‘NUTRITIONAL WATER PRODUCTIVITY OF *CUCUMIS MYRIOCARPUS* IN CONTEXT OF CLIMATE-SMART AGRICULTURE

by

PONTSHO EDMUND TSEKE

THESIS

Submitted in fulfilment of the

Requirements for the degree of

Doctor of Philosophy

in

Plant Production

in the

FACULTY OF SCIENCE AND AGRICULTURE

(School of Agricultural and Environmental Sciences)

at the

UNIVERSITY OF LIMPOPO

SUPERVISOR : PROFESSOR P.W. MASHELA

CO-SUPERVISOR : PROFESSOR K.M. POFU

2022

TABLE OF CONTENTS

DECLARATION

I, Pontsho Edmund Tseke, declare that this thesis, hereby submitted to the University of Limpopo for the degree Doctor of Philosophy in Plant Production has not been previously submitted by me or anybody for a degree at this or any other University. That this is my work in design and in execution and that all material contained herein had been acknowledged.

Candidate: Pontsho Edmund Tseke	Signature	Date
Supervisor: Professor P.W. Mashela	Signature	Date
Co-supervisor: Professor K.M. Pofu	Signature	Date

DEDICATION

To my mother (Daisy Kanyane Tseke), son (Tumisho Lotanang Latakgomo) and his mother (Ramaisela Elizabeth Latakgomo) with courteous love.

ACKNOWLEDGEMENTS

Primarily, I would like to appreciate and extend words of thankfulness and heartfelt appreciation to the Almighty God for protection and mercy to me on achieving this milestone in my lifetime. I am deeply grateful to my supervisory team Professors P.W. Mashela and K.M. Pofu for their inspiration, leadership and advice towards the achievement of this goal, may the Almighty God eternally bless you on this path of transferring skills with absolute determination and love for what you are doing, difficult as it might be! I am truly inspired! To my friend and research colleague Dr MY Maila, you will always be appreciated for the role you played during my research studies. I acknowledge the l assistance that I received during laying and harvesting of experiments from service workers at the Green Biotechnologies Research Centre of Excellence, namely, Ms Mawasha M.A., Mr Letsoalo L.T., Mr Letsoalo E.M., Ms Seabelo S.M., Ms Makwela E.M. and Ms Modingoane R.T. In addition, I am grateful to the Land Bank Chair of Agriculture – University of Limpopo, the National Research Foundation of South Africa (grant no.136124), the Flemish Interuniversity Council (VLIR) of Belgium and the Agricultural Research Council-Universities Collaboration Centres, for financial support for financial support in the form of bursaries and research consumables, without which I could not have completed the study.

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABSTRACT

Malnutrition continues to pose a serious threat to humankind and their animals, and this is inextricably exacerbated by the irrevocable incident of climate change. Due to the latter, adaptation efforts for achieving the first Goal of the eight Millennium Development Goals appear to be unequivocally remote. The first Goal intends to eliminate hunger by ensuring that humankind is provided with nutritional food, thereby eliminating hidden hunger, which is malnutrition. Globally, malnutrition is widespread since best agricultural practices that increase yield do not necessarily correlate with increased nutrition in plant produce. The concept of nutriconclusiotional water productivity (NWP) links the accumulation of certain functional nutrients to the amount of water (m^3) used in their bio-accumulation. Due to high nutrient deficiencies in exotic crops that were bred for high yield under best agricultural practices, attention is being redirected to certain indigenous underutilised crops, which had for centuries survived marginal conditions. Such underutilised crops had been, without empirically-based information, viewed as potential candidates for positively contributing towards the achievement of the first Goal of the eight Millennium Development Goals *in lieu* of biofortification. In the northern region of Limpopo Province, South Africa, one such underutilised crop is wild cucumber (*Cucumis myriocarpus*), a leaf vegetable indigenous to the northern semi-arid region of South Africa. The plant, due to its short stem, has attributes of a “stolon”, with drought-tolerance and nematode-resistant attributes, and had served as a leaf vegetable with various medicinal uses among the local people. The plant, therefore, has inherent potential benefits of promoting functional nutritional security under various conditions. Due to a lack of empirical information to advance the production of this underutilised crop, a decision was taken to investigate the production of the

crop in relation to NWP of mineral malnutrition (MMN) elements and micronutrient malnutrition (MNMN) substances under conditions that had been predicted through modelling to be threatening inland South Africa with the advent of increasing intensity of global warming. The objectives of the study were, therefore, five-fold, namely, to investigate the responses of NWP of MMN elements in *C. myriocarpus* leaf tissues to (1) planting density, (2) irrigation interval, (3) population density of root-knot (*Meloidogyne* species) nematodes, (4) chloride salinity and (5) inoculation with arbuscular vascular mycorrhiza (VAM) under various conditions. In all objectives, soil water content was measured using soil moisture profile probes, connected to an HH2 Moisture Meter (Delta-T Devices, UK). At 56 days after initiating the treatments, 20 healthy mature leaves were harvested per plant, oven-dried at 70°C for 72 h and ground in a Wiley mill, with the extraction of nutrient elements performed using the digestion method from 0.4 g material. Selected MMN elements were quantified using the Atomic Absorption Spectrophotometer ICPE-9000, with related NWP values. In three of the five objectives, MMN elements versus independent variables (*i.e*. planting density, irrigation interval or salinity) exhibited significant ($P \leq 0.05$) quadratic relationships, which offered the opportunity to compute the optimisation point using $x = -b_1/2b_2$ relations, derived from $Y = ax^2 + bx + c$ quadratic equation. In contrast, in the remaining two objectives, MMN elements versus independent variables (*i.e*. nematodes or Biocult) did not exhibit significant relationships, except that occasionally, population densities of *M. javanica* linearly reduced NWP of Fe. In conclusion, the findings in the current study demonstrated for the first time that nutritional quality in harvestable produce, as articulated using the concept of NWP of MMN elements is not aligned with the philosophy of high yield (quantity) as pronounced in best agricultural practices. The provided models showed

that although certain cultural practices could be disadvantageous to the producers in terms of quantity, stressful conditions could to a certain extent be advantageous to consumers in terms of the nutritional value of the produce.

JOURNAL ARTICLES ORIGINATING FROM WORK IN THE THESIS

1. Tseke, P.E., Mashela, P.W. and K.M. Pofu. 2018. Influence of planting density per hole drip irrigation on selected nutrient elements in indigenous leafy vegetable wild cucumber (*Cucumis myriocarpus*). *Transylvanian Review* 26:24-29.
2. Tseke, P.E**.** and P.W. Mashela. 2018. Growth response of leaf vegetable *Cucumis myriocarpus* to chloride salinity. *Research on Crops* 19:72-79.
3. Tseke, P.E. and P.W. Mashela. 2018. Influence of arbuscular mycorrhiza fungi on growth of *Cucumis myriocarpus* indigenous leaf vegetables. *Research on Crops* 19:289-293.

CHAPTER 1
RESEARCH PROBLEM

1.1 Background

1.1.1 Description of the research problem

Climate-smart agriculture focuses on mitigation strategies that match crops with predicted adverse scenarios related to climate change (Morton, 2007). Climate change inland South Africa presents a gloomy future with respect to the availability of mineral malnutrition (MMN) elements and micronutrient malnutrition (MNMN) substances, which might completely change when crops are subjected to extremes such as drought, high ambient temperatures and salinity induced by floods. The uncertainties induced by climate change call for mitigation strategies that include research and development of drought-tolerant indigenous crops for sustainable and functional food security, using innovative tools and concepts (Chivenge *et al*., 2015; FAO, 2014; Mashela *et al*., 2016). The concept nutritional water productivity (NWP) has since the inception of climate-smart philosophy gained popularity in functional food security in order to help in separating food quality from quantity (Mabhaudhi *et al*., 2016a; Renault and Wallender, 2000). As an innovative concept, NWP provides empirical-based information on the amount of water used in the assimilation of MMN elements and MNMN substances in edible parts of the crops (Mabhaudhi *et al*., 2016a; Renault and Wallender, 2000). Generally, MMN includes Ca, Mg, Na, K, P, Cl, Zn and S, which are central to human health and could be unavailable in crops cultivated for high volume as prescribed by high market demand. Indigenous crops with limited market demand (Hoque and Butler, 2016) might then supply such MMN elements and MNMN substances. In reality, MNMN substances from crops include protein, fat, moisture, carbohydrate and fibre (Renault and Wallender, 2000), along

with vitamin A, B and C and other unidentified factors, which are collectively referred to as adaptogens (Renault and Wallender, 2000). In most cases, high-volume yielding crops do not provide consumers with most of the listed elements and substances.

Historically, indigenous leafy vegetables are known to be excellent sources of MMN elements, with limited information regarding the accumulation capabilities using the least amount of available water under extreme conditions that had been predicted under climate change (FAO, 2014). For example, wild cucumber (*Cucumis myriocarpus* Naude.), with a wide range of pharmacological properties had been widely used as a leafy vegetable in Africa, whereas roots and fruit are used in traditional medicine (Mphahlele *et al*., 2012). The plant is indigenous to Botlokwa, in Limpopo Province, South Africa (23°39’55”, 28°97’46”) as a perennial plant, with a robust and deep root system, but a very short stem that does not dieback under extreme winter conditions (Kristkova *et al*., 2003). The plant is a drought-tolerant runner and leaves can be shade-dried to serve as indispensable in functional food security during uncertainties presented by climate change. However, to date *C. myriocarpus* had not been characterised in terms of NWP under biotic and abiotic factors that had been viewed to predict conditions that would prevail inland South Africa due to climate change (Steyn *et al*., 2014).

1.1.2 Impact of the research problem

The concept, NWP explains the interconnection of water-food-nutrition-health as a way of developmental agriculture based on the strategies intended to improve human nutrition and health (Chibarabada *et al*., 2017). Additionally, the NWP

concept proposes that the connections between water-food-nutrition-health should be made unambiguous in designing agricultural interventions aimed at improving food and nutrition security. Despite this acknowledgment, global insufficiencies of MMN elements in food production continue to affect people across all socio-economic groups (WHO, 1996). Worldwide, 795 million people, particularly children and pregnant women, die annually due to deficiencies of one or more MMN elements and MNMN substances (FAO, 2014). Worldwide, as well as in South Africa, functional food security differs across different geographical areas and socio-economic spectrums (Wenhold *et al*., 2007). Diseases associated with deficiencies of MMN elements are numerous, with pellagra, rickets, anaemia, high blood pressure, sugar diabetes and scurvy, being commonly encountered (Dean *et al*., 2019; WHO, 1992).

1.1.3 Possible causes of the research problem

The main causes include what had been termed best agricultural practices, which in a contest of climate-smart agriculture are unsustainable for producing high yields, where nutrient elements had been proportionally reduced in favour of increased assimilation of carbohydrates, especially starch (Harmon and Swanson, 2020). Traditionally, water-use efficiency has been focusing on biomass and arguably, at the exclusion of MMN elements. Incidentally, NWP is an essential concept in functional food security. Historically, in many countries, indigenous plants had been viewed as weeds (Mashela and Mollel, 2001). Climate-smart agriculture is an integrated tactic for managing lands such as cropland in order to address the interspersed challenges of climate change and food security (Gamba *et al*., 2020; Lipper *et al*., 2014; Thierfelder *et al*., 2017). Climate-smart agriculture, when

encompassing NWP, could serve an important role in ensuring that available food meets the minimum requirements of food security in the context of using cultural practices that would, instead of diminishing, improve food nutrition. In the NWP context, most indigenous plants are being assessed as prescribed in various government-sponsored policies for their nutritional and functional food benefits under the auspices of underutilised crops (Mabhaudhi *et al*., 2016b). Generally, NWP related to MMN elements in most indigenous plants had not been assessed due to the availability of exotic crops prior to the advent of the predicted conditions of climate change.

1.1.4 Possible solution to the research problem

Subjecting underutilised crops to conditions simulating those under climate change in properly designed experiments would generate appropriate knowledge with respect to climate-smart agriculture. Incidentally, NWP could also be used as an assessment tool of the potential of underutilised indigenous plants as alternative crops for enhancing sustainable functional food security inland South Africa, where conditions to be imposed by climate change had been reasonably predicted (Steyn *et al*., 2014).

1.1.5 General focus of the study

In rural and semi-rural regions of Limpopo Province, South Africa, the deficiency of MMN elements is a major challenge, particularly in low-income indigent households. In such regions, low-input indigenous leaf vegetables such as *C. myriocarpus* could play indispensable roles in nutrition, food security and traditional medicine. In the current study, the focus had been a deliberate move of matching certain cultural

practices for successful production of *C. myriocarpus* to the previously predicted abiotic and biotic stresses with the intended aim of improving the NWP of this underutilised crop, which affect food security in the world, with the use of a little amount of irrigation water.

1.2 Problem statement

Cucumis myriocarpus has the potential of serving as an indigenous leaf vegetable that could supplement MMN elements. Cultural practices such as managing planting density, extremes in soil moisture content, salinity and using vesicular-arbuscular mycorrhiza (VAM) could be some of the cultural practices that could have a direct bearing on NWP of *C. myriocarpus*, thereby affecting NWP of MMN elements. Increasing planting density using the 3S planter improved certain plant variables in indigenous vegetable nightshade (*Solanum retroflexum* Dun.) (Mabotja, 2019). Growth variables of *C. myriocarpus* versus increasing salinity or VAM previously exhibited positive quadratic relations (Legong, 2017). Salinity invariably results in imbalances of certain MMN elements and MNMN substances (Nedjimi, 2009). In certain plants, root-knot nematodes (*Meloidogyne species*) negatively affect some MMN elements (Mashela *et al*., 2016), and possibly MNMN substances. In addition, VAM is known to improve some MMN elements (Abbott *et al*., 1992; Begum *et al*., 2019; Liu *et al.,* 2014; Liu *et al*., 2018). In addition, abiotic and biotic factors are known to affect water use efficiency (WUE) in plants (Steyn *et al*., 2014), but stimulate NWP of Ca, K, Mg, P, Na, Fe and Zn (Hadebe *et al*., 2021).

1.3 Rationale of the study

Predictions of climate change inland South Africa suggested that high temperatures, extended droughts and intermittent floods would abound (Steyn *et al*., 2014). Generally, when floodwater evaporates, the resultant effect is the accumulation of salinity ions (Li and Shi, 2019). Inland maximum temperatures would increase by 2°C from 38°C to as high as 46°C by 2030, which would be instrumental to the accumulation of salinity ions. In the context of climate-smart agriculture, *C. myriocarpus* had the potential to add value to functional food security in terms of MMN elements and MNMN substances inland South Africa. Thus, empirically derived information on the NWP of *C. myriocarpus* under diverse environmental conditions would invariably increase the potential acceptance of this underutilised plant as a future crop for functional food security.

1.4 Purpose of the study

1.4.1 Aim

Establishment of agronomic protocols for improving NWP of *C. myriocarpus* in the context of climate-smart agriculture to improve its adoption for serving functional food security.

1.4.2 Objectives

1. To determine whether planting density/hole of drip irrigation system would affect NWP of MMN elements in *C. myriocarpus* under field conditions.
2. To investigate whether irrigation interval would influence NWP of MMN elements in *C. myriocarpus* under field conditions.
3. To test whether infection with *Meloidogyne* species would affect NWP of

MMN elements in *C. myriocarpus* under greenhouse and microplot conditions.

4. To establish whether increasing salinity would affect NWP of MMN elements in *C. myriocarpus* under greenhouse conditions.
5. To assess whether inoculation with increasing levels of VAM would affect NWP of MMN elements in *C. myriocarpus* under greenhouse conditions.

1.4.3 Null hypotheses

1. The planting density/hole of drip irrigation system would not affect plant growth and NWP of MMN elements in C*. myriocarpus*, under field conditions.
2. Irrigation interval would not influence NWP of MMN elements in *C. myriocarpus* at optimum planting density/hole of drip irrigation system under field conditions.
3. Infection with *Meloidogyne* species would not affect NWP of MMN elements in *C. myriocarpus* under greenhouse and microplot conditions.
4. Increasing salinity level would not affect NWP of MMN elements in *C. myriocarpus* under greenhouse conditions.
5. Inoculation with increasing VAM levels would not affect NWP of MMN elements in *C. myriocarpus* under greenhouse conditions.

1.5 Reliability, validity and objectivity

Reliability was ensured by the use of statistical levels of significance as derived with analysis of variance. Validity was achieved through conducting experiments using randomised completely block design arrangements (Little and Hills, 1978). Objectivity was achieved by ensuring that the findings were discussed based on

empirical evidence in order to eliminate potential subjectivity (Leedy and Omrad, 2005).

1.6 Bias

The bias would be minimised by ensuring that the experimental error in each experiment was reduced through adequate replications and randomisation. In addition, treatments were assigned at random within the selected research designs (Leedy and Ormrad, 2005).

1. 7 Structure of the thesis

The thesis has seven chapters, where Chapter 1 introduced the research problem and Chapter 2 presents a Literature Review on the work done on the problem statement. Subsequent research chapters addressed the five objectives sequentially. Each research chapter ended with the synthesis regarding the new findings and then the conclusion, along with a statement unambiguously stating whether the null hypothesis was accepted or rejected based on the overall findings. In order to improve the flow from one chapter to the other, a sentence was included to link up the current chapter and the next by summarising the major theme of the subsequent chapter. In Chapter 8, the significance of the study findings was summarised and then integrated to provide their significance, followed by the identification of potential gaps and recommendations for future research. In addition, in the final chapter, the take-home message with respect to the major cultural practices that would improve nutrition security through the test crop was amplified. Citations in text and subsequent reference listing adopted the Harvard style of using the author-alphabet.

In the subsequent chapter to the current, work done on the problem statement with regards to improving NWP through cultural practices was reviewed.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Nutritional food security exists when human beings have access to nutritious food that confers minimum daily dietary needs for a healthy life (FAO, 2013). Nutritional status is known as the physiological state of an explicit individual, which results from the affiliation between nutrient intake and requirements (Annandale *et al.*, 2012). The potential crops for human consumption had been considered on the basis of nutrient composition in edible portions, whereas the current aim in crop production is to focus on high volume of edible portions (Chibarabada *et al*., 2017). Essential nutrient elements and substances are those without which human beings would not survive. In most cases, vegetables serve as the main source of essential micronutrients and vitamins. Micronutrient deficiencies in food affect both rural and urban dwellers equally, with designated groups such as children and pregnant women enduring the most of such deficiencies (Nyathi *et al*., 2018). In broad terms, deficiencies in the intake of nutrients are referred to as malnutrition (Müller and Krawinkel, 2005). Malnutrition is common in situations where poverty is prominent, social isolation, or substance abuse. Specifically, deficiency of mineral malnutrition (MMN) elements causes diseases such as anaemia, diabetes, hypertension, hypokalaemia and osteoporosis, whereas deficiencies of micronutrient malnutrition (MNMN) substances had been associated with diseases like beriberi, night blindness and pellagra (Primm, 2013).

2.2 Work done on the problem statement.

The major focus of the review was based on five objectives of the study, reduced to planting density, irrigation interval, nematode infection, salinity and filamentous fungus inoculation, all with reference to MMN elements.

2.2.1 Effects of planting density on nutritional water productivity

Although studies on effects of planting density on NWP are hard to come by, the general belief had been that increasing planting density would duly increase interspecific competition for soil moisture. The latter would in turn affect the availability of essential nutrient elements, along with photosynthetically active radiation (PAR) (Hartmann *et al*., 2011; Kamel *et al*., 1983; Yarnia, 2010). In contrast, low planting density might result in high chlorophyll content due to less competition for nutrient and limited shading.

Adaptation of agronomic practices in relation to planting density could improve water use efficiency (Debaeke and Aboudrare, 2004), which would be commendable in the use of 3S planter (Chapter 1). The gadget was developed to improve cultural practices in smallholder farming systems where one plant was previously raised per hole drip irrigation system. In general, most of the available studies focused on the effects of planting density on plant vegetative and reproductive growth, without information on how this factor would influence NWP in various crops. In most cases, crops in the Graminae Family, like maize (*Zea mays* L.), are subjected to high planting densities.

2.2.2 Effects of irrigation interval on nutritional water productivity

Irrigation interval has direct effects on the degree of the availability of soil moisture and therefore nutrient elements, on various plants. Generally, an extended irrigation interval might result in the accumulation of salinity ions, thereby reducing osmotic potential, with water being restricted to entering root cells (Salisbury and Ross, 1992), along with various imbalances of essential nutrient elements and malnutrition substances. Extended irrigation interval in grain legumes such as groundnut (*Arachis hypogea* L.), Bambara groundnut (*Vigna subterranea* L. Verdc.) and dry bean (*Phaseolus vulgaris* L.), was observed to be related in the reduction of total proteins in seeds (Chibarabada *et al*., 2017). Previously, Nyathi *et al*. (2016) observed that extended irrigation intervals in Amaranths (*Amaranthus cruentus* L.*)*, spider flower (*Cleome gynandra* L.) and Swiss chard (*Beta vulgaris* L.) reduced NWP of certain MMN elements. In contrast, extended irrigation interval was observed to increase NWP of Al, Fe, Mn, Na, P, Ca, K, Mg, total N and total S in taro (*Colocasia esculenta* L. Schott) plants (Shelembe, 2020). In chamomile (*Matricaria chamomilla* L.) increasing water stress enhanced the accumulation of Zn in leaf tissues (Ullah *et al*., 2019). Hadebe *et al.* (2021) observed that increased irrigation interval augmented NWP_{-Zn} in three sorghum cultivars, namely, 'PAN8816', 'Marcia' and 'Ujiba'. Thus, the three cultivars could, in terms of nutrition, be ideal when bred for drought tolerance. In marigold (*Tagetes erecta* L.), high Zn content in leaf tissues was observed in cultivars with high drought tolerance (Pirzad and Shokrani, 2012). In certain sorghum genotypes, it was also observed that increased Fe accumulation in leaf tissues was associated with extended irrigation interval (Abdelhalim *et al*., 2019). Additionally, certain nutritional substances in sweet sorghum, such as sugar content

and organic acids, increased when crops were exposed to extended water stress (Ripoll *et al*., (2014). As observed in certain fruit crops, certain MMN elements and MNMN substances responded favourably to extended irrigation intervals (Schopfer, 2001), which contradict the common belief that water stress is not a best agricultural practice. Renault and Wallender (2000), in the review of NWP among different crops, without linking the NWP values with any cultural practice, provided detailed information which showed that plant produce differ widely in this attribute.

2.2.3 Effects of nematode infection on nutritional water productivity

Plant nematodes are widely destructive in crops. The root-knot nematodes (*Meloidogyne* species) had been widely investigated due to its wide host range and visible destructive-related symptoms (Fourie *et al*., 2001; Jagadeesh, 2011). In certain crops without resistance to *Meloidogyne* species such as watermelon (*Citrullus lanatus* L.) and potato (*Solanum tuberosum* L.) yield reduction due to nematodes is from as high as 50% to complete crop failure (Lima *et al*., 2018). The first observation that linked nematode infection with the deficiency of nutrient elements in leaf tissues was in citrus trees infected by the citrus nematode (*Tylenchulus semipenetrans* Cobb) in Florida, USA (Mashela, 1992). Subsequent investigations demonstrated that infection by *T. semipenetrans* actually increased Na and Cl ions in leaf tissues, but reduced the two osmoticum ions in root tissues, along with K in both root and leaf tissues (Mashela and Nthangeni, 2002; Mashela *et al*., 2016). Infection of *Meloidogyne* species on beetroot (*Beta vulgaris* var. cicla) cultivar was shown to reduce K in leaf tissues (Mashela *et al*., 2016). Similarly, infection of banana (*Musa paradisiaca* L.) plants by *Meloidogyne* species was believed to have some effects on uptake of nutrient elements (Talwana *et al*., 2003). The influence of

accumulation of nutrient elements in crops could be affected by factors such as the degree of plant resistance, nematode species, nematode population densities and the duration of exposure to infection (Abbasi and Hisamuddin, 2014; Mashela, 1992).

In the first report on NWP-nematode relations (Ramputla, 2018), in berries of chilli pepper (*Capsicum annuum* L.), NWP of MMN elements versus increasing densities of *Meloidogyne* species exhibited highly significant density-dependent growth (DDG) patterns. In DDG patterns, invariably have three distinct phases, namely, the stimulation phase, neutral phase and inhibition phase (Liu *et al*., 2003). Ramputla (2018) demonstrated that at low population densities *M. incognita* stimulated NWP for Ca, Mg, K, Fe and Zn in chilli pepper berries. In contrast, at high population densities *M. incognita* inhibited NWP of Ca, Mg, K, Fe and Zn. Since NWP is a proportion of accumulate nutrient to water (m^3) used to accumulate that particular element, during the stimulation phase, it could imply that when the accumulated nutrient remains constant, water used decreased, whereas on the inhibition phase the opposite could be true. The mechanism involved in decreasing water uptake at low population densities and increasing water uptake in high nematode population densities in order to help in conceptualising NWP-nematode relations is currently not clear. Most importantly, in terms of DDG patterns, should the DDG patterns not hold, it could imply that the relation was either in the neutral phase or outside the stimulation phase as conceptualised previously (Mashela *et al*., 2017).

In another study by Mashela *et al*. (2016), which was not designed for NWP, it was demonstrated that Ca, Cl, Fe, K, Na and Zn in leaf tissues of African ginger (*Siphonochilus aethiopicus* (Schweif.) B.L. Burt) versus increasing population

densities of *M. incognita* exhibited DDG patterns. Since *C. myriocarpus* was shown to be highly resistant to *Meloidogyne* species in South Africa, but moderately resistant to *M. incognita* in China (Liu *et al.*, 2015), it would be interesting to observe how NWP would be affected by *M. incognita* in the current study.

2.2.4 Effects of chloride salts on nutritional water productivity

Excessive floods associated with climate change are renowned for their excessive salinity when the flood water eventually evaporates or recedes (Li and Shi, 2019). High salt concentration in soil solution induces reverse water potential, where water moves down the osmotic gradient from root tissues into soil solution (Munns, 2002). Mashela and Nthangeni (2002) showed that salinity induces high reverse osmotic potential in soil, resulting in disproportionate accumulation of osmoticum ions (Na, Cl and K) in certain organs, particularly roots and leaves. Generally, the accumulation of many Na and Cl ions in soil solutions invariably inhibits the uptake of most mineral ions (Acosta-Motos *et al*., 2014; Lee and Van Iersel, 2008). In most plant species, salinity damages plants by preventing elongation of cell growth, ion toxicity, affecting the rate of photosynthesis, inhibiting shoot and root lengths and overall, inhibition of leaf development (Läuchli and Grattan, 2007). Salinity also causes nutrient deficiency by decreasing P uptake since phosphate ion precipitates with Ca ion and oxidative stress into unavailable forms to plants (Bano and Fatima, 2009). Taiz and Zeiger (2002) demonstrated that the accumulation of salinity ions in plant organs also inhibits protein synthesis and photosynthesis, damages chloroplasts and deactivates certain enzymes, which could be viewed to be affecting biosynthesis and subsequent accumulation of certain MNMN substances. Mashela and Nthangeni (2002), using a split-root system in citrus seedlings, provided the conceptualisation

mechanism through which the osmoticum ions are displayed from roots, with Na and Cl ions accumulating in leaf tissues. Due to low toxicity tolerance to Cl (0.25%) and Na (0.1%), leaf abscission that results in sparse foliage in citrus trees with slow decline is part of avoidance mechanism to avoid ion phytotoxicity (Mashela, 1992). The model also provided the potential mechanism through which K is reduced in both leaf and root tissues, while starch accumulated in affected root tissues, which contributed to the desired achievement of the mechanism output (Mashela and Nthangeni, 2002).

Observation in other studies confirmed that lower levels of salinity stimulate the accumulation of Ca, Mg, Fe, Mn, Cu, Zn and Na in amaranth (*Amaranthus tricolor* L.) leaf tissues when plants were exposed to increasing salt concentration (Legong, 2017; Sarker *et al*., 2018). Legong (2017), without assessing the accumulation of nutrient elements, observed that growth variables of *C. myriocarpus* versus increasing chloride level exhibited positive quadratic relations, characterised by DDG patterns. In most plants, photosynthesis rate or yield produce versus salinity exhibited positive correlation (Sudhir and Murthy, 2004). Tawfik (2008) observed that increasing levels of salinity, despite the reduction in plant growth, increased amino acids, especially collagen content in leaf tissues. Soil salinity also affects biochemical processes such as photosynthesis, therefore, interfering with carbohydrates, which are supplied exclusively through the process of photosynthesis (Sultana *et al*., 2000; Taffouo *et al*., 2010). The effects of salinity on MMN elements and MNMN substances depend on salt concentration as well as crop plant species, but with limited information on the effects of soil salinity on NWP of MMN elements.

2.2.5 Effects of vesicular arbuscular mycorrhizae on nutritional water productivity

Generally, vesicular arbuscular mycorrhizae (VAM) have mutual symbiotic relationships with roots of certain plants which benefit the host plant in improving MMN elements and MNMN substances, particularly in soils that have low nutrient elements (Haryantini *et al*., 2019, Legong, 2017). Plants with VAM can be able to adjust their root systems by spreading VAM hyphae beyond the physical reach of roots, which therefore diversifies the capacity to absorb nutrient elements and therefore the biosynthesis of MNMN substances, increasing the performance of plants under marginal conditions (Sukmasari *et al*., 2021; Wang and Qui, 2006). Plants associated with mycorrhizae are more effective in nutrient absorption, particularly for P elements, with increased absorption of N, S and Zn (Ingraffia *et al*., 2019). Generally, VAM colonisation of roots is well-known to increase P element uptake, particularly in soils with low available P (Goswami *et al*., 2018). Since VAM extends its mycelia from the root system into soil solutions, facilitating the movement of substances from soil solution into plants, while beneficially receiving carbohydrates from the plant (Al-Karaki, 2000; Jakobsen and Rosendahl, 1990; Sukmasari *et al*., 2021). Although plant growth variables of *C. myriocarpus* versus increasing VAM exhibited significant quadratic relations (Legong, 2017), the experiments were not designed to assess NWP of MMN elements.

2.3 Work not yet done on the problem statement.

Nutritional water productivity of *C. myriocarpus* as affected by various biotic and abiotic factors, primarily those that could be managed by the farmer, had not been documented. Most abiotic and biotic factors that were eventually investigated in the

current study were selected due to their predicted association with climate change in attempts to establish how such practices could be aligned with the production of *C. myriocarpus* in context of climate-smart agriculture. In addition to improving the understanding of how such abiotic and biotic factors interact with the test plant in relation to improving produce quality, the aim was mainly to enhance the formulation of best management strategies that would result in improved produce quality under marginal conditions imposed by climate change. In the following chapter, the NWP of MMN elements in harvestable and edible leaves of *C. myriocarpus* was investigated in relation to the planting density of the test plant.

CHAPTER 3

RESPONSE OF NUTRITIONAL WATER PRODUCTIVITY TO PLANTING DENSITY IN *CUCUMIS MYRIOCARPUS*

3.1 Introduction

Planting density is an important cultural practice that influences the microenvironment and inherently disrupts progression, development, and yield potential of crops. This cultural practice can be manipulated by the farmer or could be ascribed to failure of seed germination or fatal damaging of seedlings. Inadvertently, above, and below optimum planting density can drastically reduce plant growth and the harvestable produce. Above the optimum planting density, various factors could affect plant performance through competition for soil moisture and light, whereas below optimum density could also affect moisture by increasing evapotranspiration and the accumulation of salts in the rhizosphere (Zhao *et al*., 2011). A 3S planter gadget was developed with the aim of maximising water use efficiency per drop of water released by drip irrigation system. The gadget allows the production of at least one plant to a maximum of nine per hole drip irrigation (Mabotja, 2019). Generally, smallholder farmers raise one plant/hole drip irrigation (HDI), which exacerbates the incidence of poor yield in such farming systems. Preferably, several plants/HDI should be raised to improve water use efficiency per drop of water. However, as indicated above, high planting density/HDI could be a source for competition, thereby depriving plants of requisite nutrients in edible produce. Nutritional water productivity (NWP) had been used to link the accumulated mineral malnutrition (MMN) elements and macronutrient malnutrition (MNMN) substances in produce per m^3 water used during

assimilation (Chibarabada *et al*., 2017; Nyathi *et al*., 2018; Renault and Wallender, 2000). Due to the generally adverse conditions in which most indigenous leaf vegetables grow, such plants had been adapted for such conditions and might at any time contain high MMN elements. A good example of such crops is wild cucumber (*Cucumis myriocarpus* Naude.), which is an indigenous leaf vegetable to northern region of South Africa (Kristkova *et al.,* 2003). The plant has a deep root system, with a short erect stem, but with creeping attributes, all conferring drought-tolerance to the plant (Shiundu, 2002). In *C. myriocarpus*, cucurbitacin A ($C_{32}H_{46}O_9$) is localised in fruit and root tissues, without any trace of the chemical in leaf tissues (Jørn *et al*., 2006; Shadung, 2016).

Intra-specific competition for resources such as water, nutrient elements, photosynthetically active radiation and carbon dioxide in high planting density systems could decrease the productivity in cropping systems (Procópio *et al*., 2013). The latter could be a challenge when cropping is targeted to curb malnutrition elements in marginalised communities, where crops are intended for use in food security programmes. High planting density/HDI could reduce productivity per plant, whereas total productivity could be much higher as observed in most large-scale commercial farming systems. Limited information is currently available on how high planting density affects MMN elements in crops. Information on increasing planting density/HDI in relation to NWP of Ca, K, Mg, P, Zn, Fe and Na in edible portion of *C. myriocarpus* is yet to be documented. The objective of the study was therefore to determine whether planting density/HDI system would affect NWP of MMN elements in *C. myriocarpus* under field conditions. The null

hypothesis suggested that the planting density/HDI system would not affect NWP of MMN elements in *C. myriocarpus* under field conditions.

3.2 Materials and methods

3.2.1 Description of the study site

A field trial was conducted at the Green Biotechnologies Research Centre of Excellence, University of Limpopo, South Africa (23°53'10"S, 29°44'15"E). The location has hot and dry summers (Nov-Jan), with daily maximum temperatures ranging from 28 to 38°C. The average annual rainfall had previously been less than 500 mm, with the distribution skewed towards summers. The area had a slope of 0.05%, containing Hutton soil with loamy soil (65% sand, 30% clay, 5% silt), 1.6% organic C and EC_e 0.148 dS/m). Six months before sowing, dolomitic lime, comprising Ca carbonate and Mg carbonate, was evenly spread on the field and incorporated into the soil using a mouldboard plough. The experiment was initiated during autumn (Feb-April) 2018 and validated in 2019.

