# SEASONAL RESPONSES OF TOTAL ANTIOXIDANT LEVEL IN CULTIVATED TEA TO FERTILIZER

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A research project submitted in partial fulfilment of the requirements for the award of the degree of Bachelor of Science (Applied Statistics with computing) in the school of science of Maasai Mara University.

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## DECLARATION

This project is my original work and has not been presented for a degree in any other university.

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**DEDICATION**

I would like to dedicate this project to my mother, father and siblings.

**ACKNOWLEDGMENT**

I would like to thank God for He has been the one helping us through the project. My gratitude goes to all Maasai Mara university fraternity for the support during this project. Furthermore, my regards goes to all my classmates for being supportive, all my lecturers more so my supervisor Mr. Kipkoech Cheruiyot for his tireless help.

I also thank my family for the humble time they gave me during the project.

## ABSTRACT

The research was conducted to find out whether applying fertilizer to tea at specific season of the year will affect the level of antioxidant. The main objective of the research was to determine the correlation between the level of antioxidant in tea and the season at which fertilizer was applied. Secondary data was obtained from James Finlay Kenya Limited with the authority of Applied Research Department. Trials of NPK fertilizer were conducted during the four seasons of the year: dry season, high rains season, medium rain season and low rains season. The treatments were replicated four times in a Complete Randomized Design (CRD). The harvested leaves were then freeze dried and ground for total antioxidant using 2, 2 diphenyl-1-picrylhydrazyl (DPPH) method. Analysis of Variance (ANOVA) and correlation analysis were done using SPSS. The results of this study showed that there was a significant difference between the levels of antioxidant during the four seasons of the year with the highest being during the low rains and lowest during the dry and high rains seasons.

## Contents

LIST OF TABLES

## ABBREVEATIONS AND ACRONYMS

ANOVA- Analysis of Variance

ARD –Applied Research Department

DPPH- 2,2dyphenyl -1- picryhydrzyl

N- Nitrogen

P- Phosphorus

K- Potassium

# CHAPTER ONE

# INTRODUCTION

## 1.1 Background of the Study

Tea plant is an evergreen shrub or small tree of the *Camelia* genus, native to China, with dark green shiny leaves and white flowers. *Camelia sinensis* var. *sinensis* and *Camelia sinensis* var. *assamica* are the two varieties most commonly used for the production of different types of tea. Freshly prepared infusion of dried leaves is a beverage consumed on a daily basis worldwide. Tea beverage is prepared at home by pouring boiling water over processed tea leaves. Tea is valued for its taste, aroma, health benefits, and cultural practices. Fresh tea leaves are plucked manually or using a plucking machine. Most preferably, just two fresh leaves and the buds should be used, since it influences the quality and therefore the price of the tea. The younger the leaves used for the production are, the more expensive is the final product. Processing of tea leaves allows the production of various types of tea: white, yellow, oolong, green, black, and pu-erh, depending on the extent of the oxidation/fermentation process. Generally, leaves of the *sinensis* variety are used mainly for the production of green tea, while black and pu-erh tea are produced from *assamica* (Zhang *et al.*, 2012)

### 1.1.1 Antioxidants in tea

An antioxidant is a substance that inhibits oxidation. In the recent past, determination of antioxidant activity and the total content of antioxidants in foods, beverages, dietary supplements and herbal extracts have been on wide demand. This relates to the fact that antioxidants can prevent free radicals, primarily highly reactive oxygen and nitrogen species.

The steady increase of free radicals in cells creates the condition of oxidative stress, wherein free radicals oxidize blood vessel walls, protein molecules, DNA and lipids. The most active radicals break bonds in DNA molecules and damage the cells' genetic apparatus regulating their growth which can result to cancerous cells.

Harmful effects of free radicals and oxidative stress can be reduced by regular consumption of foods and beverages which exhibit ant oxidation activity. Tea is one of the beverages and therefore has prompted us to study whether the application of fertilizer in tea at specific seasons of the year in Kenya affected the level of antioxidant in tea.

