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Research Article

Optimizing the Proportions of Essential Oils as *Aedes aegypti* Repellent using Response Surface Methodology

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ABSTRACT

Mosquito-borne diseases, particularly those transmitted by *Aedes aegypti*, pose significant global health risks. Chemical repellents such as DEET are widely used; however, concerns regarding toxicity and environmental safety have increased interest in natural alternatives. Essential oils, with diverse bioactive properties, represent a promising option, though their repellent efficacy depends on the type and proportion of active compounds. This study employed Response Surface Methodology (RSM) to optimize the proportions of neem, lemongrass, sesame, and mint oils for developing an effective plant-based repellent. A total of 27 formulations were prepared and tested against *A. aegypti*. Analysis of Variance (ANOVA) confirmed the statistical significance of the model, demonstrating that repellent activity was strongly influenced by essential oil composition. The optimized formulation consisted of 37.931 parts neem oil, 69.338 parts lemongrass oil, and 2.828 parts sesame oil, with no mint oil. This combination achieved a predicted effectiveness score of 51.548 and a desirability index of 1.00, indicating high performance and stability. Results highlighted neem and lemongrass oils as the primary contributors to repellency, while sesame oil enhanced formulation stability. Overall, the study successfully optimized an essential oil-based repellent, offering a safe, natural, and sustainable alternative to synthetic chemicals with potential for further development and commercialization.

Keywords: *Aedes aegypti*, Essential oils, Plant-based repellent, Response Surface Methodology, Sustainable vector control.

Article History

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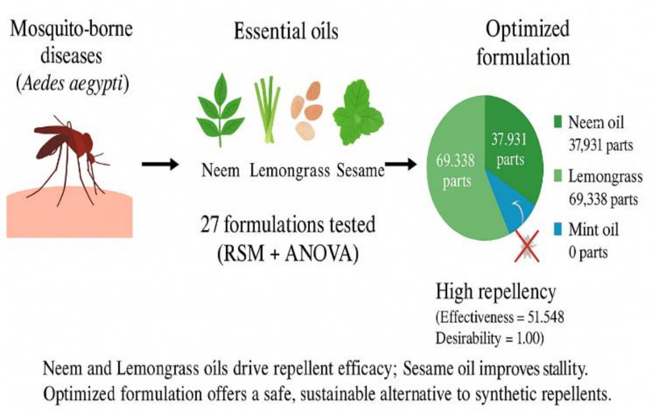
Graphical Abstract



INTRODUCTION Mosquitoes spread different diseases, which pose a significant threat to public around the world. Among the mosquitoes species *Aedes aegypti* well know species for spreading and transmitting different diseases including yellow fever, dengue fever, chikungunya and Zika virus (Näslund et al., 2021). Every year due to these diseases, millions of people lost their live, which cause a negative effect on health especially in tropical and sub-tropical areas. According to World Health Organization (WHO), every year due to dengue alone mostly 390 million people affecting, while in severe cases people hospitalized and sometime leading to death (Salehi et al., 2025). Repellent is most effective strategies to lesser mosquitoes bites. To keep away mosquitoes DEET (N, N-Diethyl-meta-toluamide) and other chemical used as a repellent for long time. According to Zheng et al. (2025), the most powerful and long lasting protection from mosquitoes bite is the use of DEET. Due to DEET and other chemicals possible toxicity, environmental hazardous and sometime mosquitoes and other insect developed resistance have sparked a greater interest in safer and natural substitutes (Akbar & Khan, 2021; Khan et al., 2015).

Major concern about the DEET's potential that it has detrimental health impacts. But when frequently use it for long time it can cause health problem, although it is believed to be safe when use as directed (Zhu et al., 2025). When the concentration of DEET is overdoses, rashes and blisters are common symptoms of skin irritation (Sudhakaran et al., 2024). DEET causing serious allergy problems, which you need to call for medical attention. The neurotoxic effect of DEET are more concerning. According to Sudhakaran et al. (2024), children experience neurological problems including headaches, light-headedness but some time if they got maximum DEET the result may even convulsions.

Environmental Protection Agency (EPA) of US divided DEET into category II (moderately harmful) compound for ocular exposure and a category III (somewhat toxic) substance for cutaneous exposure, emphasizing the significance of cautious application (Wu et al., 2024). Other drawbacks of DEET that it has substances, which are not easily, break down resulting negative impact on the environment. These substances pollutes lakes, rivers and entering to ground ecosystem by different means including runoff, washing, or swimming. Worries about these problems with DEET, found in variety of water source, which have negative effect on aquatic ecosystem have been raised by different researchers (Sridhar & Parimalarenganayaki, 2025; Leal et al., 2013). DEET revelations in aquatic ecosystem have harmful effect on the health and behaviour of aquatic community including fishes and amphibians, although the long lasting ecological effect are still being investigated (Das, 2025).

There is a possibilities that the mosquitoes species will exhibit resistance to DEET is also an alarming situation for scientific community. There is a evidence of decline that which showed that certain mosquitoes populations become less sensitive to DEET (Lazzari, 2024). Even though the real processes are not well assured, but the changes in the behaviour and genetic adaptation in mosquitoes species are probably a main reason to this resistance. In order to achieve a sustainable mosquito’s control, these prospective show the needs of other repellent and integrated pest management strategies. Thinking about the above disadvantages there is significant growing environmental friendly DEET substitutes. Plant based extracts i.e. citronella, eucalyptus, and lavender etc. have showed promising and efficient with lowest harmful effect on human health and ecosystem (Yadav et al., 2024; Maia & Moore, 2011).

For mosquitoes control, essential oil are plant based which draw attention and interest as natural repellent and these plant based oil not only safer to ecosystem but can also easily degraded and have less or fever health hazards as compared to synthetic chemicals (Hazarika & Krishnatreyya, 2025). In citronellal oil which is produced from lemon grass contain abundant citronellol, and geraniol, that restrict with mosquitoes species to find their host (Marinrojes & Uthumange, 2025). According to Özokan et al. (2024), eucalyptus oil contain p-menthane-3,8-diol (PMD) which are effective as a DEET at low concentration. Blending oil frequently enhanced repellence by creating synergistic effect (Fang et al., 2025). As compare to DEET's potent chemical scent, essential oil have more pleasant and natural aroma and these pleasing scents only serve to increase their allure (Zhang et al., 2024). This aromatic quality can make the use of mosquito repellents more pleasant and socially acceptable, increasing the likelihood of consistent use and ensuring more effective protection against bites (Yadav et al., 2024).

In rural and developing countries, essential oil extractions can have a positive and encouraging impact because the extraction of oil from plant species enhance local community’s economy by providing jobs. Customers can help these areas by selecting repellents that contain essential oils and encourage fair trade practices. Effectiveness of essential oils is often restricted by different factors such as rapid evaporation, minimizing skin persistence, and differences in repellent potency between oil kinds and species. To increase the potential efficacy of oil extracts, mix many oils in ideal ratio, which work as best repellent against mosquitoes species. The strength and durability of combining various essential oil is depends on the active ingredients presents in the oil. Prolonging protection period some oil might aid in slowing the rate at which maximum volatile evaporate. Essential oil which work like DEET or any synthetic pesticides, find best essential oil ratios that can lead to the development of natural repellents which are effective and have lesser environmental haphazard and health risk (Hazarika & Krishnatreyya, 2025). This strategy might result in safer, more environmentally friendly mosquito repellent products that are more widely accepted (Dmello et al., 2025).

In order to optimize procedures and outputs, a statistical technique called Response Surface Methodology (RSM) inspect the relation among the many independents variables and one or more dependent variable (Chen & Chen, 2025). To identify the ideal circumstances reaching to particular and correct results. The main objective of this study was to use Response Surface Methodology to evaluate different essential oil combinations for repellence against *A. aegypti*, and to know which ratios of neem, lemongrass, mint, and sesame oils are best for keeping *Ae. aegypti* away from a mixture. to assess the length of time that neem, lemongrass, mint, and sesame oils protect against *A. aegypti* bites.

