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OPTIMIZATION OF GERMINATION CONDITIONS OF *Melia volkensii* BY RESPONSE SURFACE METHODOLOGY

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Abstract

Germination of *Melia volkensii* was modelled using response surface methodology (rsm) whereby second order models were developed and the associated response surfaces analyzed. The factors under investigation were soil pH, temperature, chemical concentration and length of time of seed pre-treatment. Four chemicals were used for seed pre-treatment. These were potassium nitrate (KNO_3), hydrogen

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peroxide (H_2O_2), gibberellic acid (GA_3) and sulphuric acid (H_2SO_4). A four factor rotatable central composite design was used in the study. We established that in general, germination rates of *Melia volkensii* seeds are low. For the four chemicals used in the experiment, the germination rates were found to be 31.67% for KNO_3, 39.08% for H_2O_2, 42.00% for GA_3 and 28.25% for H_2SO_4. The germination rate was optimized at 65.98% for KNO_3 whereby the soil pH was 3.95, the temperature was 31.13°C, the KNO_3 concentration was 0.37% and the seed treatment time was 8.33 hours. For H_2O_2, germination rate was optimized at 65.24% whereby the soil pH was 4.83, the temperature was 30.43°C, the H_2O_2 concentration was 2.92% and the seed treatment time was 8.76 hours. The germination rate was optimized at 76.49% for GA_3 whereby the soil pH was 5.52, the temperature was 26.77°C, the GA_3 concentration was 0.03% and the seed treatment time was 7.65 hours. For H_2SO_4, germination rate was optimized at 57.26% whereby the soil pH was 4.70, the temperature was 27.21°C, the H_2SO_4 concentration was 38.13% and the seed treatment time was 5.43 hours.

1. Introduction

Melia volkensii (Melia) is an indigenous tree species in the plant family Meliaceae. Melia has been heavily exploited because it is highly valued as a timber tree. This trend has been worsening over the last decade owing to shortage of alternative hardwood species. As a result, the tree growers are now striving to grow Melia as a plantation species. Propagation of Melia has, however, been a major bottle neck and hindered planting of the species on large scale. The seeds of Melia fail to germinate when placed under normal conditions of air, moisture and warm temperature. Seed dormancy therefore constitutes a problem for nursery management. Some research has been done on the germination of Melia. Milimo [12] studied factors which maintain seed dormancy and conditions that lead to its release. The influence of temperature on germination of *Melia volkensii* seeds was examined by Milimo and Hellum [11]. The effects on germination of alternating day and night temperature and constant temperature between 22°C and 42°C

were studied. There were significant differences in total germination and germination rates between temperatures in both regimes. Most seeds failed to germinate at temperature above 37°C.

Mwamburi et al. [15] researched on the traditional methods used by farmers to break seed dormancy of *Melia volkensii* in Eastern and Coastal provinces of Kenya. The methods found include burning of nuts, use of troughs, cracking of nuts, long-term beds, sunken beds, direct sowing of seeds, sowing of nuts. These achieved germination of between 5%-20% in 1-10 weeks depending on the method used. Indieka and Odee [4] studied vegetative propagation of *Melia volkensii*. They concluded that *Melia volkensii* is amenable to propagation by rejuvenated leafy stem cuttings and tissue culture and proposed rooting experiments to develop an *in vitro* multiplication protocol for *Melia volkensii*.

Response surface methodology (rsm) is a collection of mathematical and statistical techniques that is useful for the modelling and analysis of problems in which a response of interest is influenced by several variables and the objective is to optimize this response (Montgomery [13]). rsm was initially developed and described by (Box and Wilson [2]). Hill and Hunter [3] conducted an extensive review of the literature for rsm emphasizing especially on the practical applications of the method. Mead and Pike [10] examined the state of rsm from the biostatistician's point of view and investigated the extent to which the methodology is used in applied research with particular emphasis on biometric applications. Myers et al. [16] evaluated the use of rsm between 1966 and 1988. Over the years, rsm has been applied in a wide variety of fields. Examples of the recent applications include Madamba [9], Hussain et al. [6], Pishgar-Komleh et al. [17], Anwar et al. [1], Hussain and Uddin [5], Krishna et al. [7] and Zainal et al. [19].

