APPLICATION OF BOX-BEHNKEN DESIGN AND RESPONSE SURFACE METHODOLOGY FOR OPTIMISATION OF BUTTERNUT (*CUCURBITA MOSCHATA*) FRUIT YIELD USING FERTILISERS AND PINCHING

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Abstract

Butternut (*Cucurbita moschata*) production is constrained by poor agronomic practices such as suboptimal application of fertilizers and lack of appropriate pinching practices aimed at improving the number of female flowers. Moreover, many farmers mix animal manures and inorganic fertilisers in one hill without any recommended rates. The objective of this study was to apply Box-Behnken Design and Response Surface Methodology to optimize butternut fruit yield using poultry manure, NPK fertiliser and pinching. The experiments were conducted in two trials in 2019 and 2020 at Karingani ward, Chuka. A Factorial experiment laid down in Randomised Complete Block Design was used. The factors included poultry manure at three levels (0, 5 and 10 tons/ha), NPK at three levels (0, 100 and 200 kg/ha of NPK 17:17:17) and pinching at three levels (0, 4th node and 6th node). Butternut variety Atlas F_1 was used. Data was collected on the fruit yield. The input variables were modelled and maximised using Box-Behnken design (BBD) and Response Surface Methodology. The optimisation of the input variables revealed that the optimal levels of application of NPK and poultry manure that can lead to maximum yield of butternut fruits were 505 kg/ha of NPK and 8102 kg/ha poultry manure. The model also showed that pinching should be conducted 30 days after planting (equivalent to pinching at 3rd node) for optimal butternut fruit yield. The study demonstrated that BBD can serve as an inexpensive tool in optimization of the butternut fruit production. However, there is need for further field studies to validate the findings of this study in order to accurately advice farmers on optimum combined application of manure, NPK and pinching time.

Key words: Optimisation, Butternut, Yield, Response Surface Methodology, Pinching, Female Flowers

INTRODUCTION

To mitigate current climate trends, one of the management strategies is to maximize crop production while minimizing environmental degradation (Shah and Wu, 2019). This entails adopting farming practices that are eco-friendly. To abate the negative impacts of soil fertility management there is need to substitute chemical fertilizers with organic fertilizer (Shah and Wu, 2019). Therefore, there is need to evaluate the effect of combined application of organic and inorganic fertilizers as a means of substituting a certain amount of inorganic with organic fertilisers, while maintaining sustainable yields. Moreover, most of agronomic practices used in butternut production are borrowed from pumpkin cultural practices. However, there are inherent genetic differences between the two species, and therefore, they may be expected to have different optimum agronomic practices.

Despite many studies on effect of soil nutrition on cucurbit production, cucurbit production is also constraint by production of female flowers (OECD, 2016), a problem which has been largely ignored. Pinching has been found to increase the number of female flowers in cucumbers (Beura *et al*., 2016; Nayak *et al*., 2018). Pinching maintains a proper balance between the vegetative and reproductive growth to maximize production

(Patel *et al*., 2017). In cucumbers (*Cucumis sativus* L), pinching significantly influenced the yield parameters, for example, fruit length, number of fruits per vine, yield per vine and yield per ha, but not fruit weight (Nayak *et al*., 2018). This demonstrates that pinching could lead to increased productivity per area. In watermelon, different pinching were shown to significantly influence the yield and yield components (Naglaa *et al*., 2019). However, there is little adoption of pinching on butternuts by most farmers, which can be attributed to limited information on effect of pinching on butternut plants performance (Beura *et al*., 2016). Therefore, this study sought to incorporate pinching as one of variable that can be optimised in order to increase the yield of butternut fruits. This requires careful design and analysis of experiments that combine the effects of fertilizer application and pinching to optimise growth and development of butternut.

