Investigating the performance and quality of the *Cucumis metuliferus* E. Mey. Ex Naudin (African horned cucumber) under different growing environments for potential commercialisation

by

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ABSTRACT

This study was carried out to investigate the performance and quality of *Cucumis metuliferus* E. Mey. Ex Naudin (African horned cucumber) under protected and open environment with the aim of comparing yield and quality for the purpose of commercialisation of the crop. Therefore, the overall objective was to determine a suitable growing environment for *C. metuliferus* between greenhouse, shade net and open field, so that a comparative yield and quality analysis could be done for the purpose of commercialisation of the crop.

Cucumis metuliferus seeds are difficult to germinate under the normal suitable environmental conditions in which most farmers operate. Germination was evaluated with respect to growth medium, scarification and seed certification. These factors ultimately control yield and fruit quality. The main aim of the study was to investigate the effect or impact of seed certification, growth medium (sand and vermiculite, peat TS1 and seedling mix) and scarification on germination success of *C. metuliferus* seeds. The seeds were classified under four different categories *viz.* treated certified, non-treated certified, treated uncertified, and non-treated uncertified.

Of the 540 certified and uncertified seeds sown in the three-growth media, 80% germinated, significantly more that those that failed. The treatment combination of treated certified seeds (TC) and peat demonstrated high germination success rate of 93.6%, followed by the treatment combination of treated certified seeds (TC) and sand+ vermiculite with germination success rate of 91.3%. The treatment combination of uncertified untreated (UTU) seeds and potting mix illustrated low germination success rate at 37.2%. In general, the study results revealed that certified seeds scarified with warm water combined had a higher germination rate that unscarified seeds, irrespective of the growth media. Since the seedling root-ball integrity is essential for transplant survival, this study suggests peat and certified seeds as the best combination for propagation and good quality plants.

Water scarcity, population growth and climate change are the major factors affecting agricultural productivity in the 20th century. *Cucumis metuliferus* grows naturally in the wild; however, its yield response to water stress, different cultivation environment and soil types, has not been assessed. A study was carried out to determine water use efficiency of the *C. metuliferus* grown the greenhouse, shade net and open field under varying soil types and irrigation water levels, so that a comparative analysis could be done on productivity levels. The research was conducted at the University of South Africa (Unisa) Science Campus, in Florida, Gauteng (-26.157831 S, 27.903364 E) during the 2017/2018 and 2018/2019 growing seasons. A factorial experiment with two factors – soil (loamy soil and sandy loam soil) and water stress levels (no water stress, moderate water stress and severe water stress). The pot experiments were a completely randomised design with nine (9) replicates per treatment. Data collected included total biomass, aboveground biomass, harvest index and water use efficiency. Results illustrated that treatment of moderate water stress combined with loamy soil and shade net decreased WUE from 6.2 to 1.4 kg m^{-3}, whereas treatment combination of no water stress combined with sandy loam and open field environment demonstrated increase in WUE from 1.4 to 6.2 kg m^{-3}.

Nutritional concentration of most crops depends on factors such as amount of water, growing environment, light intensity and soil types. However, factors influencing nutritional concentration of *C. metuliferus* fruits is not yet known. Another objective of the study was to determine the effect of different water stress levels, soil types and growing environment (greenhouse, shade net and open field) on the concentration of nutrients in *C. metuliferus* fruit. Freeze-dried fruit samples were used in the quantification of *β-carotene*, vitamin C, vitamin E, total soluble sugars, crude proteins, total flavonoids, total phenols, macro-nutrients (Ca, Mg, P, K, Na and S), and micro-nutrients (Cu, Fe, Mn and Zn).

Results demonstrated that plants grown under shade net, combined with severe water stress level and loamy soil, had increased total soluble sugars (15.8 ˚Brix) compared to other treatments. Plants under shade net environment, combined with moderate water level and loamy soil, resulted in increased crude protein content (6.31 ˚Brix). The severe water stress treatment combined with loamy soil under greenhouse conditions resulted in increased β-carotene content (1.65 mg 100 g^{-1} DW) when compared to other treatments.

Regarding vitamin C, the treatment of no water stress combined with loamy soil under shade net environment showed higher content of (33.1 mg 100 g^{-1} DW). The severe water stress treatment combined with sandy loam soil under greenhouse environment, increased vitamin E content (35.1 mg 100 g^{-1} DW) when compared to other treatments. The treatment of open field under severe water stress level and loamy soil increased total flavonoids content (0.85 mg CE/g^{-1} DW) in the fruit when compared to other treatments. The results thus imply that this plant bears better-quality fruit in terms of concentration of nutrients and biochemical constituents when grown under no to moderate water stress treatment on the loamy or sandy loam substrate in the shade net and open field environment.