3.2.2 Experimental design

Nine treatments, namely, 1, 2, 3, 4, 5, 6, 7, 8 and 9 plants/HDI, were arranged in a randomised complete block design, with eight replications. Treatments were blocked for shading by windbreak trees in the morning and in the afternoon. The experimental site measured 12.3 m long and 6.0 m wide (73.8 m^2), with plant spacing being at 0.60 m × 1.0 m. A 3S planter, was used to align holes around the drip hole in three parallel rows. The first row was alongside the drip line with three spikes, whereas the other two rows were each opposite the drip line (Figure 3.1). All spacing within the spikes was at 6 cm.

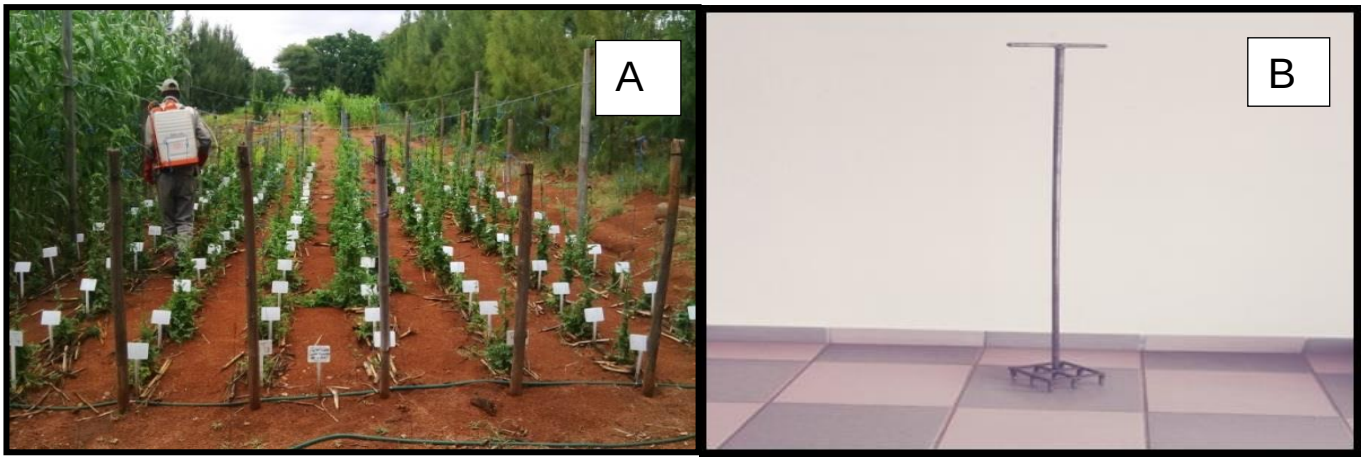

Figure 3.1 **A**-Field layout and **B**-3S planter tool at the Green Biotechnologies Research Centre of Excellence, University of Limpopo.

3.2.3 Procedures and cultural practices

Matured fruit from *C. myriocarpus* were collected from the local cultivated field, washed in tapwater, cut into pieces and seeds removed from the fruit. Seeds were shade-dried for 72 h prior to sowing in 200-hole seedling trays containing Hygromix-T (Hygrotech, Pretoria) growing medium and germinated in a dark room with temperature averaging 38ºC. Etiolated seedlings had emerged in 5 days, with trays transferred to the greenhouse. At three-leaf stage, seedlings were hardened-off under direct sunlight outside the greenhouse by withholding irrigation water. Seedlings were monitored until at least 50% seedlings were wilted, with trays transferred to a shaded area and irrigated to allow for the recovery of wilting seedlings. After recovery, trays were returned to direct sunlight to initiate the wilting process. At night, trays were left outside the greenhouse. A week after hardening-off

through intermittent withdrawal of irrigation and re-irrigation, uniform seedlings were transplanted in the field with the aid of a 3S planter used for making holes per HDI. A PR2 Profile probe access tubes (PR2/4 Model, UK) was installed at each drip irrigation station to monitor soil moisture content per planting density. A rain-gauge was installed at 2 m away from the edge of the cultivated plot. A drip irrigation system was designed to irrigate the field trials at 1 000 ml output water/hour. Approximately 6-cm deep holes were made at each drip hole and seedlings were inserted at one per hole. Soon after transplanting, 5 g cutworm bait (Sodium Fluosillicate 100 g/kg) was applied around the stem of each seedling. Five days after transplanting, each treatment/HDI was fertilised by placing 5 g NPK 2:3:2 (26) + 0.5% Zn + 5% S and 5% Ca in a 5 cm hole at 5 cm away from the stem to provide macro- and micro-elements. During the same time, seedlings were supplemented with 2 g 2:1:2 (43) Multifeed (Nulandis, Witfield, South Africa) in a different hole to provide 0.70 mg N, 0.64 mg K and 0.64 mg P, 1.8 mg, 1.5 mg Fe, 0.15 mg Cu, 0.7 mg Zn, 2 mg B, 6 mg Mn and 0.14 mg Mo/ml tapwater. The plot was irrigated with 2 000 ml for 2 h each in the morning and in the afternoon every other day. The field capacity (FC) of the soil was 28.3% and permanent wilting point (PWP) was 11.7%. Scouting for greenhouse whiteflies (*Trialeurodes vaporariorum* Westwood) was done daily and plants sprayed with 1 ml Kemprin 200 EC (active ingredient cypermethrin) per 10 L water when populations increased to above 10 whiteflies per plant. Red spider mites were managed with 400 ml per ha Seizer® 100 EC (bifenthrin). Plants were staked to allow for upward movement of the vines.

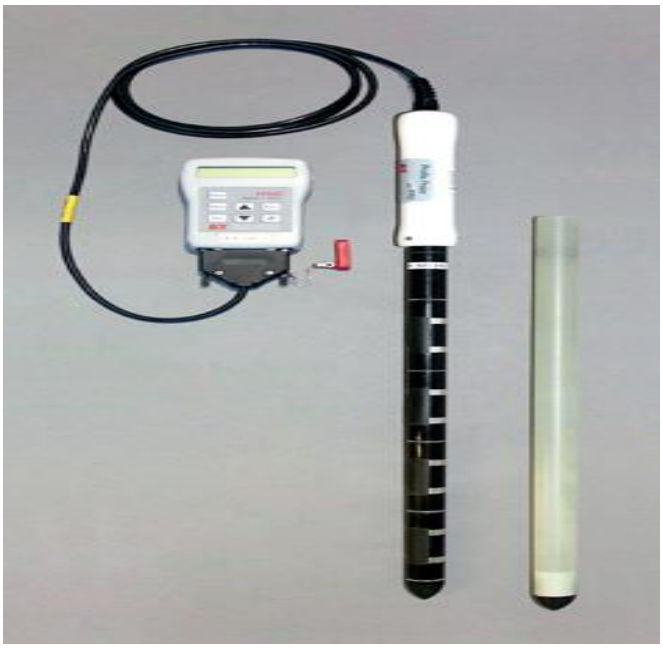

Figure 3.2. HH2 moisture meter and PR2 profile probe for soil moisture measurement.

3.2.4 Data collection

Soil water content was measured weekly prior to irrigation at 300-400 mm depths using Profile Probe that was connected to HH2 Moisture Meter (Delta-T Devices, UK). Intact soil clods collected from each planting station were weighed and recorded for fresh mass and then oven-dried at 105°C for 24 h for dry soil mass. The bulk density was then determined using the intact core method (Blake and Hartge, 1986), the particle size distribution was established using the Bouyoucos method (Bouyoucos, 1962) and soil water content through the gravimetric method (Little *et al*., 1998). Volumetric soil water content was calculated by multiplying the gravimetric soil water content (SWC) with the soil bulk density; each calculated as follows (Carter and Gregorich, 2008).

Bulk density = $(Ms)/(V_{soil})$…….…… (1),

where Ms is a mass of dry soil and V_{soil} is the volume of soil. Soil water balance is the amount of irrigation applied during the lifespan of the experiment plus any rainfall during the growing season minus the available water at harvest (Nyathi *et al*., 2018):

Eta = I + P – (D + R + water at harvest + ΔSWC) ……. (2),

where Eta = the evapotranspiration (mm), I = irrigation (mm), P = precipitation (mm), D = drainage, R = runoff and ΔSWC = changes in soil water content (%). In most cases, drainage and runoff are assumed negligible. Rainfall (mm) was obtained from the rain-gauge on site. Water productivity (WP) was calculated from dry yield harvest (kg/ha) and evapotranspiration (m^3/ha) by first computing water use efficiency or water productivity as follows (Renault and Wallender, 2000) (Appendix 3.1):

WP = Ya/ETa……. (3)

At 56 days after transplanting, 20 healthy mature leaves were harvested, washed with distilled water to remove contagious cross-contamination and to reduce infectious load, insecticide chemical residues and dust particles (Joshi *et al*., 2013). Leaves were padded dry using a laboratory tissue paper, and then oven-dried at 70°C for 72 h for dry biomass, weighted and the nutrient content (NC) quantified from 0.4 g powdered leaf samples further prepared using the digestion method (Zygmunt and Namiesnik, 2003). The

powdered material was dissolved in the microwave digestion vessels containing 5.0 ml nitric acid (HNO_3) and 3.0 ml hydrogen peroxide (H_2O_2). The mixture was carefully stirred with a clean glass bar. The vessels were capped and placed in the microwave digestion system at 190°C for 46 minutes, cooled at room temperature for about 10 minutes. The digested vessels were opened in the fume hood since a large amount of gas was produced during the process, and then transferred to 50 ml vials, with each solution diluted using deionised water. Post digestion, samples were submitted to Limpopo Agro-food Technology Station for analytical work of (Ca, K, Mg, P, Fe, Na and Zn) using an Atomic Absorption Spectrophotometer ICPE-9000 (Jones and Case, 1990). Thereafter various proportions were calculated to convert nutrient concentration to nutrition unit per kg (mg/kg), with the NWP calculated using the equation described by Renault and Wallender (2000).

$$NWP = WP \times NC \quad \text{.....(4)}$$

3.2.5 Statistical analysis

The Shapiro - Wilk test was performed to determine the normality of distribution of the data (Ghasemi and Zahediasl, 2012; Shapiro and Wilk, 1965). The NWP of various elements was normally distributed, and the data were subjected to analysis of variance using SAS software (SAS Institute, 2008). The degrees of freedom and their mean sum of squares (MSS) were partitioned to determine the contribution of sources of variation in total treatment variation (TTV) of the variables. Waller-Duncan multiple range test was used to separate means that were significant at the probability level of at least 5%. Significant

NWP were subjected to lines of the best fit, with generated quadratic equations used to compute optimum planting density for NWP of the test element using $x = -b_1/2b_2$ from the $Y = ax^2 + bx + c$ quadratic equation. Unless otherwise stated, treatment means were discussed at the probability level of 5%.

3.3 Results

In Experiment 1, treatments had highly significant ($P \leq 0.01$) effects on NWP-$_{Ca}$, NWP-$_{K}$, NWP-$_{Mg}$, NWP-$_{P}$, NWP-$_{Fe,}$ NWP-$_{Na}$ and NWP-$_{Zn}$ of *C. myriocarpus*, contributing 56, 54, 59, 58, 68, 74 and 66% in TTV of the respective variables (Table 3.1; Appendix 3.1-3.7). Means of both Experiments 1 and 2 for NWP-$_{Ca}$, NWP-$_{K}$, NWP-$_{Mg}$, NWP-$_{P}$, NWP-$_{Fe,}$ NWP-$_{Na}$ and NWP-$_{Zn}$ versus planting density were summarised (Table 3.2). In Experiment 1, NWP-$_{Ca}$, NWP-$_{K}$, NWP-$_{Mg}$, NWP-$_{P}$, NWP-$_{Fe,}$ NWP-$_{Na}$ and NWP-$_{Zn}$ versus increasing planting density/HDI exhibited positive quadratic relations, with the models explained by 74, 78, 67, 62, 84, 79 and 79%, respectively (Figure 3.3-3.6). In Experiment 2, treatments also had highly significant effects on NWP-$_{Ca}$, NWP-$_{K}$, NWP-$_{Mg}$, NWP-$_{P}$, NWP-$_{Fe,}$ NWP-$_{Na}$ and NWP-$_{Zn}$, contributing 69, 69, 73, 71, 79, 88 and 79% in TTV of the respective variables (Table 3.1; Appendix 3.8-3.14). Similarly, NWP-$_{Ca}$, NWP-$_{K}$, NWP-$_{Mg}$, NWP-$_{P}$, NWP-$_{Fe,}$ NWP-$_{Na}$ and NWP-$_{Zn}$ versus planting density/DHI exhibited positive quadratic relationships, with models explained by 85, 89, 88, 87, 85, 89 and 82%, respectively (Figure 3.3-3.6). In Experiment 1, the maximum planting density/DHI for optimum NWP for NWP-$_{Ca}$, NWP-$_{K}$, NWP-$_{Mg}$, NWP-$_{P}$, NWP-$_{Fe,}$ NWP-$_{Na}$ and NWP-$_{Zn}$ were 10, 9, 10, 9, 7, 7 and 7 respectively (Table 3.3). In Experiment 2, the maximum planting density/DHI for optimum NWP for NWP-$_{Ca}$, NWP-$_{K}$, NWP-$_{Mg}$, NWP-$_{P}$, NWP-$_{Fe,}$ NWP-$_{Na}$ and NWP-$_{Zn}$ were 10, 9, 10, 9, 7, 7

and 7, respectively. Generally, in Experiment 1 and Experiment 2, for all the test variables, NWP values were optimised at 8 plants/HDI (Table 3.3). In the derivation of the average optimum planting density, values that were beyond 9 plants were viewed as outliers since the 3S planter could only allow for 9 plants/HDI. Outliers were observed for NWP-$_{Ca}$ and NWP-$_{Mg}$ as being each 10 and 10, respectively, in Experiment 1, In contrast, outlier observed for NWP-$_{Ca}$, NWP-$_{K,}$ NWP-$_{P}$ and NWP-$_{Na}$ were respectively 90, 12, 61 and 32 plants/HDI in Experiment 2.

Table 3.1 Partitioning mean sum of squares (MSS) on nutritional water productivity of selected mineral malnutrient elements of *Cucumis myriocarpus* in response to planting density at 56 days after transplanting under field conditions (n = 72).

Sources	DF	NWP-$_{Ca}$		NWP-$_{K}$		NWP-$_{Mg}$		NWP-$_{P}$		NWP-$_{Fe}$		NWP-$_{Na}$		NWP-$_{Zn}$	
		MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
		Experiment 1													
Block	7	39.49	23	44.169	26	6.9145	22	6.625	22	0.21102	17	0.23337	17	0.36966	22
Treatment	8	95.20	56***	92.811	54***	19.046	59***	1.175	58***	0.84489	68***	1.01930	74***	1.09400	66***
Error	56	35.10	21	36.241	21	6.2006	19	6.026	20	0.18421	15	0.12934	9	0.20664	12
Total	71	169.79	100	173.221	100	32.1611	100	29.826	100	1.24012	100	1.38201	100	1.6703	100
		Experiment 2													
Block	7	30.08	18	25.43	19	4.48	15	2.82	15	0.16439	12	0.06539	5	0.22179	13
Treatment	8	113.1	69***	91.44	69***	22.35	73***	13.99	71***	1.09301	79***	1.26059	88***	1.35104	79***
Error	56	20.00	12	16.29	12	3.60	12	2.81	14	0.11903	9	0.09954	7	0.13240	8
Total	71	163.17	100	133.16	100	30.43	100	19.62	100	1.37643	100	1.42552	100	1.70523	100

***Significant at P ≤ 0.01.

Table 3.2 Nutritional water productivity *Cucumis myriocarpus* in response to increasing planting density/hole drip irrigation (HDI) under field conditions at 56 days after transplanting under field conditions (n = 72).

Planting density/HDI	NWP-$_{Ca}$	NWP-$_{K}$	NWP-$_{Mg}$	NWP-$_{P}$	NWP-$_{Fe}$	NWP-$_{Na}$	NWP-$_{Zn}$
	----------%----------				----------mg m^{-3}----------		
	Experiment 1						
1	0.888^{c} ±0.4	0.595^{c} ±0.4	0.329^{c} ±0.2	0.225^{c}±0.1	2.018^{d} ±0.1	2.445^{c} ±0.1	1.150^{c} ±0.1
2	3.000^{bc} ±1.6	3.939^{bc} ±2.2	1.229^{bc} ±0.9	1.263^{bc}±0.9	2.532^{bc} ±0.2	3.073^{b} ±0.2	1.888^{b} ±0.2
3	3.288^{bc} ±1.1	3.324^{bc}±1.5	1.375^{bc} ±0.4	1.338^{bc}±0.5	2.340^{cd} ±0.2	3.003^{b} ±0.2	1.851^{b} ±0.2
4	9.663^{a} ±4.6	9.186^{ab}±3.7	4.140^{a} ±2.3	3.838^{a}±1.9	2.850^{ab} ±0.2	3.521^{a} ±0.1	2.120^{ab} ±0.2
5	7.663^{ab} ±3.2	6.884^{ab}±3.1	3.031^{ab} ±1.4	2.725^{ab}±1.0	2.792^{ab} ±0.2	3.320^{ab} ±0.1	2.073^{ab} ±0.2
6	5.863^{abc} ±7.4	6.458^{abc}±4.3	2.659^{abc} ±2.8	2.475^{abc}±2.1	2.820^{ab} ±0.1	3.335^{ab} ±0.1	2.183^{ab} ±0.1
7	8.850^{ab} ±3.7	10.227^{a}±7.8	3.940^{a} ±2.1	4.325^{a}±2.9	3.032^{a} ±0.1	3.613^{a} ±0.1	2.387^{a} ±0.2
8	6.025^{abc} ±5.8	6.080^{abc}±5.4	2.632^{abc} ±2.4	2.113^{abc}±2.0	2.820^{ab} ±0.1	3.296^{ab} ±0.2	2.021^{ab} ±0.2
9	11.400^{a} ±7.2	10.965^{a}±4.2	5.104^{a} ±2.6	4.450^{a}±1.6	2.929^{ab} ±0.1	3.496^{a} ±0.1	2.365^{a} ±0.2
Planting density/HDI	NWP-$_{Ca}$	NWP-$_{K}$	NWP-$_{Mg}$	NWP-$_{P}$	NWP-$_{Fe}$	NWP-$_{Na}$	NWP-$_{Zn}$
	----------%----------				----------mg m^{-3}----------		
	Experiment 2						
1	0.554^{e} ±2.4	0.465^{e}±0.9	0.237^{e}±0.7	0.159^{d}±0.4	1.905^{d}±0.2	1.905^{d}±0.1	1.036^{d}±0.1
2	3.470^{cde}±1.6	3.470^{cde}±1.6	1.271^{de}±0.8	1.281^{cd}±0.6	2.580^{bc}±0.1	2.580^{bc}±0.04	1.935^{bc}±0.2
3	2.275^{de}±1.9	3.010^{de}±1.1	0.984^{de}±0.6	1.129^{cd}±0.7	2.401^{c}±0.2	2.401^{c}±0.2	1.910^{c}±0.2
4	6.612^{bcd}±1.7	7.141^{abc}±0.6	3.271^{bc}±0.6	2.960^{ab}±0.4	2.826^{ab}±0.1	2.826^{ab}±0.1	2.096^{abc}±0.2
5	8.262^{ab}±1.3	7.352^{abc}±0.7	3.244^{bc}±0.4	2.790^{abc}±0.1	2.869^{ab}±0.1	2.869^{ab}±0.01	2.138^{abc}±0.03
6	6.400^{bcd}±1.8	5.736^{bcd}±2.0	2.624^{cd}±0.8	2.326^{bc}±0.8	2.781^{ab}±0.2	2.781^{ab}±0.03	2.133^{abc}±0.003
7	7.262^{bc}±1.4	9.796^{ab}±2.4	3.428^{abc}±0.6	3.751^{ab}±0.8	2.941^{a}±0.4	3.522^{a}±0.1	2.298^{ab}±0.05
8	8.900^{ab}±2.5	9.711^{ab}±2.1	4.554^{ab}±1.0	3.275^{ab}±0.8	3.087^{a}±0.1	3.562^{a}±0.3	2.281^{ab}±0.3
9	12.638^{a}±1.5	10.351^{a}±1.7	5.296^{a}±0.6	4.144^{a}±0.7	3.017^{a}±0.2	3.585^{a}±0.1	2.453^{a}±0.1

[y]Column means ± standard error followed by the same letter were not different (P ≤ 0.05) according to Waller-Duncan multiple range test.

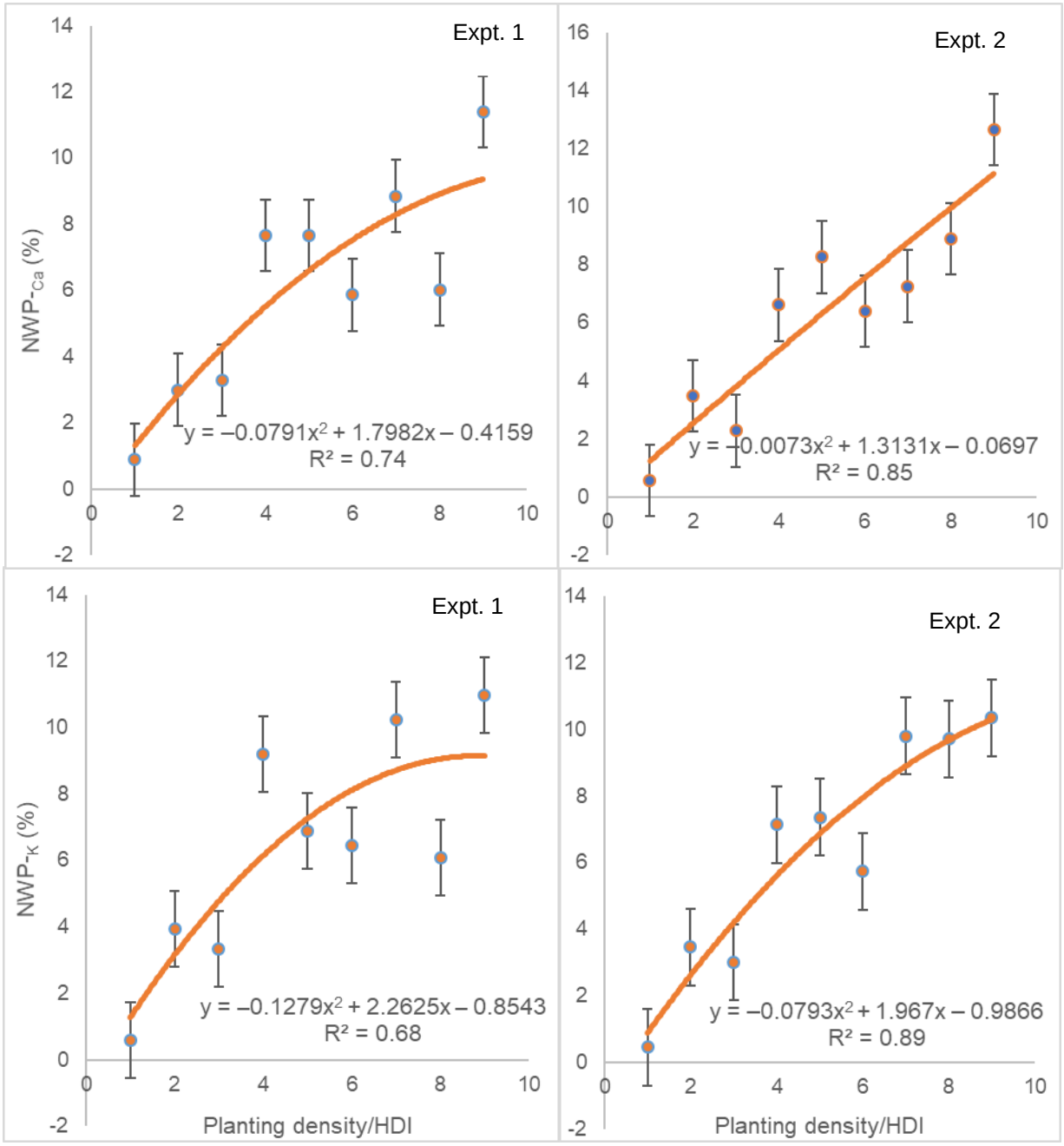

Figure 3.3 Response of nutritional water productivity of Ca (NWP_{-Ca}) and K (NWP_{-K}) each to planting density of *Cucumis myriocarpus* at 56 days after initiating treatments under field conditions.

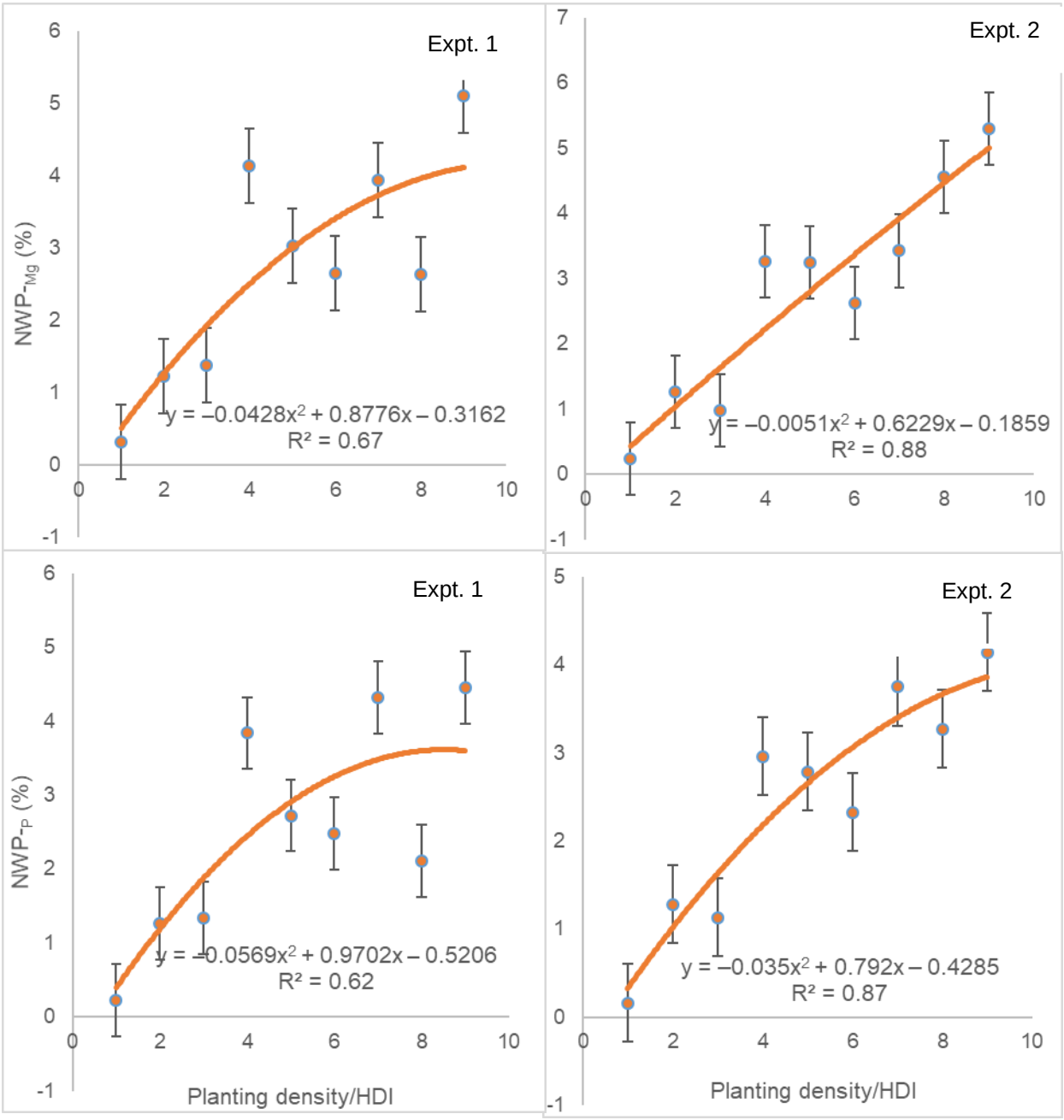

Figure 3.4 Response of nutritional water productivity Mg (NWP-$_{Mg}$) and P (NWP-$_{P}$) to planting density/HDI of *Cucumis myriocarpus* at 56 days after initiating treatments under field conditions.

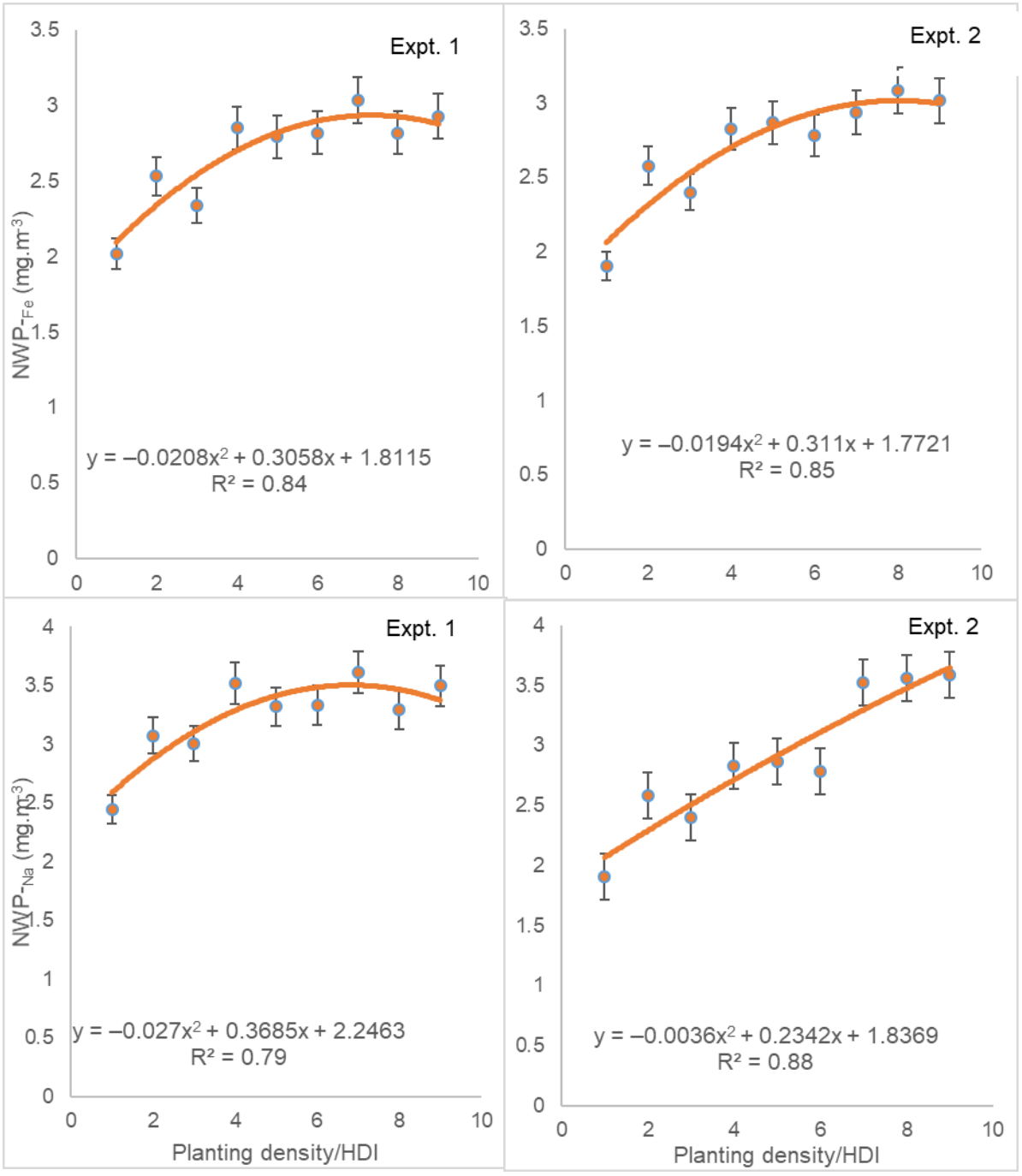

Figure 3.5 Response of nutritional water productivity Fe (NWP-$_{Fe}$) and Na (NWP-$_{Na}$) to planting density/HDI of *Cucumis myriocarpus* at 56 days after initiating treatments under field conditions.

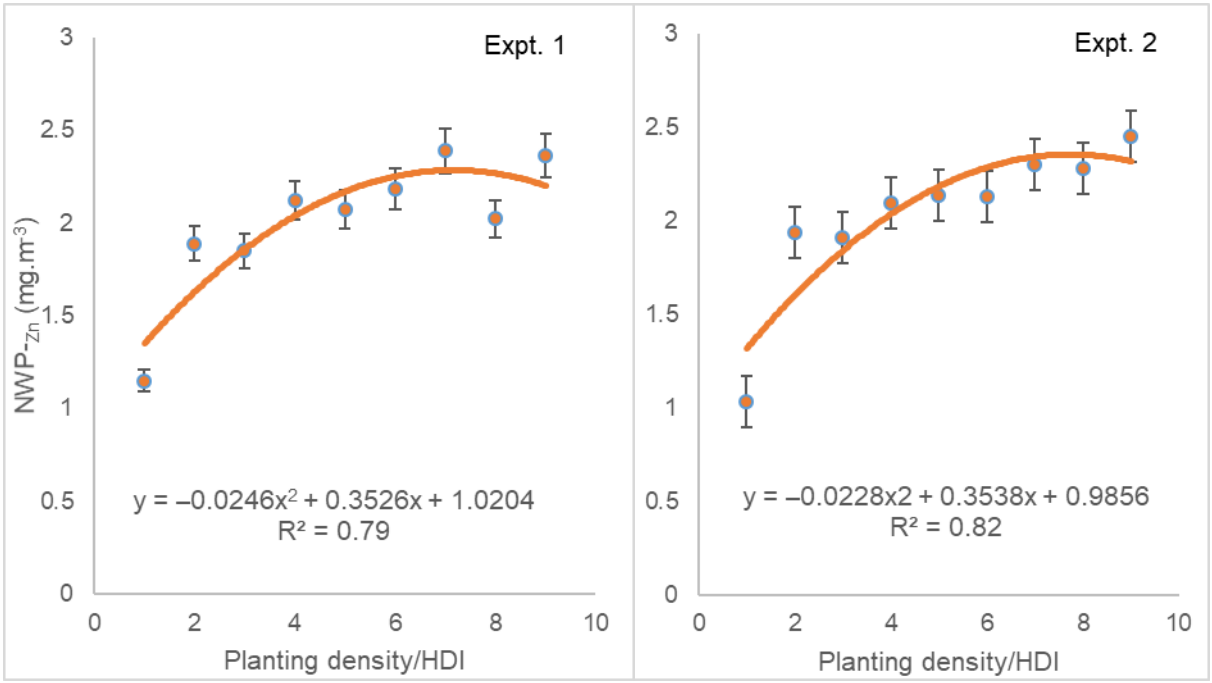

Figure 3.6 Response of nutritional water productivity Zn (NWP-$_{Zn}$) to planting density/HDI of *Cucumis myriocarpus* at 56 days after initiating treatments under field conditions.

