Original Research

Effects of phosphorus and potassium fertilization and fruit canopy position on sugar accumulation in mangifera indica cv. 'Kent' pulp

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Abstract: Sugar content is the key to mango quality and growers are constantly seeking methods to optimize it. In this study, fructose, glucose, sucrose, and maltose sugars were evaluated to determine how phosphorus and potassium fertilizers in varying amounts could affect these sugar levels and how sunlight exposure effects fruit. The experiments were conducted in the KALRO Orchard of Kiambu County, Kenya. Thirty-five healthy 7- to 8-year-old 'Kent' trees were selected and divided into five treatment groups with five replicates each. Treatment with 1 kg, 2 kg P, K and combined PK per tree resulted in a significantly higher fructose content than the control.

In contrast, neither 1 kg nor 2 kg P resulted in significantly higher glucose contents than the control. Combined PK fertilizers at 0.5+0.5 kg per tree reduced fructose and increased glucose, sucrose, and maltose. A combination of PK fertilizers at 1.0 +1.0 kg significantly increased only fructose, but not other sugars. Even after applying P and K fertilizers, the position of the fruit canopy did not affect fructose, glucose, or maltose contents. However, there was a trend towards a higher sucrose content in fruits exposed to sunlight $(5.17 \text{ g}/100 \text{ g})$ than in canopy fruits $(2.29 \text{ g}/100 \text{ g})$, although this difference was not statistically significant. The same trend was observed for the other sugars. The t-test revealed no significant differences between the two canopy positions ($t = 1.01$, $p = 0.344$). A significant difference in sugar content ($p < 0.05$) between fruits harvested at different canopy positions suggests that fruits harvested at different canopy positions did not ripen simultaneously.

Keywords: Fertilizer, Tree canopy, Mango, Sugars

1. Introduction

 Fruit quality is essential for the production of commercial fruit and affects marketability and consumer purchase choices [1]. The purchase choice is based on morphological characteristics such as appearance, colour, shape, and size [2]. However, sugar concentration often influences the sweetness of fruits, at least in part [2- 4]. The concentrations of fruit sugar and organic acids are decisive for the taste, flavour and quality of the

fruit, among other factors [3, 5]. In addition, the sugar concentration of fruit varies from the base to the apex and from sun to shade [6]. Sucrose, glucose, fructose and maltose are the primary sugars found in Mangifera indica fruit, and their contents vary depending on plant nutrition, environmental factors and the fruit canopy [3]. Rational use of fertilizers and appropriate P and K fertilization can significantly improve the quality of Mango fruit [7].

 Mineral fertilizers are used to meet the nutrient requirements. However, continuous use of mineral fertilizers leads to deterioration of soil fertility and

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accumulation of heavy metals in the plant tissues, which affects the nutritional value and quality of the fruit [5]. Phosphorus (P) and potassium (K) fertilizers are two essential macronutrients that influence fruit sugar content [8]. In the Mangifera indica orchard, considerable amounts of soil elements drain annually because of increased fruit yields and leaf pruning [9]. Good fertilization programmes must compensate for such considerable annual reductions in soil mineral content to maintain high yields and quality of fruits [9]. However, due to the high cost of these fertilizers, most farmers in underdeveloped African countries usually do not apply P or K fertilizers [10, 11].

 Phosphorus and potassium are necessary for plant growth, development, and various metabolic processes, including photosynthesis, respiration, and sugar metabolism [12]. Studies have reported that applying P and K fertilizers can significantly improve fruit pulp and sugar content in various crops, including apples, grapes, and peaches [13]. For instance, Li et al. [8] reported that applying P and K fertilizers increased the sucrose content in satsumas fruits. In addition, Zhang et al. [14] reported that applying K fertilizer increased glucose and fructose content in apple fruits. Furthermore, P-K combinations have emerged as the anions and cations with the most significant effects on fruit quality, an attribute that determines the marketability of mango fruit and consumer preferences [15].