Primary metabolites are biological compounds that are essential to the growth and development of a plant during its life cycle. They have a direct impact on the yield and biochemical constituents in plants. Quantities of the primary metabolites were determined using the LC-MS-8040 triple quadrupole mass spectrometer (Shimadzu) from fruits harvested from treatments mentioned above. The results showed that the no water stress treatment combined with sandy loam under shade net environment significantly ($P \leq 0.05$) increased asparagine content from 10×10^6 to $80x10^6$ peak intensity when compared to other treatments. The severe water stress treatment combined with sandy loam soil under open field environment during the 2017/2018 season, significantly increased dopa content from 12,030 to 324,240 peak intensity, while during the 2018/2019 season, 4-hydroxyproline from 10×10^6 to $90x10^6$ peak intensity the was significantly increased.

The study suggests that the treatment combination of water stress levels (no water stress and severe water stress) and soil substrates (loamy soil and sandy loam) under greenhouse and shade net significantly affected the shift of primary metabolites profile of *C. metuliferus* fruit as opposed to individual factors, respectively. There is therefore great potential to commercialise this crop; however, there is still a great deal that is not well understood of its growth habits and biological/biochemical constituents as a future alternative crop.

Keywords: *Cucumis metuliferus*, germination, nutritional composition, primary metabolites, water use efficiency.

NKOMISI LOWU NGA NA VUXOKOXOKO BYA NDZAVISISO WA DYONDZO

Ndzavisiso lowu wu endliwe ku lavisisa hi matirhelo na khwaliti ya *Cucumis metuliferus* E. Mey. Ex Naudin (African horned cucumber) eka mimbangu na mavala lama sirheleriweke na hi xikongomelo xa ku kotlanisa ntshovelo na vuxopaxopi bya khwaliti hi xikongomelo xa ku endla minxaviso ya ximila. Xikongomelonkulu xa ku vona ku faneleka ku kula ka *C. metuliferus* exikarhi ka ti-greenhouse, nete ya ndzhuti na mimbangu ya le rivaleni ku endlela ku pfuneta nxopaxopo.

Timbewu ta *C. metuliferus* ta nonon'hwa ku tihlukisa ehansi ka swiyimo swa mbangu leswi faneleke laha varimi va tirhaku eka tona. Ku hlukisa swi kamberiwe hi ku langutana na midiyamu ya ku kula, skarifikhexini na switifiketi swa timbewu. Swilo leswi swi lawula ntshovelo na khwaliti ya muhandzu. Xikongomelonkulu xa ndzavisiso lowu a ku ri ku lavisa hi vuyelo bya ku nyikiwa ka switifiketi, midiyamu ya ku kula (sand + vermiculite, peat TS1 and seedling mix) na skarifikhexini eka ku humelela ku hlukisa timbewu ta *C. metuliferus* E. Mey. ex naudin. Timbewu ti klasifayiwile ehansi ka tikhathegori ta mune to hambana, ku nga, treated certified, non-treated certified, treated uncertified, na non-treated uncertified.Hi vunharhu ka timediya leti ti ve na nhlukiso wa xiyenge hi 80%. Vuyelo byi kombise leswo treated certified na non-treated certified ti ve na ku humelela ka le henhla ka nhlukiso hi 93.6% na 91.3% hi ku landzelelana. Vuhumeleri bya nhlukiso wa le hansi ku ve timbewu ta treated uncertified hi vuyelo bya 37%. Vulehi bya 12 cm byi voniwe eka certified seedlings tanihi bya le henhla swinene. Swimilani swa unscarified na swa uncertified swi ve na timbewu ta le hansi, ta vulehi bya 3.44 cm eka vhiki ra vumune.

Hikokwalaho, seed certification swi ve na vuyelo ngopfu ku tlula scarification hi majini ya le henhla swinene. Ku khomaniseka ka ximila eka bolo ya misava i swa nkoka eka ku pona no ya emahlweni ka ximila loko xi transplantiwa, kasi ndzavisiso lowu wu tlakusa leswo ku va na peat na timbewu leti nga na switifiketi tanihi ndlela yo antswa swinene ya ku kurisa swimila na ku va na swimila swa khwaliti.