Various statistical methods exist that can be used to optimize the dependent variable (yield and yield components) in the design of experiments, for example, the Central Composite Design and the Box-Behnken Design (Sadhukhan *et al*., 2016; Ferreira *et al*., 2019). The two designs are classified under Response Surface Methodology (RSM), which is a collection of mathematical and statistical techniques effective for empirical modelling, optimization of process conditions, and it can determine the influence of various factors (independent variables) and their interactions on the dependent variable under investigation (Myers 2016; Montgomery, 2017). Response Surface Methodology has been widely used in agricultural sciences (Danbaba *et al*., 2015; Zarins *et al*., 2018; Masai *et al*., 2020). This study will use Response Surface Methodology to optimize the yield of butternut using treatments range applied. Box-Behnken Design will be used to fit a quadratic polynomial model, whereby, the response (butternut yield) will be expressed as a function of independent variables (fertilizer applications and pinching), giving an idea of the shape of the response surface under investigation. Box-Behnken design can generate higher order response surfaces using fewer required runs than a normal factorial design.

MATERIALS AND METHODS

Study Site

The study was conducted within two sites at Karingani Ward, Chuka, i.e., Chuka University Horticultural demonstration field in October 2019 to January 2020 and at a farmer's field, adjacent to Chuka University in January to April 2020. The study area lies within a latitude of 0° 19'60.00"N and a longitude 37° 38′59.99′E. The area has an altitude of 1500 m above sea level with an annual mean temperature of 20℃ and a total annual rainfall of 1200–1400 mm. The rainfall is bi-modally distributed with long rains from March to June and short rains from October to December. The area lies in the Upper Midland Zone (Jaetzold *et al.*, 2006) on the eastern slopes of Mount Kenya. The main crops grown in the area include beans, coffee, maize, cowpeas, pigeon peas, cassava, sorghum, millet and there is also keeping of livestock such as cattle, goats, sheep and poultry (Ogolla *et al*., 2019). The soils are mainly humic Nitisols (Jaetzold *et al*., 2006), which are deep and well weathered with moderate to high inherent fertility, but their fertility has declined due to intensive cultivation without adequate replenishment of soil nutrients.

Experimental Design

The study was carried out using a factorial experiment laid out in Randomised Complete Block Design (RCBD) and replicated three times. There were three factors: poultry manure at three levels (0, 5 and 10 tons/ha), inorganic fertiliser at three levels (0, 100 and 200 Kg/ha NPK fertilizer 17:17:17) and pinching at three levels (non-pinching, pinching at 4^{th} node and pinching at 6^{th} node). Atlas F_1 butternut variety was used in the study.

Land Preparation, Planting Material and Planting

The experimental plots was prepared to a fine tilth and divided into three blocks each with 30 plots each measuring 2 m x 2 m and a path of 1 m. Butternut certified seeds were sourced from Simlaw Seed Company, Meru branch, while the fertilizer was sourced from an Agrovet within Chuka town. The poultry manure was obtained from an identified poultry farmer in Karingani Ward, Chuka. Soil and poultry manure analyses was done at the beginning of each planting trial to determine the total N (following Kjeldahl method), available P, and K (according to Mehlich 1984 and Wortmann *et al*. 2014). Three butternut seeds were sown directly in the centre of the hill after which thinning was done when the plant attained two to three true leaves to leave one plant per hill. Cultural practices were performed as per recommended rates.

Data Collection

Data was collected on butternut fruit yield. The total number of fruits harvested from each plot were weighed using an electric weighing balance after which their weights were recorded and given in the units of grams. The total harvest from the experimental plot was then summed up and converted into hectare basis to give the fruit yield.

