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EVALUATING THE EFFECT OF COWPEA VARIETY ON THE INSECTICIDAL RESPONSE AND ANTIOXIDANT ENZYMES OF *CALLOSOBRUCHUS MACULATUS* (FABRICIUS) TREATED WITH TWO PLANT POWDERS

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ABSTRACT

Callosobruchus maculatus (Fabricius), a notorious pest wreaking havoc during the storage phase of cowpea, has remained a serious menace militating against cowpea production in sub-Saharan Africa for years. This study investigated the effects of cowpea varieties [Gombe (GBV) and Sokoto (SKV)] on the insecticidal response and antioxidant enzymes of adult *C. maculatus* treated with powders of *Piper guineense* (Schum and Thonn) and *Syzygium aromaticum* (L.). The study was conducted at an ambient temperature (28 ± 3ºC) and relative humidity (75 ± 6). Bruchids were exposed to dosages ranging from 0.05 to 0.55 g/20 g cowpea. The morphometrics of the cowpea seeds and emerged bruchids were also subjectively determined. GBV generally had significantly longer, wider, and thicker seeds than SKV. Similarly, GBV-reared bruchids were significantly ($P < 0.001$) wider and longer in body size than SKV-reared bruchids. Regardless of cowpea variety, *S. aromaticum* was generally more toxic to the bruchids than *P. guineense*. GBV-reared bruchids were more susceptible to both plant powders than SKV-reared bruchids. The specific activities of Superoxide Dismutase, Catalase, and Glutathione also varied with cowpea varieties and plant powders. Cowpea variety, experimental dosage, and their interaction (V*D) significantly ($P < 0.001$) influenced the specific activities of the three enzymes. Hence, the two plant powders, especially *S. aromaticum*, could be used as effective protectants of cowpea seeds against *C. maculatus* infestation. The findings in this study could ultimately contribute to ensuring the security and integrity of cowpea seeds, sustaining them as a staple food crop.

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INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp), commonly known as black-eyed pea, is an essential leguminous crop with global significance due to its nutritional value and adaptability to diverse agro-ecological zones (Tan et al., 2012; Urgesa, 2023). In many developing countries, it is regarded as vital source of dietary protein, essential amino acids, vitamins, and micronutrients for millions of people (Oyeniyi et al., 2015a). In fact, the amino acid and vitamin profiles of cowpea grains have make it a good compliment to low-protein staple cereals and tuber crops in many countries (Tengey et al., 2023). Consequently, the crop has helped in achieving food security among the poor people in sub-Saharan Africa (Horn et al., 2022). The production of cowpea has increased over the past few decades, with over 72% of the world's production coming from West and Central Africa (Oladipo *et al.*, 2019). Conversely, post-harvest losses of food crops, including cowpea seeds, in sub-Saharan Africa have been rising recently, with 2021 recording the highest loss of about 20% (FAO, 2023).

Most of the post-harvest losses associated with stored cowpea seeds have been linked to the presence of *Callosobruchus maculatus* (Fabricius), a formidable bruchid beetle known for causing significant damage to stored legumes (Idoko and Adesina, 2013; Oyeniyi et al., 2015b). The bruchid is a multivoltine beetle, and its life cycle involves egg-laying on the surface of cowpea grains, leading to larval infestation and subsequent damage. In fact, within a few months of infestation, stored cowpea seeds may be completely destroyed if the infestation is allowed to continue unchecked (Hossain *et al*., 2014). In many developing nations where cowpea seeds are a cheap source of essential nutrients for survival, their total destruction could have a detrimental effect on food security. The economic losses mostly incurred due to reduced grain quality have prompted a search for sustainable methods to mitigate the impact of this pest.

Various methods have been explored to minimize the infestation of this vital crop by *C. maculatus*. Among the well-known tactics are the use of chemical pesticides, mechanical, cultural, and biological control, among others (Ofuya, 2001). Synthetic pesticides have proven to be the most successful in controlling the insect pest; however, they have a number of unfavorable side effects that affect the environment, humans, and non-target organisms (Rajput *et al*., 2023). In light of these concerns, there has been a growing interest in exploring ecologically benign and economically viable methods for managing *C. maculatus* infestations in stored cowpea seeds. A viable way forward for tackling the complex challenge is to employ botanicals with insecticide properties and introduce cowpea varieties that are moderately resistant to bruchid attack (Gbaye and Holloway, 2011; Oyeniyi et al., 2015a).

Recent research has highlighted the existence of varietal differences in cowpea regarding resistance or susceptibility to *C. maculatus* (Castro et al., 2013; Tengey et al., 2023). The variations are ascribed to changes in the morphological traits of the seeds as well as genetic and metabolic components within the plants (Gbaye and Holloway, 2011; Tripathi et al., 2012; Miesho et al., 2018). Consequently, most available cowpea varieties are known to possess a low to high degree of susceptibility to bruchid damage, with various effects observed on their fertility, eclosion and fecundity behaviour, developmental period, progeny survival, and weight of emerged adults (Fatokun et al., 2012; Ahetor and Coulibaly, 2017; Tamò and Kergoat, 2017). However, none of the introduced cowpea varieties have complete resistant to *C. maculatus* (except perhaps TVu2027) (Ofuya, 2001). Similarly, the application of edible plant materials has been a subject of substantial research attention as a protective mechanism for safeguarding cowpea seeds from *C. maculatus* attack (Njoku et al., 2019). Powders of *Piper guineense* (Schum and Thonn) fruit and *Syzygium aromaticum* (L.) flower buds are two plant materials with known insecticidal properties (Idoko and Adesina, 2013; Fajinmi et al., 2015; Oyeniyi et al., 2015a, b). Both powders present an intriguing avenue for enhancing the resilience of cowpea varieties against *C. maculatus*. Recent research has hinted at the involvement of antioxidant enzymes in the defense mechanisms of *C. maculatus* infesting cowpea seeds (Kolawole et al., 2014). Antioxidant enzymes, such as superoxide dismutase (SOD) and catalase, play pivotal roles in countering oxidative stress induced by insect feeding (Oni et al., 2019). Exploring the interplay between cowpea varieties protected with two plant powders and the activation of antioxidant enzymes in *C. maculatus* in response to toxins in cowpea varieties and plant powders is a novel avenue that promises to deepen our understanding of plant-insect interactions.

Presently, information on the possible effect of two commonly consumed cowpea varieties (i.e., Sokoto and Gombe) in Nigeria on the insecticidal response of *C. maculatus* to powders of *P. guineense* fruit and *S. aromaticum* flower buds is scanty. Similarly, there is a paucity of knowledge on the effect of both cowpea varieties on the antioxidant enzymes of *C. maculatus* exposed to powders of *P. guineense* and *S. aromaticum*. In this study, we hypothesize that cowpea variety, plant powders and their interaction could influence the insecticidal response and antioxidant enzymes of *Callosobruchus maculatus* under laboratory condition. Consequently, this study seek to investigate the main and interactive effects of cowpea varieties and plant powders (i.e. *P. guineense*, and *S. aromaticum*) on the insecticidal response and antioxidant enzyme activities of *C. maculatus*. This multidimensional approach will provide a holistic understanding of how different intrinsic factors interact in bruchids micro-environment, with potential implications for the development of integrated pest management strategies.

MATERIALS AND METHODS

The experimental location

The experiments were conducted from September 2023 to January 2024 at the Research Laboratories of the Biology and Biochemistry Departments, Federal University of Technology Akure (FUTA), Nigeria.

Source of the substrate

Two clean and non-infected cowpea varieties, Gombe (designated: GBV) and Sokoto (designated: SKV), used in this study, were obtained from Oba market, Akure, Nigeria. They were disinfested in the freezer at -4°C for four weeks and thereafter allowed to equilibrate for three days in the laboratory at ambient temperature (28 ± 3ºC) and relative humidity (75 ± 6%), prior to use, to prevent mould formation.

