Chemical Thinning of Morula Fruit (Sclerocarya birrea Subspecies Caffra Hochst.) Using Benzyladenine

Master of Science

(Crop Science Horticulture)

By

Onkgolotse G. Moatshe

August 2009

MoA

UNIVERSITY OF BOTSWANA
BOTSWANA COLLEGE OF AGRICULTURE

CHEMICAL THINNING OF MORULA FRUIT (*Sclerocarya birrea* subspecies *caffra* Hochst.) USING BENZYLADENINE

A Dissertation Presented to the Department of Crop Science and Production

In Partial Fulfillment of the Requirements for the Degree of Masters of Science (MSc) in Crop Science (Horticulture)

By

ONKGOLOTSE G MOATSHE (ID No: 200200075)

August 2009

Main Supervisor:
Prof V Emongor

Co-supervisor:
Dr O Oagile

Department of Crop Science and Production
Botswana College of Agriculture

CERTIFICATION

PROF VALLANTIND EMONGOR _____ 18/09/2009

Main Supervisor's Name and Signature Date

DR OTSOSENG OAGILE _____ 18-09-2009

Co-Supervisor's Name and Signature Date

MR SIFANGLE MACHETHA _____ 18/09/2009

Head of Department's Name and Signature Date

APPROVAL

PROF VALENTINO EMONGOR 18/09/2009

Main Supervisor's Name and Signature Date

DR OTSOSENG OAGILE 18-09-2009

Co-Supervisor's Name and Signature Date

MR. SIPHAMLE MACHACHA 18/09/2009

Head of Department's Name and Signature Date

PROF E B IKHONGA 18/09/2009

Dean of Faculty's Name and Signature Date

STATEMENT OF ORIGINALITY

The work contained in this dissertation was compiled by the author at the University of Botswana, Botswana College of Agriculture between August 2007 to June 2009. It is original except where the references are made and it will not be submitted for the award of any other degree or diploma of any other University.

OG Mocdshe

Author's Signature

ACKNOWLEDGEMENTS

I would like to express my gratitude to my mother, Violet M. Moatshe for believing that I can make it this far, with love and care you managed to fully support me financially for all my studies and research. "You are the best mom"!

It is a great pleasure to thank my supervisors, Prof V. Emongor and Dr O. Oagile for the great support throughout all the stages of my research work. They supervised me well with patience, appreciation, kindness and cooperation. Additional thanks go to Veld Products Research and Development for allowing me to use their orchard and equipments for carrying out my research, without their morula orchard this project would not have been successful.

I am grateful to my brother, William Moatshe and my uncle, Francis Moatshe for their hard work as my research assistants throughout my field work. Additional thanks to all Lab Assistants from Botswana College of Agriculture (BCA) - Crop Science and Production Department and Animal Science and Production Department and not forgetting Mr Mosekiemang from University of Botswana (UB)-Environmental Science Department for the assistance they gave me. Thanks to BCA and UB for use of their facilities during my research. My sincere appreciation also goes to my friends and collegues, for all the support and assistance, their contribution is highly appreciated.

All thanks to God Almighty for giving me hope and strength to keep going, even when I stumbled I knew that by His grace I shall overcome.

DEDICATION

This work is dedicated to my family, which has been tremendously supportive financially, morally and socially throughout my studies; my mother Violet Moatshe, my brother William, my sister Thandy, my uncle Francis and not forgetting my grandmother for inspiring me throughout all my education. All this is for you.

ABSTRACT

A field experiment was conducted at Gabane Veld Product Research and Development (VPR&D) morula orchard from August 2008 to May 2009. to evaluate the effects of benzyladenine (BA) on fruit set and quality of morula (*Sclerocarya birrea* subspecies *caffra* L.). Benzyladenine was applied when the fruitlets were 8-10 mm in diameter at concentrations of 0, 50, 100 or 150 mg/l. Benzyladenine application significantly ($p < 0.001$) reduced fruit set of morula trees by between 48-67 %. Application of BA to morula fruit trees significantly ($p < 0.001$) increased fruit size (length and diameter), density and weight. However, BA application had no significant effect on the fruit length-to-diameter (L:D) ratio. Benzyladenine significantly ($p < 0.01$) increased the fruit endocarp but decreased mesocarp of morula fruit by a margin of (1.1-6.3 %), suggesting that BA-induced increase in fruit weight was attributed to increase in the endocarp. Application of BA to morula trees resulted in fruits with significantly higher soluble solids content (SSC) ($p < 0.05$), titratable acidity ($p < 0.01$), citric acid equivalent ($p < 0.01$) and vitamin c content ($p < 0.001$) compared to control (unsprayed) trees. Morula trees sprayed with BA had significantly ($p < 0.001$) higher leaf area, total leaf chlorophyll content, chlorophyll a and chlorophyll b respectively than unsprayed control trees. Benzyladenine application significantly ($p < 0.05$) increased vegetative growth (terminal shoot growth and number of shoots) of morula trees. Morula trees sprayed with BA had significantly higher leaf and fruit potassium, nitrogen, magnesium, calcium, sodium and phosphorus contents than control trees. Benzyladenine also promoted morula fruit colour development at maturation. The results of this study showed that BA has the potential to be used as a chemical thinner of morula or an agent that improves fruit quality. However, further research on concentration and timing of application needs to be done.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF ABBREVIATIONS

a.i/Litre: active ingredient/Litre

BA: Benzyladenine

Ca: Calcium

DAFB: Days After Full Bloom

g/cm^3: grams/cubic centimeter

GA: Gibberellin

HCL: Hydrochloric acid,

K: Potassium

L:D: length: diameter

LSD: Least Significant Design

mg/g: milligram/gram

mg/L: milligram/Litre

Mg: Magnesium

N: Nitrogen

Na: Sodium

NAA: Naphthalene Acetic Acid

NAAm: Naphthaleneacetamide

NAOH: Sodium hydroxide

nm: nanometer

P: Phosphorus

P: Probability

PROC GLM: Protected General Linear Model

SSC: Soluble Solid Content

TTA: Total Titratable Acidity

VPR&D: Veld Product Research and Development

CHAPTER 1

INTRODUCTION

1.1. General Introduction

Morula is a drought tolerant, dioecious, deciduous tree which grows wild in the Northern parts of South Africa and parts of eastern Botswana. It grows in various types of woodlands in sandy to sandy-loam soils to a height of above 9-20 m depending on the growing conditions (Fox and Norwood-Young, 1982; Peters, 1988). The morula tree is widely distributed in the African continent (of which in Southern Africa the subspecies found is *caffra*). In Botswana it is commonly named as morula, South Africa other names are morula in pedi, moroela in Afrikaans and marula in Shona, Kenya in meru its named mura and in Boran its named didissa. It is also found in countries such as Zaire, Ethiopia, Zambia, Zimbabwe, and has been introduced to Mauritius, Oman and Tamil Nadu (Kokwaro and Gillet, 1980).

Morula is a multi purpose tree with various uses ranging from its role as a highly nutritive valued fruit consumed fresh by human beings, game and livestock, to commercial use such as processing into a potent alcoholic drink (such as amarula), good quality semi sweet to sweet wine, jam, juice, nectar and sweets (Faine and Venter, 1996; VanWyk *et al.*, 1997).

Morula fruit has an excellent nutritive value. It contains citric acid, malic acid and sugars. The vitamin C content is four times as much as oranges. The mean fruit nutritional

composition from the selected trees in g/100g is: moisture-74, fibre-1.2, proteins-8.0, fat-15.7, carbohydrates-75, edible-portion-51, calcium-20, iron-0.5 vitamins-127 mg/100g and energy-225 KJ (Mateke, 1999). It also contains other nutrients such as magnesium, phosphorus, potassium, calcium, fructose, glucose and sucrose (Quin, 1959; Wehmeyer, 1976). Each fruit of morula contains two to three edible nuts (seeds) which contain oil valuable for food preservation and for processing into cosmetic products. The embryo is rich in oils and the kernel has proteins, fats, magnesium, copper, zinc, phosphorus and is high in energy (Faine and Venter, 1996).

Both leaves and bark of morula can be used as herbal medicines. These include treatment of circulatory system disorders and haemorrhoids. Digestive system disorders such as dysentery and diarrhoea can also be treated using morula. Other disorders such as malaria, fever, inflammation injuries and poisoning from insect stings can also be cured (Kokwaro and Gillet, 1980). Morula wood can be used to make furniture, beams and general purposes but its less durable (Kokwaro and Gillet, 1980; Fox and Norwood-Young, 1982). The economic value of these products is increasingly becoming recognized both nationally and internationally, which has led to their commercialization (Botswana Technology Center, 1997).

In planting fruit trees for both commercial orchards and home gardens, the major goal is to produce a large crop of high quality fruits each year after the tree has reached a productive age. Plants are expected to bloom and then set fruits. This period of fruit set is long and exhaustive, starting with flower induction to fruit ripening and abscission,

during which the environmental factors and management practices can have a major impact on the success of fruiting (Goldwin, 1992). The aim of fruit production is to attain commercially marketable fruits of a normal size, appearance and desirable flavour. This is particularly so with perennial crops such as pome fruits, stone fruits and citrus where the period from anthesis to ripening can last for as long as 60 weeks (Goldwin, 1992). There are several steps that are critical in the production of large quantities of high quality fruits. These steps include flower bud initiation and development which is overcome by chilling winter temperatures for most deciduous trees, flower opening and pollination in spring, fertilization of a flower, fruit setting, fruit growth and development, fruit maturation, ripening and harvest (McMahon *et al.*, 2002).

Fruit growth is part of the integrated growth of a plant; therefore, fruit yield is determined by interaction between growing conditions and morphological characteristics as well as physiological activities of the whole plant (Ho, 1992). The improvement of fruit yield is dependent on factors that control both production of leaf (assimilate production) and sink strength of the fruit (assimilate partitioning). Apart from the supply of assimilates both cell number (sink size) and physiological activities (sink activity) within a fruit may determine its sink strength in attracting assimilates to sustain fruit growth (Ho, 1992).

Fruit yield in terms of drymatter production is related to assimilate supply and hence to irradiation intercepted by the crop. However, increase in fruit yield is not only determined by improvement of photosynthesis for most crops, changing drymatter partitioning under defined growing conditions can also enhance yield (Gifford and Evans,

1981), since the rate of drymatter accumulation in fruits of the same potential size is related to the rate of concurrent photosynthesis in leaves (Tanaka *et al.*, 1974). The improvement of fruit yield can be achieved by manipulating sink strength of the fruit (Gifford and Evans, 1981; Ho, 1988). Fruit as the final growth stage of reproductive organ is commonly a strong sink for assimilates than flowers and vegetative organs, and so competition of fruits for assimilates is mainly with other adjacent fruits. This competition is most common when fruits grow in clusters such as morula, apples and grapes or in trusses as in tomatoes, since such fruits obtain assimilates from the same group of leaves (Monselise and Goldsmidt, 1982). For such fruit trees, competition between fruits can be very intense and hence resulting in fruit drop. Furthermore, competition between fruit growth and floral initiation for next year's crop may be the cause of biennial/alternate bearing habit in most of perennial fruit crops (Monselise and Goldsmidt, 1982).

Manipulation of fruiting behavior of fruit trees is one of the dominant aspects of management practices necessary to modify the balance between vegetative growth and fruiting, hence adjustment of leaf-to-fruit ratio to a desirable level (Jackson, 1985). The first approach to reducing the number of fruits per tree is by pruning, where the thin fruit shoots are removed and fruiting spurs reduced. The extent to which the pruning can be used as the main technique for fruit thinning depends largely on the consistency of cropping from year to year. Thinning of the fruitlets after blossoming provides a more flexible technique for adjusting the actual fruit load in any particular year. This is the technique practiced in plants with a heavy crop load where a portion of the crop is

removed before it matures (Jackson, 1985). Fruit thinning is done with the aim of reducing the within fruit competition for carbohydrates, improving the fruit quality in terms of its firmness, soluble solids content and anthocyanin formation hence desirable skin colour (Horscroft and Sharples, 1987; Byers and Carbaugh, 1991). It is also essential to reduce the incidence of biennial bearing (Jackson, 1985; Byers and Carbaugh, 1991). The earlier the thinning is carried out, greater are its benefits per fruitlet removed. At any given time, the individual fruitlet size is a good indicator of potential fruit size at maturity and so small fruits should preferentially be removed (Williams and Edgerton, 1981). The most common methods of fruit thinning are hand, mechanical and chemical thinning. In hand thinning, excess fruits/ fruitlets are removed by hand, fruit clusters should be broken and fruits be spaced in limbs and weakened fruits removed. It is the most effective method and yet expensive for improvement of fruit quality because of its precision and absence of side effects. It is commonly practiced in peaches, plums and pears. In mechanical thinning, mechanization and labour saving devices such as almond mallets for pre-thinning and tree shakers are used. The problem with this method is failure to accurately control the amount of fruitlets to be removed (Janick, 1986).

Chemical thinning is a method where chemical sprays are used for fruitlet thinning. These chemical thinners are widely used either to replace hand thinning totally or to reduce the amount of hand thinning required and they are often used in commercial orchards. Different chemicals have been used over the years which act differently on different crops, but all with the one objective of reducing the crop load. Chemical thinning can be performed prior to fertilization (i.e. flower thinning) or after fertilization

(i.e. fruit thinning) depending on the type of chemical used (Janick, 1986). Therefore, the materials effective in thinning may be divided into two groups depending on their mode of action. There are flower thinning compounds which are composed of both caustic and toxic substances such as phenols, asernols and dinitro compounds such as sodium 4,6-dinitro-ortho-cresylate. These are compounds which kill off blossoms or render them sterile. Fruit thinning compounds on the other hand are mostly hormone type materials especially auxins and their derivatives particularly naphthaleneacetic acid (NAA) and naphthaleneacetamide (NAAm). These compounds bring about thinning mostly through embryo abortion. Auxins are mostly effective in thinning of apples, pears, grapes, olives, peaches and some stone fruits (Janick, 1986). The other important hormone compound used is benzyladenine (cytokinin) which works mainly in cell division (Emongor, 1995). Ethephon is generally used late in the thinning season when prior thinning application has not worked. There are some insecticides which at certain concentrations can be used as thinning compounds and will reduce fruit set, and the most important being carbaryl (sevin) (Edgerton, 1981).

There are variations in responses to chemical thinners, because thinning results are affected by fruit set, climate, weather, tree age and vigour, orchard management practices and most importantly the type of the chemical thinner used, timing, method of application and its concentration (Childers, 1984; Emongor, 1995; Emongor and Murr, 1999; 2001; Byers, 2002). Most often two or more chemical thinners are combined to increase thinning strength on hard-to-thin cultivars. Surfactants and oils can be added to chemical thinners in some circumstances where the leaf permeability is low (Williams, 1979).

Among all chemical thinners, the most commonly used are ethephon (ethrel), benzyladenine (Accel), naphthaleneacetic acid (NAA), naphthaleneacetamide (NAAm), carbaryl (sevin) and oxamyl (Vrydate). Vrydate and Accel are the two most recent post bloom chemical thinners for most fruit trees (Cutwright and Pfeiffer, 1997).

1.2. Justification of Study

The greatest challenge facing Botswana is to improve food security, rural employment and income under semi-arid environments. This requires efficient use of natural resources and management skills. The challenge of preserving the environment and making prudent use of natural resources is vital for survival and future prosperity. This can help remedy the problems of poverty, poor health and disease outbreak such as HIV/AIDS, heart diseases and poor infrastructure.

Natural resources of Botswana are the most important assets and at the moment, diamonds are the most beneficial resource for development in Botswana. The discoveries of other resources not only minerals can impact in diversification of the economy. Almost half of the 300 useful plants in Southern Africa are food plants and only a few have been explored for their commercial uses, yet they are the resources which can most improve sustainable agriculture (FAO, 2002). African food plants should therefore be fully explored with regard to finding viable alternatives to arable agriculture especially for regions like Botswana which encounter low once erratic rainfall, poor soils, high temperatures and droughts.

Morula is one of the most important resources that can be of high benefit if it can be well utilized and conserved as it can thrive under arid and semi-arid conditions of Botswana. There are numerous enterprises in Southern Africa which produce jam, jelly, dried fruit rolls, sweets and cosmetics from morula. In South Africa, the Mitsubishi Cooperation brewed a morula beer locally known as 'Afreeke' in 1997 (Leakey, 1999). The Distillers

Cooperation is marketing the popular morula liquer-Amarula internationally while 'Marulaan' wine in Zambia is marketed commercially (Leakey, 1999). There is also a pasteurised juice which is marketed in Botswana (Taylor and Kwerepe, 1995). Morula is a drought tolerant indigenous fruit tree which frequently forms too many flowers and sets too many fruits which leads to a heavy crop load per cluster (Fox and Norwood, 1982; Booyers, 1996). In heavy fruiting seasons, a single morula tree can produce between 21000 and 91000 fruits (HerbalGram, 2008). The heavy fruit set results in alternate or biennial bearing, low quality fruits in terms of size, colour and soluble solids content (Faust, 1989). The fruit of morula is popular in Southern Africa but its small size hinders commercialization especially for the fresh market fruit. In Botswana, there are few upcoming Community Based Organisations such as Kgetse ya tsie, Botswana craft, The Taylors and Veld Products Research and Development which are interested in commercialization of morula.

The research on domestication of morula and improvement of morula began in the late 1980s in Botswana (Holzhausen, 1989). Veld Products Research and Development started work with morula in the early 1990s. The researches that have been done include domestication and development of appropriate agronomic techniques of morula. Through grafting, superior genotypes were identified and domesticated. This included "swarula" and "parula" all of which originated from different parts of Botswana (Mateke, 1995). Studies on the identification and control of diseases and pests in the hard veld of Botswana were also undertaken. It was reported that Aceria mite attack trees of both sexes at all ages indiscriminately. The mite infection prevents the normal fruit production

and can cause severe damage to the tree (Sunstrum, 1997). Other researches done include the use of mycorrhizal inoculation in seedlings under nursery conditions (Mateke, 1996). The seedlings artificially inoculated with isolated cultures of arbuscular micorrhizal fungi (AMF) promoted growth than non inoculated ones. Therefore it was concluded that artificial introduction of the right AMF at planting of seedlings can play a significant role in the promotion of initial plant growth and development (Mateke, 1996). In the research based on the germination response of morula seeds to season of sowing and seed pre-treatment, the best time of germination was found to be in spring and summer (September to December) (Mateke, 1995).

No research dealing with fruit set of morula has been reported in Botswana and elsewhere. This calls for a need to come up with technologies that will improve fruit size of morula to a desirable level for commercial marketing both locally and internationally. Plant breeding and use of plant growth regulators are technologies that can be used to increase fruit size. For example, Professor Holtzhausen of the University of Pretoria (Republic of South Africa) has recently selected improved clones of morula producing large fruits up to 100 g in weight and with a variety of skin colours (Nerd *et al.*, 1990). Thinning of flowers or fruitlets can improve fruit yield and quality in terms of size, taste and colour, hence increasing marketability of the remaining fruits and reducing or eliminating the problem of alternate bearing (Byers and Carbaugh, 1991; Emongor, 1995; Robinson *et al.*, 1998; Emongor and Murr, 1999; 2001). The success of this study could benefit Botswana's economy in industrialization, job creation, sustainable agriculture, food security and self reliance.

1.3. Objectives

The objective of this study was to evaluate the effects of benzyladenine on fruit set and quality of morula (*Sclerocarya birrea* subspecies *caffra L.*).

1.4. Hypothesis

H_A: BA has an effect on the fruit set and quality of morula.

H_o: BA has no effect on the fruit set and quality of morula.

CHAPTER 2

LITERATURE REVIEW

2.1. Botany of morula

Morula (*Sclerocarya birrea* subspecies *caffra* L) belongs to the family Anacardiaceae the same family which mango (*Mangifera indica*), cashew nut (*Anarcardium occidentale)* and pistachio nut (*Pistacia vera)* belong. Morula is a medium to large sized dioecious tree, indigenous to the miombo woodlands of Southern Africa and the Sudano-Sahelian range of West Africa. The name *Sclerocarya* is derived from a greek word *"Skleros"* for hard and *"karyon"* nut or kernel in illusion to a large, woody kernel of the fruit (Roodt, 1998).

Morula is a single stemmed tree with dense deciduous foliage (Roodt, 1998). The trunk is well developed, with a large spreading round-topped crown (Roodt, 1998). It has a rough, grey bark with a disc shaped flakes which gives the trunk a mottled appearance. The leaves are alternate compound with a pale green colour but turn pale yellow prior to shedding. They are aggregated at the end of the branch, with 7 to 13 pairs of opposite to sub opposite leaflets plus a terminal leaflet. The leaflets are ovate to elliptic, the apex is tapering while the base is tapering to round. Older leaves have entire margins while young ones are toothed. The leaves have a long and slender petiole and often tinged with pink colour (Faine and Venter, 1996).

Morula has unisexual flowers borne on separate trees, and arranged in sprays five to eight cm long. The floral parts are in four or five clusters with red sepals and yellow petals (Booyers, 1996). These flowers are produced in small sprays just before appearance of young leaves. The male flowers are conspicuous and borne on spur drooping racemes and pinkish in colour while the female flowers are smaller, red, purple and white and they are borne on long penducles from July to January (Rood, 1994; Roodt, 1998).

The fruits are borne in clusters at the end of twigs always on new growth. They have a thick leathery exocarp, enclosing a white juicy flesh, which adheres tightly to the stone. The fruits are oblong and about 50 mm in diameter, they fall from the tree during the period of January to March, while they are still pale green and ripen to pale waxy yellow on the ground. They are refreshing with a very juicy, sweet/acid flavour and a rich scent (Fox and Norwood-Young, 1982; Rood, 1994), when decay they release a pungent smell (Booyers, 1996). Each fruit contains a hard, often bilocular or trilocular seed, each seed locule contains a single nut in each cavity. These locules have a sealed opening by a round hard disk that protects the seed until germination (Booyer, 1996). Morula tree is a prolific bearer, it bears an abundance of fruits with yields up to 2 metric tones per tree (Booyer, 1996). The fruit abscisses when ripening commences so that it takes place on the ground. This fruits are harvested by picking the fallen ones (Booyer, 1996).

Most morula trees are dioecious, and the monoccious ones are predominantly male. The start of flowering period in Southern Africa is from September to November, and fruiting is from January to March (Faine and Venter, 1996). Like many riverine species, it is

dispersed by water streams and shows adaptation to water dispersed by having air spaces in the fruits (Fox and Norwood-Young, 1982). Propagation is easily done by sowing seeds (Roodt, 1998), but grafting also gives satisfactory results (Mateke, 1995).

2.2. Fruit set of fruit trees

The development of a fruit is normally a consequence of pollination, unpollinated flowers fall and the pollinated ones will show a fruit set. The germinating pollen is a rich source of auxins which are very important for fruit set. In some species application of auxins exogenously to unpollinated flowers can lead to development of parthernocarpic fruits (Ho, 1979). Gibberellins can also be used to induce parthernocarpic fruits in some species which cannot set fruit by auxins (Ho, 1979). Seed development in most fruit species is important in inducing fruit size because they are centers of hormone synthesis such as gibberellins, auxins and cytokinins (Ho, 1979; Looney *et al.*, 1985). The importance of seeds to fruit development is controlling the influence of auxins, cytokinins and gibberellins. These plant growth regulators are the ones that make fruits a stronger sink for food materials synthesized in the leaves, since the fruit development is normally associated with the rate of vegetative growth (Street and Opik, 1983). Thus fruit growth is principally sustained by the supply of photoassimilates, hence the rate of dry matter accumulation in the fruit relates to concurrent photosynthesis in the leaves. This implies that the fruit size is determined by the leaf-to- fruit ratio (Ho, 1979).

Fruit development following pollination involves the growth of the seed in the endocarp to maturity and the enlargement of the ovary. Cell division, cell expansion and

accumulation of food reserves are also involved in seed development. Fruit growth on the other hand involves mostly cell enlargement and in many cases there is short phase of cell division (Street and Opik, 1983). The development of fleshy fruits involves accumulation of organic metabolites (organic acids and sugars) into the succulent pericarp and associated tissues (Street and Opik, 1983).