Table 3.3 Response of planting density per hole drip irrigation of *Cucumis myriocarpus* plants at 56 days under field condition (n = 72).

Experiment 1			
Variable	Model	R^2	x
NWP_{-Ca}	$Y = -0.0791x^2 + 1.7982x - 0.4159$	0.85	10
NWP_{-K}	$Y = -0.1279x^2 + 2.2625x - 0.8543$	0.68	9
NWP_{-Mg}	$Y = -0.0428x^2 + 0.8776x - 0.3162$	0.67	(10)
NWP_{-P}	$Y = -0.0569x^2 + 0.9702x - 0.5206$	0.62	9
NWP_{-Fe}	$Y = -0.0208x^2 + 0.3058x + 1.8115$	0.84	7
NWP_{-Na}	$Y = -0.027x^2 + 0.3685x + 2.2463$	0.79	7
NWP_{-Zn}	$Y = -0.0246x^2 + 0.3526x + 1.0204$	0.79	7
		Average	8
Experiment 2			
Variable	Model	R^2	x
NWP_{-Ca}	$Y = -0.0073x^2 + 1.3131x - 0.0697$	0.85	(90)
NWP_{-K}	$Y = -0.0793x^2 + 1.967x - 0.9866$	0.89	12
NWP_{-Mg}	$Y = 0.0051x^2 + 0.6229x - 0.1859$	0.88	(61)
NWP_{-P}	$Y = -0.035x^2 + 0.792x - 0.4285$	0.87	11
NWP_{-Fe}	$Y = -0.0194x^2 + 0.311x + 1.772$	0.85	8
NWP_{-Na}	$Y = -0.0036x^2 + 0.2342x + 1.8369$	0.88	(32)
NWP_{-Zn}	$Y = -0.0228x^2 + 0.3538x + 0.9856$	0.82	8
		Average	8

The x values in brackets were outliers and therefore not used in computing the average.

$x = -b_1/2b_2$.

3.4 Discussion

Nutritional water productivity of all test MMN elements versus planting density/HDI exhibited positive quadratic relations in both experiments, where 3S planter was used to increase planting density. Observations in the current study are unique in relation to planting density since similar studies had not been conducted using 3S planter. However, DDG patterns observed in the current study were important since the produced information could assist in decision-making in relation to planting density of *C. myriocarpus* that would optimise NWP when 8 plants were sown using 3S planter. Since the gadget makes 9 holes/HDI, it implies one hole should not be sown. Since the generated information does not provide information as to which hole of 3S planter in particular should not have a plant, perhaps the one next to the drip hole should not be cultivated, allowing the rest to share equal quantities, depending on whether the surface was not having a slope.

The observed DDG patterns of NWP of MMN elements versus planting density/DHI suggested the existence of three phases, namely, stimulation, neutral and inhibition phases, as observed in most entities subjected to stresses that comply with density-dependent relationships (Salisbury and Ross, 1992). Notable examples of such relationships had been observed in allelochemical-related studies (Liu *et al*., 2003; Maile 2015; Mashela *et al*., 2017; Mashela *et al*., 2015; Pelinganga, 2013; Pelinganga *et al*., 2012). In such studies, DDG patterns of entities subjected to increasing concentration of allelochemicals had been quantified using the Curve-fitting

Allelochemical Response Dose (CARD) computer algorithm model (Liu *et al*., 2003). The model had since been used to describe various effects of allelochemicals on bioactivities of nematodes (Dube, 2016), with Mashela *et al*. (2017) providing a conceptualisation of the model, which had since assisted in providing a clear picture of how various biological entities behave when interacting with the environment.

Nutritional water productivity, described as a proportion of a specific element to the amount of water used to assimilate that element in edible portion of the test plant (Renault and Wallender, 2000). As a result, during the stimulation phase of NWP for a given nutrient element as observed in the current study, the implication could be that the amount of water decreased during a range of planting density/DHI for *C. myriocarpus*, thereby resulting in stimulation of NWP of the test MMN elements. In the neutral phase, over that range of planting density/DHI for *C. myriocarpus*, the amount of water required for assimilation of a particular nutrient element remained constant, resulting in the flattening of the quadratic curves. In contrast, beyond the neutral phase, the inhibition phase sets in, resulting in the inhibition of NWP of the test MMN elements, which could be viewed on the basis of increasing water requirement for the assimilation of a particular nutrient element.

Optimisation of planting density/DHI was important in the current study for two reasons. First, the quadratic models showed that the various test MMN elements had a wide range of optimisation values of planting density/DHI, which was biologically acceptable since the test MMN elements have diverse chemistries and bioactivities (Salisbury and

Ross, 1992). Just to emphasise, NWP of MMN elements in *C. myriocarpus* was shown to be nutrient element-specific when plants were cultivated using 3S planter, also when plants were staked. The findings could perhaps be different under certain other conditions. Staking, for example, exposes a higher percentage of soil surface to evaporation, which could have been minimal if plants were covering the soil surface, as is usually the case when *C. myriocarpus* is under cultivation. Second, the quadratic models provided information, which was previously non-existent when using 3S planter in production of *C. myriocarpus*, which on average, when using NWP of MMN elements optimised the planting density at 8 *C. myriocarpus* plants/DHI. In the derivation of the average optimum, values that were beyond 9 plants were viewed as outliers since the 3S planter could only allow for sowing 9 plants/HDI. Outliers observed in Experiment 1 for NWP_{-Ca} and NWP_{-Mg} as being 10 and 10 plants/HDI. An outlier is an observation that lies at an abnormal distance from other values in a random sample of a population. Consequently, optimisation of planting density/HDI versus NWP of test nutrient elements could not be necessary since the optimum range falls beyond the expected planting density/HDI. It could be impossible for a 3S planter to hold more than nine plants per station.

3.5 Synthesis and conclusion

In addition to loosing much water through evapotranspiration, much more water is required to assimilate nutrient elements when *C. myriocarpus* plants are sparsely cultivated. In contrast, at high planting densities/DHI, the amount of water required to assimilate elements was reduced. Currently, the two observations, increases of water

for assimilation at low population densities and the opposite at high population densities are not easy to explain unless additional experiments are conducted to account for the amount of water. However, planting density is a powerful tool in the hands of a farmer to manipulate improved nutrition content of produce.

Planting density of C*. myriocarpus*/DHI in drip irrigation system was optimised at approximately 9 plants/DHI. At the derived optimisation, *C. myriocarpus* would be in a position to provide consumers with optimum MMN elements, which would be translated to being high quality produce for human nutrition. The null hypothesis, which suggested that planting density/DHI would not have effects on NWP of MMN elements in C. myriocarpus under field conditions, was therefore rejected. In the next research chapter, effects of increasing irrigation interval on the NWP of MMN elements in *C. myriocarpus* would be investigated.

CHAPTER 4

RESPONSE OF NUTRITIONAL WATER PRODUCTIVITY TO IRRIGATION INTERVAL IN *CUCUMIS MYRIOCARPUS*

4.1 Introduction

Irrigation interval is dependent upon the plant species, soil type, the amount of water to be applied and the evapotranspiration rate (Jensen *et al*., 1990). Irrigation interval greatly affects both plant growth and quantity of the edible portion. In most high yielding cultivars, proper irrigation intervals for achieving optimum yield had been empirically-established, without due regards for the quality of the edible portion. Nutritional water productivity (NWP) was introduced to close the gap between quantity and quality of crop produce and it measures the quantity of water (m^3) required to assimilate a given mineral malnutrition (MMN) elements and/or a micronutrient malnutrition (MNMN) substance (Chibarabada *et al*., 2017; Nyathi *et al*., 2018; Renault and Wallender, 2000). Attempts have been made to quantify NWP of exotic vegetables such as onion (*Allium cepa* L.), Swiss chard (*Beta vulgaris* subsp. *vulgaris*) and beetroot (*Beta vulgaris* L.) (Ndlovu and Afolayan, 2008; Steyn *et al*., 2001), along with certain indigenous vegetables, which include nightshade (*Solanum retroflexum* Dun.), Amaranths (*Amaranthus cruentus* L.) and spider plant (*Cleome gynandra* L.) (FAO, 2017; Mulandana *et al*., 2010). In both exotic and indigenous vegetables tested, none was drought-tolerant. The test crops could therefore not be suitable for use under extended irrigation intervals as expected under climate change inland South Africa, where droughts would be common (Steyn *et al*., 2014). Thus, it became imperative that NWP

for crops with drought tolerance be investigated to establish the performance of such crops in relation to variables that are used in NWP, particularly, the MMN elements under different regimes of irrigation intervals. Wild cucumber (*Cucumis myriocarpus* Naude.), an indigenous plant to northern South Africa, is one such drought tolerant plant, which had been used by local people as a leaf vegetable (Kristkova *et al*., 2003). Harvested fresh leaves are usually shade-dried for use during winter months, but without information on the influence of irrigation on NWP of MMN elements, with the focus of the current study being exclusively on MMN elements. The objective of the study therefore was to investigate whether irrigation interval would influence NWP of MMN elements in *C. myriocarpus* under field conditions. The null hypothesis stated that irrigation interval would not influence NWP of MMN elements in *C. myriocarpus* under field conditions.

4.2 Materials and methods

4.2.1 Description of the study site

The location of the study was as described previously (Chapter 3). A rectangular plot comprising 10.8 m × 5 m was demarcated and subdivided into six strips using lines of drip irrigation system, with each line representing a treatment, with its own control valve.

4.2.2 Experimental design

Optimum plants/hole drip hole irrigation (DHI), as derived in Objective 1 (chapter 3, T_8 = 8 plants) were transplanted in each DHI using a 3S planter (Chapter 3) in plots that constituted an integrated drip irrigation system (IDIS). The IDIS consisted of six subsystems, each with a control valve for six irrigation intervals, namely, 2-, 4-, 8-, 16-,

32- and 64-day-irrigation-intervals. The irrigation intervals were arranged in a randomised complete block design, with 7 replications (Figure 4.1).

Figure 4.1 Integrated Drip irrigation system (IDIS) at the University of Limpopo experimental field.

4.2.3 Procedures

Procedures for raising seedlings, including hardening-off, land preparation, preparation of IDIS, transplanting, fertilisation, pest monitoring and control, were as describe previously (Chapter 3). However, in the current study, plants were irrigated as prescribed by the treatments. Three moisture probes were inserted at random in each treatment, for the determination of moisture.

4.2.4 Data collection

Soil water content was measured weekly prior to irrigation at 300-400 mm depths using Profile Probe that was connected to HH2 Moisture Meter (Delta-T Devices, UK). Intact soil clods collected from each planting station were weighed and recorded for fresh mass and then oven-dried at 105°C for 24 h for dry soil mass. The bulk density was then determined using the intact core method (Blake and Hartge, 1986), the particle size distribution was established using the Bouyoucos method (Bouyoucos, 1962) and soil water content through the gravimetric method (Little *et al*., 1998). Volumetric soil water content was calculated by multiplying the gravimetric soil water content (SWC) with the soil bulk density; each calculated as described on previously (chapter 3). Fresh edible leaves per plant were harvested, prepared and for nutrient elements (Ca, K, Mg, P, Na, Zn and Fe) were extracted using the digestion method and quantified using an Atomic Absorption Spectrophotometer ICPE-9000 as described previously (Chapter 3). Nutritional water productivity of MMN elements was computed for each level of increasing irrigation intervals as previously described (Chapter 3).

4.2.5 Statistical analysis

Prior to analysis, the x-axis data, which comprised a geometric series of 2, 4, 8, 16, 32 and 64 irrigation interval (days), were transformed using $\log_2 2^x$, which resulted in a series of 1, 2, 3 ,4 , 5 and 6 days (Causton, 1977). The normality of data was confirmed using the Shapiro - Wilk test (Ghasemi and Zahediasl, 2012; Shapiro and Wilk, 1965), with data subjected to analysis of variance through SAS software (SAS Institute, 2008). The degree of freedom and related mean sum of squares (MSS) were partitioned to

provide the total treatment variation (TTV) for each variable (Appendices 4.1-4.14). Mean separation was achieved using Fisher's Least Significant Difference (LSD) test at the probability level of 5%. Significantly different means were subjected to lines of the best fit. The generated relationships were further modelled by the regression curve estimates from the quadratic equation, ($Y = b_2x^2 + b_1x + c$), where Y = response for NWP of test nutrient element and x being the optimum or minimum irrigation intervals, derived from $x = – b_1/2b_2$ (Mamphiswana *et al*., 2010). Unless otherwise stated, treatment means were discussed at the probability level of 5%.

4.3 Results

In both Experiment 1 and Experiment 2, treatments had highly significant ($P \leq 0.01$) effects on NWP-$_{Ca}$, NWP-$_{K}$, NWP-$_{Mg}$, NWP-$_{P}$, NWP-$_{Fe}$, NWP-$_{Na}$ and NWP-$_{Zn}$ (Appendix 4.1-4.14). In Experiment 1, treatment effects contributed 81, 34, 82, 83, 89, 91 and 89% in TTV of the respective variables. In Experiment 2, treatment effects on NWP-$_{Ca}$, NWP-$_{K}$, NWP-$_{Mg}$, NWP-$_{P}$, NWP-$_{Fe}$, NWP-$_{Na}$ and NWP-$_{Zn}$ contributed 74, 87, 77, 85, 95, 94 and 95% in TTV of the respective variables (Table 4.1). In both Experiment 1 and Experiment 2, NWP values versus application interval exhibited short negative quadratic relations, which after the minimum, exhibited stimulation effects, which appeared to be indefinite within the irrigation interval in the current study. In Experiment 1, NWP-$_{Ca}$, NWP-$_{K}$, NWP-$_{Mg}$, NWP-$_{P}$, NWP-$_{Fe}$, NWP-$_{Na}$ and NWP-$_{Zn}$ models were explained by 80, 78, 80, 87, 83, 85 and 84% associations, respectively (Figure 4.2-4.5). Similarly, in Experiment 2, models were explained by 80, 80, 80, 81, 95, 96 and 95% associations, respectively (Figure 4.2-4.5). In Experiment 1 the minimum irrigation intervals for NWP-$_{Ca}$, NWP-$_{K}$, NWP-$_{Mg}$, NWP-$_{P}$, NWP-$_{Fe}$, NWP-$_{Na}$ and NWP-$_{Zn}$ were attained at 2 (4-day

interval), 3 (8-day interval), 3 (8-day interval), 3 (8-day interval), 1(2-day interval), 1(2-day interval) days and 1(2-day interval), respectively, with the average of 2 (4-days interval). Similarly, in Experiment 2 the minimum irrigation intervals for NWP-$_{Ca}$, NWP-$_{K}$, NWP-$_{Mg}$, NWP-$_{P}$, NWP-$_{Fe}$, NWP-$_{Na}$ and NWP-$_{Zn}$ were accomplished at 3 (8-day interval), 3 (8-day interval), 3 (8-day interval), 3 (8-day interval), 2 (4-day interval), 2 (4-day interval) and 2 (4-day interval) days, respectively, with the average of 3 (8-days interval).

Table 4.1 Partitioning sources of variation on nutritional water productivity of selected nutrients of *Cucumis myriocarpus* in response to irrigation interval at 64 days after initiating treatments under field conditions (n = 42).

Sources	DF	NWP-$_{Ca}$		NWP-$_{K}$		NWP-$_{Mg}$		NWP-$_{P}$		NWP-$_{Fe}$		NWP-$_{Na}$		NWP-$_{Zn}$	
		MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
EXPERIMENT 1															
Block	6	2230	10	1761	35	528	9	143	9	0.435	10	0.310	8	0.379	9
Trt	5	18283	81***	1701	34***	4568	82***	1368	83***	4.001	89***	3.673	91***	3.650	89***
Error	30	2071	9	1576	31	477	9	128	8	0.059	1	0.053	1	0.058	2
Total	41	22584	100	5038	100	5573	100	1639	100	4.495	100	4.036	100	4.087	100
EXPERIMENT 2															
Block	6	10711	13	1403	6	17120	11	1932	7	0.1219	3	0.201	4	0.109	2
Trt	5	60873	74***	2025	87***	11704	77***	2326	85***	4.6747	95***	4.624	94***	4.780	95***
Error	30	11141	13	1528	7	18260	12	2071	8	0.1174	2	0.105	2	0.123	3
Total	41	82724	100	2318	100	15242	100	2726	100	4.9141	100	4.930	100	5.012	100

***Significant at P ≤ 0.01.

Table 4.2 Effects of increasing irrigation interval ± standard error of a mean (SEM) on nutritional water productivity of *Cucumis myriocarpus* at 64 days after initiating treatments under field conditions (n = 42).

Irrigation interval	NWP-$_{Ca}$	NWP-$_{K}$	NWP-$_{Mg}$	NWP-$_{P}$	NWP-$_{Fe}$	NWP-$_{Na}$	NWP-$_{Zn}$
	----------%----------				----------mg m^{-3}----------		
				Experiment 1			
1 (2)	0.63^{b}±0.2	0.86^{b}±0.2	0.321^{b}±0.1	0.248^{b}±0.1	2.2080^{d}±0.2	2.4564^{d}±0.2	1.9486^{d}±0.2
2 (4)	3.84^{b}±1.0	2.84^{b}±0.8	1.760^{b}±0.5	0.847^{b}±0.2	2.8292^{c}±0.1	2.9958^{c}±0.1	2.5675^{c}±0.1
3 (8)	3.71^{b}±1.1	5.55^{b}±0.6	1.446^{b}±0.4	0.735^{b}±0.2	2.8457^{c}±0.1	2.9582^{c}±0.1	2.5331^{c}±0.1
4 (16)	4.59^{b}±0.8	8.69^{b}±0.4	2.161^{b}±0.3	1.104^{b}±0.1	3.1110^{b}±0.1	3.1715^{bc}±0.1	2.7065^{bc}±0.1
5 (32)	7.10^{b}±1.9	5.31^{b}±1.4	3.396^{b}±1.0	1.563^{b}±0.4	3.0754^{bc}±0.1	3.2784^{b}±0.1	2.8405^{b}±0.1
6 (64)	129.06^{a}±42	123.74^{a}±37	64.340^{a}±20	35.124^{a}±10	4.4873^{a}±0.2	4.6053^{a}±0.1	4.1219^{a}±0.2
Irrigation interval	NWP-$_{Ca}$	NWP-$_{K}$	NWP-$_{Mg}$	NWP-$_{P}$	NWP-$_{Fe}$	NWP-$_{Na}$	NWP-$_{Zn}$
	----------%----------				----------mg m^{-3}----------		
				Experiment 2			
1 (2)	4.84^{b}±1.8	4.38^{b}±1.0	2.45^{b}±0.7	1.50^{b}±0.4	2.9666^{c}±0.1	3.1695^{c}±0.1	2.6967^{c}±0.1
2 (4)	4.63^{b}±1.8	3.42^{b}±1.1	2.29^{b}±0.8	1.06^{b}±0.3	2.9390^{c}±0.1	3.0659^{c}±0.1	2.5907^{c}±0.1
3 (8)	2.71^{b}±0.6	2.38^{b}±0.5	1.40^{b}±0.3	0.74^{b}±0.2	2.8413^{c}±0.1	2.9931^{c}±0.1	2.4935^{c}±0.1
4 (16)	18.79^{b}±5.0	13.21^{b}±3.4	9.40^{b}±2.4	4.47^{b}±1.1	3.4253^{b}±0.2	3.5511^{b}±0.2	3.1508^{b}±0.2
5 (32)	19.36^{b}±6.4	17.77^{b}±3.7	11.52^{b}±3.2	5.85^{b}±1.3	3.6142^{b}±0.1	3.7587^{b}±0.1	3.2828^{b}±0.1
6 (64)	732.17^{a}±31	424.61^{a}±11	321.98^{a}±12	143.84^{a}±42	5.0148^{a}±0.1	5.1615^{a}±0.1	4.7144^{a}±0.2

^{y}Column means ± standard error followed by the same letter were not significantly different at P ≤ 0.05 according to Waller-Duncan multiple range test.

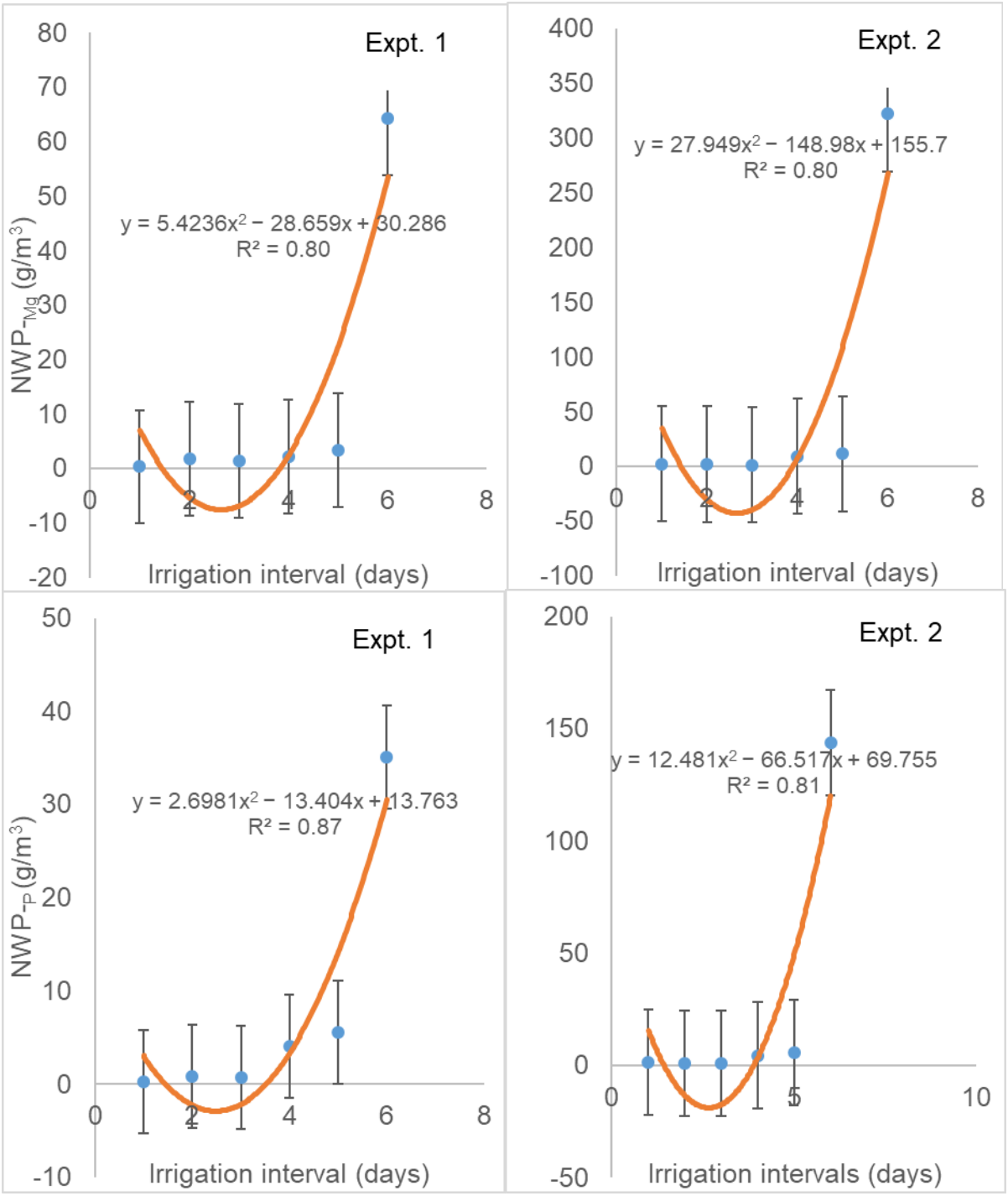

Figure 4.3 Response of increasing irrigation interval on nutritional water productivity Mg (NWP-$_{Mg}$) and P (NWP-$_{P}$) in the leaf tissues of *Cucumis myriocarpus* at 64 days after initiating treatments under field conditions.

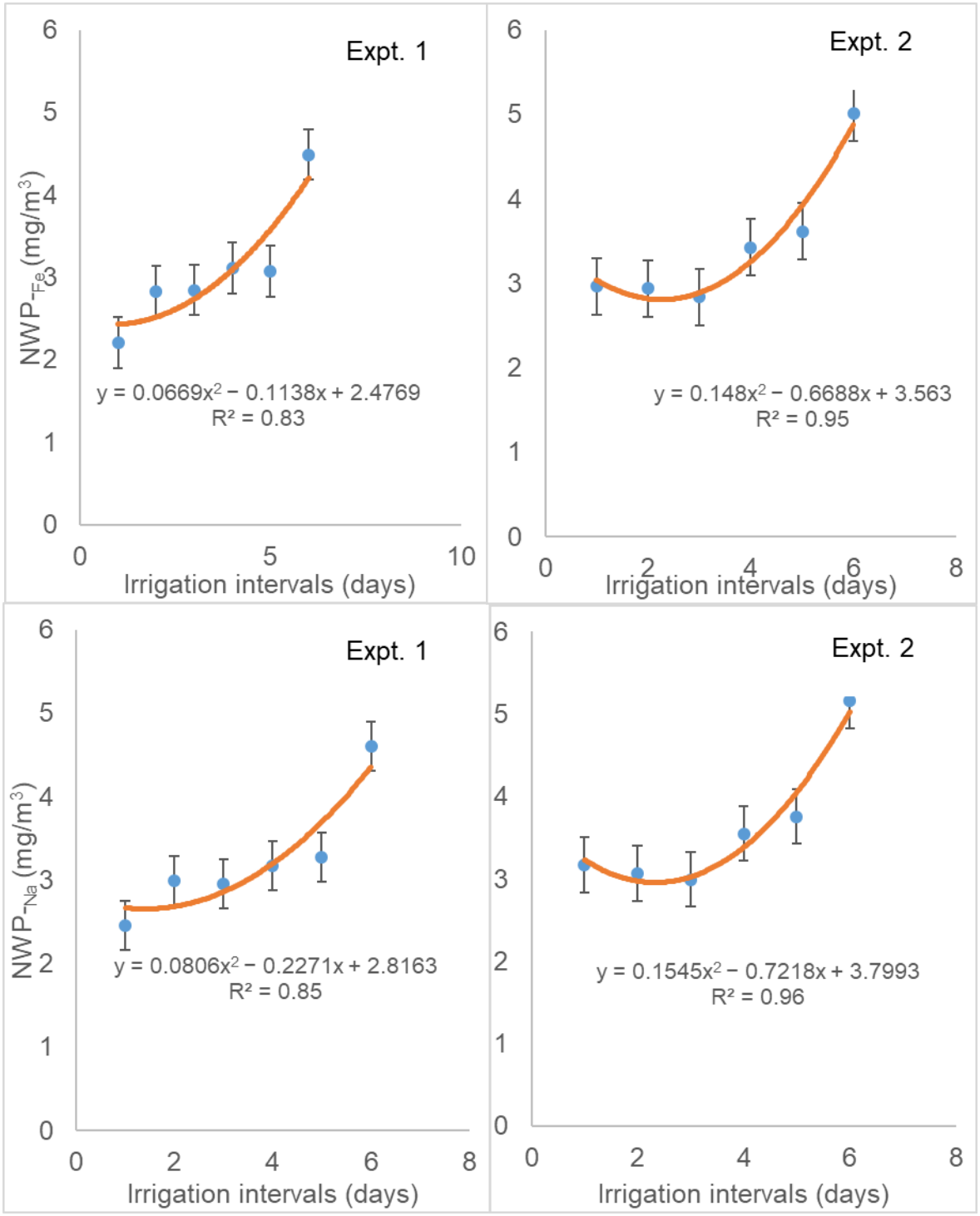

Figure 4.4 Response of increasing irrigation interval on nutritional water productivity Fe (NWP-$_{Fe}$) and Na (NWP-$_{Na}$) in the leaf tissues of *Cucumis myriocarpus* at 64 days after initiating treatments under field conditions.

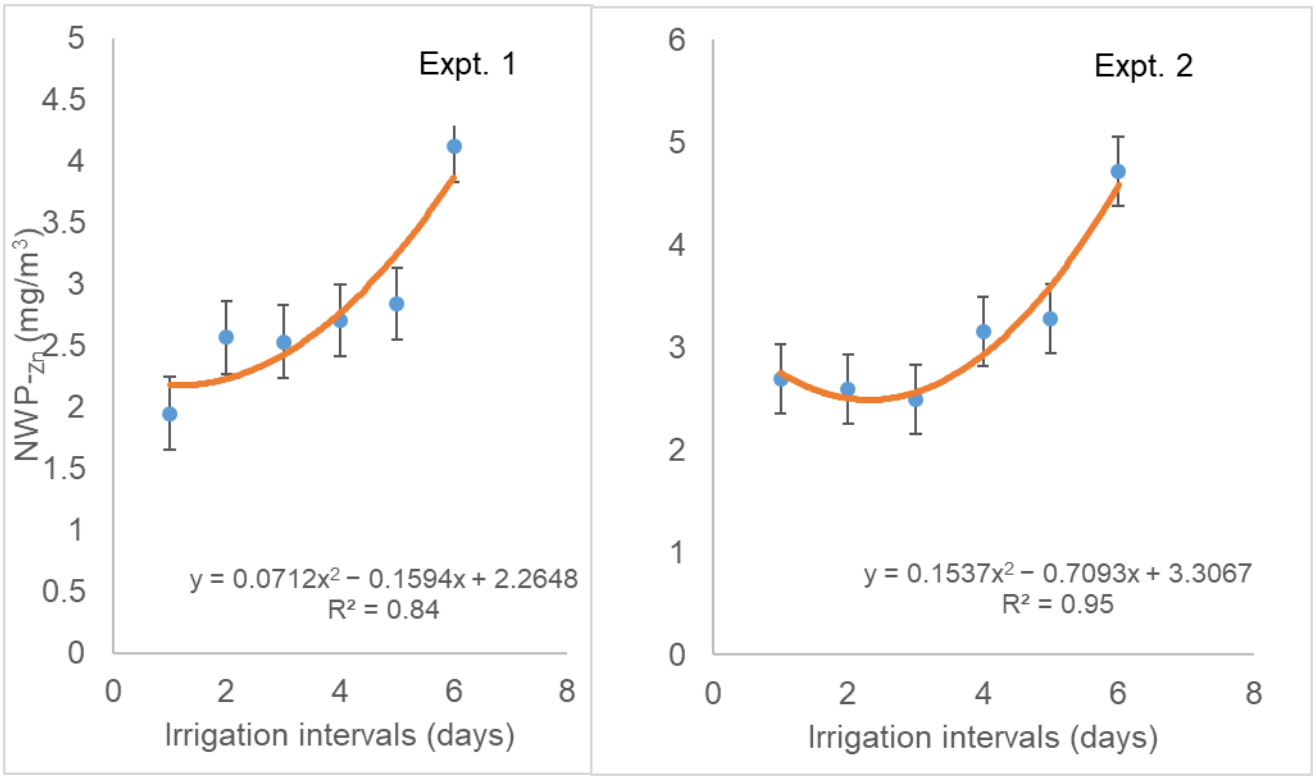

Figure 4.5 Responseof increasing irrigation interval on nutritional water productivity Zn (NWP-$_{Zn}$) in the leaf tissues of *Cucumis myriocarpus* at 64 days after initiating treatments under field conditions.

Table 4.3 Quadratic equations of nutritional water productivity (NWP) of selected mineral malnutrition elements in response of planting density per hole drip irrigation in dry shoot mass of *Cucumis myriocarpus* plants at 64 days after imposing different water deficit regimes under field conditions (n = 42).

Experiment 1			
Variable	Model	R^2	x
NWP_{-Ca}	$y = 10.791x^2 - 56.887x + 60.259$	0.80	2 (4)
NWP_{-K}	$y = 9.9623x^2 - 51.881x + 54.985$	0.78	3 (8)
NWP_{-Mg}	$y = 5.4236x^2 - 28.659x + 30.286$	0.80	3 (8)
NWP_{-P}	$y = 2.6981x^2 - 13.404x + 13.763$	0.87	3 (8)
NWP_{-Fe}	$y = 0.0669x^2 - 0.1138x + 2.4769$	0.83	1 (2)
NWP_{-Na}	$y = 0.0806x^2 - 0.2271x + 2.8163$	0.85	1 (2)
NWP_{-Zn}	$Y = -0.0712x^2 + 0.1594x + 2.2648$	0.84	1 (2)
		Mean	2 (4)
Experiment 2			
Variable	Model	R^2	x
NWP_{-Ca}	$y = 63.84x^2 - 341.26x + 356.57$	0.80	3 (8)
NWP_{-K}	$y = 36.811x^2 - 196.1x + 205.69$	0.80	3 (8)
NWP_{-Mg}	$y = 27.949x^2 - 148.98x + 155.7$	0.80	3 (8)
NWP_{-P}	$y = 12.481x^2 - 66.517x + 69.755$	0.81	3 (8)
NWP_{-Fe}	$y = 0.148x^2 - 0.6688x + 3.563$	0.95	2 (4)
NWP_{-Na}	$Y = -0.1545x^2 + 0.7218x + 3.7993$	0.96	2 (4)
NWP_{-Zn}	$Y = -0.1537x^2 + 0.7093x + 3.3067$	0.95	2 (4)
		Mean	3 (8)

The x values in brackets are geometric series before subjected to $\log_2 2$ transformed data

$x = -b_1/2b_2$.