MATERIALS AND METHODS

Essential Oils

Essential oils of Neem, mint, lemon grass and sesame were used using their leaves. The plants were obtained from Haripur region and oils of these four plants were extracted by Steam distillation according to the methods of (Akbar, Manzoor, et al., 2024; Manzoor et al., 2025) with a little modification. In this process, steam was passed through a mixture containing the desired substance. The heat from the steam helped release volatile components without degrading them. Initially, water was heated in a separate chamber to generate steam. This steam was then directed into a flask containing the material to be distilled. As the steam interacted with the substance, the volatile compounds evaporated along with the water vapor. The resulting mixture of steam and essential oils ascended into a condenser, where it cooled down and condensed into liquid form. The condensed liquid was gathered in a separator, where the essential oil, being less dense than water, floated on top. The liquid was then carefully separated from the water and stored properly. For the extractions of delicate compounds, this method allow without subjecting them to excessive heat, stopping degradation and preserving their natural properties. At 4 °C, these oils were stored until tested for their mosquitoes repellency.

Mosquitoes

After verifications that the tested *Ae. aegypti* mosquitoes were disease-free strains were reared and then used for the experiment (Farid et al., 2025) . In controlled environmental conditions, in which these mosquitoes were rear ensured continued lighting, humidity, and temperature. Following the instructions provided by Sasmita et al. (2025), the mass-rearing process produced healthy mosquito populations for the studies in a dependable and consistent manner.

Human Subject

For repellency tests, human volunteers were used as subjects. The subjects' history of skin allergies, sensitivity to essential oils, or sensitivity to insect repellents was checked. Three, there were no active diseases or skin disorders that would have affected the assay's outcome.

Oil Mixtures Preparation

In accordance with the technique of (Khizar et al., 2025), 5 mL of each mixture was made using a micropipette, and a fresh pipette tip was used for each component to prevent contamination. Once mixed, the final solution was transferred into an airtight container, labelled with details (e.g., composition and date), and stored it in a cool, dry place away from sunlight to ensures precise and consistent preparation for these formulations.

Skin Preparation and Application of Oils

The tested skin was cleaned with a mild soap and let to dry, before using the essential oil treatments. To guarantee consistent application, the oil mixture was applied to the hand using a cotton swab. A standardized quantity (2.5 ml) of essential oil mixture was applied to prevent variations in dosage across trials.

Exposure to Mosquitoes

After applying the oil, the hand was exposed to a mosquito cage (30 cm × 30 cm × 30 cm, made of a transparent acrylic frame with fine mesh sides to allow for ventilation and prevent mosquito escape. Each cage had twenty adult *Ae. aegypti* which were non-blood-fed and starved for 12 hours to ensure they were actively seeking a host. The exposure occurred in controlled environment (27 ± 2°C, 70 ± 5% RH with a 12-hour light/dark cycle). Higher and lower concentrations of the four oils were used as an input range in the software design for creating combinations are shown in Table 1.

Table 1. List of higher and lower concentrations

		Coded levels		
Independent variable	Symbol	-1	0	+1
Neem oil	A	25.00	42.50	60.00
Lemon grass	B	35.00	52.5	70.00
Sesame oil	C	1.00	3.00	5.00
mint oil	D	0 .00	2.00	4.00

In the light of above concentrations, the treatment combinations proposed by the Design Expert included 27 percent combinations (Table 2). Based on the proposed combinations, various oil mixtures were prepared, each with a total volume of 5 ml.

Table 2. List of combinations

Treatment	neem oil	lemon grass	sesame oil	mint oil
1	60.00	70.00	3.00	2.00
2	42.50	35.00	5.00	2.00
3	42.50	70.00	5.00	2.00
4	42.50	70.00	3.00	0.00
5	42.50	70.00	1.00	2.00
6	42.50	52.50	3.00	2.00
7	42.50	52.50	5.00	4.00
8	60.00	52.50	1.00	2.00
9	25.00	52.50	3.00	4.00
10	60.00	52.50	5.00	2.00
11	42.50	35.00	3.00	4.00
12	42.50	35.00	3.00	0.00
13	42.50	70.00	3.00	4.00
14	42.50	52.50	1.00	0.00
15	25.00	52.50	3.00	0.00
16	60.00	35.00	3.00	2.00
17	25.00	52.50	5.00	2.00
18	25.00	70.00	3.00	2.00
19	42.50	52.50	1.00	4.00
20	25.00	52.50	1.00	2.00
21	42.50	52.50	5.00	0.00
22	42.5	52.50	3.00	2.00
23	60.00	52.50	3.00	0.00
24	60.00	52.50	3.00	4.00
25	42.50	35.00	1.00	2.00
26	42.50	52.50	3.00	2.00
27	25.00	35.00	3.00	2.00

The relative proportions of each oil in the mixtures were determined and mixed according to the percentages specified above. Approximately 2.5 ml of a combination was applied on the upper portion of hand area using a cotton swab. The hand was then introduced into the cage and held for 30 seconds. Number of mosquitoes landing on the hand were recorded after which the hand was removed. The same process was repeated at an hourly interval where the hand was exposed again. In between the exposures, the hand was neither wiped nor washed. There was a total of six exposures (0, 1, 2, 3, 4, and 5 h).

DATA ANALYSIS

Data analysis was conducted using Response Surface Methodology (RSM) employing a Box-Behnken Design (BBD) to evaluate the effects and interactions among neem, lemongrass, sesame, and mint oils on mosquito repellency (Lamidi et al., 2022; Al-Batty et al., 2023). All experiments were performed in triplicate to ensure statistical reliability. Analysis of Variance (ANOVA) was used to determine the significance of model terms, and 3D response surface plots were generated to visualize factor interactions. Model adequacy was assessed using R^2, adjusted R^2, and lack-of-fit tests. Experiments involving human participants received ethical approval from the relevant bodies, and informed consent was obtained from all volunteers prior to testing.

RESULTS

Amongst the models tested, quadratic model showed the best fit. Table 3 shows the overall ANOVA fir the models. The analysis of variance revealed that the overall model was significant (F = 2.65, p = 0.0494), indicating that at least one factor or interaction significantly influenced the response. However, individual main effects of Neem (p = 0.8717), Lemon Grass (p = 0.1633), Sesame (p = 0.5783), and Mint (p = 0.1614) were not statistically significant. Among the interaction terms, only the interaction between Neem and Mint (AD) exhibited a significant effect (p = 0.0399), suggesting a synergistic relationship between these two factors.

Table 3. ANOVA for Quadratic model

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	1272.24	14	90.87	2.65	0.0494
A-neem	0.9352	1	0.9352	0.0272	0.8717
B-lemon grass	75.75	1	75.75	2.21	0.1633
C-sesame	11.21	1	11.21	0.3265	0.5783
D-mint	76.51	1	76.51	2.23	0.1614
AB	3.9	1	3.9	0.1136	0.7419
AC	69.72	1	69.72	2.03	0.1797
AD	182.25	1	182.25	5.31	0.0399
BC	6.76	1	6.76	0.1968	0.6652
BD	6.76	1	6.76	0.1968	0.6652
CD	0.0025	1	0.0025	0.0001	0.9933
A^2	402.13	1	402.13	11.71	0.0051
B^2	111.83	1	111.83	3.26	0.0963
C^2	2.54	1	2.54	0.0738	0.7904
D^2	45.57	1	45.57	1.33	0.2718
Residual	412.13	12	34.34		
Lack of Fit	392.61	10	39.26	4.02	0.2154
R^2	0.7553				

Model fit statistics showed a coefficient of determination (R^2) of 0.7553, meaning that approximately 75.53% of the variability in the response was explained by the model. The adequate precision value (6.5465) exceeded the threshold of 4, indicating that the signal-to-noise ratio was sufficient for the model to be used for exploratory analysis. Additional information on the variability of the model may be found in the standard deviation (5.86) and coefficient of variation (C.V. = 15.77%). These findings imply that even if the model accounts for a significant amount of the data's variability, it might still be improved.

The model appears to sufficiently explain the data without systematic bias, as indicated by the lack-of-fit test's lack of significance (F = 4.02, p = 0.2154). A good signal-to-noise ratio for exploratory analysis was indicated by the adequate precision (6.5465), which was higher than the suggested threshold of 4.

