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Full Length Research Paper

Optimization of operating conditions for the application of *Moringa oleifera (Zogale)* seeds extract in water disinfection using response surface methodology

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Good quality dry seeds of *Moringa oleifera* were selected and the seed coat and wings were removed manually. The kernel was ground to fine powder using the coffee mill attachment of the Moulinex domestic food blender. The ground powder was then sieved through 210 µm sieve. The seed powder was de-fatted using hexane in electro-thermal Soxhlet extractor. *Moringa* seeds extract was obtained using the de-fatted seed cake and aqueous extraction. Different preparations of *Moringa* extract were added to 10 mL of the *Escherichia coli* suspension and incubated for 2 h without agitation. Survival of bacterial cells was assessed by making dilution series of bacterial suspensions, plating on non-selective LB medium agar dishes, and incubating for 48 h at 37°C. Duplicates were made of every individual assay. Colonies were counted on dishes and the bacterial cell survival ratio was estimated by comparison to a control experiment where no Moring extract was added. The bacterial removal was optimized by varying the mixing time, mixing speed, and *Moringa* seeds extract dosage. Statistical optimization was conducted by using central composite design (CCD). The experimental data was analyzed using statistical software DESIGN EXPERT, V6.4.8 DEC 10 2002 for Windows. The response surface model was used to determine the optimum operating condition that yields the highest antimicrobial compounds activities from *M. oleifera* seeds extracts. A cubic model was fitted to the data. The standard deviation for the cubic model was 0.56, with R^2 = 0.9999 and adjusted R^2 = 0.9994. The analysis of variance (ANOVA) showed that the effects of mixing time, mixing speed and *Moringa* dosage were significant ($p < 0.05$) in the extraction process. The Quadratic model was used in predicting the responses and the optimal conditions were determined as 31 min mixing time, 85 rpm mixing speed and 3.25 mg/mL *Moringa* dosage. The results show that the predicted and experimental values were not significantly different and it was thus concluded that the model obtained can be used to optimize the process of antimicrobial bioactive compound extraction from de-fatted *M. oleifera* seeds.

Key words: *Moringa oleifera,* seeds extract, bacterial inactivation, optimization, response surface model.

INTRODUCTION

Many researchers (Bina, 1991; Muyibi et al., 2003) have reported the great potential of *Moringa oleifera* (*Zogale*) seeds extract in water treatment. Several researchers have reported its use as a coagulant (Jahn and Dirar, 1979; Muyibi and Evison, 1995; Jahn, 1986, 1988; Folkard et al., 1992); a softening agent (Muyibi et al., 2003); and a bactericidal agent (Madsen et al., 1987; Eilert et al., 1981; Kalogo et al., 2000). Thilza et al. (2010) reported that *Moringa* leaf stalk extract had

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mild activities against *E. coli* and *Entrobacter aerogenes*. Bukar et al. (2010) also studied the antimicrobial activities of *Moringa* seed chloroform extract and *Moringa* seed ethanol extract. They found both show inhibitory effects on the growth of *E. coli* and the minimum Inhibitory Concentration (MIC) was > 4 mg/ml.

The safety of using *M. oleifera* in water treatment has also received some attention. Sani (1990) reported the use of leaves as vegetables and for medicinal purposes in Northern Nigeria. Berger et al. (1984) in a study on the toxicology of *M. oleifera* seed concluded that it may not constitute a serious health hazard. Muyibi and Evison (1995) suggested that further studies need to be carried out to ensure the complete safety of using *M. oleifera* in water treatment. Folkard et al. (1989) however reported that to date, all the studies have concluded that there is no evidence to suggest any acute or chronic effects on humans, particularly at the low doses required for water treatment.

The mechanism of the action of *M. oleifera* seeds extract in water disinfection is yet to be fully understood (Suarez et al., 2005). Bichi (2011) studied the effect of the method of seed processing on the disinfection action of *Moringa*. It was found that the largest zone of inhibitions of 9 mm (using agar well method) and 12.38 mm (using Disc Diffusion method) were produced with the *Moringa* Disinfection Solution which was produced using the de-fatted *Moringa* cake and aqueous extraction method. In comparison, Thilza et al. (2010) found that extract from *Moringa* leaf stalk, 1000 mg/mL, inhibited *E. coli* with the zone of inhibition of 10 mm. The operating conditions for the extraction of the bioactive compounds, however, needs to be optimized to obtain the best result for subsequent application. The aim of this research was to determine the optimum operating conditions for the application of *M. oleifera* seeds extract in water disinfection. This involved optimizing the operating conditions (*Moringa* extract dosage, mixing time and mixing speed), for the extraction of the bio-active constituents of the *M. Oleifera* seeds, for application in the disinfection of portable water. The optimized parameter was the percentage of *E. coli* removal with *M. oleifera* seeds extract.

MATERIALS AND METHODS

Preparation of *Moringa oleifera* seeds extract

The dry *M. oleifera* seeds used for the studies were obtained locally from the villages surrounding the Bayero University (New Campus) Kano, Nigeria. The seeds were air freighted to the Biotechnology Engineering Research Unit (BERU) of the Department of Biotechnology Engineering, Kulliyya of Engineering, International Islamic University Malaysia (IIUM) Kuala Lumpur, Malaysia where the laboratory investigation was carried out. Good quality dry seeds of *M. oleifera* were selected and the seed coat and wings were removed manually. The kernel was ground to fine powder using the coffee mill attachment of the Moulinex domestic food blender. The ground powder was then sieved through 210 µm sieve. The seed powder was de-fatted using hexane in electro-thermal Soxhlet extractor (Ali, 2010) and the procedure was as follows: Weighing 10 g of *M. oleifera* seed powder and setting it in the thimbles of the electro thermal soxhlet extraction chamber; addition of 170 mL of hexane in the heating chamber; evaporating of hexane within three cycles each for 30 min to ensure the extraction of oil from the seeds (until the hexane became colorless); drying of *M. oleifera* cake residue from the soxhlet thimbles and weighting the dry sample (Muyibi et al., 2003).

The *M. oleifera* cake residue stock after oil extraction was used in the preparation of the *Moringa* seeds extract. Measured quantities of the de-fatted Moring seed powder was dissolved in a beaker and made up to 1000 mL with distilled water. The active ingredients were extracted by mixing with a stirrer at a pre-set mixing speed and for a pre-set mixing time as outlined in the experimental design. The mixture was filtered through No.1 Whatt-man filter paper and the extract was used for the disinfection studies.