Teas have antioxidant properties of a wide range of amphipathic molecules termed phenolic compounds (Ivanova *et al.* 2004). The antioxidant activity of phenolics are mainly due to their redox properties which allow them to act as reducing agents, hydrogen donators, singlet oxygen quenchers and metal chelators (Morer *et al.* 1994; Rice-Evans *et al.* 1997).

## 1.2 Statement of the Problem.

The level of antioxidant in tea is affected by several factors including method of processing, agronomic practices, Owuor *et al.* (2012), type of tea among others. A single application of 300 kilograms per hector of nitrogen or phosphorous fertilizer and 200kg/ha of calcium maximized shoot growth Mudau et al. (2015) whereas the combined application of 300kg/ha Nitrogen or Phosphorus and 200kg/ha of calcium doubled growth and total polyphenal content of cultivated bush tea hence high antioxidant level. Correlation between fertilizer application seasons and antioxidant level had not been greatly researched on and this study found this as a problem to be researched on

## 1.3 Justification

Farmers and companies should not just apply fertilizer at their own time as this could end up not producing the anticipated output. Tea of less quality will end up being rejected in the market and therefore affect Kenya's economy at large. This study therefore, gives farmers guidelines on the right season to apply their fertilizer to their tea.

## 1.4 Objectives of the study

### General objective

To determine the relationship between the level of antioxidant and season of application of fertilizer to tea

### Specific objectives

1. To determine whether there is a difference between the levels of antioxidants in tea during the different seasons of the year
2. To determine the appropriate season for the application of fertilizer in order to attain a high antioxidant level

## 1.5 Significance of the study.

The findings of the study will ensure production of tea with highest antioxidant level. This will improve the market of the Kenyan tea hence boosting the economy.

Large companies as well as small scale farmers will use the findings to determine the right season for application of fertilizer.

## 1.6 Scope of the study

The study was carried out in James Finlay Kenya located in Kericho County. The experimental farms were situated in Applied Research Department.

## 1.7 Assumptions

It was assumed that the only factor causing changes in antioxidant level was time of fertilizer application. All other factors were considered negligible.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Introduction

This chapter has acquired and reviewed information from research studies, journals, newspapers, seminar papers, conference and books on the factors affecting level of antioxidant in different types of tea.

## 2.2 Research on factors affecting level of antioxidant

Agronomic practices such as plucking of leaves Owuor *et al.* (2012) and mineral nutrition Owuor( 2013); Owuor and Odhiambo (2012) increased the concentration of total polyphenals and total antioxidant contents in green tea. Total polyphenals in leaves of tea were lowest in March, April and September and highest in June and July according to Mudau et al. (2014). Antioxidant activity of green tea, as measured by different methods is usually higher in green tea than black tea Yoshan ( 2011). However, it was determined that theaflavins in black tea and catechins in green tea are equally effective antioxidants

The major hypothesis on the beneficial health effects of green tea is associated with its antioxidant properties Duan (2010)

In a research done by the Tea Research Foundation Kenya TRFK (2013) has established that overall "green and white teas" had significantly higher antioxidant activity as compared to black tea.

The composition of bioactive compounds, and therefore antioxidant capacity of tea, might be influenced by several parameters associated with growth conditions: genetic strain, climatic conditions, soil profile, growth altitude, horticultural practices, or plucking season. Interesting results on the effects of shade growth on tea phytochemical composition and antioxidant capacity were presented by Ku *et al.(* 2010). Tencha, shade grown tea, showed generally lower antioxidant capacity (and epicatechin, epigallocatechin, and galloylquinic acid content) but higher content of amino acids than green tea.

The manufacturing process evokes profound changes in the phenolic compound profile, in the content of individual compounds as well as in the antioxidant capacity of final tea products. Therefore, depending on the type of tea, the final products contain highly distinct profiles of antioxidant compounds. The identification of 92 phenolic compounds in a total of 66 non-fermented and fermented teas collected from all around the world allowed the classification of all types of tea into five groups, Lin *et al.*, (2008).
A number of studies carried out have aimed to characterize of different types of commercially available teas in specific regions or around the world.

