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Insecticidal Properties of Blended Crude Extracts Using a Four-Factor Three-Level Box-Behnken Design Against *Callosobruchus maculatus* and GC-MS Characterization of the Active Components

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ABSTRACT

In this study, characterization of plant extracts and evaluation of the insecticidal activity of blended formulations comprising *Calotropis procera* (Cc), *Hyptis suaveolens* (He), *Striga hermonthica* (St), *and Cymbopogon citratus* (Cy*)* essential oils against *Callosobruchus maculatus (C. maculatus),* a cowpea pest, were evaluated using Box-Behnken Design (BBD). GC-MS analysis showed that the Cy contained mostly terpenoid compounds, while the crude extracts of Cc, He and St, were rich in fatty acid methyl esters. The blended formulations showed varying insecticidal activities against *C. maculatus* with mortality ranging from 0.00 to 100% at 24 h, 0.00 to 30% at 48 h and 0 to 70% at 72 h indicating time dependence. Oviposition deterrence ranged from 0.00 to 77.77% indicating variation in reproductive inhibition between formulations. Analysis of variance (ANOVA) and model fitting revealed that the reduced quadratic model for 24 h mortality was significant ($p < 0.05$) whereas the linear models for 48 and 72 h mortality were non-significant ($p > 0.05$). Among the model terms, only He factor ($p = 0.0106$) and the quadratic effect of A^2 ($p = 0.0002$) influenced significantly 24 h mortality. These findings suggest that some blended formulations are potential eco-friendly substituents to synthetic insecticides against *C. maculatus.*

Key words: Insecticidal properties, Blended crude extracts, *Callosobruchus maculatus,* GC-MS characterization

INTRODUCTION

Insect infestation after harvest is a serious issue for food security and sustainable storage of grains in tropical and sub-tropical regions. Cowpea (*Vigna unguiculata)* is an essential source of dietary protein and income for households in Africa [1,2]. However, the crop is highly perishable during storage, mainly due to infestation by *C. maculatus.* This storage pest causes a lot of damage to

grain, loss of seed viability, contamination, nutritional losses and serious economic losses and reduction in germination percentage during storage [3,4]. In severe infestations, the stored grains may be totally destroyed in a matter of months, threatening food and planting materials for subsequent agricultural seasons [5]. Synthetic insecticides have been the significant tool of storage insect control due to their rapid knockdown and wide-ranging spectrum.

However, there are serious concerns about the long-term use of synthetic chemicals, which include insect resistance, environmental persistence, toxic residues in food products, ecological disruption, and health hazards to humans and non-target organisms [6]. These limitations have raised global interest in the search for safer, biodegradable and environmental sustainable alternatives, particularly plant-derived bioactive constituents [7, 8]. Botanical crude extracts and essential oils (EOs) have been suggested as potential alternatives to synthetic pesticides due to their avaiility, degradability, non-toxicity, and complex phytochemical compositions [7, 9].

Plant-derived compounds have been reported to exhibit different beneficial activities such as insecticidal, repellent, antifeedant, ovicidal, antimicrobial and antioxidant activities [10]. These features make them a potential candidate for the protection of stored grains and preservation of grain quality during the storage period [11]. Efficacy of botanical extracts has been promising, but single plant formulations may be limited due to rapid volatilization, short residual activity, variability in potency and concentration-dependent phytotoxicity [12]. Therefore, the present study focuses on the development of blended botanical formulations to enhance possible synergistic interactions among phytochemicals. Combining crude extracts and essential oil may improve the insecticidal efficacy, broaden the spectrum activity, enhance persistence during storage, and reduce the quantity of extract needed for effective protection. Synergistic interaction of bioactive components may therefore provide better preservative effect than single plant treatments used alone.

To optimize blended botanical formulations, there is a need for reliable statistical tools capable of evaluating the effects of multiple variables and their interactions simultaneously**.** Response surface methodology (RSM) has become an important statistical tool in biological and agricultural research because of its efficiency in experimental design, process optimization, and predictive modeling [13]. Among the various RSM designs, the BBD is widely used because it requires fewer experimental runs while providing reliable estimates of the linear, quadratic, and

interaction effects of the independent variables. This design is particularly useful for identifying the optimum combination of botanical crude extracts and essential oil to enhance their insecticidal and preservative efficacy against *Callosobruchus maculatus*. Furthermore, identification and characterization of phytochemical constituents of botanical products are necessary for understanding of the compounds possibly responsible for the biological efficacy and for practical implementation of their use in pest control strategies.

Hence, the present study aims to characterize the phytochemical profiles of selected plant extracts and evaluate the insecticidal potential of their combined formulations against *C. maculatus* using Box-Behnken design optimization approach.

MATERIAL AND METHODS

Sample collection, and preparation

Approximately 3 kg of each selected plant leaf samples from Calotropis *procera*, *Hyptis suaveolens*, *Striga hermonthica*, and *Cymbopogon citratus* along with cowpea grains, were collected from the Nigerian Defence Academy in Kaduna State using clean bags. About 30 kg of uninfected cowpea was sourced from farms. The infected cowpea was collected from Mondo Market in the Igabi Local Government of Kaduna State, Nigeria. All the leaves were subjected to authentication at the herbarium, Department of Biological Sciences, at the Nigerian Defence Academy. The samples were washed with water, air-dried for two weeks, pulverized into a fine powder using a mechanical grinder, filtered, and stored in labeled airtight bottles to prevent weakening of the bioactive compounds. The cowpea grains were cleaned by removing unwanted materials, transferred into zip-lock bags, and frozen for 72 h to eliminate *C. maculatus* or any existing eggs [14]. Then, the frozen cowpea grains were exposed to air to remove residual moisture and transferred into airtight storage bags to avoid exposure to dampness and attack by *C. maculatus.*

