INFLUENCE OF NPK FERTILIZER RATES ON GROWTH, FLOWER ABORTION, CONCENTRATION OF SECONDARY METABOLITES AND QUALITY OF FIELD AND GREENHOUSE GROWN PEPINO MELONS (*Solanum muricatum* Aiton)

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EGERTON UNIVERSITY

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DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been presented in this University or any other for the award of a degree.

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Recommendation

This thesis has been submitted with our approval as University supervisors.

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DEDICATION

This work is dedicated to my mum, Magdalene Mutua and my son James Musau who have been and will always be my source of strength and encouragement.

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ABSTRACT

Pepino melon (*Solanum muricatum* Aiton) is a plant that was originally grown in South America, mainly for its juicy fruits. It was introduced in Kenya in 2013 and is gaining a lot of popularity due to its high nutritive, medicinal and economic value. Despite its popularity and multiple uses, production of pepino is constrained by flower abortion and inadequate knowledge on the effect of nutrients on growth, yield, quality, nutritional composition and concentration of secondary metabolites. The main objective of this study was to investigate the effects of NPK fertilizer rates on growth, concentration of secondary metabolites, nutritional composition, flower abortion, yield and postharvest quality of field and greenhouse grown pepino melons. The study was conducted in the open field and greenhouse at the Horticulture Training Field three of Egerton University, Njoro in two trials. The experimental design in the field and greenhouse was Randomized Complete Block Design (RCBD) with five treatments (0, 100, 200, 300 and 400 kg NPK ha^{-1}) with three replications. Results indicated that at 100 Days after planting (DAP) plants grown in the greenhouse and supplied with 200 kg NPK ha^{-1} had a stem diameter of 14.01 mm which was significant ($p \leq 0.05$) compared to a stem diameter of 11.71mm obtained from open field grown plants supplied with 300 kg NPK ha^{-1} in trial two. Application of 300 kg NPK ha^{-1} for field grown pepino melons gave the highest yield of 1102.48 kg ha^{-1} and 1060.55 kg ha^{-1} in trial one and two respectively. Application of 200 kg NPK ha^{-1} favoured accumulation of lutein, lycopene and β-carotene in both growing environments and trials. Fruits from greenhouse grown plants which were not supplied with fertilizer (control) had a Total phenolic content (TPC) content of 174.3 and 145.5 mg Gallic acid equivalent (GAE) $100g^{-1}$ fresh weight (FW) in trial one and two, respectively. Application of 200 and 300 kg NPK ha^{-1} enhanced copper (Cu), manganese (Mn), molybdenum (Mo) and iron (Fe) content of greenhouse and field grown pepino melon fruits respectively. Application of 200 kg NPK ha^{-1} enhanced vitamin C content in field and greenhouse grown pepino melon fruits. Application of 200 and 300 kg NPK ha^{-1} to greenhouse and field grown pepino melons increased the number of flowers per truss, reduced flower abortion, increased pollen viability and *in vitro* pollen germination in both trials. Field grown fruits from the control treatment stored at room temperature had the highest TSS of 8.67 °Brix and 8.13 °Brix in trial one and two, respectively after 28 days of storage. Application of 100 kg NPK ha^{-1} to both field and greenhouse grown pepino melon plants and storage at low temperature (7°C) enhanced the quality and shelf life of pepino melon fruits. It is concluded that NPK fertilizer rates and growing environment have an effect on growth, secondary metabolites, flower abortion, yield and postharvest quality of pepino melon.

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LIST OF ABBREVIATIONS AND ACRONYMS

ABTS	2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)
ARA	Aldose Reductase Activity
CAN	Calcium Ammonium Nitrate
CESAAM	Centre of Excellence in Sustainable Agriculture and Agribusiness Management
CRD	Completely Randomized Design
DAP	Days After Planting
DNA	Deoxyribonucleic Acid
DSP	Double superphosphate
DPPH	2, 2-Diphenyl-1-(2, 4, 6-trinitrophenyl) hydrazyl
EC_{50}	Half Maximal Effective Concentration
FAO	Food and Agriculture Organization
FRAP	Ferric Reducing Antioxidant Power
FW	Fresh Weight
GAE	Gallic Acid Equivalent
HPLC	High-performance Liquid Chromatography
HUVEC	Human Umbilical Vein Endothelial Cells
LD_{50}	Lethal Dose 50%
NPH	Normal Pressure Hydrocephalus
NPK	Nitrogen Phosphorous Potassium
PAE	Pepino Aqueous Extract
PAL	Phenylalanine Lyase
PARP	Poly ADP Ribose Polymerase
PWL	Percentage Weight Loss
PC3	Prostate Cancer3-lines
PEE	Pepino Ethanol Extract
PSY	Phytoene Synthase
RCBD	Randomized Complete Block Design
RE	Rutin Equivalent
RIL	Renal Interleukin
ROS	Reactive Oxygen Species
SAS	Statistical Analysis System

SA	Sugar acid ratio
SOD	Super oxidase Dismutase
TA	Titratable Acidity
TPC	Total Phenolic Content
TSS	Total Soluble Solids

CHAPTER ONE
INTRODUCTION

1.1 Background information

Pepino melon (*Solanum muricatum* Aiton) is a fruit vegetable that was mostly grown in the Andes. The centre of origin of pepino is thought to be Southern Colombia or Northern Ecuador (Blanca *et al*., 2007). Pepino melon belongs to the family Solanaceae together with other vegetables like tomato (*Solanum lycopersicum*), pepper (*Capsicum annum*) and potato (*Solanum tuberosum*) (Francke, 2010). In Kenya, pepino melon was introduced in 2013 (Kahuro, 2017) and its demand is increasing due to its nutritional composition, flavour and health benefits. Pepino melon is a herbaceous vegetable that is grown for its fruits (Anderson *et al*., 1996). The fruit is low in calories, contains minerals such as calcium, phosphorus, potassium, and vitamins B1, B2, B3, and C (Diaz, 2006). Pepino fruits contain approximately 92% of water (Sanchez *et al*., 2010) and they vary in size, shape and colour (Herraiz *et al*., 2015), but often the weight ranges between 80 and 250 g. Fruit shape ranges from round to elongated and immature or unripe fruits are yellow in colour while fully mature fruits are brown or yellow in colour and in both there are purple stripes (Rodriguez *et al*., 2011). Pepino fruits have high moisture content and have a mixture of melon and pear aroma when ripe (Rodriguez *et al*., 2011).

Pepino melon can be consumed when raw or cooked and the skin is removed because it has a bitter flavour (Yildiz & Kalkan, 2014). On the contrary, Maheshwari *et al.* (2014) reported that both the exocarp and the mesocarp can be consumed. The fruit stalk is removed and the fruit is cooked for a short time to make it easy to remove the exocarp (Huyskens-Keil *et al*., 2006). Mature green fruits are cooked as a vegetable in stews (Sudha *et al*., 2011). Ripe pepino fruit has a flavor resembling the cantaloupe melon and it can be consumed as a dessert fruit, as an ingredient of fruit salads, in juices, or in ice cream (Martinez-Romero *et al*., 2003). Pepino melon is an emerging crop and there is limited information on production statistics worldwide but in Ecuador it is estimated to be 400 ha^{-1} (Hildago, 2006). In Turkey, the yield of pepino melon was found to be 36.52 t ha^{-1} although the yield was affected by environmental conditions (Cavosoglu *et al*., 2009). Yields of up to 40-50 t ha^{-1} have been achieved in Peru (Popenoe, 1990).

Flower development is a very important process in horticultural crops. Flower abortion due to high temperature stress leads to reduction in yield of many crops (Warner & Erwin, 2005). High temperature stress in tomato plants caused abortion of most of the flowers but 4% of the flowers remained on the plant and later developed into parthenocarpic fruits (Sato *et al*., 2000). Parthenocarpic fruit development in tomatoes is caused by flower abortion (Sato *et al*., 2001) while in pepino melon it's due to failure to produce viable pollen (Ruiz *et al*., 1996). Flower bud activation and formation of inflorescence can occur due to environmental factors, even with nutritional imbalance but, if the high nutrient demand by flowers is not met, then this can lead to flower or fruit abortion (Puthur & Kumar, 2006). Application of NPK (10:20:20) at a rate of 100 g/ plant per year significantly increases the number of inflorescence per plant, number of fruits per plant and decreases flower and fruit abortion of vanilla (Diaz *et al*., 2016). Increasing NPK fertilizer from 0 to 300 kg ha^{-1} leads to a decrease in the number of aborted flowers per plant in okra production (Iyagba *et al*., 2013). In sweet pepper (*Capsicum annum* L.) flower abortion occurs if they are grown at temperatures lower than 15°C or higher than 32-38°C (Rylski & Spigelman, 1986). Floral abortion in *Arabidopsis thaliana* plants varies depending on temperature with complete flower abortion occurring at 36°C after exposure for 72 hours (Warner & Erwin, 2005). Failure of flower buds to develop may be due to reduced photo-assimilate production during high temperature exposure (Warner & Erwin, 2005).

Secondary metabolites are organic compounds which are produced by plants but they are not directly involved in growth and development (Jimenez-Garcia *et al*., 2013). The number and quantitative variation of plant secondary metabolites are affected by the growing environment and abiotic factors which include temperature, soil fertility, water and light (Szakiel *et al*., 2011). Crop production and consequently secondary metabolites depend on nutritional management which is an important factor for success in agriculture (Valiki *et al*., 2015). Both the primary and secondary metabolism of higher plants is influenced by mineral nutrition (Caretto *et al.*, 2015). Species growing in nutrient-poor habitats often have traits that lead to high nutrient retention and high levels of secondary metabolites (Lillo *et al.*, 2008). In a study by Diana *et al*. (2007) it was found that application of NPK fertilizer ($N_{60}P_{60}K_{60}$) produced the highest amount of phenolic compounds in Export II and Campell 1327 varieties of tomatoes. Nitrogen nutrition is of great importance as it influences both the primary and secondary metabolic pathways thus secondary plant metabolites accumulation (Chen *et al*., 2011). Deficiency of crucial elements for instance nitrogen, has been found to enhance accumulation of phenolics compounds in the plant tissues (Ibrahim *et al*., 2011). Different rates of nitrogen

application can influence phytochemical build up in plant tissues (Argyropoulou *et al.,* 2015; Salahas *et al.,* 2011), however very little research has been done on effects of NPK fertilizer on concentration of secondary metabolites in vegetables. Most of the secondary metabolites are synthesized from the intermediates of primary carbon metabolism via phenylpropanoid, shikimate, mevalonate or methyl erythritol phosphate (MEP) pathways (Wahid & Ghazanfar, 2006). High-temperature stress induces production of phenolic compounds such as flavonoids and phenylpropanoids. Phenylalanine ammonia-lyase (PAL) is considered to be the principal enzyme of the phenylpropanoid pathway. Increased activity of PAL in response to thermal stress is considered as the main acclimatory response of cells to heat stress. Thermal stress induces the biosynthesis of phenolics and suppresses their oxidation, which is considered to trigger the acclimation to heat stress for example as in watermelon, *Citrulus vulgaris* (Rivero *et al*., 2001).

1.2 Statement of the problem

Pepino melon demand and popularity is increasing due to its medicinal, nutritional, health and economic value. Though a popular vegetable with multiple uses, pepino melon has no clearly defined agronomic package in terms of fertilizer recommendation. Pepino melon was introduced in Kenya in 2013 and most growers use blanket fertilizer rates borrowed from tomato. Pepino melon differs from the other solanaceous vegetables in that it is vegetatively propagated from stem cuttings, it grows luxuriantly and hence vegetative growth may interfere with flowering, some cultivars are parthenocarpic and it has an extended growing period of about seven months. The effect of fertilizer application and growing environment on postharvest quality attributes is not fully understood. The vegetable is also characterized by flower abortion which leads to reduction in yields. Very little has been documented on the effect of fertilizer application on the concentration of secondary metabolites. Despite many investigations in the area of nutrition, there is limited knowledge on how inorganic fertilizers especially NPK fertilizer influence growth, yield, secondary metabolite concentration, nutrient content, flower abortion and postharvest quality of the fruit vegetable.

1.3 Objectives

1.3.1 General objective

To contribute towards improved production of field and greenhouse grown pepino melon through the use of NPK fertilizer.

1.3.2 Specific objectives

The specific objectives of the study were to determine the:

i) Effects of NPK fertilizer rates on growth and yield of field and greenhouse grown pepino melon.
ii) Effects of NPK fertilizer rates on flower abortion of field and greenhouse grown pepino melon.
iii) Effects of NPK fertilizer rates on the concentration of secondary metabolites of field and greenhouse grown pepino melon.
iv) Effects of NPK fertilizer rates on nutrient content of field and greenhouse grown pepino melon.
v) Effects of NPK fertilizer rates and storage temperature on postharvest quality of field and greenhouse grown pepino melon.

1.4 Hypotheses

The null hypotheses of the study were:

i) NPK fertilizer rates have no effect on growth and yield of field and greenhouse grown pepino melon.
ii) NPK fertilizer rates have no effect on flower abortion of field and greenhouse grown pepino melon.
iii) NPK fertilizer rates have no effect on the concentration of secondary metabolites of field and greenhouse grown pepino melon.
iv) NPK fertilizer rates have no effect on nutrient content of field and greenhouse grown pepino melon.
v) NPK fertilizer rates and storage temperature have no effect on postharvest quality of field and greenhouse grown pepino melon.

1.5 Justification of the study

Pepino melon has currently gained a lot of attention because of its increasing demand in the Kenyan local market. The high demand is due to its nutritional value, medicinal value, health benefits, flavor and attractive appearance. The consumption of pepino melon is increasing around the world owing to the increasing popularity of natural products (Oczan & Arslan, 2011). Pepino fruit has antioxidant, antidiabetic, anti-inflammatory, and antitumor activities (Shathish & Guruvayoorappan, 2014; Sudha *et al.*, 2011). The crop also has a very good market in Japan and Europe thus could be a source of foreign exchange. The vegetable can also grow well in the tropical climate and soils of Kenya and it can be grown outdoors and in the greenhouse. Pepino melon also adapts well to greenhouse environment where the plants are trained as they grow up to 2m tall and the yields obtained are two to three times higher than those obtained outdoors (Mahato *et al*., 2016). Although the biosynthesis of secondary metabolites is genetically controlled, it can be affected by environmental factors such as high temperature, light, drought stress and nutrient elements (Azeizeh, 2012). The evaluation of the content of nutrients and secondary metabolites may contribute to the enhancement of neglected crops including pepino melon as this may result to the discovery of significant or high levels for certain nutrients or secondary metabolites that can contribute to the medicinal properties of the vegetable. Pepino melon might have a potential application prospect in healthy food, nutritional supplements and even pharmaceuticals as it contains high amounts of calcium, sodium, potassium and phosphorous (Oczan & Arslan, 2011). This study sought to investigate the effects of NPK fertilizer rates on growth, secondary metabolite concentration, nutrient content, flower abortion, yield and postharvest quality of pepino melon. It is anticipated that the findings of this study will contribute to the existing scientific knowledge of pepino melon.

1.6 Scope and Limitation of the Study

The study was conducted using one pepino variety; Ecuadorian Gold with respect to its response to different rates of NPK fertilizer on growth, yield, secondary metabolites, postharvest quality, nutritional composition and flower abortion. The study was carried out at Egerton University Njoro but the findings can be applied to other areas with similar conditions. Egerton University lies at a latitude of 0º 23’ South, longitudes 35º 35’ East in the Lower Highland III Agro Ecological Zone (LH3) at an altitude of approximately 2,238 m above sea

level (Jaetzold & Schimdt, 2006). Average maximum and minimum temperatures range from 19 ºC to 22 ºC and 5 ºC to 8 ºC, respectively, with a total annual rainfall ranging from 1200 to 1400 mm. The soils are predominantly mollic andosols (Jaetzold & Schimdt, 2006) There are many varieties of pepino melon but the study only focused on one variety because of limited time and resources. Further studies should explore different varieties in different ecological conditions to ascertain fertilizer requirements, growth and yield. Genetic and molecular studies on the genes which are either upregulated or downregulated in aborted and non-aborted flowers and the effects of either moisture or temperature stress on the concentration of secondary metabolites in pepino melon fruits.

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin of pepino melon

Pepino melon is a well-known cultigen of the Andes. Pepino is described by early Spanish chroniclers as being cultivated along the coast; especially the Moche Valley in Peru. They were used as a popular decorative motif in Moche art (Mahato *et al.,* 2016). Its cultivation was important during pre-Columbian times, but since the decline of the Inca Empire it became a neglected crop (Mahato *et al.,* 2016). However, during the past few decades, there has been renewed interest in pepino cultivation both in the Andean region and in several other countries of Central America, Morocco, Spain, Israel and the highlands of Kenya (Munoz *et al.,* 2014). Pepino melon is a crop with potential for diversification of horticultural production (Munoz *et al.,* 2014). The plant is mainly grown in Chile, New Zealand and Western Australia. In Chile, production is carried out on more than 400 hectares specifically in the Longotoma Valley with most of the produce being exported (Mahato *et al.,* 2016). Recently, pepino has been reported in Colombia, Ecuador, Bolivia, Peru and Chile markets. Commercial production is carried out in Ecuador, Peru, and Chile for export to USA and Europe while in New Zealand pepino is exported to Japan (Prohens *et al.,* 1996).

Taxonomically, pepino melon is placed within *Solanum* subgenus *Potatoe* section *Basarthrum* (Anderson *et al*., 1996). The section, *Basarthrum* is characterized by plants whose flowers fall off with pedicels attached to leave only scars on the inflorescence axis and it includes 11 species, the cultivated pepino and 10 wild species distributed through Central and South America (Mahato *et al.,* 2016). The wild species bear green fruits, 1 to 5 cm in length and typically ovate or round (Plate 2.1). Species from *Solanum* section *Basarthrum* are divided into four series: Series *Muricata*, of which pepino melon (*Solanum muricatum* Aiton) is the only member; series *Caripensia*, which includes eight species; series *Suaveolentia*, whose only member is *Solanum suaveolens* Kunth and Bouche.

Plate 2.1 Diversity of pepino fruits in England

Source: Mahato *et al*. (2016)

2.2 Ecological requirements for cultivation of pepino melon

Pepino melon is relatively hardy and grows at altitudes ranging from close to sea level up to 3,000 m (Mahato *et al*., 2016). However, it performs best in a warm, relatively frost-free climate. The plant can survive low temperature of -2.5 ℃ if the freeze is not prolonged, although this may lead to leaf abscission (Mahato *et al*., 2016). For optimum fruit set pepino requires cool days and nights with temperatures of 12-15 ℃ (Bravo & Arias, 1983). If the temperatures are below 10 ℃ or above 30 ℃ fruit set will be reduced (Prohens *et al*., 2000). Pepino grows well within a temperature range of 10-30 ℃ and the optimum temperature for growth is between 15 and 25 ℃ (Lim, 2013). The crop also adapts well to greenhouse cultivation where training of the plants up to 2 m tall is practiced and the yields obtained are 2-3 times higher than those obtained outdoors (Mahato *et al*., 2016).

Pepino melon can grow on a wide variety of soils as long as they are well drained (Nemati *et al*., (2009). According to Lim (2013), pepino performs best in well drained loamy soils but is intolerant to saline soils. The optimum soil pH range is 6-7.5 (Lim, 2013). It is also drought tolerant and has the ability to recover well after undergoing stress (Popenoe, 1990). The crop does well in areas with an annual rainfall of 500-2000 mm and the optimum range is 800 and 1400 mm (Lim, 2013). In areas with an average rainfall of 1000 mm, irrigation is not necessary

(Popenoe, 1990). Field capacity of 70% and 60 - 65% is the best for growth and pollination, respectively (Nemati *et al*., 2007).

2.3 Health benefits and uses of pepino melon

Pepino melon has antioxidant, antidiabetic, anti-inflammatory, and antitumor properties (Shathish & Guruvayoorappan, 2014; Sudha *et al.,* 2011). Pepino fruits are traditionally used in the management of diabetes mellitus, hypertension, sprue, stroke, high blood pressure, indigestion, cancer, kidney, constipation, and haemorrhoids (Ahmad *et al.,* 2014; Anonim, 2007). Studies in diabetic mice revealed that PAE (Pepino Aqueous Extract) could attenuate the progression of diabetes (Hsu *et al*., 2011). PAE and PEE (Pepino Ethanol Extract) were found to exert similar effects on total phenolic acids but PAE had higher ascorbic acid and total flavonoids than PEE. PAE treatments with pepino extract at 2% and 4% administered for 5 weeks significantly lowered high levels of plasma insulin. PAE treatments also significantly decreased the levels of malonyldialdehyde and Reactive Oxygen Species in kidney and significantly reduced oxidized glutathione formation, increased glutathione level and retained catalase and renal glutathione activities (Maheshwari *et al*., 2014).

Pepino melon has a high antioxidant capacity (Chun *et al.,* 2005). Pepino fruits are rich in vitamin C and carotenoids (Hsu *et al.,* 2011). The fruits and leaves contain alkaloids, flavonoids, and tannins (Saptarini *et al.,* 2011). Phenols and flavonoids can counteract free radicals in the humans. However, the human body needs consumption of antioxidants to prevent accumulation of too many free radicals. It has been found that the phenolic content of the pepino fruit is high (Di Scala *et al.,* 2011) hence the bioactive properties of pepino. It has a total phenolic content of 24.68 mg gallic acid equivalent (GAE)/g and the total flavonoid contents 53.60 mg RE/g, with IC_{50} of 0.44 mg/ml (Sudha *et al*., 2011).

The ethyl acetate extract of ripe pepino melon fruit has a scavenging activity with Half Maximal Effective Concentration (EC_{50}) values of 0.16, 0.82, 39.51, 1.06 and 0.26mg/ml for DPPH radical, reducing power, iron chelation, 2, 2'- azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS radical), Ferric Reducing Antioxidant Power (FRAP) and hydroxyl radical respectively (Maheshwari *et al*., 2014). The antioxidant properties are attributed to the presence of polyphenols in the fruit extracts (Sudha *et al*., 2011). The total phenol and flavonoid content of pepino melon extract were found to be 20.43 mg GAE/g dry weight, 53.85mg/g dry

weight respectively (Maheshwari *et al*., 2014). The total antioxidant capacity observed in the ripe ethyl acetate extract of pepino fruit was 238.27 nM GAE/g (Maheshwari *et al*., 2014). Additionally, pepino extracts have anticancer selective activity against the entire human tumour cell lines which were tested with normal cells having Lethal Dose 50% (LD_{50}) values ranging from 561-825 µg/ml (Maheshwari *et al*., 2014). The extract was found to show a much lower cytotoxicity to Normal Pressure Hydrocephalus (NPH), Human Umbilical Vein Endothelial (HUVEC) and Winstar Institute foetus 38 (WI38) normal cell lines with LD_{50} values of 2.8-3.2 mg/ml which was 3-6 fold higher than on tumour cells. Injection of pepino extract (100 µg) directly into tumour mass was found to reduce tumour volume dramatically in mice inoculated with MKN45 gastric cancer cells. Pepino extract mediated tumour growth inhibition through induction of apoptotic morphology, Deoxyribonucleic Acid (DNA) formation and Poly ADP Ribose Polymerase (PARP) cleavage assay (Rana *et al*., 2014).

The fruit is eaten raw or cooked. When ripe, the fruit is eaten raw as a fresh fruit with a melon taste. The ripe fruit is highly juicy, moderately sweet, has an aromatic fragrance and it can be consumed as a dessert fruit, and as an ingredient of fruit salads, in juices, or in ice cream (Martinez-Romero *et al*., 2003). More frequently, it is eaten as a dessert of fruit in syrup (Yildiz & Kalkan, 2014). The completely pedunculated fruit is cooked for a short time in water so that the skin can easily be removed (Huyskens-Keil *et al*., 2006). When the fruit is mature green, it can be cooked as a vegetable in stews (Anderson *et al*., 1996). Pepino pairs well in savoury salads with avocado and as an accompaniment to cured meats (Maheshwari *et al*., 2014). As a dessert, pepino can be poached in simple syrup and served with ice cream. It is also used as a garnish or pureed and combined with other fruits like mango to make sorbet (Maheshwari *et al*., 2014).

Pepino melon can be used in any part of the meal as an appetizer, entree or dessert (National Research Council [NRC], 1989). South Americans and Japanese consume it frequently as a fresh dessert. It is highly suited in culinary experimentation. In New Zealand it is served with soups, seafood, sauces, prosciutto, meat, fish, fruit salads and desserts (Maheshwari *et al*., 2014). The fruits can also be frozen, jellied, dried, canned or bottled (Prohens *et al*., 1996). The skin of some varieties like 'El Camino' has a disagreeable flavour. The seeds are soft, tiny and edible (NRC, 1989). Prohens *et al*. (1996) reported that the Spanish use the fruit juice mixed with pink ointment against kidney heat and the fruit is also a good ascorbutic. Pepino melon is also a good source of α-galactosidase (Sen *et al*., 2011). Three-phase partitioning was found to

be a very rapid, simple and highly efficient method of bioseperation of pepino α- galactosidase making the enzyme a good choice for industrial applications (Sen *et al*., 2011).

2.4 Nutritional composition of pepino melon

Pepino melon breaks down into glucose to provide energy. It is rich in β-carotene which is converted in the body to vitamin A, which plays a role in the processes of vision, reproduction, and metabolism (Maheshwari *et al*., 2014). Moreover, β-carotene is an antioxidant whose function is to reduce the concentration of peroxyl radicals (Maheshwari *et al*., 2014). The antioxidant ability of β-carotene is derived from its ability to stabilize carbon-core radicals. β-carotene is effective at low concentrations of oxygen and this could complement the antioxidant properties of vitamin E which is effective at high concentrations of oxygen (Hsu *et al*., 2011). The antioxidant property of β-carotene also aids in cancer prevention, especially skin and lung cancer. β-carotene can reach more parts of the body in a relatively longer time than vitamin A, thereby providing optimal protection against cancer. β-carotene is good for children's intelligence and also prevents dementia for adults (Maheshwari *et al*., 2014).

Pepino melon is rich in Vitamins A, B, C and K, proteins and minerals like Fe, Cu, Ca, and K and contains fibre which ranges from 1 to 1.5 g/ 100 g (Maheshwari *et al*., 2014). In the digestive tract, fibre binds bile acids and also aids the digestive system. According to Nemati *et al*. (2009), ripe pepino fruits contain 9.5% soluble solids, 4.6% carbohydrates, 0.06% acids and 34.25 mg (%) vitamin C (Bravo & Arias, 1983). Aromatic and volatile compounds such as esters, aldehydes, ketones, terpenes, alcohols, mesifuran and beta-damascenone are found in pepino fruits (Rodriguez- Burruezo *et al*., 2004). The mineral content of pepino melon is shown in Table 2.1

Table 2.1 Mineral contents of pepino melon fruits

Minerals	Values (mg/kg)
Aluminium	90.46
Boron	27.23
Calcium	3256.96
Copper	17.17
Iron	79.73
Potassium	43465.59
Magnesium	2541.12
Manganese	7.39
Molybdenum	1.14
Sodium	1496.13
Nickel	1.66
Phosphorous	7906.32
Selenium	2.23
Zinc	29.67

Source: Oczan and Arslan (2011)

2.5 Effect of fertilizer on nutrient content of vegetables

Fertilizer affects the productivity and nutrient quality of crops (Oloyede, 2012). Weak vegetative growth, poor fruit setting, undesirable fruit quality and low nutritional quality result from inadequate levels of the primary nutrients namely: nitrogen, phosphorus and potassium (Liu *et al*., 2010). Pepino fruits from plants which were fertilized with the lowest magnesium rate (0.5 mg g^{-1} per plant) produced fruits with the highest total nitrogen and potassium while the fruits of plants fertilized with the highest Mg rate (1.5 mg g^{-1} per plant) had the highest amounts of Ca and Mg (Francke, 2010a). Fruits from plants which were not fertilized had the highest P content. It was also noted that fully ripe fruits contained significantly more N and Mg while unripe fruits had a higher content of P, K and Ca. (Francke, 2010a). When Potassium fertilizer was used, fruits from plants fertilized with 2 g K $plant^{-1}$ had the highest total N while those fertilized with 1 g K $plant^{-1}$ accumulated the highest amount of calcium (Francke, 2010b). Non fertilized plants had the highest potassium content and fully ripe fruits contained more N and Mg while unripe fruits had a high content of P, K and Ca (Francke, 2010b).

Tomaszewska and Mazur (2007) carried out a study on pepino melon using two multicomponent fertilizers namely: Polifoska S and Polifoska 5 and found that higher doses of the fertilizer significantly decreased extract content and acidity of fruits but increased vitamin C and total sugars. Potassium fertilization in the form of KNO_3 at a dosage of 8 g per plant resulted in the highest dry matter and vitamin C content in eggplant (Michalojc & Buczkowska, 2009). Neshev and Manolov (2015) reported that increasing rates of KCl decreased more severely dry matter, starch and vitamin C contents of potato tubers. However, the potassium source (KCl and K_2SO_4) did not affect the content of reducing sugars. The use of $Ca(NO_3)_2$ on sweet pepper had a positive effect on the accumulation of vitamin C and carotenoids compared to other fertilizers. Reduced calcium spraying proved to be beneficial in sweet pepper fruit yield and carotenoid concentration (Buczkowska *et al*., 2016).

Nitrogen application significantly increased the fat, protein, carbohydrate, crude fibre, ash contents, vitamin C and mineral nutrients of hot pepper fruits (Ayodele *et al*., 2015). Potassium application at 60 kg ha^{-1} significantly increased the yield, fruit weight, firmness, acidity, dry matter, mineral matter, total soluble solids and total invert sugars of tomato fruit over control while higher levels of K (90 and 120 kg ha^{-1}) did not significantly increase yield and quality parameters (Ahmad *et al*., 2015). Ascorbic acid was not affected by the application of potassium (Ayodele *et al*., 2015). The application of NPK fertilizer on *Amaranthus spp* increased the protein content. *Amaranthus hybridus* had the highest protein composition (Oyejedi *et al*., 2014) compared to the other *Amaranthus* species. Makinde (2013) found that crude protein in the leaves of *Moringa oleifera* increased as the fertilizer rate increased from 8.18% in the control to 19.01% in plants that received 120 kg NPK ha^{-1}. Makinde (2013) concluded that the application of 30 kg NPK ha^{-1} recorded the highest amount of calcium (6.30 ppm) and iron (0.3 ppm) while the plants which received 60 kg NPK ha^{-1} had the highest phosphorous content (401.74ppm). Recent findings by Ligoriya and Nithiya (2015) revealed that the application of NPK fertilizer resulted to low amount of proteins but increased ascorbic acid content of *Solanum nigrum* compared to the application of cattle dung manure and vegetable waste compost. Increasing NPK (20:10:10) fertilizer rates did not have a significant effect on proteins, vitamins and fat contents of okra fruit and also increasing the storage period led to an increase in weight loss and deterioration of vitamins, fat and protein content of okra fruits (Adewoyin, 2012). Information on the effect of NPK fertilizer on nutrient content of pepino melon is scanty.

2.6 Effect of NPK fertilizer and storage temperature on Postharvest quality of vegetables

The type and amount of fertilizer supplied to tomato plants can influence not only its yield but also its nutrient content, taste and postharvest storage quality (Sainju *et al*., 2003). Good tomato fruit qualities such as excellent red colour, low pH, high titratable acidity, high soluble solids, firm fruits, low seed content, and long shelf life largely depend on genetic control are influenced by the type, amount and time of fertilizer application (Ogunlela *et al*., 2005). Tomato fruits fertilized with chicken manure had a longer shelf life than those fertilized with NPK (15:15:15) fertilizer (Appiah, 2015). On the contrary, Nyamah *et al*. (2011) reported that tomato fruits from NPK fertilized plants had a longer shelf life than those from poultry manure treatments. Increasing NPK (20:10:10) fertilizer led to a significant increase in the percentage weight loss of okra (*Abelmoschus esculentus* L. Moen) fruits (Adewoyin, 2012). Babatola and Adewoyin (2004) found that application of NPK (15:15:15) did not have a significant effect on fruit firmness, weight loss and colour change of cucumber. Similarly, Zegbe *et al*. (2015) reported that the application of different rates of NPK fertilizer did not have a significant effect on fruit firmness and total soluble solids of 'Cristalina' cactus pear.

Soil amendments have an effect on the postharvest quality of tomato fruits (Nyamah *et al*., 2011). The application of 250 kg ha^{-1} of NPK (15-15-15) and top dressing with 250 kg ha^{-1} of 'Asasewura' cocoa fertilizer can be used to improve the postharvest quality of tomato by reducing fruit weight loss, fruit membrane ion leakage, fruit decay and improve fruit firmness, fruit general appearance, fruit shelf life and total soluble solids (Nyamah *et al*., 2011). Application of the recommended amount of NPK (N=253, P=90 and K= 125 kg ha^{-1}) led to the highest shelf life (9-12 days) compared to 50% less and 50% more than the recommended NPK rate in tomato production (Salam *et al*., 2010). Tomatoes fertilized with NPK recorded longer shelf life than those fertilized with poultry manure (Nyamah *et al*., 2011). On the contrary, Appiah (2015) reported that poultry manure was more superior to NPK fertilizer because tomato fruits from NPK (15:15:15) treated plots took a short time to become watery as compared to poultry manure.

Long term storage of pumpkin at room temperature and humidity led to a decrease in ascorbic acid, beta carotene and total soluble solids (Rahman *et al*., 2013). In another study, apple stored at 0°C had the highest firmness while fruits stored at 12°C had the lowest firmness (Khorshidi

et al., 2010). Total soluble solids and titratable acidity also reduced in apple fruits stored at 12°C while weight loss % increased as storage temperature increased. Weight loss % was not significant in apple fruits stored at 0° and 5°C but it was significant in fruits stored at 12°C (Khorshidi *et al*., 2010). It was therefore concluded that storage of apple fruits at 0°C could maintain better produce quality (Khorshidi *et al*., 2010). Hailu (2016) reported that as storage temperature increased titratable acidity of mango cv. Keitt decreased after three weeks of storage while total soluble solids decreased as temperature decreased. Average weight loss of mango fruits increased as storage temperature increased from 7°, 10°, 13° and 21-24°C (Hailu, 2016). Storage of okra fruits in cold storage at 4°C decreased the cumulative weight loss and this was attributed to the reduction in respiration and transpiration. Fruits stored at room temperature decayed within a period of less than 2 weeks (Adewoyin, 2012). Gabiroba fruits (*Campomanesia pubescens*) should be stored at 12°C for short term storage because vitamin C and phenols were retained at this temperature and 6°C for long term storage (Silva *et al*., 2013). There is limited information on the effect of NPK fertilizer and temperature on postharvest quality of pepino melon.

2.7 Effect of fertilizers on secondary metabolites

Secondary metabolites are organic compounds which are produced by plants and are not directly involved in growth and development (Hounsome *et al*., 2008). According to the British Nutrition Foundation, secondary metabolites can be classified into four major groups: phenolic and polyphenolic compounds (about 8000 compounds), terpenoids (about 25,000 compounds), alkaloids (about 12,000 compounds) and sulphur containing compounds (Goldberg, 2003). Some secondary metabolites are natural antioxidants and they are produced by plants in different concentrations (Chanwitheesuk *et al*., 2005). Nutrient supply can influence the occurrence of secondary metabolites in plants (Fine *et al*., 2006). The accumulation of secondary metabolites is determined by fertilization with nutrients and by maintaining a favourable C/N balance together with proper environmental conditions, light intensity, plant species and age (Azaizeh, 2012).

Phuong and Emily (2008) observed that high total phenolic content, rosmarinic acid and caffeic acid concentration in basil (*Ocimum basilicum*) was low at the lowest nitrogen treatment. On the contrary, Zamani *et al*. (2014) reported that secondary metabolites increased with an increase in nitrogen and phosphorous treatments in the salt stressed madder. In another study,

nitrogen fertilization significantly increased rosmarinic acid of *Satureja hortensis* L. (Mesbah *et al*., 2010). In the study it was concluded that the application of 150 kg N ha^{-1} was the best for the production of rosmarinic acid. The application of calcium carbonate had no effect on rosmarinic acid (Mesbah *et al*., 2010).

Nitrogen nutrition is of great importance as it influences both the primary and secondary metabolic pathways thus secondary plant metabolites accumulation (Chen *et al.,* 2011). Deficiency of nitrogen has been found to enhance accumulation of phenolics compounds in the plant tissues (Ibrahim *et al.* 2011). Low nitrogen fertilization resulted to high content of flavonoids and anthocyanin's in *Labisia pumila* (Ibrahim *et al*., 2012). The application of high amounts of nitrogen led to a decrease in the production of secondary metabolites in *Labisia pumila* and this was attributed to the correlation of reduced phenylalanine lyase (PAL) activity with low C/N ratio, photosynthetic rates and total non-structural carbohydrates (Ibrahim & Jafaar, 2011). On the contrary, the application of a nitrate fertilizer on tobacco (*Nicotiana tabacum*) induced the biosynthesis of nicotine by inhibiting the biosynthesis of phenylpropanoid and flavonoid (Fritz *et al*., 2006). The application of sole ammonium fertilizer and no nitrogen application resulted to the accumulation of total phenolic and total flavonoid contents in amaranth when compared to nitrate and ammonium nitrate mixture (Munene *et al*., 2017). On the contrary, Salahas *et al.* (2011) observed that deficiency of nitrogen stimulated the biosynthesis of total phenolics and betacyanins in red beet (*Beta vulgaris* L.). The concentration of total phenolics significantly increased in nitrogen starved sweet basil plants (*Ocimum basilicum* L.), indicating that biosynthesis of plant secondary metabolites are stimulated by nitrogen deficiency (Argyropoulou *et al.,* 2015). Apple plants grown under high nitrogen supply had low levels of phenolics and flavonoid content (Leser & Treutter, 2005).

Deficiency in mineral elements such phosphorous and potassium have been reported to up-regulate the amounts of polyphenols either as existing pools or by inducing their *de novo* synthesis (Glynn *et al.,* 2008). Lack of phosphorous and water increased the amount of flavonoids in *Arabidopsis* (Nakabayashi *et al*., 2014). Ligoriya and Nithiya (2015) found that the use of organic fertilizers increased flavonoids and total phenolic as compared to the use of NPK fertilizer in *Solanum nigrum.* On the contrary, Oloyede (2012) reported that the application of 180 kg NPK ha^{-1}led to increase in phenolic content in pumpkin leaves compared to the other NPK levels. It was also observed that further increase of the NPK fertilizer led to a decline in phenolic compounds. Flavonoids were not significantly affected by the NPK

fertilizer (Oloyede, 2012). Most of the studies have only majored on nitrogen and its effect on the accumulation of secondary metabolites in vegetables but there is inadequate information on the effect of NPK on accumulation of secondary metabolites in pepino melon.

2.8 Flower abortion in vegetables

Abortion is defined as the cessation of development and growth of an organ, after which it usually abscises (Wubs *et al*., 2009). Reproductive organs that abort can be buds, flowers, or young fruits (Wubs *et al*., 2009). Successful flower development is critical for production of many agronomic and horticultural crops (Warner & Erwin, 2005). Exposure to high temperatures results in floral abortion on many species, including tomato (*Solanum lycopersicum* L.), sweet pepper *(Capsicum annum* L.), beans (*Phaseolus vulgaris* L.), avocado (*Persea americana* Mill.), cowpea (*Vigna unguiculata* (L) Walp) and cotton (*Gossypium hirsutum* L.). In addition, a physiological disorder termed 'blindness' in roses is due to abortion of the flower at an early stage of development under low irradiance or high temperature conditions (Hubbell, 1934). High temperature-induced floral bud abortion can result to reduced yield of many crops (Warner & Erwin, 2005).

Temperatures and the timing of exposure causing floral bud abortion vary across species (Warner & Erwin, 2005). When day/night temperatures are increased from 25/20°C to 30/25°C inflorescence development in *Phaleonopsis amabilis* Blume was inhibited (Chen *et al*., 1994). Cowpea floral development was suppressed at night temperatures above 24°C, whereas day temperatures of 33°C did not impair floral development (Ahmed & Hall, 1993). On the contrary, cotton flower bud and boll abscission was low under day/night temperatures of 35/27°C but increased to over 90% at day/night temperatures of 40/32°C (Reddy *et al*., 1992). This is because high temperature is the environmental factor that induces abortion of reproductive organs by increasing ethylene production in the reproductive organs (Huberman *et al*., 1997). In a study by Warner and Erwin (2005) on the basis of high temperature-induced floral bud abortion using naturally occurring variation for heat-tolerance of floral development among *Arabidopsis thaliana* (L.). High temperature-induced floral bud abortion was dependent on both temperature and duration of exposure. Normalizing high temperature exposures to degree-hours(C-h) above 33° C indicated that abortion of flower buds increased as exposure increased between 200 and 300 °C-h above 33°C and exposures > 300 °C-h above 33°C resulted in abortion of the entire primary inflorescence (Warner & Erwin, 2005). The entire

inflorescence aborted because of the long period of exposure to high temperature because under high temperatures, auxin transport from the flowers decreases while the production of ethylene increases and this leads to flower abortion (Aloni *et al*., 1994).

Abortion of reproductive organs is common in sweet pepper and it occurs even when the sweet peppers are grown in a controlled environment (Wubs *et al*., 2009). Flower abortion may be caused by environmental factors like light, carbon dioxide and temperature, the effects of plant growth and development like competition with other fruits and management practices like pruning, or cultivar choice (Wubs *et al*., 2009). Abortion levels in the bell-type pepper cultivar 'Delphin', over a 19-week period, increased from 59% to 83% when the 24 h mean temperature was increased from 16ºC to 24ºC (Bakker, 1989) and also increasing daytime as well as night temperatures increased abortion, but the effect of night temperature was more significant. Marcelis *et al*. (2004) found that a constant temperature of 33ºC for 4 days caused 100% abortion of buds and flowers of cultivar 'Mazurka' of bell pepper because of the long period of exposure which leads to an increase in ethylene production hence abortion of flower buds (Aloni *et al*., 1994).

Physiologically, flower abortion takes place at the abscission zone, which consists of a morphologically distinguished layer of cells in the pedicel (Roberts *et al*., 2000). Flower or fruit abortion takes place when the middle lamina and cell wall in the abscission zone breakdown and the cells separate and then flower or fruit abscission occurs (Roberts *et al*., 2000). According to Lieth *et al*. (1986) four stages are involved in the abscission of cotton bolls: stage 1, application of the stimulus; stage 2, separation layer starts to form, stage 3, completion of the layer; and stage 4, abscission of the boll. Abscission is reversible in the first two stages, but stage 3 and stage 4 are irreversible. Information on flower abortion in pepino melon is insufficient.

CHAPTER THREE

EFFECT OF NPK FERTILIZER RATES ON GROWTH AND YIELD OF FIELD AND GREENHOUSE GROWN PEPINO MELON (*Solanum muricatum* Aiton)

Abstract

Pepino melon (*Solanum muricatum* Aiton) is an exotic vegetable whose consumption is on the increase in Kenya due to its health and nutritional benefits. Pepino melon was introduced in Kenya in 2013, has no fertilizer recommendations and the effect of NPK fertilizer on growth and yield is not documented. A study was conducted at Egerton University, Kenya in 2018-2020 to investigate the effects of NPK fertilizer rates (0, 100, 200. 300 and 400 kg ha^{-1}) on growth and yield of field and greenhouse grown pepino melons. The experiment was laid in a randomized complete block design with three replications. Data were recorded on plant height, stem diameter, number of leaves per bush, number of branches, days to 50% flowering, fruit weight, dry weight and total yield. Data were analysed using analysis of variance with the SAS statistical package. Significant means were separated using Tukey's honestly significant difference at $p \leq 0.05$. Results indicated that NPK fertilizer rates and growing environment influenced growth and yield of pepino melon. At 100 DAP plants grown in the greenhouse and supplied with 200 kg NPK ha^{-1} had a stem diameter of 14.01 mm which was significantly higher $p \leq 0.05$ compared to plants grown in the open field and supplied with 300 kg NPK ha^{-1} which recorded a stem diameter of 11.71 mm in trial two. Application of 300 kg NPK ha^{-1} for field grown pepino melons gave the highest total yield of 1102.48 kg ha^{-1} and 1060.55 kg ha^{-1} in trial one and two, respectively. In conclusion, application of 300 kg ha^{-1} of NPK fertilizer for field grown pepino melon is recommended.

3.1 Introduction

Pepino melon (*Solanum muricatum* Aiton) is an exotic vegetable which belongs to the family solanaceae (Sudha *et al*., 2012). The fruit was initially grown in South America but its cultivation has extended to Australia, New Zealand, USA (Huyskens *et al*., 2006) Central America, Morocco, Spain, Israel and the highlands of Kenya (Munoz *et al*., 2014). The vegetable was introduced in Kenya in 2013 and its consumption in Kenya is increasing due to its health, nutritional and economic value (Kahuro, 2017). The edible part is the fruit which is aromatic and juicy (Martinez-Romero *et al*., 2006). The fruit can be eaten when mature green as a vegetable in stews (Sudha *et al*., 2012) and the ripe fruit is eaten as a dessert fruit, in salads

and ice creams (Martinez-Romero *et al*., 2006). Pepino is low in calories but very rich in minerals such as calcium, phosphorous and potassium and vitamins A, B1, B2, B3 and C (Diaz, 2006). The fruit varies in size and shape depending on the cultivar and the colour ranges from completely purple, solid green or green with purple stripes, or cream coloured with or without purple stripes (Oczan & Arslan, 2011). Pepino melon is quite different from other solanaceous vegetables because it is vegetatively propagated by use of cuttings, grows luxuriantly and vegetative growth may compete with fruit set, most pepino melon cultivars tend to be parthenocarpic with some being obligately parthenocarpic and the fruits take a long time of up to 70 days to fully ripen (Herraiz *et al*., 2015). Pepino melon is an emerging crop and there is little information on production statistics worldwide but in Ecuador production is estimated to be carried out on 400 ha^{-1} (Hildago, 2006). In Turkey, pepino melon yield was found to be 36.32 t ha^{-1} and it was reported that growth and yield of pepino melon is influenced by environmental conditions (Cavosoglu *et al*., 2009). In Peru, the yields of up to 40-50 t ha^{-1} have been reported (Popenoe, 1990). Pepino melon grows luxuriantly and therefore nitrogen fertilizer application should be controlled to avoid excessive vegetative growth at the expense of fruit set (Herraiz *et al*., 2015). Several studies have been carried out on pepino melon but there are no studies on the effects of fertilizers on growth and yield of pepino melon. In addition, fertilizer recommendations for pepino melon production are not documented.