Collection and preparation of plant material

Dry flower buds of *S. aromaticum* (cloves) and dry seeds of *P. guineense* (West African black pepper) were bought from a traditional herbal stall at Oba market, Akure, Ondo State, Nigeria. The plant materials were pulverized in the laboratory using a Mascot Mixer Grinder (AN ISO 9001:2000; Titan Scales, Thane, Maharashtra, India). The powder obtained was sieved with a 180 µm mesh before being stored in plastic containers with airtight lids. This procedure was carried out separately to avoid plant materials contaminating each other.

Insect culture

The cowpea bruchids used in the present study were obtained from the existing stock culture of *C. maculatus* in the Research Laboratory, Department of Biology, FUTA. To eliminate maternally inherited dietary effects, the adult *C. maculatus* was reared separately on previously disinfested GBV and SKV for two generations using 1.65-liter plastic containers (Gbaye *et al.*, 2012). About 60–80 bruchids were introduced into 100 g of each variety of cowpea in each container. The containers were covered with a perforated cover sealed with muslin cloth to prevent the escape of the insect and allow air into the container. The insects were maintained on each cowpea variety without exposure to either synthetic insecticides or plant materials at an ambient temperature (28 ± 3ºC) and relative humidity (75 ± 6%).

Experimental procedure

Determination of morphometrics of cowpea seeds and bruchids that emerged from them

Twenty cowpea seeds from each variety were randomly selected for measurement of length, width, and thickness using a digital vernier caliper (RQHS NORM 2002/95/EC). Similarly, twenty adult *C. maculatus* (10 pairs) were randomly selected from GBV and SKV-reared bruchids. The lengths and widths of 20 bruchids/cowpea variety were thereafter determined using the digital vernier caliper. The male and female bruhcids were distinguished using the various differences described by Ofuya (2001). The seed and bruchid morphometrics were measured in millimeters (mm).

Exposure procedure for *C. maculatus* reared on GBV and SKV to two plant powders

Twenty grams of GBV and SKV seeds were weighed into separate pre-labeled 170-ml plastic containers (8.7 cm in diameter) using a Metler beam PB 3002 weighing scale. The seeds were then thoroughly mixed with 0.05, 0.15, 0.25, 0.35, 0.45, or 0.55 g of *P. guineense* powder. The control (0.00 g) was also set up. In each treatment container, twenty adult *C. maculatus* (1-3 days old) were added and covered. Blocked randomization technique was employed in assigning the bruchids from each variety to treatment blocks. Every treatment was replicated four times in a randomized complete block design. Bruchid responses to this plant material were assessed at 24, 48, 72, and 96 h post-treatment, using dead insects as indicators. Bruchids were confirmed dead when they elicited no response to a tiny needle gently poked into their abdomen. The above procedure was repeated for *S. aromaticum* using similar doses.

In vivo biochemical assay

In vivo bioassay and preparation of enzyme extract

The pre-determined LD_{50} values of the powders of *P. guineense* and *S. aromaticum* were used for the biochemical assays. Pre-labelled plastic containers (170 ml) were dosed with pre-determined LD_{50} at 48 h of exposure to *P. guineense* (GBV - 0.530 and SKV - 0.489 g/20 g cowpea) and *S. aromaticum* (GBV- 0.075 and SKV- 0.087 g /20 g cowpea) following the earlier method explained under the section "Exposure procedure for *C. maculatus* reared on GBV and SKV to two plant powders". The control (0.00 g) was also set up. A total of 40 unsexed adult *C. maculatus* were introduced into each container. The containers were then kept in the incubator and observed for 48 h. Each treatment was replicated four times in a randomized complete block design, and the treatment was done for both varieties. About 20–25 surviving adults of *C. maculatus* weighing about 52–55 mg after exposure were quickly kept at -4°C for 5 h and allowed to freeze to death. Each replicate was then separately homogenized for 3 min with ice-cold buffer [25 mM potassium phosphate buffer (pH 7.2) EDTA and 1 mM 2-mercaptoethanol] using a hand-held glass homogenizer previously kept in an ice box (Nathan, 2008). Each homogenate from each treatment was made up to 1.5 ml in ice-cold buffer before being quickly centrifuged at 13,000 rpm for 10 min at 4°C (Oni *et al*., 2019). The resulting supernatants/cowpea variety/plant material were carefully stored in aliquots at a temperature below -4°C and served as an enzyme source until needed. All the supernatants were used within 3 days.

Protein determination

The protein concentration of the insect's homogenate was determined using the method of Bradford (1976), and bovine serum albumin was used as the standard. Enzyme activities were expressed in terms of μmol/min (U) and presented as specific activities (μmol/min/mg protein, i.e., U/mg protein).

Superoxide dismutase (SOD) activity

Superoxide dismutase activity was determined according to the method of Beauchamp and Fridovich (1971), modified for *C. maculatus* by Oni *et al*. (2019). A 250 μL of tissue homogenate was treated with 1500 μL of SOD reagent (1.17 mM ribofavin, 0.1 M methionine, 20 mM potassium thiocyanide, 56 mM nitro blue tetrazolium). The mixture was incubated for 60 min at room temperature. Blank was also prepared simultaneously with distilled water replacing the insect's homogenate and incubated for the same period of time in a dark cupboard. The absorbance was read at 560 nm using the UV/visible spectrophotometer. The SOD activity was expressed as units (U) per mg of protein. One unit is defined as the amount of change in the absorbance by 0.1 h^{-1} mg^{-1} protein. Protein concentrations were measured as described by Bradford (1976), using BSA as a standard. SOD activity was calculated with the equation below:

$$SOD = \frac{R_4}{A}$$

$$A = R_1(\frac{50}{100})$$

$$R_4 = R_3 - R_2$$

$$R_3 = OD\ of sample$$

$$R_2 = OD\ of\ blank$$

$$R_1 = OD\ of\ reference$$

Where R_1 is the absorbance of the reference solution, R_2 is the absorbance of the blank, and R_3 is the absorbance of sample when enzyme has been added at a particular level.

Catalase (CAT) activity

The activity of catalase (EC. 1.11.1.6) was determined according to the method of Aebi (1984). To 1.8 ml of potassium phosphate buffer (100 mM, pH 7.0) was added 140 μL of bruchids homogenate, and the enzyme reaction started with the addition of 20 μL of H_2O_2 solution (38 mM). The decrease in absorbance was measured at 240 nm over a 3-min period at 25°C against the blank using the UV/visible spectrophotometer. The enzyme blank was run simultaneously, and distilled water was added to replace the insect homogenate used. Two readings were taken at 0 and 3 min. The enzyme activity was expressed as units (U) per mg of protein. CAT activity was calculated with the equation below:

$$CAT = \frac{R_1 - R_2}{T}$$

Where R_1 is the initial reading at 0 min, R_2 is the final reading after 3 min, and the T is the time intervals.

Level of reduced glutathione (GSH)

The level of reduced glutathione was estimated by the method of Ellman (1961). A mixture containing 200 μL of insect's homogenate, 200 μL of distilled water, 200 μL of 5, 5′-dithiobis nitro benzoic acid (DTNB), 1.38 mL of nicotinamide adenine dinucleotide phosphate hydrogen (NADPH), and 20 μL of glutathione reductase (GR) were incubated for 15 min at room temperature. The intensity of the yellow color formed was measured at 420 nm using the UV/visible spectrophotometer along with a blank containing 1.0 mL of distilled water. The amount of GSH was expressed as nmol GSH/mg protein.