Extraction of bioactive compounds from selected plants

Powdered leaves of *Calotropis procera*, *Striga hermonthica*, and *Hyptis suaveolens* (300 g each) were extracted separately by maceration. Each sample was transferred into a 2 L glass bottle and immersed in 1.5 L of 70% ethanol. The bottles were sealed and agitated at 250 rpm for 1 h daily using an orbital shaker. Extraction was carried out for seven days following the AOAC [15]

procedure. The plant residues were then re-extracted twice with fresh solvent, with the final extraction continued for an additional three days, resulting in a total extraction period of 10 days [16]. Filtrates from each extraction were pooled for the respective plant species and filtered through Whatman No. 1 filter paper. The extracts were concentrated under reduced pressure at 40 °C using a rotary evaporator (benchtop model IKA RV 3) and further dried at room temperature to constant weight. The resulting semi-solid crude extracts were stored until required for the bioassays. Essential oil from *Cymbopogon citratus* was obtained by hydrodistillation using a modified Clevenger method [17]. Fresh leaves, cut into 1 to 2 cm pieces, were mixed with distilled water (1:2, w/v) in a 1-L round-bottom flask connected to a Clevenger apparatus. The mixture was heated at 80 °C for 1.5 h, after which the essential oil was collected and stored for subsequent analyses.

Mass rearing of *C. maculatus* for bioassay activity

About 300 g of infected cowpea collected from Mondo Market in Igabi Local Government of Kaduna State, Nigeria was weighed and transferred into 500 ml plastic jar. Using a modified method by Zewde, and Jembere [18], 30 pairs of adult *C. maculatus* were collected after 7 days of incubation and transferred in the three different 400 ml plastic jars containing 300 g of uninfected cowpea for mass rearing. The investigational jars were covered with muslin cloth and tied with a rubber band to allow air flow. The insects (dead and alive) were removed by sieving after 7 days of oviposition and the cowpea grains were retuned back to the incubation jar under the same condition for the development of the first generation (F1) and repeated with F1 for the emergency of the F2 generation.

Insecticidal bioassay of Box-Behnken design for blended plant extract formulations against *C. maculatus*

A four-factor, three-level Box–Behnken Response Surface Design was employed to evaluate the insecticidal and preservative effects of blended Cy essential oil and the ethanol crude extracts of Cc, He, and St against *C. maculatus* in stored cowpea. The factors and their levels were as follows: Cy essential oil (factor A) at 5%, 10%, and 15% (v/v); Cc crude extract (factor B) at 7.5, 13.75, and 20 mg/mL; He crude extract (factor C) at 7.5, 13.75, and 20 mg/mL; and St crude extract (factor D) at 7.5, 13.75, and 20 mg/mL. In coded units, these corresponded to low (-1), medium

(0), and high (+1) levels for each factor. Clean cowpea (100 g) was weighed into triplicate 250 cm^3 plastic jars and treated with 2 mL extract blended concentrations of 29 different runs. The procedure followed the method by Ekeh et al [19], with minor modifications. Grains were manually stirred for uniform coating, air-dried for 1 h at room temperature to evaporate solvents, and then infested with 10 pairs of 2 to 3 day-old *C maculatus* (F2). The jars were covered with muslin cloth secured with rubber bands to allow proper ventilation while preventing insect escape. Observations were recorded at 24 h, 48 h, and 72 h for insect mortality and oviposition count (7 days).

Gas chromatography-mass spectrometry (GC-MS)

GC-MS analysis of the crude extracts and essential oils was carried out using an Agilent 7890B gas chromatograph coupled with an Agilent 5977A Mass Selective Detector (MSD). Separation was performed on an HP-5MS Ultra Inert capillary column (30 m × 0.25 mm i.d. x 0.25 µm film thickness) with helium (99.999% purity) serving as the carrier gas at a constant flow rate of 1.13 mL min^{-1}. Sample injections (1 µL) were made in splitless mode at an injector temperature of 260 ^oC. The oven temperature program was initiated at 110 ^oC and held for 2 min, increased to 200 ^oC at a rate of 10 ^oC min^{-1}, and subsequently raised to 280 °C at 5 °C min^{-1}, where it was maintained for 9 min. The transfer line temperature was set at 260 ^oC. The mass spectrometer was operated in electron ionization (EI) mode at 70 eV, with the ion source and quadrupole temperatures maintained at 230 °C and 150 °C, respectively. Mass spectra were acquired in full-scan mode within a mass range of m/z 50 to 650 following a solvent delay of 5 min. Prior to analysis, crude extracts (0.5 g) were dissolved in ethanol and filtered, whereas essential oils were diluted (1:10, v/v) with hexane. A 1 µL aliquot of each prepared sample was then analyzed under the specified GC-MS conditions. Phytochemical constituents were determined by comparing the sample mass spectra with those in the National Institute of Standards and Technology (NIST) Mass Spectral Library (NIST 23). Compound identification was based on mass spectral matching, retention time, relative peak area, and library match quality using established GC–MS analytical procedures [20, 21].

Data analysis

A four-factor, three-level Box–Behnken design was generated using Design Expert software (Version 13.0.15) to evaluate the interactions and combined insecticidal and preservative effects of the plant-based treatments. The experimental data were analyzed using ANOVA alongside model fitting for reduced linear and quadratic models. Differences among the factors were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

The result of the 4-factors 3-level BBD (Table 1) presented a significant variation in the insecticidal activity of the combined botanical extracts against *C. maculatus*. Both mortality and ovipositon deterrence activity varied between the formulation, demonstrating that the biological activity of the blends was influenced by the concentration and combination of *Cymbopogon citratus* essential oil, *Calotropis procera*, *Hyptis suaveolens*, and *Striga hermonthica*. The insecticidal activity followed a time dependent pattern, with mortalities ranging from 0 to 100% at 24 h, 0 to 30% at 48 h, and 0 to 70% at 72 h, depending on the treatment combination. Similarly, oviposition deterrence (OD%) differed clearly between the treatments ranging from -55.55 to 77.77 %, demonstrating differences in the ability of the formulation to suppress egg laying in *C. maculatus.* At 24 h, the highest mortality (100 %) was recorded in Run 6, which consisted of low Cy (5 %) with low Cc (7.5 mg/mL) and moderate He and St (13.75 mg/mL each).