Vegetable including pepino melon play a vital role in food and nutrition security (Dunsin *et al*., 2019). Soil fertility is an important factor for improving crop growth and yield (Kolodzeij, 2006). The amount of fertilizer applied has a positive impact on the availability of nutrients for the crop and nutrient status of the soil (Bijlsma *et al*., 2000). Fertilizers are compounds that are added to the soil to supply one or more elements needed by crops for growth and development (Masarirambi *et al*., 2012). The three primary macronutrients are nitrogen, phosphorous and potassium, secondary macronutrients include calcium, sulphur and magnesium, while the minor elements include copper, zinc, iron, manganese, molybdenum and boron (De, 1988). Fertilizers improve soil fertility or replenish elements which are removed from the soil through harvesting, leaching or soil erosion (Ginindza *et al*., 2015). Most inorganic fertilizers have an appropriate concentration of nitrogen, phosphorous and potassium for various crops and growth conditions (Ginindza *et al*., 2015). Nitrogen promotes leaf growth and is involved in protein and chlorophyll synthesis while phosphorous promotes root, flower and fruit development and potassium is involved in stem and root growth and protein synthesis (Ginindza *et al*., 2015). Nitrogen deficiency in the soil reduces number of fruits, fruit size,

storage quality, colour and taste of tomato (Savvas *et al*., 2008). On the other hand, application of excess nitrogen delays fruit set and maturity of tomato and this leads to low yields (Kaniszewki & Elkner, 1990). Availability of these elements to crops has a significant effect on growth and yield of most vegetables (Aluko *et al*., 2014). Crop yield is as a result of the interactions between genotype, climate, soil conditions and management practices carried out on the crop (Jing *et al*., 2008). Therefore, adequate soil fertility is essential in for sustainable vegetable production. The main challenge in vegetable production is nutrient deficiency due to improper use of fertilizers (Shaheen *et al*., 2010). Proper vegetable growth requires ideal nutrient supply (Liu *et al*., 2010). In the tropics, soil fertility is declining due to excessive rainfall and continuous cultivation has led to lack of essential nutrients in the soil (Obalum *et al*., 2012). NPK fertilizer has the ability to release nutrients very fast into the soil and thus help sustain soil fertility and crop production (Uyovbisere *et al*., 2012). The present study sought to investigate the effects of different rates of NPK fertilizer on growth and yield of field and greenhouse grown pepino melons.

3.2 Materials and methods

3.2.1 Site Description

The experiment was conducted at the Horticulture Research and Teaching Field, Egerton University, Njoro. The field lies at a latitude of 0º 23' South, longitudes 35º 35' East in the Lower Highland III Agro Ecological Zone (LH3) at an altitude of approximately 2,238 m above sea level (Jaetzold & Schimdt, 2006). Average maximum and minimum temperatures range from 19 ºC to 22 ºC and 5 ºC to 8 ºC, respectively, with a total annual rainfall ranging from 1200 to 1400 mm. The soils are predominantly mollic andosols (Jaetzold & Schimdt, 2006). The mean monthly temperatures in the greenhouse and field during the experiment are presented in Table 3.1.

Table 3.1 Average monthly field and greenhouse temperature (°C) in trial one and two

		2018		2019					
		Nov	Dec	Jan	Feb	Mar	Apr	May	June
Trial one	Field	20.9	19.7	20.9	21.7	22.8	22.6	21.2	18.9
	Greenhouse	30.3	21.0	33.4	30.2	29.4	34.0	35.8	28.0
		2019						2020	
		July	Aug	Sept	Oct	Nov	Dec	Jan	Feb
Trial two	Field	19.1	19.2	20.5	19.3	19.3	18.9	19.1	22.6
	Greenhouse	18.5	29.4	30.0	28.0	32.0	28.0	35.3	36.7

Source: Department of Agricultural Engineering, Egerton University

3.2.2 Experimental design, treatment application, crop establishment and maintenance

The experimental design was Randomized Complete Block Design (RCBD) with five treatments and three replications. The five treatments were different rates of NPK fertilizer (17:17:17) (0, 100, 200, 300 and 400 kg ha^{-1}). Pepino melon seedlings (Ecuadorian Gold variety) were obtained from Garlic and Pepino Farm, Nakuru, Kenya. For the field experiment, each experimental unit was 3.2m × 3.2m (Figure 3.1) and the seedlings were planted in rows 80 cm apart and 50 cm from plant to plant within the rows (Food and Agriculture Organization [FAO], 1994) to give a total of 24 plants per unit. In the greenhouse experiment, each experimental unit was 2m × 5 m (Figure 3.2) at the same spacing as in the field experiment to give a total of 25 plants per experimental unit. Soil samples were collected from the experimental units in the field and greenhouse and analysed for total N, P, K and pH before onset of the experiment using the method described by Okalebo *et al*. (2002) and the results are presented in Table 3.2. NPK fertilizer was applied and thoroughly mixed with the soil before placing the seedlings in the transplanting holes. Weeding was done uniformly to all experimental units. Field capacity was determined as described by Cong *et al*. (2014) thereafter tensiometers were placed in two experimental units in each block. Irrigation was done when the field capacity fell below 60% because pepino requires a field capacity of 60-65% (Lim, 2013). Drip irrigation was used in the greenhouse when the field capacity fell below 60% and it supplied about 180 litres to the 375 plants in the greenhouse. The experiment was carried out in two trials. Trial one was carried out from November 2018 to June 2019 and trial two from July 2019 to February 2020.

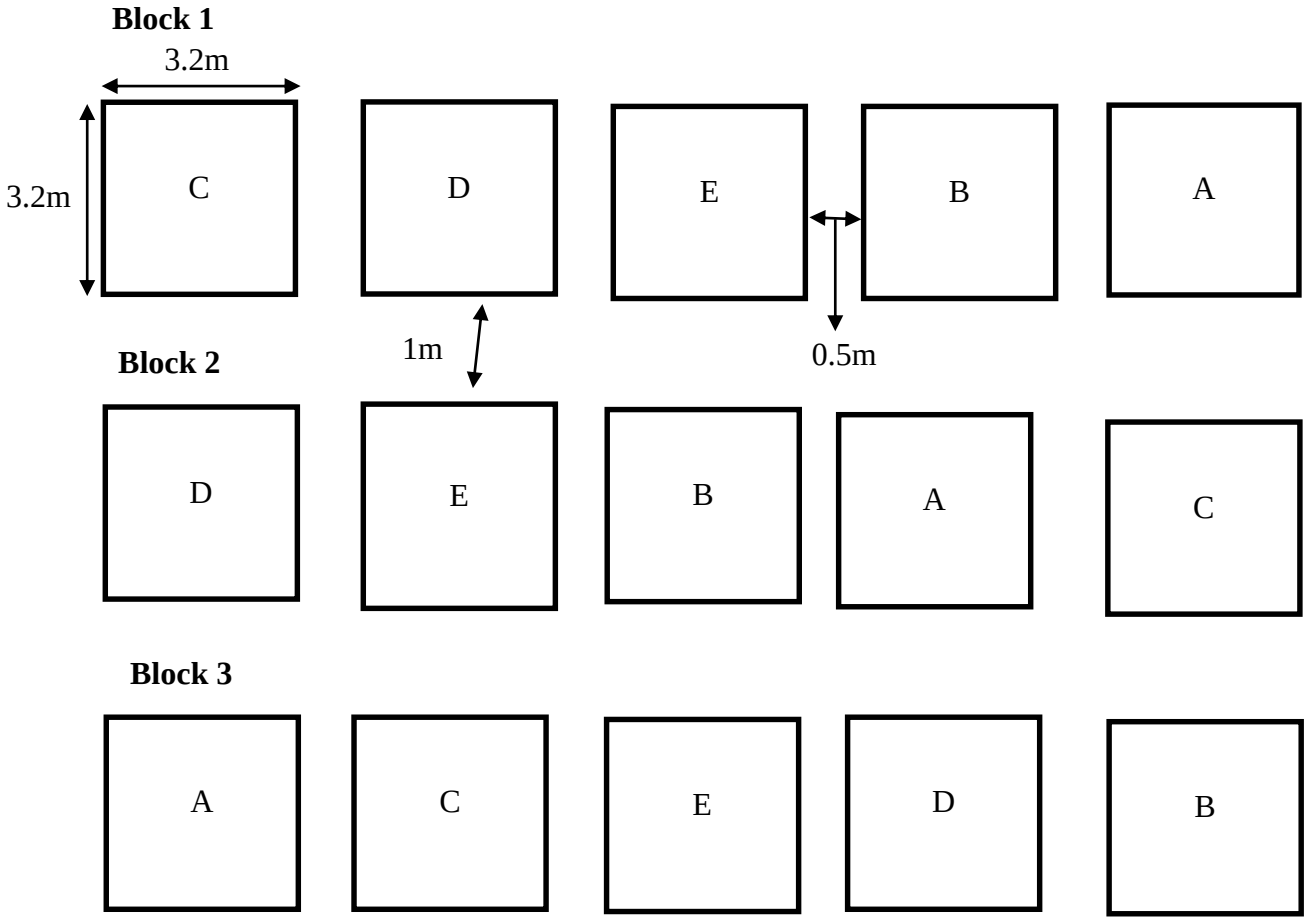


Figure 3.1 Experimental layout for the field experiment

KEY: A- 0kg NPK ha^{-1} **B-**100kg NPK ha^{-1} **C-**200kg NPK ha^{-1} D- 300kg NPK ha^{-1} **E-**400 kg NPK ha^{-1}

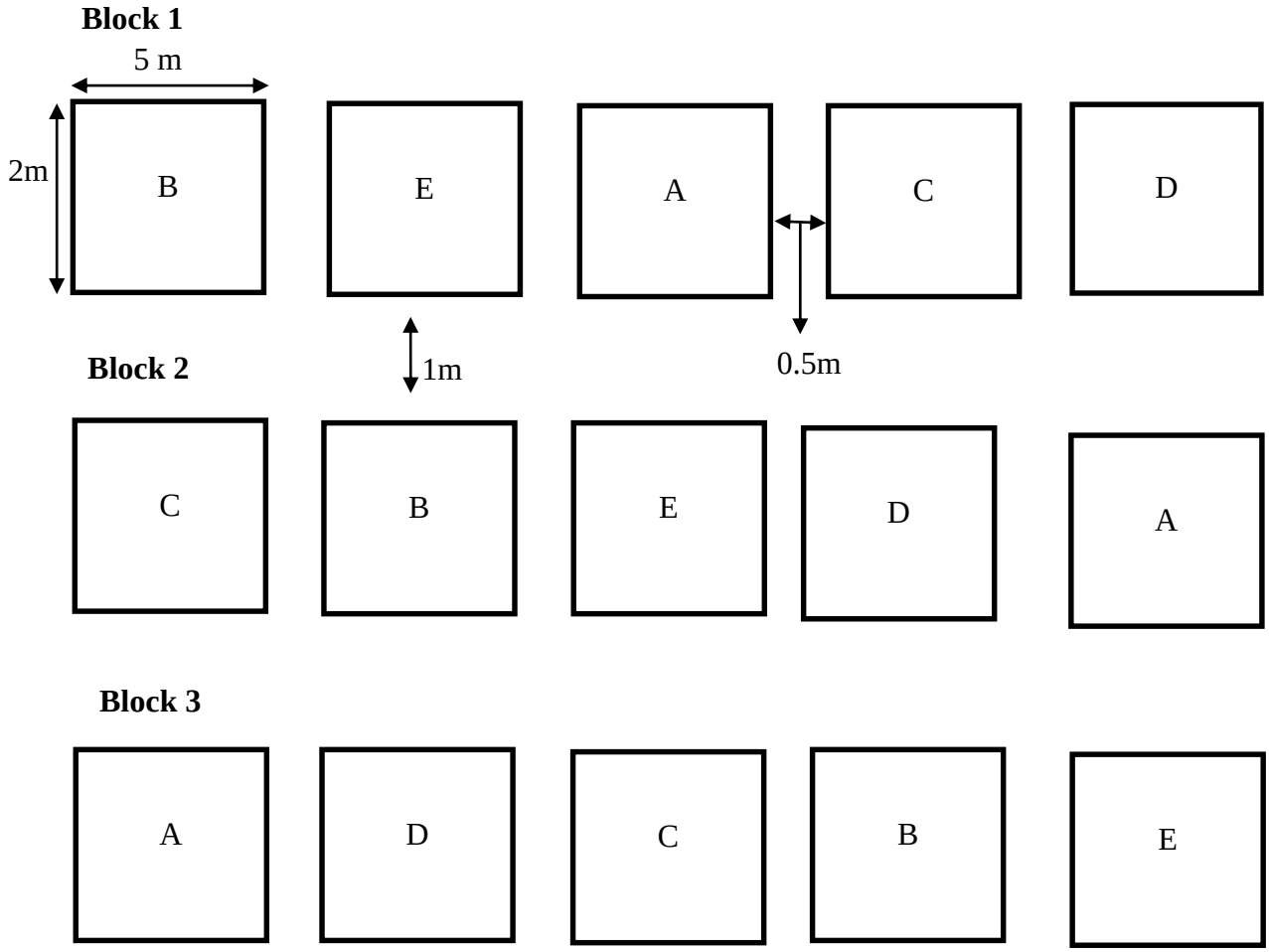


Figure 3.2 Experimental layout for the greenhouse experiment

KEY: A- 0kg NPK ha^{-1} **B**-100kg NPK ha^{-1} **C**-200kg NPK ha^{-1} **D**- 300kg NPK ha^{-1} **E**-400 kg NPK ha^{-1}

Table 3.2 Pre-planting soil analytical results in trial one and two

Soil properties	Field		Greenhouse	
	Trial 1	Trial 2	Trial 1	Trial 2
Total Nitrogen (%)	0.28-0.45	0.21-0.40	0.21-0.78	0.20-0.72
Potassium (mg/kg)	12.6-22.13	10.2-20.45	19.4-48.6	17.5-38.7
Available P (mg/kg)	1.31-1.72	1.23-1.63	1.96-3.06	1.88-2.99
pH(water)	4.8-5.7	4.6-5.2	4.38-6.03	4.25-6.00

3.2.3 Data collection

Data were collected and recorded on the following variables from a total of 8 plants from the two middle rows of each experimental unit. The outer rows in each experimental unit served as guard rows.

i) Plant height: This was determined from the ground to the tip of the plant by means of a tape measure in centimeters. This was done at an interval of 14 days starting 30 DAP up to 100 DAP.

ii) Stem diameter: The same pepino melon plants used to measure plant height were used to measure stem thickness at the ground level using vanier callipers in millimeters once after every 14 days starting from 30 DAP up to 100 DAP.

iii) Number of leaves per bush before flowering: Number of leaves were counted from the selected plants just before flowering. A mean number of leaves was computed for each experimental unit.

iv) Number of branches per plant: The number of primary branches per plant was determined by counting from the selected plants from each experimental unit at the peak of vegetative phase, just before flowering. A mean number of branches was computed for each plot from the 8 selected plants.

v) Days to 50% flowering: The number of days from planting to when 50% of the plants in each treatment had at least one flower was monitored and recorded for each experimental unit. Data obtained was used to compute the mean number of days to 50% flowering for the different treatments.

vi) Fruit Weight: Mature fruits were harvested from the different experimental units and weighed separately using an electronic weighing machine (JA10003) to determine fruit weight in grams (g). This was done on individual fruits from the selected plants in each experimental unit.

vii) Total yield: Mature fruits from each experimental unit were harvested and weighed on an electronic weighing machine (JA10003) to determine their weight in grams (g) which was later converted to kg ha^{-1}. Fruits from each experimental unit were pooled together before being weighed.

viii) Dry weight: Fresh ripe fruits were harvested, weighed to determine initial fresh weight, cut into small pieces and placed in a drying oven at 105°C for 24 h until a constant weight was achieved. Dry weight was expressed in g.

3.2.4 Data analysis

Data collected were subjected to Analysis of variance (ANOVA) and significant means separated using Tukey's honestly significant difference (Tukey's HSD) test at $p \leq 0.05$. The SAS statistical package (SAS Institute, 2005) was used for data analysis.

The basic RCBD model fitted for the experiment was:

$Y_{ijk} = \mu + \alpha_i + \beta_j + \tau_k + \beta\tau_{jk} + \varepsilon_{ijk}$

i=1, 2, 3; j = 1, 2, 3, 4, 5; k = 1, 2

Where; Y_{ijkl} – Pepino melon response

μ – Overall mean

α_i – Effect due to the i^{th} block

β_j– Effect due to j^{th} fertilizer rate

τ_k- Effect due to k^{th} growing environment

$\beta\tau_{jk}$- Interaction effect of the j^{th} fertilizer rate and k^{th} growing environment

ε_{ijk}– Random error component which was normally and independently distributed about zero mean with a common variance σ^2.

3.3 Results

3.3.1 Effect of NPK fertilizer rates and growing environment on plant height of pepino melon plants

NPK fertilizer rates and growing environment had a significant effect on plant height in both trials. Plants which were supplied with 200kg NPK ha^{-1} and grown in the greenhouse were significantly taller at $p \leq 0.05$ than those supplied with other NPK rates except field grown plants supplied with 300 kg NPK ha^{-1} in trial one and two (Table 3.3 and 3.4) The same trend was observed from 30 to 100 DAP. In trial two, at 44 DAP greenhouse grown plants supplied with 200 kg ha^{-1} were significantly taller than field grown plants supplied with 300 kg ha^{-1} (Table 3.4). Generally, it was noted that plant height increased from 30 DAP to 100 DAP. Plants which were planted in the greenhouse were generally taller than those grown in the field in both trials (Table 3.3 and 3.4).

Table 3.3 Effect of NPK fertilizer rates and growing environment on plant height (cm) of pepino melon in trial one (Nov 2018–June 2019)

Environment	Fertilizer (kg ha^{-1})	30 DAP	44 DAP	58 DAP	72 DAP	86 DAP	100 DAP
Greenhouse	0	16.79e	26.13c	36.38c	44.54d	52.66cd	56.50bcd
	100	23.92bcd	39.34b	50.63b	55.34c	62.00b	52.63cd
	200	35.79a	55.58a	62.88a	69.34a	76.67a	77.00a
	300	25.08bc	38.50b	48.00b	56.92bc	60.09bc	63.67b
	400	26.54b	38.83b	46.55b	55.25c	61.25bc	60.38bc
Field	0	18.46de	21.54c	25.25d	31.87e	41.83e	48.58d
	100	21.08bcde	23.96c	28.13d	36.92de	47.58de	52.92cd
	200	21.79bcde	23.87c	28.83cd	36.25de	46.38de	52.84cd
	300	35.35a	52.50a	61.44a	66.29ab	72.63a	74.83a
	400	20.92cde	22.29c	29.25cd	38.25de	48.63de	51.50d

*Means followed by the same letter (s) within a column are not significantly different according to Tukey's HSD at $p \leq 0.05$. DAP – Days after planting

Table 3.4 Effect of NPK fertilizer rates and growing environment on plant height (cm) of pepino melon in trial two (July 2019-Feb 2020)

Environment	Fertilizer (kg ha^{-1})	30 DAP	44 DAP	58 DAP	72 DAP	86 DAP	100 DAP
Greenhouse	0	13.28d	16.61f	19.93e	23.05e	28.49e	33.00e
	100	22.99bc	28.50cd	33.22c	36.16c	40.39c	57.22bc
	200	38.78a	44.83a	48.34a	62.00a	70.06a	81.33a
	300	25.16bc	29.56c	33.11c	37.22c	43.82bc	48.94bc
	400	25.67b	29.44c	32.17c	35.67c	43.05c	50.34bc
Field	0	18.88cd	20.88ef	23.71de	25.38e	29.59de	30.92e
	100	21.67bc	23.59de	27.75cd	31.84cd	36.17cde	37.21de
	200	23.56bc	24.84cde	29.09cd	31.29cd	40.46c	44.04cde
	300	32.82a	38.92b	41.06b	51.29b	51.58b	62.00b
	400	22.38bc	24.63cde	25.11de	33.75c	37.46cd	42.17de

*Means followed by the same letter (s) within a column are not significantly different according to Tukey's HSD at $p \leq 0.05$. DAP – Days after planting

3.3.2 Effect of NPK fertilizer rates and growing environment on stem diameter of pepino melon plants

NPK fertilizer rates and growing environment had a significant effect on stem diameter of pepino melon plants. Plants which were grown in the field and supplied with 300 kg NPK ha^{-1} had a significantly high stem diameter at 30 DAP but not significantly different at $p \leq 0.05$ from those grown in the greenhouse and supplied with 200 kg NPK ha^{-1} in trial one. From 44 DAP to 100 DAP plants grown in the greenhouse and supplied with 200 kg NPK ha^{-1} had a significantly high stem diameter at $p \leq 0.05$ though not different from field grown plants supplied with 300 kg NPK ha^{-1} in trial one (Table 3.5). In trial two, plants which were grown in the greenhouse and supplied with 200 kg NPK ha^{-1} had a significantly higher stem diameter at $p \leq 0.05$ though not significantly different from plants grown in the field and supplied with 300 kg NPK ha^{-1} from 30 DAP to 86 DAP. At 100 DAP plants grown in the greenhouse and supplied with 200 kg NPK ha^{-1} had a stem diameter of 14.01 mm which was significantly different at $p \leq 0.05$ from plants grown in the field and supplied with 300 kg NPK ha^{-1} with a stem diameter of 11.71 mm (Table 3.6).

Table 3.5 Effect of NPK fertilizer rates and growing environment on stem diameter(mm) of pepino melon in trial one Nov 2018-June 2019)

Environment	Fertilizer (kg ha^{-1})	30 DAP	44 DAP	58 DAP	72 DAP	86 DAP	100DAP
Greenhouse	0	4.91c	5.91de	7.33d	4.84d	5.51c	6.92d
	100	5.72bc	8.37bc	9.35c	8.02bcd	9.03bc	9.83c
	200	8.56ab	9.96a	12.55a	14.30a	15.63a	13.11ab
	300	4.89c	8.21bc	9.64bc	6.09cd	6.99bc	9.75c
	400	4.31c	7.08cd	9.91bc	5.82cd	6.49bc	9.37cd
Field	0	4.52c	4.08f	5.21e	7.93bcd	5.51c	10.40c
	100	3.94c	4.79ef	5.85de	8.78bc	8.62bc	9.99c
	200	4.88c	4.51ef	5.64de	8.29bcd	9.87b	10.70bc
	300	9.05a	9.41ab	11.34ab	11.09ab	14.32a	15.32a
	400	4.41c	4.69ef	6.32de	8.59bcd	9.30b	10.64bc

*Means followed by the same letter (s) within a column are not significantly different according to Tukey's HSD at $p \leq 0.05$. DAP – Days after planting

Table 3.6 Effect of NPK fertilizer rates and growing environment on stem diameter (mm) of pepino melon in trial two (July 2019-Feb 2020)

Environment	Fertilizer (kg ha^{-1})	30 DAP	44 DAP	58 DAP	72 DAP	86 DAP	100DAP
Greenhouse	0	3.38d	4.11c	5.30c	6.64c	8.25b	8.94c
	100	4.29cd	4.97bc	6.66bc	7.73c	8.58b	9.50c
	200	5.91a	7.61a	9.05a	10.85a	11.93a	14.01a
	300	4.49bc	5.30bc	6.51bc	7.33c	8.31b	8.99c
	400	4.57bc	5.19bc	6.74bc	7.86bc	8.25b	9.12c
Field	0	1.32e	1.77d	2.23d	2.83d	3.43d	3.21f
	100	1.72e	2.07d	2.36d	3.04d	4.26cd	4.48ef
	200	2.28e	2.02d	2.76d	3.15d	4.79cd	5.76de
	300	5.37ab	6.20b	7.81ab	9.45ab	11.00a	11.71b
	400	1.71e	2.37d	2.98d	3.47d	5.45c	6.06d

*Means followed by the same letter (s) within a column are not significantly different according to Tukey's HSD at $p \leq 0.05$. DAP – Days after planting

3.3.3 Effect of NPK fertilizer rates and growing environment on number of leaves per bush before flowering of pepino melon plants

NPK fertilizer rates and growing environment had a significant effect $p \leq 0.05$ at on number of leaves. In both trials plants which were supplied with 400 kg NPK ha^{-1} whether grown in the field or greenhouse had the highest number of leaves per bush just before flowering compared to the control in both environments. In trial one, greenhouse and field grown plants supplied with 400 kg NPK ha^{-1} had 128.79 and 126.67 leaves per bush respectively (Figure 3.1). Field grown and greenhouse grown plants supplied with 100kg NPK ha^{-1} had 89 and 86 leaves per bush in trial one, respectively. Field grown and greenhouse grown plants supplied with 300 kg NPK ha^{-1} had 99.67 and 94.95 leaves per bush respectively. Field and greenhouse grown plants not supplied with NPK fertilizer had 64 and 59.22 leaves per bush respectively. In trial two, greenhouse and field grown plants supplied with 400 kg NPK ha^{-1} had 124.17 and 110 leaves per bush respectively. Greenhouse and field grown plants supplied with 100 kg NPK ha^{-1} had 77.39 and 73.33 leaves per bush respectively. Greenhouse and field grown plants supplied with 200 kg NPK ha^{-1} recorded 86.05 and 78.67 leaves per bush respectively. Plants supplied with 300 kg NPK ha^{-1} and grown in the greenhouse had 88.28 leaves while field grown plants had

84.67 leaves per bush. Greenhouse and field grown plants not supplied with NPK ertilizer had 52.67 and 46.67 leaves per bush respectively. It was observed that as the fertilizer rate increased the number of leaves also increased in both environments and trials.

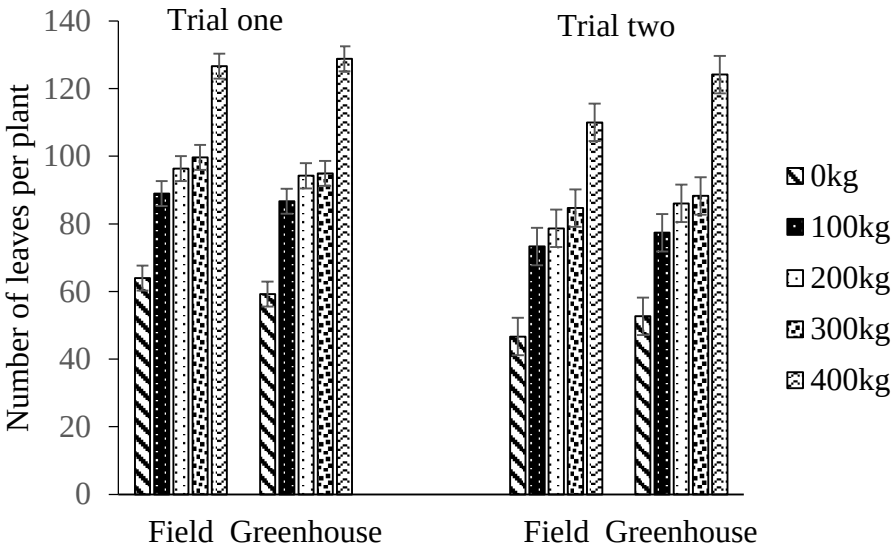


Figure 3.3 Effect of NPK fertilizer rates and growing environment on number of leaves of pepino melon in trial one (Nov 2018-June 2019) and trial two (July 2019-Feb 2020)

3.3.4 Effect of NPK fertilizer rates and growing environment on number of branches of pepino melon plants

NPK fertilizer rates and growing environment had a significant effect at $p \leq 0.05$ on the number of primary branches. In trial one plants supplied with 200kg NPK ha^{-1} and grown in the greenhouse had 23.05 branches while those supplied with 300 kg NPK ha^{-1} and grown in the field had 19.35 branches and were not significantly different at $p \leq 0.05$. Control recorded the lowest number of branches with 8.99 and 7.13 branches for greenhouse and field grown pepino melons respectively. However, plants supplied with 300 kg NPK and grown in the field were not significantly different from greenhouse plants supplied with 400 and 300 kg NPK ha^{-1}. In trial two, field grown plants supplied with 300 kg NPK ha^{-1} had 18.46 branches while greenhouse grown plants supplied with 200 kg NPK ha^{-1} had 15.03 branches and they were not significantly different at $p \leq 0.05$. Greenhouse grown plants that received 200 kg NPK ha^{-1} were not significantly different at $p \leq 0.05$ from field grown plants that received 400, 100 and 200 kg NPK ha^{-1} and greenhouse grown plants that received 100, 300 and 400 kg NPK ha^{-1} (Figure 3.2). The control plants had the lowest number of branches with 9.13 and 7.06 branches

for field and greenhouse grown pepino plants respectively. Generally, it was observed that plants had higher number of branches in trial one compared to trial two.

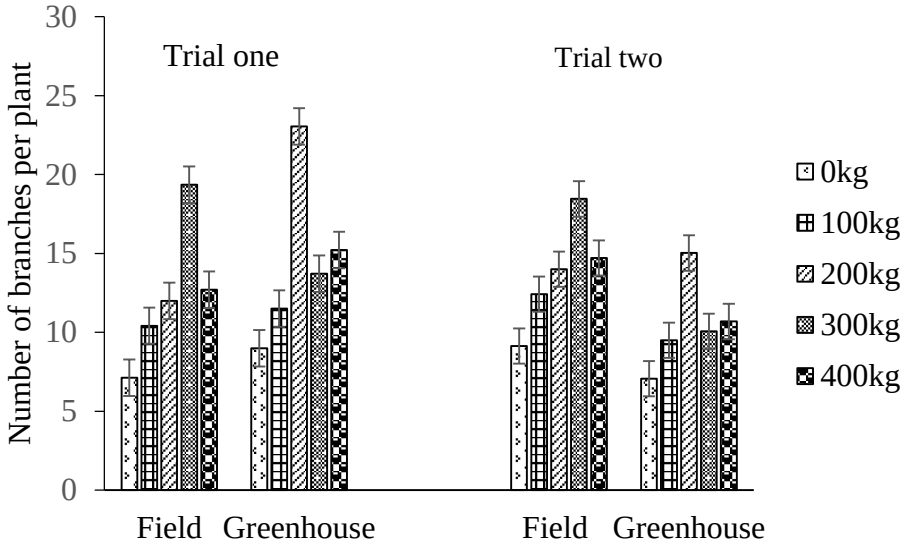


Figure 3.4 Effect of NPK fertilizer rates and growing environment on number of branches of pepino melon in trial one (Nov 2018-June 2019) and trial two (July 2019-Feb 2020)

3.3.5 Effect of NPK fertilizer rates and growing environment on number of days to 50% flowering of pepino melon plants

NPK fertilizer rates and growing environment had a significant effect at $p \leq 0.05$ on days to 50% flowering. Plants which were supplied with NPK fertilizer took longer to flower compared to the control which took the shortest time to flower in both environments and trials. In trial one, control plants grown in the field took 33 days to achieve 50% flowering while those grown in the greenhouse took 31 days to 50% flowering. In trial one, field and greenhouse grown plants supplied with 400 kg NPK ha^{-1} took 63 and 60 days to achieve 50% flowering respectively. Field grown and greenhouse grown plants supplied with 300 kg NPK ha^{-1} took 44 and 40 days to achieve 50% flowering respectively. Field grown plants supplied with 200 kg NPK ha^{-1} took 39 days while greenhouse grown plants took 38 days to achieve 50% flowering. In trial two, plants supplied with 400 kg NPK ha^{-1} took 65 and 60 days in the field and greenhouse respectively (Figure 3.3). Field grown plants supplied with 200, 300 kg NPK ha^{-1} and greenhouse grown plants supplied with 300 kg NPK ha^{-1}were not significantly different in number of days to 50% flowering with 41, 46 and 42 days respectively. Generally, plants in the greenhouse took a shorter time to flower compared to those grown in the field regardless of the fertilizer rate.

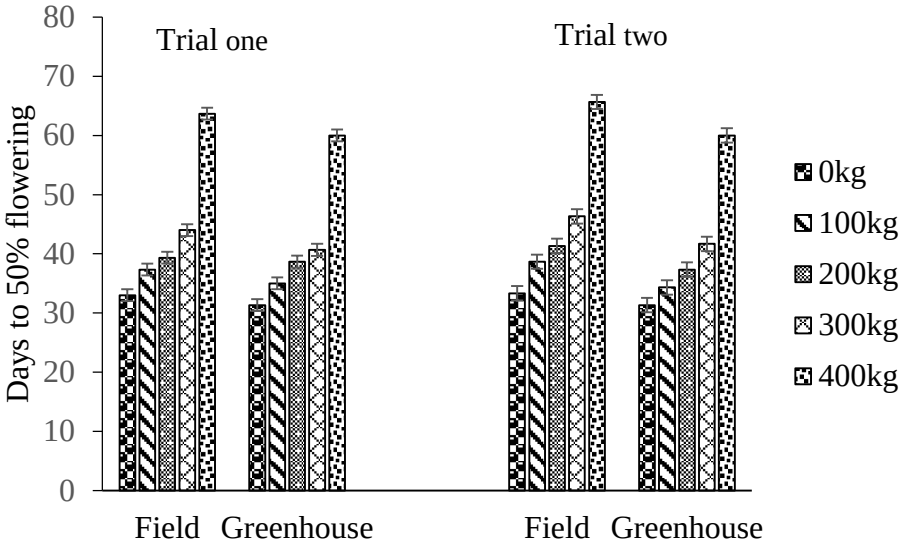


Figure 3.5 Effect of NPK fertilizer rates and growing environment on number of days to 50% flowering of pepino melon in trial one (Nov 2018-June 2019) and trial two (July 2019-Feb 2020)

3.3.6 Effect of NPK fertilizer rates and growing environment on pepino melon fruit weight

NPK fertilizer rates and growing environment had a significant effect at $p \leq 0.05$ on fruit weight of pepino melon. Field grown plants supplied with 300 kg NPK ha^{-1} had the highest fruit weight of 380.23 g and 361.46 g in trials one and two respectively. In trial one, field and greenhouse grown pepino melon plants which received 0 kg NPK ha^{-1} had a fruit weight of 137.04 g and 68.53 g respectively (Figure 3.4). Field grown fruits from plants which received 400 kg NPK ha^{-1} had a fruit weight of 231.33 g though this was not significantly different from field grown fruits from plants supplied with 100, 200 and greenhouse grown fruits from plants supplied with 200 kg NPK ha^{-1}. In trial two, greenhouse grown fruits from plants supplied with 200 kg NPK ha^{-1} had a fruit weight of 224.61g but this was not significantly different from field grown fruits from plants supplied with 200, 400 kg NPK ha^{-1} and greenhouse grown fruits from plants supplied with 300 kg NPK ha^{-1}. Field grown fruits from plants supplied with 0, 100 kg NPK ha^{-1} and greenhouse grown fruits from plants not supplied with NPK fertilizer had a fruit weight which was not significantly different. Generally, field grown pepino melons had a higher fruit weight compared to greenhouse grown pepino plants regardless of the NPK fertilizer rate. It was also observed that as the NPK fertilizer rates increased the fruit weight also increased up

to 300 kg NPK ha^{-1} for field grown plants and 200 kg NPK ha^{-1} for greenhouse grown pepino melons. Increasing NPK fertilizer rates above 300 kg NPK ha^{-1} for field grown plants and 200 kg NPK ha^{-1} for greenhouse grown did not lead to a significant increase in fruit weight.

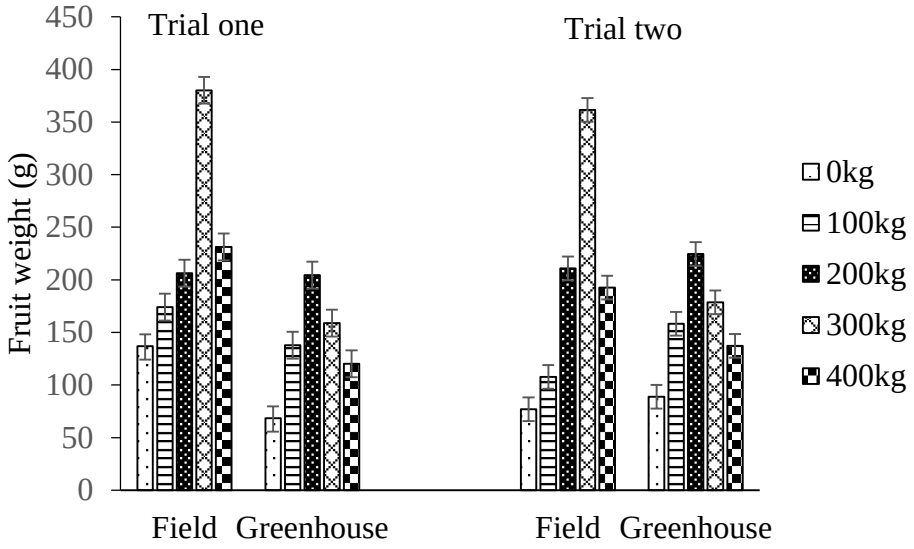


Figure 3.6 Effect of NPK fertilizer rates and growing environment on fruit weight of pepino melon in trial one (Nov 2018-June 2019) and trial two (July 2019-Feb 2020)

3.3.7 Effect of NPK fertilizer rates and growing environment on total yield of pepino melon plants

NPK fertilizer rates and growing environment had a significant effect at $p \leq 0.05$ on total yield of pepino melon. Increasing rates of NPK fertilizer led to an increase in the total yield of pepino melon in both growing environments (Figure 3.5). However, it was noted that increasing the rate above 300 kg NPK ha^{-1} for field grown plants and 200 kg NPK ha^{-1} for the greenhouse grown pepino plants led to an insignificant increase or decrease in yield. The control (no fertilizer) recorded the lowest total yield in both environments and trials. Field grown pepino melons that were supplied with 300 kg NPK ha^{-1} had the highest yield of 1102.48 kg ha^{-1} and 1060.55 kg ha^{-1} in trial one and two respectively (Figure 3.5). Greenhouse grown plants supplied with 200 kg NPK ha^{-1} had a total yield of 782.64 kg ha^{-1} and 648.8 kg ha^{-1} in trial one and two respectively. In trial one, plants not supplied with fertilizer (control) had a total yield of 411.22 kg ha^{-1} and 211.36 kg ha^{-1} in the field and greenhouse respectively. In trial two, the control recorded 209.4 kg ha^{-1} and 212.7 kg ha^{-1} in the field and greenhouse respectively.

Generally, it was observed that the yield was higher in the field grown compared to the greenhouse grown pepino melons regardless of the NPK fertilizer rate. It was also observed that the total yield was higher in trial one compared to trial two.

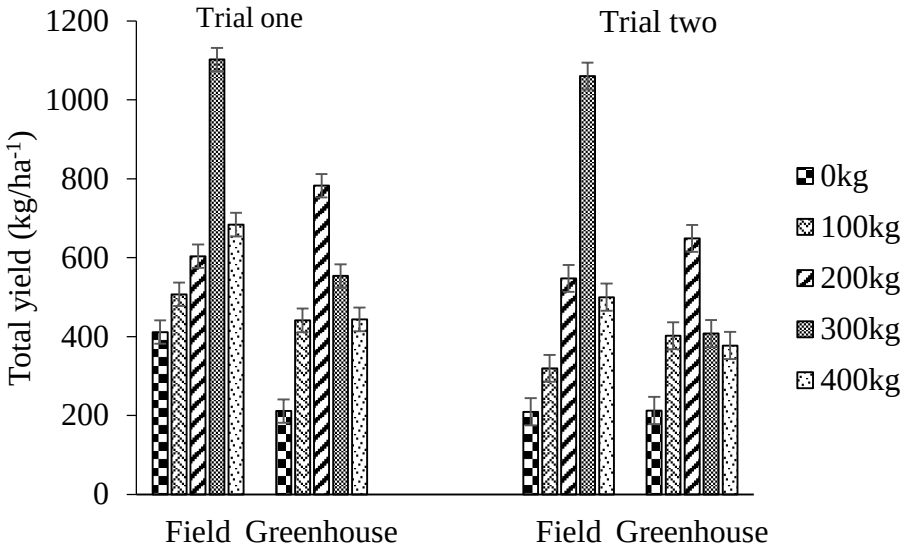


Figure 3.7 Effect of NPK fertilizer rates and growing environment on total yield of pepino melon in trial one (Nov 2018-June 2019) and trial two (July 2019-Feb 2020)

3.3.8 Effect of NPK fertilizer rates and growing environment on dry weight of pepino melon fruits

NPK fertilizer rates and growing environment had a significant effect at $p \leq 0.05$ on dry weight of pepino fruits. In trial one, field grown pepino melon fruits from plants supplied with 300 kg NPK ha^{-1} had the highest dry weight of 14.14 g and this was not significantly different from greenhouse grown fruits from plants supplied with 200 kg NPK ha^{-1} with a dry weight of 13.76 g. Greenhouse grown fruits from plants supplied with 300 kg NPK ha^{-1} had a dry weight of 9.62 g which was not significantly different from field grown fruits from plants supplied with 200 kg NPK ha^{-1}. Field grown fruits from plants supplied with 200 kg NPK ha^{-1} were not significantly different from both greenhouse and field grown fruits from plants supplied with 100 kg NPK ha^{-1} with a dry weight of 8.23 g and 8.22 g respectively. The lowest dry weight was recorded in field and greenhouse grown fruits from plants not supplied with NPK fertilizer

(control) with a dry weight of 4.38 g and 4.3 g respectively (Figure 3.6). In trial two, greenhouse grown fruits from plants supplied with 200 kg NPK ha^{-1} had the highest dry weight of 14.42g. Field grown fruits from plants supplied with 300 kg NPK ha^{-1} had a dry weight of 12.23g (Figure 3.6). The dry weight of greenhouse grown fruits from plants supplied with 300 and 100 kg NPK ha^{-1} was not significantly different from the dry weight of field grown fruits from plants supplied with 100 and 200 kg NPK ha^{-1}. The lowest dry weight was recorded in field and greenhouse grown fruits from plants not supplied with NPK fertilizer (control) with a dry weight of 4.28g and 4.29g respectively. Generally, fruit dry weight increased as the fertilizer rates increased but reached its peak at 200 kg NPK ha^{-1} after which it decreased for field grown fruits while for greenhouse grown fruits the dry weight also increased as the fertilizer rates increased and reached its peak at 300 kg NPK ha^{-1} after which the dry weight decreased in both trials.

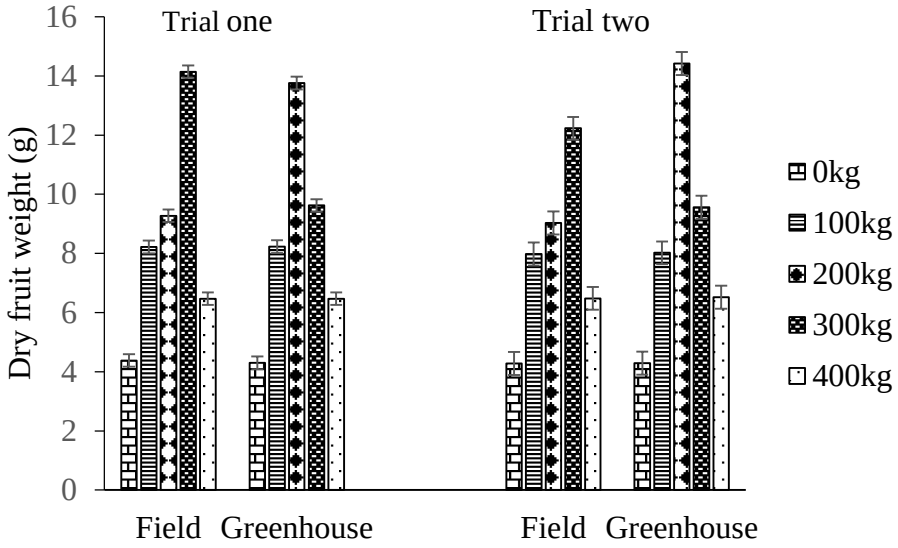


Figure 3.8 Effect of NPK fertilizer rates on dry weight of field and greenhouse grown pepino melon fruits in trial one (Nov2018-June 2019) and trial two (July 2019-Feb 2020)

3.4 Discussion

In the present study NPK fertilizer rates significantly influenced plant height of field and greenhouse grown pepino melon plants. Similar results were reported by Lego *et al*. (2016) in which capsicum plants that received NPK 150:120:60 kg ha^{-1} fertilizer resulted to increased plant height of 60.10 cm compared to other NPK fertilizer rates and the control. Similarly, Dhaliwal *et al*. (2007) reported that bell pepper plants that were grown in the polyhouse were taller than those grown in the open field. There was a significant increase in plant height in the

plots that were supplied with 200 kg NPK ha^{-1} in the greenhouse and 300 kg NPK ha^{-1} in the field compared to the control. Adequate supply of NPK fertilizer regulates plant physiological functions and favors morphological responses (Shree *et al*., 2014). Increase in plant height following the application of NPK fertilizer might be due to the fact that phosphorous is a constituent of nucleoprotein which plays a vital role in cell division and tissue formation (Singh & Sanganna, 2000). Phosphorous also aids in mitotic division of the shoot apical meristem and this led to increase in plant height (Kareem *et al*., 2020). In addition, increase in plant height in pepino melon plant supplied with NPK fertilizer in both growing environments may be due to release of nutrients from the fertilizer and this led to an increase in photosynthesis (Iqtidar *et al*., 2006). Plant height is very important for determining growth of crops (Sanni, 2016). Optimum plant height is positively correlated with plant productivity (Saeed *et al*., 2001). On the other hand, the low plant height recorded in the control in both environments was due to lack of nutrients which led to reduced growth. Increasing NPK fertilizer rates above 200 kg NPK ha^{-1} in the greenhouse and 300 kg NPK ha^{-1} in the field did not result into an increase of plant height. This could be due to luxurious consumption of nutrients by the crops leading to no increase in growth.

Stem diameter is one of the most important sites for the storage of food materials from photosynthesis and can be influenced by the nutrients present in the soil (Godia, 2014). The average stem diameter of 4.08cm in capsicum plants which were supplied with NPK 150:120:60 kg ha^{-1} compared to the control (Lego *et al*., 2016). Plants which were grown in the greenhouse had a larger stem diameter compared to those grown in the open field. This may be due to the favourable micro climatic conditions in the greenhouse (Naik *et al*., 2011). Increase in stem diameter following application of NPK might have resulted from the role of phosphorous in metabolic activities such as cell division, cell expansion and cell enlargement (Kareem *et al*., 2020). The low stem diameter in the control in both environments might be due to low phosphorous which resulted to reduced growth, cell division, cell expansion and cell enlargement (Kareem *et al*., 2020).