Preparation of *Escherichia coli* culture

The *E. coli* culture was prepared as described in Obire et al. (2005). Nutrient broth (130.0 gm) was dissolved in 1000 mL distilled water by heating slightly. The mixture was sterilized at 130°C for 15 min at 15 SPT in autoclave. The sterilized broth was cooled to room temperature and was used to prepare the *E. coli* culture. *Escherchia coli* (ER2566) strain, obtained from the Department of Biological Sciences, Bayero University, Kano-Nigeria, was grown in 10 mL broth at 37°C overnight to obtain an exponential growth phase. This gave an *E. coli* concentration of 1.0×10^5 cfu/mL and was used for disinfection studies as the synthetic water.

Disinfection studies

The procedure for the disinfection study described in Suarez et al. (2003) and Fisch et al. (2004) was used. The study was conducted according to the experimental matrices developed as presented in Tables 1 and 2. One milliliter (mL) each of the *Moringa* extract dosage (Table 2) was added to 10 mL of the *E. coli* suspension and incubated for 2 h without agitation. The various cell survivals were assessed by making dilution series of bacterial suspensions, plating on non-selective LB medium agar dishes, and incubating for 48 h at 37°C. Duplicates were made of every individual assay. Colonies were counted on dishes and the cell survival ratio was estimated by comparison to a control experiment where no *Moringa* extract was added. The same procedure was applied to surface water obtained from Rimin Gado dam reservoir, about 13 km from Bayero University New Campus, for the validation of the results.

Experimental design

Optimization parameters

The aim here was to determine the responses of a dependent variable on some chosen independent variables. The dependent variable selected for this study was the residual *E. coli* count expressed in bacterial population removal (%). The independent variables chosen were mixing speed (rpm), mixing Time (min), and *M. oleifera* extracts dosage (mg/L). The range of values of each of these parameters used for the optimization study was chosen based on the following criteria:

(i) Mixing time (A): Ali (2010) considered 2 to 6 min mixing time for low speed and 15 to 35 min for high speed and found optimum at 41 min mixing time at low mixing speed of 40 rpm for the application of *Moringa* seed extract in coagulation. Thus, a mixing time of

Table 1. Experimental domain of central composite design (CCD).

X_j	Factor level				
	-1.682	**-1**	**0**	**1**	**+1.682**
Mixing time (min)	1.00	2.00	31.00	60.00	79.77
Mixing speed (rpm)	15.00	20.00	85.00	150.00	194.32
Moringa dosage (mg/mL)	0.25	0.50	3.25	6.00	7.87

2 to 60 min was selected for this study to cover this range in the disinfection study.

(ii) Mixing speed (B): Ali (2010), using 20 to 60 rpm low speed and 100 to 150 rpm high speed mixing, found that high speed mixing was not significant for coagulation and obtained highest turbidity removal at 100 rpm and 60 min mixing time. The study also found optimum turbidity removal at 40 rpm mixing speed and 41 min mixing time. Thus, a mixing speed covering this range of 20 to 150 rpm was chosen for the disinfection study.

(iii) *Moringa* dosage (C): Suarez et al. (2003) reported that 2 mg/mL of *Moringa* had the strongest inhibition and 6 mg/mL of Flo had the highest effect on *E. coli* growth. Thilza et al. (2010), using extracts from *Moringa* leaf stalk, found 1000 mg/mL had mild activity against *E. coli* and *Entrobacter aerogenes*. Bukar et al. (2010) reported 4 mg/mL as the minimum inhibitory concentration (MIC) for chloroform extract of *M. oleifera* seeds. Ali (2010) found 0.75 mg/L *Moringa* seeds extract as optimum concentration for coagulation of low turbidity waters. In this study therefore, a concentration range of 0.5 to 6.0 mg/L *Moringa* seeds extract was considered for the optimization of the operating conditions for the application of *Moringa* seeds extract in water disinfection.

Experimental design

The optimization study was carried out using response surface methodology (RSM), which is a statistical method that uses quantitative data from appropriate experiments to determine the regression model equation and the operating conditions (Gan and Latiff, 2011). The dependent variable selected for the study is the residual *E. coli* count expressed in percentage bacterial removal, and the independent variables chosen are: i) mixing time[A] (min); ii) mixing speed [B] (rpm); and iii) processed *M. oleifera* seed extract dose [C] (mg/mL).

Statistical optimization was conducted by using central composite design (CCD). The experimental data was analyzed using statistical software DESIGN EXPERT, V6.4.8 DEC 10 2002 for Windows. Using the range of values for the three parameters selected, Design expert generated the following experimental design. The range and centre point values of the three independent variables were presented in Table 1. These were based on the results of Suarez et al. (2003) and Ali (2010). The experimental design consisted of eight factorial points, six axial points at a distance of ±1.682 from the centre and six replicates of the central point. Five experimental runs were added based on point results obtained by earlier researchers (Suarez et al., 2003; Ali, 2010) investigating other applications of *Moringa* in water treatment. The extreme lowest values were negative, and hence were adjusted to the next lower positive values.

The design summary and the design layout for the 25 experiments were given in Table 2 for surface response, central composite design. Each experiment was replicated three times (Suarez et al., 2003) and the mean values selected as observed response.

As presented by Gan and Latiff, (2011), the variables were coded according to the equation:

$$X = (X_i - X_o) / \Delta X \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

Where X = coded value, X_i = actual value, X_o = actual value in the centre of the domain, and ΔX= the increment of X_i corresponding to a variation of 1 unit of X.
The mathematical model corresponding to the composite design is:

$$Y = \beta_o + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=1+1}^{3} \beta_{ij} X_i X_j + \varepsilon \quad \ldots\ldots\ldots\ldots \quad (2)$$

Where Y = dependent variable (Percentage Coliform reomoval), β_o = model constant, β_i , β_{ii}, β_{ij} = model coefficients, and ε = error.

The equation represents the linear, quadratic and interaction effects of the variables. Design expert software (Version 6.0, Stat-Ease, Inc., Minneapolis, MN) was used to analyze the experimental data and calculate the predicted responses. The validity of the experimental design was verified using additional experiments thereafter.

Statistical analysis

The experimental data was analyzed using statistical software (DESIGN EXPERT, V6.4.8 DEC 10 2002 for Windows) to develop the factorial regression model for determining the optimum conditions. The optimal extraction conditions were estimated from the regression analysis and three-dimensional (3D) response surface plots of the independent variable and each of the three dependent variables.