In comparison, some formulations resulted little or no mortality indicating substantial difference in insecticidal activity among the blends. Mortality generally declined at 48 h and 72 h in most treatments, suggesting that the insecticidal activity of several formulations was strongest shortly after application. The oviposition deterrence results showed a similar variation among formulations. The highest OD value (77.77%) was recorded in Runs 1, 9 and 18, indicating strong inhibition of egg laying by *C. maculatus*. Conversely, negative OD values were observed in Run 14 (-55.55%) and Run 16 (-33.33%), suggesting that these formulations were ineffective in suppressing oviposition and may have been less deterrent than the untreated control. The wide variation observed in both mortality and oviposition deterrence indicates that the biological activity of the blended formulations depended on the specific combinations and concentrations of the botanical components.

Table 1: Insecticidal properties of blended plant extracts using a 4-factor, 3-level Box–Behnken Design (29 runs) for mortality and oviposition (OD%)

Std	Run	A:Cy (%v/v)	B:Cc (mg/ml)	C: He (mg/ml)	D: St (mg/ml)	24 h %	48 h %	72 h %	OD %
1	6	5	7.5	13.75	13.75	100	-	-	77.77
2	13	15	7.5	13.75	13.75	60	10	10	11.11
3	8	5	20	13.75	13.75	80	20	-	66.6
4	16	15	20	13.75	13.75	30	20	50	11.11
5	15	10	13.75	7.5	7.5	40	10	30	44.44
6	9	10	13.75	20	7.5	30	10	30	33.33
7	27	10	13.75	7.5	20	20	0	40	44.44
8	11	10	13.75	20	20	10	0	40	-11.11
9	10	5	13.75	13.75	7.5	60	10	30	77.77
10	5	15	13.75	13.75	7.5	30	20	20	22.22
11	22	5	13.75	13.75	20	30	30	20	22.22
12	3	15	13.75	13.75	20	70	0	20	44.44
13	7	10	7.5	7.5	13.75	60	10	10	66.6
14	18	10	20	7.5	13.75	30	20	50	-55.55
15	17	10	7.5	20	13.75	20	10	30	66.6
16	23	10	20	20	13.75	0	20	40	-33.33
17	29	5	13.75	7.5	13.75	50	30	10	33.33
18	14	15	13.75	7.5	13.75	60	20	20	77.77
19	25	5	13.75	20	13.75	20	10	70	44.44
20	4	15	13.75	20	13.75	10	20	20	33.33
21	19	10	7.5	13.75	7.5	10	0	60	44.44
22	21	10	20	13.75	7.5	10	0	0	22.22
23	28	10	7.5	13.75	20	10	20	20	11.11
24	26	10	20	13.75	20	20	0	20	22.22
25	24	10	13.75	13.75	13.75	20	30	10	33.33
26	12	10	13.75	13.75	13.75	20	20	10	22.22
27	2	10	13.75	13.75	13.75	10	10	20	0.00
28	1	10	13.75	13.75	13.75	0	0	20	22.22
29	20	10	13.75	13.75	13.75	30	10	50	0.00

The ANOVA results (Tables 2-6) indicated that the reduced quadratic model for 24 h mortality was significant ($F = 5.28$, $p = 0.0014$), which suggests that the model was able to adequately explain the variability in insect mortality across the blended formulations. The non-significant lack-of-fit ($p = 0.1762$) also supported the adequacy of the model for prediction of the response. Among the terms in the models, the concentration of *Hyptis suaveolens* was significantly affecting the mortality ($p = 0.0106$) and the quadratic effect of *Cymbopogon citratus* essential oil (Cy2) was

highly significant (p = 0.0002). These data indicated a non-linear response of essential oil concentration to the level of *Hyptis suaveolens* and these were the main factors affecting the mortality. Regression equations fitted for 24 h mortality were: Y = 20.00 - 6.67A - 7.50B - 14.17C - 1.67D - 5.00AC + 17.50AD + 30.00A2 where A = Cy essential oil, B = Cc, C = He and D = St. The magnitude of the coefficients shows the relative influence of the factors on the mortality action. Between the main effect *Hyptis suaveolens* (C = - 14.17), demonstrated largest coefficient, signifying that it contributed most strongly to 24 h mortality. The positive quadratic coefficient of A2 (+30.00) also showed that the effect of *Cymbopogon citratus* essential oil was concentration-dependent and was not a linear response. Although the model retained the interaction terms AC (−5.00) and AD (+17.50) but the ANOVA indicated that these interactions were not statistically significant (p > 0.05). The AD interaction (Cy×St) was marginal (p = 0.0585) suggesting a possible, but weak effect on the mortality response.

The model indicated that *Hyptis. suaveolens* and the concentration-dependent effect of *Cymbopogon citratus* essential oil were the main factors controlling mortality at 24 h. If the final model retains only linear terms (A, B, C, and D) and no interaction (AB, AC, BC) or quadratic terms (A^2, B^2, etc.), it means that the software found no significant interaction or curvature effects among the factors, as shown in Tables 4 and 5. It suggests that the software did not find any significant interaction or curvature effects among the factors. The linear models for the 48 h and 72 h mortality were not significant (p = 0.4008 and p = 0.8423, respectively), suggesting that the factors selected did not sufficiently explain the variation in mortality observed at these exposure periods. This suggests that the insecticidal response was not significantly influenced by interactive or non-linear effects at later exposure times, and may reflect reduced complexity of factor influence under these conditions.

Thus, optimization and predictive modeling are better addressed to the 24 h mortality response where factor effects were more pronounced and statistically meaningful. The quadratic model was significant (F = 3.30, p = 0.0217) for oviposition deterrence, with a non-significant lack-of-fit (p = 0.1075), indicating satisfactory model performance. The concentration of *Calotropis procer* significantly affected the oviposition deterrence (p=0.0146). The quadratic effect of *Cymbopogon citratus* essential oil was also significant (p=0.0264). The results show that the inhibition of egg laying in *C. maculatus* was mainly controlled by the concentration of

Calotropis procera and concentration-dependent response pattern of essential oil, and not by the interaction effects between factors.

Table 2: Model fitting and ANOVA for reduced quadratic model (24 h % Mortality).

