OPTIMIZATION OF *Pleurotus ostreatus* PRODUCTION THROUGH RESPONSE SURFACE METHODOLOGY AND SIMPLEX CENTROID MIXTURE DESIGNS

By

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DECLARATION

Declaration by the Candidate

This thesis is my original work and has not been presented for a degree in any other institution. No part of this thesis may be reproduced without the permission of the author and/or Moi University.

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DEDICATION

This research work is dedicated to my students, the current and potential mushroom farmers.

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ABSTRACT

Despite the increased recognition of the nutritional value of the Oyster mushroom, coupled with its ability to tolerate a wide range of climatic conditions, its production is still at infancy stage with low adoption rate in Kenya. The low uptake could be attributed to lack of skills for substrates and spawns preparations, cost of buying substrates and spawns coupled with poor knowledge on its production and consumption benefits. The objective of this study was to optimize *Pleurotus ostreatus* (Oyster mushroom) production through response surface methodology and simplex centroid mixture designs. The specific objectives were to optimize the spawns production, screen the local suitable substrates for the oyster mushroom cultivation, establish yield as a function of proportions of mixture components and then conduct the economic return analysis for the oyster mushroom farming in Machakos County. To achieve the objectives the spawns propagation was optimized by varying the temperature level, sterilization time and culture media concentration in order to establish the feasible levels which minimized the days of mycelium full development using central composite designs. One factor at a time approach was used to determine the local suitable substrate among them, the star grass, euphorbia, cattle manure, sugarcane bagasse and sawdust. Simplex-centroid mixture design was used to determine the substrates mixture that maximized the yield and lastly the contribution margin formula was used to determine the economic returns on the oyster mushroom production. Based on the study findings 26.30^0C, 17.40 minutes and and 60.89g/L of temperature level, sterilization time and culture media concentration level respectively minimized the days to full coverage of mycelium in a petri dish. There was no pinning on the cattle manure and the euphorbia substrates hence they were eliminated at the screening stage. The results showed significant variability on the different substrate compositions used under the study. Sawdust yielded the most under the pure blend at 1.1 kg per experimental unit while on the mixed blend sugarcane bagasse and sawdust produced the highest yield at 1.3 kg per experimental unit (1kg of dry substrate), giving 10% and 30% biological efficiency respectively. The economic returns analysis indicated that, the break-even point was at 54 kilograms of the oyster mushroom production, beyond that point each succeeding kilogram was produced at a diverging profit. Therefore oyster mushroom production was economically viable against the continued arable land decrease in Machakos County coupled with the rainfall unreliability. Central Composite Designs in controlling the temperature level, sterilization time and culture media were recommended for spawns maximum production. Since the mixture response was found to be more valuable than the pure blend responses then simplex- centroid mixture design for rightly proportioned substrates was recommended for improved oyster mushroom production. A further research on determining suitability of alternative locally found substrates which may be more cost effective and multiple response optimizations aimed at achieving maximal nutritional value and yield against minimal cost of spawns and substrates are recommended.

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ABBREVIATION AND ACRONYMS

ANOVA	**:**	Analysis Of Variance
ASP	:	Average Selling Price
AVC	:	Average Variable Cost
BE	:	Biological Efficiency
CCC	:	Circumscribed Composite designs
CCD	:	Central Composite Designs
CCF	:	Face Centered Composite
CCI	:	Inscribed Composite Designs
DOE	:	Designs of Experiment
EPS	:	Exo Poly Saccharide
FO	:	First Order
HDP	:	Host Defense Potentiators
JKUAT	:	Jomo Kenyatta University of Agriculture and Technology
MEA	:	Malt Extract Agar
NAFIS	:	National Farmers Information Service
NCD	:	Non Communicable Diseases
OFAT	:	One Factor At a Time
PDA	:	Potato Dextrose Agar
PQ	:	Pure Quadratic
PVC	:	Poly Vinyl Chloride, (plastic Pipe)
RCCD	**:**	Rotatable Central Composite Design
RSM	:	Response Surface Methodology
SCMD	:	Simplex Centroid Mixture Designs
SPV	:	Scaled Prediction Variance

TFC	:	Total Fixed Cost
TOC	:	Total Organic Carbon
TWI	:	Two Way Interaction

OPERATIONAL DEFINITION OF TERMS

Autoclaves: use of high pressure and high temperature steam for sterilization.

Casing run: is the period in which the mycelia are left to grow on the casing soil.

Centroid: is the mean position of all the points on all of the coordinate directions.

Culture media: is a substance that supports the growth of microorganisms eg agar plate or gel

Epigeous: Organisms that grow on the soil surface

Experimental units: are the spaces or containers to which treatments are applied for comparison

Hygrometer: it is an instrument of measuring relative humidity in the air

Hypogeous: Organisms that grow under the soil surface

Mother culture: it is the pure culture prepared by inoculating a tissue culture from a grown mushroom on PDA or MEA

Orthogonality: the degree by which the main effect and interaction estimates of interest are dependent/independent of one another

Relative humidity (RH): is the percentage of moisture in the air compared to the maximal amount that the air can hold at that level of temperature and pressure.

Responses: observed outcomes after applying a treatment to an experimental unit.

Rotatability: a design whose variance of estimated response$(\hat{y})$, is a function of distance from the centre only but not the direction.

Spawn: it is a planting material equivalent of farmers' seed for starting mushroom cultures. It is made from mycelia of mushroom grown on a carrier such as grains and is produced in specialized laboratories under sterile conditions.

Straw: is the medium for growing the mushrooms substrates

Substrate: it is an organic-based material on which mushrooms grow.

Thermometer: it is an instrument of measuring temperature

Treatments: are the different procedures compared in a study.

CHAPTER ONE

INTRODUCTION

1.0 Overview

The chapter covered the study introductions; section 1.1 presents the background information and motivation, sub-sections 1.1.1 and 1.1.2, presents the Central Composite Designs and Simplex Centroids Designs respectively. Section 1.2 and sub-section 1.2.1 presents the Spawns and Oyster mushroom production and Oyster mushroom farming in Kenya respectively, while sections 1.3, 1.4 and 1.5 present the problem statement, justification of the study, and the study objectives respectively. The scope of the study is covered in section 1.6.

1.1 Background and Motivation

Response Surface Methodology (RSM) is a collection of mathematical and statistical techniques that are useful for modeling and analysis of problems in which a response variable of interest is influenced by several variables and the objective is to optimize the response variable, such that if $y = f(x_1, x_2, ..., x_k) + \varepsilon$, then the expected value of y, which is given as $E(y) = f(x_1, x_2, ..., x_k) = \eta$ is referred to as the response surface (Montgomery, 2001). Therefore RSM can be referred to as a body of methods for exploring the optimum operating conditions through experimental methods. Typically, this involves doing several experiments, using the preceding results of one experiment to provide a direction for the subsequent experiments. This next action could be to undertake the experiment around a different set of conditions, or to collect more data in the current experimental region in order to fit a higher-order model or confirm the previous observation, (Myers & Montgomery, 1995).

The response surface methodology is most appropriate with a quantitative response affected by continuous factors and it works best with only a handful of critical factors, those that pass the screening phase of the experimental program. However, the design can be customized for nearly any situation whether categorical factors, continuous factors or constrained design space. Its primary role is to produce the optimal settings for the process factors so as to maximize, minimize, or stabilize the responses of interest especially where such input variables have a significant influence on the quality characteristics of the product or the production process.

The initial step in the application of RSM is to establish the true functional relationship between the response variable and the set of predicting variables. In cases where the response variable can be aptly modeled by a linear function of the regressors, then the approximating function is the first order model (Montgomery, 2001). Otherwise a polynomial of higher degree such as the second-order model is used, especially with appearance of a curvature in the system.

1.1.1 Central Composite Designs

Central Composite Designs (CCD) and the Box-Behnken designs are common forms of response surface methodology. Central composite designs are mostly used when the design appropriately fits sequential experimentation since the designs obtain information from a correctly planned factorial experiment and can accommodate up to five levels per factor. On the other hand Box-Behnken designs usually have fewer design points than central composite designs, and they can perform as composite central designs but they can neither include runs from a factorial nor run above three levels per factor, (Anderson, Montgomery, & Myers, 2009).

It is against that backdrop that CCD has become one of the most popular models of response surface methodology following the pioneering work by Box and Wilson (1951).

1.1.2 Simplex Centroid Mixture Designs

Simplex Centroid Mixture Design (SCMD) is a method of determining a unique set of components combination at various centroids that maximize or minimize the response variable depending on the objective function. Scheff$\acute{e}$ (1963) gave the simplex centroid designs consisting of $2^q - 1$ points with q permutations of $(1,0,0,...0)q$ pure blends, ${}^qC_2$ permutations of $\left(\frac{1}{2},\frac{1}{2},0,...,0\right)$ giving binary blends and the overall centroid $\left(\frac{1}{q},\frac{1}{q},...,\frac{1}{q}\right)$ giving a mixture blend of every component at equal proportions.

1.2 Spawns and Oyster Mushroom Production

Mushrooms are macrofungi with a distinctive fruiting body which can be either epigeous or hypogeous. The macrofungus has fruiting body large enough to be seen with the naked eye and can be picked up by hand (Chang, 1996).

Spawn production is the first stage in mushroom farming and spawn quality is counted the most important part in mushroom production (Ansari & Elhami, 2008)

Spawn, which is the reproducing part of the mushrooms, should be multiplied in a pure culture media in a sterilized environment.The pure culture can either be raised by tissue culture or spore culture. In tissue culture a well grown mushroom with membrane covering the gills is selected, from which a small bit of mushroom from gill portion is taken using forceps and inoculated on Potato Dextrose Agar (PDA) or

Malt Extract Agar (MEA). Under spore culture method, the spores are collected from well-developed fruiting body by ‘spore mapping technique’ and then the spores are inoculated to the PDA or MEA slants as in tissue culture under aseptic condition. The mycelia (spawns) are supposed to cover the entire surface after which they are placed in a sterilized jars or bottles with a boiled grain seeds like wheat or millet for further multiplication. It is at this stage when the seeds can be used as mushroom spawn and can produce mushrooms if mixed with a well compost substrate.

Beside the correctly compost substrate, the oyster mushroom does well under certain levels of temperature, humidity, light and ventilation, while the quality of spawn is affected by the mother culture, media culture and spawn substrate preparations, (Sharma, Kumar R., Gupta, Kumar S., and Singh, 2013).

Hoa and Chun-Li, (2015) research finding indicated that oyster mushrooms yield depends on the type of substrate used and the quality of the spawns.

1.2.1 Oyster Mushroom Farming in Kenya

Mushroom Farming in Kenya is currently valued at KSh 340 million, whereby the large scale producers account for over 95% and less than 5% is accounted for by the small scale producers. Globally and all over Kenya, button is the most produced mushroom, and then Shitake, though not common in Kenya, it is globally rated second after button. Oyster mushroom production is a distance third despite the fact that, it is easy to grow and has higher yields (Wambua, 2014).

1.3 Problem Statement

Despite the increased recognition of the nutritional value of the Oyster mushroom, coupled with its ability to tolerate a wide range of climatic conditions, its production in Kenya is still at infancy stage with low adoption rate Wambua also mentioned. The

low uptake could be attributed to lack of skills for substrates and spawns preparations, cost of buying substrates and spawns coupled with poor knowledge on its production and consumption benefits.

According to the Institute of Biotechnology Research (IBR) of Jomo Kenyatta University of Agriculture and Technology (JKUAT) report (2016), Kenya produces 500 tons of mushrooms per annum against an annual demand of 1200 tons both in hotels and home consumption. Wambua also mentioned that the few farmers producing mushroom in Machakos County uses commercially available oyster mushroom spawn and wheat straw or rice straw as the main substrate component which are not locally available, hence making it an expensive venture.

Therefore this study focused on enhancing the oyster mushroom production and yield in Machakos County and Kenya in general to bridge the gap by using the central composite designs and simplex centroids mixture design. The findings would help in reducing the oyster mushroom spawn production process and the costs of growing the mushroom hence improve on its overall production and consumption.

1.4 Justification

The central composite design has been used successfully in optimizing the processes and evaluating the relationship, and the relative significance between a set of independent variables even in the presence of complex factor-factor interactions, as highlighted in chapter two of this study. Likewise simplex centroid mixture design has been used in formulating and optimizing many agricultural and pharmaceutical processes and products.

Oyster mushroom (*Pleurotus ostreatus)* which is an edible fungus has received a wide research attention due to its nutritional and medicinal value with various species

which are edible and environmental cleaning by recycling the agricultural products (Cohen, Persky & Hadar, 2002).

Pleurotus species presents high adaptability for growth and fructification within a wide variety of agro –industrial lingo cellulosic waste due to their production of lignolytic and hydrolytic enzymes, (Gry & Anderson, 2014)

Bihal (2010) established that Oyster mushroom has stupendous advantages over most of the other species, such as its ability to grow on any kind of agricultural waste containing lignin, cellulose or hemicelluloses. Bihal also claimed that, Oyster mushroom has the highest number of species with a varied shape, colour, texture, and aroma. It tolerates a wide range of environmental conditions such as the temperature, humidity and carbon dioxide. Its productivity is higher compared to the rest of the species and lastly it can easily be dried up and kept for future use.

Jiyul (1993) established that oyster mushroom is more advantageous over the rest with regard to its tolerance to harsh climatic conditions and diseases, taste quality and it is widely distributed almost all over around the world.

Oyster mushroom is one among the few mushrooms that offer a long list of health benefits. It occupies 14% of the global market and ranks third in the global trade. It tolerates a temperature of 7 - 37°C with an optimal range of 26 - 28°C and it is rich in protein, fiber, iron, vitamins and minerals (Wani. A.H, Bodha, & Wani. B.A., 2012)

Past studies (Wachira, 2003 & Fermont, 2008) indicates that poverty in the rural areas is on increase because of high population growth rate and diminishing land fertility due to subdivision into small holder farms which have been subjected into intensive cultivation.

Hence given the challenges posed by the fast rate of population growth coupled with the increased land subdivision in Kenya, and rainfall unreliability on arable farming, research on Oyster mushroom production which requires little space and inputs but still caters for food security is not only timely but necessary.

Definitely the increased production of *oyster mushroom* is a strategic undertaking to realizing vision 2030 on agricultural sector and sustainable development goals on zero hunger, good health and well-being that will have a huge impact of turning around the fortunes of small scale farmers in Machakos County. The findings will form the basis for further research in this area.

1.5 Objectives of the Study

1.5.1 General Objective

Optimization of *pleurotus ostreatus* production through response surface methodology and simplex centroid mixture designs

1.5.2 Specific Objectives

The specific objectives for this study were to;

i. Apply Central Composite Design (CCD) to optimize spawns propagation and hence develop an empirical model that relates the spawn's production to the factors.
ii. Carry out factor screening to identify the local suitable substrates that support the cultivation of oyster mushroom *(Pleurotus ostreatus).*
iii. Model the Oyster mushroom (Pleurotus ostreatus) yield as a function of the proportions of the mixture components (Substrates) using simplex centroids design.

iv. Establish the economic returns and benefits of oyster mushroom production through focused group discussions.

1.6 Scope of the Study

The study applied three factors Central Composite Designs (CCD) against a single response variable for spawns propagation. The experiment was conducted in Kenya National Museum, department of mycology laboratory. Simplex-Centroid Mixture Design (SCMD) was applied to determine the optimal substrates mix for *Pleurotus ostreatus* maximum yield. The substrates' mixture experiment was carried out at the Machakos University ground in Machakos County.

The CCD varied levels of temperature, sterilization time and the culture media concentration on the mother substrate for spawn's propagation, with the objective function being to minimize the time in days to full colonization of the media as evidenced by mycelium full coverage in the Petri dish as the experimental unit. While the SCMD varied the proportions of substrates, with the objective function of maximizing the Oyster mushroom yield under which a dry kilogram of the substrate in a polythene paper was the experimental unit.

CHAPTER TWO

LITERATURE REVIEW

2.0 Introduction

The chapter focused on reviewing and understanding the past and the current knowledge on response surface methodology, simple centroid mixture design and Oyster mushroom production. Literature related to optimization and central composite design is presented in section 2.1 and sub-section 2.1.1 respectively. The reviewed literature on the screening of the local suitable substrates is given in section 2.2 while in section 2.3 is the reviewed literature on simplex centroids mixture design and lastly the economic return analysis on oyster mushroom production literature is given in section 2.4.

2.1 Optimization Design of experiments

Designs of Experiment (DOE) methods cover a range of activities that relate to the logical choice of experiments with which to explore a system or test hypotheses about a system, with an aim of optimization. (Derek, Rob and Mark, 2017)

In this study, two different forms of response surface methodology for optimization were deployed; central composite designs and simplex centroid mixture designs.

2.1.1 Central Composite Designs

Central Composite Designs (CCD) is popular and the most commonly used response surface design experiment. The CCD is composed of three design points: square or edge points as in two level designs (± 1), star points at $\pm\alpha$; $|\alpha| \geq 1$ that take care of the quadratic effect and then the centre points (Diamond, 1981).

CCD has widely been used in various fields of research; Thiagarajan and Kayaroganam (2012) applied CCD in optimizing machine parameters in drilling

hybrid metal matrix composites, and found that the predicted values and measured values were fairly close, which indicated that the developed models could effectively be used to predict the responses in the drilling of hybrid metal matrix composites. Al-Shingiti and Huda (2004), asserts that CCD's are very popular 2^{nd}-order designs because; they are extremely simple to use, and allows estimation of all the parameters in a full second-order model coupled with the fact that among the exact (integer) designs, the CCD's often have high efficiencies under the commonly used A-, D- and E-optimality criteria.

Bahrim, Horincar, Popa, and Vincetiu, (2017) applied Central Composite Design and Response Surface Methodology to optimize the conditions of submerged cultivation of the Fomes fomentarius mushroom. Concentration of dextrose, yeast extract and time of cultivation in days were selected and their correlative effect on mushroom multiplication established. The maximum yield of dry weight biomass was 23.74 g/L with 0.8-7.5 g/L concentration after eleven (11) days of submerged cultivation.

Dressaire (2016) used CCD to show that evaporative cooling of the air surrounding the pileus created convective airflows capable of carrying spores at speeds of centimeters per second. That work revealed how mushrooms tolerate and even benefit from crowding and explained their high water needs. It was evident that spores continuously flow out from thin gaps, even in the absence of external winds.

As Sarrai *et al.,* (2016) noted CCD was very effective in optimizing the Degradation of Tylosin from Aqueous Solution by Photo-Fenton Reaction. The interaction effects and optimal parameters were obtained and the significance of the independent variables and their interactions was tested by means of analysis of variance (ANOVA) at 95% confidence level. Results showed that the concentration of the ferrous ion and

pH were the main parameters affecting Total Organic Carbon (TOC) removal, while peroxide concentration had a slight effect on the reaction. The optimum operating conditions to achieve maximum TOC removal were determined. There was a good agreement between the model prediction and experimental results confirming the soundness of the developed model.

Peiqin *et al.,* (2012) applied response surface methodology to optimize the main factors which significantly affected exo poly saccharide (EPS) production. The concentrations of glucose and peptone were found to be the main effective factors for EPS production by the fractional factorial design (FFD) and central composite design, experimental analysis. Verification experiment confirmed the validity with the actual EPS yield as 13.97 g/L, which was 6.29-fold in comparison with that (2.22 g/L) in the original basal medium.

(Vahabzadeh, Ahmadi, Bonakdarpour, Mofarrah and Mehranian, 2005) applied central composite design and response surface methodology to the advanced treatment of olive oil processing wastewater using Fenton's peroxidation, in a second-order polynomial multiple regression model. Analysis of variance (ANOVA) showed a high coefficient of determination (R^2) value of 0.902–0.998, thus ensuring a satisfactory, adjustment of the second-order regression model with the experimental data.

2.2 Locally Available Suitable Substrates for Oyster Mushroom Cultivation

Screening is the exercise of finding out the few significant factors from a list of several potential ones through experimentation (Vine, 2004).

Several studies have carried out screening experiments prior to the main experiment involving the few significant factors.

Mekonnen & Semira (2014) investigated the suitability of selected substrates for oyster mushroom production in Northern Ethiopia, Mekelle city. Five different substrates namely; wheat straw, teff straw, barley straw, cotton hull and sawdust were tested. During the experiment plastic bags were filled with 1 kg of each substrate in eight trials. The effect of the substrates on its yield, pinning and harvesting time were measured regularly. The result indicated that cotton hull, wheat and teff straw had short pinning durations of 18, 21 and 23 days, respectively. Maximum (6 days) were recorded in sawdust substrate for the first harvesting time after putting out from the dark room. Cotton hulls and wheat straw took minimum (4 days) at the second harvesting time. Maximum yield of 11.2 kg was recorded in wheat straw followed by cotton hulls (8.7 kg). Spawn running time and mushroom yield of the trialed substrates had not found significant association during the experiment ($p = 0.490$). It was concluded that with the exception of teff straw the rest of the organic substrates were found excellent for oyster mushroom production.