The study aimed to investigate optimum conditions that are favourable for the germination of *Melia volkensii* using response surface methodology.

2. Materials and Methods

2.1. Materials

Four chemicals were used for seed pre-treatment. The chemicals were sulphuric acid (H_2SO_4), gibberellic acid (GA_3), hydrogen peroxide (H_2O_2) and potassium nitrate (KNO_3). Seeds for *Melia volkensii* were purchased at Kenya Forestry Research Institute (KEFRI) in Muguga, Kiambu County, Kenya. Chemicals of analytical grade were purchased from Chemicals and School Supplies Limited, Nairobi, Kenya.

2.2. Methods

The experiment was performed by soaking 20 seeds of *Melia volkensii* in a chemical solution for a specified period of time. The seeds were then placed in a Petri dish containing soil of a particular pH. They were then placed in germination chambers of a defined temperature. The outcome was the number of seeds that germinate in a particular Petri dish.

2.2.1. Experimental design

A four factor rotatable central composite design was used in this study. There were two replicates each for the cubic and star portions of the design. The centre point was replicated 12 times. Therefore, there were 60 experiments for each of the four chemicals used for seed treatment. The chemical concentrations were unique to each chemical but were set as per the requirements of the design. However, temperature, soil pH and length of seed pre-treatment were uniform among all the chemicals. The coded values in conformity to the design and the corresponding raw actual value setting are summarized in Table 1.

Table 1. Coded variable setting and corresponding actual values

Coded value	Temperature	Soil pH	Pre-treatment time	Chemical concentration (%)			
			(hours)	H_2SO_4	GA_3	H_2O_2	KNO_3
–2	15.0	3.0	4.0	20.0	0.01	1.0	0.1
–1	20.0	5.0	6.0	35.0	0.02	2.0	0.2
0	25.0	7.0	8.0	50.0	0.03	3.0	0.3
1	30.0	9.0	10.0	65.0	0.04	4.0	0.4
2	35.0	11.0	12.0	80.0	0.05	5.0	0.5

2.2.2. Statistical analysis

Response surface methodology was used both to fit second order models for the germination of *Melia volkensii* and also to analyse the fitted models. The analyses were executed in R 3.1.1 (R Core Team [18]) using the rsm package (Lenth [8]). We formulated four models corresponding to each one of the chemicals used for seed pre-treatment. The fitted second order model was of the form:

$$\hat{y} = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + B_{11}x_1^2 + B_{12}x_1x_2 + B_{13}x_1x_3 + B_{14}x_1x_4 + B_{22}x_2^2 + B_{23}x_2x_3 + B_{24}x_2x_4 + B_{33}x_3^2 + B_{34}x_3x_4 + B_{44}x_4^2. \quad (1)$$

Here x_1 denotes the temperature, x_2 denotes the soil pH, x_3 denotes the chemical concentration and x_4 denotes the length of time of seed pre-treatment. $\hat{y}$ the estimated response was the number of seeds that germinated in a given Petri dish that contains 20 seeds.

In matrix form, (1) can be written as:

$$\hat{y} = b_0 + x'\boldsymbol{b} + x'\boldsymbol{B}x, \text{ where } \boldsymbol{b} = (b_1, b_2, b_3, b_4)' \text{ and}$$

$$\boldsymbol{B} = \begin{bmatrix} B_{11} & B_{12} & B_{13} & B_{14} \\ B_{12} & B_{22} & B_{23} & B_{24} \\ B_{13} & B_{23} & B_{33} & B_{34} \\ B_{14} & B_{24} & B_{34} & B_{44} \end{bmatrix}. \quad (2)$$

For each fitted model, we showed the estimated coefficients, their standard errors, *t* values and their corresponding *p* values. A parameter was deemed to be significant if the associated *p* value was less than 0.05.

Analyses of variance were performed to check the adequacy of the models. The output included the multiple R^2, adjusted R^2, the *F*-statistic and the *p* value of this statistic. A model was classified as being significant if the *p* value of its *F*-statistic was less than 0.05.

