickness of the seed in mm.

The surface area (Sa) in cm² of each cowpea seed was determined using the relationship given by Conskuner & Karababa (2007).

$$S_a = \pi(D_g)^2$$

Where S_a Is the Surface Area in cm^2 and D_g Is the geometric mean diameter in mm.

The volume (V) of the cowpea varieties was determined using equations adopted by Hauhouout-O'Hara et al. (2000)

$$V = \frac{\pi B^2 L^2}{6(2L-B)}$$

Where; $B = (WT)^{1/2}$; L is the length of the seeds; W is the width of the cowpea seeds; T is the thickness of the seeds in mm.

To determine the bulk density and true density of seeds, a sample size of 100 g was used. The bulk density of the seed was calculated by dividing the weight of each sample by its volume, measured using a graduated cylinder, as described by Ayman et al. (2010). True density and volume were determined by the toluene solution displacement method (Mohamed,

2005). Bulk density and true density were expressed as g/cc at given seed moisture conditions.
The porosity was determined as the percentage of the densities of the bulk seeds
Therefore, porosity (ε) was determined using Equation:

$$\varepsilon = 100 \text{ x } \left(1 - \frac{\text{Bulk Density}}{\text{True Density}}\right)$$

Moisture content analysis was performed using the AOAC 2019 method in a hot-air oven. Approximately 2.0 g of pulverised sample was weighed into a pre-weighed moisture dish and placed in the oven at 130 ± 2 °C for 1 hour. After cooling in a desiccator, the dried sample with the dish was re-weighed, and the moisture content was calculated using the equation.

Moisture content (%) = [(W1+W2) -W3]/W2 X 100%

Where W1 = weight of empty dish (g), W2 = weight of wet sample (g), W3 = weight of dish + dried sample.

Susceptibility test

Two hundred healthy seeds from each variety were selected and placed in nylon bags to form seed lots. These seeds were cold-shocked to remove any initial infestations and then stored in glass jars covered with muslin cloth. Adult *Callosobruchus maculatus* beetles were sourced from laboratory cultures and sexed under an electron microscope. The beetles were introduced to the seed lots at male-to-female ratios of 1:1, 2:2, and 3:3. The experiment was conducted with five replications using a Completely Randomised Block Design.
Following a 45-day infestation period, a 5-day cold shock treatment was applied to halt further insect activity. The seeds were subsequently inspected to determine the extent of damage by recording the number of damaged kernels and their weight.

Seed damage (%) was calculated using the formula by Idowu (2005):
Seed Damage (%) = Number of damaged seeds ÷ Total number of seeds × 100

Weight loss (%) was determined following Idowu (2005) & AOAC (2019) formula:

$$\text{Weight Loss (\%)} = \frac{(\text{U}\times\text{Nd})-(\text{D}\times\text{Nu})}{\text{U x (Nd x Nu)}} \text{ X } 100$$

Where: U = weight of undamaged seeds, D = weight of damaged seeds, Nd = number of damaged seeds, Nu = number of undamaged seeds.

Infestation data were analysed using hierarchical cluster analysis in SPSS and MS Excel to classify cowpea varieties based on resistance or susceptibility levels. Results were visualised using dendrograms, tables, and charts to illustrate varietal groupings and resistance patterns.

Results and Discussion

Physical characteristics test

The dendrogram revealed three main groups of bean varieties based on physical similarities. The first group, including *Agwugwu*, Lima Silver, Lima Black, and *Shamjir Cream*, aligns in terms of overall physical profiles despite individual differences. This grouping supports the observations by Shams et al. (2012), who found that physical and biochemical traits play a crucial role in resistance to *Callosobruchus maculatus*.
The dendrogram analysis provided significant insights into the variability of physical properties among different bean varieties, demonstrating patterns of similarity and variation based on their characteristics (Figure 1). *Shamjir Cream* exhibited the greatest variability among the varieties in its physical traits. This unique profile could have implications for its processing, culinary uses, or breeding potential, especially since it is distantly related to all other varieties in the study. This finding aligns with those of Mogbo et al. (2014), who emphasised that distinct physical traits, such as seed coat thickness, are crucial in determining pest resistance in cowpea varieties.
In contrast, Butter beans displayed less variability than *Shamjir Cream* but still showed noticeable divergence from other varieties. Its close clustering with varieties such as *Achi Shiru*, Kidney Beans, and Sampea-7 suggests a shared set of physical traits. This result resonates with the work of Kpoviessi et al. (2020), who found that physical characteristics, such as seed size and hardness, influence the level of resistance to *Callosobruchus maculatus*.
The relationships between these groups highlight the importance of comparing physical traits among cowpea varieties, as noted by Audu et al. (2019), who reported distinct variations in seed characteristics across different varieties. The close relationship between *Agwugwu*, Lima varieties, *Dan-Yinusa*, *Ire-Ire*, and Butter beans in this study suggests that these varieties could share similar resistance profiles, providing useful information for future breeding and selection programs. The clustering supports the work of Coskuner & Karababa (2007), who suggested that seed properties such as size, density, and surface area significantly impact pest resistance. These findings

