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Evaluation of phytotoxicity indicators of cowpea seed treated with selected botanical insecticides

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Abstract

Phytotoxicity is a major problem associated with the preservation of seed with synthetic insecticides. Thus, the aim of this study was to evaluate the safety of some selected plant derived insecticides on treated seed. Seed of SAMPEA 11, SAMPEA 14 and SAMPEA 12 were each divided into five (5) lots of 50 grams/lot identified, as L1, L2, L3, L4 and L5. L1 and L2 were treated with 50 and 12.5 µg/mL of myristicin and alpha-humulene based-insecticides respectively. L3 was treated with 6.25 µg/mL of azadirachtin based insecticide. L4 was not treated, while L5 was treated with diluted chlorpyrifos. In SAMPEA 12 and 11, the catalase activity and seed protein content were significantly ($P<0.05$) reduced compared to what obtained for L4. While in SAMPEA 14, catalase activity was not significantly ($P>0.05$) different from that reported for L4. Similar trend was observed on the protein content of the said cultivars treated with the aforementioned botanical insecticides. This study established similarity and variation in varietal responses to the effect the aforementioned insecticides to which the studied seed of cowpea cultivars were exposed to.

Keywords: Botanical insecticide, Cowpea, Seed, Phytotoxicity, Catalase, Protein

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INTRODUCTION

A pesticide is said to be phytotoxic, if it wields the potential to permanently or temporarily damage the vegetative or generative structure in a sensitive cultivar or genetic line usually by oxidative stress mechanism (Sharma *et al*., 2018). It is mainly associated with the extensive use of the synthetic pesticides in pursuit of food

security and has been implicated in poor crop performance and consequent declined productivity (Edwards *et al*., 2023).
The essence of treating seed with pesticides is basically to preserve it against pests and pathogens. Although, its indispensability to effective crop production cannot be overemphasized, it is considered the major factor that predisposes treated seed to phytotoxicity owing to enhanced seed-pesticide contact (Guedes *et al*., 2023). Nigeria has been the largest producer and consumer of cowpea in African and is responsible for approximately 40.2% of its production annually estimated at 8.9 million metric tons (FAO, 2021). However, the impressive record on Nigeria's massive cowpea production capability is heavily threatened by the the deleterious attack of *Callosobruchus maculatus* (Togola *et al*., 2020) a cosmopolitan insect pest that has been implicated in substantial loss of stored cowpea seed in the tropics (Mekoneem *et al*., 2022).

The use of chemical method of controlling insect infestation on stored seed is extensive and productive (Leivas *et al*., 2020). Unfortunately, its application is discouraged mainly due to phytotoxicity (Obidola *et al*., 2019) on cowpea seed to which they are exposed to. Therefore, it becomes imperative to intensify efforts to develops safer options.
Insecticidal plants have been used locally to protect agricultural products against insect attacks and research has revealed their ease of degradability with minimal capacity to inflict harm (Srinivasan *et al*., 2021).

Extracts of plant sources of azadirachtin, myristicin and alpha-humulene have demonstrated activity against *C. maculatus* (Ito and Ighere, 2017).
Although plant derived active ingredients such as Azadirachtin, myristicin and alpha-humulene based insecticides have demonstrated activity against *C. maculatus* (Ewa *et al*., 2024). Their safety as seed treatment chemicals is yet to be determined. Thus, the imperativeness of this study is defined.

MATERIALS AND METHODS

Seed collection

SAMPEA14 (IT99K-1-1), SAMPEA 11 (IT89KD-288) and SAMPEA 12 (IT89KD-391) bought from the Seed Unit of the Institute for Agricultural Research (IAR) Samaru Zaria, Kaduna State were certified.

Active compounds

Azadirachtin A7430-5MG, alpha-humulene PHL83351-100 MG (Sigma Aldrich, USA), Myristicin 09237-10MG-F (Fluka, Germany), Phosphoric acid (Fluka, England), Sodium chloride, hydrogen peroxide, dichromate acetic acid (AR GHTECH, Sigma Aldrich, USA).