 The fruit canopy regulates light interception and photosynthesis, which are essential for plant's sugar production [16]. Various factors, including canopy density, leaf area index, and leaf angle distribution, influence light interception by fruit canopies [17]. Studies have reported that fruit canopy manipulation can significantly affect the sugar content of fruits of various crops, including apples, avocados and loquats [17-20]. Pellegrino et al. [21] reported that pruning fruit leaves increased the sucrose content in the vine. Although Umar et al. [22] observed that unilaterally pruned "Kinnow" mandarin plants had the highest total sugar levels in their fruit juice, the opposite was true for unpruned plants.

 Similarly, Amarantea et al. [23] reported that shade netting increased the glucose and fructose content in 'Gala' and 'Fuji' apple trees by up to 15%. In addition, fruit canopy management significantly affects fruit sugar content by regulating light interception and photosynthesis [17]. Therefore, understanding the effects of P and K fertilizers and fruit canopies on fruit sugar content is crucial for optimizing fruit quality and productivity.

 Mango is a highly promising fruit in Kenya and wellsuited for various agroecological zones that span from sub-humid to semi-arid [7]. In a 5-year-old fruit orchard, mango trees can produce 12,000 to 18,000 tons of fruits, which is equivalent to approximately 500 to 800 tons per tree per year [7]. In addition, the country exports mangoes to France, Germany, and the United Kingdom, and the country has considerable potential to significantly increase mango production [24]. Nevertheless, the availability of

fresh and processed mangoes are insufficient now in terms of both quality and quantity to meet the demand in local and export markets. However, accurate data on this issue is limited. Consequently, mangoes are used as a model fruit for experimentation regarding quality control.

 In this study, we examined the effect of different amounts of phosphorus and potassium fertilizer on fruit sugars and the effect of fruit canopy position on fruit sugars.

2. Materials and Methods

2.1 Plant material and fertilizer application

 Field research was conducted in KALRO Orchad, Kiambu County, Kenya (29.23°N, 102.36°E; 780 m above sea level) during the time period from 25 Feb 2018 to 31 Dec 2019. Thirty-five healthy 7-to 8-year-old 'Kent' trees were chosen and divided into five treatment groups with five replicates as follows: 1 kg triple superphosphate, 1 kg potassium sulphate; 2 kg triple superphosphate, 2 kg potassium sulphate; 0.5 kg triple superphosphate and 0.5 kg potassium sulphate; 1 kg triple superphosphate and 1 kg potassium sulphate and control (0 kg). The selected cultivar is widely grown in Kenya with high yields and is readily available across KALRO orchids [24]. Soil chemical analyses were conducted in February 2015 (Table 1) before the experiment with the application of P and K. Exchangeable cations were analyzed with the method developed by Sparks [25]. The annual rainfall in Kiambu county varies with altitude, the higher regions receive as much as 2,000 millimeters of rainfall annually, while the lower regions near Thika Town receive only 600 millimeters [26].

 Fertilizer was applied at the beginning of 'Kent' tree flowering on luminol soils with high subsurface clay concentrations in 10 m long rows spaced 10 m apart (Figure 1). This open-pollinated Brooks seedling was brought to Kenya from Miami, Florida in 1944. Kent has an earlier ripening period, typically occurring in March. The fruits have a greenish-yellow color and a scarlet or crimson blush on the shoulder. They reach an average length of 12.4 cm, a width of 9.7 cm and an average weight of 545 g [7] (Figure 1b). The 'Kent' tree has a robust growth, characterized by a substantial and compact vertical canopy (Figure 1a). In addition, it demonstrates high productivity throughout the later part of the midseason [7].