Optimization analyses were done to find optimal conditions for the germination of *Melia volkensii*. Response surfaces were plotted to give a visual display of the nature of the responses. Stationary points were then obtained for each of the fitted models. A stationary point is the one in which the response has an optimum value (maximum or minimum). Canonical analyses were carried out to examine the fitted second order response surface. This involved transforming the models into their canonical equivalent forms. The canonical model was of the form:

$$\hat{y} = \hat{y}_s + \sum_{i=1}^{p} \lambda_i w_i^2, \quad (3)$$

where $\hat{y}_s$, the estimated stationary point, is the centre of the contours and w_is are a new set of axes called the *principal axes*. The coefficients λ_is are the eigenvalues of $\hat{\beta}$ and give the shape of the surface such that:

If $\lambda_1, \lambda_2, ..., \lambda_p$ are all negative, then the stationary point is a point of maximum response.

If $\lambda_1, \lambda_2, ..., \lambda_p$ are all positive, then the stationary point is a point of minimum response.

If $\lambda_1, \lambda_2, ..., \lambda_p$ are mixed in sign, then the stationary point is a saddle point.

Verification tests were done by performing three experiments at the derived optimum conditions and comparing the outcome with the predicted values at these conditions.

3. Results and Discussion

3.1. Fitting the models

Sixty observed responses were used to fit each of the four models corresponding to the chemicals applied for seed treatment. From the data, second order models were obtained. These are shown in (4), (5), (6) and (7) corresponding to the models for KNO_3, H_2O_2, GA_3 and H_2SO_4, respectively,

$$\hat{y} = 10.500 + 1.875x_1 - 1.875x_2 + 0.292x_3 + 0.208x_4 - 2.208x_1^2 - 1.938x_1x_2 + 0.813x_1x_3 + 0.250x_1x_4 - 1.396x_2^2 - 0.063x_2x_4 - 0.896x_3^2 - 0.563x_3x_4 - 0.708x_4^2, \quad (4)$$

$$\hat{y} = 11.667 + 1.229x_1 - 1.271x_2 + 0.063x_3 + 0.146x_4 - 1.766x_1^2 - 2.094x_1x_2 - 0.156x_1x_3 + 0.844x_1x_4 - 1.516x_2^2 + 0.219x_2x_3 + 0.719x_2x_4 - 1.203x_3^2 + 0.406x_3x_4 - 0.328x_4^2, \quad (5)$$

$$\hat{y} = 14.250 + 1.583x_1 - 2.167x_2 + 0.125x_3 + 0.375x_4 - 2.969x_1^2 - 0.813x_1x_2 + 1.313x_1x_3 + 0.438x_1x_4 - 1.781x_2^2 + 0.875x_2x_3 + x_2x_4 - 1.969x_3^2 - 0.250x_3x_4 - 0.594x_4^2, \quad (6)$$

$$\hat{y} = 9.667 + x_1 - 1.208x_2 - 0.375x_3 - 1.125x_4 - 2.115x_1^2 - 1.375x_1x_2 + 0.188x_1x_3 + 0.438x_1x_4 - 1.427x_2^2 + 0.938x_2x_3 + 0.563x_2x_4 - 0.865x_3^2 - 0.615x_4^2. \quad (7)$$

Table 2 shows the p values associated with each factor. A factor was deemed significant if the corresponding p value was at most 0.05.

Tables 2 shows that for KNO_3, the significant factors were the intercept, x_1, x_2, x_1^2, x_1x_2, x_2^2 and x_3^2. For H_2O_2, the significant factors were the intercept, x_1, x_2, x_1^2, x_1x_2, x_2^2 and x_3^2. For GA_3, the significant factors were the intercept, x_1, x_2, x_1^2, x_1x_3, x_2^2, x_2x_3, x_2x_4 and x_3^2. For H_2SO_4, the significant factors were the intercept, x_1, x_2, x_1^2, x_1x_2, x_2^2 and x_3^2. Hence, the factors that were significant across the models were the intercept, x_1, x_2, x_1^2, x_2^2 and x_3^2.