^{x}*Calculated optimum treatment level (day-irrigation intervals)* $x = -b_1/2b_2$*, where* b_1 *= coefficient of x and* b_2 *= coefficient of* x^2 *on the quadratic equation, then x was the optimum inoculum level.*

4.4 Discussion

In all test NWP of MMN elements versus irrigation interval, exhibited brief incidents of negative quadratic relations, with inhibition of NWP from 2–day irrigation interval to a minimum of 4-day interval in Experiment 1 and to a minimum of 8-day interval in Experiment 2. In all test NWP, after the minimum the NWP values were stimulated as shown by the curves, with the curves showing the classical example of approaching infinity as it is becoming increasingly difficult to absorb water to assimilate nutrient elements. The uniqueness of the current observations is that there are no comparative observations in literature in terms of NWP. Although most biological entities versus gradually-imposed environmental conditions exhibit quadratic relations (Salisbury and Ross, 1992). However, in most empirically-based studies such observations hardly occur due to limited interval, which did not cover all three phases of density-dependent growth (DDG) patterns as outlined in the Curve-fitting Allelochemical Response Dose (CARD) computer algorithm model (Liu *et al.,* 2003). Mashela *et al*. (2017) demonstrated that to achieve in order to comply with DDG patterns that are articulated by CARD model, the x-axis values should straddle all three DDG phases, namely, stimulation, neutral and inhibition phases (Liu *et al*., 2003).

In both Experiment 1 and Experiment 2, the minimum values for test NWP were achieved at 2- and 8-day interval for the test MMN elements, with the average of 5-day interval. The decrease to the minimum values for all test MMN elements, followed by sharp increases in MMN elements need to be contextualised in terms of DDG principles (Liu *et al*., 2003; Mashela *et al*., 2017; Salisbury and Ross, 1992). At short irrigation

intervals, where plant stress is not deliberately allowed, NWP as a proportion of the test MMN element to M^3 water used in assimilation of the test MMN element, is placed in the inhibition phase as observed in allelochemical studies (Mashela *et al*., 2017). In the present study, NWP can decrease in one of two ways, when the test MMN element decreases with application time or when the availability of water measured in m^3 increase at the given time. The increase in available water at short irrigation time is more plausible than the decrease in MMN elements in the edible portion of the plant, which at the end comes to the same thing – a decrease in NWP of the test MMN element. In contrast, after the minimum, the stimulation phase was exhibited, where M^3 decreased – approaching zero, where NWP of test MMN elements approached infinity. This observation, without getting into the mechanisms, which were not investigated, in the current study, clearly demonstrate the role played by irrigation interval in *C. myriocarpus* production.

In context of NWP, the current observations suggested that in order to increase the accumulation of MMN elements in edible leaves of *C. myriocarpus*, longer irrigation intervals are preferable. In nightshade (*Solanum retroflexum* Dun.), another drought tolerant indigenous plant to South Africa, Mabotja (2019) demonstrated that the ideal average irrigation interval for acquiring high NWP in edible leaves was 8 days. A similar application interval was proposed in okra (*Abelmoschus esculentus* (L.) Moench), when NWP was based on MMN elements in fruit pods (Mutshekwa *et al.*, 2019). Observations in the current study confirmed assertions by Mutshekwa *et al*. (2019) that in most cases, optimum crop yield as promoted by best agricultural practices does not correlate with

quality of edible produce. The latter is in agreement with the philosophy related to what NWP was advanced for – to provide sufficient nutrition in terms of MMN elements (Chibarabada *et al*., 2017; Mabhaudhi *et al*., 2016a; Nyathi *et al*., 2016; Renault and Wallender, 2000). The philosophy is supported by active participation of various research teams in biofortification (Marijke, 2020), the technology intended to breed crop cultivars that contain high MMN elements or MNMN substances. Biofortification, as shown in various sweet potato (*Ipomoea batatas* (L.) Lam.) cultivars at the Agricultural Research Council in South Africa (Laurie *et al*., 2015) and elsewhere (Dhuique-Mayer *et al*., 2018), can be a lengthy breeding process when compared with selection of indigenous underutilised crops such as *C. myriocarpus,* which are already adapted to the areas where investigations are made. However, once identified, as shown in the current study, cultural practices that would promote the accumulation of high MMN elements should have to be investigated. In the current study, observations had since shown that planting density and irrigation interval could play a major role in promoting the accumulation of MMN elements in edible leaves of *C. myriocarpus.*

Although in the current study MNMN substances were not quantified for NWP, Kanda *et al*. (2020) observed that NWP of carbohydrates was related to water stress. However, in most such studies, limited irrigation intervals were used, to the extent that the three DDG phases were not straddled by the treatment of interest as observed in citrus seedlings subjected to various levels of salinity or the citrus nematode (*Tylenchulus semipenetrans* Cobb) (Mathabatha *et al.,* 2017), where treatments had no significant effects in NWP of test MMN elements.

4.5 Synthesis and conclusion

Irrigation interval is one of the cultural practices that is affected by many abiotic and biotic factors – and had been based on the quantity of crop yield, with limited focus on nutritional quality as articulated by NWP. All test MMN elements in the current study demonstrated that irrigation interval was important in ensuring that *C. myriocarpus* of desired nutritional quality could only be produced under water stress. Although the study did not measure crop yield, it would be important to balance this variable with NWP of the test MMN elements in order to provide a compromise in terms of when *C. myriocarpus* is in the test region, where other factors such as soil type, depth and other equally important factors such as quality of irrigation water should be taken into account. In conclusion, the null hypothesis, which suggested that irrigation interval would not influence NWP of MMN elements in *C. myriocarpus* did not hold and on the basis of the findings, the null hypothesis was rejected. In the subsequent chapter, the effects of population densities of *Meloidogyne* species on NWP of MMN elements in harvestable leaves of *C. myriocarpus* were investigated.

CHAPTER 5

RESPONSE OF NUTRITIONAL WATER PRODUCTIVITY TO INFECTION BY *MELOIDOGYNE* SPECIES IN *CUCUMIS MYRIOCARPUS* LEAF VEGETABLE

5.1 Introduction

Root-knot (*Meloidogyne* species) nematodes are the most serious and economic pests of all cultivated crops around the world. *Meloidogyne* species have a wide host range and are mainly distributed in both tropical and temperate regions (Coyne *et al.,* 2007). Following the withdrawal of fumigant nematicides from the agrochemical markets due to their environment-unfriendliness, with alternatives such as systemic synthetic nematicides being highly toxic to humans and other non-target organisms, alternatives such as nematode resistance in crops have been widely investigated.

Pofu (2012) demonstrated that wild cucumber (*Cucumis myriocarpus* Naude.) was resistant to thermophilic *Meloidogyne* species in South Africa, including *M. incognita* race 2, *M. incognita* race 4 and *M. javanica*. In a subsequent study, *M. incognita* race 4 was classified using molecular techniques *as M. enterolobii* (Maleka, 2020). In China, Liu *et al*. (2015) observed that *C. myriocarpus* was moderately resistant to the Chinese isolate of *M. incognita*. In *C. myriocarpus* the active ingredient cucurbitacin A ($C_{32}H_{46}O_9$) occurs primarily in root and fruit tissues (Jeffery, 1978), with leaves used as green vegetable in Limpopo Province, South Africa. Due to its resistance to local isolates *Meloidogyne* species, the plant could serve an important role in amelioration of nutrient-

induced malnutrition due to its high level of nutrient elements, especially Fe (Mashela, 2002). The concept nutritional water productivity (NWP) had been used to assess nutritional content (mg) per unit of water (m^3) required to assimilate the test element in edible portion of the test crop (Chibarabada *et al*., 2017; Nyathi *et al*., 2016; 2018; Renault and Wallender, 2000). Previously (Chapter 3), it was shown that NWP of mineral malnutrient (MMN) elements in edible foliage of *C. myriocarpus* versus planting density exhibited positive quadratic relations, whereas NWP of MMN elements versus irrigation interval exhibited negative quadratic relations (Chapter 4). Similarly, Ramputla (2018) demonstrated that NWP of MMN elements in berries of nematode susceptible chilli pepper (*Capsicum annuum* L.) cv. Serrano versus increasing population densities of *M. incognita and M. javanica,* exhibited positive quadratic relations. However, NWP of MMN elements in edible foliage in nematode-resistant *C. myriocarpus* had not been documented. The objective of this study was therefore to investigate whether subjecting *C. myriocarpus* to increasing population densities of *Meloidogyne* species would affect NWP of MMN elements in edible leaves. The null hypothesis suggested that subjecting *C. myriocarpus* to increasing population densities of *Meloidogyne* species would not affect NWP of MMN elements in edible leaves.

5.2 Materials and methods

5.2.1 Description of the study site

Both greenhouse and microplot experiments were conducted under both greenhouse and microplot conditions at the Green Biotechnologies Research Centre of Excellence,

University of Limpopo (23°53'10"S, 29°44'15"E). Microplot conditions (Chapter 3) were as described previously.

5.2.1.1 Greenhouse trials

The experiment was conducted in autumn (February-April) 2018 and validated in 2019. Ambient day/night temperatures averaged 28/21^{o}C, with maximum temperatures controlled using thermostatically activated fans. The length and width of the greenhouse were 130 m and 15 m, respectively, with the roof covered with a green net that allowed 80% photosynthetically active radiation. Conditions inside the greenhouse were not homogenous due to wind streams induced by heat-extracting fans. A wet wall on the southern side of the greenhouse and heat-extracting fans on the northern wall were intended to maintain relative humidity at 60-70%. Nematode inocula were prepared, when required, by extracting eggs and freshly hatched second-stage juveniles (J2) of *M. incognita* from roots of greenhouse-grown nematode-susceptible kenaf (*Hibiscus cannabinus* L.) in 1% NaOCl solution (Hussey and Barker, 1973).

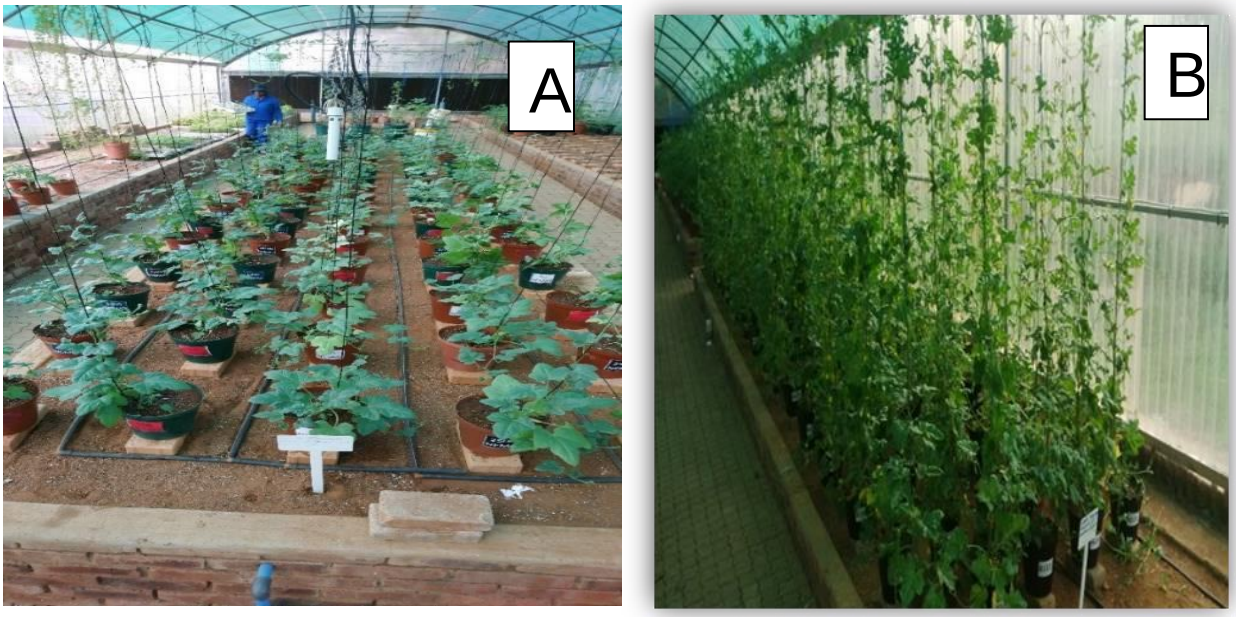

Figure 5.1 *Cucumis myriocarpus* seedlings (A) and mature plants (B) inoculated with *Meloidogyne incognita* under greenhouse conditions.

Preparation of experimental units and materials: Growing mixture, sowing seeds and hardening-off of seedlings were as described previously (Chapter 4). At five-leaf stage seedlings were transplanted into 20-cm-diameter plastic pots, filled with 2.7 L steam-pasteurised (300°C for 1 h) loam soil and river sand, with unpasteurised Hygromix-T at 3:1:1 (v/v) ratio. Pots were placed on greenhouse benches at 0.3 m × 0.4 m spacing. Growing mixture in pots was irrigated to field capacity and hardened-off seedlings transplanted after 12 h. Seedlings were irrigated with 300 ml tap water every other day. Three days after transplanting, seedlings were fertilised and at three weeks after transplanting staked as described previously (Chapter 4).

Treatments, experimental design and procedures: Five treatments, namely, 0, 10, 100, 1 000 and 25 000 eggs and J2 of *M. incognita* were arranged in a randomised complete block design (RCBD), with 18 replications during autumn (Feb-April) 2018 and validated in 2019. Four sets of iTuin Metre (Institute of Opto-Electronic Technology, Beijing) were inserted to 15-cm depth in randomly selected pots to monitor soil moisture tension, with plants irrigated to full capacity using 300 ml chlorine-free tapwater at 50% moisture depletion. Scouting for greenhouse whiteflies (*Trialeurodes vaporariorum* Westwood) was done daily and plants sprayed with 1 ml Kemprin 200 EC (active ingredient cypermethrin) per 10 L water when populations increased to above 10 whiteflies per plant. Red spider mites were managed with 400 ml per ha Seizer® 100 EC (bifenthrin).

Data collection: At 56 days after harvesting, intact soil clods collected from each pot were weighed and recorded for fresh mass and then oven-dried at 105°C for 24 h for dry soil mass. The bulk density was then determined using the intact core method (Blake and Hartge, 1986), the particle size distribution was established using the Bouyoucos method (Bouyoucos, 1962) and soil water content through the gravimetric method (Little *et al*., 1998). Volumetric soil water content was calculated by multiplying the gravimetric soil water content (SWC) with the soil bulk density; each calculated as follows (Carter and Gregorich, 2008).

Twenty healthy mature leaves were collected per plant prior to termination of experiments and placed in clean brown bags. For each treatment, leaves were lightly

dipped in distilled water, shaken to remove possible contaminants and padded dry using a laboratory paper (Joshi *et al*., 2013). Leaves were oven-dried at 70°C for 72 h and ground as described previously (Chapter 3). Leaf material for MMN elements were subjected to the digestion method and then quantified as described previously (Chapter 3).

Data analysis: The Shapiro - Wilk test was performed to determine the normality of distribution of data (Ghasemi and Zahediasl, 2012; Shapiro and Wilk, 1965). The collected NWP data exhibited normality and data were subjected to an analysis of variance using SAS software (SAS Institute, 2008). Mean separation was achieved using Fisher's Least Significant Difference (LSD) test at the probability level of 5%. Unless otherwise stated, only treatment means significant at 5% level of probability were discussed.

5.2.1.2 Microplot trials

Microplot trials were conducted during autumn (February-April) 2019 and validated in 2020. The plot size was 20 m in length and 5.2 m in width. A rain gauge was installed at 2 m distance from the plots for measuring rainfall. Artificial microplots were established by inserting 30-cm-diameter plastic pots into 20-cm-deep holes at 1.0 m × 0.6 m spacing.

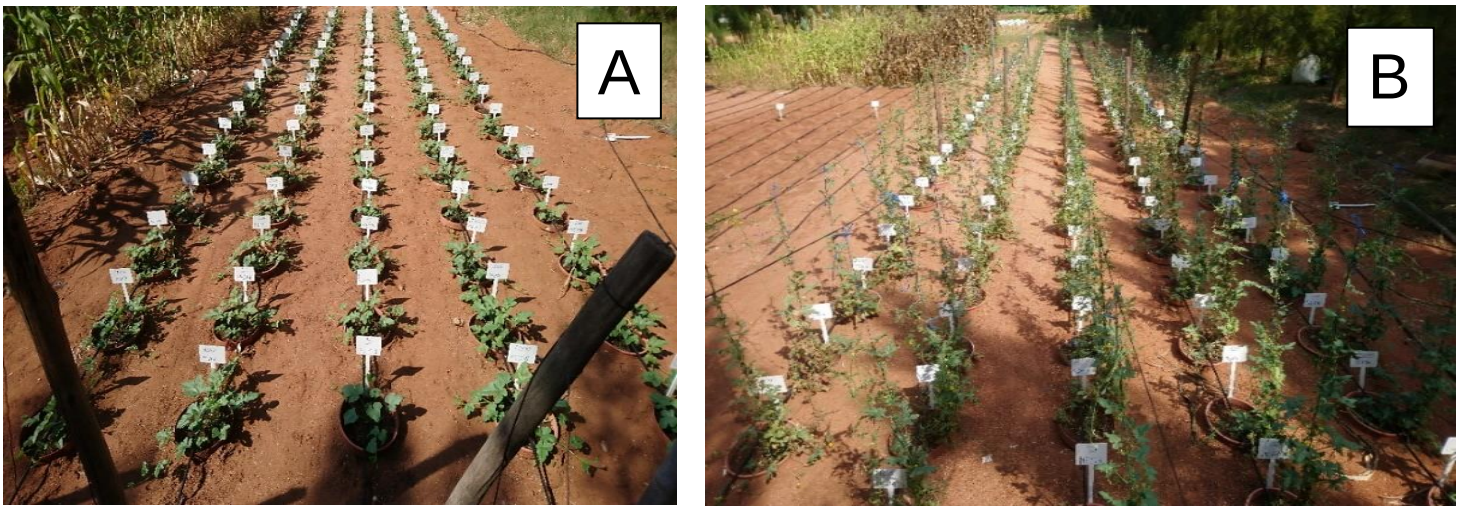

Figure 5.2 *Cucumis myriocarpus* seedlings at transplanting (**A**) and mature plants inoculated with *Meloidogyne javanica* under microplot conditions (**B**).

Preparation of experimental units and material: *Cucumis myriocarpus* seedlings were prepared as outlined in the greenhouse trial. A week after hardening-off at five-leaf stage, seedlings were transplanted into 30-cm-diameter plastic pots, filled with 10 L steam-pasteurised (300°C for 1 h) loam soil, sand and Hygromix-T at 3:1:1 (v/v) ratio. Treatments, *viz*. 0, 10, 100, 1 000 and 25 000 eggs and J2 of *M. javanica* were arranged in an RCBD, with 18 replicates. A week after transplanting, plants were each inoculated by dispensing an approximate number of eggs and J2 using a 20-ml plastic syringe and placing into 2½-cm-deep holes around the stem. Fertilisation was as previously described under greenhouse conditions. Plants were irrigated with 1 000 ml chlorine-free tap water as soon as 50% of the moisture meters had readings just below 2 units. Plants were staked to allow the vines free upward moments. Weeds among pots were removed using hand-held hoes.

Data collection and analysis: In microplot, data collection and analysis were as explained for greenhouse experiments.

5.3 Results

Meloidogyne incognita treatment levels did not have any significant ($P \leq 0.05$) effects on NWP_{-Ca}, NWP_{-K} NWP_{-Mg}, NWP_{-P}, NWP_{-Fe}, NWP_{-Na} and NWP_{-Zn} in both Experiment 1 and Experiment 2 under both greenhouse and microplot conditions were not significant (Appendix 5.1-5.14). Data for treatment means, without subjecting them to any post-hoc test, were further summarised for completeness (Table 5.1). Similarly, *M. javanica* did not have any significant effects on NWP_{-Ca}, NWP_{-K}, NWP_{-Mg}, NWP_{-P}, NWP_{-Na} and $NWP_{-Zn,}$ however, significant effects were observed on NWP_{-Fe} (Appendix 5.8-5.14), as illustrated in the summarised format, with increasing level of *M. javanica* in Experiment 2 gradually reducing NWP of Fe (Table 5.2). Relative to untreated control, the highest inoculation of 25 000 eggs + J2 of *M. javanica* reduced NWP_{-Fe} by 22%.

Table 5.1 Partitioning sources of variation on nutritional water productivity of selected malnutrient elements of *Cucumis myriocarpus* in response to increasing *Meloidogyne incognita* at 56 days after initiating treatments (n = 45).

Variables	NWP-$_{Ca}$	NWP-$_{K}$	NWP-$_{Mg}$	NWP-$_{P}$	NWP-$_{Fe}$	NWP-$_{Na}$	NWP-$_{Zn}$
	----------%----------				----------mg m^{-3}----------		
				Experiment 1			
0	2.85 ± 0.08	2.45 ± 0.3	2.94 ± 0.11	2.69 ± 0.07	1.69 ± 0.05	2.46 ± 0.10	1.97 ± 0.28
10	2.90 ± 0.08	2.48 ± 0.27	2.93 ± 0.13	2.68 ± 0.06	1.44 ± 0.19	2.51 ± 0.10	2.04 ±0.29
100	2.99 ± 0.10	2.32 ± 0.23	3.07 ± 0.12	2.72 ± 0.06	1.58 ± 0.15	2.61 ± 0.12	2.23 ± 0.30
1000	2.98 ± 0.09	2.20 ± 0.25	3.17 ± 0.11	2.71 ± 0.06	1.75 ± 0.07	2.66 ± 0.09	2·44± 0.28
25000	2.82 ± 0.10	2.56 ± 0.21	2.85 ± 0.14	2.70 ± 0.09	1.69 ± 0.02	3.39 ± 0.14	1.78 ± 0.31
P-value	NS	NS	NS	NS	NS	NS	NS
Variables	NWP-$_{Ca}$	NWP-$_{K}$	NWP-$_{Mg}$	NWP-$_{P}$	NWP-$_{Fe}$	NWP-$_{Na}$	NWP-$_{Zn}$
	----------%----------				----------mg m^{-3}----------		
				Experiment 2			
0	3.14 ± 0.08	1.94 ± 0.14	3.31 ± 0.11	2.66 ± 0.02	1.70 ± 0.05	2.80 ± 0.08	2.81 ± 0.21
10	2.70 ± 0.31	1.87 ± 0.32	2.85 ± 0.30	2.32 ± 0.29	1.35 ± 0.23	2.36 ± 0.30	2.24 ± 0.34
100	3.02 ± 0.22	1.60 ± 0.17	3.20 ± 0.20	2.47 ± 0.20	1.58 ± 0.17	2.66 ± 0.21	2.81 ± 0.20
1000	3.09 ± 0.08	2.06 ± 0.21	3.22 ± 0.11	2.70 ± 0.06	1.63 ± 0.18	2.74 ± 0.09	2.60 ± 0.24
25000	3.09 ± 0.05	1.97 ± 0.21	3.27 ± 0.08	2.64 ± 0.07	1.68 ± 0.06	2.75 ± 0.06	2.74 ± 0.16
P-value	NS	NS	NS	NS	NS	NS	NS

NS = Not significant at P ≤ 0.05.

Table 5.2 Partitioning sources of variation on nutritional water productivity of selected malnutrient elements of *Cucumis myriocarpus* in response to increasing *Meloidogyne javanica* at 56 days after initiating treatments (n = 45).

Variables	NWP-$_{Ca}$	NWP-$_{K}$	NWP-$_{Mg}$	NWP-$_{P}$	NWP-$_{Fe}$	NWP-$_{Na}$	NWP-$_{Zn}$
	----------%----------				----------mg m^{-3}----------		
				Experiment 1			
0	4.01 ± 0.08	3.91 ± 0.06	3.74 ± 0.03	3.49 ± 0.06	1.89 ± 0.10	3.31 ± 0.06	2.23 ±0.08
10	3.99 ± 0.11	3.93 ± 0.08	3.79 ± 0.05	3.51 ± 0.07	1.93 ± 0.12	3.30 ± 0.07	2.21 ±0.08
100	4.10 ± 0.07	4.05 ± 0.05	3.82 ± 0.03	3.57 ± 0.04	1.77 ± 0.07	3.38 ± 0.04	2.34 ±0.06
1000	4.07 ± 0.10	3.96 ± 0.06	3.76 ± 0.05	3.53 ± 0.05	1.91 ± 0.10	3.34 ± 0.05	2·26 ±0.06
25000	4.06 ± 0.06	4.01 ± 0.05	3.82 ± 0.04	3.53 ± 0.04	1.74 ± 0.08	3.37 ± 0.04	2.31 ±0.05
P-value	NS	NS	NS	NS	NS	NS	NS
Variables	NWP-$_{Ca}$	NWP-$_{K}$	NWP-$_{Mg}$	NWP-$_{P}$	NWP-$_{Fe}$	NWP-$_{Na}$	NWP-$_{Zn}$
	----------%----------				----------mg m^{-3}----------		
				Experiment 2			
0	3.87 ± 0.10	3.86 ± 0.16	3.78 ± 0.06	3.56 ± 0.04	2.06^{b} ± 0.06	3.28 ± 0.09	2.31 ± 0.19
10	3.89 ± 0.06	4.01 ± 0.05	3.73 ± 0.02	3.58 ± 0.02	1.69^{c} ± 0.08	3.27 ± 0.05	2.21 ± 0.06
100	3.95 ± 0.08	4.13 ± 0.04	3.73 ± 0.04	3.63 ± 0.04	1.96^{bc} ± 0.11	3.34 ± 0.05	2.33 ± 0.05
1000	3.97 ± 0.09	4.06 ± 0.07	3.77 ± 0.05	3.61 ± 0.04	1.88^{bc} ± 0.08	3.34 ± 0.05	2.32 ± 0.05
25000	3.97 ± 0.06	3.98 ± 0.09	3.75 ± 0.05	3.56 ± 0.04	1.60^{a} ± 0.17	3.31 ± 0.05	2.24 ± 0.07
P-value	NS	NS	NS	NS	0.05	NS	NS

NS = Not significant at P ≤ 0.05.

5.4 Discussion

Under both greenhouse and microplot conditions, the two nematode species did not have significant effects on NWP of MMN except for *M. javanica* on NWP of Fe in Experiment 2, where the effects were significant. Observations in the current study contradicted observations where NWP of MMN elements versus increasing population density of *M. incognita* or *M. javanica* exhibited significant quadratic relations on chili pepper berries under microplot conditions (Ramputla, 2018). Most chili pepper cultivars are moderately resistant to *Meloidogyne* species, whereas *C. myriocarpus* was shown to be resistant to *M. incognita* race 2, *M. incognita* race 4 *and M. javanica* (Pofu and Mashela, 2011). However, when *C. myriocarpus* was subjected to another isolate of *M. incognita* in China, the resistance was viewed as moderate (Liu *et al*., 2015). In all other plant-nematode relation studies on *C. myriocarpus-Meloidogyne* species in South Africa, regardless of the condition under which the study was conducted, the plant exhibited high resistance attributes to *Meloidogyne* species (Pofu, 2012). Thus, in the current study, it could be implied that the nematode effects on NWP of MMN elements on *C. myriocarpus* were not significant due to the resistance ability of the plant.

In the context of density-dependent growth (DDG) patterns, which were observed in NWP of MMN elements versus planting density (Chapter 3) or irrigation interval (Chapter 4), observations in the current study suggested that the stimulation threshold density (Liu *et al*., 2003) for NWP of MMN elements as induced by *Meloidogyne* species was not achieved in the current study. Such a condition condition can occur, as suggested by Liu *et al*. (2003), when the population density is below the range of the

three phases or the density was within the neutral phase (Mashela *et al*., 2017). Mathabatha *et al.* (2017), when investigating the non-phytotoxic values of cucurbitacin phytonematicides in various citrus rootstocks, observed that as long as the three phases of DDG patterns were not straddled by the treatments on the x-axis, the quadratic relations would hardly be significant at the probability level of 5%.

In Experiment 2 *M. javanica* had significant effects on NWP of Fe in *C. myriocarpus*, with treatment means showing a gradual decline, with the highest inoculation (25 000 eggs + J2) having a reduced relative impact of 22%. In terms of DDG patterns (Liu *et al*., 2003), NWP of Fe was therefore already in the inhibition phase (Mashela *et al*., 2017). In this phase the water (m^3) required to achieve NWP of Fe was increasing, a mechanism that is not yet clearly understood, although previously confirmed in planting density (Chapter 3) and extended irrigation interval (Chapter 4) studies. Generally, leaf tissues of *C. myriocarpus* are known to be high in Fe content (Mashela, 2002), a heavy metal that is involved in various biochemical processes/ activities in plants. The mechanism through which this plant accumulates Fe in leaf tissues is not yet understood, but it is important to note that as population densities of *M. javanica* increased, the nutritional quality of harvestable leaves of *C. myrioarcpus* in terms of Fe declined.

5.5 Synthesis and conclusion

Increasing levels of *Meloidogyne* species had no significant effect on NWP of most test MMN elements, which suggested that under both greenhouse and microplot conditions nematodes did not feed and reproduce on *C. myriocarpus.* Although not confirmed under other conditions, it should be noted that under low population densities *M. javanica* had a tendency of reducing NWP of Fe in *C. myriocarpus*, which translated into the reduction of nutritional quality of harvestable leaves. The null hypothesis in this study suggested that *Meloidogyne* species would not affect NWP of MMN elements in *C. myriocarpus* and since it was so in most elements, except for one incident of Fe, the null hypothesis could as well be accepted the following research chapter, the effects of the increasing concentration of chloride salts on NWP of MMN elements in *C. myriocarpus* were investigated.

CHAPTER 6

RESPONSE OF NUTRITIONAL WATER PRODUCTIVITY TO INCREASING CONCENTRATION OF CHLORIDE SALTS IN *CUCUMIS MYRIOCARPUS*

6.1 Introduction

Globally, among many other natural incidents, global warming is characterised by incidents of floods. The subsiding water due to both seepage and evaporation, leaves large quantities of salts on soil surface (Mashela, 2017). In cultivated land, the resulting salinity lowers water potential in the soil, making it difficult for plants to absorb water and certain essential nutrient elements (Nedjimi, 2009). Other sources of soil salinity include soil type, irrigation with poor quality water and high evapotranspiration (Mashela, 1992; Shrivastava and Kumar, 2015). In certain exotic crops such as *Citrus* species and relatives, salinity challenges had been responsible for as high as 33% yield losses to complete crop failure (Shrivastava and Kumar, 2015). The response of most indigenous underutilised crops to increasing salinity had hardly been investigated. Legong (2017) demonstrated that vine length, dry shoot mass, dry root mass and chlorophyll content of wild cucumber (*Cucumis myriocarpus* Naude) versus increasing concentration of chloride salt exhibited positive quadratic relations. Although salinity affects the accumulation of essential nutrient elements in leaf tissues of most plants, the potential effects of this factor on nutritional water productivity (NWP) of mineral malnutrition (MMN) elements in *C. myriocarpus* had not been documented. The objective of the study was therefore to investigate whether subjecting *C. myriocarpus* to increasing concentration of chloride salinity would affect NWP of MMN elements in edible leaves of

the test crop. The null hypothesis suggested that subjecting *C. myriocarpus* to increasing concentration of chloride salinity would not affect NWP of MMN elements in edible leaves of the test crop.

6.2 Materials and methods

6.2.1 Description of the study site

The experiments were conducted in the greenhouse at the Green Biotechnologies Research Centre, University of Limpopo, South Africa (23°53'1''S, 29°44'1''E) in spring (August-December) 2017 and validated in spring 2018. Minimum/maximum ambient temperatures averaged 13/25°C, with maximum temperatures controlled using thermostatically activated fans. The length and width of the greenhouse were 100 m and 20 m, respectively, with the roof covered with a green net that allowed 80% photosynthetically-active radiation. Conditions inside the greenhouse were not homogenous due to wind streams induced by heat-extracting fans. A wet wall on the southern side of the greenhouse and heat-extracting fans on the northern wall were intended to maintain relative humidity at 60-70% and maximum temperature at 28°C.

6.2.2 Treatments, experimental design and procedures

Seven treatments comprised 0, 2, 4, 8, 16, 32 and 64 mM NaCl with $CaCl_2/m^3$ water at 3:1 ratio, respectively. Treatments were arranged in a randomised complete block design, with 18 replications. *Cucumis myriocarpus* seedlings were raised in 200-hole seedling trays containing Hygromix-T (Hygrotech, Pretoria) growing medium under greenhouse conditions (Chapter 3). A week after hardening-off outside the greenhouse

through the intermittent withdrawal of irrigation (Chapter 3), uniform seedlings at five-leaf stage were transplanted into 20-cm-diameter plastic pots, filled with 2 700 ml steam-pasteurised (300°C for 1 h) loam soil, river sand and Hygromix-T at 3:1:1 (v/v) ratio. Pots were placed at an inter-row and intra-row spacing of 0.30 m × 0.30 m (111 000 plants ha^{-1}) on greenhouse benches as illustrated (Figure 6.1). Fertilisation at three days after transplanting was as described previously (Chapter 3). Seven iTuin Metre moisture probes (Institute of Opto-Electronic Technology, Beijing) each was inserted in pots with different treatments. Pest management involved daily monitoring and when necessary greenhouse whiteflies (*Trialeurodes vaporariorum* Westwood) were sprayed as described previously (Chapter 3). Prior to the treatments, each plant was irrigated with 300 ml chlorine-free tapwater, which was substituted for salt treatment at five-leaf stage. Plants were staked to allow for free upward moment of the vines.

