Optimization Of Bio-Fertilizer Production From Watermelon Peels Using Response Surface Methodology.

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ABSTRACT

The continuous use of chemical fertilizer affects the environment and soil fertility. Bio-fertilizer helps in safeguarding soil fertility and reduce environmental pollution. This research focuses on the optimization of production of bio-fertilizer from watermelon peels. Central Composite Design (CCD) under the Response Surface Methodology (RSM) of the Design Expert software version 6.0 was used to optimize the retention days and water ratio, (factors), while Nitrogen(N), Phosphorous (P) and potassium (K) were the responses. Watermelon peel was blended and fermented for different days based on the experimental design obtained. The optimum values obtained after optimization for retention days and water ratio are 20 days and 400 ml respectively, while N. P and K contents are 26.2824, 94.5099 and 0.0005, respectively. The use of bio-fertilizers will be more economical and safer for farming activities.

Keywords: Bio-Fertilizer, Watermelon peels, Plastic rubber.

1.0 BACKGROUND

Bio-fertilizers are products from agro-waste which consist of some micro-organism like bacteria, fungi and algae. Basically they are cost efficient, not harmful to the environment & natural source of nutrients [8].

Presently, there is a growing concern about the negative impact and threats to sustainable agriculture resulting for chemical agents such as chemical fertilizer. The continuous use of bio-fertilizers proves to be economical in terms of production, eco-friendly in terms of use, more efficient, productive and attainable to farmers over chemical fertilizers.[5]

Bio-fertilizer is favourably the need for present day agriculture, since it is safe and residue free food is increasing on daily basis. In view of the shifting focus towards organic farming and reduction of negative effects and residues in the ecosystem, it is necessary to promote the production of bio-fertilizers in large scale in order to meet the present demand. Bio-fertilizers became popular due to high negative impact of chemical fertilizers [16].

Environmentalists are requiring immediate action by society for a shift to more environmentally friendly method of farming. Organic farming tends to be more environmentally sustainable form of agricultural production. similarly it emphasizes on good physical and mental state of animal and the prevention of synthetic chemical inputs such as fertilizers and genetically modified organisms (GMOs).[10]

For a sustainable agriculture system, it is essential to use natural resources, renewable inputs which are more helpful to the plant and cause less environment hazard[10]. Agro-wastes are made up of waste which is formed from various agricultural products. Agro-wastes consist of fruits,vegetables, weeds and organic manure. The accumulation of agro-waste is harmful to the ecosystem and causes some environmental hazards. In order to avoid this, proper and safe waste management are undertaken.

The agro-wastes can be used in the production of Bio-fertilizer using fermentation method such as solid state fermentation which is a simple and cost efficient method [14].

In the times past, the farmers were keen in the usage of chemical fertilizer as it result to high yield. But eventually, they realized that chemical fertilizer affects the soil nutrients and kills the essential microbes which enhance the growth of crops. The negative impact faced using chemical fertilizers were affecting not only the soil but also human beings who eat these farm products[13]. Generally agricultural products which includes bio-fertilizer are used to replace the usage of chemical fertilizers as it does not contain any toxic substance and makes the soil enriched[1]

1.1 Sustainability of Bio-fertilizers.

The environmental hazards resulting from the frequent use and application of mineral fertilizers, and the negative environmental impacts of chemical fertilizers and their rising costs, enhances the application of bio-fertilizer which is valuable in the sustainable agricultural practices. The continuous application of bio-fertilizer encouraged plant growth and productivity [5] due to the presence of different strain groups such as nitrogen fixer, nutrient mobilization microorganisms which help in increasing the availability of minerals and their forms in composted minerals and increase levels of extractable of macro or micro-nutrients has increased significance effect of bio-fertilizers in different crop plants[2] [4]. Bio-fertilizer can be processed through Solid-state fermentation[5][12]. Usually the retention period for the anaerobic digestion of agro-wastes at mesophilic temperature ranges between 20 to 40 days [8].