Table 2. *p* values for the fitted models

Factor	*p* value			
	HNO_3	H_2O_2	GA_3	H_2SO_4
Intercept	<0.0001	<0.0001	<0.0001	<0.0001
x_1	<0.0001	0.0050	0.0001	0.0245
x_2	<0.0001	0.0038	<0.0001	0.0073
x_3	0.4759	0.8814	0.7257	0.3875
x_4	0.6101	0.7279	0.2952	0.0120
x_1^2	<0.0001	<0.0001	<0.0001	<0.0001
x_1x_2	0.0003	0.0002	0.0675	0.0122
x_1x_3	0.1090	0.7608	0.0041	0.7233
x_1x_4	0.6173	0.1051	0.3185	0.4102
x_2^2	0.0006	0.0003	<0.0001	0.0009
x_2x_3	>0.9999	0.6701	0.0496	0.0816
x_2x_4	0.9005	0.1657	0.0258	0.2909
x_3^2	0.0226	0.0034	<0.0001	0.0369
x_3x_4	0.2636	0.4300	0.5672	>0.9999
x_4^2	0.0685	0.4041	0.0798	0.1333

3.2. ANOVA analysis

The tests of significance for second order models were performed using the ANOVA procedure. The adequacy of the fitted models was determined by the *p* values of the realized *F* values together with the multiple R^2 and adjusted R^2. Multiple R^2 is used for evaluating how well the model fits the data. They tell how much of the variance in the dependent variable (*the predicted variable*) can be explained by the independent variables (*the predictor variables*). Adjusted R^2 is a modified version of R^2 that has been adjusted for the number of predictors in the model. Further, the models were broken down to the first order, two way interaction, pure quadratic, lack of fit and pure error components. A component was deemed significant if its *p*

value was less than 0.05. Table 3 summarizes the ANOVA analysis for the four models developed.

Table 3. ANOVA table for the fitted second order models

Model	Source	Sum of squares	Degrees of freedom	Mean square	*F* value	*p* value
KNO_3	Model	843.84	14	60.274	7.630	<0.0001
$R^2 = 0.7036$	First order	343.67	4	85.917	10.876	<0.0001
$Adj.R^2$	Two way interaction	153.50	6	25.583	3.238	0.0099
$= 0.6114$	Pure quadratic	346.67	4	86.667	10.971	<0.0001
	Residuals	355.50	45	7.900		
	Lack of fit	130.50	10	13.050	2.030	0.0598
	Pure error	225.00	35	6.429		
	Total	1199.34	59			
H_2O_2	Model	636.28	14	45.449	5.458	<0.0001
$R^2 = 0.6294$	First order	151.25	4	37.812	4.541	0.0036
$Adj.R^2$	Two way interaction	187.19	6	31.198	3.747	0.0042
$= 0.5141$	Pure quadratic	297.84	4	74.459	8.942	<0.0001
	Residuals	374.71	45	8.327		
	Lack of fit	64.54	10	6.454	0.728	0.6929
	Pure error	310.17	35	8.862		
	Total	1010.99	59			
GA_3	Model	1189.57	14	84.969	14.117	<0.0001
$R^2 = 0.8145$	First order	353.17	4	88.292	14.670	<0.0001
$Adj.R^2$	Two way interaction	140.88	6	23.479	3.901	0.0032
$= 0.7569$	Pure quadratic	695.52	4	173.881	28.891	<0.0001
	Residuals	270.83	45	6.019		
	Lack of fit	61.08	10	6.108	1.019	0.4476
	Pure error	209.75	35	5.993		
	Total	1460.40	59			
H_2SO_4	Model	618.77	14	44.198	4.986	<0.0001
$R^2 = 0.6080$	First order	185.58	4	46.396	5.234	0.0015
$Adj.R^2$	Two way interaction	106.00	6	17.667	1.993	0.0865
$= 0.4861$	Pure quadratic	327.19	4	81.798	9.228	<0.0001
	Residuals	398.88	45	8.864		
	Lack of fit	166.71	10	16.671	2.513	0.0214
	Pure error	232.17	35	6.633		
	Total	1017.65	59			

Table 3 shows that all the models were significant with p values of <0.0001. The value of R^2 ranged between 0.6080 for the H_2SO_4 model to 0.8145 for the GA_3 model. The adjusted R^2 values were 0.4861, 0.5141, 0.6114 and 0.7569, respectively, for the H_2SO_4, H_2O_2, KNO_3 and GA_3 models, respectively. For all the models, the first order and the pure quadratic components were significant. Lack of fit was only significant for the H_2SO_4 model. The results indicated that the second order model adequately represented the germination of *Melia volkensii*. The GA_3 model was found to be the most reliable one with the H_2SO_4 model being the least reliable for modelling germination of *Melia volkensii*.