Data Analysis

The second- order models representing the yield of the butternut fruit (Y_1) expressed as a function of poultry manure (X_1), NPK (X_2) and pinching (X_3) which were the independent variables of the yield of the butternut fruit response. The appropriate second-order models were expressed as;

$$Y_1 = \alpha_0 + \alpha_1 X_1 + \alpha_2 X_2 + \alpha_3 X_3 + \alpha_{11} X_1^2 + \alpha_{22} X_2^2 + \alpha_{33} X_3^2 + \alpha_1\alpha_2 X_1 X_2 + \alpha_1\alpha_3 X_1 X_3 + \alpha_2\alpha_3 X_2 X_3 + \varepsilon_1$$

where Y_i = Response value [Yield of butternut per plot], X_1 = Poultry manure, X_2 = NPK, X_3 = pinching, α_0 = constant; α_i = linear coefficients, α_{ii} = quadratic coefficients, $\alpha_i\alpha_j$ = cross product ($i<j$) and e_i = error terms.

Parameter Estimation

The regression coefficients in equation 2 were estimated using least square method (Montgomery, 2013). The equation 2 can be written in matrix form as follows,

$$\boldsymbol{Y} = \boldsymbol{X\beta} + \boldsymbol{\varepsilon}$$

where ***Y*** is the response vector, ***X*** is a matrix of the chosen experimental design, ***β*** is the vector constituted by parameters of the model and ***e*** is the residual. Assuming that $E(\boldsymbol{e}) = 0$ and $var(\boldsymbol{e}) = \delta^2 I_{20}$,

The analysis of a second-order model was implemented using R Software.

Checking the Adequacy of the Model

The model adequacy was tested using the lack of fit test statistic and the adjusted R^2(Myers *et al*., 2004).

Optimization

A general mathematical solution for the location of the stationary point was obtained according to Montgomery (2013). The optimal parameter setting for the second order model was found by use of matrix algebra. The second order model can be written in matrix form as;

$$\hat{Y} = \hat{\alpha}_0 + X^T b + X^T B X$$

The derivatives of $\hat{Y}$ with respect to the elements of the vector X are;

$$\frac{\partial \hat{Y}}{\partial X} = b + 2BX = 0$$

Therefore the solution to stationary point is;

$$X_s = -\frac{1}{2} B^{-1} b$$

where $b = \begin{bmatrix} \alpha_1 \\ \alpha_2 \\ \alpha_1 \end{bmatrix}$ and $B = \begin{bmatrix} \alpha_{11} & \frac{\alpha_{12}}{2} & \frac{\alpha_{13}}{2} \\ \frac{\alpha_{21}}{2} & \alpha_{22} & \frac{\alpha_{23}}{2} \\ \frac{\alpha_{31}}{2} & \frac{\alpha_{32}}{2} & \alpha_{33} \end{bmatrix}$

Finally, the estimated response value of the stationary points was calculated as;

$$\hat{y}_s = \hat{a}_0 + \frac{1}{2} x_s^T \text{ b}$$

RESULTS AND DISCUSSION

Preliminary Analysis

The results of the study showed that the average yield of butternut fruit produced was 3790.82 g per plot with a median of 3636.50 g and a standard deviation of 2098.21 g (Table 1). The skewness for weight of butternut was -0.0245, while the kurtosis value was -1.4123.

Table 1: Preliminary analysis

Mean	Standard deviation	Median	Skewness	Kurtosis	Maximum	Minimum
3790.82	2098.21	3636.50	-0.0245	-1.4123	7023.50	612.00

Skewness and Kurtosis test can be used to determine the normality of the data. The results showed that the yield of butternut fruits was normally distributed. This is because skewness and Kurtosis test values fell within the range of ±3 and ±2, respectively (George and Mallery, 2010). Test for normality is important since several statistical tests and models assume that the underlying data is normally distributed.

Fitting a Second Order Model for Butternut Fruit Yield

The second order model was fitted using the yield of butternuts per plot as the response and the pinching, poultry manure and NPK as the independent variables. There was significant effect of pinching, NPK and poultry manure on butternut fruit yield for Atlas F_1 (Table 2).