The major source of organic matter in soil is agro-wastes, because of the decaying part of plants. Agricultural wastes are the cheapest form of raw material that can be used by farmers to improve the fertility of soil due to it availability. [3]

1.2 Problem Statement

The continuous use of chemical fertilizers affects the environment, soil fertility and kills the beneficial microbes which enhances the growth of the crops. Hence, the use of natural products like bio-fertilizers in crop cultivation will help in safeguarding the soil health, reduce environmental pollution and yield quality products.

1.3 Justification

Presently, most communities in the rural areas in Nigeria are exposed to environmental issues, as a result of accumulation of agro-waste and continuous use of chemical fertilizer. In order to overcome the problems caused by continuous use of chemical fertilizers to the environment and soil, the focus of this research is to produce bio-fertilizer as a natural source against synthetic chemicals thereby reducing side effect resulting from the use of chemical fertilizer and waste in our environment through the concept of waste to wealth.

The application of Response surface methodology (RSM) to design optimization is aimed at reducing the cost of expensive analysis methods (e.g. finite element method or CFD analysis) and their associated numerical noise.

This research work is based optimization Of bio-fertilizer production from watermelon peels, in which Central Composite Design (CCD) under the Response Surface Methodology (RSM) was used to optimize the retention days and water ratio, (factors), while Nitrogen(N), Phosphorous (P) and potassium (K) were the responses.

2.0 MATERIAL AND METHOD

The raw material used for this study were sourced from yelwa market bauchi.while the chemicals used were of analytic reagent grade.

2.1 Materials

Watermelon peels,Water, Plastic rubber.

2.2 Experimental Design

Central composite design (CCD) method under the response surface methodology (RSM) of the design expert software were employed in the optimization of the production conditions such as retention time and water ratio. So as to determine the optimal condition for production of biofertilizer from watermelon peels.

2.2.1 Experimental design table

Run	Factor 1 Retention days (days)	Factor 2 Water ratio (ml)
1	40.00	1.00
2	30.00	4.00
3	20.00	7.00
4	15.86	4.00
5	3.00	4.00
6	3.00	8.25
7	20.00	1.00
8	44.14	4.00
9	30.00	-0.24
10	30.00	4.00
11	40.00	7.00
12	30.00	4.00
13	30.00	4.00

Table 1: Experimental design used for analysis.

2.2.2 Production Condition.

In order to study and determine the most feasible local environmental conditions for optimal production of biofertilizer from watermelon peel, 500 g of watermelon peel and different water ratio were used based on the experimental design table.

2.3 Preparation of Raw materials

Watermelon peels were collected from yelwa market,Bauchi. The raw materials were pre-treated by removal of unwanted materials, the watermelon peels were washed with water in order to remove impurities. The watermelon peels were blended using commercial blender.

Five hundred gram (500 g) of watermelon peel was measured using a weighing balance. The biomass were blended and mixed with water based on the experimental design values as stated. The essence of mixing the biomass with water is to allow the bacteria to move freely inside the fermenter (airtight container). The blended feed-stock was fed into the fermenter (airtight container) and closed after charging, so as to ensure airtight condition of the fermentation process. And it were allowed to ferment for different retention days at mesophilic temperature(with an optimum growth range from 20 to 45 °C). After the retention period of each of the sample, the substrate were collected and filtered. Then a sample of the soluble product were taken for elemental analysis.

2.4 Determination of nitrogen (N)

2.4.1 *Digestion*

10 ml of the sample were measured into a beaker and 8 g of K_2SO_4 and 1g of $CuSO_4$ were add to the sample, then 15 ml of H_2SO_4 was slowly added and then the beaker was then placed on heating mantle and was heated until it boiled. The boiled sample was allowed to cool and were diluted with 100 ml of distill water, which was then filtered using filter paper.

2.4.2 *Distillation*

50 ml of the filtered sample solution were measured and 30ml of NaOH were added and used for distillation. After distillation, 15 ml of the distillate were used for titration.

2.4.3 *Titration*

10 ml of boric acid and 10ml of sodium hydroxide (NaOH) were added to the 15 ml of the distillate and 2-3 drops of methyl orange were droped into it and mixed, then titration were carried out until the titrant turns pale pink. The burrete reading were then taken and percentage Nitrogen were calculated.