3.3. Optimization analysis

The key objective of the study was to find optimal conditions for the germination of *Melia volkensii*. First, we plotted the response surfaces to visualize their nature. These are displayed in Figures 1, 2, 3 and 4.

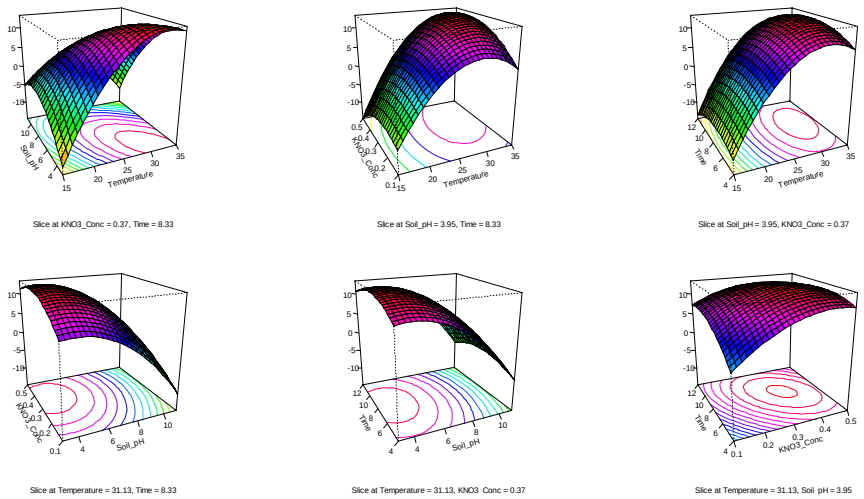

Figure 1. Response surface plot for the KNO_3 model.

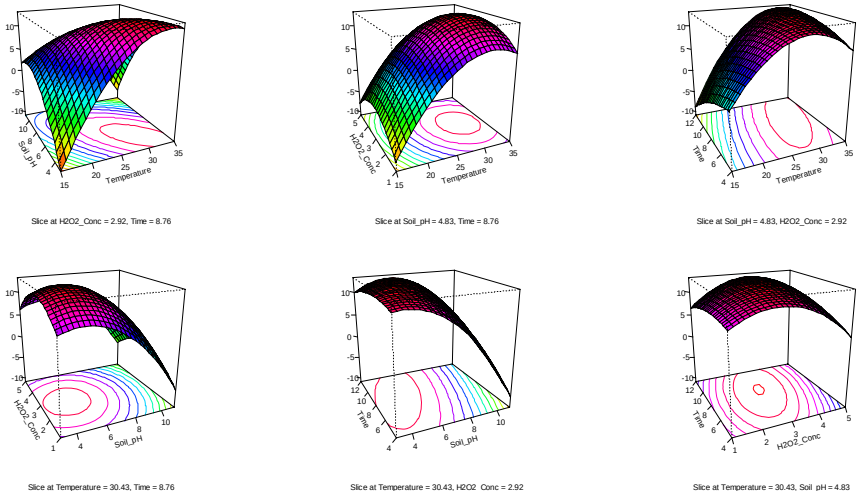

Figure 2. Response surface plot for the H_2O_2 model.