Statistical analysis

All data on dosage-mortality bioassay were converted to percentage mortality and thereafter subjected to a one-way analysis of variance (ANOVA). Data on biochemical assays were also subjected to a one-way ANOVA. A single-blind technique was used for all the treatments on biochemical assays. Block randomization and single-blind techniques were employed in order to minimize bias. Tukey's test was used to separate means at α = 0.05. A student T test was used to compare the morphometrics of cowpea varieties and the bruchids that emerged from them. Regression analysis was done by subjecting adult mortality data and doses of both plant powders to probit and log transformation, respectively, to determine the dose of each plant powder lethal to 50% of *C. maculatus* that emerged from each variety (Finney, 1971). General Linear Model (GLM) was used to investigate main and interactive effects of cowpea variety, experimental doses and exposure time on the response of *C. maculatus* to *S. aromaticum* and *P. guineense*. Also, pairwise comparisons (Tukey's Test) were used to investigate the degree of difference between both cowpea varieties. All analyses were done using the Statistical Package for Social Sciences (SPSS) version 22 software package at α = 0.05.

RESULTS

Morphometrics of the two cowpea varieties and *C. maculatus*

The morphometrics of the two cowpea varieties and the bruhcids that emerged from each variety are presented in Table 1 and Figure 1, respectively. The Gombe variety (GBV) seeds were significantly ($P < 0.001$) wider, longer, and thicker than the Sokoto variety (SKV). Similarly, bruchids that emerged from GBV had significantly ($P < 0.05$) longer and wider bodies than their counterparts that emerged from SKV.

Table 1: Morphometrics of the two cowpea varieties.

Seed morphometrics (mm)	Variety	*N*	Mean ± S.E	*Sig.*	Remark
Width	SKV	20	4.63 ± 0.06	< 0.001	Significant
	GBV	20	6.17 ± 0.12		
Length	SKV	20	8.44 ± 0.17	< 0.001	Significant
	GBV	20	11.14 ± 0.15		
Thickness	SKV	20	6.42 ± 0.07	< 0.001	Significant
	GBV	20	7.96 ± 0.12		

Key: GBV: Gombe variety; SKV: Sokoto variety.

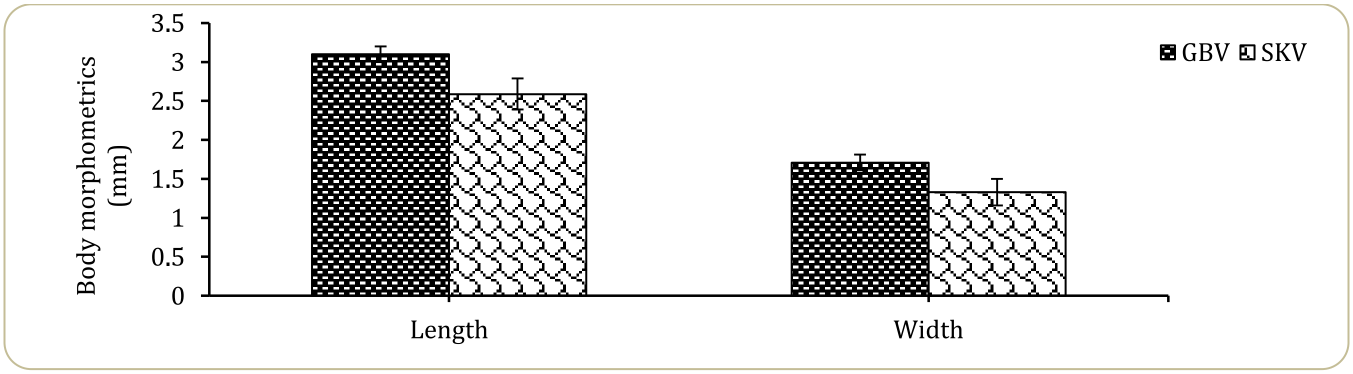

Figure 1. Body morphometrics (mean± SE) of *C. maculatus* that emerged from the two cowpea varieties.

Main and interactive effects of cowpea variety, experimental dosage and exposure time

The effects of cowpea variety (V), experimental dosage (D) and exposure time (T) on the insecticide toxicity of both plant powders were generally significant ($P < 0.001$ in all cases). Irrespective of cowpea variety, complete mortality (100%) was observed only in bruchids exposed to *S. aromaticum* (Figure 2), while *P. guineense* did not evoke up to 100% (Figure 3). The two-way interactions of cowpea variety with dose (V*D) and cowpea variety with exposure time (V*T) significantly ($P < 0.001$ in all cases) influenced the response of bruchids to *S. aromaticum* (V*D: $F_{5,\ 144} = 36.21$; V*T: $F_{3,\ 144} = 12.12$) (Figure 2) and *P. guineense* (V*D: $F_{5,\ 144} = 8.30$; V*T: $F_{3,\ 144} = 136.82$) (Figure 3). Similarly, the two-way interactions of dose with exposure time (D*T) significantly ($P < 0.001$ in all cases) influenced the toxicity of *S. aromaticum* ($F_{15,\ 144} = 68.50$) and *P. guineense* ($F_{15,\ 144} = 3.38$) to cowpea bruchids. Also, the three-way interaction of cowpea variety with dose and exposure time (V*D*T) significantly affected the mortality of bruchids exposed to *S. aromaticum* ($F_{15,\ 144} = 2.54$; $P = 0.002$) and *P. guineense* ($F_{15,\ 144} = 7.16$; $P < 0.001$).

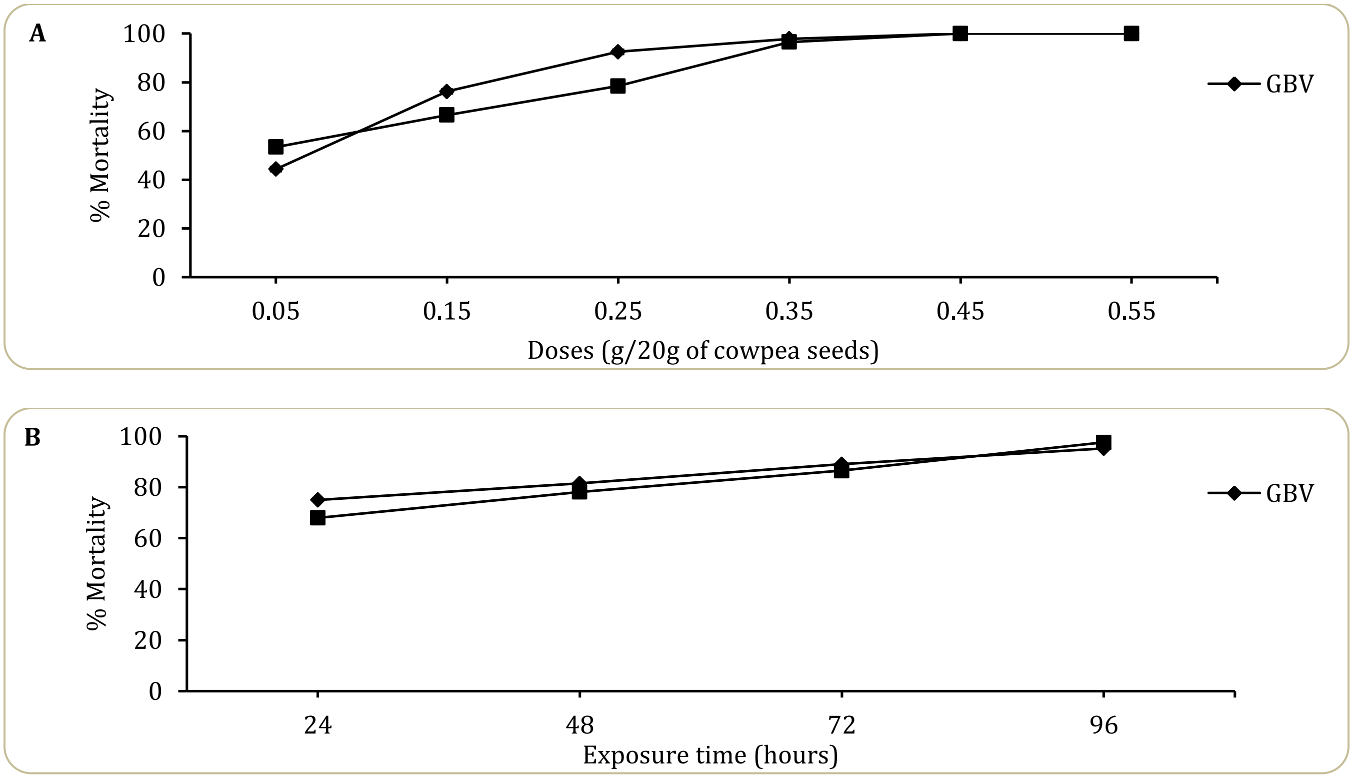

Figure 2. Interactive effects of (A) cowpea variety-by-dose and (B) cowpea variety-by-exposure time on the response of *C. maculatus* to *S. aromaticum* powder.

