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RESEARCH

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Assessment of phytotoxicity of selected botanical insecticides on treated cowpea (*Vigna unguiculata*) seed

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Abstract

Background: Synthetic insecticides employed in seed treatment are often phytotoxic, especially at slightest misapplications. Cowpea seed is mainly attacked by *Callosobruchus maculatus*. It is established that azadirachtin, myristicin and α-humulene based insecticides (botanical insecticides) are eco-friendly and have activity against *C. maculatus* and thus are considered ideal candidates in research efforts targeted at developing plant based options for protecting cowpea seed against *C. maculatus* attack. Therefore, the aim of this study was to evaluate the toxicity of selected botanical insecticides on treated cowpea (*Vigna unguiculata*) seed.

Results: Electrical conductivity of leachate obtained from cowpea seed treated with botanical insecticides was significantly ($P < 0.05$) lower than that recorded on seed treated with chlorpyrifos. Malondialdehyde levels in seed of cowpea cultivars; SAMPEA 11 and 12 was significantly ($P < 0.05$) higher than that recorded on seed treated with chlorpyrifos. However, malondialdehyde levels in seed of SAMPEA 14 treated with myristicin and azadirachtin based insecticides were not significantly ($P > 0.05$) different from that reported for cowpea seed treated with chlorpyrifos. Less than 50% of the embryo recovered from seed treated with botanical insecticides was unstained contrary to the observation made on seed of SAMPEA 14 dressed with chlorpyrifos.

Conclusion: This study reveals similarity as well as variation in varietal sensitivity to phytoxicity among the various cultivars of cowpea seed studied implying that a farmer's choice of botanical insecticides for the protection of cowpea seed against *Callosobruchus maculatus* would strictly depend on the cultivars involved.

Keywords: Phytotoxicity, Cowpea seed, Synthetic insecticides, Botanical insecticides, *Callosobruchus maculatus*

Background

Globally, cowpea seed production predominates in Africa and Nigeria is the largest consumer and producer of the said crop accounting for 61% of its annual production estimated at 4.5 million metric tons (FAOStat 2017). Unfortunately, Nigeria's impressive cowpea production status is seriously threatened by *Callosobruchus maculatus*, a cosmopolitan field to store insect pest that has been implicated in substantial loss of stored cowpea seed (Ayodele et al. 2014).

Seed treatment refers to the exposure of seed to certain agents including physical, chemical or biological to reduce, control or repel disease causing organisms, insects or other pests which pose threat to seed (Rakesh et al. 2013). It ranges from basic dressing to coating and pelleting (Dubey et al. 2008). Seed treatment is critical to farmer's quest for increased crop yield. The application of chemical pesticides is the most widely adopted measure to protect crops from pests. This is evident by the fact that about two million tonnes of pesticides are consumed annually across the globe (De et al. 2014).

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Synthetic pesticides employed in seed dressing are often phytotoxic, a phenomenon defined by the capacity of pesticides to inflict damage either temporary or permanent to vegetative or generative organ which consequently translates to declined physiological qualities in sensitive plant species, certain varieties or genetic lines through mechanisms such as oxidative stress induction (Stanković 1972; Sharma et al. 2018). For instance, cypermethrin, a synthetic pyrethroid insecticide applied to cowpea seed to protect it against attack by *C. maculatus* was found to inhibit early germination (Obidola et al. 2019).

Owing to the problem of phytotoxicity impacted on treated cowpea seed by different synthetic insecticides applied to protect it against cowpea weevil attack, it is imperative to explore botanical options as potential alternatives to the deleterious synthetic insecticides in use. Azadirachtin, myristicin and α-humulene, derivatives of *Azadirachta indica* (neem), *Myristica fragrans* (nutmeg), and *Lantana camara* (red sedge), respectively, are ecofriendly and effective against *C. maculatus* and therefore are considered suitable candidates in research efforts towards developing plant based options for protecting cowpea seed against *C. maculatus* attack (Ito and Ighere 2017).

Methods

Collection of cowpea seed

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

Active ingredients

Azadirachitin (A7430-5MG), α-humulene (PHL83351-100MG) and Myristicin (09237-10MGF) were purchased from Sigma Aldrish, USA.

Seed treatment

Each cultivar of cowpea seed was divided into six lots of 50 g per lot labeled Lot 1, Lot 2, Lot 3, Lot 4, Lot 5 and Lot 6. Lot 1, Lot 2 and Lot 3 were dressed with 2 mL of 12.5 μg/mL α-humulene, 50 μg/mL myristicin and 6.25 μg/mL azadirachtin based insecticides, respectively. Lot 4 was dressed with fatty acid methyl ester (solvent carrier) and Lot 5 was dressed with 15 mL of chlorpyrifos in 25 L of distilled water/kg of cowpea seed. Treatment was by quick wet method (Okunola et al. 2004; Igor et al. 2020).

Determination of seed viability

Twenty (20) cowpea seed from each lot was hydrated between sheets of paper towel moistened to approximately 2.5 times their dry weight for 3 h at 40 °C. After preconditioning, seed was bisected longitudinally through the midsection of the embryonic axis and then placed in plastic cups, covered with a 0.075% 2, 3, 5 triphenyl tetrazolium chloride solution and incubated at 40 °C for 1 h, 2 h and 3 h. After each of these periods, the triphenyl tetrazolium solution was discarded, the seed rinsed thoroughly with cool, running tap water and left immersed in water until evaluation. Seed was evaluated individually using microscope of magnification (40 ×) and was classified into two categories: viable seed; exhibiting firm and turgid embryo tissues, light red colour in all extension of embryo and endosperm. Non-viable seed; tissues appear flaccid unstained or whitish in more than 50% of cotyledon or endosperm areas. After seed interpretation, image acquisition was established using a custom digital Nikon DI camera and software processing of images, Nikoncapture Powerful Imaging Software adjusted with the software, photoshop 6 (Santos et al. 2007).