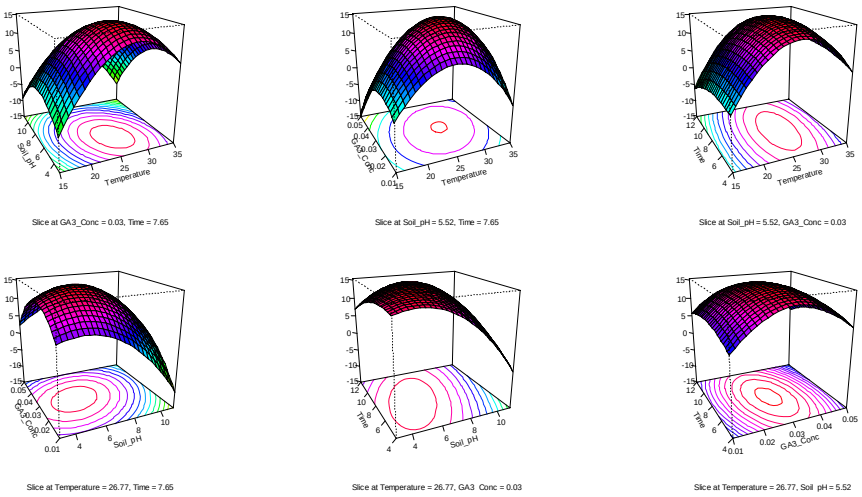

Figure 3. Response surface plot for the GA_3 model.

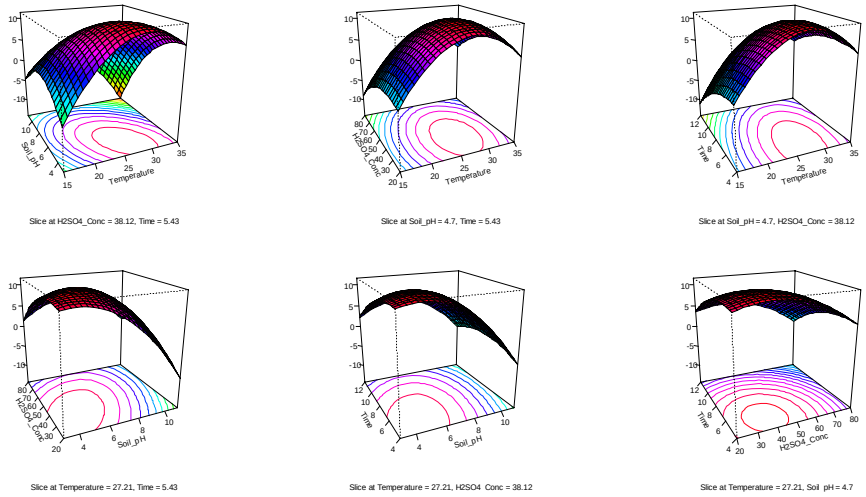

Figure 4. Response surface plot for the H_2SO_4 model.

We then performed canonical analyses of the four models. The points that yielded maximum predicted values (stationary points) and the associated optimal predicted germination rates are shown in Table 4.

Table 4. Stationary points for the germination of *Melia volkensii* models

Model	Stationary point (coded)				Stationary point (actual)				Optimal germination rate
	x_1	x_2	x_3	x_4	Temp (^oC)	pH	Conc. (%)	Time (hours)	
KNO_3	1.2262	–1.5263	0.6667	0.1661	31.13	3.95	0.37	8.33	65.98%
H_2O_2	1.0857	–1.0843	–0.0786	0.3819	30.43	4.83	2.92	8.76	65.24%
GA_3	0.3541	–0.7390	–0.0033	–0.1753	26.77	5.52	0.03	7.65	76.49%
H_2SO_4	0.4423	–1.1497	–0.7922	–1.2840	27.21	4.70	38.13	5.43	57.26%

Table 4 shows that germination rate was optimized at 65.98% for KNO_3 whereby the soil pH was 3.95, the temperature was 31.13°C, the KNO_3 concentration was 0.37% and the seed treatment time was 8.33 hours. For H_2O_2, germination rate was optimized at 65.24% whereby the soil pH was 4.83, the temperature was 30.43°C, the H_2O_2 concentration was 2.92% and the seed treatment time was 8.76 hours. The germination rate was optimized

at 76.49% for GA_3 whereby the soil pH was 5.52, the temperature was 26.77°C, the GA_3 concentration was 0.03% and the seed treatment time was 7.65 hours. For H_2SO_4, germination rate was optimized at 57.26% whereby the soil pH was 4.70, the temperature was 27.21°C, the H_2SO_4 concentration was 38.13% and the seed treatment time was 5.43 hours.

Table 5 depicts the eigenvalues realized from the canonical analyses of the fitted models.