(Musieba, Okoth, Mibey, Wanjiku & Moraa, 2012) sought to find out the Suitability of locally available substrates among the; bean straw (*Phaseolus vulgaris*), sawdust of African mahogany (*Khayaanthotheca*), rice straw (*Oryza sativa*), maize cobs (*Zea mays*), wheat straw (*Triticumaestivum*), sugarcane bagasse (*Saccharumofficinarum*) and banana leaves (*Musa sp.*) for the growth and yield performance of Golden Oyster Mushroom (*Pleurotus citrinopileatus Singer*). The best performance was obtained from the bean straw substrate. Maximum yield (397.71 g kg-1 wet substrate) and biological efficiency of 148% were obtained from bean straw at spawn rate of 5%.

(Prakash, Anuradha, Niveditha & Dhanalakshmi, 2010) conducted a research to investigate sustainable alternatives to grow Oyster mushroom (Pleurotus ostreatus) using paddy straw, bagasse, wheat bran, urea and humic acid at varying composition.

The study optimizes the composition of the substrates, other than the paddy which is the main raw material, for the maximum yield of Pleurotus ostreatus and analyses the nutrient content of the biomass produced. Bagasse composition (17.5g/l -32.5g/l), Wheat bran composition (3.5g/l – 6.5 g/l), Urea composition (3.0 g/l -7.0 g/l) and Humic acid (2% -6%) were chosen as the process variables for optimization. A five (5) level four (4) variable central composite design was used to evaluate the effects of these parameters on the yield of Pleurotus ostreatus. After the process of optimization, the significant interaction among the process variables studied, humic acid was key in the determination of the yield. Depending on the different process parameters the yield of mushroom varied from 74 – 204g. Optimum process parameters for maximum yield of Pleurotus ostreatus were found to be Bagasse 21.25g/l, Wheat bran 3.5g/l, Urea 5.0g/l and Humic acid 4%. The process parameters also showed significant effect on yield, productivity and biological efficiency. Mycelia colonization of compost bags and subsequent growth of oyster mushroom was faster in high Humic acid-based substrates. Hence they produced larger and firmer fruiting bodies. The response surface methodology provided here could be used as a strategy to grow Oyster mushrooms under adverse conditions and limited resources.

This study employed one-factor-at-a-time (OFAT) approach in order to determine the most important factors among Star grass ,Sawdust , Cattle manure , Euphorbia , and Sugarcane bagasse substrates on which the oyster mushroom grew.

2.3 Simplex Centroids Mixture Designs

Simplex centroids designs have been utilized variedly in optimization experiments successfully;

The studies by Bahra (2013) applied simplex centroids mixture design to optimize stabilizer combination for ice cream manufacturing. Based on the optimization criteria, it was found that the most excellent combination was 84.43 % basil seed gum and 15.57 % guar gum at concentration of 0.15 %. This research proved the capability of basil seed gum as a novel stabilizer in ice cream stabilization. (Rongzhi, Zhenya, Norio & Chuanping, 2009) used simplex centroid mixture design in developing and optimizing ceramic adsorbent for As (V) removal from water solution,

The analysis of variance (ANOVA) results and the predicted values were in good agreement with the experiment data, indicating that the simplex centroid mixed designs was a reliable method for determining the optimum mixture proportion of ceramic. (Gabriel, Carmen, Silva, Karina, Coppo & Dionisio, 2014) applied simplex centroid designs to optimize conditions for obtaining B100 biodiesel from sunflower oil using different catalysts with methanol and ethanol as process variable. Reaction yield was optimized only to 89.65% when ethanol as process variable and KOH as catalyst were employed.

(Mucheru-Muna, Mugendi, Pyres, Mugwe, Kungu, and Vanlauwe, 2013) conducted two sites trials in different soil fertility to determine the combined effects of organic, mineral fertilizers and their combination on maize grain yield. Maize grain yield were significantly lower in fields with only mineral fertilizers compared to the combined cases (mineral and organic) with generally low economic returns. Treatments that gave the highest maize grain yield returns, resulted to reduced soil fertility. The study concluded that there was need for a trade-off between high maize grain yield and soil fertility soil management options a gap whose solution lies with the robust statistical testing and modeling.

(Zhang, Liu, Meagher, & Ian, 2004) used response surface methodology to optimize cell density and dilution rate in chemostat for a Pichiapastoris continuous fermentation for extracellular production of a recombinant proteins, interferon. The objective was accomplished where the optimal for cell density and dilution rate was 328.9 grams per liter and 0.0361 liters per hour.

Dengwu (2018) used simplex centroid mixture design to strike a balance among workability, compressive strength, durability, economic efficiency, and sustainability on cement and concrete composites. The findings indicated that the optimal ratio among the paste, fine aggregate and course aggregate were optimized using the simplex centroid design method based on rheological properties. That is the optimal content of the total cementitious materials in concrete could be obtained as per the associations between the workability, yield stress, plastic viscosity and the paste volume fraction.

Different substrates on oyster mushroom cultivation have been tried and applied, (Kimenju, Odera, Mutitu, Wachira, Narla & Muiru, 2009) tried bean straw, water hyacinth, rice straw and maize straw and according to his findings bean straw had the best yield.

Ajonina, Samuel, Tatah and Eugene, (2012*)* performed experiments using wheat straw, coffee husks and sawdust and according to the findings the wheat straw had the highest biological efficiency of over 75%, implying wheat straw would convert at 75 to 100%, which meant 75-100 kg of fresh mushrooms were expected from 75-100 kg of a dried wheat straw. However, given the scarcity of wheat straw, bean straw and water hyacinth in most parts within the country otherwise potentially suitable for oyster mushroom production, it makes it a costly venture hence need for alternative

locally available and suitable substrates for comparative advantage gain. The simplex centroid mixed design was applied to investigate an optimal mix of the sawdust, star grass, poultry manure, sugarcane bagasse and euphorbia substrates.

2.4 Oyster Mushroom Production Benefits

Oyster mushroom is loaded with many health-boosting vitamins, minerals, and antioxidants. Oyster mushroom production can also create an extra income stream from a small scale to large scale as discussed in the following subsection.

2.4.1 Economic Returns Analysis on Oyster Mushroom Production

The section aimed at understanding the recent past benefit/cost analysis of oyster mushroom production generally in Kenya but particularly in Machakos County, especially as a diversification of rural income. This was important since the uptake of oyster mushroom farming would depend largely on its production benefits among them, the profitability of the oyster mushroom enterprise.

Several farmers failed to succeed in mushroom cultivation because of inadequate knowledge about the marketing system, low capital and the underlying principles on oyster mushroom cultivation (Odendo, Kirigua, Kimenju, Musieba, & Wasilwa, 2012).

To win over consumers, farmers must struggle to identify the product value. Farmers must look for ways that eases production and add value to increase consumption of mushroom. Other than price and quality, value addition now includes reliability and outlook (Wanjiru, 2014). To establish the economic returns on oyster mushroom production a breakeven point was determined by contribution margin method.

2.4.2 Health Benefits of Oyster Mushroom Consumption

Oyster mushrooms are a rich source of nutrients, particularly proteins, minerals as well as vitamins B, C and D (Panjikkaran & Mathew, 2013).

Mushrooms contain 20–40% of protein depending on the type when dry, they are low in lipids and contain all the nine essential amino acids (Kalac, 2009).

Sabiha (2014) established that, Oyster mushrooms are probiotic compounds classified as host defense potentiators (HDP) with inherent properties of enhancing immune systems, yet to date, the inherent biological power embodied within the mycelia network of edible mushrooms remains untapped resource.

Muhammad, Iqball, Abdul and Sheikh (2005) found that, mushrooms are rich in protein (4-44%) depending on the species, implying some mushrooms' protein is higher than beef protein (16%). Muhammad *et al.,* also concluded that Oyster mushroom production and consumption would help in healthy balanced diet which helps in protecting against malnutrition in all its forms, as well as non-communicable diseases (NCDs), including diabetes, heart disease, stroke and cancer.

In addition, many research projects (Agrawal, 2010; Jednak & Sliva, 2008) reveal that oyster mushrooms could prevent and reduce several serious diseases, including high blood pressure, cholesterols, breast cancer and prostate cancer.

CHAPTER THREE

METHODOLOGY

3.0 Introduction

The chapter explains how each of the study specific objectives was achieved. In section 3.1 and sub-section 3.1.1, optimization process and the central composite design methods of establishing the temperature level, sterilization time and the culture media concentration that shortened mycelia colonization time (days), are discussed respectively. Section 3.2 presents the method of determining the significant substrates for the oyster mushroom cultivation. In section 3.3 the simplex centroid application and procedure for generating substrate mixture that maximized the oyster mushroom yield is presented and section 3.4 presents the economic returns analysis formula for oyster mushroom cultivation.

3.1 Optimization Design of Experiments

The main objective of optimization is to achieve the best outcome of a given operation while satisfying certain restrictions. This objective has always been central to the design process, but in the recent past it has assumed greater significance than ever because of the maturity of mathematical and computational tools available for design (Stander, 2014)

Mathematically optimization by minimizing can be represented by

$$\begin{aligned} & \min\ f(x) \\ \text{subject to} \quad & g_{(j)}(x) \le 0; \quad j=1, 2, ..., m \\ \text{and} \quad & h_{(k)}(x) = 0; \quad k=1, 2, ..., n \end{aligned}$$

Where *f* is the objective function which identifies the quality or quantity to be either minimized or maximized, while *g* and *h* are the constraint functions representing the design restrictions.

Graphically the theory of optimization can be represented by the input-output model figure 3.1

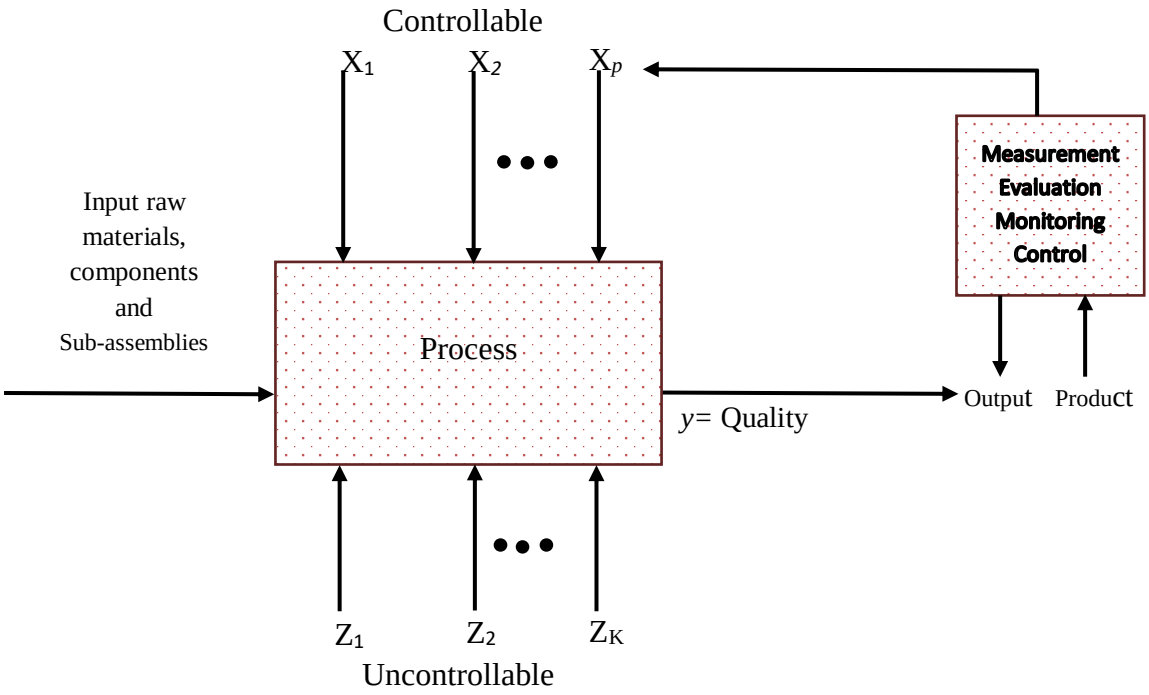

Figure 3.1 Input output model (Source: Montgomery, p.15, 2001)

The Input-Output (IPO) Model is a functional graph that identifies the inputs, outputs, and required processing tasks required to transform inputs into outputs. The optimization is realized through variation of the controllable factors while observing the quality characteristics of the output products. The model is ideal for optimization or decision problems where multiple decisions need to be made in the best way possible, while simultaneously satisfying a number of logical conditions or improve efficiency of operations.

The current study used three factor central composite designs to optimize the spawns' propagation of *Pleurotus ostreatus*. Table 3.1 shows the factors and their levels.

Table 3.1: Factors and their levels in the experiment

Factors	Type	Levels
Culture media concentration	Variable	2
Temperature level	Variable	2
Sterilization time	Variable	2

Since spawns production must be done under sterile condition which is usually difficult for an ordinary farmer or researcher. The spawns propagation trials for this study were done from the national museum laboratory in Nairobi, at which the optimal condition for their development were determined through the CCD procedures. The growth process of the spawns was a function of temperature level, Sterilization time and the culture media concentration level as put in equation 3.1

$$y = f(x_1, x_2, x_3) + \varepsilon \tag{3.1}$$

$x_1 = Temperature\ level$

$x_2 = Sterilization\ Time$

$x_3 = Culture\ media\ Concentration\ level$

The objective function was to determine the level of the regressors' experimental setting that minimized the mycelia full coverage in the petri dish area.

3.1.1 Central Composite Design

The Central Composite Design (CCD) is a factorial or fractional factorial design composed of square points or cube points, star points also referred to as axial points and the center points whereby;

i. The cube points consist of, 2^k factorial design (±1, ±1,…,±1), where k is the number of independent variables. The design can be replicated n_f times.

ii. The star points consisting of $2k$ units on the axis of each factor at a distance α, from the centre of the design [(±α, 0,…0), (0, ±α, 0,…,0)], and its selection is based on orthogonality and rotatability criterion, which takes one observation at each of the vector $\pm\alpha e_i$ and can be replicated n_s times. Where e_i is the *i-th* Euclidean unit vector and $\alpha > 0$.

iii. The centre points are designated as "0" points and can be replicated n_c times.

Then by letting *n* denote the total number of experimental runs in the CCD, based on *k* design factors, we have

$$n = 2^{k-p} n_f + 2kn_s + n_c \tag{3.2}$$

where;

n is the desired sample size

$2^{k-p} n_f$ is factorial component of the design, replicated n_f times, where k is the number of factors varied and p is the number of factors subtracted from *k.*

$2kn_s$ is the axial component of the design replicated n_s times and

n_c is the number of times the centre points were replicated.

There are various types of central composite designs namely; circumscribed, inscribed and face centered central composite designs depending on where the star points are placed. Circumscribed Composite Designs (CCC) has circular, spherical or hyperspherical symmetry. In the circumstances where the limits specified for settings are truly the experimental limits then it is referred to as the Inscribed Composite Design (CCI) which uses the factor settings as the star points and creates a factorial or

fractional factorial design within those limits. Then lastly Face Centered Composite Design (CCF) has an alpha of 1, such that the axial points are at the center of each face of the factorial space, hence $\alpha \pm 1$. Figure 3.2 shows the graphical representation of the three variants of central composite designs; circumscribed (CCC), face centered (CCF) and inscribed (CCI) with two level factorial experiments for three factors.

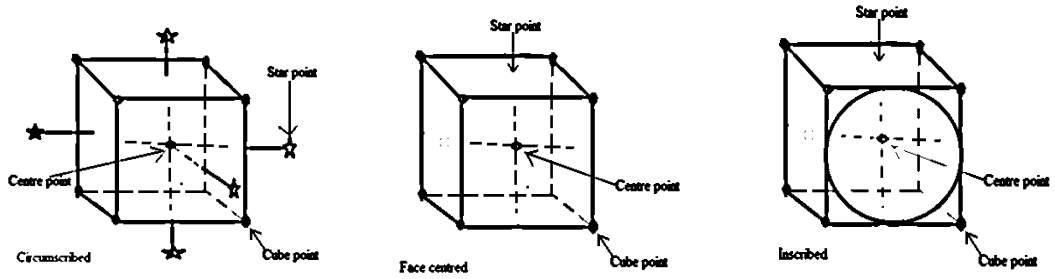

Figure 3.2: Three Factor two levels (Source; the author)

Box and Hunter (1957) suggested that a second order response surface design should be rotatable, to make it possible to extract the most information regarding the dependent variable, and leave the least amount of uncertainty for the prediction of future values.

Rotatable design provides the preferred property of constant prediction variance at all points that are equidistant from the design center, hence improving the quality of the prediction. It is a desirable property, especially when there is a need to optimize $\hat{y}(x)$ over the region of interest.

A design is said to be orthogonal if it can provide independent information about the effects of the various terms in the model.

In order to ensure orthogonality and rotatability of the design, circumscribed composite design was preferred. This ensured that any non-allowable operating conditions at two or more of the extremes of the design region were encompassed.

3.1.2 Rotatability Conditions

The design matrix for a CCD experiment involving k independent variables of L levels, is a matrix derived from the values corresponding to the three types of experimental runs stated in section 3.1 to form a design with N of L^N treatment combinations matrix, such that

$$X = \begin{bmatrix} \pm 1_{1,1}, & \pm 1_{1,2} & ,\cdots, & \pm 1_{1,m} \\ \vdots & \vdots & \ddots & \vdots \\ \alpha & 0 & ,\cdots, & 0 \\ -\alpha & 0 & ,\cdots, & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & ,\cdots, & \alpha \\ 0 & 0 & ,\cdots, & -\alpha \\ 0 & 0 & ,\cdots, & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 0_{N,1} & 0_{N,2} & ,\cdots, & 0_{N,m} \end{bmatrix} \tag{3.3}$$

usually referred to as the design matrix.

Where, the treatment combinations are the points of the design, such that x_i denotes the i^{th} factor at j^{th} treatment in a given level. Box and Hunter (1957) asserted that, a design of the above form will be a rotatable design of order *d* if a response polynomial surface

$$y_u = \beta_{0u} + \sum_{i=1}^{n} \beta_{iu} x_{ui} + \sum_{i=1}^{n} \beta_{iiu} x_{ui}^2 + \sum_{i=1}^{n-1} \sum_{i<j=2}^{n} \beta_{iju} x_{ui} x_{uj} + \sum_{i=1}^{n-2} \sum_{i<j=2}^{n-1} \sum_{i<j<k=3}^{n} \beta_{ijku} x_{iu} x_{ju} x_{ku} +, \cdots, + \varepsilon_u \tag{3.4}$$

is so fitted such that the variance, $Var\left[\hat{y}(x)\right]$ is a function of the distance of x from the origin but not the direction and therefore constant at all points that are equidistant from the design center, such that $r_u^2 = x_{1u}^2 + x_{2u}^2 +, \cdots, + x_{ku}^2$, where $\hat{y}(x)$ is the estimated response at the points $r = (x_1, x_2, \cdots, x_k)$.

When the model is of the second degree, that is $d = 2$, then such constancy of variance is attained if the design points are chosen to satisfy the following conditions;

$$\sum_{u==1}^{n} x_{iu} = 0, \quad \sum_{u=1}^{n} x_{ui} x_{uj} = 0, \quad \sum_{u=1}^{n} x_{iu} x_{ju}^2 = 0, \text{ for i} \neq \text{j=0,1,}\cdots\text{,k} \qquad \text{(i)}$$

$$\sum_{u=1}^{n} x_{ui}^2 = \text{constant=N}\lambda, \ \forall \text{i} \qquad \textbf{(ii)}$$

$$\sum_{u=1}^{n} x_{ui}^4 = \text{constant=3N}\lambda, \ \forall \text{i} \qquad \textbf{(iii)}$$

$$\sum_{u=1}^{n} x_{ui}^2 x_{uj}^2 = \text{constant=N}\lambda \ \forall \text{i} \neq \text{j} \qquad \textbf{(iv)}$$

where λ is a constant of any design.

Hence, $\sum_{u=1}^{n} x_{iu}^4 = 3\sum_{u=1}^{n} x_{iu}^2 x_{ju}^2 \qquad \text{for i} \neq \text{j} = 1,2,\cdots,\text{k}$ (3.5)

where the summation in the relations is over the design points $u = 1, 2, \cdots, N$.

The choice of axial distance α is based on the region of interest and operability. This study considered rotatable central composite design (RCCD) under which the value of α depends on the number of experimental runs in the factorial portion of the central composite design, such that $\alpha = \left(f\right)^{\frac{1}{4}}$, where f is the square points in a central composite design , making the design rotatable.

This was due to; (Box & Hunter, 1957; Box & Draper, 1987; Cornell & Khuri, 1987; and Montgomery, 1991).