Source	Sum of Squares	Df	Mean Square	F-value	p-value	Remark
Model	11306.03	7	1615.15	5.28	**0.0014**	Significant
A-Cy	533.33	1	533.33	1.74	0.2009	
B-Cc	675.00	1	675.00	2.21	0.1523	
C-He	2408.33	1	2408.33	7.87	**0.0106**	
D-St	33.33	1	33.33	0.1089	0.7446	
AC	100.00	1	100.00	0.3268	0.5736	
AD	1225.00	1	1225.00	4.00	0.0585	
A^2	6331.03	1	6331.03	20.69	**0.0002**	
Residual	6425.00	21	305.95			
Lack of Fit	5905.00	17	347.35	2.67	0.1762	not significant
Pure Error	520.00	4	130.00			
Cor Total	17731.03	28				

Terms with p-values < 0.050 were considered significant and are shown in bold.

Table 3: Coefficients in Terms of Coded Factors for 24 h

Factor	Coefficient Estimate	Df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	20.00	1	4.24	11.18	28.82	
A-Cy	-6.67	1	5.05	-17.17	3.83	1.0000
B-Cc	-7.50	1	5.05	-18.00	3.00	1.0000
C-He	-14.17	1	5.05	-24.67	-3.67	1.0000
D-St	-1.67	1	5.05	-12.17	8.83	1.0000
AC	-5.00	1	8.75	-23.19	13.19	1.0000
AD	17.50	1	8.75	-0.6878	35.69	1.0000
A^2	30.00	1	6.59	16.29	43.71	1.0000

Table 4: Model fitting and ANOVA for linear model for 48 h % mortality

Source	Sum of Squares	Df	Mean Square	F-value	p-value	Remark
Model	516.67	5	103.33	1.07	0.4008	not significant
A-Cy	8.33	1	8.33	0.0866	0.7712	
B-Cc	75.00	1	75.00	0.7790	0.3866	
C-He	33.33	1	33.33	0.3462	0.5620	
D-St	0.0000	1	0.0000	0.0000	1.0000	
AD	400.00	1	400.00	4.15	0.0532	
Residual	2214.37	23	96.28			
Lack of Fit	1694.37	19	89.18	0.6860	0.7459	not significant
Pure Error	520.00	4	130.00			
Cor Total	2731.03	28				

Table 5: Model fitting and ANOVA for linear model for 72 h % mortality

Source	Sum of Squares	Df	Mean Square	F-value	p-value	Remakk
Model	500.00	4	125.00	0.3487	0.8423	not significant
A-Cy	8.33	1	8.33	0.0232	0.8801	
B-Cc	75.00	1	75.00	0.2092	0.6515	
C-He	408.33	1	408.33	1.14	0.2965	
D-St	8.33	1	8.33	**0.0232**	0.8801	
Residual	8603.45	24	358.48			
Lack of Fit	7523.45	20	376.17	1.39	0.4103	not significant
Pure Error	1080.00	4	270.00			
Cor Total	9103.45	28				

Table 6: Model fitting and ANOVA for quadratic model for OD%

Source	Sum of Squares	Df	Mean Square	F-value	p-value	Remark
Model	11773.63	5	2354.73	3.30	**0.0217**	Significant
A-Cy	1243.39	1	1243.39	1.74	0.2000	
B-Cc	4975.98	1	4975.98	6.97	**0.0146**	
C-He	504.01	1	504.01	0.7058	0.4095	
D-St	1028.60	1	1028.60	1.44	0.2423	
A^2	4021.65	1	4021.65	5.63	**0.0264**	
Residual	16423.62	23	714.07			
Lack of Fit	15534.91	19	817.63	3.68	0.1075	not significant
Pure Error	888.71	4	222.18			
Cor Total	28197.25	28				

The RSM contour and three-dimensional (3D) response surface plots were generated to evaluate the interaction between Cy and He on the 24-h mortality of C. maculatus, with Cc and St fixed at their center-point concentrations (13.75 mg/mL). These plots were used to visualize factor interactions and identify the optimum response region (Figures 1-3).

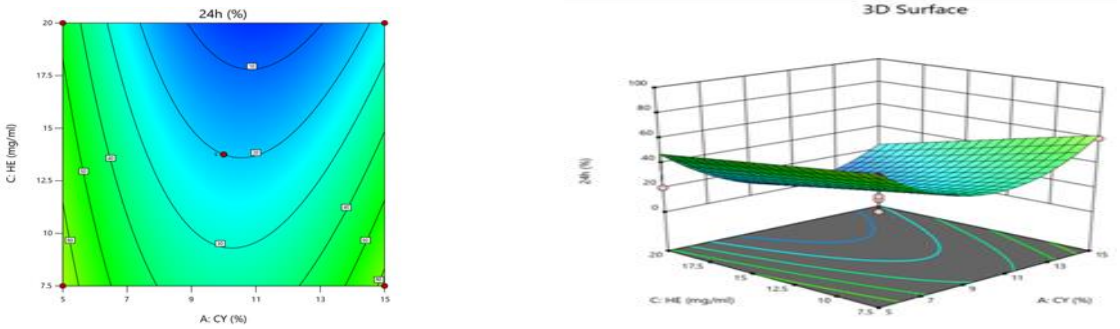

Figure 1: Response surface methodology contour and 3D surface plots of 24-hour mortality (A × C Interaction).

The interaction between Cy and St on the 24-h mortality of *C. maculatus* was evaluated using contour and three-dimensional (3D) response surface plots, while the concentrations of Cc and He were maintained at their center-point levels (13.75 mg/mL).