Generally, fruit dry matter accumulation is determined by cell number, size and cell content (Coombe, 1976; Zhang *et al.*, 2005). Final fruit volume particularly fleshy ones is determined by the extent of cell enlargement (Coombe, 1976; Faust, 1989). While actual fruit size is mainly determined by final cell number in the fruit, duration of cell division after anthesis varies among species and between tissues of the same fruit (Bollard, 1970). Therefore, it is important to assess relative importance of cell division activity before and after anthesis in determining the final cell number. Once cell number is finalized in the fruit, cell enlargement is a dominating event which involves cell wall extensibility, accumulation of water and solutes particularly the vacuole. This is affected by the number of metabolic activities inside the fruit. In most fruits such as stone fruits (peaches), 80% of their fresh weight and/or 60% dryweight is accumulated during cell enlargement. In apple cell division in the flesh (pith and cortex) ceases about three to four weeks after anthesis and more than 80% fruit growth period is due to cell enlargement (Bollard,1970). Fruit thinning can be used to prolong the process of cell division, resulting in a bigger fruit size. Alteration of cell division at early post bloom is more effective than late period in determining final cell number hence final fruit size (Emongor and Murr, 2001).

. Fruit thinning by chemical thinning agents

satisfactory thinning spray will remove enough fruits to ensure an adequate return om the following season or year (Emongor, 1995). Fruit thinning is mostly achieved nmercially by the use of chemical thinners. Chemical thinning agents are both cost and e effective (Emongor and Murr, 2001). Generally fruit development is dependent on interactions of five major classes of plant hormones which are auxins, gibberellins, okinins, abscisic acid and ethylene (Zhang *et al.*, 2005). Auxins, cytokinins, berellins, an ethylene inhibitor, Amino Vinyl Glycine (AVG) and mixtures of regulators are effective in the increase of fruit set (Miller, 1988; Zhang, 2005). wever, bioregulators are not always effective. Lack of consistent response and desirable side effects such as reduced fruit size, phytotoxicity, fruit russeting, abnormal it shape and excessive abscission of parthenocarpic fruit have limited commercial use bioregulators (Miller, 1988).

uit thinning serves slightly different purposes in different fruit species. In stone fruits, ge fruit size is almost impossible without proper thinning especially with early turing cultivars (Faust, 1989). In apples, in addition to moderate increase in fruit size, : main purpose of thinning is to remove the source of gibberellins, the seeds, which :vent flower bud formation (Luckwill, 1970; Tromp, 1982; Mclaughlin and Greene, 84). Chemical thinning enables growers to overcome alternate bearing habits of some ple cultivars and to improve the size and quality of the fruit in years of heavy set. emical thinning using plant growth regulators is characterized by a narrow time of

response. This narrow window may reflect rapid internal changes in growth substance balance or in the tissue sensitivity to the thinning compounds. Gibberellins (GAs) have been shown to diffuse from flowers/fruits into spurs (Marino and Greene, 1981) and more GAs move out of fruit of biennially bearing cultivars than out of annual cultivars (Hoad, 1978). Williams and Edgerton (1981) and Grochowska *et al.* (1984), reported that seeds from annual and biennial cultivars produce GAs which diffuse from developing fruits, this causes alternate bearing (Pharis and King, 1985). The amount of GAs produced per individual seed is not known, but there is strong evidence that GA_7 rather than GA_4 inhibits flower bud formation in apples (Tromp 1982; Looney *et al.*, 1985).

The return of bloom the next year improved by chemical thinning is related to the effects of gibberellins produced by the endosperm of developing seeds of the fruit such as apples. The endosperm of the apple seeds develops very rapidly in the weeks following fertilization. Production of gibberellic acid by the growing endosperm results in their movement from seeds into the tissues of the subtending spur to which the fruitlet is attached. The presence of gibberellins at that time probably results in the inhibition of flower induction in the lateral meristem of the spur (Tromp, 1982). The exogenous application of gibberellins in apples following flowering can inhibit flower bud formation (Chan and Cain, 1967; Tromp, 1982). The presence of fruitlets at the time of gibberellin application greatly enhances the inhibitory responses. This shows that exogenous application of gibberellins may promote inhibition of flower formation by supplementing the natural internal balance of gibberellins (Eflving and Cline, 1993).

The mechanism(s) whereby chemicals are used to selectively thin fruits has been the subject of many experiments and review (Landsberg and Brain, 1978; Williams, 1979; Bangerth, 1990; Emongor, 1995; Emongor and Murr, 1999). The understanding of scientists about physiological mechanisms that lead to fruit drop and how these mechanism are influenced by chemical thinners lags far behind the empirical applications of plant growth regulators (PGRs) in the orchard (Emongor, 1995). Theories proposed include reduced translocation of auxin from the fruitlet (Crowe, 1965); stress-induced ethylene production, which interfers with transport of "vital chemicals" to the seed (Williams and Batjer, 1964; Williams and Edgerton, 1981); the diversion of nutrients to fruit growth and auxin production, and abscission (Bangerth, 1990).

Bangerth (1990) reported that primigenic dominance of the "King fruit", and auto stimulation of transport pathway by indole acetic acid (IAA) from the earlier developing fruit, while inhibiting the IAA export of later developing fruit (autoinhibiting), could explain the thinning activity of most chemical thinners used in the apple industry. Emongor and Murr (1991; 1994) reported that the response of "Empire" apples to chemical thinners may be mediated by ethylene. Greene *et al.* (1992), working with "McIntosh/M26" apple, reported a significant increase in ethylene production in fruitlets and leaves one day after BA application. Magg (1963) suggested that the mechanism by which chemical thinners reduce fruit set is probably involved with the alteration in the supply of assimilates to fruitlets. The consequence of greater limitation on the supply of photosynthesis to the fruitlets is that a larger proportion of the fruitlets are unable to sustain sufficient growth and eventually abscise (Magg, 1963). The thinning action of

chemicals is due to their effects in desiccating stigmas and styles of flowers thereby preventing pollination and hence fruit set (Williams *et al.*, 1995). Blossom thinners cause physical damage to flowers thereby impending fertilization. Auxins, cytokinins and ethylene and their derivatives are all capable of reducing fruit set when applied during a short time interval after anthesis (Magg, 1963; Webster and Spencer, 1999).

2.4. Chemical thinners used in the fruit industry worldwide

There is a wide range of chemical thinners used in fruit trees. Thinning can be accomplished at bloom time and during the early post bloom period. The post bloom period is the time at which the fruit tree has reached 80% of full bloom stage (Williams, 1979). The strongly biennial cultivars may require both the bloom and the post bloom program for adequate thinning and return bloom. The commonly used chemical thinners include the following:

2.4.1. Benzyladenine

Benzyladenine (BA) is a synthetic cytokinnin compound which provides very little risk to the environment. A precondition of precocious bearing in young trees is the development of a canopy structure which has good cropping potential and this can be achieved by using BA. Fruit thinning with BA in mature trees can result in larger fruit size and increased return bloom. However, the temperature dependence of the thinning response remains a problem to be resolved. The efficiency of benzyladenine is determined by the physiological age of trees, environmental conditions at application and the application methods used (Buban, 2000). The advantage of BA as a synthetic cytokinin is its

influence on hastened cell division so that the fruit enlargement should be greater than could expected from its thinning action (Stoppa, 1999). BA has the ability to promote carbohydrate metabolism and create new source-sink relationships and hence lead to increased sink strength and fruit size at harvest (Dyer *et al.*, 1990; Emongor and Murr, 2001). BA causes increased fruit size not just from thinning due to reduced competition, but also from more cell division hence stimulating additional fruit growth. It also increases the fruit soluble solids and the fruit colour (William, 1995).

2.4.2. Accel

Accel (mixture of benzyladenine and GA_{4+7} in a ratio of 10:1) was introduced in 1994 by Abbot Laboratories USA. This synthetic cytokinin 6-benzyladenine (BA) has been found to be a good thinning agent. It is a post-bloom thinner and it thins fruitlets best at 10 mm diameter and has a positive influence on return bloom (Robinson *et al.*, 1998). Accel is effective when applied to the leaves but its more effective in the increased fruit size when applied to the fruit. The maximum concentration of Accel depends on the size of the tree. Gibberellins (GAs) as part of Accel also play a role in inducing fruit set and expansion of the pericarp. The exogenous application of GAs is known to increase fruit enlargement in grapes (*Vitis spp*) and Japanese pear *(Pyrus serotina)*. It also enhances fruit set in seedless cultivars of mandarin (*Citrus reculata* L.) (Zhang *et al.*, 2005). Accel thins over a wide window, probably from bloom to 30 days after full bloom, and is reported to promote cell division. It is a gentle, mild thinner and is dose-dependent. It is efficient in most fruit tree cultivars (Zhang *et al.*, 2005). At the present time, Accel is not recommended to be used with NAA or NAAm on the same tree within the same year.

The combined use of Accel and NAA or NAAm on Red Delicious apples in the same year has resulted in more pygmy fruits (small, mishappen fruits) (Garcia *et al.*, 2003).

2.4.3. Sodium 4,6-dinitro-ortho-creyslate or Dinitrocyclohexylphenol [(DNOC), (Elgetol)]

It is one of the early bloom time spray used especially in apples. This chemical thinner is a caustic material applied at mid bloom, it burns the flower petals and pistils preventing pollination and/or fertilization hence no fruit set (Williams, 1979). Caustic materials damage the pistils on the side of the fruit, this activity is physical but not physiological. The stigma is damaged so there is no pollen germination (Dennies, 2000). This chemical is no longer used in most parts of the world (Garcia *et al.*, 2003).

2.4.4. Naphthaleneacetic acid (NAA)

This is a fairly effective thinning agent and the response is rate dependent. It is equally effective when applied to both leaves and fruits. The absorption of NAA takes place when the spray dries and the unabsorbed material is rapidly broken down by sunlight which reduces the potential for additional uptake caused by rewetting. The use of NAA may induce abscission of the young fruit immediately or delay the process and fruits may fall later. In "Golden Delicious" apple cultivars, a combination of NAA and Sevin applied at petal fall usually gives better results than either material alone. Application of NAA on Starkrimson and other spur-type Red Delicious strain of apples has occasionally caused problems of pygmy fruits (small, mishappen fruits). Instead of dropping off, these seedless pygmy fruit remain on the tree until harvest, growing to about a quarter of the

normal size. In cases where pygmy fruit presents a problem, sevin should be used for thinning. (Garcia *et al.*, 2003).

When NAA is the only thinning agent applied, its absorption and effectiveness are greatly increased by use of an activator. The concentration of NAA can be reduced in to half if wetting agents are used, such additives help to counteract the variable effects of weather conditions on the absorption and effectiveness of Amid-Thin and Sevin. Because of varying growing conditions, NAA sprays are best timed according to fruit size rather than days after full bloom. NAA is most effective on apple fall and winter apple varieties when most of the fruits are 8 -10 mm, the king blossom fruit is 10 -13 mm, and the smallest fruits are less than eight mm in diameter (Garcia *et al.*, 2003).

2.4.5. Naphthaleneacetamide (NAAm)

NAAm is similar to NAA, but milder. Its use should be limited to petal fall as later use can result in pygmy fruit. It is recommended for use on apple cultivars that ripen before McIntosh apple and works best when applied under slow drying conditions. Frequently, follow-up thinning is required (Garcia *et al.*, 2003). The physiological basis of flower and fruit thinning by auxins (NAA and NAAm) is probably by causing fruitlets to drop due to alteration of the balance of auxins in the outer and inner ends of the fruit stem which induces the development of the abscission layer, or due to the embryo abortion or the elimination of natural pollination by the thinning process (Datta, 1994).

2.4.6. Sevin

The preferred formulation of Sevin for fruit thinning is XRL Plus. Sevin is the mildest and safest insecticidal thinner which is toxic to bees. Before application of sevin, the bees should be removed from the orchard. It is dose-dependent up to a certain point, however, it seldom over-thins and it is more effective when applied on the fruit provided photosynthates are not limiting. It can be used from petal fall to about 21 days after bloom. It is frequently combined with Accel or NAA for heavy thinning late in the thinning window. The limit is about 25 mm fruit size. Over-thinning of these combinations can occur (Garcia *et al.*, 2003).

2.4.7. Ethephon

This is a chemical thinner which releases ethylene on the hydrolysis within the treated tissues. It acts through the promotion of abscission zone development and it enhances return of bloom through the reduction in the shoot growth following application. The response can vary depending on the weather and over thinning results especially at high temperatures after application. Ethephon is more effective when applied into the fruits than leaves (Dennies, 2000).

2.5. Factors affecting the efficacy of chemical thinners

The two most important factors to consider during thinning is when and how to thin fruit trees. Thinning is affected by timing of application, type of thinning material used in relation to species/cultivar being thinned and concentration of thinning agent (Forshey, 1990; Emongor, 1995; Emongor and Murr, 2001).

2.5.1. Timing, Concentration and Materials

If thinning is done at petal fall or shortly thereafter, sevin, or a combination of sevin and NAA (5mg/L) can be applied. Accel on the other hand, should not be applied at petal fall, instead its more effective when applied 7-10 days after petal fall and this is mostly when the application coincides with active cell division (Williams, 1979). For chemicals such as DNOC in apples (Washington cultivar), application should be made when there are three blossoms or spur cluster opening on the north side of the tree or at full bloom. Only one spray should be used and low temperature will increase its effectiveness and can result in over thinning. DNOC as a blossom spray is not adapted for use in post bloom thinning and it can only be used where post bloom frosts are not likely to occur. Timing is a critical factor especially when hormones like NAA and NAAm are used on summer varieties at the petal fall stage. If it is used later than 10 days after full bloom, stimulating effects manifested by premature ripening and fruit splitting is likely to occur.

Sevin, NAA and NAAm are usually applied from 10-25 days after full bloom (Williams, 1979), they may thin fall and winter varieties of apples effectively during bloom (Childers, 1984). Sevin effectively thins apples when applied 5-30 days after full bloom, with consistent and uniform results when applied at 15-27 days after full bloom. Thinning is also dependent on weather conditions (Emongor and Murr, 2001), during a cool postbloom period, natural fruit drop is delayed and when it occurs sprays are effective later in the season than if the weather is warmer (Childers, 1984).

Ethephon on the other hand is used on the non bearing trees to induce flower bud formation. It is always effective when it is combined with alar, which controls growth by inducing spur development. These chemicals should be avoided on moderately vigorous trees which may be stunted by over blooming and setting such as M9 apple varieties. Application is effective 14-21 days after full bloom and ethephon is delayed until after drop begins or five to six weeks after bloom to avoid over thinning (Childers, 1984).

Effective concentration of thinning sprays is related to weather conditions, tree vigour and tree species or cultivar (Forshey, 1990; Emongor, 1995; Emongor and Murr, 2001). NAA has a greater reduction in fruit set with stronger spray application (Childers, 1984). DNOC is used at the concentrations of 160-480 mg/100 water, while NAA is applied at concentrations from 2-15 mg/L. Ethephon (300 mg/L) combined with NAAm at 10-21 days after bloom is effective in Golden Delicious apples. The post bloom thinners are effective within one to two weeks or more, depending on weather conditions (Miller, 1988).

2.5.2. Penetration of Chemical Thinners

For thinning chemicals to be effective they must diffuse across cuticles which cover aerial portions of the plant. This includes leaves, flowers and young fruits. The cuticular wax provides a major barrier to penetration. There is little wax secreted on the leaf surface when the weather is cold and cloudy, this permit greater penetration. Sunny and dry weather causes more wax to be deposited and secreted, thereby restricting the

penetration. This means that chemical thinners are less effective after warm, dry periods and more effective after cool moist periods (Dennies, 2000).

2.5.3. Environmental Factors

The thinning response varies depending on the climate, cultivar and weather conditions before and after thinning.

2.5.3.1. Temperature

Temperature during atmospheric application and after application is critical to successful thinning. Different chemical thinners have a greater response as the temperature increases because for them to work they have to be absorbed, thus absorption increases as the temperature increases. At higher temperatures, foliage waxy cuticle increases hence reduce chemical absorption. This will lead to application of the chemical with a surfactant to increase the leaf permeability. However, application made in the morning or evening when the temperatures are low have a longer drying period on the leaf resulting in a slow and yet sustained uptake of chemical, while at a higher temperature during mid-day drying times are shorter, resulting in a short but rapid uptake of chemical (Byers, 2002; Garcia *et al.*, 2003). NAA generally gives best results under fast drying conditions and also when the temperature is between 21^{O}C and 27 ^{O}C. Amid-Thin gives the best results under slow drying conditions and is often applied in the evening. Accel is most effective when air temperature is 21 ^{O}C to 27 ^{O}C (Garcia *et al.*, 2003).

2.5.3.2. Light

The factors that decrease photosynthesis rate such as cloud cover or within tree shade increase the effectiveness of thinners and the sunny weather which decreases the sensitivity of the fruitlets to the thinners. The light intensity 3-5 days before the application is important in the uptake of the chemical due to a thin leaf cuticle and reduced carbohydrate and their supply for fruit growth. The interaction of temperature and sunlight with chemical thinners creates stress in the tree, which is necessary to make some fruit drop off (Byers, 2002; Garcia *et al.*, 2003).

2.5.3.3. Stress

Regardless of the mode of action of chemical thinners, plants require some type of stress imposed by weather for ideal thinning response. Competition among flowers, fruits, leaves and growing points occur for nutrients, water, metabolites, photosynthates and growth regulators. The chemical thinners increase this stress on fruits causing small fruits and those with a small number of seeds to abscise. In some cases where chemical thinners have worked poorly the weather imposed stress has occurred before the thinner application (Dennies, 2000). Generally stress on the tree increases the period of response. The stress can occur before bloom such as winter damage, heavy crop the previous year, vigorous trees or it can occur during bloom such as frost damage (Garcia *et al.*, 2003).

Basing on efficacy of thinning, materials commonly used are BA, Accel, NAA, Amid-Thin (naphthaleneacetamide), and Sevin (carbaryl). These materials vary among species and cultivars. If seed numbers are small, or trees are weak, or if the weather is cool and

cloudy, lower concentrations of thinner chemicals are used. Generally young trees are easier to thin than mature trees. All thinner applications should be based on average fruit diameter. All hand-gun applications should be made to the point of runoff at a rate of chemical use that is one-third to half the rate recommended for an airblast application to enhance efficiency on thinning (Garcia *et al.*, 2003).

CHAPTER 3

MATERIALS AND METHODS

3.1. Experimental site

A field experiment was conducted at Gabane Veld Product Research and Development (VPR&D) morula orchard (S24 39.140'; E25 46.390), at an altitude of 1110 above sea level, from August 2008 to May 2009. The experimental site was on an uncultivated land classified as shrub and tree savanna with major vegetation grouping of *Acacia negrescens, Combretum apeculatum* and *Acacia tortilis* (Bekker and De Wilt, 1991). Soils are shallow sands with poor phosphorus values for most agricultural crops (De Wilt and Nachtengaele, 1996). Mean annual rainfall is 500 mm but erratically distributed (Campbell, 1990). The orchard was planted in January 1995 on rootstocks of superior phenotypes of morula selected from different areas in Botswana. The morula trees are free standing and trained in a central leader system with spacing of 12 m inter row and 6 m intra row. The orchard was under sod.

3.2. Experimental design

Single, whole tree plots were used in all trials in randomized complete block design with six replications. The treatments were 0, 50, 100 or 150 mg/litre of BA. Treatments were randomized within the block and a new randomization was made for each block.

3.3. Cultural practices

Clearing and pruning of trees was done, random tree selection was then carried out and tree trunk circumference recorded at 10 cm above graft union. Before full bloom, two limbs per tree, 10-15 cm in circumference (measured at five cm from the crotch angle), were selected and tagged, circumference recorded and blossom clusters counted. The selected limbs were from opposite sides of the tree and met the following criteria: well exposed to sunlight; free from severe or unusual pruning cuts; and bloom representative of the entire tree. Full bloom was considered to be a day when 80 % of spur bloom flowers on one year old wood are open on the north side of the tree and bees are active. Benzyladenine in the form of Maxcel^{R} (Vaalent Biosciences USA) was applied to the whole tree as dilute spray to runoff with a pressurized knapsack sprayer [Guarany Industria e Comercio Ltda, CEP 13308-200-Itu-SP-Brazil] at 0, 50, 100, or 150 mg/litre BA [Valent Biosciences USA; liquid concentrate containing 19g a.i/litre (w/w) benzyladenine]. Control trees of morula were sprayed with distilled water.

3.4. Data collection: Dependent variables determined

Fruit number, fruit growth curve, fruit diameter, fruit length, fruit length: diameter (L:D) ratio, fruit density, fruit weight, fruit endocarp and mesocarp drymatter content, leaf, fruit dry matter and water content, total titratable acidity (TTA), citric acid equivalent, vitamin C content, soluble solids content, fruit chlorophyll, anthocynin + carotenoids content, leaf chlorophyll content, shoot number per limb circumference, terminal shoot length, fruit and leaf mineral analysis were determined.

3.4.1. Determination of fruit set

After natural drop was completed (January 2009), all fruit persisting on two tagged limbs per tree were counted using a hand counter. Fruit number per cm limb circumference was calculated.

3.4.2. Determination of fruit quality

3.4.2.1. Fruit size

Fruits were sampled every week to measure the diameter and length in order to establish the growth pattern of morula fruit. At commercial harvest, 10 representative fruit from each tree were harvested, weighed and evaluated for fruit diameter (D), length (L) and L/D ratio. Fruit length was measured by laying each fruit end-to-end and side-to-side in a "V"-shaped trough and measuring total length and diameter. The L/D ratio was calculated from the readings of fruit length and diameter.

Five fruits per replicate were weighed using the laboratory scale (*ADAM* AFP 400L) to determine their mass (fruit weight at harvest) before volume determination. A 500 ml measuring cylinder was filled with distilled water, the readings were then taken. After taking the readings five fruit samples per replicate were added into the measuring cylinder, the readings taken were subtracted from the volume of water displaced to derive the fruit volume. The fruit volume was then divided by the fruit weight to determine the fruit density (g/ (cm^3).

3.4.2.2. Soluble solids content

Soluble solids content were determined using a refractometer, 10 fruits per replication were pinched using a razor blade and the juice squeezed directly into the hand refractometer (0-32 %) and then the average sugar content determined in % brix.

3.4.2.3. Titratable acidity

The fruit pulp and skin were cut and 100g of the sample weighed, then 400ml of distilled water added to the sample and the mixture blended for five minutes. The sample was homogenized and filtered with five layers of cheese cloth to extract the fruit juice. 20 ml of the filtrate was put in a 50 ml conical flask and two drops of phenolphthalein indicator added. The sample was then titrated with 0.1 N NaOH to end point, and this was done in triplicates. The results were expressed as citric acid and total titratable acidity equivalents.

$$\text{g citric acid/100ml juice} = \frac{\text{(ml base x normality base x 0.0064 x 100 x5)}}{\text{ml sample}}$$

3.4.2.4. Vitamin C content

Vitamin C content was determined using 2,6-dichloroindophenol titrimetric method according to AOAC (1996). Ascorbic acid reduces oxidation-reduction indicator dye (2,6-dichloroindophenol) to colourless solution. At end point, excess unreduced dye is rose pink in acid solution. Vitamin is extracted and titration performed in presence of metaphosphoric acid-acetic acid solution to maintain proper acidity for reaction and to avoid antioxidation of ascorbic acid at high pH (AOAC, 1996).

3.4.2.5. Fruit dry matter content

Ten representative fruit per treatment per replicate were weighed to determine fresh weight. Then they were oven-dried at 66 °C to a constant weight for 72 hours and dry weight determined (expressed in % dry matter). The fresh and dry weight was then used to determine the % fruit dry matter and water content.

$$\% \text{ Dry matter} = \frac{\text{Dry weight}}{\text{Fresh weight}} \times 100$$

$$\% \text{ Water Content} = \frac{\text{(Fresh weight- Dry weight)}}{\text{Fresh weight}} \times 100$$

After determining the fresh and dry weight the dried fruit mesocarp (pulp) was removed by scrabbing the dried fruits and the endocarp (kernel) was weighed. The weight was used to determine the % endocarp and mesocarp weight by subtracting the dry weight after scrabbing from the weight before scrabbing.

3.4.2.6. Fruit anthocyanin, carotenoids and chlorophyll content

Anthocyanin plus carotenoids and chlorophyll content of the skin was measured from two discs (11 mm diameter) per fruit (five morula fruit per replicate) cut from yellowest and greenest portion of the skin using a cork borer and scapel. The 10 discs were extracted in 4 ml of 0.1 N HCl in methanol at 23 °C in the dark for 24 hours. Absorbance of extracts was measured using a UV Visible Spectrophotometer [UV-160 1PC, Shimadzu, RSA Pty Ltd. Co REG No: 90/07641/07] at various wavelengths of 530, 620, 645, 650 and 663 nm. Anthocyanin plus carotenoids content was determined according to Creasy (1966)

and Proctor and Creasy (1971), using a molar extinction coefficient of 4.62×10^4 (Zapsalis and Francis, 1965).

Anthocyanin plus carotenoids (nmole/ cm^2 morula skin) = $\frac{[(A_{530}-A_{620}) - 0.1(A_{650}-A_{620})]}{(4.62 \times 10^4)}$

Skin chlorophyll content was measured as absorbance at 653 nm (Holden, 1965; Douglas, 1983). The following equation was used to calculate the relative total chlorophyll content (Douglas, 1983).