2.5 Determination of phosphorus (P)

50 ml of the sample were measured and 1 drop of phenolphthalein indicator were added to it, then 1 ml of 10 N H_2SO_4 and 0.5g of $K_2S_2O_8$ were added to adjust the colour.

Then the sample were boiled for 30 minutes until a final volume was reached. The boiled sample solution were cooled and diluted with 20 ml of distill water, and 1 drop of phenolpthalein indicator solution were added to it. then the faint pink colour were neutralize with 5 ml of NaOH and distill water were added to the sample solution to make it up to 100 ml.

50 ml of the digested sample were measured and 1 drop of phenolphthalein and 8 ml of combined reagent were added to it and mixed. Then the absorbance and concentration of the sample solution were measured at 880 nm.

2.6 Determination of potassium (K)

20 ml of the sample were measured into a beaker and 5 ml of Hcl and 5ml of HNO_3 were added to the sample, then 50 ml of distill water were added as well.

Then the sample solution were then heated until totally digested, after digestion it was then diluted with 50 ml of distill water and filtered.

Then the concentration of potassium, were then determined using atomic absorption spectrophotometer.

3.0 RESULTS AND DISCUSSIONS

3.1 Results

Table 2: Determination of N.P.K Content in fermented watermelon peel samples

Run	Factor 1 A:Retention days (days)	Factor 2 B:water ratio (mL)	Response 1 N content (%)	Response 2 P content (%)	Response 3 K content (%)
1	20.00	1.00	9.072	19.6	0.000244
2	30.00	8.24	16.352	46.76	6.45E-005
3	30.00	4.00	17.192	94.44	7.4E-005
4	30.00	4.00	17.136	94.48	7.37E-005
5	30.00	4.00	17.024	94.4	7.3E-005
6	30.00	4.00	16.912	94.5	7.34E-005
7	40.00	7.00	24.13	57.54	3.55E-005
8	30.00	4.00	17.192	94.5	7.35E-005
9	40.00	1.00	18.592	38.92	0.0001354
10	20.00	7.00	8.512	21.22	0.0002662
11	15.86	4.00	18.312	19.54	0.000225

The result of experiments are given in table 2, the result obtained from each sample shows that, the concentration of phosphorus in each of the sample is higher compared to nitrogen, while the concentration of potassium is in trace. This a clear indication that biofertilizer produced from watermelon peel is a phosphorous solubilizing biofertilizer and Nitrogen fixing biofertilizer.

3.2 Summary and ANOVA for Response Surface Cubic Model for N.P.K content.

Table 3: Lack of Fit Tests Nitrogen content

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Linear	498.9524	6	83.15874	5642.011	< 0.0001	
2FI	489.656	5	97.9312	6644.269	< 0.0001	
Quadratic	412.7925	3	137.5975	9335.479	< 0.0001	
Cubic	0	0				Aliased
Pure Error	0.058957	4	0.014739			

Table 4: Model Summary Statistics

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	7.064074	0.15142	-0.0183	-0.79062	1052.979	
2FI	7.376501	0.167229	-0.11036	-1.76589	1626.491	
Quadratic	7.679764	0.297937	-0.20354	-3.99207	2935.608	
Cubic	0.121405	0.9999	0.999699		+	Aliased

Table 5: Analysis of variance table (ANOVA for Response Surface CubicModel)

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	587.9956	8	73.49945	4986.665	< 0.0001	significant
A	279.4843	1	279.4843	18961.97	< 0.0001	
B	70.0715	1	70.0715	4754.091	< 0.0001	
A^2	79.10107	1	79.10107	5366.714	< 0.0001	

B^2	6.851241	1	6.851241	464.8313	< 0.0001		
AB	9.296401	1	9.296401	630.7263	< 0.0001		
A^3	307.8179	1	307.8179	20884.3	< 0.0001		
B^3	70.0149	1	70.0149	4750.251	< 0.0001		
A^2B	70.10218	1	70.10218	4756.173	< 0.0001		
AB^2	0	0					
Pure Error	0.058957	4	0.014739				
Cor Total	588.0545	12					
R-Squared	0.9999		Adj R-Squared	0.9997	Adeq Precision	228.976	

