

Evaluation of maize tassel ash and *Xylopia aethiopica* powder as protectants against cowpea seed infestation by *Callosobruchus maculatus* Fab. (Coleoptera: Bruchidae)

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Abstract

Callosobruchus maculatus insect is a highly destructive insect pest of cowpea that causes significant damage and threatens global food security. This study aimed to evaluate the protectability potential of maize tassel ash and *Xylopia aethiopica* powder against *Callosobruchus maculatus*. Maize tassel ash (MTA) and *Xylopia aethiopica* powder (XAP) were mixed with 20g of sterilized cowpea seeds at sole concentrations: 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0g and a mixture of 1:1, 2:1, 1:2, 3:1, 1:3. Adult mortality, number of eggs laid, adult emergence and weight loss were recorded to evaluate the insecticidal activity. Adult mortality at 24 h in MTA sole application did not differ significantly (p<0.05) despite an increase in the dosage, compared to XAP, which exerted significant corresponding adult mortality. Seeds treated with 0.4g MTA and above resulted in 100% adult mortality at 48 h, and seeds treated with 0.2g – 0.8g XAP did not significantly differ from control. In MTA and XAP mixture ratios of 1:1, 2:1, and 3:1, 100% adult mortality was observed at 48 h. Sole MTA did not significantly suppress oviposition and adult emergence, compared to 0.2 0.6 and 0.8 XAP. While the treatment mixture significantly subdued oviposition, adult emergence and weight loss contrasted with the sole application. The treatment mixture proved effective against *C. maculatus* infestation and seed damage, hence recommended as a suitable alternative to synthetic chemical control. Further study is envisaged to evaluate increased application rates to develop an appropriate dosage that offers good protection.

Keywords: Maise Tassel Ash; Mixture Ratio; Seeds Protectant; Sole Application; Synergetic Action.

1. Introduction

Cowpea Vigna unguiculata (L) being an important source of dietary protein plays an important role in the nutrition of vegetarians and the common man of emerging countries. *Callosobruchus maculatus* (Fab.) (Coleoptera: Bruchidae) infestation in Nigeria accounts for the astronomical losses during post-harvest storage as the insect multiplies rapidly and causes significant loss ranges between 40 and 100% in

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unprotected cowpea, thus leading to importation from neighbouring countries to supplement local production to guarantee food security. Callosobruchus maculatus infestation causes quantifiable and qualitative damages exhibited by seed damage, seed weight reductions, reduced farmer's income, decreased market worth, seed germination impairment and reduction in nutritional value (Shunmugadevi and Radhika, 2020; Adesina et al., 2022; Adesina, 2022) as a result of the larvae feeding activities (Taiwo et al., 2023). Damaged seeds are unfit for human consumption, and unsuitable for agricultural and commercial purposes (Adesina, 2022; Idoko et al., 2024b). Consequently, these losses of more

than 50% prior to consumption constitute a main risk to food security, self-sufficiency and availability (Seberini, 2020; Adesina, 2022).

Effective management of this all-important agricultural insect pest has been dominated by the adoption of synthetic chemical approaches. Presently used chemical control products suffer from countless disadvantages in terms of low effectiveness due to insect resistance to conventional insecticides or non-eco-friendly formulations which have a lower fringe of safety to non-target creatures (Sandeep et al., 2023). In addition, the toxicity effects of synthetic insecticides on the environment due to soil and water pollution and residue in stored food grains (Adesina, 2022) play a grave impact in the occurrence of several diseases affecting human beings and livestock (Haggag and Shalab, 2022). Owing to the deleterious effect of chemical insecticides and fumigants, exploring other unconventional management approaches that are operative, ecological and affordable is necessary. Utilization of alternate control strategies offers an environmentally approachable and non-toxic substitute to the use of synthetic insecticides for controlling insect pest infestation in the field and postharvest (Haggag and Ali, 2019; Anusha et al., 2020). In search of new control tactics, scientists have deepened their attention on botanicals and other alternative approaches which confer benefits in terms of a higher level of safety to decomposable non-target animals, with efficiency almost at par with synthetic insecticides. In addition, plant products are readily available, require little or no skill or technology for processing and application, have low mammalian toxicity, non-phytotoxicity, and systemic, and are easily biodegradable, resulting in their eco-friendly nature (Ileke et al., 2020; Katarzyna et al., 2021; Gupta et al., 2023). They leave little to no residues in food chains and often mixtures of bioactive chemical contain compounds with multiple modes of action, ranging from acute toxicity, repellent, deterrent or inhibitory properties on oviposition, feeding, growth and development of insect developmental stages (Adesina, 2022; Adesina *et al.*, 2022). The present effort is expected to provide insight into the ability of sole and combined maize tassel ash and *Xylopia aethiopica* powder for the control of *Callosobruchus maculatus* infestation on stored cowpea.