align with the broader understanding that seed characteristics, such as physical properties and structural features, play a crucial role in the natural resistance of cowpea varieties to pest infestations, as observed in previous studies (Josephine et al., 2022). Future breeding strategies can leverage these insights to enhance resistance in cowpea varieties, ensuring better storage, adaptability, and sustainability in pest management.

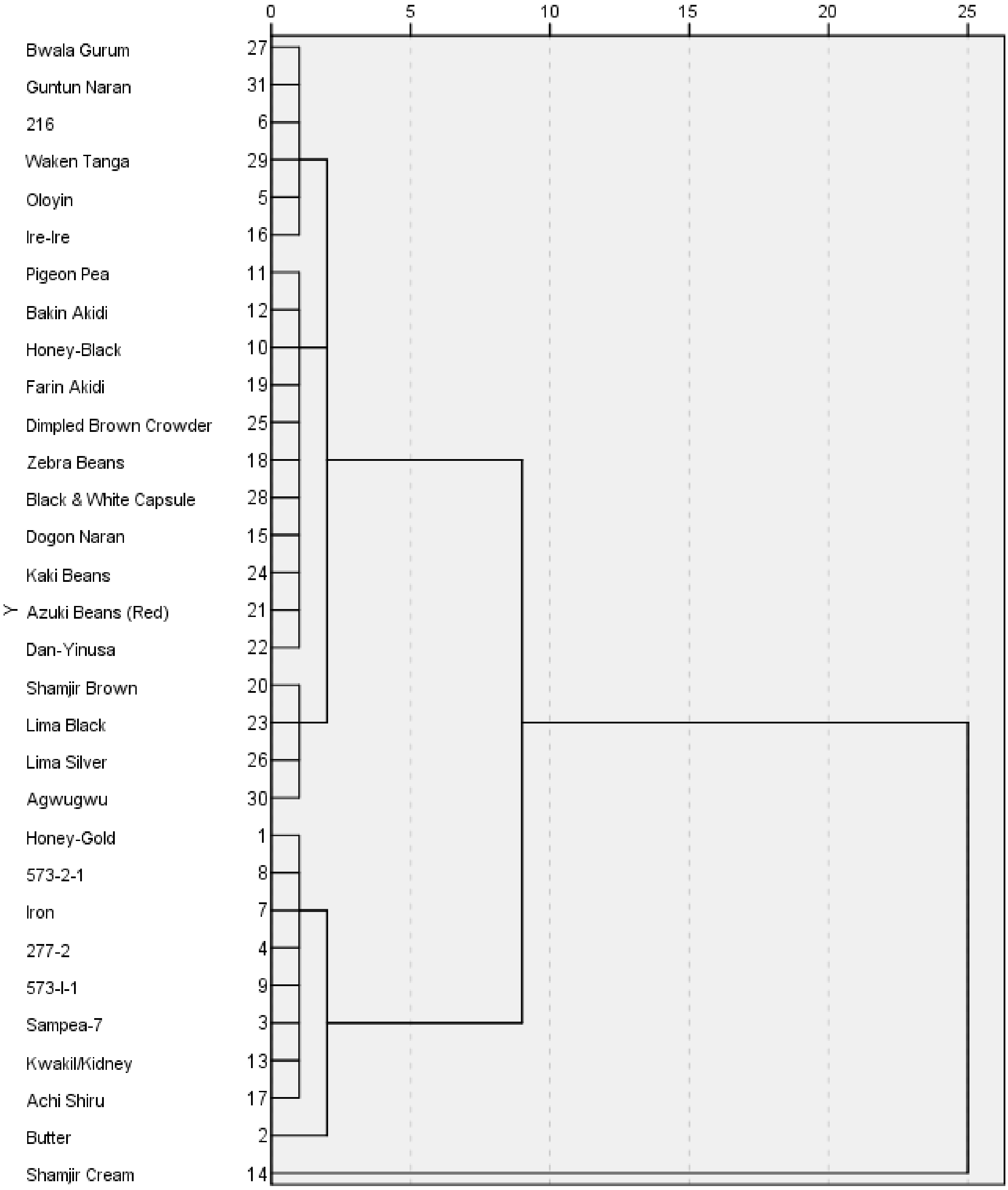

Figure 1: Dendrogram for Physical Properties of 31 Varieties of Cowpea

Percentage Seed Damage

The percentage seed damage for 31 cowpea varieties reveals significant variation in their susceptibility to *Callosobruchus maculatus* infestation, a crucial factor in postharvest management (Figure 2). *Farin Akidi* emerged as the most vulnerable variety, followed by *Agwugwu and Zebra Beans*, all of which showed the highest levels of seed damage. Other moderately susceptible varieties, including *Butter Beans, Achi Shiru, and Bakin Akidi*, exhibited similar trends in damage, suggesting lower resistance levels and may require additional pest control measures during storage. In contrast, several varieties, including *Gutun Naran*, Pigeon Pea, *Iron Beans*, and Sampea-7, also showed moderate susceptibility, albeit to a lesser extent, with their seed damage percentages lower than those of the most susceptible group. These varieties may still benefit from pest management strategies, but are more resilient than others. The most promising varieties in terms of pest resistance were *Dan-Yinusa*, *Dogon Naran*, *Dimpled Brown Crowder*, *Bwala Gurun*, *Ire-Ire*, *Kidney Beans*, *Shamjir Cream*, *Shamjir Brown*, Lima Black, Lima Silver, and *Kaki Beans*. These varieties, exhibiting the least susceptibility, are prime candidates for breeding programs aimed at developing pest-resistant cowpeas for sustainable production and reduced postharvest losses, as demonstrated by Mogbo et al. (2014), who identified key traits linked to resistance against *Callosobruchus maculatus* infestations. Their study highlights the potential of selecting such resistant varieties to enhance legume production and minimise postharvest losses.