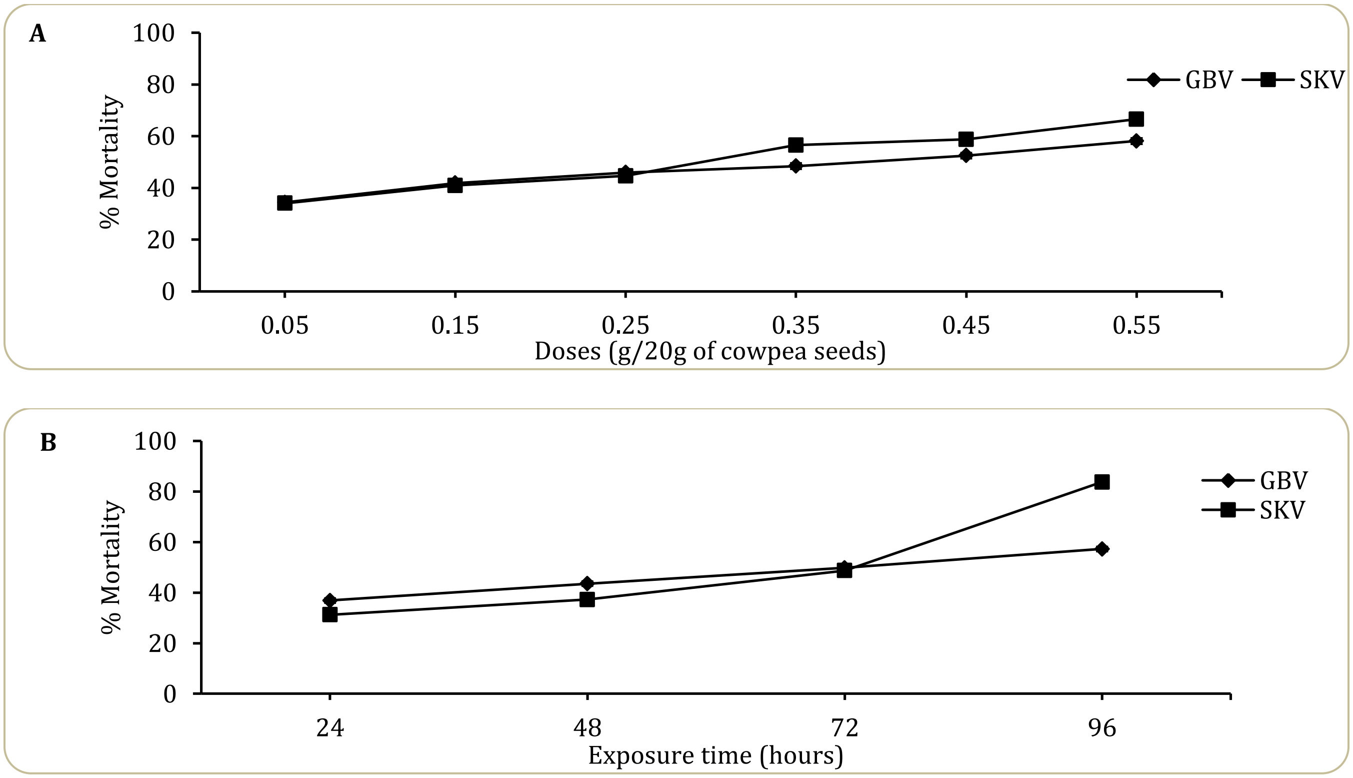

Figure 3. Interactive effects of (A) cowpea variety-by-dose and (B) cowpea variety-by-exposure time on the response of *C. maculatus* to *P. guineense* powder.

Lethal doses (LD) of *S. aromaticum* and *P. guineense* at 48 and 72 h post-treatments

The lethal doses of *S. aromaticum* and *P. guineense* that killed 50% of the exposed insects at 48 and 72 h post-treatment are presented in Table 2. The positive intercept and slope of regression, irrespective of cowpea variety and plant material, showed that bruchid mortality generally increased with an increase in doses of *S. aromaticum* and *P. guineense*. Table 2 also shows that *S. aromaticum* was more toxic than *P. guineense*, irrespective of cowpea variety and exposure time. Based on the fiducial limits, there were significant ($P < 0.05$) differences in the LD_{50} values of *S. aromaticum* and *P. guineense* for GBV- and SKV-reared bruchids, irrespective of exposure time. Also, the highest susceptibility at 48 (0.075 g/20g cowpea) and 72 (0.044 g/20g cowpea) h post-treatments was observed in GBV- and SKV-reared bruchids, respectively, exposed to *S. aromaticum*. However, the least susceptibility at 48 (0.53 g/20g cowpea) and 72 (0.255 g/20g cowpea) h post-treatments was observed in GBV- and SKV-reared bruchids, respectively, exposed to *P. guineense*.

Table 2: Lethal dose (LD) result at 48 and 72 h post treatment for *P. guineense* and *S. aromaticum* powder.

Exposure time	Cowpea	Plant	Slope±S.E	Intercept±S.E	χ^2	LD_{50}
48	GBV	*SA*	2.85±0.11	3.19±0.11	61.71	0.075 (0.065 - 0.085)
		PG	0.47±0.08	0.13±0.05	16.34	0.53 (0.394 - 0.851)
	SKV	*SA*	2.37±0.10	2.52±0.08	172.88	0.087 (0.065 - 0.107)
		PG	1.11±0.08	0.34±0.06	16.58	0.489 (0.428 - 0.574)
72	GBV	*SA*	2.67±0.13	3.47±0.14	87.87	0.05 (0.039 - 0.061)
		PG	0.32±0.07	0.19±0.05	22.68	0.243 (0.162 - 0.371)
	SKV	*SA*	1.84±0.10	2.50±0.09	147.76	0.044 (0.025 - 0.062)
		PG	0.95±0.08	0.56±0.05	66.42	0.255 (0.202 - 0.326)

Note: S. E = Standard Error, χ^2 = Chi-square, LD_{50} = Lethal dose at which 50% population response. Values in parenthesis represent 95% confidence interval

Main and interactive effects of cowpea variety and experimental doses on the specific activities of anti-oxidant enzymes of *C. maculatus*

Generally, cowpea variety, experimental dosage, and their interaction (V*D) significantly ($P < 0.001$ in all cases) influenced the specific activities of catalase (cowpea variety: $F_{1,18} = 65.01$; Dose: $F_{2,18} = 467.36$; V*D: $F_{2,18} = 45.90$), SOD (cowpea variety: $F_{1,18} = 6858.57$; Dose: $F_{2,18} = 271,175.59$; V*D: $F_{2,18} = 41,066.89$), and GSH (cowpea variety: $F_{1,18} = 257.41$; Dose: $F_{2,18} = 4297.87$; V*D: $F_{2,18} = 206.74$). The specific activities of catalase, SOD, and GSH also varied significantly ($P < 0.001$) with cowpea varieties and plant materials.