Chlorophyll (mg/cm^2 of morula skin) = $24.88 \times A_{653}$.

3.4.3. Vegetative growth determination:

3.4.3.1. Leaf area:

Five leaves (rachi) from each treatment per replicate were collected to determine the average leaf area (cm^2) using the Delta T Scan (Delta T Devices: Splash Cover) leaf area meter.

3.4.3.2. Total leaf chlorophyll, chlorophyll a and chlorophyll b

Ten leaves per tree of the same age (leaf five and six) were collected and one disc was cut from each leaf using 11 mm cork borer. The 10 discs were extracted in 4 ml of 0.1 N HCl in methanol at 25 °C in the dark for 24 hours. Absorbance of extracts was measured using a UV Visible Spectrophotometer (UV-160 1PC) at various wavelengths of 530, 620, 645, 653 and 663 nm. The following formulae were used to estimate chlorophyll content:

Chlorophyll a (mg/cm^2) = $12.7A_{663} - 2.069A_{645}$

Chlorophyll b (mg/cm^2) = $22.9\ A_{645} - 4.68A_{663}$

Total Chlorophyll (mg/cm^2) = $24.88A_{653}$

Where, A is the absorbance at 645, 653 and 663 nm, respectively and the coefficients are the molar extinction coefficients at the respective wavelengths at 25 °C.

3.4.3.3. Terminal shoot length and number

The numbers and length of terminal shoots on marked limbs per tree was determined in May 2009. The length was measured using a five meter tape while the number of shoots was counted using a hand counter on the two tagged limbs. The terminal shoot length was expressed in cm while the shoot number was expressed as number per cm limb circumference.

3.4.4. Mineral analysis

Midshoot leaves (leaf five and six) from current season growth were collected in the first week of January 2009. The samples were oven-dried at 66 °C to constant weight (72 hours). Seven days before commercial harvest time, 10 representative fruits per tree were also harvested for mineral determination. Fruit epidermis and mesocarp were cut finely from 10 samples per tree and the mixture oven dried at 66 °C to constant weight for three days. The dried samples were ground using a sieve of size two and 1.25 g composite sample digested in 20ml sulphuric acid (98 %) and 4 ml hydrogen peroxide (30 %) in a BD block at 330 °C for 7hrs.

Nitrogen (N) was determined through distillation and titration using the micro kjeldahl method (AOAC, 1996). Phosphorus (P) was determined calorimetrically using sodium phenol and ammonium molybdate plus ascorbic acid method (AOAC, 1996). The

absorbance was read on the UV Visible Spectrophotometer (Model of spectrophotometer). Calcium (Ca), magnesium (mg) and potassium (K) were determined by atomic absorption spectrophotometry (Varian SpectrAA 300). Data was expressed as total mineral content in mg/g on dry weight basis.

3.5. Statistical analysis

Analysis of variance was performed on the data collected using general linear model (PROC GLM) procedure of Statistical Analysis System (SAS 2009, Carey, NC) program package. Appropriate regression models were used to examine the response of morula fruits to increasing benzyladenine concentration. Multiple comparisons among means was done using Protected Least Significant Difference (LSD) at $p = 0.05$. Proc univariate procedure was carried out on residuals to support assumptions of normality made.

CHAPTER 4

RESULTS

4.1. Fruit set

Application of benzyladenine significantly ($p < 0.0001$) reduced fruit set of morula compared to the control trees (Figure 1). The response of morula fruit set to increasing BA concentration was quadratic (Figure 1). The reduction in fruit set by benzyladenine ranged between 48-67 %. However, there was no significant difference between 50, 100 and 150 mg/L BA in their ability to reduce fruit set of morula trees (Figure 1).

4.2. Fruit Size

The morula fruit growth curve in terms of fruit diameter and length are shown in Figures 2 and 3. Both Figures 2 and 3 showed that morula fruit has got a simple sigmoid growth pattern which is characterized by two phases of growth. Phase I is characterized by cell division and phase II is characterized by cell enlargement. In the current results, phase I commenced after pollination to 65 days after full bloom (DAFB) (Figure 2 and 3). The period between 65 DAFB to until 86 DAFB (four weeks) was characterized by slow fruit growth (length and diameter), this was the period of endocarp growth (Figure 2 and 3). From 86 DAFB until 128 DAFB was the last stage of morula fruit growth and development (Figure 2 and 3) which was characterized mainly by cell elongation and enlargement. Benzyladenine application significantly increased morula fruit size [length ($p < 0.01$), diameter ($p < 0.001$), L:D ratio ($p < 0.01$), weight ($p < 0.001$) and density ($p < 0.05$)] (Figure 4,5,6,7,8).

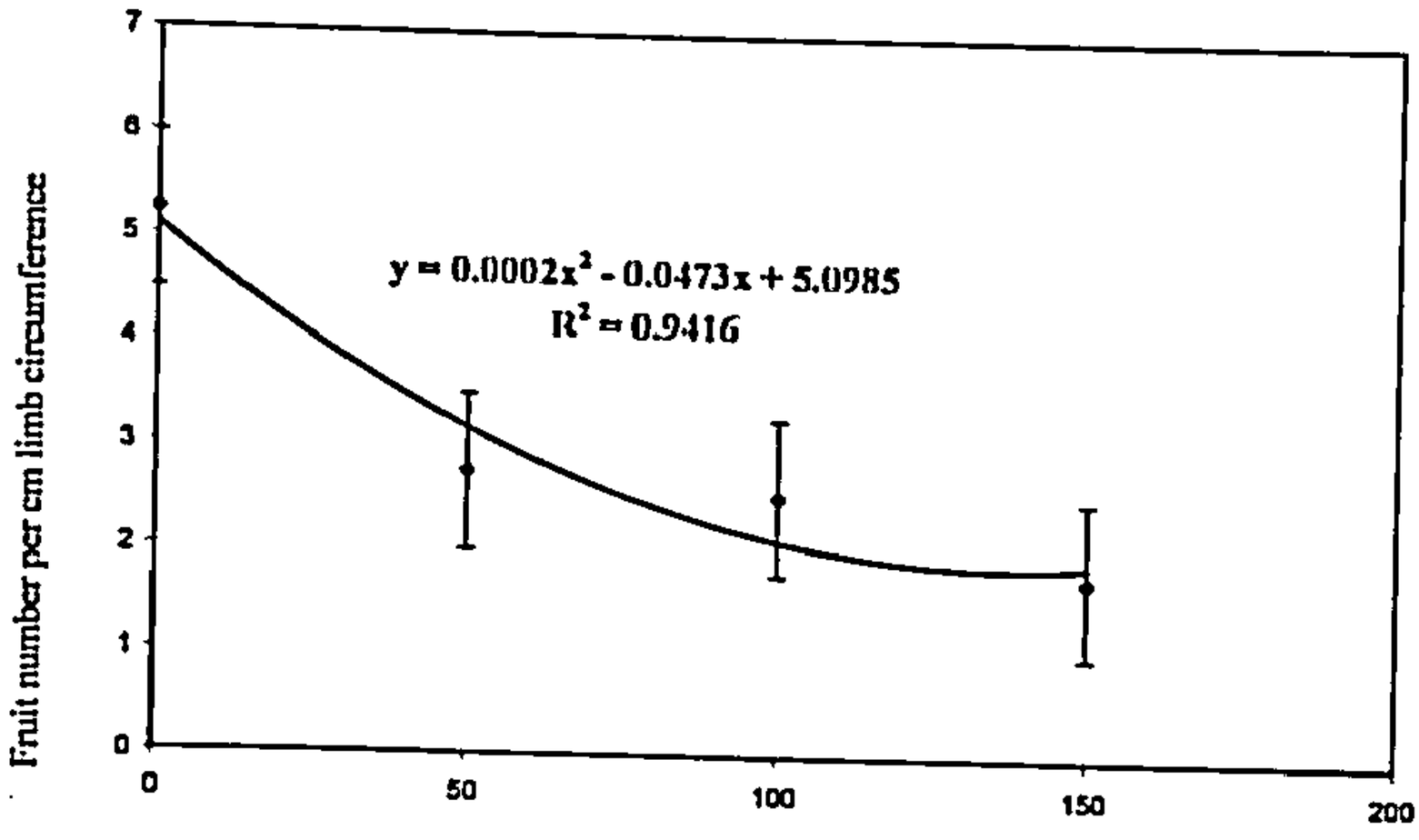

Figure 1: Effect of benzyladenine on fruit set of morula; I represent standard error bars.

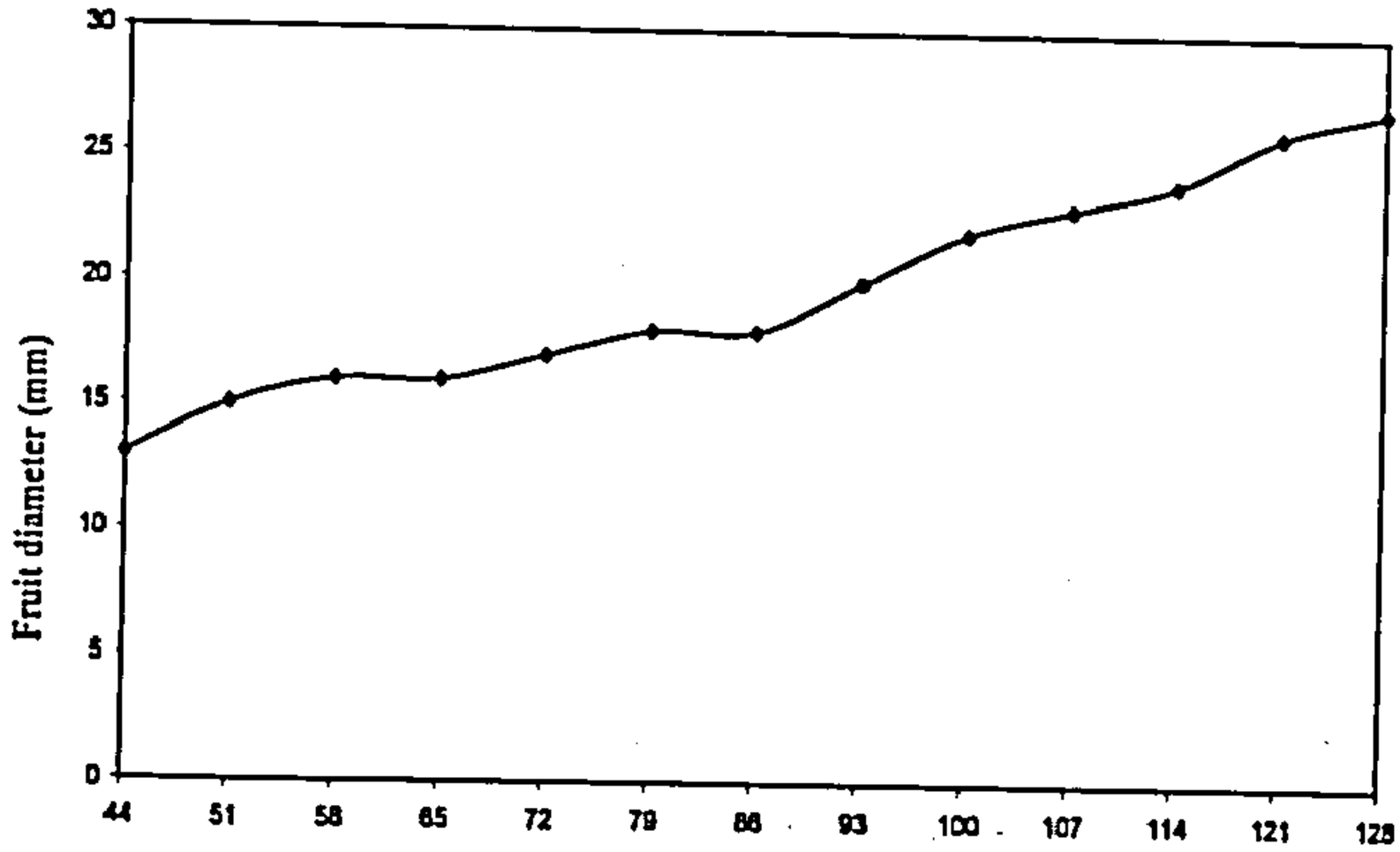

Figure 2: Morula fruit diameter growth over time

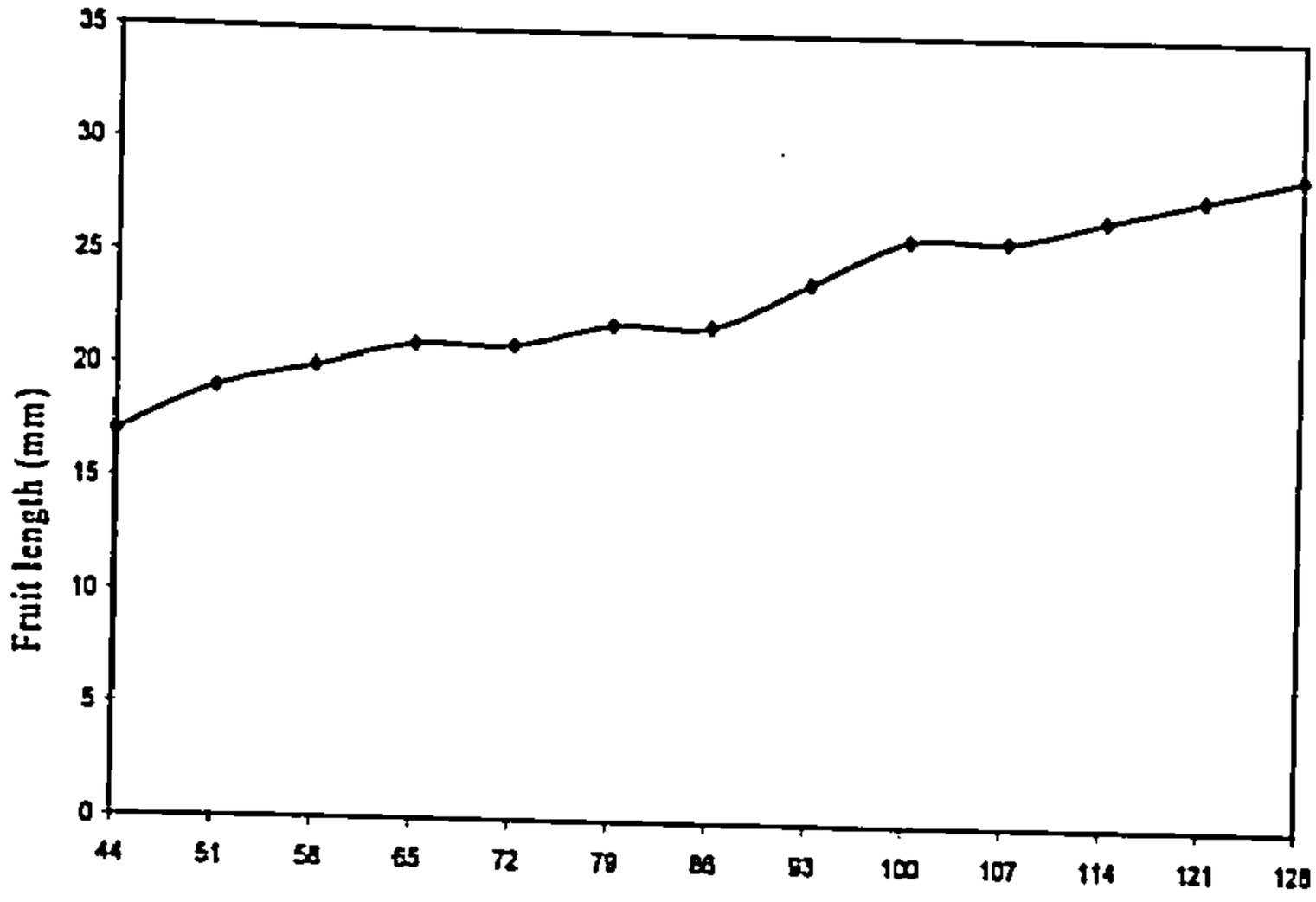

Figure 3: Morula fruit length growth over time

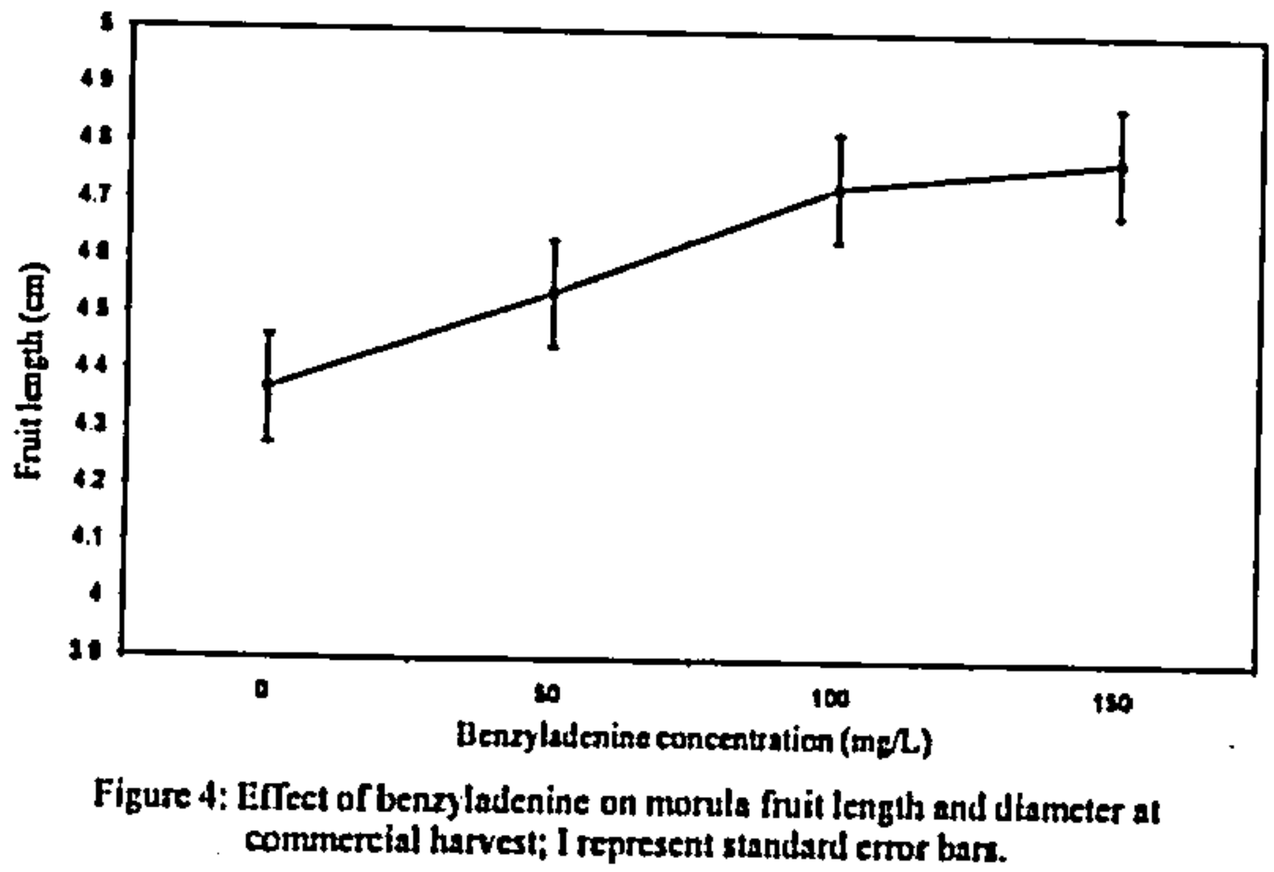

Figure 4: Effect of benzyladenine on morula fruit length and diameter at commercial harvest; I represent standard error bars.

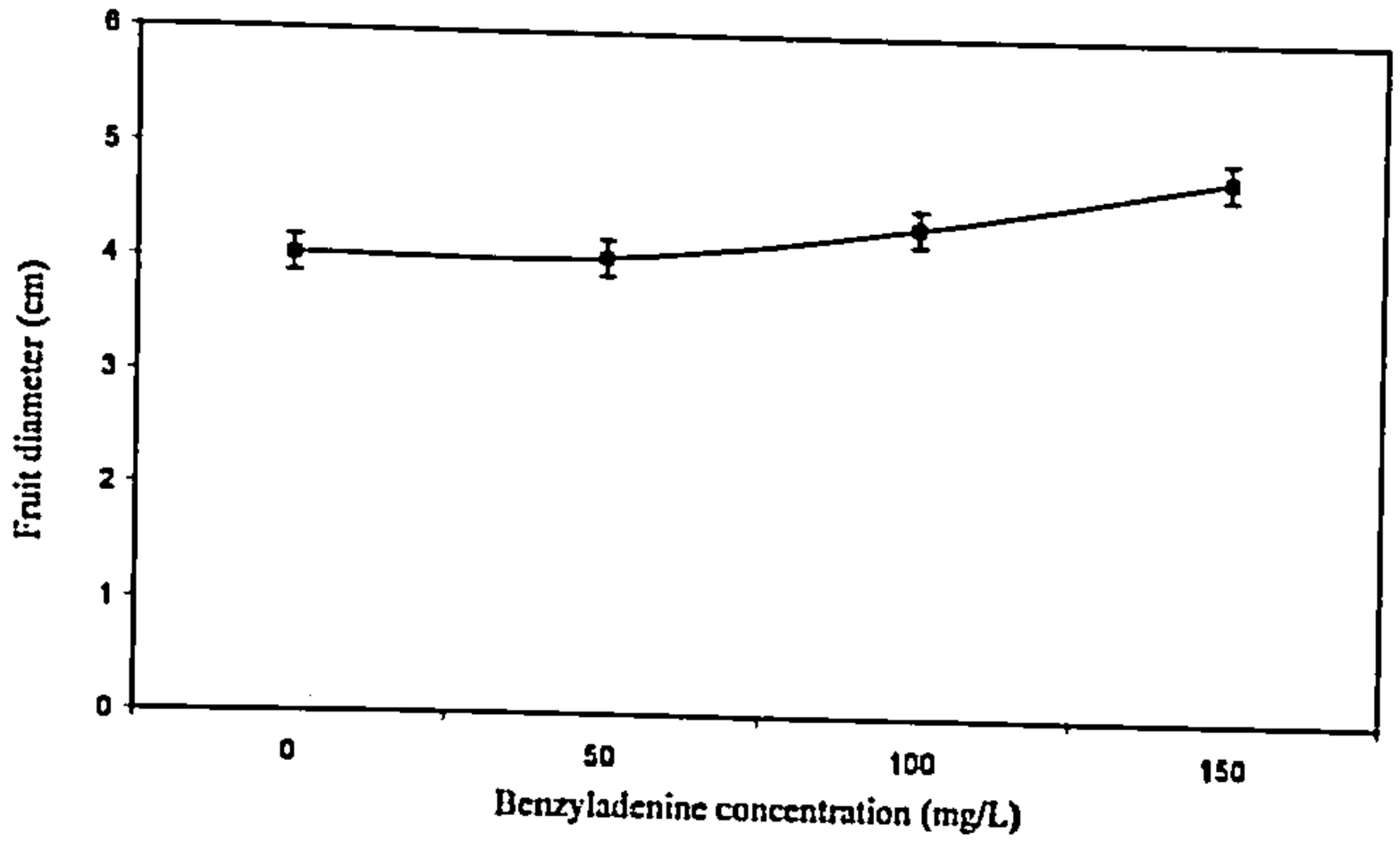

Figure 5: Effect of benzyladenine on morula fruit diameter at commercial harvest; I represent standard error bars.