2. Materials and methods

2.1. Experimental Setting

The investigation was carried out under ambient settings of $28 \pm 2^{\circ}$ C temperature, $70 \pm 5\%$ relative humidity and 12 h day: light at the Entomology Laboratory, Department of Crop, Soil and Pest Management Technology, Federal University of Technology, Akure, Ondo State, located in the South-Western part of Nigeria and lies between latitude 50 45" and 80 15" North and longitude 40 30" and 60 East. This treatment consists of sole application pulverized maize tassel ash (MTA) and X. aethiopica (XAP) at the rate of 0.2, 0.4, 0.6, 0.8 and 1.0 g and a mixture of MTA and XAP using the following ratios 1:1, 2:1, 1:2, 3:1, and 1:3 at 0.4 g of the various ratios admixed with 20 g of disinfested cowpea respectively arranged in a completely randomised design replicated thrice.

2.2. Culturing of test insect

The C. maculatus used was obtained from an established culture in the Entomology Laboratory, Department of Crop, Soil and Pest Management, Federal University of Technology, Akure, Nigeria. The emerged adults were subcultured in a 1 L wide-mounted plastic container containing susceptible 'oloyin' local cowpea cultivar in the laboratory under ambient conditions (28 \pm 3°C and 70 \pm 5% relative humidity) until the emergence of adult C. maculatus. The culture container was covered with a fine mesh cloth and a cut cover to allow the flow of air and to also stop the adult cowpea weevils from escaping (Umeanaeto et al., 2020) and this was kept on a shelve in the laboratory. After five (5) days, all introduced adult beetles

were removed; by this time, eggs had been laid on most of the seeds. A day-old freshly emerged adults from the cultures were used for the trial.

2.3. Preparation of Maize Tassel Ash

Maize tassels, a waste part after harvesting were obtained from the Teaching and Research Farm, Federal University of Technology, Akure. The maize tassels were oven-dried, pre-ashed in a cooking pot and the pre-ashed tassels were put in crucibles which were afterwards placed in the furnace and left to ash for 30 minutes at a temperature of 500 °C and allowed to cool. The ashes were collected via the crucibles and stored in 250 ml plastic bottles.

2.4. Preparation of X. aethiopica Powder

Xylopia aethiopica fruits were procured from the local herbal stall at Erekasan market, Akure, Ondo State, Nigeria, the fruit was air dried and blended into powder by using an electric blender (Daewoo Blender BDL849 model), and the blended powder was sieved with 2 mm sieve to obtain fine powder particles of *X. aethiopica* which was packed into black polythene bad and kept in a cool dry place till use for the experiment.

2.5. Preparation of Sample Cowpea Seeds

Oloyin a susceptible local cowpea seeds cultivar was sourced from Teaching and Research Farm, Federal University of Technology, Akure, Nigeria. The sample cowpea seeds with no history of synthetic insecticide preservatives were cleaned of foreign materials, and broken seeds were stored in a tightly covered container and kept in the deep freezer for two weeks for disinfection until needed for the bioassay to eradicate any hidden developmental stage, because the insect developmental stages, in particular the eggs sensitive to cold (Adegbola *et al.*, 2024).

2.6. Protectability Effect of Maize Tassel Ash and X. aethiopica Powder

The protectability effect of both maize tassel ash (MTA) and *X. aethiopica* (XAP) was investigated by exposing 10 unsexed 1-2-day old adult *C. maculatus* to 0.2, 0.4, 0.6, 0.8 and 1.0 g of the pulverized MTA and XAP in 250 ml plastic containers, thoroughly mixed with 20 g

disinfested cowpea seeds to achieve uniform coating of the seeds with the ash and powder. Untreated control was set up and each treatment was replicated three times.