Determination of seed vigour

Exactly 2 g of clean seed was immersed in 50 mL of water at 28 ± 1 °C for 12 h before being removed with forceps. The conductivity meter was warmed for 30 min before testing. First, the conductance of distilled water was determined. The electrode was then cleaned with a tissue paper and conductance of the leachate determined. The electrode was thoroughly washed and wiped with a clean tissue paper before reusing. While recording the conductance, the lower bulb of the electrode was fully immersed in the leachate. In order to obtain the electrical conductivity of leachate, the electrical conductivity of distilled water was subtracted from that of the sample. The value was multiplied by the cell constant factor. The result was expressed as μs/cm/g of seed. Reduced value of electrical conductivity is an indication of an impressive level of seed vigour (Matthews and Bradnock 1968).

Data analysis

Data generated were analysed using Statistical Package for the Social Sciences (SPSS) Version 23 and results expressed as Mean ± Standard Deviation Where necessary, data were analysed using one way Analysis of Variance (ANOVA) and differences in mean compared using Turkey's post hoc test. p-values less than 0.05 were considered statistically significant.

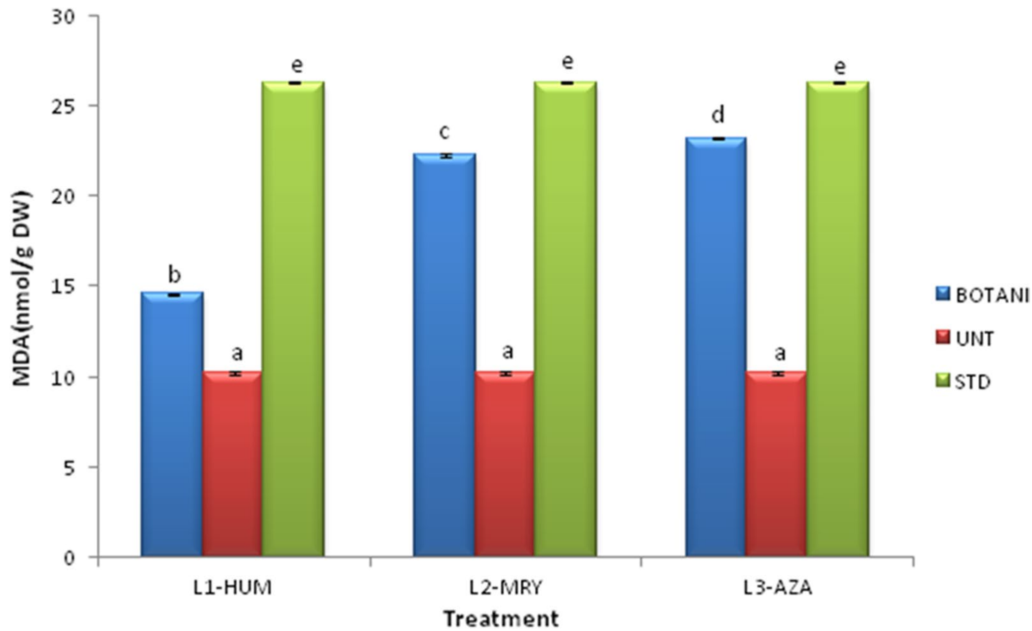


Fig. 1 Levels of malondialdehyde in SAMPEA 11 seed treated with botanical insecticides. Keys: BOTANI = Botanical insecticides, L4-UNT = LOT 4 (Untreated seed), L5-STD = LOT 5 (Standard insecticide), L1-HUM = LOT 1(α-Humulene), L2-MRY = LOT 2 (Myristicin), L3-AZA = LOT 3 (Azadirachitin)

Results

Figure 1 shows the level of malondialdehyde in the seed of cowpea cultivar SAMPEA 11 dressed with botanical insecticides; α-humulene, myristicin and azadirachtin-based insecticides. The level of malondialdehyde in the seed of SAMPEA 11 dressed with botanical insecticides is significantly ($P < 0.05$) lower than that reported for seed dressed with chlorpyrifos but significantly ($P < 0.05$) higher than that reported for the untreated seed. Figure 2 shows the level of malondialdehyde in