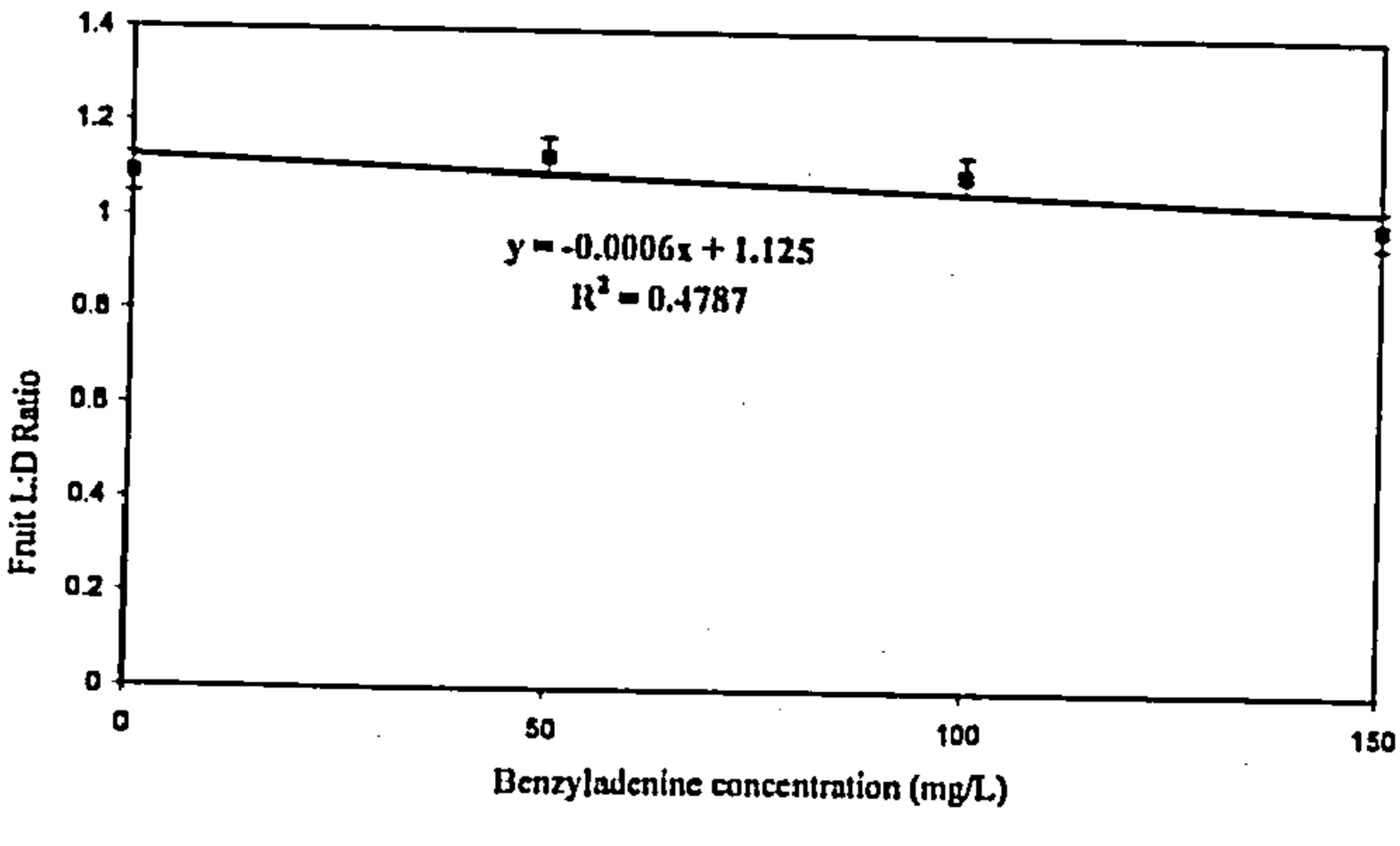

Figure 6: Effect of benzyladenine on morula fruit length: diameter (L:D) ratio; I represent standard error bars.

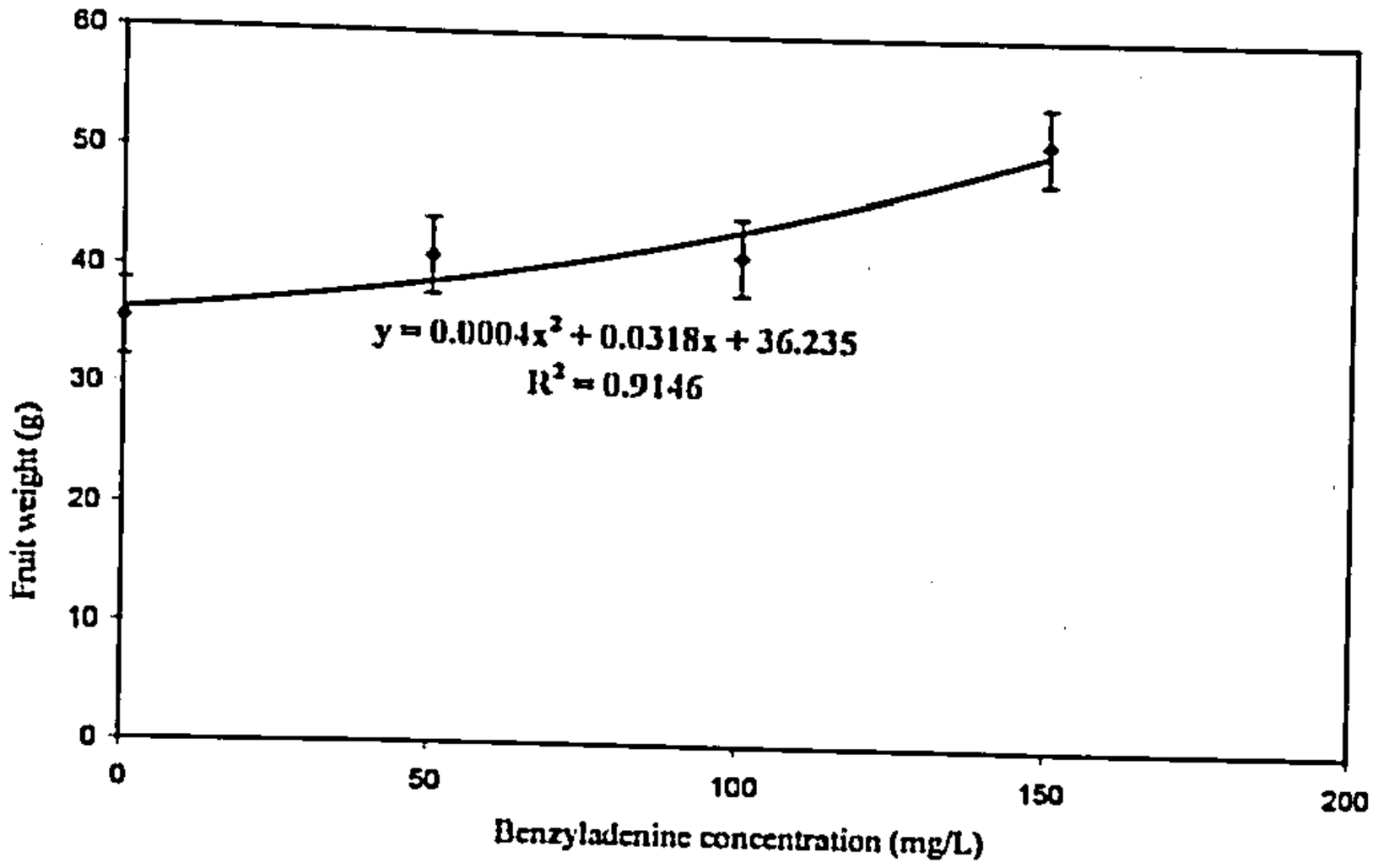

Figure 7: Effect of benzyladenine on morula fruit weight at commercial harvest; I represent standard error bars.

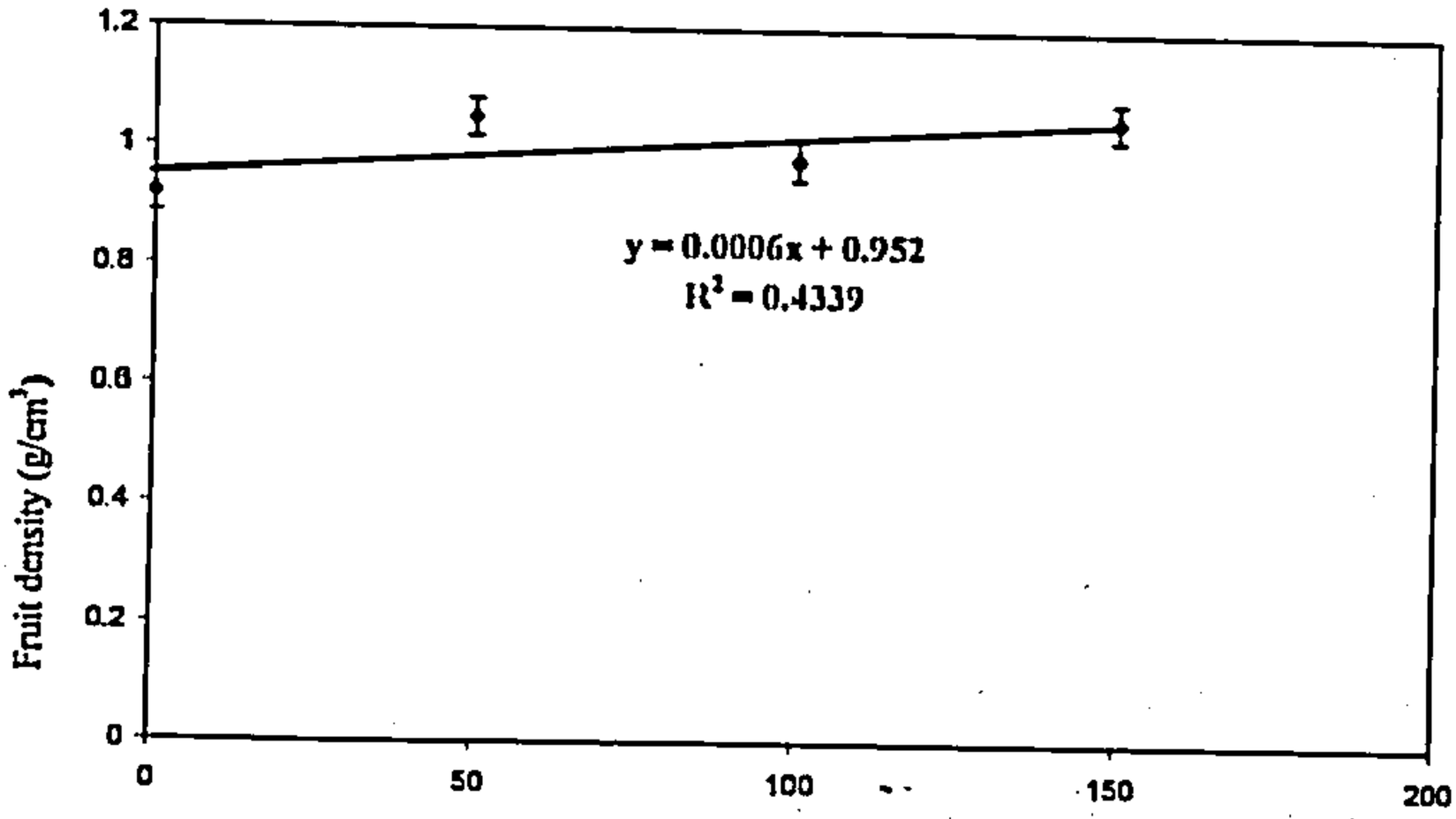

Figure 8: Effect of benzyladenine on the morula fruit density at commercial harvest; I represent standard error bars.

The maximum BA-induced fruit length and diameter was 9% and 16%, respectively, which occurred on morula trees sprayed with 150 mg/L BA (Figure 4 and 5). Morula trees sprayed with 50, 100 or 150 mg/L BA significantly ($p < 0.001$) increased fruit weight compared to control trees (Figure 7). Benzyladenine application increased fruit weight by between 14-31 % compared to the control fruits (Figure 7). The response of morula fruit weight to increasing BA concentration was quadratic (Figure 7). There was no significant difference in fruit weight from morula control trees and those sprayed with 50 and between 50 or 100 mg/L BA (Figure 7). Morula fruit density was significantly ($p < 0.05$) increased by BA application compared to control fruit (Figure 8). However, there was no significant difference among the BA concentrations in their ability to increase fruit density (Figure 8). The BA-induced increase in density ranged between 6-12 % compared to control fruit (Figure 8). Benzyladenine application at 50, 100 and 150 mg/L significantly ($p < 0.01$) increased the endocarp weight of morula fruit (Figure 9). The increase in the endocarp weight increased with increasing BA concentration, while the mesocarp (pulp) weight significantly ($p < 0.01$) decreased with the increasing BA concentration (Figure 9). Benzyladenine decreased mesocarp weight by 1.1- 6.3 % compared to control fruit. The BA-induced increase in fruit weight was positively related to the endocarp weight increase induced by BA at the expense of the mesocarp (Figure 9). Figure 10 showed the dry matter and water content of leaves and fruits as affected by BA application. There was no significant effect of BA appplication to both fruit and leaf dry matter contents. Similarly BA did not have any significant effect on both the fruit and leaf water contents (Figure 10).

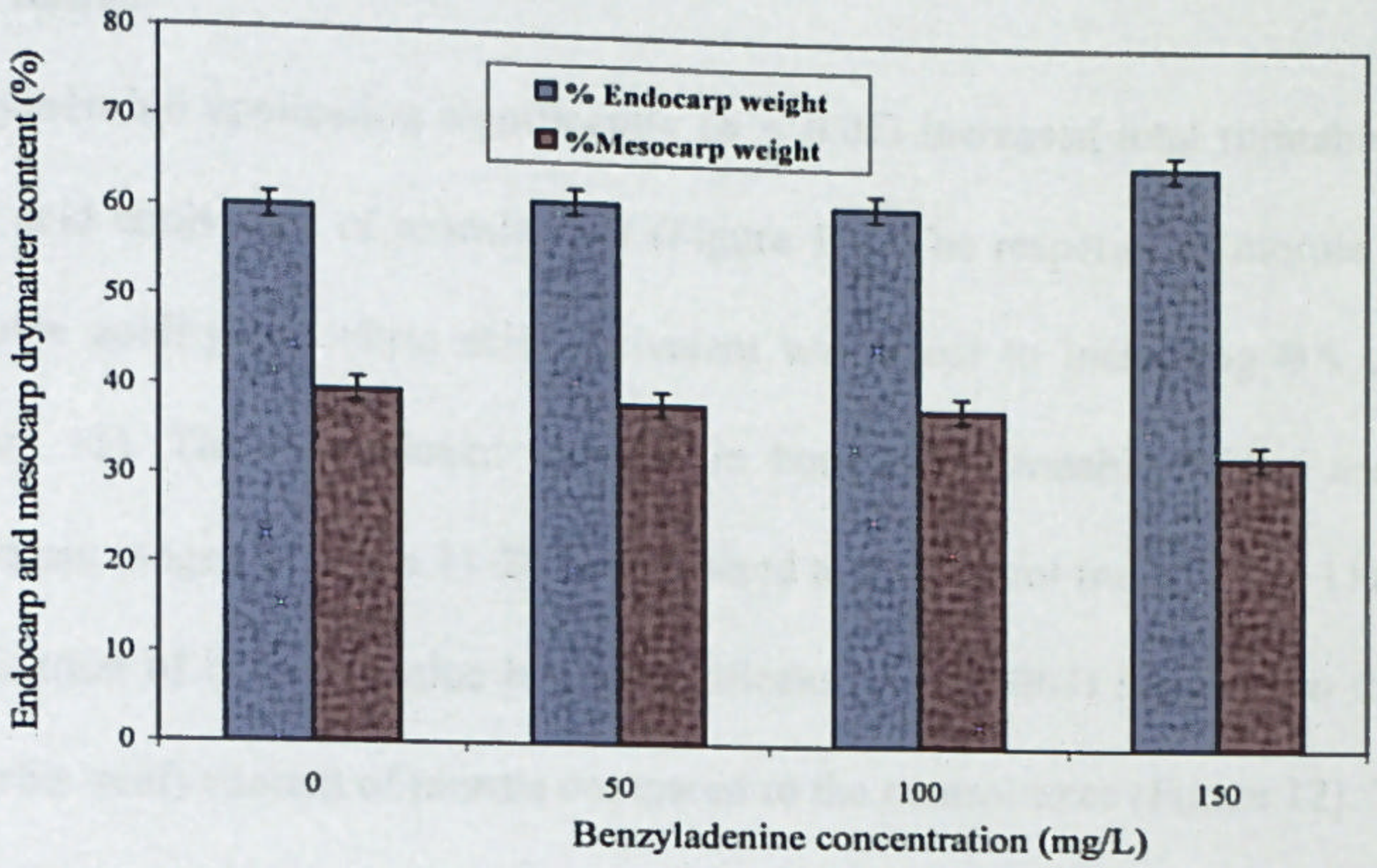

Figure 9: Effect of benzyladenine on the fruit endocarp and mesocarp weight; I represent standard error bars.

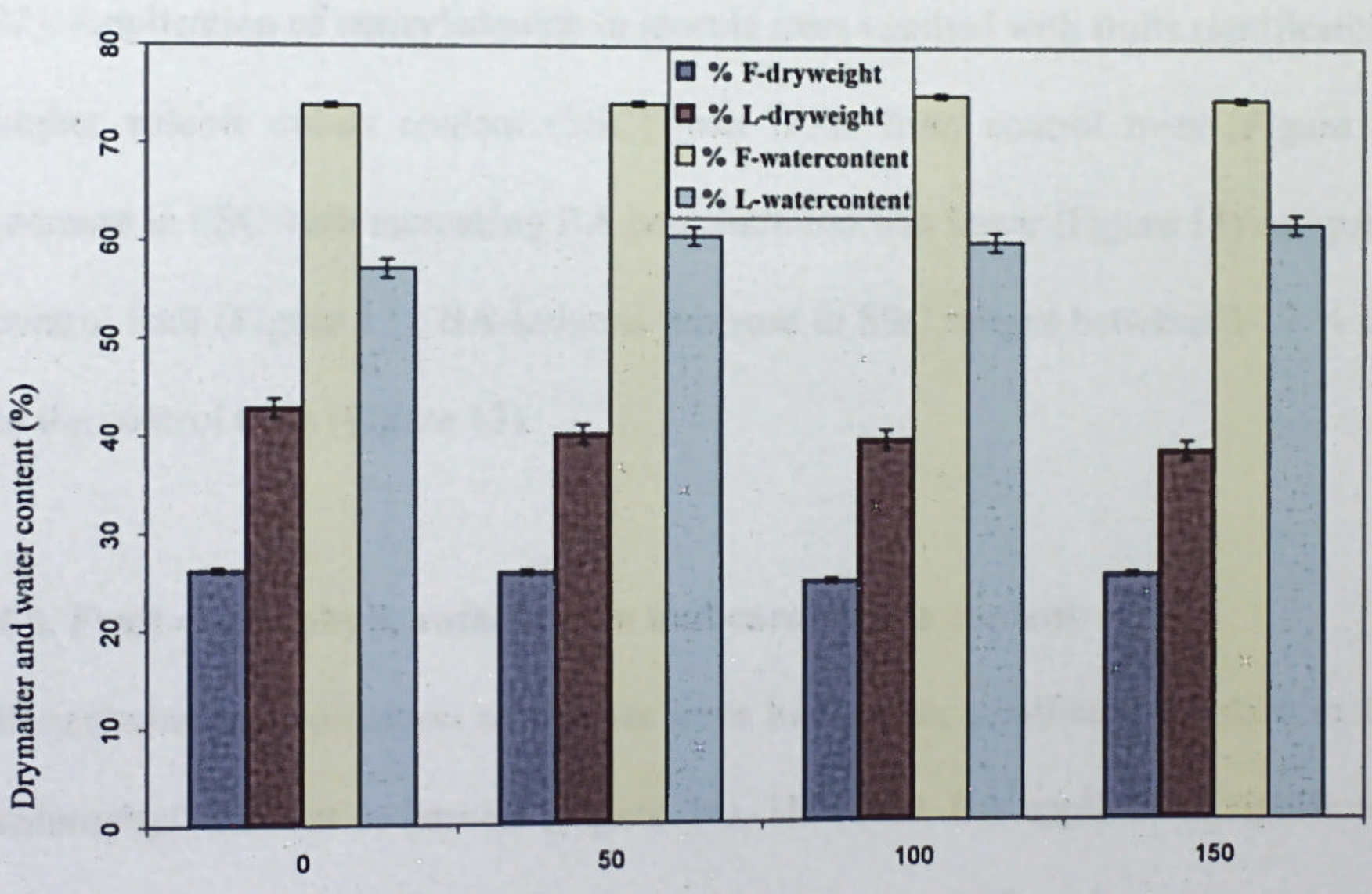

Figure 10: Effect of benzyladenine on the fruit (F), leaf (L) dry matter and water content; I represent standard error bars.

4.3. Fruit titratable acidity, citric acid equivalent, vitamin C content and soluble solid content

Benzyladenine application significantly ($p < 0.05$) increased total titratable acidity and citric acid equivalent of morula fruit (Figure 11). The response of morula fruit to total titratable acidity and citric acid equivalent was linear to increasing BA concentration (Figure 11). The BA-induced increase in both total titratable acidity and citric acid equivalent ranged between 11-20 % compared to the control fruit (Figure 11).

Application of benzyladenine had a significant ($p < 0.0001$) increase on the vitamin C (ascorbic acid) content of morula compared to the control trees (Figure 12). The response of fruit vitamin C content was linear with increasing BA concentration (Figure 12). The increase in the morula vitamin C content induced by BA ranged between 16-18 % (Figure 12). Application of benzyladenine to morula trees resulted with fruits significantly having higher soluble solids content (SSC) than fruits from control trees (Figure 13). The increase in SSC with increasing BA concentration was linear (Figure 13) compared to the control fruit (Figure 13). BA-induced increase in SSC ranged between 3-12 % compared to the control trees (Figure 13).

4.4. Fruit chlorophyll, anthocyanin and carotenoids content

Benzyladenine application to morula trees had a non significant increase in fruit total chlorophyll content at harvest (Figure 14). However, BA application significantly ($p < 0.05$) increased fruit colour (anthocyanin + carotenoids) at harvest compared to the control fruit (Figure 15). The increase in fruit colour (anthocynin + carotenoids) increased linearly with increasing BA concentration (Figure 15).

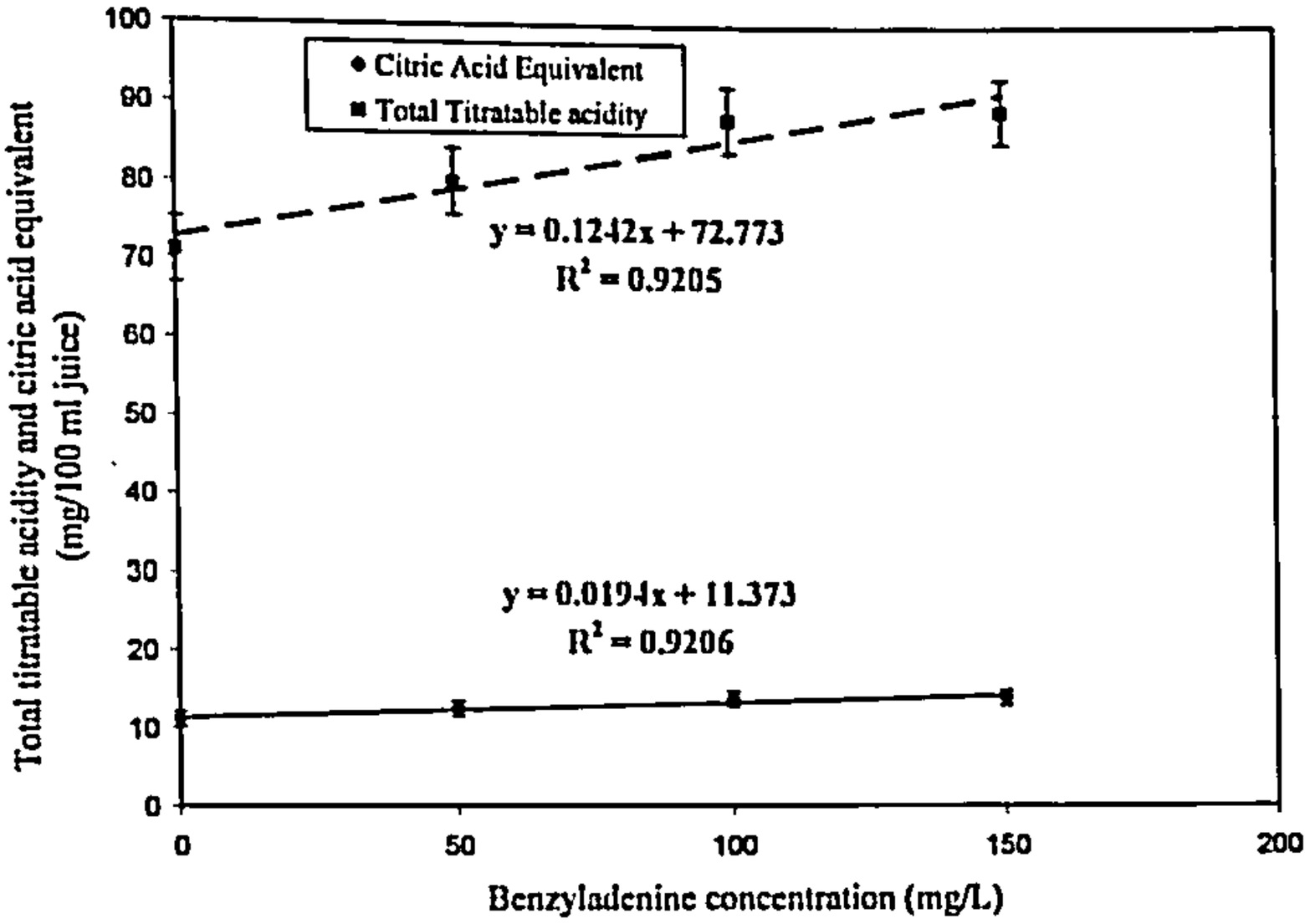

Figure 11: Effect of benzyladenine on the fruit titratable acidity and citric acid equivalent; I represent standard error bars.