Model verification

The predictive model was verified using surface water collected from Rimin Gado dam reservoir about 15 km from the Bayero University New Campus. The procedure was as described in Suarez et al. (2003), Fisch et al. (2004) and Gan and Latiff (2011) as earlier presented in Section 2.3.

RESULTS AND DISCUSSION

Observed and predicted *E. coli* removal

The detailed results for the 25 experimental runs are given in Table 2, together with the observed responses and predicted values. The percentage of *E. coli* removal ranged from 44.8 to 100.0%. The highest *E. coli* removal

Table 2. Observed and predicted responses (*E. coli* removal).

Standard order	Run order	Block	Mixing time (Min)	Mixing speed (Rpm)	*Moringa* dosage (mg/mL)	Observed *E. coli* removal (%)	Predicted *E. coli* removal (%)
15	1	Block 1	31.00	85.00	3.25	99.3	99.42
25	2	Block 1	31.00	85.00	3.25	98.5	99.42
11	3	Block 1	31.00	15.00	3.25	75.09	75.09
19	4	Block 1	31.00	85.00	3.25	100	99.42
9	5	Block 1	1.00	85.00	3.25	100	100
12	6	Block 1	31.00	194.32	3.25	97.7	97.7
10	7	Block 1	79.77	85.00	3.25	100	100
8	8	Block 1	60.00	150.00	6.00	40	40
18	9	Block 1	31.00	85.00	3.25	100	99.42
1	10	Block 1	2.00	20.00	0.50	95.9	95.9
6	11	Block 1	60.00	20.00	6.00	76.7	76.7
14	12	Block 1	31.00	85.00	7.87	44.8	44.8
7	13	Block 1	2.00	150.00	6.00	53.2	53.2
4	14	Block 1	60.00	150.00	0.50	89.05	89.5
13	15	Block 1	31.00	85.00	0.25	48.3	48.3
2	16	Block 1	60.00	20.00	0.50	91.75	91.75
5	17	Block 1	2.00	20.00	6.00	45.8	45.8
16	18	Block 1	31.00	85.00	3.25	99.3	99.42
17	19	Block 1	31.00	85.00	3.25	99.4	99.42
3	20	Block 1	2.00	150.00	0.50	97	97
24	21	Block 1	30.00	100.00	2.00	46	46
23	22	Block 1	40.00	150.00	4.00	64.9	64.9
22	23	Block 1	30.00	150.00	2.00	47	47
20	24	Block 1	41.00	40.00	0.75	71.4	71.4
21	25	Block 1	60.00	100.00	2.50	88.7	88.7

was obtained at mixing speed of 85 rpm, mixing time of 31 min, and *Moringa* dosage of 3.25 mg/mL. Figure 1 shows the plot of the predicted versus actual responses and a close agreement between experimental and predicted values was indicated. The normal plot of residuals (Figure 2) also indicated good normality of the residuals.

The highest *E. coli* removal was obtained under the experimental conditions of mixing time (A) = 31 min, mixing speed (B) = 85 rpm, and *moringa* dosage (C) = 3.25 mg/mL. All the six axial points fall at this value (Figure 3) and the point prediction gave percentage *E. Coli* removal of 99.4167% with 98.83% at 95% CI low and 100% at 95% CI high. The standard error of the mean was 0.23 which was low enough (Table 5).

Model fitting

Table 3a, b and c shows the model fit summary. A cubic model was indicated with the sum of squares of 7220.76, 10 degrees of freedom, and an F value of <0.0001 which is <0.05 and thus the model is significant (Table 3a). The lack-of-fit test result (Table 3b) shows that the model has insignificant lack-of fit. With 0.0 sum of squares and < 0.0001 p-value. Table 3c shows that the standard deviation for the cubic model was 0.56, R^2 = 0.9999 and adjusted R^2 = 0.9994 which was the highest value of adjusted R^2 and thus was adequate.

Table 4 shows the analysis of variance (ANOVA) for the response surface cubic model. The ANOVA indicated that the contribution of the cubic model was significant. The fitted cubic model in coded variable was given in equation (3) and in actual values in equation (4).

E. coli **Removal (%)** = +99.42 + 91.93 *A -111.31* B + 72.92* C +34.62 *A^2 -50.20 *B^2 -10.17* C^2 -5.99*A*B +3.72*A*C -3.46*B*C -52.97*A^3 +68.84*B^3 -31.22*C^3 -67.99*A^2 *B +61.55 *A^2*C +152.17*A*B^2 -190.44*A*C^2 -123.00*B^2 *C +106.59*B*C^2 -5.04*A*B*C …(3)

Where: A=Mixing Time (min); B=Mixing Speed (rpm); and C=*Moringa* Dosage (mg/mL)

E. coli **Removal (%)** = +40.16576 -7.18449* MT +7.59540*MS -120.74497*MD +0.26237 *MT^2 -0.079895*MS^2+21.77717 *MD^2 -0.13404 *MT * MS+4.12350 * MT * MD +0.40090* MS * MD -2.17176E-003 * MT^3 +2.50664E-004 * MS^3-1.50103* MD^3

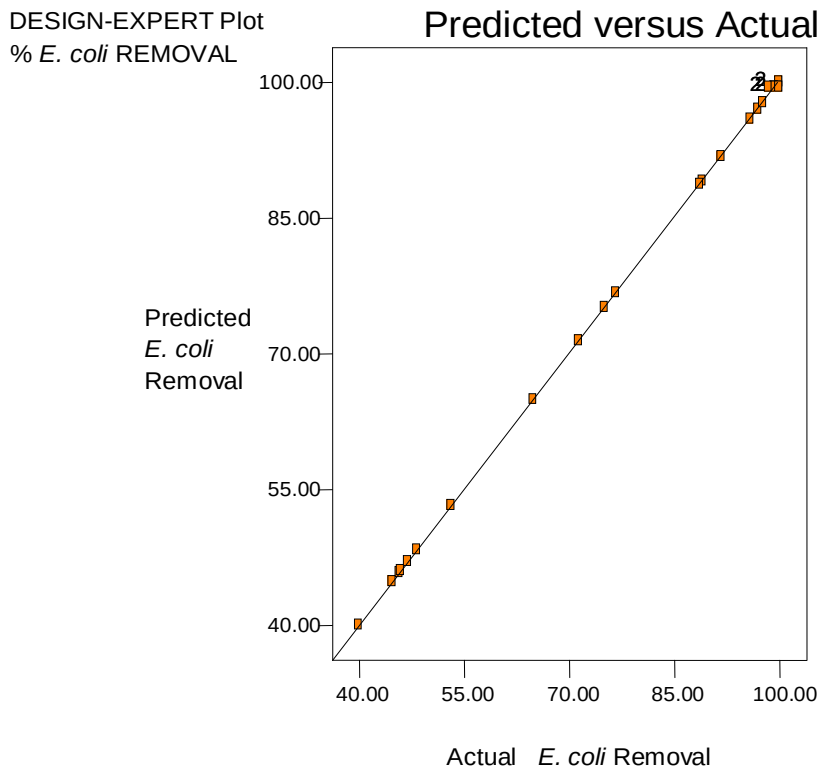

Figure 1. Predicted versus actual responses.