Adult mortality was observed at 24 and 48 h after infestation respectively and insects that did not move any of the appendages or react to a mild pinch of safety pin were considered dead (Adesina and Aderibigbe, 2021). Observed deaths of the adult beetles were modified by Abbott's (1925) formula. Both live and dead insects were removed on the second day after the mortality count. Five days after infestation, the number of eggs laid by treated and untreated cowpea seeds was observed with the aid of a hand lens and eggs present on these grains were counted and recorded (Adesina, 2022). The experimental setup was left undisturbed until the emergence of adult insects, 28 days after infestation and seed weight loss was documented after no emergence was detected for four consecutive days with the grains properly sieved through a 3 mm sieve to remove the emerging insects and frass. Thereafter, the stored grains in each treatment were reweighed to determine weight loss (Adesina and Mobolade-Adesina, 2020). The percentage of adult emergence and weight loss was determined using the formula of Adesina et al. (2015) and Adesina (2012) respectively.

2.7. Protectability Effect of Mixture of Maize Tassel Ash and X. aethiopica Powder on C. maculatus

Following the procedure above, a mixture of MTA and XAP was combined using the following ratios 1:1, 2:1, 1:2, 3:1, and 1:3 for toxicity against *C. maculatus* at 0.4 g of the various ratios admixed with 20 g of disinfested cowpea.

2.8. Data analysis

Before the analysis of data, percentages were normalized and homogenised by subjecting the same to arc sine transformation (Asiry and Zaitoun 2020). Thereafter, the data were subjected to one-way analysis of variance (ANOVA) using the Statistical Package for the Social Science (SPSS 16.0) and new Duncan's Multiple Range Test version 17 was used to compare significant treatment means at p < 0.05 significant levels.

3. Results

3.1. Protectability Effect of Sole Application of Maize Tassel Ash and X. aethiopica Powder on C. maculatus

The result as observed in Table 1 shows that at 24 h after treatment, adult mortality in cowpea seeds treated with sole application of MTA and the control did not differ significantly despite the increase in mortality with the increase in the application rate. But, at 48 h the adult mortality of *C. maculatus* was significantly (p<0.05) higher in the treated seeds than the control, with 100% death documented for insects exposed to other dosages of MTA.

Table 1. Percentage of adult mortality of *Callosobruchus maculatus* in cowpea seeds treated with sole application of maize tassel ash and *X. aethiopica* powder

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Application rates (g/20g)	Maize tassel ash		X. aethiopica	
	24 h	48 h	24 h	48 h
0.0	53.3±3.33a	73.3±6.67a	0.0±0a	10.00±0a
0.2	56.7±3.33a	96.7±3.33b	13.33±3.33 b	23.33±3.33b
0.4	66.7±3.33a	100.0±0.0b	20.00±0.67bc	26.67±3.33b
0.6	70.0±0.00a	100.0±0.0b	23.33±0bcd	30.00±0bc
0.8	73.3±3.33a	100.0±0.0b	30.00±0cd	36.67±0.67bc
1.0	90.0±5.77a	100.0±0.0b	36.67±8.82d	46.67±0.33c

Means in each column bearing the same letter is not significantly different at the 5% level of probability by the Tukey test.

Mean adult mortality of *C. maculatus* exposed to XAP indicated that at 24 h post-treatment, XAP exerted significantly (p<0.05) corresponding adult mortality of *C. maculatus* in response to the increase in XAP application rates (Table 1). A similar inclination was observed at 48 h post-treatment with the highest mortality observed from *C maculatus* exposed to 1.0 g of XAP. The non-significant difference in mortality was documented between 0.6 - 0.8 g and 0.2 - 0.4 at 24 and 48 h post-application correspondingly but statistically different compared to the control.

The mean number of eggs laid and F1 adult emergence in cowpea seeds treated with sole application of MTA and XAP was presented in Table 2. The results revealed that the number of eggs laid on seeds treated with MTA was correspondingly significantly lower (P < 0.05) than the number of eggs laid on unprotected seeds (control). However, the number of eggs laid on seeds treated with 0.2 - 0.8 g of MTA was not statistically significantly different but was different compared to number of eggs laid on cowpea seeds in control and 1.0g dishes (Table 2). In contrast, XAP-treated cowpea seeds recorded significantly (p<0.05) higher number of eggs laid with increasing application rates with cowpea treated with 1.0 g of XAP having the highest number of eggs laid (95.67) compared to the lowest number of eggs laid on cowpea seeds treated with 0.2 g XAP (Table 2).