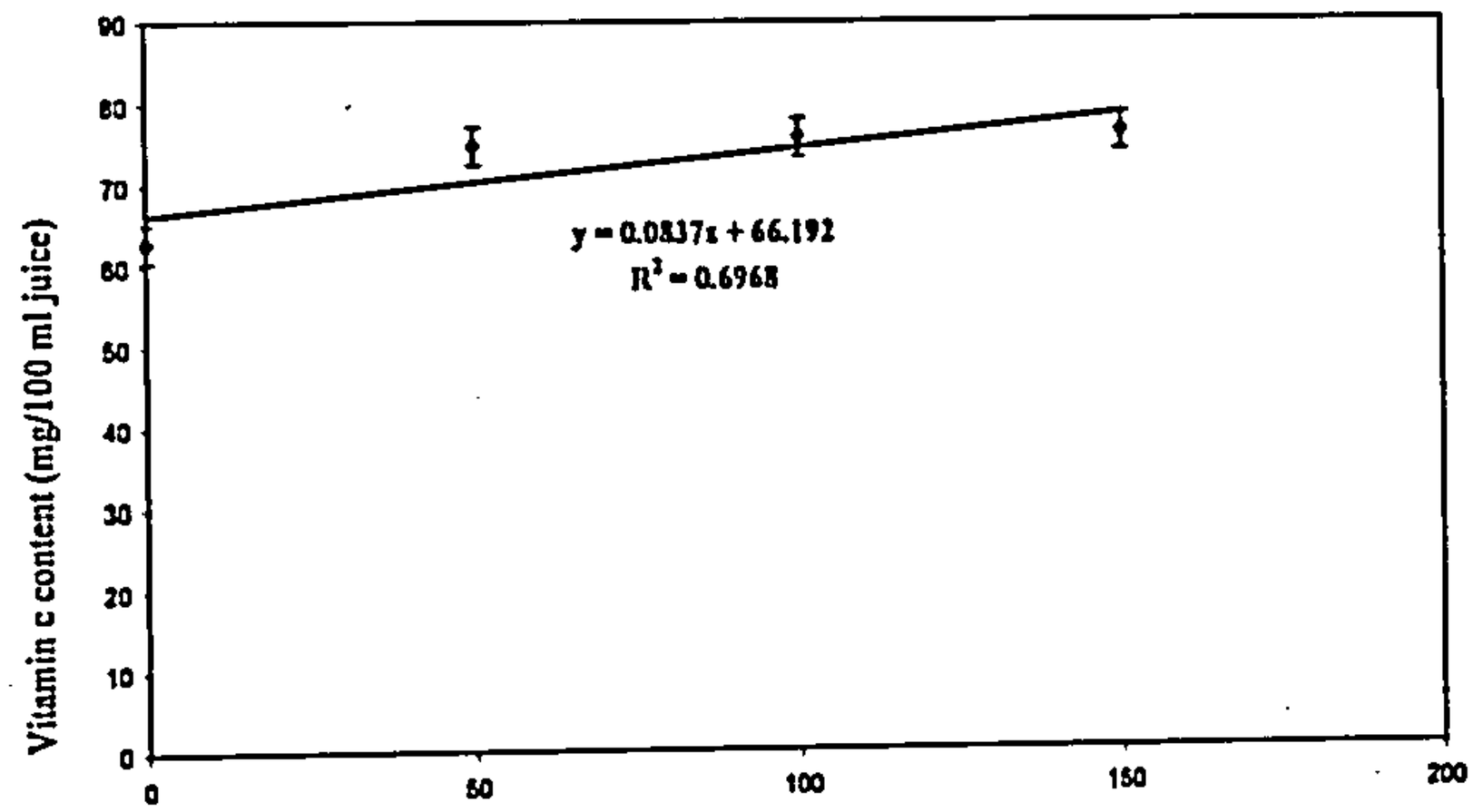

Figure 12: Effect of benzyladenine on the fruit vitamin C content at commercial harvest; I represent standard error bars.

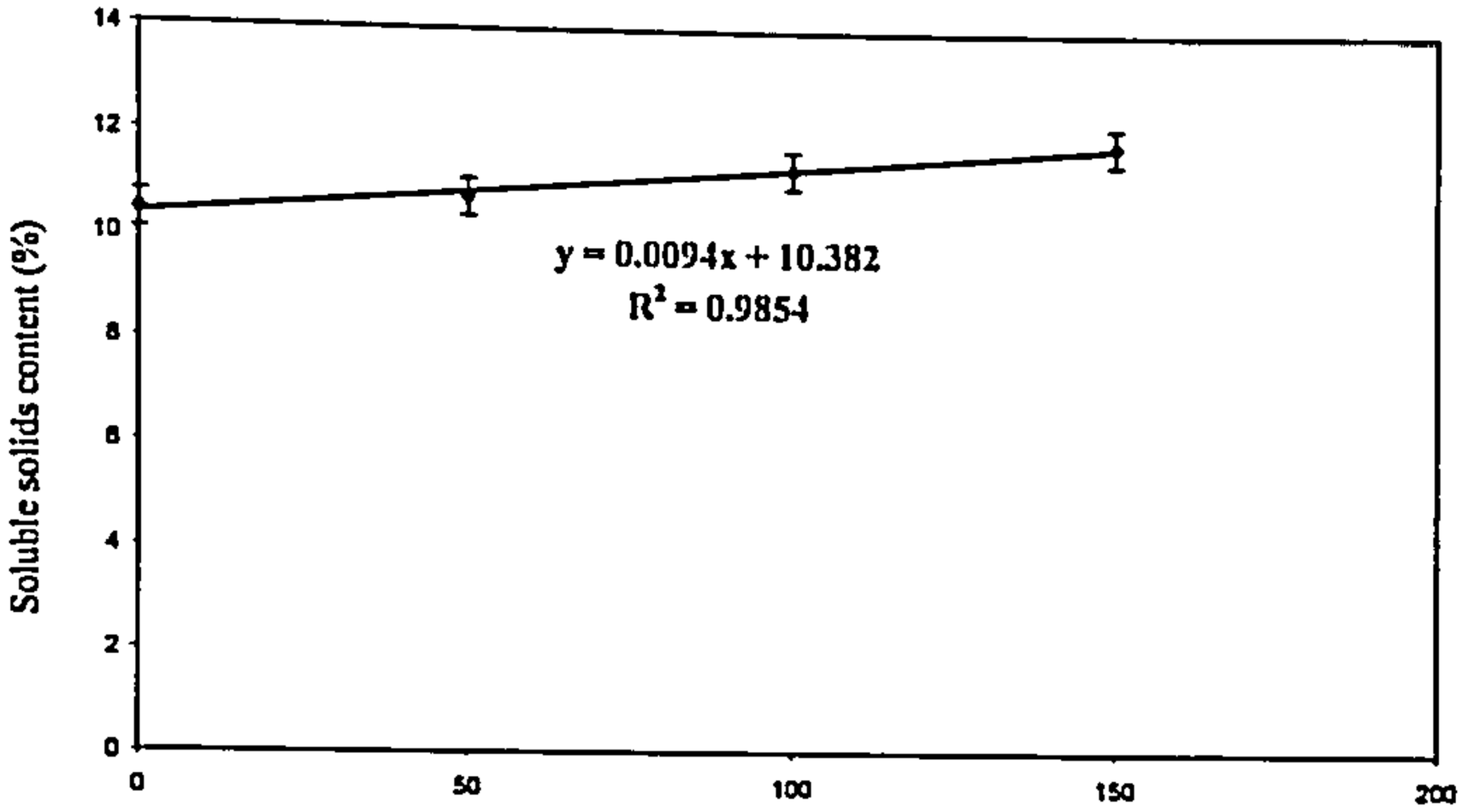

Figure 13: Effect of benzyladenine on the fruit soluble solids content; I represent standard error bars.

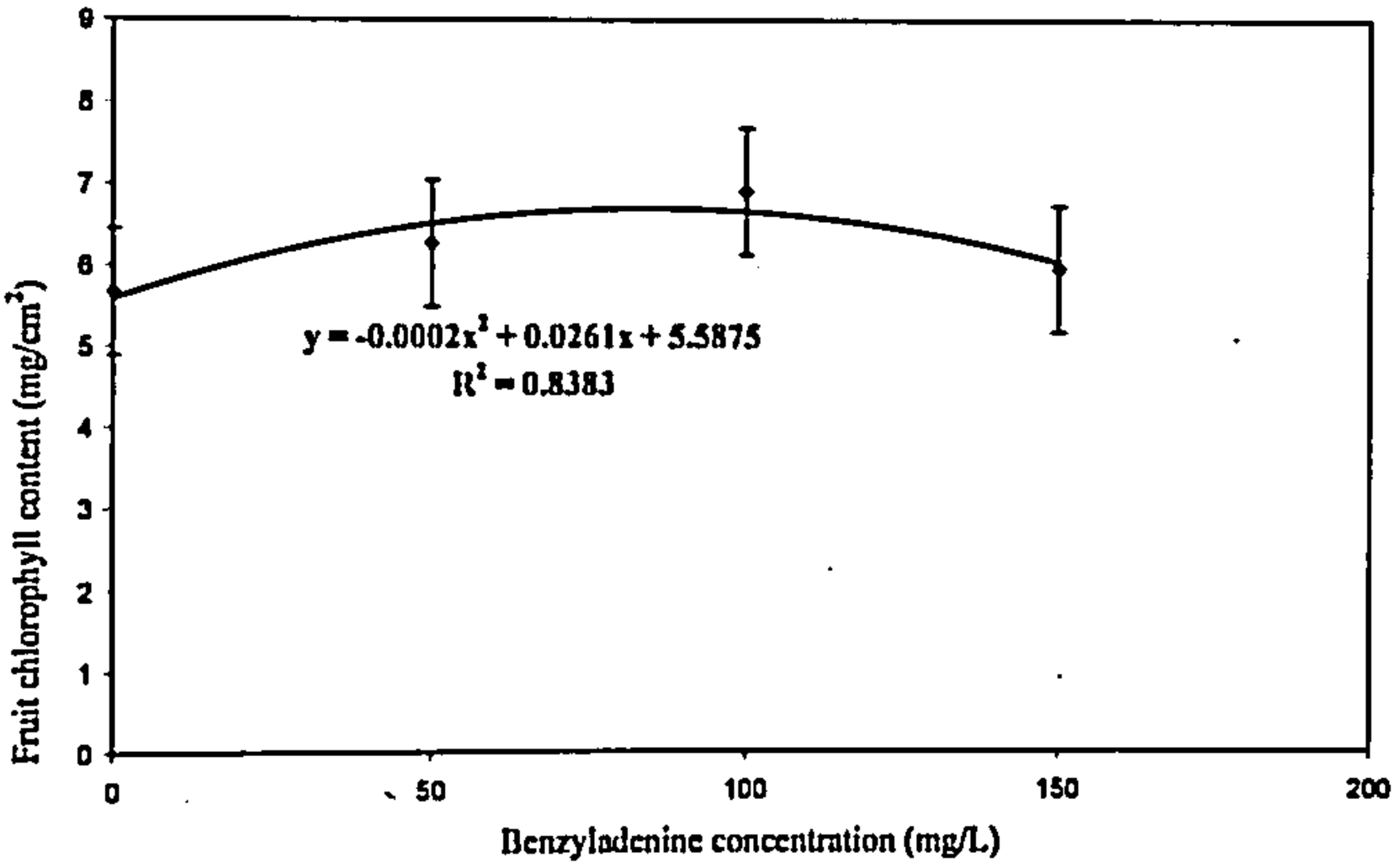

Figure 14: Effect of benzyladenine on the fruit chlorophyll content at harvest; I represent standard error bars.

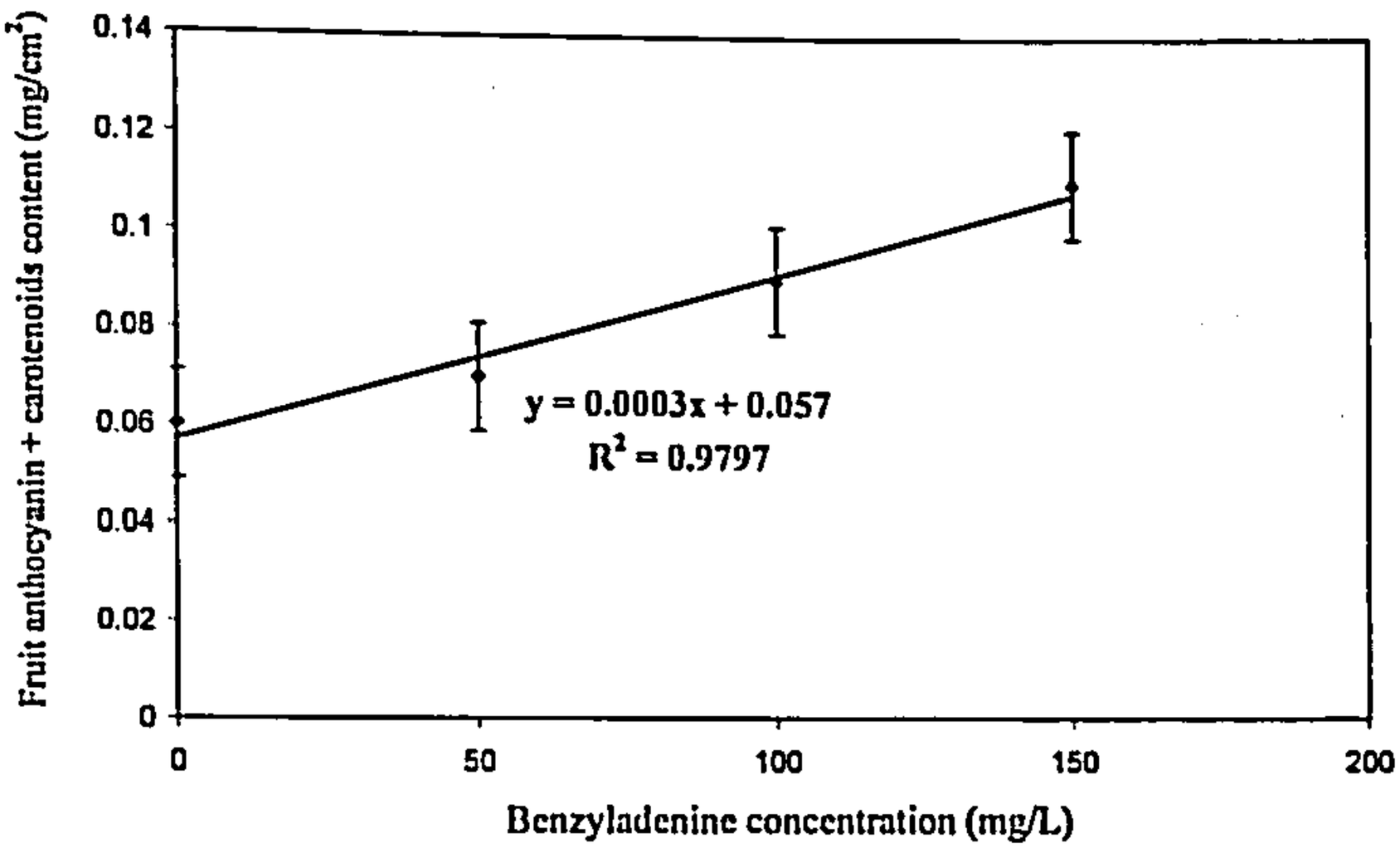

Figure 15: Effect of benzyladenine on the fruit anthocyanin and carotenoids content at harvest; I represent standard error bars.

4.5. Leaf chlorophyll content

Application of BA to morula trees significantly ($p < 0.01$) increased leaf total chlorophyll, chlorophyll a and chlorophyll b contents compared to leaves of control trees (Figure 16, 17). The total leaf chlorophyll content showed a quadratic response to the increasing BA concentration (Figure 16). The BA-induced increase in total leaf chlorophyll content ranged from 5-23 % compared to the control morula trees (Figure 16). Benzyladenine induced increase in both chlorophyll a and chlorophyll b content ranged between 9-32 % and 10-35 % respectively (Figure 17). The response of morula leaf chlorophyll a and chlorophyll b content was quadratic to the increasing BA concentration (Figure 17).

4.6. Leaf area

Application of benzyladenine at 50, 100 or 150 mg/l significantly ($p < 0.0001$) increased the leaf area of morula trees compared to control trees (Figure 18). The increase in morula leaf area was quadratic with increasing BA concentration (Figure 18). However, there were no significant differences in leaf area of morula trees sprayed with either control or 50 mg/L BA (Figure 18).

4.7. Vegetative growth

Application of benzyladenine at 50, 100 and 150 significantly ($p < 0.05$) increased the terminal shoot length compared to the control morula trees (Figure 19). The response of morula terminal shoot length was linear with increasing BA concentration (Figure 19).

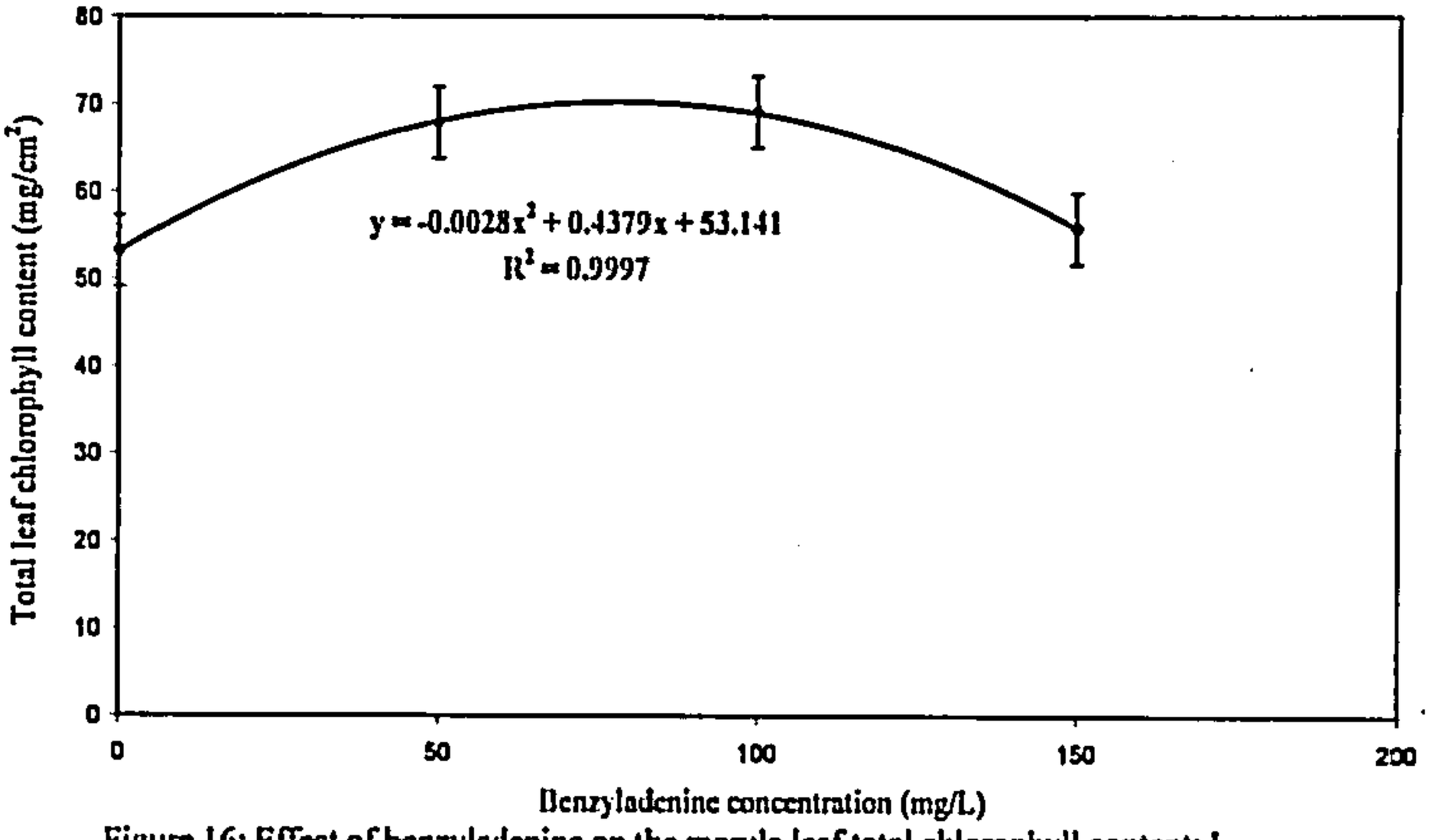

Figure 16: Effect of benzyladenine on the morula leaf total chlorophyll content; I represent standard error bars.