Table 6: Lack of Fit Tests for Phosphorous content

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Linear	16487.97	6	2747.995	1461699	< 0.0001	
2FI	16415.72	5	3283.144	1746353	< 0.0001	
Quadratic	1683.522	3	561.1741	298496.9	< 0.0001	Suggested
Cubic	0	0				Aliased
Pure Error	0.00752	4	0.00188			

Table 7: Model Summary Statistics

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	40.60539	0.057708	-0.13075	-0.55083	27136.1	
2FI	42.70796	0.061837	-0.25088	-0.89199	33105.55	
Quadratic	15.5082	0.903786	0.835062	0.315889	11970.4	Suggested
Cubic	0.043359	1	0.999999		+	Aliased

Table 8 Analysis of variance table (ANOVA for Response Surface Cubic Model)

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	17497.73	8	2187.217	1163413	< 0.0001	significant
A	945.7965	1	945.7965	503083.3	< 0.0001	
B	597.5713	1	597.5713	317857.1	< 0.0001	
A^2	10128.88	1	10128.88	5387704	< 0.0001	
B^2	765.3576	1	765.3576	407105.1	< 0.0001	
AB	72.25	1	72.25	38430.85	< 0.0001	
A^3	838.336	1	838.336	445923.4	< 0.0001	
B^3	597.078	1	597.078	317594.7	< 0.0001	
A^2B	597.7567	1	597.7567	317955.7	< 0.0001	
AB^2	0	0				
Pure Error	0.00752	4	0.00188			
Cor Total	17497.74	12				
R-Squared	1		Adj R-Squared	1	Adeq Precision	2590.692

Table 9: Lack of Fit Tests for potassium content

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Linear	4.00E-08	6	6.66E-09	48649.44	< 0.0001	Suggested
2FI	3.63E-08	5	7.25E-09	52938.3	< 0.0001	
Quadratic	2.28E-08	3	7.60E-09	55447.68	< 0.0001	
Cubic	0	0				Aliased
Pure Error	5.48E-13	4	1.37E-13			

Table 10: Model Summary Statistics

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	6.32E-05	0.574803	0.489763	0.109053	8.38E-08	Suggested
2FI	6.35E-05	0.614431	0.485908	-0.52043	1.43E-07	
Quadratic	5.71E-05	0.75769	0.584612	-0.72264	1.62E-07	
Cubic	3.70E-07	0.999994	0.999983		+	Aliased

Table 11: Analysis of variance table (ANOVA for Response Surface cubic Model)

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	9.41E-08	8	1.18E-08	85812.78	< 0.0001	significant
A	6.49E-09	1	6.49E-09	47386.07	< 0.0001	
B	1.92E-08	1	1.92E-08	139944.5	< 0.0001	
A^2	2.17E-09	1	2.17E-09	15838.93	< 0.0001	
B^2	1.40E-08	1	1.40E-08	102526.2	< 0.0001	

AB	3.73E-09	1	3.73E-09	27205.13	< 0.0001	
A^3	5.56E-11	1	5.56E-11	405.9738	< 0.0001	
B^3	1.92E-08	1	1.92E-08	139915.8	< 0.0001	
A^2B	1.92E-08	1	1.92E-08	140022.1	< 0.0001	
AB^2	0	0				
Pure Error	5.48E-13	4	1.37E-13			
Cor Total	9.41E-08	12				
R-Squared	1		Adj R-Squared	1	Adeq Precision	864.367