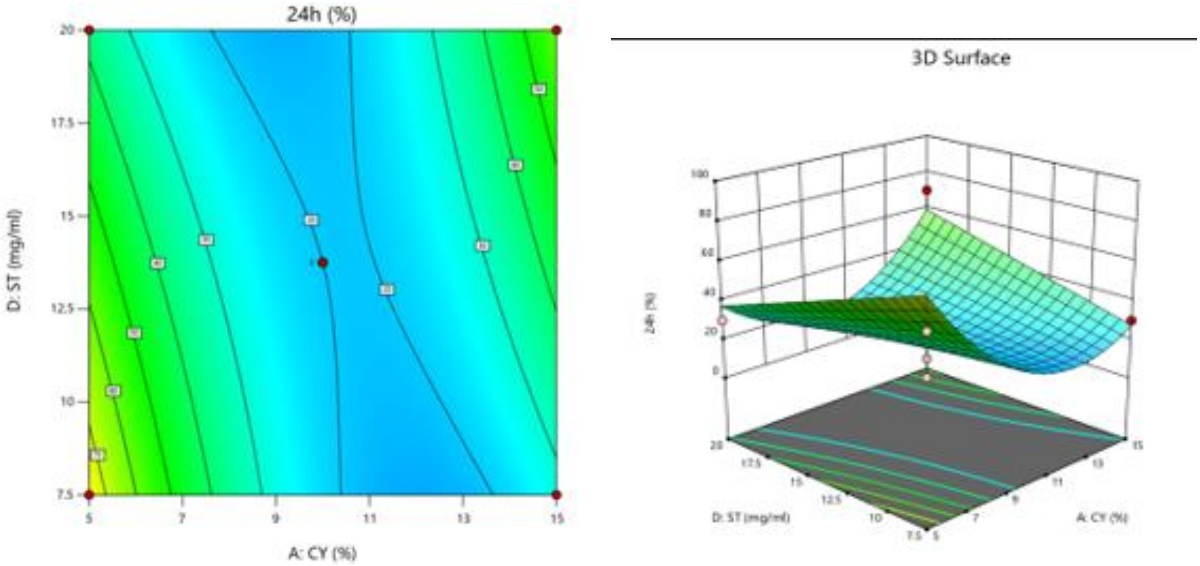

Figure 2: Response surface methodology contour and 3D surface plots of 24-hour mortality (A × D Interaction)

The RSM contour and three-dimensional (3D) response surface plots were used to evaluate the interaction between Cy and Cc on the oviposition deterrence (OD%) of *C. maculatus*. He and St were maintained at their center-point concentrations (13.75 mg/mL) to illustrate the combined effects of Cy and Cc on oviposition deterrence.

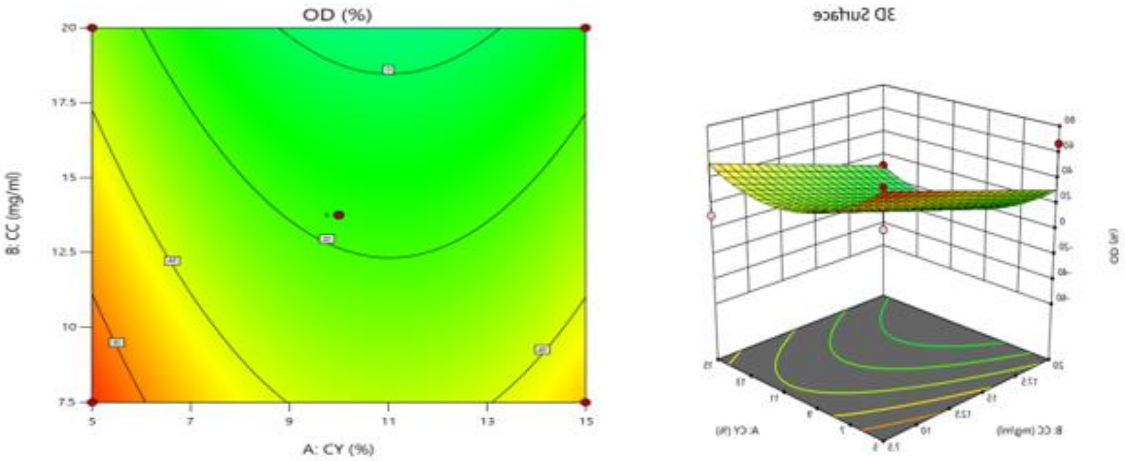

Figure 3: Response surface methodology contour and 3D surface plots of OD% (A × B interaction).

The contour and 3D response surface plots (Figure 1) illustrating the interaction between Cy essential oil and He at fixed levels of the other factors (B and D = 13.75 mg/mL) revealed a generally smooth and gently curved surface. The contour and 3D response surface plots (figure 1) illustrating the interaction between Cy essential oil and He extract at fixed levels of the other factors (B and D = 13.75 mg/mL) revealed a generally smooth and gently curved surface. The numerical values displayed on the contour plot (60, 50, 40, 30, 20, and 10) represent predicted 24-hour mortality percentages generated from the response surface model. The contour plot exhibits broadly spaced, slightly elliptical contour lines, confirming a weak interaction between Cy and He.

The response pattern shows a clear U-shaped trend along the Cy axis, where mortality is higher at both low (5 %) and high (15 %) Cy levels ranged 60 to 40 while intermediate concentrations (around 10 to11 %) correspond to the lowest mortality region (approximately 10–30%). This pattern reflects the strong quadratic effect of Cy observed in the ANOVA results. In contrast, variation along the He axis produces only a gradual change in mortality, indicating a weaker, predominantly linear contribution. The 3D surface plot further confirms this behavior, forming a shallowed U-shaped surface dominated by curvature along the Cy axis. The surface dips in the central region and rises toward both ends of the Cy range, supporting the non-linear response pattern. Along the He axis, the surface remains relatively flat with mild gradients, indicating limited influence on the overall response shape. The non-significant interaction term (A x C) is visually supported by the lack of twisting or strong directional coupling between the two variables on the surface. The overall shape of the surface indicates that the interaction between Cy and He was weak, as reflected by the non-significant AC term ($p > 0.05$). Therefore, the variation in 24 h mortality in this region of the design space is mainly driven by the individual and nonlinear effect of Cy rather than a strong synergistic interaction between the two factors. The contour and 3D surface plots (figure 2) of the Cy x St interaction exhibited a response pattern similar to that observed for Cy x He, mortality was highest at low Cy concentrations (approximately 5%), declined markedly at intermediate concentrations (9 to 11 %), and increased again at higher Cy levels (13 to 15 %), confirming the significant quadratic effect of Cy observed in the ANOVA. In contrast, St exerted only a modest influence on the response, slightly modifying the depth and shape of the mortality trough. Thus, the Cy x St interaction was weak but detectable, consistent

with the borderline significance of the A x D term (p = 0.0585). Overall, the contour and 3D surface plots demonstrate that 24-hour mortality was governed mainly by the nonlinear effect of Cy rather than by a strong Cy × St interaction. The interaction between Cy and Cc on OD % was analyzed using contour and 3D surface plots (figure 3) derived from the reduced quadratic model. The ANOVA results indicated that Cc (p = 0.0146) and the quadratic term of Cy (Cy^2, p = 0.0264) were the only significant contributors to OD %, confirming a predominantly nonlinear Cy effect with a modulatory influence of Cc.