A second order model was then fitted for weight of butternuts (Table 1). The response surface second order model revealed that pinching, poultry manure and NPK were significant ($p < 0.05$) in explaining the yield of butternut fruits (Table 3). The interaction between pinching and NPK and pinching and poultry manure was not significant ($p > 0.05$) in explaining the yield of butternut. All the quadratic terms were significant ($p < 0.05$) in explaining the yield of butternut fruits (Table 3).

Analysis of variance was used to check the adequacy of the model for the response in the study at 5% significance level (Table 4). The analysis of variance results showed that the combined first order terms were significant ($p < 0.05$), implying that pinching, NPK and poultry manure individually are significant in explaining variation in yield of butternut fruits. Thus applying them alone can still lead to significant increase in the yield of butternut fruits. The combined two way interactions were not significant ($p < 0.05$), but the combined purely quadratic terms were all significant ($p < 0.05$).

Table 2: Analysis of variance for each factor and their combined effect (treatment effect) for yield of butternut fruit for two trials

Source of variation	df	SS	MS	F-value	p-value
Pinching	2	1001.0537	500.526859	139.4728	<0.00001
NPK	2	1329.5966	664.798293	185.2473	<0.00001
Manure	2	13115.2412	6557.620619	1827.2938	<0.00001
Residuals	38	136.3708	3.588706		<0.00001

Table 3: Model for the butternut fruit weight

Variable	Estimate	Std. Error	t value	p-value
(Intercept)	3.06699663	0.03794079	80.8364	<0.000000001
x_1	-0.03252091	0.01601373	-2.0308	0.04971
x_2	0.49139592	0.01512279	32.4937	<0.000000001
x_3	0.77358465	0.01512279	51.1536	<0.000000001
$x_1: x_2$	-0.00136325	0.01014742	-0.1343	0.89388
$x_1: x_3$	-0.00089615	0.01014742	-0.0883	0.93012
x_1^2	-0.08360088	0.00879692	-9.5034	<0.000000001
x_2^2	0.06065144	0.02512023	-2.4144	0.02097
x_3^2	0.62355211	0.02512023	-24.8227	<0.000000001

Where x_1 = pinching, x_2 = NPK and x_3 = manure

The models in Table 3 can be summarised in a statistical equations as;

$$Y = 3.07 - 0.03x_1 + 0.49x_2 + 0.77x_3 - 0.001x_1x_2 - 0.001x_1x_3 - 0.08x_1^2 + 0.06x_2^2 + 0.62x_3^2$$

where, Y = yield of the butternut fruits, X_1 = pinching, X_2 = NPK and X_3 = poultry manure

Table 4: The analysis of variance for the second order model for the yield of butternuts

Terms	Df	SS	MS	F-value	p-value
Linear	3	12.7595	4.2532	1123.3457	<0.0000001
Two way interaction	2	0.0001	0.0000	0.0091	0.9909010
Pure quadratic	3	2.6864	0.8955	236.5114	<0.0000001
Residuals	36	0.1363	0.0038		
Lack of fit	6	0.0735	0.0122	5.8443	0.0003975
Pure error	30	0.0628	0.0021		

The results of this study indicate that a unit positive change in NPK and poultry manure or cattle manure had a positive effect on the yield of butternut fruits, while a unit change in pinching have a negative effect on yield of butternut fruits. These findings are in agreement with those Purbajanti *et al*. (2019), which found a significant effect of cattle manure and NPK fertilizer on various traits of peanuts. Kimtai *et al*. (2020) also found a significant effect of the poultry manure, goat and cattle manure on the yield of common beans. A unit change of the quadratic term of the NPK and poultry manure had a positively effect on the yield of the butternut fruits, while a unit change in the quadratic term of pinching had a negatively effect. These results can be interpreted that an increase in NPK and poultry manure will lead to an increase in yield of butternut fruits, while an increase in days to pinching will lead to a decrease in yield of butternut. This can be supported by the finding of this studies that showed that pinching at 34 days (i.e., pinching at 4th node) after sowing gave significantly high yield of butternut fruits compared by pinching at 49 days (i.e., pinching at 6th node) after sowing. Beura *et al*. (2016) showed that pinching significantly influences the flowering patterns and yield of butternut. In cucumber, pinching has also been found to significantly influence the yield parameters (Nayak *et al*., 2018).