Table 4. Coefficients in Terms of Coded Factors

Factor	**Coefficient Estimate**	**df**	**Standard Error**	**95% CI Low**	**95% CI High**	**VIF**
Intercept	38	1	3.38	30.63	45.37	
A-neem	-0.2792	1	1.69	-3.97	3.41	1.00
B-lemon grass	2.51	1	1.69	-1.17	6.2	1.00
C-sesame	0.9667	1	1.69	-2.72	4.65	1.00
D-mint	-2.53	1	1.69	-6.21	1.16	1.00
AB	0.9875	1	2.93	-5.4	7.37	1.00
AC	-4.17	1	2.93	-10.56	2.21	1.00
AD	6.75	1	2.93	0.3656	13.13	1.00
BC	-1.3	1	2.93	-7.68	5.08	1.00
BD	-1.3	1	2.93	-7.68	5.08	1.00
CD	-0.025	1	2.93	-6.41	6.36	1.00
A^2	-8.68	1	2.54	-14.21	-3.15	1.25
B^2	4.58	1	2.54	-0.9499	10.11	1.25
C^2	-0.6896	1	2.54	-6.22	4.84	1.25
D^2	2.92	1	2.54	-2.61	8.45	1.25

From the Response Surface Methodology, Table 4 presents the results of the regression analysis including quadratic terms, coefficient estimates for coded factors and their interaction. The scale values are coded factors, in which each factor changed to have a mean of 0 and a standard deviation of 1. The table showed that lemon grass factor B and Sesame factor C, had a positive influence on the response, as seen by their positive coefficient values. On the other hand, factor A (neem) had a negative coefficient, suggesting an inverse relationship with the response. Additionally, several interaction effects (e.g., AD) and quadratic terms (e.g., A^2 and B^2) were found to be significant. The 95% confidence intervals showed that factors B, C, and D, along with various interaction and quadratic effects, were statistically significant, whereas factor A was not. The overall regression model is as follows:

Response = $38 - 0.2792A + 2.51B + 0.9667C - 2.53D + 0.9875AB - 4.17AC + 6.75AD - 1.3BC - 1.3BD - 0.025CD - 8.68A^2 + 4.58B^2 - 0.6896C^2 + 2.92D^2$.

Final Equation in Terms of Actual Factors

Table 5. Actual factor

Effectiveness	=
22.17646	
2.19697	Neem
-1.37776	lemon grass
8.54985	Sesame
-10.4131	Mint
0.003224	neem * lemon grass
-0.119286	neem * sesame
0.192857	neem * mint
-0.037143	lemon grass * sesame
-0.037143	lemon grass * mint
-0.00625	sesame * mint
-0.028354	neem^2
0.014952	lemon grass^2
-0.172396	sesame^2
0.730729	mint^2

The analysis produced a regression equation that described effectiveness as a function of four actual factors: neem, lemon grass, sesame, and mint. The intercept of the equation was 22.17646. The linear coefficient for neem was +2.19697, indicating that an increase in neem led to an increase in effectiveness. In contrast, the coefficient for lemon grass was -1.37776, suggesting a negative relationship. Sesame had a positive linear effect with a coefficient of +8.54985, while mint exhibited a negative linear effect with a coefficient of -10.41310. Interaction effects were also observed. Neem and lemon grass had a small positive interaction effect (+0.003224), whereas neem and sesame showed a negative interaction effect (-0.119286). Neem and mint exhibited a positive interaction effect (+0.192857), while both lemon grass and sesame, as well as lemon grass and mint, demonstrated negative interaction effects (-0.037143 in both cases). Additionally, sesame and mint had a negative interaction effect (-0.006250). The equation also included quadratic terms. Neem squared (-0.028354) and sesame squared (-0.172396) had negative coefficients, while lemon grass squared (+0.014952) and mint squared (+0.730729) had positive coefficients Table 5. The report concluded that the equation was appropriate for making predictions within the original units of the factors but was not suitable for comparing the relative impact of individual factors due to differences in coefficient scaling.

By contrasting expected values (vertical axis) with actual values (horizontal axis), the scatter plot graphically assessed the model's efficacy (Figure 1). A high level of predicted accuracy was exhibited by the data points, which are shown as orange squares, being mostly concentrated close to the diagonal centerline. The majority of the points closely packed around the line, indicating a high degree of agreement between the actual and anticipated values. Nonetheless, a few spots were found to be farther from the line, indicating slight differences in prediction accuracy. This clustering pattern shows that the model's overall prediction accuracy was satisfactory.

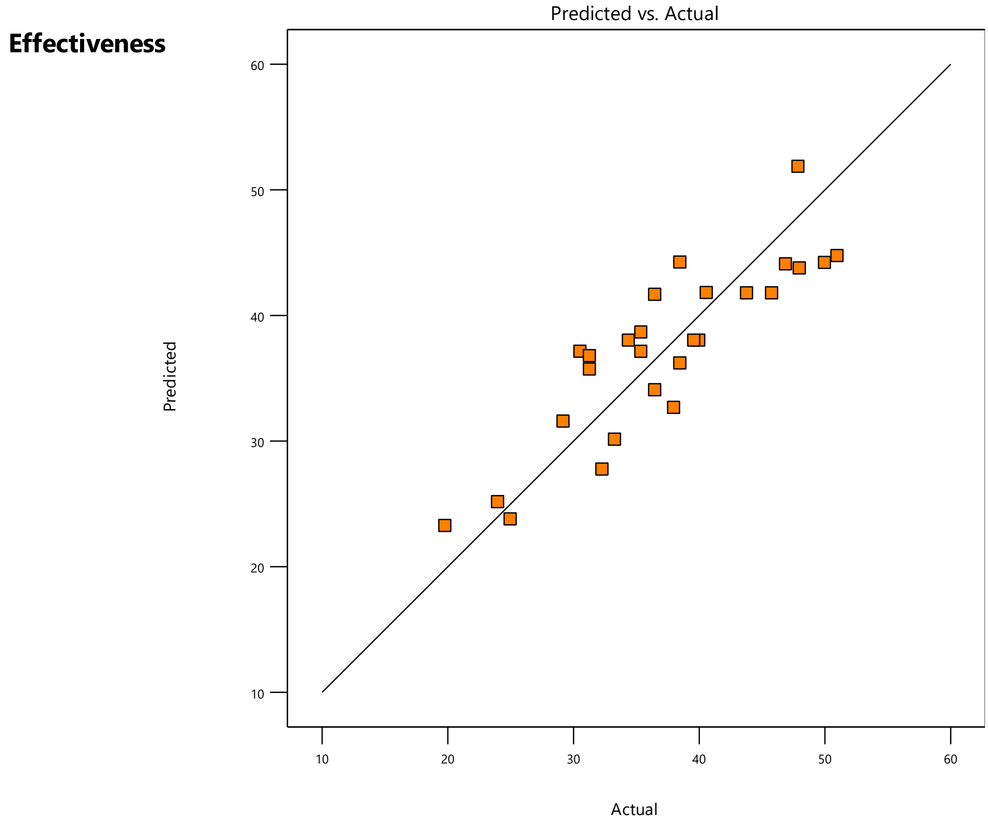


Figure 1. Predicted vs Actual model graph

Figure 2 is the perturbation plot showing the effect of individual factors on the response while keeping all other factors constant at the center of the design space. The perturbation plot shows how four factors (A, B, C, and D) individually affected the "Effectiveness". Each line traces how the effectiveness changed when a specific factor was adjusted from its low to high setting (represented by coded units of -1 to +1), while the other factors remained constant at a reference point. Factor A exhibited a non-linear, inverted-U shaped influence, where the effectiveness peaked near its reference point and decreased with higher or lower adjustments. Factors B demonstrated a positive linear relationship, with increasing levels of B corresponding to increases in effectiveness. Factors C and D, on the other hand, had smaller effect on the response, their variations produced no effect on the effectiveness. Notably, at the reference point where all factors had no deviation (coded units of 0), the actual values were A=42.5, B=52.5, C=3, and D=2, with factor B having the largest impact at this point in comparison with the other factors. Overall, the graph displayed the sensitivity of effectiveness to each factor, revealing the most influential factors and whether their effects were linear or non-linear.