Pesticide application to cowpea seed

Exactly 300 g each of SAMPEA 14 (IT99K-1-1), SAMPEA 12 (IT89KD-391) and SAMPEA 11 (IT89KD-288) were divided into six (6) lots of 50 grams/lot named LOT 1, LOT 2, LOT 3, LOT 4, LOT 5 and LOT 6. While 12.5 µg/mL of α-humulene and 50 µg/mL of myristicin based insecticides were applied to LOT 1 and LOT 2 respectively, 6.25 µg/mL of azadirachtin based insecticide was applied to LOT 3. LOT 4 was untreated. LOT 6 was treated with insecticide 15 mL/kg of diluted chlorpyrifos (synthetic). Treated seed was subsequently dried at room temperature (Okunola *et al*., 2004; Igor *et al*., 2020).

Evaluation of oxidation stress makers of treated cowpea seed

Sample preparation

Two (2 g) of cowpea seed sample was homogenized in ice cold 10 mL of 50 mM phosphate buffer (pH 7.8) and afterwards, homogenated and resulting homogenate centrifuged for 10 minutes at 10,000 xg. The supernatant collected was subsequently used for the assay (Iffat *et al*., 2010).

Determination of catalase activity

A mixture of 1.0 mL of 50 mM phosphate buffer (pH 7), 100 µL of seed extract and 0.5 mL of 0.2 M hydrogen peroxide solution was halted at 0, 30 and 60 seconds following the addition of 2.0 mL of dichromate acetic acid reagent. This mixture was subsequently heated for 10 minutes, cooled and read at 570 nm against a blank. Expression of catalase activity was thus, µ moles of hydrogen peroxide decomposed/min/mg protein (Sivaprakasam and Sambasivam, 2018). Determination of catalase activity was performed with the aid of the

formular: Catalase = OD × Volume of buffer (V) × volume of enzyme extract (v) × volume of sample in cuvet (A)/ε × weight. Where, ε = 39.4 mM^{-1} cm^{-1}.

Determination of seed protein content

Sample preparation

Two (2 g) of cowpea seed sample was crushed with a homogenizer in 10 mL of 0.1 M sodium hydroxide (NaOH) in 3.5% sodium chloride (NaCl). Subsequently, the homogenate was incubated at the temperature of 60 ^{o}C for 90 minutes prior to centrifugation at 4000×g for 30 minutes at 4 ^{o}C. The resulting supernatant was preserved at -20 ^{o}C (Maehre *et al*., 2016).

Procedure

One hundred (100 mg) of Coomassie Brilliant Blue G-250 was introduced into a conical flask bearing 50 mL 95% ethanol. This was followed by the addition of 100 mL of 85% phosphoric acid (H_3PO_4) which. The mixture was thoroughly stirred before distilled water was added to a total volume of 1 L. The solution produced was filtered and preserved at the temperature of 4 ^{0}C. 100 µL of extract and 5 mL Bradford solution were mixed and incubated for 5 minutes. A standard curve was made of Bovine Serum Albumin (BSA) (0, 0.0625, 0.125, 0.25, 0.5 and 1 g L^{-1}) and absorbance was read at 595 nm (Bradford, 1976).

Data analysis

Data were expressed as Mean ± Standard error of mean. Each set of treatment was separately subjected to statistical analysis with the aid of One-Way Analysis of Variance (ANOVA) and comparison of variation among mean values was achieved with turkey's test. *P-values* below 0.05 were significant statistically.

RESULTS

The catalase activity in the seed of cowpea cultivar "SAMPEA 11" treated with botanical insecticides (α-humulene, myristicin and azadirachtin based insecticides) is displayed in Figure 1. Catalase activity was significantly ($P<0.05$) higher in seed treated with botanical insecticides compared to that reported for cowpea seed treated with the standard insecticide (chlorpyrifos) but was significantly ($P<0.05$) lower compared to that of its untreated counterpart.