The emergence of F1 progeny of *C. maculatus* in seeds treated with MTA and XAP powder was significantly suppressed with increasing application rates, with 1.0 g rate having the significantly (p<0.05) lowest adult emergence compared to the highest emergence recorded from control. However, within the treated cowpea seeds, adult *C. maculatus* emergence from 0.2 – 1.0 g of MTA was not statistically different when compared. Likewise, 0.2 – 0.4 and 0.6 – 0.8 g of XAP (Table 2).

The percentage of seed weight loss of cowpea seeds treated with the sole application of MTA and *X. aethiopica* powder revealed that seeds treated with MTA and XAP were not

significantly different from the untreated seeds (control). The highest percentage of weight loss was noted in untreated seeds (control) and the lowest percentage of weight loss was observed in those cowpeas admixed with 1. 0 g of both treatments (Table 3).

Table 2. Mean number of eggs laid and Adult emergence of *C. maculatus* in cowpea seed treated with maize tassel ash and *X. aethiopica* powder

	maize tassel ash		X. aethiopica	
Application rates (g/20 g)	Oviposition	Adult	Oviposition	Adult emergence
		emergence		
0.0	23.33±1.43b	13.33±0.88b	34.67±5.70 a	39.67 <u>+</u> 1.76d
0.2	19.67±5.67ab	7.00±1.53a	27.33±0.88a	27.67 <u>+</u> 1.33c
0.4	18.67±2.52ab	6.33±1.67a	77.67±13.20b	21.33 <u>+</u> 0.88bc
0.6	17.67±1.20ab	4.67±0.33a	63.00±6.11b	15.33 <u>+</u> 1.20b
0.8	13.00±4.37ab	3.67±0.33a	78.00±6.51b	15.00 <u>+</u> 1.53b
1.0	9.33±0.88a	3.33±0.67a	95.67±2.4b	7.33 <u>+</u> 1.45a

Means in each column bearing the same letter is not significantly different at the 5% level of probability by the \overline{T} ukey test.

Table 3. Mean percentage weight loss of cowpea seed treated with maize tassel ash and X. aethiopica powder

Application rates (g/20 g)	maize tassel ash	X. aethiopica
0.0	7.33±0.93a	13.4±1.94a
0.2	7.23±0.18a	13.18±0.67a
0.4	6.50±0.45a	12.87±0.60a
0.6	6.33±0.84a	11.73±0.3a
0.8	6.23±0.47a	11.62±0.79a
1.0	5.88±0.19a	10.81±0.35a

Means in each column bearing the same letter is not significantly different at the 5% level of probability by the Tukey test.

3.2. Protectability effect of Maize Tassel Ash and X. aethiopica Powder mixture on C. maculatus

The result in Table 4 shows that at 24 hours, *C.* maculatus adult mortality in seeds treated with a 3:1 mixture of MTA and XAP induced, the highest mortality narrowly trailed by a 2:1 ratio dosage and the least mortality was observed in seeds treated with ratio 1:2 dosages. However, despite the appreciable mortality recorded with the mixture of MTA and XAP; observed adult mortality was not significantly different when compared. At 48 h, adult mortality of *C.* maculatus in seeds treated with a mixture of MTA and XAP at treatments 1:1, 2:1, 3:1, and 3:1 was not significantly different in comparison but there existed a significant difference (p<0.05) when compared with seeds treated with a mixture of MTA and XAP at 1:2 ratio dosage (Table 4). Table 5 revealed that a mixture of MTA and XAP powder at different treatment ratios significantly subdued the number of eggs laid in treated seeds. Seeds treated with 1:1 ratio treatment recorded significantly fewer laid eggs and 1:3 ratio dosage documented a significantly higher (p<0.05) number of eggs laid. However, the number of eggs laid in seeds treated with a mixture of MTA and XAP at treatment ratios 2:1, 1:2, and 3:1 was not statistically different.

Adult emergence from the treated seeds followed the pattern observed for several eggs laid, though with significant (p<0.05) adult emergence to treatments; with least F1 progeny emergence recorded from 1:1 treatment ratio faithfully followed by 2:1 and 3:1 ratio that was not significantly different. While significant (p < 0.05) maximum adult emergence was noted in seeds treated with a 1:3 treatment ratio (Table 5).