2.2 Extraction and analysis of the sugars

 Mango fruits that had reached maturity were harvested, than transported to the laboratory and left to ripen at ambient temperature (20 \degree C). The ripeness of the fruit was evaluated subjectively by observing the colour and

Table 1. Soil chemical parameters of the KALRO-Kandara Mango experimental farm, Kiambu county, Kenya

Soil Property	Units	Min	Max.	Mean \pm Std
pH		5.43	6.02	5.73 ± 0.21
m3.B	$mg \, kg^{-1}$	0.71	0.96	0.84 ± 0.13
m3.Cu	$mg\,kg^{-1}$	9.22	9.25	9.24 ± 0.12
m3.Fe	$mg\,kg^{-1}$	166.20	158.20	162.20 ± 3.26
m3.Mn	$mg \, kg^{-1}$	231.01	224.66	227.84 ± 6.06
m3.P	$mg\,kg^{-1}$	20.00	22.02	21.01 ± 0.06
m3.S	$mg \, kg^{-1}$	17.81	18.00	17.91 ± 2.02
m3.Zn	$mg\,kg^{-1}$	12.66	13.70	13.18 ± 0.06
ExNa	cmolc kg^{-1}	0.12	0.16	0.14 ± 0.01
ExCa	cmolc kg^{-1}	8.44	11.34	9.89 ± 0.07
ExMg	cmolc kg^{-1}	2.88	2.85	2.87 ± 0.02
ExK	cmolc kg^{-1}	1.37	2.11	1.74 ± 0.01

 $m3.$ = Mehlich 3 extractable; Ex = exchangeable

Figure 1. (a) Experimental field of 7-8-year-old mango trees 'Kent' cultivars and tree spacings; (b) Kent fruits

softness of the rind. To obtain two sample sets per tree, five sun-exposed and five within the canopy mango fruits were individually combined [27]. The obtained fruit pulp was then freeze-dried and stored for sugar extraction.

 Sugars (glucose, fructose, sucrose and maltose) were extracted using the method described by Medlicott and Thompson [28], with minor modifications as described by Olale et al. [27]. For HPLC analysis, the mobile phase was acetonitrile: water (75:25) at a flow rate of 1.0 ml/min. The column was SupelcosilTM LC-NH2, 5 μm (25 cm × 4.6 mm) (Bonna-Agela, Inc., China), the column temperature was 35 °C, and the eluted peaks were identified using a refractive index detector (Agilent 1200 model). The individual sugars were quantified by comparing their peak areas with those of standard sugars.

2.3 Statistical analysis

 Data were presented as mean and standard deviation. Statistical analysis was performed using IBM SPSS statistics software (version 23.0), and one-way analysis of variance (ANOVA) was conducted to determine any significant differences between the means. Tukey's test was applied with a significance level of $p < 0.05$ to compare the differences between means. Furthermore, principal component analysis (PCA) and hierarchical cluster analysis were performed using Ward's linkage method.

3. Results

3.1 Fertilizer application rates on fruit sugar content

 Fruit sugar is an essential parameter for quality measurement in mango fruit and is a source of energy in different biosynthesis pathways. The effects of varying fertilizer application rates on fruit sugar content are shown in Figure 2. The results revealed that fertilizer type and application rate significantly influenced sugars (glucose,

sucrose, and maltose) regardless of fruit position in the tree canopy. However, variations in terms of fructose content and the control (No fertilizer application) were not statistically significant ($P > 0.05$, Figure 2). Glucose concentrations were significantly higher in trees treated with 1 kg of phosphorous than in control and other rates.

 The combined PK fertilizers at 0.5+0.5 kg significantly increased sucrose more than any other combination. However, the trees under control still had substantially higher sucrose content than those fertilized with PK fertilizers at either rate. For maltose sugars, the increased rate of combined PK fertilizers (1.0+1.0 kg) did not result in increased concentrations, nor did the combined higher rate of P and K. However, significantly higher rates were reported for the control. In contrast, increased K fertilizer application had no significant effect on sugar content (fructose, glucose, sucrose and maltose). These findings may be ascribed to differences between cultivars in absorption and response to PK fertilization and environmental factors such as light, water and nutrient supply [29]. Another reason for the lower sugar content could be that higher fertilizer application create a barrier to mango plant nutrition or other constraints in the soil [30].