Ku pfumaleka ka mati, nkulo wa swilo hinkwaswo na ku cinca ka tlayimete i swa nkoka leswi khumbaka ku tirheka ka vurimi eka malembexidzana ya 20. *Cucumis metuliferus* yi kula hi ntumbuluko enhoveni; kambe ntshovelo wa yona wu angula eka ku kala ka mati, tindhawu to hambana ta ku rimiwa na mixaka ya misava, a swi si kamberiwa. Ku endliwe ndzavisiso ku vona ku faneleka ka mafambiselo ya ku kurisa ximila eka greenhouse, nete ya ndzhuti eka swiyimo swa mavala lama pfulekeke, leswo nxopaxopo wu ta kotlanisiwa eka tilevhele ta vuyelo bya ntshovelo loku nga endliwaka.

Ndzavisiso wu endliwe eka greenhouse, nete ya ndzhuti na swiyimo swa mavala lama pfulekeke eKhempasi ya Sayense eUniversity of South Africa (Unisa) eFlorida, eGauteng (26.157831 S, 27.903364 E) hi nkarhi wa 2017/2018 na 2018/2019 hi tisizini ta ku byala. Ekspirimente leyi nga na swilo swimbirhi – ku nga misava ya loamy na misava ya misava ya sava ya loam) na levhele ya ncheleto wa mati (laha ku nga ri ku na mati kahle, laha ku nga na matinyana na laha ku kalaka mati). Xipirimente xa le mapotweni xi endliwe hi ndlela yo ka yi nga kunguhatiwangi hi ku tirhisa ku phindaphinda ka nkaye (9), na dizayini ya kona leyi nga kombisiwa laha henhla. Tipharamita ta ku pimiwa ti katsa chlorophyll content, stomatal conductance na xiyenge xa ntshovelo, xo fana na ku tirhisa mati, vuheleri bya biomass, biomass ehenhla ka bayomasi ya misava, indeksi ya ntshovelo, vulehi bya muhandzu, nhlayo ya mihandzu, na ku tirhisiwa ka mati hi ndlela yo hlayisa.

Vuyelo byi kombise leswo tirhelo ra mavala lama pfulekeke swi pfanganisiwa na ndhawu yo kala mati na misava ya sava ya loam, swi ngetela nhlayo ya mihandzu. Ku tirhiwa ka swiyimo swa mavala lama nga pfuleka, swi hlanganisiwa na ndhawu yo kalanyana mati na misava ya sava ya loam, swi kombise ku tirhisiwa kahle ka mati ka le henhla hi (6. 2 kg m^{-}3) loko swi kotlanisiwa na ku tirhiwa ku n'wana. I swa nkoka ku lemuka leswaku a ku va ngi na ku hambana ku kulu exikarhi ka misava ya sava ya loam na misava ya loam eka ntirhiso wa mati lowu ku nga water use efficiency (WUE). Kambe, misava ya sava ya loam yi kombise xiyenge xa le henhla xa WUE loko swi kotlangisiwa na misava ya loam. Hikokwalaho ku nga fikeleriwa eka mhaka ya leswo ku pfanganisa ku tirhana na mavala yo pfuleka, tilevhele ta ncheleto wa mati (kahle na le xikarhi) na misava ya sava ya loam swa bumabumeriwa eka varimi leswo ku ta fikelekeleriwa xiyenge xa le henhla xa WUE na ku humelela ka ntshovelo wa *C. metuliferus*.

Ku hlengeletana ka tinutriyente eka ndhawu yin'we (nutritional concentration) ka swimila swi titshege hi swilo swo fana na leswi kumekaka eka mati, mbangu wa ku kula, masana ya dyambu na mixaka ya misava. Kambe, swilo swo fana na ku hlengeletana ka tinutriyente ta mihandu ya *C. metuliferus* a swi si tiveka. Xikongomelo xa ndzhavisiso a ku ri ku vona vuyelo bya tilevhele to hambana ta ku kala ka mati (ku pfumaleka ka mati, ku pfumalekanyana, na ku pfumaleka swinene ka mati), mixaka ya misava (misava ya loam na misava ya sava) mbangu wa ku kula (greenhouse, nete ya ndzhuti na mavala yo pfuleka) hi ku pfangana na tinutriyente eka mihandzu ya C. metuliferus E. Mey. ex naudin. Tisampuli ta mihandzu leyi nga omisiwa yi friziwa ti tirhisiwe eka ku endla vunyingi bya β-carotene, Vhitamini C, Vhitamini E, na total soluble sugars, ti-crude protein na ti-total flavonoids, total phenols, na micro-nutrients (Cu, Fe, Mn na Zn).