Figure 6.1. Cucumis myriocarpus experiment at 56 days before harvesting **(A),** seedling stage of *Cucumis myriocarpus* under greenhouse conditions **(B).**

6.2.3 Data collection

At 56 days after initiating the salt treatments, 20 mature healthy leaves were harvested and prepared for extraction of nutrient elements using the digestion method and quantified as described previously (Chapter 3). Intact soil clods were collected from each pot using soil core, weighed for wet soil mass and oven-dried at 105°C overnight for water volume and bulk density measurement. Bulk density and volumetric soil water content were determined as described previously (Chapter 3). Evapotranspiration, water productivity (WP) and NWP were calculated as previously described (Chapter 3).

6.2.4 Statistical analysis

The collected data were subjected to Shapiro- Wilk test for normality distribution (Ghasemi and Zahediasl, 2012; Shapiro and Wilk, 1965), after confirmation of normality, data were subjected to analysis of variance using (SAS Institute, 2008) software. The degrees of freedom and their mean sum of squares (MSS) were partitioned to determine the contribution of sources in total treatment variation (TTV) of the variables. Waller-Duncan multiple range test was used to separate treatment means that were significant at the probability level of 5%. Prior to analysis, treatments were transformed using $\log_2 2$ (Causton, 1977), resulting in 1, 2, 3, 4, 5, 6 and 7 mM NaCl with $CaCl_2/m^3$ water. Significant means were further subjected to lines of the best fit. The relationships were modelled by the regression curve estimates from the quadratic equation ($Y = b_2x^2 + b_1x + c$), where Y = response in NWP of selected malnutrient elements and $x = -b_1/2b_2$ being the optimum value (Mamphiswana *et al*., 2010). Unless otherwise stated, treatment effects were discussed at the probability level of 5%.

6.3 Results

In Experiment 1, treatment effects had highly significant ($P \leq 0.01$) effects on NWP-$_{Ca}$, NWP-$_{Mg}$, NWP-$_{P}$, NWP-$_{Fe}$, NWP-$_{Na}$ and NWP-$_{Zn}$ in leaf tissues of *C. myriocarpus*, contributing 79, 78, 73, 79, 55 and 36% in TTV of the respective variables (Table 6.1). However, treatments had no significant effects on NWP-$_{K.}$ Similarly, in Experiment 2, treatment effects had highly significant effects on NWP-$_{Ca}$, NWP-$_{K}$, NWP-$_{Mg}$, NWP-$_{P}$, NWP-$_{Fe}$, NWP-$_{Na}$ and NWP-$_{Zn}$, contributing 84, 89, 90, 92, 56, 94 and 90% in TTV of the respective variables (Appendix 6.1-6.14). Treatment means of NWP for test MMN elements were summarised for both experiments (Table 6.2). Nutritional water productivity of NWP-$_{Ca}$ over salinity exhibited negative quadratic relations with the model explained by 94% association in Experiment 1. Similarly, In Experiment 2 NWP-$_{P}$ over increasing chloride salinity exhibited negative quadratic relations with the model explained by 82% association. In contrast, in Experiment 1 NWP-$_{K}$, NWP-$_{Mg}$, NWP-$_{P}$, NWP-$_{Fe}$, NWP-$_{Na}$ and NWP-$_{ZN}$ versus increasing chloride salinity exhibited positive quadratic relations, with models explained by 74, 93, 92, 96, 84 and 93% associations, respectively. Similarly, in Experiment 2, NWP-$_{Ca}$, NWP-$_{K}$, NWP-$_{Mg}$, NWP-$_{Fe}$, NWP-$_{Na}$ and NWP-$_{ZN}$ versus increasing chloride salinity exhibited positive quadratic relations, with models explained by 88, 93, 86, 69, 78 and 85% associations, respectively (Figure 6.2-6.5). Nutritional water productivity of K, NWP-$_{Mg}$, NWP-$_{P}$, NWP-$_{Fe}$, NWP-$_{Na}$ and NWP-$_{Zn}$ in Experiment 1 was optimised at 1.3, 0.24, 0.3, 1.4, 1.3, and 0.5 mM/m^3 ,respectively but and NWP-$_{Ca}$, was minimised at 14. In Experiment 2, NWP-$_{Ca}$, NWP-$_{K}$,

NWP-$_{Mg}$, NWP-$_{P}$, NWP-$_{Fe}$, NWP-$_{Na}$ and NWP-$_{Zn}$ were optimised at 1.6, 1.1, 13.7, 8.2, 1.8, 1.1 and 1.8, respectively (Table 6.3).

Table 6.1. Partitioning sources of variation on nutritional water productivity of selected malnutrient elements of *Cucumis myriocarpus* in response to chloride ions at 56 days under greenhouse conditions (n = 126).

Sources	DF	NWP-$_{Ca}$		NWP-$_{K}$		NWP-$_{Mg}$		NWP-$_{P}$		NWP-$_{Fe}$		NWP-$_{Na}$		NWP-$_{Zn}$	
		MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
EXPERIMENT 1															
Block	17	0.0045	14	29.809	85	21.511	15	1.6293	20	0.0607	13	0.2000	30	0.9958	57
Trt	6	0.0261	79***	2.1751	6^{ns}	108.56	78***	6.0379	73***	0.3672	79***	0.3685	55***	0.6380	36***
Error	102	0.0023	7	3.0775	9	9.255	7	0.6034	7	0.0370	8	0.1021	15	0.1225	7
Total	125	0.0329	100	35.062	100	139.33	100	8.2706	100	0.4649	100	0.6706	100	1.7563	100
EXPERIMENT 2															
Block	17	207.86	12	68.962	7	14.212	6	4.9693	5	0.1801	37	0.0184	4	0.0422	7
Trt	6	1454.9	84***	861.81	89***	205.50	90***	98.695	92***	0.2725	56***	0.4728	94***	0.5354	90***
Error	102	63.03	4	34.511	4	7.970	4	3.3600	3	0.0318	7	0.0124	2	0.0146	3
Total	125	1725.8	100	965.28	100	227.68	100	107.02	100	0.4844	100	0.5036	100	0.5922	100

^{ns}Not significant at P ≤ 0.05; ***Significant at P ≤ 0.01.

Table 6.2 Response of nutritional water productivity of *Cucumis myriocarpus* to increasing chloride salts on at 56 days after initiating treatments under greenhouse conditions (n = 126).

$NaCl+CaCl_2$ mM/m^3	NWP-Ca	NWP-K	NWP-Mg	NWP-P	NWP-Fe	NWP-Na	NWP-Zn
	----------%----------				---------- mg m^{-3}----------		
	Experiment 1						
0 (0)	0.13^{a}±0.02	1.59±0.8	10.9^{a}±0.9	2.83^{a}±0.3	3.43^{a}±0.05	4.61^{a}±0.1	4.43a±0.1
1 (2)	0.12^{ab}±0.01	1.98±0.9	10.7^{a}±0.9	2.92^{a}±0.2	3.46^{a}±0.03	4.67^{a}±0.05	4.40a±0.1
2 (4)	0.13^{abc}±0.02	1.94±0.5	9.98^{ab}±0.7	2.45^{ab}±0.2	3.44^{a}±0.04	4.64^{a}±0.05	4.43a±0.1
3 (8)	0.10^{bc}±0.01	1.43±0.6	8.22^{ab}±0.8	2.17^{ab}±0.2	3.38^{a}±0.06	4.50^{ab}±0.1	4.23ab±0.1
4 (16)	0.08^{bc}±0.01	1.64±0.7	8.63^{ab}±0.9	2.40^{ab}±0.2	3.36^{a}±0.04	4.62^{a}±0.1	4.27a±0.1
5 (32)	0.05^{cd}±0.01	1.14±0.4	7.09^{b}±0.8	1.88^{bc}±0.2	3.27^{a}±0.05	4.43^{ab}±0.1	4.14ab±0.1
6 (64)	0.02^{d}±0.004	1.08±0.5	3.64^{c}±0.4	1.24^{c}±0.1	3.05^{b}±0.07	4.28^{b}±0.1	3.91b±0.1
$NaCl+CaCl_2$ mM/m^3	NWP-Ca	NWP-K	NWP-Mg	NWP-P	NWP-Fe	NWP-Na	NWP-Zn
	----------%----------				---------- mg m^{-3}----------		
	Experiment 2						
0 (0)	38.99^{a}±2.3	29.09^{a}±1.6	14.78^{a}±0.7	9.34^{a}±0.6	3.85^{a}±0.1	4.69^{a}±0.02	3.78^{a}±0.03
1 (2)	36.01^{a}±1.9	28.33^{ab}±1.5	13.40^{ab}±0.7	9.21^{a}±0.5	3.82^{a}±0.04	4.70^{a}±0.02	3.77^{a}±0.03
2 (4)	37.28^{a}±1.9	26.88ab±1.8	13.43^{ab}±0.6	9.01^{a}±0.02	3.85^{a}±0.03	4.69^{a}±0.02	3.78^{a}±0.02
3 (8)	34.96^{ab}±2.5	24.73b±1.3	12.87^{bc}±0.7	3.72^{ab}±0.03	3.82^{a}±0.1	4.64^{ab}±0.03	3.72^{ab}±0.03
4 (16)	34.82^{ab}±2.4	24.49b±1.6	12.75^{bc}±0.7	3.73^{ab}±0.03	3.78^{a}±0.1	4.67^{ab}±0.02	3.73^{ab}±0.03
5 (32)	29.93^{b}±2.3	20.36c±1.4	11.09^{c}±0.8	3.67^{b}±0.03	3.84^{a}±0.04	4.61^{b}±0.03	3.67^{b}±0.03
6 (64)	12.73^{c}±1.6	9.04d±1.1	4.59^{d}±0.5	3.29^{c}±0.1	3.51^{b}±0.1	4.225^{c}±0.04	3.29^{c}±0.1

[y]Column means ± standard error followed by the same letter were not different (P ≤ 0.05) according to Waller-Duncan multiple range test.

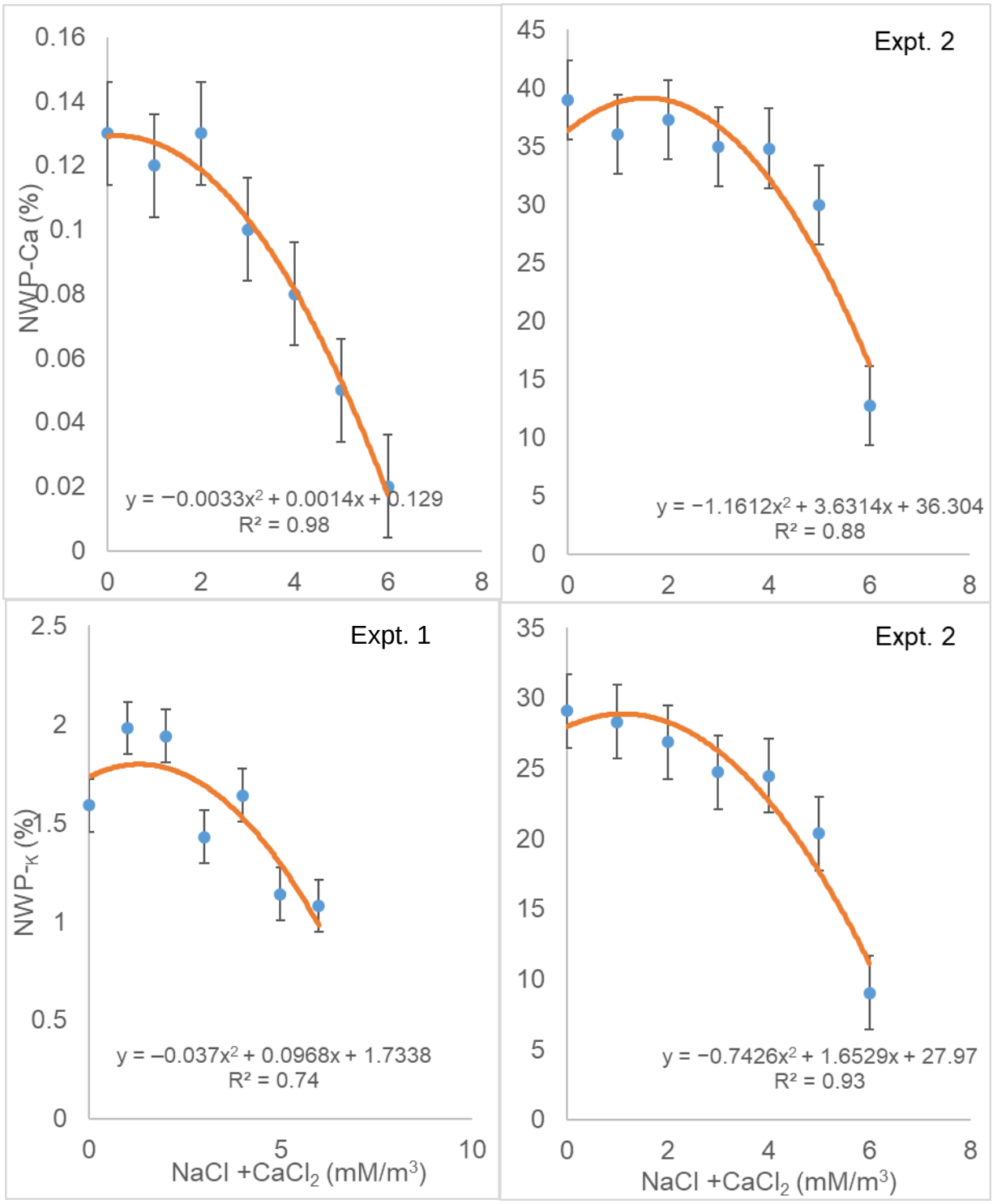

Figure 6.2. Response of nutritional water productivity Ca (NWP_{-Ca}) and K (NWP_{-K}) in *Cucumis myriocarpus* to sodium chloride salinity under greenhouse conditions at 56 days after initiating salinity.

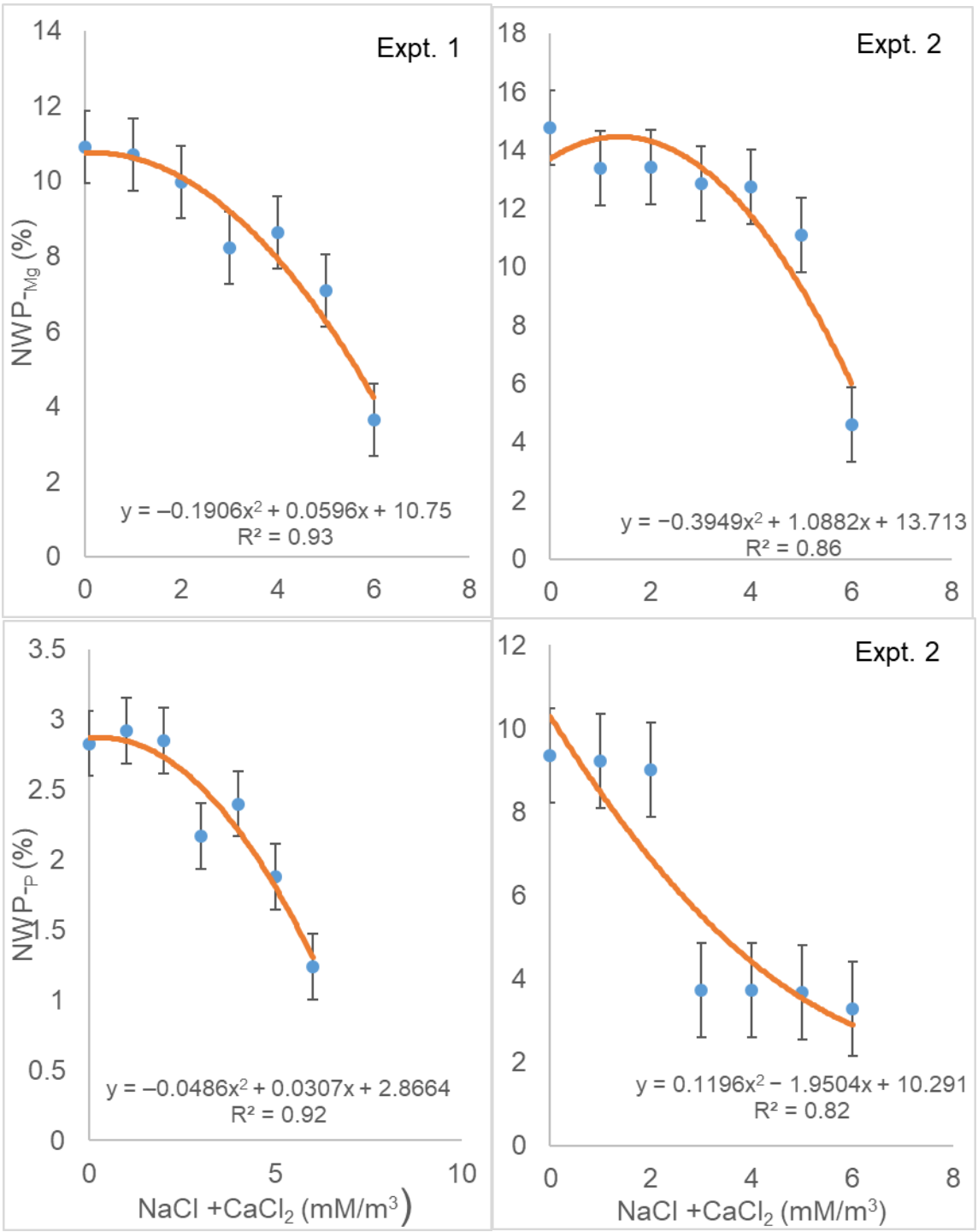

Figure 6.3. Response of nutritional water productivity Mg ($NWP-_{Mg}$) and P ($NWP-_{P}$) to chloride ions in the leaf of *Cucumis myriocarpus* under greenhouse conditions at 56 days after initiating salinity.

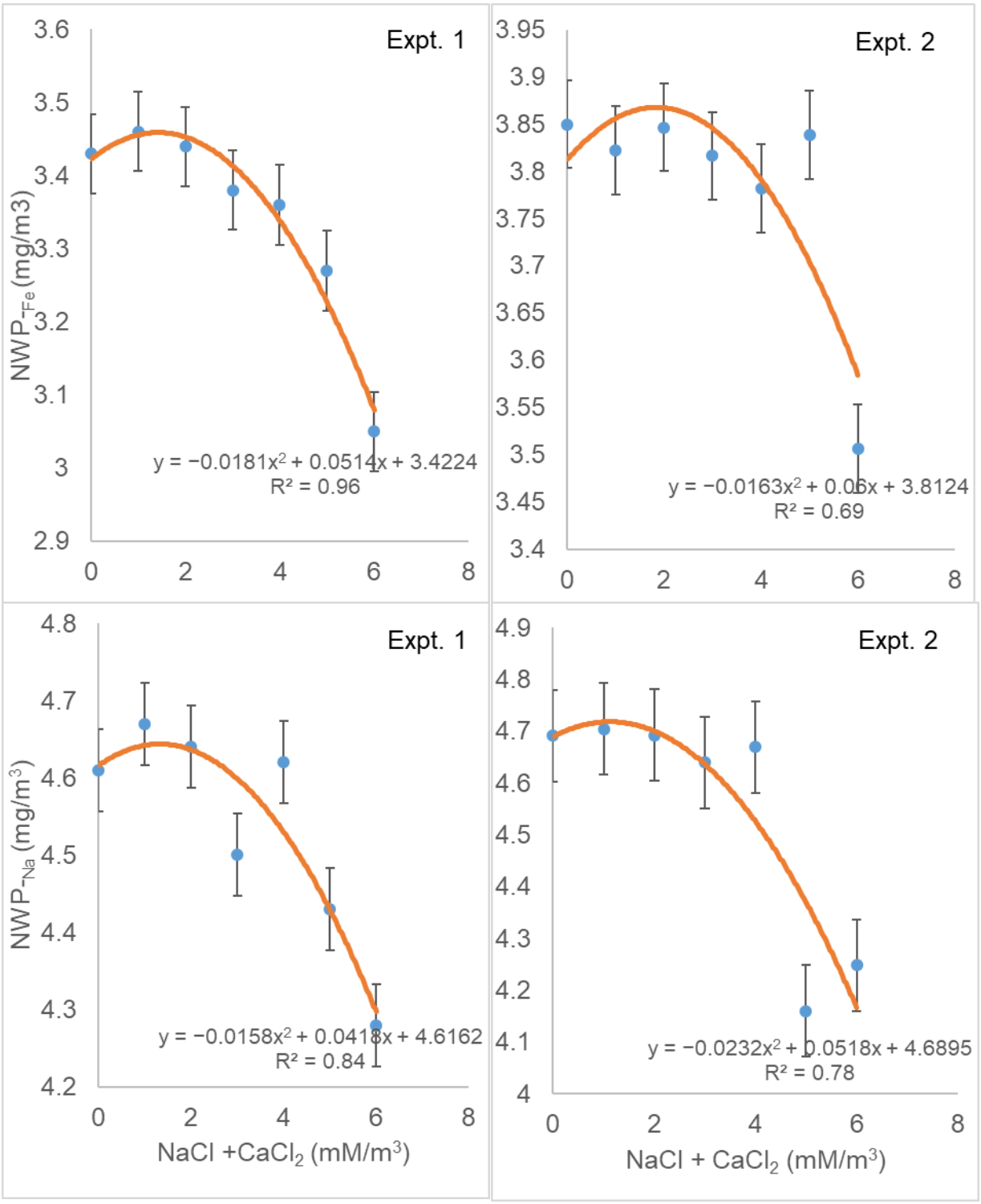

Figure 6.4. Response of nutritional water productivity Fe (NWP-$_{Fe}$) and Na (NWP-$_{Na}$) to chloride ions in the leaf of *Cucumis myriocarpus* under greenhouse conditions at 56 days after initiating salinity.

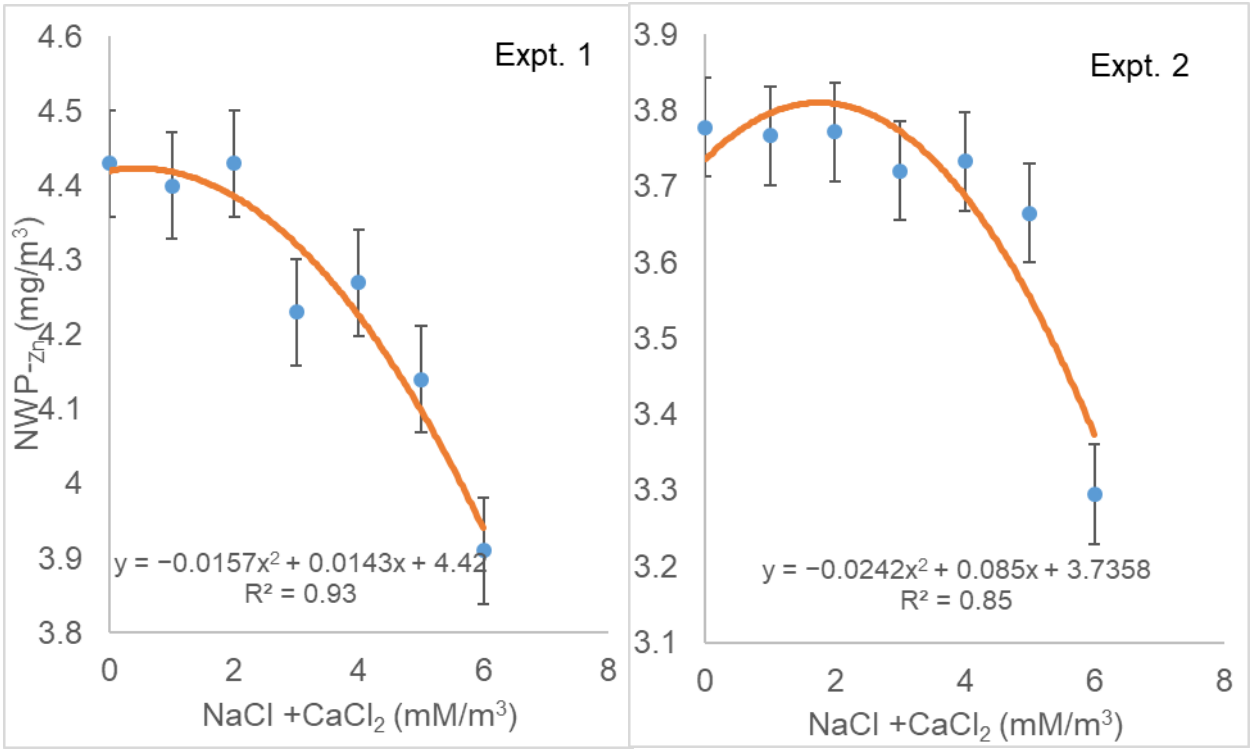

Figure 6.5. Response of nutritional water productivity Zn (NWP-$_{Zn}$) to chloride ions in the leaf of *Cucumis myriocarpus* under greenhouse conditions at 56 days after initiating salinity.

Table 6.3. Optimisation of chloride salts to nutritional water productivity of selected nutrient contents in the indigenous leaf vegetable of *Cucumis myriocarpus* at 56 days after initiating treatments (n = 126).

Experiment 1			
Variable	Model	R^2	x
NWP-$_{Ca}$	$y = -0.0033x^2 + 0.0014x + 0.129$	0.98	0.2
NWP-$_{K}$	$y = -0.037x^2 + 0.0968x + 1.7338$	0.74	1.3
NWP-$_{Mg}$	$y = -0.1906x^2 + 0.0596x + 10.75$	0.93	0.2
NWP-$_{P}$	$y = -0.0486x^2 + 0.0307x + 2.8664$	0.92	0.3
NWP-$_{Fe}$	$y = -0.0181x^2 + 0.0514x + 3.4224$	0.96	1.4
NWP-$_{Na}$	$y = -0.0158x^2 + 0.0418x + 4.6162$	0.84	1.3
NWP-$_{Zn}$	$y = -0.0157x^2 + 0.0143x + 4.42$	0.93	0.5
		Average	0.7
Experiment 2			
Variable	Model	R^2	x
NWP-$_{Ca}$	$y = -1.1612x^2 + 3.6314x + 36.304$	0.88	1.6
NWP-$_{K}$	$y = -0.7426x^2 + 1.6529x + 27.97$	0.93	1.1
NWP-$_{Mg}$	$y = -0.03949x^2 + 1.0882x + 13.713$	0.86	13.7
NWP-$_{P}$	$y = 0.1196x^2 – 1.9504x + 10.291$	0.82	8.2
NWP-$_{Fe}$	$y = -0.0163x^2 + 0.06x + 3.8124$	0.69	1.8
NWP-$_{Na}$	$y = -0.0232x^2 + 0.0518x + 4.6895$	0.78	1.1
NWP-$_{Zn}$	$y = -0.0242x^2 + 0.085x + 3.7358$	0.85	1.8
		Average	.2

$x = -b_1/2b_2$.

6.4 Discussion

Nutritional water productivity of MMN elements versus increasing salinity all except for P (Experiment 2), exhibited density-dependent growth (DDG) patterns. In the stimulation phase (Liu *et al*., 2003; Mashela *et al*., 2017), salinity stimulated NWP of MMN elements, followed by exhibition of the neutral phase where the maximum values were computed using. After the maxima, the inhibition phase was in operation. The later demonstrated that high salinity inhibited NWP in *the in C. myriocarpus.* The current results supported those in the study where the plant was, in terms of growth variables, viewed as being tolerant to chloride salinity (Legong, 2017).

During stimulation, less water (NWP = mg/m^3 water) was gradually required to accumulate MMN elements and MNMN substances, whereas in the neutral phase the amount of water required became constant. Thereafter, the amount of water required gradually increased, resulting in gradually reduced NW. Usually, at high salinity, it becomes increasingly difficult for plants to have access to available water (Claeys *et al*., 2014; Grattan and Grieve, 1999; Shannon and Grieve, 1999). Consequently, it is not easy to comprehend how at high salinity the amount of water is increasing. However, since information on NWP of MMN elements is not available in literature, in the current study, the observations on planting density (Chapter 3) and irrigation interval (Chapter 4) were used to further discuss the observation in the current study.

Both high planting density and extended irrigation interval reduced the quantity of available water to the plant, which is analogous to high salinity (Claeys *et al*., 2014;

Grattan and Grieve, 1999; Shannon and Grieve, 1999). Thus, the stimulation and inhibition in NWP of MMN elements requires a deeper context than the physical decrease and increase of water as contained in the NWP = mg/m^3 water relation (Mabhaudhi *et al*., 2016a; Nyathi *et al*., 2016; 2018; Renault and Wallender, 2000).

At least in salinity, Ca from $CaCl_2$ was used to protect the integrity of the membranes in root cells, particularly at high salinity. Generally, chloride salinity decreases Ca^{2+}/Na^+ ratio, thereby causing excessive uptake of other salt ions, particularly Na^+, thereby causing the deterioration of membrane permeability (Villora *et al*., 2000). The negative effects on cell membranes induced by salt stress could be attributed to extensive oxidative damage and the disturbing of physiological processes which might result in drastic reduction in nutritional quality, chlorophyll content, protein and sugar content (Asaadi, 2009; Ghorbanpour *et al*., 2011). Soil salinity leads to a significant change in ion imbalance, ion uptake, ion toxicity, water potential and oxidative stress in plants (Grattan and Grieve, 1999). Plaut *et al*. (2013) demonstrated that chloride salts in irrigation water affected soil-water-plant relations, with the emphasis being on the physiological activities that eventually limit the productive capacity of the crops.

In both high planting density (Chapter 3) and extended irrigation interval (Chapter 4), the availability of water, for example, mimics that in high salinity, where it becomes increasingly difficult to access the available water due to the accumulation of salts in the soil, thus, reducing osmotic potential (Salisbury and Ross, 1992), where water tends to move from the root cells into the soil. Notwithstanding, it was an important observation that NWP of MMN elements versus the stresses imposed induced on

the availability of soil moisture, regardless of the source as observed in planting density (Chapter 3), irrigation interval (Chapter 4) and salinity in the current study (Chapter 6), exhibited negative quadratic relationships. Although the mechanisms involved are currently obscure, the observations open new frontiers of knowledge in nutrition, in relation to the quality of produce. In all three cases, it was important to observe that at lower levels, planting density (Chapter 3), irrigation interval (Chapter 4) and salinity (Chapter 6), could improve NWP of MMN elements– a very important attribute in improving the nutrition of edible portions of crops, which should be extended to other crops. Additionally, although NWP of MMN elements versus population densities of root-knot (*Meloidogyne* species) nematodes (Chapter 5) did not have significant effects in *C. myriocarpus*, it should be noted that in chili pepper (*Capsicum annuum* L.) cv. Serrano (Ramputla, 2018), the effects exhibited quadratic relationships as observed in planting density, application interval and salinity in the current study. In NWP versus nematodes (Chapter 5), it was emphasised that the significant relationship was not observed probably because *C. myriocarpus* was known to be resistant to *Meloidogyne* species (Pofu *et al*., 2012; Liu *et al*., 2015). In the two experiments, NWP of P versus salinity exhibited contradictory relations, negative (Experiment 1) and positive (Experiment 2) quadratic relations. Since the two were contradictory, it is important that not much emphasis be placed on the observation, except to quickly look at how P is absorbed by roots from soil solutions. Notably, roots from soil solutions primarily as simple cations absorb all MMN elements except for P. In contrast, plants acquire P from soil solution predominately as inorganic phosphate (Pi) ($H_2PO_4PO_4$/HPO−24HPO_4^{2-}), which has a maximum uptake rate at narrow pH of 5 to 6 (Holford, 1997; Marschner, 2012; Rae *et al*., 2003). The uptake occurs by a plasma membrane-localised phosphate transporter-

mediated process, which has been suggested to operate as an H^+co-transporter (Rae *et al*., 2003; Raghothama, 2005). Generally, due to the complexity of Pi, most plants are assisted by mycorrhiza in the optimal absorption of phosphorus (Campos *et al*., 2018). In the current study, their plants were not inoculated with mycorrhiza and although soil pH was not measured, it is well-established that salinity alters soil pH (Mashela, 1992).

6.5 Synthesis and conclusion

Nutritional water productivity of MMN elements versus salinity exhibited negative quadratic relationships, characterised by DDG patterns with three phases: namely, stimulation, neutral and inhibition phases. Observations in the current study corroborated those in planting density (Chapter 3) and irrigation interval (Chapter 4), but without providing any clue as to how the stresses operate to stimulate or inhibit NWP of the test MMN elements. In the context of DDG patterns, salinity stress at low concentration was also beneficial in the MMN of the harvestable leaves of *C. myriocarpus*, whereas the stress was detrimental at high concentrations. The null hypothesis suggesting increasing salinity would not affect the NWP of MMN elements in *C. myriocarpus* under greenhouse and conditions was therefore rejected. In the following research chapter, the effects of increasing vascular arbuscular mycorrhiza on NWP of MMN elements in harvestable leaves of *C. myriocarpus* vegetables were investigated.

CHAPTER 7

EFFECTS OF RESPONSE OF NUTRITIONAL WATER PRODUCTIVITY TO VESICULAR ARBUSCULAR MYCORRHIZA IN *CUCUMIS MYRIOCARPUS*

7.1 Introduction

Vesicular arbuscular mycorrhiza (VAM) are filamentous fungi that develop mutual symbiosis with roots to acquire carbohydrates in exchange for providing the host plant with certain essential nutrient elements and at times with water (Özbay and Newman, 2004; Campos *et al*., 2018; Chandanie *et al*., 2009). Generally, plant growth variables are important in the determination of crop yield (quantity), whereas nutritional water productivity (NWP) (mg/m^3) quantifies the quality of the edible portion of plants in relation to mineral malnutrition (MMN) elements and micronutrient malnutrition (MNMN) substances (Mabhaudhi *et al*., 2016a; Nyathi *et al*., 2016; 2018; Renault and Wallender, 2000). Most plants are poor absorbers of large nutrient elements, which are absorbed in inorganic forms like P, and are dependent upon VAM in absorbing such nutrient elements (Campos *et al*., 2018). Plant growth variables of wild watermelon (*Cucumis myriocarpus* Naude.) versus increasing levels of VAM exhibited positive quadratic relations (Legong, 2017), but without reporting on the responses on NWP of MMN elements, which exclusively focuses on the nutrition of harvestable produce per m^3 water use in their accumulation (Mabhaudhi *et al*., 2016a; Nyathi *et al*., 2016; 2018; Renault and Wallender, 2000). The objective of the study was therefore to determine whether NWP of MMN elements in harvestable leaves of *C. myriocarpus* would be affected by increasing application levels of VAM in the rhizosphere. The null hypothesis stated that NWP of

MMN elements in harvestable leaves of *C. myriocarpus* would not be affected by increasing application levels of VAM in the rhizosphere.