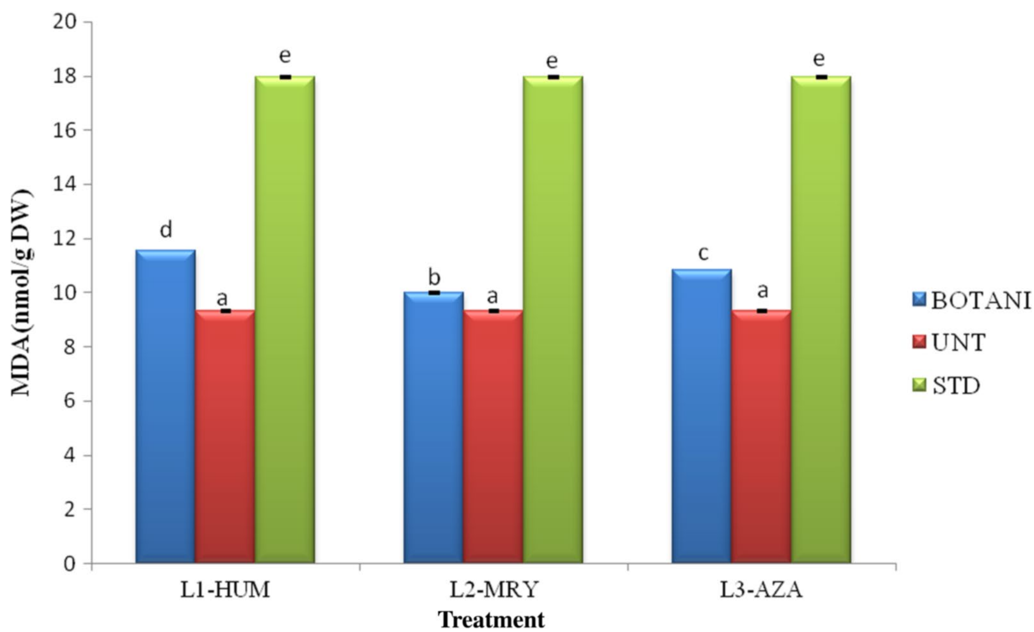


Fig. 2 Levels of malondialdehyde in SAMPEA 12 seed treated with botanical insecticides. Keys: BOTANI = Botanical insecticides, L4-UNT = LOT 4 (Untreated seed), L5-STD = LOT 5 (Standard insecticide), L1-HUM = LOT 1(α-Humulene), L2-MRY = LOT 2 (Myristicin), L3-AZA = LOT 3 (Azadirachitin)

seed of cowpea cultivar SAMPEA 12 dressed with botanical insecticides; α-humulene, myristicin and azadirachtin-based insecticides indicating that the level of malondialdehyde reportedly present in seed of SAMPEA 12 dressed with botanical insecticides is significantly ($P < 0.05$) lower than that reported for seed treated with chlorpyrifos but is significantly ($P < 0.05$) higher than that reported for its untreated counterpart. Figure 3 shows the level of malondialdehyde in seed of cowpea cultivar SAMPEA 14 dressed with botanical insecticides; α-humulene, myristicin and azadirachtin-based insecticides. The malondialdehyde level in the seed of cowpea cultivar SAMPEA 14 dressed with α-humulene based insecticide is not significantly ($P > 0.05$) different from that reported for its untreated counterpart. However, the malondialdehyde level in seed dressed with myristicin and azadirachtin based insecticides is higher, than that reported for its untreated counterpart. The malondialdehyde level in seed dressed with botanical insecticides is significantly ($P < 0.05$) lower than that reported for seed dressed with chlorpyrifos. Plates 1–3 show the photomicrograph of the embryo and cotyledon of seed of cowpea cultivars SAMPEA 11, 12 and 14 dressed with α-humulene, myristicin and azadirachitin based-insecticide, respectively, and plates 4 and 5, the photomicrograph of the embryo and cotyledon of the untreated seed and seed dressed with chlorpyrifos respectively. A small portion of the embryo and cotyledon which however is less than 50% of the total tissue surface is unstained as shown on plates 1, 2, 3 and 5. However, a contrary observation is made on the seed of the SAMPEA 14 dressed with chlorpyrifos (Lot 5) which presented conspicuously unstained tissue surface surpassing 50% of the entire tissue surface contrary to the observation made on the embryo and cotyledon of their untreated counterpart which stained light red in all extension of the tissue surface. Table 1 shows the electrical conductivity of leachate obtained from seed of SAMPEA 11, 12 and 14 treated with botanical insecticides. Electrical conductivity recorded for leachate obtained from seed dressed with α-humulene, myristicin and azadirachitin based insecticides is significantly ($P < 0.05$) lower than that reported for seed dressed with the chlorpyrifos and higher than that recorded for the untreated seed (Fig. 4).

Discussion

High quality seed is pivotal to improved crop yield. Healthy seed is characterized by impressive level of viability and vigor. One of the most important characteristics of seed deterioration is progressive loss of membrane phospholipids and consequent loss of vigor and viability (Samama and Pearce 1993). Although there was an indication of oxidative stress induction in cowpea seed treated with botanical insecticides which could have been as a result of reactive oxygen species generated due to pesticide stress. This finding was consistent with the outcome of a study carried out by Dilip and Badre (2014) which showed that azadirachitin based insecticide (Achook) increased lipid peroxidation and reduced catalase activity