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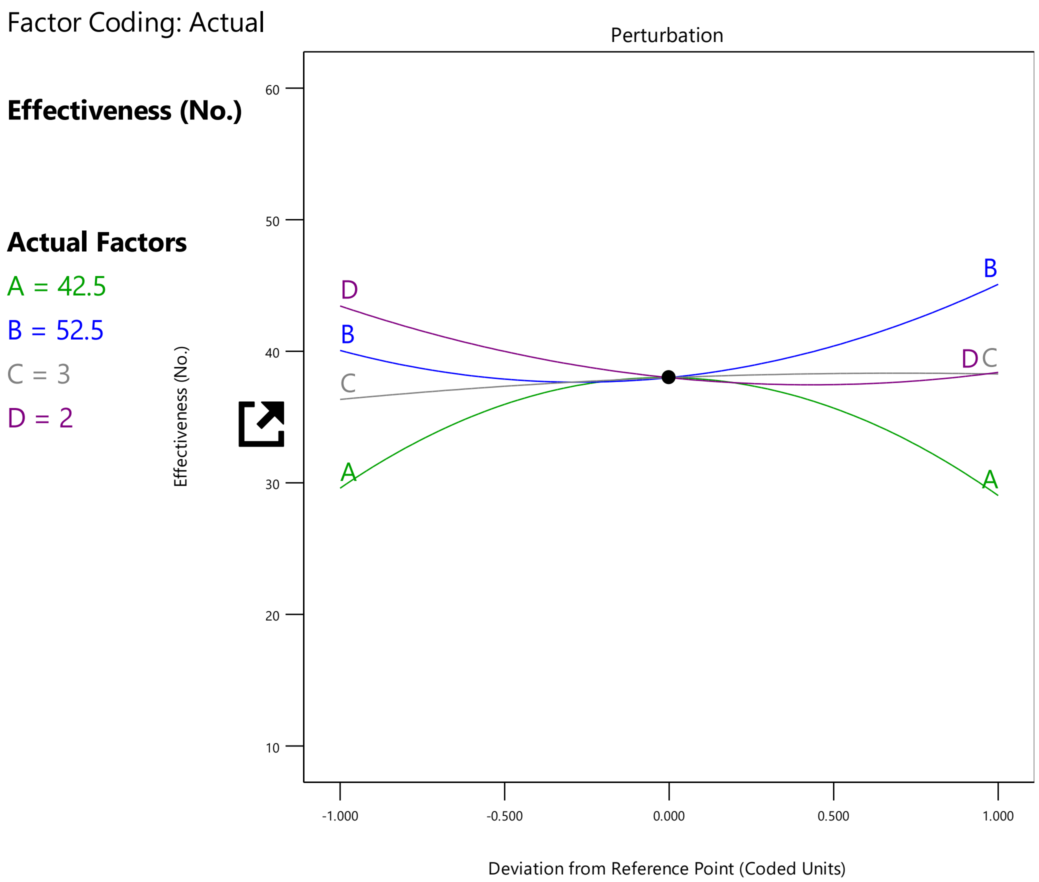

Figure 2. Perturbation

The image show 3D surface plot, demonstrating the relationship between two independent variables and their effect on the dependent variable, "Effectiveness". The plot depicted the influence of neem percentage (variable A, on the x-axis) and lemon grass percentage (variable B, on the y-axis), with the corresponding effectiveness measured on the z-axis. The 3D surface was color-coded, where higher, greener regions indicated higher predicted effectiveness values and lower, yellowish areas corresponded to lower predicted effectiveness. A contour plot directly beneath the 3D plot displayed the same data in a two dimensional format where each line represents points of equal effectiveness. The model identified X1 as A and X2 as B, with actual factors C and D given as 3 and 2 respectively. The experimental design points were marked with red and pink circles, to indicate whether the model under or over-predicted those results. The plot provided a visual way to assess the interaction between neem and lemon grass percentages on the resulting effectiveness.

Here are four lines explaining the key information from the plots 1. Both the 2D and 3D surface plots (Figure 3a, Figure 3b). Show the effectiveness of different combinations of lemongrass and neem percentages 2. The plots revealed that Effectiveness generally increases as the percentages of both lemongrass and neem increase. 3, the highest Effectiveness represented by the peak or warmest colors on. Both plots is achieved with higher percentages of both lemongrass and neem 4. Conversely, the lowest Effectiveness shown by The Valleys or cooler colors occurs at lower concentrations of both substances.