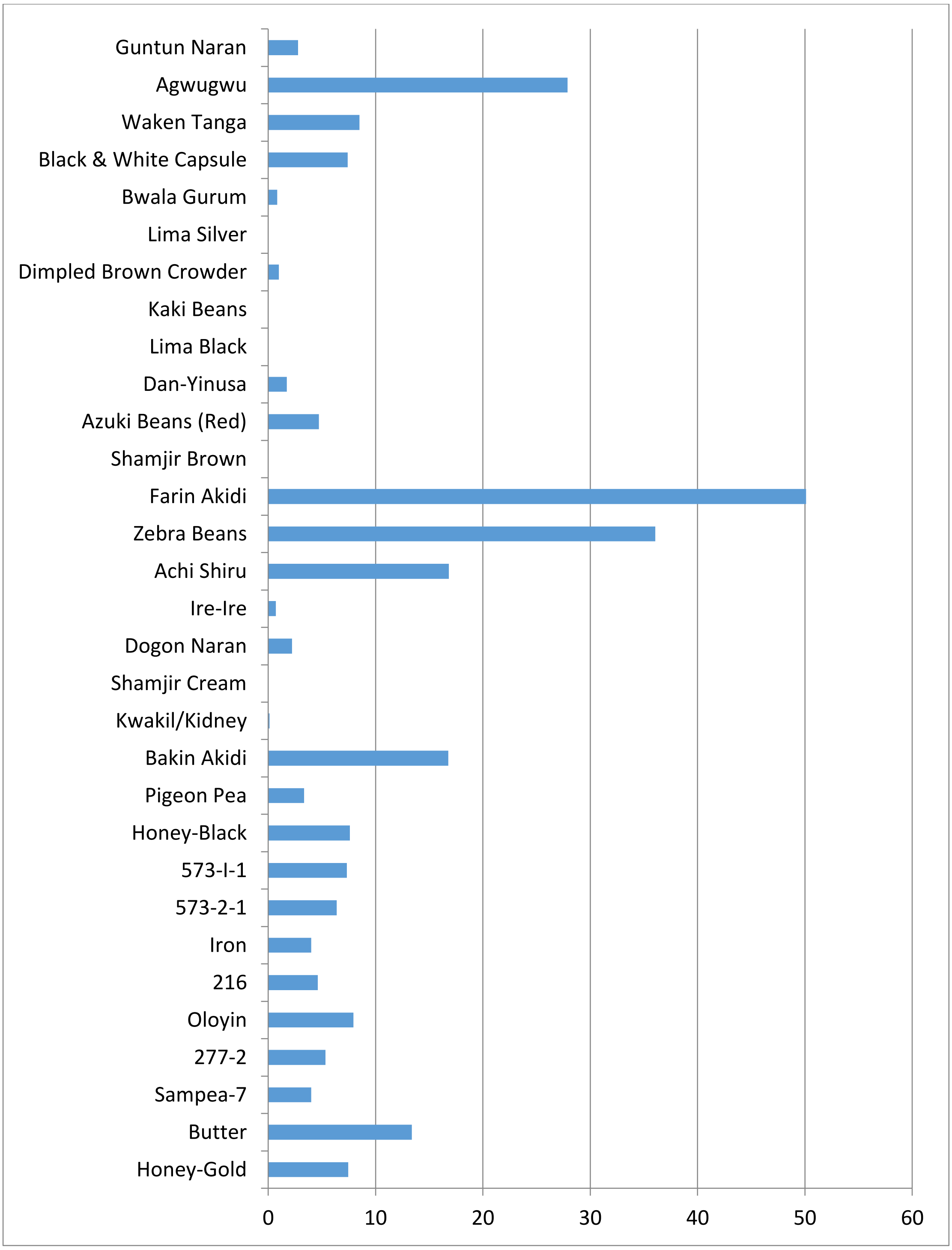

Figure 2: Percentage Seed Damage for 31 Varieties of Cowpea

Correlation between seed damage and physical characteristics

Table 1: Overview of Correlation between Seed Damage and Physical Parameters

Variable	Correlation with Seed Damage
Seed Damage	1
L (Length in mm)	-0.318
W (Width in mm)	-0.340
T (Thickness in mm)	-0.379 *
Ds (Diameter in mm)	-0.383 *
Sa (Surface Area in cm^2)	-0.361 *
V (Volume in mm^3)	-0.349
Bd (Bulk Density in kg/m^3)	-0.223
Td (True Density in kg/m^3)	-0.235
Ø (Porosity in %)	-0.026
ε (Moisture Content in %)	-0.306
Ws (Weight in g)	-0.359 *

*Correlation is significant at the 0.05 level.

The table provides an overview of the correlation matrix, and it provides a valuable snapshot of the relationships between susceptibility and various measured physical characteristics (Table 1). Thickness (T), Diameter (Ds), Surface Area (Sa), and Weight (Ws) all show significant negative correlations with susceptibility. Variables such as length, width, volume, bulk density, true density, moisture content, and porosity exhibit weak or no significant correlations.

The results reveal several correlations between seed characteristics and susceptibility to bruchid attack, with varying degrees of significance. A moderate negative correlation of -0.318 suggests that as seed length increases, susceptibility tends to decrease, although this correlation is not statistically significant. Similarly, a moderate negative correlation of -0.340 suggests that increased seed width may be associated with lower susceptibility; however, this relationship is not statistically significant. However, a significant negative correlation of -0.379* indicates that increased seed thickness is likely linked to decreased susceptibility to bruchid attack.