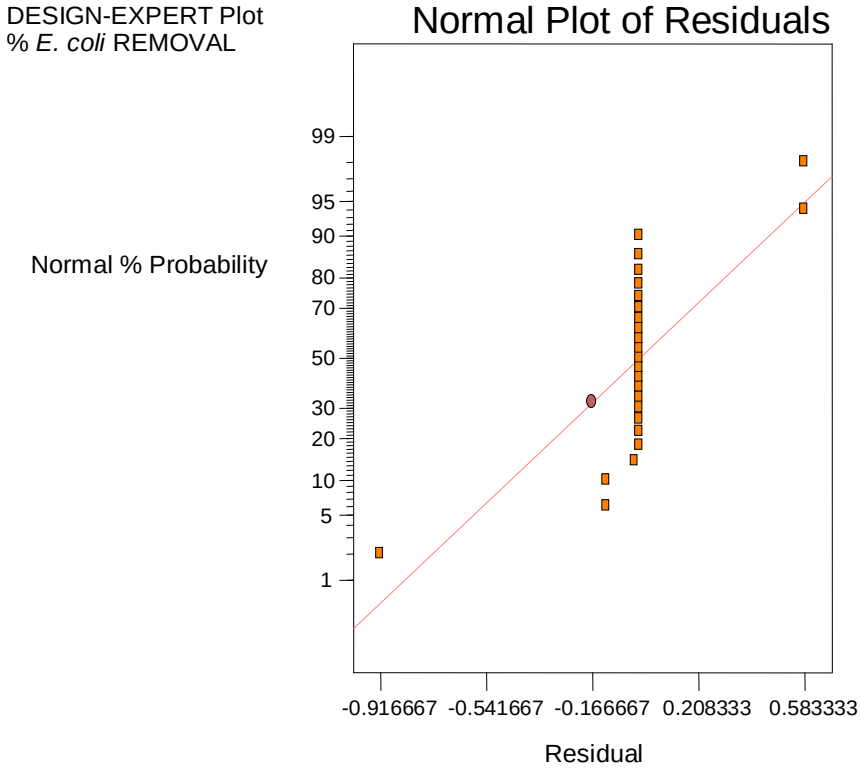

Figure 2. Normal plot of residuals.

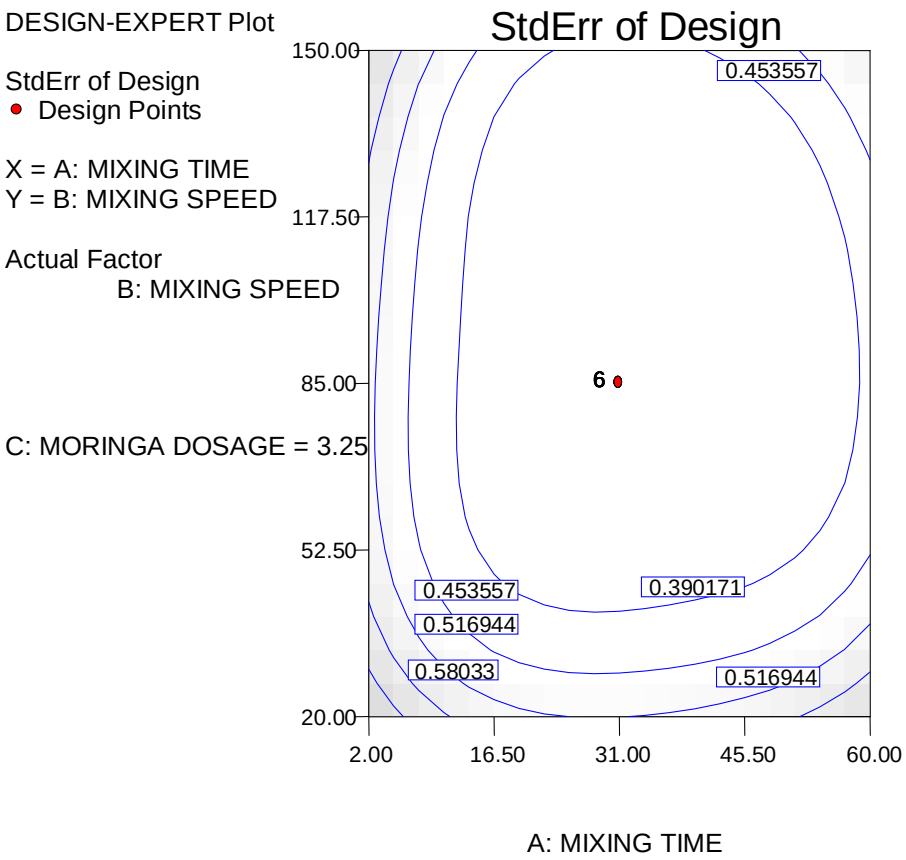

Figure 3. Axial points for CCD surface response model.

Table 3a. Model fit summary: sequential model sum of squares.

Source	Sum of squares	DF	Mean squares	F Value	Prob > F	
Mean	1.552E + 005	1	1.552E + 005			Suggested
Linear	1863.94	3	621.31	1.23	0.3245	
2FI	416.77	3	138.92	0.24	0.8639	
Quadratic	2988.60	3	996.20	2.07	0.1474	
Cubic	7220.76	10	722.08	2331.78	< 0.0001	Suggested
Residual	1.55	5	0.31			
Total	1.677E + 005	25	6707.78			

Sequential Model Sum of Squares": Select the highest order polynomial where the additional terms are significant and the model is not aliased.

Table 3b. Lack of Fit Tests

Source	Sum of squares	DF	Mean square	F-Value	Prob > F	
Linear	10626.13	16	664.13	2144.67	< 0.0001	
2FI	10209.36	13	785.34	2536.07	< 0.0001	
Quadratic	7220.76	10	722.08	2331.78	< 0.0001	
Cubic	0.000	0				Suggested
Pure Error	1.55	5	0.31			

"Lack of Fit Tests": Want the selected model to have insignificant lack-of-fit.