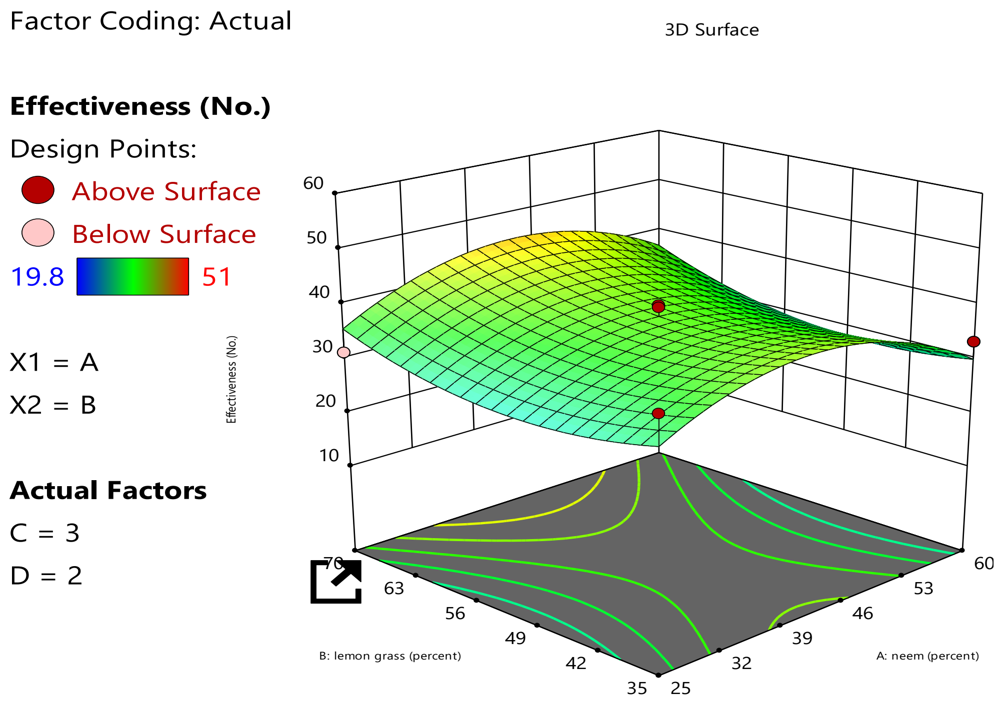

Figure 3a. 3D surfaces plot of AB

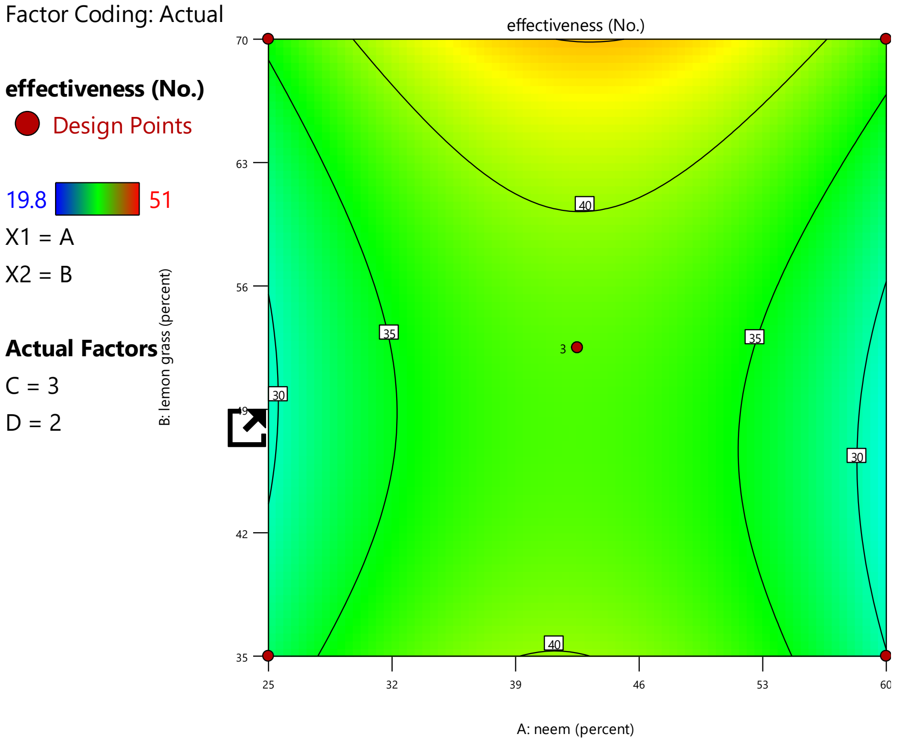

Figure 3b. surfaces plot of AB

Both plots visualize the effectiveness of a process based on varying concentrations of neem and sesame oil (Figure 4a, Figure 4b). The 3D surface plot reveals that the highest Effectiveness is achieved with moderate levels of both specifically, with a neem value of 52.5 percent and a sesame oil value of 2%, both parameters, have an impact on the effectiveness, which decreases sharply, as either increases or decreases from these values, the two D surface plot provides a top-down view. Further highlighting the same Trend, the green area in the center, shows the region with the highest Effectiveness, which corresponds to the optimal range identified in the 3D plot, the concentric Rings show. A gradual decline in Effectiveness As you move away from the center towards the regions with lower concentrations of neem and sesame oil as shown in the 3D plot.

The image displayed a 3D surface plot, generated by Design-Expert 13 software, which depicted the relationship between two independent variables and their effect on the dependent variable "Effectiveness (No.)". The plot showed the effects of the percentages of sesame (variable C) and neem (variable A) on the y-axis and x-axis, respectively, and quantified the effectiveness along the z-axis. Predicted effectiveness over the variable ranges was represented by the color-coded 3D surface; higher, greener portions denoted better effectiveness, while lower, yellowish regions denoted lower effectiveness. A contour plot displayed the same data from a bird's eye perspective with each line showing areas of the same effectiveness value. The model identified X1 as A and X2 as C, with actual factors B and D given as 52.5 and 2 respectively. To show if the model overestimated or underestimated the outcomes, red and pink circles were used to denote the design points. An examination of the interaction of neem and sesame percentages on efficacy was made possible by this visual model.

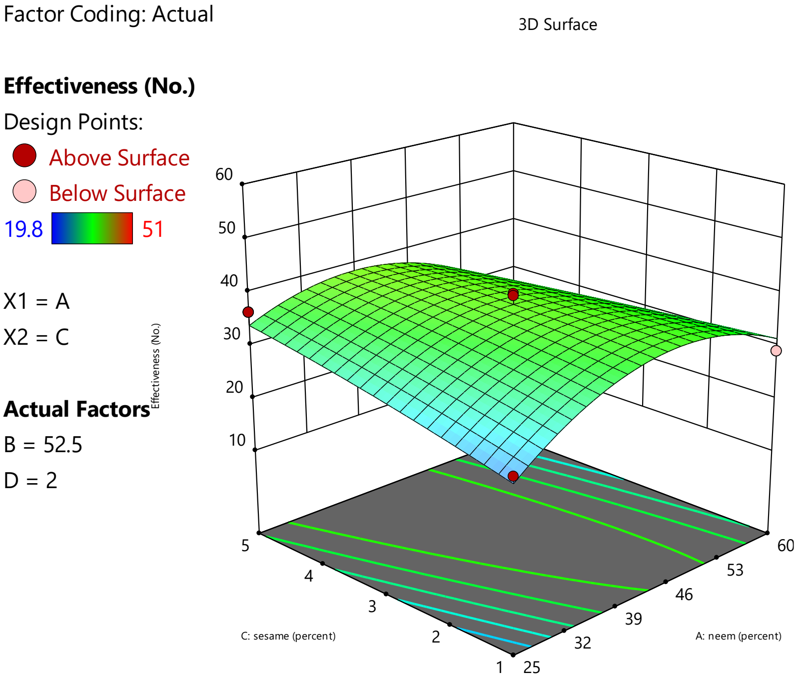

Figure 4a. 3D surface plot of AC

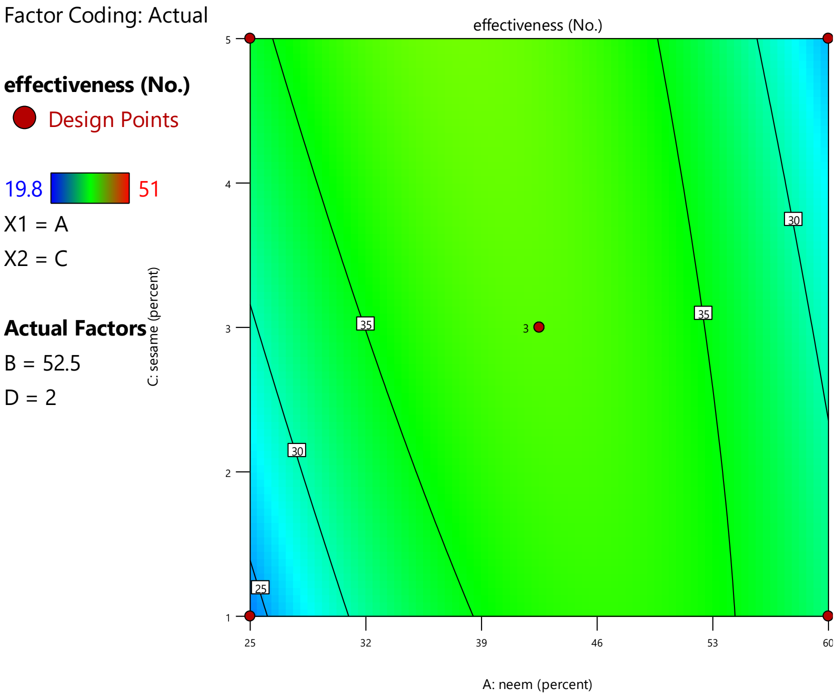

Figure 4b. surface plot of AC

The image showed a 3D surface plot, (Figure 5a, Figure 5b) which shown the relationship between two independent variables and their effect on the dependent variable, "Effectiveness". Sesame percentage (variable C, on the y-axis) and lemon grass percentage (variable B, on the x-axis) are two factors were plotted against the predicted effectiveness on the z-axis. The 3D surface was color-coded, with maximum, greener regions showing maximum effectiveness values and minimum, yellowish regions exhibiting lower effectiveness. There is a complex interaction between sesame and lemon grass percentage, with effectiveness increasing with increased lemon grass.

. Red and pink circles were used to mark experimental data points, indicating whether the model under-predicted or over-predicted a given outcome. The plot identified X1 as B and X2 as C, with actual factors A and D given as 42.5 and 2 respectively. This plot visualized the combined effects of the percentages of lemon grass and sesame on the effectiveness values.