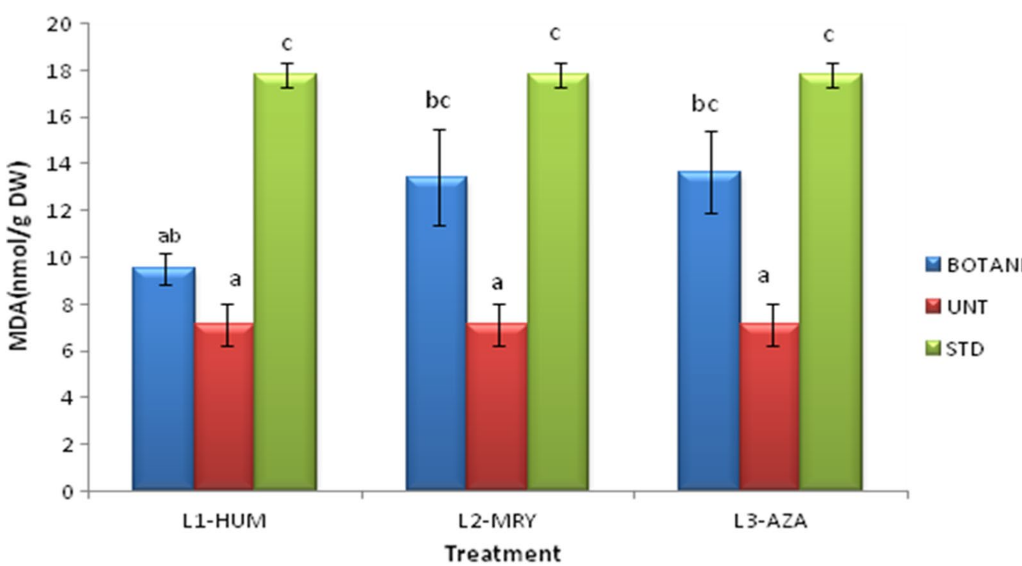


Fig. 3 Levels of malondialdehyde in SAMPEA 14 seed treated with botanical insecticides. Keys: BOTANI = Botanical insecticides, L4-UNT = LOT 4 (Untreated seed), L5-STD = LOT 5 (Standard insecticide), L1-HUM = LOT 1(α-Humulene), L2-MRY = LOT 2 (Myristicin), L3-AZA = LOT 3 (Azadirachitin)

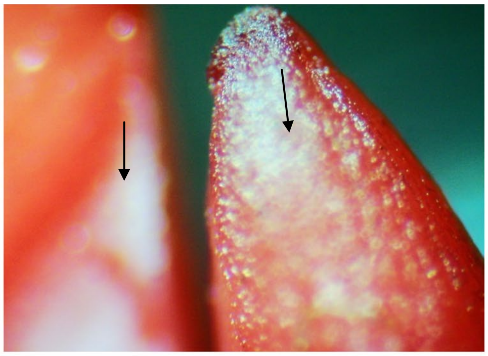


Plate 1: L1-HUM

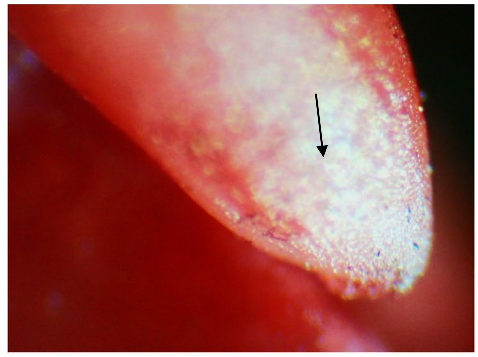


Plate 2: L2-MRY

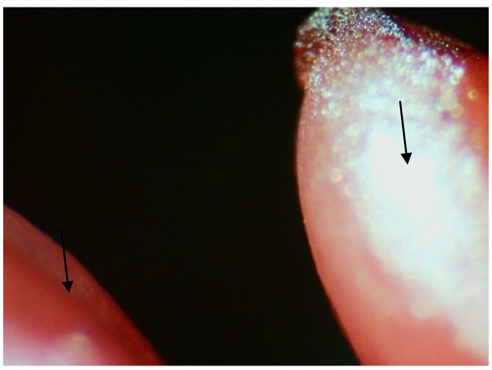
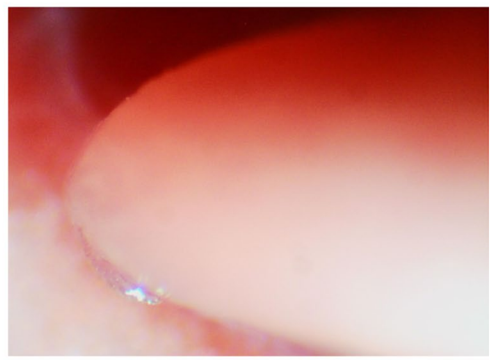


Plate 3: L3-AZA



Plates 1-3 show the photomicrographs of the embryo and cotyledon of cowpea seed (SAMPEA 11) treated with different botanical insecticides, while plate 4 and 5 show the photomicrographs of the embryo and cotyledon of the untreated seed and seed treated with standard insecticide (chlorpyrifos) respectively.

Key: L1-HUM = LOT-1α-Humulene
L2-MRY = LOT-2 Myristiciin
L3-AZA = LOT-3 Azadirachitin
L4-UNT = LOT-4 Untreated
L5-STD = LOT –Standard insecticide

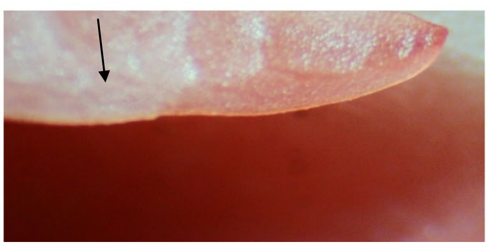


Plate 5: L5-STD

Fig. 4 Photomicrograph of the Embryo and Cotyledon of Seed of Cowpea Cultivars treated with Botanical Insecticides