Table 3c. Model summary statistics.

Source	Std Dev.	R-squared	Adjusted R-squared	Predicted R-squared	Press	
Linear	22.50	0.1492	0.0277	-0.1441	14292.08	
2FI	23.82	0.1826	-0.0899	-0.5333	19153.20	
Quadratic	21.94	0.4218	0.0749	-1.5448	31788.46	
Cubic	0.56	0.9999	0.9994		+	Suggested

+ Case(s) with leverage of 1.0000: PRESS statistic not defined.

Table 4. ANOVA for response surface cubic model.

Source	Sum of squares	DF	Mean square	F-Value	p = Prob>F	
Model	12490.07	19	657.37	2122.84	<0.0001	Significant
A	*60.73*	1	60.73	*196.12*	< 0.0001	
B	*93.36*	1	93.36	*301.49*	< 0.0001	
C	*354.19*	1	354.19	*1143.76*	< 0.0001	
A^2	*57.91*	1	57.91	*187.01*	< 0.0001	
B^2	*178.44*	1	178.44	*576.23*	< 0.0001	
C^2	*95.90*	1	95.90	*309.69*	< 0.0001	
AB	*286.80*	1	286.80	*926.16*	< 0.0001	
AC	*111.00*	1	111.00	*358.47*	< 0.0001	
BC	*95.91*	1	95.91	*309.72*	< 0.0001	
A^3	*59.62*	1	59.62	*192.52*	< 0.0001	
B^3	*119.57*	1	119.57	*386.13*	< 0.0001	
C^3	*247.25*	1	247.25	*798.43*	< 0.0001	
A^2B	*36.37*	1	36.37	*117.45*	0.0001	
A^2C	*17.07*	1	17.07	*55.14*	0.0007	
AB^2	*22.07*	1	22.07	*71.28*	0.0004	
AC^2	*26.18*	1	26.18	*84.54*	0.0003	
B^2C	*84.61*	1	84.61	*273.21*	< 0.0001	
BC^2	*42.22*	1	42.22	*136.34*	< 0.0001	
ABC	*203.01*	1	203.01	*655.58*	< 0.0001	
Pure Error	1.55	5	0.31			
Cor Total	12491.62	24				
Std. Dev.		0.56		R-Squared		0.9999
Mean		78.79		Adj R-Squared		0.9994
C.V.		0.71		Pred R-Squared		N/A
PRESS		N/A		Adeq Precision		120.548

Case(s) with leverage of 1.0000: Pred R-Squared and PRESS statistic not defined.

$$-1.24373E\text{-}003 * MT^2 * MS + 0.026612 * MT^2 * MD + 1.24194E\text{-}003 * MT * MS^2 - 0.86833 * MT * MD^2 - 0.010586 * MS^2 * MD + 0.21684 * MS * MD^2 - 9.71787E\text{-}004 * MT * MS * MD \quad (4)$$

Where: MT=Mixing Time (min); MS=Mixing Speed (rpm); and MD=*Moringa* Dosage (mg/mL)

The significance of each coefficient was determined using the F-test and P-value (Table 4).

According to Atkinson and Doney (1992), the corresponding variables would be more significant if the absolute F-value becomes greater and the P-value becomes smaller. Values of p (Prob >F) greater than 0.100 indicates the model terms are not significant. The model F-value of 2122.84 implies the model is significant. There is only 0.01% chance that a 'model F-value' this large could occur due to noise. The p-values (Prob. >F) less than 0.0500 indicate model terms are significant. Values greater than 0.1000 indicate the model terms are

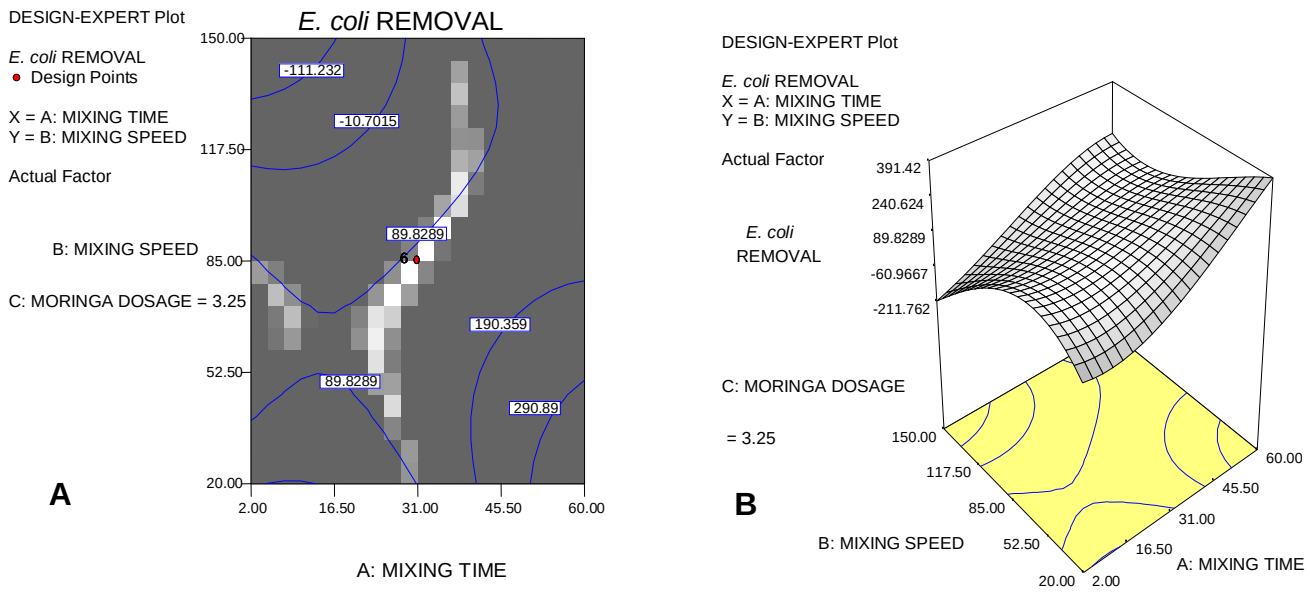

Figure 4. Mixing speed and *Moringa* dosage interaction plots (a) Contour plots (b) 3D surface response plots.