Varietal effect of cowpea on the activities of catalase, SOD and GSH

Figure 4, 5 and 6 show the effect of cowpea varieties on the specific activities of catalase, SOD and GSH, respectively, in bruchids treated with both plant powders. The specific activities of catalase and SOD in bruchids exposed to control were significantly ($P < 0.05$) lower than those exposed to *S. aromaticum* and *P. guineense (*Figure 4 and 5*).* Figure 4 shows that only SKV-reared bruchids exposed to *S. aromaticum* had significantly ($P < 0.05$) higher catalase activity than their counterparts reared on GBV. Figure 5 however shows that SKV-reared bruchids exposed to *P. guineense* and control had significantly ($P < 0.05$) higher specific activity of SOD than their counterparts reared on GBV. On the contrary, GBV-reared bruchids exposed to *S. aromaticum* had significantly ($P < 0.05$) higher specific activity of SOD than their counterparts reared on SKV (Figure 5). Figure 6 shows that in GBV-reared bruchids, the activity of GSH in control was significantly ($P < 0.05$) lower than those exposed to *S. aromaticum* and *P. guineense*. However, for SKV-reared bruchids, the activity of GSH in control and *P. guineense* was significantly ($P < 0.05$) higher than those exposed to *S. aromaticum*.

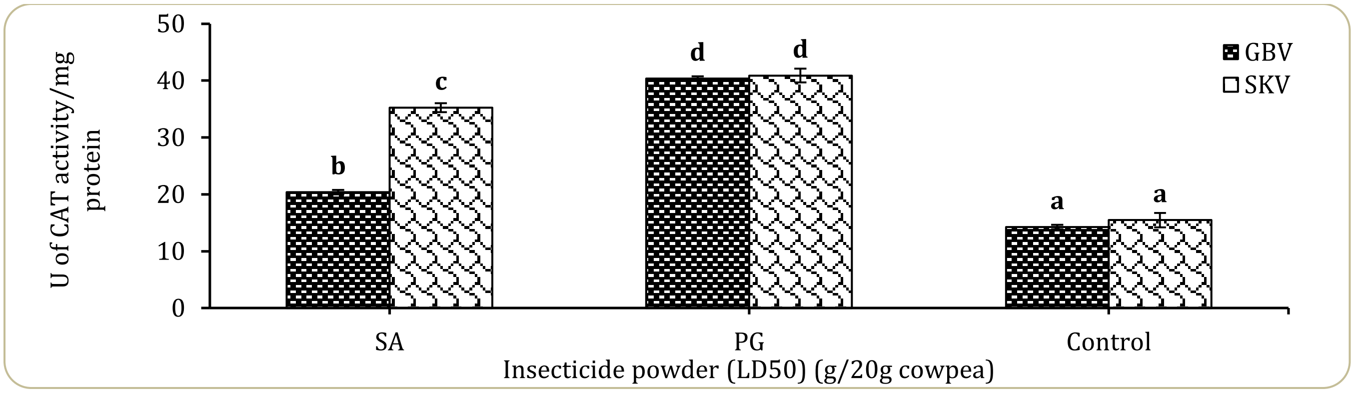

Figure 4. Varietal effect of cowpea on the specific activity of catalase in *C. maculatus* exposed to LC_{50} of two plant derived insecticides. Each bar represents Mean ± standard deviation. Bars with different alphabets are significantly different using Tukey's test at P < 0.05.

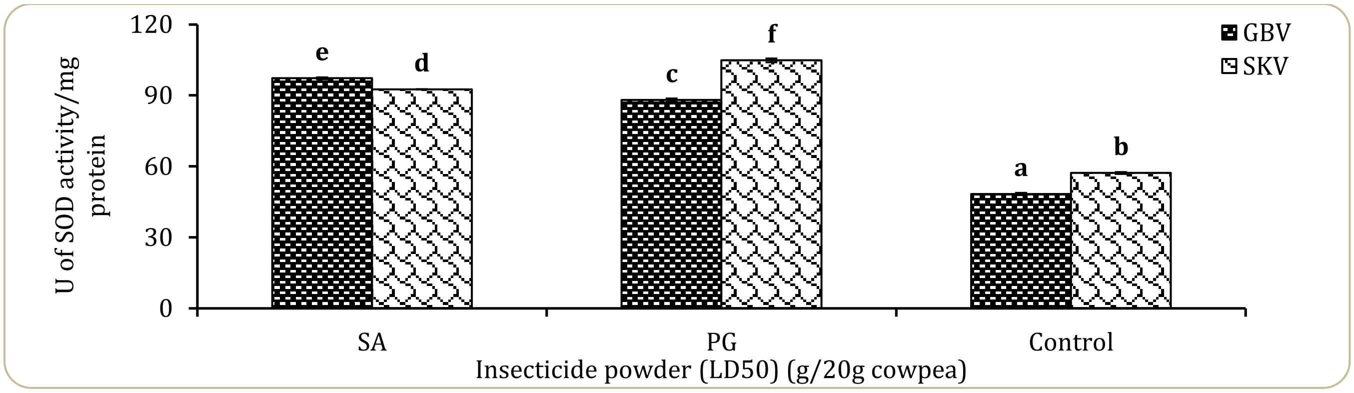

Figure 5: Varietal effect of cowpea on the specific activity of SOD in *C. maculatus* exposed to LC_{50} of two plant derived insecticides. Each bar represents Mean ± standard errors. Bars with different alphabets are significantly different using Tukey's test at P < 0.05.

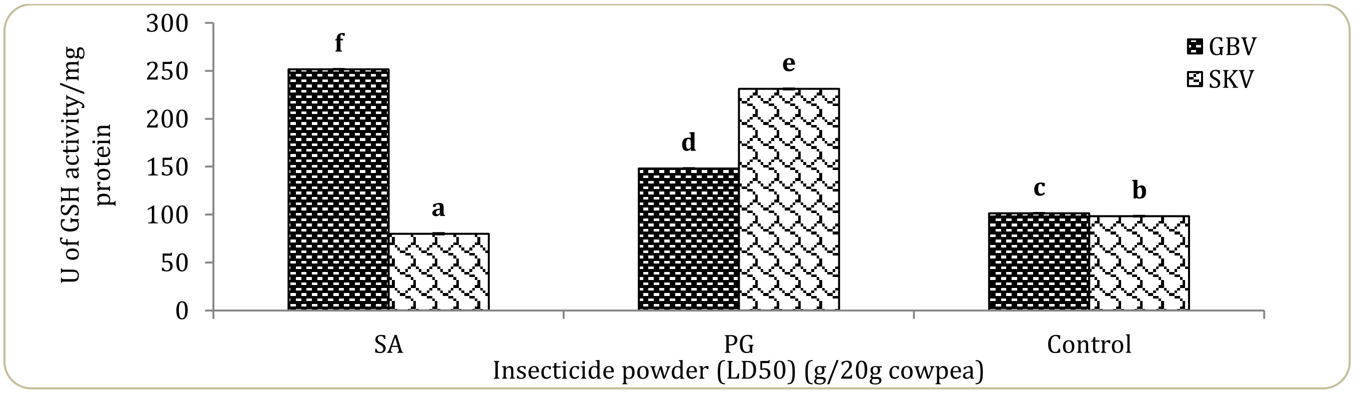

Figure 6: Varietal effect of cowpea on the specific activity of GSH in *C. maculatus* exposed to LC_{50} of two plant derived insecticides. Each bar represents mean ± standard error. Bars with different alphabets are significantly different using Tukey's test at P < 0.05.