Seed weight loss was highest in cowpea seeds treated with 0.4g of combination treatment of

MTA and XAP at a ratio of 1:3 followed by combination treatment 1:2 respectively (Table 5). While seeds treated with a 3:1 treatment ratio suffered the least weight loss and the percentage weight loss was not significantly different compared to a 2:1 treatment combination.

Table 4. Mean percentage Adult mortality of *Callosobruchus maculatus* in cowpea seeds treated with a combination mixture of maize tassel ash and *Xylopia aethiopica* powder

	% mortality (n = 10)	% mortality (n = 10)
Application rates (0.4 g/20 g)	24 h	48 h
1:1	66.67±5.77a	100±0b
2:1	73.33±8.82a	100±0b
1:2	43.33±8.82a	86.67±3.33a
3:1	80.00±13.33a	100±0b
1:3	63.33±3.33a	96.67±3.33b

Means in each column bearing the same letter is not significantly different at the 5% level of probability by the Tukey test.

Table 5. Mean number of eggs laid and F1 Adult emergence of Callosobruchus maculatus and weight loss in Cowpea seeds treated with Maize Tassel Ash and Xylopia aethiopica powder

Application rates	Oviposition	F1 adult emergence	% weight loss
(0.4g/20 g)			
1:1	1.33±0.33a	1.00±0a	8.1±1.40ab
2:1	8.00±0.60ab	2.66±0.88a	7.98±0.97a
1:2	18.67±0.88b	9.00±1.73b	8.33±0.83bc
3:1	7.00±3.06 ab	2.66±1.2a	5.65±0.30a
1:3	56.33±2.33c	29.33±2.73c	8.72±0.41c

Means in each column bearing the same letter is not significantly different at the 5% level of probability by the Tukey test.

4. Discussion

Lately, there has been a firm intensification in the usage of botanical products as a nontoxic approach to safeguarding stored products against insect infestation and this provides a clue to the present investigation. The results obtained from this research showed that sole application of MTA at 48 hours of treatment was highly effective in the increase of adult mortality across the treatment concentrations in comparison with XAP. The MTA attained higher adult mortality even at lower concentrations. Combinations of MTA and XAP at different ratios exerted significant (p<0.05) adult mortality to treatment ratios and exposure durations with 100%

2:1 and 3:1 respectively. The non-considerable percentage of *C. maculatus* adult mortality noted with XAP is in disagreement with the effectiveness of plant products in evoking significant adult beetle mortality as expressed by several researchers (Ito and Anigboro, 2019; Adesina, 2022; Anaele *et al.*, 2024). The efficacy obtained from the ash may be a result of its physical poison action which ordinarily affects the insect cuticle and causes waterlessness in the insect body thus resulting in their death. This result validates the report of Rahman and Talukdar (2006) where Bablash ash was found to be more effective than nishinda, eucalyptus and Bankhami leaf powder in inducing adult *C*.

observed at 48 h in seeds treated with ratios 1:1,

A blend of MTA and XAP powder in varying

maculatus death. Besides, both ash and powders tend to obstruct the insect respiratory tract thus causing oxygen deprivation, which eventually causes fatality. This result authenticates the outcomes of Oparaeke and Bunmi (2006) where Mahogany wood ash caused *C. maculatus* suffocation and led to their subsequent death. The result resultant effect of the MTA and XAP mixture observed from this study is due to the synergetic potentials of the bioactive chemical substance it contains and this validates the findings of Ileke *et al.* (2016) who reported that a mixture of *M. fragrans* and *A. melegueta* products evoked between 80-100% *S. zeamais* mortality at 24 and 48 h of treatment.

MTA sole treatment concentrations inhibited C. maculatus oviposition and their antiovipositional effect was directly compared to the increase in their application rates. This is opposed to the result obtained from XAP-treated seeds whereby the number of eggs laid increased with the increase in application rates. In both treatments, non-significant oviposition was noted in 0.2 - 0.8 and 0.4 - 1.0 treatment concentrations for MTA and XAP respectively. This result disagreed with the previous work of Shifa et al. (2010) and Orunoye and Okrikata (2010) where plant or botanical products had a reducing effect on the oviposition of insects and that reduction in insect oviposition increased with an increase in treatment concentrations. The efficacy of MTA as revealed in this result conforms with Idoko et al. (2024a) who reported that the greater the amount of wood ash applied for the control of the beetle, C. maculatus, the greater the adverse effect on the beetle oviposition. The significant reduction in oviposition by female C. maculatus might be occasioned by the physical barrier of the ash which prohibited the adult insects from mating and gaining access to the seeds (Wolfson et al., 1991); where the insects are opportune to mate and gain access to the treated seeds, the presences of the ash impaired the attachment of the eggs to the surface of the seed coat.