The findings of this study also revealed that a unit change in the interaction between the pinching and NPK and pinching and poultry manure has no significant effect on yield of butternut fruits. Implying that the effect of pinching on yield of butternut fruit is independent of application of NPK and poultry manure. Nayak *et al*. (2018) found non-significant of factor combination on various traits studied. However, the results of this study suggest that early pinching in combination of low rate of NPK and high rate of poultry manure would give the best results. This can partly be attributed to fact that pinching enhances branching and subsequent support of enhanced plant growth by increased plant nutrition. Therefore, pinching operation would be an efficient practices in production of butternuts fruits.

Optimization of the Yield of Butternut Fruit Yield using Response Surface Methodology

The aim of this study was to find the optimal combined pinching time and, amount of NPK and poultry manure that can produce a maximum yield of butternut fruits. Three dimension (3D) plots for different combination of variables (pinching, NPK and poultry manure) exhibiting the trend of variation of responses within the selected range of input variables are as shown in Figure 1, 2 and 3.

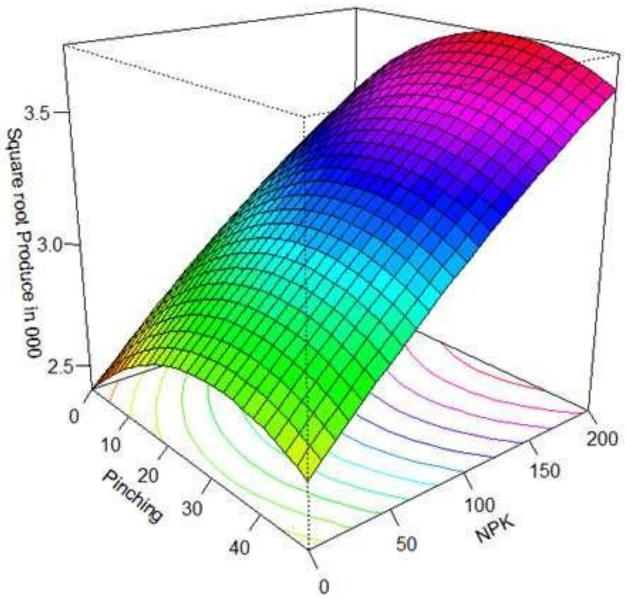

Figure 1: Response surface for weight of butternut fruit as a function of pinching and NPK at a constant level of poultry manure.

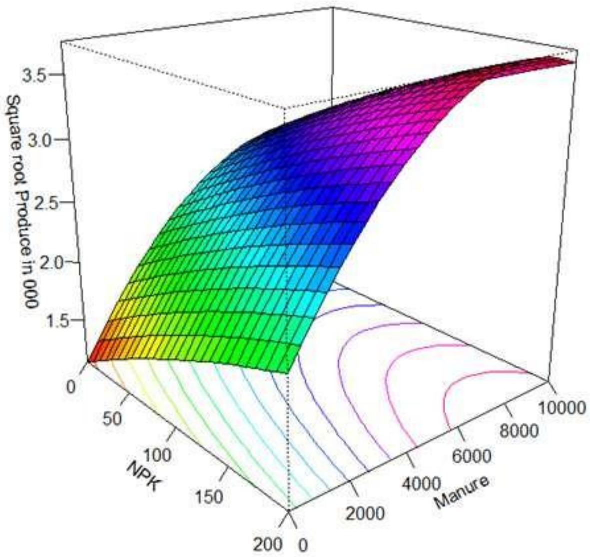

Figure 2: Response surface for weight of butternut fruit as a function of pinching and poultry manure at a constant level of pinching.