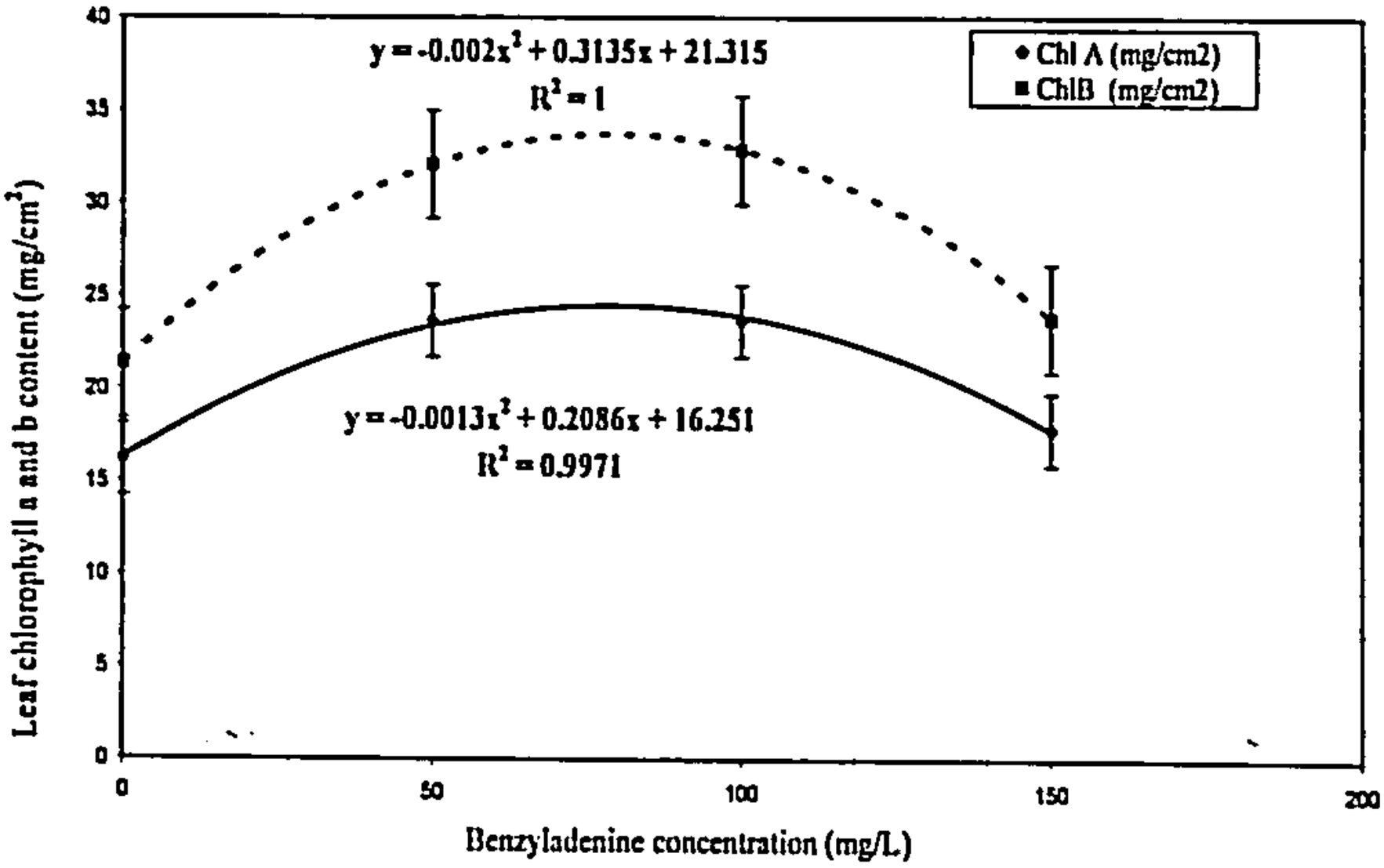

Figure 17: Effect of benzyladenine on the morula leaf chlorophyll a and chlorophyll b content; I represent standard error bars

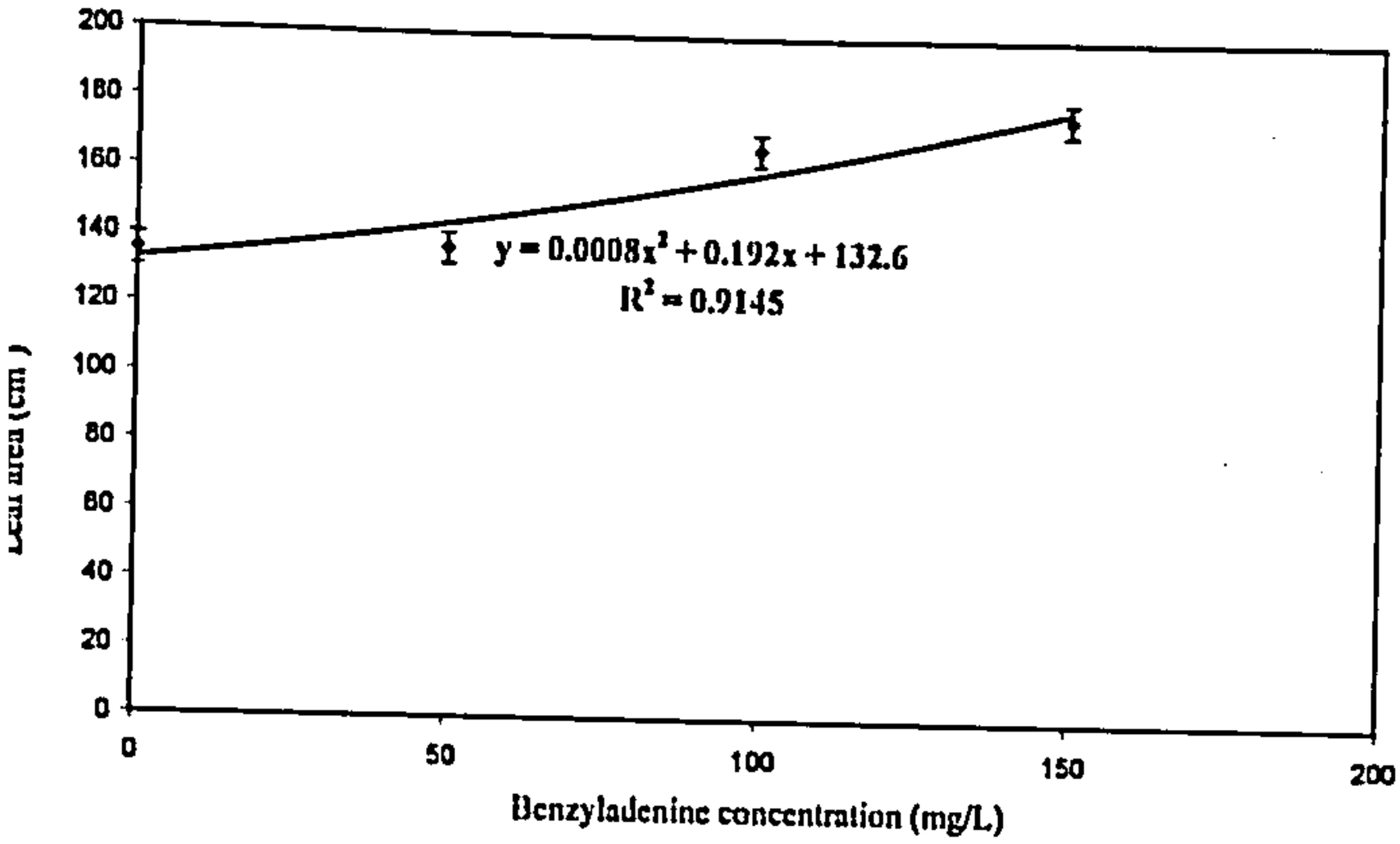

Figure 18: Effect of benzyladenine on the morula leaf area; I represent standard error bars.

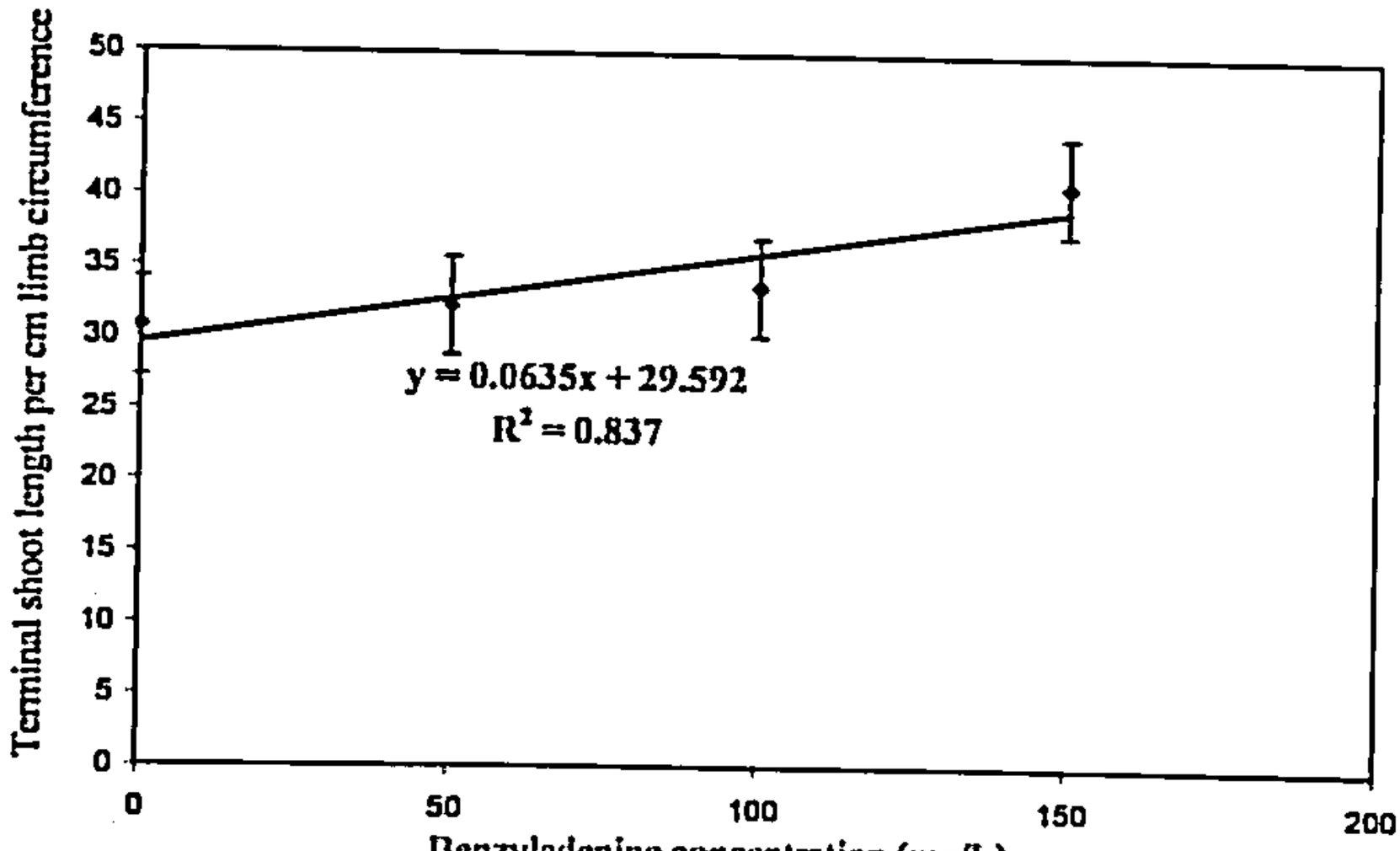

Figure 19: Effect of benzyladenine on the terminal shoot length of morula; I represent standard error bars.

The increase in the morula terminal shoot length induced by BA ranged between 22-24 % (Figure 19). Application of benzyladenine at 50, 100 and 150 significantly ($p < 0.0001$) increased the shoot number of morula compared to the control trees (Figure 20). The response of morula trees to BA application with respect to shoot number per limb circumference was linear with the increasing BA concentration (Figure 20). The BA–induced increase in the shoot number per limb circumference between 33-50 % compared to the control (Figure 20).

4.8. Interaction between vegetative growth and fruit set

The relationship between shoot number and fruit set (Figure 21), and between terminal shoot length and fruit set (Figure 22) were both negatively correlated. Increase in morula fruit set significantly ($p < 0.01$) reduced shoot number per cm limb circumference (Figure 21). The increase in fruit set also reduced the terminal shoot length of morula trees (Figure 22).

4.9. Fruit and leaf mineral content

Application of benzyladenine at 50, 100 or 150 mg/L to morula trees significantly increased the morula fruit mineral content compared to the control trees (Table 1). The fruit calcium (Ca) ($p < 0.05$), magnesium (Mg) ($p < 0.05$), nitrogen (N) ($p < 0.01$) and potassium (K) ($p < 0.001$) were significantly increased with increasing BA concentration reaching the highest mineral content at 150 mg/l BA except for potassium (Table 1). Differences in fruit Na, Ca, Mg and K content from trees sprayed with either 100 or 150 mg/L BA were not significant (Table 1). With respect to fruit P, Na, Ca, Mg and N

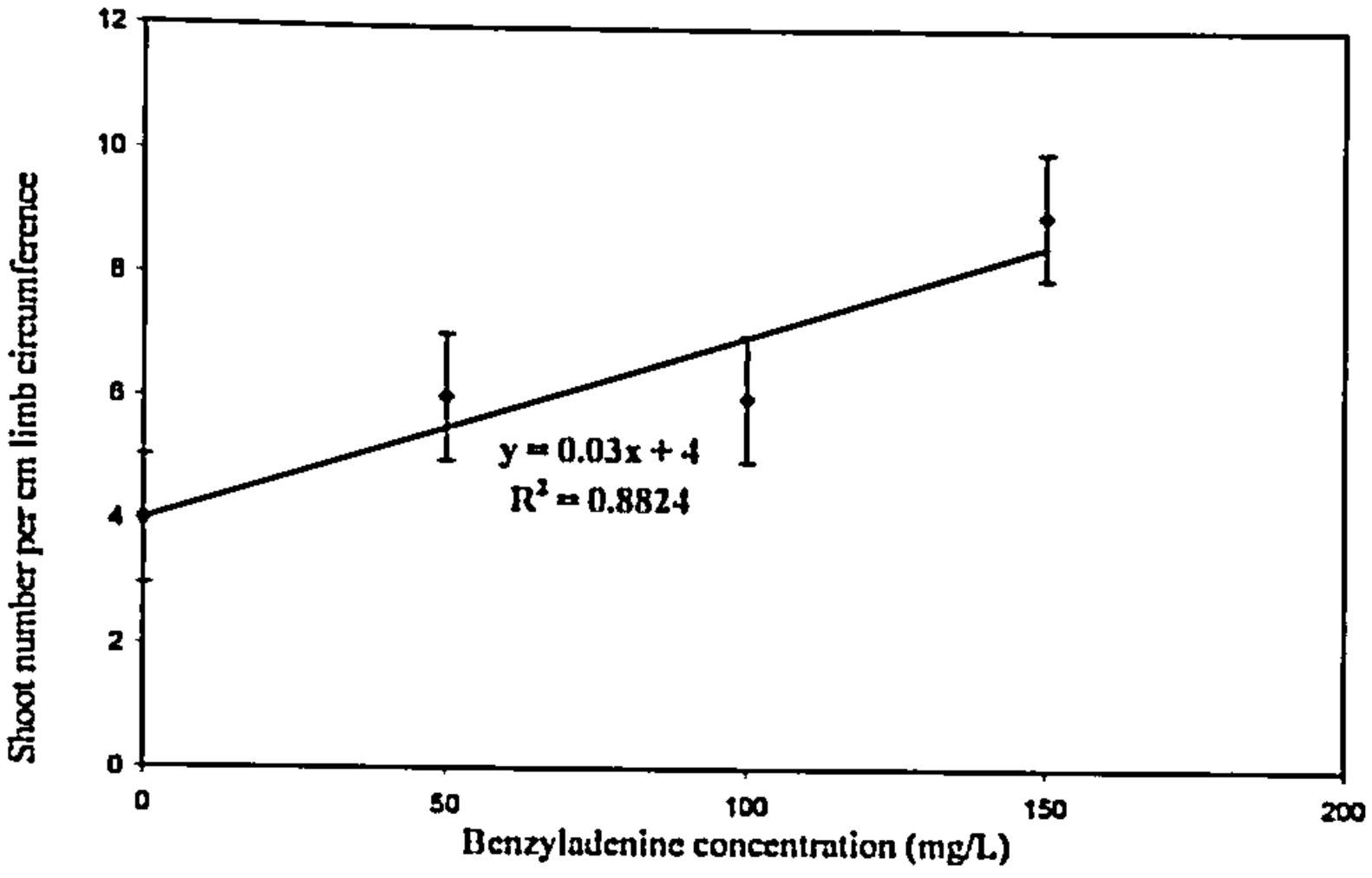

Figure 20: Effect of benzyladenine on the terminal shoot number of morula; I represent standard error bars.

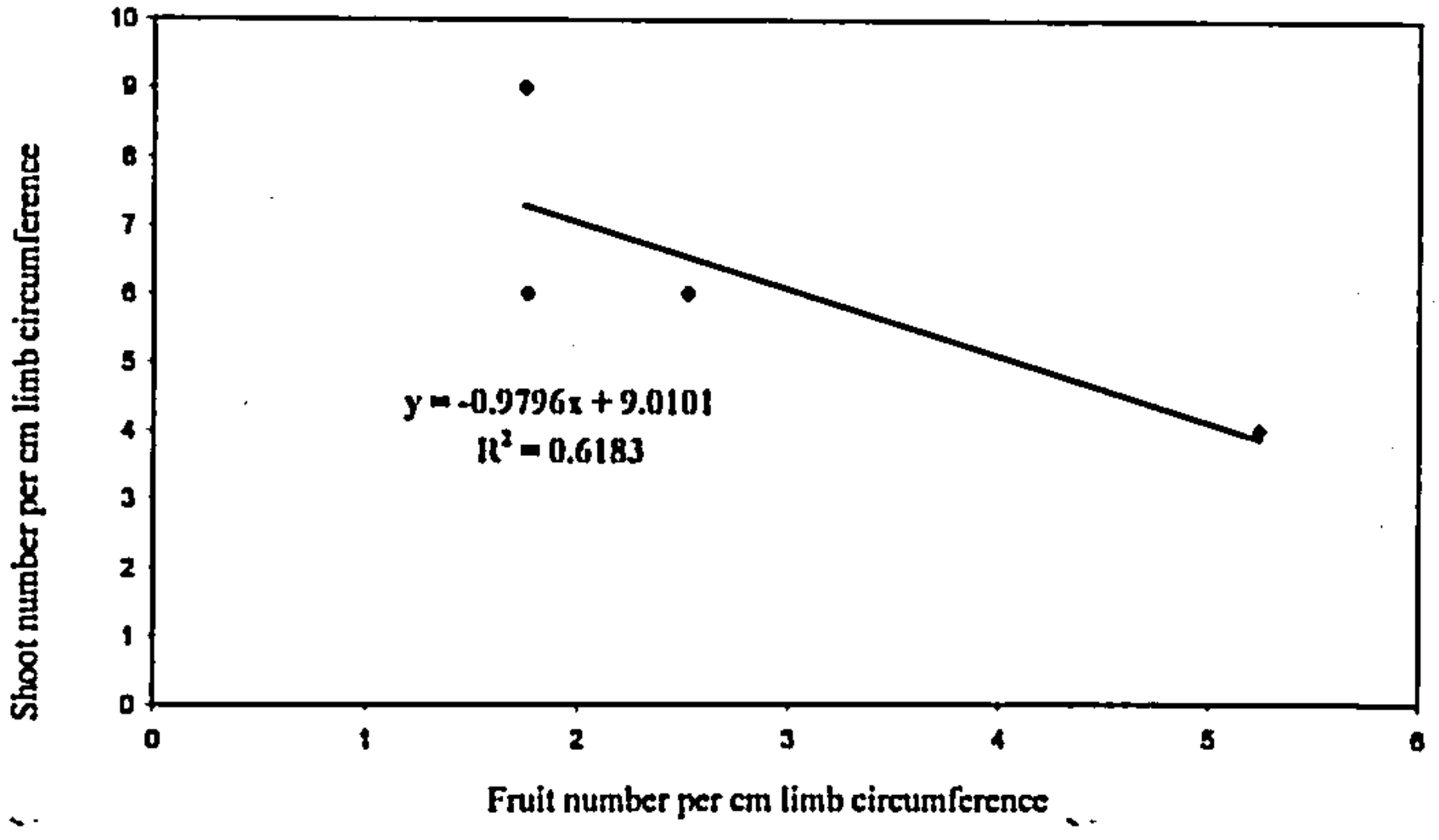

Figure 21: Relationship between morula shoot number and fruit set

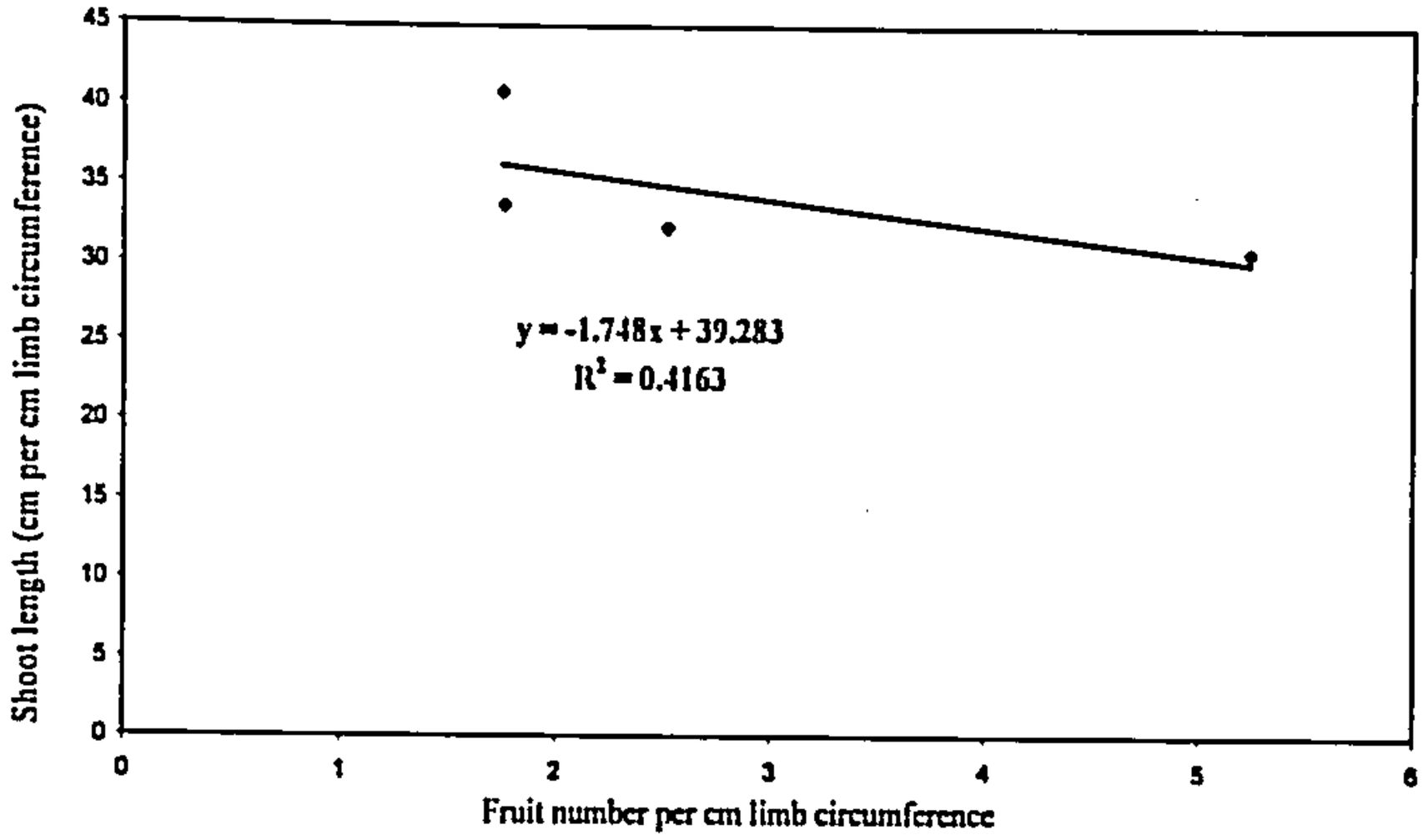

Figure 22: Relationship between morula terminal shoot length and fruit set

Table 1: Morula fruit mineral content (mg/g) as affected by benzyladenine

BA (mg/L)	Phosphorus (mg/g)	Sodium (mg/g)	Calcium (mg/g)	Magnesium (mg/g)	Nitrogen (mg/g)	Potassium (mg/g)
0	0.51	0.90	1.09	1.18	3.55	10.23
50	0.67	1.00	1.27	1.24	3.75	11.36
100	0.95	1.12	1.45	1.48	3.77	11.49
150	1.54	1.05	1.80	1.55	4.21	10.76
Significance	****	*	*	*	**	*
LSD	0.28	0.14	0.49	0.28	0.35	0.83

Significance level: * 0.05, ** 0.01, *** 0.001, **** 0.0001

morula content, there were no significant differences among the BA concentrations between control (unsprayed) trees and 50 mg/L in their ability to influence P, K and Na translocation to the fruit (Table 1). In comparison to other minerals, phosphorus had the highest response to BA applications with fruit content between 24-67 % compared to the control, thus the overall increase of 43% (Table 1). Potassium had the lowest response compared to other mineral elements ranging from 11-15 % thus an overall increase of 6 % (Table 1). The percentage increase of calcium was between 14-40 %, while Na percentage increase was between 10-20% (Table 1).

Similar to fruit mineral content, BA application to morula trees increased the leaf mineral content (Table 2). Application of 150 mg/L BA significantly increased the morula tree mineral uptake of P, Na, Ca, Mg, N and K compared to control trees (Table 2). However, application of 50 or 100 mg/L BA did not significantly increase the leaf content of P, Na, N and K compared to control (Table 2). With respect to leaf Ca and Mg content, application of 100 mg/L BA significantly increased their contents compared to leaf content in the control trees (Table 2).

Table 2: Morula leaf mineral content (mg/g) as affected by benzyladenine

BA (mg/L)	Phosphorus (mg/g)	Sodium (mg/g)	Calcium (mg/g)	Magnesium (mg/g)	Nitrogen (mg/g)	Potassium (mg/g)
0	0.38	0.96	1.76	3.22	3.80	7.22
50	0.45	1.04	2.07	3.41	3.84	7.40
100	0.52	1.22	2.52	3.67	3.85	8.83
150	0.78	1.67	2.89	3.94	4.17	16.00
Significance	**	**	****	*	*	***
LSD	0.23	0.33	0.4	0.44	0.29	3.4

Significance level: * 0.05, ** 0.01, *** 0.001, **** 0.0001

CHAPTER 5
DISCUSSION

5.1. Fruit Set

Fruit growth commences after successful pollination and fertilization of ovules within a flower. Flowers with unfertlilised ovules die and those remaining begin to grow into morula fruits. Some fruits drop off and after that the retained fruits continue to develop to maturity with different physiological processes taking place. Morula fruit growth and development showed a simple sigmoid curve. This means that it follows the same pattern of growth with fruits such as mango (*Mangifera indica L*), jack fruit (*Artocarpus heteropyllus*), apples *(Malus domestica)*, Japanese pear *(Pyrus serotina)*, pecan *(Carya illinoinensis)* and filberts *(Corylus avellana)* (El-Sharkawy *et al.*, 2007; Ullah and Haque, 2008).