DISCUSSION

Effect of cowpea variety on the morphometrics of cowpea seeds and *C. maculatus*

Generally, the morphometrics (viz; length, width and thickeness) of cowpea seeds influenced the sizes of individuals that emerged on each variety. For instance, bruchids that emerged from GBV were observed to be generally bigger than their counterparts that emerged from SKV, and this could be linked to a higher surface area on GBV seeds than SKV seeds. Bruchids are known to distribute their eggs evenly on cowpea seeds; thereafter, larvae that emerge from the seeds compete for food within the cowpea micro-environment (Mitchell, 1991; Arong and Usua, 2006). The larvae are known to pupate after they exhaust the food in the cowpea and then emerge as small adults. Consequently, larger cowpea seeds are expected to offer the bruchids better food reserves than small-sized cowpea, and this is expected to influence the size of the bruchids that will eventually emerge from such seeds. Earlier, Willmer *et al.* (2000) opined that adult insects that emerge from larger cowpea seeds are expected to be bigger than their counterparts from small cowpea seeds. The large size of the GBV-reared bruchids could therefore be linked to the bigger size of GBV seeds relative to SKV seeds.

Main and interactive effects of cowpea varieties and plant powders on the insecticidal response of *C. maculatus*

Both plant powders were able to evoke bruchid mortality regardless of the experimental dosage and cowpea varieties. This agrees with previous studies where the protective abilities of both plant powders against *C. maculatus* infesting cowpea seeds were reported (Ofuya *et al.*, 2010; Idoko and Adesina, 2013; Fajinmi *et al.*, 2015). The protective ability of both powders may be linked to secondary bioactive compounds in the plant powders (Gajger and Dar, 2021). For instance, the major bioactive compounds within *P. guineense* are α-pinene, β-pinene, limonene, and β-caryophyllene (Francois *et al.*, 2009), while those of *S. aromaticum* are the volatiles eugenol and β-caryophyllene (Srivastava *et al.*, 2005; Haro-González *et al.*, 2021). However, regardless of cowpea variety, the LD_{50} values show that *S. aromaticum* was more toxic to bruchids than *P. guineense*. This also agrees with the previous findings by Oyeniyi *et al.* (2015a) that *P. guineense* was less toxic than *S. aromaticum* to *C. maculatus*, regardless of the cowpea variety on which the bruchids were reared.

Similarly, this study showed that bruchids that emerged from GBV were generally more susceptible to both plant powders than their counterpart that emerged from SKV, especially after 48 h post-treatment. This confirms the varietal effect of cowpea on the response of bruchids to both plant powders. The higher mortality observed in GBV-reared bruchids exposed to both plant powders could be attributed to several factors such as the size of cowpea seeds and the chemical constituents of the seeds on which they were reared. As stated earlier, GBV-reared bruchids are bigger in size than SKV-reared bruchids. The larger size of the GBV-reared bruchids could have enabled them to pick up more plant powder inside the containers than small-sized bruchids from SKV. This could be responsible for the higher mortality observed in GBV-reared bruchids relative to SKV-reared bruchids, irrespective of plant material and dosage at 48 h post-treatment. This is contrary to what was reported by Oyeniyi *et al.* (2015a), where bruchids that emerged from larger cowpea seeds were more tolerant of plant powders than their counterparts that emerged from small cowpea seeds. At 72 h post-treatment, the toxic effect of *S. aromaticum*, however, outweighs the varietal effect due to a non-significant difference in their LD_{50} values.

Also, the variation in the chemical composition of cowpea seeds could be responsible for the differences in the responses of bruchids to *P. guineense* and *S. aromaticum*. The coat of cowpea seeds is known to contain secondary compounds such as tannins, flavonoids, and phenolic acids (Egounlety and Aworh, 2013). The energy used by bruchids to detoxify most of the secondary compounds in cowpea grains could ultimately influence the physiology of adults emerging from them (Gbaye *et al.*, 2012). For instance, life history characters such as the survival of adult bruchids exposed to synthetic or botanical insecticides are usually affected by the grains used in rearing them (Povey and Holloway, 1992). Similarly, the cowpea variety used in rearing bruchids was also shown to have influenced their insecticidal response to malathion (Gbaye and Holloway, 2011) and *E. aromatica* (Oyeniyi *et al.*, 2015b). Although the secondary compounds in both cowpea varieties were not determined in this study, the high mortality observed in GBV-reared bruchids regardless of plant powder could suggest the presence of more secondary compounds in GBV than SKV. The energy used in the detoxification of these compounds in GBV by bruchids could have reduced the energy reserve in adults that emerged from them. This could have ultimately made GBV-reared bruchids more susceptible to both plant powders than SKV-reared bruchids and this agrees with the findings of Gbaye and Oyeniyi (2014).

In addition, the degrees of interactions among cowpea variety, plant material, and experimental dosage have different impacts on the susceptibility of *C. maculatus* to both botanicals. Although all the factors significantly influenced the bruchids response to both plant powders, the effect of plant material was the most pronounced, while that of cowpea variety was the least pronounced based on their F-values. The high effect of plant materials may be responsible for the high mortality observed in both GBV- and SKV-reared bruchids, irrespective of experimental dosages. Also, the two-way interactions of cowpea variety with dose or exposure time considerably influenced the response of bruchids to both plant powders. This shows that the degree to which cowpea variety affected the mortality of bruchids was greatly influenced by the experimental dosage of *S. aromaticum* and *P. guineense* as well as the time of exposure to the both plant powders (Oyeniyi et al., 2015a).

Main and interactive effects of cowpea variety and plant powders on the antioxidant enzymes of *C. maculatus*

The grains used in rearing insects could play a major role in their physiological responses to insecticides due to the induction of various arrays of enzymes to differing degrees (Gbaye et al., 2012). In this study, the varietal effect of cowpea on the antioxidant enzymes of bruchids exposed to plant powders of *S. aromaticum* and *P. guineense* was also investigated. The result of this work showed that the activity of the antioxidant enzymes in adult *C. maculatus* varied with the cowpea variety and plant material used. The increase in the specific activities of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione in bruchids treated with powders of *S. aromaticum* and *P. guineense* relative to those in the control could indicate an adaptive response to the insecticide-induced oxidative stress. All the variations observed in the activities of antioxidant enzymes of *C. maculatus* based on the variety of cowpea and type of plant powders showed how the interactive effects of cowpea variety and plant powders could have influenced the response of bruchids to *S. aromaticum* and *P. guineense* under laboratory conditions.

The antioxidant enzyme SOD is essential in the defense of insects against oxidative stress. It catalyzes the conversion of superoxide radicals (O_2^-) into molecular oxygen and hydrogen peroxide (Leung *et al*., 2006; Kolawole *et al*., 2014; Oni et al., 2019). An increase in SOD activity relative to controls, regardless of cowpea variety, suggests that the bruchids are experiencing elevated levels of superoxide radicals, possibly due to the presence of reactive oxygen species (ROS) generated by both plant powders. ROS are the contributors to oxidative stress that cause different diseases and disorders in insects (Buyukguzel, 2006). Insect proteins, lipids, mitochondria, and DNA are damaged by the overproduction of ROS, which prevents the cell’s natural defense mechanisms from neutralizing them (Kolawole *et al*., 2014; Juan et al., 2021; Sule et al., 2022). Eventually, this causes the insects to die. The increase in SOD activity is an attempt by the insect to counteract the harmful effects of oxidative stress, possibly induced by both plant powders, in order not to die. In this study, GBV-reared bruchids had a higher specific activity of SOD than their counterparts reared on SKV when treated with *S. aromaticum*. However, for *P. guineense* and control, SKV-reared bruchids had higher specific activity of SOD than their GBV counterparts. This suggests that phytochemicals in SKV and GBV might be interacting with *S. aromaticum* and *P. guineense*, respectively, to reduce the activity of SOD in the exposed bruchids. This shows that the cowpea variety influences the rate at which superoxide radicals are produced in response to the toxic effects of *S. aromaticum* and *P. guineense*.