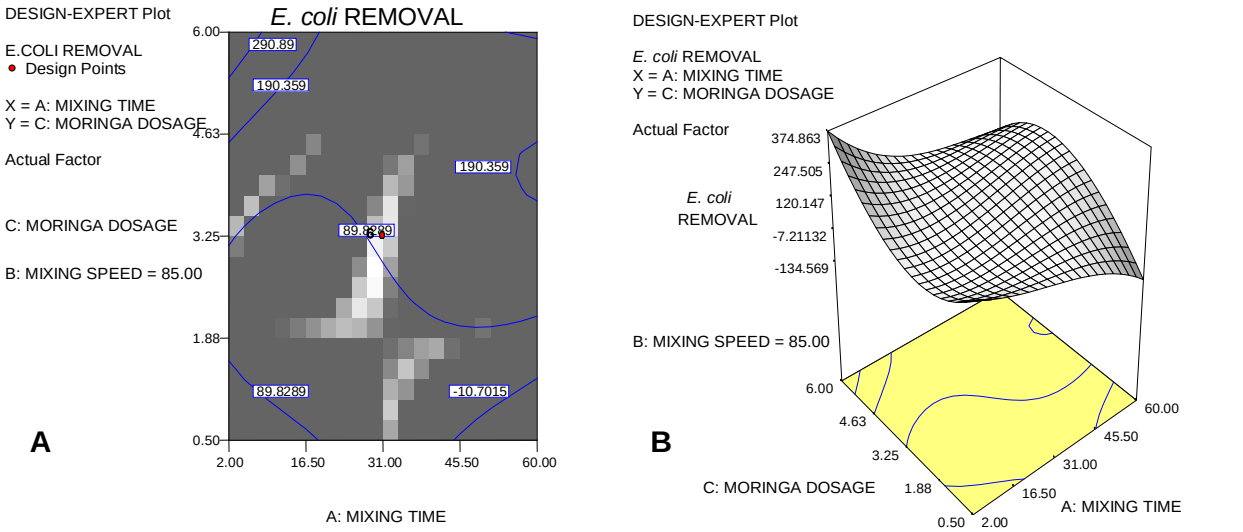

Figure 5. Mixing time and *Moringa* dosage interaction plots (a) Contour plots (b) 3D surface response plots.

not significant. Thus A, B, C, A^2, B^2, C^2, AB, AC, BC, A^3, B^3, C^3, A^2B, A^2C, AB^2, AC^2, B^2C, BC^2, ABC are significant model terms.

"Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. In this case, ratio of 120.548 indicates an adequate signal and thus the model can be used to navigate the design space.

From Table 4, the variable with the largest effect on the *E. coli* removal was the *Moringa* dosage (F = 1143.76), followed by the interaction term of mixing time and mixing speed (AB: F = 926.16) and the cubic term of *moringa* dosage (C^3 : F = 798.43). The p-values (Prob.>F) of < 0.05 indicated that all the model terms were significant.

Response surface model

The 3D response surface and contour plot as a function of mixing speed and Moringa dosage mixing time and *Moringa* dosage, and mixing speed and *Moringa* dosage are given in Figures 4a, b, 5a, b, and 6a, b respectively. Figure 4a and b shows that the region of 85 rpm mixing speed and 31 to 35 min mixing time would give a higher *E. coli* removal, compared to higher or lower mixing speed and mixing time. The removal of *E. coli* decreased with increasing or decreasing mixing speed from this region. Figure 5a and b show that *E. coli* removal was maximum at around 3.0 to 3.5 mg/mL *Moringa* dosage

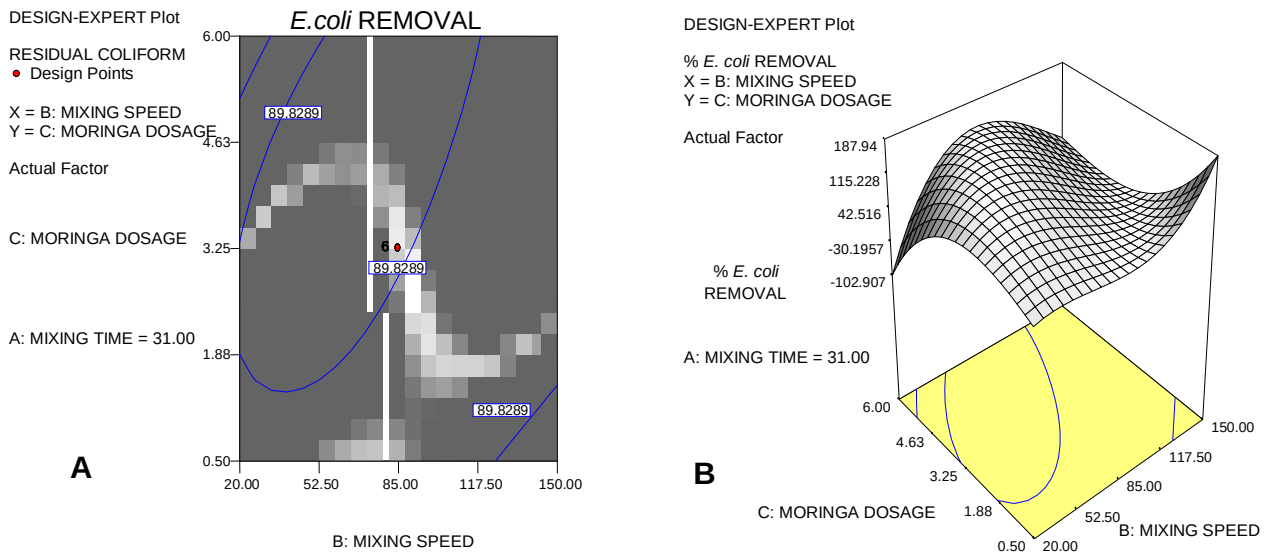

Figure 6. *Moringa* dosage and Mixing speed interaction plots (a) Contour plots (b) 3D surface response plots.

Table 5. Centre Point Prediction for Response Surface Model

Factor	Name	Level	Low Level	High Level	Std. Dev.		
A	Mixing Time	31.00	2.00	60.00	0.000		
B	Mixing Speed	85.00	20.00	150.00	0.000		
C	*Moringa* Dosage	3.25	0.50	6.00	0.000		
	Prediction	**SE Mean**	**95% CI Low**	**95% CI High**	**SE Pred**	**95% PI Low**	**95% PI High**
% *E. coli* Removal	99.4167	0.23	98.83	100.00	0.60	97.87	100.96

Table 6a. Constraints for Targetted Variables

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
Mixing Time (mins)	is target = 31.00	2	60	1	1	3
Mixing Speed(rpm)	is target = 85.00	20	150	1	1	3
Moringa Dosage(mg/mL)	is target = 3.25	0.5	6	1	1	3
E.Coli Removal (%)	is target = 100.00	40	100	1	1	3

and 31 to 45 min mixing time, whereas it decreases with low values of mixing speed and higher or lower *Moringa* dosage.