7.2 Materials and methods

7.2.1 Description of the study site

The experiments were conducted in the greenhouse at the Green Biotechnologies Research Centre of Excellence, University of Limpopo, South Africa (23°53'1" S, 29°44'1" E) during autumn (Feb-April) and spring (August-December).

7.2.2 Treatments, experimental design and procedures

Eight treatments, viz., 0, 10, 20, 30, 40, 50, 60 and 70 g/pot mycorrhiza Biocult® (Biocult (Pty) Ltd, (Sommerset West, Republic of South Africa), were arranged in a randomised complete block design, with nine replications. *Cucumis myriocarpus* seedlings were raised in 200-hole seedling trays containing Hygromix-T (Hygrotech, Pretoria) growing medium under greenhouse conditions and hardened-off as described previously (Chapter 3). At 5-leaf stage, uniform seedlings were transplanted into 20-cm-diameter plastic pots, filled with 2 700 ml steam-pasteurised (300°C for 1 h) loam soil, river sand and Hygromix-T at 3:1:1 (v/v) ratio. Pots were placed at 0.40 m × 0.40 m spacing on greenhouse benches. A day after transplanting seedlings were each fertilised with 5 g 2:3:2 (26) + 0.5% Zn + 5% S and 5% Ca per plant and 2 g 2:1:2 (43) and 2 g 2:1:2 (43) Multifeed (Nulandis, Witfield, South Africa) to provide 0.70 mg N, 0.64 mg K and 0.64 mg P, 1.8 mg, 1.5 mg Fe, 0.15 mg Cu, 0.7 mg Zn, 2 mg B, 6 mg Mn and 0.14 mg Mo/ml tapwater. Biocult was dissolved in chlorine-free tap water as per label instruction, stirred and then applied into circular 5-cm deep furrow around the stem of each seedling at

three days after transplanting. Moisture meters were placed in pots with different treatments and plants were irrigated with 300 ml chlorine-free tapwater as soon as 50% of the moisture meters had readings just below 2 units (Chapter 5). Plants were staked to allow the vines free upward moments.

7.2.3 Data collection

At 56 days after inoculation with VAM, 20 mature leaves per plant were manually-harvested and placed in brown paper bags and then oven-dried at 70°C for 72 h and ground in a Willy mill and nutrient elements extracted from 0.4 g materials using the digestion method and quantified using Atomic Absorption Spectrophotometer ICPE 9000 as described previously (Chapter 3). Intact soil clods were collected from each pot, weighed and recorded for wet mass. Soil clods were oven-dried at 105°C for 24 h for dry soil mass. Bulk density and volumetric soil water content were determined as described previously in Chapter 3. Nutritional water productivity was calculated as described previously (Chapter 6).

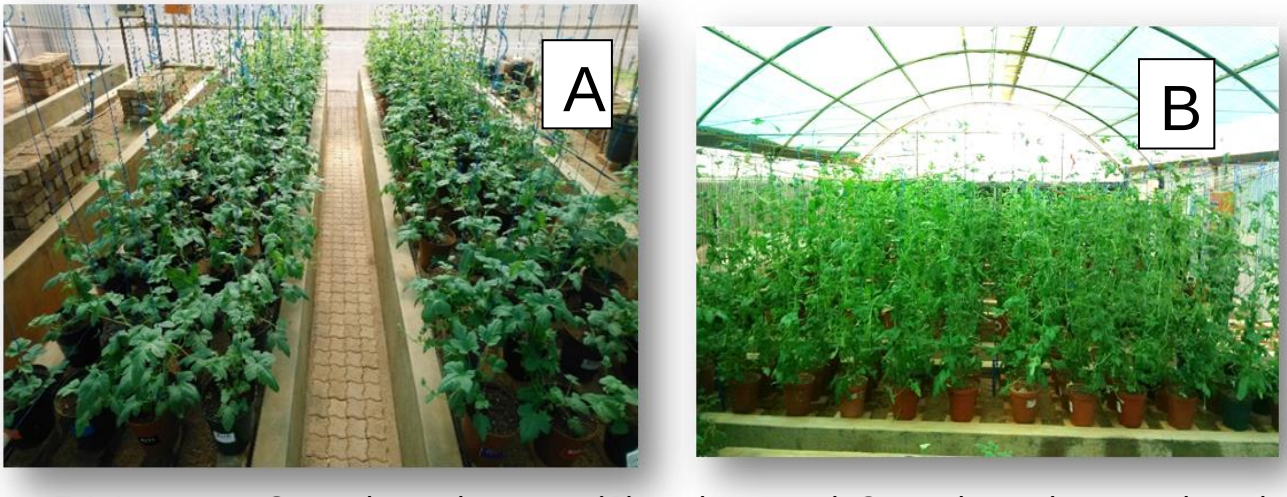

Figure 7.1 Immature Cucumis myriocarpus (**A**) and matured *Cucumis myriocarpus* inoculated with Biocult® mycorrhiza (**B**).

7.2.4 Statistical analysis

The Shapiro - Wilk test was performed to determine the normality of distribution of the data (Ghasemi and Zahediasl, 2012; Shapiro and Wilk, 1965). Since the collected data on NWP of selected malnutrient elements were normally distributed, the data were subjected to analysis of variance (ANOVA) using SAS software (SAS Institute, 2008). However, since the treatment effects were not significant, treatment means were not subjected to any post hoc test.

7.3 Results

Application of Biocult did not have significant effects on NWP of test MMN elements and MNMN substances in in both Experiment 1 and Experiment 2 (Appendix 7.1-7.14). However, treatment means were summarised on tabular format with an indication that the effects were not significant (Table 7.1).

Table 7.1 Partitioning sources of variation on nutritional water productivity of selected malnutrient elements of *Cucumis myriocarpus* in response to increasing vascular arbuscular mycorrhiza at 56 days after initiating treatments (n = 72).

VAM	NWP-$_{Ca}$	NWP-$_{k}$	NWP-$_{Mg}$	NWP-$_{p}$	NWP-$_{Zn}$	NWP-$_{Fe}$	NWP-$_{Na}$
	----------%----------				---------- mg m^{-3}----------		
	Experiment 1						
0	0.95 ± 0.2	1.57 ± 0.2	0.41 ± 0.1	0.43 ± 0.1	2.23 ± 0.1	2.63 ± 0.1	2.56 ± 0.1
10	1.21 ± 0.2	1.82 ± 0.2	0.52 ± 0.1	0.54 ± 0.1	2.27 ± 0.2	2.79 ± 0.2	2.52 ± 0.2
20	1.05 ± 0.1	1.36 ± 0.2	0.39 ± 0.1	0.39 ± 0.1	2.15 ± 0.1	2.62 ± 0.1	2.36 ± 0.1
30	1.20 ± 0.2	1.60 ± 0.3	0.52 ± 0.1	0.43 ± 0.1	2.22 ± 0.1	2.78 ± 0.1	2.45 ± 0.1
40	1.19 ± 0.1	1.63 ± 0.2	0.51 ± 0.1	0.48 ± 0.1	2.23 ± 0.1	2.83 ± 0.1	2.45 ± 0.1
50	1.00 ± 0.2	1.43 ± 0.2	0.44 ± 0.1	0.40 ± 0.1	1.87 ± 0.1	2.32 ± 0.1	2.13 ± 0.1
60	1.01 ± 0.2	1.63 ± 0.2	0.50 ± 0.1	0.44 ± 0.1	2.19 ± 0.2	2.87 ± 0.2	2.47 ± 0.2
70	0.70 ± 0.1	0.84 ± 0.2	0.29 ± 0.1	0.24 ± 0.1	2.07 ± 0.1	2.38 ± 0.1	2.21 ± 0.1
P-value	NS	NS	NS	NS	NS	NS	NS
VAM	NWP-$_{Ca}$	NWP-$_{K}$	NWP-$_{Mg}$	NWP-$_{p}$	NWP-$_{Zn}$	NWP-$_{Fe}$	NWP-$_{Na}$
	----------%----------				---------- mg m^{-3}----------		
	Experiment 2						
0	1.40 ± 0.2	2.60 ± 0.2	0.63 ± 0.1	0.74 ± 0.1	2.42 ± 0.1	2.68 ± 0.1	2.96 ± 0.1
10	1.43 ± 0.2	2.60 ± 0.2	0.67 ± 0.1	0.73 ± 0.1	2.43 ±0.04	2.68 ± 0.1	3.00 ± 0.1
20	1.22 ± 0.2	1.78 ± 0.2	0.52 ± 0.1	0.54 ± 0.1	2.31 ± 0.1	2.60 ±0.1	2.70 ± 0.1
30	1.21 ± 0.1	2.13 ± 0.2	0.57 ±0.04	0.58 ± 0.1	2.32 ±0.03	2.56 ±0.04	2.80 ±0.04
40	1.23 ± 0.2	2.15 ± 0.2	0.64 ± 0.1	0.61 ± 0.1	2.35 ±0.03	2.69 ±0.04	2.84 ± 0.1
50	0.90 ± 0.2	2.14 ± 0.3	0.50 ± 0.1	0.55 ± 0.1	2.21 ± 0.1	2.50 ± 0.1	2.79 ± 0.1
60	1.44 ± 0.2	2.57 ± 0.2	0.69 ± 0.1	0.73 ± 0.1	2.36 ±0.04	2.64 ± 0.1	2.95 ± 0.1
70	0.95 ± 0.1	2.31 ± 0.2	0.63 ± 0.1	0.66 ± 0.1	2.34 ± 0.1	2.61 ± 0.1	2.94 ± 0.1
P-value	NS	NS	NS	NS	NS	NS	NS

NS = Not significant at P ≤ 0.05.

7.4 Discussion

Observations of NWP of MMN elements versus increasing levels of Biocult were similar to those of NWP of MMN elements versus increasing levels of root-knot (*Meloidogyne* species) nematodes (Chapter 5). Generally, in cases were test entities versus increasing factor levels exhibit significant quadratic effects, relations could either exhibit a positive or negative relationship – provided the relations complied with density-dependent growth (DDG) principles (Dube, 2016; Mashela *et al*., 2017; Liu *et al*., 2003). Most of the time, these relationships occur only when the values of the x-axis straddle all three phases of DDG patterns. Mashela *et al.* (2017) demonstrated that to straddle the three DDG phases, the a-axis should at least use a geometric series of 2^x, for example, (0, 2, 4, 8, 16, 32 and 64 units), which to comply with normality and therefore inference should be transformed to using $\log 2^{2x}$, which results into 0, 1, 2, 3, 4, 5 and 6 units (Causton, 1977). The transformation promoted normality, thereby enhancing the feasibility of inference, which is central in scientific methods, which rely on parametric tests for the analysis of data.

In the current and nematode study (Chapter 5), the principle of straddling the three phases of DDG patterns has not complied. Additionally, when the principle was not observed for variables versus allelochemicals, curves of the Curve-fitting Allelochemical Response Dose (CARD) algorithm computer model, which advanced the frontiers of knowledge in quadratic relations (Mashela *et al*., 2022), were invariably skewed to the right (Liu *et al*., 2003, Mafeo, 2012). The latter implied that the principle of inference about the population mean was infringed, suggesting that the model was not a parametric test. In a citrus study, when the principle was not complied for in citrus versus cucurbitacin phytonematicides, outputs of CARD model

were not significant (Mathabatha *et al*., 2017), but when corrected, the model had significant outputs for the test variables.

In terms of CARD model, it indicated that before the stimulation threshold density (D_m) which occurs just prior to the stimulation phase, responses to the factor would not occur (Liu *et al*., 2003). Similarly, when the entity is saturated by the factor, which occurs in the neutral phase (Salisbury and Ross, 1992), treatment effects would not be significant as observed in the current study. However, to be able to make a principled pronouncement, it would be important to later on repeat the study using Biocult levels that would inherently straddle the three DDG phases in order to rule out interference from other factors.

In *C. myriocarpus*, the active ingredient, namely, cucurbitacin A ($C_{32}H_{48}O_8$), accumulates mainly in roots and in fruit (Chen *et al*., 2005). The active ingredient, in extracted form, had been shown to consistently suppress population densities of nematodes (Dube, 2016; Mashela *et al*., 2017). In a recent study, it was shown that cucurbitacin A suppressed the efficacy of Biocult in relation to plant growth and nutrient element variables in tomato plants (Pofu and Mashela, 2022). Others (Olawuyi *et al*., 2011; Nzanza *et al*., 2012; Maboko *et al*. (2013) observed that various other factors in tomato production could affect the efficacy of filamentous fungi in promoting nutrient uptake. Such factors included the level of nutrient elements in soil solution, with the product being more effective under marginal nutrient element regimes. Other factors that influence the efficacy of the product included root volume (Kucey and Janzen, 1987), exposure period (Koch *et al*., 2004; Jakobsen *et al*., 1992, 2002; Smith *et al*., 2004) and soil pH (Salem, 2003). However,

Mashela (2002), confirmed in many other studies (Mashela *et al*., 2017), demonstrated that cucurbitacin phytonematicides did not interfere with soil pH, thus expounding the complexity of the factors that influence the efficacy of VAM. Apparently, observations of non-significant effects in the current study could be attributed to both technical issues and other abiotic factors.

7.5 Synthesis and conclusion

In the current study, failure to comply with DDG principle of straddling all three phases with the treatment level, which is a technical issue, could have resulted in the failure of the treatments to induce significant effects on NWP of MMN elements. Thus, observations in the current study constitute a gap that needs to be addressed in the future. The null hypothesis, which suggested that increasing VAM would not have effects on NWP of MMN elements in harvestable leaves of *C. myriocarpus* was therefore accepted. In the following chapter, the summary, the significance of the findings, the future recommendations and the overall conclusions of the study were provided.

CHAPTER 8

SUMMARY OF FINDINGS, SIGNIFICANCE, RECOMMENDATIONS AND CONCLUSIONS

8.1 Summary of findings

Nutritional water productivity (NWP) of mineral malnutrition (MMN) elements in harvestable leaves of wild cucumber (*Cucumis myriocarpus* Naude) versus planting density, irrigation interval or salinity level, each exhibited significant ($P \leq 0.05$) negative quadratic relations, straddling three phases, namely, stimulation, neutral and inhibition phases. In contrast, NWP of MMN elements in the test plant versus population densities of root-knot (*Meloidogyne* species) nematode or increasing level of Biocult, did not exhibit significant ($P \geq 0.05$) quadratic relationships. The observed effects complied with density-depended growth (DDG) patterns, which spelt outabstract the relationships as being negative or positive quadratic relationships, or no relationship at all. Generally, when relations were exhibiting negative quadratic relations, such allowed optimisation, which was generated using $x = -b_1/2b_2$ relation, derived from the generated quadratic relations. Most NWP of MMN elements versus planting density, irrigation interval or salinity, conformed to negative quadratic relations, which allowed the establishment of the optimisation values, where the independent variable would generate an optimum value for the test MMN elements. Maximisation is important in decision making regarding the attribute being investigated. For example, beyond the maximum point, the independent variables would invariably reduce the dependent variables. Except for NWP_{-P} versus salinity in one of the two experiments, which exhibited positive quadratic relationships, with the

capability of using the $x = -b_1/2b_2$ relation to derive the maximum salinity concentration for P under salinity, the other had negative relation.

8.2 Significance findings

Optimisation of NWP for MMN elements in the current study when *C. myriocarpus* was subjected to various conditions, which were previously predicted to result in extremes of water scarcity had been of quite significance since they could be used in decision-making. First, the stimulation phase, prior to the optimisation point, is an ideal phase in terms of linking the produce of *C. myriocarpus* to the nutritional value of the crop. In contrast, the inhibition phase, post the optimisation point, is not an ideal phase in which the crop should be produced. Observations in the study provided empirically-based averages of optimisation for the test MMN elements versus a particular factor, which would be important for ensuring that *C. myriocarpus*, would produce food of high nutritional value. Explained differently, the crop should never be produced, for the three test independent variables, beyond the optimum average value, since it would be detrimental to some of the MMN elements in the produce of *C. myriocarpus*. Overall, the findings in the current study demonstrated for the first time that nutritional quality in crops is not aligned with the philosophy of high yield (quantity), which is profit-driven. Additionally, the test crop, under marginal growing conditions, would be important in providing MMN elements in marginal communities.

8.3 Recommendations

The influence of M*eloidogyne* species and Biocult on MMN elements in *C. myriocarpus* should be further investigated in compliance with the principle of

independent variables straddling all three DDG phases. Additionally, since *C. myriocarpus* is resistant to *M. incognita*, attempts should be made to use *M. enterolobii*, which is a highly aggressive thermophilic nematode. Additionally, other conditions that impose stress on plants, such as soil type, salt type and infestation with foliar pests, could also be investigated in relation to NWP of MMN elements in harvestable produce of *C. myriocarpus*. In the current study, although under planting density, irrigation interval or salinity the stimulation of NWP of MMN elements, along with their inhibition was associated with increased and decreased water (M^3) in NWP = mg/m^3 water in *C. myriocarpus*, the study was not designed to provide the related mechanisms at the membrane or cellular level. The latter, in unravelling the mechanism involved in relation to stimulating or inhibiting NWP of MMN elements in *C. myriocarpus*, would advance the frontiers of knowledge in enhancing the nutritional values of crops under stress induced by extreme moisture conditions which would be part of a cultural practice intended to conserve moisture.

8.4 Conclusions

Findings in the study demonstrated that *C. myriocarpus* could serve as an ideal leaf vegetable under conditions that had been predicted through modelling to be some of the factors that would prevail as climate change conditions intensify inland South Africa and in many parts of the world. Overall, most of the predicted conditions are characterised by water scarcity and therefore food scarcity. In the context of climate-smart agriculture, it is therefore of uttermost importance that studies in terms of preserving the produce from *C. myriocarpus* for use during times of food scarcity like winter, be investigated as a matter of urgency.

REFERENCES

Abbasi, A. and S. Hisamuddin. 2014. Effect of different inoculum levels of *Meloidogyne incognita* on growth and biochemical parameters of*Vigna radiata*. *Asian Journal of Nematology* 3:15-20.

Abbott, L.K., Robson, A.D. and C. Gazey. 1992. Selection of inoculant vesicular arbuscular mycorrhizal fungi. *Methods in Microbiology* 24:1-21.

Abdelhalim, T.S., Kamal, N.M. and A.B. Hassan. 2019. Nutritional potential of wild sorghum: Grain quality of Sudanese wild sorghum genotypes (*Sorghum bicolour* L. Moench). *Food Science and Nutrition* 1-11.

Acosta-Motos, J.R., Alvarez, S., Barba-Espin, G., Hernandez, J.A. and M.J. Sanchez-Blanco. 2014. Salts and nutrients present in regenerated waters induce changes in water relations, antioxidative metabolism, ion accumulation and restricted ion uptake in *Myrtus communis* L. plants. *Plant Physiological Biochemistry* 85:41-50.

Al-Karaki, G. N. 2000. Growth of mycorrhizal tomato and mineral acquisition under salt stress. *Mycorrhiza* 10:51-54.

Annandale, J.G., Steyn, J.M. and M.K. Nyathi. 2012. Water use and nutritional water productivity of selected food products. In: Wenhold, F., Annandale, J.G., Faber, M. and T. Hart (Eds.). Water use and micronutrient content of crop and animal food products for improved household food security: A scoping study, WRC Report No TT 537/12.

Asaadi, A.M. 2009. Investigation of salinity stress on seed germination of *Trigonella foenum-graecum*. *Research Journal of Biological Sciences* 4:1152-1155.

Bano, A. and M. Fatima. 2009. Salt tolerance in Zea mays (L.) following inoculation with *Rhizobium* and *Pseudomonas*. *Biology and Fertility of Soils* 45:405-413.

Begum, N., Qin, C., Ahanger, M.A., Raza, S., Khan, M.I., Ashraf, M., Ahmed, N. and L. Zhang. 2019. Role of arbuscular mycorrhizal fungi in plant growth regulation: Implications in abiotic stress tolerance. *Frontiers in Plant Science* 10:1068.

Blake, G.R. and K.H. Hartge. 1986. Bulk density. In: Klute, A. (Ed.). Methods of Soil Analysis, Part 1—Physical and Mineralogical Methods, Agronomy Monograph 9, American Society of Agronomy—Soil Science Society of America, Madison, 363-382.

Bouyoucos, G.J. 1962. Hydrometer method improved for making particle size analysis of soils. *Agronomy Journal* 54:464-465.

Campos, P., Borie, F., Cornejo, P., López-Ráez, J.A., López-García, Á. and A. Seguel. 2018. Phosphorus acquisition efficiency related to root traits: Is mycorrhizal symbiosis a key factor to wheat and barley cropping? *Frontiers in Plant Science* 9:752.

Carter, M.R. and E.G. Gregorich. 2008. Soil Sampling and Methods of Analysis. CRC Press, Taylor and Francis Group, Boca Raton.

Causton, D.R. 1977. Contemporary biology: a biologist's mathematics (23). Barrington, E.J.W. and J. Willis (Eds.). 41 Bedford Square, London WCIB 3DO.

Chandanie, W.A., Kubota, M. and M. Hyakumachi. 2009. Interaction between the arbuscular mycorrhizal fungus *Glomus mosseae* and plant growth-promoting fungi and their significance for enhancing plant growth and suppressing damping-off of cucumber (*Cucumis sativus* L.). *Applied Soil Ecology* 41:336-341.

Chen, J.C., Chiu, M.H., Nie, R.L., Cordell, G.A. and X.S. Qiu. 2005. The cucurbitacins

and cucurbitane glycosides: Structures and biological activities. *Natural Product Report* 22:386-399.

Chibarabada, T.P., Modi, A.T. and T. Mabhaudhi. 2017. Nutrient content and nutritional water productivity of selected grain legumes in response to production environment. *International Journal of Environmental Research and Public Health* 14:1-17.

Chivenge, P., Mabhaudhi, T., Modi, A.T. and P. Mafongoya. 2015. The potential role of neglected and underutilised crop species as future crops under water scarce conditions in Sub-Saharan Africa. *International Journal of Environmental Research and Public Health* 12:5685-5711.

Claeys, H., Van Landeghem, S., Dubois, M., Maleux, K. and D. Inze. 2014. What is stress? Dose response effects in commonly used *in vitro* stress assays. *Plant Physiology* 165:519-27.

Coyne, M., McCoach, D. and S. Kapp. 2007. Vocabulary intervention for kindergarten

students: Comparing extended instruction to embedded instruction and incidental exposure. *Learning Disability Quarterly* 30:74-88.

Dean, T., Kaushik, N., Williams, S., Zinter, M. and P. Kim. 2019. Cardiac arrest and pulmonary hypertension in scurvy: a case report. *Pulmonary circulation* 9:1-4.

Debaeke, P. and A. Aboudrare. 2004. Adaptation of crop management to water-limited

environments. *European Journal of Agronomy* 21:433-446.

Dhuique-Mayer, C., Servent, A., Messan, C., Achir, N., Dornier, M. and Y. Mendoza. 2018. Bioaccessibility of biofortified sweet potato carotenoids in baby food: Impact of manufacturing process. *Frontiers in Nutrition* 5:98.

Dube, Z.P. 2016. Nemarioc-AL and Nemafric-BL phytonematicides; bioactivities in *Meloidogyne incognita*, tomato crop, soil type and organic matter. PhD Thesis, University of Limpopo. Sovenga, South Africa.

FAO. 2017. The Future of Food and Agriculture–Trends and Challenges*. Annual Report* 296.

FAO. 2014. The state of food and agriculture, innovation in family farming. FAO: Rome, Italy.

FAO. 2013. FAO Statistical Yearbook: World food and agriculture, food and agriculture organsation of the United Nations, Rome.

Fourie, H., Mc Donald, A. and G. Loots. 2001. Plant-parasitic nematodes in field crops in South Africa. 6. Soybean. *Nematology* 3:447-454.

Gamba, A.M., Kimaro, A.A. and K.M. Mtei. 2020. Effects of climate smart agricultural practices and planting dates on maize growth and nutrient uptake in semi-arid Tanzania. *International Journal of Biosciences* 16:98-109.

Ghasemi, A. and S. Zahediasl. 2012. Normality tests for statistical analysis: A guide for non-statisticians. *International Journal of Endocrinology and Metabolism* 10:486-489.

Ghorbanpour, A., Mami, Y., Ashournezhad, M., Abri, F. and M. Amani. 2011. Effect of salinity and drought stress on germination of Fenugreek. *African Journal of Agricultural Research* 6:5529-5532.

Goswami, B.R., Parakhia, M.V. Golakiya, B.A. and C.R. Kothari. 2018. Morphological
and molecular identification of arbuscular mycorrhizal (AM) fungi. *International Journal of Current Microbiology and Applied Sciences* 7:2336-2347.

Grattan, S.R. and M.C. Grieve. 1999. Salinity-mineral nutrient relations in horticultural
crops. *Scientia Horticulturae* 78:127-157.

Hadebe, S.T., Modi, A.T. and T. Mabhaudhi. 2021. Assessing suitability of sorghum to alleviate Sub-Saharan nutritional deficiencies through the nutritional water productivity index in semi-arid regions. *Foods* 10:385.

Harmon, D.L. and K.C. Swanson. 2020. Review: Nutritional regulation of intestinal starch and protein assimilation in ruminants. *Animal* 14:17-28.

Hartmann, G.L., West, E.D. and T.K. Herman. 2011. Crops that feed the World 2. Soybean – worldwide production, use, and constraints caused by pathogens and pests. *Food Security* 3:5-17.

Haryantini, B., Yuwariah, Y., Amien, S., Fitriatin, B., Setiawati, M.R., Nurbaity, A. Kamaluddin, N., and T. Simarmata. 2019. Community of Indigenous Arbuscular Mycorrhizal Fungi (Amf) of Chili Rhizosphere and Natural Forest Ecosystem. *Malaysian Journal of Soil Science* 23:135-147.

Holford, I.C.R. 1997. Soil phosphorus: its measurement, and its uptake by plants. *Australian Journal of Soil Research* 35:227-239.

Hoque, M.A. and A.P. Butler. 2016. Mineral nutrients and toxicants in Bangladesh groundwater? Do we need a holistic approach to water quality? Arsenic research and global sustainability. CRC Press, New York.

Hussey, R.S. and K.R. Barker. 1973. A comparison of methods of collecting inoculants of *Meloidogyne* species including a new technique. *Plant Disease Report* 7:1025-1028.

Ingraffia, R., Amato, G., Frenda, A.S. and D. Giambalvo. 2019. Impacts of arbuscular mycorrhizal fungi on nutrient uptake, N2 fixation, N transfer, and growth in a wheat/faba bean intercropping system. *PloS One* 14:1-16.

Jagadeesh, P. 2011. Effect of root-knot nematode, *Meloidogyne graminicola* on the nutrient uptake and water use efficiency of rice plant and quality and vigour of seed. Division of Nematology Indian Agricultural Research Institute, New Delhi.

Jakobsen, I. and L. Rosendahl. 1990. Carbon flow into soil and external hyphae from roots of mycorrhizal cucumber plants. *New Phytologist* 115:77-83.

Jakobsen, I., Abbott, L.K. and A.D. Robson. 1992. External hyphae of vesiculal~arbuscular mycorrhizal fungi associated with Subterranean clover *(Trifolium subterraneum* L.*)* spread of hyphae and phosphorous inflow into roots*. New Phytologist* 120:371-380.

Jakobsen, I., Smith, S.E. and F.A. Smith. 2002**.** Function and diversity of arbuscular mycorrhizae in carbon and mineral nutrition. In: van der Heijden, M.G.A and I.R. Sanders. (Eds.). *Mycorrhizal ecology.* Heidelberg, Springer Verlag, Germany.

Jeffery, C. 1978. Cucurbitaceae. In: Launert, E. (Ed.). Flora Zambesiaca Managing Committee: London, UK.

Jensen, M.E., Burman, R.D. and R.G. Allen. 1990. Evapotranspiration and irrigation water requirements. ASCE Manuals and Reports on Engineering Practice 70. New York.

Joshi, K., Mahendran, R., Alagusundaram, K., Norton, T. and B. Tiwari. 2013. Novel disinfectants for fresh produce. *Trends in Food Science and Technology* 34:54-61.

Jones, J.B. and V.W. Case. 1990. Sampling, handling and analysing plant tissue samples. In: Westerman, R.L. (Ed.). Soil testing and plant analysis. Third edition, Soil Science Society of America, Book Series No. 3, Madison, Wisconsin.

Jørn, G. Inge, S. and C.A. 2006. Cucurbitacins in plant food. In: Kole, C. and H. Matsumura (Eds.). The Bitter Gourd Genome. New Deihi, India.

Kanda, E.K., Senzanje, A., Mabhaudhi, T. and S.C. Mubanga. 2020. Nutritional yield and nutritional water productivity of cowpea (*Vigna unguiculata* L. Walp) under varying irrigation water regimes. *Water SA* 46:410-418.

Kamel, M.S., Rauf, A., Mahmood, M.S. and S. Amer. 1983. The effect of plant population on local Roumi maize grain yield when grown under irrigation. *Annals of Agricultural Science* 19:79-93.

Koch, A.M., Kuhn, G., Fontanillas, P., Fumagalli, L., Goudet, I. and I.R. Sanders. 2004. High genetic variability and low local diversity in a population of arbuscular mycorrhizal fungi. *Proceedings of the National Academy of Sciences of the United States of America* 101:2369-2374.

Kristkova, E., Lebeda, A., Vinter, V. and O. Blahousek. 2003. Genetic resources of genus *Cucumis* and their morphological description. *HortScience* 30:14-42.

Kucey, R.M.N. and H. Janzen. 1987. Effects of VAM and reduced nutrient availability on growth and phosphorus and micronutrient uptake of wheat and field beans under greenhouse conditions. *Plant and Soil* 104:71-78.

Läuchli, A. and S.R. Grattan. 2007. “Plant growth and development under salinity stress. In: Jenks, M.A., Hasegawa, P.A. and S.M. Jain. (Eds.). Advances in molecular-breeding towards salinity and drought tolerance, Springer-Verlag, New York.

Laurie, S., Faber, M., Adebola, P. and A. Belete. 2015. Biofortification of sweet potato for food and nutrition security in South Africa. *Food Research International* 76:962-970

Lee, M. and M. Van Iersel. 2008. Sodium chloride effects on growth, morphology, and physiology of chrysanthemum (*Chrysanthemum morifolium*). *HortScience* 43:1888-1891.

Leedy, P.D. and J.E. Ormrad. 2005. Practical research: Planning and design. UpperSaddle River, Prentice Hall, New Jersey.

Legong, N.E. 2017. Interactive effects of salinity and vesicular arbuscular mycorrhiza in growth and accumulation of essential nutrient elements in indigenous *Cucumis myriocarpus.* MSc mini-dissertation, University of Limpopo. Sovenga, South Africa.

Li, X. and F. Shi. 2019. The effect of flooding on evaporation and the groundwater table for a salt-crusted soil. *Water* 11:1003.

Lima, F.S., Mattos, V.S., Silva, E.S., Carvalho, M.A., Teixeira, R.A., Silva, J.C. and V.R. Correa. 2018. Nematodes affecting potato and sustainable practices for their management. In: Mustafa, Y. (Ed.). Potato: From Incas to all over the world. Intech open, London, United Kingdom.

Lipper, L., Thornton, P., Campbell, B.M., Baedeker, T., Braimoh, A., Bwalya, M., Caron, P., Cattaneo, A., Garrity, D., Henry, K., Hottle, R., Jackson, L., Jarvis, A., Kossam, F., Mann, W., McCarthy, N., Meybeck, A., Neufeldt, H., Remington, T., Sen, P.T., Sessa, R., Shula, R., Tibu, A. and E.F. Torquebiau. 2014. Climate-smart agriculture for food security. *Nature Climate Change* 4:1068-1072.

Little, T.M. and F.J. Hills. 1978. Agricultural experimentation: Design and analysis. Wiley, New York.

Little, K.M. Metelerkamp, B. and C.W. Smith. 1998. A comparison of three methods of soil water content determination. *South African Journal of Plant and Soil* 15:80-89.

Liu, D.L., An, M., Johnson, I.R. and J.V. Lovett. 2003. Mathematical modelling of allelopathy. III. A model for Curve-fitting Allelochemical Response Dose. *Non-linearity in Biology, Toxicology and Medicine* 1:37-50.

Liu, L.Z., Gong, Z.Q., Zhang, Y.L. and P.J. Li. 2014. Growth, cadmium uptake and accumulation of maize (*Zea mays* L.) under the effects of arbuscular mycorrhizal fungi. *Ecotoxicology* 23:1979-1986.

Liu, B., Ren, J., Zhang, Y., An, J., Chen, M. and H. Chen. 2015. A new grafted rootstock against root-knot nematode for cucumber, melon, and watermelon. *Agronomy for Sustainable Development* 35:251-259.

Liu, C., Ravnskov, S., Liu, F., Rubæk, G.H. and M.N. Andersen. 2018. Arbuscular mycorrhizal fungi alleviate abiotic stresses in potato plants caused by low phosphorus and deficit irrigation/partial root-zone drying. *Journal of Agricultural Science* 156:46-58.

Mabhaudhi, T., Chibarabada, T. and A. Modi. 2016a. Water-Food-Nutrition-Health Nexus: Linking water to improving food, nutrition and health in Sub-Saharan Africa. *International Journal of Environmental Research of Public Health* 13:1-19.

Mabhaudhi, T., Chibarabada, T. and A. Modi. 2016b. Water-Food-Nutrition-Health Nexus: Linking water to improving food, nutrition and health in Sub-Saharan Africa. *International Journal of Environmental Research and Public Health* 13:107.

Maboko, M.M., Bertling, I. and C.P. du Plooy. 2013. Arbuscular mycorrhiza has limited effects on yield and quality of tomatoes grown under soiless cultivation. *Acta Agriculturae Scandinavica, Section B — Soil and Plant Science* 63: 261-270.

Mabotja, T.C. 2019. Interactive effects of irrigation interval and planting density on vegetative yield and chemical composition of nightshade (*Solanum retroflexum*) in Limpopo Province, South Africa. MSc Dissertation. University of Limpopo, Sovenga.