 The results are in agreement with those of Azam [31], who reported an inconsistent effect of fertilization rate on the accumulation of sugars in mango fruit. In addition, Khan [9] reported an improved performance due to the higher dose of muriate of potash. In contrast, Jakhar et al. [4] reported that an increase in K fertilizer application rate could increase the total sugar and ascorbic acid contents. Compared with other fruits, Zielinski et al. [32] reported that apple trees receiving P and K had the highest contents of sucrose and total sugar. The differences between these results and this experiment may be due to different agronomic conditions.

 Figure 2. Effect of fertilizer application rate on mango fruit sugar content. Data points represent the mean \pm standard deviation. (n=21). ns, *, **, and *** indicate significant differences at $P > 0.05, 0.01 < P \le 0.05, 0.001 < P \le 0.01$, or $P \le 0.001$ level, respectively

Sugars $(g/100 g)$	Fruit Position	N	$Mean \pm SD$	F		Sig. (2-tailed)
Fructose	Sun exposed	19	$10.94{\pm}4.0$	0.654	1.192	0.242
	Within Crown	14	9.41 ± 3.08		1.240	0.224
Glucose	Sun exposed	6	1.69 ± 1.31	0.019	0.085	0.934
	Within Crown	6	1.63 ± 1.43		0.085	0.934
Sucrose	Sun exposed	6	5.17 ± 2.59	2.214	1.015	0.344
	Within Crown	3	2.29 ± 1.97		1.316	0.230
Maltose	Sun exposed	5	6.05 ± 2.45	0.946	1.000	0.351
	Within Crown	4	4.56 ± 1.89		1.032	0.336

Table 2. T-test effect of fruit position on mango fruit sugars

3.2 Effect of fruit canopy position on sugar content

 The results of the relationship between the position of a fruit on the tree and its sugar content are presented in Table 1. Fruits on the outermost part of the canopy, which receive the most sunlight, generally contain higher levels of sugar than fruits nestled within the canopy [26]. This difference was not observed in fructose, glucose, or maltose content even after applying P and K fertilizers. However, there was a trend towards a higher sucrose content in sun-exposed fruits than in canopy fruits, although this difference was not statistically significant (Table 2). Conversely, fruits shaded by the canopy had lower sugar content, suggesting that sunlight exposure plays an important role in sugar production of the fruit. The increased sugar content in outer canopy fruits means higher sweetness and lower acidity; this difference is usually reflected in consumer preference.

 The sucrose content was higher in the sun-exposed fruits $(5.17 \pm 2.59 \text{ g}/100 \text{ g})$ than in crown fruits $(2.29 \pm 1.97 \text{ g})$ $g/100$ g), but the t-test showed no significant difference between the two positions (t = 1.015, p = 0.344) (Table 2). On the other hand, maltose content was higher in the sunexposed fruits $(6.05 \pm 2.45 \text{ g}/100 \text{ g})$ than in fruits within the crown (4.56 \pm 1.89 g/100 g), although the difference was not significant ($t = 1.0$, $p = 0.351$). These results agree with those previously reported by other authors [26, 32-34], who found that sun-exposed fruits contained more sugars. A significant difference in sugar content ($p < 0.05$) observed in fruits from different canopy positions suggests that fruits picked from different canopy positions may not ripen simultaneously [20].

 Light exposure in the canopy could explain why our inner-canopy fruit had lower sucrose levels because sucrose is transferred from the source leaves to the fruit in apples [18].

3.3 Hierarchical cluster analysis and heat map of fertilizer rate and sugars

 Hierarchical cluster analysis identified four primary clusters based on fertilizer types and rates (Figure 3). Cluster 1 consisted mainly of potassium (1kg and 2 kg) and combined PK at 2 kilograms and showed a low correlation with glucose. In contrast, cluster 4 correlated strongly with fructose and glucose. This is similar to the outcomes of the PC analysis (Figure 4), which also drew attention to clusters within the measured variables. Cluster 4 is comprised exclusively of phosphorous, suggesting that a significant number of P molecules are interconnected with glucose and fructose. An additional cluster 2, consisting only of PK at 1 kg, was observed in association with each sugar content; however, it suggested the possibility of a low correlation between the aforementioned fertilizer types and application rate with the sugar content. Cluster 3 comprises control (no fertilizer) and correlates strongly with fructose, maltose and sucrose.