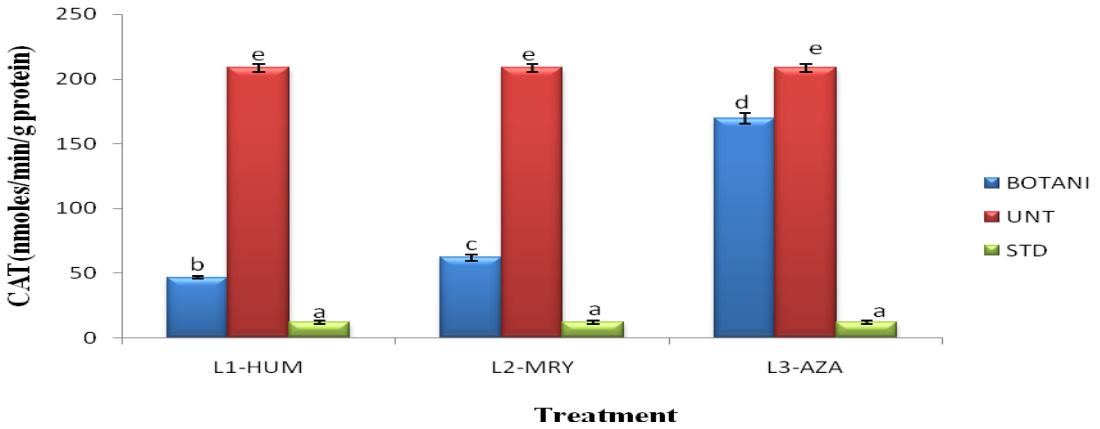

Figure 1: Catalase activity of cowpea seed cultivar (SAMPEA 11) exposed to botanical insecticides
Key: BOTANI = Botanical insecticide, L4-**UNT** (Untreated seed), L5-**STD** (Standard insecticide), L1-HUM (α-Humulene), L 2-MRY (Myristicin), L3-AZA (Azadirachtin)

Displayed on Figure 2 is the activity of catalase in the seed of cowpea cultivar "SAMPEA 12" treated with botanical insecticides (α-humulene, myristicin and azadirachtin based insecticides). The activity of the aforementioned enzyme in the seed of SAMPEA 12 was significantly ($P<0.05$) higher in seed treated with the botanical insecticides compared to that recorded on cowpea seed treated with chlorpyrifos (standard insecticide) but was significantly ($P<0.05$) lower than that reported for its untreated counterpart.