Mafeo, T.P. 2012. Responses of economically important crops to crude extracts of *Cucumis myriocarpus* fruit when used as a pre-emergent bio-nematicide. PhD Thesis, University of Limpopo, Sovenga, South Africa.

Maile, K.D. 2015. Responses of *Tylenchulus semipenetrans* to crude extracts of indigenous *Cucumis* fruits with and without effective micro-organisms in citrus production. Masters Dissertation. University of Limpopo, Sovenga.

Maleka, K.G. 2020. Interactive effects of *Meloidogyne* species and sugarcane aphid (*Melanaphis sacchari)* on nematode resistance in sweet stem sorghum

and effects of terpenoid-containing phytonematicides on both pests. PhD Thesis, University of Limpopo, Sovenga, South Africa.

Mamphiswana, N., Mashela, P.W. and L. Mdee. 2010. Distribution of total phenolics and antioxidant activity in fruit, leaf, stem and root of *Monsonia burkeana*. *African Journal of Agricultural Research* 5:2570-2575.

Marijke, H. 2020. Biofortification for better nutrition: developing and delivering crops with more impact. PhD Thesis, National University of Ireland, Galway.

Marschner, H. 2012. Marschner's mineral nutrition of higher plants. Marschner, P. (Ed.). Academic Press, London.

Mashela, P.W. 2017. Chloride and carbonate salinity tolerance in *Mimusops zeyheri* seedlings during summer and winter shoot flushes. *Acta Agriculturae Scandinavica, Section B-Soil and Plant Science* 67:734-42.

Mashela, P.W. 2002. Ground wild cucumber fruits suppress numbers of *Meloidogyne incognita* on tomato in microplots. *Nematropica* 32:13-19.

Mashela, P.W. 1992. Interactions of *Tylenchulus semipenetrans*, citrus rootstocks and salinity. PhD thesis, University of Florida, Gainesville, Florida, USA.

Mashela, P.W. and M.E. Nthangeni. 2002. Efficacy of *Ricinus communis* fruit meal with and without *Bacillus* species on suppression of *Meloidogyne incognita* and growth of tomato. *Journal of Phytopathology* 150:399-402.

Mashela, P.W. and N. Mollel. 2001. Farmer-identified indigenous fruit tree with suitable attributes for the semi-arid Northern Province of South Africa. *South African Journal of Agricultural Extension* 30:1-12.

Mashela, P.W., Dube, Z.P. and K.M. Pofu. 2015. Managing the phytotoxicity and inconsistent nematode suppression in soil amended with

phytonematicides. In: Meghvansi, M.K. and A. Varma (Eds.). Organic amendments and soil suppressiveness in plant disease management. Springer International Publishing, Switzerland.

Mashela, P.W., Pofu, K.M. Ndhlala, A.R. and Z.P. Dube. 2016. Response of mineral malnutrition elements in African ginger pseudo-stems to nematode infection. *Acta Agriculturae Scandinavica, Section B – Soil and Plant Science* 66(5):387-390.

Mashela, P.W., De Waele, D., Dube, Z.P., Khosa, M.C., Pofu, K.M. Tefu, G., Daneel, M.S. and H. Fourie. 2017. Alternative nematode management strategies. In: Fourie, H., Spaull, V.W., Jones, R.K., Daneel, M.S. and D. De Waele. (Eds.). Nematology in South Africa: A view from the 21st century. Springer International Publishing, Heidelberg, Switzerland.

Mashela, P.W., Shokoohi, E., Ndhlala, A.R., Pofu, K.M. and D. Raphasha. 2022. Bioremediation of cucurbitacins from cucurbitacin phytonematicides, in *Pesticide Bioremediation.* Solana, A.C., Vieira de Castro, L.F. M. and N. Nayling. (Eds.). (Cham: Springer). Springer International Publishing.

Mathabatha, R.V., Mashela, P.W. and M.N. Mokgalong. 2017. Non-phytotoxic concentration of cucurbitacin-containing phytonematicides and the overall sensitivities to Swingle citrumelo seedling rootstock. *Research on Crops* 18(3):518-522.

Morton, J.F. 2007. The impact of climate change on smallholder and subsistence agriculture. *Proceedings of the National Academy of Sciences of the United States of America* 104:19680-19685.

Mphahlele, R.R., Mashela, P.W. and K.M. Pofu. 2012. Post-harvest fruit decay-inducing pathogen in medicinally important *Cucumis* species indigenous to South Africa. *African Journal of Agricultural Research* 6:3786-379.

Mulandana, N.S., Mamadi, N.E., Beletse, Y.G. and C.P. du Plooy. 2010. Effect of spacing, transplanting time and harvesting methods on Amaranths (*Amaranthus cruentus*) production. *Abstracts of the combined congress*. Bloemfontein.

Müller, O. and M. Krawinkel. 2005. Malnutrition and health in developing countries. *Canadian Medical Association Journal* 173:279-86.

Munns, R. 2002. Comparative physiology of salt and water stress. *Plant Cell and Environment* 28:239-250.

Mutshekwa, N., Mashela, P.W. and M.S. Mphosi. 2019. Effects of irrigation interval on vegetative growth and productivity of okra (*Abelmoschus esculentus*). *Research on Crops* 20:748-752.

Ndlovu, J. and A.J. Afolayan. 2008. Nutritional analysis of the South African wild vegetable *Corchorus olitorius* L. *Asian Journal of Plant Sciences* 7:615-618.

Nedjimi, B. 2009. Salt tolerance strategies of *Lygeum spartum* L.: A new fodder crop for Algerian saline steppes. *Flora* 204:747-754.

Nyathi, M.K., Van Halsema, G.E., Beletse, Y.G., Annandale, J.G. and P.C. Struik. 2018. Nutritional water productivity of selected leaf vegetables. *Agricultural Water Management* 209:111-122.

Nyathi, M., Annandale, J., Beletse, Y., Beukes, D., du Plooy, C.P., Pretorius, B. and G.E. van Halsema. 2016. Nutritional water productivity of

traditional vegetable crops. Water Research Commission, Pretoria, South Africa.

Nzanza, B., Marais, D. and P. Soundy. 2012. Effect of arbuscular mycorrhizal fungal inoculation and biochar amendment on growth and yield of tomato. *International Journal of Agriculture and Biology* 14:965-969.

Olawuyi, O.J., Babatunde, F.E., Akinbode, O.A., Odebode, A.C. and S.A. Olakojo. 2011. Influence of Arbuscular mycorrhizal and N.P.K fertilizer on the productivity of cucumber (*Cucumis sativus*). *International Journal of Organic Agriculture Research and Development 3:*22-31.

Özbay, N., and S. Newman. 2004. Effect of *Trichoderma harzianum* strains to colonize tomato roots and improve transplant growth. *Pakistan Journal of Biological Sciences* 7:253.257.

Pelinganga, O.M. 2013. Developing bio-nematicides using indigenous *Cucumis africanus* and *Cucumis myriocarpus* fruits for tomato production systems. PhD Thesis, University of Limpopo, Sovenga, South Africa.

Pelinganga, O.M., Mashela, P.W., Nzanza, B. and M.S. Mphosi. 2012. Baseline information on using fermented crude extracts from *Cucumis africanus* fruit for suppression of *Meloidogyne incognita* and improving growth of tomato plant. *African Journal of Biotechnology* 11:11407-11413.

Pirzad, A. and F. Shokrani. 2012. Effects of iron application on growth characters and flower yield of *Calendula officinalis* L. under water stress. *World Applied Sciences Journal* 18:1203-1208.

Plaut, J.A., Wadsworth, W.D., Pangle, R., Yepez, E.A., McDowell, N.G. and W.T. Pockman. 2013. Reduced transpiration response to precipitation

pulses precedes mortality in a pinon juniper woodland subject to prolonged drought. *New Phytologist* 200:375-387.

Pofu, K.M. 2012. Potential uses of indigenous *Cucumis africanus* and *Cucumis myriocarpus* as root-knot nematode-resistant rootstocks in watermelon (*Citrullus lanatus*) husbandry. PhD Thesis, University of Limpopo. Sovenga, South Africa.

Pofu, K.M. and P.W. Mashela. 2011. Using relative penetration and maleness indices in *Meloidogyne incognita* to establish resistance type in *Cucumis myriocarpus*. *African Journal of Biotechnology* 10:390-393.

Pofu, K.M. and P.W. Mashela. 2022. Interactive effects of filamentous fungi and cucurbitacin phytonematicide on growth of cowpea and suppression of *Meloidogyne enterolobii*. *Frontiers in Microbiology* 13:765051.

Pofu, K.M., Mashela, P.W. and H. Shimelis. 2012. Intergeneric grafting in watermelon for managing *Meloidogyne* species: a review. *Scientific Research and Essays* 7:107-113.

Primm, A. 2013. Four-diseases caused by a lack of essential vitamins and minerals. Accessed date March 2018. www.mentalfloss.com/article/50697/4-diseases caused-lack-essential-minerals-and vitamins.

Procópio, S.O., Balbinot A.A., Debiasi, H. Franchini, J.C. and F. Panison. 2013. Cross-planting in soybean using a cultivar with a habit of undetermined growth. Journal of Agricultural Sciences 56:319-325.

Rae, A.L., Cybinski, D.H., Jarmey, J.M., and F.W. Smith. 2003. Characterization of two phosphate transporters from barley; evidence for diverse function and kinetic properties among members of the Pht1 family. *Plant Molecular Biology* 53:27-36.

Raghothama, K.G. 2005. Phosphorus and plant nutrition: An overview. In: Sims, T. and A.N. Sharpley (Eds.). Agronomy Monographs. American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc.

Ramputla, M.J. 2018. Nutritional water productivity of hot chilli (*Capsicum annuum*) under infection with *Meloidogyne javanica* and *Meloidogyne incognita* race 4. MSc mini-Dissertation. University of Limpopo, Sovenga.

Renault, D. and W.W. Wallender. 2000. Nutritional water productivity and diets. *Agricultural Water Management* 45:275-296.

Ripoll, J., Urban, L., Staudt, M., Lopez-Lauri, F., Bidel, L.P.R. and N. Bertin. 2014. Water shortage and quality of fleshy fruits-making the most of the unavoidable. *Journal of Experimental Botany* 65:4097-5117.

Salem, S.F. 2003. Impact of mycorrhizal fungi and other symbiotic microbes as biocontrol agents on soil borne pathogens and some ecophysiological changes in tomato roots. PhD Thesis, Szent Stván University.

Salisbury, F.B. and C.W. Ross. 1992. Plant physiology, hormones and plant regulators: Auxins and gibberellins. Wadsworth Publishing, Belmont.

Sarker, U., Islam, T. and S. Oba. 2018. Salinity stress accelerates nutrients, dietary fibre, minerals, phytochemicals and antioxidant activity in *Amaranthus tricolor* leaves. PLoS ONE13 (11):e0206388.

SAS Institute INC. 2008. *SAS/STAT® 9.2 Qualification Tools User's Guide*. SAS Institute Inc, Cary, North Carolina.

Schopfer, P. 2001. Hydroxyl radical-induced cell-wall loosening in vitro and in vivo: Implications for the control of elongation growth. *Plant Journal* 28:679-688.

Shadung, K.G. 2016. Quality Protocols for Nemarioc-AL and Nemafric-BL phytonematicides and potential chemical residues in tomato fruits. PhD Thesis, University of Limpopo, Sovenga, South Africa.

Shannon, M.C. and C.M. Grieve. 1999. Tolerance of vegetable crops to salinity. *Scientia Horticulturae* 78:5-38.

Shapiro, S.S. and M.B. Wilk. 1965. An analysis of variance test for normality (complete samples). *Biometrika 52*:591-611.

Shelembe, S.C. 2020. Water use and nutritional water productivity of taro (*Colocasia esculenta* L. schott) landraces. MSc Dissertation, University of KwaZulu-Natal Pietermaritzburg South Africa.

Shiundu, K.M. 2002. Role of African leafy vegetables (ALVs) in alleviating food and nutrition insecurity in Africa. *African Journal of Food and Nutritional Sciences* 2:96-97.

Shrivastava, P. and R. Kumar. 2015. Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. *Saudi Journal of Biological Sciences* 22:123-131.

Smith, S.E., Smith, F.A. and I. Jakobsen. 2004. Functional diversity in arbuscular mycorrhizal (AM) symbioses: The contribution of the mycorrhizal P uptake pathway is not correlated with mycorrhizal responses in growth or total P uptake. *New Phytologist* 162:511-524.

Steyn, J.M., Haverkort, A.J., Franke, A.C., Engelbrecht, F.A., van der Waals, J.E. and K. Kruger. 2014. Impact of different climate change scenarios on future potato production in South Africa: Eastern Free State, Limpopo and Sandveld. *Potatoes South Africa*, Final Report: Pretoria.

Steyn, N.P., Burger, S., Monyeki, K.D., Alberts, M. and G. Nthangeni. 2001. Seasonal variation in dietary intake of the adult population of Dikgale. *South African Medical of Clinical Nutrition* 14:140-145.

Sudhir, P. and S.D.S. Murthy. 2004. Effect of salt stress on basic processes of photosynthesis. *Photosynthetica* 42:481-486.

Sukmasari, M.D., Dani, U., and A.A. Wijaya. 2021. Arbuscular mycorrhiza inoculation for increasing the tolerance index and productivity of soybean on marginal soils. *Earth and Environmental Science* 748.

Sultana, N., Ikeda, T. and R. Itoh. 2000. Effect of NaCl salinity on photosynthesis and dry matter accumulation in developing rice grains. *Environmental and Experimental Botany* 42:211-220.

Taffouo, V.D., Nouck, A.H., Dibong, S.D. and A. Amougou. 2010. Effects of salinity stress on seedling growth, numeral nutrients, and total chlorophyll of some tomato (*Lycopersicum esculentum* L.) cultivars. *African Journal of Biotechnology* 9:5366-5372.

Taiz, L. and E. Zeiger. 2002. Sinauer Association. *Annual of Botany* 91:750-751.

Talwana, H.A.L., Speijer, P.R., De Waele, D. and R.L. Swennen. 2003. Effect of nematode infection and damage on nutrient concentrations in leaves of three banana cultivars commonly grown in Uganda. *African Crop Science Conference Proceedings* 6:182-190.

Tawfik, K.M. 2008. Evaluating the use of rhizobacterin on cowpea plants grown under salt stress. *Research Journal of Agriculture and Biological Sciences* 4(1):26-33.

Thierfelder, C., Chivenge, P., Mupangwa, W., Rosenstock, T.S., Lamanna, C. and J.X. Eyre. 2017. How climate-smart is conservation agriculture (CA)? – Its

potential to deliver on adaptation, mitigation and productivity on smallholder farms in southern Africa. *Food Security* 9:537-560.

Ullah, S., Ashraf, M., Asghar, H.N., Iqbal, Z. and R. Ali. 2019. Plant growth promoting rhizobacteria mediated amelioration of drought in crop plants. *Soil Environmental Science* 38:01-20.

Villora, G., Moreno, D.A., Pulgar, G. and L. Romero. 2000. Salinity affects phosphorus uptake and partitioning in zucchini. *Communications in Soil Science and Plant Analysis* 31:501-507.

Wang, B. and Y. L. Qiu. 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza* 16:299-363.

Wenhold, F.A.M., Faber, M., van Averbeke, W. Oelofse, A., Van Jaarsveld, P. Jansen van Rensburg, W.S., van Heerden, I. and R. Slabbert. 2007. Linking smallholder agriculture and water to household food security and nutrition. *Water South Africa* 33:327-36.

World Health Organisation (WHO). 1992. Global programme on AIDS: consensus statement from the WHO/UNICEF consultation on HIV transmission and breastfeeding. *Weekly Epidemiological Records* 67:177-184.

World Health Organization (WHO). 1996. Trace elements in human nutrition and health. *Geneva: World Health Organisation*.

Yarnia, M. 2010. Sowing dates and density evaluation of Amaranth (cv. Koniz) as a new crop. *Advances in Environmental Biology* 4:41-46

Zhao, D., Kane, M. and B.E. Borders. 2011. Growth responses to planting density and management intensity in loblolly pine plantations in the south eastern USA Lower Coastal Plain. *Annals of Forest Science* 68:625-635.

Zygmunt, B. and J. Namiesnik. 2003. Preparation of samples of plant material for chromatographic analysis. *Journal of Chromatographic Science* 39(10):109-116.

APPENDICES

Appendix 3.1. Collected data and calculations of bulk density (BD), Evapotranspiration (Eta) and water productivity (WP) in *Cucumis myriocarpus* in response to planting density/hole drip irrigation at 56 days under field conditions, in Experiment 1 (n = 72).

Rep	PD	Ww	Dw	Gravimetric	BD	Vol (%)	Vol (mm/m)	Moisture (mm)	Moisture (mm)	Eta = I + R -(SWC)	DSM	Dry Yield	WP
R1	T1	141.6	130.91	8.17	1.34	10.91	109.08	21.82	50.3	462.2	6.58	109.89	0.25
R1	T2	152.33	144.02	5.77	1.47	8.48	84.80	16.96	49.6	462.9	6.58	109.89	0.25
R1	T3	133	118.23	12.49	1.21	15.07	150.71	30.14	56.0	456.5	2.65	44.26	0.10
R1	T4	132.1	118.23	11.73	1.21	14.15	141.53	28.31	57.0	455.5	21.07	351.87	0.82
R1	T5	136.46	117.55	16.09	1.20	19.30	192.96	38.59	56.8	455.7	18.51	309.12	0.74
R1	T6	143.99	129.52	11.17	1.32	14.77	147.65	29.53	58.8	453.7	21.2	354.04	0.83
R1	T7	140.15	130.59	7.32	1.33	9.76	97.55	19.51	56.6	455.9	13.05	217.94	0.50
R1	T8	135.98	126.59	7.42	1.29	9.58	95.82	19.16	48.4	464.1	23.95	399.97	0.90
R1	T9	153.79	130.98	17.41	1.34	23.28	232.76	46.55	58.8	453.7	22.04	368.07	0.90
R2	T1	131.06	129.4	1.28	1.32	1.69	16.94	3.39	56.6	455.9	6.97	116.40	0.26
R2	T2	138.61	130.08	6.56	1.33	8.70	87.04	17.41	48.4	464.1	5.45	91.02	0.20
R2	T3	131.44	120.9	8.72	1.23	10.76	107.55	21.51	50.3	462.2	14.33	239.31	0.54
R2	T4	153.8	120.46	27.68	1.23	34.02	340.20	68.04	58.8	453.7	111.92	1869.06	4.85
R2	T5	138.56	129.2	7.24	1.32	9.55	95.51	19.10	49.6	462.9	39.06	652.30	1.47
R2	T6	133.8	109.32	22.39	1.12	24.98	249.80	49.96	57.0	455.5	9.8	163.66	0.40
R2	T7	123.06	109.82	12.06	1.12	13.51	135.10	27.02	56.0	456.5	24.71	412.66	0.96

R2	T8	134.81	127.1	6.07	1.30	7.87	78.67	15.73	49.6	462.9	34.15	570.31	1.28
R2	T9	138.86	123.06	12.84	1.26	16.12	161.22	32.24	56.8	455.7	35.54	593.52	1.40
R3	T1	135.69	127.1	6.76	1.30	8.77	87.65	17.53	48.4	464.1	5.42	90.51	0.20
R3	T2	141.22	125.37	12.64	1.28	16.17	161.73	32.35	56	456.5	11.85	197.90	0.47
R3	T3	155.97	124.63	25.15	1.27	31.98	319.80	63.96	58.8	453.7	6.75	112.73	0.29
R3	T4	132.58	122.68	8.07	1.25	10.10	101.02	20.20	50.3	462.2	90.37	1509.18	3.41
R3	T5	131.18	121.36	8.09	1.24	10.02	100.20	20.04	56.6	455.9	5.69	95.02	0.22
R3	T6	149.55	125.37	19.29	1.28	24.67	246.73	49.35	50.3	462.2	44.19	737.97	1.79
R3	T7	135.88	126.79	7.17	1.29	9.28	92.76	18.55	48.4	464.1	60.83	1015.86	2.28
R3	T8	131.97	118.97	10.93	1.21	13.27	132.65	26.53	56.8	455.7	27.4	457.58	1.07
R3	T9	153.38	118.51	29.42	1.21	35.58	355.82	71.16	57	455.5	31.4	524.38	1.36
R4	T1	150.99	135.64	11.32	1.38	15.66	156.63	31.33	56.8	455.7	11.2	187.04	0.44
R4	T2	147.47	132	11.72	1.35	15.79	157.86	31.57	50.3	462.2	52.1	870.07	2.02
R4	T3	157.21	147.32	6.71	1.50	10.09	100.92	20.18	49.6	462.9	17.64	294.59	0.67
R4	T4	127.7	113.7	12.31	1.16	14.29	142.86	28.57	56	456.5	136.95	2287.07	5.34
R4	T5	148.85	113.7	30.91	1.16	35.87	358.67	71.73	57	455.5	47.95	800.77	2.09
R4	T6	144	125.29	14.93	1.28	19.09	190.92	38.18	50.3	462.2	29.2	487.64	1.15
R4	T7	138.4	127.45	8.59	1.30	11.17	111.73	22.35	48.4	464.1	78.97	1318.80	2.99
R4	T8	128.25	119.5	7.32	1.22	8.93	89.29	17.86	56.6	455.9	29.23	488.14	1.11
R4	T9	153.59	135.58	13.28	1.38	18.38	183.78	36.76	58.8	453.7	30.22	504.67	1.21
R5	T1	134.91	129.01	4.57	1.32	6.02	60.20	12.04	58.8	453.7	3.42	57.11	0.13

R5	T2	130.42	116.2	12.24	1.19	14.51	145.10	29.02	56.8	455.7	62.03	1035.90	2.43
R5	T3	140.79	125.29	12.37	1.28	15.82	158.16	31.63	56	456.5	17.63	294.42	0.69
R5	T4	147.29	128.15	14.94	1.31	19.53	195.31	39.06	56.6	455.9	10.16	169.67	0.41
R5	T5	164.45	154.1	6.72	1.57	10.56	105.61	21.12	49.6	462.9	49.45	825.82	1.87
R5	T6	152.87	140.74	8.62	1.44	12.38	123.78	24.76	48.8	463.7	24.69	412.32	0.94
R5	T7	153.92	143.02	7.62	1.46	11.12	111.22	22.24	50.3	462.2	113.93	1902.63	4.32
R5	T8	135.24	121.83	11.01	1.24	13.68	136.84	27.37	56	456.5	73.55	1228.29	2.86
R5	T9	148.62	125.29	18.62	1.28	23.81	238.06	47.61	57	455.5	32.08	535.74	1.31
R6	T1	133.01	129.26	2.90	1.32	3.83	38.27	7.65	58.8	453.7	1.5	25.05	0.06
R6	T2	143.65	126.1	13.92	1.29	17.91	179.08	35.82	56	456.5	20.58	343.69	0.82
R6	T3	148.37	121.83	21.78	1.24	27.08	270.82	54.16	57	455.5	11.04	184.37	0.46
R6	T4	152.73	142.74	7.00	1.46	10.19	101.94	20.39	49.6	462.9	31.12	519.70	1.17
R6	T5	136.97	122.86	11.48	1.25	14.40	143.98	28.80	56.8	455.7	55.91	933.70	2.19
R6	T6	135.1	125.82	7.38	1.28	9.47	94.69	18.94	48.4	464.1	73.31	1224.28	2.75
R6	T7	130.16	120.79	7.76	1.23	9.56	95.61	19.12	50.3	462.2	93	1553.10	3.51
R6	T8	143.32	130.4	9.91	1.33	13.18	131.84	26.37	56.6	455.9	8.09	135.10	0.31
R6	T9	133.34	118.56	12.47	1.21	15.08	150.82	30.16	56.8	455.7	87.83	1466.76	3.45
R7	T1	141.7	139.28	1.74	1.42	2.47	24.69	4.94	56.6	455.9	2.44	40.75	0.09
R7	T2	137.59	131.95	4.27	1.35	5.76	57.55	11.51	57	455.5	22.45	374.92	0.84
R7	T3	136.98	128.36	6.72	1.31	8.80	87.96	17.59	49.6	462.9	62.55	1044.59	2.35
R7	T4	133.55	126.1	5.91	1.29	7.60	76.02	15.20	57	455.5	36.12	603.20	1.37

R7	T5	136.74	123.57	10.66	1.26	13.44	134.39	26.88	56.8	455.7	93.7	1564.79	3.65
R7	T6	143.99	121.78	18.24	1.24	22.66	226.63	45.33	58.8	453.7	42.44	708.75	1.74
R7	T7	144.88	131.95	9.80	1.35	13.19	131.94	26.39	56	456.5	73.4	1225.78	2.85
R7	T8	143.03	133.1	7.46	1.36	10.13	101.33	20.27	48.4	464.1	79.72	1331.32	3.00
R7	T9	149.09	115.66	28.90	1.18	34.11	341.12	68.22	50.3	462.2	143.52	2396.78	6.08
R8	T1	134.12	122.87	9.16	1.25	11.48	114.80	22.96	48.4	464.1	2.59	43.25	0.10
R8	T2	137.33	125.32	9.58	1.28	12.26	122.55	24.51	56.6	455.9	5.07	84.67	0.20
R8	T3	149.89	125.05	19.86	1.28	25.35	253.47	50.69	57	455.5	22.9	382.43	0.94
R8	T4	129.55	117.2	10.54	1.20	12.60	126.02	25.20	56.8	455.7	32.91	549.60	1.28
R8	T5	137.45	125.46	9.56	1.28	12.23	122.35	24.47	50.3	462.2	58.87	983.13	2.25
R8	T6	145.52	122.5	18.79	1.25	23.49	234.90	46.98	58.8	453.7	23.22	387.77	0.95
R8	T7	128.5	118.47	8.47	1.21	10.23	102.35	20.47	49.6	462.9	57.5	960.25	2.17
R8	T8	136.88	124.05	10.34	1.27	13.09	130.92	26.18	56.6	455.9	18.05	301.44	0.70
R8	T9	147.06	125.05	17.60	1.28	22.46	224.59	44.92	56	456.5	57.69	963.42	2.34

Appendix 3.2. Analysis of variance for nutritional water productivity of Ca ($NWP\text{-}_{Ca}$) in *Cucumis myriocarpus* in response to planting density/hole drip irrigation at 56 days under field conditions, in Experiment 1 (n = 72).

Source	**DF**	**SS**	**%**	**F**	**P ≤**
Block	7	276.44	10		
Density	8	761.62	25***	2.71	0.0134
Error	56	1965.33	65		
Total	71	3003.39	100		

*** = Significant at P ≤ 0.01.

Appendix 3.3. Analysis of variance for nutritional water productivity of K ($NWP\text{-}_{K}$) in *Cucumis myriocarpus* in response to planting density/hole drip irrigation at 56 days under field conditions, in Experiment 1 (n = 72).

Source	**DF**	**SS**	**%**	**F**	**P ≤**
Block	7	309.18	10		
Density	8	742.48	24***	2.56	0.0187
Error	56	2029.51	66		
Total	71	3081.18	100		

*** = Significant at P ≤ 0.01

Appendix 3.4. Analysis of variance for nutritional water productivity of Mg (NWP-$_{Mg}$) in *Cucumis myriocarpus* in response to planting density/hole drip irrigation at 56 days under field conditions, in Experiment 1 (n = 72).

Source	**DF**	**SS**	**%**	**F**	**P**
Block	7	48.401	9		
Density	8	152.369	28***	3.07	0.0061
Error	56	347.232	63		
Total	71	548.002	100		

*** = Significant at P ≤ 0.01

Appendix 3.5. Analysis of variance for nutritional water productivity of P (NWP-$_{P}$) in *Cucumis myriocarpus* in response to planting density/hole drip irrigation at 56 days under field conditions, in Experiment 1 (n = 72).

Source	**DF**	**SS**	**%**	**F**	**P ≤**
Block	7	46.378	9		
Density	8	137.399	26***	2.85	0.0099
Error	56	337.427	65		
Total	71	521.204	100		

*** = Significant at P ≤ 0.01

Appendix 3.6. Analysis of variance for nutritional water productivity of Zn ($NWP\text{-}_{Zn}$) in *Cucumis myriocarpus* in response to planting density/hole drip irrigation at 56 days under field conditions, in Experiment 1 (n = 72).

Source	**DF**	**SS**	**%**	**F**	**P**
Block	7	2.5876	11		
Density	8	8.7520	38***	5.29	0.0001
Error	56	11.5721	51		
Total	71	22.9116	100		

*** = Significant at P ≤ 0.01

Appendix 3.7. Analysis of variance for nutritional water productivity of Fe ($NWP\text{-}_{Fe}$) in *Cucumis myriocarpus* in response to planting density/hole drip irrigation at 56 days under field conditions, in Experiment 1 (n = 72).

Source	**DF**	**SS**	**%**	**F**	**P**
Block	7	1.4772	8		
Density	8	6.7591	36***	4.59	0.0002
Error	56	10.3157	56		
Total	71	18.5520	100		

*** = Significant at P ≤ 0.01

Appendix 3.8. Analysis of variance for nutritional water productivity of Na ($NWP\text{-}_{Na}$) in *Cucumis myriocarpus* in response to planting density/hole drip irrigation at 56 days under field conditions, in Experiment 1 (n = 72).

Source	**DF**	**SS**	**%**	**F**	**P**
Block	7	1.6336	10		
Density	8	8.1544	48***	7.88	0.0000
Error	56	7.2430	42		
Total	71	17.0310	100		

*** = Significant at P ≤ 0.01

Appendix 3.9. Analysis of variance for nutritional water productivity of Ca ($NWP\text{-}_{Ca}$) in *Cucumis myriocarpus* in response to planting density/hole drip irrigation at 56 days under field conditions, in Experiment 2 (n = 72).

Source	**DF**	**SS**	**%**	**F**	**P**
Block	7	210.51	9		
Density	8	905.01	41***	5.65	0.0000
Error	56	1120.28	50		
Total	71	2235.80	100		

*** = Significant at P ≤ 0.01

Appendix 3.10. Analysis of variance for nutritional water productivity of K (NWP_K) in *Cucumis myriocarpus* in response to planting density/hole drip irrigation at 56 days under field conditions, in Experiment 2 (n = 72).

Source	**DF**	**SS**	**%**	**F**	**P**
Block	7	178.01	10		
Density	8	731.55	40***	5.61	0.0000
Error	56	912.28	50		
Total	71	1821.83	100		

*** = Significant at P ≤ 0.01

Appendix 3.11. Analysis of variance for nutritional water productivity of Mg (NWP_{Ca}) in *Cucumis myriocarpus* in response to planting density/hole drip irrigation at 56 days under field conditions, in Experiment 2 (n = 72).

Source	**DF**	**SS**	**%**	**F**	**P**
Block	7	19.729	7		
Density	8	111.912	39***	4.99	0.0001
Error	56	157.063	54		
Total	71	288.704	100		

*** = Significant at P ≤ 0.01

Appendix 3.12. Analysis of variance for nutritional water productivity of P (NWP_P) in *Cucumis myriocarpus* in response to planting density/hole drip irrigation at 56 days under field conditions, in Experiment 2 (n = 72).

Source	**DF**	**SS**	**%**	**F**	**P**
Block	7	31.326	8		
Density	8	178.799	43***	6.20	0.0000
Error	56	201.784	49		
Total	71	411.909	100		

*** = Significant at P ≤ 0.01

Appendix 3.13. Analysis of variance for nutritional water productivity of Fe (NWP_{Fe}) in *Cucumis myriocarpus* in response to planting density/hole drip irrigation at 56 days under field conditions, in Experiment 2 (n = 72).

Source	**DF**	**SS**	**%**	**F**	**P**
Block	7	1.1507	7		
Density	8	8.7441	53***	9.18	0.0000
Error	56	6.6656	40		
Total	71	16.5605	100		

*** = Significant at P ≤ 0.01.

Appendix 3.14. Analysis of variance for nutritional water productivity of Zn ($NWP\text{-}_{Zn}$) in *Cucumis myriocarpus* in response to planting density/hole drip irrigation at 56 days under field conditions, in Experiment 2 (n = 72).

Source	**DF**	**SS**	**%**	**F**	**P**
Block	7	0.4577	3		
Density	8	10.0847	63***	12.66	0.0000
Error	56	5.5741	34		
Total	71	16.1166	100		

*** = Significant at P ≤ 0.01

Appendix 3.15. Analysis of variance for nutritional water productivity of Na ($NWP\text{-}_{Na}$) in *Cucumis myriocarpus* in response to planting density/hole drip irrigation at 56 days under field conditions, in Experiment 2 (n = 72).

Source	**DF**	**SS**	**%**	**F**	**P**
Block	7	1.5525	8		
Density	8	10.8083	55***	10.20	0.0000
Error	56	7.4144	37		
Total	71	19.7752	100		

*** = Significant at P ≤ 0.01

Appendix 4.1. Analysis of variance for nutritional water productivity of Ca ($NWP\text{-}_{Ca}$) in *Cucumis myriocarpus* in response to irrigation interval at 64 days under field conditions, in Experiment 1 (n = 42).

Source	**DF**	**SS**	**%**	**F**	**P**
Block	6	13380	8		
Trt	5	91417	55***	8.83	0.0000
Error	30	62114	37		
Total	41	166911	100		

*** = Significant at P ≤ 0.01

Appendix 4.2. Analysis of variance for nutritional water productivity of K ($NWP\text{-}_{K}$) in *Cucumis myriocarpus* in response to irrigation interval at 64 days under field conditions, in Experiment 1 (n = 42).

Source	**DF**	**SS**	**%**	**F**	**P**
Block	6	1761.3	9		
Trt	5	17007.8	84***	10.79	0.0000
Error	30	1576.2	7		
Total	41	20345.3	100		

*** = Significant at P ≤ 0.01

Appendix 4.3. Analysis of variance for nutritional water productivity of Mg (NWP_{-Mg}) in *Cucumis myriocarpus* in response to irrigation interval at 64 days under field conditions, in Experiment 1 (n = 42).

Source	**DF**	**SS**	**%**	**F**	**P**
Block	6	3170.4	8		
Trt	5	22838.3	57***	9.58	0.0000
Error	30	14305.5	35		
Total	41	40314.2	100		

*** = Significant at P ≤ 0.01

Appendix 4.4. Analysis of variance for nutritional water productivity of P (NWP_{-P}) in *Cucumis myriocarpus* in response to irrigation interval at 64 days under field conditions, in Experiment 1 (n = 42).