3.3 Interaction Between Water Ratio And Retention Days On N.P.K. Content

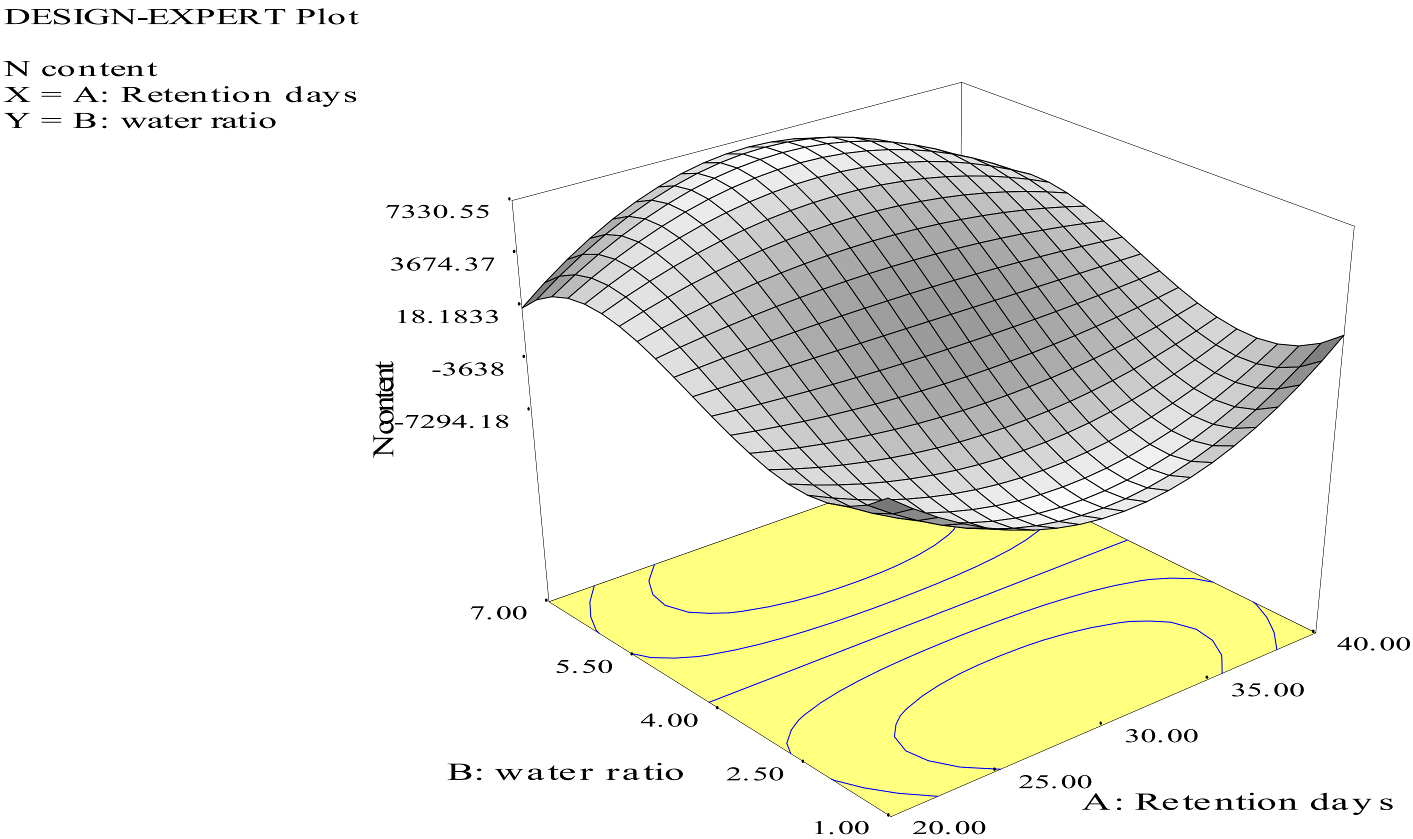

Figure 1: *Interaction between water ratio and retention days on Nitrogen content.*