Therefore by using equation (3.2) and the rotatability conditions in equation (3.5) equation (3.6) was generated.

$$\alpha^4 = \frac{2^{k-p} n_c}{n_s} \tag{3.6}$$

3.1.3 The First- order Model

The first order model aids in getting the direction of the relationship between the response and independent variables. It leads the experimenter sequentially and efficiently along the path of steepest ascent/descent. The first order model is a lower-order polynomial. A first order model with *k* variables takes the form.

$$y = \beta_0 + \beta_1 x_1 + \cdots + \beta_k x_k + \varepsilon \tag{3.7}$$

Where x_i and β_k's are the design variables and regression coefficients respectively.

When the curve turns, the second-order model is used to approximate the response precise values.

3.1.4 The Second- order Model

The central composite design was used to explore the region for fitting in the first and second order models in this study. The prevailing region of exploration was 20^0-30^0 degree Celsius for temperature, 10-20 minutes of sterilization time and 35-65 grams of agar per 1000 cubic centimeter of PDA. To simplify the calculations the independent variables were coded (-1, 1) interval such that given ξ_1, ξ_2 and ξ_3 as the natural variables for temperature, time and culture media concentration levels respectively, then the coded variables should be derived as;

$$x_1 = \frac{\xi_1 - 25}{5}, \quad x_2 = \frac{\xi_2 - 15}{5} \quad \text{and} \quad x_3 = \frac{\xi_3 - 50}{15} \tag{3.8}$$

As established by Box and Wilson (1951)

Therefore the experiments were generated and tried on a coded scale as summarized in table 3.2

Table 3.2: Design matrix for RSM Spawn Propagation

Std	**Natural Variable**			**Coded Variable**		
#	ξ_1	ξ_2	ξ_3	x_1	x_2	x_3
1	20	10	35	-1	-1	-1
2	30	10	35	1	-1	-1
3	20	20	35	-1	1	-1
4	20	10	65	-1	-1	1
5	30	10	65	1	-1	1
6	30	20	35	1	1	-1
7	20	20	65	-1	1	1
8	30	20	65	1	1	1
9	$-\alpha$	0	0	-1.682	0	0
10	α	0	0	1.682	0	0
11	0	$-\alpha$	0	0	-1.682	0
12	0	α	0	0	1.682	0
13	0	0	$-\alpha$	0	0	-1.682
14	0	0	α	0	0	1.682
15	25	15	50	0	0	0
16	25	15	50	0	0	0
17	25	15	50	0	0	0
18	25	15	50	0	0	0
19	25	15	50	0	0	0

Given k = 3 and p=0, a CCD was made up of at least fifteen distinct points: eight points for the 2^3 factorial part (±1, ±1, ±1), i.e. the cube points or square points.; then six points for the 2(3) axial points, which were at equal distance, α, from the centre of the design, [(±α, 0, 0), (0, ±α, 0) and ((0, 0, ±α], i.e. the star and at least one centre point. To enable in the identification of the best CCD by creating a grid of all combinations of the design choices, the information function for the second-order (quadratic) model had to be rotatable, the α values needed for orthogonality and

rotatability were computed. Ideally, in order to make an unbiased estimate of pure error, the CCD should comprise of three to five centre points (Russell, 2012).The central composite design is commonly used to fit the second order response surface model of the form

$$y_u = \beta_0 + \sum_{i=1}^{k} \beta_i x_{ui} + \sum_{i=1}^{k} \beta_{ii} x_{ui}^2 + \sum_{i=1}^{k-1} \sum_{i<j=2}^{k} \beta_{ij} x_{ui} x_{uj} + \varepsilon_u \tag{3.9}$$

where;

y_u is the response obtained from the *u*th combination of factors (*u* = 1, 2, …, *n)*

x_{ui} denotes the level of the i^{th} factor (i=1,2,⋯,k) in the u^{th} run (u=1,2,⋯,n) of the experiment,

$\beta_{i's}$ are the model coefficients to be determined; β_0 being a constant, β_i being the i^{th} linear regression coefficient, β_{ii} being the i^{th} quadratic regression coefficient and β_{ij} being the $(i, j)^{th}$ interaction coefficient and

$\varepsilon_{u's}$ are the uncorrelated random errors in the u^{th} observation with a mean zero and a constant variance σ^2.

3.1.5 The Significant Regression Coefficients

To test the significance of the individual regression coefficient β_j, the *P-Value* and the *t* test were used. The t test statistics is based on the t distribution and has the form

$$t = \frac{\hat{\beta}_j}{s\left(\hat{\beta}_j\right)} \tag{3.10}$$

where $\hat{\beta}_j$ is the least square estimator of the parameter β_j (j = 1, 2..., q) and $s\left(\hat{\beta}_j\right)$ is the estimated standard error of $\hat{\beta}_j$. The standard error of each parameter $\hat{\beta}_j$ is

given by the square root of the diagonal elements of the variance-covariance matrix, $\hat{\beta}$

$$\mathrm{Cov}\left(\hat{\beta}_j\right) = \sigma^2 \left(X^{'} X\right)^{-1} \tag{3.11}$$

and X is the matrix of values of explanatory variables referred to as the design matrix, such that.

$$\mathrm{X} = \begin{bmatrix} 1 & x_{1,1} & x_{1,2} & \cdots & x_{1,p-1} \\ 1 & x_{2,1} & x_{2,2} & \cdots & x_{2,p-1} \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ 1 & x_{n,1} & x_{n,2} & \cdots & x_{n,p-1} \end{bmatrix}$$

3.1.6 Location and Characterization of the Stationary Points

The temperature level (x_1), Sterilization time (x_2) and Culture media concentration level (x_3), that minimized the days of *Pleurotus ostreatus* spawns development were established by carrying out the following partial derivatives from the function $f(x_1, x_2, x_3) = \hat{y}$

$$\partial\hat{y}/\partial x_1 = \partial\hat{y}/\partial x_2 = \partial\hat{y}/\partial x_3 = 0 \tag{3.12}$$

and by letting b_0, b_i, b_{ii}, b_{ij} denote the least square estimators of $\beta_0, \beta_i, \beta_{ii}, \beta_{ij}$ respectively we have

$$y = b_o + b_i x_i + b_{ii} x_i^2 + \sum_{i<j} b_{ij} x_{ij} \tag{3.13}$$

whereby the second order model in matrix notation was

$$\hat{y} = \hat{\beta}_0 + x^{'} b + x^{'} B x \tag{3.14}$$

such that

$$\mathrm{X} = \begin{bmatrix} x_1 \\ x_2 \\ \vdots \\ x_k \end{bmatrix},\ b = \begin{bmatrix} \hat{\beta}_1 \\ \hat{\beta}_2 \\ \vdots \\ \hat{\beta}_k \end{bmatrix} \text{ and } B = \begin{bmatrix} \hat{\beta}_{1,1} & \hat{\beta}_{1,2}/2 & \cdots & \hat{\beta}_{1,k}/2 \\ & \hat{\beta}_{2,2} & \cdots & \hat{\beta}_{2,k}/2 \\ \vdots & \vdots & \ddots & \vdots \\ sym & & \cdots & \hat{\beta}_{k,k} \end{bmatrix} \tag{3.15}$$

Whereby b is a (k×1) vector of first order regression coefficients and B is a (k×k) symmetric matrix whose main diagonal elements are pure quadratic coefficients $(\hat{\beta}_{ii})$ and whose off-diagonal elements are one-half the mixed quadratic coefficients $(\hat{\beta}_{ij}, i \neq j)$

Therefore

$$\partial\hat{y}/\partial x = b + 2Bx = 0 \tag{3.16}$$

then the stationary points would be

$$x_s = \frac{1}{2} B^{-1} b \tag{3.17}$$

and the predicted response at the stationary points would be

$$\hat{Y}_s = \hat{\beta}_0 + \frac{1}{2} x_s' b \tag{3.18}$$

The second order model normally takes the maximum, minimum, saddle point or stationary ridge. The coordinates and the nature of each stationary point were determined by carrying out the partial derivatives and represented by using the contour maps.

3.1.7 Canonical Analysis

The canonical analysis objective was to determine the nature of the stationary point and the entire response surface. If the signs of the eigenvalues of matrix $\hat{B}$ are all positive then the stationary point is minimum otherwise it is a maximum response surface if all the signs of the eigenvalues of $\hat{B}$ are all negative. A saddle point would exist if there is a mixed sign, which is both positive and negative.

The canonical equation takes the form

$$\hat{y} = \hat{y}_s + \lambda_1 w_1^2 + \lambda_2 w_2^2 + \ldots + \lambda_k w_k^2 \tag{3.19}$$

Where $\{\lambda_i\}$ are the eigenvalues or the characteristic roots of the matrix $\hat{\beta}$, while $\{w_i\}$ are the transformed independent variables**.** The nature of the response surface is determined from the stationary points and the signs of the magnitudes λ_i. If all λ_i are positive then x_s is a minimum response surface, otherwise x_s is a maximum response surface if all λ_i are negative, while mixed sign indicates a saddle response surface and if at least one eigenvalue is zero then it is a ridge surface. The response surface is steepest in the $\{w_i\}$ direction corresponding to the largest absolute eigenvalue. Canonical analysis is very instrumental in systems of maxima and minima determination in many dimensions and, in particular to identify complicated ridge systems, where direct geometric representation is not possible.

3.1.8 Design Criteria

Optimality criteria give a summary of how good a design is, and they are maximized or minimized by an optimal design.The optimal criterion design can be; information based, distance-based or compound based criterion.

The design criteria try to extract the best set of points, the most popular ones attempt to select the points so as to minimize the variance of the coefficients of the response surface, where a key to that variance is the moment matrix. The core business of the optimal design is to establish the best with respect to the chosen criteria. This study considered information based criteria related to the information matrix, $\mathrm{M}(\xi)=\frac{1}{\sigma^2}\mathrm{X}'\mathrm{X}$ for the design. All the same, the model, region of interest, number of runs, and the design points should be specified and justified. The goodness or efficiency of an experimental design can be quantified. Mostly the goodness of design matrix X is based on the information matrix $\mathrm{X'X}$. Discussed in the following section are the mostly used design optimality criteria and their efficiencies;

3.1.8.1 D- Optimality Criterion

A design is said to be D-optimal if $\left|(\mathrm{X}'\mathrm{X})^{-1}\right|$ is minimized or $\left|\mathrm{X'X}\right|$ is maximized, that is $\max_{x_i, i=1,\cdots,k}\left|X'X\right|$, provided the moment matrix is nonsingular. The optimality focuses on the estimation of model parameters through good attributes of the moment matrix defined as $\mathrm{M}=\left(\frac{\mathrm{X'X}}{n}\right)$, where n, the total number of experimental runs. The efficiency can also be scaled to range from 0 to 100 percent such that

$$\mathrm{D_e}=100\left(\frac{\left|\mathrm{X'X}\right|^{\frac{1}{\mathrm{p}}}}{n}\right) \tag{3.20}$$

Where p is the total number of parameters in the model, including the intercept, if the value is 100% the design is balanced and orthogonal, implying all the parameters can be estimated, inversely if the value is 0% they can't be estimated. Any value between

0% and 100% implies that, the parameters can be estimated but with less than the optimal precisions.

Basically the efficiency measures the goodness of a design relative to hypothetical orthogonal design. However, since it may never exist, then efficiency measures a relative comparison of one design to another under the same condition; a measure of the relative efficiency of design 1 to design 2 based on the D-optimality is given by

$$D_e = \left(\frac{\left|(X_2^{'} X_2)^{-1}\right|}{\left|(X_1^{'} X_1)^{-1}\right|} \right)^{1/p} \tag{3.21}$$

Where X_1 and X_2 are the X matrices for the two designs while p is the number of parameters in the model.

3.1.8.2 A- Optimality Criterion

A design is said to be A-optimal if it minimizes the sum of the main diagonal element of the inverse of the information matrix denoted as $\text{tr}((X^{'}X)^{-1})$, that is $\min_{x_i=1,\cdots,k} trace(X'X^{-1})$. In effect A-optimal criterion minimizes the sum of the variances of the regression coefficients. Measure of the relative efficiency of a design based on the A-optimality is given by

$$A_e = 100\left(\frac{p}{\text{trace}(N(X'X)^{-1}} \right) \tag{3.22}$$

3.1.8.3 E- Optimality Criterion

A design is said to be E-optimal if it maximizes the minimum eigenvalue of $(X'X)$ or equivalently, minimize the maximum eigenvalue of $(X'X)^{-1}$, denoted as $\max \lambda_{\min}(X'X)$ or $\min \lambda_{\max}(X'X)^{-1}$ respectively. Its aim is to minimize the

maximum variance of all possible normalized linear combinations of parameter estimates.

3.1.8.4 G- Optimality Criterion

This is one among the prediction variance criteria. A design is said to be G-optimal if it minimizes the maximum Scaled Prediction Variance (SPV) over the design region. The scaled prediction variance is defined as

$$\frac{\mathrm{NV}[\hat{y}(x)]}{\sigma^2} = \mathrm{N} f'(x)\left(X'X\right)^{-1} f(x) \tag{3.23}$$

That is the maximum value over the design region is a minimum, where N is the number of points in the design, σ^2 ,the process variance is assumed to be 1 and $f(x)$ is the vector of coordinates of point in the region of interest expanded to model form, such that

$$f'(x) = \left[1, x_1, \cdots, x_k, x_1^2, \cdots, x_k^2, x_1x_2, \cdots, x_{k-1}x_k\right]. \tag{3.24}$$

If the model has p-parameters, the G-efficiency of the design is as given by equation

$$\mathrm{G_e} = \frac{\mathrm{p}}{\max \mathrm{NV}[\hat{y}(x)]/\sigma^2} \tag{3.25}$$

Equivalently equation 3.25 can be expressed as

$$\mathrm{G_e} = 100\frac{\sqrt{\mathrm{P}/\mathrm{N}}}{\sigma_m} \tag{3.26}$$

Where σ_m stands for the maximum standard error for prediction across the list of candidate points.

3.1.8.5 V- Optimality Criterion

This criterion is nested in the G-optimality criterion. It considers the prediction variance at a specific set of points of interest within the feasible design region. A

design that minimizes the average prediction variance over the specified set of points is the V-optimal design.

3.1.8.6 I- Optimality Criterion

This criterion considers the smallest possible value of the average prediction variance. That is min average var(ŷ)

3.2 Suitable Local Substrates Screening for Oyster Mushroom Cultivation

In order to select the significant substrates for the oyster mushroom cultivation, one-factor-at-a-time (OFAT) screening approach was adopted, which was pure blend experimentation to select the best substrate from the locally available selected substrates.

The five components tried were; Star grass, Sawdust, Cattle manure, Euphorbia, and Sugarcane bagasse substrates in which the oyster mushroom grew. The actual data value for each input variable/s was noted, and the response surface observed.

The next step of the experimental work shifted from screening to substrates mixture and Oyster yield optimization.

3.3 Simplex Centroid Methodology

A lot of products are formed by putting together two or more ingredients at predetermined proportions to arrive at a desired quality product. Examples of such products include; Fruit juices, building construction concrete, paints and fertilizers, (Montgomery, 2001)

Mixture component is a product of two or more ingredients mixed together, such that, the components of a mixture and the response varies as the proportions vary, (Cornel, 2002).

The design factors in a mixture experiment are the proportions of the components of a blend, by which the response variables vary as a function of these proportions making the total and not the actual quantity of each component. Therefore, the total amount of the mixture is normally fixed in a mixture experiment and the component settings are proportions of the total amount. Hence, the component proportions in a mixture experiment cannot vary independently as in factorial experiments since they are constrained to a constant sum of 1 or 100% for standard designs.

The sum of the proportions is equal to one, such that for a q-component mixture;

$$0 \le x_i \le 1, \text{ for } i = 1, 2, \cdots, q \quad \text{and} \quad \sum_{i=1}^{q} x_i = 1 \tag{3.27}$$

Where x_i represents the proportions of the i^{th} component in the mixture of q-components

Among the different mixture designs such as Simplex Lattice, Simplex Centroid and Axial designs. The study adopted Simplex Centroid Mixture Design (SCMD).

The $\{q, m\}$ simplex lattice designs and simplex centroid designs were introduced by Scheff*é* (1958, 1963).

In this study substrates mixture of ingredients $\left(x_1, x_2, ..., x_q\right)$, with $\left(x_i \ge 0\right)$ and further restriction of $\sum x_i = 1$ were investigated to establish their influence in the oyster mushroom yield through their ratios or proportions variation.

3.3.1 Simplex Centroid Application Procedure

Procedurally all the input materials were gathered prior to the starting of the process. The substrate materials were weighed and shred into small pieces to easy mixing, packing and soaking.

The substrate materials were soaked for an overnight to absorb enough water content that could sustain the whole process of mycelia colonization and fruition. The substrates were mixed with wheat bran, lime and as designated with other substrate components.

The mixed substrates were packed into polythene paper bags per kilogram and labelled as per the substrates' composition. The Polyvinyl Chloride (PVC) plastic pipes were fixed and sealed with the cotton wool which was fastened with the rubber bands to avoid external contaminations.

The sealed bags were steam boiled for five hours to sterilize them. The bags were allowed to cool and then the spawns were placed into the substrates through the spooning chambers, in a sterilized germ free environment with the attendants' hands and mouth gloved and covered respectively. The inoculated bags were then placed on the shelves in a dark room where the temperature and humidity were controlled for at least a month for substrates' full colonization. The PVC and the cotton wool were detached to allow the pinheads to sprout out which finally transformed into the oyster fruits.

To ensure ANOVA test statistics assumptions were not violated during the experimentation period, complete randomization of the polythene bags was doneand the polythene bags were labelled as per the substrates composition but randomly and independently placed.

3.3.2 Optimal Mixture for the Significant Factors

The significant substrates were tried repetitively and randomly. Table 3.3 shows the possible substrates combinations for q components which survived the screening stage.

Table 3.3: Simplex Centroid Design for k Substrates Mix Ratio

Design Points	Substrates proportions					Designated Blend	Expected Yield
#	x_1	x_2	x_3	• • •	x_q	X	$\bar{y}$
1	1	0	0	• • •	0	x_1	$\bar{y}_1$
2	0.5	0.5	0	• • •	0	x_1x_2	$\bar{y}_{1,2}$
3	0	0	1	• • •	0	x_3	$\bar{y}_3$
•	•	•	•	•	•	•	•
•	•	•	•	•	•	•	•
•	•	•	•	•	•	•	•
nth	0	0	0	• • •	1	x_q	$\bar{y}_q$

The simplex centroid mixture design was used to establish an optimal mix among the significant substrates; sawdust, star grass, cattle manure, sugarcane bagasse and euphorbia.

Each of the designated design had one kilogram polythene paper of the substrate for each experiment set up and for which the yield in kilograms was noted throughout the harvesting and fruition period. Assuming q number of components or substrates in this case, the simplex centroid design had $2^q - 1$ points, corresponding to q permutations of (1, 0, 0,…, 0) of the pure blends consisting of only one substrate (k single-substrate blends). And there were $\binom{q}{2}$ permutations of $\left(\frac{1}{2}, \frac{1}{2}, 0, \cdots, 0\right)$ that is the binary blends involving a mixture of two substrates in the ratio 1:1. Then there

was $\binom{q}{3}$ permutations of $\left(\frac{1}{3},\frac{1}{3},\frac{1}{3},0,\cdots,0\right)$ for the triad blends involving a mixture of the three substrates in the ratio 1:1:1 until the overall centroid point $\left(\frac{1}{q},\frac{1}{q},\cdots,\frac{1}{q}\right)$ involving one *q-th* proportions of all the substrates when in equal proportions (assuming equal significance) consisting of every subset of each component was conducted.

3.3.3 Parameter estimate in the polynomials

The constraint $\sum x_i = 1$ in the mixture models makes them differ from the usual polynomials employed in response surface methodologies.

At the points of simplex centroids design, the response variable data was fitted onto a polynomial that had the same number of parameters to be estimated as there were points in the associated design. The standard form of a mixture polynomial model is defined as

$$\eta = \sum_{i=1}^{q}\beta_i x_i + \sum\sum_{i<j}^{q}\beta_{ij}x_i x_j + \sum\sum\sum_{i<j<k}^{q}\beta_{ijk}x_i x_j x_k + \cdots + \beta_{12\cdots q}x_1 x_2 \cdots x_q \qquad (3.28)$$

The parameters as shall be shown are expressible as linear functions of the expected responses at the points of the simplex centroid design. The parameter β_i represents the expected response to the pure blend. In case of a curvature due a nonlinear blending between component pairs, the parameter β_{ij} represents either synergistic or antagonistic blending otherwise it is a mere additive blending.