Vuyelo bya ndzavisiso byi kombise leswo swimila leswi nga kurisiwa eka nete ya ndzhuti, swi pfanganisiwa na levhele ya nkalo wa mati swinene na misava ya loam, swi ngetele ti-soluble sugars hi (15.8 ˚Brix) loko ku kotlanisiwa na ku tirhiwa kun'wana. Swimila leswi nga hansi ka mbangu wa nete ya ndzhuti, swi pfanganisiwa na nkayivelonyana wa mati hi vuxikarhi na misava ya loam, swi ve na vuyelo bya ku ngetela crude protein content hi (6.31˚Brix).

Ku tirhiwa ka nkayivelo wa mati swinene swi pfanganisiwa na misava ya loam ehansi ka swiyimo swa greenhouse swi ngetelele *β-carotene* content (1.65 mg/100 g^{-1} DW) loko swi kotlanisiwa na ku tirhiwa kun'wana. Ku tirhiwa ka ku kayivela ka mati swi pfanganisiwa na misava ya loam ehansi ka mbangu wa nete ya ndzhuti swi kombise ku ngeteleleka ka vhitamini C hi (33.1 mg 100 g^{-1} DW). Ku tirhiwa ka nkayivelo wa mati swinene swi pfanganisiwa na misava ya loam ehansi ka swiyimo swa mbangu wa greenhouse swi ngetelele vhitamin E hi (35.1 mg 100 g^{-1} DW) loko swi kotlanisiwa na ku tirhiwa kun'wana. Ku tirhiwa ka mavala lama nga rivaleni ehansi ka nkayivelo swinene wa mati na misava ya loam, swi ngetelele ti-total flavonoids content (0.85 mg

CE g^{-1} DW) loko swi kotlanisiwa na ku tirhiwa kun'wana. Ku tirhana na nkayivela mati ka levhela ya le xikarhi na misava ya sava ya loam ehansi ka mbangu wa nete ya ndzhuti swi kombise ku ngeteleleka ka Zn content (12.7 µg g^{-1} DW) loko swi kotlanisiwa na ku tirhiwa kun'wana.

Vuyelo byi kombisa leswaku ximila lexi xi na mihandzu ya khwaliti yo antswa hi ku landza ku hlengeletana ka tinutriyeente na tikhonstituwenti ta bayokhemikali, loko xi kurisiwa ehansi na ku ka ku nga ri na nkayivela mati kumbe ku kayivelanyana ka mati, hi ku tirhisa misava ya loam kumbe misava ya sava eka nete ya ndzhuti na le ka mavala ya le rivaleni.Ti-primary betabolites ti tlhela titiviwa tanihi biological compounds leti ti faneleke eka ku kula na ku hluvuka ka ximila hi nkarhi wa vutomi bya xona. Ti na vuyelo byo kongoma eka ntshovelo na tikhonsticuwenti ta bayokhemikala eka swimila. Vunyingi bya primary metabolites swi vekiwe hi ku tirhisa LC-MS-8040 triple quadrupole mass spectrometer (Shimadzu) eka mihandzu leyi nga ntshovelo wa ku tirhiwa kun'wana loku ku nga vuriwa laha henhla. Vuyelo byi kombe leswo ku tirhana na nkala nkayivelo wa mati, swi pfanganisiwa na misava ya loam ehansi ka mbangu wa nete ya ndzhuti, swi ngetelele swinene asparagine content from 10×106 to 80x106 mz loko swi kotlangisiwa na ku tirhiwa kun'wana.

Ku tirhana na nkayivelo wa mati swinene, swi pfanganisiwa na misava ya sava ya loam ehansi ka mbangu wa mavala lama pfulekeke hi nkarhi wa sizini ya 2017/2018, swi ngetelele swinene dopa content ku suka eka 12,030 to 324,240 peak intensity, kasi hi nkarhi wa sizini ya 2018/2019 season, 4-hydroxyproline ku 10×106 to 90x106 peak intensity swi ngeteleleke swinene. Ku tirhana ko fanana ehansi ka mbangu wa greenhouse, swi ngetelele swinene acetylcarnitine content ku suka eka 3,761 to 82,841 area under the curve hi nkarhi wa sizini ya 2018/2019. Ku tirhiwa ka ku nga ri na ku kayivela ka mati ka le xikarhi swi pfanganisiwa na misava ya loam ehansi ka mbangu wa mavala lama nga rivaleni swi ngetelele swinene norepinephrine content from 71,577 to 256,1045 peak intensity. Ndzavisiso wu pimanyete leswo mpfanganyiso wa ku tirhana na tilevhele ta ncheleteo wa mati (laha ku nga ri ku na ku kayivela ka mati na le ku nga na nkayivelo wa mati) na misava ya loam na misava ya sava ya loam) ehansi ka greenhouse na nete ya ndzhuti swi khumbe swinene ku xifta ka mihandzu ya primary metabolites profile of *C. metuliferus* E. Mey. ex naudin loko ku langutaniwa na tifekthara ha yin'we yin'we hi ku landzelelana.