In recent years there has been more and more research on the effect of consumer preparation on composition and activity of tea infusions Kyle *et al.*,( 2007); Lakenbrink *et al.*, 2000; Molan *et al.*, 2009; Su *et al.*, (2006). The studies have examined the different culinary methods used in the domestic preparation of tea infusions, taking into account factors such as water temperature, infusion time, stirring and dosage form, i.e., loose-leaf tea *versus* tea bag.

The extraction temperature, extraction time, water quality and water-to-tea ratio, tea particle size, extraction pH, and the number of extractions are all important factors which directly affected the efficiency of the extraction of antioxidants Vuong *et al.*,( 2011)

In the case of green tea it was reported that the higher temperature of brewing, the higher the reducing power of the infusion; however, no difference was noticed between extraction carried out for 10 min and 30 min Molan *et al.*,( 2009). Similarly, for black and oolong tea, the longer the time of brewing was, the higher the antioxidant activity as well as the total phenolic and catechin contents were Kyle *et al.*,( 2007). Oolong tea prepared at 95°C for 3 min had the highest scores of aroma, bitterness, astringency, and sweetness, but the total polyphenol and radical-scavenging activity in tea solutions increased with higher temperatures and longer brewing times. The brewing conditions optimal for maximal TEAC values of green tea were reported to involve 80°C for 3–5 min Samaniego-Sanchez *et al.*, (2011). In terms of the maximal extraction of catechins per gram of green tea, the best extraction efficiency was achieved with water extraction at 80°C for 30 min Vuong *et al.*, (2011).

Antioxidant activity and catechin content of green tea depends on form of powder, loose leaves, or tea bags, as well as brewing conditions Komes *et al.*, (2010). Powder and bagged green tea allowed the extraction of more phenolic compounds than leaves, regardless of the extraction temperature. Total flavan-3-ols amounted to around 1 g/l, whereas for loose-leaf teas ranged from 0.35 to 0.9 g/l for different tea brands. The highest concentration of flavon-3-ols was obtained for extractions performed at 80°C for 5 min (powder), 15 min (bagged), and 30 min (loose leaf). Generally, higher antioxidant activity was noted for tea powder and tea bags than tea leaves. Antioxidant activity was positively influenced by increased extraction time and temperature. However, other studies showed that the extraction efficacy of phenolic compounds from white Rusak *et al.*,( 2008) and green tea Samaniego-Sanchez *et al.*, (2011) was not significantly affected by form of loose leaf *versus* tea bag.

Addition of milk can reduce antioxidant potential of black tea ,Ryan and Petit(2010). In the case of green tea the results are contradictory: The addition of milk to powdered Matcha green tea mildly decreased its antioxidant activity, whereas for two other types of tea (Gyokuro and Twinings) the opposite effect was observed Komes *et al.*, (2014).
The antioxidant activity of green tea was enhanced after addition of lemon juice, which might be explained by the synergistic effect of ascorbic acid on the polyphenols as well as increased stability of polyphenols due to lower pH.
A single application of 300 kilograms per hector of nitrogen or phosphorous fertilizer and 200kg/ha of calcium maximized shoot growth Mudau et al. (2015) whereas the combined application of 300kg/ha Nitrogen or Phosphorus and 200kg/ha of calcium doubled growth and total polyphenal content of cultivated bush tea hence high antioxidant level. Correlation between fertilizer application seasons and antioxidant level had not been greatly researched on and this study tried to address this gap.

# CHAPTER 3

# METHODOLOGY

## 3.1 Introduction

In this chapter we outlined the methods used for data collection and analysis

## 3.2. Experiment procedure

### 3.2.1. Sample

At harvest, a sample of 200 young leaves was taken then freeze dried and analyzed for antioxidant level using the 2, 2 diphenyl-1-picrylhydrazyl (DPPH) method.

A Completely Randomized Design was used. Trials of NPK fertilizer were conducted during the four seasons of the year: dry season from January to March (precipitation rate <0.25mm per hour), heavy rains(precipitation rate 4.0-16.0mm/hr)from April to June, medium rains from July to August(precipitation rate 1.0-4.0mm/hr) and low rains from September to December(precipitation rate 0.25-1.0mm/hr).