The results of this study showed that BA reduced the fruit set of morula fruit trees. The response of morula to increasing BA concentration was quadratic, with no significant differences between 100 or 150 mg/L BA in their ability to reduce crop load or fruit set. Emongor (1995) reported similar results in Empire apples. He found that application of 50, 100 or 200 mg/L BA to "Empire" apples significantly reduced fruit set and the response was quadratic. However, Elfving and Cline (1993) using several apple cultivars on different rootstocks reported a linear relationship between BA concentration and reduction of fruit set. Bound *et al.* (1993) reported no linear fruit thinning response to increasing BA concentration on 5-year old red "Fuji" apple trees on seedling rootstocks.

Benzyladenine has been reported to be effective in thinning fruit trees and its effectiveness varies among different species/ cultivars, fruitlet size, timing of application and application rates (Greene and Autio, 1989; Greene *et al.*, 1992; Elfving and Cline, 1993; Ferree, 1996). In some apple cultivars, BA effectively thins fruits at the concentration of 50 to 150 mg/L and beyond that the chemical may not thin the fruitlets or may over thin them (Elfving and Cline, 1993; Emongor, 1995; Ferree, 1996; Emongor and Murr, 2001).

The reduction of crop load may be induced by the increase in ethylene production in the BA-treated fruitlets and leaves than the control trees (Greene, *et al.*, 1992; Emongor and Murr, 2001; Stopar, 2002). Ethylene releasing compounds have been shown to be very effective in the formation of abscission layer in fruits such as cherries, apples, walnuts, macadamia nuts and tangerines (Arteca, 1996). This may explain morula fruitlet abscission and dropping a few days after benzyladenine application. This was demonstrated by Kondo and Mizuno (1989) and Emongor (1995) where BA spray increased ethylene evolution from apple fruitlets followed by fruit abscission. Benzyladenine may also inhibit the export of auxins on the fruitlets intended to drop of which may lead to the accumulation of the extractable auxins (Bangerth, 1986) and increased ethylene production, hence leading to formation of the abscission layer (Reid, 1985). The formation of the abscission layer can also be due to fruitlet sensitivity to ethylene, interference of BA with the synthesis, transport or action of auxins and thereby leading to the abscission of fruitlets (Emongor and Murr, 1999).

5.2. Fruit Quality: Fruit size

Benzyladenine increased fruit size in terms of fruit weight, length, diameter, length-to-diameter ratio and fruit density. Fruit thinning has been associated with increased fruit size in peach (Byers, 1989), apples (Forshey and Elfving, 1990; Emongor and Murr, 1999; Emongor and Murr, 2001; Stopar, 2002) and grapes (Reynolds *et al.*, 1986). This was also the case in some studies where benzyladenine showed the highest response towards increase in fruit size of apple cultivars (Elfving and Cline, 1993; Ferree, 1996; Greene, 2005) The general increase in fruit size (weight, length, diameter and density) of morula contributed by BA was associated with the reduction in the fruit set since there is a strong relationship between the number of fruits in a tree and fruit size (Forshey, 1986). Fruit size (weight) of morula increased with the increasing BA application and the response was quadratic. This suggests that there was a reduced competition between fruits and within fruits for photosynthetic assimilates, metabolites, mineral elements, water and other growth factors of the remaining fruits leading to the leaf:fruit balance (Williams and Edgerton, 1981; Wismer, 1994; Elfving *et al.*, 1996). The BA-induced increase in morula fruit size (length, diameter, L:D ratio and weight) could also be attributed to increased number of cells in the BA-treated fruits. Benzyladenine has been shown to increase cell division in the young fruitlets (Wismer, 1994) resulting in more cell number per unit fruit weight or volume than control fruit. Benzyladenine has also been reported to increase fruit enlargement (Stoppa, 1999) due to increased cell division and elongation.

Costa *et al.* (2004) reported that in "Gala" and "Fuji" apple varieties treated with BA and naphthalene acetic acid (NAA), the average fruit weight of treated apples increased in response to BA and naphthalene acetic acid (NAA). They further reported that BA treated apples were heavier in weight than the NAA treated ones even though the thinning action was slightly lower. This confirms that BA increases fruit size by both increasing cell division and enlargement, and reduction in inter-and-intra fruit competition for assimilates and fruit growth requirements. In delicious apples, the application of BA promoted fruit enlargement and development of calyx lobes (Arteca, 1996). Benzyladenine has been reported to improve the grading index of apples with the largest apple of diameter greater than 90 mm hence increasing the percentage of marketable apples (Basak, 2004).

Benzyladenine has also been reported to have the ability to promote carbohydrate metabolism and create new source-sink relationship which increases the sink strength and fruit size at harvest (Dyer *et al.*, 1990; Emongor and Murr, 2001). The increase in morula fruit size in the current study was attributed to the BA-induced increase in the endocarp. This suggests that BA may have prolonged the period of endocarp development. Within a fruit, seeds are stronger sinks than mesocarp; therefore, they may have directed metabolites to the endocarp as their site of growth and development. The BA-induced increase in morula fruit size was also attributed to the BA-induced increase in fruit dry matter. In most fruits usually 50-80 % of dry matter yield is carbohydrates (Kays and Paull, 2004), of which have been indirectly contributed by the reduction of competition for photosynthates leading to enough accumulation of photoassimilates. In the current

study, BA increased the leaf area of morula treated trees hence explaining the increase in fruit dry matter and fruit size in the BA-treated morula trees. BA-induced mineral composition may have also influenced the increase in the morula fruit size. Potassium and sugars act in a complementary manner to produce the tugor potential for cell extension. Nitrogen also plays a role in osmoregulation and cell division, this may have influenced the increase in fruit size of morula treated fruits (Marshner, 1995). Pacheco *et al.*, (2004) reported that N and K fertilization showed significant effect on the leaf mineral composition at fruit enlargement in Kiwi fruit "Hayward". In cucumber cotyledons, K^+ supply enhances cell extension in response to application of cytokinnins (Green and Muir, 1979).

5.3. Soluble solid content, organic acid content

Carbohydrates are the most abundant biochemical constituents in plants, representing 50-80 % of the total dry weight (Kays and Paull, 2004). Stored energy reserves make up much of the structural framework of the cells. Simple carbohydrates such as sugar, sucrose, glucose and fructose, impart important quality attributes to harvested horticultural produce. In fruits, it is primarily the sugars and organic acids which contribute to the fruit taste although the astringent nature of some fruits can be attributed to their phenolic and tannin content. However, the characteristic flavour of individual fruit usually derived through our sense of smell is due to the production of specific volatiles. Thus, the flavour of the fruit depends on the complex interaction of sugars, organic acids, phenolics and more specialized flavour compounds including a wide range of volatiles (Kays and Paull, 2004).

The morula trees treated with benzyladenine produced fruit high in soluble solids content (sugars), citric acid equivalent and total titratable acidity compared to fruit from untreated trees. Sugars and organic acids are used as respiratory substrates and both originate from photosynthetic assimilates. Benzyladenine increased soluble solids content, citric acid equivalent, total titratable acidity and ascorbic acid content because of the increased morula leaf area, chlorophyll a, chlorophyll b and total chlorophyll content which might have increased photosynthetic ability of the BA-treated morula trees, hence increased assimilates production. The increased photosynthetic rate may also be attributed to the BA-induced increase in the leaf nitrogen (N) content, because leaf N is one of the factors that determine the photosynthetic rate per leaf area, thus the higher the leaf N the higher the leaf photosynthesis. Dejong (1983) reported a linear correlation between the leaf N and photosynthetic rate per leaf area in peach and other stone fruits. Benzyladenine increased morula fruit SSC because BA reduced the within and between fruit competition and increased the leaf-to-fruit ratio resulting from the reduction in fruit set as there would be more leaves to support fruit growth. However, this may provide only a partial explanation, because Greene *et al.* (1990) reported that BA, NAA and carbaryl reduced crop load to a comparable level in "McIntosh" and "Empire" apples, yet SSC was increased only in BA-treated fruit. This suggests that BA could have altered the source-sink relationship by increasing sink strength, promoting carbohydrate metabolism and altering tree physiology (Emongor, 1995; Emongòr and Murr, 2001). Basak (2004) reported that higher BA concentrations caused a significant increase of soluble solid

content (SSC) compared to non-thinned trees, but as compared to the hand thinned trees there was a similar response towards the sugar content.

Morula fruits treated with benzyladenine significantly increased the vitamin C content with a linear response to the increasing BA concentration. Vitamin C as an organic acid is an early product of photosynthesis, due to the increase in the total leaf chlorophyll, chlorophyll a, chlorophyll b content and leaf area of morula, there was an increased photosynthetic activity which led to the increase in vitamin C content. BA also induced the balance between leaf-to-fruit ratio of morula and therefore reducing the competition within the fruits, hence leading to increased photoassimilates and metabolites essential for vitamin C synthesis. Potassium is one of the most important minerals in the leaf tissues since photosynthesis requires adequate levels of K in the leaf tissues (Smid and Peaslee, 1976). The synthesis of adenosine triphosphate (ATP) needed for photosynthesis reactions such as activities and efficiencies of the enzymes involved in photosynthesis (RUBp carboxylase), carbon dioxide uptake into the leaves (stomatal openings) and the balance of electrical charges needed for phosphorylation in chloroplasts are influenced by K (Marshner, 1995). This is one of the contributing factors to the improvement of the fruit soluble solid content and organic acids content (total titratable acidity, citric acid equivalent and vitamin C) on the BA treated fruits since leaves as the major source of photoassimilates have to synthesize enough assimilates to sustain the fruit. Potassium has also been proven to have a role in many crop quality parameters (Usherwood, 1985). It promotes phloem transport of photosynthates (mainly sucrose and amino acids) and their

mobilization to the physiological sinks (fruits, roots, tubers, seeds and grains) and even their conversion into starch, proteins, vitamins and oils (Mengel, 1997).

With proper K nutrition, tomato fruit is generally high in total solids, sugars, organic acids, carotene and lycopene as well as longer shelf life (Mengel, 1997). In citrus, K is reported to increase vitamin C and soluble solids content (Usherwood, 1985) and improves the acid/sugar ratio (Koo, 1995). Similar results have been reported in peaches where the increase in K^+ led to an increase in the fruit titratable acidity (Kwong and Fischer, 1962; Mengel and Haeder, 1974). The presence of K^+ and other ions help to maintain osmotic concentration necessary to keep the cell turgid and for translocation of assimilates and soluble solids to the stronger sinks (fruits) (Maschner, 2002), which caused the increase in fruit soluble solids content, citric acid equivalent, total titratable acidity and vitamin C content. Phosphorus also plays a role in the formation of sugars and starch, therefore, the BA-induced increase in phosphorus content may have also contributed to the increased SSC of morula fruit. This suggests that apart from the direct effects of BA to the improvement of fruit quality, the BA-induced increase in mineral content such as K and P indirectly also improved morula fruit quality.

5.4. Fruit colour at harvest:

Many fruits change colour during ripening and it represents a key attribute along with texture for determination of quality of fruits. Colour change in fruits can result from degradation of chlorophyll, which in turn unmasks previously present pigments such as a β-carotene (Emongor, 1995). However, in most fruits the loss of chlorophyll is

accompanied by the biosynthesis of either anthocyanins or carotenoids (Kays and Paull, 2004). In the current study, application of BA to morula trees at 50, 100 or 150 mg/L increased fruit colour (anthocyanins and carotenoids content), but had no significant effect on the fruit chlorophyll content. Benzyladenine might have increased the concentration of morula fruit colour at harvest because of the increased SSC induced by BA. Emongor (1995) reported greater anthocyanin content in BA-treated apple fruits and attributed it to high SSC in the BA-treated fruit. The biosynthesis of anthocyanins during maturation is closely related to carbohydrate accumulation (Winkler *et al.*, 1974; Goldschmidt, 1980). The link between carbohydrates and anthocyanins may be via the pentose phosphate pathway which leads to the shikimic acid pathway used in anthocyanin biosynthesis (Goldschmidt, 1980). Cytokinins have also been reported to enhance anthocyanin accumulation in olive fruit (Goldschmidt, 1980).

Carotenoids are terpenoid compounds derived from acetyl CoA via the mevalonic acid pathway. The BA-induced increase in anthocyanins and carotenoids may also be related to accumulation of organic acids especially acids involved in the tricarboxylic acid cycle (TCA). In the current study BA-treated fruit had significantly higher citric acid equivalent, total titratable acidity, ascorbic acid content than control fruit. Anthocyanin, carotenoids and organic acids all require acetyl CoA in their biosynthetic pathways. Benzyladenine may have possibly influenced anthocyanins, carotenoids and organic acids biosynthesis by influencing the transcription of acetyl CoA. Cytokinins also play a vital role in enhancing phenylalanine ammonia layase (PAL) and tyrosine ammonia layase (TAL). These are the key enzymes in controlling anthocyanin biosynthesis from

phenylalanine and/or phenol synthesis (Tucker, 1993). PAL enzyme catalyses the elimination of ammonia from phenylalanine to give trans-cinnamic acid (parent compound for anthocyanin biosynthesis) (Tena *et al.*, 1984). This may have partly influenced the BA increased anthocyanin content in morula fruits at harvest. El-Meleigy (1989) found that BA enhanced the biosynthesis of anthocyanin content in roselle *(Rosa spp)* leaves and carlyx. The PAL and TAL activities in roselle plants increased with more response pronounced when BA was applied at 100mg/L and/or 100mg/L GA (Raifa *et al.*, 2005). Similar results were also found in strawberry fruits (Montero *et al.*, 1998) and periwinkle (Ohlsson and Berglund, 2001).

5.5. Leaf area, water content and drymatter content

Benzyladenine increased vegetative growth of morula tree. The leaf area of morula in response to BA application was quadratic. Benzyladenine as a synthetic cytokinin has been reported to stimulate leaf enlargement by promoting cell expansion of the leaves (Taiz and Zeiger., 2002). It has been reported that with enhanced cytokinnin content, transgenic tobacco plants produced more leaf cells and bigger leaves than the control leaves (Werner *et al.*, 2001). Mineral content of both the fruits and leaves may have increased the leaf area. The cell extension of leaves is related to their potassium and nitrogen content because potassium plays a role in cell enlargement due to osmoregulation and nitrogen is essential for cell division. Though the leaf area was significantly increased by BA application, the leaf dry matter and water content results were not significantly increased. Taiz and Zeiger (2002) reported that cytokinin treatment promoted additional cell expansion but with no increase in the dry weight of the treated

plants. Jackson (1985) observed that the thinned trees can have either the same or greater stomatal conductance than the unthinned trees but a higher water potential suggesting that the presence of fruits may be affecting the turgor or osmotic potential of the leaves and hence their water content.

5.6. Leaf chlorophyll content

The results of the current study showed that BA significantly increased chlorophyll content (chlorophyll A, chlorophyll B and total chlorophyll) of morula leaves. The chlorophyll content increased with increasing BA concentration with a quadratic response. Benzyladenine as a cytokinnin plays a role in the light regulated development such as stimulation of chlorophyll synthesis (Salopeck-Sondi *et al.*, 2002) and differentiation (Taiz and Zeiger, 2002). Benzyladenine has been reported to promote chloroplast development and chlorophyll synthesis in several plant structures (Salisbury and Ross, 1996; Emongor and Murr, 2004) This suggests that besides light interception, benzyladenine stimulated chlorophyll synthesis in the leaves. The increased mineral content of morula leaves and fruits induced by BA may have also influenced the increase in the chlorophyll content. Nitrogen and magnesium are important components of chlorophyll (Marschner, 1995), which were all increased by BA in the current study.

5.7. Terminal shoot growth and shoot number

Benzyladenine increased the terminal shoot length and shoot number of morula trees significantly. The increase was linear with increasing BA application. Emongor (1995)

working with "Empire" apples reported that application of 50, 100 and 200 mg/L BA increased vegetative growth. The increase in vegetative growth in the current study induced by BA was attributed directly to the reduction in crop load (fruit set) caused by BA depending on the concentration used. Benzyladenine increased vegetative growth and reduced fruit set, thus increasing the leaf-to-fruit ratio, resulting in availability of more photoassimilates and growth factors for vegetative growth.

Benzyladenine has been reported to increase the formation and activity of shoot apical meristems (Taiz and Zeiger, 2002). Normally after fruit set, vegetative growth continues to slow down due to a high demand of the assimilates and metabolites for fruiting since fruits are stronger sinks, but under circumstances of abundant water, nutrients such as nitrogen and potassium, plant growth regulators and photoassimilates a high vigour is maintained and hence the shoot growth may continue at a steady rate throughout the season (Robinson *et al*, 1998). The increased shoot number per cm limb circumference was also attributed to BA overcoming apical dominance of morula trees, a characteristic cytokinin correlative effect (Emongor, 1995).

Studies have been carried out on different plant species and cultivars and it has been shown that BA has an effect on vegetative growth in terms of shoot growth and what varies is the concentration of response to benzyladenine. Imai *et al.* (1995) reported that BA promoted the release of suppressed lateral buds and increased the number of long shoots on *Larix occidentals nutt* which were suitable for stem tip cuttings. Benzyladenine at 10-20 ppm significantly increased the number of shoots of potatoes in a study done on the BA effect on vegetative growth and tuber production. While in bengonia leaf discs

where kinetin was used as a synthetic cytokinin suppressed root growth but shoots appeared sooner in larger quantities (Badizadegan *et al.*, 1971). The exogenous supply of benzyladenine to *Bengonia x Cheimantha* also promoted shoot formation at a constant temperature of 24 °C (Leomadi *et al.*, 2006). Benzyladenine has been used in foliage plants such as *Synqoniump podophyllum 'White Butterfly', Schlumbergera truncate, Peperomia obtusifolia, Cordyline terminalis, Hedera helix* 'English Ivy' to induce lateral shooting (Henny, 1989). The increased mineral nutrition in the current study due to BA application especially nitrogen may have contributed to the increased terminal shoot length and number because nitrogen increases the rate of shoot growth and development (Marschner, 1995).

5.8. Interaction of vegetative and reproductive growth

The results of this study showed that there was a negative correlation between vegetative and reproductive growth of morula trees. Several studies suggest that a wide range of species have a reduction in dry matter partitioning to shoots, leaves and roots due to fruiting. Hansen (1971) and Magg (1963) reported a reduction in shoot and leaf production with increasing fruit load. Heim *et al.* (1979) reported similar results on the effects of fruiting over the stem dry matter accumulation, thus over 40 % of the stem dry matter was fixed in the non-fruiting apple cultivars compared with 10 % for heavily fruiting trees. Sanz *et al.* (1987) reported an interaction between the shoot and fruit growth in citrus and between crop weight and increase in the trunk cross sectional area in McIntosh apples (Webster and Brown., 1980). This suggests that there should be a balance between the shooting and fruiting of the tree. The leaves as a major source produce all the carbohydrates through photosynthesis to be mediated and utilized by the

fruit (sink) for growth and development hence contributing to the fruit quality. Forshey. (1986) reported that optimum fruit size and quality require about 30 leaves per fruit, and fruit thinning improves the leaf: fruit ratio by increasing the leaf number and size available to each persistent fruit. Even though thinning improved the leaf area available to the persisting fruit not all of the increase in carbohydrate supply goes to the fruit, part of it is diverted to sustain vegetative growth for shoot growth and development, chlorophyll synthesis, root strength and activity.

5.9. Mineral nutrition

Generally fruiting reduces growth of all parts of the plant but with a greater effect on the roots (Forshey, 1986). This reduces development, strength and activity of the roots and hence reducing the supply of reserves and essential growth regulators such as water, plant growth regulators (since others such as cytokinins are synthesized in the roots) and the mineral elements. Due to enough supply of assimilates in response to benzyladenine application, the root activity was improved and hence promoting the translocation of minerals to the sinks (fruits and leaves) and other growth regulators. This correlates well with the current results of this study in which BA significantly increased the mineral content of morula leaves and fruits compared to the control (untreated) trees.

In some trees especially heavy fruiting cultivars, it has been found that fruiting reduces the root growth, development and activity which affects the mineral nutrient uptake (Wright, 1985). This implies that with a reduced crop load the root activity was increased, and thereby enhancing their ability to transport minerals to the sinks. Because

the fruits are stronger sinks than the leaves, the results of the current study showed that they had a greater amount of total minerals compared to the leaves. Apart from the reduction in fruit set, BA also increased mineral nutrient mobilization, a phenomenon known as the cytokinin-induced nutrient mobilization (Taiz and Zeiger., 2002). The nutrients will move from the site of production and/or storage (roots and/or leaves) to the site of utilization (leaves/ fruits). Among all macro-elements, calcium (Ca), nitrogen (N), potassium (K), and phosphorus (P) and among microelements, boron (B) have been more frequently associated with various fruit quality attributes (Fallahi and Fallahi, 2007). These elements responded to the benzyladenine application and therefore improved fruit quality. The effect of BA on the increased potassium mineral content in both the leaves and fruits may have influenced the increased uptake of other minerals (N, P, Mg, Na, Ca). Potassium plays an important role in osmo-regulation and translocation of nutrients into plant cells (Atwell *et al.*, 1999). The uptake of minerals through the transpiration stream is driven by diffusion/ mass flow through the osmotic gradient, thus water availability in the transpiration stream is a major drive for mineral translocation through the phloem and xylem vessels. Calcium also plays a role in the uptake of nitrogen and other minerals, due to their role in the osmotic balance. The increased phosphorus content might have also increased uptake of other minerals because through cell division, P stimulates the formation and growth of roots (Marschner, 1995). The rate of root elongation and surface area of the roots are important factors for root activity because larger surface area is vital for the mineral uptake from the soil and their translocation to the leaves and fruits. Phosphorus might have improved the ability of plants to absorb water and nutrients.

Increased mineral uptake in the morula leaves and fruits may also be attributed to the increased photosynthetic factors such as high leaf chlorophyll content, increased leaf area resulting in high sugar content and organic acids. These assimilates are essential for ATP and carbondioxide fixation (energy compounds), the high energy is required for nutrient uptake and their assimilation in the plant cells.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

Benzyladenine reduced the morula fruit set. It has increased vegetative growth, improved fruit quality and increased nutrient partitioning to the leaves and fruits. The heavy demands of fruiting distort carbon, minerals and growth requirements among vegetative parts, including the root/shoot balance and fruiting. Heavy fruiting depletes storage reserves leaving the tree susceptible to winter injury. From the results of this study, application of benzyladenine to morula trees reduces the fruit set and increase vegetative growth, thus increasing the leaf-to-fruit ratio and vigour of the fruit trees. These results suggest that BA can be used to manipulate fruiting behavior of morula fruit trees to improve the fruit quality, and vigour (vegetative growth) of the morula trees, and marketable fruit size.

6.2. Recommendations

Based on the results of this study, further research on concentration and timing of BA application to improve the efficacy of thinning should be done. The research should also include different sites of Botswana for example the Southern region, Northern region, Central region, Eastern region and Western parts of Botswana to represent the different agricultural regions of the country.

REFERENCES

Arteca, R.N. (1996). Plant growth substances: Principles and practices. Chapman and Hall. Newyork. Pp: 42-219.

Atwell, B., Kridemann, P. and Turnbull, C. (1999). Plants in action: Adaptation in Nature, Performance in Cultivation. Macmillian Education. South Yarra, Victoria, Australia. Pp: 103-200.

AOAC, (1996). Association of Official Analytical Chemists Official Methods of Analysis.17th Edition, Arlington, Virginia, USA.

Badizadegan, M. Tafazoli, E. Khednam, M. (1971). Effect of N^6 BA on vegetative growth and tuber production in potato. *Journal of Potato Research.* 49 (3): 109-116.

Basak, A, (2004). Fruit thinning using BA with ethephon, ATS, NAA, urea and carbaryl in some apple cultivars. *Acta horticulturae.* 653: 99-106.

Bangerth, F. (1986). Thinning of apple fruits and relations to endogenous hormones. *Acta horticulturae.* 179: 303-322.

Bangerth, F.B. (1990). Polar auxin transport in fruit trees in relation to fruit drop. *Acta horticulturae.* 275: 416-468.

Bekker, R.P. and De wilt, P.V. (1991). Contribution to the vegetation classification of Botswana. Soil mapping and advisory services. Food and Agriculture Organisation of the UN. Gaborone, Botswana. Pp: 34-66.

Bollard, E.G. (1970). The physiology and nutrition of developing fruits. In The Biochemistry of Fruits and their Products. Academic Press. London 1: 387-425.

Booyers, G. (1996). Veld Products marketing study: Natural resource management program. Botswana USAID Project. Pp: 23-30.

Botswana Technology Center. (1997). Food Technology Research Services, BTC/FTR.

Bound, S.A., Jones, K.M., Oakford, M.J., and Tichon, M. (1993). Assessing interactive effects between Cytolin and ethephon on cropping red 'Delicious'. *Journal of Horticultural Science.* 68: 209-213.

Buban, T. (2000). The use of benzyladenine on orchard fruit growing. *Plant Growth Regulation.* 32: 381-390.