Figure 6a, and b shows that up to 100% *E. coli* removal could be achieved around 3.0 to 3.5 mg/mL *Moringa* dosage and 80 to 90 rpm mixing speed. This efficiency, however, decreased with higher or lower mixing speed and *Moringa* dosage.

Optimization

The centre point prediction from the model is given in Table 5. This showed the centre point for the optimized design solution at a mixing time of 31.0 min, mixing speed at 85.0 rpm, and *Moringa* dosage of 3.25 mg/mL within the range of values of the variables considered. All the six design points yielded *E. coli* removal of around 99.4167 and 98.83% at 95% Confidence Interval fall within this centre point. This design point was indicated in the overlay plots of mixing speed and mixing time given in Figure 7. Using this centre point prediction as a constraint (Table 6a), the response surface model yielded two design point solutions as shown in Table 6b. The two design solutions consisted of mixing time of 31.00 and 31.25 min, 84.66 and 85.00 rpm mixing speed, and 3.25

Table 6b. Optimized numerical solutions.

Solution number	Mixing time (min)	Mixing Speed (rpm)	*Moringa* Dosage (mg/mL)	*E. coli* removal (%)	Desirability	
1	31.00	84.66	3.25	99.9999	0.999	Selected
2	31.25	85.00	3.24	99.4177	0.960	

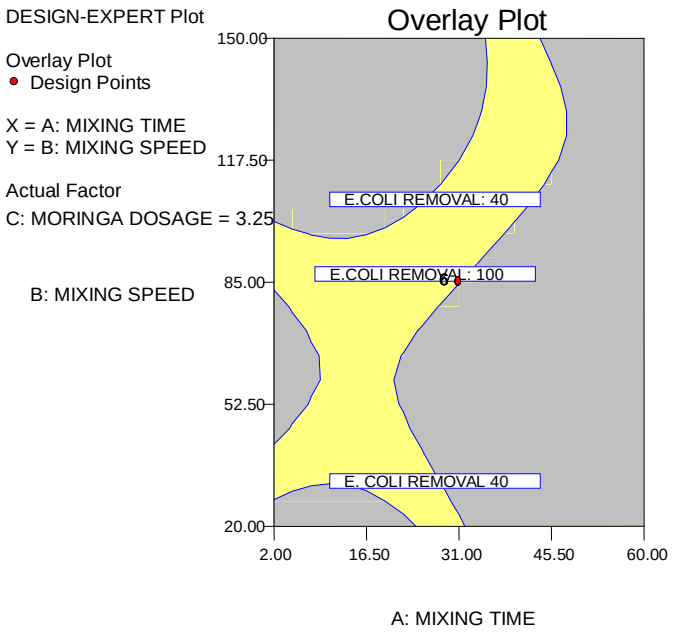

Figure 7. Overlay plots of design points for mixing time and mixing speed.

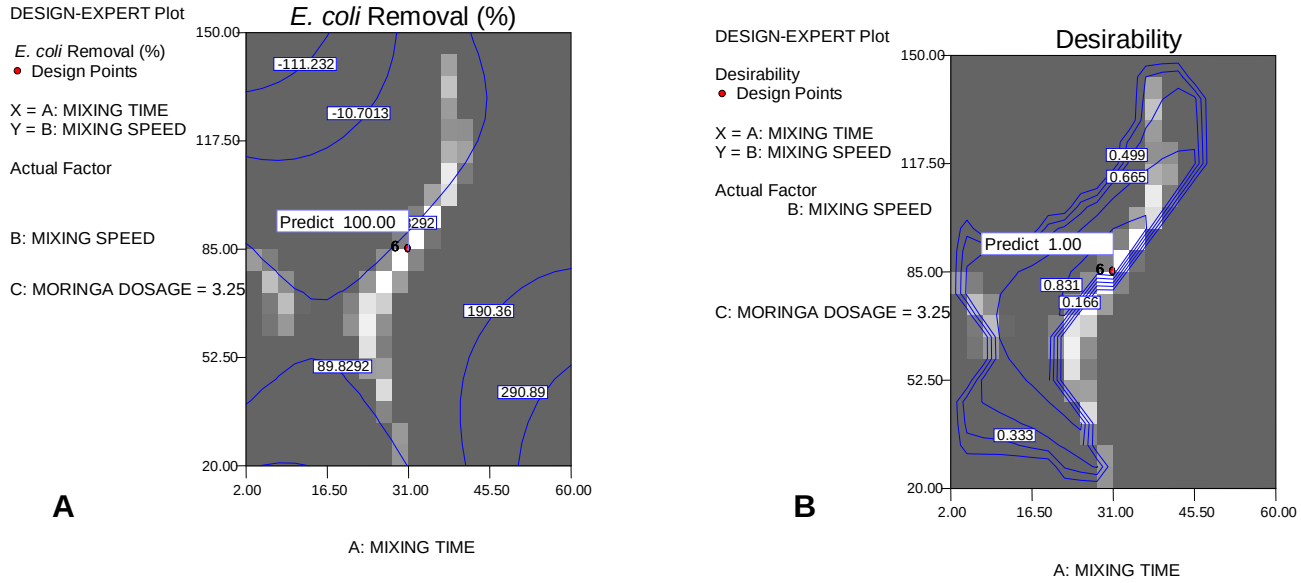

Figure 8. Optimized solution number 1 design point (a) percentage of *E. coli* removal (b) desirability plot.

and 3.24 mg/mL *Moringa* dosage. This would yield 99.999 and 99.4177% *E. coli* removal with desirability of 0.999 and 0.960 as shown in Table 6b and indicated in Figures 8a, b and 9a, b respectively.

Table 7. Validation of Percentage *E. coli* removal of models using synthetic water.