This finding contrasts with Gemechu et al. (2012), who reported that improvements in seed size inadvertently led to higher infestation levels and seed damage in chickpeas, highlighting the complex relationship between seed traits and pest resistance. On the other hand, the results align with those of Guiyun et al. (2008) and Prakit et al. (2008), which suggest that smaller seed sizes or specific morphological traits may confer higher resistance to insect attacks, emphasizing the nuanced role of seed characteristics in pest resistance strategies.

Seed diameter (Ds) shows a significant negative correlation of -0.383*, indicating that larger seed diameters are associated with lower susceptibility to *C. maculatus*. Similarly, a correlation of -0.361* suggests that larger seed surface areas may also correspond with reduced susceptibility, indicating a statistically significant relationship. These findings are supported by the work of Guiyun et al. (2008), who identified correlations between plant, pod, and seed traits and resistance to pests such as *Leguminivora glycinivorella*. Additionally, Prakit et al. (2008) mapped quantitative trait loci for bruchid resistance in *Vigna nepalensis*, highlighting the role of seed traits in pest resistance. These studies highlight the significance of seed morphology as a crucial factor in developing pest-resistant legume varieties.

The correlation of -0.349 between seed volume and susceptibility indicates a moderately negative correlation. This means that, generally, as seed volume increases, its susceptibility to *Callosobruchus maculatus* infestation tends to decrease. In other words, larger seeds might be somewhat less vulnerable to infestation (Hagstrum & Subramanyam, 2017). However, this correlation is not statistically significant, meaning the relationship could be due to chance, and further research would be needed to confirm this trend.