The contour plot showed that OD % was highest (approximately 60 %) at low Cy and Cc levels, but declined as Cy increased, reaching minimum values (0 to 20 %) in the mid-range region (Cy: 9 to 11 %; Cc: 13 to 19 mg/mL). A partial recovery to approximately 40 % was observed at higher Cy levels (13 to 14 %), indicating a U-shaped response along the Cy axis. The 3D surface plot supported this pattern, revealing a pronounced depression at intermediate Cy-Cc combinations and a secondary elevation at higher Cy concentrations. Overall, the contour and 3D surface plots demonstrate that OD % was influenced primarily by the quadratic effect of Cy, with Cc contributing to the suppression of the response, particularly within the intermediate concentration range. The GC-MS analysis of the ethanoic extract of *Calotropis procera* , *Hyptis suaveolens, Striga hermonthica* and essential oil of *Cymbopogon citratus* showed the total ion chromatogram (TIC) in Figures 4 to 7.

The GC-MS analysis (Table 7) revealed distinct differences in phytochemical profiles of botanical extracts that may explain their different contribution to the response surface methodology (RSM) optimization model

Table 7: Gas chromatography-mass spectrometry analysis of He, Cc and St

Extracts	Major constituent	Molecular Formula	Retention Time	Relative abundance (%)	Quality (%)
Cy	Neric acid	$C_{10}H_{16}O_2$	5.218	9.96	38
	2,6-Octadienal	$C_{10}H_{16}O$	14.990	2.32	64
He	Octadecenoic acid methyl ester	$C_{19}H_{36}O_2$	13.327	53.49	98
	Pentadecanoic acid methyl ester	$C_{16}H_{30}O_2$	11.559	17.64	93
St	13-Octadecenoic acid	$C_{19}H_{36}O_2$	13.057	44.22	95

	Pentadecanoic acid,methyl ester	$C_{17}H_{34}O_2$	11.227	17.75	96
Cc	Octadecenoic acid methyl ester	$C_{19}H_{36}O_2$	13.072	37.65	96
	Hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	11.225	17.49	95

The major components of the essential oil of *Cymbopogon citratus* were oxygenated monoterpenes, namely the citral isomers neral and geranial, which are known to exhibit insecticidal, fumigant, repellent and neurotoxic activities against stored-product insects [7, 22-25]. The abundance of these bioactive constituents likely accounts for the higher insecticidal efficacy of *C. citratus* and its major contribution to the optimized botanical blends. On the contrary, *Hyptis suaveolens*, *Calotropis procera* and *Striga hermonthica* were identified predominantly by fatty acid methyl esters (FAMEs) like hexadecanoic acid methyl ester, 9-octadecenoic acid methyl ester and pentadecanoic acid methyl ester with relatively lower quantities of terpenoid constituents. Such compounds, although they have insecticidal activity, generally work by disrupting membranes and causing physiological stress, rather than the rapid neurotoxic effects that are observed with oxygenated monoterpene [7]. The difference in phytochemical composition might explain the comparatively low contributions of these botanicals to the optimization model.

The agreement between the GC-MS profiles and the RSM optimization results indicates that the enhanced efficacy of the optimized blends was principally related to the phytochemical richness of *Cymbopogon citratus*, while the other botanicals probably played complementary or synergistic roles. Furthermore, the high desirability values and the good predictive performance of the fitted quadratic model confirm the reliability and robustness of the optimized formulations, highlighting the importance of coupling RSM with GC-MS characterization for the development of effective botanical insecticides for controlling C. maculates. GC-MS analysis revealed the presence of a wide range of phytochemical constituents in the botanical extracts which correlated with the observed insecticidal activities. The present study is mainly focused on the optimization and biological efficacy of the botanical formulations.

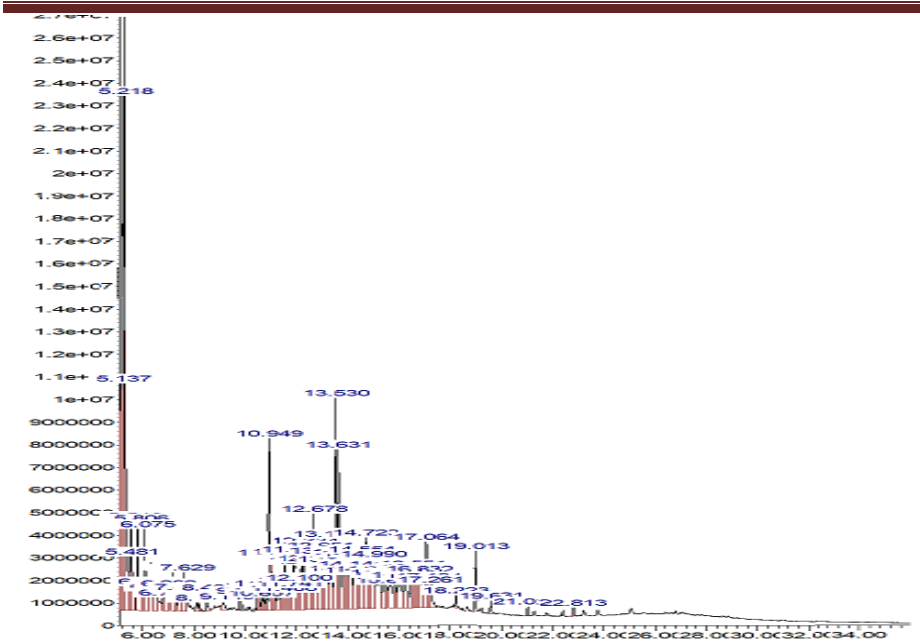

Figure 4: Total ion chromatogram (TIC) of *Cymbopogon citratus* essential oil obtained by GC–MS analysis.