By substituting η_i, η_{ij} and η_{ijk} into equation 3.28 for the responses $x_i = 1$, $x_j = 0, i \neq j$ to $x_i = x_j = \frac{1}{2}$ and $x_i = x_j = x_k = \frac{1}{3}$ respectively, for all *i*, *j* and *k* then the parameters were;

$$\beta_i = \eta_i,\ \beta_{ij} = 2\{2^1\eta_{ij} - 1^1(\eta_i + \eta_j)\} \text{ and}$$

$$\beta_{ijk} = 3\{3^2\eta_{ijk} - 2^2(\eta_{ij} + \eta_{ik} + \eta_{jk}) + 1^2(\eta_i + \eta_j + \eta_k)\} \quad (3.29)$$

and by extension,

$$\beta_i = \eta_i = \bar{y}_i,\ (i = 1, 2, ..., k)$$

$$\beta_{ij} = 4\bar{y}_{ij} - 2(\bar{y}_i + \bar{y}_j),\ (ij = 1, 2, ..., n)_{i<j} \quad (3.30)$$

Oyster mushroom (*Pleurotus ostreatus*) was cultivated on Star grass (*cynodonplectostachyus*), locally known as *ikoka*, then Sawdust, Cattle manure, Euphorbia and Sugarcane bagasse substrates, complemented at different proportions such that;

$$x_i \geq 0,\ x_1 + x_2 + x_3 + \cdots + x_q = 1 \quad (3.31)$$

Where; x_l, for $i = 1 \cdots q$ represented the substrate components and each component proportion x_i took the values zero to unity and all the blends among the ingredients were tried. Since the experiment used the simplex centroid design the mixtures were located at the centroid of the $(q-1)$ dimensional simplex and at the centroids of all the lower dimensional simplexes contained within the $(q-1)$ dimensional simplex.

3.3.4 Contour Plots

The outcome produced an empirical polynomial model which gave an approximation of the true response surface over a factor region. By overlaying contour maps from the experimental responses, it was possible to find the ideal "window" of operability.

3.4 Economic Returns Analysis for Oyster Mushroom Cultivation

The contribution margin of determining the breakeven point was applied to estimate the level of oyster mushroom production output that would pay-off all the production

costs. A purposeful sampling method was applied to identify the key informants and the oyster mushroom farmers within the study targeted area to share their experience and challenges on oyster mushroom production, marketing and consumption benefits.

CHAPTER FOUR

RESULTS AND DISCUSION

4.0 Introduction

The chapter focuses on the analysis, interpretation and discussion of the results emanating from the study objectives. Section 4.1 and sub-section 4.1.1 presents the results on spawn propagation by using the central composite designs. The first order, second order and the rotatable second order designs results are presented in sub-sections; 4.1.2, 4.1.3 and 4.1.4 respectively. The results on optimality criteria, second order model regression equation and canonical analysis are presented in sub-sections 4.1.5, 4.1.6 and 4.1.7 respectively. The stationary points and the contour plots for second order model are presented in sub-sections 4.1.8 and 4.1.9 respectively. Section 4.2 presents the most important substrates in Oyster mushroom production out of the potential substrates tried. while section 4.3 presents the results on simplex centroid mixture designs from the established significant substrates. Lastly the economic returns analysis on Oyster mushroom production results is presented in section 4.4.

4.1 Optimization of Spawns Propagation

The operating optimal levels of temperature, sterilization time and culture media concentration that minimized time in days of the mycelia full coverage in a petri dish area were determined through central composite designs. The colonised media in a petri dish is displayed in figure 4.1.

4.1.1 Central Composite Design

The central composite design was used to explore the region for fitting in the first and second order models. The second order design of 3 factors; temperature level, sterilization time and culture media concentration at 2 levels were chosen as the process variables for optimization, by investigating their effect on time to full

colonization of a substrate, in a petri dish for spawn production of oyster mushroom (*Pleurotus ostreatus).*The CCD comprised a total of 19 experiments: with a full factorial design 8 experiments, 6 axial points, and central points replicated five times. In order to measure the effect of, incubation temperature, the sterilization time and culture media concentration on the time (days) to full colonization of the substrate for spawn multiplication, by using the central composite design the following procedure was adopted.

Three levels of PDA extract agar concentration (35g, 50g and 65g) were weighed separately using laboratory analytical balance. Each concentration was dissolved in 1000 milliliter of distilled water and boiled for 2 minutes to dissolve the media. The media solution was then autoclaved for 10, 15 and 20 minutes each time but separately. This was followed by aseptically pouring of the cooled media into petri dishes in the laminar air flow which had been thoroughly sterilized using 70% alcohol. Once the media solidified in the petri dishes, the oyster mushroom (*Pleurotus ostreatus*) was inoculated. This was achieved by cutting $2 \times 2\text{mm}^2$ pieces of pure mycelia which was centrally placed on the cooled media. Each concentration was replicated 5 times making 15 plates for the three autoclaving and incubation temperatures per batch. The plates were completely sealed with a parafilm and incubated separately in dark condition at $20^0 C$, $25^0 C$ and $30^0 C$ temperature level each until the mycelium developed and covered the full area or otherwise. A complete randomized design was used to place the petri plates in the incubator. The mycelia of *Pleurotus ostreatus* species were observed daily and measurements of the colony diameter of mycelia was noted after inoculation using a clear (transparent) ruler until the plates were fully colonized or otherwise.

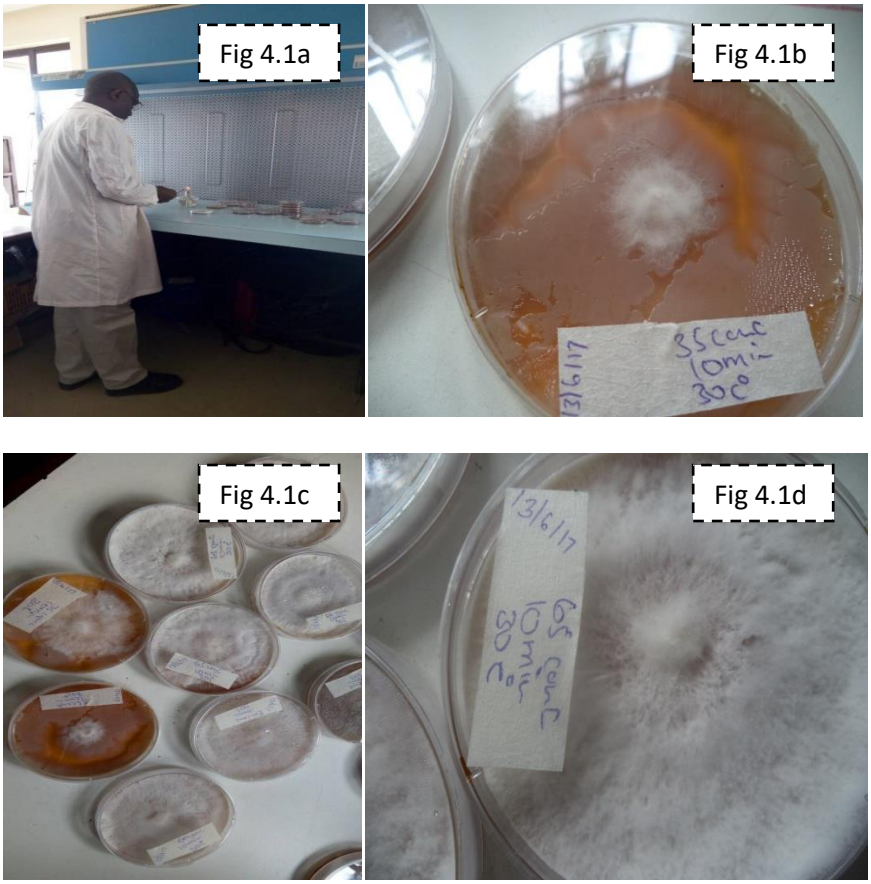

Figure 4.1 Spawns Propagation

The petri dishes were regularly inspected and the contaminated ones were immediately discarded. The inset 4.1b of figure 4.1 shows a contaminated petri dish while the inset 4.1d is a fully colonized petri dish. The mycelia growth covered the entire substrate within the 10-20 days of inoculation, by which the spawns were ready for use.

4.1.2 Analysis of a First- order Model

Method of steepest descent was used to establish a point of local minimum, where the gradient was converging to zero.

Each day the mycelium coverage was observed and the days to total coverage in the petri dish were noted. Table 4.1 gives the summary of the average number of days per run replicated five times. Given that the Response Surface Methodology (RSM) is a

sequential procedure the first-order model $\hat{y} = \hat{\beta}_0 + \sum_{i=1}^{k} \hat{\beta}_i x_i$ was fitted to lead rapidly and efficiently along the path of improvement towards the general vicinity of the optimum.

The level of temperature, time of sterilization and the culture media concentration level were sequentially altered along the path of steepest descent until there was no further decrease of days to full colonization. The findings from the experiment are summarized in table 4.1. At the final stages of process operations, CCD illuminated the desired spot where the largest mycelia coverage could be achieved at fewest possible days and low cost. It produced statistically validated predictive models on spawn production. Table 4.1 shows the experimental design output in a convenient layout that sorted the predictor variables by level. The actual run order for experiments was sequentially done to observe the curvature.

Table 4.1: Three Factors CCD on Spawn Production

	Natural Variable			**Coded Variable**			**Days**
Steps	ξ_1	ξ_2	ξ_3	x_1	x_2	x_3	**y**
Original	**25**	**15**	**50**	**0**	**0**	**0**	**11**
Δ	**05**	**05**	**15**	**1**	**1**	**1**	**-**
Original+ Δ	**30**	**20**	**65**	**1**	**1**	**1**	**10**
Original+ 2Δ	**35**	**25**	**80**	**2**	**2**	**2**	**17**
Original+ 3Δ	**40**	**30**	**95**	**3**	**3**	**3**	**19**
Original+ 4Δ	**45**	**35**	**110**	**4**	**4**	**4**	**20**
Original $-\Delta$	**20**	**10**	**35**	**-1**	**-1**	**-1**	**09**
Original -2Δ	**15**	**05**	**20**	**-2**	**-2**	**-2**	**23**
	30	**10**	**65**	**1**	**-1**	**1**	**10**
	20	**20**	**35**	**-1**	**1**	**-1**	**15**
	30	**10**	**35**	**1**	**-1**	**-1**	**14**

The coded values were such that $x_1 = \frac{\xi_1 - 25}{5}$, $x_2 = \frac{\xi_2 - 15}{5}$ and $x_3 = \frac{\xi_3 - 50}{15}$

The objective function was to determine the operating condition that minimized the days of mycelia full coverage in a petri dish. Appendix IV summarizes the observed

measurements of the colony diameter of mycelia after inoculation using a clear (transparent) ruler until the plates were fully colonized. There was no significant lack of fit in this experiment as it could be inferred by inspection of figure 4.2. The response surface for mycelia full coverage occurred at varying Temperature levels, Sterilization time and malt extract agar concentration levels.

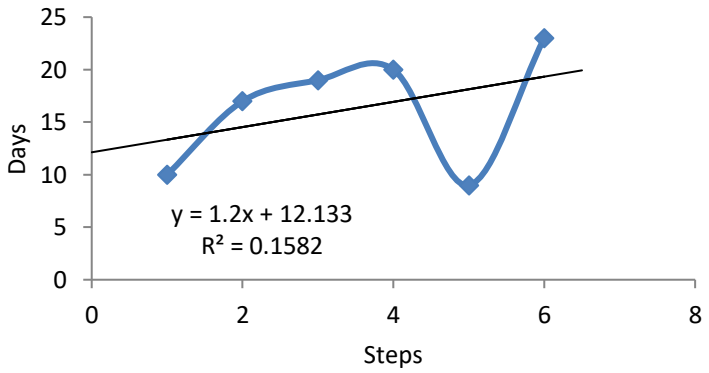

Figure 4.2 Steps and Days to Mycelium full colonization

Figure 4.2 displays the initial stages of finding out the number of days to full mycelium colonization at varied levels of media culture concentrations, sterilization time and temperature, labeled as steps along the path of steepest descent. The gradient of the straight line was the substrate rate of colonization. All the three variables were important and affected the colonization period. They clearly gave information on the direction of the steepest descent.

The results indicated that the vicinity of optimum was within step 4 and step 6. However, the small value of the coefficient of determination $\left(R^2 = 0.1582\right)$ coupled with the existence of a curvature, the first order model could not adequately explain the variations in the response surface due to varied levels of media culture concentrations, sterilization time and temperature, necessitating need to fit in a second order model to obtain a more precise estimate of the optimum.

4.1.3 Analysis of a Second- order Model

A model that incorporated the curvature was appropriate to approximate the response, given that the region of optimization was identified, then a second order model was fitted to determine the optimum set of operating levels for the temperature, time of sterilization and the culture media concentration that minimized the days to full mycelium colonization. The second order model in the form of equation (3.9) was adopted.

4.1.4 Rotatable Second-order Designs

To make a design both (approximately) orthogonal and rotatable, the axial distance for rotatability was chosen, and then the center points were added, in compliance with Cornell and Khuri, (1987) and Box and Hunter (1957) who introduced rotatability as a desirable and necessary property in response surface methodology for second order models exploration.

Under this design criterion, $Var\left[\frac{\partial \hat{y}(x)}{\partial x_i}\right]$ was constant $\forall x \in X$ that were equidistant from the design center $(i = 1, 2, \cdots, k)$. Therefore by applying equation (3.2) the experimental runs were 19 and it was possible to have a rotatable and orthogonal design by adding the star- points and centre points replicated five times to the simple square factorial design points;

Such that given k = 3, p= 0, $n_f = n_s = 1$and $n_c = 5$ for the design to be rotatable it had

$$\alpha = \left(\frac{2^{3-0}(1)}{1}\right)^{\frac{1}{4}} \Leftrightarrow (8)^{\frac{1}{4}} = \pm 1.682 \qquad (4.1)$$

Since the value of α for a rotatable CCD depended on the fourth root of the cube (Montgomery, 1991).

To simplify the calculations and for the convenience sake the region of interest was defined by simple lower and upper limits on each of the design variables and coded as (-1, 1) interval hence making all the variables to be bound in the cube, $-1 \le x_i \le 1$, with the average of these two values being assigned to "0".

By letting ξ_1, ξ_2 and ξ_3 be the natural variables of temperature level, sterilization time and culture media concentration levels respectively, then the provided datasets used were factor settings of $Temperature = (25 \pm 5)^0 C$, $Sterilization\ Time = (15 \pm 5)$ minutes and the *Culture media concentration level* $= 50 \pm 15$ grams per liter with five center point. Thus the coded variables were derived as; $x_1 = \frac{\xi_1 - 25}{5}, x_2 = \frac{\xi_2 - 15}{5}$ and $x_3 = \frac{\xi_3 - 50}{15}$

for temperature, sterilization time and culture media concentration respectively.

The actual data value for each input variable was sequentially altered and noted, as the process and the response surface was observed.

The length of the colony diameter of mycelia was noted daily and the time to full colonization was observed as summarized in table 4.2.

Table 4.2: Coded and Original Variable Data

	Natural Variable			Coded Variable			Days
Std Run	ξ_1	ξ_2	ξ_3	x_1	x_2	x_3	y
1	25	15	50	0	0	0	07
2	30	10	35	1	-1	-1	14
3	20	20	35	-1	1	-1	15
4	20	10	65	-1	-1	1	13
5	30	10	65	1	-1	1	08
6	25	15	50	0	0	0	07
7	20	20	65	-1	1	1	10
8	30	20	65	1	1	1	06
9	33.41	15	50	1.682	0	0	13
10	16.59	15	50	-1.682	0	0	12
11	25	19.205	50	0	1.682	0	08
12	25	10.795	50	0	-1.682	0	12
13	25	15	58.41	0	0	1.682	09
14	25	15	41.59	0	0	-1.682	11
15	20	10	35	-1	-1	-1	19
16	30	20	35	1	1	-1	11
17	25	15	50	0	0	0	07
18	25	15	50	0	0	0	08
19	25	15	50	0	0	0	07

Table 4.2 shows the runs and the factor levels. The rows represent the runs and the columns represent the levels of the factors. The first row was run at the traditionally normal level of all of the controllable factors, the second row was run at the 'high' level of temperature as a factor but the 'low' levels of sterilization time and agar media concentration.

By default, the variable names remained x_1 , x_2 and x_3 with the design order 1-19, however, the actual experiment implementation was randomized. All the necessary properties for CCD second-order (quadratic) model such as rotatability and orthogonality were observed. To make an unbiased estimate of pure error, the CCD comprised of five centre points. Alteration of the temperature level, sterilization time and the culture media concentration that shortened the mycelia colonization time (days), were as summarized in table 4.3.

Table 4.3: Coded Variable Design Data

y	x_1	x_2	x_3	x_1x_2	x_1x_3	x_2x_3	x_1^2	x_2^2	x_3^2
08	0	0	0	0	0	0	0	0	0
14	1	-1	-1	-1	-1	1	1	1	1
15	-1	1	-1	-1	1	-1	1	1	1
13	-1	-1	1	1	-1	-1	1	1	1
07	0	0	0	0	0	0	0	0	0
11	1	1	-1	1	-1	-1	1	1	1
10	-1	1	1	-1	-1	1	1	1	1
06	1	1	1	1	1	1	1	1	1
07	0	0	0	0	0	0	0	0	0
12	-1.682	0	0	0	0	0	2.829	0	0
08	0	1.682	0	0	0	0	0	2.829	0
12	0	-1.682	0	0	0	0	0	2.829	0
07	0	0	0	0	0	0	0	0	0
11	0	0	-1.682	0	0	0	0	0	2.829
08	1	-1	1	-1	1	-1	1	1	1
13	1.682	0	0	0	0	0	2.829	0	0
09	0	0	1.682	0	0	0	0	0	2.829
19	-1	-1	-1	1	1	1	1	1	1
07	0	0	0	0	0	0	0	0	0

The shortest time observed was six (6) days with nineteen (19) days being the longest time. By using the coded second model data, it was possible to get the design matrix (X), the model equation and carry out the computation of the criteria and their efficiencies as discussed in the following section.

4.1.5 Optimality Criteria

The general three factors rotatable CCD was modified by varying the number of times the center point was replicated. The optimality criteria D-, A-, and E which are best suited for the model parameters estimation were chosen to test the effect of varying the number of centre points in the design.

Given the design matrix X, it was possible to determine the design optimality criterion at varied number of center points given that;

$$X'X = \begin{pmatrix} 19 & 0 & 0 & 0 & 0 & 0 & 0 & 13.66 & 13.66 & 13.66 \\ 0 & 13.66 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 13.66 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 13.66 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 8 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 8 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 8 & 0 & 0 & 0 \\ 13.66 & 0 & 0 & 0 & 0 & 0 & 0 & 24.01 & 8 & 8 \\ 13.66 & 0 & 0 & 0 & 0 & 0 & 0 & 8 & 24.01 & 8 \\ 13.66 & 0 & 0 & 0 & 0 & 0 & 0 & 8 & 8 & 24.01 \end{pmatrix}$$

By using A-criterion, whereby a design is said to be A-optimal if it minimizes the sum of the main diagonal element (trace) of $(X`X)^{-1}$ denoted as $tr((X`X)^{-1})$. The sum of the diagonal was 1.0137 in all the designs. The value changed only due to the number of experimental runs.

With regard to the D-optimal, the value that maximized $|X'X|$ was 1.5e-11 and it was constant among different number of design centre points.

Finally, regarding the E-optimal the value that minimized the maximum eigenvalue of $(X'X)^{-1}$, denoted as $\min \lambda_{\max}(X'X)^{-1}$, was 0.2762, and it was constant among the three different chosen center points.

Table 4.4: Optimality Criteria

Criterion and its efficiency	**Number of the Centre Points**		
	1	3	5
D-Optimality	1.5e-11	1.5e-11	1.5e-11
A-Optimality	2.0786	1.1927	1.0137
E-Optimality	0.2762	0.2762	0.2762

Therefore the addition of center points on CCD second-order designs did not significantly affect the optimality. However, the five center points were chosen since

according to Chigbu and Orisakwe (2000) to make an unbiased estimate of pure error, the CCD should comprise of three to five centre points.

4.1.6 The Second Order Model Regression Equation

The second degree order model was computed using the R commands and the outputs were as given in tables; 4.5, 4.6, 4.7 and 4.8.

Table 4.5: Second Order Model Coefficients

Factors	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	7.1635e+00	9.5210e-01	7.5238	3.602e-05 ***
x_1	-1.1947e+00	5.7674e-01	-2.0715	0.068194 .
x_2	-1.3712e+00	5.7674e-01	-2.3775	0.041398 *
x_3	-1.8570e+00	5.7674e-01	-3.2199	0.010491 *
x_1:x_2	2.5000e-01	7.5358e-01	0.3317	0.747674
x_1:x_3	-1.9694e-16	7.5358e-01	0.0000	1.000000
x_2:x_3	2.5000e-01	7.5358e-01	0.3317	0.747674
x_1^2	2.0753e+00	5.7680e-01	3.5978	0.005768 **
x_2^2	1.1916e+00	5.7680e-01	2.0658	0.068826 .
x_3^2	1.1916e+00	5.7680e-01	2.0658	0.068826 .

*Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1*
Multiple R-squared: 0.8075, Adjusted R-squared: 0.615
F-statistic: 4.195 on 9 and 9 DF, p-value: 0.02201

Therefore based on the second order model regression output the model equation was

$$\hat{y} = 7.16 - 1.20x_1 - 1.37x_2 - 1.86x_3 + 2.08x_1^2 + 1.20x_2^2 + 1.20x_3^2 + 0.25x_1x_2 + 0.00x_1x_3 + 0.25x_2x_3 \quad (4.2)$$

4.1.6.1 Significant Predictors

Based on the *t* and the *p-values* it was observed that the entire predictor variables were statistically significant for influencing the time to spawn full development but at a varied levels of significance. Temperature was only significant at 5% but sterilization time and media culture concentration at 1% significance level. The temperature quadratic term was statistically significant at 1% significance level. The coefficient of determination R^2 indicated that the model could approximate the data at the design points at 80.75% (after adjusting 61.5%).

Table 4.6: ANOVA Table 1

Source of variance	Df	Sum Sq	Mean Sq	F value Computed	F Value Critical	Pr(>F)
FO(x_1, x_2, x_3)	3	92.277	30.7591	5.8761	3.86	0.011020
TWI(x_1, x_2, x_3)	3	1.000	0.3333	0.0637	3.86	0.972778
PQ(x_1, x_2, x_3)	3	60.348	20.1160	3.8597	3.86	0.050015
Residuals	9	47.111	5.2346			
Lack of fit	5	40.311	8.0623	4.7835		0.07835
Pure error	4	0.800	0.2000			

The table 5 outputs display the first-order models (FO), which specifies the first-order response surface (i.e., a linear function), the canonical analysis of the response surface with two-way interaction model (TWI) and the pure quadratic terms. The table also included lack-of-fit test and the pure error values.

Since the lack of fit was statistically insignificant $(\Pr = 0.078 > 0.05)$ then the model could predict the response variable appropriately.

Table 4.7: Stationary point of response surface

x_1	x_2	x_3
0.2594475	0.4715914	0.7297619

The response surface stationary points output was in coded form, which could be transformed into their natural value through the equations;

$$x_1 = \frac{\xi_1 - 25}{5}, x_2 = \frac{\xi_2 - 15}{5} \text{ and } x_3 = \frac{\xi_3 - 50}{15}$$

such that; $\xi_1 = 26.29^0 C$, $\xi_2 = 17.36$ Minutes and $\xi_3 = 60.95 g/L$

Table 4.8: Eigen values and vectors

	$values		
	λ_1=2.092927	λ_2=1.306832	λ_3=1.058665
	$vectors		
x_1	0.98996363	-0.1096008	0.08921697
x_2	0.13998264	0.6737557	-0.72557435
x_3	0.01941311	0.7307810	0.68233584

The nature of the stationary points is determined by the signs of the eigenvalues. All the eigenvalues were positive implying the stationary points were minimum optimising regions.

The information in tables; 4.5, 4.6, 4.7 and 4.8 was computer generated. However, the same results could be arrived at through manual calculations as discussed in the following section.

4.1.7 Canonical Analysis

The fitted quadratic model provided a noticeable response variation with clarity. The canonical analysis gave the coordinates of the estimated stationary points and the canonical directions from the points.

The fitted canonical form model is characterized by $\hat{y}(w_i) = \hat{y}_s + \lambda_1 w_1^2 + \lambda_2 w_2^2 + \ldots + \lambda_k w_k^2$ Where the fitted value at the stationary point was $\hat{y}_s$, thenλ_j were the eigenvalues obtained by getting the roots of the determinant of the matrix $|B - \lambda I| = 0$ and $w_j^2 \ , (j = 1, \cdots, k)$ were the eigenvectors.

With reference to the second degree model earlier computed in (equation 4.2) which can be transformed into matrix form as;

$$X = \begin{bmatrix} x_1 \\ x_2 \\ x_3 \end{bmatrix}, \ b = \begin{bmatrix} -1.20 \\ -1.37 \\ -1.86 \end{bmatrix} \text{ and } \hat{B} = \begin{bmatrix} 2.08 & 0.125 & 0.00 \\ 0.125 & 1.20 & 0.125 \\ 0.00 & 0.125 & 1.20 \end{bmatrix} \text{ with}$$

$$b_0 = 7.16$$

for completing the second order model matrix notation in equation (3.15) was used. The eigenvalues and the eigenvectors output calculated using the R commands were;

$$\lambda_1 = 2.10, \ \lambda_2 = 1.32 \text{ and } \lambda_3 = 1.07 \qquad (4.3)$$

$$V_1 = \begin{bmatrix} 0.990 \\ 0.141 \\ 0.020 \end{bmatrix} V_2 = \begin{bmatrix} -0.110 \\ 0.694 \\ 0.731 \end{bmatrix} \text{and} V_3 = \begin{bmatrix} -0.090 \\ 0.726 \\ -0.682 \end{bmatrix} \tag{4.4}$$

Now note the operation;

$$\hat{B}V_1 = \lambda_1 V_1 = \begin{bmatrix} 2.077 \\ 0.295 \\ 0.042 \end{bmatrix}, \ \hat{B}V_2 = \lambda_2 V_2 = \begin{bmatrix} -0.145 \\ 0.886 \\ 0.961 \end{bmatrix} \text{ and } \hat{B}V_3 = \lambda_3 V_3 = \begin{bmatrix} -0.963 \\ 0.777 \\ 0.730 \end{bmatrix} \tag{4.5}$$

$$\text{Now suppose } p = \begin{bmatrix} V_1' \\ V_2' \\ V_3' \end{bmatrix} = \begin{bmatrix} 0.990 & 0.141 & 0.020 \\ -0.110 & 0.674 & 0.731 \\ -0.090 & 0.726 & -0.682 \end{bmatrix}$$

$$\text{so that, } p^{-1} = \begin{bmatrix} 0.989 & 0.141 & 0.019 \\ -0.111 & 0.673 & 0.731 \\ -0.089 & 0.725 & -0.682 \end{bmatrix}$$

then

$$pp' = pp^{-1} = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix} \tag{4.6}$$

Therefore P is an orthogonal matrix since its columns and rows are orthogonal unit vectors (orthogonal vectors).

$$\text{Suppose } \Lambda = \begin{bmatrix} \lambda_1 & 0 & 0 \\ 0 & \lambda_2 & 0 \\ 0 & 0 & \lambda_3 \end{bmatrix} = \begin{bmatrix} 2.10 & 0 & 0 \\ 0 & 1.32 & 0 \\ 0 & 0 & 1.07 \end{bmatrix} \tag{4.7}$$

$$\text{let } \quad x = Pw \Rightarrow \begin{bmatrix} x_1 \\ x_2 \\ x_3 \end{bmatrix} = \begin{bmatrix} 0.990 & -0.110 & 0.090 \\ 0.141 & 0.674 & 0.726 \\ 0.020 & 0.731 & -0.682 \end{bmatrix} \begin{bmatrix} w_1 \\ w_2 \\ w_3 \end{bmatrix}$$

implying

$$w = P^{-1}x \Rightarrow \begin{bmatrix} w_1 \\ w_2 \\ w_3 \end{bmatrix} = \begin{bmatrix} 0.990 & -0.110 & -0.090 \\ 0.141 & 0.673 & 0.725 \\ 0.019 & 0.731 & -0.682 \end{bmatrix} \begin{bmatrix} x_1 \\ x_2 \\ x_3 \end{bmatrix} = \begin{bmatrix} 0.990x_1 - 0.110x_x - 0.090x_3 \\ 0.141x_1 + 0.673x_2 + 0.725x_3 \\ 0.019x_1 + 0.731x_2 - 0.682x_3 \end{bmatrix} \quad (4.8)$$

Now using equation 4.7 and 4.8 the following is obtained

$$w'\Lambda w = \lambda_1 w_1^2 + \lambda_2 w_2^2 + \lambda_3 w_3^2$$

$$= \begin{bmatrix} 0.990x_1 + 0.141x_2 + 0.020x_3, & -0.110x_1 + 0.674x_2 + 0.731x_3, & -0.090x_1 + 0.726x_2 - 0.682x_3 \end{bmatrix}$$
$$\begin{bmatrix} 2.10 & 0 & 0 \\ 0 & 1.32 & 0 \\ 0 & 0 & 1.07 \end{bmatrix} \begin{bmatrix} 0.990x_1 + 0.141x_2 + 0.020x_3 \\ -0.110x_1 + 0.674x_2 + 0.731x_3 \\ -0.090x_1 + 0.726x_2 - 0.682x_3 \end{bmatrix}$$

$$= 2.10(0.990x_1 + 0.141x_2 + 0.020x_3)^2 + 1.32(-0.110x_1 + 0.674x_2 + 0.731x_3)^2$$
$$1.07(-0.090x_1 + 0.726x_2 - 0.682x_3)^2$$

$$\square\ 2.08x_1^2 + 1.20x_2^2 + 1.20x_3^2 + 0.25x_1x_2 + 0.00x_1x_3 + 0.25x_2x_3 = x'\hat{B}x \quad (4.9)$$

Therefore $\hat{y} \square\ x'\hat{B}x \square\ w'\Lambda w$ and in canonical form $\hat{y} = \lambda_1 w_1^2 + \lambda_2 w_2^2 + \lambda_3 w_3^3$

Then given that all the eigenvalues of $\hat{B}$ were positive, it implied the quadratic form $x'\hat{B}x$ was positive definite, and the stationary point of the response surface was a point of minimum.

4.1.8 Stationary Points

To determine the levels of temperature (x_1), sterilization time (x_2) and culture media concentration level (x_3) that minimized time to full colonization of the agar medium, the partial derivatives were conducted such that;

$$\partial\hat{y} / \partial x_1 = \partial\hat{y} / \partial x_2 = \partial\hat{y} / \partial x_3 = 0$$

That is the stationary point were obtained by $\frac{d\hat{y}}{dx} = b + 2\hat{B}x = 0$

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

$$\text{and } \hat{y}_s = b_0 + \frac{1}{2}x_s'b$$

Therefore given $\hat{B} = \begin{bmatrix} 2.08 & 0.125 & 0.00 \\ 0.125 & 1.20 & 0.125 \\ 0.00 & 0.125 & 1.20 \end{bmatrix}$, $\hat{B}^{-1} = \begin{bmatrix} 0.484 & -0.051 & -0.005 \\ -0.051 & 0.848 & -0.088 \\ 0.005 & -0.088 & 0.843 \end{bmatrix}$

$$\text{and } b = \begin{bmatrix} -1.20 \\ -1.37 \\ -1.86 \end{bmatrix}$$

Then

$$X_s = -\frac{1}{2}\begin{bmatrix} 0.484 & -0.051 & -0.005 \\ -0.051 & 0.848 & -0.088 \\ -0.005 & -0.088 & 0.843 \end{bmatrix}\begin{bmatrix} -1.20 \\ -1.37 \\ -1.86 \end{bmatrix} = \begin{bmatrix} 0.260 \\ 0.480 \\ 0.726 \end{bmatrix} \quad (4.10)$$

$$x_1 = \frac{\xi_1 - 25}{5} \Rightarrow 0.260 = \frac{\xi_1 - 25}{5} \qquad \therefore \quad \xi_1 = 26.3$$

$$x_2 = \frac{\xi_2 - 5}{5} \Rightarrow 0.480 = \frac{\xi_2 - 15}{5} \qquad \therefore \quad \xi_2 = 17.4$$

$$x_3 = \frac{\xi_3 - 50}{15} \Rightarrow 0.726 = \frac{\xi_3 - 50}{15} \qquad \therefore \quad \xi_3 = 60.89$$

Table 4.9: Stationary Points

Variables	x_1	x_2	x_3
Coded Values	0.260	0.480	0.726
Variable Names	Temperature	Sterilization Time	Culture media Concentration
Variables (Original Units)	0^0C	minutes	g/L
Original Values	26.30	17.40	60.89

Therefore the response surface could be predicted by using the following equation.

$$\hat{y}_s = b_0 + \frac{1}{2}x_s^{'}b \text{ such that}$$

$$\hat{y}_s = 7.16 + \begin{bmatrix} 0.260 & 0.480 & 0.726 \end{bmatrix}\begin{bmatrix} -1.20 \\ -1.37 \\ -1.86 \end{bmatrix} \quad (4.11)$$

$$= 7.16 - 1.152 = 6.008 \text{ Days}$$

4.1.9 Diagnostics and Plots of the Estimated Response Surfaces

The basic assumptions on errors which include, independence, normality and the constancy of variance for errors for applying the second order statistical model and ANOVA test statistics were tested. The findings are summarized in figure 4.3.

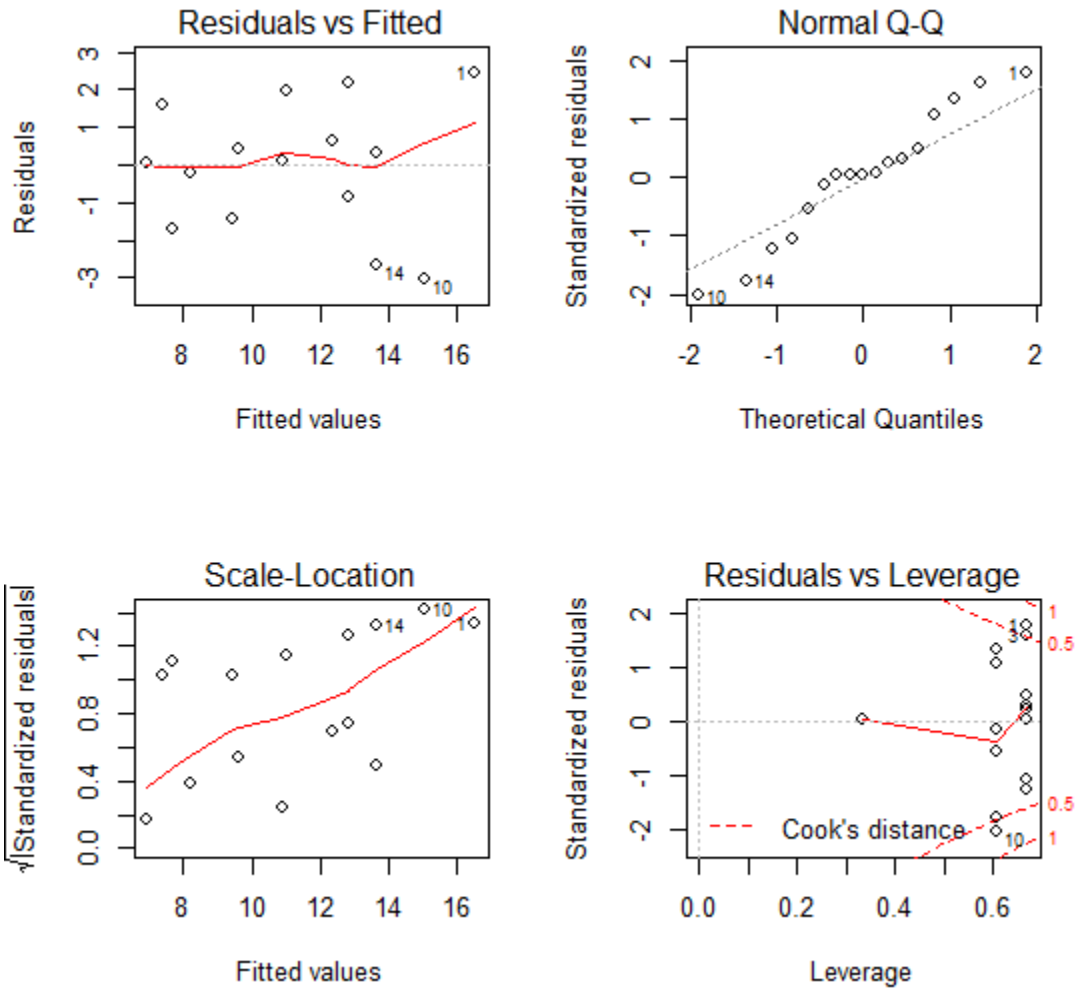

Figure 4.3: Regression Diagnostic Plots

The fact that there were equally spread residuals around the horizontal line without any distinct patterns in the residual versus fitted values plot, then that was an indication of residuals linear or non-linear relationships nonexistence, thereby satisfying the assumption of residuals independence. The normal quantile-quantile

plot shows the residuals were normally distributed given that the observed values were along the reference line hence ascertained normality.

The scale-Location plot showed that the residuals were equally spread along the ranges of predictors and hence the assumption of equal variance (homoscedasticity) was not violated. Based on the residuals versus leverage plot, most of the predictor variables were well inside of the Cook's distance lines hence few identified outliers. The following response surface graphs display the predictor variables on the x- and y-axes and then a continuous surface that represents the fitted response values on the z-axis. For each of the surface map, two variables were displayed at a time holding the third variable constant.

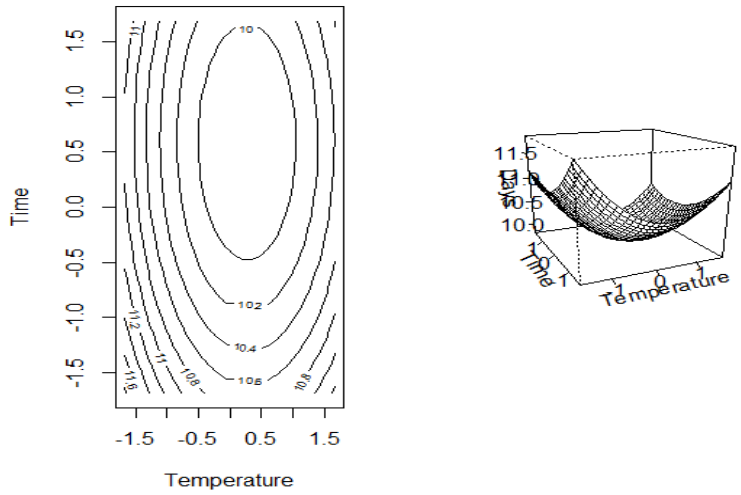

Figure 4.4: Response Surface for Time and Temperature

Figure 4.4: Shows the response surface map and a contour plot for temperature and time of substrate sterilization for spawn's multiplication.

They represent a minimum response surface thus indicating a point of optimum operating conditions reaching a minimum. By inspection the plots are non-linear, implying a strong temperature and sterilization interaction effect on the time to

spawns maturity and the response surface is curved due to the fact that the model contains quadratic terms that were statistically significant.

The contour lines appeared to follow the direction of movement along the path of minimum response from a reference point. The contour lines were within the response values in the data which ranged between 6 to19 nevertheless it is worth noting that the accuracy of the response surface plot depends on how well the model represents the true relationships among the variables.

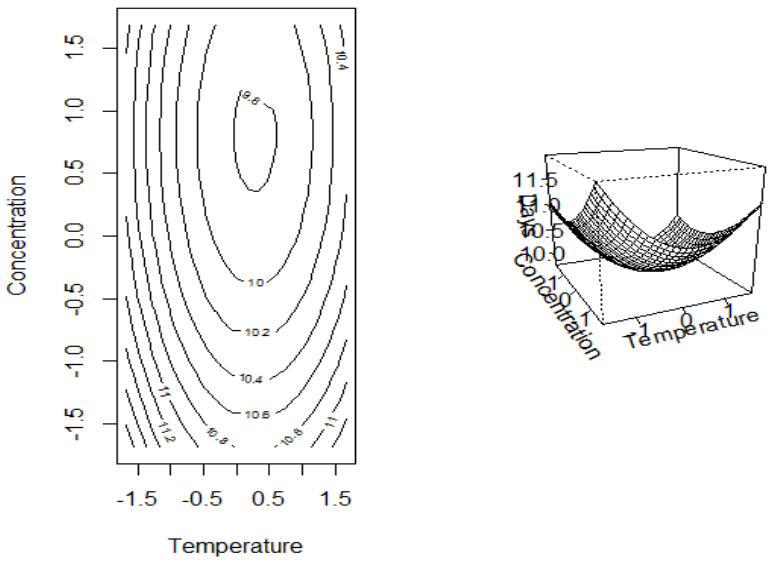

Figure 4.5: Response Surface for Agar concentration and Temperature

The lowest values of days to full colonization of the substrate in the petri dish were in the upper side of the contour plot, which corresponds with high values of both agar media concentration and temperature, time of sterilization was held constant for this plot.

As with the contour plot the lowest values of the response surface were along the side opposite the temperature axis, which corresponded with high values agar media concentration and temperature

The following is the contour plot and then the response surface map for agar media concentration and time of sterilization.