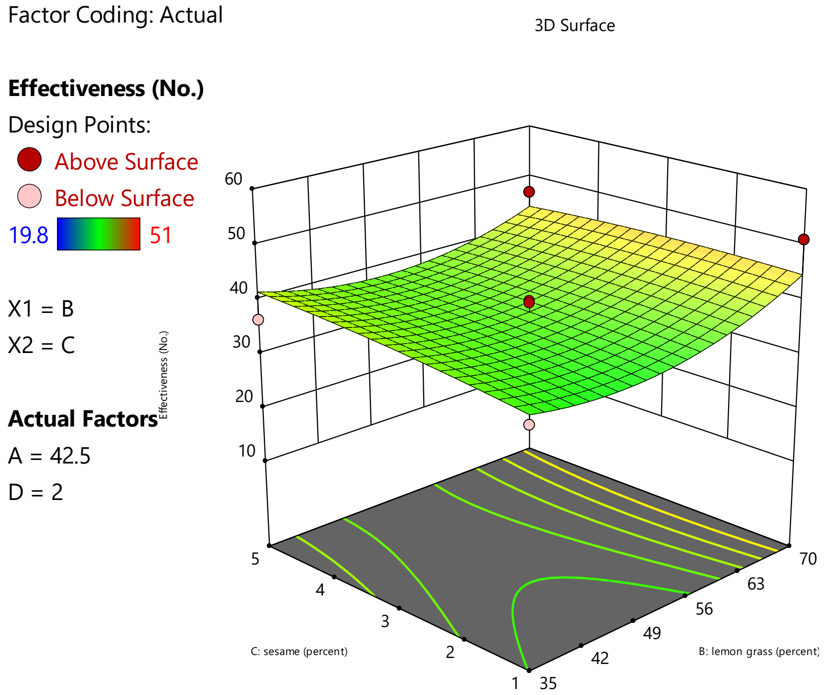

Figure 5a. 3D Surface plot of BC

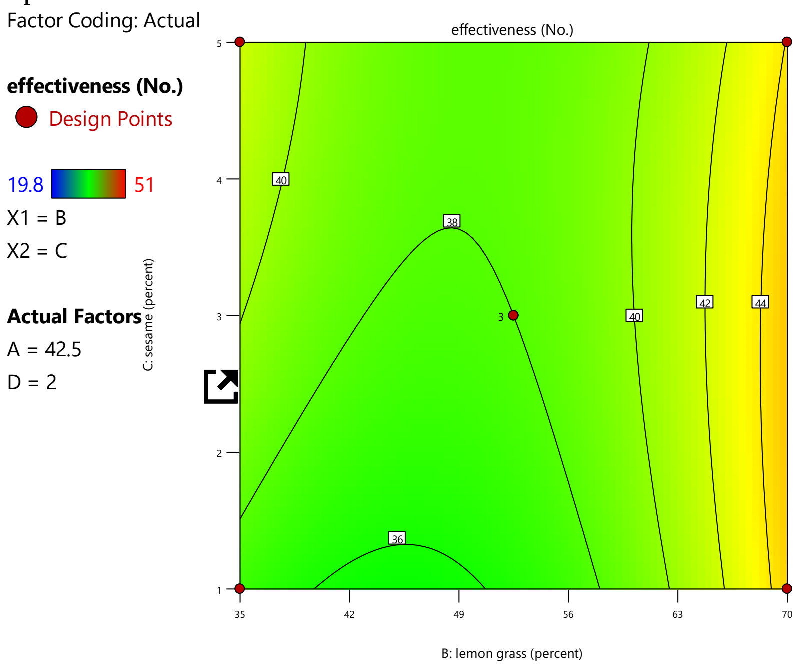

Figure 5b. Surface plot of BC

The 3D surface plot (Figure 6a, Figure 6b) show the combined effects of neem (A, x-axis) and mint (D, y-axis) percentages on effectiveness (z-axis). The green-to-yellow surface exhibits predicted effectiveness values, with maximum, greener regions at high neem and moderate mint levels, and lower, yellow areas indicating reduced effectiveness. The contour plot below provides a top-down view with lines of equal effectiveness. In the model, X1 and X2 correspond to A and D, while factors B and C were fixed at 52.5 and 3, respectively. Red and pink circles mark experimental points where the model under- or over-predicted the data.

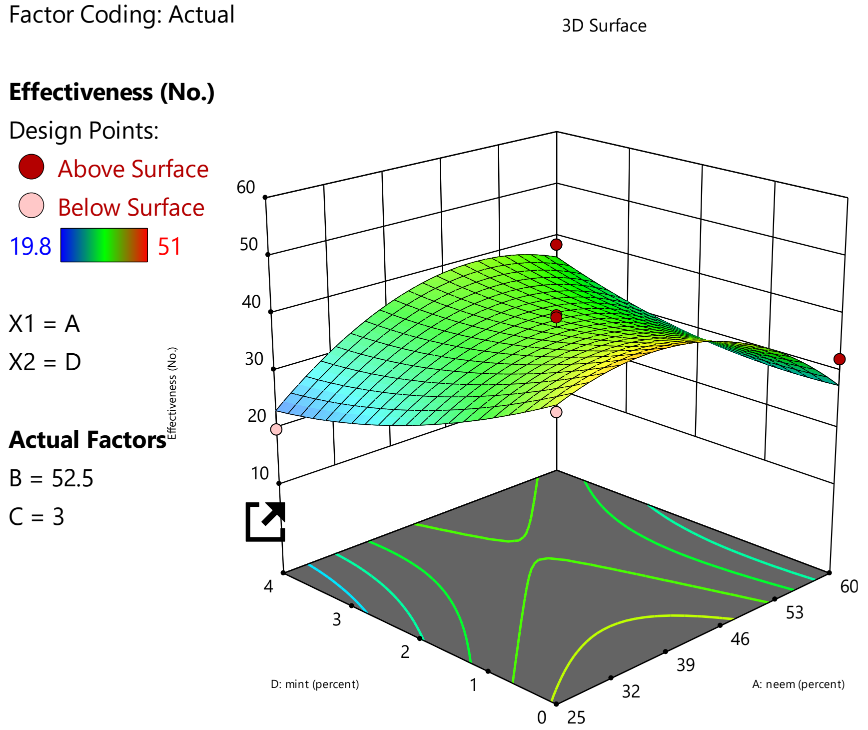

Figure 6a. 3D Surface plot of AD

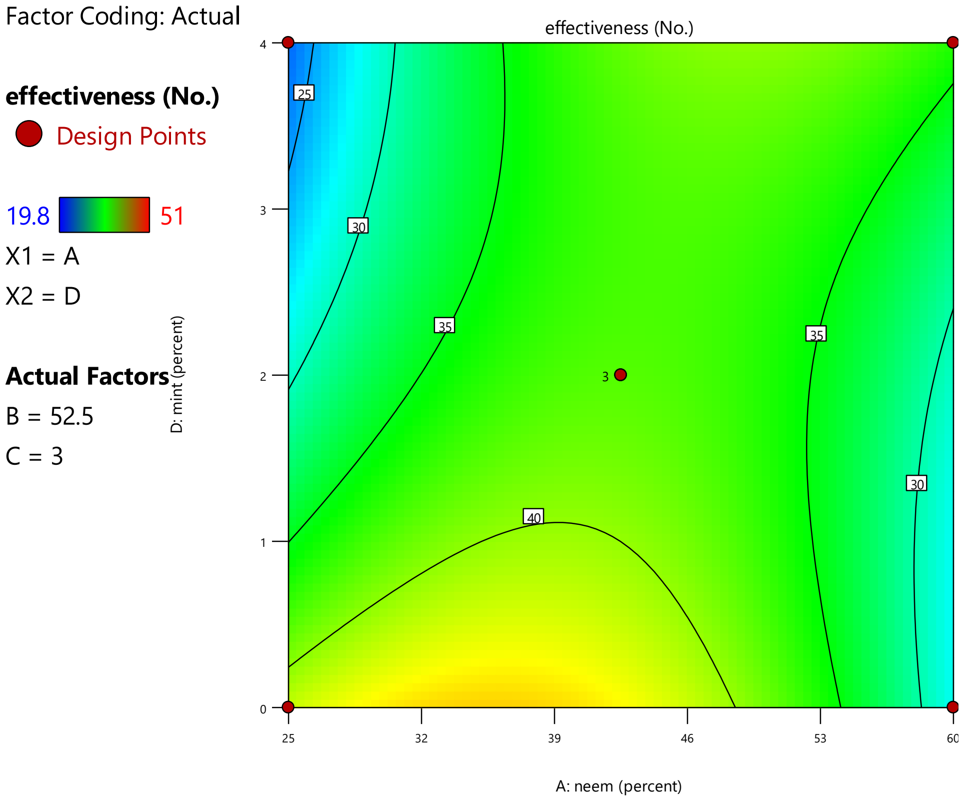

Figure 6b. Surface plot of AD

The 3D surface plot (Figure 7a, Figure 7b) illustrates the combined effects of lemon grass (B, x-axis) and mint (D, y-axis) percentages on effectiveness (z-axis). The green-to-yellow surface represents predicted effectiveness, with higher, green regions showing greater effectiveness and lower, yellow areas indicating reduced values. Effectiveness increased with higher lemon grass levels. The contour plot below provides a top-down view, confirming this trend. Red and pink circles denote points where the model under- or over-predicted outcomes. The model defined X1 and X2 as B and D, with A and C fixed at 42.5 and 3, respectively.