Byers, R. E. (2002). Influence of temperature and darkness on apple fruit abscission and chemical thinning. *Journal of Tree Fruit Production.* 3 (1): 41-53.

Byers, R.E. (1989). Effects of bloom-thinning chemicals on peach fruit fruit set. *Journal of Tree Fruit Production.* 2(2): 54-78.

Byers, R. E. and Carbaugh, D. H. (1991). Effect of chemical thinning sprays on apple fruit set. *Horticultural Technology.* Pp: 41-48.

Campbell, A. (1990). The Nature of Botswana: A guide to conservation and development. Field operations division. International Union for the conservation of nature and natural resources. Switzerland. 87p.

Chan, B and Cain. J.C. (1967). The effect of seed formation on the subsequent flowering in apple. *Proceedings of the American Society for Horticultural Science.* 91: 63-68.

Childers, N.F. (1984). Morden Fruit Science: Orchard and small fruit culture. Library of Congress. Institutê of pomolgy. Skiemiewice, Poland. Pp: 146-160.

Coombe, B.G. (1976). The grape berry as a sink. *Acta Horticulturae.* 239: 149-158.

Costa, G. Bucchi, F. Montefiori. M. Bregoli, A. Corelli, M. and Grappadelli, L. (2004). Thinning activity and fruit quality of 'Gala' and 'Fuji' apple varieties as affected by cytokinins. *Acta Horticulturae*. 653: 107-113.

Creasy, L.L. (1966). The effect of temperature on anthocynin synthesis in McIntosh apple skin. *Proceedings of Anthocyanin Synthesis of New York State. Horticultural Society.* 11: 93-96.

Crowe, A.D. (1965). Effect of thinning sprays on metabolism of growth substances in the apple. *Proceedings of American Society of Horticultural Science*. 86: 23-27.

Cutwright, R. and Pfeiffer, D.G. (1997). Apple fruit thinning. Department of Entomology. Virginia. Blacksburg. 4987p.

Datta, S.C. (1994). Plant Physiology. New Age International (P) Limited, Publishers. New Delhi, London. 486p.

Davies, P.J. (1995). Plant hormones, physiology, biochemistry and molecular biology. Dorchecht. Netherlands.

Dejong, T.N. (1983). Effect of leaf nitrogen on photosynthesis. *Journal of American Society of Horticultural Science*. 108: 303-307.

Dennies, F.G. (2000). The History of Fruit Thinning. *Plant Growth Regulators*. 31:1-16.

De-Wilt, P.V. and Nachtengaele, F.O. (1996.) Explanatory notes on soil map of Republic of Botswana. Soil Mapping and Advisory Services. Botswana. 48p.

Douglas, J.B. (1983). An evaluation of harvest indices for indices for "McIntosh" apples in two orchards. *HortScience*. 18(2): 216-218.

Dyer, D. Cotterman, J.C. Cotterman, C.D. Kerr, P.S. and Carlson, D.R. (1990). Cytokinins as metabolic stimulants which induce pod set. In Pharis, R.P. and S.B. Roods (Eds). Plant Growth Substances. Springer-verlag. Pp: 457-467.

Edgerton, L.J. (1981). Some effects of aminoethoxyvinylglcine, 6-benzylamino purine and gibberellins on fruit set and vegetative growth of apple. *Acta Horticulturae.* 120: 125-130.

Elfving, D.C. and Cline, R.A. (1993). BA and other chemicals for thinning "Empire" Apple trees. *Journal of American Society of Horticultural Science.* 5: 593-598.

Elfving, D.C., Schechter, I. and Bom, M. (1996). Effects of root pruning and benzyladenine applicationon tree growth and fruit size in 'Empire' and 'McIntosh' apple. *Journal of Tree Fruit Production.* 1 (2): 1-7.

El-Meigy, S. (1989). Physiological studies on roselle plant (*Hibiscus saboloriffe)* L.PhD. Ain Shams University.

El-Sharkawy, W., Kim, S., El-Kereamy, A., Jayasankar, S., Svircev, A.M. and Brown, D. C. W.. (2007). Isolation and characterization of 4 ethylene signal transduction elements in plums *(Prunus salicina L). Journal of Experimental Botany.* 58(13): 3631-3643.

Emongor, V.E. (1995). Thinning activity of Benzylanine on "Empire" apples: Application, timing and fruit storage. PhD Thesis, University of Guelph. Faculty of Agriculture, Horticultural Science Department. 174p.

Emongor, V.E. (2004). Effect of promalin on growth and development of Kale (*Brassica oleraceae* L. var. acephala D.C). *Journal of Agronomy.* 3(3): 208-214.

Emongor, V.E. and Murr, D.P. (1999). Effects of time of application of Benzylanine on fruit set and fruit characteristics of "Empire" apples. *African Crop Science Journal.* 7(2): 173-184.

Emongor, V.E. and Murr, D.P. (2001). Effect of benzyladenine on fruitset, quality, and vegetative growth of "Empire" apples. *East African Agriculture and Forestry Journal.* 67(2): 83-91.

Emongor, V.E. and Murr, D.P. (2004). Response of apple trees to aminoethoxyvinylglycine (AVG) with emphasis on apical dominance, fruit set and mechanism of action of fruit thinning chemicals. *Acta Horticulturae.* 120: 137-142.

Faine, and Venter, J. (1996). Making the most of indigenous trees. Briza publications. Pretoria. South Africa. Pp: 457-458.

Fallahi, E and Fallahi, B. (2007). Apple fruit quality as influenced by mineral nutrition. University of Idaho. Research and Extension Center. USA.

FAO, (2002). Physical situation about indigenous fruit trees in Southern African Region. FAO Desk Study Report.

Faust, M. (1989). Physiology of temperate zone fruit trees. A Wiley-Interscience Publication, John Wiley and Sons, Newyork, Chichester, Brisbane, Toronto, Singapore. 222p.

Ferree, D.C. (1996). Performance of Benzyladenine as a chemical thinner on eight apple cultivars. *Journal of Tree Fruit Production.* 1(2): 164-195.

Forshey. C.G. (1986). Chemical fruit thinning of apples. Newyork's food and life sciences bulletin. Newyork's State Agricultural Experiment Station. Geneva. Pp: 1-7.

Forshey, C.G. (1990). The use of alar on vigorous "McIntosh" apple trees. *Journal of the American Society for Horticultural Science.* 95: 66-67.

Forshey, C.G. and Elfving, D.C (1990). Fruit numbers, fruit size and yeld relationships in McIntosh apples. *Journal of the American Society for Horticultural Science.* 115: 394-400.

Fox, F.W. and Norwood-Young, M.E. (1982). Food from the veld: Edible wild plants of Southern Africa. South African institute of medicinal research. Delta books (pty) Ltd. Johannesburg. South Africa. Pp: 79-80.

Garcia, M.E., Berkett, L., Bradshow, T., Benedict, C., Kuitems, C., (2003). Apple thinning pointers. Vermont Apple newsletter. Department of Plant and Soil Science, University of Vermont, Burlington. 20p.

Gifford, R.M. and Evans, L.T. (1981). Photosynthesis, carbon partitioning and yield. *Annual Review of Plant Physiology.* 32: 485-509.

Goldschmidt, E.E. (1980). Pigment changes associated with fruit maturation and their control; pp 207-217. In: K.V. Thimann, R.C. Adelman, and D.S. Roth (Eds). Senescence in Plants. CRC Press, Boca Raton, Florida.

Goldwin, G.K. (1992). Environmental and internal regulation of fruiting with particular references to Cox's Orange, Pippin Apple. Pp: 74-100.

Greene, D.W. (2005). Time of aminoethoxyvinylglycine application influences preharvest drop and fruit quality of 'McIntosh' apples. *HortScience.* 40: 2056-2060.

Greene, D.W. and Autio, W.R. (1989). Evaluation of Benzyladenine as a chemical thinner on 'McIntosh' apples. *Journal of the American Society for Horticultural Science.* 114: 68-73.

Greene, D.W., Autio, W.R. and Miller, D. (1990). Thinning activity of benzyladenine on several apple cultivars. *Journal of American Society of Horticultural Science.* 115: 394-400.

Greene, D.W., Autio, W.R., Ert, J.A. and Mao, Z. F. (1992). Mode of action of benzyladenine when used as a chemical thinner on apples. *Journal of the American Society for Horticultural Science.* 117 (5): 775-779.

Greene, J.F. and Muir, R.M. (1979). Analysis of the role of potassium in the growth effects of cytokinnin, light and abscisic acid on cotyledon expansion. *Physiology of Plants.* 46: 19-24.

Grochowska, M.J., Buta, G.J., Steffens, G.L. and Faust, M. (1984). Exogenus auxin and gibberellin level in low and high vigour apple. *Acta Horticulturae.* 146: 125.

Hansen, P. (1971). ^{14}C studies on apple trees. The early seasonal growth in leaves, flowers and shoots as dependent upon current photosynthates and existing reserves. *Physilogia Plantarum.* 25: 469-473.

Heim, G. Landsberg, J.J. Watson, R.L. and Brain, P. (1979). The ecophysiology of apple trees; dry matter production and partitioning by young Golden Delicious trees in France and England. *Journal of Applied Ecology.* 16: 179-194.

Henny, R.J. (1989). A review of literature concerning the use of growth regulators to induce lateral or basal shoot production in ornamental tropical foliage plants. University of Florida. USA.

HerbalGram, (2008). Tree of life: The use of Marula oil in Southern Africa. American Botanical Council. 79: 32-41.

Ho. L.C. (1992). Fruit growth and Sink strength. Society for Experimental Biology. Seminar Series. Cambridge University press. 47: 101-124.

Ho. L.C. (1988). Metabolism and compartmentation of imported sugars in sink organs in relation to sink strength. *Annual Review of Plant Physiology and Plant molecular Biology*. 39: 355-378.

Hoad, G.V. (1978). The role of seed derived hormones in the control of flowering in apple. *Acta Horticulturae*. 80: 93-103.

Holden, M. (1965). Chlorophylls. In: T.W. Goodwin (eds), Chemistry and biochemistry of plant pigments. Academic Press. New York. 462p.

Holzhausen, L.C. (1989). Propagation of Morula. *Acta Horticulturae*. 275(1): 323-229.

Hosrtcroft, J.C. and Sharples, R.O. (1987). The effect of modern production systems on apple quality. *Report of the East Mailing Research Station 1986*. 111p.

Imai, K. Okamoto, M. Yamaya, H. Kudo, N. Tamada, T. and Mikami, T. (1995). Effect of carbaryl on thinning apples. *Bulletin of the Aomori Apple Experimental Station*. 28: 23-51.

Jackson, J.E. (1985). The manipulation of fruiting. AFRC Institute of Horticultural Research. East Malling. Maidstone. Kent. UK. Pp: 1-25.

Janick, J. (1986). Horticultural Science. 2nd edition. W.H. Freeman and Company. San Franscisco. U.S.A. Pp: 270-271.

Kays, J.S. and Paull, R.E. (2004). Postharvest Biology, Exin Press Anthens, G.A. 568p.

Kokwaro, J.O. and Gillet, J.O. (1980). Notes on the Anarcadiaceae of Eastern Africa. Kew Bulletin. 34 (4): 756.

Kondo, S. and Mizuno, N. (1989). Relation between early drop of apple fruit and endogenous growth regulators, and effects of MCPB, GA_3 plus GA_4 and BA sprays on fruit abscission. *Journal of Japanese Society of Horticultural Science*. 58: 9-16.

Koo, R.C.J. (1985). Potassium Nutrition of Citrus. In: Potassium in Agriculture. R.S. Munson and W.I. Madison (Eds). Pp: 1077-1086.

Kwong. S.S. and Fisher, E.G. (1962). Potassium effects on titratable acidity and soluble nitrogenous content compounds of Jerseyland peach. *Proceedings American Society of Horticultural Science*. 81: 168-171.

Lakso, A.N. (1980). Correlation of fisheye photography to canopy structure, light climate, and biological response to light in apple trees. *Journal of the American Society for Horticultural Science*. 105: 43-46.

Landsberg, J.J. and Brain, P. (1978). Model of apple tree growth. Fruit drop. Annual Report Long Ashton Research station. Pp: 119-120.

Leakey, R.R.B. (1999). Potential for novel food products from agroforestry trees: *A Review in Food chemistry*. 66: 1-14.

Leornadi, C. Ruggeri, A. Malfa, S. (2006). Homorne effects on in vitro proliferation and rooting of Grevillea explants. *Scientia Horticulturae*. 90(3-4): 335-341.

Lenz, F. (1986). Fruit effects on transpiration and drymatter production in apples. In: The regulation of photosynthesis in fruit trees. A. Lakso and F. Lenz (Eds). *Symposium Proceedings Publication*, NY State Agricultural Experimental Station, Geneva, New York. Pp: 92-95.

Lidster, P. D. and S. W. Porritt. (1978). Some factors affecting uptake of calcium by apples dipped after harvest in calcium chloride solution. *Canadian Journal of Plant Science*. 58: 35-40.

Looney, N.E. Pharis, R.P. and Noma, M. (1985). Promotion of flowering in apple trees with gibberellin (GA_4) and C-3 epi-gibberellin (GA_4). 165: 292-294.

Luckwill, L.C. (1970). The control of growth and fruitfulness of apple trees. In: L.C. Luckwill and C.V. Cutting (Eds). Physiology of tree crops. Academic Press, New York. Pp: 237-254.

Magg, D.H. (1963). The reduction in growth of apple trees brought about by fruiting. *Journal of Horticultural Science*. 38: 119-128.

Manson, J. L., Jasmin, J.J. and Granger, R. L. (1975). Softening of 'McIntosh' apples reduced by a postharvest dip in calcium chloride solution plus thickener. *HortScience* 10: 524-525.

Marino, F. and Greene, D. W. (1981). Involvement of gibberellins in the biennial bearing of "Early McIntosh" apples. *Journal of the American Society for Horticultural Science*. 106: 593-596.

Marschner, H. (1995). Mineral Nutrition of Higher plants. 2nd Edition. Academic Press. London. Pp: 105-250.

Mateke, S.M. (1995). The horticultural annual report. Veld Products Research and Development. Gaborone. Botswana. Pp: 1-38.

Mateke, S.M. (1996). Indigenous fruit tree research programme: Progress Report of Indigenous Food Research Work. Veld Products Research and Development. 63.001.6. Gaborone. Botswana.

Mateke, S.M. (1999). Indigenous fruit trees and non timber forest products: Management of resources. Horticultural Section. Veld Products Research and Development. Gabane. Botswana. Pp: 19,20.

McLaughlin, J.W. and Greene, D.W. (1984). Effects of BA, GA_{4+7}, and Diaminozide on fruitset, fruit quality, vegetative growth, flower initiation, and flower quality of "Golden Delicious" apples. *Journal of the American Society of Horticultural Science.* 116(3): 450-453.

McMahon, M.J., Kofranek. A.M. and Rubatzky, V.E. (2002). Hartmann Plant Science: Growth, Development and Utilization. Pearson Education, Inc., Uppersaddle River, New Jersey. U.S.A. Pp: 282-283.

Mengel, K. (1997). Impact of Potassium on crop yield and quality with regard to economical and ecological aspects. In: Food security in the WANA region, the essential needs for balanced fertilization. A.E Johnston (Ed).

Mengel, K. and Haeder, H.E. (1974). Photosynthese und assimilatransport bei weizen wahred der kornausbildung bei unterchiedlielichet, kaliumernahrung. Z. Aker-u. *PflBau.* 140: 206-213.

Miller, S.S. 1988. Low –volume sprays of BA and Promalin increase branching in apple trees. *HortScience.* 20: 730-732.

Monselise, S.P. and Goldsmidt, E.E. (1982). Alternate bearing in fruit trees. *Horticultural reviews.* 4:128-173.

Montero, T., Molla, E., Martin-Cabrejas, M and Lopez-Andreu. (1998). Effect of Gibberellic acid (GA_3) on strawberry PAL (Phenylanine Ammonia Lyase) and TAL

(Tyrosine Ammonia Lyase) enzyme activities. *Journal of Science Food Agriculture*. 77: 230-234.

Nerd, A., Aronson, J.A. and Mizrahi, Y. (1990). Introduction and domestication of rare and wild fruit and nut trees for desert areas. In: J. Janick and J.E. Simon (Eds). Advances in new crops. Timber Press, Portland, Oregon. Pp: 355-363.

Ohlsson, A.B. and Bergland, T. (2001). Gibberellic acid-induced changes in gluthione metabolism and anthocynin content in plant tissue. Plant Tissue and Organ Culture. 64: 77-80.

Pacheco, C., Vieira, S., Santos, F., Neves, N., Curado, F., Rodrigures, S and Caulouro, F. (2004). Exportacoes de nutrientes pelos frutos elenha depode em pomores de actinidia deliciosa de beira litoral. *Proceedings X. Simposio Iberico de Nutricio Mineral das Plantas*. Lisbon. Pp: 263-269.

Peters, C.R. (1988). Notes on the distribution and relative abundance of *Sclerocarya birrea* (a. Rich) Hochst (Anacardiaceae). *Monogram System of Botany in Missouri Botanical Garden*. 25: 403-410.

Pharis, R.P. and King, R.W. (1985). Gibberellins and reproductive development in seed plants. *Annual Review of Plant Physiology*. 36: 517-568.

Proctor, J. T. A. and Creasy, L.L. (1971). Anthocynin synthesis in "Mc Intosh" apples. *Journal of the American Society of Horticultural Science*. 96 (4): 523-526.

Quin, P.J. (1959). Foods and feeding habits of the Pedi. Witwatersrand University Press. Johannesburg. 19(12): 49-53.

Raifa, A., Hemmat, K.I.K. Hala, M.S., EL-Bassiouny, M.S. and Mervat, S.S. (2005). Increasing the active constituents of sepals of Rosselle "Hibiscus sabdariffa L." Plant

by applying Gibberellic Acid and BA. *Journal of Applied. Science Research.* 1 (2): 137-146.

Reid, M.S (1985). Ethylene and abscission. *HortScience.* 20: 45-50.

Reynolds, A.G., Pool, R.M., and L.R. Mattick, L.R. (1986). Effect of shoot density and crop control on growth, yield, fruit composition and wine quality of "Seyval blanc". *American Journal Society of Horticultural Science.* 111(1): 55-63.

Robinson, T., Lakso, A.,Stover, E. and Hoying, S. (1998). Practical apple thinning programs for New york. New York Fruit Quarterly. 6(1): 14-18.

Rood B (1994). Uit die veldapteek. Cape town, South Africa. Tafelberg. Pp: 1- 50.

Roodt, V. (1998). Trees and shrubs of the okavango delta. Medicinal uses and nutritional value. Shell Oil Botswana (Pty) Ltd. Gaborone, Botswana. Pp: 151-153.

Salisbury, F.B. and Ross, C.W. (1996). Plant physiology. 5th edition. Oxford University. Wadsworth, Belment. Pp: 309-360.

Salopeck-Sondi, B. Kovac, B. Preberg, T. and Magnus, V. (2002). Developing fruits direct postfloral mophorgenesis in *Helleborus niger aestrium* L. *Journal of Experimental Botany.* 53: 1949-1957.

Sanz, A. Monerri, G. Gonzalez-Ferrer, J. and Guardiola, J.L. (1987). Changes in carbohydrates and mineral elements in citrus leaves during flowering and fruitset. *Physiologia Plantarum*, 69: 93-98.

Smid, A.E. and Peaslee, D.E. (1976). Growth and carbondioxide assimilation by corn as related to K nutrition and simulated canopy shading. *Agronomy Journal.* 68: 904-908.

Stopar, M. (2002). Thinning of 'gala' and 'Golden' delicious apples with BA, NAA nad other combinations. Agricultural Institute of Solvenia. Hacqueta. 17p.

Stoppa, M. (1999). NAA thinning of "small fruited" apple cultivars in combination with CPPU. *Phyton.*39 (3): 85-90.

Street, H.E. and Opik, H. (1983). The physiology of flowering plants: Their growth and development. 3rd Edition. Edward Arnold (Publishers) Ltd. London. Pp: 247-250.

Sunstrum, R. (1997). Intergrating capacity enhancement with research and development projects. V PR&D (Programme memorandum of 5yr plan, 1997-2001). Indigenous Natural Resources for Economic and Environmental Development (INREED). Botswana. Pp: 1-25.

Taiz, L and Zeiger, E. (2002). Plant physiology. 3rd Edition. Sinauer Associates, Inc. Publishers. Sunderland, Massachusetts. Pp: 250-400.

Takana, A., Fujita, K. and Kikuchi, K. (1974). Nutrio-physiological studies on the tomato plant III. Photosynthetic rate of individual leaves in relation to drymatter production of plants. *Soil Science and Plant nutrition.* 20: 173-183.

Taylor, F.W and Kwerepe, B. (1995) Towards domestication of some indigenous fruit trees in Botswana. In J.A. Maghembe Y. Ntupanyama and P.W. Chirwa (Eds) Improvement of indigenous fruit trees of the Miombo woodlands of Southern Africa Nairobi, Kenya. Pp: 113-134.

Tena, M., Lopez valbuena R and Jorrin, J. (1984). Induction of PAL in hypocotyls seedlings by light, excision and sucrose. *Physiology of Plants*. 60: 159-166.

Tromp, J. (1982). Flower bud formation in apple as influenced by various gibberellins. *HortScience*. 57: 277-282.

Turker, G.A. (1993). Introduction in biochemistry of fruit ripening. Eds. Seymour, G. Taylor. J and Tucker, G.A. Eds. Chapman and Hall. London UK. Pp: 1-15.

Ullah, M.A. and Haque, M.A. (2008). Studies on fruiting, bearing habit and fruit growth of Jackfruit germplasm. *Bangaladesh Journal of Agricultural Research*. 33 (3): 391-397.

Usherwood, N.R. (1985). The role of potassium in crop quality. In: Potassium in Agriculture (Ed: R.S. Munson). ASA-CSSA-SSSA, Mandison. WI. Pp: 489-513.

Van Wyk, B. and Van oudtsshoorn, B. and Gericke, N. (1997). Medicinal plants of South Africa. Briza publications. Pretoria, South Africa. 234p.

Webster, D.H. and Brown, G.L. (1980). Trunk growth of apple tree as affected by crop load. *Canadian Journal of Plant Science*. 60: 1383-1391.

Webster, A.D. and Spencer, J.E. (1999). New strategies of chemical thinning of apple (*Malus domestica* Borkh.) cultivars, Queen cox and Royal Gala. *Journal of Horticultural science and Biotechnology*. 74(3): 337-346.

Wehmeyer, A.S. (1976). Edible wild fruits of the Transvaal. Food industry in South Africa. 19(12): 149-153.

Werner, T. Motyka, V. Stmad, M. Schmuling, T. (2001). Regulation of plant growth by cytokinins. *Plant Biology*. 98: 10487-10492.

Williams, M. W. (1979). Chemical thinning of apples. *Horticultural. Review.* 1: 270-300.

Williams, M. W. (1981). Response of apple trees to Aminoethoxyvinylglycine (AVG) with emphasis on apical dominance, fruit set and mechanism of action of fruit thinning chemicals. *Acta Horticulturae.* 120: 137-142.

Williams, M. W. and Batjer, L.P. (1964). Site and mode of action of 1-naphthyl-N-methylcarbamate (Sevin) in thinning apples. *Proceedings American Society of Horticultural Science.* 85: 1-10.

Williams, M.W., Bond, S.A., Hughes, J. and Justin, H.M. (1995). Endothall: A blossom thinner for apples. *Journal of Horticultural Technology.* 5: 257-259.

Williams, M.W. and Edgerton, L.J. (1981). Fruit thinning of Apples and Pears with chemicals. United States Department of Agriculture, Information Bulletin. 289p.

Winkler, A.J., Cook, J.A., Kliewer, W.W. and Lider, L.A. (1974). General viticulture, 2nd edition. University of Carlifornia Press. Berkley. Pp: 138-196.

Wismer, P.T. (1994). Benzyladenine as a fruit thinning agent. Application and effects on cell division and cell size. Msc Thesis. Faculty of Agriculture, Department of Horticultural Science, University of Guelf. Pp: 2-48.

Wright, C.J. (1985). Interactions between vegetative and reproductive growth. School of agriculture. University of Nottingham, Loughborough, UK.

Zapsalis, C. and Francis, F.J. (1965). Cranberry anthocyanins. *Journal of food science.* 30: 396-399.

Zhang, C., Tanabe, K., Tamara, F., Itai, A. and Wang, S. (2005). Spur characteristics, fruit growth and carbon partitioning in to late maturing Japanese pear (*Pyrus*

pyrifolia) cultivars with contrasting fruit size. *Journal of American Society for Horticultural Science*. 130: 252-260.