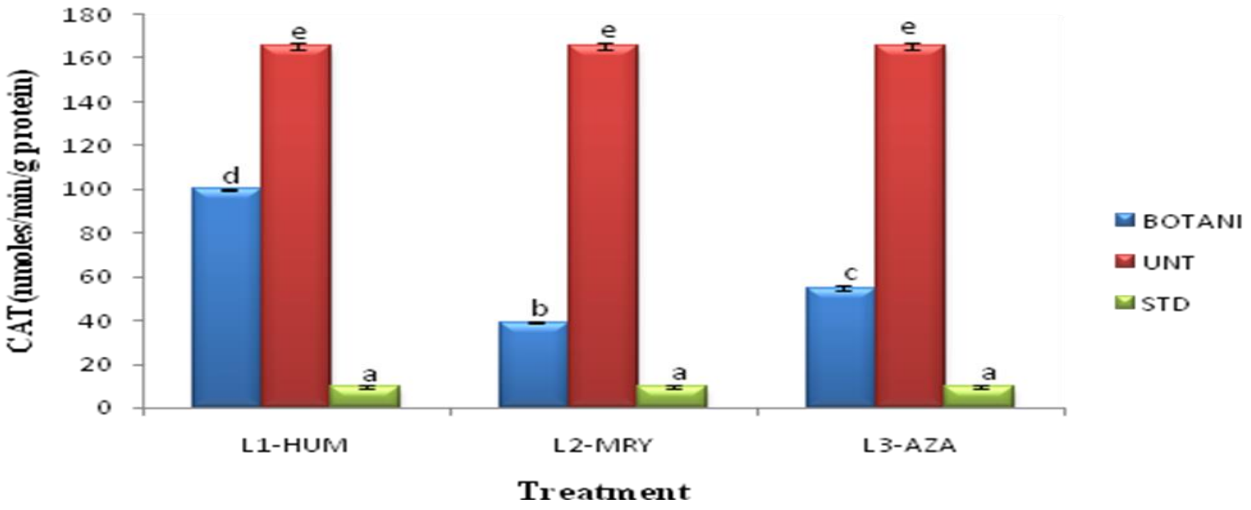

Figure 2: Catalase activity of cowpea seed cultivar (SAMPEA 12) exposed to botanical insecticides

Key: BOTANI = Botanical insecticides, L4-UNT (Untreated seed), L5-STD (Standard insecticide), L1-HUM (α-Humulene), L 2-MRY (Myristicin), L3-AZA (Azadirachtin)

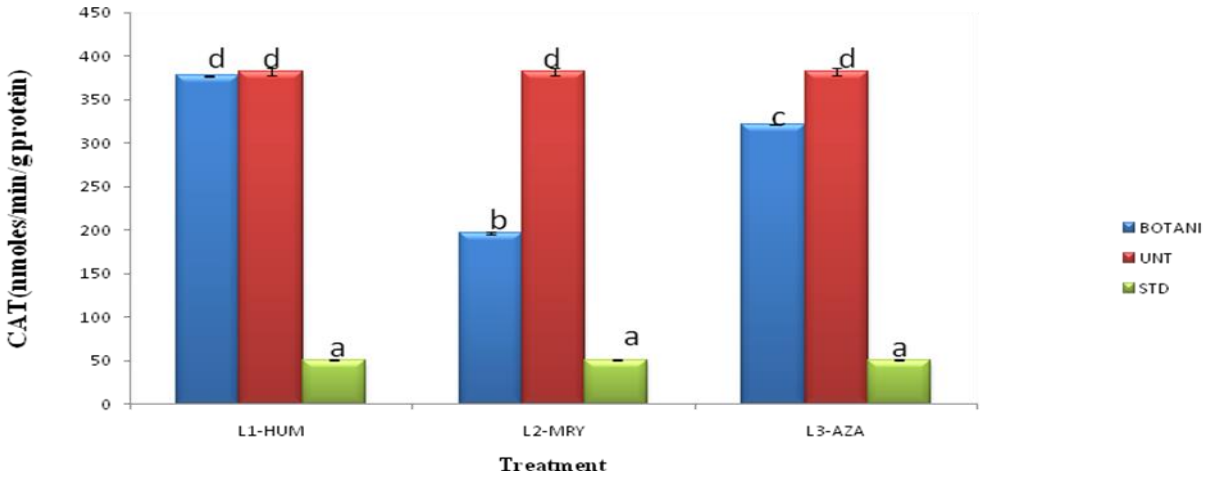

Figure 3: Catalase activity of cowpea seed cultivar (SAMPEA 14) exposed to botanical insecticides
Key: BOTANI = Botanical insecticides, L4-UNT (Untreated seed), L5-STD (Standard insecticide), L1-HUM (α-Humulene), L 2-MRY (Myristicin), L3-AZA (Azadirachtin)