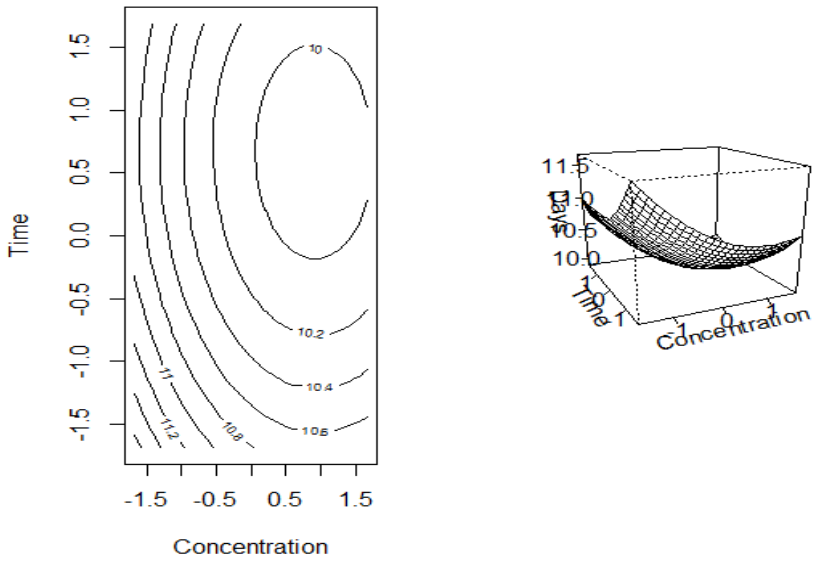

Figure 4.6: Contour plot & Response Surface for Agar concentration and Time

As in the contour plot the lowest values of days to full substrate colonization are in the lower right corner of the response surface, which corresponded with high values of agar media concentration. The lowest values of days to full substrate colonization are in the upper right corner of the plot, which corresponds with high values of agar media concentration but average time of sterilization, temperature was held constant in this particular analysis and representation.

4.2 Suitable Local Substrates Screening for Oyster mushroom Cultivation

To select the potentially important substrates among the locally available selected substrates one- factor-at-a-time screening methods was used, which provided information on direct effects of each substrate on oyster mushroom yield.

Five mixture components were tried for oyster mushroom growth on pure blend basis but under similar conditions. The five components included; Sawdust (x_1), Sugarcane bagasse (x_2), Cattle manure (x_3), Euphorbia (x_4), and Star grass (x_5) substrates.

Factors that could be considered as candidates for mixture experiment were chosen based 0n the individual component performance.

The results showed significant variability on the different substrate compositions used under this study. Table 4.10 gives the summary of the findings.

Table 4.10: Screening Experiments Results

Substrate	Fruition Weight (Kgs)	$\bar{y}$
x_1	0.5, 0.7,0.9,0.4,0.5	0.6
x_2	0.4, 0.7, 0.2, 0.6, 0.9, 0.2	0.6
x_3	0.1	0.1
x_4	-	-
x_5	0.2, 0.4, 0.5, 0.3, 0.1	0.3

The Sugarcane bagasse and sawdust recorded the highest yield of o.6kg on average while cattle manure recorded the least 0.1kg for the whole fruition period. There was no single pinning on the euphorbia substrate. The last two substrates were eliminated from further trials and the study proceeded with a three component mixture experiment to establish the substrate mixture that maximized the oyster mushroom yield. That is Sugarcane bagasse (x_1), Sawdust (x_2), and Star grass(x_3).

4.3 Simplex Centroid Mixture Design

The significant substrates were tried under pure blends, binary and ternary combinations repetitively and randomly. The substrate materials for the three components which included; Sawdust (x_1), Sugarcane bagasse (x_2), and Star grass (x_3) were shred to sizable pieces to easy mixing, weighing, packing and soaking.

They were soaked for an overnight to absorb enough water content that could sustain the whole process of mycelia colonization and fruition. The substrates were mixed with wheat bran, lime and as designated with the substrate components, they were

rationed to try the desired outcome. Figures 4.7 and 4.8 for the three components indicate the designated design points.

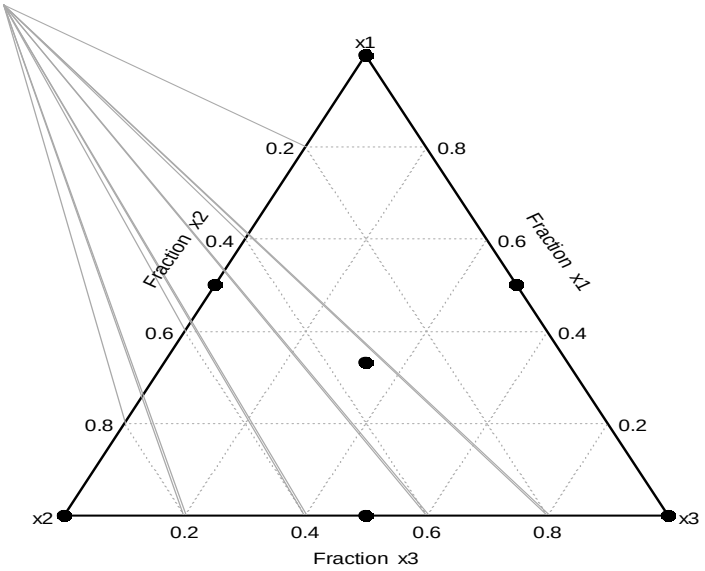

Figure 4.7: Distinct Experimental Points

Each point in the graph represented a design point for the three components; the vertices represented the pure component blends; x_1, x_2 and x_3. Binary blends, half combinations of any two substrates occurred at the midpoints of the sides on the triangle, while interior dot represent the bary centre which is also a geometric centroid of the three blends mixed at equal proportions. The measuring units were kilograms; for pure blends the spawns were inoculated in a kilogram of each substrate while for binary half of two different substrates. Then for the ternary a third of the three substrates was mixed to form a kilogram in which the spawns were inoculated. Figure 4.8 displays the response surface for the three components.

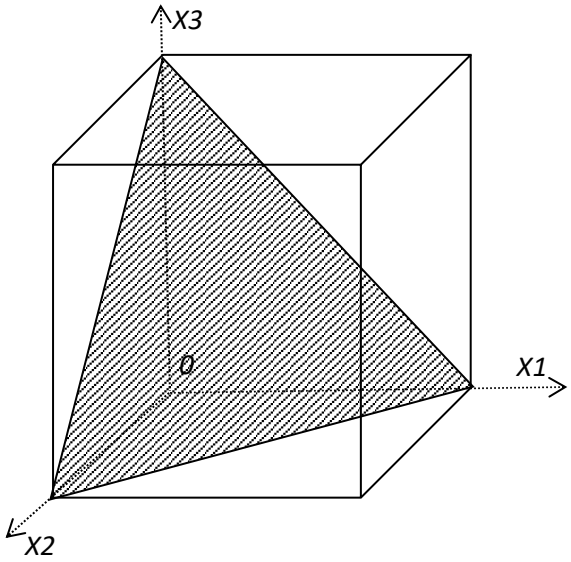

Figure 4.8: Planar Surface Spaces

The shaded region gives the possible responses as a function of substrates setting. It was assumed each substrate increased its value from the vertex towards the centre of the planar surface space.

Given the three component simplex centroid design, the number of distinct points were 2^3-1, which corresponded to 3 permutations of (1,0, 0) on the three single component blends, $\binom{3}{2}$ permutations of $\left(\frac{1}{2},\frac{1}{2},0\right)$ i.e the binary mixtures and then the overall centroid point $\left(\frac{1}{3},\frac{1}{3},\frac{1}{3}\right)$ trinary mixture. Figure 4.9 shows the substrates combinations process; mixing, soaking and steaming. The substrates were soaked for the whole night having been cut into small sizes after which the blending was done. It was then mixed with bran and lime.

The growing room was cleaned and dimly lit to retain moisture in the air and simultaneously provide airflow when ventilation is needed. To prepare the room for the inoculations, it was sprayed with a solution of bleach along the walls and corners.

Figure 4.9: Mixing the Substrates

The dry mixed substrates were packed into polythene paper bags per kilogram. The PVC plastic pipes were fixed and sealed with the cotton wool which was fastened with the rubber bands to reduce the chances of contamination and insect infestation.

The sealed bags were steam boiled for five hours to sterilize them. The bags were allowed to cool and then the spawns were placed into the substrates through the spooning chambers, in a sterilized germ free environment with the attendants' hands and mouth gloved and covered respectively.

The inoculated bags were then placed on the shelves in a dark, temperature and humid controlled room to incubate for at least a month. During this time the spawn ran (mycelium spreads) throughout the substrate, implying it was fully colonized.

The air temperature in the spawn run room was maintained at 18–25°C. Relative humidity was maintained at 95 to 98 percent to minimize drying of the substrate surfaces.

The bags were regularly checked for any mould contamination and any infected bag was immediately removed from the growing area

The PVC and the cotton wool were detached for the pinheads to sprout out which finally transformed to the oyster fruit.

Figure 4.10: Packing and Spraying

Figures 4.10a and 4.10c shows the parked substrates in the cropping area while figure 4.10b shows the spraying of the polythene bags for sanitation taking place. A successful cultivation of mushroom requires proper sterilization of the substrates prior to inoculation with the quality spawn (Musieba, Okoth, Mibey, Wanjiku, & Moraa,

2012). Figure 4.10d shows an infected substrate after the inoculation. The infected bags were discarded to avoid further contamination.

The polythene bags were arranged in a completely randomized design on shelves in the mushroom growing room and incubated at ambient temperature and relative humidity controlled by manually spraying water on the walls and placing open containers filled with water in the corners of the room.The fruition continued reproducing for a period of 3-4 months, and the harvesting was done daily by plucking the whole fruit with sterilized hands. The fruit was sold fresh and dry. Table 4.11 gives the summary of the fruition and harvested yield per experimental unit.

Table 4.11: Experimental output

Design Points	Substrates Proportions							Observed weight (kg) values	Average
	x_1	x_2	x_3	x_1x_2	x_1x_3	x_2x_3	$x_1x_2x_3$	y	$\bar{y}$
η_i	1	0	0	0	0	0	0	1.0,1.2,1.3,1.1,1.1,0.9	1.1
η_j	0	1	0	0	0	0	0	1.1,0.7,0.9,0.9,0.5	0.8
η_k	0	0	1	0	0	0	0	0.4,0.2	0.3
η_{ij}	0.5	0.5	0	0.25	0	0	0	1.1,1.4,1.5,1.4,1.6,1.2,0.8	1.3
η_{ik}	0.5	0	0.5	0	0.25	0	0	0.4,0.6,0.5,0.7,1.1,0.3	0.6
η_{jk}	0	0.5	0.5	0	0	0.25	0	1.0,0.8,0.4,0.6,0.1,0.2	0.5
η_{ijk}	0.33	0.33	0.33	0.1089	0.1089	0.1089	0.0360	0.6,0.5,0.9,1.0,0.7,0.4	0.7

The design points in Table 4.11 refers to one polythene paper with one setting for each of the substrate/s of the experiment and for which a single value for the response was observed, that is the yield in kilogram per polythene bag. Each setting was replicated five times and randomized. Therefore the single output value was the expected value per setting. The results indicated that there was a synergism of the binary mixture between the sugarcane bagasse and the sawdust in excess of 0.3 kg, while the binary mixture between the star grass and the sawdust as well as between

the Sugarcane bagasse and the Star grass registered antagonism of the mixture amounting to 0.4kg and 0.5kg respectively.

Figure 4.11: Harvesting and Packing

Figure 4.11a shows the plucked out flesh mushroom fruits and figure 4.11b shows the packed flesh oyster mushroom. The harvested fruit were packed into 200g, 1kg or 2kg units. The average price was ksh 600 per kg when fresh while about ksh 4000 when dry. Implying one kilogram of dry oyster was approximately equal to seven kilograms when fresh in terms of both the quantity and value.

4.3.1 ANOVA Test Statistics for Substrates

The one way between groups Analysis of Variance (ANOVA) was conducted to explore the impact of substrates mixture variation on the mushrooms yield. The subjects were categorized into seven groups based on the mixture blend (pure blends, binary blends and the triad blend)

To ensure ANOVA test statistics assumptions were not violated during the experimentation period, complete randomization of the polythene bags was doneand the polythene bag labelled but randomly and independently placed. The precautionary actions were taken prior to the data analysis to ensure the data conformed to the parametric test statistics assumptions.

The levene's test for the assumption of homogeneity of variance was conducted whose significance value was 0.370 (≥0.05) implying the assumption of homogeneity of variance was not violated.

4.3.1.1 The ANOVA Table

The results for one way ANOVA conducted to explore the impact of mixing different proportions of substrates on Oyster mushroom yield are summarized in table 4.13.

Table 4.12: ANOVA Table 2

Source of variance	Sum of Square	Df	Mean Square	F	Sig.
Between Groups	3.484	6	0.581	8.767	0.000
Within Groups	2.053	31	0.066		
Total	5.537	37			

There was a statistical significant difference among the mean yield for the seven mixture groups at $\alpha = 0.05$ as was evident by both the p-value and the computed F-value. AS indicated in table 4.12 the $p = 0.000$, which was less than the critical value 0.05 in the expected yield. The computed $F = 8.767 > F_{0.05,\ 6,\ 31} = 2.42$, therefore the null hypothesis (H_0) was rejected with a conclusion that the seven substrates mixture groups differed significantly in their yielding amount as measured by the average size of their yield. This meant that the yield difference per mixture blend could not be attributed to chance but the proportions of the substrates included in the mixture.

4.3.1.2 Post Hoc Tests

The post hoc tests were carried out among the component means with a significant difference from each other. The differences were revealed by Tukey's Highest Significant Difference (HSD) analysis.

Table 4.13: Significant Mean Difference

Source of Difference	Mean Difference	Sig.	Std. error
x_1 □ x_3	0.8000	0.010	0.21
x_{13}	0.5000	0.030	0.15
x_{23}	0.5833	0.007	0.15
x_{12} □ x_3	0.9857	0.001	0.21
x_{13}	0.6857	0.001	0.14
x_{23}	0.7690	0.000	0.14
x_{123}	0.6024	0.003	0.14

The highest mean yield difference was between the sawdust and sugarcane bagasse binary blend and the star grass at 0.9857. The second highest mean yield difference was between the sawdust and the star grass from star grass pure blends at 0.8000.

4.3.2 Parameter estimate in the polynomials

The fitted polynomial equation for parameter estimation was

$$\eta = \sum_{i=1}^{q} \beta_i x_i + \sum \sum_{i<j}^{q} \beta_{ij} x_i x_j + \sum \sum \sum_{i<j<k}^{q} \beta_{ijk} x_i x_j x_k \qquad (4.12)$$

Where β_i was the linear blending of component i which represented the expected response to pure component i. While β_{ij} was the coefficient of the non-additive blending of components i and j and then finally β_{ijk} was the coefficient of the ternary blending among the components i, j and k such that β_i were nonnegative quantities representing the height of the surface above the simplex at the vertex where $x_i = 1$, for $i = 1, 2$ and 3

The $2^q - 1$ parameters in the polynomial equation were expressed as linear functions of the average responses at the points of the simplex centroids design. The parameters could be computer generated or manually computed.

4.3.3 Computer Generated Parameter Estimate in the Polynomials

The coefficients of the simplex centroid mixture model could be obtained through the R statistical computer package. The computer output summary of the oyster mushroom yield as influenced by varying the substrate's mixture component is summarized in table 4.14.

Table 4.14: Polynomial Parameter Estimate Output

					95% CI for Mean			
Mix	N	Est	Std. Dev	Std. Error	Lower Bound	Upper Bound	Min	Max
x_1	6	1.100	0.1414	0.0577	0.952	1.248	0.9	1.3
x_2	5	0.820	0.2280	0.1020	0.537	1.103	0.5	1.1
x_3	2	0.300	0.1414	0.1000	-0.971	1.571	0.2	0.4
x_{12}	7	1.386	0.2734	0.1033	1.033	1.539	0.8	1.6
x_{13}	6	-0.400	0.2828	0.1155	0.303	0.897	0.3	1.1
x_{23}	6	-0.200	0.3488	0.1424	0.151	0.883	0.1	1.0
x_{123}	6	-3.248	0.2317	0.0946	0.440	0.926	0.4	1.0

The highest onetime yield recorded was 1.6 kgs from the sawdust and sugarcane bagasse binary blend set, from which the best average mean was also realized of 1.286 kgs with a 95% confidence interval of 1.033 to 1.539 mean values. The best pure blend was the sawdust with a mean yield of 1.1kgs and a 95% confidence interval mean value of 0.952 to 1.248. The sawdust pure blend also registered the smallest standard error of 0.0577, an indication that the sample mean was a more accurate reflection of the actual population mean.

The minimum average yield was 0.3 kgs, obtained from Star grass pure blend with a 95% confidence interval of -0.971 to 1.571 mean values. The minimum one set single yield was 0.1kgs from the sugarcane bagasse and Star grass binary blend. Therefore from the output in table 4.14, the yield could be predicted using the model.

$$\hat{y}(x) = 1.1x_1 + 0.8x_2 + 0.3x_3 + 1.4x_1x_2 - 0.4x_1x_3 - 0.2x_2x_3 - 3.1x_1x_2x_3 \quad (4.13)$$

4.3.4 Computed Parameters Estimate in the Polynomials

The polynomial parameters could also be calculated follows. By definition the formulas for the parameter estimate are;

$$\beta_i = \eta_i \text{, hence from table 4.11}$$

$$\beta_i = \eta_i = 1.1$$

$$\beta_j = \eta_j = 0.8 \qquad (4.14)$$

$$\beta_k = \eta_k = 0.3$$

$$\beta_{ij} = 2\left\{2^1\eta_{ij} - 1^1(\eta_1 + \eta_j)\right\} \text{ Hence from table 4.11, it } \Rightarrow$$

$$\beta_{ij} = 2\left\{2^1(1.3) - 1^1(1.1+0.8)\right\} = 1.4$$

$$\beta_{ik} = 2\left\{2^1(0.6) - 1^1(1.1+0.3)\right\} = -0.4 \qquad (4.15)$$

$$\beta_{jk} = 2\left\{2^1(0.5) - 1^1(0.8+0.3)\right\} = -0.2 \text{ and then lastly,}$$

$$\beta_{ijk} = 3\left\{3^2\eta_{ijk} - 2^2(\eta_{ij} + \eta_{ik} + \eta_{jk}) + 1^2(\eta_i + \eta_j + \eta_k)\right\}$$

hence by using table 4.11 values, it $\Rightarrow$

$$\beta_{ijk} = 3\left\{3^2(0.7) - 2^2(1.3+0.6+0.5) + 1^2(1.1+0.8+0.3)\right\} = -3.3 \qquad (4.16)$$

hence the fitted model in the three components is;

$$\hat{y}(x) = 1.1x_1 + 0.8x_2 + 0.3x_3 + 1.4x_1x_2 - 0.4x_1x_3 - 0.2x_2x_3 - 3.3x_1x_2x_3 \qquad (4.17)$$

Which is the same as equation 4.13 to a great extent.