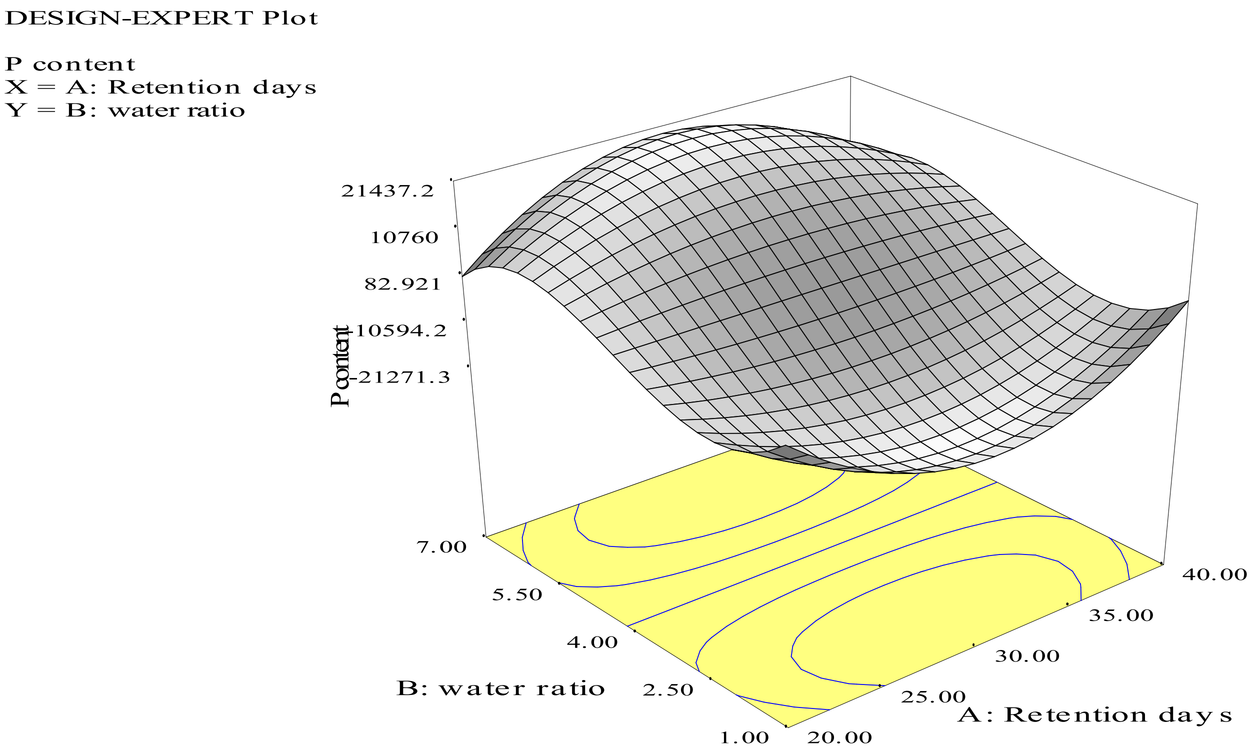

Figure 2: Interaction between water ratio and retention days on phosphorus content.