NPK fertilizer treatments significantly influenced the vegetative performance of pepino melon plants. Plants which were supplied with 400 kg NPK ha^{-1} had the highest number of leaves in both environments and this could be due to the high amount of nitrogen in the fertilizer. Nitrogen is a component of the chlorophyll molecule and promotes vegetative growth by providing a sufficient photosynthetic area which in turn helps in flowering and fruit set (Kumar *et al*., 2013). Similar results were reported by Babatunde *et al*. (2019) where application of

NPK fertilizer (15:15:15) had a significant effect on number of leaves of tomato plants. Nafiu *et al*. (2011) also reported that application of 300 kg NPK ha^{-1} on okra led to increased number of leaves compared to the control and the other treatments, in this experiment 300 kg NPK ha^{-1} was the highest fertilizer treatment. Furthermore, Okonwu and Monsah (2012) reported that application of 350 kg NPK ha^{-1} led to increased number of leaves of pumpkin. On the contrary, Gloria *et al*. (2017) reported that application of NPK fertilizer rates did not have a significant effect on the number of leaves of okra. The increase in number of leaves in this study can be attributed to nitrogen supply in the fertilizer applied and the fact that nutrient availability especially nitrogen influenced plant vegetative development which is in agreement with Babatunde *et al*. (2019). The low number of leaves in the control treatments could be due to low levels of nitrogen and low levels of potassium have also been reported to limit plant growth (Preciado-Rangel *et al*., 2018).

Number of branches was greatly influenced by the growing environment and NPK fertilizer application. Naik *et al*. (2018) also reported increased number of branches of tomato plants grown in the greenhouse. Similarly, Lego *et al*. (2016) reported that application of NPK 150:120:60 kg ha^{-1} led to an increase in the number of branches of capsicum (*Capsicum annuum* L.) cv Asha. The significant difference observed in the number of branches can be due to the fact that the NPK fertilizer applied to the soil was readily available in a form that is readily absorbed by plant roots and thereby resulting to a significant increase in the morphological growth of the plants (Kanneh *et al*., 2017). Phosphorous stimulates root growth and this enables roots to absorb nutrients from the soil (Tsige *et al*., 2022). Potassium is involved in movement of water, nutrients and carbohydrates in plant tissues and this affects ATP production which in turn regulates photosynthesis and in addition plants receiving sufficient nitrogen have high rates of photosynthesis and have vigorous growth which in turn in turn results to an increase in number of branches (Tsige *et al*., 2022). Increase in number of branches as phosphorous levels in the NPK fertilizer increased might be due to the role of phosphorous in cell division, cell expansion and cell enlargement (Tsige *et al*., 2022). The number of branches is positively correlated to yield (Kumar *et al*., 2013). On the contrary, Nafiu *et al*. (2011) reported that an increase in the number of branches did not lead to increased okra fruit production as expected. This might be due to the fact that other shoot characteristics were favoured in growth at the expense of fruit production. Nafiu *et al*. (2011) reported that okra plants that received an application of 300 kg NPK ha^{-1} had the highest number of primary branches.

Statistically significant results were observed for days taken by plants attain 50% flowering. Plants which were not supplied with NPK fertilizer (control) took the shortest time to flower while those supplied with the highest fertilizer rate 400 kg NPK ha^{-1} took the longest time to flower. Kumar *et al*. (2013) reported that increasing levels of NPK delayed flowering of tomato plants. On the contrary, Imran *et al*. (2014) reported that cucumber plants which were not supplied with any fertilizer took the longest time (38 days) to flower compared to application of 1000 g $fertigation^{-1}$. In this study it was observed that greenhouse plants flowered earlier compared to field grown plants and this is due to the fact that plant physiological processes and growth rate increases 2-3 times for every 10°C increase in temperature between 15ºC and 25ºC. Similarly, Nkansah *et al*. (2017) reported that sweet pepper plants grown in the greenhouse flowered earlier than those grown in the open field. This could be due to the micro climate in the greenhouse. Pepino plants supplied with 400 kg NPK ha^{-1} took the longest time to flower in the field and greenhouse. This was attributed to excessive nitrogen which favoured vegetative growth at the expense of flower development. Phosphorous stimulates floral cluster formation but increase in phosphorous level delays flowering hence the longer period taken by both field and greenhouse grown plants supplied with 400 kg NPK ha^{-1} (Kumar *et al*., 2013). In addition, both greenhouse and field grown plants supplied with NPK fertilizer did not have stress and therefore lack of stress led to extended vegetative phase (Gelaye *et al*., 2021). On the other hand, potassium increases the availability of soil nitrogen and this in turn extended the vegetative phase and delayed flowering (Gelaye *et al*., 2021). The short time taken by pepino plants which were not supplied with NPK fertilizer (control) to attain 50% flowering in both environments could be due to lack of essential nutrients which are needed by plants for growth. Early flowering in field and greenhouse grown plants not supplied with NPK fertilizer could be due to nutrient stress which triggered the signal for sex hormone that hastens flowering (Gelaye *et al*., 2021).

There was a significant effect of NPK fertilizer rates and growing environment on fruit weight of pepino melon. Field grown plants supplied with 300 kg NPK ha^{-1} had the highest fruit weight in both trials. Greenhouse grown plants supplied with 200 kg NPK ha^{-1} had a higher fruit weight compared to the other fertilizer rates. However, it was noted that application of NPK fertilizer beyond 300 kg NPK ha^{-1} did not significantly increase fruit weight of pepino plants grown in the field. This could be due to the fact that potassium plays a role in fruit filling and fruits that receive enough potassium have improved water efficiency due to increasing osmotic pressure of cells making them more turgid and increasing the size and weight of the fruits (Preciado-

Rangel *et al*., 2018). In addition, phosphorous promotes flowering and fruit set hence under optimum phosphorous levels hasten fruit maturity and increase fruit size (Kumar *et al*., 2013). Results of this study for both greenhouse and field grown pepino melons are in agreement with those of Oloyede *et al*. (2013) who found that increasing NPK fertilizer rate led to an increase in fruit weight of pumpkin up to a point beyond which there was no significant increase pumpkin fruit weight. Temperature plays a very important role in growth and development of fruits in most fruit vegetables (He *et al*., 2019). High air temperature encourages early fruit production compared to vegetative growth and this might eventually lead to less fruit production (De Koning, 1988). The air temperature in the greenhouse ranged from 21-35.8°C and 18.5- 36.7°C in trial one and two respectively. The air temperature in the field ranged from 18.9-22.8°C and 18.9-22.6°C in trial one and two respectively. Low air temperatures lead to increased number of flowers, late ripening and large fruits (Sawhney & Polowick, 1985). This might be the reason why field grown fruits had a higher fruit weight compared to greenhouse grown fruits.

Papadopoulus and Hao (2001) reported that day temperatures of 19°C led to early yields, total yield and the best fruit weight for greenhouse grown tomatoes. Therefore, if the temperature is above 19°C fruit size will be reduced and this is due to decreased pollination and fertilization at high temperatures (Khah & Passam, 1992). The temperature in the greenhouse were above 19°C most of the months and this may be the cause for low fruit weight of greenhouse grown pepino melons. Increase in temperature leads to a decrease in fruit weight and this could be due to the fact that respiration doubles for every 10°C and thus the stored sugars are used up and there is also reduced translocation of sugars (Went, 1944). Results of this study are in agreement with those of Ahumada and Cantwell (1996) who reported that the fruit weight of pepino melon ranged from 100 to 500 g. On the contrary, lower fruit weight of 268 g, 80-250g and 181-330 g of pepino melon have been reported (Cavusoglu *et al*., 2009; Gonzalez *et al*., 2000; Rodriquez-Burruezo *et al*., 2011). Similarly, Martinetti and Paganini (2006) reported that increased fertilizer rates led to an increase in fruit weight up to a point beyond which there was no significant increase in fruit weight. The low fruit weight obtained following application of 400 kg NPK ha^{-1} in both growing environments could be attributed to excess nitrogen which leads to reduced plant growth, small leaves, stunted root systems and in severe cases death (Sanchez *et al*., 2004) and also due to the fact that most of the photosynthates are directed towards vegetative growth and very little to the fruits hence decreased fruit size and weight. In addition, fruits receiving excess potassium have reduced water efficiency and thus decrease

osmotic potential and hence decrease in size and weight of the fruits (Preciado-Rangel *et al*., 2018). Excess phosphorous delays fruit maturity and decreases fruit size (Kumar *et al*., 2013). The low fruit weight recorded in plants not supplied with NPK fertilizer could be due to low potassium which led to reduced filling of the fruits and this decreased fruit size and weight (Preciado-Rengel *et al*., 2018). Low phosphorous level in the control decreased growth and hence low fruit weight (Zhu *et al*., 2017).

NPK fertilizer rates and growing environment had a significant effect on total yield of pepino melon. Field grown pepino melon plants had the highest yield compared to greenhouse grown plants. Application of 300 kg NPK ha^{-1} for field grown pepino melons gave the highest total yield in both trials. This could be due to the fact that fruits receiving enough potassium have improved water efficiency due to increased osmotic pressure of cells, making them more turgid and thus increase in fruit size, weight and eventually high yields (Preciado-Rengel *et al*., 2018). Potassium from the NPK fertilizer is also involved in translocation of photosynthates from the leaves to the fruits and hence increase in yield in greenhouse and field grown plants supplied with 200 and 300 kg NPK ha^{-1}, respectively (Malvi, 2011). However further increase in potassium leads to luxurious uptake of potassium with no effect on yield (Malvi, 2011). In addition, phosphorous is a component of nucleic acids, phospholipids and energy rich phosphate compounds and it plays a role in fruit growth and development, therefore under conditions of optimum phosphorous, there is increase in fruit weight resulting to increase in yield (Kareem *et al*., 2020). Similar trend of results was obtained by Gloria *et al*. (2017) who reported that application of NPK fertilizer increased the yield of okra plants. In the study, okra plants which were supplied with 13 g of NPK had the highest yield and control had the lowest yield. The results of this study are in harmony with the findings of Omotoso and Shitu (2007) who concluded that increasing NPK fertilizer rates led to an increase in the yield of okra plants. In this study field grown pepino plants had a high total yield compared to greenhouse grown plants. On the contrary, Nkansah *et al*. (2017) reported high yield for greenhouse grown sweet pepper compared to open field grown sweet pepper plants. In a study by Cavusoglu *et al*. (2009) the yield of pepino melon was found to be 3.68 t ha^{-1} to 14.03 t ha^{-1}. This was higher than the yield recorded in this study, however, Cavusoglu *et al*. (2009) reported that the yield of pepino melon depends on climatic conditions. The low yield recorded in the control experiment could be due to low levels of potassium and the low yield following the application of 400 kg NPK ha^{-1} might be due to excess potassium which has been reported to decrease crop yield (Preciado-Rengel *et al*., 2018). In addition, low yield recorded in the control (no NPK fertilizer)

could be due to low levels of phosphorous which led to reduced growth and this in turn reduces yield (Zhu *et al*., 2017) and also due to low potassium which leads to reduction in the accumulation of soluble carbohydrates and this reduces yield (Malvi, 2011). The reduction in greenhouse grown plants supplied with 300 and 400 kg NPK ha^{-1} and field grown plants supplied with 400 kg NPK ha^{-1} could be due to excess nitrogen which promotes vegetative growth by providing a sufficient photosynthetic area which in turn helps in flowering and fruit set but excess nitrogen delays fruit maturity, decreases fruit size hence low yields (Kumar *et al*., 2013). Excess phosphorous also leads to decreased fruit size hence low yields (Kumar *et al*., 2013).

In this study, the low yield recorded in the greenhouse might be due to increased flower abortion as compared to field where flower abortion was minimal (chapter seven). Flower abortion occurred in the greenhouse because of high temperatures. In trial one the average temperature in the greenhouse ranged from 21°C to 35.8°C, whereas in trial two it ranged from 18.5°C to 36.7°C. It was noted that flower abortion occurred when the temperature in the greenhouse was above 30°C. Prohens *et al*. (2000) reported that temperatures above 30°C contribute to poor conditions for pepino melon fruit formation. Temperature is vital for fruit set in most solanaceous vegetables (Karaparos *et al*., 2008). In tomato, a mean daily temperature of 29°C leads to a decrease in fruit number and fruit weight (Peet *et al*., 1997). This could be due to the fact that the reproductive organs of most solanaceous vegetables are highly sensitive to high temperature compared to the vegetative organs (Raja & Kakani, 2007). Furthermore, temperatures above 23°C affect one or more processes involved in successful pollen fertilization (Pressman *et al*., 2007).

Metabolic processes in tomato are favoured by temperatures of between 25-30°C while fruit set requires a mean daily temperature of 21-24°C (Suzuki *et al*., 2001). The reduced yield in the greenhouse grown pepino fruits could be due to high temperatures which could have led to impaired pollen germination, pollen tube growth and fertilization resulting to flower abortion and hence reduced fruit set (Erickson & Markhart, 2002). High temperature in tomato plants led to a decrease in sucrose uptake to the developing flower buds and this causes flower abortion and this may also have been the case in greenhouse grown pepino melons (Dinar & Rudich, 1985). Additionally, high day time temperatures in pepper lead to a decrease in the activity of acid invertase in the developing flower buds and this explains the in ability of reproductive organs to take up assimilates (Aloni *et al*., 1991). Apart from reducing up take of

carbohydrates in the developing flower buds, high temperature also leads to increased production of ethylene by the flowers and this will also lead to flower abortion but this will depend on the cultivar (Aloni *et al*., 1994).

Under high temperatures conditions low fruit set is caused by reduced pollen viability (Kanayama & Kochetov, 2015). Decrease in pollen viability may be due to changes which occur during the development of the anthers (Suzuki *et al*., 2001). During the tetrads stage, high temperature affects the structure and function of the tapetum which plays a vital role of providing nutrients to the pollen mother cells and regulating the release of pollen grains (Suzuki *et al*., 2001). Fruit set in tomato was reduced under high temperatures due to reduced release of pollen grains (Sato *et al*., 2000). The malfunctioning of the tapetum causes pollen sterility and this have led to the low fruit set leading to low yields for greenhouse grown plants. High temperature during the tetrads stage in tomatoes causes enlargement of the tapetal cells and this leads to pollen sterility (Iwahori, 1965).

In summary the results of this study are in agreement with the findings of (Iwahori, 1965; Kanayama & Kochetov, 2015; Peet *et al*., 1997; Sato *et al*., 2000) and also with another study which was carried out in a glasshouse in Turkey, where it was observed that when the temperature was above 25°C flower formation and fruit set of pepino melon was negatively affected (Cavusoglu *et al*., 2009). The field temperature during this study ranged from 19.7°C to 22.8°C in trial one and 18.9°C to 20.5°C in trial two, thus reduced flower abortion and hence the high yield recorded in the field grown pepino melons in the two trials. The low temperature recorded in the field might have led to an increase in the number of floral organs possibly due to the initiation of a high number of flower primordia (Lozano *et al*., 1998). This might explain the high total yield recorded for the field grown pepino melons because high yield depends on profusion of flowering, successful pollination and fertilization.

Dry weight of pepino melon fruits was greatly enhanced by increasing NPK fertilizer rates in both growing environments and trials. In the absence of the NPK fertilizer fruits from the control plants had the lowest dry weight. Similarly, Oyinlola and Jinadu (2012) reported high dry weight of tomatoes from plants fertilized with 90 kg N ha^{-1} and low dry weight in the control. Results of this study are also in harmony with the findings of Kebede and Woldewahid (2014) who reported high dry matter content of tomato fruits following the application of 230 kg N ha^{-1} compared to the control which had the lowest dry matter content. Hermans *et al*.

(2006) and Poorter *et al*. (2012) also reported higher dry matter allocation to the roots than other organs under limited soil resources like nitrogen supply. The low dry weight in the control could be due to lack of additional nutrients for plant utilization to improve cell formation and hence decrease in fruit dry weight. Dry matter accumulation in plants is as a result of nutrient uptake and it is one of the measures of plant growth (Noggle & Fritz, 1983).

From the results of this study, it is evident that fruit dry weight increased as fertilizer rates increased and reached a peak at 200 kg NPK ha^{-1} for greenhouse grown fruits and 300 kg NPK ha^{-1} for field grown fruits. The low NPK fertilizer application for greenhouse grown pepino melon plants might be due to the controlled environment such as reduced evaporation of soil water and lack of leaching by rainfall compared to open field (Yu *et al*., 2005). Increase in dry weight as fertilizer rates increased might be due to additional plant nutrients for plant utilization supplied by the NPK fertilizer which could have led to improved cell formation and thus increase in fruit dry weight. The distribution of dry matter occurs between source and sink organs. The high dry matter in greenhouse and field grown fruits from plants supplied with 200 and 300 kg NPK ha^{-1} could be due to allocation of more dry matter to the fruits while the low dry matter in the control and 400 kg NPK ha^{-1} could be due to favoured dry matter accumulation in the leaves, stems and roots than the fruit (Ronga *et al*., 2019). Increase in potassium from the NPK fertilizer increases dry weight due to increase in accumulation of photosynthates but further increase in potassium does not lead to increase in dry weight hence the low dry weight following application of high fertilizer rates in both growing environments (Hawkesford *et al*., 2012). The source strength in the control was weak while application of 400 kg NPK ha^{-1} resulted to excessive and imbalanced crop growth due to luxurious vegetative growth (Ronga *et al*., 2019). The high dry weight could probably be due to enhanced vegetative growth resulting to more photosynthetic products being partitioned to the fruits. Wang *et al*. (2012) reported that dry matter partitioning to leaves, fruits and yield formation are not only affected by light, temperature but also by fertilizer application.

3.5 Conclusion

NPK fertilizer rates and growing environment have an effect on growth and yield of pepino melon. Field grown pepino melons supplied with 300 kg NPK ha^{-1} gave the highest fruit weight and total yield.

CHAPTER FOUR

EFFECT OF NPK FERTILIZER RATES ON FLOWER ABORTION OF FIELD AND GREENHOUSE GROWN PEPINO MELON (*Solanum muricatum* Aiton)

Abstract

Flower abortion is the detachment of flowers from the plant. A study was conducted at Egerton University, Kenya in 2018-2020 to investigate the effect of NPK fertilizer rates (0, 100, 200. 300 and 400 kg ha^{-1}) on flower abortion of field and greenhouse grown pepino melons. The experiment was laid out in a randomized complete block design with three replications. Data were collected on number of flowers, number of aborted flowers, viable and non-viable pollen and *in vitro* pollen germination. Data were analysed using Analysis of variance at $p \leq 0.05$ using the SAS statistical package. Significant means were separated using Tukey's honestly significant difference at $p \leq 0.05$. Results indicated that field grown plants supplied with 200 and 300 kg NPK ha^{-1} had on average 10.28 and 11.18 flowers per truss, respectively in trial one. In trial two, field grown plants supplied with 300 kg NPK ha^{-1} had 11.32 flowers per truss. Greenhouse grown plants supplied with 300 kg NPK ha^{-1} had 20.61 and 14.19 aborted flowers in trial one and two respectively. Pollen viability tests using iodine-potassium iodide test revealed a high pollen viability for pollen from non-aborted flowers obtained from field grown plants supplied with 300 kg NPK ha^{-1} with a pollen viability of 94.48% and 93.97% in trial one and two, respectively. The lowest pollen viability was recorded in pollen obtained from flowers of greenhouse grown plants not supplied with NPK fertilizer (control) with a pollen viability of 83.39% and 82.29% in trial one and two respectively. On the other hand, pollen from aborted flowers from field grown plants supplied with 300 kg NPK ha^{-1} had the highest pollen viability of 58.25% and 58.44% in trial one and two respectively. In vitro pollen germination test using Brewbacker and Kwack's medium revealed that pollen from non-aborted flowers obtained from field grown plants supplied with 300 kg NPK ha^{-1} had the highest pollen germination of 68.72% and 67.72% in trial one and two respectively. Pollen grains of aborted flowers obtained from field grown plants supplied with 300 kg NPK ha^{-1} had the highest pollen germination of 48.14% and 48.39% in trial one and two respectively. Application of 200 and 300 kg NPK ha^{-1} for field and greenhouse grown pepino melon plants lead to reduced flower abortion, high number of flowers per truss, high pollen viability and pollen germination.

4.1 Introduction

Successful flower development is vital in the production of many horticultural crops (Warner & Erwin, 2005). Abortion is the cessation of development of an organ after which it detaches from the main body of the plant (Wubs *et al*., 2009). Flower buds, flowers and fruits are the main reproductive organs that abort and this leads to reduction in yields of most horticultural crops (Nyoka *et al*., 2015). In an earlier study, Stephenson (1981) reported that flower, fruit and seed abortion is caused by pollination failure, limited photo assimilates, adverse weather conditions including moisture stress and predation. Flower abortion is also caused by high temperatures on the male reproductive organs (Kafizadeh *et al*., 2008). Flower abortion causes serious economic problems in flowering horticultural plants. High temperature in pepper causes flower abortion by increasing ethylene production (Huberman *et al*., 1997). Abscisic acid, salicylic acid and ethylene production increases as a result of high temperature (Kotak *et al*., 2007). Taylor and Whitelaw (2001) reported that ABA accelerates abscission by enhancing senescence and hence ethylene climacteric which eventually leads to abscission. Wubs *et al*. (2009) reported that before flower abortion takes place there is reduction of auxin from the flower while ethylene production increases. In sweet pepper, high flower abortion occurred three weeks after anthesis (Wubs *et al*., 2009). Abortion of reproductive organs in sweet pepper occurs even when grown in a greenhouse (Wubs *et al*., 2009).

Reproduction in plants is highly affected by environmental factors such as temperature and may have significant effects on the reproductive phase hence serious implications in agricultural crops (Thunar, 2010). The reproductive phase in flowering plants is very sensitive to hot or cold temperature stress (Zinn *et al*., 2010). Exposure to high temperatures results to flower and floral bud abortion in many crop species including tomato (Levy *et al*., 1978). Reduction in fruit set at high temperatures is mostly due to poor pollen viability, reduced pollen production and poor pollen tube growth and all result to poor flower fertilization (Prasad *et al*., 2003). Pollen development, fertilization, and asynchrony of stamen and gynoecium's development are sensitive to temperatures during flowering (Boote *et al.*, 2005; Croser *et al.*, 2003; Prasad *et al*., 1999). Sexual reproduction in plants is more delicate to high temperatures than vegetative cycle, and thus reproductive organs will be more sensitive to changes in short periods of high temperatures preceding and flowering (Reddy & Kakani, 2007).

Reproductive processes such as pollen grain production, pollen tube growth, and fruit set are adversely affected by temperature than any other environmental factor when water is not a limiting factor (Thunar, 2010). In addition, environmental stress during pollen development, germination and pollen tube growth affects the functioning of pollen and eventually fruit and seed set (Thunar, 2010). Once pollen grains are released from the anthers they are exposed to environmental factors and thus high temperatures during flowering adversely affect pollen more compared to the ovules (Thunar, 2010). High temperatures inhibit pollen germination and pollen tube growth but the sensitivity differs between different crops (Huan *et al.,* 2000; Kakani *et al.,* 2002). Temperatures above 33°C in tomato inhibit pollen formation, reduce pollen viability and eventually leads to reduced fruit set (Adams *et al*., 2001; Dominguez *et al*., 2005). High temperature will accelerate the production of reactive oxygen species (ROS) which at high levels can lead to oxidative damage and even cell death (Apel & Hirt, 2004). Exposure of sweet pepper to a temperature of 33°C for four days led to 100% abortion of flower buds and flowers (Marcelis *et al*., 2004). Several studies have reported abortion of flower buds, flowers and young fruits due to high temperatures above 30°C (Aloni *et al*. 1991; Erickson & Markhart, 2001, 2002; Huberman *et al*. (1997).

NPK fertilizers have been reported to have an effect on flower abortion in many crops. Application of 200 and 300 kg NPK ha^{-1} reduced flower abortion in okra compared to the control and application of 100 kg NPK ha^{-1} (Iyagba *et al*., 2013). On the contrary, Makinde *et al*. (2016) reported that NPK fertilized tomato plants had more flower abortion compared to the control which had the lowest flower abortion. Low nutrient supply will increase abortion (Wubs *et al*., 2009). There is insufficient information on the effects of NPK fertilizer and temperature on flower abortion of pepino melon. The present study sought to investigate the effect of NPK fertilizer rates and temperature on flower abortion of field and greenhouse grown pepino melons.

4.2 Materials and methods

4.2.1 Experimental Site description

The experiment was conducted at the Horticulture Research and Teaching Field, Egerton University, Kenya. The field lies at a latitude of 0º 23’ South, longitudes 35º 35’ East in the Lower Highland III Agro Ecological Zone (LH3) at an altitude of approximately 2,238 m above sea level. Average maximum and minimum temperatures range from 19 ºC to 22 ºC and 5 ºC

to 8 ºC, respectively, with a total annual rainfall ranging from 1200 to 1400 mm. The soils are predominantly mollic andosols (Jaetzold & Schmidt, 2006). The greenhouse used was 8m by 60m and the covering material was polythene with a thickness of 12×150 microns purchased from Amiran Kenya Ltd. The mean monthly temperatures in the greenhouse and field during the experiment are presented in Table 3.1.

4.2.2 Experimental design, treatment application, crop establishment and maintenance

The experimental design was randomized complete block design (RCBD) with five treatments and three replications. The five treatments were (0, 100, 200, 300 and 400 NPK (17:17:17) kg ha^{-1}). Pepino melon seedlings (Ecuadorian Gold variety) were obtained from Garlic and Pepino Farm, Nakuru. For the field experiment, each experimental unit was 3.2m × 3.2m (Figure 3.1) and the seedlings were planted in rows 80 cm apart and 50 cm within the plants (FAO, 1994) to give a total of 24 plants per experimental unit. In the greenhouse experiment, each experimental unit was 2m × 5 m (Figure 3.2) at the same spacing as in the field experiment to give a total of 25 plants per unit. Soil samples were collected from the experimental units in the field and greenhouse and analysed for total N, P, K and pH before the experiment was carried out. Soil sampling was done at a depth of 0-40cm using a soil auger, bulked to form a composite sample and taken for analysis of selected nutrients and soil pH. Soil samples were air dried and crushed to pass through a less than 1mm sieve. Analysis was carried out using the method described by Okalebo *et al*. (2002) and the results are presented in Table 3.2. The NPK fertilizer was applied and thoroughly mixed with the soil before placing the seedlings in the transplanting holes. Weeding was done uniformly to all experimental units. Field capacity was determined as described by Cong *et al*. (2014) thereafter tensiometers were placed in two experimental units in each block. Irrigation was done when the field capacity fell below 60% since pepino melon requires a field capacity of 60-65% (Lim, 2013). Drip irrigation was used in the greenhouse experiment and this was done when the field capacity fell below 60%. Trial one was carried out from November 2018 to June 2019 and trial two from July 2019 to February 2020.

4.2.3. Data collection

Data were collected and recorded on the following variables from a total of 8 plants from the two middle rows of experimental unit. The outer rows in each experimental unit served as guard rows.

i) Number of flowers per truss: This was determined by counting the number of flowers per truss from the Eight selected plants in each experimental unit.

ii) Number of aborted flowers per plant: This was determined by counting aborted flowers on the surface of the soil in each experimental unit.

iii) Pollen viability: This was done by use of the iodine-potassium iodide test as described by Rathod *et al*. (2018) where 1 g of potassium iodide and 0.5 g of iodine was dissolved in distilled water to make a final volume of 100 ml. Pollen was dusted on a microscope slide using a brush and about 200 pollen grains were counted on a light microscope after which 1-2 drops of the dye was added and mixed thoroughly. A cover slip was placed on the slide and after 5-10 minutes the number of viable pollen grains (darkly stained) and non-viable pollen grains (lightly stained) were examined under a light microscope (Motic Type 102 M) and images were taken using Moticam X camera. This was done for the three replications and the five NPK fertilizer treatments in the field and greenhouse. Percentage pollen viability was then calculated using the formular:

$$\text{Pollen viability (\%)} = \frac{\text{Number of stained pollen grains}}{\text{Total number of pollen grains on slide}} \times 100$$

iv) *In vitro* pollen germination: This was done by use of Brewbacker and Kwack's (1963) medium (10% sucrose, 100 mg l^{-1} boric acid, 300 mg l^{-1} calcium nitrate, 200 mg l^{-1} magnesium sulfate and 100 mg l^{-1} potassium nitrate). Pollen was collected from fresh open flowers and aborted flowers from the field and greenhouse at 7.00 to 8.00 a.m. A drop of the medium was placed on a microscope slide, pollen grains were then dusted onto the medium using a brush and the slide covered with a cover slip and its periphery sealed with Vaseline to maintain the required humidity. The slide was then placed inversely over a petri dish lined with moist filter paper. Germination of pollen was counted after 24 hours at 25°C on germination medium. Pollen grains were considered to have germinated only if the length of the pollen tube was at least equal to the diameter of the pollen tube. Germinated pollen grains were examined and counted under a light microscope. Percentage pollen germination was computed using the formula below:

$$\text{Pollen germination (\%)} = \frac{\text{Number of germinated pollen}}{\text{Total number of pollen grains on slide}} \times 100$$

4.2.4 Data analysis

Data collected were subjected to Analysis of variance (ANOVA) and significant means separated using Tukey's honestly significant difference test (Tukey's HSD) at $p \leq 0.05$. The SAS statistical package (SAS Institute, 2005) was used for data analysis.

The basic model fitted for the experiment:

$Y_{ijk} = \mu + \alpha_i + \beta_j + \tau_k + \beta\tau_{jk} + \varepsilon_{ijk}$

i=1, 2, 3; j = 1, 2, 3, 4, 5; k = 1, 2

Where; Y_{ijkl} – Pepino melon response

μ – Overall mean

α_i – Effect due to the *i*th block

β_j– Effect due to *j*th fertilizer rate

τ_k- Effect due to *k*th growing environment

$\beta\tau_{jk}$- Interaction effect of the *j*th fertilizer rate and *k*th growing environment

ε_{ijk}– Random error component which is normally and independently distributed about zero mean with a common variance $\sigma 2$.

4.3 Results

4.3.1 Effect of NPK fertilizer rates and growing environment on number of flowers per truss of pepino melon plants

NPK fertilizer rates and growing environment had a significant effect ($p \leq 0.05$) on the number of flowers per truss in both trials. In trial one, field grown plants supplied with 200 and 300 kg NPK ha^{-1} had 10.28 and 11.18 flowers per truss respectively. The lowest number of flowers per truss was recorded in both field and greenhouse grown plants supplied with 400 kg NPK ha^{-1} and they had 4.53 and 4.2 flowers per truss respectively. In trial two, field grown plants supplied with 300 kg NPK ha^{-1} had 11.32 flowers per truss and this was significantly higher than the flower number per truss from the other fertilizer rates regardless of the growing environment. The lowest number of flowers per truss was recorded in both field and greenhouse grown plants supplied with 400 kg NPK ha^{-1} which had 4.26 and 4.14 flowers per truss respectively. Generally, it was observed that field grown plants had more flowers per truss compared to greenhouse grown plants (Figure 4.1).

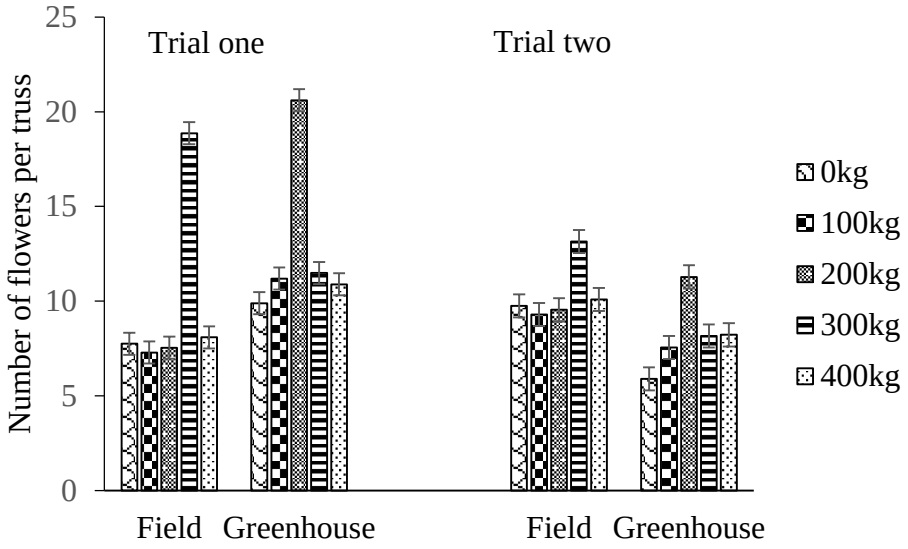


Figure 4.1 Effect of NPK fertilizer rates and growing environment on number of flowers per truss of pepino melon in trial one (Nov 2018-June 2019) and trial two (July 2019-Feb 2020)

4.3.2 Effect of NPK fertilizer rates and growing environment on number of aborted flowers of pepino melon plants

NPK fertilizer rates and growing environment had a significant effect ($p \leq 0.05$) on the number of aborted flowers in both trials. In trial one, greenhouse grown plants supplied with 300 kg NPK ha^{-1} had the highest number of aborted flowers (20.61 per plant) while field grown plants not supplied with NPK fertilizer (control) had 5.42 aborted flowers per plant although this was not significantly different from field grown plants supplied with 100 and 200 kg NPK ha^{-1} with 6.29 and 7.54 aborted flowers respectively. In trial two, greenhouse grown plants supplied with 300 kg NPK ha^{-1} had 14.19 aborted flowers and this was significantly different from all the other treatments. The lowest number of aborted flowers was recorded in field grown plants not supplied with NPK fertilizer with 5.24 aborted flowers but this was not significantly different from field grown plants supplied with 100, 200 and 400 kg NPK ha^{-1}. It was noted that the number of aborted flowers increased as the fertilizer rates increased and reached a peak at 300 kg NPK ha^{-1} after which the number dropped in both growing environments and trials. Trial, one had the highest number of aborted flowers compared to trial two in both environments. Generally, greenhouse grown plants had the highest number of aborted flowers compared to filed grown plants (Figure 4.2).

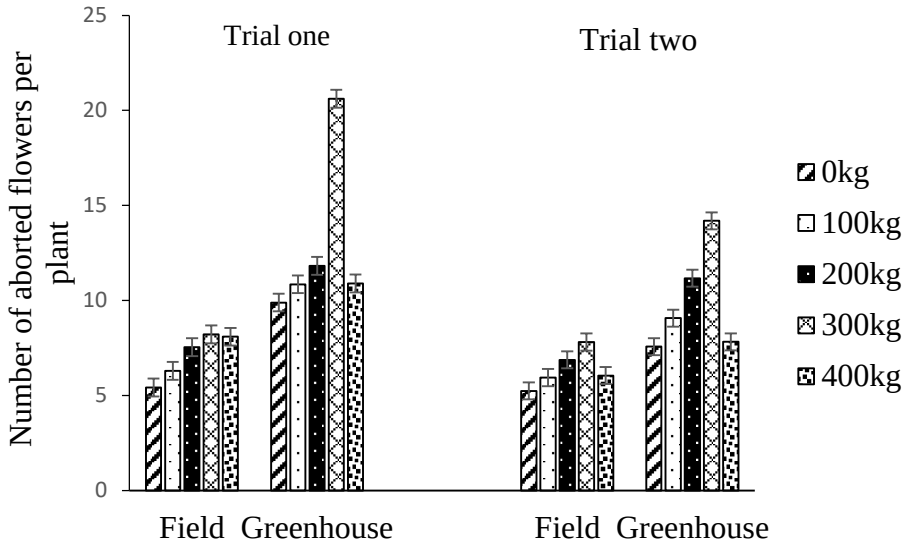


Figure 4.2 Effect of NPK fertilizer rates and growing environment on flower abortion of pepino melon in trial one (Nov 2018-June 2019) and trial two (July 2019 –Feb 2020)

4.3.3 Effect of NPK fertilizer rates and growing environment on pollen viability of non-aborted and aborted flowers of pepino melon plants

NPK fertilizer rates and growing environment had a significant effect on pollen viability of pepino melon flowers. In trial one, non-aborted flowers from field grown plants supplied with 300kg NPK ha^{-1} had the highest pollen viability of 94.48% while the lowest pollen viability of 83.39% was recorded in flowers from greenhouse grown plants not supplied with NPK fertilizer (control). Flowers from field grown plants supplied with 200 kg NPK ha^{-1} had a pollen viability of 91.22% but this was not significantly different from flowers obtained from field grown plants supplied with 100 kg NPK ha^{-1} (Table 4.1). In trial two, flowers from field grown plants supplied with 300 kg NPK ha^{-1} had the highest pollen viability of 93.97% while the lowest pollen viability was recorded in flowers from greenhouse grown plants not supplied with NPK fertilizer. Flowers from field grown plants supplied with 100 and 200 kg NPK ha^{-1} had a pollen viability of 89.34% and 90.37% respectively. Pollen viability of flowers from plants supplied with 100 kg NPK ha^{-1} was not significantly different from flowers obtained from field grown plants supplied with 400 kg NPK ha^{-1} and greenhouse grown plants supplied with 200 kg NPK ha^{-1} with a viability of 88.65% and 88.12% respectively. Generally, pollen viability increased as the NPK fertilizer rates increased but reached a peak at 200 and 300 kg NPK ha^{-1} for flowers from greenhouse and field grown plants respectively (Table 4.1). Flowers from field grown plants had a higher pollen viability compared to flowers from greenhouse

grown plants in both trials. Viable pollen grains were darkly stained while non-viable pollen grains were lightly stained (Plate 4.1).

Table 4.1 Effect of NPK fertilizer rates on pollen viability of field and greenhouse grown pepino melon non-aborted flowers in trial one (Nov 2018-June 2019) and trial two (July 2019-Feb 2020)

		Pollen viability (%)	
Environment	Fertilizer (kg ha^{-1})	Trial 1	Trial 2
Field	0	87.44ef*	86.30d
	100	90.20bc	89.34bc
	200	91.22b	90.37b
	300	94.48a	93.97a
	400	89.45cd	88.65c
Greenhouse	0	83.39g	82.29f
	100	88.40de	85.33de
	200	89.37cd	88.12c
	300	89.19cd	86.41d
	400	87.23f	84.47e

*Means followed by the same letter (s) within a trial are not significantly different according to Tukey's test at ($p \leq 0.05$).

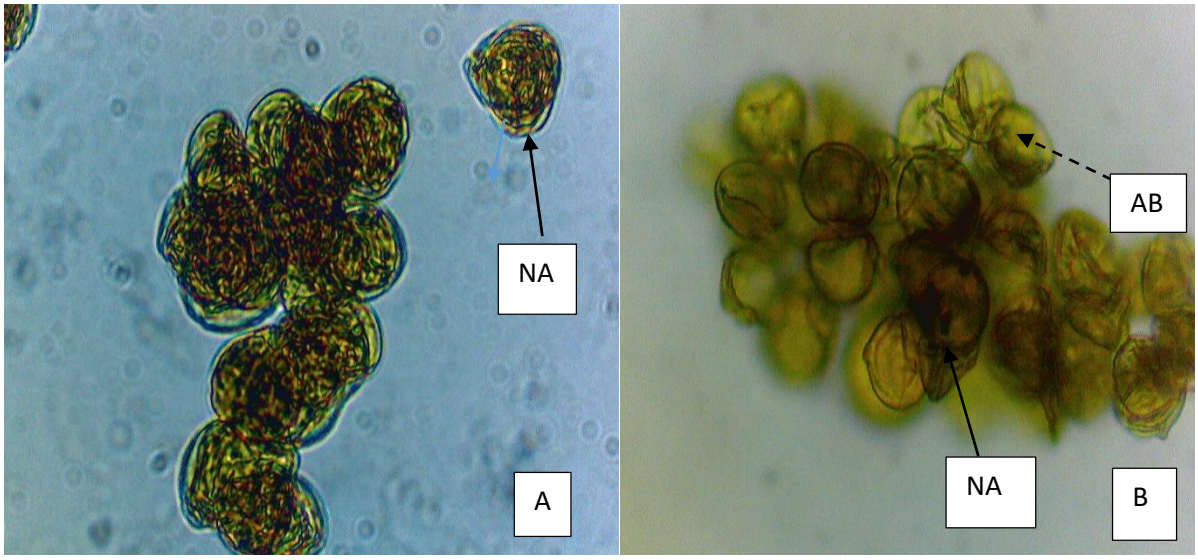


Plate 4.1 Pollen viability of non-aborted flowers: A- non-aborted (NA) pollen grains of flowers from field grown plants and B-Aborted (AB) and non-aborted (NA) pollen grains of flowers from greenhouse grown plants. Magnification ×400.

NPK fertilizer rates and growing environment had a significant effect at $p \leq 0.05$ on pollen viability of field and greenhouse grown pepino melon aborted flowers. In trial one, the highest pollen viability of 58.25% was recorded in aborted flowers from field grown plants supplied with 300 kg NPK ha^{-1} while the lowest pollen viability of 38.70% was in aborted flowers from greenhouse grown plants not supplied with NPK fertilizer. Aborted flowers from field grown plants supplied with100 and 200 kg NPK ha^{-1} had a pollen viability of 53.68% and 54.99% which was not significantly different. Pollen viability of aborted flowers from field grown plants supplied with 100 and 400 kg NPK ha^{-1} was also not significantly different (Table 4.2).

In trial two, aborted flowers from field grown plants supplied with 300 kg NPK ha^{-1} had the highest pollen viability of 58.44%. Aborted flowers from field grown plants supplied with 100 and 200 kg NPK ha^{-1} were not significantly different at $p \leq 0.05$ with a pollen viability of 53.2% and 54.56% respectively. However, the pollen viability of 53.2% recorded in aborted flowers from field grown plants supplied with 100 kg NPK ha^{-1} was not significantly different from that of aborted flowers from field grown plants supplied with 400 kg NPK ha^{-1} which had a pollen viability of 51.83% (Table 4.2). The lowest pollen viability of 38.92% was recorded in aborted flowers from greenhouse grown plants not supplied with NPK fertilizer. Generally, aborted flowers from greenhouse grown plants had the lowest pollen viability in both trials. Viable pollen grains were darkly stained while non-viable pollen grains were lightly stained (Plate 4.2).

Table 4.2 Effect of NPK fertilizer rates on pollen viability of field and greenhouse grown pepino melon aborted flowers in trial one (Nov 2018-June 2019) and trial two (July 2019-Feb 2020)

		Pollen viability (%)	
Environment	Fertilizer (kg ha^{-1})	Trial 1	Trial 2
Field	0	46.38d*	48.33d
	100	53.68bc	53.20bc
	200	54.99b	54.56b
	300	58.25a	58.44a
	400	52.02c	51.83c
Greenhouse	0	38.70g	38.92g
	100	42.82ef	41.61f
	200	44.54de	43.45e
	300	46.29d	47.16d
	400	42.21f	41.19f

*Means followed by the same letter (s) within a trial are not significantly different according to Tukey's test at ($p \leq 0.05$).

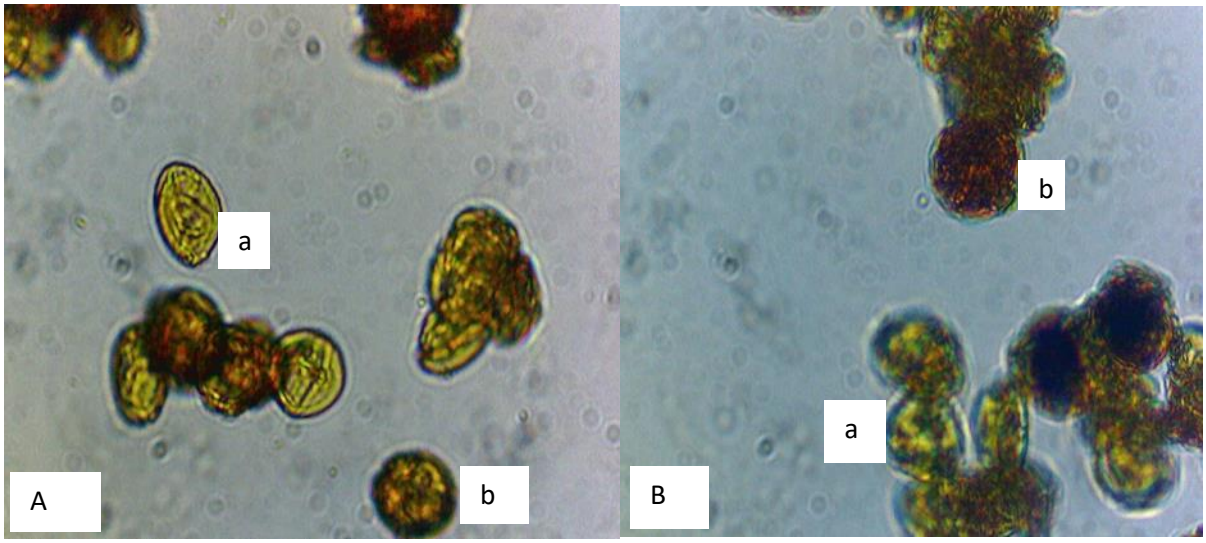


Plate 4.2 Pollen viability of aborted flowers: A- pollen from aborted flowers from greenhouse grown plants, B-pollen from aborted flowers from field grown plants. a-lightly stained non-viable pollen and b-darkly stained viable pollen. Magnification ×400.

4.3.4 Effect of NPK fertilizer rates and growing environment on *in vitro* germination of pollen from non-aborted and aborted flowers of pepino melon

NPK fertilizer rates and growing environment had a significant effect germination of pollen from non-aborted pepino melon flowers. In trial one, the highest pollen germination of 68.26% was recorded in flowers from field grown plants supplied with 300 kg NPK ha^{-1} and this was significantly higher from all the other treatment combinations. Pollen germination of flowers from field grown plants supplied with 100, 200 and 400 kg NPK ha^{-1} were not significantly different with a pollen germination of 61.7%, 63% and 60.63%, respectively. The lowest pollen germination of 52.61% was recorded in flowers from greenhouse grown plants not supplied with NPK fertilizer although the pollen germination was not significantly different from that of flowers of greenhouse grown plants supplied with 300 and 400 kg NPK ha^{-1} with a pollen germination of 54.63% and 52.73% respectively (Table 4.3).