in the brain and muscle of zebra fish (*Danio rerio*). The study which was restricted to some indicators of phytotoxicity revealed that a small unstained portion of the embryo and cotyledon which however was less than 50% of the total tissue surface area of cowpea seed treated with botanical insecticides. It can be inferred that treatment with the aforementioned botanical insecticides did not erode viability from treated seed which could be attributed to the damage mitigating potential of antioxidants inherent in the seed which although was not within the scope of this study to be evaluated. Electrical conductivity was inversely proportional to seed vigor. Thus, the fact that the electrical conductivity of the seed lots is as follows Lot 1 < Lot 2 < Lot 3 implies that the seed treated with azadirachtin-based insecticide was least vigorous while that treated with α-humulene-based insecticide the most vigorous. High electrical conductivity observed on the leachate obtained from some seed lot could be as a result of loss of membrane integrity and consequent electrolyte leakage that characterises lipid peroxidation in seed. This observation was in tandem with the findings of Shakir et al. (2018) which showed that enhanced reactive oxygen species generation resulting from pesticide application caused membrane damage.

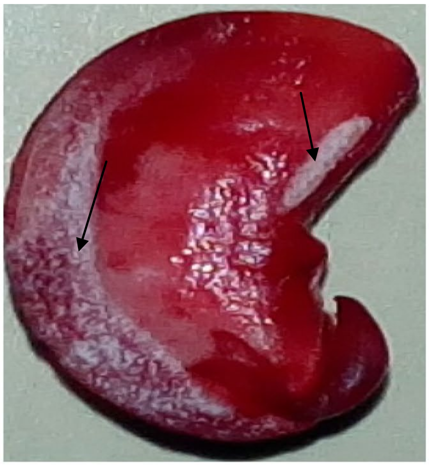


Plate 1: L1-HUM

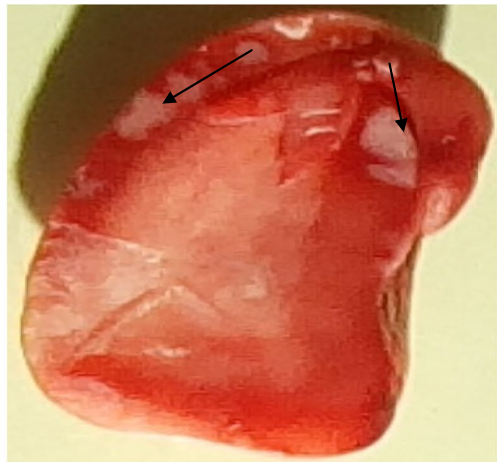


Plate 2: L2-MRY

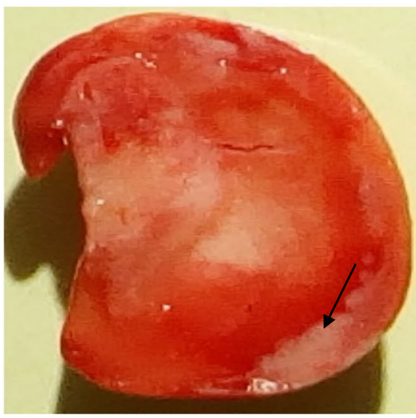


Plate 3: L3-AZA

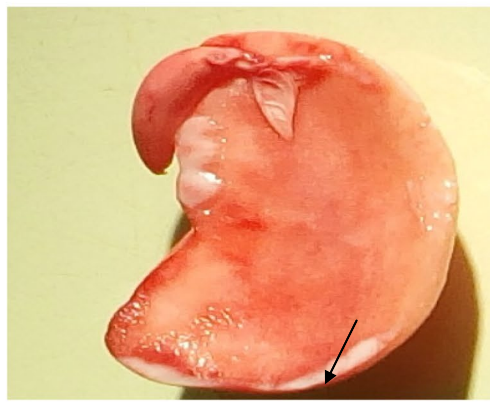


Plate 4: L4-UNT

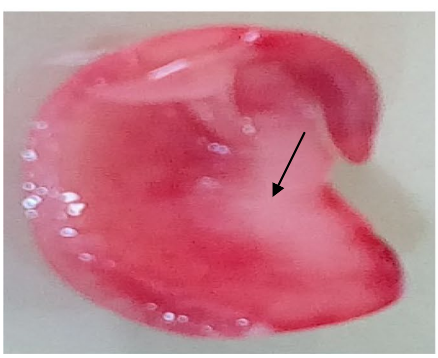


Plate 5: L5-AZA

Plates 1-3 show the photo image of the embryo and cotyledon of cowpea seed (SAMPEA 11) treated with different botanical insecticides, while plate 4 and 5 show the photo images of the embryo and cotyledon of the untreated seed and seed treated with the standard insecticide (chlorpyrifos) respectively.

Key: L1-HUM = LOT-1α-Humulene
L2-MRY = LOT-2 Myristiciin
L3-AZA = LOT-3 Azadirachitin
L4-UNT = LOT-4 Untreated
L5-STD = LOT Standard insecticide

Fig. 4 continued

Conclusions

This work shows that the application of azadirachtin, myristicin and α-humulene based insecticides had no adverse effects on SAMPEA 11(IT89KD-288) and SAMPEA 12 (IT89KD-391). Similarly, α-humulene based insecticide had no adverse effect on SAMPEA 14 (IT99K-1-1), while azadirachtin and myristicin based insecticides impacted negatively on the later although viability was preserved. This implies that prospects abound for botanical insecticides as a plant based options in the protection of stored cowpea seed against *Callosobruchus maculatus* infestation with adequate information on cultivar-insecticide compatibility.