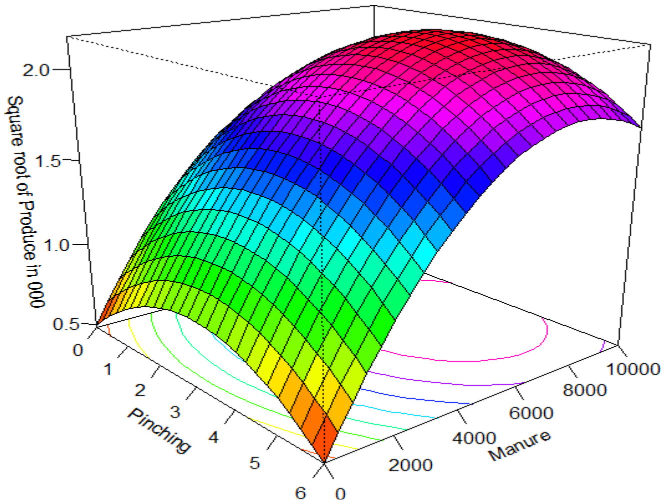

Figure 3: Response surface for weight of butternut fruit as a function of pinching and NPK at a constant level of poultry manure. Plotting was done using number of nodes instead on number of days to pinching.

The pilato plots aided in the study of the response surface. They characterize the shape of the surface and locate the optimum with reasonable precision. The results of this study revealed that the optimal number of days to pinching is 30 days (which is equivalent to pinching at 3rd node, Figure 3), with optimal NPK rate at 505 kg/ha and poultry manure at 8102 kg/ha. The results of this study demonstrate that it is possible to determine the optimum settings of the independent variables that result in a maximum response variable. These results are in agreement with those of Muriithi *et al*. (2018), which showed that low rates cattle manure and high rates poultry manure results in enhanced production of water melons.

In this study, a combined application low rate of NPK and high rate of poultry manure would be expected to enhance the yield of butternut fruits. This can be attributed to the fact that poultry manure is rich in nutrient concentration, especially nitrogen, and is steadily released throughout the growing season, which enhance crop growth and development. The nutrients analysis of the poultry manure used in this study showed that it contained high content of nitrogen. In watermelon, the rich nutrient concentration in poultry manure have been attributed to increased growth and productivity (Enujeke, 2013). The application of poultry manure and NPK fertilizer and their combination have been shown to increase productivity and yield components of French beans (Arjumandbanu *et al*., 2013).

The application of the optimal levels of poultry manure, NPK fertilizer and pinching is key in order to achieve the maximum yield. To determine optimal combination of different input variables used in this study (poultry manure, NPK and pinching), response surface methodology was used to optimize butternut fruit yield. The study found that a unit increase in amount of poultry manure and NPK, and earlier pinching of butternut plant lead to an increase in butternut fruit yield until optimal amount were achieved. These optimum amount were 505 kg/ha NPK and 8102 kg/ha poultry manure and pinching at 3rd node. The results demonstrated that response surface methodology can be a cheaper alternative to optimise input variables, before setting out field experiments for validation of predicted values.

Conclusion

Response surface methodology was successfully used to predict the response of a butternut yield using a set of input variables, i.e., poultry manure, NPK and pinching. The optimal settings of these input variables were obtained by solving the fitted second order models using the response surface pilato plots. It can be concluded that use of statistical models for understanding effects of various input factors is the new way forward in order to cut of experimental costs. Once a setting of predicted variables are arrived at, then small experiments can be carried out under field conditions to validate the settings.

Recommendation for Further Studies

Further study is needed to:

i. Validate the findings of predicted input variables by response surface methodology under field conditions using the studied butternut variety.
ii. Conduct further studies using the recommended input variables and predicted input variables in this study under different agro ecological zones and farmer conditions.

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COMPETING INTERESTS

Authors have no competing interests.

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