In trial two, the highest pollen germination of 67.72% was recorded in flowers from field grown plants supplied with 300 kg NPK ha^{-1} and this was significantly different from all the other treatments. Pollen germination of flowers from field grown plants supplied with 100, 200 and 400 kg NPK ha^{-1} were not significantly different and it was 60.65%, 62.07% and 59.83% respectively. However, pollen germination of flowers from field grown plants supplied with 400 kg NPK ha^{-1} was not significantly different from that of flowers from greenhouse grown plants supplied with 200 kg NPK ha^{-1} with a pollen germination of 57.59%. The lowest pollen germination of 51.64% was recorded in flowers from greenhouse grown plants not supplied with 400 kg NPK ha^{-1} although this was not significantly different from pollen germination of flowers from greenhouse grown plants not supplied with NPK fertilizer and those supplied with 300 kg NPK ha^{-1} with a pollen germination of 51.71% and 53.36% respectively (Table 4.3). Generally, flowers from field grown plants had the highest pollen germination percentage in both trials.

Table 4.3 Effect of NPK fertilizer rates on pollen germination of non-aborted flowers of field and greenhouse grown pepino melons in trial one (Nov 2018-June 2019) and trial two (July 2019-Feb 2020)

		Pollen germination (%)	
Environment	Fertilizer (kg ha^{-1})	Trial 1	Trial 2
Field	0	57.66d*	56.61de
	100	61.70b	60.65b
	200	63.00b	62.07b
	300	68.26a	67.72a
	400	60.63bc	59.83bc
Greenhouse	0	52.61f	51.71g
	100	55.80de	54.90ef
	200	58.50cd	57.59cd
	300	54.63ef	53.36fg
	400	52.73f	51.64g

*Means followed by the same letter (s) within a trial are not significantly different according to Tukey's test at ($p \leq 0.05$).

NPK fertilizer rates had a significant effect at $p \leq 0.05$ on *in vitro* pollen germination of aborted flowers of field and greenhouse grown pepino melon. In trial one, aborted flowers from field grown plants supplied with 300 kg NPK ha^{-1} had the highest pollen germination of 48.14% and this was significantly different from the other treatment combinations. Aborted flowers from field grown plants supplied with 100 and 200 kg NPK ha^{-1} had a pollen germination of 43.33% and 44.6% respectively and this was not significantly different. In addition, the pollen germination of aborted flowers from field grown pepino plants supplied with 400 kg NPK ha^{-1} was not significantly different from that of aborted flowers from field grown plants not supplied with NPK fertilizer. The lowest pollen germination of 30.92% was recorded in aborted flowers from greenhouse plants supplied with 400 kg NPK ha^{-1} but this was not significantly different from greenhouse grown plants not supplied with NPK fertilizer and those supplied with 300 kg NPK ha^{-1} with a pollen germination of 32.06% and 33% respectively (Table 4.4).

In trial two, aborted flowers from field grown plants supplied with 300 kg NPK ha^{-1} had the highest pollen germination of 48.39% and this was significantly different from all the other

treatment combinations. Aborted flowers from field grown plants supplied with 100 and 200 kg NPK ha^{-1} had a pollen germination of 42.37% and 43.69% respectively and this was not significantly different (Table 4.4). The lowest pollen germination of 29.98% was recorded in aborted flowers from greenhouse grown plants supplied with 400 kg NPK ha^{-1} although this was not significantly different from that of aborted flowers not supplied with NPK fertilizer and those supplied with 300 kg NPK ha^{-1}. Generally, aborted flowers from field grown pepino melon plants had a higher pollen germination compared to aborted flowers from greenhouse grown plants.

Table 4.4 Effect of NPK fertilizer rates on *in vitro* pollen germination of aborted flowers of field and greenhouse pepino melon plants in trial one (Nov 2018-June 2019) and trial two (July 2019-Feb 2020)

		Pollen germination (%)	
Environment	Fertilizer (kg ha^{-1})	Trial 1	Trial 2
Field	0	39.95de*	38.75de
	100	43.33bc	42.37bc
	200	44.60b	43.69b
	300	48.14a	48.39a
	400	41.69cd	40.43cd
Greenhouse	0	32.06fg	30.89g
	100	34.34f	33.30f
	200	38.29e	36.95e
	300	33.00fg	31.95fg
	400	30.92g	29.98g

*Means followed by the same letter (s) within a trial are not significantly different according to Tukey's test at ($p \leq 0.05$).

4.4 Discussion

In the present study, NPK fertilizer rates and growing environment had a significant effect on number of flowers per truss of pepino melon in both trials. Application of 400 kg NPK ha^{-1} in both field and greenhouse grown plants had the lowest number of flowers. This could be attributed to nitrogen from the NPK fertilizer which promotes vegetative growth providing a

sufficient photosynthetic surface which in turn helps in flowering but excess nitrogen delays flowering and hence reduced number of flowers (Kumar *et al*., 2013). In addition, phosphorous stimulates flowering but further increase in phosphorous delays flowering (Kareem *et al*., 2020). Field grown plants had higher number of flowers per truss compared to greenhouse grown plants (Figure 6.1). This could be due to low temperature recorded in the field compared to the high temperature in the greenhouse. High temperature has been reported to cause flower abortion in tomato and pepino plants (Sato *et al*., 2000). There was more flower abortion in the greenhouse compared to the field and this led to reduced number of flowers in greenhouse grown plants. The number of flowers per truss increased as the NPK fertilizer rates increased. Similarly, Khetran *et al*. (2016) also reported an increase in the number of flowers of okra plants as the NPK fertilizer rates increased. Results of the current study are also in harmony with the findings of Iyagba *et al*. (2013) who reported that application of 100, 200 and 300 kg NPK ha^{-1} to okra plants produced the highest number of flowers compared to the control. Cavusoglu *et al*. (2009) stated that air temperatures above 25°C cause a negative effect on flower formation of pepino melon.

NPK fertilizer rates and growing environment had a significant effect on flower abortion of pepino melon. Greenhouse grown plants had the highest number of aborted flowers. This could be attributed to the high temperature in the greenhouse. Similarly, Ascough *et al*. (2005) reported that abortion of reproductive organs in sweet pepper occurred even when they were grown in a controlled environment. Abortion of reproductive organs is mainly caused by temperature (Huberman *et al*., 1997). Temperature has a direct effect on respiration even when plants have adequate supply of water and nutrients (Ascough *et al*., 2005). High temperature leads to increased metabolism resulting to an increase in use of energy reserves and this leads to abortion (Guinn, 1974). Similarly, Marcelis *et al*. (2004) reported that exposure of sweet pepper to a temperature of 33°C resulted to 100% abortion of buds and flowers.

Several studies have also reported increased abortion of flowers by high temperatures above 30°C (Aloni *et al*., 1991; Erickson & Markhart, 2001, 2002; Huberman *et al*., 1997). Increase in temperature from 33-35°C led to an increase in respiration and sugar accumulation in buds and flowers (Aloni *et al*., 1997) and it was concluded that abortion was caused by decrease in dry matter partitioning rather than a decrease in photosynthesis. At high temperatures auxin concentration and transport to the pedicels of flowers and fruitlets is decreased (Huberman *et al*., 1997). Consequently, the levels of ethylene precursor 1-aminocyclopropane-1-carboxylic

acid (ACC) increase after long exposure to high temperatures (Wien *et al*., 1993) resulting to an increase in ethylene concentration in buds and flowers and this increases susceptibility to abortion (Wubs *et al*., 2009). High temperature causes a decrease in photosynthesis and consequently a decrease in availability of carbohydrates (Sato *et al*., 2000). Carbohydrate content affects the expression of flower abortion genes through hexokinases which play a role in sugar metabolism and signal transduction to other genes (Jang & Sheen, 1997). Flower abortion in chilli pepper was caused by high temperatures when chilli was planted at different times of the year (Mends-Cole *et al*., 2019). Similarly, Van and Stead (1997) reported that flower retention was highly sensitive to environmental factors particularly temperature.

Pollen production is a significant stage in plants and fertile pollen is critical for plant multiplication. Abiotic stresses decrease synthesis of photosynthates and genotypes likewise reduce mobilization of reserves to the tapetum cells and this decreases pollen fertility (Razzaq *et al*., 2019). The low pollen viability recorded in flowers from greenhouse grown plants could be due to high temperature. High temperature affects reproductive growth, flower growth, fertilization and fruit maturity (Prasad, 1999). Prasad *et al*. (2002) reported that the number of pollen grains in kidney bean reduced from 2000 to almost zero when the air temperature increased from 28/18°C to 40/30°C day/night temperatures. Similarly, in tomato the number of pollen grains per flower reduced from 700,000 to less than 400,000 when temperature increased from 28/22°C to 32/26°C day/night temperatures (Pressman *et al*., 2002). In addition, flower and fruit development is sensitive to high temperature but the response depends on the stage of flower growth and plant genotype (Kafizadeh *et al*., 2008). Most crops do not respond in a similar manner to high temperature but lack of viable pollen is the major cause for fruit formation failure (Abdul-Baki, 1992; Atherton & Rudish, 1986). Both male and female gametophytes are sensitive to high temperatures but the male gametophyte is more vulnerable because at high temperature, pollen germination and tube growth are greatly reduced (Kakani *et al*., 2005). Several studies have indicated that high temperature on male reproductive organ leads to flower abortion and subsequent yield reduction (Kafizadeh *et al*., 2008). Similarly, Pressman *et al*. (2002) reported decreased pollen production per flower and decreased pollen viability in tomato when the temperatures were increased to 32°/26°C day/night temperatures.

The high pollen viability recorded in flowers from field grown plants could be due to the low temperatures in the field during the growing season. Low temperature favoured the male gametophyte and thus reduced flower abortion, increased viability and increased yield for field

grown plants. Increase in pollen viability as the fertilizer rates increased could be attributed to increase in absorbed nutrients which had a positive effect on photosynthesis, flowering and consequently pollen viability. The low pollen viability in flowers from plants not supplied with NPK fertilizer could be due to reduced absorption of nutrients which led to reduced photosynthesis, flowering and pollen viability. Lau and Stephenson (1993) also reported that zucchini plants supplied with high nitrogen produced 1.15 times more pollen than plants supplied with low nitrogen levels. Pollen grains are packed with inorganic nutrients and energy reserves such as lipids and starch which are utilized during germination and pollen tube growth (Lau & Stephenson, 1993). Pollen grains from *Cucurbita pepo* plants supplied with high nitrogen levels were larger compared to pollen grains from plants supplied with low nitrogen levels (Lau & Stephenson, 1993). Therefore, pollen grains from plants supplied with high nitrogen levels had more reserves compared to those from low nitrogen plants and this had an effect on pollen viability and germination. On the other hand, flowers from plants which were supplied with 400 kg NPK ha^{-1} had low pollen viability because most of the absorbed nutrients were directed towards vegetative growth rather than to the flowers. In addition, the reduction in pollen viability could also be attributed to low soluble sugar content in the developing pollen grains (Pressman *et al*., 2002). Sucrose protects pollen grains from desiccation thus pollen grains which have high sucrose levels do not dehydrate and are therefore viable. Hoekstra *et al*. (1989) reported that the survival rate of pollen from dehydration was positively associated with sucrose concentration. Pollen viability is important because pollen should be viable at the time of pollination for fruit set to occur.

Flower abortion is mainly caused by high temperature and the pollen grains are extremely sensitive to high temperature. The low pollen viability in the aborted flowers could be due to damage of pollen grains as a result of high temperature especially in the greenhouse. The high pollen viability of aborted flowers from field grown plants could be due to low temperature in the field. The low pollen viability in aborted flowers from plants not supplied with NPK fertilizer could be due to reduced absorption of nutrients which led to reduced photosynthesis, flowering and pollen viability. On the other hand, aborted flowers from plants supplied with intermediate nutrients had high pollen viability because of the availability of nutrients which led to increased photosynthesis, flowering and pollen viability. Aborted flowers from plants supplied with 400 kg NPK ha^{-1} had the lowest pollen viability probably due to allocation of most of the photosynthates for vegetative growth rather than to the flowers. Pollen viability

tests using dyes are easier and faster but they tend to overestimate viability. Therefore, truly viable pollen can be quantified by use of *in vitro* pollen germination tests.

NPK fertilizer rates had a significant effect at $p \leq 0.05$ on *in vitro* pollen germination of non-aborted and aborted flowers from field and greenhouse grown pepino melon plants. Flowers from greenhouse grown plants had a low pollen germination compared to flowers from field grown plants. The low pollen germination in flowers from greenhouse grown plants could be due to the high temperatures in the greenhouse. Similarly, Kafizadeh *et al*. (2008) reported reduced pollen germination of pepper when plants were grown in 38°C compared with pepper plants grown at 25°C. Findings of the current study are in harmony with those of Kakani *et al*. (2005) who reported that temperature above 28°C for 12 hours during flowering in tomato led to reduced pollen germination. High temperatures lead to reduced pollen production, pollen viability and pollen tube growth (Kafizadeh *et al*., 2008). The low pollen germination in flowers from greenhouse grown plants could be attributed to reduction in pollen moisture due to the high temperature (Kafizadeh *et al*., 2008). When the relative humidity is normal, pollen grains perform their metabolic activities normally but at low relative humidity due to high temperature, pollen desiccation occurs and thus affect metabolic activities and membrane integrity (Kafizadeh *et al*., 2008). The low pollen germination under high temperature conditions in flowers obtained from greenhouse grown plants could also be due to under-utilization or unavailability of carbohydrates in pollen grains during pollen formation (Kakani *et al*., 2005). In addition, Pressman *et al*. (2002) reported a reduction in starch and soluble sugars in the anther walls and pollen grains of tomato when exposed to 32/26°C day/night temperatures. Therefore, the low pollen germination in flowers from greenhouse grown plants could be due to reduced starch and soluble sugars in the pollen grains. Pollen tube growth and development are energy requiring processes and carbohydrates act as the main energy sources and thus a reduction in the two will lead to low pollen germination. In addition, high temperatures have a negative impact on pollen development and this leads to decreased *in vitro* pollen germination percentage. High temperatures also lead to formation of reactive oxygen species (ROS) in the pollen grains and this may have led to reduced pollen viability for pollen from greenhouse grown flowers and hence reduced *in vitro* pollen germination percentage.

The low pollen germination in flowers from plants not supplied with NPK fertilizer could be due to reduced absorption of nutrients which led to reduced photosynthesis, flowering and pollen germination. On the other hand, flowers from plants supplied with intermediate nutrients

had high pollen germination because of the availability of nutrients which led to increased photosynthesis, flowering and pollen germination. Flowers from plants supplied with 400 kg NPK ha^{-1} had the lowest pollen germination probably due to allocation of most of the photosynthates for vegetative growth rather than to the flowers.

4.5 Conclusion

NPK fertilizer rates and growing environment have an effect on number of flowers per truss, number of aborted flowers, pollen viability and pollen germination of pepino melons. Application of 200 kg NPK ha^{-1} and 300 kg NPK ha^{-1} for greenhouse and field grown pepino melons had high number of flowers per truss, reduced flower abortion, high pollen viability and pollen germination respectively.

CHAPTER FIVE

EFFECT OF NPK FERTILIZER RATES ON CONCENTRATION OF SECONDARY METABOLITES OF FIELD AND GREENHOUSE GROWN PEPINO MELONS (*Solanum muricatum* Aiton)

Abstract

Secondary metabolites are bioactive compounds which are synthesized naturally in all plant parts. The quantities of secondary metabolites produced by plants differ depending on the plant and environmental conditions under which they are produced. The present study aimed at investigating the effects of different rates (0, 100, 200, 300 and 400 kg ha^{-1}) of NPK fertilizer (17:17:17) on the quantity of secondary metabolites produced in field and greenhouse grown pepino melons (*Solanum muricatum* Aiton). The experimental design was Randomized Complete Block Design with three replications. The five NPK fertilizer rates comprised the treatments. Fruits were analysed for lutein, β-carotene, lycopene and total phenolic content (TPC). Data were subjected to Analysis of variance (ANOVA) using the SAS statistical package and significant means were separated using Tukey's honestly significant difference at $p \leq 0.05$. Results indicated that an increase in NPK fertilizer rate led to an increase of carotenoids (lutein, lycopene and β-carotene) up to a maximum at 200 kg NPK ha^{-1} beyond which the contents decreased regardless of the growing environment and trial. The control treatment (no NPK fertilizer application) favoured the accumulation of TPC in both growing environments and trials. Greenhouse grown pepino melon fruits which were not supplied with NPK fertilizer (control) had a total phenol content of 174.3 and 145.5 mg GAE /100g fresh weight (FW) in trial one and two respectively. NPK fertilizer rates did not enhance production of Total Phenolic Content in pepino melon fruits and application of 200 kg NPK ha^{-1} is recommended for maximum accumulation of carotenoids (lycopene, lutein and β-carotene).

5.1 Introduction

Plants produce a wide variety of organic compounds which can be grouped as primary and secondary metabolites. Primary metabolites include organic acids, amino acids and phytosterols and they play vital roles in respiration, photosynthesis, growth and development in plants. On the other hand, secondary metabolism is a process through which small molecule products are produced and they are not involved in growth and development of plants (Yang

et al., 2018a). Secondary metabolites enable plants to adapt to both biotic and abiotic stresses and also as a means of communication with symbiotic microorganisms as well as to attract pollinators and seed dispersal agents (Wink, 2003). Plant secondary metabolites are classified based on their chemical structure and they include flavonoids, terpenoids, steroids and alkaloids (Yang *et al*., 2018a). Secondary metabolites have been used as traditional medicine, and in perfumery and raw materials for industries (Balandrin *et al*., 1985). Currently, carotenoids and phenolic compounds which are associated with secondary metabolites are of commercial importance because of their wide application in pharmaceutical, nutraceutical and cosmetic industries (Zheng *et al*., 2014).

Carotenoids are an extensive group of lipophilic yellow-orange pigments which are derivatives of tetraterpenes (Becerra *et al*., 2020). They are the most abundant pigments in nature and are needed by photosynthetic organisms (Sandmann, 2015). Carotenoids are classified as xanthophyll's (lutein and zeaxanthin) and carotenes (β-carotene, lycopene) (Becerra *et al.*, 2020). Carotenoids are found in plant tissues such as leaves, roots, flowers and fruits and they possess an antioxidant activity which can protect humans against cardiovascular diseases, arthritis and cancer (Maiani *et al*., 2009). Additionally, β-carotene acts as pro-vitamin A while lutein protects the eye from UV radiation and is vital for brain development (Becerra *et al*., 2020).

Phenolic compounds are synthesized from the shikimate-phenylpropanoids-flavonoids pathways and are needed by plants for growth, reproduction, pigmentation, resistance to pathogens, resistance to influences of heavy metal-salts or in a general form to biotic and abiotic stresses (Ferrari, 2010). Phenolics can be broadly divided into non-soluble compounds such as condensed tannins, lignins, and cell-wall bound hydroxycinammic acids, and soluble phenolics such as phenolic acids, flavonoids and quinones (Krzyzanowska *et al*., 2010). Phenolics are an important human dietary component and they possess enormous natural antioxidant activity and other health benefits (Kumar & Goel, 2019). Phenolics have many biological and pharmacological properties such as antiviral, anticancer, anti-inflammatory, antimicrobial, antiallergic, antithrombotic, antidiabetic, hepatoprotective and food additive (Kumar & Goel, 2019).

Plant mineral nutrition not only promotes growth but also influences secondary metabolite content (Yang *et al*., 2018a). Most studies carried out show that nutrient deficiency can increase flavonoid accumulation specifically anthocyanin. Suitable nutrient supply is required for accumulation of secondary metabolites (Gaude *et al*., 2007). The type and quantity of secondary metabolites produced by plants depends on the nutrients available in the soil (Wei *et al*., 2019). For instance, nitrogen deficiency in the soil favours accumulation of non-nitrogen secondary metabolites such as terpenoids and phenols whereas nitrogen sufficiency favours accumulation of nitrogenous secondary metabolites such as alkaloids and cyanogenic glycosides (Gershenzon, 1984). Anthocyanins, proanthocyanaidin and phenols accumulation was enhanced by application of 0, 50 and 100 kg nitrogen, phosphorous and potassium (NPK) ha^{-1} while application on 0 and 50 kg NPK ha^{-1} favoured accumulation of flavonoids in pumpkin seeds (Oloyede *et al*., 2012). In another study, Ibrahim *et al*. (2013) found that application of NPK fertilizer above 90 kg NPK ha^{-1} resulted in reduction in TPC and flavonoids in *Labisia pumila* herb. β-carotene content in tomatoes was high in NPK treated plots compared to plots treated with organic fertilizers (Aina *et al*., 2019).

The synthesis and accumulation of secondary metabolites in plants is largely dependent on environmental conditions too such as light, temperature, soil water, soil fertility and salinity (Yang *et al*., 2018a). Plant secondary metabolites can be generated in response to environmental stresses and hence they play a role in adaptation and survival of plants in response to stimuli (Berini *et al*., 2018). Temperature is one of the major environmental factors that significantly affect the composition of plant secondary metabolites, increasing temperature generally enhances the concentration of all secondary metabolites (Yang *et al*., 2018a). Increase in temperature increased phenolic compounds in three *Ribes nigrum* cultivars (Zheng *et al*., 2012). High temperatures were also reported to induce the biosynthesis of alkaloids (Yang *et al*., 2018a).

Most of the studies have only majored on nitrogen and its effect on the accumulation of secondary metabolites in other vegetables but there is inadequate information on the effect of NPK fertilizer on accumulation of secondary metabolites in pepino melon. Pepino melon has been reported to contain several secondary metabolites with many health benefits. The present study is therefore, aimed at investigating the effect of NPK fertilizer rates on accumulation of carotenoids and total phenolic content of field and greenhouse grown pepino melons.

5.2 Materials and methods

5.2.1 Experimental site description

The experiment was conducted at the Horticulture Research and Teaching Field, Egerton University, Njoro. The field lies at a latitude of 0º 23' South, longitudes 35º 35' East in the Lower Highland III Agro Ecological Zone (LH3) at an altitude of approximately 2,238 m above sea level. Average maximum and minimum temperatures range from 19 ºC to 22 ºC and 5 ºC to 8 ºC, respectively, with a total annual rainfall ranging from 1200 to 1400 mm. The soils are predominantly mollic andosols (Jaetzold & Schimdt, 2006). The greenhouse used was 8m by 60m and the covering material was UV stabilized polythene with a thickness of 12×150 microns purchased from Amiran Kenya Ltd. The mean monthly temperatures in the greenhouse and field during the experiment are presented in Table 3.1.

5.2.2 Experimental design, treatment application, crop establishment and maintenance

The experimental design was Randomized Complete Block Design (RCBD) with five treatments and three replications. The five treatments included (0, 100, 200, 300 and 400 kg ha^{-1} NPK (17:17:17) fertilizer. Pepino melon seedlings (Ecuadorian Gold variety) were obtained from Garlic and Pepino Farm, Nakuru, Kenya. For the field experiment, each experimental unit was 3.2m × 3.2m (Figure 3.1) and the seedlings were planted in rows at 80 cm between rows and 50 cm from plant to plant within the rows (FAO, 1994) to give a total of 24 plants per unit. In the greenhouse experiment, each experimental unit was 2m × 5 m (Figure 3.2) at the same spacing as in the field experiment to give a total of 25 plants per unit. Soil samples were collected from the experimental units in the field and greenhouse and analysed for total N, P, K and pH before the experiment was carried out. The nutrient analysis was carried out using the method described by Okalebo *et al*. (2002) and the results obtained are presented in Table 3.2. NPK fertilizer was applied and thoroughly mixed with the soil before placing the seedlings in the transplanting holes. Weeding was done uniformly to all experimental units. Field capacity was determined as described by Cong *et al*. (2014) thereafter tensiometers were placed in two experimental units in each block. Irrigation was done when the field capacity fell below 60% since pepino melons require a field capacity of 60-65% (Lim, 2013). Drip irrigation was used in the greenhouse when the field capacity fell below 60% and it supplied about 180 litres to the 375 plants in the greenhouse. The experiment was carried out in two trials. Trial one was carried out from November 2018 to June 2019 and trial two from

July 2019 to February 2020. Ripe pepino fruits collected from selected plants in the field and greenhouse were used for analysis of secondary metabolites.

5.2.3 Determination of carotenoid content

The carotenoids of interest in this study were β-carotene, lycopene, and lutein. Carotenoids were extracted as described by Fish *et al*. (2002) using acetone/hexane (4:5, V: V). An aliquot of 0.5 g fresh frozen material was homogenized with acetone/hexane (4:5, V: V) for 1 min and centrifuged for 10 min (1789 ×g). The supernatants were collected in a 25 mL volumetric flask and brought to volume with the same acetone/hexane mixture. β-carotene determination was done by spectrophotometric analysis at 453 nm (β-carotene) and lutein at 445nm. β-carotene and lutein content were calculated using the formula as described by Nagata and Yamashita (1992), (E_x x V)/FW where E_x is absorbance depending on the carotenoid, V is volume of the solution (25 ml) and FW is the fresh weight of the sample. β-carotene and lutein content were expressed in mg/g FW. Lycopene was extracted from pepino melon fruits from the different treatments using acetone as described by Babitha (2006). The fruit was cut into small pieces and macerated in a blender. Thereafter, 2g of the fruit pulp was taken in a 100 ml stoppered conical flask containing 5 ml of 0.05% (W/V) Butylated hydroxytoluene (BHT) in acetone, 5 ml of 95% ethanol and 10 ml of hexane. The contents were stirred for 15 minutes in a magnetic stirrer and later kept in an orbital shaker in ice for 15 minutes. Afterwards 3 ml of deionized water was added and later shaken for another 15 minutes. The contents were kept for 5 minutes to allow for phase separation. Absorbance was measured at 503nm in a spectrophotometer. Lycopene content (mg/100g fresh weight) was then calculated using the formula by Ranganna (1977) where:

$$\text{Lycopene content } (\frac{\text{mg}}{100\text{g}} \text{fresh weight}) = 3.1206 \times A \times V \times D \times 100 \times 1000$$

Where: A=Absorption, V=Volume made up, D= Dilution, W= Weight of Sample

5.2.4 Determination of total phenolic content

Sample extractions were done according to Ndhlala *et al*. (2008). Fresh fruit samples (2 g) were extracted with cold 50% aqueous methanol (10 ml) twice. The two extracts were combined and centrifuged at 3000 rpm for 10 min. The collected filtrate was used for analysis of total phenolic content. Total phenolic content (TPC) in the methanol extract was analysed by Folin Ciocalteau method as described by Singleton *et al*. (1999). An aliquot of 0.1 ml of extract was mixed with

6 ml of distilled water and 0.5 ml of Folin-Ciocalteu reagent. After 4 minutes, 1.5ml of sodium carbonate solution (7.5%) was added and the samples brought to final volume of 10ml with distilled water. After 30 minutes at room temperature, absorbance was measured at 760nm using a UV/Vis spectrophotometer SP-756PC Spectrum Instruments Shanghai China in the Plant Molecular Biology and Biotechnology laboratory, Egerton University, Kenya. Total phenolics were quantified by a calibration curve obtained by measuring the absorbance of gallic acid standard. The concentrations were expressed as milligrams of gallic acid equivalents (GAE) per 100g of fresh weight (FW).

5.2.5 Data analysis

Data collected were subjected to Analysis of Variance (ANOVA) and significant means separated using Tukey's honestly significant difference (Tukey's HSD) test at $p \leq 0.05$. The SAS (Version 9.1; SAS Institute, Cary, NC) statistical package was used for data analysis.

The basic model fitted for the experiment:

$Y_{ijk} = \mu + \alpha_i + \beta_j + \tau_k + \beta\tau_{jk} + \varepsilon_{ijk}$

$i=1, 2, 3; j = 1, 2, 3, 4, 5; k = 1, 2$

Where; Y_{ijkl} – Pepino melon response

μ – Overall mean

α_i – Effect due to the ith block

β_j– Effect due to jth fertilizer rate

τ_k- Effect due to kth growing environment

$\beta\tau_{jk}$- Interaction effect of the jth fertilizer rate and kth growing environment

ε_{ijk}– Random error component which was normally and independently distributed about zero mean with a common variance $\sigma 2$.

5.3 Results

5.3.1 Effect of NPK fertilizer rates and growing environment on lutein content of pepino melon fruits

NPK fertilizer rates and growing environment had a significant effect on lutein content of pepino melon fruits in both trials. In trial one, fruits from greenhouse grown plants which were supplied with 200 kg NPK ha^{-1} had the highest lutein content of 56.16 mg/g FW while the control fruits from field grown plants had the lowest lutein content 23.23 mg/g FW (Table 5.1).

In trial two, fruits from field grown plants supplied with 200 kg NPK ha^{-1} had the highest lutein content. Generally, it was observed that as the fertilizer rate increased lutein content also increased and reached its peak at 200 kg NPK ha^{-1} after which the content dropped in both growing environments and trials.

Table 5.1 Effect of NPK fertilizer rates and growing environment on lutein (mg/g FW) content of pepino melon fruits in trial one (Nov 2018-June 2019) and trial two (July 2019-Feb 2020)

		Lutein (mg/g FW)	
Environment	Fertilizer(kg ha^{-1})	Trial 1	Trial 2
Greenhouse	0	34.78cd	30.39f
	100	41.62b	35.92ef
	200	54.16a	47.88bc
	300	42.04b	38.34de
	400	39.64bc	34.90ef
Field	0	23.23e	36.97de
	100	30.86d	42.45cd
	200	42.53b	67.25a
	300	32.16d	52.35b
	400	31.43d	44.05c

*Means followed by the same letter (s) within a trial are not significantly different according to Tukey's test ($p \leq 0.05$).

5.3.2 Effect of NPK fertilizer rates and growing environment on β-Carotene content of pepino melon fruits

NPK fertilizer rates and growing environment had a significant effect on β-carotene content of pepino melon in both trials. In trial one, β-carotene content of fruits from greenhouse grown plants supplied with 200 kg NPK ha^{-1} were significantly different at $p \leq 0.05$ compared to the other fertilizer rates in both growing environments. Field grown plants supplied with 200 kg NPK ha^{-1} had a β-carotene content of 14.28 mg/g FW which was significantly different from all the other NPK fertilizer rates except greenhouse grown fruits from plants supplied with 200 kg NPK ha^{-1} in trial one (Table 5.2). In trial two, fruits from field grown plants supplied with 200 kg NPK ha^{-1} had a β-carotene content which was of 21.59 mg/g FW and this was

significantly different at $p \leq 0.05$ from the other fertilizer rates in both growing environments. β-carotene content of greenhouse grown pepino melon fruits from plants supplied with 200 kg NPK ha^{-1} were not significantly different at $p \leq 0.05$ from field grown fruits supplied with 300 kg NPK ha^{-1} in trial two. It was observed that as the fertilizer rate increased the β-carotene content also increased and reached its peak at 200 kg NPK ha^{-1} after which the content dropped in both growing environments and trials.

Table 5.2 Effect of NPK fertilizer rates and growing environment on β-carotene (mg/g FW) content of pepino melon in trial one (Nov 2018-June 2019) and trial two (July 2019-Feb 2020)

		β-carotene (mg/g FW) content	
Environment	Fertilizer(kg ha^{-1})	Trial 1	Trial 2
Greenhouse	0	2.18fg	1.18g
	100	2.74ef	1.63fg
	200	17.31a	13.23b
	300	9.49c	6.49d
	400	6.86d	4.51e
Field	0	0.86g	1.74fg
	100	1.25fg	3.39ef
	200	14.28b	21.59a
	300	5.71d	9.85c
	400	3.97e	6.97d

*Means followed by the same letter (s) within a trial are not significantly different according to Tukey's test at ($p \leq 0.05$).

5.3.3 Effect of NPK fertilizer rates and growing environment on lycopene content of pepino melon fruits

NPK fertilizer rates and growing environment had a significant effect on lycopene content of pepino melon fruits in both trials. Greenhouse grown plants supplied with 200 kg NPK ha^{-1} had the highest lycopene content of 14.25 mg/g FW compared to the other treatments in trial one (Table 5.3). In trial two, both greenhouse and field grown plants supplied with 200 kg NPK ha^{-1} had the highest lycopene content compared to the other fertilizer rates. It was observed that

as the fertilizer rate increased the lycopene content also increased and reached its peak at 200 kg NPK ha^{-1} after which lycopene content decreased.

Table 5.3 Effect of NPK fertilizer rates and growing environment on lycopene (mg/g FW) content in pepino melon in trial one (Nov 2018-June 2019) and trial two (July 2019-Feb 2020)

		Lycopene (mg/g) content	
Environment	Fertilizer(kg ha^{-1})	Trial 1	Trial 2
Greenhouse	0	4.05def	2.30d
	100	4.47cde	2.67d
	200	14.25a	11.16a
	300	7.71bc	4.65cd
	400	6.19bcd	3.43d
Field	0	1.39f	2.39d
	100	2.37ef	2.81d
	200	8.98b	12.87a
	300	5.76bcde	7.96b
	400	4.39cdef	6.49bc

*Means followed by the same letter (s) within a trial are not significantly different according to Tukey's test ($p \leq 0.05$).

5.3.4 Effect of NPK fertilizer rates and growing environment on total phenolic content of pepino melon fruits

NPK fertilizer rates and growing environment had a significant effect on Total phenolic content (TPC) of pepino melon fruits in both trials. Greenhouse grown pepino melon fruits from plants which were not supplied with any fertilizer (control) recorded the highest TPC compared to the other fertilizer rates (Table 5.4). Greenhouse grown pepino melon fruits from plants which were not fertilized had a TPC of 174.3 mg GAE/100g FW and 148.5 mg GAE/100g FW in trial one and two respectively. Field grown pepino melon fruits from plants not supplied with fertilizer also had a TPC which was not significantly different from greenhouse grown plants supplied with 100, 200 and 300 kg NPK ha^{-1} in both trials. Field grown fruits from plants supplied with 400 kg NPK ha^{-1} had the lowest TPC though not significantly different from pepino melon fruits from plants that received 300 kg NPK ha^{-1} in both trials. Generally,

greenhouse grown fruits had a higher TPC compared to field grown pepino plants. It was also observed that as the NPK fertilizer rate increased from 0 kg ha^{-1} to 400 kg ha^{-1} the TPC also decreased in both growing environments and trials.

Table 5.4 Effect of NPK fertilizer rates and growing environment on Total Phenolic Content (mg GAE/100g FW) content of pepino melon in trial one (Nov 2018-June 2019) and trial two (July 2019-Feb 2020)

		Total phenolic content (mg GAE/100g FW)	
Environment	Fertilizer(kg ha^{-1})	Trial 1	Trial 2
Greenhouse	0	174.3a	148.5a
	100	128.3b	104.7b
	200	104.2bc	92.0bc
	300	93.8bc	87.0bc
	400	83.6cd	85.1bc
Field	0	129.1b	105.4b
	100	72.6cde	70.5cd
	200	50.1de	61.7cd
	300	39.6ef	46.5de
	400	14.0f	19.4e

*Means followed by the same letter (s) within a trial are not significantly different according to Tukey's test ($p \leq 0.05$).

5.4 Discussion

The current study revealed that an increase in NPK fertilizer led to an increase in lutein content of pepino melon fruits. Similar results were reported by Kopsell *et al.* (2007), Neugart *et al*., (2018) and Zhang *et al.* (2016) who reported that increasing nitrogen fertilizer led to an increase in the lutein content of kales, cv Winterbor and tomatoes respectively. Furthermore, the findings of this study are in agreement with those of Chenard *et al.* (2005) who reported that lutein content of parsley increased with an increase in nitrogen fertilizer rates. Barickman *et al.* (2009) also reported that a positive correlation existed between nitrogen fertilizer rates and the concentration of antioxidant carotenoids like lutein in watercress (*Nasturtium officinal* R. Br.). Lutein is a lipid soluble tetraterpenoid and is found in the plastids (Baslam *et al*., 2013). Lutein is a xanthophyll pigment of the light harvesting photosystem II and light harvesting antenna and it plays the function of dissipating excess heat from the photosystem and is a Reactive

Oxygen Species (ROS) scavenger (Jahns & Holzwarth, 2012). Based on these functions the concentration of lutein and other xanthophylls may decrease due to nitrogen deficiency because the photosystem will lack nitrogen for chlorophyll synthesis and this explains the low content of lutein in the control (no fertilizer).

The low lutein content in fruits from the control could also be due to the fact that plants which are grown in areas with low resources have reduced growth, low production and decreased production of secondary metabolites (Fanciullino *et al*., 2014). The soil in this study had low nutrient resources according to Horneck *et al*. (2011) and hence the low lutein content in the control. On the other hand, plants growing in areas with intermediate resources including fertilizers will have the highest allocation of secondary metabolites and this could explain why lutein content was high and reached its peak in fruits which were supplied with 200 kg NPK ha^{-1} compared to plants supplied with higher fertilizer rates. Under intermediate nutrient resources, high production of secondary metabolites occurs due to the availability of an excess pool of carbon to synthesize carbon-based secondary metabolites like carotenoids and lutein being one of them (Fanciullino *et al*., 2014). The low lutein content in plants supplied with 400 kg NPK ha^{-1} could be due to high fertilizer rate resulting to the allocation of most of the photosynthates to growth and development and thus low accumulation of secondary metabolites.

Phytoene synthase enzyme catalyses the first rate limiting step in carotenoid biosynthesis which involves the condensation of two geranylgeranyl diphosphate (GGPP) molecules into one phytoene molecule (Fanciullino *et al*., 2014). Phytoene synthase (PSY) enzyme is sensitive to temperature and this means that the carotenoid biosynthetic pathway may be involved in temperature stress response (Stanley & Yuan, 2019). High temperature leads to the production of reactive oxygen species (ROS) and this increases the activity of phytoene synthase enzyme and hence in lutein content (Yang *et al*., 2018a). The increase in ROS leads to increase in biosynthesis of carotenoids through redox signalling by increasing the expression of genes and enzymes involved in carotenogenesis (Fanciullino *et al*., 2014). This might explain why lutein content was high for greenhouse grown pepino melons in trial one because of the high temperature in the greenhouse. In trial two, field grown pepino melon supplied with 200 kg NPK ha^{-1} had the highest lutein content. The temperatures in the field ranged from 18.9-22.6°C. This contrasts a previous study which reported that temperatures of 18.5°C led to a decrease in carotenoids in tobacco leaves because of decrease in PSY enzyme (Yang *et al*., 2018b).

Results of this study revealed that an increase in NPK fertilizer application led to an increase in β-carotene content of pepino melon fruits. This is in harmony with the findings of Boskovic-Rakocevik *et al.* (2012) who reported that an increase in nitrogen fertilizer application led to an increase in β-carotene content of carrot roots. Similarly, Chenard *et al*. (2005) found that β-carotene content of parsley leaves was affected by increasing nitrogen rates. On the contrary, Musa *et al.* (2010) reported that the applied nitrogen did not have a significant effect on β-carotene of *Corchorus olitorius* at fruiting. Similar results were reported by Sorensen (1999) who reported that a decrease of nitrogenous fertilizer from 240 to 60 kg N ha^{-1} resulted to a 12% decrease in β-carotene content of carrots. Increase in temperature from 15°C to 30°C led to an increase in the β-carotene content of kales and spinach (Lefsrud *et al*., 2005). Vitamin A carotenoids especially β-carotene are significantly influenced by NPK nutrition but the results vary between vegetable and fruit types (Jones *et al*., 2015). On the contrary, Neugart *et al*. (2018) reported that increase in nitrogen fertilizer did not have a significant effect in β-carotene content of kales. In trial two, field grown plants supplied with 200 kg NPK ha^{-1} had the highest β-carotene content of 21.59 mg/g FW. This could be due to the fact that β-carotene content decreases with increasing temperature because the activity of enzymes phytoene synthase and phytoene desaturase catalysing the synthesis of β-carotene is influenced by temperature above 30°C (Lurie *et al*., 1996). At temperatures above 30°C phytoene synthase levels are reduced and hence the reduced levels of β- carotene in the greenhouse in trial two. In trial one, the average monthly temperature in the field was 18.9°C to 22.8°C and in trial two the temperature was18.9-22.6°C while in the greenhouse the temperature was 21°-35.8°C and 18.5-36.7°C in trial one and two respectively (Table 3.1). On the contrary, Menegol *et al.* (2017) reported that carotenes (β-carotene and lycopene) are not affected by temperature.

The present study indicated an increase in lycopene content as the NPK fertilizer rates increased with a peak at 200kg NPK ha^{-1} in both growing environments and trials. On the contrary, Dorais (2007) reported that increased nitrogen application led to a decrease in lycopene content of tomatoes. This explains the decrease in lycopene content when NPK fertilizer rates exceeded 200 kg NPK ha^{-1}. Lycopene content is influenced by genetic and environmental factors. In trial one, lycopene content was high for greenhouse grown pepino fruits and the temperature in the greenhouse ranged from 21° to 35.8°C. Helyes *et al*. (2003) also found out that greenhouse grown indeterminate tomatoes had a high lycopene content compared to field grown tomatoes. Brandt *et al*. (2006) stated that maximum lycopene content occurs at temperatures of 25° to 30°C and is completely inhibited at above 32°C. Fruits grown at high temperatures have a low

lycopene content although temperature regulation of carotenoids is crop specific. This could be due to the fact that fruits which are exposed to high temperatures as in the case of greenhouse grown pepino fruits had low lycopene content. When the air temperature is 30°C, the surface temperature of the fruit may range between 40-50°C and this decreases lycopene synthesis (Adegoroye & Joliffe, 1983). In the greenhouse the temperature ranged from 18.5 -36.7°C and therefore when the temperature was above 30°C the lycopene content was low due to conversion to β-carotene. In the field the fruits were exposed to direct sunlight and this led to an increase in the surface temperature of the fruit and hence low lycopene content. In trial two, temperature in the field ranged from 18.9° to 22.6°C and the lycopene content was higher than that for the greenhouse grown fruits. Results of the current study are in agreement with Abushita *et al*. (2000) who also reported that field grown tomatoes have a higher lycopene content than greenhouse grown tomatoes.

Lycopene content increases with an increase from low to medium temperature then drastically declines from medium to high temperatures. Hamauzu *et al.* (1998) stated that high temperatures above 35°C inhibit the accumulation of lycopene by converting it into β-carotene. This further explains the high lycopene content in the field grown pepino fruits in this study in trial two because the temperatures were lower in the field compared to the greenhouse. Fruits which were exposed to high temperatures had low lycopene content. Lycopene content is high in fruits which are exposed to light compared to those which are shaded (Dumas *et al*., 2003). It should be noted that the carotenoid content varies depending on different growing seasons, locations and cultivars and this depends on the regulation of genes particularly Zeoxanthin epoxidase (ZEP) and Violaxanthin de-epoxidase (VDE) and other genes involved in biosynthesis of carotenoids (Othman *et al*., 2014). Several studies have shown that potassium has an effect on the concentration of carotenoids (Constan-Aguilar *et al*., 2015; Kaur *et al*., 2018; Tavallali *et al*., 2018;). Some of the studies have reported increasing levels of carotenoids with increasing potassium fertilization (Constan-Aguilar *et al*., 2015; Tavallali *et al*., 2018) while other studies have shown no effect or decrease in carotenoids (Fanasca *et al*., 2006; Taber *et al*., 2008). The observed differences could be due to differences in abiotic factors or differences in cultivars. Application of 200 kg NPK ha^{-1} led to accumulation of carotenoids (lutein, β-carotene and lycopene) in both growing environments because potassium may catalyse some enzymes which are involved in synthesis of carotenoids such as phytoene synthase which produces phytoene the first component in carotenoid pathway (Rodriguez-Amaya, 2001). Further increase in potassium doesn't increase carotenoids because the

synthesis of carotenoids reached its maximum level while additional accumulation of potassium in the cytosol has no effect on carotenoids (Rodriguez-Amaya, 2001).

The current study revealed increased total phenolic content where no NPK fertilizer (control) was supplied to pepino melon plants. The results are in harmony with the findings of Ibrahim *et al*. (2011) which showed that accumulation of phenolic content in plant tissues was increased under conditions of low nitrogen. Nitrogen deficiency stimulated the accumulation of secondary metabolites like phenolics and betacynins in red beet plants (Sahalas *et al*., 2011). Similar trend of results was obtained by Munene *et al.* (2017) who reported high total phenolic content in amaranthus plants which were not supplied with any fertilizer (control).

Similarly, Argyropoulou *et al*. (2015) reported that the synthesis of secondary metabolites was stimulated by nitrogen deficiency and this enhanced the accumulation of total phenolic content of sweet basil (*Ocimum basilicum* L.). The results are also in agreement with Vanitha and Mehalai (2016) who reported the total phenolic content of ripe pepino fruits to be 93.02 mg GAE/100g FW. On the contrary, Kola (2010) reported that the TPC of pepino melon was 480-540 mg GAE/100g FW which is quite high compared to the results obtained in the current study. This could be due to differences in environmental conditions in Turkey and Kenya. It has been reported that environmental conditions play a vital role in the quality of pepino melon plants (Kola, 2010). Phenylalanine is a precursor in the biosynthesis of phenolics and is also an amino acid used in protein synthesis. Therefore, there might be a competition for phenylalanine between protein synthesis and secondary metabolite synthesis and therefore biosynthesis of secondary metabolites might be inhibited due to incorporation of phenylalanine into protein synthesis (Margna, 1977). A positive correlation exists between activity of phenylalanine lyase (PAL) an enzyme of the phenylpropanoid pathway and accumulation of carbon-based secondary metabolites in plants (Jeyaramraja *et al*., 2003). Nitrogen deficient plants increase the availability of ammonia by enhancing PAL activity hence an increase in the accumulation of polyphenolic compounds (Margna, 1977). Nitrogen is efficient for protein synthesis and thus phenolic content decreased for a given amount of phenylalanine. In addition, the expression of phenylalanine genes increases under nitrogen depletion (Larbat *et al.*, 2012). Potassium catalyses production of phenols under stress conditions because plants strive to produce more phenols as a defence mechanism against abiotic stresses (Daoud *et al*., 2018). Greenhouse grown plants had a higher TPC due to high temperature in the greenhouse. Temperature might have different responses depending on the species and their tolerance or sensitivity to high temperatures. Exposure of tomato plants a temperature of 35°C led to a

significant increase of total phenols, while a decrease in TPC was observed in watermelon (Toscano *et al*., 2019). The increase or decrease of TPC could be due to high or low PAL enzyme activity suggesting a vital role for this enzyme in regulating plant stress response (Toscano *et al*., 2019).