 The application of a heat map and hierarchical clustering analysis to the data matrix revealed informative patterns related to sugar content and fertilizer application, suggesting a potential interaction effect between fertilizer type and rate. This provided valuable insights to identify fertilizer types and application rates that could optimize sugar production. Additionally, a preliminary understanding of the potential interactions between fertilizer type and sugar content was gained. These results could help in the development of targeted fertilization strategies for different crop types or desired sugar profiles. These clusters potentially captured different effects of fertilizer application and may have grouped fertilizer types with high sugar content associated with a specific fertilizer application rate compared to others. However, the clusters could not show a trend of increasing or decreasing sugar content with varying fertilizer application rates.

3.4 Principal component analysis of sugars and fertilizers at different application rates

 PCA showed that fertilizer application rates and fruit sugars were segregated between different rates and controls, while obvious clustering was evident among the sugar types (Figure 4). The first two principal components, PC1 and PC2, explained 83.8% of the variation among the four sugar types (Figure 4). A biglot showed a positive correlation between glucose, maltose, and fructose levels in PC1. Glucose and maltose levels were positively correlated with phosphorus at 1 and 2 kg, respectively. Furthermore, there was an obvious correlation between sucrose and combined potassium and phosphorus fertilizers. We also found a significant inverse relationship between fertilizer application rates (0, 1, and 2 kg) and sucrose, suggesting the possibility of glucose conversion to sucrose.

 The score plot revealed the distinct groupings of sugars based on fertilizer application rates and types. Mango trees that treated with similar fertilizer rates tended to cluster together, indicating a clear association between fertilizer application rate, type, and sugar profile of mango trees. The clustering by fertilizer treatment highlighted the influence of fertilization on sugar composition and, thus, the contribution of individual sugar components and fertilizer elements to each PC. From the PC loadings, it is possible to identify key sugars that responded significantly to fertilizer application and the fertilizer elements that most prominently influenced the variations of sugar profiles across the mango trees. The findings reported here provide valuable insights for optimizing fertilizer application strategies to achieve the desired sugar content and composition of a specific crop.

4. Conclusion

 This study investigated the effects of phosphorus (P) and potassium (K) fertilizers in different application

Figure 3. Heat map and hierarchical clustering analysis using Euclidean distance and Ward's method generated from sugars, fertilizer type, and fertilizer rate

Figure 4. PCA of sugars (fructose, glucose, sucrose, and maltose) and the P, K, and PK rates in Kent mangoes.

rates and fruit canopy position (sun-exposed/within the crown) on fruit sugar content. The findings demonstrate the effectiveness of specific fertilization strategies in optimizing sugar profiles. Treatments with combined PK fertilizers (0.5 kg P + 0.5 kg K or 1.0 kg P + 1.0 kg K per tree) significantly increased fructose content compared with the control and other fertilizer treatments. Interestingly, combined PK application at a lower rate $(0.5 \text{ kg P} + 0.5)$ kg K) also increased glucose, sucrose and maltose levels, suggesting a broader impact on sugar composition.

 Although P fertilization alone did not significantly increase glucose content, the combined PK treatments suggest a potential interaction between these elements in influencing sugar profiles. Notably, even after fertilizer application, the fructose, glucose and maltose levels of fruits located within the canopy (sun-exposed vs. crown) did not significantly changed. However, we observed a trend towards higher sucrose content in sun-exposed fruits

than in crown fruits, suggesting that sunlight potentially plays a role in sucrose accumulation. The significant difference in overall sugar content between fruits harvested from different canopy positions highlights the possibility of uneven ripening of the fruits within the canopy.

 This finding suggests it is important to consider fruit location during harvest to ensure optimal sugar content and quality. Future studies could explore the relations between canopy position, light exposure, and ripening dynamics in 'Kent' mangoes to provide more targeted harvesting recommendations.

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Conflict of interests

 The author has no relevant financial or non-financial interests to disclose.

Data availability statement

All data are included in the manuscript.

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