The hydrogen peroxide produced due to the conversion of superoxide radicals into molecular oxygen and hydrogen peroxide by SOD is usually broken down into water and oxygen by catalase, thus protecting cells from the toxic effects of hydrogen peroxide (Liochev and Fridovich, 2007; Fujii *et al*., 2022). In this study, an increase in catalase activity, irrespective of cowpea variety, suggests that the bruchids are trying to eliminate extra hydrogen peroxide, which may be produced as a by-product of detoxification of the toxin in the plant powders or in reaction to the insecticide-induced oxidative stress by the powders. Varietal effects of cowpea were not pronounced on the activities of catalases because SKV-reared bruchids had higher catalase activity than GBV-reared bruchids, irrespective of plant materials. In fact, the difference in the activities of catalase in bruchids from both varieties was only significant in those exposed to *S. aromaticum,* and this further confirms the higher toxic effects of *S. aromaticum* than *P. guineense*. It also shows that phytochemicals in GBV might be interacting more with toxic compounds in *S. aromaticum* in order to lower catalase activity in the exposed bruchids. However, this needs to be further investigated. An essential antioxidant for cellular protection against oxidative stress is glutathione (Basil Ribeiro, 2023). In addition to its direct antioxidant action, it aids in the antioxidants' renewal. An increase in glutathione activity or levels may be a sign that the insect is trying to maintain redox homeostasis (Kwon *et al*., 2019). In this study, it is possible that glutathione is being utilized to neutralize reactive oxygen species produced in response to the plant powders, and an increase in their activity in treated bruchids relative to control could signify an adaptive response to oxidative stress.

CONCLUSION

From the various results obtained from this research, it could be concluded that both varieties of cowpea have influenced the response of *C. maculatus* to both plant powders (*S. aromaticum* and *P. guineense*). Generally, bruchids that emerged from GBV were more susceptible to both plant powders than their counterparts that emerged from SKV. It could also be established that, irrespective of the cowpea variety, bruchids were more susceptible to *S. aromaticum* than *P. guineense*. Cowpea variety, experimental dosages, and their interaction also influenced the response of bruchids to both plant powders. The higher activities of the three antioxidant (superoxide dismutase, catalase, and glutathione) enzymes of cowpea bruchids exposed to both plant powders relative to controls is an indication of stress or physiologic harm to the bruchids. In conclusion, the present investigation into the varietal effect of cowpea on the insecticidal response and antioxidant enzymes of *C. maculatus*, particularly when exposed to *P. guineense* and *S. aromaticum* powders, represents a novel and multifaceted approach towards sustainable pest management in agricultural systems. This research not only contributes to our understanding of plant-insect interactions but also holds promise for the development of practical solutions to enhance the resilience of cowpea seeds in the face of storage pest challenges. This study also adds to the knowledge base of insect physiology, providing a foundation for further research in the field of applied entomology and aligns with the global shift towards more sustainable and environmentally friendly agricultural practices. Although the various results obtained in this study are promising, it is important to state that the various findings may be specific to the chosen cowpea varieties and plant powders. Extrapolating the results to different cowpea varieties or powders may require caution. In a similar vein, the interaction between cowpea varieties, plant powders, and *C. maculatus* is likely complex. Thus, pinpointing the precise mechanisms involved could necessitate further investigation and might not be entirely clarified by this study.

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AUTHORS' CONTRIBUTIONS

EAO conceived idea, supervised research, wrote manuscript, analyzed data; EAO and YAO conducted research and edited the manuscript; YAO helped in data collection and analysis.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Aebi, H., 1984. Catalase, *in vitro*. Methods in Enzymology 105, 121-126.

Ahetor, M.O., Coulibaly, O.N., 2017. Bruchid resistance in cowpea: a review. African Journal of Agricultural Research 12(22), 1894-1904.

Arong, G.A., Usua, E.J., 2004. Comparative development of cowpea bruchid, (*Callosobruchus maculatus* (F.) (Coleptera: Bruchidae) on pods and seeds of come cowpea cultivars (*Vigna unguiculata*). Global Journal of Pure and Applied Sciences 12(1), 35-40.

Basil Ribeiro, M.D., 2023. Glutathione: the master antioxidant. Ozone Therapy Global Journal 13(1), 175-197.

Beauchamp, C., Fridovich, I., 1971. Improve assays and an assay applicable to acrylamide gels. Analytical Biochemistry 44, 276-287.

Bradford, M.M., 1976. A rapid and sensitive method for the quantification of microgram quantities of proteins utilizing the principle of protein dye binding. Annals of Biochemistry 72, 248-252.

Büyükgüzel, K., 2006. Malathion-induced oxidative stress in a parasitoid wasp: effect on adult emergence, longevity, fecundity, and oxidative and antioxidative response of *Pimpla turionellae* (Hymenoptera: Ichneumonidae). Journal of Economic Entomology 99(4), 1225-34.

Castro, M.J.P., Baldin, E.L.L., Cruz, P.L., Souza, C.M., Da Silva, P.H.S., 2013. Characterization of cowpea genotype resistance to *Callosobruchus Maculatus*. Pesquisa Agropecuária Brasileira 48(9), 1201-1209.

Egounlety, M., Aworh, O.C., 2003. Effect of soaking, dehulling, cooking and fermentation with *Rhizopus oligosporus* on the oligosaccharide, trypsin inhibitor, phytic acid and tannins of soybean (*Glycine max*), cowpea (*Vigna uguiculata* L.Walp) and ground bean (*Marcotyloma geocarpa* Harms). Journal of Food Engineering 50, 249-254.

Ellman, G.L., Courtney, K.D., Andres, V.J., Featherstone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology 7, 88-95.

Fajinmi, A., Fayemiro, S.S., Okonji, C.J., Fajinmi, O.B., 2015. Protectant effects of *Eugenia aromatica* and *Piper guineense* against *Callosobruchus maculatus*. Tropical Agriculture (Trinidad) 92(1), 27-34.

FAO., 2023. World Food and Agriculture - Statistical Yearbook 2023. Rome. https://doi.org/10.4060/cc8166en

Fatokun, C.A., Boukar, O., Muranaka, S., 2012. Genetic diversity among cowpea varieties and their response to bruchid infestation. Plant Genetic Resources: Characterization and Utilization 10(3), 199-204.

Finney, D.J., 1971. "Probit Analysis". Cambridge University Press, Cambridge, London, 333pp.

François, T., Michel, J.D.P., Lambert, S.M., Ndifor, F., Vyry, W.N.A. Henri, A.Z.P., Chantal, M., 2009. Comparative essential oils composition and insecticidal effect of different tissues of *Piper capense* L., *Piper guineense* Schum. et Thonn., *Piper nigrum* L. and *Piper umbellatum* L. grown in Cameroon. African Journal of Biotechnology 8(3), 424-431.

Fujii, J, Homma, T., Osaki, T., 2022. Superoxide radicals in the execution of cell death. *Antioxidants* 11(3), 501.

Gajger, I.T., Dar, S.A., 2021. Plant allelochemicals as sources of insecticides. *Insects* 12(3), 189.

Gbaye, O.A., Holloway, G.J., 2011. Varietal effects of cowpea, *Vigna Unguiculata*, on tolerance to malathion in *Callosobruchus Maculatus* (Coleoptera: Bruchidae). Journal of Stored Products Research 47 (4), 365-371.

Gbaye, O.A., Millard J.C., Holloway, G.J., 2012. Synergistic effects of geographical strain, temperature and larval food on insecticide tolerance in *Callosobruchus maculatus* (F.) Journal of Applied Entomology 136, 282-291.

Gbaye, O.A., Oyeniyi, E.A., 2015. Variation in the tolerance of Osun state *Callosobruchus maculatus* (Fabricius) populations to *Eugenia aromatica* (Baill). Nigeria Journal of Plant Protection 28(1), 21-33.