To summarize, increase in temperature may enhance the accumulation of most secondary metabolites in plants (Yang *et al*., 2018a). This explains why greenhouse grown fruits which were not supplied with NPK fertilizer had the highest TPC compared to field grown pepino melon fruits because in the greenhouse the temperatures were higher than in the field. In trial one, the average monthly temperature in the greenhouse temperature was 21°-35.8°C and in trial two the temperature was 18.5°-36.7°C while in the field the temperature was 18.9°-22.8°C and 18.9°-22.6°C in trial one and two respectively. There is a negative correlation between proteins and phenols because phenylalanine is used in protein synthesis and not phenolics under conditions of excess nitrogen supply (Li *et al*., 2008). This might be the reason for the low total phenolics recorded in this study when high NPK fertilizer rates were used. NPK fertilizer rates have an effect on the accumulation of secondary metabolites in pepino melon fruits grown in the field and greenhouse.

5.5 Conclusion

NPK fertilizer rates and growing environment have an effect on the concentration of secondary metabolites in pepino melon. Greenhouse and field grown pepino melons supplied with 200 kg NPK ha^{-1} and 300 kg NPK ha^{-1}, respectively had the highest concentration of lutein, β-carotene and lycopene. Greenhouse and field grown pepino melons not supplied with NPK fertilizer had the highest concentration of total phenolic content.

CHAPTER SIX

EFFECT OF NPK FERTILIZER RATES ON MICRONUTRIENT AND VITAMIN C CONTENT OF FIELD AND GREENHOUSE GROWN PEPINO MELONS (*Solanum muricatum* Aiton)

Abstract

Proper plant nutrition is essential for growth, development and quality of vegetable crops. Micronutrients are essential nutrients required by plants in small quantities. Mineral malnutrition and vitamin deficiencies in humans are considered as serious global challenges. The present study aimed at investigating the effect of NPK fertilizer (17:17:17) rates (0, 100, 200, 300 and 400 kg ha^{-1}) on the on selected micronutrients and vitamin C content of field and greenhouse grown pepino melons. The experimental design was Randomized Complete Block Design with five rates of NPK fertilizer as the treatments replicated three times. Results indicated that application of 200 and 300 kg NPK ha^{-1} enhanced copper (Cu), manganese (Mn), molybdenum (Mo), nickel (Ni) and iron (Fe) content of whether grown in the greenhouse or open field. Application of 200 kg NPK ha^{-1} enhanced vitamin C content in field and greenhouse grown pepino melon fruits.

6.1 Introduction

Pepino melon (*Solanum muricatum* Aiton) is a small bushy plant with a woody base and fibrous roots (Oczan & Arslan, 2011). It belongs to the family Solanaceae and is also known as pepino dulce, melon pear or mishqui. Pepino fruits vary in size and shape which varies from round to elongate and oval (Ahumada & Cantwell, 1996). Fruit colors also vary from purple, solid green or green with purple stripes, or cream colored with or without purple stripes and the flesh is greenish to white and yellowish-orange (Vanitha & Mehalai, 2016). Pepino melon fruits are rich in Vitamins A, B, C, K and proteins (Maheshawari *et al*., 2014) and minerals like iron, manganese, molybdenum, copper, nickel, calcium, selenium, potassium, magnesium, aluminium, sodium, boron and zinc (Oczan &Arslan, 2011). Pepino can grow on a wide variety of soils as long as they are well drained (Nemati *et al*., (2009). According to Lim (2013), pepino performs best in well drained loamy soils but is intolerant to saline soils. The optimum soil pH range is 6-7.5 (Lim, 2013). It is also drought tolerant and has the ability to recover well after undergoing stress (Popenoe, 1990). The crop does well in areas with an annual rainfall of 500-2000 mm and the optimum range is between 800 and 1400 mm (Lim, 2013). Oczan and Arslan

(2011) reported that pepino melon fruits contain copper (17.17mg kg^{-1}), iron (79.83 mg kg^{-1}), molybdenum (1.14 mg kg^{-1}), nickel (1.66 mg kg^{-1}) and manganese (7.39 mg kg^{-1}). The fruit can be eaten when ripe, as a fresh fruit with a melon taste while green mature fruits are cooked like squash (Anderson *et al*., 1996). The ripe fruit is highly juicy, moderately sweet, has an aromatic fragrance and it can be consumed as a dessert fruit, and as an ingredient of fruit salads, in juices, or in ice cream (Martinez-Romero *et al*., 2003).

Vegetables play a vital role in food and nutrition security and are the cheapest sources of vitamins and minerals required for human health (Dunsin *et al*., 2019). Worldwide over 2 billion people suffer from iron and other micronutrients deficiency (WHO, 2016). Severe micronutrient deficiencies have been reported in Sub-Saharan Africa with 1.5-12% of the total disability adjusted life years being as a result of micronutrient deficiencies (Muthayya *et al*., 2013). Iron deficiency is more severe and it affects over 50% females in countries like Democratic Republic of Congo, Ghana, Senegal, Mali and Togo leading to 115,000 maternal deaths per year (International Food Policy Research Institute [IFPRI], 2015). Anaemia is primarily caused by iron deficiency in the diet (Devalenca & Bake, 2016). Widespread deficiencies in micronutrients have been reported in Ivory Coast, Nigeria, Togo, Democratic Republic of Congo, Kenya, Sudan, Ethiopia, Ghana, Malawi, Sierra Leone, Tanzania, Zambia as well as Burkina Faso (Kihara *et al*., 2017).

Micronutrient deficiencies affects crop production and consequently human nutrition. Deficiency of micronutrients in the soil affects crop production and nutritional quality and both may affect human health (Marschner, 2012). Most soils in Africa have multiple micronutrient deficiencies in Zn, Fe, Cu, Mn and B (Vanlauwe *et al*., 2015). Soil micronutrient deficiencies are severe in sub-Saharan Africa with about 75% of the total arable land having soil fertility problems (Toenniessen *et al*., 2008). Micronutrient deficiencies thus lead to low crop productivity and poor nutritional quality of the crops. Most of diets of poor people in Sub-Saharan Africa are mainly staple foods such as maize, sorghum, cassava, rice and sweet potato and these diets have low micronutrients and therefore micronutrient deficiencies are common in these populations especially in women and children (FAO, 2015).

Micronutrients are required by plants in relatively small quantities and also referred to as trace or minor elements (Sidhu *et al*., 2019). The micronutrients include copper (Cu), iron (Fe), zinc (Zn), chlorine (Cl), boron (B), manganese (Mn), Molybdenum (Mo) and Nickel (Ni). Although

they are required by plants in small quantities they are as important as the macro nutrients for good growth, yield and quality in plants (Yadav *et al*., 2018). In the recent past there has been increase in micronutrient deficiencies in crops due to intensive cultivation, leaching of nutrients, soil erosion and unbalanced application of fertilizers (Aske *et al*., 2017). Micronutrients are absorbed by plant roots from the soil and translocated to the other parts of the plant including the edible parts. Iron acts as a catalyst in chlorophyll synthesis and assists in absorption of other nutrients (Pandav *et al*., 2016), a structural component of molecules like cytochrome, hemes, hematin, ferrichrome and leg hemoglobin which are involved in oxidation-reduction reactions in respiration and photosynthesis (Borlotti *et al*., 2012). Iron is also involved in DNA and protein synthesis and reduction of nitrates and sulphates (Sidhu *et al*., 2019). In humans, iron is required for synthesis of haemoglobin and myoglobin (Huskisson *et al*., 2007).

Copper is involved in various biochemical reactions in plants, a cofactor of enzymes and proteins, regulates several metabolic and physiological processes in plants (Sidhu *et al*., 2019) and helps in utilization of iron during chlorophyll synthesis (Harris & Lavanya, 2016). In humans, copper is involved in enzyme functions, development of connective tissue and nerve coverings (Huskisson *et al*., 2007). Deficiency of copper is not common in humans but it can lead to hypochromic anaemia, leucopenia, neutropenia and skeletal disturbances (Huskisson *et al*., 2007). Manganese activates enzymes which are involved in photosynthesis and respiration (Pankaj *et al*., 2018), accelerates germination and maturity, increases the availability of phosphorous and calcium and enhances root growth and fruit development (Sidhu *et al.*, 2019). In humans, manganese is an enzyme cofactor in antioxidant reactions related to glucose metabolism and gluconeogenesis (Huskisson *et al*., 2007). In plants, molybdenum is needed for assimilation of nitrates, fixation of atmospheric nitrogen, protein synthesis, sulphur metabolism, absorption and translocation of iron in plants (Sidhu *et al*., 2019). In humans, molybdenum assists in removal of nitrosamines, cofactor for oxidant enzymes like sulphite oxidase and xanthine oxidase (Shenkin, 2008). Manganese deficiency leads to severe neurodegeneration resulting to early childhood death (Schwartz, 2005). Nickel plays a role in activation of enzyme urease which is involved in nitrogen metabolism, controls senescence, iron uptake and it can substitute zinc and iron as an enzyme cofactor (Sidhu *et al*., 2019).

Vitamin C also known as L-Ascorbic acid is an essential antioxidant in both plant and animal metabolism and plays a vital role as an enzyme cofactor (Fenech *et al*., 2019). Vitamin C was

first isolated in 1923 by a Hungarian biochemist Szent-Gyorgyi and later synthesized by Howart and Hirst (1933). It exists in reduced form as ascorbate and the oxidized form as dehydroascorbic acid which are inter-convertible and biologically active giving the vitamin its antioxidant properties (Chambial *et al*., 2013). Deficiency of vitamin C causes anemia, scurvy, poor wound healing, capillary haemorrhage, muscle degeneration, atherosclerotic plaques and neurotic disturbances (Chambial *et al*., 2013). More than 90% of vitamin C in human diets is supplied by fruits and vegetables (Valejo *et al*., 2002). Vitamin C is a very important and vital antioxidant and it has many biological roles in the human body (Lee & Kader, 2000). Davies and Hobson (1981) stated that the vitamin C content of tomatoes varies depending on season, nutrients available and environment. High light leads to increased ROS as a result of increased photo reduction and photorespiration and this leads to increase in vitamin C (Asada, 1999). On the contrary, low light leads to reduced vitamin C content in plants (Fenech *et al*., 2019). Vitamin C content of tomatoes varies between varieties and even greatly due to growing conditions (Hamner *et al*., 1945). Production of vitamin C depends on plant metabolism, nutrient and water supply from the soil (Premamali *et al*., 2019). At first mineral nutrition was presumed not to have any effect on vitamin C content of several species (Hamner *et al*., 1945). However, recent studies have shown that application of high rates of nitrogenous fertilizers reduces vitamin C indirectly by enhancing growth of foliage and this leads to shading of the fruits from direct sunshine (Dumas *et al*., 2003). Application of high rates of nitrogen fertilizers resulted to reduction in vitamin C content of tubers (Lin *et al*., 2004). On the other hand, increase in phosphorous and potassium fertilization increases vitamin C content of vegetables (Bongekike *et al*., 2016). Recently, micronutrients and vitamins are gaining popularity among vegetable crops because of their nutritional importance and the roles they play in human health. The current study aimed at investigating the effect of NPK fertilizer rates on selected micronutrients from the ones reported in pepino melon and vitamin C content of field and greenhouse grown pepino melon fruits.

6.2 Materials and methods

6.2.1 Experimental site description

The experiment was conducted at the Horticulture Research and Teaching Field, Egerton University, Njoro. The field lies at a latitude of 0º 23’ South, longitudes 35º 35’ East in the Lower Highland III Agro Ecological Zone (LH3) at an altitude of approximately 2,238 m above sea level. Average maximum and minimum temperatures range from 19 ºC to 22 ºC and 5 ºC to 8 ºC, respectively, with a total annual rainfall ranging from 1200 to 1400 mm. The soils are

predominantly mollic andosols (Jaetzold & Schimdt, 2006). The greenhouse used was 8m by 60m and the covering material was polythene with a thickness of 12×150 microns purchased from Amiran Kenya Ltd. The mean monthly temperatures in the greenhouse and field during the experiment are presented in Table 3.1.

6.2.2 Experimental design, treatment application, crop establishment and maintenance

The experimental design was Randomized Complete Block Design (RCBD) with five treatments and three replications. The five treatments were different rates of NPK fertilizer (17:17:17) at (0, 100, 200, 300 and 400 kg ha^{-1}). Pepino seedlings (Ecuadorian Gold variety) were obtained from Garlic and Pepino Farm, Nakuru. For the field experiment, each experimental unit was 3.2m × 3.2m (Figure 3.1) and the seedlings were planted in rows 80 cm apart and 50 cm within the plants (FAO, 1994) giving a total of 24 plants per unit. In the greenhouse experiment, each experimental unit was 2m × 5 m (Figure 3.2) at the same spacing as in the field experiment to give a total of 25 plants per experimental unit. Soil samples were collected from the field and greenhouse experimental units and analysed for total N, P, K and soil pH before the experiment was carried out. Analysis was done using the method described by Okalebo *et al*. (2002) and results are presented in Table 3.2. The NPK fertilizer was applied and thoroughly mixed with the soil before placing the seedlings in the transplanting holes. Weeding was done uniformly to all experimental units. Field capacity was determined as described by Cong *et al*. (2014) thereafter tensiometers were placed in two experimental units in each block. Irrigation was done when the field capacity fell below 60% since pepino melon requires a field capacity of 60-65% (Lim, 2013). Drip irrigation was used in the greenhouse experiment when the field capacity fell below 60%. Trial one was carried out from November 2018 to June 2019 and trial two from July 2019 to February 2020. Ripe pepino melon fruits harvested from selected plants in the field and greenhouse were used for analysis of selected micronutrients and vitamin C.

6.2.3 Determination of micronutrients (Mn, Mo, Ni, Cu and Fe)

The analysis of mineral composition was performed using the modified method of Babalola and Akinwande (2014) by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) on wet digested samples. Duplicate aliquots of approximately 1 g of homogenized samples were digested with 10 mL of 65% HNO_3 in a tightly closed screw cap glass tubes for 16 h at room temperature, and then for a further 4 h at 90 °C. In order to determine the levels of Mn, Mo, Ni, Fe and Cu, 3 mL of the digested solution was added to 6 mL of deionized water and 1 mL of 1 g L^{-1} scandium solution. The instrumental analysis was performed using atomic emission spectrometer. Instrument operating conditions will be: radiofrequency power, 1400 W; plasma gas flow, 15.0 L min^{-1}; auxiliary gas flow, 0.2 L min^{-1}; nebulizer gas flow 0.75 L min^{-1}, crossed flow; standard axial torch with 2.0 mm injector of silica; peristaltic pump flow, 1 mL min^{-1}; number of replicates, 3. Results were expressed as mg kg^{-1}.

6.2.4 Determination of ascorbic acid

Ascorbic acid content was estimated by using 2, 6 - diclorophenolindophenol dye method (AOAC, 1990). Briefly, 10g of the sample was extracted in 30 ml of 5% oxalic acid in a mortar and pestle and then filtered. Standard indophenol solution was prepared by dissolving 0.05g of 2, 6 - dichlorophenolindophenol in distilled water, diluted to 100 ml and then filtered. Ascorbic acid standard solution was prepared by dissolving 0.05g of pure ascorbic acid in a small volume of 5% oxalic acid solution and then diluted to 250ml with the same oxalic acid solution. Thereafter, 10 ml of ascorbic acid standard solution was titrated as blank. The amount of ascorbic acid corresponding to 1ml of indophenol solution was then calculated and10 ml of the filtered sample was pipetted into a 50ml flask and made to the mark with the 5% oxalic acid solution and then filtered quickly through glass wool after the first few millilitres of the filtrate were discarded. The standard indophenol solution was used to titrate 10ml of the filtrate. Ascorbic acid content was calculated as mg/100g sample.

6.2.5 Data analysis

Data collected were subjected to Analysis of variance (ANOVA) and significant means separated using Tukey's honestly significant difference (Tukey's HSD) test at $p \leq 0.05$. The SAS (Version 9.1; SAS Institute, Cary, NC) statistical package was used for data analysis.
The basic model fitted for the experiment:

$Y_{ijk} = \mu + \alpha_i + \beta_j + \tau_k + \beta\tau_{jk} + \varepsilon_{ijk}$

i=1, 2, 3; j = 1, 2, 3, 4, 5; k = 1, 2

Where; Y_{ijkl} – Pepino melon response

μ – Overall mean

α_i – Effect due to the *i*th block

β_j– Effect due to *j*th fertilizer rate

τ_k- Effect due to *k*th growing environment

$\beta\tau_{jk}$- Interaction effect of the *j*th fertilizer rate and *k*th growing environment

ε_{ijk}– Random error component which is assumed to be normally and independently distributed about zero mean with a common variance $\sigma 2$.

6.3 Results

6.3.1 Effect of NPK fertilizer rates and growing environment on copper content of pepino melon fruits

NPK fertilizer rates and growing environment had a significant effect on the concentration of copper in pepino melon fruits at $p \geq 0.05$ in both trials. In trial one, greenhouse grown pepino fruits from plants supplied with 200 kg NPK ha^{-1} and field grown fruits from plants supplied with 300 kg NPK ha^{-1} had the highest copper content of 13.41 mg kg^{-1} and 15.69 mg kg^{-1}, respectively (Figure 6.1) The same trend recurred in trial two, greenhouse grown fruits from plants supplied with 200 kg NPK ha^{-1} and field grown fruits from plants supplied with 300 kg NPK ha^{-1} had the highest copper content of 12.49 and 13.51 mg kg^{-1}, respectively (Figure 6.1). The lowest copper content of 5.24 mg kg^{-1} was recorded in the control (no fertilizer) in both growing environments although it was not significantly different from greenhouse grown and field grown fruits from plants supplied with100 kg NPK ha^{-1}. In both trials, field grown fruits had higher copper content compared to greenhouse grown fruits. It was observed that the copper content increased as the fertilizer rate increased and reached its peak at 200 kg NPK ha^{-1} and 300 kg NPK ha^{-1} for greenhouse and field grown fruits respectively.

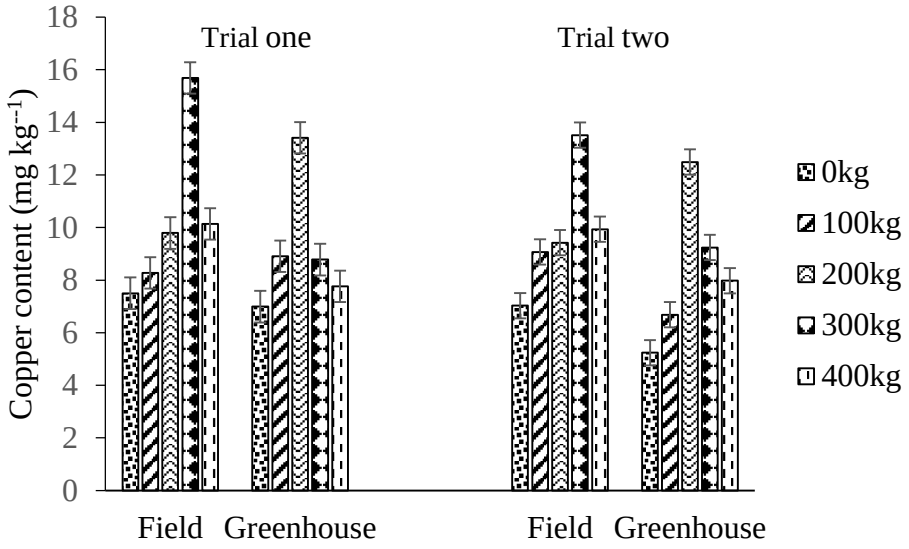


Figure 6.1 Effect of NPK fertilizer rates and growing environment on copper content of pepino melon fruits in trial one (Nov 2018-June 2019) and trial two (July 2019-Feb 2020)

6.3.2 Effect of NPK fertilizer rates and growing environment on manganese content of pepino melon fruits

NPK fertilizer rates and growing environment had a significant effect on the concentration of manganese (Mn) in pepino melon fruits at $p \geq 0.05$ in both trials. In trial one, field and greenhouse grown pepino fruits from plants which were supplied with 300 and 200 kg NPK ha^{-1} had the highest manganese content of 5.34 and 4.47 mg kg^{-1} respectively (Figure 6.2). The lowest Mn content was recorded in the control (no fertilizer) in both growing environments though this was not significantly different from greenhouse grown fruits obtained from plants supplied with 100 and 400 kg NPK ha^{-1} and field grown fruits from plants supplied with 100 kg NPK ha^{-1}. In trial two, field grown pepino fruits from plants supplied with 300 kg NPK ha had the highest Mn content of 5.57 mg kg^{-1}. Fruits from the control (no fertilizer) had the lowest Mn content in both growing environments. It was observed that the Mn content increased as the fertilizer rate increased and reached its peak at 200 and 300 kg NPK ha^{-1} for greenhouse and field grown fruits after which the content decreased. In both trails, field grown pepino fruits recorded the highest Mn content.

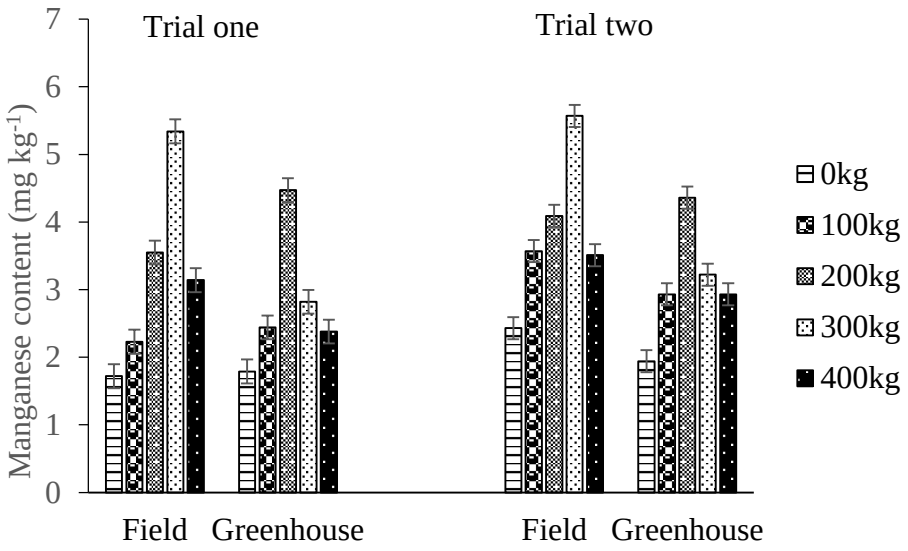


Figure 6.2 Effect of NPK fertilizer rates and growing environment on manganese content (mg kg^{-1}) of pepino melon fruits in trial one (Nov 2018-June 2019) and trial two (July 2019-Feb 2020)

6.3.3 Effect of NPK fertilizer rates and growing environment on molybdenum content of pepino melon fruits

NPK fertilizer rates and growing environment had a significant effect on the concentration of molybdenum (Mo) in pepino melon fruits at $p \geq 0.05$ in both trials. In trial one, greenhouse grown fruits from plants supplied with 200 kg NPK ha^{-1} and field grown fruits from plants supplied with 300 kg NPK ha^{-1} had the highest Mo content of 4.17 and 4.31mg kg^{-1} respectively (Figure 6.3). Field grown fruits from the control had the lowest Mo content of 1.15 mg kg^{-1} though this was not significantly different from field grown fruits from plants supplied with100 and 400 kg NPK ha^{-1} and greenhouse grown fruits from the control (no fertilizer). In trial two, the same trend was repeated where greenhouse and field grown fruits from plants supplied with 200 and 300 kg NPK ha^{-1} had the highest Mo content of 3.82 and 3.69 mg kg^{-1} respectively (Figure 6.3). The lowest Mo content was recorded in field grown fruits from plants which were not supplied with NPK fertilizer but this was not significantly different from field grown fruits obtained from plants supplied with 100 and 200 kg NPK ha^{-1} and greenhouse grown fruits from the control. In both trials greenhouse grown fruits recorded higher Mo contents compared to field grown fruits. Generally, it was observed that the Mo content increased as the fertilizer

rates increased and reached its peak at 200 and 300 kg NPK ha^{-1} for greenhouse and field grown fruits respectively.

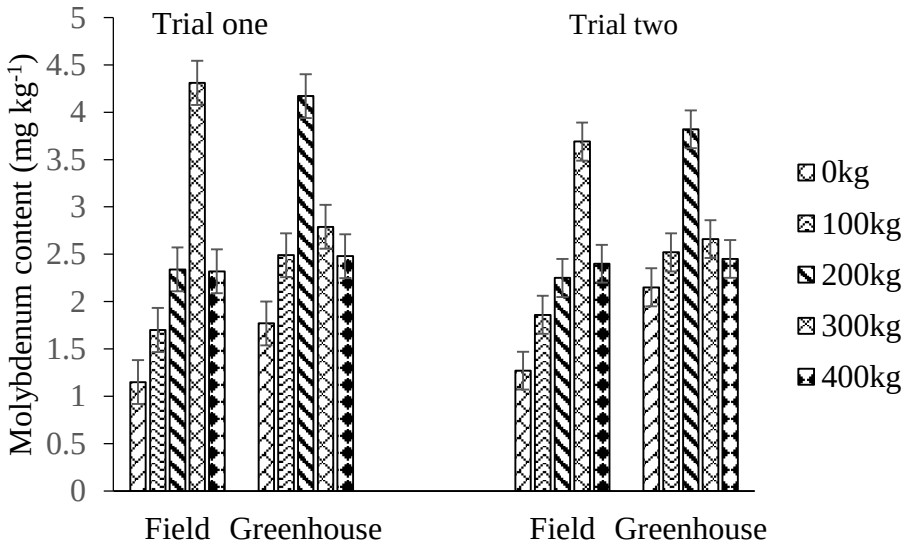


Figure 6.3 Effect of NPK fertilizer rates and growing environment on molybdenum content (mg kg^{-1}) of pepino melon fruits in trial one (Nov 2018-June 2019) and trial two (July 2019-Feb 2020)

6.3.4 Effect of NPK fertilizer rates and growing environment on nickel content of pepino melon fruits

NPK fertilizer rates and growing environment had a significant effect on the concentration of nickel (Ni) in pepino melon fruits at $p \geq 0.05$ in both trials. In trial one, greenhouse grown pepino fruits from plants supplied with 200 kg NPK ha^{-1} had the highest Ni content of 4.24 mg kg^{-1} but this was not significantly different from field grown fruits from plants supplied with 300 kg NPK ha^{-1} (Figure 6.4). In trial two, greenhouse grown fruits from plants supplied with 200 kg NPK ha^{-1} had the highest Ni content of 3.89 mg kg^{-1}. The lowest Ni content was recorded in the control in both growing environments but this was not significantly different from field grown fruits from plants supplied with 100 and 200 kg NPK ha^{-1}. In both trials, greenhouse grown fruits recorded higher Ni content compared to field grown fruits. Generally, it was observed that the Ni content increased as the fertilizer rates increased and reached its peak at 200 kg NPK ha^{-1} for greenhouse grown fruits and 300 for field grown fruits after which the content decreased.

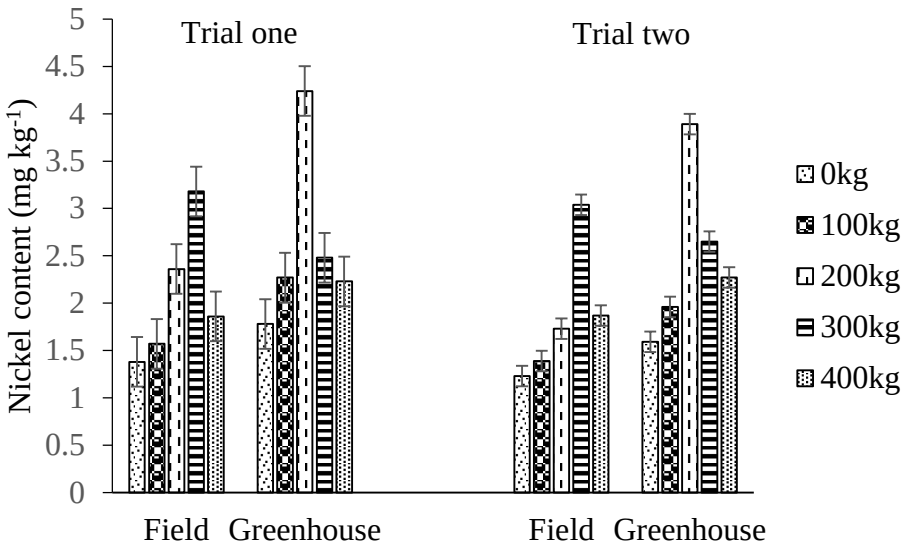


Figure 6.4 Effect of NPK fertilizer rates and growing environment on nickel content (mg kg^{-1}) of pepino melon fruits in trial one (Nov 2018-June 2019) and trial two (July 2019-Feb 2020)

6.3.5 Effect of NPK fertilizer rates and growing environment on iron content of pepino melon fruits

NPK fertilizer rates and growing environment had a significant effect on the concentration of iron (Fe) in pepino melon fruits at $p \geq 0.05$ in both trials. In trial one, greenhouse grown fruits from plants supplied with 200 kg NPK ha^{-1} and field grown fruits from plants supplied with 300 kg NPK ha^{-1} had the highest Fe content of 18.09 and 18.84 mg kg^{-1} respectively (Figure 6.5). In trial two, greenhouse and field grown fruits from plants supplied with 200 kg NPK ha^{-1} and 300 kg NPK ha^{-1} had the highest Fe content of 16.96 and 17.23 mg kg^{-1} respectively. Fruits from the control (no fertilizer) in both growing environments had the lowest Fe content but this was not significantly different from fruits which were obtained from greenhouse grown plants supplied with 100 and 400kg NPK ha^{-1} and field grown plants supplied with 100 kg NPK ha^{-1}. Generally, it was observed that the Fe content increased as the fertilizer rates increased in both growing environments and reached its peak at 200 kg NPK ha^{-1} and 300 kg NPK ha^{-1} for greenhouse and field grown fruits respectively.

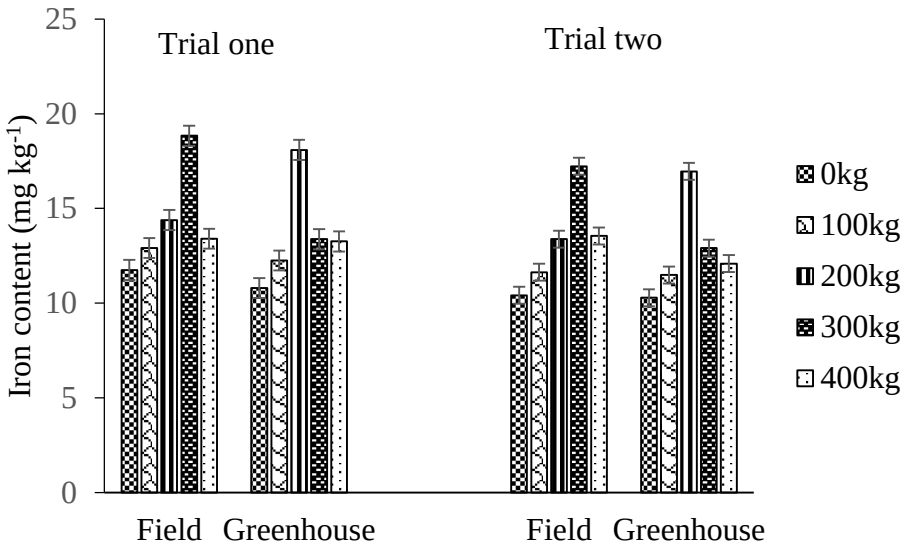


Figure 6.5 Effect of NPK fertilizer rates and growing environment on iron content (mg kg^{-1}) of pepino melon fruits in trial one (Nov 2018-June 2019) and trial two (July 2019-Feb 2020)

6.3.6 Effect of NPK fertilizer rates and growing environment on vitamin C (Ascorbic acid) content of pepino melon fruits

NPK fertilizer rates and growing environment had a significant effect on vitamin C content of pepino melon fruits. In trial one, field and greenhouse grown pepino melon fruits from plants supplied with 200 kg NPK ha^{-1} had the highest vitamin C content of 97.70 and 85.77 mg $100g^{-1}$ sample respectively (Figure 6.6). The lowest vitamin C content was recorded in field grown fruits from plants which were not supplied with NPK fertilizer (control) although this was not significantly different from greenhouse grown fruits from control, field grown fruits from plants supplied with 100 kg NPK ha^{-1} and field grown fruits supplied with 400 kg NPK ha^{-1}. In trial two, field grown pepino fruits from plants supplied with 200 kg NPK ha^{-1} had the highest vitamin C content of 90.30 mg $100g^{-1}$ sample (Figure 6.6). Greenhouse grown fruits from plants which were not supplied with fertilizer (control) recorded the lowest vitamin C content though this was not significantly different from field grown pepino fruits not supplied with NPK fertilizer (control) and greenhouse fruits supplied with 100 kg NPK ha^{-1}. It was noted that vitamin C content increased as the NPK fertilizer rate increased and reached its peak at 200 kg NPK ha^{-1} after which the content decreased in both growing environments and trials.

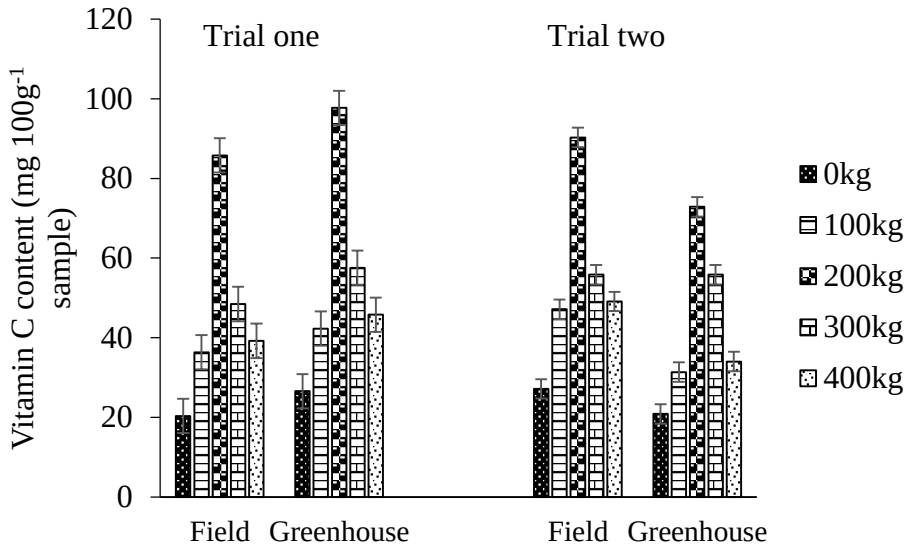


Figure 6.6 Effect of NPK fertilizer rates and growing environment on vitamin C content (mg $100g^{-1}$) of pepino melon fruits in trial one (Nov 2018-June 2019) and trial two (July 2019-Feb 2020)

6.4 Discussion

In this study, NPK fertilizer rates and growing environment had a significant effect on copper concentration of pepino melon fruits. NPK fertilized experimental units had a higher copper content compared to the control. Similarly, copper concentration in maize was high in NPK fertilized plots compared to the control (Kihara *et al*., 2020). In this study, copper content ranged from 7.5-15.69 mg kg^{-1} and 7.03-13.51 mg kg^{-1} in trial one and two, respectively. Results of the current study are in agreement with those of Oczan and Arslan (2011) who reported that pepino melon fruits grown in Turkey had a copper content of 16.53-18.81 mg kg^{-1}. Wyszkowski and Brodowska (2020) reported that nitrogen fertilizers increased Cu content in the soil by 9%. Similarly, Rutkowska *et al*. (2014) found that application of nitrogen fertilizers increased the mobility of Cu from the soil to the plants. Li *et al*. (2007) observed an increase in trace elements available to plants in plots fertilized with NPK fertilizer. This explains the low Cu content recorded in the control because the mobility of Cu was low. The high Cu content with an increase in NPK fertilizer application may be due to the fact that increased NPK fertilizer application increases Cu content in the soil (Huang & Jin, 2008). Czarnecki and During (2015) reported that application of NPK fertilizer increased the total content and mobile form of Cu in the soil and hence increase in plant uptake.

In the current study, NPK fertilizer rates and growing environment had a significant effect ($p \leq 0.05$) on Mn content of pepino melon fruits. Similar results were reported by Dunsin *et al*. (2019) who reported that summer squash from plots supplied with NPK fertilizer had higher Mn content. Moreno *et al*. (2003) also reported that Mn content in cucumber was high in NPK fertilized plots compared to the control. In this study, Mn content increased as fertilizer rates increased but reached its peak at 200 and 300 kg NPK ha^{-1} for greenhouse and field grown pepino melon fruits respectively. On the contrary Chenard *et al*. (2005) found that Mn content in the leaves of parsley decreased with an increase in nitrogen concentration in the nutrient solution. Increase in Mn with increase in NPK fertilizer could be due to enhanced uptake of Mn. At high NPK rates there is better utilization and availability of plant nutrients (Ilupeju *et al*., 2015). Control treatment recorded the lowest Mn levels and this could be due to low availability of nutrients from the soil and thus low availability of plant nutrients. The control relied on nutrients in the soil which apparently were not sufficient and most likely decreased over time because there was no replenishment. Heidari and Mohammad (2012) reported an increase in Mn content of *Momordica charantia* fruits when nitrogen from an inorganic fertilizer was increased from 75-225kg N ha^{-1}. Oczan and Arslan (2011) reported higher content of Mn in pepino melon fruits (6.167-8.613 mg kg^{-1}) grown in Turkey. In addition, Mn is not readily available in acidic soils because it readily binds to free iron and aluminium when the soil pH is less than 5.5 (Gong *et al*., 1998). Mn content increases in the soil following the application of NPK fertilizers but the proportion of Mn available to plants decreases with an increase in soil pH (Huang & Jin, 2008). The soil in this study was acidic (Table 3.2). Nitrogen fertilization increased Mn content in the soil by 12% (Wyskowski & Brodowska, 2020) which in turn increases its uptake by plants because nitrogen increases the mobility of Mn (Rutkowska *et al*., 2014). Czarnecki and During (2015) reported that application of NPK fertilizer increased the total content and mobile form of Mn in the soil and hence increase in plant uptake.

In this study, NPK fertilizer rates and growing environment had a significant effect ($p \leq 0.05$) on Mo content of pepino fruits. The present results are higher than those reported by Oczan and Arslan (2011) on the Mo content of pepino fruits grown in Turkey which was in the range of 0.82-1.46 mg kg^{-1}. This could be due to differences in environmental conditions and methods of analysis used (Guil *et al*., 1998). On the contrary, Chenard *et al*. (2005) reported that Mo content in parsley leaves decreased with an increase in nitrogen concentration in nutrient solution. Plants absorb micronutrients from the soil and the availability of Mo for plants is highly dependent on soil pH, concentration of adsorbing oxides like Fe oxides, water drainage

and organic compounds present in soil colloids (Kaiser *et al*., 2005). In alkaline soils, the solubility of Mo increases and it is readily available to plants (Kaiser *et al*., 2005) while in acidic soils (pH < 5.5) Mo availability decreases as adsorption by anion oxides increases (Reddy *et al*., 1997). The soils in this study were acidic and therefore the solubility of Mn was low hence not readily available to the plants.

NPK fertilizer rates and growing environment had a significant effect on Ni content of pepino melon fruits. The current study reported higher values of Ni compared to those reported by Oczan and Arslan (2011) in the range of 1.58-1.74 mg kg^{-1}. This could be due to the fact that Ni availability increases in acidic soils as was the case in this study because low pH facilitates uptake of Ni from the soil to plant roots (Li *et al*., 2009). Soluble Ni was high at pH 5.1 to 5.6 suggesting high solubility of Ni in acidic soils (Molas & Baran, 2004). The soil in this study was acidic as shown in Table 3.2. Nickel availability in the soil could be associated with soil pH, base cation saturation and soil inherent characteristics (Shahzad *et al*., 2018). Wyszkowski and Brodowska (2020) reported that nitrogen fertilization reduced Ni content in the soil by 24% thus its availability for absorption by plants was also reduced. This might explain the low Ni content in pepino fruits from plants which were supplied with high NPK fertilizer rates.

Results from the present study revealed that NPK fertilizer rates and growing environment had a significant effect ($p \leq 0.05$) on Fe content of pepino fruits. Similarly, Dunsin *et al*. (2019) reported high Fe content of summer squash in plots which were supplied with NPK fertilizer. Prom-u-thai and Rerkasem (2003) also reported an increase in Fe concentration in rice as the nitrogen nutrition increased. In this study, Fe content increased as the NPK fertilizer rates increased but reached a peak at 200 and 300 kg NPK ha^{-1} for greenhouse and field grown pepino fruits respectively. On the contrary, Chenard *et al*. (2005) reported decrease in Fe content of parsley leaves as the nitrogen concentration increased. The increase in Fe content as the fertilizer rates increased could be due to high levels of nitrogen from the NPK fertilizer and this might have enhanced uptake of Fe. Nitrogen fertilization enhances mobility of Fe from the soil to the plants (Rutkowska *et al*., 2014). Wyskowski and Brodowska (2020) reported that nitrogen fertilizers increased Fe content in the soil by 3%. Plant available Fe is largely influenced by nitrogen availability for root growth which in turn promotes uptake and accumulation of Fe in plant tissues (Tisdale *et al*., 1995). In parsley, nitrogen nutrition

improved plant biomass and uptake of Fe from the soil (Chenard *et al*., 2005). Similar results were observed in spinach (Mark *et al*., 2007), rice (Hao *et al*., 2007) and wheat (Shi *et al*., 2010). The decrease in Fe content at high fertilizer rates could be due to decrease in light intensity at the surface of the leaves because the plants had dense vegetation and thus decrease in Fe content (Panda *et al*., 2012). This might be due to the slow movement of iron in the phloem and most of accumulates in the roots and leaves. Similarly, decrease in micronutrient concentration due to application of high nitrogen content has been reported in wheat (Shi *et al*., 2010) and rice (Hao *et al*., 2007). High iron content of 76.46-83mg kg^{-1} in pepino melon fruits was reported by Oczan and Arslan (2011). The low Fe content recorded in this study could be due to the fact that application of NPK fertilizers increases trace elements in the soil but their available forms to plants decrease with an increase in soil pH and in this study the soil was acidic. In summary, the quantity of nutrients that accumulate in fruits depends on the nutrients available in the soil and the ability of the plant to absorb and translocate these nutrients to the respective sinks (Barunawati *et al*., 2013). High amounts of phosphorous and potassium lead to low Fe content.

NPK fertilizer rates and growing environment had a significant effect ($p \leq 0.05$) on vitamin C content of pepino melon fruits. As the NPK fertilizer rates increased the vitamin C content increased and reached a peak at 200 kg NPK ha^{-1} after which the content decreased. Similar results were reported by Boskovic-Rakocevic *et al.* (2012) where increased nitrogen rates led to decrease in vitamin C content of carrots. Increased nitrogen levels lead to an increase in vegetative growth and larger fruits and the reduction in vitamin C content could be due to dilution effect (Stefanelli *et al*., 2010). Findings of this study are also in agreement with Mozafar (1993), who reported that increase in nitrogen content led to a decrease in vitamin C content of most fruits and vegetables. Increased use of synthetic fertilizers leads to the accumulation of NH_4 in plant tissues and this leads to reduced vitamin C content (Dusin *et al*., 2019). Application of excess nitrogenous fertilizers leads to an increase in NO_3 and this results to decreased vitamin C content (Mozafar, 1993). On the contrary, Dusin *et al.* (2019) reported that high vitamin C content was recorded in summer squash fruits from plants which were supplied with *Tithonia diversifolia* compared to those supplied with NPK fertilizer. Skwarylo-Bednarz and Krzepilko (2008) also reported that nitrogen fertilizer application increases vitamin C content of fruits and vegetables.

Field grown pepino fruits had a higher vitamin C content compared to field grown pepino fruits in trial two. Similarly, Lopez-Andreu *et al.* (1986) reported that greenhouse grown tomatoes had a lower vitamin C content compared to open field grown tomatoes and this was due to low light intensity in the greenhouse compared to open field. Similarly, Rana *et al*. (2014) reported 14.5 mg and 12.82mg ascorbic acid $100g^{-1}$ FW of field and greenhouse grown tomatoes respectively. Tomato fruits grown under shady conditions or artificial cover had a 15-20% decrease in vitamin C content compared with tomatoes that were directly exposed to sunlight (Venter, 1977). Fruits which are directly exposed to sunlight have high amounts of vitamin C compared to those which are inside and shaded on the same plant (Lee & Kader, 2000). Luminosity is not directly involved in the synthesis of vitamin C but it affects accumulation during plant growth and consequently its accumulation in the resulting fruits (Rana *et al*., 2014). Vitamin C is synthesized from sugars which are produced during photosynthesis (Lee & Kader, 2000). During photosynthesis, luminosity intensity plays a vital role and the low vitamin C recorded in greenhouse grown fruits is due to low light intensity in the greenhouse which led to a reduction in sugar which is used as a substrate during vitamin C synthesis (Rana *et al*., 2014). Additionally, relative humidity was high in the greenhouse and this led to reduced transpiration leading to increase of water in the xylem vessels and this was favourable to the fruits because the fruits act as drains for high concentrations of organic molecules and, consequently, low water potential (Bertin *et al*., 2000). The low water potential in the fruits resulted to absorption of water by greenhouse grown fruits leading to a "dilution effect" and hence reducing vitamin C content of greenhouse grown fruits compared to field grown fruits (Rana *et al*., 2014).

Pepino fruits from plants supplied with higher NPK fertilizer rates had a low vitamin C content. This could be due to the fact that high nitrogen content in the NPK fertilizer led to dense foliage and thus the fruits were shaded from direct sunlight and hence the decrease in vitamin C content. Dumas *et al.* (2003) reported that increase of nitrogen fertilizer from 160 to 320 kg N ha^{-1} led to a decrease in vitamin C content of tomatoes and this might be due to decrease in monosaccharides due to increase in N-compounds and organic acids regardless of the increase in the photosynthetic surface. Decrease in vitamin C content with increase in NPK fertilizer might be due to increase in protein synthesis and decrease in carbohydrate synthesis resulting from application of NPK fertilizer (Worthington, 2001) and since vitamin C is formed from carbohydrates its synthesis is also reduced (Singh, 2005).

6.5 Conclusion

NPK fertilizer rates and growing environment have an effect on micronutrient and vitamin C content of pepino melon. Greenhouse and field grown pepino melons supplied with 200 and 300 kg NPK ha^{-1} respectively, had the highest copper, manganese, molybdenum, nickel and iron content. Application of 200 kg NPK ha^{-1} in both greenhouse and field grown pepino melons enhanced vitamin C content.