Experimental run order	Factor 1	Factor 2	Factor 3	Control *E. coli* count (x 10^5 cfu/mL)	Experimental *E. coli* Count (x 10^5 cfu/mL)				Response: Observed *E. coli* Removal (%)*	Predicted *E. coli* Removal (%)
	Mixing time (Min)	Mixing speed (rpm)	*Moringa* dosage (mg/mL)		Plate 1	Plate 2	Plate 3.	Average+		
Centre Point	31.00	85.00	3.25	36	0	0	0	0	100	99.4167
Predictive Model 1	31.00	84.66	3.25	32	0	0	1	0.33	99.06	99.9999
Predictive model 2	31.25	85.00	3.24	190	4	8	11	7.66	95.96	99.4177

*After 2 h incubation time; +Average *E.coli* count from Plates 1, 2, and 3.

Table 8. Validation of percentage *E. coli* removal of models using surface water.

Experimental Run Order	Factor 1	Factor 2	Factor 3	Control *E. coli* Count (x 10^5 cfu/mL)	Experimental *E. coli* Count (x 10^5 cfu/mL)				Response: Observed *E. coli* Removal (%)*	Predicted *E. coli* Removal (%)
	Mixing Time (Min)	Mixing Speed (rpm)	*Moringa* dosage (mg/mL)		Plate 1	Plate 2	Plate 3.	Average+		
Centre Point	31.00	85.00	3.25	24	0	0	0	0	100	99.4167
Predictive Model 1	31.00	84.66	3.25	5	0	0	0	0	100	99.9999
Predictive model 2	31.25	85.00	3.24	34	0	1	1	0.66	98.03	99.4177

*After 2 h incubation time. +Average *E. coli* count from Plates 1, 2 and 3.

These response surface model predictions were used to verify the model.

Verification of predictive model

Based on the findings above, an optimization study was carried out to evaluate the optimal operating conditions for the responses. The target was to obtain high *E. coli* removal within the extraction parameters where conside-ration of efficiency, energy conservation and feasibility of experiment were taken into account. Two solutions were generated by the software as indicated in Table 6b. Experiments were conducted at these conditions and comparison was made between the experimental results and the predicted results. The experiments were repeated using synthetic water and surface water.

Table 7 shows the percentage of *E. coli* removal for both the centre point model and the predictive models using synthetic water, and the optimal condition for the *E. coli* removal with the predicted and experimental values. These showed that only small deviations were found between the experimental and predicted values, for the centre point and the first predictive model. There was, however, a difference of 3.47% between the experimental and predicted values for the second predictive model which was also within the 5% CI.

Table 8 shows the percentage of *E. coli* removal for both the centre point and predictive models using river water obtained from Rimin Gado dam reservoir, and the comparison of experimental versus predicted results in the two cases. Both results in Tables 7 and 8 indicated very close agreement between experimental and pre-dicted results using synthetic and surface waters for the centre point and the predictive models. Thus the optimum values of the process variables were: mixing time – 31.0 min; mixing speed – 85 rpm; and *Moringa* dose of 3.25 mg/mL. These results also fall within the range of results obtained by Ali (2010) for optimization of operating conditions for application of *M. oleifera* in water coagulation. The model can therefore be used to optimize the process of bioactive antimicrobial compounds extraction from *M. oleifera* seeds.

Conclusion

The response surface model was used to determine the optimum operating condition that

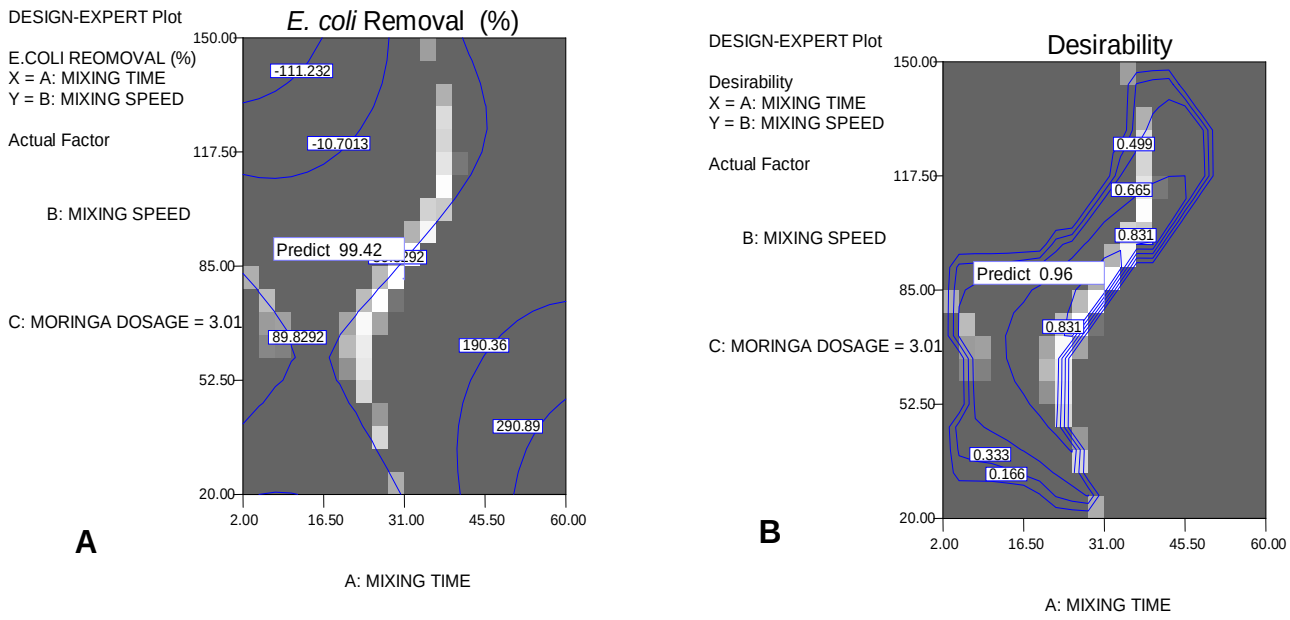

Figure 9. Optimized solution number 2 design point (a) percentage of *E. coli* removal (b) desirability plot.

yielded the highest antimicrobial compounds activities from *M. oleifera* seeds extracts. The analysis of variance (ANOVA) showed that the effects of mixing time, mixing speed and *Moringa* dosage were significant in the extraction process ($p < 0.05$). Quadratic model was used in predicting the responses and the optimal conditions were determined as 31 min mixing time, 85 rpm mixing speed and 3.25 mg/mL *Moringa* dosage. The results show that the predicted and experimental values were not significantly different. Thus, it can be concluded that the model obtained can be used to optimize the process of antimicrobial bioactive compound extraction from de-fatted *M. oleifera* seeds.

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