Haro-González, J.N., Castillo-Herrera, G.A., Martínez-Velázquez, M., Espinosa-Andrews, H., 2021. Clove essential oil (*Syzygium aromaticum* L. Myrtaceae): Extraction, chemical composition, food applications, and essential bioactivity for human health. Molecules 26, 6387.

Horn, L.N., Nghituwamhata, S.N., Isabella, U., 2022. Cowpea production challenges and contribution to livelihood in Sub-Saharan region. Agricultural Sciences 13, 25-32.

Hossain, M.A., Bachchu, M.A.A., Ahmed, K.S., Haque, M.A., 2014. Effectiveness of indigenous plant powders as grain protectant against *Callosobruchus chinensis* (L.) in stored chickpea (*Cicer arietinum*). Bangladesh Journal of Agricultural Research 39(1), 93-103.

Idoko, J.E., Adesina, J.M., 2013. Effect of *Callosobruchus maculatus* (FAB.) (Coleoptera: Chysomelidae) infestation level on control using different particle sizes of *Eugenia aromatic* and piper guineense powders. Journal of Biodiversity and Environmental Sciences 3(1), 54-60.

Juan, C.A., Pérez de la Lastra, J.M., Plou, F.J., Pérez-Lebeña, E., 2021. The Chemistry of reactive oxygen species (ROS) revisited: outlining their role in biological macromolecules (DNA, Lipids and Proteins) and induced pathologies. International Journal of Molecular Sciences *22*, 4642.

Kolawole, A.O., Olajuyigbe, F.M., Ajele, J.O., Adedire, C.O., 2014. Activity of the antioxidant defense system in a typical bioinsecticide-and synthetic insecticide-treated cowpea storage beetle *Callosobrochus maculatus* F. (Coleoptera: Chrysomelidae). International Journal of Insect Science 6, 99-108.

Kwon, D.H., Cha, H.J., Lee, H., Hong, S.H., Park, C., Park, S.H., Kim, G.Y., Kim, S., Kim, H.S., Hwang, H.J., Choi, Y.H., 2019. Protective effect of glutathione against oxidative stress-induced cytotoxicity in RAW 264.7 macrophages through activating the nuclear factor erythroid 2-related factor-2/heme oxygenase-1 pathway. Antioxidants (Basel) 8(4), 82.

Leung, H.W-C., Kuo, C-L., Yang, W-H., Lin, C-H., Lee, H-Z., 2006. Antioxidant enzymes activity involvement in luteolin-induced human lung squamous carcinoma CH27 cell apoptosis. European Journal of Pharmacology 534, 12-18.

Liochev, S.I. and Fridovich, I., 2007. The effects of superoxide dismutase on H2O2 formation. Free Radical Biology and Medicine 42, 1465-1469.

Miesho, W. B., Gebremedhin, H. M., Msiska, U. M., Mohammed, K. E., Malinga, G. M., Sadik, K., Odong, T. L., Rubaihayo, P., Kyamanywa. S., 2018. New sources of cowpea genotype resistance to cowpea bruchid *Callosobruchus Maculatus* (F.) in Uganda. International Journal of Agronomy Agricultural Research 12 (4), 39-52.

Mitchell, R., 1991. The traits of a biotype of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) from South India. Journal of Stored Products Research 27, 221-224.

Nathan, S.S., Choi, M.Y., Seo, H.Y., Paik, C.H., Kalaivani, K., Kim, J.D., 2008. Effect of azadirachtin on acetylcholinesterase (AchE) activity and histology of the brown planthopper *Nilaparvata lugens* (Stal). Ecotoxicology and Environmental Safety 70, 244-250.

Njoku, K.L., Ogunwolu, E.O., Adeyemi, A.A., 2019. Potential of edible plant materials as protectants against cowpea bruchid, *Callosobruchus maculatus*. Journal of Stored Products and Postharvest Research 10(2), 23-30.

Ofuya, T.I, Olotuah, O.F., Akinyoade, D.O., 2010. The effect of storage on the efficacy of *Eugenia aromatica* (Baill) in the control of *Callosobruchus maculatus* (F) (Coleoptera: Bruchidae) pest. Journal of Applied Science and Environmental Management 14(1), 97-100

Ofuya, T.I., 2001. Biology, ecology and control of insect pests of stored food legumes in Nigeria. In: Ofuya, T.I. and Lale, N.E.S. (Eds) Pest of stored cereals and pulses in Nigeria. Published by TDave Collins Publications, Akure, Ondo state, Nigeria. 24-58 pp.

Oladipo, O.E., Oyeniyi, E.A., Aribisala, T.E., 2019. Toxicity and biochemical mechanisms underlying the insecticidal efficacy of two plant extracts on *Callosobruchus chinensis* (Coleoptera: Chrysomelidae) infesting cowpea seeds. Journal of Crop Protection 8(3), 259-274.

Oni, M.O., Ogungbite, O.C, Oguntuase, S.O., Bamidele, O.S., Ofuya, T.I., 2019. Inhibitory effects of oil extract of green Acalypha (*Acalypha wilkesiana*) on antioxidant and neurotransmitter enzymes in *Callosobruchus maculatus*. The Journal of Basic and Applied Zoology 80, 47.

Oyeniyi, E.A., Gbaye, O.A. and Holloway, G.J., 2015a. Interactive effect of cowpea variety, dose and exposure time on bruchid tolerance to botanical pesticides. African Crop Science Journal 23(2), 165-175.

Oyeniyi, E.A., Gbaye, O.A., Holloway, G.J., 2015b. The influence of geographic origin and food type on the susceptibility of *Callosobruchus maculatus* (Fabricius) to *Piper guineense* (Schum and Thonn). Journal of Stored Product Research 63, 15-21.

Povey, S.R., Holloway, G.J., 1992. The effect of energy tradeoffs on life history and fitness in the rice weevil, *Sitophilus oryzae*. Oikos 64, 441-450.

Rajput, K., Gilal, A.A., Rajput, L.B., Hajano, J-U-D., Magsi, S., Sahto, J.G.M., Panhwar, Z., Jatoi, G.H., 2023. Evaluation of plant essential oils against *Sitophilus Oryzae* Linnaeus (Coleoptera: Curculionidae) under laboratory conditions. Plant Protection 7(03), 417-426.

Srivastava, A.K., Srivastava, S.K., Yamsundar, K.V., 2005. Bud and leaf essential oil composition of *Syzygium aromaticum* from India and Madagascar. Flavour and Fragrance Journal 20(1), 51-53.

Sule, R.O., Condon, L., Gomes, A.V., 2022. A Common feature of pesticides: oxidative stress-the role of oxidative stress in pesticide-induced toxicity. Oxidative Medicine and Cellular Longevity, 5563759.

Tamò, M., Kergoat, G.J., 2017. Cowpea bruchids: occurrence, biology, and management in sub-Saharan Africa. Annual Review of Entomology 62(1), 101-118.

Tan, H., Tie, M., Luo, Q., Zhu, Y., Lai, J., Li, H., 2012. A review of molecular makers applied in cowpea (*Vigna unguiculata* L. Walp.) breeding. Journal of Life Sciences 6, 1190-1199.

Tengey, T.K., Alidu, S.M., Moro, A., Nboyine, J., Affram, E.I., 2023. Resistance to *Callosobruchus maculatus* among cowpea (*Vigna unguiculata* (L.) Walp) genotypes. Journal of Crop Improvement 37(3), 309-322.

Tripathi, K., Bhalla, S., Prasad, T.V., Srinivasan, K., 2012. Differential reaction of cowpea (*Vigna Unguiculata*) genotypes to pulse-beetle (*Callosobruchus maculatus*). International Journal of Plant Research 25(2), 367-374.

Urgesa, L., 2023. Review on cowpea production, utilization and research achievements in Ethiopia. International Journal of Advanced Research in Biological Sciences 10(1): 172-180

Willmer, P., Stone, G., Johnston, I., 2000. Environmental physiology of animals. Blackwell Science, Oxford.