CHAPTER SEVEN

POSTHARVEST QUALITY OF PEPINO MELON (*Solanum muricatum* Aiton) AS INFLUENCED BY NPK FERTILIZER RATES, GROWING ENVIRONMENT AND STORAGE TEMPERATURE

Abstract

Over the last decade, consumers are becoming increasingly aware about the quality of the vegetables they are consuming. Plant nutrition affects the quality of most vegetables. The present study aimed at investigating the effect of NPK fertilizer (17:17:17) rates (0, 100, 200, 300 and 400 kg ha^{-1}) on the postharvest quality of field and greenhouse grown pepino melons (*Solanum muricatum* Aiton) stored at room temperature (15-22°C) and at low temperature (7°C). The experimental design was Randomized Complete Block Design with five NPK fertilizer treatments, two growing environments and two storage temperatures with three replications. Green mature pepino melon fruits were kept at room temperature (15-22°C) and at low temperature (7°C). Data were collected on percentage weight loss (PWL), total soluble solids (TSS), firmness, titratable acidity (TA), sugar acid ratio (SA ratio) and shelf life. Data were subjected to Analysis of variance (ANOVA) using the SAS statistical package and means for significant treatments at F-test separated using Tukey's honestly significant difference at $p \leq 0.05$. Results indicated that greenhouse and field grown fruits supplied with 200, 300 and 400 kg NPK ha^{-1} had the highest PWL compared to the control and fruits from plants supplied with 100 kg NPK ha^{-1} at both storage temperatures. Field grown fruits from the control treatment stored at room temperature had the highest TSS of 8.67 °Brix and 8.13 °Brix in trial one and two, respectively after 28 days of storage. Field grown and greenhouse grown fruits from the control treatment stored at low temperature were firmer with a firmness of 3.83 kg F and 3.40 kg F respectively in trial one after 28 days of storage. Field grown fruits from the control stored at low temperature had the lowest TA of 0.32% and 0.29% in trial one and two respectively after 28 days of storage. Field grown fruits not supplied with NPK fertilizer and stored at low temperature had a shelf life of 27 and 26 days in trial one and two respectively. It is therefore recommended that application of 100 kg NPK ha^{-1} and storage of pepino melon fruits at low temperature can be used to enhance quality and shelf life.

7.1 Introduction

Pepino melon (*Solanum muricatum* Aiton) is a little- known crop which belongs to the family Solanaceae and originated from the tropical and subtropical region of Andes and is grown for its edible fruits (Heiser, 1964). Pepino melon fruits are aromatic, juicy, scented, mild sweet, and they vary in size, shape and colour depending on the cultivar (Martinez-Romero *et al*., 2003). The fruit matures 30 to 80 days after pollination and the skin is usually golden yellow with purple stripes (Nuez *et al*., 1996). Pepino melon has been reported to contain around 92 % water, 6-12% soluble solids, vitamin B and C (>200mg/kg) and minerals (Gonzalez *et al*., 2000). Several studies have reported significant losses in horticultural produce after harvest (Toktam *et al*., 2019). The losses are caused by dehydration, decay, and physiological disorders during postharvest handling. Fresh fruits and vegetables also undergo rapid transformation in nutritional and sensory quality after harvest, some of which contribute to loss of market value (Ahmad & Siddiqui, 2015). The losses can be reduced through good management of pre- and postharvest factors (Toktam *et al*., 2019).

Postharvest quality is also affected by climatic factors such as temperature and light intensity, and other pre-harvest factors like soil type, fertilization, irrigation, mulching, and other cultural practices (Toktam *et al*., 2019). Temperature affects growth and development of fruits and vegetables as well as the cellular compounds and their structure and this in turn affect firmness (Toktam *et al*., 2019). Fertilizers have also been shown to influence postharvest quality of most fruits and vegetables. The type of fertilizer used and the amount applied will dictate the quality of the resulting vegetables (Arah *et al*., 2015). Application of potassium fertilizers on tomato has been shown to improve fruit colour, reduce the occurrence of yellow shoulder and enhance titratable acidity (Passam *et al*., 2007). Adequate soil nitrogen is essential for development of vegetable colour, flavour, texture and nutritional quality (Benkeblia *et al*., 2011). Application of excess nitrogen tends to lower fruit sugar content and titratable acidity while in green leafy vegetables it leads to accumulation of nitrates in the leaves (Benkeblia *et al*., 2011). On the other hand, application of high doses of nitrogenous fertilizers to greenhouse grown tomatoes reduces fruit quality by reducing total soluble solids (Passam *et al*., 2007). Temperature management between the time of harvesting and consumption has been shown to be effective in maintaining the quality of harvested vegetables. High temperatures increase metabolic activities and ethylene production but this is dependent on other factors like oxygen or carbon dioxide levels, time of exposure and the ripening stage (De Wild *et al*., 2003). Storage of vegetables at low temperature slows down metabolic processes and hence give more time for

postharvest handling of produce (Arah *et al*., 2015). The present study sought to investigate the effect of NPK fertilizer rates, growing environment and storage temperature on the postharvest quality of pepino melons.

7.2 Materials and methods

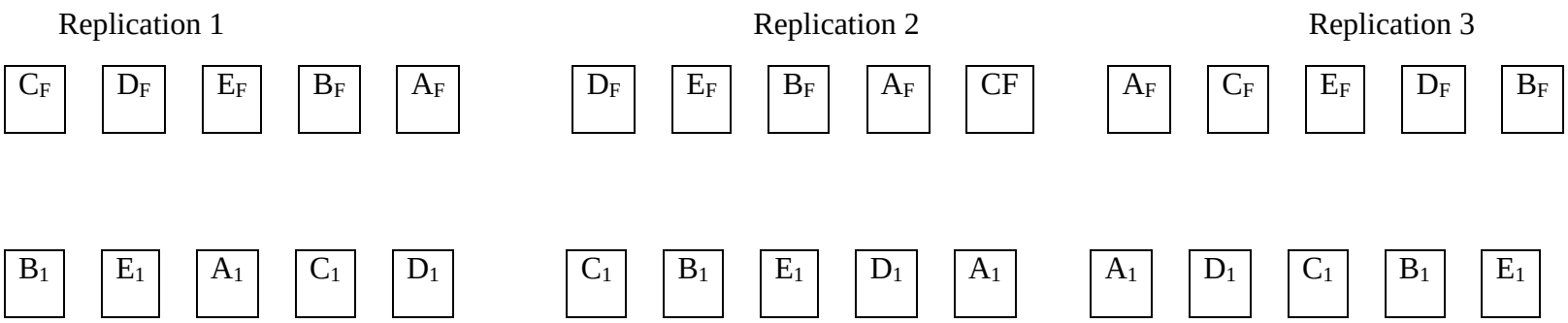
7.2.1 Site description

The experiment was conducted at the Horticulture Research and Teaching Field, Egerton University, Njoro. The field lies at a latitude of 0º 23' South, longitudes 35º 35' East in the Lower Highland III Agro Ecological Zone (LH3) at an altitude of approximately 2,238 m above sea level. Average maximum and minimum temperatures range from 19 ºC to 22 ºC and 5 ºC to 8 ºC, respectively, with a total annual rainfall ranging from 1200 to 1400 mm. The mean monthly temperatures in the greenhouse and field during the experiment are presented in Table 3.1.

7.2.2 Plant material and experimental design

Mature green pepino fruits were harvested from the five NPK fertilizer rate treatments in the field and in the greenhouse and stored at low temperature (7 °C) in a refrigerator and at room temperature (15-22°C) in the biotechnology laboratory of Egerton University. The experimental design was RCBD with treatments consisting of fruits harvested from the five NPK treatments in the field and greenhouse with three replications (Figure 7.1). Each experimental unit comprised of twenty pepino fruits randomly selected from the harvest of the individual respective treatments in the field and greenhouse experiments.

Low temperature (7°C)



Room temperature (15-22°C)

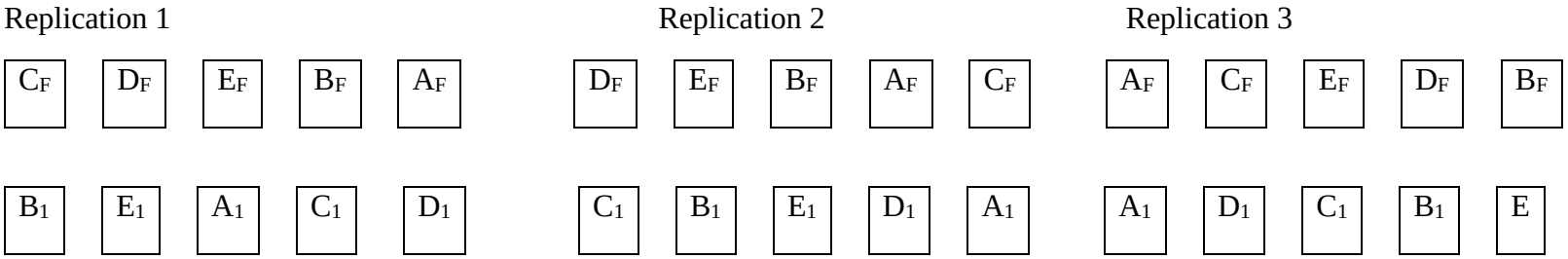


Figure 7.1 Experimental layout for the postharvest experiment for the field and greenhouse grown pepino melon fruits

KEY: A- 0kg NPK ha^{-1} **B**-100kg NPK ha^{-1} **C**-200kg NPK ha^{-1} **D-** 300 kg NPK ha^{-1} **E-** 400 kg NPK ha^{-1}- fruits from the field experiment

A_1- 0kg NPK ha^{-1} **B_1-**100kg NPK ha^{-1} **C_1-**200kg NPK ha^{-1} **D_1-** 300 kg NPK ha^{-1} **E_1-** 400 kg NPK ha^{-1}- fruits from the greenhouse experiment

7.2.3 Data collection

Data were collected and recorded on the following variables from five fruits in each treatment.

i) Percentage weight loss: For determining the weight loss, five fruits in each replication for each treatment were marked before storage and weighed in grams using a digital balance (HANGPIN JA, 12002 Japan). The same fruits were weighed at the beginning of the experiment and at an interval of seven days for 28 days. The results were expressed as the percentage loss of initial weight using the formula:

$$\text{Percentage weight loss} = \frac{\text{Initial weight-final weight}}{\text{Initial weight}} \times 100$$

ii) Total Soluble Solids (TSS): Total Soluble Solids was determined using a hand-held refractometer (0-30 ºBrix) (RHW refractometer, Optoelectronic Technology Company Ltd. UK) as per the procedure described by Tigchelaar (1986). Results were expressed as °Brix. This was done at 7 days' intervals.

iii) Fruit firmness: This was done using hand held penetrometer (model 62/DR, UK) from the beginning of the experiment and continued at an interval of seven days for 28 days. The results were reported in kg Force.

iv) Titratable acidity: This was determined by titrating 10g of fruit pulp with 0.1 N NaOH to the phenolphthalein end point at an interval of seven days for 28 days. Percentage TA was calculated using the formula (Association of Official Analytical Chemists [AOAC], 1995) where:

$$\%\ \text{TA} = \frac{\text{Titre value} \times \text{Normality} \times \text{milli} - \text{equivalent of citric acid}}{\text{Volume of sample}} \times 100$$

Milli-equivalent of citric acid which is 0.0064.

v) Fruit Sugar: Acid Ratio: The values obtained for TSS and TA were used to compute sugar acid ratio of fruit using the formula by Moneruzzaman *et al*. (2008) where:

$$\text{Sugar: acid ratio} = \frac{\%\ \text{TSS of fruit pulp}}{\%\text{TA of fruit pulp}} \times 100$$

vi) Shelf-life: The shelf life of pepino fruits was determined by counting the number of days at which at least 50% of the fruits had reached senescence and were not marketable (too soft, wrinkled or with fungal rots). Quality evaluation was done using a rating scale of 1-5 (Miguel & Marita, 1996). Shelf life was expressed in days.

7.2.4 Data analysis

Data collected were subjected to Analysis of variance (ANOVA) and significant means separated using Tukey's honestly significant difference (Tukey's HSD) test at $p \leq 0.05$. The SAS statistical package (SAS Institute, 2005) was used for data analysis.

The basic model fitted for the experiment was:

$Y_{ijkl} = \mu + \alpha i + \beta j + \tau k + \rho l + \beta\tau_{jk} + \beta\rho_{jl} + \tau\rho_{kl} + \beta\tau\rho_{jkl} + \varepsilon_{ijk}$

i=1, 2, 3; j = 1, 2, 3, 4, 5; k = 1, 2; l=1, 2

Where; Y_{ijkl} – Pepino melon response

μ – Overall mean

αi – Effect due to the *i*th block

βj– Effect due to *j*th fertilizer rate

τk- Effect due to *k*th growing environment

ρl – Effect due to *l*th storage temperature

$\beta\tau_{jk}$ – interaction effect of the *j*th fertilizer rate and *k*th growing environment

$\beta\rho_{jl}$ – interaction effect of the *j*th fertilizer rate and *l*th storage temperature

$\tau\rho_{kl}$ – interaction effect of *k*th growing environment and *l*th storage temperature

$\beta\tau\rho_{jkl}$- Interaction effect of the *j*th fertilizer rate, *k*th growing environment and *l*th storage temperature

$\varepsilon_{ij\kappa l}$– Random error component which is normally and independently distributed about zero mean with a common variance $\sigma 2$.

7.3 Results

7.3.1 Effect of NPK fertilizer rates, growing environment and storage temperature on Percentage weight loss (PWL) of pepino melon fruits

NPK fertilizer rates, growing environment and storage temperature did not have a significant effect at $p \leq 0.05$ on PWL of pepino fruits from day seven to day 21 in trial one. On day 28, NPK fertilizer rates, growing environment and storage temperature had a significant effect on PWL of pepino melon. In trial one, the highest PWL was recorded in greenhouse grown fruits from plants supplied with 300 and 400 kg NPK ha^{-1} and stored at room temperature and field grown fruits from plants supplied with 400 kg NPK ha^{-1} and stored at low temperature and room temperature (Table 7.1). The lowest PWL was recorded in greenhouse and field grown fruits from the control stored at low temperature though this was not significantly different

from other treatment combinations. In trial two, NPK fertilizer rates, growing environment and storage temperature had a significant effect on PWL from day 7 to day 28. Greenhouse grown fruits from plants supplied with 400 kg NPK ha^{-1} and stored at room temperature had the highest PWL throughout the storage period. On day 28, greenhouse and field grown fruits from plants supplied with 400 kg NPK ha^{-1}, stored at room temperature had the highest PWL of 19.38% and 15.54% respectively (Table 7.2). It was noted that as the fertilizer rates increased the PWL also increased in fruits regardless of the growing environment under which they were produced and storage temperatures in both trials. Generally, fruits stored at low temperature had lower PWL compared to those stored at room temperature in both trials. Greenhouse grown fruits also had a higher PWL compared to field grown fruits in trial two.

Table 7.1 Effect of NPK fertilizer rates, growing environment and storage temperature on percentage weight loss of pepino fruits stored at room temperature trial one (Nov 2018-June 2019)

Storage temperature	Environment	Fertilizer (kg ha^{-1})	Day 7	Day 14	Day 21	Day 28
Room temp.	Field	0	1.067	2.050	2.837	3.987def*
		100	1.423	2.800	3.620	5.303def
		200	1.980	3.580	4.713	6.383cdef
		300	2.500	5.147	5.717	9.120bcd
		400	4.417	7.280	11.353	12.457ab
	Greenhouse	0	1.213	1.840	2.820	3.413ef
		100	2.037	4.317	5.590	7.047cde
		200	2.673	5.540	7.160	8.913bcd
		300	3.167	6.400	8.720	10.863abc
		400	5.810	9.327	11.410	15.770a
Low temp.	Field	0	0.523	1.043	1.283	1.557f
		100	0.917	1.720	1.997	4.300def
		200	1.057	2.170	2.763	2.310ef
		300	1.740	3.883	4.040	7.120cde
		400	2.737	5.497	8.020	15.123a
	Greenhouse	0	0.543	1.087	1.630	2.173ef
		100	1.063	1.353	2.070	2.633ef
		200	1.543	2.417	2.867	4.012def
		300	2.240	2.977	5.080	3.183ef
		400	3.950	6.107	9.067	8.630bcd

*Means followed by the same letter (s) in a given day are not significantly different according to Tukey's test at $p \leq 0.05$. Room storage temperature varied between 15 and 22°C and low temperature was 7°C.

Table 7.2 Effect of NPK fertilizer rates, growing environment and storage temperature on percentage weight loss of pepino fruits stored at room temperature trial two (July 2019-Feb 2020)

Storage temperature	Environment	Fertilizer (kg ha^{-1})	Day 7	Day 14	Day 21	Day 28
Room temp.	Field	0	0.94ij*	1.17kl	1.44kl	4.18fghi
		100	1.89fghi	2.63fghijk	3.12fghij	7.10defg
		200	2.51efgh	3.13defghi	4.59cdefg	8.72cde
		300	3.67bcd	4.77cde	5.65cd	11.69bc
		400	4.55b	7.36b	10.88b	15.54ab
	Greenhouse	0	1.16hij	1.67ijkl	1.94ijkl	3.36fghi
		100	3.07cdef	3.08efghi	4.23defgh	7.29def
		200	3.70bcd	4.33cdef	5.33cd	9.29cd
		300	4.11bc	4.81cd	6.19c	11.60bc
		400	6.62a	10.48a	14.70a	19.39a
Low temp.	Field	0	0.66j	0.92l	1.28l	1.65i
		100	1.05ij	1.42ijkl	1.99ijkl	2.24hi
		200	1.97efghi	2.17hijkl	2.78hijkl	2.48hi
		300	2.72defg	2.89fghij	3.53efghi	3.01ghi
		400	4.12bc	3.99cdefg	4.74cdef	5.96defgh
	Greenhouse	0	1.61ghij	1.34jkl	1.72jkl	2.19hi
		100	2.31efgh	2.47ghijkl	2.99ghijk	4.59efghi
		200	3.12cde	3.52defgh	4.22defgh	5.33defghi
		300	3.69bcd	4.16cdefg	4.82cde	4.88efghi
		400	4.47b	5.49c	6.20c	5.93defgh

*Means followed by the same letter (s) in a given day are not significantly different according to Tukey's test at $p \leq 0.05$. Room storage temperature varied between 15 and 22°C and low temperature was 7°C.

7.3.2 Effect of NPK fertilizer rates, growing environment and storage temperature on Total Soluble Solids (TSS) of pepino melon fruits

NPK fertilizer rates, growing environment and storage temperature had a significant effect at $p \geq 0.05$ on TSS of pepino fruits from day seven to day 28 in both trials. In trial one, field grown fruits from plants which were not supplied with fertilizer (control) and stored at room temperature had the highest TSS from day seven to day 28. The highest TSS was recorded after 28 days of storage where field grown fruits from plants not supplied with fertilizer and stored at room temperature had a TSS of 8.67°Brix and it was significantly different from all the other treatment combinations. Greenhouse grown fruits from plants supplied with the highest fertilizer rate of 400 kg NPK ha^{-1} and stored at low temperature had the lowest TSS of 4.40°Brix (Table 7.3). In trial two, field grown fruits from plants not supplied with fertilizer (control) and stored at room temperature had the highest TSS from day 14 to day 28. On day seven, field grown fruits from plants not supplied with NPK fertilizer and stored at room or under low temperature had the highest TSS of 5.6 and 5.27°Brix respectively (Table 7.4). The highest TSS was recorded after 28 days of storage where field grown fruits from plants not supplied with NPK fertilizer and stored at room temperature had a TSS of 8.13°Brix which was significantly higher than for greenhouse grown fruits from plants supplied with 400 kg NPK ha^{-1} and stored at low temperature with a TSS of 4.3°Brix. It was observed that TSS increased as the storage time progressed and decreased as the fertilizer rates increased in both environments and storage temperatures. Generally, field grown fruits had higher TSS compared to greenhouse grown fruits in both storage temperatures and trials. On the other hand, fruits stored at low temperature had lower TSS values compared to those stored at room temperature in both trials

Table 7.3 Effect of NPK fertilizer rates, growing environment and storage temperature on TSS (°Brix) of pepino melon fruits in trial one (Nov 2018-June 2019)

Storage temperature	Environment	Fertilizer (kg ha^{-1})	Day 7	Day 14	Day 21	Day 28
Room temp.	Field	0	6.00 a*	6.93a	7.83a	8.67a
		100	4.37cde	5.73b	6.13c	6.40bcde
		200	4.53cde	4.97c	5.73cd	6.13cdef
		300	4.53cde	4.90c	5.47de	5.80defg
		400	4.17de	4.67cde	4.80ghi	5.20ghi
	Greenhouse	0	5.00b	6.00b	6.77b	7.07b
		100	4.40cde	4.90c	5.47de	5.80defg
		200	4.47cde	4.90c	5.23ef	5.67fgh
		300	4.17de	4.63cde	4.86fghi	5.10ghij
		400	4.10e	4.30ef	4.53hijk	4.80ij
Low temp.	Field	0	5.00b	5.63b	6.00c	6.83bc
		100	4.60bcd	4.83c	5.13efg	5.77efg
		200	4.43cde	4.63cde	4.97fg	5.27ghi
		300	4.30de	4.40def	4.73ghij	4.97hij
		400	4.10e	4.13f	4.33jk	4.56ij
	Greenhouse	0	4.77bc	5.00c	5.90c	6.50bcd
		100	4.60bcd	4.77cd	4.93fghi	5.23ghi
		200	4.47cde	4.63cde	4.80ghi	4.97hij
		300	4.30de	4.33ef	4.50ijk	4.73ij
		400	4.17de	4.10f	4.20k	4.40j

*Means followed by the same letter (s) in a given day are not significantly different according to Tukey's Honestly Significant Difference Test at $p \leq 0.05$. Room storage temperature varied between 15 and 22°C and low temperature was 7°C.

Table 7.4 Effect of NPK fertilizer rates, growing environment and storage temperature on TSS (°Brix) of pepino melon fruits in trial two (July 2019-Feb 2020)

Storage temperature	Environment	Fertilizer (kg ha^{-1})	Day 7	Day 14	Day 21	Day 28
Room temp.	Field	0	5.60a*	6.70a	7.60a	8.13a
		100	4.50cde	5.36c	5.67d	6.10cd
		200	4.33defg	4.97cde	5.26ef	5.77def
		300	4.17efg	4.53fghi	4.80ghi	5.00hij
		400	4.60g	4.13ijk	4.27j	4.50jkl
	Greenhouse	0	4.70bc	5.23c	6.07c	6.50bc
		100	4.50cde	4.80def	5.10efg	5.50efgh
		200	4.33defg	4.97cde	4.87ghi	5.13ghi
		300	4.13fg	4.53fghi	4.60ij	4.90ijk
		400	4.03g	4.07k	4.27j	4.47kl
Low temp.	Field	0	5.27a	5.83b	6.47b	6.93b
		100	4.60bcd	5.00cde	5.37de	5.83def
		200	4.30defg	4.70efg	4.93fghi	5.43fgh
		300	4.13fg	4.50fghij	4.70hi	5.00hij
		400	4.07fg	4.10jk	4.27j	4.60jkl
	Greenhouse	0	4.87b	5.17cd	5.67d	6.00cde
		100	4.50cde	4.77defg	5.00efgh	5.53efg
		200	4.30defg	4.53fghi	4.73ghi	5.00hij
		300	4.13fg	4.37ghijk	4.60ij	4.83ijk
		400	4.03g	4.13ijk	4.23j	4.30l

*Means followed by the same letter (s) in a given day are not significantly different according to Tukey's Honestly Significant Difference Test at $p \leq 0.05$. Room storage temperature varied between 15 and 22°C and low temperature was 7°C.

7.3.3 Effect of NPK fertilizer rates, growing environment and storage temperature on firmness of pepino melon fruits

NPK fertilizer rates, growing environment and storage temperature had a significant effect on firmness of pepino melon fruits in day seven and day 28 in trial one, and day 21 and 28 in trial two. In day seven of trial one, field grown pepino melon fruits from plants which were not supplied with NPK fertilizer and stored at either room or low temperature had the highest firmness of 4.67 kg F and 4.83 kg F, respectively. However, this was not significantly different from the firmness of 4.57 kg F recorded for greenhouse grown fruits harvested from plants not supplied with NPK fertilizer and maintained under low temperature during storage. On day 28, the highest firmness was recorded in field grown fruits from plants not supplied with fertilizer and stored at low temperature with a firmness of 3.83 kg F (Table 7.5). The lowest firmness of 0.52 kg F was recorded in greenhouse grown fruits from plants supplied with 400 kg NPK ha^{-1} and stored at room temperature after 28 days of storage.

In trial two, NPK fertilizer rates, growing environment and storage temperature had a significant effect on firmness of pepino melon fruits after 21 and 28 days of storage. On day 21, field grown fruits from plants not supplied with fertilizer and stored at low temperature had the highest firmness of 4.13 kg F but this was not significantly different from greenhouse fruits from plants not supplied with fertilizer and stored at low temperature and field grown fruits from plants not supplied with fertilizer stored at room temperature and field grown plants from plants supplied with 100 kg NPK ha^{-1} (Table 7.6). The lowest firmness was recorded in greenhouse grown fruits from plants supplied with 400 kg NPK ha^{-1} but this was not significantly different from other treatment combinations.

Generally, it was observed that fruits from field grown plants were firmer compared to greenhouse grown fruits and fruits stored at low temperature were firmer compared to those stored at room temperature. Firmness also decreased as the fertilizer rates and storage days increased.

Table 7.5 Effect of NPK fertilizer rates, growing environment and storage temperature on firmness (kg F) of pepino melon fruits in trial one (Nov 2018-June 2019)

Storage temperature	Environment	Fertilizer (kg ha^{-1})	Day 7	Day 14	Day 21	Day 28
Room temp.	Field	0	4.67a*	4.03	3.60	3.03b
		100	3.90c	3.27	3.03	2.77bc
		200	3.57cde	3.20	2.87	2.37cdef
		300	3.03ef	2.57	2.00	1.57hijk
		400	2.83f	2.33	1.50	1.02kj
	Greenhouse	0	3.97bc	3.57	2.90	2.47cdef
		100	3.73cd	3.13	2.23	2.07efgh
		200	3.43cdef	2.83	2.13	1.80ghij
		300	2.93ef	2.20	1.83	1.50ijk
		400	2.17g	1.87	1.27	0.52l
Low temp.	Field	0	4.83a	4.77	4.33	3.83a
		100	3.97bc	3.67	3.37	2.70bcd
		200	3.83cd	3.40	3.07	2.50bcde
		300	3.57cde	3.20	2.83	2.17defg
		400	3.33cdef	2.97	2.17	1.50ijk
	Greenhouse	0	4.57ab	4.40	3.30	3.03b
		100	3.53cde	3.30	2.73	2.27cdefg
		200	3.20def	2.93	2.40	1.93fghi
		300	3.00ef	2.57	2.13	1.57hijk
		400	2.87f	2.20	1.80	1.27jk

*Means followed by the same letters are not significantly different according to Tukey's Honestly Significant Difference Test at $p \leq 0.05$. Room storage temperature varied between 15 and 22°C and low temperature was 7°C.

Table 7.6 Effect of NPK fertilizer rates, growing environment and storage temperature on firmness (kg F) of pepino melon fruits in trial two (July 2019-Feb 2020)

Storage temperature	Environment	Fertilizer (kg ha^{-1})	Day 7	Day 14	Day 21	Day 28
Room temp.	Field	0	4.63	4.07	3.70abc*	2.73bc
		100	3.90	3.67	3.10def	2.60bc
		200	3.80	3.17	2.87efgh	2.13def
		300	3.43	2.87	2.17ijk	1.87fg
		400	3.00	2.43	1.90jk	1.20ij
	Greenhouse	0	4.27	3.80	3.07def	2.47cde
		100	3.93	3.27	2.80fgh	2.03efg
		200	3.60	3.00	2.40hij	1.70fgh
		300	3.13	2.63	2.00jk	1.30hi
		400	2.90	2.20	1.70k	0.85j
Low temp.	Field	0	4.70	4.37	4.13a	3.83a
		100	4.40	3.93	3.67abc	2.83bc
		200	4.13	3.60	3.33cde	2.93b
		300	3.93	3.37	3.07def	2.63bc
		400	3.63	3.07	2.77fgh	2.07defg
	Greenhouse	0	4.27	4.07	3.93ab	3.40a
		100	4.03	3.87	3.50bcd	2.83bc
		200	3.83	3.20	2.97efg	2.50bcd
		300	3.43	2.87	2.53ghi	2.10def
		400	3.20	2.53	2.17ijk	1.63ghi

*Means followed by the same letter (s) in a given day are not significantly different according to Tukey's Honestly Significant Difference Test at $p \leq 0.05$. Room storage temperature varied between 15 and 22°C and low temperature was 7°C.

7.3.4 Effect of NPK fertilizer rates, growing environment and storage temperature on Titratable Acidity (TA) of pepino melon fruits

NPK fertilizer rates, growing environment and storage temperature had a significant effect ($p \leq 0.05$) on TA of pepino fruits after 28 days of storage in both trials. There was no significant difference from day zero to day 21 in both trials. In trial one, the highest TA of 0.32% was recorded in field grown fruits from plants which were not supplied with fertilizer (control) while the lowest (0.01 %) was recorded from fruits which were obtained from greenhouse grown plants supplied with 400 kg NPK ha^{-1} and stored at low temperature after 28 days of storage. The TA ranged from 0.01 % to 0.32 % in trial one (Table 7.7). In trial two, the highest TA of 0.29 % was recorded in field grown fruits from the control stored at low temperature while the lowest TA was recorded in greenhouse grown fruits supplied with 400 kg NPK ha^{-1} and stored at room temperature after 28 days of storage. The TA ranged from 0.03 % to 0.29 % in trial two (Table 7.8). It was noted that the TA decreased as the storage time advanced. The highest TA was recorded on day zero while the lowest TA was recoded after 28 days of storage in both trials. Generally, field grown pepino melon fruits had a higher TA compared to greenhouse grown fruits in both storage temperatures and trials. Fruits stored at low temperature had a higher TA compared to those stored at room temperature in both trials.

Table 7.7 Effect of NPK fertilizer rates, growing environment and storage temperature on titratable acidity (%) of pepino melon fruits in trial one (Nov 2018-June 2019)

Storage temperature	Environment	Fertilizer (kg ha^{-1})	Day 7	Day 14	Day 21	Day 28
Room temp.	Field	0	0.31	0.26	0.21	0.17cde*
		100	0.27	0.23	0.18	0.14efgh
		200	0.24	0.19	0.16	0.12hij
		300	0.20	0.17	0.13	0.09jkl
		400	0.17	0.13	0.09	0.06lmn
	Greenhouse	0	0.22	0.18	0.15	0.09jkl
		100	0.19	0.16	0.12	0.07klm
		200	0.17	0.13	0.09	0.05mno
		300	0.14	0.11	0.07	0.02no
		400	0.12	0.10	0.04	0.01o
Low temp.	Field	0	0.36	0.34	0.33	0.32a
		100	0.32	0.29	0.27	0.25b
		200	0.30	0.27	0.23	0.20c
		300	0.28	0.23	0.19	0.16cdef
		400	0.24	0.20	0.17	0.13fghi
	Greenhouse	0	0.33	0.30	0.28	0.18cd
		100	0.29	0.27	0.25	0.19cd
		200	0.26	0.24	0.21	0.16defg
		300	0.23	0.20	0.17	0.12ghij
		400	0.21	0.17	0.13	0.10ijk

*Means followed by the same letter (s) in a given day are not significantly different according to Tukey's Honestly Significant Difference Test at $p \leq 0.05$. Room storage temperature varied between 15 and 22°C and low temperature was 7°C.

Table 7.8 Effect of NPK fertilizer rates, growing environment and storage temperature on titratable acidity (%) of pepino melon fruits in trial two (July 2019-Feb 2020)

Storage temperature	Environment	Fertilizer (kg ha^{-1})	Day 7	Day 14	Day 21	Day 28
Room temp.	Field	0	0.32	0.28	0.26	0.16de*
		100	0.27	0.24	0.21	0.13ef
		200	0.25	0.22	0.19	0.11fg
		300	0.20	0.18	0.15	0.10fgh
		400	0.17	0.14	0.11	0.08ghi
	Greenhouse	0	0.26	0.19	0.16	0.11fg
		100	0.21	0.16	0.13	0.09gh
		200	0.17	0.13	0.11	0.07hi
		300	0.15	0.11	0.09	0.05ij
		400	0.13	0.09	0.07	0.03j
Low temp.	Field	0	0.36	0.34	0.31	0.29a
		100	0.33	0.31	0.29	0.25b
		200	0.30	0.28	0.26	0.22bc
		300	0.26	0.24	0.22	0.19c
		400	0.23	0.21	0.19	0.15de
	Greenhouse	0	0.32	0.30	0.27	0.18cd
		100	0.28	0.26	0.23	0.15de
		200	0.24	0.22	0.19	0.13ef
		300	0.21	0.19	0.17	0.11fg
		400	0.18	0.16	0.13	0.10fgh

*Means followed by the same letter (s) in a given day are not significantly different according to Tukey's Honestly Significant Difference Test at $p \leq 0.05$. Room storage temperature varied between 15 and 22°C and low temperature was 7°C.

7.3.5 Effect of NPK fertilizer rates, growing environment and storage temperature on Sugar acid ratio (SA ratio) of pepino melon fruits

NPK fertilizer rates, growing environment and storage temperature did not have a significant effect at $p \leq 0.05$ on SA ratio of pepino melon fruits from day zero to day 21 in trial one and day zero to day 28 in trial two. A significant effect was only recorded in trial one after 28 days of storage. In trial one, greenhouse grown fruits from plants supplied with 400 kg NPK ha^{-1} and 300 kg NPK ha^{-1} and stored at room temperature had the highest sugar acid ratio of 110 and 98.3 respectively. The SA ratio ranged from 10.26 to 110.00 in trial one (Table 7.9). In trial two, there was no significant difference throughout the storage period. The SA ratio ranged between 10.26 and 97.00 (Table 7.10). Generally, SA ratio increased as the storage days advanced in both trials. It was also noted that greenhouse grown pepino fruits had a higher SA ratio compared to field grown fruits in both trials. Fruits stored at room temperature had a higher SA ratio compared to those stored at low temperature in both trials.

Table 7.9 Effect of NPK fertilizer rates, growing environment and storage temperature on sugar acid ratio of pepino melon fruits in trial one (Nov 2018-June 2019)

Storage temperature	Environment	Fertilizer (kg ha^{-1})	Day 7	Day 14	Day 21	Day 28
Room temp.	Field	0	19.58	27.15	37.54	49.01c*
		100	16.46	25.20	32.98	44.71c
		200	18.99	25.78	36.81	51.11c
		300	22.84	29.56	42.29	62.83c
		400	24.79	35.64	49.78	88.24c
	Greenhouse	0	22.58	32.88	45.48	73.33c
		100	22.91	31.58	45.72	84.31c
		200	25.91	36.99	59.76	91.67bc
		300	31.01	40.98	76.19	98.3ab
		400	35.25	43.00	98.67	110.00a
Low temp.	Field	0	13.77	16.28	18.39	21.61c
		100	14.23	16.50	19.03	23.09c
		200	14.78	17.56	21.53	26.74c
		300	15.54	18.86	24.56	30.24c
		400	16.86	20.33	25.74	33.75c
	Greenhouse	0	14.62	16.72	21.13	35.47c
		100	15.74	17.79	19.91	27.22c
		200	17.36	19.86	23.42	31.37c
		300	18.80	21.43	26.48	37.54c
		400	20.36	24.29	32.99	44.00c

*Means followed by the same letter (s) in a given day are not significantly different according to Tukey's Honestly Significant Difference Test at $p \leq 0.05$. Room storage temperature varied between 15 and 22°C and low temperature was 7°C.

Table 7.10: Effect of NPK fertilizer rates, growing environment and storage temperature on sugar acid ratio of pepino melon fruits in trial two (July 2019-Feb 2020)

Storage temperature	Environment	Fertilizer (kg ha^{-1})	Day 7	Day 14	Day 21	Day 28
Room temp.	Field	0	17.35	23.79	28.81	51.94
		100	16.63	22.47	26.77	47.07
		200	17.69	22.31	28.16	52.42
		300	20.81	22.39	32.27	50.00
		400	25.09	30.20	37.83	52.99
	Greenhouse	0	18.49	28.59	39.21	57.25
		100	22.13	30.79	41.44	59.00
		200	25.92	36.89	43.90	70.12
		300	29.89	38.67	51.46	87.11
		400	33.60	47.64	61.32	97.00
Low temp.	Field	0	14.64	17.16	20.63	23.65
		100	13.82	15.98	18.76	23.33
		200	14.49	16.58	19.24	25.09
		300	16.09	18.49	21.36	25.93
		400	17.56	19.55	22.95	30.03
	Greenhouse	0	15.25	17.27	21.07	32.88
		100	16.20	18.53	22.18	36.94
		200	17.89	20.94	24.71	38.89
		300	19.63	22.81	28.11	44.64
		400	22.80	26.69	32.94	41.76

*Means followed by the same letter (s) in a given day are not significantly different according to Tukey's Honestly Significant Difference Test at $p \leq 0.05$. Room storage temperature varied between 15 and 22°C and low temperature was 7°C.

7.3.6 Effect of NPK fertilizer rates, growing environment and storage temperature on shelf life of pepino melon fruits

NPK fertilizer rates, growing environment and storage temperature had a significant effect ($p \leq 0.05$) on the shelf life of pepino melon fruits in both trials. In trial one, field grown pepino fruits from plants which were not supplied with NPK fertilizer (control) and stored at low temperature (7°C) had the longest shelf life of 27 days, followed by field grown fruits from plants supplied with 100 kg NPK ha^{-1} stored at low temperature though they were not significantly different from greenhouse grown fruits from plants not supplied with fertilizer and those supplied with 100kg NPK ha^{-1} (Table 7.11). The lowest shelf life of 11 days was recorded in greenhouse grown fruits from plants supplied with 400 kg NPK ha^{-1} and stored at room temperature although this was not significantly different from field grown fruits from plants supplied with 300 and 400 kg NPK ha^{-1} and stored at room temperature, greenhouse grown fruits from plants supplied with 200 and 300 kg NPK ha^{-1} and stored at room temperature and field grown fruits from plants supplied with 400 kg NPK ha^{-1} and stored at low temperature.

In trial two, field grown pepino fruits from plants not supplied with NPK fertilizer and stored at low temperature had the longest shelf life of 26 days, followed by field grown fruits from plants supplied with 100 kg NPK ha^{-1} stored at low temperature with a shelf life of 21 days although this was not significantly different from greenhouse grown fruits from plants not supplied with fertilizer and those supplied with 100 kg NPK ha^{-1} and stored at low temperature and field grown fruits from the control and stored at room temperature (Table 7.11). The lowest shelf life was recorded in greenhouse grown fruits from plants supplied with 400 kg NPK ha^{-1} stored at room temperature with a shelf life of 10 days but this was not significantly different from greenhouse grown fruits from plants supplied with 200 and 300 kg NPK ha^{-1} stored at room temperature and field grown fruits from plants supplied with 400 kg NPK ha^{-1} and stored at room temperature. Generally, it was observed that fruits stored at low temperature had a longer shelf life than those stored at room temperature. Field grown fruits had a longer shelf life compared to greenhouse grown fruits. The shelf life decreased as the NPK fertilizer rates increased.

Table 7.11: Effect of NPK fertilizer rates, growing environment and storage temperature on shelf life (days) of pepino melon fruits in trial one (Nov 2018-June 2019) and trial two (July 2019-Feb 2020)

Storage temperature	Environment	Fertilizer (kg ha^{-1})	Shelf life (Days)	
			Trial 1	Trial 2
Room temp.	Field	0	18cd*	20bcd
		100	17cde	18cdef
		200	15defg	16fghij
		300	14fghi	14ijk
		400	12hi	11lm
	Greenhouse	0	18cd	17efgh
		100	16defg	14hijk
		200	13ghi	12klm
		300	12hi	10lm
		400	11i	10m
Low temperature	Field	0	27a	26a
		100	22b	21b
		200	18cd	17defg
		300	16def	15ghij
		400	14fghi	13jkl
	Greenhouse	0	21b	21bc
		100	19bc	19bcde
		200	17cdef	16efghi
		300	16defg	15ghij
		400	15efgh	14ijk

*Means followed by the same letter (s) in a given trial are not significantly different according to Tukey's Honestly Significant Difference Test at $p \leq 0.05$. Room storage temperature varied between 15 and 22°C and low temperature was 7°C.

7.4 Discussion

In the present study, NPK fertilizer rates, growing environment and storage temperature had a significant effect on PWL of pepino melon. On the contrary, Kodithuwakku and Kirthisinghe (2009) reported that nitrogen fertilizer treatments did not have a significant effect on weight loss of cauliflower curds stored under open conditions. It was observed that there was a progressive increase in percentage weight loss as the storage days advanced. Fruits which were not supplied with any fertilizer in both growing environments and storage temperatures had the lowest PWL. On the other hand, fruits from field and greenhouse grown plants which received the highest fertilizer rate of 400 kg NPK ha^{-1} and stored at room temperature had the highest PWL. Similar results were reported in sweet potato in which excessive application of nitrogen led to an increase in percentage weight loss during storage (Mark *et al*., 2003). Nitrogen fertilizer rates affect the rate of water loss in fruits and vegetables (Warner *et al*., 2004). The initial water content in the fruits will determine the weight loss. Fruits with a high initial water content will tend to lose more water because they have a high vapour pressure deficit compared to those with low initial water content (Kays, 1991). This could be the reason for the high PWL in fruits which were obtained from plants supplied with 400 kg NPK ha^{-1} because high nitrogen levels lead to accumulation of more water in the fruits. Dehydration and shrivelling were observed on pepino fruits stored at room temperature. This is because weight loss is as a result of water loss from the fruit surface.

Transpiration is the main cause of deterioration because it results in direct loss of weight. Weight loss is the major cause of softening and shrivelling of fruits and vegetables damaging the appearance of fruits and loss of market value (Wilson *et al*., 1999). Weight loss of horticultural produce differs based on the variety, size, texture, length and method of storage. The quality of most fruits and vegetables is affected by weight loss but this depends on the temperature and humidity during storage (Perez *et al*., 2003). Storage of pepino fruits at room temperatures could also have resulted in production of high levels of ethylene and increased respiration and subsequent weight reduction. Loss of water reduces visual quality because the fruits appear shrivelled and most consumers will not buy them. The high PWL for the fruits stored at room temperature compared to those stored at low temperature could be associated with the high room temperature (15-22°C) during storage. High temperatures during storage lead to increased water loss resulting to shrivelling and loss of fresh appearance of the fruits (Wills *et al*., 1989). Fruits lose weight when metabolic activities increase. Metabolic activities increase with an increase in temperature around the produce resulting in loss of water and

reduction in weight. In this study pepino melon fruits were harvested when green mature and as ripening progressed there was an increase in ethylene production which led to senescence and shrivelling of the fruits during storage (Wills *et al*., 1989). Greenhouse fruits had a higher percentage weight loss probably because of the high preharvest temperature during growth of the pepino fruits. Similar results were reported by Figas *et al.* (2018) who reported that greenhouse grown tomatoes had a high weight loss compared to open field grown tomatoes.

At room temperature the temperatures were higher than at low temperature (7°C) and this could have resulted to faster ripening, increased respiration rates and hence high PWL. It was noted that the percentage weight loss for fruits stored at low temperature was lower compared to those stored at room temperature. Similar findings by Song and Thornalley (2007) reported that broccoli stored at low temperatures of 4-8°C had a weight loss of 1.4% while those stored at ambient temperatures of 12-22°C had a weight loss of 9%. Vanitha and Mehalai (2016) also reported that pepino fruits stored at room temperature had a higher weight loss compared to those stored at low temperature. Mutari and Debbie (2011) reported that the weight loss of tomatoes was higher at 20°C than at 12°C. Temperatures above 20°C can lead to abnormal physiological processes in fresh produce, respiration occurs and water is lost to the surrounding environment and hence reduction in weight. Although there was an increase in PWL as the storage days increased, the rate was much lower in pepino fruits stored at low temperature compared to those stored at room temperature. Similar findings were reported by Seyoum (2002) in which reduced storage temperatures of vegetables led to a significantly lower PWL.

Low temperature reduces respiration and metabolic processes thereby slowing down the rate of fruit weight loss during storage. Low temperature also reduces the sensitivity of fruits to ethylene and senescence is reduced (Wills *et al.*,1989). In both trials, field and greenhouse grown pepino fruits supplied with the highest NPK fertilizer rate had the highest PWL. This is in agreement with the findings of Hailu *et al*. (2008) and Mark *et al*. (2003) where application of highest nitrogen fertilizer rates had the highest physiological weight loss of carrots and sweet potatoes respectively during storage. The increased PWL due to increased level of nitrogen supply may be attributed to the higher moisture content in the fruits which may lead to decreased shelf-life due to rapid metabolic activity, moisture loss and shrinkage in storage (El-Tantawy & El-Beik, 2009). On the other and, fruits from the control (no NPK fertilizer) had the lowest PWL this could be due to low moisture content in the fruits, slowed metabolic activities and hence reduced moisture loss. The PWL decreased as the phosphorous and potassium rates in the NPK fertilizer increased. This could be due to the fact that potassium

plays a role in maintaining fruit firmness but high rates do not result to further increase in firmness. The firmer the fruit the less the PWL and reduction in firmness results to more PWL. On the other hand, high nitrogen levels coupled with high phosphorous levels reduce fruit quality because most of the carbohydrates are translocated to the shoots rather than to the developing fruits resulting to dense vegetative growth. Fruits produced by plants which have dense vegetative growth tend to be less firm resulting to high PWL while fruits from plants with less vegetative growth are firmer and hence low PWL.

In summary PWL progressively increased with increase in storage time in both room and low temperatures. Weight loss is due to loss of water from the fruit surface due to transpiration and this leads to shrivelling of the fruits and reduces consumer acceptability. As the fruit continues to ripen the rate of respiration also increases and this also leads to increase in weight loss. However, low temperature leads to reduced ripening and hence reduced respiration resulting to low PWL compared to ambient room temperatures.