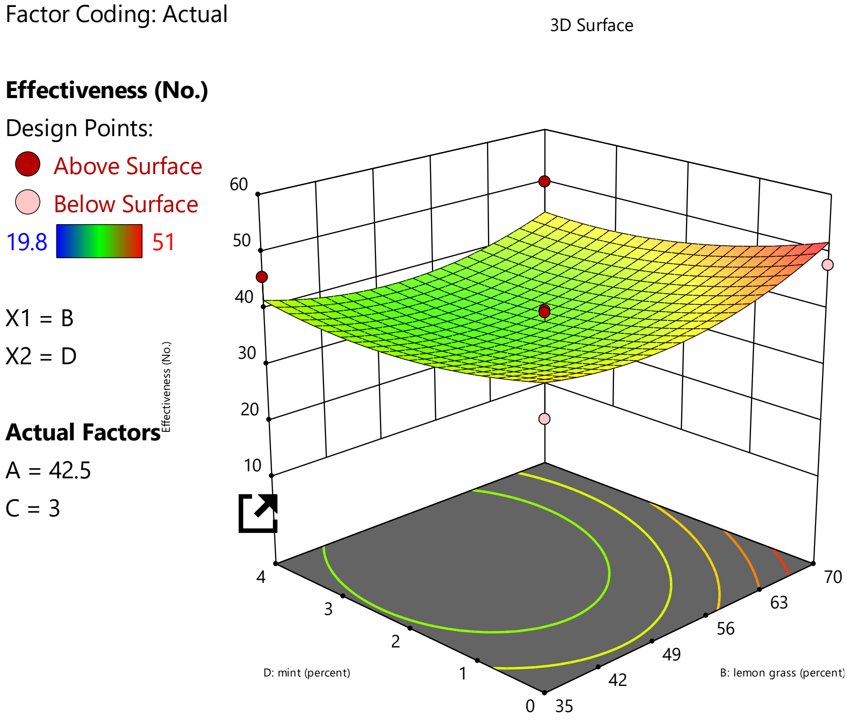

Figure 7a. 3D Surface plot of BD

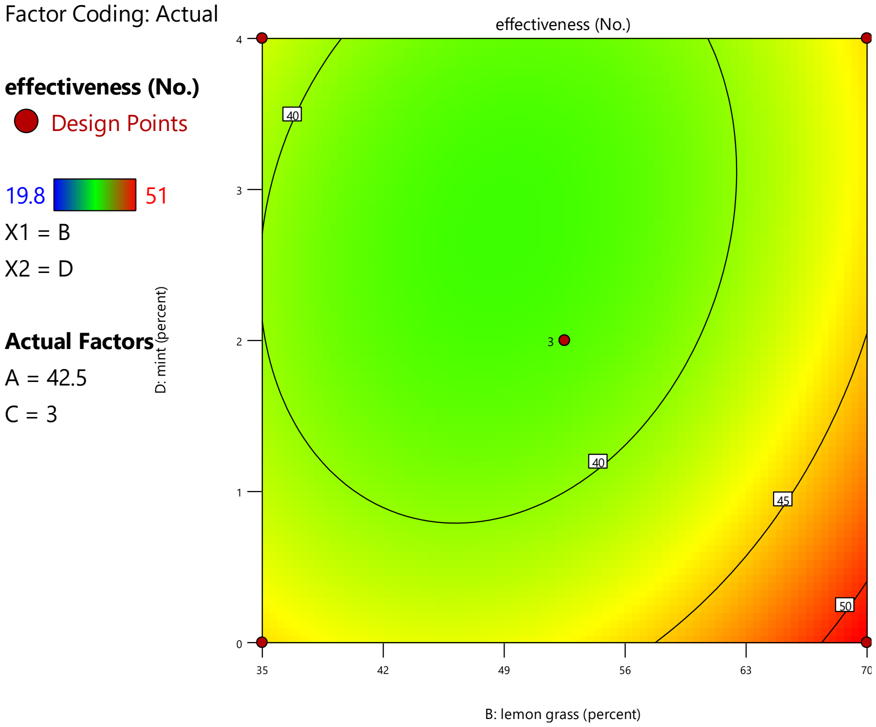

Figure 7b. Surface plot of BD

The 3D surface plot, visualized the relationship between sesame and mint percentages (factors C and D, respectively) and their impact on 'Effectiveness' (Figure 8a, Figure 8b). The green-to-yellow shaded surface represented the predicted effectiveness across the factor ranges, with higher, greener areas indicating increased effectiveness, showing the combination of higher sesame and mint amounts trended toward higher effectiveness. The 2D contour plot below further reinforced this observation, indicating areas of highest effectiveness where both factors were at higher concentrations. The design points, marked as red and pink circles, indicated whether the model under or over-predicted the data, while actual factors A and B were also given as additional experimental design information. Overall, the plot allowed for a clear assessment of the interplay between the two factors on the observed effectiveness.