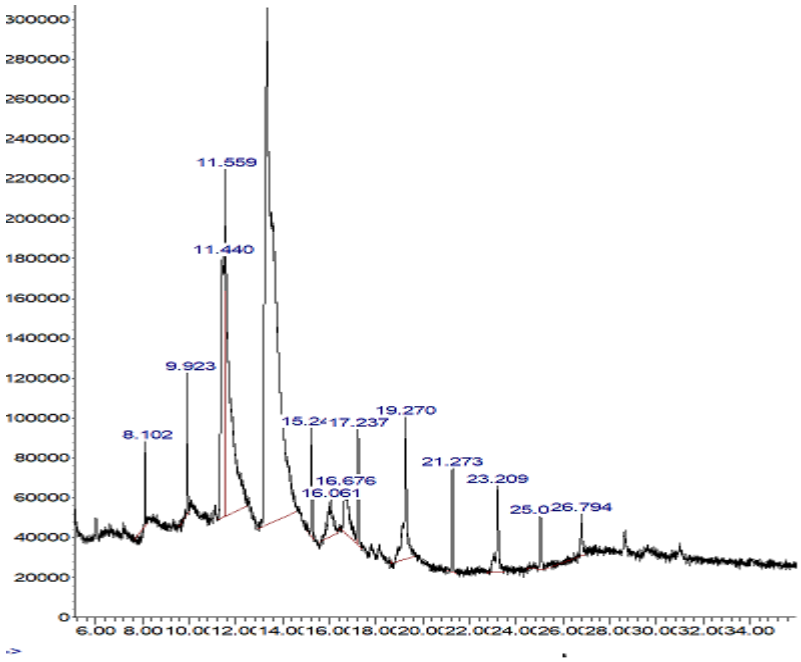

Figure 5: Total ion chromatogram of the ethanoic extract of He obtained by GC-MS

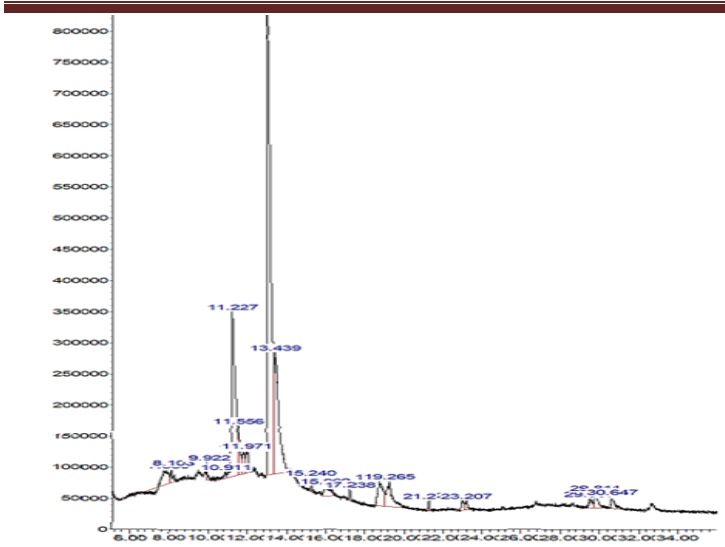

Figure 6: Total ion chromatogram of the ethanoic extract of *Striga hermonthica* obtained by GC–MS analysis.

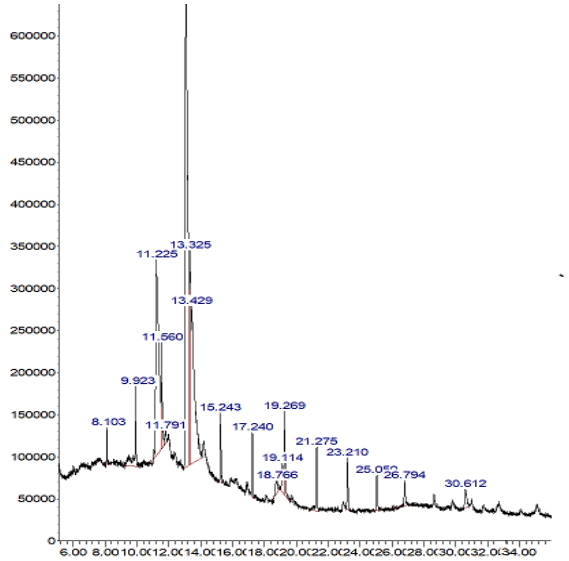

Figure 7: Total ion chromatogram of the ethanoic extract of *Calotropis procera* obtained by GC-MS analysis.

The chromatogram obtained showed several peaks at different retention times, confirming the presence of different volatile phytochemical constituents. The observed insecticidal activity is in accordance with the chemical complexity of the extracts.

CONCLUSION

Response surface analysis showed that mortality was influenced mainly by the individual and nonlinear effects of the botanical extracts, with *Cymbopogon citratus* exerting the greatest effect, while interaction effects among the extracts were generally weak. Box–Behnken Design successfully optimized the botanical formulations, with the predicted mortality exceeding 85%, demonstrating the robustness of the model and the effectiveness of the optimized extract combinations. GC-MS analysis revealed that the optimized formulation contained terpenoid-rich Cy essential oil and fatty acid methyl ester (FAME)-rich extracts of Cc, He, and St. The optimized blend (run 6) functions as an effective botanical insecticide against *Callosobruchus maculatus.*

REFERENCES

[1] Idoko, J. E. & Ileke, K. D. (2020). Comparative evaluation of insecticidal properties of essential oils of selected botanicals as biopesticides against the cowpea bruchid, *Callosobruchus maculatus* (Fabricius), in stored cowpea. *Bulletin of the National Research Centre, 44*, 119.