In the present study, NPK fertilizer rates, growing environment and storage temperature had a significant effect on total soluble solids (TSS) of pepino fruits. TSS increased as the storage days increased. Harman *et al*. (1986) also reported that as pepino melon fruits mature TSS increases significantly during maturation and ripening. Hailu (2016) reported similar findings in mango. The increase in TSS might be due to alteration of cell wall structure and the breakdown of complex carbohydrates into simple sugars. At room temperature, the temperatures were high and this led to an increase in metabolic processes, respiration and ripening resulting to high TSS. During ripening of climacteric fruits, enzyme phosphorylase converts starch to sugars leading to an increase in TSS (Hailu, 2016). Increase in TSS could also be due to excessive moisture loss of fruits which led to increased concentration of pepino fruits stored at room temperature (Nath *et al*., 2011). At high temperatures the rate of ripening is higher than at low temperatures and this increases TSS.

Field grown pepino fruits had a higher TSS compared to greenhouse grown pepino fruits in both storage temperatures. Similarly, Yeshiwas and Tolessa (2018) reported that field grown tomatoes had a higher TSS compared to greenhouse grown tomatoes during storage. The high TSS recorded in field grown pepino fruits could be due to high light intensity and thus high photosynthesis leading to more accumulation of sugars in the fruit compared to greenhouse grown fruits where light intensity was low leading to reduced photosynthesis and hence low accumulation of sugars in the fruits (Beckmann *et al*., 2006). Any factor that interferes with

photosynthesis will affect glucose and sucrose accumulation in the fruit and thus alter TSS (Rana *et al*., 2014). High relative humidity in the greenhouse may also have led to reduced transpiration and this enhances flow of water in the xylem vessels and this is good for the fruits because fruits act as drains for high concentrations of organic molecules leading to low water potential (Bertin *et al*., 2000). The low water potential in the fruits promotes absorption of water by the fruits leading to "dilution effect" making the fruits to have low TSS compared to those grown in the field (Rana *et al*., 2014). The low TSS recorded in greenhouse grown fruits could also be due to the fact that high temperatures during ripening of pepino melon reduce sugar content of the fruits (Pluda *et al*., 1993).

Fruits stored at low temperature had a lower TSS compared to that of fruits stored at room temperature. Botrel and Melo (2020) also reported that pepino melon fruits stored at cold temperatures of 10±2°C had higher TSS values compared to those stored at room temperature. The low TSS values in pepino fruits stored at low temperature could be due to delayed fruit ripening as a result of low temperature. During ripening there is breakdown of complex carbohydrates into simple sugars and this increase TSS. At high temperatures the conversion of carbohydrates into simple sugars is accelerated and this results to high TSS whereas at low temperature ripening is delayed and the hydrolysis of carbohydrates to sugars is slower, resulting to low TSS. In the present study, TSS ranged from 4.00-7.07 and 4.00-8.13 °Brix in trial one and two respectively. Other studies have reported lower TSS of 4.91-5.40 °Brix (Kola, 2010) and 5.04-5.46 °Brix (Maruapey & Yuwono, 2016). The low TSS was attributed to high water content in pepino fruits in the range of 90-92% (Gonzalez *et al*., 2000) and the fact that the quality of pepino melon fruits is greatly influenced by the environment in which this study was conducted which is quite different from the environment in the current study.

TSS decreased as the fertilizer rates increased in both growing environments and storage temperatures in both trials. Field grown fruits from control plants had the highest TSS and this could be due restriction of vegetative growth because no NPK fertilizer was applied and thus the fruits became the only sink for sugars and hence increase in TSS (Pluda *et al*., 1993). The fruits from the control plants had a low water content because they were not supplied with NPK fertilizer and this led to increased concentration of sugars in the fruit. Greenhouse grown fruits from plants supplied with the highest fertilizer rate of 400 kg NPK ha^{-1} had the lowest TSS and this might be due to excessive vegetative growth of both the main and side shoots therefore most of the photosynthates were directed to the young developing shoots rather than to the fruits leading to low sugar concentration in the fruits (Pluda *et al*., 1993). Excess nitrogen

fertilizers make plants be more succulent, thus fruits from plants supplied with 400 kg NPK ha^{-1} had a high-water content and this might have led to dilution of sugars in the fruit resulting to low TSS. In addition, potassium increases TSS due to its involvement in carbohydrate assimilation and transfer of photosynthates from the leaves to the fruits and this leads to high concentration of sugars in the cytosol, however further increase in potassium does not result to an increase in TSS (Daoud *et al*., 2018).

NPK fertilizer rates, growing environment and storage temperature had a significant effect at $p \leq 0.05$ on firmness of pepino melon fruits. Firmness decreased as the storage days advanced. Similarly, Vanitha and Mehalai (2016) reported that the force needed for penetration of pepino melon fruits decreased during storage. Lana *et al.* (2005) also reported that firmness of tomatoes decreased during storage. Decrease in firmness is strongly related to increased weight loss. In this study, firmness decreased as the NPK fertilizer rates increased. This could be attributed to the fact that potassium maintains firmness but high rates don't result to further increase in firmness because as potassium increases calcium uptake decreases and calcium is a constituent of the cell wall (Rather *et al*., 2019). Low calcium levels will affect cell wall formation and this will have implications on fruit firmness (Rather *et al*., 2019). Warner *et al.* (2004) reported that nitrogen rates did not affect firmness of tomato fruits. There is a negative correlation between increase in nitrogen content and firmness because firmness is associated with cell turgor and cell wall characteristics (Knee, 2002). Nitrogen is involved in fruit growth rate with consistency effects on cell properties (Knee, 2002). On the contrary, Toktam *et al*. (2019) reported that nitrogen fertilizer application can be effective in improving fruit quality.

Fruits from plants which were supplied with the highest fertilizer rate (400 kg NPK ha^{-1}) had the lowest firmness and this could be due to the fact that plants with dense vegetative growth are less firm than those with low or moderate vegetative growth (Toktam *et al*., 2019). This is true considering fruits which were not supplied with any fertilizer (control) and those that were supplied with the highest fertilizer rate of 400kg NPK ha^{-1}. In addition, potassium maintains firmness but further increase in potassium leads to decrease in calcium which is a constituent of the cell wall and hence a decrease in firmness in fruits from plants supplied with 400 kg NPK ha^{-1} (Rather *et al*., 2019). Fruits from the control (no fertilizer) were firmer compared to those supplied with the highest fertilizer rate after 28 days of storage in both trials. Loss of moisture and enzymatic changes results to change in firmness (Ball, 1997). Hemicelluloses and pectins become more soluble and this cause changes and loosening of the cell wall (Paul *et al*., 1999). Temperature affects cellular compounds and their structure and this in turn affects

firmness (Toktam *et al*, 2019). In both trials, field grown pepino fruits were firmer than greenhouse grown fruits. Figas *et al*. (2018) also reported that open field grown tomatoes were firmer compared to greenhouse grown tomatoes. This could be due to the fact that lower temperature during the growing season increases firmness (Anagnostou & Vasilakakis, 1995). In the greenhouse the temperatures were high and it has been reported that high temperatures tend to decrease firmness (Paul *et al*., 1999). Khorshidi *et al*. (2010) reported that apple fruits stored at low temperature were firmer than those stored at high temperature. Pepino fruits stored at low temperature were firmer compared to those stored at room temperature. Results of the present study are in agreement with those of Botrel and Melo (2020) who reported that pepino fruits stored at a temperature of 10±2°C were firmer than those stored at room temperature. Previous studies reported that loss of firmness in pepino melon is due to softening which is caused by breakdown of structural cell wall carbohydrates and an increase in soluble pectic substances during storage (Heyes *et al*., 1994). Increase in pectic substances leads to weakening of cell walls and reduction of cohesive forces binding cells together (Heyes *et al*., 1994).

In summary, fruit softening is caused by structural as well as compositional changes in various components of the cell wall carbohydrates partly as a result of fruit softening enzymes (Abbasi *et al*., 2011). Other studies have reported that fruit softening is as a result of cell wall digestion by pectinesterase, polygalacturonase and other enzymes and this is increased by an increase in storage temperature (Ahmed *et al*., 2009). Low temperature storage-maintained firmness of pepino melon fruits.

In the present study, TA of pepino melon fruits decreased during storage in both trials. Similar results were reported by Yeshiwas and Tolessa (2018) where the TA of tomatoes decreased during storage. Chaudhary *et al*. (2016) also reported decrease in TA of red grapefruits during storage. Decrease in TA could be due to higher respiration as ripening advanced and organic acids were used as substrates for respiration. Field grown fruits had a higher TA compared to greenhouse grown fruits. The effect of environment on TA is complex (Yeshiwas & Tolessa, 2018) because organic acids can be produced from within the fruit from stored carbohydrates (Sakayama & Stevens, 1976) or can be translocated from the leaves and roots to the fruits (Bertin *et al*., 2000). The low TA recorded in greenhouse grown pepino fruits may be due to low photosynthesis in the plants because of shading leading to low carbohydrate accumulation in the fruits (Caliman *et al*., 2010). Reduction in TA is a function of temperature and respiration during storage. Greenhouse and field grown fruits stored at room temperature (15-22°C) had a

low TA this could be due the high temperature which led to increased respiration and ripening hence an increase in the utilization of acids in the fruit hence leading to a decrease in TA. Pepino fruits stored at low temperature had a higher TA compared to those stored at room temperature. Similarly, Botrel and Melo (2020) reported that pepino melon fruits stored at a temperature of 10±2°C had a higher TA compared to fruits stored at room temperature. The lower TA for the pepino fruits stored at low temperature (7°C) could be due to decreased ripening and respiration as a result of low temperatures and hence decreased utilization of acids in the fruit resulting to a slow decrease in TA.

NPK fertilizer rates, growing environment and storage temperature did not have a significant effect on TA of pepino fruits from day zero to day 21 in both trials. This is in harmony with the findings of Harman *et al*. (1986) who reported that as pepino melon fruit matures TA does not vary significantly. In this study, TA ranged from 0.01-0.32 and 0.03-0.29% in trial one and two respectively. Ahumada and Cantwell (1996) reported higher values of pepino fruits TA in the range of 0.041-0.045% while Kola (2010) recorded lower TA of 0.090-0.124% in pepino fruits. This could be due to difference in environment since the quality of pepino melon fruits is greatly influenced by the environment. As the fertilizer rates increased the TA decreased in both growing environments and trials. The lowest TA was recorded in field grown fruits from the control (no fertilizer) after 28 days of storage. This could be due to restriction of vegetative growth because no NPK fertilizer was applied and thus the fruits became the major sink leading to an increase in acid concentration in the fruits (Pluda *et al*., 1993). On the other hand, greenhouse grown fruits from plants supplied with the 400 kg NPK ha^{-1} stored at room temperature had the lowest TA in both trials. This might be due to excessive vegetative growth due to high NPK supply leading to growth of the main and side shoots and therefore most of the photosynthates were directed towards the young developing shoots rather than the fruits hence low acid concentration (Pluda *et al*., 1993). Plants with optimum potassium levels have increased production of organic acids so as to balance cation-anion ratio and further increase in potassium does not lead to an increase in organic acids hence the low TA in plants receiving 400 kg NPK ha^{-1} (Etienne *et al*., 2013).

Sugar acid ratio increased with increase in storage time. Similar results were reported by Yeshiwas and Tolessa (2018) were the sugar acid ratio of tomatoes increased throughout the storage period. Sugar acid ratio is used to determine harvesting time, ripeness and taste (Zolfaghari *et al*., 2010). The highest SA ratio was recorded in fruits stored at room temperature

compared to those stored at low temperature. Results of the present study indicated that pepino melon ripening is affected by storage temperature as ripening is more pronounced at higher storage temperature. Further increase in SA ratio was correlated with increase in TSS and reduction in TA. Studies done on different fruits have shown that at the beginning of the ripening process the SA ratio is low because the sugar content is low while fruit acid content is high and this makes the fruit to have a sour taste but as the ripening process continues, starch gets converted to sugars gradually leading to an increase in TSS, reduction in TA and subsequently increase in SA ratio that forms an important indicator of flavour (Shyam & Matsuoka, 2004). Greenhouse grown fruits had a higher SA ratio compared to field grown fruits. Similar results were reported by Yeshiwas and Tolessa (2018) in which greenhouse grown tomatoes had a higher SA compared to field grown tomatoes. The high SA ratio for greenhouse grown fruits could possibly be due to the low TA. The high SA ratio for greenhouse grown pepino fruits indicates that greenhouse grown fruits have a good flavour compared to field grown fruits (Yeshiwas & Tolessa, 2018). The flavor of most fruits is influenced by a balance between sugars and acids.

NPK fertilizer rates, growing environment and storage temperatures had a significant effect on shelf life of pepino melon fruits. Pepino fruits stored at low temperature had a longer shelf life compared to those stored at room temperature. Similarly, mango fruits stored at high temperature had a shorter shelf life than those stored at low temperature (Ezz & Awad, 2011). Results of the present study are in agreement with those of El-Zeftawi *et al*. (1988) who reported that the shelf life of pepino fruits stored at ambient temperatures is one to two weeks. In a recent study, Lei Yi *et al*. (2019) reported that mango fruits stored at 13°C had a longer shelf life than those stored at room temperature.

Temperature is the most important factor in shelf life of fruits and vegetables (Lei Yi *et al*., 2019). Pepino fruits stored at low temperature had a longer shelf life because low temperature storage reduces ethylene production, respiration, ripening, weight loss, senescence, retains firmness and other metabolic activities and this enhances shelf life and quality of produce (Lei Yi *et al*., 2019). On the other hand, pepino fruits stored at room temperature had a shorter shelf life because high temperature results to increased ethylene production, respiration, ripening, weight loss, senescence, loss of firmness and other metabolic processes and this reduces shelf life (Mutari & Debbie, 2011).

Field grown fruits had a longer shelf life compared to greenhouse grown fruits. This could be attributed to lower temperature in the field during the growing season and this increases firmness (Anagnostou & Vasilakakis, 1995). In the greenhouse the temperatures were high and it has been reported that high temperatures tend to decrease firmness (Paul *et al*., 1999). Therefore, field grown fruits remained firmer than greenhouse grown fruits and hence the former had a longer shelf life. Fruits from the control had the longest shelf life and this could be attributed to low nitrogen levels and low water content in these fruits hence they remained firmer. On the other hand, fruits from plants supplied with high NPK fertilizer rates had a short shelf life and this might be due to high water content in the fruits due to excess nitrogen which also leads to postharvest decay especially fruits which were stored at room temperature.

7.5. Conclusion

NPK fertilizer rates, growing environment and storage temperature have an effect on postharvest quality of pepino melons. Application of 100 kg NPK ha^{-1} for both field and greenhouse grown pepino melons and storage at low temperature (7°C) enhanced postharvest quality and shelf life of pepino melon fruits.

CHAPTER EIGHT

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

8.1 General discussion

Since pepino melon is a vegetable which was recently introduced in Kenya, most farmers are not aware of the fertilizer requirements of the vegetable. Pepino melon has numerous health, medicinal, nutritional and economic benefits for both small- and large-scale farmers in Kenya. The present study evaluated the effect of NPK fertilizer rates on growth and yield of field and greenhouse grown pepino melons (Chapter three). Growth and yield of most vegetable crops depends on the quality and quantity of fertilizers used. The use of fertilizers has significantly led to increased crop yields (Fageria *et al*., 2008). Worldwide there has been 50% increase in crop yields following the application of chemical fertilizers (Borlaug & Dowswell, 1994). There exists a linear relationship between relative growth rate and nitrogen concentration in plants (Ddamulira *et al*., 2019). Nitrogen is an essential nutrient for plant growth including pepino melon. Horticultural crops also need potassium for quality fruits yet these two nutrients are rarely applied in vegetable crops (Savvas *et al*., 2008). Potassium has been reported to increase growth and yield of tomato (Gupta & Sengar, 2000). In this study, the highest yield of pepino melon was obtained from field grown plants supplied with 300 kg NPK ha^{-1} compared to control which had the lowest yield. Low levels of NPK fertilizer negatively influenced yield because the resulting fruits are small hence compromising yield. On the other hand, application of high fertilizer rates (400 kg NPK ha^{-1}) did not translate into increased yield possibly due to luxurious consumption of nutrients and increased vegetative growth at the expense of fruits. Most of the growth parameters were favoured in the greenhouse and this could be due to the microclimate in the greenhouse. However, yield was not high in the greenhouse and this could be attributed to the high temperatures in the greenhouse which led to increased flower abortion. In contrast, Nkansah *et al*. (2017) reported that greenhouse grown capsicums had a yield which was four to ten times the yield of open field grown capsicums. Based on the findings of this study, it was observed that increasing the rates of NPK fertilizer increased the yield of pepino melon but this increment did not go beyond 300 kg NPK ha^{-1} because above this rate a decreasing trend in pepino melon yield was observed.

Effect of NPK fertilizer rates and growing environment on number of flowers per truss, number of aborted flowers, pollen viability and *in vitro* pollen germination of pepino melon was also evaluated (Chapter four). Application of 400 kg NPK ha^{-1} for both field and greenhouse grown pepino melon plants had the lowest number of flowers per truss in both trials. This could be

attributed to high nitrogen from the NPK fertilizer which favoured the vegetative phase at the expense of flowering. Field grown plants had higher number of flowers per truss compared to greenhouse grown plants. This could be due to the low temperatures in the field compared to the high temperatures recorded in the greenhouse. High temperatures in the greenhouse led to flower abortion hence the decrease in the number of flowers per truss. It has also been reported that air temperatures above 25°C in pepino melon interfere with flower formation (Cavusoglu *et al*., 2009). NPK fertilizer rates and growing environment had a significant effect on flower abortion in pepino melon. More flower abortion occurred in the greenhouse and this could be due to the high temperatures recorded in the greenhouse. Several studies have also reported increased abortion of flowers by high temperatures above 30°C (Aloni *et al*., 1991; Erickson & Markhart, 2001;2002; Huberman *et al*., 1997). At high temperatures auxin concentration and transport to the pedicels of flowers and fruitlets is decreased (Huberman *et al*., 1997). Consequently, the levels of ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) increase after long exposure to high temperatures (Wien *et al*., 1993) resulting to an increase in ethylene concentration in buds and flowers and this increases susceptibility to abortion (Wubs *et al*., 2009). High temperature causes a decrease in photosynthesis and consequently a decrease in availability of carbohydrates (Sato *et al*., 2000). Carbohydrate content affects the expression of flower abortion genes through hexokinases which play a role in sugar metabolism and signal transduction to other genes (Jang & Sheen, 1997).

The low pollen viability recorded in flowers from greenhouse grown plants could be due to high temperature. Both male and female gametophytes are sensitive to high temperatures but the male gametophyte is more vulnerable because at high temperature, pollen germination and tube growth are greatly reduced (Kakani *et al*., 2005). Several studies have indicated that high temperature on male reproductive organ leads to flower abortion and subsequent yield reduction (Kafizadeh *et al*., 2008). Similarly, Pressman *et al*. (2002) reported decreased pollen production per flower and decreased pollen viability in tomato when the temperatures were increased to 32°/26°C day/night temperatures. The high pollen viability recorded in flowers from field grown plants could be due to the low temperatures in the field during the growing season. Low temperature could have favoured the male gametophyte and thus reduced flower abortion, increased viability and increased yield for field grown plants. Increase in pollen viability as the fertilizer rates increased could be attributed to increase in absorbed nutrients which had a positive effect on photosynthesis, flowering and consequently pollen viability. The low pollen viability in flowers from plants not supplied with NPK fertilizer could be due to

reduced absorption of nutrients which led to reduced photosynthesis, flowering and pollen viability. Flowers from plants which were supplied with 400 kg NPK ha^{-1} had low pollen viability because most of the absorbed nutrients were directed towards vegetative growth rather than to the flowers. In addition, the reduction in pollen viability could also be attributed to low soluble sugar content in the developing pollen grains (Pressman *et al*., 2002). Sucrose protects pollen grains from desiccation thus pollen grains which have high sucrose levels do not dehydrate and are therefore viable. Hoekstra *et al*. (1989) reported that pollen dehydration survival rate had a positive association with sucrose concentration. Pollen viability is important because pollen should be viable at the time of pollination for fruit set to occur.

The low pollen germination in flowers from greenhouse grown plants could be due to reduced starch and soluble sugars in the pollen grains. Pollen tube growth and development are energy requiring processes and carbohydrates act as the main energy sources and thus a reduction in the two will lead to low pollen germination. In addition, high temperatures have a negative impact on pollen development and this leads to decreased *in vitro* pollen germination percentage. High temperatures also lead to formation of reactive oxygen species (ROS) in the pollen grains and this may have led to reduced pollen viability for pollen from greenhouse grown flowers and hence the reduced *in vitro* pollen germination percentage. The low pollen germination in flowers from plants not supplied with NPK fertilizer could be due to reduced absorption of nutrients which led to reduced photosynthesis, flowering and pollen germination. On the other hand, flowers from plants supplied with intermediate nutrients had high pollen germination because of the availability of nutrients which led to increased photosynthesis, flowering and pollen germination. Flowers from plants supplied with 400 kg NPK ha^{-1} had the lowest pollen germination probably due to allocation of most of the photosynthates for vegetative growth rather than to the flowers.

The effect of NPK fertilizer rates on accumulation of secondary metabolites of greenhouse and field grown pepino melon fruits was also evaluated (Chapter five). The concentration of secondary metabolites is influenced by environmental conditions such as light, carbon dioxide levels, fertilization and biotic factors (Ibrahim *et al*., 2012). However, the changes in concentration of secondary metabolites due to environmental factors are as a result of changes in the rate of synthesis and rate of catabolism (Ibrahim *et al*., 2011). Accumulation of lutein, β-carotene and lycopene was high in greenhouse grown pepino melon fruits supplied with 200kg NPK ha^{-1} in trial one while in trial two field grown fruits from plants supplied with 200

kg NPK ha^{-1} had the highest content of the three carotenoids. Under intermediate nutrient resources, high production of secondary metabolites occurs due to the availability of an excess pool of carbon to synthesize carbon-based secondary metabolites like carotenoids (Fanciullino *et al*., 2014). The low carotenoid content in plants supplied with 400 kg NPK ha^{-1} could be due to high fertilizer rate resulting to the allocation of most of the photosynthates to growth and development and thus low accumulation of secondary metabolites. Phytoene synthase enzyme catalyses the first-rate limiting step in carotenoid biosynthesis which involves the condensation of two geranylgeranyl diphosphate (GGPP) molecules into one phytoene molecule (Fanciullino *et al*., 2014). The high carotenoid content in the greenhouse could be due to the fact that Phytoene synthase (PSY) enzyme is sensitive to temperature and this means that the carotenoid biosynthetic pathway may be involved in temperature stress response (Stanley & Yuan, 2019). High temperature leads to the production of ROS and this increases the activity of PSY enzyme and hence increase in carotenoid content including lutein content (Yang *et al*., 2018a). The increase in ROS leads to increase in biosynthesis of carotenoids through redox signalling by increasing the expression of genes and enzymes involved in carotenogenesis (Fanciullino *et al*., 2014).

Field grown fruits from plants supplied with 200 kg NPK ha^{-1} had a high β-carotene content in trial two. This could be due to the fact that β-carotene content decreases with increasing temperature because the activity of enzymes PSY and phytoene desaturase catalysing the synthesis of β-carotene is influenced by temperature above 30°C (Lurie *et al*., 1996). At temperatures above 30°C PSY levels are reduced and hence the reduced levels of β- carotene in the greenhouse in trial two because the temperatures were above 30°C. In the field the temperatures were not above 30°C and hence the high β-carotene content in trial two.

Lycopene content was high for greenhouse grown pepino fruits in trial one and the temperature in the greenhouse ranged from 21° to 35.8°C. Brandt *et al*. (2006) stated that maximum lycopene content occurs at temperatures of 25° to 30°C and is completely inhibited at temperatures above 32°C. Fruits grown at high temperatures have a low lycopene content although temperature regulation of carotenoids is crop specific. When the air temperature is 30°C, the surface temperature of the fruit may range between 40-50°C and this decreases lycopene synthesis (Adegoroye & Joliffe, 1983). Lycopene content increases as temperature increases from low to medium then drastically declines from medium to high temperatures. Hamauzu *et al*. (1998) stated that high temperatures above 35°C inhibit the accumulation of lycopene by converting it into β-carotene. This further explains the high lycopene content for

field grown fruits in the second trial because the temperatures in the field were lower compared to the greenhouse.

The current study revealed increased concentration of total phenolic content (TPC) in greenhouse grown fruits where no NPK fertilizer (control) was applied. Phenylalanine is a precursor in the biosynthesis of phenolics and is also an amino acid used in protein synthesis. Therefore, there might be a competition for phenylalanine between protein synthesis and secondary metabolite synthesis and therefore biosynthesis of secondary metabolites might be inhibited due to incorporation of phenylalanine into protein synthesis (Margna, 1977). A positive correlation exists between activity of phenylalanine lyase (PAL) an enzyme of the phenylpropanoid pathway and accumulation of carbon-based secondary metabolites in plants (Jeyaramraja *et al*., 2003). Plants grown in nitrogen deficient soils increase the supply of ammonia by enhancing PAL activity hence a rise in the accumulation of polyphenolic compounds (Margna, 1977). Temperature enhances the accumulation of most secondary metabolites and this explains why greenhouse grown fruits not supplied with NPK fertilizer had high TPC compared to field grown fruits not supplied with NPK fertilizer. Similarly, Wang and Zheng (2001) reported that increase in growing temperature from 18°C to 30°C led to an increase in phenolic content of strawberry fruits. The low TPC content when NPK fertilizer was applied could be due to the negative correlation between proteins and phenols because phenylalanine is used in protein synthesis and not phenolics under conditions of excess nitrogen supply (Li *et al*., 2008). Similarly, Stefaneli *et al*. (2010) reported that nitrogen availability reduces phenolic content in most cases.

The effect of NPK fertilizer rates on micronutrient content (copper, manganese, molybdenum, nickel and iron) and vitamin C content of field and greenhouse grown pepino melon fruits was also evaluated (Chapter six). The micronutrient content of pepino melon fruits increased as the NPK fertilizer rates increased and reached a peak at 200 kg NPK ha^{-1} and 300 kg NPK ha^{-1} for greenhouse and field grown fruits respectively. The high Cu content with an increase in NPK fertilizer application may be due to the fact that increased NPK fertilizer application increases the mobility of Cu from the soil to the plants (Huang & Jin, 2008). Czarnecki and During (2015) reported that application of NPK fertilizer increased the total content and mobile form of Cu in the soil and hence increase in plant uptake. The low copper content in the control could be due to reduced mobility of Cu because no fertilizer was applied in the control experiment. Increase in Mn with increase in NPK fertilizer could be due to enhanced uptake of Mn. At high NPK rates there is better utilization and availability of plant nutrients (Ilupeju *et al*., 2015).

Control treatment recorded the lowest Mn levels and this could be due to low availability of nutrients from the soil and thus low availability of plant nutrients. The control relied on nutrients in the soil which apparently were not sufficient and most likely decreased over time because there was no replenishment. In addition, Mn is not readily available in acidic soils because it readily binds to free iron and aluminium when the soil pH is less than 5.5 (Gong *et al*., 1998). Plants absorb micronutrients from the soil and the availability of Mo for plants is highly dependent on soil pH, concentration of adsorbing oxides like Fe oxides, water drainage and organic compounds present in soil colloids (Kaiser *et al*., 2005). In alkaline soils, the solubility of Mo increases and it is readily available to plants (Kaiser *et al*., 2005) while in acidic soils (pH < 5.5) Mo availability decreases as adsorption by anion oxides increases (Reddy *et al*., 1997). The soils in this study were acidic and therefore the solubility of Mn was low hence not readily available to the plants. The increase in Fe content as the fertilizer rates increased could be due to high levels of nitrogen from the NPK fertilizer and this might have enhanced uptake of Fe. Nitrogen fertilization enhances mobility of Fe from the soil to the plants (Rutkowska *et al*., 2014). The decrease in Fe content at high fertilizer rates could be due to decrease in the intensity of photosynthetic active radiation at the surface of the leaves because the plants had dense vegetation and thus decrease in Fe content (Panda *et al*., 2012). This might be due to the slow movement of iron in the phloem and most of accumulates in the roots and leaves. High iron content of 76.46-83mg kg^{-1} in pepino melon fruits was reported by Oczan and Arslan (2011). The low Fe content recorded in this study could be due to the fact that application of NPK fertilizers increases trace elements in the soil but their available forms to plants decrease with an increase in soil pH and in this study the soil was acidic.

Vitamin C content of pepino melon fruits increased as the NPK fertilizer rates increased and reached a peak at 200 kg NPK ha^{-1} for greenhouse and field grown fruits. Increase in nitrogen content leads to a decrease in vitamin C content of most fruits and vegetables (Mozafar, 1993). In the present study, it was noted that field grown pepino melon fruits had a high vitamin C content in trial two compared to greenhouse grown pepino fruits. During photosynthesis, luminosity intensity plays a vital role and the low vitamin C recorded in greenhouse grown fruits can be attributed to low light intensity in the greenhouse which could have led to a reduction in sugar which is used as a substrate during vitamin C synthesis (Rana *et al*., 2014). Additionally, relative humidity was high in the greenhouse and this led to reduced transpiration leading to increase of water in the xylem vessels and this was favourable to the fruits because the fruits act as drains for high concentrations of organic molecules and, consequently, low

water potential (Bertin *et al*., 2000). The low water potential in the fruits resulted to absorption of water by greenhouse grown fruits leading to a "dilution effect" and hence reducing vitamin C content of greenhouse grown fruits compared to field grown fruits (Rana *et al*., 2014). Pepino fruits from plants supplied with higher NPK fertilizer rates had a low vitamin C content. This could be due to the fact that high nitrogen content in the NPK fertilizer led to dense foliage and thus the fruits were shaded from direct sunlight and hence the decrease in vitamin C content. Decrease in vitamin C content with increase in NPK fertilizer might be due to increase in protein synthesis and decrease in carbohydrate synthesis resulting from application of NPK fertilizer (Worthington, 2001) and since vitamin C is formed from carbohydrates its synthesis is also reduced (Singh, 2005).

Postharvest quality of pepino melon fruits as influenced by NPK fertilizer rates, growing environment and storage temperature was also evaluated (Chapter seven). Percentage fruit weight loss (PWL) increased as the storage days advanced for fruits harvested from both growing environments and in both storage temperatures. Fruits from plants which received the highest NPK fertilizer rate (400 kg NPK ha^{-1}) and stored at room temperature had the highest PWL. The initial water content in the fruits will determine the weight loss. Fruits with a high initial water content will tend to lose more water because they have a high vapour pressure deficit compared to those with low initial water content (Kays, 1991). This could be the reason for the high PWL in fruits which were obtained from plants supplied with 400 kg NPK ha^{-1} because high nitrogen levels lead to accumulation of more water in the fruits. Storage of pepino fruits at room temperature (15-22°C) could also have resulted in production of high levels of ethylene and increased respiration and subsequent weight reduction. High temperatures lead to increased water loss because metabolic activities also increase. Although there was a progressive increase in PWL the rate was lower in fruits stored at low temperature (7°C).

Total soluble solids (TSS) increased as storage days advanced. At room temperatures, the temperatures were high resulting to an increase in metabolic activities, respiration and ripening hence high TSS. Increase in TSS might also be due to the conversion of complex carbohydrates into simple sugars. Additionally, fruits stored at room temperature had a high PWL and this could have increased the sugar concentration of pepino melon fruits resulting into high TSS. On the other hand, fruits stored at low temperature had a low TSS because metabolic activities, respiration and ripening are slowed down. Field grown fruits had a higher TSS compared to greenhouse grown fruits. This could be due to the reduction in sugar content of the fruits

because of the high temperatures in the greenhouse during growth. The high TSS recorded in field grown pepino fruits could be due to high light intensity and thus high photosynthesis leading to more accumulation of sugars in the fruit compared to greenhouse grown fruits where the light intensity was low leading to reduced photosynthesis and hence low accumulation of sugars in the fruits (Beckmann *et al*., 2006). Pepino melon fruits from plants that were not supplied with NPK fertilizer had a high TSS probably because of reduced vegetative growth thus fruits were the only sink for photosynthates leading to accumulation of more sugars in the fruit (Pluda *et al*., 1993). On the contrary, fruits which received the highest fertilizer rate of 400 kg NPK ha^{-1} had a low TSS because the plants had dense vegetative growth therefore the photosynthates were directed to the growing shoots rather than the fruit leading to low sugar concentration in the fruits (Pluda *et al*., 1993). In addition, high nitrogen fertilizer rates make the plants to be succulent, thus fruits from plants supplied with 400 kg NPK ha^{-1} had a high-water content and this might have led to dilution of sugars in the fruit resulting to low TSS.

Fruit firmness decreased as the storage days advanced. Decrease in firmness is strongly related to increase in weight loss because as fruits lose weight, they become soft. It was observed that as the NPK fertilizer rates increased, fruit firmness decreased. Pepino melon fruits from plants that were supplied with 400 kg NPK ha^{-1} had the lowest firmness and this could be attributed to the fact that fruits from plants with dense vegetative growth are less firm compared to those from plants with moderate vegetative growth. Field grown fruits were firmer compared to greenhouse grown fruits probably because of the low temperature during the growing season. It has been reported that high temperatures during the growing season tend to decrease fruit firmness (Paul *et al*., 1999). Fruit softening is as a result of cell wall digestion by pectinesterase, polygalacturonase and other enzymes and the activity of such enzymes is increased by an increase in storage temperature (Ahmed *et al*., 2009). Fruits stored at low temperature were firmer than those stored at room temperature.

Titratable acidity (TA) of pepino melon fruits decreased during storage. Decrease in TA could be due to higher respiration rates as ripening advanced and organic acids were used as substrates for respiration. The low TA recorded in greenhouse grown pepino fruits may be due to low photosynthesis in the plants because of shading leading to low carbohydrate accumulation in the fruits (Caliman *et al*., 2010) whereas the high TA in field grown fruits could be due to increased photosynthesis leading more carbohydrate accumulating in the fruits. Both field and greenhouse grown fruits stored at room temperature had a low TA because high temperature led to increased respiration and ripening hence an increase in the utilization of

acids in the fruit hence leading to a decrease in TA. The low TA recorded in fruits stored at low temperature could be due to reduced respiration and ripening resulting to decreased utilization of organic acids.

Sugar acid (SA) ratio of pepino melon fruits increased as storage days advanced. Increase in SA ratio was correlated with increase in TSS and reduction in TA. At the beginning of the ripening process the SA ratio is low because the sugar content is low while fruit acid content is high and this makes the fruit to have a sour taste but as the ripening process continues, starch gets converted to sugars gradually leading to an increase in TSS, reduction in TA and subsequently increase in SA ratio that forms an important indicator of flavour (Shyam & Matsuoka, 2004).

Pepino fruits stored at low temperature had a longer shelf life compared to those stored at room temperature. Low temperature storage led to reduced ethylene production, respiration, ripening, weight loss, senescence, retention of firmness and reduction of other metabolic activities and this enhances shelf life and quality of produce (Lei Yi *et al*., 2019). On the other hand, pepino fruits stored at room temperature had a shorter shelf life because high temperature results to increased ethylene production, respiration, ripening, weight loss, senescence, loss of firmness and other metabolic processes and this reduced shelf life (Mutari & Debbie, 2011). Field grown fruits had a longer shelf life compared to greenhouse grown fruits. This could be due to lower temperature in the field during the growing season as low temperatures have been reported to increase firmness (Anagnostou & Vasilakakis, 1995). In the greenhouse the temperatures were high and it has been reported that high temperatures tend to decrease firmness (Paul *et al*., 1999). Therefore, field grown fruits remained firmer than greenhouse grown fruits and hence the former had a longer shelf life. Fruits from the control had the longest shelf life and this could be attributed to low nitrogen levels and low water content in these fruits hence they remained firmer. On the other hand, fruits from plants supplied with high NPK fertilizer rates had a short shelf life and this might be due to high water content in the fruits due to excess nitrogen which also leads to postharvest decay especially for fruits which were stored at room temperature.

8.2 Conclusions

From the results of this study, the following conclusions can be drawn:

i) NPK fertilizer rates have an effect on growth and yield of field and greenhouse grown pepino melons.
ii) NPK fertilizer rates have an effect on the concentration of secondary metabolites of field and greenhouse grown pepino melons.
iii) NPK fertilizer rates and growing environment have an effect on micronutrients and vitamin C content of field and greenhouse grown pepino melons.
iv) NPK fertilizer rates have an effect on flower abortion of field and greenhouse grown pepino melons.
v) NPK fertilizer rates and storage temperature have an effect on postharvest quality of field and greenhouse grown pepino melon fruits stored at room and low temperature.

8.3 Recommendations

Based on the results of this study the following recommendations can be formulated:

i) Application of 300 kg NPK ha^{-1} for optimum fruit weight and yield at the location where this experiment was conducted.
ii) Application of 200 kg NPK ha^{-1} for both field and greenhouse grown pepino melons is recommended for increased concentration of carotenoids. No fertilizer application is recommended for maximum concentration of total phenolic content of greenhouse and field grown pepino melons.
iii) Application of 200 and 300 kg NPK ha^{-1} is recommended for the accumulation of copper, manganese, molybdenum, nickel and iron content of greenhouse and field grown pepino melon fruits respectively. Application of 200 kg NPK ha^{-1} to enhance vitamin C content of field and greenhouse grown pepino melon fruits.
iv) Application of 200 and 300 kg NPK ha^{-1} for greenhouse and field grown pepino melon is recommended for high number of flowers per truss, reduced flower abortion, high pollen viability and pollen germination of pepino melon respectively.
v) Application of 100 kg NPK ha^{-1} for both field and greenhouse grown plants and storage at low temperature (7°C) is recommended to enhance postharvest quality and shelf life of pepino melon fruits.

8.4 Areas for further studies

The following areas of study can be highlighted from the current study:

i) Cultivation of pepino melon in different ecological areas in order to ascertain the nutritional requirements and yield potential.

ii) Cultivation and exposure of pepino melon fruits to different environmental stresses to determine how different stresses affect the accumulation of secondary metabolites.

iii) Evaluation of NPK fertilizer rates on growth, yield, postharvest quality and flower abortion of different varieties of pepino melon.

iv) Genetic and molecular studies on the genes which are either upregulated or downregulated in aborted and non-aborted pepino melon flowers.

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APPENDICES

Appendix I: Key data analysis output for objective one

Fruit weight ANOVA trial one

Source	SS	MS	DF	F Ratio	Prob>F
Total	201292.47		29		
Fertilizer	91183.60	22795.9	4	46.5518	<.0001*
Env	57920.00	57920.0	1	118.2791	<.0001*
Env*Fertilizer	43190.20	10797.6	4	22.0498	<.0001*
Block	184.28	92.1	2	0.1882	
Error	8814.40	489.7	18		

Fruit weight ANOVA trial two

Source	SS	MS	DF	F Ratio	Prob>F
Total	194264.93		29		
Fertilizer	127270.00	31817.40	4	83.8600	<.0001*
Env	7922.53	7922.53	1	20.8811	0.0002*
Env*Fertilizer	51074.40	12768.60	4	33.6537	<.0001*
Block	1168.95	584.47	2	1.5405	
Error	6829.40	379.40	18		

Total yield ANOVA trial one

Source	SS	MS	DF	F Ratio	Prob>F
Total	1742729.8		29		
Fertilizer	974553.0	243638.00	4	92.1141	<.0001*
Env	273718.0	273718.00	1	103.4867	<.0001*
Fertilizer*Env	436752.0	109188.00	4	41.2815	<.0001*
Block	10097.5	5048.75	2	1.9088	
Error	47609.3		18		
		2645.00			

Total yield ANOVA trial two

Source	SS	MS	DF	F Ratio	Prob>F
Total	1828284.5		29		
Fertilizer	1023146.0	255787.00	4	72.9480	<.0001*
Env	124806.0	124806.00	1	35.5936	<.0001*
Fertilizer*Env	613752.0	153438.00	4	43.7592	<.0001*
Block	3464.1	1732.06	2	0.4940	
Error	63115.6	3506.00	18		

Days to 50% flowering ANOVA trial one

Source	SS	MS	DF	F Ratio	Prob>F
Total	3330.30		29		
Fertilizer	3196.47	799.12	4	263.4451	<.0001*
Env	40.83	40.83	1	13.4615	0.0018*
Env*Fertilizer	9.00	2.25	4	0.7418	0.5759
Block	29.40	14.70	2	4.8462	
Error	54.60	3.03	18		

Days to 50% flowering ANOVA trial two

Source	SS	MS	DF	F Ratio	Prob>F
Total	3666.00		29		
Fertilizer	3383.00	845.75	4	192.7025	<.0001*
Env	128.13	128.13	1	29.1949	<.0001*
Env*Fertilizer	10.87	2.72	4	0.6190	0.6547
Block	65.00	32.50	2	7.4051	
Error	79.00	4.39	18		

Plant height ANOVA 30 days after planting trial one

Source	SS	MS	DF	F Ratio	Prob>F
Total	1211.5128		29		
Fertilizer	617.524	154.381	4	42.0291	<.0001*
Env	33.159	33.159	1	9.0273	0.0076*
Env*Fertilizer	482.675	120.669	4	32.8512	<.0001*
Block	12.037	6.0186	2	1.6385	
Error	66.117	3.673	18		

Plant height ANOVA 44 days after planting trial one

Source	SS	MS	DF	F Ratio	Prob>F
Total	4632.935		29		
Fertilizer	1712.920	428.231	4	25.6706	<.0001*
Env	882.051	882.051	1	52.8752	<.0001*
Env*Fertilizer	1717.130	429.283	4	25.7337	<.0001*
Block	20.556	10.278	2	0.6161	
Error	300.272	16.682	18		

Plant height ANOVA 58 days after planting trial one

Source	SS	MS	DF	F Ratio	Prob>F
Total	5492.689		29		
Fertilizer	1951.220	487.805	4	69.0981	<.0001*
Env	1535.030	1535.030	1	217.4391	<.0001*
Env*Fertilizer	1868.400	467.101	4	66.1653	<.0001*
Block	10.959	5.479	2	0.7761	
Error	127.073	7.060	18		

Plant height ANOVA 72 days after planting trial one

Source	SS	MS	DF	F Ratio	Prob>F
Total	4969.592		29		
Fertilizer	1818.010	454.503	4	44.3245	<.0001*
Env	1546.860	1546.860	1	150.8542	<.0001*
Env*Fertilizer	1410.320	352.580	4	34.3846	<.0001*
Block	9.828	4.914	2	0.4792	
Error	184.572	10.254	18		

Plant height ANOVA 86 days after planting trial one

Source	SS	MS	DF	F Ratio	Prob>F
Total	3814.174		29		
Fertilizer	1273.060	318.265	4	31.3330	<.0001*
Env	928.465	928.465	1	91.4068	<.0001*
Env*Fertilizer	1410.780	352.694	4	34.7225	<.0001*
Block	19.035	9.518	2	0.9370	
Error	182.835	10.158	18		

Plant height ANOVA 100 days after planting trial one

Source	SS	MS	DF	F Ratio	Prob>F
Total	2832.819		29		
Fertilizer	1379.380	344.844	4	38.7530	<.0001*
Env	261.075	261.075	1	29.3392	<.0001*
Env*Fertilizer	1014.330	253.583	4	28.4972	<.0001*
Block	17.864	8.932	2	1.0038	
Error	160.173	8.899	18		

Plant height ANOVA 30 days after planting trial two

Source	SS	MS	DF	F Ratio	Prob>F
Total	1452.531		29		
Fertilizer	849.791	212.448	4	40.4435	<.0001*
Env	15.059	15.059	1	2.8668	0.1077
Env*Fertilizer	488.687	122.172	4	23.2577	<.0001*
Block	4.441	2.220	2	0.4227	
Error	94.553	5.253	18		

Plant height ANOVA 44 days after planting trial two

Source	SS	MS	DF	F Ratio	Prob>F
Total	1959.714		29		
Fertilizer	1055.270	263.817	4	70.5174	<.0001*
Env	77.747	77.747	1	20.7815	0.0002*
Env*Fertilizer	751.757	187.939	4	50.2355	<.0001*
Block	7.601	3.801	2	1.0159	
Error	67.341	3.741	18		

Plant height ANOVA 58 days after planting trial two

Source	SS	MS	DF	F Ratio	Prob>F
Total	1987.503		29		
Fertilizer	1115.700	278.925	4	66.8800	<.0001*
Env	120.641	120.641	1	28.9270	<.0001*
Env*Fertilizer	670.898	167.725	4	40.2166	<.0001*
Block	5.195	2.597	2	0.6228	
Error	75.069	4.171	18		

Plant height ANOVA 72 days after planting trial two

Source	SS	MS	DF	F Ratio	Prob>F
Total	3794.132		29		
Fertilizer	1940.020	485.004	4	88.0060	<.0001*
Env	126.526	126.526	1	22.9587	0.0001*
Env*Fertilizer	1626.260	406.566	4	73.7730	<.0001*
Block	2.127	1.063	2	0.1930	
Error	99.199	5.511	18		

Plant height ANOVA 86 days after planting trial two

Source	SS	MS	DF	F Ratio	Prob>F
Total	3996.269		29		
Fertilizer	2358.280	589.569	4	73.3497	<.0001*
Env	280.082	280.082	1	34.8457	<.0001*
Env*Fertilizer	1199.670	299.919	4	37.3136	<.0001*
Block	13.555	6.778	2	0.8432	
Error	144.680	8.038	18		

Plant height ANOVA 100 days after planting trial two

Source	SS	MS	DF	F Ratio	Prob>F
Total	6837.484		29		
Fertilizer	3179.580	794.895	4	30.9735	<.0001*
Env	890.966	890.966	1	34.7170	<.0001*
Env*Fertilizer	2157.860	539.465	4	21.0205	<.0001*
Block	147.133	73.566	2	2.8665	
Error	461.947	25.664	18		

Number of leaves before flowering ANOVA trial one

Source	SS	MS	DF	F Ratio	Prob>F
Total	14621.018		29		
Fertilizer	13422.600	3355.6500	4	81.5337	<.0001*
Env	41.489	41.4893	1	1.0081	0.3287
Env*Fertilizer	47.388	11.8469	4	0.2878	0.8820
Block	368.713	184.3570	2	4.4794	
Error	740.819	41.1600	18		

Number of leaves before flowering ANOVA trial two

Source	SS	MS	DF	F Ratio	Prob>F
Total	16371.415		29		
Fertilizer	14042.600	3510.650	4	162.0261	<.0001*
Env	371.994	371.994	1	17.1685	0.0006*
Env*Fertilizer	109.025	27.256	4	1.2579	0.3226
Block	1457.800	728.902	2	33.6408	
Error	390.009	21.670	18		