The catalase activity in the seed of cowpea cultivar "SAMPEA 14" is shown in Figure 3 indicating that catalase activity in cowpea seed treated with α-humulene based insecticide was significantly (P<0.05) higher than that reported for cowpea seed treated with azadirachtin based insecticide which in turn was significantly (P<0.05) higher than that reported for cowpea seed treated with myristicin based insecticide. However, while catalase activity in cowpea seed treated with α-humulene based insecticide was not significantly (P>0.05) different from that reported for its untreated counterpart, the activity of the said enzyme in seed treated with myristicin and azadirachtin based insecticides was significantly (P<0.05) lower than that reported for its untreated counterpart. The protein content of the seed of SAMPEA 11 treated with botanical insecticides (α-humulene, myristicin and azadirachtin based insecticides) is presented in Figure 4. The protein content of the seed of the said cowpea cultivar was

significantly (P<0.05) higher in seed treated with α-humulene based insecticide than that reported for seed treated with the synthetic insecticide but however was significantly (P<0.05) lower compared to that of the untreated seed. Similar observation was made on seed treated with myristicin and azadirachtin based insecticides respectively.

The protein content of the seed of "SAMPEA 12" treated with botanical insecticides is shown in Figure 5. The protein content of the seed of the said cultivar was significantly (P<0.05) higher in seed treated with α-humulene based insecticide compared to that recorded for seed treated with the standard insecticide but however, was not significantly (P>0.05) different from that of the untreated seed. The protein content of seed treated with myristicin and azadirachtin based insecticides was significantly (P<0.05) higher than that reported for seed treated with the standard insecticide but lower than that reported for the untreated seed

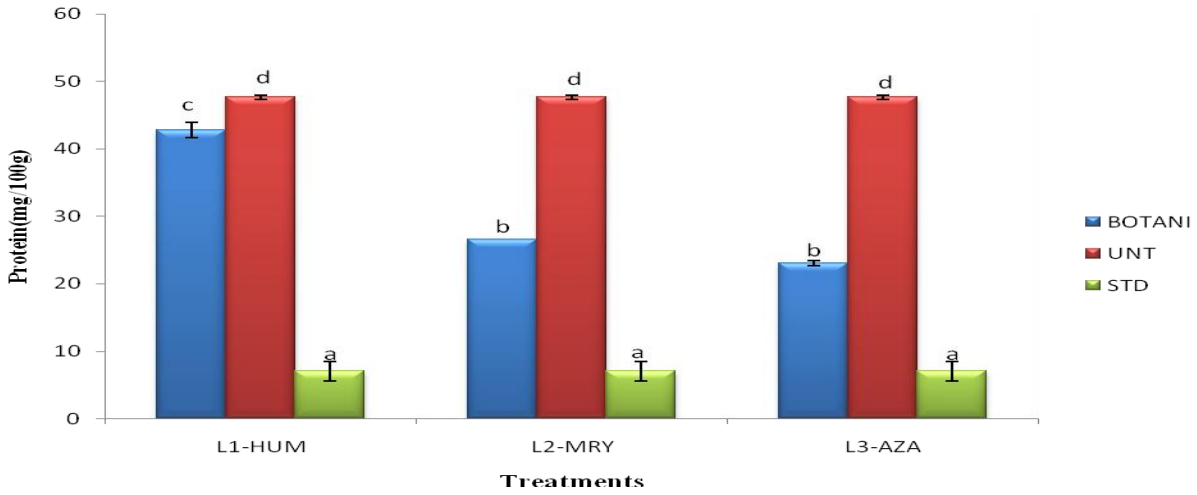

Figure 4: Protein content of cowpea seed cultivar (SAMPEA 11) exposed to botanical insecticides
Key: BOTANI = Botanical insecticides, L4-UNT (Untreated seed), L5-STD (Standard insecticide), L1-HUM (α-Humulene), L 2-MRY (Myristicin), L3-AZA (Azadirachtin)

The amounts of protein reported for seed of "SAMPEA 12" treated with botanical insecticides is displayed in Figure 6. In the cowpea seed treated with α-humulene based insecticide, protein was significantly (P<0.05) higher than that reported for seed treated with chlorpyrifos (standard insecticide). However, no significant (P<0.05) difference was observed between the treated seed and its untreated counterpart. While, the protein content of cowpea seed treated with myristicin based insecticides was significantly (P<0.05) higher than that of the seed treated with the standard insecticide. There was no significant (P>0.05) difference in the protein content of the seed treated with azadirachtin based insecticide and that, treated with the standard insecticide. The protein content of cowpea seed treated with myristicin and azadirachtin based insecticides was significantly (P<0.05) lower than that recorded on the untreated seed.