[2] Obembe, O. M., Ojo, D. O. & Ileke, K. D. (2020). Efficacy of *Kigelia africana* (Lam.) Benth. Plant extracts against the cowpea seed beetle, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae), infesting stored cowpea (*Vigna unguiculata*) seeds. *Heliyon, 6*(10), e05256.

[3] Mofunanya, A. A. & Nta, A. I. (2016). Effect of Callosobruchus maculatus (Coleoptera: Bruchidae) and Acanthoscelides obtectus Say infestation on the qualitative and quantitative phytochemicals of Vigna unguiculata, Vigna aconitifolia, Phaseolus vulgaris, Phaseolus lunatus, and Phaseolus acutifolius. *IOSR Journal of Environmental Science, Toxicology and Food Technology*, 10(12), 13 - 18.

[4] Ileke, K. D., Adesina, J. M., Abidemi-Iromini, A. O. & Abdulsalam, M. S. (2021). Entomocidal effect of Alstonia boonei De Wild. on the reproductive performance of Dermestes maculatus (Coleoptera: Dermestidae) infesting smoked African catfish (Clarias gariepinus*). International Journal of Tropical Insect Science,* 41(2), 1293 - 1304.

[5] Fox, T. (2013). Global food: Waste not, want not. Institution of Mechanical Engineers.

[6] Alzahrani, S. M. & Ebert, P. R. (2023). Pesticidal toxicity of phosphine and its interaction with other pest control treatments. *Current Issues in Molecular Biology*, 45(3), 2461 - 2473.

[7] Isman, M. B. (2020). Botanical insecticides in the twenty-first century? *Annual Review of Entomology*, 65, 233-249.

[8] Okagu, I. U., Okeke, E. S., Ezeorba, W. C. F. & Ndefo, J. C., (2023). Overhauling the ecotoxicological impact of synthetic pesticides using plants' natural products: A focus on Zanthoxylum metabolites. *Environmental Science and Pollution Research*, 30, 77063 - 77087.

[9] Lengai, G. M. W., Muthomi, J. W. & Mbega, E. R. (2022). Use of botanical pesticides in agriculture as an alternative to synthetic pesticides. *Agriculture*, 12(5), 600. https://doi.org/10.3390/agriculture12050600.

[10] Chaudhari, A. K., Singh, V. K., Kedia, A., Das, S. & Dubey, N. K. (2021). Essential oils and their bioactive compounds as eco-friendly novel green pesticides for management of storage insect pests: Prospects and retrospects. *Environmental Science and Pollution Research*, 28, 18918 - 18940.

[11] Nair, A., Mallya, R., Suvarna, V., Khan, T. A., Momin, M. & Omri, A. (2022). Nanoparticles: Attractive carriers of antimicrobial essential oils. *Antibiotics*, 11(1), 108.

[12] Pavela, R. (2016). History, presence and perspective of using plant extracts as commercial botanical insecticides and farm products for protection against insects: A review. *Plant Protection Science,* 52(4), 229 - 241.

[13] Mvumi, B. M. & Stathers, T. E. (2003). Challenges of grain protection in sub-Saharan Africa: The case of diatomaceous earths. In *Proceedings of the Food Africa Internet-Based Forum.*

[14] Babarinde, S. A., Oyedeji, S. O. & Adebayo, M. A. (2021). Eco-friendly management of cowpea bruchid, Callosobruchus maculatus (F.) (Coleoptera: Chrysomelidae), using botanical powders. *International Journal of Tropical Insect Science*, 41(2), 1073 - 1083.

[15] AOAC International. (2019). Official methods of analysis (21st ed.). AOAC International.

[16] Azwanida N. N. (2015). A review on the extraction methods used in medicinal plants: Principle, strength and limitation. *Medicinal & Aromatic Plants*, 4(3), 196.

[17] Amaral W., Deschamps C., Bizzo, H. R., Pinto, M. A. S., Biasi, L. A. & Silva, L. E. (2017). Essential oil yield and composition of native tree species from the Atlantic Forest, southern Brazil. *Journal of Essential Oil Bearing Plants*, 20(6), 1525 - 1535.

[18] Zewde D. K. & Jembere B. (2010). Evaluation of orange peel (Citrus sinensis L.) as a source of repellent, toxicant and protectant against Zabrotes subfasciatus (Coleoptera: Bruchidae). *Momona Ethiopian Journal of Science*, 2(1).

[19] Ekeh F. N., Okoli, C. G. & Ezenwaji N. E. (2013). Comparative efficacy of five botanical powders and pirimiphos-methyl against *Callosobruchus maculatus* in stored cowpea seeds. *Journal of Biopesticides, 6*(1), 36 - 44.

[20] Lovestead T. M. & Urness, K. N. (2019). Gas chromatography–mass spectrometry (GC/MS). In *ASM Handbook* (Vol. 10, *Materials Characterization*, pp. 235 to 241). ASM International.

[21] National Institute of Standards and Technology. (2023). NIST/EPA/NIH Mass Spectral Library (NIST 23). U.S. Department of Commerce.

[22] Alves M. S., Campos I. B., Brito D. M. C., Cardoso C. W., Pontes E. G. & Souza M. A. A. (2019). Efficacy of lemongrass essential oil and citral in controlling *Callosobruchus maculatus* (Coleoptera: Chrysomelidae), a postharvest cowpea insect pest. *Crop Protection, 119*, 191 - 196.

[23] Gupta H., Deeksha, Urvashi, & Reddy S. G. E. (2023). Insecticidal and detoxification enzyme inhibition activities of essential oils for the control of pulse beetle, *Callosobruchus maculatus (F*.) and *Callosobruchus chinensis* (L.) (*Coleoptera: Bruchidae*). *Molecules*, 28(2), 492.

[24] Abdelgaleil S. A. M., Gad H. A., Ramadan G. R. M., El-Bakry A. M. & El-Sabrout A. M. (2024). Monoterpenes: Chemistry, insecticidal activity against stored product insects and modes of action-a review. *International Journal of Pest Management*, 70(3), 267-289.