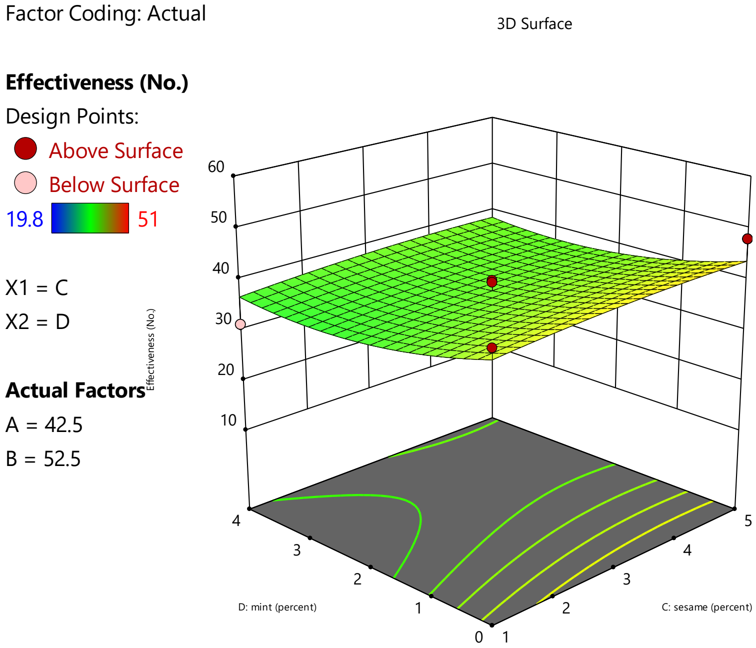

Figure 8a. 3D Surface plot of CD

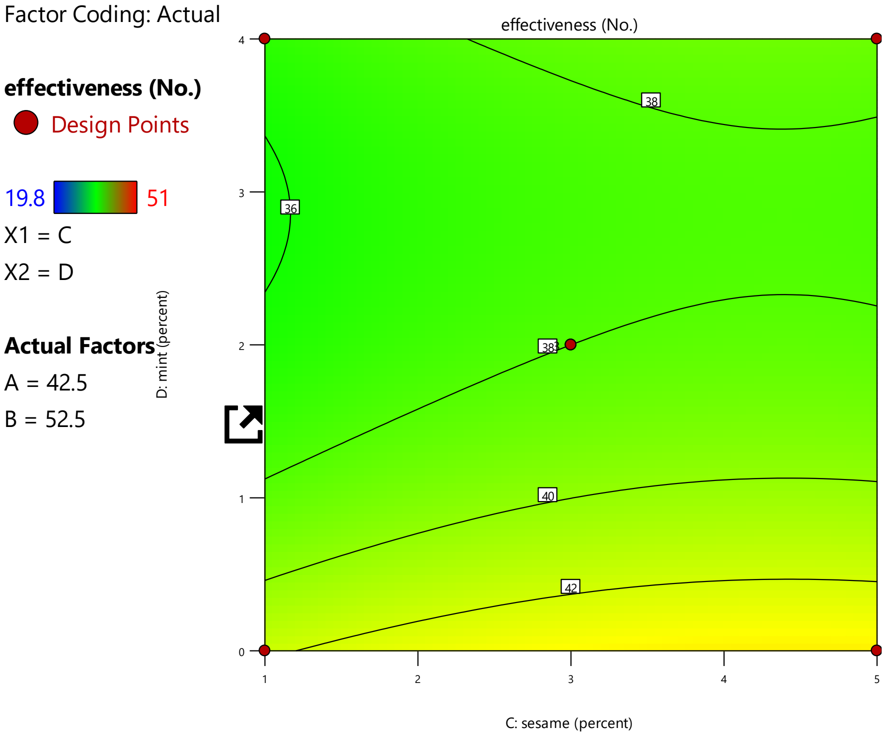

Figure 8b. Surface plot of CD

Response surface methodology (RSM) software, presented the optimized proportions of various essential oils for a mosquito repellent formulation. The study aimed to identify the combination of oils that maximized effectiveness and reached a high level of overall desirability. Table 6 showed that the optimal formulation was comprised of 37.931 parts of neem essential oil, 69.338 parts of lemon grass essential oil, and 2.828 parts of sesame essential oil, with no mint oil included. This particular mixture was predicted to yield an effectiveness score of 51.548, while also scoring 1.00 on the desirability scale, showing it was close to the desired outcome in the study. Result of these findings, this specific combination of oil proportions was marked as "selected", indicating that it was the chosen mixture from the analysis. This indicated that the software determined the mixture had the most potential for high performance, based on the tested factors.

Table 6. Optimizing proportion of essential oils

Neem	lemon grass	sesame	Mint	Effectiveness	Desirability	
37.931	69.338	2.828	0.131	51.548	1.00	Selected

DISCUSSION

The recent work aimed to evaluate the effectiveness of neem, sesame essential oils as mosquito repellents using Response Surface Methodology (RSM). Over 5 hours exposure time with significant findings confirmed through ANOVA analysis, neem oil exhibited (37.931%), lemongrass oil (69.338%), sesame oil (2.828%), and mint oil (0%). The recent findings, when compared with earlier research findings, discuss potential explanations for the observed effectiveness of the selected essential oils.
The repellent properties of essential oils were examined especially lemon grass and neem against mosquitoes (Peach et al., 2019) and other insect pests (Akbar, Faheem, et al., 2024). For example at 25% concentrations up to 6 hours exposure period, neem oil showed highest repellent activity against *Ae.* a*egypti* Aswin (Jeno et al., 2022; Silapanuntakul et al., 2016). The same results were noted in our research that the repellent effect was increased at 30% in neem oil. Azadirachtin and salannin are the active ingredients present in need, which can change the mosquitoes species behaviour and deter other insects and discourage biting (Akbar, Afzal, et al., 2024; Keerthanadevi et al., 2025). Azadirachtin a well-known insect growth regulator that inhibits with molting, feeding, and reproduction, resulting to minimize survival and repellency in insect pests (Akbar et al., 2022).

Lemongrass extracts also widely applied for its repelling properties against mosquitoes. According Manimaran et al. (2012), at 50% concentration lemon grass oil showed maximi efficacy against *Anopheles stephensi* and *Culex quinquefasciatus*. These finding support our current results, which exhibited 70% lemongrass oil significantly enhanced the repellent effect. Citral, an active ingredients present in lemongrass oil which interfere with mosquito olfactory receptors making it hard for them find its host (Tang et al., 2024). It may be possible that that the repellent's duration enhanced by the higher concentrations of lemongrass oil making a stronger hurdle against mosquitoes' species. Citral, contain a mixture of geranial and neral, present in Lemongrass oil acts as a potent repellent and neurotoxic agent by disrupting mosquito olfactory receptors and affecting their host-seeking behaviour (Nerio et al., 2010).

There is little research on sesame oil, against mosquito's repellent species has been reported when combine with other essentials oils reported to have synergistic effects. According to the research by Wei et al. (2022), sesame oil did not showed strong repellent properties but when used in mixtures with other plant oil showed significantly improved the longevity of other essential oils. The presence of sesame oil (5%) in the current research ideal formulation may have contributed to prolonged efficacy by acting as a fixative, reducing the evaporation rate of neem and lemongrass oils. This is in the line with previous findings by (Marshall et al., 2023), who reported that carrier oils like sesame could enhance the stability and persistence of volatile repellent compounds.

The exclusion of mint oil (0%) in the optimal formulation also aligns with previous research. The effectiveness of mint oil is inconsistent across different studies but mostly is investigated for its repellent potential. According to Pohlit et al. (2011), mint oil need frequent reapplication because it only provide short-term protection against mosquitoes species. Our recent findings are aligned with previous research. Also mint oil's quick volatilization could decrease its longevity compared to neem and lemongrass oils (Gupta et al., 2023).

Although the overall ANOVA model indicated a significant effect of treatments, some individual factors and interactions were not statistically significant. This variation may result from natural biological variability, differences in experimental sensitivity, or environmental heterogeneity among replicates. Gomez & Gomez, (1984) and Quinn & Keough, (2002), were reported the same finding in ecological and agricultural experiments, where certain factors or interactions show weaker responses in spite of an overall significant model

Overall, the study's findings are consistent with prior research, reinforcing the efficacy of neem and lemongrass oils as key mosquito repellents. The synergistic role of sesame oil further supports previous findings regarding fixative agents in essential oil formulations. The results highlight the potential of natural repellents as alternatives to synthetic chemical-based products, particularly in regions where vector-borne diseases pose a significant threat. Future research could explore variations in formulation stability and field trials to validate laboratory-based results under real-world conditions. The present results were achieved under controlled laboratory conditions. Different environmental factors like temperature, humidity, wind, and mosquito density in natural conditions may influence the performance and longevity of essential oil-based repellents. To validate these results under real-world conditions field trials are recommended.

CONCLUSION

The recent research effectively enhanced a plant-based mosquito repellent formulation using Response Surface Methodology (RSM), finding the ideal combination of neem (37.9%), lemongrass (69.338%), sesame (2.828%), and mint (0%) oils, which provided approximately 51% repellency effectiveness and up to five hours of protection against *Ae. aegypti*. The statistical validity of the model was confirmed ANOVA analysis, and the 3D response surface plots effectively showed the interactive effects among the plant extracts concentrations. These outcomes supports the potential of essential oils as environmental-friendly alternatives to synthetic repellents and add to the growing body of evidence supporting natural pest management strategies. Though moderate efficacy was showed by current formulation, its optimization offers a solid foundation for future research. In future, it is recommend improving performance and longevity by involving environmental variation trials, large-scale field validation, and exploration of synergistic additives or carrier oils. For sustainable mosquito control, solution it is need to integrate advanced modelling approaches with RSM that can deepen understanding of oil interactions, while relative studies with synthetic repellents and public health assessments on user acceptability, safety, and cost-effectiveness will be crucial for translating this formulation.

Credit authorship contribution statement

AF: Writing–original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. HB: Writing–review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization, Data curation. R.A: Writing review & editing, funding acquisition, Formal analysis, Conceptualization, Data curation. A. R, Writing–review & editing, Software, Methodology, Data curation, Formal analysis, Validation. G.Z.K: Writing–review & editing, Data curation, Formal analysis, Investigation, Methodology.

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AVAILABILITY OF DATA AND MATERIAL

The datasets supporting this study are included in the article. Extended methodological details and raw data can be accessed by contacting the corresponding author, subject to ethical and institutional guidelines.

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ETHICAL APPROVAL AND INFORMED CONSENT

This study was conducted in accordance with the ethical standards of the Ethical Review Committee of the University of Haripur, Pakistan, and with the 1964 Helsinki Declaration and its later amendments. Ethical approval for the study was obtained from the Ethical Review Committee of the University of Haripur.

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