Therefore the response value can be predicted at any point, for instance $\hat{y}\left(\frac{1}{2}, \frac{1}{2}, 0\right)$ would be

$$\hat{y}\left(\frac{1}{2},\frac{1}{2},0\right)=1.1\left(\frac{1}{2}\right)+0.8\left(\frac{1}{2}\right)+0.3(0)+1.4\left(\frac{1}{2}\right)\left(\frac{1}{2}\right)-0.4\left(\frac{1}{2}\right)(0)-0.2\left(\frac{1}{2}\right)(0)-3.3\left(\frac{1}{2}\right)\left(\frac{1}{2}\right)(0)$$

 1.3

4.3.4.1 The Variance and the Standard errors

The standard errors were determined by using the sample standard deviations $\left(S^2\right)$ since the population standard deviation $\left(\sigma^2\right)$ could not be obtained;

$$S^2=\sum_{i=j=k}^{2\text{ or }5\text{ or }6}\sum_{ij=ik=jk}^{6\text{ or }7}\sum_{ijk}^{6}\frac{\left(y_{iu}-\bar{y}_i\right)^2}{\sum_{i=1}^{7}\left(r_i-1\right)} \qquad (4.18)$$

$$S^2=\frac{(1.0-1.1)^2+(1.2-1.1)^2+\cdots+(0.4-0.7)^2}{5+4+1+6+5+5+5}=0.07$$

and the estimates of the variance of the parameter were obtained by;

$\text{var}(b_i)=\frac{s^2}{r_i}$ for the pure blends, $\text{var}(b_{ij})=s^2\left\{\frac{16}{r_{ij}}+\frac{4}{r_i}+\frac{4}{r_j}\right\}$

and

$$\text{var}(b_i)=\frac{s^2}{r_i}=\frac{0.07}{6}=0.012$$

$$\text{var}(b_j)=\frac{s^2}{r_j}=\frac{0.07}{5}=0.014$$

$$\text{var}(b_k)=\frac{s^2}{r_k}=\frac{0.07}{2}=0.035$$

$$\text{var}(b_{ij})=s^2\left\{\frac{16}{r_{ij}}+\frac{4}{r_i}+\frac{4}{r_j}\right\}=0.07\left\{\frac{16}{7}+\frac{4}{6}+\frac{4}{5}\right\}=0.263$$

$$\text{var}(b_{ik}) = s^2\left\{\frac{16}{r_{ik}} + \frac{4}{r_i} + \frac{4}{r_k}\right\} = 0.07\left\{\frac{16}{6} + \frac{4}{6} + \frac{4}{2}\right\} = 0.373$$

$$\text{var}(b_{jk}) = s^2\left\{\frac{16}{r_{jk}} + \frac{4}{r_j} + \frac{4}{r_k}\right\} = 0.07\left\{\frac{16}{6} + \frac{4}{5} + \frac{4}{2}\right\} = 0.383$$

hence the fitted second- degree model to the observed data was

$$\hat{y}(x) = \underset{(0.110)}{1.1x_1} + \underset{(0.118)}{0.8x_2} + \underset{(0.187)}{0.3x_3} + \underset{(0.513)}{1.4x_1x_2} - \underset{(0.612)}{0.4x_1x_3} - \underset{(0.619)}{0.2x_2x_3} \qquad (4.19)$$

4.3.4.2 Adequacy of the Fitted Model

The adequacy of each design point in the fitted model can be tested through the null hypothesis that the estimate of the response at the designated check point is not significantly different. To estimate the variance of a fitted point, the following formula is used.

$$\text{var}\left[\hat{y}(x)\right] = s^2\left\{\sum_{i=1}^{3}\frac{a_i^2}{r_i} + \sum\sum_{i<j}^{3}\frac{a_{ij}^2}{r_{ij}}\right\} \qquad (4.20)$$

$$\text{For } a_i = x_i(2x_i - 1) \text{ and } a_{ij} = 4x_ix_j$$

For instance to estimate the variance of $\hat{y}(x)$ at the centroid point $\hat{y}\left(\frac{1}{3},\frac{1}{3},\frac{1}{3}\right)$ in the model, equation 4.17;

$$\text{var}\left[\hat{y}(x)\right] = s^2\left\{\sum_{i=1}^{3}\frac{a_i^2}{r_i} + \sum\sum_{i<j}^{3}\frac{a_{ij}^2}{r_{ij}}\right\}$$

$$\text{For } a_i = x_i(2x_i - 1) \text{ and } a_{ij} = 4x_ix_j$$

$$\text{var}\left[\hat{y}(x)\right] = 0.07\left\{\frac{\left(-\frac{1}{9}\right)^2 + \left(-\frac{1}{9}\right)^2 + \left(-\frac{1}{9}\right)^2}{6+5+2} + \frac{\left(\frac{4}{9}\right)^2 + \left(\frac{4}{9}\right)^2 + \left(\frac{4}{9}\right)^2}{7+6+6}\right\} \approx 0.034$$

To test the satisfaction and fitness to the fitted model, the test statistics in the following equation was used.

$$t = \frac{\bar{y}_{obs} - \hat{y}_{est}}{\sqrt{\text{var}\left(\bar{y}_{obs}\right) + \text{var}\left(\hat{y}_{est}\right)}}$$

$$= \frac{0.7 - 0.699}{\sqrt{0.07 + 0.034}} = 0.0031 \qquad (4.21)$$

but tabulated $t_{0.025,\ 6} = 2.447 > computed$ t=0.0031.

Hence the H_0 is not rejected at $\frac{\alpha}{2} = 0.025$ and the response at this point $\hat{y}\left(\frac{1}{3}, \frac{1}{3}, \frac{1}{3}\right)$ is not significantly different from the mean.

Equally the estimate of the variance of $\hat{y}(x)$ at the point $\hat{y}\left(\frac{1}{2}, \frac{1}{2}, 0\right)$, $\hat{y}\left(\frac{1}{2}, 0, \frac{1}{2}\right)$ and $\hat{y}\left(0, \frac{1}{2}, \frac{1}{2}\right)$ can be made.

4.3.5 Graphical Yield Representation

The yield for each type of substrate was recorded and represented in a multiple linear graph, figure 4.12. The cumulative yield was noted and summarized in figures 4.13, in the form of linear graph, bar graph, pie char and the box plots. Figure 4.14 displayed the optimal mixture proportions in the form of contours.

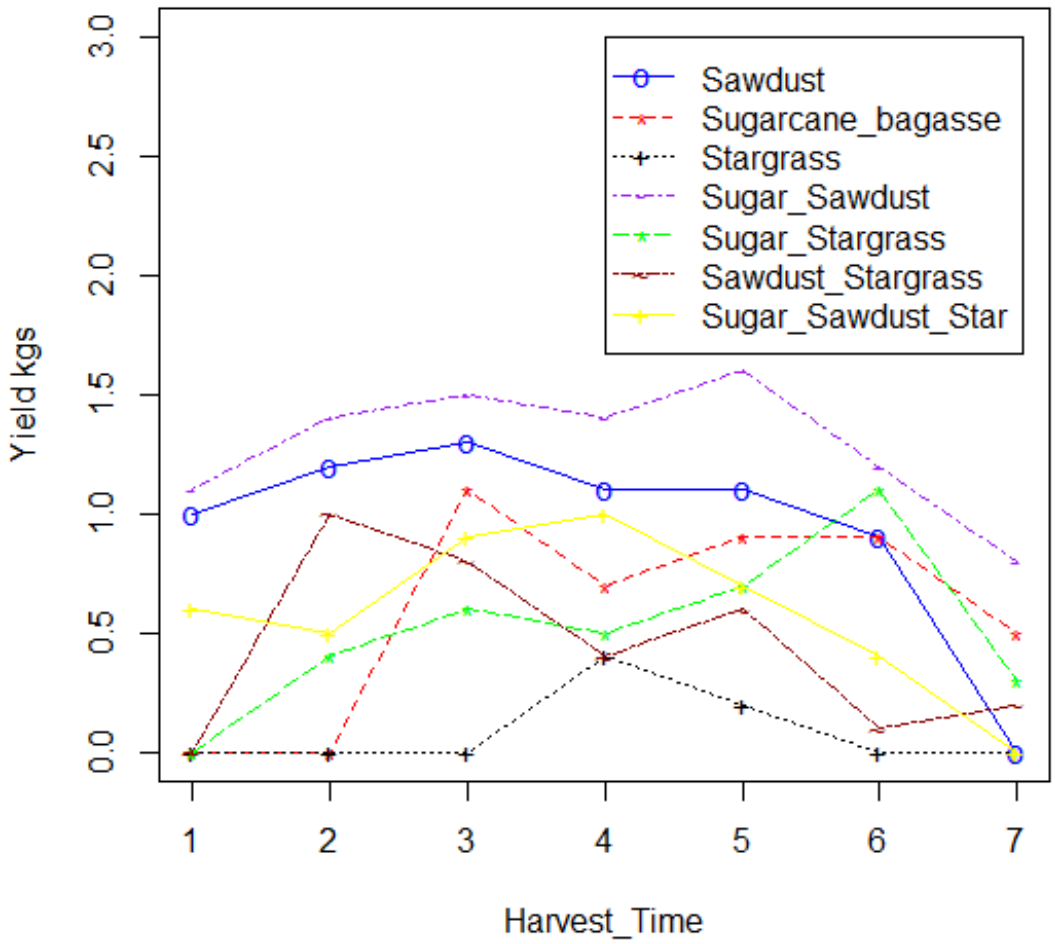

Figure 4.12: Substrates Performance

The yield from the sugarcane bagasse and sawdust binary blend was the highest all along the harvest times. Among the pure blend sawdust gave the best yield while star grass performed most dismally.

Cumulatively the mixture of sugarcane bagasse and sawdust was the best as summarized in figures 4.13a, 4.13b, 4.13c and 4.13d.

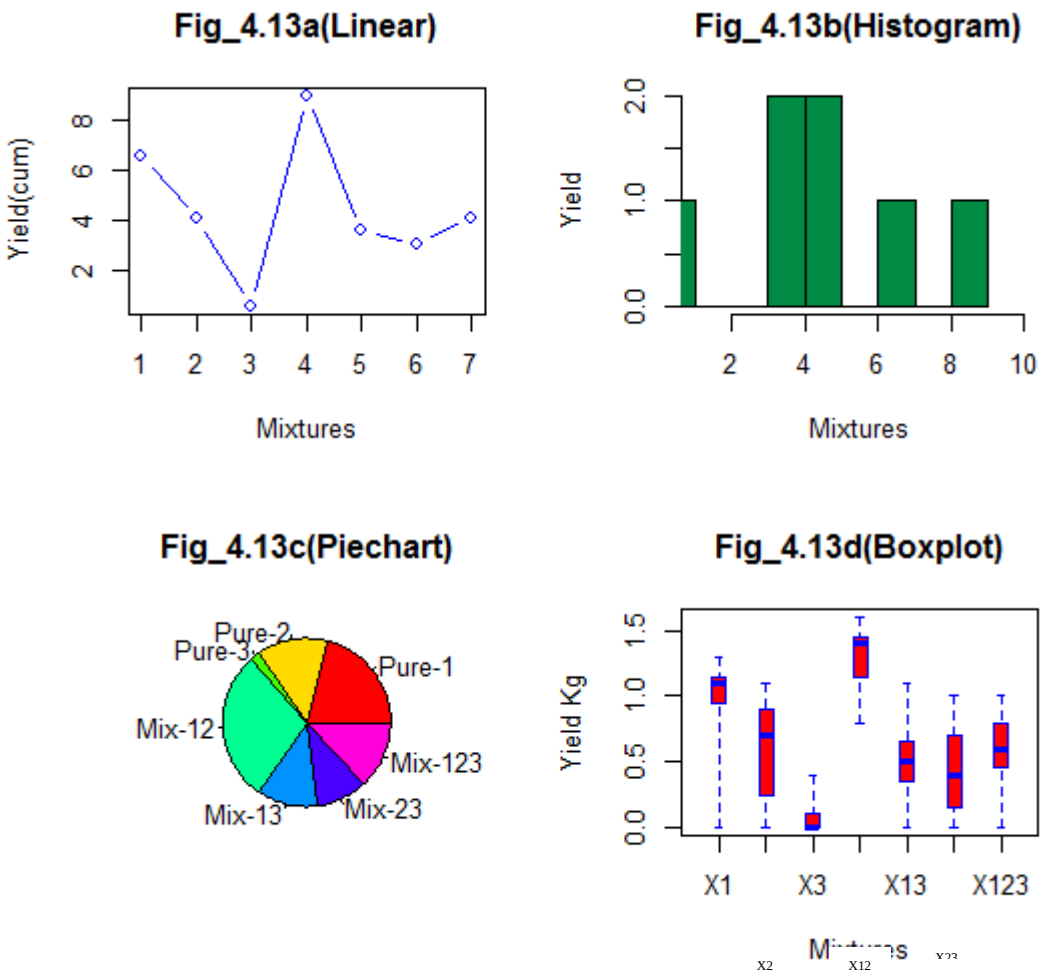

Figure 4.13: Cumulative Yield

By inspection from figures 4.13 the sugarcane bagasse and saw dust mixture recorded the highest yield while on the pure blend the star grass labeled as pure-3 in figure 4.13c recorded the lowest yield with the sawdust substrate yielding the best. In their research Shah, Ashraf and Ishtiaq (2004) sawdust performed the best among wheat straw and leaves. The box plots displayed the middle and the quartiles distribution of the yield per substrate composition. Based on the box plots displays star grass was the least dispersed while the sawdust pure blend, star grass and sawdust binary blend were among the most dispersed.

As put by, Khademi and Timmermans (2012), the mixture experiments are widely used today in formulation experiments, blending experiments, and marketing choice

experiments, where the goal is to determine the most preferred attribute composition of a product at a given price. Simplex centroid mixture design was the most appropriate in establishing the optimal significant substrates setting for maximum oyster mushroom yield.

4.3.6 Surface Contour Plot for Mixtures

Generally contour lines for a function of variables connect the points where the function has the same value. Contour plot for the yield as a function of the three mixture substrate combination is shown in figure 4.14.

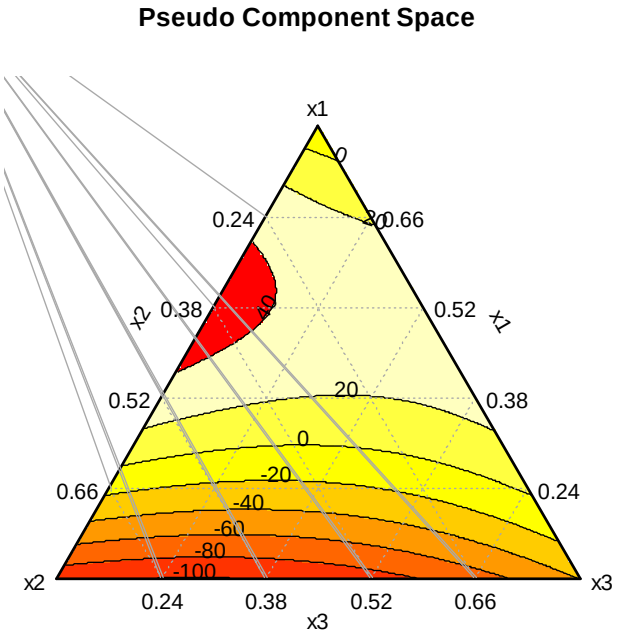

Figure 4.14: Mixture Contour Plots

The grey and yellow colours are the highest in the design space with little of maroon and red colours. The optimal mixture yield could be spotted at the around seventy percentage of sawdust , about twenty percent of sugarcane bagasse and ten percent of the star grass.

4.4 Economic Returns Analysis on Oyster Mushroom farming

The section aimed at analyzing the cost, returns and break-even point of the oyster mushroom production, vis-a-vis the existing marketing system along with marketing cost, margins and marketing efficiency.

The findings were based on the observed practices, experiences and challenges shared by purposefully sampled oyster mushroom producers in Machakos County, Kenya.

4.4.1 Composition of the Sampled Farmers

The purposeful and snowballing sampling techniques were employed in coming up with the ten oyster mushroom farmers in Machakos County. They constituted seven females and three men all within the age brackets of 29 to 51 years. None had a professional certificate but only a form four and primary school certificates. They all confessed decreased ailments from their family members for period they have made oyster mushroom as part of their regular family meal. A training session was captured in a picture displayed in appendix II.

4.4.2 Cost on Mushroom Production

The study considered the upfront, production and marketing costs assuming a startup venture. The fixed upfront costs included the construction of a simple mud-house and a few wooden shelves to utilize the vertical space available and the metallic drums to pasteurize the substrate. The variable costs covered things like hand gloves, methylated spirit and cotton wool for hygiene. Appendix III gives the summary of all the materials and their associated cost a typical mushroom grower needs for a complete cycle of growing the *Pleurotus ostreatus.*

4.4.2.1 Fixed cost

These were costs of the facilities that may last for more than two production cycles of the oyster mushroom and their cost is independent of the units produced or yield. Regarding the structure and equipment, the growth of oyster mushroom does not call for costly infrastructure facilities but can be done in a seasonal low costly room with very little expenditure like the one shown in figure 4.15, which is just a traditional hut with mud-walls and grass-thatched roof but well ventilated. The structure could last for two or three years.

Handful of machinery and equipment are necessary namely, the metallic drums, spraying pump, nozzle, trays, a thermometer, hygrometer and a knife for harvesting.

Figure 4.15: Simple Mushroom Structure

Figure 4.15a is the external side view of a simple oyster mushroom production structure while figure 4.15b is interior view of the structure stocked with bagged substrates.

4.4.2.2 Variable cost

These were the costs that varied directly proportional to the production output. They included the costs of the substrates, pest controls, the marketing cost and cropping inputs such as the wheat bran, polythene papers, PVC plastic pipes, cotton wool,

rubber bands firewood and water. Appendix III gives the summary of the income statement of a typical start up oyster mushroom farmer in Machakos County, Kenya. The money values represented the average amount from a group of ten oyster mushroom farmers purposefully sampled to share their experience and challenges in mushroom farming by the time of this study. The statement represented the actual market rates of the sales and the cost of the goods by the time of this study, however the values were highly dispersed for lack of structured common market and marketing.

4.4.3 Disposal Pattern of Mushroom

There was no organized market structure where someone could sell his/her produce or buy fresh or dry mushroom within Machakos County, nevertheless a farmer could market his produce through any of the following conventional channels, namely;

$$\text{Mushroom farmer} \rightarrow \text{Consumer}$$

$$\text{Mushroom farmer} \rightarrow \text{Retailer/wholesaler/commission agent} \rightarrow \text{Consumer}$$

Other times there was no actual money transaction but quantification of the family and friends consumed mushroom.

4.4.4 Break-even point

At break-even point there is a zero loss and/or zero profit. There exist several methods of determining the break-even point such as contribution margin. The necessary and sufficient condition for this point is that, the marginal cost and the marginal revenue must be equal.

For this study the break-even point (BEP) of output was calculated by using the contribution method formula;

$$BEP = \frac{TFC}{ASP - AVC} \quad (4.22)$$

Where;

TFC- Total fixed cost

ASP- Average selling price of the oyster mushroom in Kenya Shillings per kilogram

AVC- Average Variable Cost of the oyster mushroom in Kenya Shillings per kilogram

It should be noted that the denominator is the contribution margins; hence the fixed cost is divided by the profit.

Based on the figures in Appendix III, where the fixed costs consisted of cropping structure, metallic drums and the labour, totaling to KShs 27,000 and the rest being treated as the variable costs, amounting to KShs 25,000, then;

$$BEP = \frac{27,000}{600 - 100} = 54$$

Based on the study findings figure 4.17 (Not drawn to scale) illustrates the break-even point and the associated costs, revenues and the units produced.

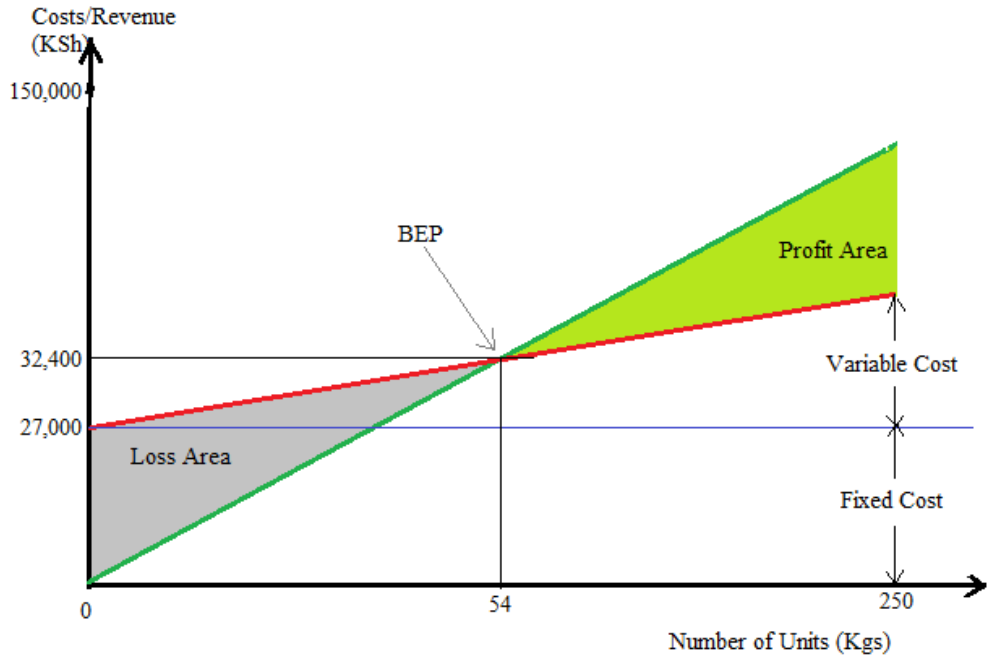

Figure 4.16 Break-Even Point

Based on the figures for this study the break-even point was at 54 kilograms of the oyster mushroom production. Below that point each unit was being produced at a loss but on a converging series, beyond that point each unit was being produced at a diverging profit. The fixed cost based on the study's classification was KSH 27,000, since the Average Variable Cost (AVC) was KSH 100, the total cost at that point was (27,000+54*100)=32,400/=. The total revenue at that point given the Average Selling Price (ASP) of KSh 600 was 54*600=32,400/= Implying the break-even point is the point at which the total cost equals the total revenue.

The break-even point (BEP) was supposed to stabilize and decrease as the farmer continues since some of the costs included in that example were one off expenses, but mandatory for the new ventures. Otherwise for the professional farmer the fixed and the variable costs are quite distinct.

4.4.5 Marketing efficiency

Market efficiency is a measure of the information availability that gives the maximum opportunities to customers and sellers to carry out the business transactions with minimum costs possible. Anybody with the entrepreneurial mind would instantaneously incorporate such information to allocate his/her capital in its highest and most productive area to maximize the output for any given level of input. It was calculated by using formula 4.23

$$\text{Marketing Efficiency} = \frac{\text{Marketing value per Kilogram}}{\text{Markeing cost per Kilogram}} - 1 \qquad (4.23)$$

Based on the farmers focused group discussion information as summarized in appendix 3, the marketing efficiency was;

$$\text{Marketing Efficiency} = \frac{600}{100} - 1 = 5$$

This meant that for every one Kenya shilling invested in the oyster mushroom production the farmer earned five times; hence the input -output ratio was highly maximized. Therefore it meant venturing into oyster mushroom farming is not hopping onto a sinking ship but can form a niche among small-scale farmers in Kenya. The same views were echoed by Marshall and Nair (2009) in his findings on making money by growing mushrooms.