ratios of combinations reduced adult C. maculatus oviposition rate (number of eggs laid) considerably. Nonetheless, more eggs were laid on seeds treated with a higher ratio of XAP powder concentrations (1:3), compared to the number of eggs laid on cowpea seeds treated with a higher mixture of MTA ratio (3:1). Whereas the seeds treated with equal ratio combination of MTA and XAP powder substantially suppressed oviposition rate of the insect. The result further stresses the relatively poor performance of XAP in the present study. This finding is at variance with previous bioassay that established the efficacy of XAP in reducing stored product insect pest oviposition rate (Ukeh et al., 2012; Adesina et al., 2015b; Ito et al., 2018; Nta and Oku, 2019). The inefficacy of XAP observed from the study The adult beetle emergence reduction was directly proportional to the increase in the concentrations of sole MTA and XAP application. However, none of the MTA application rates significantly suppressed adult beetle emergence when statistically compared. Whereas, application rates of XAP powder expressively inhibited adult beetle emergence. The result also showed that adult beetle emergence was relatively reduced in seeds treated with a mixture of MTA and XAP with treatment ratios 1:1 having the lowest adult beetle emergence and 3:1 ratio recorded the significantly highest adult beetle emergence. MTA adult beetle emergence reduction effects as observed herein were in support of Mazarin et al. (2016), Buraimoh et al. (2000), Aljouri et al. (2020) and Suleiman and Haruna (2020); who acknowledged that wood and cow dung ash reduced F1 adult emergence of C. maculatus. The reduction in adult beetle emergence might be due to the ovicidal and larvicidal potentials of both MTA and X. aethiopica powder which results in the inability of the eggs laid to hatch and the larvae to develop into adult beetles respectively. In a related development, Adesina et al. (2015) opined that the shortened adult life span of C.

maculatus ultimately contributed to oviposition reduction and in turn led to suppression of adult emergence.

Despite the decrease in the percentage of seed weight loss due to the increase in the application rates of sole MTA and XAP powder treatments. None of the treatment and their application rates was able to achieve a significant reduction in weight loss. Whereas, mixture ratio treatment gave a significant (p < 0.05) reduction in seed weight loss with 3:1 treated seeds having the significantly lowest percentage of seed weight loss. The inability of hatched larvae to penetrate the seed for survival, feeding and completion of its developmental stages might be responsible for the observed non-significant weight loss. The reduction in the number of adult beetle emergence with its resultant circular adult exit hole may equally contribute to the observed low weight loss in treated seeds.

5. Conclusion

From the results obtained, the effectiveness of both treatments is directly proportional to the increase in their application rates. Sole application of MTA does not significantly evoke mortality, suppressed adult beetle emergence and weight loss proved compared to XAP. The mixture of both treatments considerably proved effective against C. maculatus infestation, which may be a result of its synergetic action, and the poor performance of XAP can be ascribed to the low application rates employed in the study. Based on the findings from the study, the application of a mixture of MTA and XAP is recommended as a cowpea seed protectant to suppress C. maculatus infestation and seed damage. Thus, guarantee long-term cowpea seed storage to enhance food self-sufficiency and food security. Increased application rates of sole MTA and mixture ratios should be evaluated to come up with an appropriate dosage that can offer good protection against C. maculatus on stored cowpea seeds.

Authors' Contributions

A.M. Augustine conceptualization, design, execution, data collection. J.E. Idoko validation, supervision and data interpretation. J.M. Adesina data interpretation, preparation and drafting of the manuscript. F.S. Ogunsefunmi data collection, and analysis. All authors reviewed and approved the final manuscript.

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Data presented in this study are available upon reasonable request from the corresponding author.

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Consent for Publication

Not applicable.

Conflicts of Interest

The authors disclosed no conflict of interest from the study's conduct, data analysis, and writing until the publication of this research work.

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