Treatments were the four seasons of the year and the experiment was replicated four times.

The layout was as follows (Table(1)).

Table 1

| | | | | | |
|---|---|---|---|---|---|
| Seasons of the year | Dry season | | | | |
| | Heavy rains | | | | |
| | Medium rains | | | | |
| | Low rains | | | | |

At harvest leaves were freeze dried and ground for total antioxidant using 2,2-diphenyl-1-picryhydrazyl (DPPH) method. The analysis was done in the company's laboratory.

### 3.2.2Preparation of extracts for total antioxidant level

Fifteen grams of finely ground tea leaves were sieved for 5 minutes. From the sieved material, 0.5 grams was mixed with 5ml of 75% acetone for two hours in a shaker and then centrifuged for 5 minutes. The supernatant was carefully decanted and the extraction procedure repeated three times on the residue. Three supernatants were combined and made up to a volume of 15 ml of the filtrate extracts.

### 3.2.3 Determination of total antioxidant content.

Total antioxidant level was analysed using 2,2-dyphenyl-1- picryhydrazyl (DPPH) method. In this method, 24 mg of DPPH was mixed in 100 ml methanol and mechanically shaken for 20 minutes to produce a mother solution. 10 ml of the mother solution was mixed in 50 ml methanol. The absorbance of the solution was adjusted to 1.1 at 515nm by 20 ml mother solution. The extract of 2850µl of DPPH solution was added to 150µl sample extract for six hours until the completion of the reaction. The absorbance was measured at 515 nm in a spectrophotometer and expressed in percentage decimals.

### 3.2.4. Data analysis

Data was analysed statistically using SPSS. The data was subjected to Analysis Of Variance (ANOVA) and correlation analysis. The treatments were the seasons of fertilizer application. The values obtained were that of the level of antioxidant arrived at by the tea being freeze dried and then analyzed for antioxidants using the DPPH method

The model for completely randomized design is

$$Yi= \mu + t_i + e_i \quad (3.1)$$

Where

$\mu$ is the overall mean

$t_i$ is the effect due to rates of fertilizer

$e_i$ is the error component

Post Hoc test were further done to determine the different groups

Pearson correlation analysis was thereafter done to determine the relationship between the season of application of fertilizer application and level of antioxidant

The simple correlation coefficient r measures the extent to which two measured traits X(antioxidant level) and Y (season of application of fertilizer) vary linearly together. The correlation coefficient is estimated as

$$r= \frac{SP_{XY}}{\sqrt{SS_X}\ X\ \sqrt{SS_Y}} \quad (3.2)$$

Where the sums of squares of deviations of X and Y are

$$SS_X=\sum_{i=1}^{n}(x^2) - \frac{1}{n}(\sum_{i=1}^{n} x)^2 \quad (3.3)$$

$$SS_y=\sum_{i=1}^{n}(y^2) - \frac{1}{n}(\sum_{i=1}^{n} y)^2 \quad (3.4)$$

And the sum of deviation cross product is

$$Sp_{xy}=\sum_{i=1}^{n}(xy) - \frac{1}{n}\sum_{i=1}^{n} x \text{ x } \sum_{i=1}^{n} y \qquad (3.5)$$

Estimates of r may vary from -1 to +1; the extreme values indicate respectively perfect negative or positive correlation. Estimates of r =0 implies no relationship between values of the two traits