4.4.6 Challenges

Some challenges were observed along the period of experimentation coupled with an interview of the ten practicing farmers and few potential mushroom farmers. Cost of acquiring the spawns and substrates whose quality was never guaranteed, cost of pasteurization and maintaining of sanitation and then marketing of the produce were

common cited challenges. These challenges were in agreement with the Richard (2006) findings on the main challenges that faced small mushroom farmers in India. Odendo et al (2012) findings that, several farmers failed to succeed in mushroom cultivation because of inadequate knowledge about the marketing system, poor capital and the underlying principles on oyster mushroom cultivation were confirmed through this study.

It was also observed that poor knowledge of the benefits of producing and consuming mushrooms coupled with some of traditional believes of some of the Kenyan communities were hindrances to full exploitation of producing and consuming mushrooms in Machakos County.

The idea of a community's staple food was cited as a major obstacle to the successful uptake of the product by the locals.

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.0 Introduction

This chapter comprises of section 5.1 which presents the summary of the study findings, section 5.2 which presents the conclusions and then 5.3 which presents the recommendations for further research.

5.1 Summary of the Study Findings

The application of Response Surface Methodology on Oyster Mushroom showed how optimization on mixture formulation and the processing conditions could be applied to arrive at the desired quality characteristic. The study showed how CCD and simplex centroid mixture method could be applied to optimize the spawn processing conditions and the substrates mixture formulation for oyster mushroom production respectively. Experimental results showed that canonical analysis of the response surfaces was an efficient method for pinpointing the optimal points for both the inputs and the predicted output.

Response surface graphics, which could be produced with R statistical software, made it easy to find the peak performance, for both the substrates and the spawns process variables namely; temperature, substrate sterilization time and the agar media concentration.

Response surface methods (RSM) provided statistically-validated predictive models which could be manipulated to determine the optimal operating conditions. The computed experimental results showed that 26.30^0C, 17.40 minutes and 60.89g/L levels of temperature, sterilization time and culture media concentration respectively minimized the days for full coverage of mycelium in a petri dish. Regarding the

substrates mixture, there was no pinning on the cattle manure and the euphorbia substrates hence they were eliminated at the screening stage. The results showed significant variability on the different substrate compositions used under the study. Sawdust yielded most under the pure blend at 1.1 kg per experimental unit while on the mixed blend sugarcane bagasse and sawdust produced the highest yield at 1.3 kg per experimental unit. The economic returns analysis indicated that, oyster mushroom production was economically viable against the continued arable land decrease in Kenya coupled with the rainfall unreliability. The production break-even point was at 54 kg input of dry substrates used during experimentation and for that particular period of study.

5.2 Conclusions

The simplex centroid mixture design was found to be very efficient and effective in determining and discovering the optimal substrate combination for the best oyster mushroom yield.

The CCD provided the statistical elements necessary for the evaluation of temperature level, agar concentration level and sterilization time during spawn propagation.

The contour plots and the response surface maps generated characterized surface and helped a great deal in locating the optimum points precisely at a glance.

The oyster mushroom sampled famers unanimously agreed that, the income from mushrooms could supplement cash flows, providing either: a safety net during critical times, preventing people falling into greater poverty; a gap-filling activity which can help spread income and generally make poverty more bearable through improved nutrition and higher income; or a stepping stone activity to help make people less poor, or even permanently lift them out of poverty.

It was therefore proved that with the right knowledge and having the correct input materials chosen on the basis of availability, cost and preference, and proportioned correctly, it is possible to prepare quality spawn and attain oyster mushroom yield close to 100% biological efficiency. Given that the study established just handful actively mushroom producing farmers in Machakos County, it meant mushrooms are unexploited potential resources capable of improving the socio-economic status as alternative source of livelihood.

5.3 Recommendations

Venturing into oyster mushroom farming not only does provide a protein- rich food but reduces the environmental pollution and requires a very small land to operate. It is a transformative link for inedible wastes into edible biomass of high monetary value. Therefore it is recommended that most of the farmers should be exposed to the activity and be trained to understand the factors which individually or interactively affect mushroom production. However based on the highlighted challenges the study suggested a zero rated interest financial assistance to be advanced to the practicing and potential mushroom farmers through the national or county government institutional agencies. The study recommended the formation of mushroom SACCOs or produce boards to make the acquisition of the input materials and marketing infrastructure more efficient and effective. Routinely training on sanitation and how one would process the substrates and spawns using the locally available agricultural by-products or forest wastes, using the scientifically proven formulations was timely and would immensely increase the profit margins**.** A continuous research to find out a more cost effective alternative substrate for growing, oyster mushroom was recommended. Creative ways of value addition to mushrooms such as grinding dried mushrooms and divergence use was recommended.

According to Shah (2004), minimizing the mycelia colonization time narrows the gap of opportunity for competitor invasion hence improving its quality. Therefore further research on reducing the time to full colonization of the spore culture was recommended.

This study endevours to provide a solution to enough food provision for humankind and reverse the Malthus' theory of the capacity of world to feed humankind: Geometric increase in human population versus arithmetic increase in food poduction. Hence continous trials for more locally found substrates was highly recommended. Multiple response optimizations aiming to achieve maximal nutritional value and yield against minimal duration of spawns propagation and substrates cost was also recommended.

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APPENDICES

Appendix I: Sampled R Gui Commands

```
#=========================================
#Cannoniclesolutions(eigenvalues and Stationary points)
data=read.table("phd3.csv",header=TRUE,sep=",")
data
library(rsm)
fit=rsm(y~SO(x1,x2,x3),data=data)
fit
names(fit)
summary(fit)
#=====================================
#Response surface (Nets, Images & contours)
data=read.table("phd1.csv",header=TRUE, sep=",")
data
data=cbind(Temperature,Time)
data=data.frame(data)
data
attach(data)
fit<- lm(Days ~ poly(Time, Temperature, degree=2), data=data)
library(rsm)
par(mfrow=c(1,2))
image(fit, Time ~ Temperature)
contour(fit, Time ~ Temperature)
persp(fit, Time ~ Temperature, zlab = "Days")
#==================================

# Phd mixtures-multiple line graphs
Harvest_Time<- c(1,2,3,4,5,6,7)
Sugarcane<- c(1.0,1.2,1.3,1.1,1.1,0.9,0)
Sawdust<- c(0,0,1.1,0.7,0.9,0.9,0.5)
Stargrass<- c(0,0,0,0.4,0.2,0,0)
Sugar_Sawdust<- c(1.1,1.4,1.5,1.4,1.6,1.2,0.8)
Sugar_Stargrass<- c(0,0.4,0.6,0.5,0.7,1.1,0.3)
Sawdust_Stargrass<- c(0,1.0,0.8,0.4,0.6,0.1,0.2)
Sugar_Sawdust_Star<- c(0.6,0.5,0.9,1.0,0.7,0.4,0)

# plot the first curve by calling plot() function
# First curve is plotted
plot(Harvest_Time, Sugarcane, type="o", col="blue", pch="o", lty=1, ylim=c(0,3),
ylab="Yield kgs" )

# Add second curve to the same plot by calling points() and lines()
# Use symbol '*' for points.
points(Harvest_Time, Sawdust, col="red", pch="*")
lines(Harvest_Time, Sawdust, col="red",lty=2)

# Add Third curve to the same plot by calling points() and lines()
# Use symbol '+' for points.
points(Harvest_Time, Stargrass, col="black",pch="+")
lines(Harvest_Time, Stargrass, col="black", lty=3)
```

```
points(Harvest_Time, Sugar_Sawdust, col="purple",pch="-")
lines(Harvest_Time, Sugar_Sawdust, col="purple", lty=4)

points(Harvest_Time, Sugar_Stargrass, col="green",pch="*")
lines(Harvest_Time, Sugar_Stargrass, col="green", lty=5)

points(Harvest_Time, Sawdust_Stargrass, col="dark red",pch="~")
lines(Harvest_Time, Sawdust_Stargrass, col="dark red", lty=6)

points(Harvest_Time, Sugar_Sawdust_Star, col="yellow",pch="+")
lines(Harvest_Time, Sugar_Sawdust_Star, col="yellow", lty=7)

# Adding a legend inside box at the location (2,40) in graph coordinates.
# Note that the order of plots are maintained in the vectors of attributes.
legend(4,3,legend=c("Sugarcane","Sawdust","Stargrass","Sugar_Sawdust","Sugar_Stargras
s","Sawdust_Stargrass","Sugar_Sawdust_Star"),
col=c("blue","red","black","purple","green","darkred","yellow"),
pch=c("o","*","+","-","*","~","+"),lty=c(1,2,3,4,5,6,7), ncol=1)
#================================================
#Cummulative 4 graphs in a page
#cumulative yield
x0<-c(1,2,3,4,5,6,7)
x1<-c(1.0,2.2,3.5,4.6,5.7,6.6,6.6)
x2<-c(1.1,1.8,2.7,3.6,4.1,4.1,4.1)
x3<-c(0,0,0.4,0.6,0.6,0.6,0.6)
x12<-c(1.1,2.5,4.0,5.4,7.0,8.2,9.0)
x13<-c(0,0.4,1.0,1.5,2.2,3.3,3.6)
x23<-c(1.0,1.8,2.2,2.8,2.9,3.1,3.1)
x123<-c(0,0.6,1.1,2.0,3.0,3.7,4.1)

par( mfrow = c( 2, 2 ), oma = c( 0, 0, 2, 0 ) ) #Four graphs in one frame
plot(c(1,2,3,4,5,6,7),c(6.6,4.1,0.6,9.0,3.6,3.1,4.1), type="b", col="blue",
main="Fig_4.15a(Linear)",
xlab="Mixtures", ylab="Yield(cum)")

hist(c(6.6,4.1,0.6,9.0,3.6,3.1,4.1), breaks=8, col="springgreen4", xlim = c(1,10),
xlab="Mixtures",
ylab="Yield", main="Fig_4.15b(Histogram)")

yield<- c(6.6,4.1,0.6,9.0,3.6,3.1,4.1)     #picharts
pie(x = yield, main="Fig_4.15c(Piechart)", col=rainbow(length(yield)),
          label=c("Pure-1","Pure-2","Pure-3", "Mix-12", "Mix-13","Mix-23", "Mix-123"))

x1<-c(1.0,1.2,1.3,1.1,1.1,0.9,0)
x2<-c(1.1,0.7,0.9,0.9,0.5,0,0)
x3<-c(0,0,0.4,0.2,0,0,0)
x12<-c(1.1,1.4,1.5,1.4,1.6,1.2,0.8)
x13<-c(0,0.4,0.6,0.5,0.7,1.1,0.3)
x23<-c(1.0,0.8,0.4,0.6,0.1,0.2,0)
x123<-c(0,0.6,0.5,0.9,1.0,0.7,0.4)
alis<- list(x1,x2,x3,x12,x13,x23,x123)
boxplot(alis, range=0.0, horizontal=FALSE, varwidth=TRUE, notch=FALSE,
      outline=TRUE, names=c("X1","X2","X3","X12","X13","X23","X123"), boxwex=0.3,
```

```
    border=c("blue","blue","blue","blue","blue","blue","blue"),
col=c("red","red","red","red","red","red","red"), main="Fig_4.15d(Boxplot)")

title(xlab = "Mixtures")
title(ylab = "Yield Kg")
#================================================

#contour plots# for mixtures
fit<- Xvert(nfac = 3, lc = c(0.35, 0.2, 0.15), uc = c(1, 1, 1),ndm = 1, plot = FALSE)
y <- c(15.3, 20.0, 28.6, 12.5, 32.7, 42.4)
orig<- cbind(orig[1:6, ], y)
quadm<- lm(y ~ -1 + x1 + x2 + x3 + x1:x2 + x1:x3 + x2:x3, data = orig)
title<- c("Actual Component Space", "Pseudo Component Space")
option<- c(FALSE, TRUE)
for (i in 1:2) {ModelPlot(model = quadm,dimensions = list(x1 = "x1", x2 = "x2", x3 = "x3"),
+ main = title[i], lims = c(0.35, 1, 0.20, 1, 0.15, 1),
+ constraints = TRUE, contour = TRUE, cuts = 6, fill = TRUE,
+ axislabs = c("x1", "x2", "x3"), cornerlabs = c("x1", "x2", "x3"),
+ pseudo = option[i])
+ }

#==========================================
data=read.table("phd1.csv",header=TRUE, sep=",")
data
attach(data)
X<-cbind(x1,x2,x3,x12,x13,x23,x11,x22,x33)
X
D=t(X)%*%(X)
D
D0=det(D)
D0
D01=(D0^0.1)/19
D01
#therefore efficiency is 52.04%
D1=solve(D)
D1
D2=det(D1)
D2
D3=D2^0.1
D3
#efficiency is 53.25%
fit=lm(y~x1+x2+x3+x12+x13+x23+x11+x22+x33,data=data)
fit
#A-optimality criterion
D1=solve(D)
D1
D2=det(D1)
D2
D3<- sum(diag(D1))
D3
D4=D3*19
D4
D5=eigen(D1)
D5
#END
```

Appendix II: Pictorial Data for Oyster Mushroom farmers Discussion Group.

Appendix III: Income Statement for Oyster Mushroom Production Start-ups

Table 4.15: Income Statement

Small Oyster Mushroom Farmer
Income Statement
For a Period of three Months Ending 30/04/2018

Sales	**KSHs**	**KSHs**
Sales 250kg @ KSHs 600		150,000
Cost of Sales		
Cropping structure (semi-permanent)	7,000	
Labor	18,000	
Metallic Drums	2,000	
Marketing cost	10,000	
Spawns 2L @ KSHs 750	1,500	
Substrates	3,000	
Firewood & water	3,000	
Polythene bags, wheat bran, lime, spirit, PVP pp, Cotton wool, rubber bands	3,000	
Dressing, lighting & pesticides	2,500	
Miscellaneous	2,000	52,000
Income before tax		98,000
Tax @ 30%		29,400
Net income		**68,600**

Appendix IV: Central Composite Designs Experiments' Data

Media conc.	Sterilization Temp.	Incubation time	25/6/17	26/6/17	27/6/17	28/6/17	29/6/17	30/6/17	31/6/17	1/7/2017
65	20	30	0.5	1.6	2	2.5	3.1	3.8	4.3	4.3
65	20	30	0.6	1.7	2.3	2.5	3.1	4	4.3	4.3
65	20	30	0.1	1.1	2	2.5	3.2	4	4.3	4.3
65	20	30	0.6	1.4	1.8	2.4	3	3.7	4.3	4.3
65	20	30	0.6	1.6	1.9	2.5	3.4	4	4.3	4.3
			2.4	**7.4**	**10**	**12.4**	**15.8**	**19.5**	**21.5**	**21.5**
			0.48	**1.48**	**2**	**2.48**	**3.16**	**3.9**	**4.3**	**4.3**

Media conc.	Sterilization Temp.	Incubation time	25/6/17	26/6/17	27/6/17	28/6/17	29/6/17	30/6/17	31/6/17	1/7/2017
50	20	30	0.7	1.6	1.6	2.2	3.5	3.9	4.3	4.3
50	20	30	0.6	1.7	2.2	3.1	3.8	4.1	4.3	4.3
50	20	30	0.1	1.1	1.9	2.5	3.7	4	4.3	4.3
50	20	30	0.6	1.4	2	2.2	3.6	4	4.3	4.3
50	20	30	0.5	1.6	1.9	2.5	3.6	3.9	4.3	4.3
			2.5	**7.4**	**9.6**	**12.5**	**18.2**	**19.9**	**21.5**	**21.5**
			0.5	**1.48**	**1.92**	**2.5**	**3.64**	**3.98**	**4.3**	**4.3**

Media conc.	Sterilization Temp.	Incubation time	25/6/17	26/6/17	27/6/17	28/6/17	29/6/17	30/6/17	31/6/17	1/7/2017
35	20	30	0.6	1.6	1.6	2.3	3	3.5	4.1	4.3
35	20	30	0.6	1.7	2	2.5	3.2	3.7	4.3	4.3
35	20	30	0.1	1.1	1.6	2.4	2.9	3.5	4	4.3
35	20	30	0.5	1.4	1.8	2.5	3.1	3.6	4.1	4.3
35	20	30	0.6	1.6	1.6	2.4	2.9	3.5	4	4.3
			2.4	**7.4**	**8.6**	**12.1**	**15.1**	**17.8**	**20.5**	**21.5**
			0.48	**1.48**	**1.72**	**2.42**	**3.02**	**3.56**	**4.1**	**4.3**

Media conc.	Sterilization Temp.	Incubation time	6/6/2017	7/6/2017	8/6/2017	9/6/2017	10/6/2017	11/6/2017	12/6/2017	13/6/17
65	20	15	0.7	1.4	1.9	2.4	2.9	3.7	4.2	4.3
65	20	15	0.6	1.5	2	2.4	3.1	3.7	4.3	4.3
65	20	15	0.9	1.5	2	2.4	3.1	3.6	4.1	4.3
65	20	15	1.1	1.8	2.4	2.9	3.5	3.9	4.3	4.3
65	20	15	0.6	1.4	1.9	2.5	3.2	3.7	4.3	4.3
			3.9	**7.6**	**10.2**	**12.6**	**15.8**	**18.6**	**21.2**	**21.5**
			0.78	**1.52**	**2.04**	**2.52**	**3.16**	**3.72**	**4.24**	**4.3**
Media conc.	Sterilization Temp.	Incubation time	6/6/2017	7/6/2017	8/6/2017	9/6/2017	10/6/2017	11/6/2017	12/6/2017	13/6/17
35	20	15	0.9	1.6	1.9	3	3.4	3.8	4.3	4.3
35	20	15	0.6	1.5	1.9	2.6	3.3	3.7	4.2	4.3
35	20	15	0.9	1.5	2	2.7	3.4	3.9	4.3	4.3
35	20	15	0.6	0	0	0	0	0	0	0
35	20	15	0	0	0	0	0	0	0	0
			3	**4.6**	**5.8**	**8.3**	**10.1**	**11.4**	**12.8**	**12.9**
			0.6	**0.92**	**1.16**	**1.66**	**2.02**	**2.28**	**2.56**	**2.58**
Media conc.	Sterilization Temp.	Incubation time	6/6/2017	7/6/2017	8/6/2017	9/6/2017	10/6/2017	11/6/2017	12/6/2017	13/6/17
35	20	15	0.6	1.9	2.5	3.3	3.8	4.1	4.3	4.3
35	20	15	0.6	2	2.7	3.4	3.9	4.2	4.3	4.3
35	20	15	0.1	1.1	0	0	0	0	0	0
35	20	15	0.5	1	0	0	0	0	0	0
35	20	15	0	0	0	0	0	0	0	0
			1.8	**6**	**5.2**	**6.7**	**7.7**	**8.3**	**8.6**	**8.6**
			0.36	**1.2**	**1.04**	**1.34**	**1.54**	**1.66**	**1.72**	**1.72**

Media conc.	Sterilization Temp.	Incubation time	4/6/2017	5/6/2017	6/6/2017	7/6/2017	8/6/2017	9/6/2017	10/6/2017	11/6/2017
65	10	20	0.5	1.3	2.2	2.9	3.6	4.2	4.3	4.3
65	10	20	0.6	1.4	2.1	2.8	3.6	4.2	4.3	4.3
65	10	20	0.1	0	0	0	0	0	0	0
65	10	20	0.6	0	0	0	0	0	0	0
65	10	20	0.6	0	0	0	0	0	0	0
			2.4	**2.7**	**4.3**	**5.7**	**7.2**	**8.4**	**8.6**	**8.6**
			0.48	**0.54**	**0.86**	**1.14**	**1.44**	**1.68**	**1.72**	**1.72**
Media conc.	Sterilization Temp.	Incubation time	4/6/2017	5/6/2017	6/6/2017	7/6/2017	8/6/2017	9/6/2017	10/6/2017	11/6/2017
50	10	20	0.5	0	0	0	0	0	0	0
50	10	20	0.6	0	0	0	0	0	0	0
50	10	20	0.1	0	0	0	0	0	0	0
50	10	20	0.6	0	0	0	0	0	0	0
50	10	20	0.6	0	0	0	0	0	0	0
			2.4	**0**	**0**	**0**	**0**	**0**	**0**	**0**
			0.48	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Media conc.	Sterilization Temp.	Incubation time	4/6/2017	5/6/2017	6/6/2017	7/6/2017	8/6/2017	9/6/2017	10/6/2017	11/6/2017
35	10	20	0.5	0	0	0	0	0	0	0
35	10	20	0.6	0	0	0	0	0	0	0
35	10	20	0.1	0	0	0	0	0	0	0
35	10	20	0.6	0	0	0	0	0	0	0
35	10	20	0.6	0	0	0	0	0	0	0
			2.4	**0**	**0**	**0**	**0**	**0**	**0**	**0**
			0.48	0	0	0	0	0	0	0