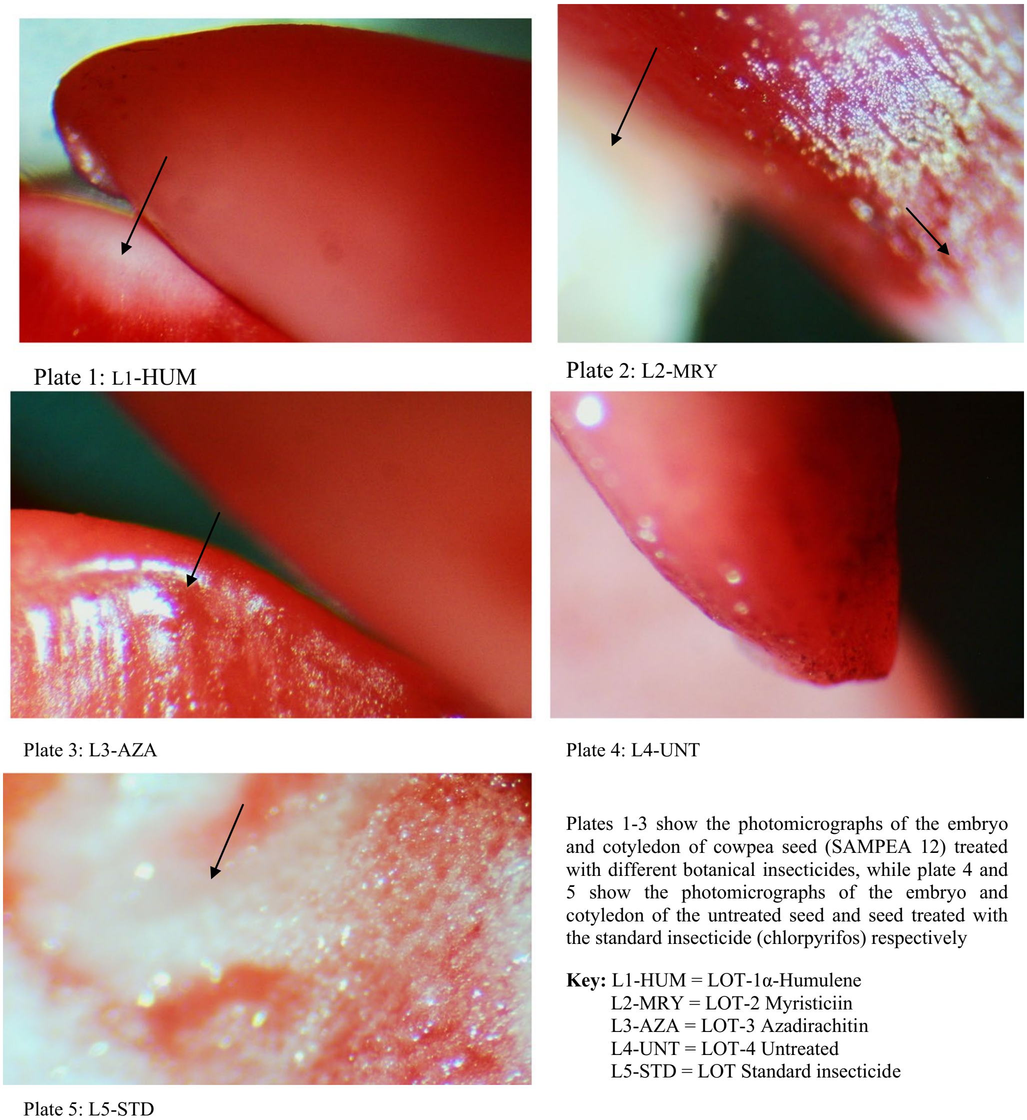


Fig. 4 continued

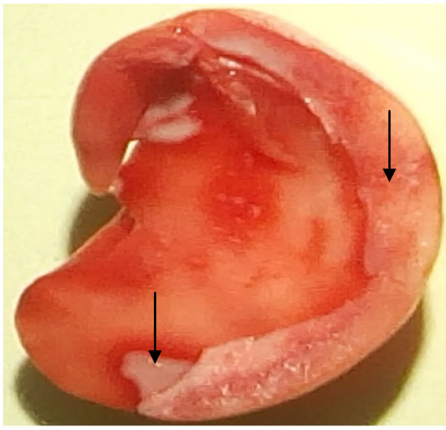


Plate 1: L1-HUM

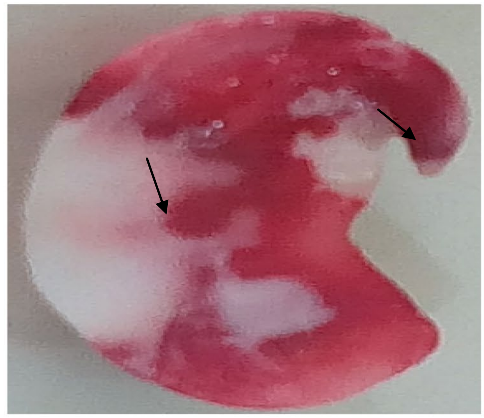


Plate 2: L2-MRY

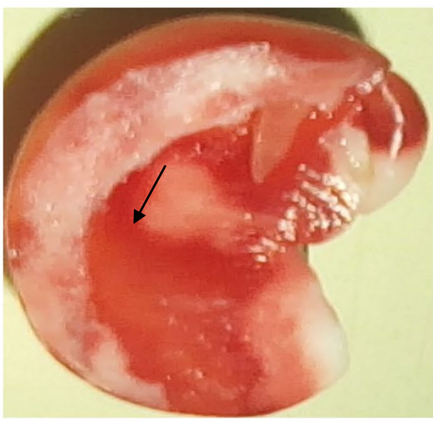


Plate 3: L3-AZA

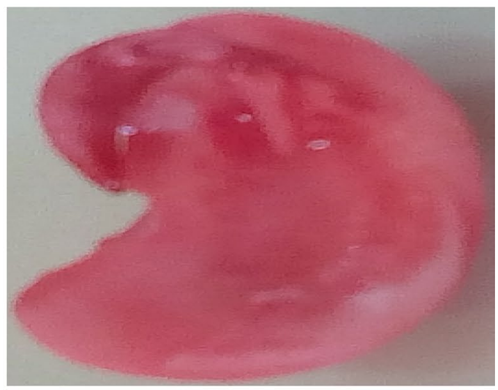


Plate 4: L4-UNT

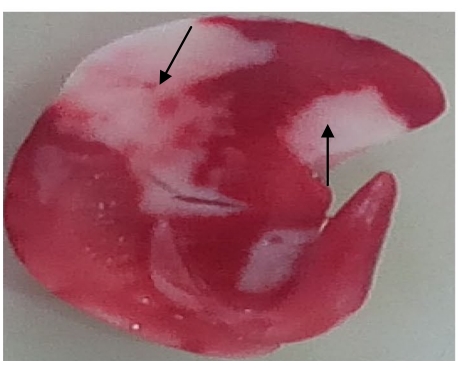


Plate 5: L5-UNT

Plates 1-3 show the photo images of the embryo and cotyledon of cowpea seed (SAMPEA 12) treated with different botanical insecticides, while plate 4 and 5 show the photomicrographs of the embryo and cotyledon of the untreated seed and seed treated with the standard insecticide (chlorpyrifos) respectively

Key: L1-HUM = LOT-1α-Humulene
L2-MRY = LOT-2 Myristiciin