## CHAPTER FOUR

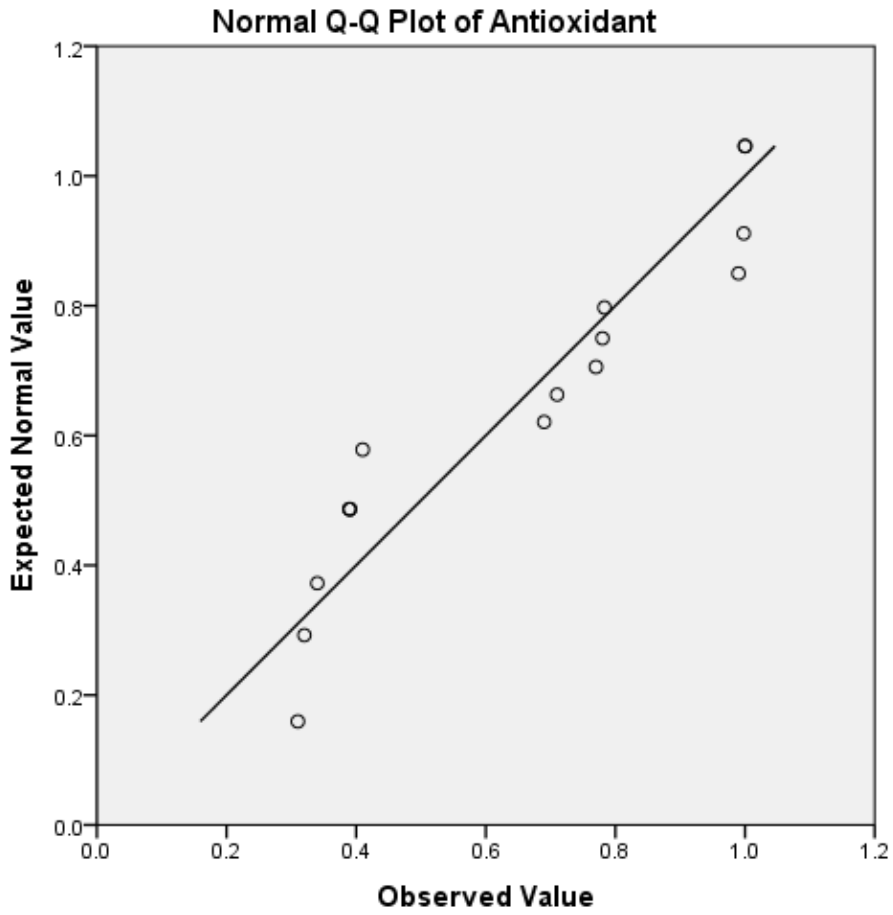
## RESULTS AND DISCUSSION

### 4.1. Introduction.

This chapter has captured the results obtained during data analysis and given the interpretation of the result.

### 4.2.1 Test for normality

The data was tested for normality using normal Q-Q plots as shown below.




From the graph, it is clear that the data entries are close to the line of fit indicating that the data is normally distributed hence allowing us to perform ANOVA

**4.2 Analysis of Variance (ANOVA).**

The analysis was done to determine whether there was a significant difference between the levels of antioxidant in the four seasons. The results are shown in Table (2) below:

Table 4.2.1

**ANOVA**
Antioxidant

| | Sum of Squares | Df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 1.011 | 3 | .337 | 38.430 | .001 |
| Within Groups | .105 | 12 | .009 | | |
| Total | 1.116 | 15 | | | |

**INTERPRETATION OF OUTPUT**

The p value 0.001<0.05 showed that there was a statistically significant difference between the seasons of application of fertilizer with the F value being 38.430.

To show the season that differed from another we did a Tukey post hoc test and are contained in the following multiple comparisons Table (3)

Table 4.2.2

**Multiple Comparisons**

Dependent Variable: Antioxidant

Tukey HSD

| (I) season | | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| dry season | high rains | .02000 | .06621 | .990 | -.1766 | .2166 |
| | medium rains | -.34575* | .06621 | .001 | -.5423 | -.1492 |
| | low rains | -.58200* | .06621 | .000 | -.7786 | -.3854 |
| high rains | dry season | -.02000 | .06621 | .990 | -.2166 | .1766 |
| | medium rains | -.36575* | .06621 | .001 | -.5623 | -.1692 |
| | low rains | -.60200* | .06621 | .000 | -.7986 | -.4054 |
| medium rains | dry season | .34575* | .06621 | .001 | .1492 | .5423 |
| | high rains | .36575* | .06621 | .001 | .1692 | .5623 |
| | low rains | -.23625* | .06621 | .018 | -.4328 | -.0397 |
| low rains | dry season | .58200* | .06621 | .000 | .3854 | .7786 |
| | high rains | .60200* | .06621 | .000 | .4054 | .7986 |
| | medium rains | .23625* | .06621 | .018 | .0397 | .4328 |

The mean difference is significant at the 0.05 level.