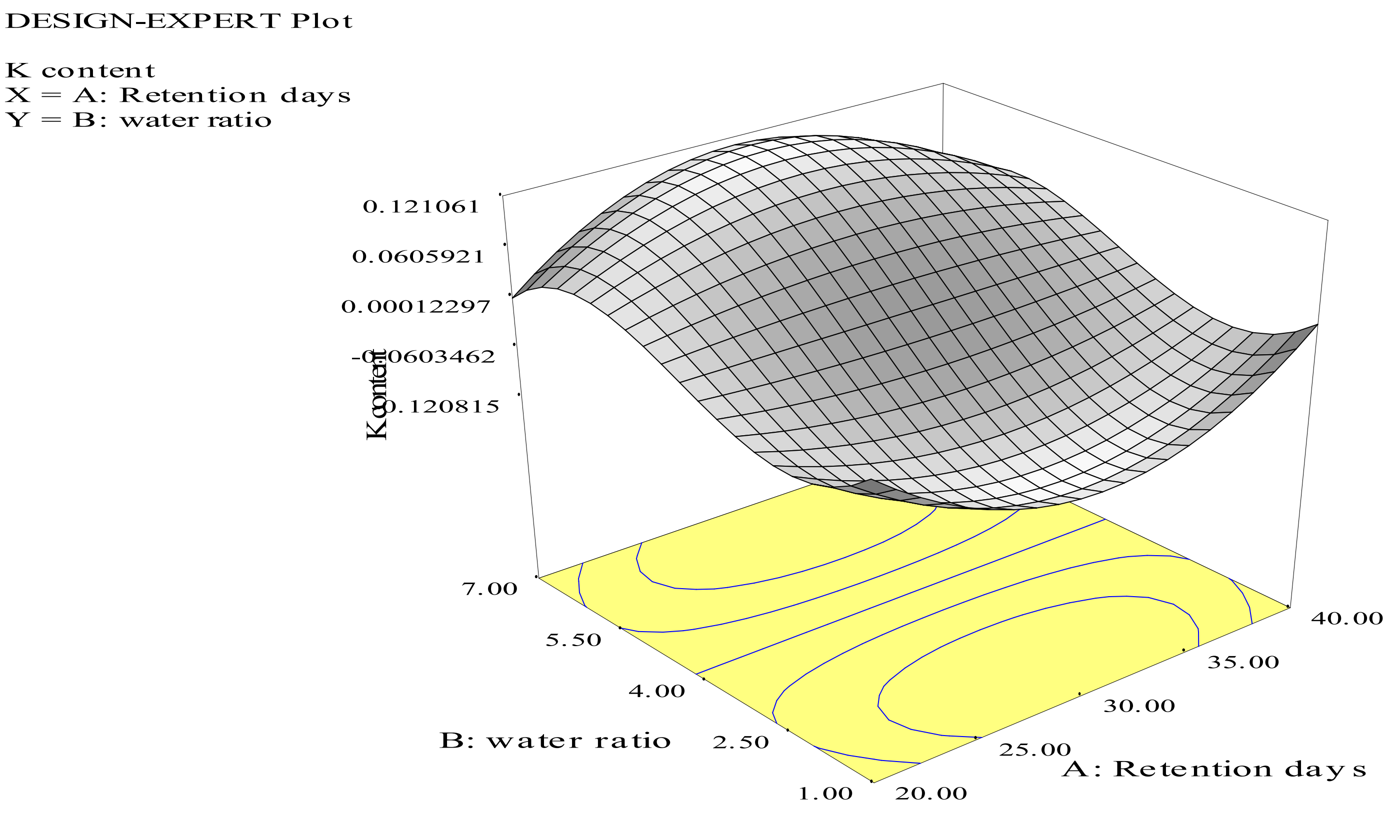

Figure 3: *Interaction between water ratio and retention days on potassium content.*

ANOVA results for the cubic model were shown in table 5, 8 and 11. Based on the CCD design, the result analysis generated the following cubic model equation based on coded factors as seen in equation 4.1, 4.2 and 4.3.

$$\text{N-content} = + 17.09 + 18.69A + 13443.23B - 3.72A^2 + 1.71B^2 + 1.52AB - 12.41A^3 - 6723.09B^3 - 6718.90A^2B \quad \ldots(4.1)$$

$$\text{P-content} = + 94.46 + 34.39A + 39257.98B - 42.11A^2 - 18.04B^2 - 4.25AB - 20.48A^3 - 19633.10B^3 - 19619.82A^2B \quad \ldots(4.2)$$

$$\text{K-content} = +7.352E\text{-}005 - 9.010E\text{-}005A + 0.22B + 1.949E\text{-}005A^2 + 7.727E\text{-}005B^2 - 3.052E\text{-}005AB + 5.275E\text{-}006A^3 - 0.11B^3 - 0.11A^2B \quad \ldots(4.3)$$

Where N.P.K content is the response and the coded term A and B represent retention days and water ratio. It can be seen from the model equation that the linear terms A and B, and the interaction term AB in equation 4.1 and 4.2 are positive which denote positive contribution to N-content and P-content, while the quadratic and cubic terms $A^{2,}$ B^2 A^3 B^3 and $A^2B$are negative, which denote negative contribution to both N and P content. While for equation 4.3, it could be seen that the cubic quadratic terms A^2 B^2 and $A^3$are positive, which denote positive contribution to K-content. While the interaction term AB and A^2B is negative which denotes negative contribution to K-content. From the numerical solution obtained these solution were picked which gave an optimum values, which are retention days20.00, water ratio4.01, N content26.2824, P content94.5099 and K content0.000495515.

4.0 CONCLUSION

Based on the analysis, design expert software 6.0, were used to optimize retention days and water ratio which gave an optimum value of N.P.K content at a good desirability level.

Bio fertilizer were produced from water melon peel after certain retention days with different water ratio based on the experimental design. The result obtained from this study shows that, the higher the retention days the higher the yield of bio-fertilize at a minimum water ratio while the lower the retention days the lower the yield. there for retention days plays a vital role in production of Bio-fertilizer from watermelon peels.

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