Number of branches ANOVA trial one

Source	SS	MS	DF	F Ratio	Prob>F
Total	706.3101		29		
Fertilizer	370.0540	92.5136	4	23.0980	<.0001*
Env	35.5341	35.5341	1	8.8719	0.0081*
Env*Fertilizer	211.652	52.9130	4	13.2109	<.0001*
Block	16.9750	8.4875	2	2.1191	
Error	72.0946	4.0053	18		

Number of branches ANOVA trial two

Source	SS	MS	DF	F Ratio	Prob>F
Total	395.7756		29		
Fertilizer	169.3800	42.3451	4	10.9224	0.0001*
Env	80.4913	80.4913	1	20.7617	0.0002*
Env*Fertilizer	70.4481	17.6120	4	4.5428	0.0103*
Block	5.6712	2.8356	2	0.7314	
Error	69.78450	3.8769	18		

Appendix II: Key data analysis output objective two

Flowers per truss ANOVA trial one

Source	SS	MS	DF	F Ratio	Prob>F
Total	138.3903		29		
Fertilizer	95.6428	23.9107	4	59.1258	<.0001*
Env	20.7002	20.7002	1	51.1870	<.0001*
Env*Fertilizer	13.6070	3.4017	4	8.4117	0.0005*
Block	1.1611	0.5806	2	1.4356	
Error	7.2793	0.4044	18		

Flowers per truss ANOVA trial two

Source	SS	MS	DF	F Ratio	Prob>F
Total	130.66835		29		
Fertilizer	98.6769	24.6692	4	156.4817	<.0001*
Env	10.9929	10.9929	1	69.7298	<.0001*
Env*Fertilizer	17.2680	4.3170	4	27.3836	<.0001*
Block	0.8929	0.4464	2	2.8318	
Error	2.8377	0.1576	18		

Number of aborted flowers ANOVA trial one

Source	SS	MS	DF	F Ratio	Prob>F
Total	509.0596		29		
Fertilizer	163.9410	40.9852	4	61.9554	<.0001*
Env	243.9030	243.9030	1	368.6970	<.0001*
Env*Fertilizer	87.2455	21.8114	4	32.9713	<.0001*
Block	2.0627	1.0313	2	1.5590	
Error	11.9075	0.6615	18		

Number of aborted flowers ANOVA trial two

Source	SS	MS	DF	F Ratio	Prob>F
Total	212.5378		29		
Fertilizer	82.9522	20.7380	4	34.3352	<.0001*
Env	96.1946	96.1946	1	159.2657	<.0001*
Env*Fertilizer	20.0994	5.02484	4	8.3194	0.0006*
Block	2.4199	1.20997	2	2.0033	
Error	10.8718	0.60400	18		

Appendix III: Key data analysis output for objective three

Lutein ANOVA trial one

Source	SS	MS	DF	F Ratio	Prob>F
Total	2047.8631		29		
Fertilizer	1171.8800	292.969	4	103.2570	<.0001*
Env	811.6160	811.616	1	286.0540	<.0001*
Env*Fertilizer	12.0012	3.000	4	1.0575	0.4059
Block	1.2977	0.649	2	0.2287	
Error	51.0711	2.837	18		

Lutein ANOVA trial two

Source	SS	MS	DF	F Ratio	Prob>F
Total	3180.1177		29		
Fertilizer	1988.6000	497.149	4	130.9567	<.0001*
Env	929.2990	929.299	1	244.7918	<.0001*
Env*Fertilizer	182.8340	45.709	4	12.0403	<.0001*
Block	11.0562	5.528	2	1.4562	
Error	68.3331	3.796	18		

β-carotene ANOVA trial one

Source	SS	MS	DF	F Ratio	Prob>F
Total	862.6725		29		
Fertilizer	802.7190	200.6800	4	579.5120	<.0001*
Env	46.9453	46.9453	1	135.5659	<.0001*
Env*Fertilizer	6.7587	1.6897	4	4.8793	0.0077*
Block	0.0160	0.0080	2	0.0231	
Error	6.2332	0.3463	18		

β-carotene ANOVA trial two

Source	SS	MS	DF	F Ratio	Prob>F
Total	1119.4535		29		
Fertilizer	973.5590	243.390	4	570.3853	<.0001*
Env	81.8850	81.885	1	191.8981	<.0001*
Env*Fertilizer	54.1382	13.535	4	31.7183	<.0001*
Block	2.1907	1.096	2	2.5670	
Error	7.6808	0.427	18		

Lycopene ANOVA trial one

Source	SS	MS	DF	F Ratio	Prob>F
Total	406.5102		29		
Fertilizer	299.5780	74.8944	4	52.3392	<.0001*
Env	56.8357	56.8357	1	39.7190	<.0001*
Env*Fertilizer	12.5140	3.1285	4	2.1863	0.1117
Block	11.8259	5.9129	2	4.1322	
Error	25.7569	1.4309	18		

Lycopene ANOVA trial two

Source	SS	MS	DF	F Ratio	Prob>F
Total	413.0252		29		
Fertilizer	363.0350	90.7588	4	116.1621	<.0001*
Env	20.9268	20.9268	1	26.7841	<.0001*
Env*Fertilizer	14.1542	3.5386	4	4.5290	0.0105*
Block	0.8452	0.4226	2	0.5409	
Error	14.0636	0.7813	18		

Total phenolic content ANOVA trial one

Source	SS	MS	DF	F Ratio	Prob>F
Total	655.1501		29		
Fertilizer	379.3880	94.8469	4	58.6376	<.0001*
Env	233.2440	233.2440	1	144.1995	<.0001*
Env*Fertilizer	4.6372	1.1593	4	0.7167	0.5914
Block	8.7664	4.3832	2	2.7099	
Error	29.1152	1.6175	18		

Total phenolic content ANOVA trial two

Source	SS	MS	DF	F Ratio	Prob>F
Total	368.8371		29		
Fertilizer	179.6430	44.9108	4	37.6078	<.0001*
Env	129.8780	129.8780	1	108.7585	<.0001*
Env*Fertilizer	9.6100	2.4025	4	2.0118	0.1384
Block	8.3703	4.1852	2	3.5046	
Error	20.3012	1.1942	18		

Appendix IV: Key data analysis output for objective four

Copper ANOVA trial one

Source	SS	MS	DF	F Ratio	Prob>F
Total	237.0927		29		
Fertilizer	106.9710	26.7426	4	24.9554	<.0001*
Env	9.2519	9.2519	1	8.6335	0.0088*
Env*Fertilizer	91.6237	22.9059	4	21.3751	<.0001*
Block	9.9575	4.9787	2	4.6460	
Error	19.2891	1.0716	18		

Copper ANOVA trial two

Source	SS	MS	DF	F Ratio	Prob>F
Total	203.7029		29		
Fertilizer	113.3900	28.3475	4	40.9160	<.0001*
Env	16.1040	16.1040	1	23.2441	0.0001*
Env*Fertilizer	44.3883	11.0971	4	16.0172	<.0001*
Block	17.3499	8.6749	2	12.5212	
Error	12.4708	0.6928	18		

Manganese ANOVA trial one

Source	SS	MS	DF	F Ratio	Prob>F
Total	39.6495		29		
Fertilizer	25.4720	6.36800	4	68.4859	<.0001*
Env	1.3021	1.30208	1	14.0035	0.0015*
Env*Fertilizer	10.4913	2.62283	4	28.2078	<.0001*
Block	0.71045	0.35522	2	3.8203	
Error	1.6737	0.09298	18		

Manganese ANOVA trial two

Source	SS	MS	DF	F Ratio	Prob>F
Total	30.5165		29		
Fertilizer	19.1761	4.79403	4	60.0173	<.0001*
Env	4.3092	4.30923	1	53.9480	<.0001*
Env*Fertilizer	5.5902	1.39755	4	17.4961	<.0001*
Block	0.0032	0.00160	2	0.0201	
Error	1.4378	0.07988	18		

Molybdenum ANOVA trial one

Source	SS	MS	DF	F Ratio	Prob>F
Total	31.0517		29		
Fertilizer	17.4648	4.36620	4	26.9876	<.0001*
Env	1.0679	1.06785	1	6.6004	0.0193*
Env*Fertilizer	8.9346	2.23365	4	13.8063	<.0001*
Block	0.6723	0.33613	2	2.0776	
Error	2.9121	0.16179	18		

Molybdenum ANOVA trial two

Source	SS	MS	DF	F Ratio	Prob>F
Total	18.49832		29		
Fertilizer	8.84569	2.21142	4	18.2956	<.0001*
Env	1.35681	1.35681	1	11.2252	0.0036*
Env*Fertilizer	5.75189	1.43797	4	11.8967	<.0001*
Block	0.36824	0.18412	2	1.5233	
Error	2.17569	0.12087	18		

Nickel ANOVA trial one

Source	SS	MS	DF	F Ratio	Prob>F
Total	26.04232		29		
Fertilizer	11.96730	2.99182	4	14.5548	<.0001*
Env	2.11205	2.11205	1	10.2748	0.0049*
Env*Fertilizer	5.13011	1.28253	4	6.2393	0.0025*
Block	3.13286	1.56643	2	7.6205	
Error	3.70001	0.20556	18		

Nickel ANOVA trial two

Source	SS	MS	DF	F Ratio	Prob>F
Total	21.28903		29		
Fertilizer	10.18440	2.54610	4	72.4400	<.0001*
Env	2.83976	2.83976	1	80.7950	<.0001*
Env*Fertilizer	5.27545	1.31886	4	37.5234	<.0001*
Block	2.35674	1.17837	2	33.5262	
Error	0.63266	0.03515	18		

Iron ANOVA trial one

Source	SS	MS	DF	F Ratio	Prob>F
Total	232.1005		29		
Fertilizer	116.1560	29.0390	4	34.5520	<.0001*
Env	3.7031	3.7031	1	4.4061	0.0502
Env*Fertilizer	63.6761	15.9190	4	18.9412	<.0001*
Block	33.4372	16.7186	2	19.8926	
Error	15.1279	0.8404	18		

Iron ANOVA trial two

Source	SS	MS	DF	F Ratio	Prob>F
Total	186.40495		29		
Fertilizer	108.82000	27.2050	4	45.2233	<.0001*
Env	1.85505	1.8551	1	3.0837	0.0961
Env*Fertilizer	48.73260	12.1832	4	20.2523	<.0001*
Block	16.16920	8.0846	2	13.4392	
Error	10.82826	0.6016	18		

Vitamin C ANOVA trial one

Source	SS	MS	DF	F Ratio	Prob>F
Total	17862.488		29		
Fertilizer	15751.000	3937.740	4	70.8825	<.0001*
Env	475.331	475.331	1	8.5563	0.0090*
Env*Fertilizer	39.069	9.767	4	0.1758	0.9479
Block	597.163	298.581	2	5.3747	
Error	999.956	55.550	18		

Vitamin C ANOVA trial two

Source	SS	MS	DF	F Ratio	Prob>F
Total	12844.426		29		
Fertilizer	10901.600	2725.39	4	152.9988	<.0001*
Env	1415.910	1415.91	1	79.4867	<.0001*
Env*Fertilizer	112.676	28.17	4	1.5814	0.2222
Block	93.656	46.83	2	2.6288	
Error	320.636	17.81	18		

Appendix V: Key data analysis output for objective five

Percentage weight loss ANOVA day 7 trial one

Source	SS	MS	DF	F Ratio	Prob>F
Total	180.7705		58		
Fertilizer	136.0890	34.0222	4	177.3180	<.0001*
Env	12.9642	12.9642	1	67.5672	<.0001*
Temp	13.3954	13.3954	1	69.8144	<.0001*
Fertilizer*Temp	2.8223	0.7056	4	3.6773	0.0126*
Fertilizer*Env	1.3411	0.3353	4	1.7474	0.1598
Temp*Env	0.0286	0.0286	1	0.1491	0.7016
Temp*Env*Fert.	0.8707	0.2177	4	1.1345	0.3549
Block	5.9682	2.9841	2	15.5527	
Error	7.2911	0.1918	38		

Percentage weight loss ANOVA day 14 trial one

Source	SS	MS	DF	F Ratio	Prob>F
Total	324.0708		59		
Fertilizer	259.9280	64.9819	4	147.4436	<.0001*
Env	18.3264	18.3264	1	41.5826	<.0001*
Temp	12.1680	12.1680	1	27.6091	<.0001*
Fertilizer*Temp	2.0058	0.5015	4	1.1378	0.3534
Fertilizer*Env	5.3573	1.3393	4	3.0389	0.0287*
Temp*Env	0.9728	0.9728	1	2.2073	0.1456
Temp*Env*Fert.	0.4204	0.1051	4	0.2384	0.9148
Block	8.1449	4.0725	2	9.2404	
Error	16.7475	0.4407	38		

Percentage weight loss ANOVA day 21 trial one

Source	SS	MS	DF	F Ratio	Prob>F
Total	499.6962		59		
Fertilizer	428.5740	107.1430	4	298.6048	<.0001*
Env	14.3375	14.3375	1	39.9581	<.0001*
Temp	29.3300	29.3300	1	81.7418	<.0001*
Fertilizer*Temp	7.4801	1.8700	4	5.2117	0.0019*
Fertilizer*Env	3.4424	0.8606	4	2.3985	0.0670
Temp*Env	0.6469	0.6469	1	1.8028	0.1873
Temp*Env*Fert.	0.0284	0.0071	4	0.0198	0.9992
Block	2.2225	1.1113	2	3.0970	
Error	13.6349	0.3588	38		

Percentage weight loss ANOVA day 28 trial one

Source	SS	MS	DF	F Ratio	Prob>F
Total	1321.6380		59		
Fertilizer	877.5160	219.3790	4	78.9321	<.0001*
Env	3.2947	3.2947	1	1.1854	0.2831
Temp	193.1060	193.1060	1	69.4792	<.0001*
Fertilizer*Temp	19.3628	4.8407	4	1.7417	0.1610
Fertilizer*Env	48.9176	12.2294	4	4.4001	0.0051*
Temp*Env	33.1527	33.1527	1	11.9283	0.0014*
Temp*Env*Fert.	29.6145	7.4036	4	2.6638	0.0471*
Block	11.0585	5.5293	2	1.9894	
Error	105.6148	2.7793	38		

Percentage weight loss ANOVA day 7 trial two

Source	SS	MS	DF	F Ratio	Prob>F
Total	138.8185		59		
Fertilizer	102.3200	25.5801	4	166.6410	<.0001*
Env	14.3082	14.3082	1	93.2102	<.0001*
Temp	8.1549	8.1549	1	53.1249	<.0001*
Fertilizer*Temp	1.4108	0.3527	4	2.2976	0.0767
Fertilizer*Env	1.1289	0.2822	4	1.8386	0.1415
Temp*Env	0.2587	0.2587	1	1.6855	0.2020
Temp*Env*Fert.	2.1733	0.5433	4	3.5395	0.0150*
Block	3.2301	1.6151	2	10.5212	
Error	5.8332	0.1535	38		

Percentage weight loss ANOVA day 14 trial two

Source	SS	MS	DF	F Ratio	Prob>F
Total	317.1077		59		
Fertilizer	212.3360	53.0840	4	174.4127	<.0001*
Env	17.7670	17.7670	1	58.3754	<.0001*
Temp	34.0657	34.0657	1	111.9264	<.0001*
Fertilizer*Temp	28.2216	7.0554	4	23.1813	<.0001*
Fertilizer*Env	6.6133	1.6533	4	5.4321	0.0015*
Temp*Env	0.0126	0.0126	1	0.0414	0.8398
Temp*Env*Fert.	3.4096	0.8524	4	2.8007	0.0393*
Block	3.1163	1.5581	2	5.1194	
Error	11.5656	0.3044	38		

Percentage weight loss ANOVA day 21 trial two

Source	SS	MS	DF	F Ratio	Prob>F
Total	617.4711		59		
Fertilizer	386.1680	96.5420	4	334.4073	<.0001*
Env	22.7797	22.7797	1	78.9055	<.0001*
Temp	84.8946	84.8946	1	294.0625	<.0001*
Fertilizer*Temp	95.7669	23.9417	4	82.9307	<.0001*
Fertilizer*Env	8.1611	2.0403	4	7.0672	0.0002*
Temp*Env	0.1739	0.1739	1	0.6023	0.4425
Temp*Env*Fert.	4.8120	1.2030	4	4.1671	0.0068*
Block	3.7445	1.8723	2	6.4852	
Error	10.9704	0.2887	38		

Percentage weight loss ANOVA day 28 trial two

Source	SS	MS	DF	F Ratio	Prob>F
Total	1371.6694		59		
Fertilizer	516.6910	129.1730	4	68.7278	<.0001*
Env	18.9956	18.9956	1	10.1068	0.0029*
Temp	538.0820	538.0820	1	286.2925	<.0001*
Fertilizer*Temp	168.9730	42.2433	4	22.4760	<.0001*
Fertilizer*Env	7.9237	1.9809	4	1.0540	0.3925
Temp*Env	2.2349	2.2349	1	1.1891	0.2824
Temp*Env*Fert.	20.7456	5.1864	4	2.7595	0.0415*
Block	26.6034	13.3017	2	7.0773	
Error	71.4203	1.8795	38		

TSS ANOVA day 7 trial one

Source	SS	MS	DF	F Ratio	Prob>F
Total	11.8073		59		
Fertilizer	7.6973	1.9243	4	90.2402	<.0001*
Env	0.3840	0.3840	1	18.0074	0.0001*
Temp	0.1500	0.1500	1	7.0341	0.0116*
Fertilizer*Temp	1.1467	0.2867	4	13.4430	<.0001*
Fertilizer*Env	0.8593	0.2148	4	10.0745	<.0001*
Temp*Env	0.2667	0.2667	1	12.5051	0.0011*
Temp*Env*Fert.	0.2967	0.0742	4	3.4780	0.0162*
Block	0.1963	0.0982	2	4.6035	
Error	0.8103	0.0213	38		

TSS ANOVA day 14 trial one

Source	SS	MS	DF	F Ratio	Prob>F
Total	27.7418		59		
Fertilizer	17.8677	4.46692	4	311.0742	<.0001*
Env	1.6335	1.63350	1	113.7563	<.0001*
Temp	4.4282	4.42817	1	308.3757	<.0001*
Fertilizer*Temp	1.4643	0.36608	4	25.4939	<.0001*
Fertilizer*Env	1.0257	0.25642	4	17.8568	<.0001*
Temp*Env	0.4002	0.40017	1	27.8674	<.0001*
Temp*Env*Fert.	0.2223	0.05558	4	3.8708	0.0098*
Block	0.1543	0.07717	2	5.3739	
Error	0.5457	0.01436	38		

TSS ANOVA day 21 trial one

Source	SS	MS	DF	F Ratio	Prob>F
Total	46.0633		59		
Fertilizer	31.7117	7.92792	4	428.5360	<.0001*
Env	2.3207	2.32067	1	125.4414	<.0001*
Temp	8.0667	8.06667	1	436.0360	<.0001*
Fertilizer*Temp	1.6317	0.40792	4	22.0495	<.0001*
Fertilizer*Env	0.2377	0.05942	4	3.2117	0.0229*
Temp*Env	0.7707	0.77067	1	41.6577	<.0001*
Temp*Env*Fert.	0.2910	0.07275	4	3.9324	0.0091*
Block	0.3303	0.16517	2	8.9279	
Error	0.7030	0.01850	38		

TSS ANOVA day 28 trial one

Source	SS	MS	DF	F Ratio	Prob>F
Total	62.7773		59		
Fertilizer	44.6623	11.1656	4	204.4125	<.0001*
Env	4.2667	4.2667	1	78.1115	<.0001*
Temp	8.2140	8.2140	1	150.3767	<.0001*
Fertilizer*Temp	0.9077	0.2269	4	4.1542	0.0069*
Fertilizer*Env	0.8350	0.2088	4	3.8217	0.0105*
Temp*Env	0.7260	0.7260	1	13.2912	0.0008*
Temp*Env*Fert.	0.7057	0.1764	4	3.2297	0.0224*
Block	0.3843	0.1922	2	3.5181	
Error	2.0757	0.0546	38		

TSS ANOVA day 7 trial two

Source	SS	MS	DF	F Ratio	Prob>F
Total	10.6765		59		
Fertilizer	8.4423	2.11058	4	177.3077	<.0001*
Env	0.3682	0.36817	1	30.9293	<.0001*
Temp	0.0015	0.00150	1	0.1260	0.7246
Fertilizer*Temp	0.0343	0.00858	4	0.7211	0.5829
Fertilizer*Env	0.9310	0.23275	4	19.5531	<.0001*
Temp*Env	0.0082	0.00817	1	0.6861	0.4127
Temp*Env*Fert.	0.1977	0.04942	4	4.1514	0.0069*
Block	0.2410	0.12050	2	10.1231	
Error	0.4523	0.01190	38		

TSS ANOVA day 14 trial two

Source	SS	MS	DF	F Ratio	Prob>F
Total	24.8818		59		
Fertilizer	18.4727	4.6182	4	259.4731	<.0001*
Env	2.2815	2.2815	1	128.1868	<.0001*
Temp	0.3682	0.3682	1	20.6856	<.0001*
Fertilizer*Temp	0.4927	0.1232	4	6.9202	0.0003*
Fertilizer*Env	1.9460	0.4865	4	27.3342	<.0001*
Temp*Env	0.3682	0.3682	1	20.6856	<.0001*
Temp*Env*Fert.	0.2460	0.0615	4	3.4554	0.0167*
Block	0.0303	0.0152	2	0.8521	
Error	0.6763	0.0178	38		

TSS ANOVA day 21 trial two

Source	SS	MS	DF	F Ratio	Prob>F
Total	41.5818		59		
Fertilizer	33.3527	8.33817	4	514.0892	<.0001*
Env	2.6882	2.68817	1	165.7388	<.0001*
Temp	0.9375	0.93750	1	57.8015	<.0001*
Fertilizer*Temp	1.1167	0.27917	4	17.2120	<.0001*
Fertilizer*Env	2.3893	0.59733	4	36.8286	<.0001*
Temp*Env	0.2282	0.22817	1	14.0676	0.0006*
Temp*Env*Fert.	0.2427	0.06067	4	3.7404	0.0116*
Block	0.0103	0.00517	2	0.3186	
Error	0.6163	0.01622	38		

TSS ANOVA day 28 trial two

Source	SS	MS	DF	F Ratio	Prob>F
Total	51.4173		59		
Fertilizer	40.8990	10.2247	4	361.2090	<.0001*
Env	3.9527	3.9527	1	139.6356	<.0001*
Temp	0.9627	0.9627	1	34.0081	<.0001*
Fertilizer*Temp	1.4157	0.3539	4	12.5028	<.0001*
Fertilizer*Env	2.5857	0.6464	4	22.8359	<.0001*
Temp*Env	0.1127	0.1127	1	3.9802	0.0532
Temp*Env*Fert.	0.4090	0.1023	4	3.6122	0.0137*
Block	0.0043	0.0022	2	0.0765	
Error	1.0757	0.0283	38		

Titratable acidity ANOVA day 7 trial one

Source	SS	MS	DF	F Ratio	Prob>F
Total	0.2675		59		
Fertilizer	0.1047	0.02618	4	75.6748	<.0001*
Env	0.0400	0.04004	1	115.7391	<.0001*
Temp	0.0952	0.09520	1	275.1772	<.0001*
Fertilizer*Temp	0.0002	5.58e-5	4	0.1614	0.9566
Fertilizer*Env	0.0005	0.00013	4	0.3734	0.8262
Temp*Env	0.0033	0.00338	1	9.7553	0.0034*
Temp*Env*Fert.	0.0006	0.00015	4	0.4456	0.7749
Block	0.0097	0.00483	2	13.9513	
Error	0.0131	0.00034	38		

Titratable acidity ANOVA day 14 trial one

Source	SS	MS	DF	F Ratio	Prob>F
Total	0.27309		59		
Fertilizer	0.10526	0.02631	4	113.9747	<.0001*
Env	0.03038	0.03038	1	131.5634	<.0001*
Temp	0.11180	0.11180	1	484.2473	<.0001*
Fertilizer*Temp	0.00166	0.00041	4	1.7939	0.1502
Fertilizer*Env	0.00132	0.00033	4	1.4257	0.2442
Temp*Env	0.00280	0.00280	1	12.1349	0.0013*
Temp*Env*Fert.	0.00082	0.00021	4	0.8915	0.4784
Block	0.01029	0.00515	2	22.2918	
Error	0.00877	0.00023	38		

Titratable acidity ANOVA day 21 trial one

Source	SS	MS	DF	F Ratio	Prob>F
Total	0.33365		59		
Fertilizer	0.13017	0.03254	4	167.7102	<.0001*
Env	0.03220	0.03220	1	165.9580	<.0001*
Temp	0.14702	0.14702	1	757.6722	<.0001*
Fertilizer*Temp	0.00329	0.00082	4	4.2432	0.0062*
Fertilizer*Env	0.00024	0.00006	4	0.3092	0.8700
Temp*Env	0.00400	0.00400	1	20.6234	<.0001*
Temp*Env*Fert.	0.00067	0.00017	4	0.8675	0.4923
Block	0.00869	0.00435	2	22.4014	
Error	0.00737	0.00019	38		

Titratable acidity ANOVA day 28 trial one

Source	SS	MS	DF	F Ratio	Prob>F
Total	0.33225		59		
Fertilizer	0.10343	0.02586	4	170.5932	<.0001*
Env	0.06208	0.06208	1	409.5666	<.0001*
Temp	0.14504	0.14504	1	956.8721	<.0001*
Fertilizer*Temp	0.00260	0.00065	4	4.2882	0.0058*
Fertilizer*Env	0.00746	0.00187	4	12.3038	<.0001*
Temp*Env	0.00014	0.00014	1	0.8906	0.3513
Temp*Env*Fert.	0.00357	0.00089	4	5.8935	0.0009*
Block	0.00217	0.00109	2	7.1690	
Error	0.00576	0.00015	38		

Titratable acidity ANOVA day 7 trial two

Source	SS	MS	DF	F Ratio	Prob>F
Total	0.27325		59		
Fertilizer	0.14346	0.03587	4	111.4233	<.0001*
Env	0.04566	0.04566	1	141.8542	<.0001*
Temp	0.05560	0.05560	1	172.7161	<.0001*
Fertilizer*Temp	0.00057	0.00014	4	0.4456	0.7749
Fertilizer*Env	0.00074	0.00018	4	0.5736	0.6834
Temp*Env	0.00028	0.00028	1	0.8590	0.3599
Temp*Env*Fert.	0.00055	0.00014	4	0.4267	0.7884
Block	0.01416	0.00708	2	21.9959	
Error	0.01223	0.00032	38		

Titratable acidity ANOVA day 14 trial two

Source	SS	MS	DF	F Ratio	Prob>F
Total	0.30091		59		
Fertilizer	0.12083	0.03021	4	79.2504	<.0001*
Env	0.06255	0.06255	1	164.0976	<.0001*
Temp	0.08980	0.08980	1	235.6017	<.0001*
Fertilizer*Temp	0.00080	0.00020	4	0.5258	0.7174
Fertilizer*Env	0.00123	0.00031	4	0.8076	0.5281
Temp*Env	0.00237	0.00237	1	6.2215	0.0171*
Temp*Env*Fert.	0.00140	0.00035	4	0.9150	0.4651
Block	0.00746	0.00373	2	9.7872	
Error	0.01448	0.00038	38		

Titratable acidity day 21 trial two

Source	SS	MS	DF	F Ratio	Prob>F
Total	0.28373		59		
Fertilizer	0.11258	0.02814	4	75.7909	<.0001*
Env	0.06172	0.06172	1	166.2133	<.0001*
Temp	0.08850	0.08850	1	238.3367	<.0001*
Fertilizer*Temp	0.00083	0.00021	4	0.5592	0.6936
Fertilizer*Env	0.00125	0.00031	4	0.8445	0.5058
Temp*Env	0.00117	0.00117	1	3.1376	0.0845
Temp*Env*Fert.	0.00192	0.00048	4	1.2929	0.2900
Block	0.00165	0.00082	2	2.2183	
Error	0.01411	0.00037	38		

Titratable acidity ANOVA day 28 trial two

Source	SS	MS	DF	F Ratio	Prob>F
Total	0.24465		59		
Fertilizer	0.06208	0.01552	4	130.3758	<.0001*
Env	0.06198	0.06198	1	520.6280	<.0001*
Temp	0.10320	0.10320	1	866.9094	<.0001*
Fertilizer*Temp	0.00222	0.00056	4	4.6711	0.0036*
Fertilizer*Env	0.00099	0.00025	4	2.0799	0.1026
Temp*Env	0.00708	0.00708	1	59.4423	<.0001*
Temp*Env*Fert.	0.00240	0.00060	4	5.0446	0.0023*
Block	0.00017	8.3e-5	2	0.6970	
Error	0.00452	0.00012	38		

Firmness ANOVA day 7 trial one

Source	SS	MS	DF	F Ratio	Prob>F
Total	27.9493		59		
Fertilizer	20.5277	5.13192	4	112.7894	<.0001*
Env	2.56267	2.56267	1	56.3223	<.0001*
Temp	0.9127	0.91267	1	20.0586	<.0001*
Fertilizer*Temp	0.8923	0.22308	4	4.9029	0.0028*
Fertilizer*Env	0.1457	0.03642	4	0.8004	0.5325
Temp*Env	0.0540	0.05400	1	1.1868	0.2828
Temp*Env*Fert.	0.5210	0.13025	4	2.8626	0.0362*
Block	0.6043	0.30217	2	6.6410	
Error	1.7290	0.04550	38		

Firmness ANOVA day 14 trial one

Source	SS	MS	DF	F Ratio	Prob>F
Total	32.516000		59		
Fertilizer	24.4927	6.12317	4	305.0878	<.0001*
Env	2.904	2.904	1	144.6923	<.0001*
Temp	2.904	2.904	1	144.6923	<.0001*
Fertilizer*Temp	0.696	0.174	4	8.6696	<.0001*
Fertilizer*Env	0.216	0.054	4	2.6906	0.0455*
Temp*Env	0.096	0.096	1	4.7832	0.0350*
Temp*Env*Fert.	0.08067	0.02017	4	1.0048	0.4171
Block	0.364	0.182	2	9.0682	
Error	0.762667	0.02007	38		

Firmness ANOVA day 21 trial one

Source	SS	MS	DF	F Ratio	Prob>F
Total	35.2525		59		
Fertilizer	23.1233	5.78083	4	123.1574	<.0001*
Env	5.4601	5.46017	1	116.3257	<.0001*
Temp	3.4082	3.40817	1	72.6090	<.0001*
Fertilizer*Temp	0.2827	0.07067	4	1.5055	0.2200
Fertilizer*Env	0.6373	0.15933	4	3.3945	0.0181*
Temp*Env	0.0882	0.08817	1	1.8783	0.1786
Temp*Env*Fert.	0.2460	0.06150	4	1.3102	0.2836
Block	0.2230	0.11150	2	2.3754	
Error	1.7837	0.04694	38		

Firmness ANOVA day 28 trial one

Source	SS	MS	DF	F Ratio	Prob>F
Total	36.4016		59		
Fertilizer	27.8053	6.95133	4	217.3606	<.0001*
Env	3.7951	3.79514	1	118.6699	<.0001*
Temp	2.0130	2.01300	1	62.9444	<.0001*
Fertilizer*Temp	0.9225	0.23063	4	7.2115	0.0002*
Fertilizer*Env	0.2654	0.06634	4	2.0745	0.1033
Temp*Env	0.0084	0.00840	1	0.2627	0.6112
Temp*Env*Fert.	0.3511	0.08778	4	2.7447	0.0423*
Block	0.0255	0.01274	2	0.3982	
Error	1.2153	0.03198	38		

Firmness ANOVA day 7 trial two

Source	SS	MS	DF	F Ratio	Prob>F
Total	17.40583		59		
Fertilizer	11.97000	2.99250	4	91.4353	<.0001*
Env	1.320170	1.32017	1	40.3374	<.0001*
Temp	1.320170	1.32017	1	40.3374	<.0001*
Fertilizer*Temp	0.32733	0.08183	4	2.5004	0.0585
Fertilizer*Env	0.12400	0.03100	4	0.9472	0.4474
Temp*Env	0.18150	0.18150	1	5.5457	0.0238*
Temp*Env*Fert.	0.06267	0.01567	4	0.4787	0.7511
Block	0.85633	0.42817	2	13.0826	
Error	1.24367	0.03273	38		

Firmness ANOVA day 14 trial two

Source	SS	MS	DF	F Ratio	Prob>F
Total	23.2498		59		
Fertilizer	17.2257	4.30642	4	97.6589	<.0001*
Env	1.4415	1.44150	1	32.6897	<.0001*
Temp	2.1282	2.12817	1	48.2616	<.0001*
Fertilizer*Temp	0.0810	0.02025	4	0.4592	0.7651
Fertilizer*Env	0.0477	0.01192	4	0.2702	0.8953
Temp*Env	0.0375	0.03750	1	0.8504	0.3623
Temp*Env*Fert.	0.2083	0.05208	4	1.1811	0.3346
Block	0.4043	0.20217	2	4.5846	
Error	1.6757	0.04410	38		

Firmness ANOVA day 21 trial two

Source	SS	MS	DF	F Ratio	Prob>F
Total	29.4818		59		
Fertilizer	19.0210	4.75525	4	164.9220	<.0001*
Env	1.9802	1.98017	1	68.6763	<.0001*
Temp	6.0802	6.08017	1	210.8728	<.0001*
Fertilizer*Temp	0.0657	0.01642	4	0.5694	0.6864
Fertilizer*Env	0.0723	0.01808	4	0.6272	0.6461
Temp*Env	0.0015	0.00150	1	0.0520	0.8208
Temp*Env*Fert.	0.3810	0.09525	4	3.3035	0.0203*
Block	0.7843	0.39217	2	13.6012	
Error	1.0957	0.02883	38		

Firmness day 28 trial two

Source	SS	MS	DF	F Ratio	Prob>F
Total	32.43913		59		
Fertilizer	18.9277	4.73192	4	230.2341	<.0001*
Env	2.4200	2.42004	1	117.7485	<.0001*
Temp	9.3220	9.32204	1	453.5692	<.0001*
Fertilizer*Temp	0.3823	0.09558	4	4.6507	0.0037*
Fertilizer*Env	0.1193	0.02983	4	1.4516	0.2361
Temp*Env	0.0184	0.01837	1	0.8940	0.3504
Temp*Env*Fert.	0.2493	0.06233	4	3.0329	0.0290*
Block	0.2190	0.10950	2	5.3278	
Error	0.7810	0.02055	38		

Shelf life ANOVA trial one

Source	SS	MS	DF	F Ratio	Prob>F
Total	937.6500		59		
Fertilizer	540.4000	135.1000	4	119.2982	<.0001*
Env	40.0167	40.0167	1	35.3362	<.0001*
Temp	244.017	244.017	1	215.4756	<.0001*
Fertilizer*Temp	21.7333	5.4333	4	4.7978	0.0031*
Fertilizer*Env	15.0667	3.7667	4	3.3261	0.0198*
Temp*Env	0.81667	0.8167	1	0.7211	0.4011
Temp*Env*Fert.	22.2667	5.5667	4	4.9156	0.0027*
Block	10.3000	5.1500	2	4.5476	
Error	43.0333	1.1325	38		

Shelf life ANOVA trial two

Source	SS	MS	DF	F Ratio	Prob>F
Total	1024.5833		59		
Fertilizer	637.6670	159.4170	4	174.5773	<.0001*
Env	88.8167	88.8167	1	97.2632	<.0001*
Temp	183.7500	183.7500	1	201.2248	<.0001*
Fertilizer*Temp	7.6667	1.9167	4	2.0989	0.1000
Fertilizer*Env	29.9333	7.4833	4	8.1950	<.0001*
Temp*Env	4.8167	4.8167	1	5.2747	0.0272*
Temp*Env*Fert.	12.6000	3.1500	4	3.4496	0.0168*
Block	24.6333	12.3167	2	13.4880	
Error	34.7000	0.9132	38		

Appendix VI: Published paper on objective one

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Effect of NPK Fertilizer Rates on Growth and Yield of Field and Greenhouse Grown Pepino Melon (*Solanum muricatum* Aiton)

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Keywords: Pepino melon, NPK fertilizer, plant growth, yield, field, greenhouse

Abstract. Pepino melon (*Solanum muricatum* Ait.) is an exotic vegetable whose consumption is on the increase in Kenya due to its health and nutritional benefits. A study was conducted at Egerton University, Kenya in 2018-2019 to investigate the effect of NPK fertilizer rates (0, 100, 200. 300 and 400 kg ha^{-1}) on growth and yield of field and greenhouse grown pepino melons. The experiment was laid in a randomized complete block design with three replications. Data was recorded on plant height, stem diameter, number of leaves per bush, number of branches, days to 50% flowering, fruit weight and total yield. Data were analyzed using analysis of variance with the SAS statistical package. Significant means were separated using Tukey's Honestly Significant Difference at $p \leq 0.05$. Results indicated that NPK fertilizer rates and growing environment influenced growth and yield of pepino melon. At 100 DAP plants grown in the greenhouse and supplied with 200 kg NPK ha^{-1} had a stem diameter of 14.01 mm which was significantly bigger $p \leq 0.05$ compared to those grown in the field and supplied with 300 kg NPK ha^{-1} with a stem diameter of 11.71 mm in trial two. Application of 300 kg NPK ha^{-1} for field grown pepino melons gave the highest yield of 1102.48 kg ha^{-1} and 1060.55 kg ha^{-1} in trial one and two respectively. In conclusion, application of 300 kg ha^{-1} of NPK fertilizer for field grown pepino melon is recommended.

Introduction

Pepino melon (*Solanum muricatum* Ait.) is an exotic vegetable which belongs to the family solanaceae [1]. The fruit was initially grown in South America but its cultivation has extended to Australia, New Zealand, USA [2] Central America, Morocco, Spain, Israel and the highlands of Kenya [3]. The vegetable was introduced in Kenya in 2013 and its consumption in Kenya is increasing due to its health, nutritional and economic value [4]. The edible part is the fruit which is aromatic and juicy [5]. The fruit can be eaten when mature green as a vegetable in stews [1] and the ripe fruit is eaten as a dessert fruit, in salads and ice creams [5].

Pepino is low in calories but very rich in minerals such as calcium, phosphorous and potassium and vitamins A, B1, B2, B3 and C [6]. The fruit varies in size and shape depending on the cultivar and the colour ranges from completely purple, solid green or green with purple stripes, or cream colored with or without purple stripes [7]. The main challenge in vegetable production is nutrient deficiency due to improper use of fertilizers [8]. Proper vegetable growth requires ideal nutrient supply [9]. In the tropics, soil fertility is declining due to excessive rainfall and continuous cultivation has led to lack of essential nutrients in the soil [10]. NPK fertilizer has the ability to release nutrients very fast into the soil and thus help sustain soil fertility and crop production [11]. The present study aimed at investigating the effects of different rates of NPK fertilizer on growth and yield of field and greenhouse grown pepino melons.

Appendix VII: Published paper on objective two

integrity Research Journals

Advanced Journal of Plant Biology
Volume 4(2), pages 21-32, April 2023
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https://doi.org/10.31248/AJPB2022.023
https://integrityresjournals.org/journal/AJPB
Full Length Research

Flower abortion of pepino melon (*Solanum muricatum* Ait.) as influenced by NPK fertilizer rates and growing environment

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ABSTRACT: Flower abortion is the detachment of flowers from the plant. A study was conducted at Egerton University, Kenya in 2018 to 2020 to investigate the effect of NPK fertilizer rates (0, 100, 200. 300 and 400 kg ha^{-1}) on flower abortion of field and greenhouse grown pepino melons. The experiment was laid out in a randomized complete block design with three replications. Data was collected on number of flowers, number of aborted flowers, viable and non-viable pollen and *in vitro* pollen germination. Data were analysed using analysis of variance with the SAS statistical package. Significant means were separated using Tukey's Honestly Significant Difference at $p \leq 0.05$. Results indicated that field grown plants supplied with 200 and 300 kg NPK ha^{-1} had 10.28 and 11.18 flowers per truss respectively in trial one. In trial two, field grown plants supplied with 300 kg NPK ha^{-1} had 11.32 flowers per truss. Greenhouse grown plants supplied with 300 kg NPK ha^{-1} had 20.61 and 14.19 aborted flowers in trial one and two respectively. High pollen viability was recorded from non-aborted flowers obtained from field grown plants supplied with 300 kg NPK ha^{-1} with a pollen viability of 94.48% and 93.97% in trial one and two respectively. Pollen from non-aborted flowers obtained from field grown plants supplied with 300 kg NPK ha^{-1} had the highest pollen germination of 68.72 and 67.72% in trial one and two respectively. Application of 200 and 300 kg NPK ha^{-1} for field and greenhouse grown pepino melon plants led to reduced flower abortion, high number of flowers per truss, high pollen viability and pollen germination.

Keywords: Aborted, Flowers, non-aborted, pollen germination, pollen viability.

INTRODUCTION

Successful flower development is vital in the production of many horticultural crops (Warner and Erwin, 2005). Abortion is the cessation of development of an organ after which it detaches from the main body of the plant (Wubs *et al*., 2009). Flower buds, flowers and fruits are the main reproductive organs that abort and this leads to reduction in yields of most horticultural crops (Nyoka *et al*., 2015). In an earlier study, Stephenson (1981) reported that flower, fruit and seed abortion is caused by pollination failure, limited photo assimilates, adverse weather conditions, moisture stress and predation. Flower abortion is caused by high temperatures on the male reproductive organs (Kafizadeh *et al*., 2008). Flower abortion causes serious economic problems in horticultural crops. High temperature in pepper causes flower abortion by increasing ethylene production (Huberman *et al*., 1997). Abscisic acid, salicylic acid and ethylene production increases as a result of high temperature (Kotak *et al*., 2007). Taylor and Whitelaw (2001) reported that ABA accelerates abscission by enhancing senescence and hence ethylene climacteric which eventually leads to abscission. Wubs *et al*. (2009) reported that before flower abortion takes place there is reduction of auxin from the flower while ethylene production increases. In sweet pepper, high flower abortion occurred three weeks after anthesis (Wubs *et al*., 2009). Abortion of reproductive organs in sweet pepper occurs even when grown in a greenhouse (Wubs *et al*., 2009).

Reproduction in plants is highly affected by environmental factors such as temperature and may have

Appendix VIII: Published paper on objective three

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ACADEMIC JOURNALS

Journal of Horticulture and Forestry

Full Length Research Paper

Effect of NPK fertilizer rates on secondary metabolites of pepino melon (*Solanum muricatum* Aiton)

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Secondary metabolites are bioactive compounds which are synthesized naturally in all plant parts. The quality and quantity of secondary metabolites produced by plants differ depending on the plant and environmental conditions under which they are produced. The purpose of the study was to investigate the effects of nitrogen, phosphorous and potassium (NPK) fertilizer (17:17:17) rates (0, 100, 200, 300 and 400 kg ha^{-1}) on the production of secondary metabolites in field and greenhouse grown pepino melons (*Solanum muricatum* Aiton). The experimental design was randomized complete block design with five NPK fertilizer treatments replicated three times. Results indicated that an increase in NPK fertilizer rate led to an increase of carotenoids (lutein, lycopene and β-carotene) up to a maximum at 200 kg NPK ha^{-1} after which the contents decreased in both growing environments and trials. The control (no fertilizer application) favored the accumulation of total phenolic content (TPC) in both growing environments and trials. Greenhouse grown pepino melon fruits which were not supplied with fertilizer (control) had a TPC content of 174.3 and 145.5 mg GAE 100g^{-1} fresh weight (FW) in trial one and two, respectively. Fertilizers could not enhance production of TPC in pepino melon fruits and application of 200 kg NPK ha^{-1} is recommended for maximum accumulation of carotenoids (lycopene, lutein and β-carotene).

Key words: Secondary metabolites, NPK fertilizer, greenhouse, field, pepino.

INTRODUCTION

Plants produce a wide variety of organic compounds which can be grouped as primary and secondary metabolites. Primary metabolites include organic acids, amino acids and phytosterols and they play vital roles in respiration, photosynthesis, growth and development in plants. In contrast, secondary metabolism is a pathway through which small molecule products are produced and they are not involved in growth and development of

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Appendix IX: Published paper on objective five

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AHS
Advances in Horticultural Science

Postharvest quality of pepino melon (*Solanum muricatum* Aiton) as influenced by NPK fertilizer rates, growing environment and storage temperature

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Key words: firmness, shelf life, total soluble solids, weight loss.

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Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing interests:
The authors declare no competing interests.

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Abstract: The present study evaluated the effect of NPK fertilizer (17:17:17) rates (0, 100, 200, 300 and 400 kg ha^{-1}) on the postharvest quality of field and greenhouse grown pepino melons (*Solanum muricatum* Ait.) stored at room temperature (15-22°C) and at low temperature (7°C). The study was carried out in randomized complete block design with fruits from the field and greenhouse, five NPK fertilizer rates as treatments and the two storage temperatures replicated three times. Data were collected on percentage fruit weight loss (PWL), total soluble solids (TSS), firmness and shelf life. Results indicated that greenhouse and field grown fruits from the control and plants supplied with 100 kg NPK ha^{-1} had low PWL at both storage temperatures. Field grown fruits from the control stored at room temperature had the highest TSS and were firmer after 28 days of storage. Field grown fruits not supplied with fertilizer and stored at low temperature had a shelf life of 27 and 26 days in trial one and two respectively. Application of 100 kg NPK ha^{-1} and storage of pepino melon fruits at low temperature can be used to enhance quality and shelf life.

1. Introduction

Pepino melon (*Solanum muricatum* Aiton) is a little-known vegetable crop which belongs to the family solanaceae. It originated from the tropical and subtropical region of Andes and is grown for its edible fruits (Heiser, 1964). Pepino melon fruits are aromatic, juicy, scented, mild sweet, and vary in size, shape and colour depending on the cultivar (Martinez-Romero *et al.*, 2003). The fruits mature 30 to 80 days after pollination and the skin is usually golden yellow with purple stripes (Nuez and Ruiz, 1996). Several studies have reported significant losses in horticultural produce after harvest (Toktam *et al.*, 2019). Such losses are caused by dehydration, decay, and physiological disorders during postharvest handling. Fresh fruits and vegetables also undergo rapid transformation in nutritional and sensory quality after harvest, some of which contribute to loss of market value (Ahmad and Siddiqui, 2015). The losses can

Appendix X: Research Permit

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