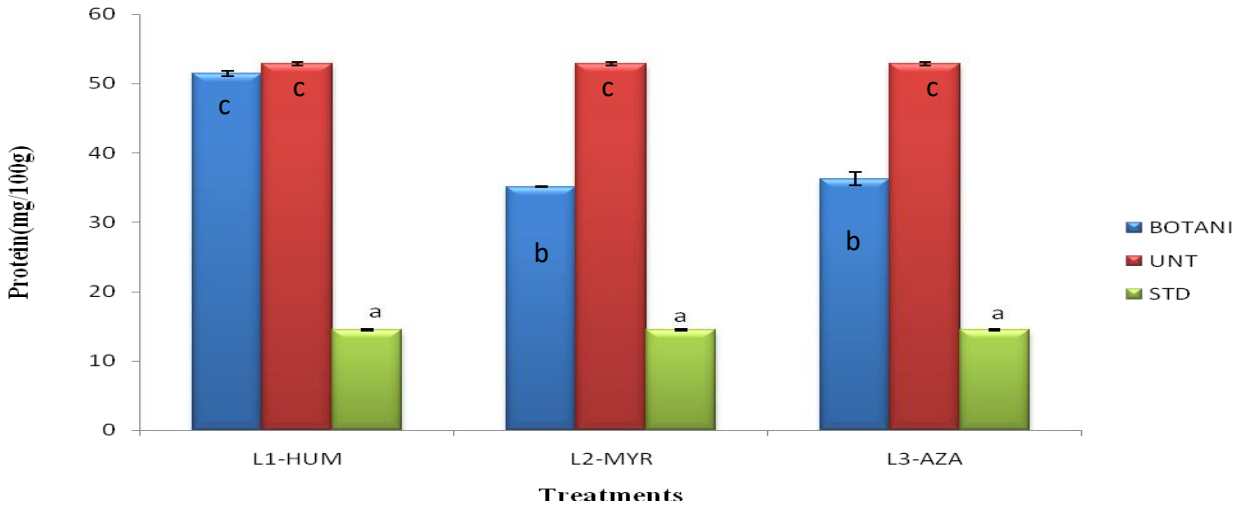

Figure 5: Protein content of cowpea seed cultivar (SAMPEA 12) exposed to botanical insecticides
Key: BOTANI = Botanical insecticides, L4-UNT (Untreated seed), L5-STD (Standard insecticide), L1-HUM (α-Humulene), L 2-MRY (Myristicin), L3-AZA (Azadirachtin)