L3-AZA = LOT-3 Azadirachitin
L4-UNT = LOT-4 Untreated
L5-STD = LOT Standard insecticide

Fig. 4 continued

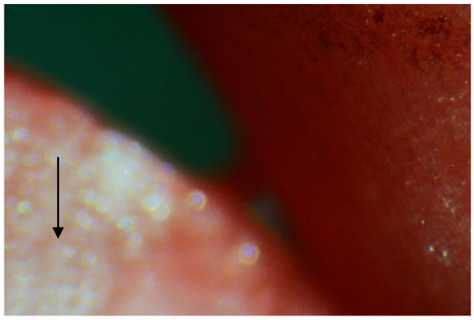


Plate 1: L1-HUM

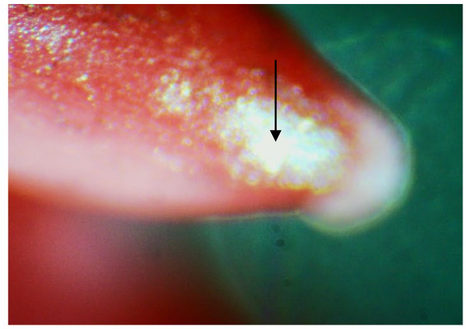


Plate 2: L1-MRY

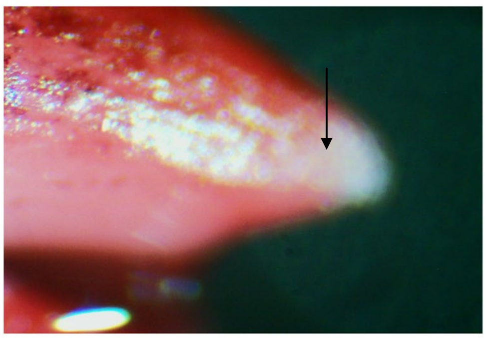


Plate 1: L3-AZA

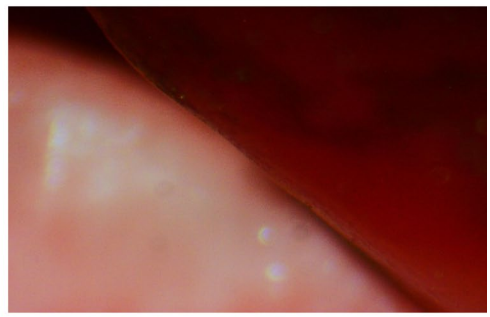


Plate 1: L4-UNT

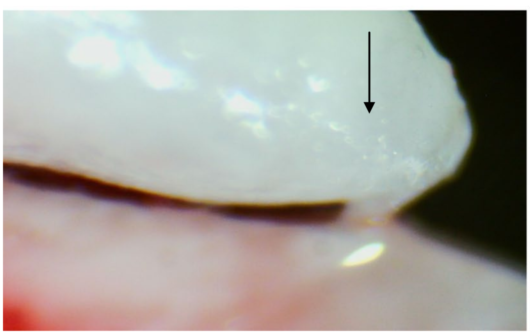


Plate 1: L5-STD

Plates 1-3 show the photomicrographs of the embryo and cotyledon of cowpea seed (SAMPEA 14) treated with different botanical insecticides, while plate 4 and 5 show the photomicrographs of the embryo and cotyledon of the untreated seed and seed treated with the standard insecticide (chlorpyrifos) respectively