A correlation of -0.223 between seed bulk density and susceptibility indicates a weak negative relationship. This means that seeds with higher bulk density might show a slight reduction in susceptibility to insect attack. However, the relationship is weak and not statistically significant**,** implying that it is not strong enough to draw a definitive conclusion. The correlation is likely too small to be of practical importance without further validation. This finding aligns with Christos & Constantin (2020), who reported that grain bulk density was not well correlated with insect numbers.

The correlation of -0.235 between true density and susceptibility shows a minor negative association. This suggests that seeds with higher true density may exhibit slightly lower susceptibility to *C. maculatus*. However, similar to the previous correlations, this relationship is not statistically significant, indicating that any effect of true density on susceptibility is minimal and could be coincidental rather than meaningful. Christos & Constantin (2020) similarly observed that grain properties, including true density, can influence insect behaviour. Still, the impact on pest susceptibility may not always be direct or significant, highlighting the complexity of pest interactions with different seed characteristics.

The correlation of -0.026 between porosity (Ø) and susceptibility suggests that there is almost no relationship between the two. In other words, the amount of porosity (the tiny holes or spaces between seeds) has very little or no effect on how likely the seeds are to be attacked by *C. maculatus*. Since the correlation value is so close to zero, it means that porosity doesn't seem to influence susceptibility in any meaningful way.

The moderate negative correlation of -0.306 between moisture content (ε) and susceptibility suggests that as the moisture content in the seeds increases, their susceptibility to *C. maculatus* tends to decrease. This finding aligns with the work of Aynadis et al. (2020), who reported that moisture content plays a significant role in insect infestation, with lower moisture levels in grains being associated with higher susceptibility to pests. Although the correlation in this study is moderate, it highlights the importance of moisture management as a factor influencing pest resistance and the overall quality of stored seeds.

The correlation of -0.359 for seed weight (Ws) reveals a moderate negative relationship between seed weight and susceptibility to bruchid attack, indicating that as seed weight increases, the likelihood of *Callosobruchus maculatus* infestation decreases. This statistically significant correlation suggests that seed weight is a meaningful factor in selecting cowpea varieties for bruchid resistance. Heavier seeds may naturally offer better protection against pest attacks, providing valuable information for breeders aiming to develop cowpea varieties less prone to bruchid infestation. This finding aligns with the research of Abdulrasak & Abiola (2017), which assessed the susceptibility of various cowpea varieties to *C. maculatus* and found that certain physical seed characteristics, including weight, influenced the degree of infestation. Their study emphasises the importance of considering seed weight in breeding programs focused on enhancing pest resistance in legumes.

Conclusion

This study evaluated the susceptibility of 31 legume varieties from Northern Nigeria to *Callosobruchus maculatus* infestation by assessing their physical characteristics and the percentage of seed damage after infestation. Results from cluster analysis revealed distinct groupings of varieties based on physical traits, such as seed size, thickness, and width, which were found to influence resistance levels significantly. Varieties, including *Farin Akidi*, *Agwugwu*, and Zebra Beans, were the most susceptible, exhibiting the highest seed damage. At the same time, *Dan-Yinusa*, *Dogon Naran*, Dimpled Brown Crowther, *Bwala Gurun*, *Ire-Ire*, Kidney Beans, *Shamjir* Cream, *Shamjir Brown*, Lima Black, Lima Silver, and *Kaki* Beans demonstrated strong resistance and are promising candidates for breeding programs. Correlation analysis confirmed that seed thickness, diameter, surface area, and width had significant negative relationships with susceptibility, whereas bulk density, true density, and porosity showed weak or no association. The findings highlight the crucial role of physical seed traits in mediating resistance to *C. maculatus*, offering valuable insights for breeding and storage management strategies aimed at minimising postharvest losses. Promoting resistant varieties in farmer adoption programs could enhance food security and sustainability in cowpea production across Northern Nigeria.

Ethical statement

All research activities were conducted in strict compliance with ethical standards and regulatory requirements. Cowpea varieties were procured through authorised and legal channels, and experimental protocols were designed to minimise environmental impact while safeguarding local biodiversity. The study was undertaken in collaboration with local farmers and agricultural experts, ensuring appropriate integration of indigenous knowledge. Insect cultures were maintained under controlled laboratory conditions to prevent unintended release, and all data were reported with integrity, free from fabrication, falsification, or inappropriate manipulation.

Funding statement
This research was funded by the Nigerian Stored Products Research Institute, which covered the materials and operational costs essential to the study.

Acknowledgement
We gratefully acknowledge the management of NSPRI for endorsing this research under the 2023 Institute-Funded Project.

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