There was a significant difference between the dry season and medium and low rain season. The p values are 0.001 and 0.00 respectively which are less than 0.05.

There was however no significant difference between the dry seasons and high rains. P value is 0.990 which is greater than 0.05. There was also no significant difference between the low rains season and medium rain season, p-value 0.18>0.05

### 4.3. Correlation analysis

The correlation analysis was aimed at finding out whether there was a relationship between the season of application of fertilizer and the level of antioxidant in tea. Since the data came from a normal population, we preferred using the Pearson correlation.

The results were as shown in Table (4).

Table 2.3.1

**Correlations**

| | | season | Antioxidant |
|---|---|---|---|
| Season | Pearson Correlation | 1 | .894** |
| | Sig. (2-tailed) | | .000 |
| | N | 16 | 16 |
| Antioxidant | Pearson Correlation | .894** | 1 |
| | Sig. (2-tailed) | .000 | |
| | N | 16 | 16 |

**. Correlation is significant at the 0.01 level (2-tailed).

The coefficient of correlation, 89%, show there was a strong correlation between the level of antioxidant and season of application of fertilizer.

# CHAPTER FIVE

# SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

## 5.1. Introduction

This chapter summarised the findings of the study. It also gave conclusions and recommendations for practice and for further studies on the same subject

## 5.2 Summary of findings.

A Q-Q plot showed that the data was normally distributed. Analysis of variance showed that there was a significant difference between the four seasons of the year with a p value of .001<0.05. This was followed by a post Hoc test to determine the groups which were different. The test showed that there was a significant difference between the dry seasons and the low rains season likewise to the medium rain season. There was however no significant difference between the dry season and the heavy rains season. In addition, there was no significant difference between the low rain season and the medium rain season

Correlation analysis exhibited a positive value of 0.84 using the Pearson correlation coefficient. This was significant at 95% level of significance.

## 5.3 Conclusion

In conclusion, there was a significant difference between the level of antioxidant in the four seasons showing that level of antioxidant in tea is affected by the season of application of fertilizer. The low rain season and medium rain seasons had the highest level of antioxidant and the heavy rains season had the lowest level of antioxidant. The best season for application of fertilizer was therefore during the low rain and medium rain seasons

## 5.4. Recommendation for policy practice

It is highly recommended that farmers put fertilizer into their tea during the low rainy season or medium rain season. It is a common practice by farmers to apply fertilizer as soon as it is dispatched by the government without considering the season of the year.

The government should also ensure that they make awareness to the rural farmers and if possible dispatch fertilizers to different places during the right season.

## 5.5 Recommendation for further research.

Due to scarcity of resources, time and skills, this research narrowed itself to studying the effect of seasonal application of fertilizer to level of antioxidant only. It did not regard the fact that it could be affected by many other factors. There are also many other components of tea that contribute to the quality of tea which this study did not cover.

It is therefore recommended that further research be done on the factors affecting level of antioxidant in tea. Further research should also be done on other factors that work together to improve the quality of tea

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## APPENDIX

### Budget

| | |
|---|---|
| Transport | KSH3000 |
| Labour | 0 |
| Miscellaneous | KSH3500 |
| Fertilizer | 0 |
| TOTAL | KSH 6500 |

#### WORK PLAN

| TIME | ACTIVITY |
|---|---|
| November to January | Proposal development |
| February to march | Data collection |
| March | Data analysis and project completion |
| April | Data analysis and development of conclusion and recommendation |

### Data showing total antioxidant level

| | | | | | |
|---|---|---|---|---|---|
| Seasons of the year | Dry season | 0.3 | 0.34 | 0.69 | 0.32 |
| | Heavy rains | 0.39 | 0.39 | 0.41 | 0.39 |
| | Medium rains | 0.78 | 0.78 | 0.77 | 0.71 |
| | Low rains | 1.0 | 1.0 | 1.0 | 0.99 |