Key: L1-HUM = LOT-1α-Humulene
L2-MRY = LOT-2 Myristiciin
L3-AZA = LOT-3 Azadirachitin
L4-UNT = LOT-4 Untreated
L5-STD = LOT Standard insecticide

Fig. 4 continued

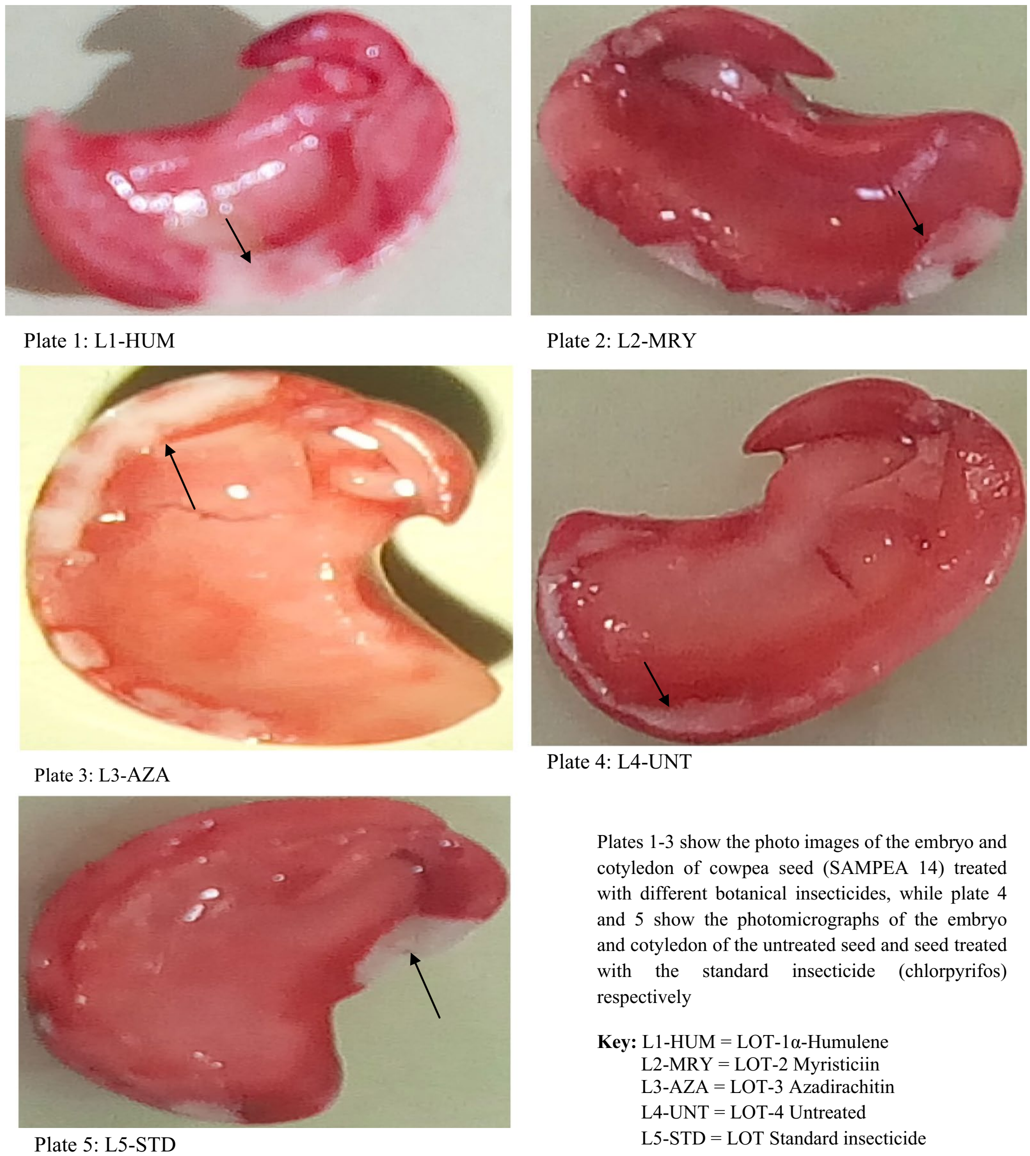


Fig. 4 continued

Table 1 Electrical conductivity of treated cowpea seed treated with botanical insecticides

Electrical conductivity (µs/cm/g of seed)			
Treatment	**SAMPEA 11**	**SAMPEA 12**	**SAMPEA 14**
L1-HUM	186.04 ± 0.16^{b}	212.70 ± 0.00^{d}	186.76 ± 0.16^{b}
L2-MRY	231.88 ± 0.28^{c}	174.21 ± 0.00^{b}	271.95 ± 0.16^{d}
L3-AZA	237.71 ± 0.28^{d}	184.71 ± 0.00^{c}	262.79 ± 0.33^{c}
L4-UNT	167.76 ± 0.00^{a}	139.88 ± 0.16^{a}	168.12 ± 0.00^{a}
L5-STD	256.76 ± 0.00^{e}	338.88 ± 0.16^{e}	354.95 ± 0.16^{e}

Results are expressed as mean ± standard error of mean

Values with different superscripts in a column are significantly ($P < 0.05$) different

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Author contributions
EO conceptualized the research problem, designed the experiment and performed the laboratory activities that produced the final results. AAR, provided the materials with which the article was written, while EIB performed the statistical analysis. VA proof read the manuscript prior to submission. All authors have read and approved the manuscript.

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Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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