Table 5. Eigenvalues for the germination of *Melia volkensii* models

Model	Eigenvalues			
KNO_3	–0.4768	–0.6421	–1.1710	–2.9184
H_2O_2	–0.1652	–0.5608	–1.2677	–2.8188
GA_3	–0.4078	–1.4899	–1.8798	–3.5350
H_2SO_4	–0.4345	–0.6668	–1.2618	–2.6578

Table 5 shows that for the four models, all the eigenvalues are negative. This indicated that all the stationary points were points of maximum response.

The canonical equivalent forms of the fitted models are given in (8), (9), (10) and (11) for the KNO_3, H_2O_2, GA_3 and H_2SO_4 models, respectively,

$$\hat{y} = 13.1951 - 0.4768w_1^2 - 0.6421w_2^2 - 1.1710w_3^2 - 2.9184w_4^2, \quad (8)$$

$$\hat{y} = 13.0486 - 0.1652w_1^2 - 0.5608w_2^2 - 1.2677w_3^2 - 2.8188w_4^2, \quad (9)$$

$$\hat{y} = 15.2979 - 0.4078w_1^2 - 1.4899w_2^2 - 1.8798w_3^2 - 3.5350w_4^2, \quad (10)$$

$$\hat{y} = 11.4534 - 0.4345w_1^2 - 0.6668w_2^2 - 1.2618w_3^2 - 2.6578w_4^2. \quad (11)$$

3.5. Verification of optimized condition and predictive models

For each of the models developed, optimal conditions for germination of *Melia volkensii* were obtained as shown in Table 4. In order to verify the adequacy of the models developed, three confirmation run experiments were performed for each model at the optimal conditions. The results are shown in Table 6.

Table 6. Confirmation experiments at optimum conditions

Chemical	Temp. (°C)	pH	Conc. (%)	Time (hours)	Predicted value	Experimental value	Residual	Error (%)
KNO_3	31.13	3.95	0.37	8.33	13.2	13	0.2	1.54
	31.13	3.95	0.37	8.33	13.2	12	1.2	10.00
	31.13	3.95	0.37	8.33	13.2	13	0.2	1.54
H_2O_2	30.43	4.83	2.92	8.76	13.0	9	4.0	44.44
	30.43	4.83	2.92	8.76	13.0	12	1.0	8.33
	30.43	4.83	2.92	8.76	13.0	14	–1.0	–7.14
GA_3	26.77	5.52	0.03	7.65	15.3	15	0.3	2.00
	26.77	5.52	0.03	7.65	15.3	15	0.3	2.00
	26.77	5.52	0.03	7.65	15.3	13	2.3	17.69
H_2SO_4	27.21	4.70	38.13	5.43	11.5	8	3.5	43.75
	27.21	4.70	38.13	5.43	11.5	13	–1.5	–11.54
	27.21	4.70	38.13	5.43	11.5	12	–0.5	–4.17

The difference between the predicted value and the actual value ranged between –1.5 to 4.3 with the percentage error ranging between –11.54% to 44.44%. Paired *t*-test was done to establish whether there was any significant difference between the experimental value and the predicted one. The *t* statistic value for the test was found to be 1.7024 with a two sided *p* value of 0.1156. This showed that there was no significant difference between the predicted and the experimental values implying that the rsm approach was appropriate for optimizing the conditions for germination of *Melia volkensii*.

4. Conclusions and Recommendations

We established that in general, germination rates of Melia seeds are low. For the four chemicals used in the experiment, the germination rates were found to be 31.67% for KNO_3, 39.08% for H_2O_2, 42.00% for GA_3 and 28.25% for H_2SO_4. Hence, the use of GA_3 for seed pre-treatment achieved the best germination rates. For this chemical, the optimum temperature was found to be 26.77°C, the optimal soil pH was 5.52, the optimal concentration was 0.03% and the optimal pre-treatment time was 7.65 hours.

The overall germination rate was found to be 35%. However, when the conditions are favourable and set correctly, germination rates can be optimized at between 55% and 75%. To maximize germination rates of Melia seeds, the appropriate combination of temperature, soil pH, chemical

concentration and treatment time needs to be formulated. Further, an investigation can be carried out to increase the reliability of second order models in predicting germination rates for *Melia volkensii*.

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