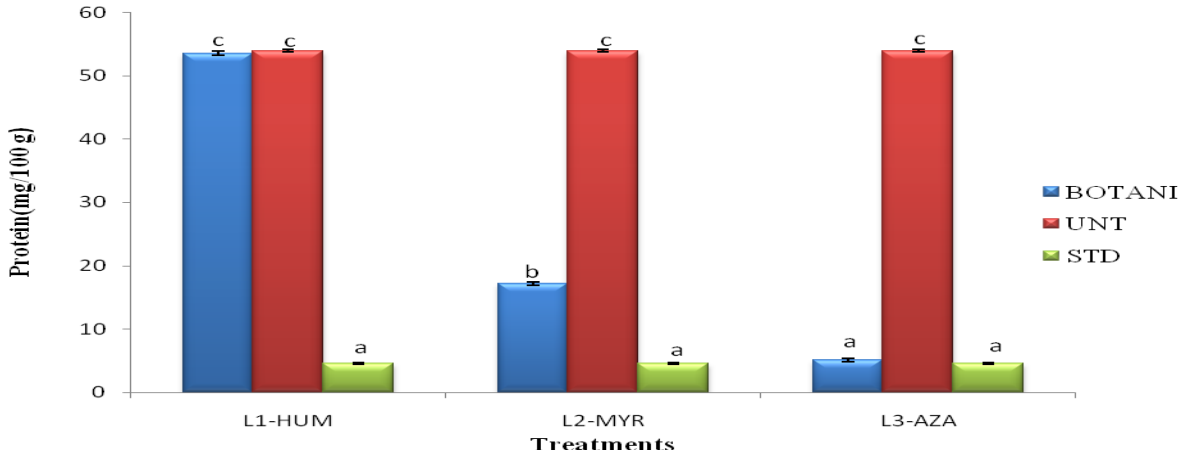

Figure 6: Protein content of cowpea seed cultivar (SAMPEA 14) exposed to botanical insecticides
Key: BOTANI = Botanical insecticides, L4-UNT (Untreated seed), L5-STD (Standard insecticide), L1-HUM (α-Humulene), L 2-MRY (Myristicin), L3-AZA (Azadirachtin)

DISCUSSION

The bulk of cowpea seed consumed in the world comes from Nigeria. An impression which is under constant threat by *C. maculatus* attack (Mekoneem *et al*., 2022). Insecticide induced oxidative stress has been implicated in the distortion of cellular redox balance by altering ascorbate-gluthatione cycle which ultimately translates to the destruction of other antioxidant defense systems (Mirza *et al*., 2019). Reduced catalase activity observed in treated seed across all cultivars studied compared to their untreated counterparts could be attributed to the inactivation of the said enzyme following excessive generation of H_2O_2 an observation, which is in tandem with the outcome of an experiment performed by Dilip and Badre (2014) which revealed the lipid peroxidation enhancing and catalase suppressing

potential of Achook, an azadirachtin base insecticide on certain organs of a specie of the zebra fish. Total soluble protein is critical in the determination of phytotoxicity posed by environmental stresses. Exposure of seed of the studied cultivars to the test insecticides caused a significant ($P<0.05$) reduction in the protein content in some of the seed. This may be attributed to the fact that reactive oxygen species interact with protein molecules at specific amino acid side chains thereby modifying its structure which ultimately translates to fragmentation of the peptide chain, alteration in electrical charges, peroxynitrite nitrate protein accumulation and consequently increased proteolysis (Blokhina *et al*., 2003). This result agrees with the finding of Shakirullah *et al*. (2018) which linked pesticide orchestrated degeneration of protein to excessive generation of reactive oxygen species in *Solanum lycopersicum* seedling. Furthermore, Tanou *et al*. (2009) also reported enhanced protein modification in stressed plants. Sharma *et al*. (2017) inferred that pesticide toxicity reduced chlorophyll and protein content in exposed seed resulting to loss of photosynthetic efficiency in affected plant.

CONCLUSION

Although SAMPEA 11 and 12 are more susceptible to the toxic effect of botanical insecticides (azadirachtin, myristicin and α-humulene based insecticides) than SAMPEA14, the activity of catalase as well as the level of protein in cowpea seed treated with chlorpyrifos (conventionally used seed treatment synthetic alternative) points overwhelmingly to the toxic influence of the said synthetic insecticide which outweighs the volume of toxic influence imposed on seed treated with botanical insecticides. However, the purification of the active ingredients' solvent carrier (methyl hexadecanoate) developed from *Jathropha curcas* seed oil which was limited to degumming could be considered a limitation, as the possibility of retaining certain toxic components in them cannot be completely set aside.

Conflict Interest

Authors have no competing interest to declare.

Author contribution

OE identified the research problem, designed the experiment and wrote the manuscript. ABC supervised the experimental set-up, MKR proof read the manuscript. AEE handled the data analysis, AKA conducted the literature review, AJN designed the study while WJ assembled the methodology.

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