Source	**DF**	**SS**	**%**	**F**	**P**
Block	6	859.5	8		
Trt	5	6839.4	59***	9.58	0.0000
Error	30	3851.4	33		
Total	41	11550.3	100		

*** = Significant at P ≤ 0.01

Appendix 4.5. Analysis of variance for nutritional water productivity of Fe ($NWP\text{-}_{Fe}$) in *Cucumis myriocarpus* in response to irrigation interval at 64 days under field conditions, in Experiment 1 (n = 42).

Source	**DF**	**SS**	**%**	**F**	**P**
Block	6	2.6091	11		
Trt	5	20.0095	82***	68.29	0.0000
Error	30	1.7580	7		
Total	41	24.3767	100		

***= Significant at P ≤ 0.01

Appendix 4.6. Analysis of variance for nutritional water productivity of Na ($NWP\text{-}_{Na}$) in *Cucumis myriocarpus* in response to irrigation interval at 64 days under field conditions, in Experiment 1 (n = 42).

Source	**DF**	**SS**	**%**	**F**	**P**
Block	6	1.8572	9		
Trt	5	18.3629	84***	69.12	0.0000
Error	30	1.5939	7		
Total	41	21.8141	100		

*** = Significant at P ≤ 0.01

Appendix 4.7. Analysis of variance for nutritional water productivity of Zn ($NWP\text{-}_{Zn}$) in *Cucumis myriocarpus* in response to irrigation interval at 64 days under field conditions, in Experiment 1(n = 42).

Source	**DF**	**SS**	**%**	**F**	**P**
Block	6	2.2740	10		
Trt	5	18.2492	82***	63.11	0.0000
Error	30	1.7350	8		
Total	41	22.2582	100		

*** = Significant at P ≤ 0.01

Appendix 4.8. Analysis of variance for nutritional water productivity of Ca ($NWP\text{-}_{Ca}$) in *Cucumis myriocarpus* in response to irrigation interval at 64 days under field conditions, in Experiment 2 (n = 42).

Source	**DF**	**SS**	**%**	**F**	**P**
Block	7	210.51	9		
Planting	8	905.01	41***	5.65	0.0000
Error	56	1120.28	50		
Total	71	2235.80	100		

***= Significant at P ≤ 0.01

Appendix 4.9. Analysis of variance for nutritional water productivity of K ($NWP\text{-}_K$) in *Cucumis myriocarpus* in response to irrigation interval at 64 days under field conditions, in Experiment 2 (n = 42).

Source	**DF**	**SS**	**%**	**F**	**P**
Block	7	642647	9		
Planting	8	3043627	43***	5.61	0.0000
Error	56	3342261	48		
Total	71	7028535	100		

*** = Significant at P ≤ 0.01

Appendix 4.10. Analysis of variance for nutritional water productivity of Mg ($NWP\text{-}_{Mg}$) in *Cucumis myriocarpus* in response to irrigation interval at 64 days under field conditions, in Experiment 2 (n = 42).

Source	**DF**	**SS**	**%**	**F**	**P**
Block	7	10568	7		
Planting	8	85039	60***	4.99	0.0001
Error	56	47285	33		
Total	71	142892	100		

***= Significant at P ≤ 0.01

Appendix 4.11. Analysis of variance for nutritional water productivity of P (NWP_P) in *Cucumis myriocarpus* in response to irrigation interval at 64 days under field conditions, in Experiment 2 (n = 42).

Source	**DF**	**SS**	**%**	**F**	**P**
Block	7	3170.4	8		
Planting	8	22838.3	57***	6.20	0.0000
Error	56	14305.5	35		
Total	71	40314.2	100		

*** = Significant at P ≤ 0.01

Appendix 4.12. Analysis of variance for nutritional water productivity of Fe (NWP_{Fe}) in *Cucumis myriocarpus* in response to irrigation interval at 64 days under field conditions, in Experiment 2 (n = 42).

Source	**DF**	**SS**	**%**	**F**	**P**
Block	7	859.5	8		
Planting	8	6839.4	59***	9.18	0.0000
Error	56	3851.4	33		
Total	71	11550.3	100		

***= Significant at P ≤ 0.01

Appendix 4.13. Analysis of variance for nutritional water productivity of Zn ($NWP\text{-}_{Zn}$) in *Cucumis myriocarpus* in response to irrigation interval at 64 days under field conditions, in Experiment 2 (n = 42).

Source	**DF**	**SS**	**%**	**F**	**P**
Block	7	0.7315	3		
Planting	8	23.3787	85***	12.66	0.0000
Error	56	3.5220	12		
Total	71	27.6323	100		

*** = Significant at P ≤ 0.01

Appendix 4.14. Analysis of variance for nutritional water productivity of Na ($NWP\text{-}_{Na}$) in *Cucumis myriocarpus* in response to irrigation interval at 64 days under field conditions, in Experiment 2 (n = 42).

Source	**DF**	**SS**	**%**	**F**	**P**
Block	7	0.6514	2		
Planting	8	23.8990	85***	10.20	0.0000
Error	56	3.6822	13		
Total	71	28.2326	100		

*** = Significant at P ≤ 0.01

Appendix 5.1. Analysis of variance for nutritional water productivity of Ca (NWP-$_{Ca}$) in *Cucumis myriocarpus* in response to increasing *Meloidogyne incognita* at 56 days after initiating experiment under greenhouse conditions, in Experiment 1 (n = 45).

Source	**DF**	**SS**	**%**	**F**	**P**
Rep	8	0.4631	15		
Treatment	4	0.2091	7^{ns}	0.68	0.612
Error	32	2.4630	78		
Total	44	3.1352	100		

^{ns}= Not significant at P ≤ 0.05

Appendix 5.2. Analysis of variance for nutritional water productivity of K (NWP-$_{K}$) in *Cucumis myriocarpus* in response to increasing *Meloidogyne incognita* at 56 days after initiating experiment under greenhouse conditions, in Experiment 1 (n = 45).

Source	**DF**	**SS**	**%**	**F**	**P**
Rep	8	6.626	30		
Treatment	4	0.704	3^{ns}	0.38	0.823
Error	32	14.946	67		
Total	44	22.276	100		

^{ns}=Not significant at P ≤ 0.05

Appendix 5.3. Analysis of variance for nutritional water productivity of Mg ($NWP-_{Mg}$) in *Cucumis myriocarpus* in response to increasing *Meloidogyne incognita* at 56 days after initiating experiment under greenhouse conditions, in Experiment 1 (n = 45).

Source	**DF**	**SS**	**%**	**F**	**P**
Rep	8	1.399	33		
Treatment	4	0.605	0.1^{ns}	1.20	0.331
Error	32	4.046	66.9		
Total	44	6.050	100		

^{ns}=Not significant at P ≤ 0.05

Appendix 5.4. Analysis of variance for nutritional water productivity of P ($NWP-_{P}$) in *Cucumis myriocarpus* in response to increasing *Meloidogyne incognita* at 56 days after initiating experiment under greenhouse conditions, in Experiment 1 (n = 45).

Source	**DF**	**SS**	**%**	**F**	**P**
Rep	8	0.477	27		
Treatment	4	0.011	1^{ns}	0.07	0.99
Error	32	1.245	72		
Total	44	1.733	100		

^{ns}=Not significant at P ≤ 0.05

Appendix 5.5. Analysis of variance for nutritional water productivity of Fe ($NWP\text{-}_{Fe}$) in *Cucumis myriocarpus* in response to increasing *Meloidogyne incognita* at 56 days after initiating treatments under greenhouse conditions, in Experiment 1 (n = 45).

Source	**DF**	**SS**	**%**	**F**	**P**
Rep	8	8.922	27		
Treatment	4	2.247	7^{ns}	0.82	0.521
Error	32	21.853	66		
Total	44	33.023	100		

^{ns}=Not significant at P ≤ 0.05

Appendix 5.6. Analysis of variance for nutritional water productivity of Na ($NWP\text{-}_{Na}$) in *Cucumis myriocarpus* in response to increasing *Meloidogyne incognita* at 56 days after initiating treatments under greenhouse conditions, in Experiment 1 (n = 45).

Source	**DF**	**SS**	**%**	**F**	**P**
Rep	Rep	8	1.025	22	
Treatment	Treatment	4	0.445	9^{ns}	1.09
Error	Error	32	3.275	69	
Total	Total	44	4.745	100	

^{ns}=Not significant at P ≤ 0.05

Appendix 5.7. Analysis of variance for nutritional water productivity of Zn ($NWP\text{-}_{Zn}$) in *Cucumis myriocarpus* in response to increasing *Meloidogyne incognita* at 56 days after initiating treatment under greenhouse conditions, in Experiment 1 (n = 45).

Source	**DF**	**SS**	**%**	**F**	**P**
Rep	8	0.586	11		
Treatment	4	0.535	10^{ns}	1.0.4	0.4034
Error	32	4.128	79		
Total	44	5.249	100		

^{ns}=Not significant at P ≤ 0.05

Appendix 5.8. Analysis of variance for nutritional water productivity of Ca ($NWP\text{-}_{Ca}$) in *Cucumis myriocarpus* in response to increasing *Meloidogyne incognita* at 56 days after initiating treatment under greenhouse conditions, in Experiment 2 (n = 45).

Source	**DF**	**SS**	**%**	**F**	**P**
Rep	8	2.662	21		
Treatment	4	1.132	9^{ns}	1.04	0.402
Error	32	8.712	70		
Total	44	12.506	100		

^{ns}= Not significant at P ≤ 0.05

Appendix 5.9. Analysis of variance for nutritional water productivity of K ($NWP\text{-}_K$) in *Cucumis myriocarpus* in response to increasing *Meloidogyne javanica* at 56 days after initiating treatments under greenhouse conditions, in Experiment 2 (n = 45).

Source	**DF**	**SS**	**%**	**F**	**P**
Rep	8	8.010	44		
Treatment	4	1.080	6^{ns}	0.96	0.443
Error	32	8.996	50		
Total	44	18.086	100		

^{ns}= Not Significant at P ≤ 0.05

Appendix 5.10. Analysis of variance for nutritional water productivity of Mg ($NWP\text{-}_{Mg}$) in *Cucumis myriocarpus* in response to increasing *Meloidogyne incognita* at 56 days after initiating treatment under greenhouse conditions, in Experiment 2 (n = 45).

Source	**DF**	**SS**	**%**	**F**	**P**
Rep	8	1.741	14		
Treatment	4	1.268	10^{ns}	1.03	0.4071
Error	32	9.851	76		
Total	44	12.859	100		

^{ns}= Not significant at P ≤ 0.05

Appendix 5.11. Analysis of variance for nutritional water productivity of Na (NWP-$_{Na}$) in *Cucumis myriocarpus* in response to increasing *Meloidogyne javanica* at 56 days after initiating treatments under greenhouse conditions, Experiment 2 (n = 45).

Source	**DF**	**SS**	**%**	**F**	**P**
Rep	8	2.932	24		
Treatment	4	1.086	9^{ns}	099	0.429
Error	32	8.814	73		
Total	44	12.057	100		

^{ns}= Not significant at P ≤ 0.05

Appendix 5.12. Analysis of variance for nutritional water productivity of Fe (NWP-$_{Fe}$) in *Cucumis myriocarpus* in response to increasing *Meloidogyne javanica* at 56 days after initiating treatments under greenhouse conditions, in Experiment 2 (n = 45).

Source	**DF**	**SS**	**%**	**F**	**P**
Rep	8	1.9247	21		
Treatment	4	0.7089	8^{ns}	12.28	0.0014
Error	32	6.6578	72		
Total	44	9.2914	100		

**= Not significant at P ≤ 0.05

Appendix 5.13. Analysis of variance for nutritional water productivity of P (NWP-$_P$) in *Cucumis myriocarpus* in response to increasing *Meloidogyne incognita* at 56 days after initiating treatments under greenhouse conditions, in Experiment 2 (n = 45).

Source	**DF**	**SS**	**%**	**F**	**P**
Rep	8	2.932	29		
Treatment	4	0.9182	9^{ns}	0.99	0.429
Error	32	6.417	62		
Total	44	10.267	100		

^{ns}= Not significant at P ≤ 0.05

Appendix 5.14. Analysis of variance for nutritional water productivity of Zn (NWP-$_{Zn}$) in *Cucumis myriocarpus* in response to increasing *Meloidogyne incognita* at 56 days after initiating treatments under greenhouse conditions, in Experiment 2 (n = 45).

Source	**DF**	**SS**	**%**	**F**	**P**
Rep	8	2.844	13		
Treatment	4	2.088	9^{ns}	0.95	0.447
Error	32	17.547	78		
Total	44	22.479	100		

^{ns}= Not significant at P ≤ 0.05

Appendix 5.15. Analysis of variance for nutritional water productivity of (NWP-$_{Ca}$) in

Cucumis myriocarpus in response to increasing *Meloidogyne javanica* at 56 days after initiating treatments on microplot conditions, in Experiment 1 (n = 45).

Source	DF	SS	%	F	P
Rep	8	1.1166	42		
Treatment	4	0.0760	3^{ns}	0.41	0.803
Error	32	1.4958	55		
Total	44	2.6884	100		

^{ns}= Not significant at P ≤ 0.05

Appendix 5.16. Analysis of variance for nutritional water productivity of K ($NWP\text{-}_K$) in *Cucumis myriocarpus* in response to increasing *Meloidogyne javanica* at 56 days after initiating treatments on microplot conditions, in Experiment 1 (n = 45).

Source	DF	SS	%	F	P
Rep	8	0.7529	54		
Treatment	4	0.1221	9^{ns}	1.86	0.1414
Error	32	0.5249	37		
Total	44	1.3999	100		

^{ns}= Not significant at P ≤ 0.05

Appendix 5.17. Analysis of variance for nutritional water productivity of Mg ($NWP\text{-}_{Mg}$)

in *Cucumis myriocarpus* in response to increasing *Meloidogyne javanica* at 56 days after initiating treatments on microplot conditions in Experiment 1 (n = 45).

Source	DF	SS	%	F	P
Rep	8	0.1658	26		
Treatment	4	0.0414	6^{ns}	0.77	0.553
Error	32	0.4305	68		
Total	44	0.6377	100		

^{ns}= Not significant at P ≤ 0.05

Appendix 5.18. Analysis of variance for nutritional water productivity of P (NWP-$_P$) in *Cucumis myriocarpus* in response to increasing *Meloidogyne javanica* at 56 days after initiating treatments on microplot conditions in Experiment 1 (n = 45).

Source	DF	SS	%	F	P
Rep	8	0.7529	54		
Treatment	4	0.1221	9^{ns}	1.86	0.1414
Error	32	0.5249	37		
Total	44	1.3999	100		

^{ns}= Not significant at P ≤ 0.05

Appendix 5.19. Analysis of variance for nutritional water productivity of Fe (NWP-$_{Fe}$)

in *Cucumis myriocarpus* in response to increasing *Meloidogyne javanica* at 56 days after initiating treatments on microplot conditions, in Experiment 1 (n = 45).

Source	**DF**	**SS**	**%**	**F**	**P**
Rep	8	0.3068	9		
Treatment	4	0.2736	8^{ns}	0.74	0.5696
Error	32	2.9443	83		
Total	44	3.5246	100		

^{ns}= Not significant at P ≤ 0.05

Appendix 5.20. Analysis of variance for nutritional water productivity of Na (NWP_{Na}) in *Cucumis myriocarpus* in response to increasing *Meloidogyne javanica* at 56 days after initiating treatments on microplot conditions, , in Experiment 1 (n = 45).

Source	**DF**	**SS**	**%**	**F**	**P**
Rep	8	0.4671	46		
Treatment	4	0.0413	4^{ns}	0.64	0.636
Error	32	0.5145	50		
Total	44	1.0229	100		

^{ns}= Not significant at P ≤ 0.05

Appendix 5.21. Analysis of variance for nutritional water productivity of Zn (NWP-$_{Zn}$) in *Cucumis myriocarpus* in response to increasing *Meloidogyne javanica* at 56 days after initiating treatments on microplot conditions, in Experiment 1 (n = 45).

Source	**DF**	**SS**	**%**	**F**	**P**
Rep	8	0.8684	49		
Treatment	4	0.1040	6^{ns}	1.05	0.3994
Error	32	0.7962	45		
Total	44	1.7686	100		

^{ns}= Not significant at P ≤ 0.05

Appendix 5.22. Analysis of variance for nutritional water productivity of Ca (NWP-$_{Ca}$) in *Cucumis myriocarpus* in response to increasing *Meloidogyne javanica* at 56 days after initiating treatments on microplot conditions, in Experiment 2 (n = 45).

Source	**DF**	**SS**	**%**	**F**	**P**
Rep	8	1.1348	47		
Treatment	4	0.0839	4^{ns}	0.56	0.696
Error	32	1.2074	49		
Total	44	2.4260	100		

^{ns}= Not significant at P ≤ 0.05

Appendix 5.23. Analysis of variance for nutritional water productivity of K ($NWP\text{-}_K$) in *Cucumis myriocarpus* in response to increasing *Meloidogyne javanica* at 56 days after initiating treatments on microplot conditions, in Experiment 2 (n = 45).

Source	**DF**	**SS**	**%**	**F**	**P**
Rep	8	1.0885	32		
Treatment	4	0.3676	11^{ns}	1.48	0.230
Error	32	1.9816	57		
Total	44	3.4376	100		

^{ns}= Not significant at P ≤ 0.05

Appendix 5.24. Analysis of variance for nutritional water productivity of Mg ($NWP\text{-}_{Mg}$) in *Cucumis myriocarpus* in response to increasing *Meloidogyne javanica* at 56 days after initiating on mocroplot conditions, in Experiment 2 (n = 45).

Source	**DF**	**SS**	**%**	**F**	**P**
Rep	8	0.2791	35		
Treatment	4	0.0192	2^{ns}	0.31	0.870
Error	32	0.4993	63		
Total	44	0.7977	100		

^{ns}= Not significant at P ≤ 0.05

Appendix 5.25. Analysis of variance for nutritional water productivity of P (NWP-$_P$) in *Cucumis myriocarpus* in response to increasing *Meloidogyne javanica* at 56 days after initiating treatments under microplot conditions, in Experiment 2 (n = 45).

Source	**DF**	**SS**	**%**	**F**	**P**
Rep	8	0.1115	22		
Treatment	4	0.0390	8^{ns}	0.87	0.495
Error	32	0.3600	70		
Total	44	0.5105	100		

^{ns}= Not significant at P ≤ 0.05

Appendix 5.26. Analysis of variance for nutritional water productivity of Fe (NWP-$_{Fe}$) in *Cucumis myriocarpus* in response to increasing *Meloidogyne javanica* at 56 days after initiating treatments under microplot conditions, in Experiment 2 (n = 45).

Source	**DF**	**SS**	**%**	**F**	**P**
Rep	8	0.8236	10		
Treatment	4	4.1938	51***	10.22	0.000
Error	32	3.2834	39		
Total	44	8.3008	100		

***= Significant at P ≤ 0.05

Appendix 5.27. Analysis of variance for nutritional water productivity of Na (NWP-$_{Na}$) in *Cucumis myriocarpus* in response to increasing *Meloidogyne javanica* at 56 days after initiating treatments under microplot conditions, in Experiment 2 (n = 45).

Source	**DF**	**SS**	**%**	**F**	**P**
Rep	8	0.6900	51		
Treatment	4	0.0365	3^{ns}	0.46	0.764
Error	32	0.6328	46		
Total	44	1.3593	100		

^{ns}= Not significant at P ≤ 0.05

Appendix 5.28. Analysis of variance for nutritional water productivity of Zn (NWP-$_{Zn}$) in *Cucumis myriocarpus* in response to increasing *Meloidogyne javanica* at 56 days after initiating treatments under microplot conditions, in Experiment 2 (n = 45).

Source	**DF**	**SS**	**%**	**F**	**P**
Rep	8	1.3854	38		
Treatment	4	0.1098	3^{ns}	0.42	0.795
Error	32	2.1064	58		
Total	44	3.6016	100		

^{ns}= Not significant at P ≤ 0.05

Appendix 6.1. Analysis of variance for nutritional water productivity of Ca ($NWP\text{-}_{Ca}$) in *Cucumis myriocarpus* in response to increasing chloride salts at 56 days under greenhouse conditions, in Experiment 1 (n = 126).

Source	**DF**	**SS**	**%**	**F**	**P**
Block	7	0.07629	16		
Planting	8	0.15663	33***	11.14	0.0000
Error	56	0.23895	51		
Total	71	0.47187	100		

*** = Significant at P ≤ 0.01

Appendix 6.2. Analysis of variance for nutritional water productivity of K ($NWP\text{-}_{K}$) in *Cucumis myriocarpus* in response to increasing chloride salts at 56 days under greenhouse conditions, in Experiment 1 (n = 126).

Source	**DF**	**SS**	**%**	**F**	**P**
Block	7	506.762	60		
Planting	8	13.051	2ns	0.71	0.6448
Error	56	313.904	38		
Total	71	833.717	100		

*** = Significant at P ≤ 0.01

Appendix 6.3. Analysis of variance for nutritional water productivity of Mg (NWP_{-Mg}) in *Cucumis myriocarpus* in response to increasing chloride salts at 56 days under greenhouse conditions, in Experiment 1 (n = 126).

Source	**DF**	**SS**	**%**	**F**	**P**
Block	7	365.68	19		
Planting	8	651.38	33***	11.73	0.0000
Error	56	944.03	48		
Total	71	1961.09	100		

*** = Significant at P ≤ 0.01

Appendix 6.4. Analysis of variance for nutritional water productivity of P (NWP_{-P}) in*Cucumis myriocarpus* in response to increasing chloride salts at 56 days under greenhouse conditions, in Experiment 1 (n = 126).

Source	**DF**	**SS**	**%**	**F**	**P**
Block	7	27.698	22		
Planting	8	36.228	29***	10.01	0.0000
Error	56	61.548	49		
Total	71	125.473	100		

*** = Significant at P ≤ 0.01

Appendix 6.5. Analysis of variance for nutritional water productivity of ($NWP\text{-}_{Zn}$) in *Cucumis myriocarpus* in response to increasing chloride salts at 56 days under greenhouse conditions, in Experiment 1 (n = 126).

Source	**DF**	**SS**	**%**	**F**	**P**
Block	7	16.9285	51		
Planting	8	3.8277	12***	9.18	0.0000
Error	56	12.4923	38		
Total	71	33.2485	100		

*** = Significant at P ≤ 0.01

Appendix 6.6. Analysis of variance for nutritional water productivity of Fe ($NWP\text{-}_{Fe}$) in *Cucumis myriocarpus* in response to increasing chloride salts at 56 days under greenhouse conditions, in Experiment 1 (n = 126).

Source	**DF**	**SS**	**%**	**F**	**P**
Block	7	1.03188	15		
Planting	8	2.20329	31***	9.92	0.0000
Error	56	3.77419	54		
Total	71	7.00936	100		

*** = Significant at P ≤ 0.01

Appendix 6.7. Analysis of variance for nutritional water productivity of Na ($NWP\text{-}_{Na}$) in *Cucumis myriocarpus* in response to increasing chloride salts at 56 days under greenhouse conditions, in Experiment 1 (n = 126).

Source	**DF**	**SS**	**%**	**F**	**P**
Block	7	3.3985	21		
Planting	8	2.2109	14***	3.61	0.0028
Error	56	10.4141	65		
Total	71	16.0235	100		

***= Significant at P ≤ 0.01

Appendix 6.8. Analysis of variance for nutritional water productivity of Ca ($NWP\text{-}_{Ca}$) of *Cucumis myriocarpus* in response to increasing chloride salts at 56 days under greenhouse conditions, in Experiment 2 (n = 126).

Source	**DF**	**SS**	**%**	**F**	**P**
Block	7	3533.6	19		
Treatment	6	8729.7	47***	23.08	0.0000
Error	102	6429.2	34		
Total	125	18692.5	100		

***= Significant at P ≤ 0.01

Appendix 6.9. Analysis of variance for nutritional water productivity of K ($NWP\text{-}_K$) in *Cucumis myriocarpus* in response to increasing chloride salts at 56 days under greenhouse conditions, in Experiment 2 (n = 126).

Source	**DF**	**SS**	**%**	**F**	**P**
Rep	17	1172.36	12		
Treatment	6	5170.89	52***	24.97	0.0000
Error	102	3520.12	36		
Total	125	9863.36	100		

*** = Significant at P ≤ 0.01

Appendix 6.10. Analysis of variance for nutritional water productivity of Mg ($NWP\text{-}_{Mg}$) in *Cucumis myriocarpus* in response to increasing chloride salts at 56 days under greenhouse conditions, in Experiment 2 (n = 126).

Source	**DF**	**SS**	**%**	**F**	**P**
Rep	17	241.61	10		
Treatment	6	1233.02	54***	25.79	0.0000
Error	102	812.90	36		
Total	125	2287.53	100		

*** = Significant at P ≤ 0.01

Appendix 6.11. Analysis of variance for nutritional water productivity of P ($NWP\text{-}_P$) in *Cucumis myriocarpus* in response to increasing chloride salts at 56 days under greenhouse conditions, in Experiment 2 (n = 126).

Source	**DF**	**SS**	**%**	**F**	**P**
Rep	17	84.48	8		
Treatment	6	592.17	58***	29.37	0.0000
Error	102	342.73	34		
Total	125	1019.37	100		

***= Significant at P ≤ 0.01

Appendix 6.12. Analysis of variance for nutritional water productivity of Zn ($NWP\text{-}_{Zn}$) in *Cucumis myriocarpus* in response to increasing chloride salts at 56 days under greenhouse conditions, in Experiment 2 (n = 126).

Source	**DF**	**SS**	**%**	**F**	**P**
Rep	17	0.71666	13		
Treatment	6	3.21262	59***	36.58	0.0000
Error	102	1.49316	28		
Total	125	5.42245	100		

*** = Significant at P ≤ 0.01

Appendix 6.13. Analysis of variance for nutritional water productivity of Fe ($NWP\text{-}_{Fe}$) in *Cucumis myriocarpus* in response to increasing chloride salts at 56 days under greenhouse conditions, in Experiment 2 (n = 126).

Source	**DF**	**SS**	**%**	**F**	**P**
Rep	17	3.06084	39		
Treatment	6	1.63523	21***	8.58	0.0000
Error	102	3.24071	40		
Total	125	7.93679	100		

*** = Significant at P ≤ 0.01

Appendix 6.14. Analysis of variance for nutritional water productivity of Na ($NWP\text{-}_{Na}$) in *Cucumis myriocarpus* in response to increasing chloride salts at 56 days under greenhouse conditions, in Experiment 2 (n = 126).

Source	**DF**	**SS**	**%**	**F**	**P**
Rep	17	0.31300	7		
Treatment	6	2.83658	64***	38.29	0.0000
Error	102	1.25936	29		
Total	125	4.40894	100		

*** = Significant at P ≤ 0.01

Appendix 7.1. Analysis of variance for nutritional water productivity of Ca ($NWP\text{-}_{Ca}$) in *Cucumis myriocarpus* in response to increasing VAM at 56 days after initiating experiment under greenhouse conditions, in Experiment 1 (n = 72).

Source	**DF**	**SS**	**%**	**F**	**P**
Rep	8	4.7027	20		
Treatment	7	1.8679	8^{ns}	0.87	0.5321
Error	56	17.0798	72		
Total	71	23.6504	100		

^{ns}= Not significant at P ≤ 0.05

Appendix 7.2. Analysis of variance for nutritional water productivity of K ($NWP\text{-}_{K}$) in *Cucumis myriocarpus* in response to increasing VAM at 56 days after initiating experiment under greenhouse conditions, in Experiment 1 (n = 72).

Source	**DF**	**SS**	**%**	**F**	**P**
Rep	8	9.3624	21		
Treatment	7	5.5852	13^{ns}	1.53	0.1767
Error	56	29.2374	66		
Total	71	44.1849	100		

^{ns}=Not significant at P ≤ 0.05

Appendix 7.3. Analysis of variance for nutritional water productivity of P (NWP_P) in *Cucumis myriocarpus* in response to increasing VAM at 56 days after initiating experiment under greenhouse conditions, in Experiment 1 (n = 72).

Source	**DF**	**SS**	**%**	**F**	**P**
Rep	8	0.8199	23		
Treatment	7	0.4620	13^{ns}	1.61	0.151
Error	56	2.2947	64		
Total	71	3.5766	100		

^{ns}=Not significant at P ≤ 0.05

Appendix 7.4. Analysis of variance for nutritional water productivity of Mg (NWP_{Mg}) in *Cucumis myriocarpus* in response to increasing VAM at 56 days after initiating experiment under greenhouse conditions, in Experiment 1 (n = 72).

Source	**DF**	**SS**	%	**F**	**P**
Rep	8	1.4411	9		
Treatment	7	2.6912	17^{ns}	1.86	0.0938
Error	56	11.5755	74		
Total	71	15.7078	100		

^{ns}=Not significant at P ≤ 0.05

Appendix 7.5. Analysis of variance for nutritional water productivity of Zn (NWP_{Zn}) in *Cucumis myriocarpus* in response to increasing VAM at 56 days after initiating experiment under greenhouse conditions, in Experiment 1 (n = 72).

Source	**DF**	**SS**	**%**	**F**	**P**
Rep	8	0.99978	12		
Treatment	7	1.06841	13^{ns}	1.33	0.2555
Error	56	6.44738	75		
Total	71	8.51557	100		

^{ns}=Not significant at P ≤ 0.05

Appendix 7.6. Analysis of variance for nutritional water productivity of Fe (NWP_{Fe}) in *Cucumis myriocarpus* in response to increasing VAM at 56 days after initiating experiment under greenhouse conditions, in Experiment 1 (n = 72).

Source	**DF**	**SS**	%	**F**	**P**
Rep	8	1.0728	10		
Treatment	7	1.4464	13^{ns}	1.32	0.2570
Error	56	8.7502	77		
Total	71	11.2694			

^{ns}=Not significant at P ≤ 0.05

Appendix 7.7. Analysis of variance for nutritional water productivity of Fe (NWP-$_{Fe}$) in *Cucumis myriocarpus* in response to increasing VAM at 56 days after initiating experiment under greenhouse conditions, in Experiment 1 (n = 72).

Source	**DF**	**SS**	%	**F**	**P**
Rep	8	0.88087	23		
Treatment	7	0.41695	11^{ns}	1.32	0.2596
Error	56	2.53306	66		
Total	71	3.83089			

^{ns}=Not significant at P ≤ 0.05

Appendix 7.8. Analysis of variance for nutritional water productivity of Ca (NWP-$_{Ca}$) in *Cucumis myriocarpus* in response to increasing VAM at 56 days after initiating experiment under greenhouse conditions, in Experiment 2 (n = 72).

Source	**DF**	**SS**	%	**F**	**P**
Rep	8	2.8628	12		
Treatment	7	2.7570	11^{ns}	1.17	0.3343
Error	56	18.8403	77		
Total	71	24.4601	100		

^{ns}=Not significant at P ≤ 0.05

Appendix 7.9. Analysis of variance for nutritional water productivity of P (NWP_P) in *Cucumis myriocarpus* in response to increasing VAM at 56 days after initiating experiment under greenhouse conditions, in Experiment 2 (n = 72).

Source	**DF**	**SS**	**%**	**F**	**P**
Rep	8	1.01405	31		
Treatment	7	0.45889	14^{ns}	2.07	0.0623
Error	56	1.77546	55		
Total	71	3.24840	100		

^{ns}=Not significant at P ≤ 0.05

Appendix 7.10. Analysis of variance for nutritional water productivity of K (NWP_K) in *Cucumis myriocarpus* in response to increasing VAM at 56 days after initiating experiment under greenhouse conditions, in Experiment 1 (n = 72).

Source	**DF**	**SS**	%	**F**	**P**
Rep	8	10.3407	24		
Treatment	7	5.3212	12^{ns}	1.52	0.1810
Error	56	28.0929	64		
Total	71	43.7549	100		

^{ns}=Not significant at P ≤ 0.05

Appendix 7.11. Analysis of variance for nutritional water productivity of Mg (NWP-$_{Mg}$) in *Cucumis myriocarpus* in response to increasing VAM at 56 days after initiating experiment under greenhouse conditions, in Experiment 1 (n = 72).

Source	**DF**	**SS**	**%**	**F**	**P**
Rep	8	0.54484	16		
Treatment	7	0.28475	8^{ns}	0.88	0.5311
Error	56	2.59981	76		
Total	71	3.42940	100		

^{ns}= Not significant at P ≤ 0.05

Appendix 7.12. Analysis of variance for nutritional water productivity of Na (NWP-$_{Na}$) in *Cucumis myriocarpus* in response to increasing VAM at 56 days after initiating experiment under greenhouse conditions, in Experiment 1 (n = 72).

Source	**DF**	**SS**	%	**F**	**P**
Rep	8	0.61530	15		
Treatment	7	0.73444	18^{ns}	2.09	0.0601
Error	56	2.81646	67		
Total	71	4.16620	100		

^{ns}= Not Significant at P ≤ 0.05

Appendix 7.13. Analysis of variance for nutritional water productivity of Zn ($NWP\text{-}_{Zn}$) in *Cucumis myriocarpus* in response to increasing VAM at 56 days after initiating experiment under greenhouse conditions, in Experiment 1 (n = 72).

Source	**DF**	**SS**	**%**	**F**	**P**
Rep	8	0.34889	15		
Treatment	7	0.30942	13^{ns}	1.43	0.2108
Error	56	1.72796	72		
Total	71	2.38626	100		

^{ns}= Not significant at P ≤ 0.05

Appendix 7.14. Analysis of variance for nutritional water productivity of Fe ($NWP\text{-}_{Fe}$) in *Cucumis myriocarpus* in response to increasing VAM at 56 days after initiating experiment under greenhouse conditions, in Experiment 1 (n = 72).

Source	**DF**	**SS**	%	**F**	**P**
Rep	8	0.22008	8		
Treatment	7	0.27286	10^{ns}	0.94	0.4860
Error	56	2.33081	82		
Total	71	2.82375	100		

^{ns}= Not significant at P ≤ 0.05