SETSOPOLWA

Thuto ye e dirilwe ka maikemišetšo a go nyakišiša tiragatšo le boleng bja *Cucumis metuliferus* E. Mey. Ex Naudin (phara ya seAfrika) mo tikologong yeo e šireleditšwego le ya mo lebaleng e le nepo ya go bapetša tshekatsheko ya kotollu le boleng go hola thekišo ya mabele. Maikemišetšo kakaretšo e le go humana tsela ya maleba ya go mediša *C. Metuliferus* dipakeng tša mokhukhutšhireletšo, nnete ya moriti le mo ditikologong tša mabala ao a bulegilego gore go nolofatšwe tshekatsheko.

Go boima go mediša dipeu tša *C. Metuliferus* ka tlase ga maemo a tikologo ya maleba ya go tlwaelega yeo e šomišwago ke bontši bja balemi. Medišo ya dipeu e lekanyeditšwe go ya le ka sedirišwa sa go mediša dimela, go fala dipeu le go hlahlobo ya boleng bja dipeu. Dikokwana tše ke tšona di laolago kotollu le boleng bja dienywa. Nepokgolo ya thuto ye e be e le go nyakišiša khuetšo ya tlhahlobo ya polokego ya dipeu tše, sedirišwa sa go mediša dimela (mohlaba+vermiculite, peat TS1 le motswako wa dipeu) le phalo ya dipeu go kgonthišiša katlego ya go mela ga dipeu tša *C. Metuliferus*. Dipeu di ile tša arolwa go ya le ka magoro a mane, bjalo ka peu ya go okobatšwa ka dikhemikhale yeo e hlahlobilwego, peu yeo e sa okobatšwago gomme e hlahlobilwe, peu ya go okobatšwa e sa hlahlobjwago le peu yeo e sa okobatšwago gomme e se ya hlahlobjwa.

Boraro bja didirišwa tše di laeditše katlego ya go mediša yeo e ka balelwago go 80%. Dipoelo di šupa gore dipeu tšeo di okobaditšwego di se a hlahlobjwa le tšeo di sa okobatšwago di hlahlobilwe di bile le katlego ya tlhogo yeo e ka balelwago go 93.6% le 91.3%. Tlhogo ya fase e bile go dipeu tšeo di okobaditšwego di sa hlahlobjwago ka poelo ya 37%. Dipeu tše di hlahlobilwego di laeditše botelele bja 12cm gomme e le bjona bja go di feta ka moka. Dipeu tšeo di sa falwago le go hlahlobjwa di bile le botelele bja fase bja go balelwa go 3.44 cm ka dibeke tše nne. Bjalo, tlhahlobo ya dipeu e tlišitše katlego go fetiša phalo. Ka ge mudu wa dipeu o le bohlokwa go tšhutišetšo ya maphelo a dimela, thuto ye e thekga mmutedi le tlhahlobo ya dipeu bjalo ka tlhakanyo ya go mediša dimela tša boleng bja maleba.

Tlhokego ya meetse, go oketšega ga baagi, le diphetogo tša klaemete ke tšona dikokwana tše di amago tšwelelo go tša temo nakong ya bjale. *C. Metuliferus* E. Mey. ex naudin e mela ka lešokeng tlhagong ya yona; efela, kotullo ya yona go tlhokego ya meetse, go mehuta ya mašemo le mehuta ya mabu ga se e ahlaahlwe. Thuto e ile ya dirwa go humana mokgwa wa go bjala/mediša dimela dipakeng tša mokhukhutšhireletšo, nnete ya moriti le boemo bja lebala le le bulegilego, gore go tle go tšweletšwe tshekatsheko yeo e laetšago diphapano tša mabato a puno.

Nyakišišo ye e diritšwe ka fase ga maemo a mokhukhutšhireletšo, nnete ya moriti le lebaleng le le bulegilego Yunibesithing ya Afrika Borwa (UNISA) Khamphasing ya tša Saense, go la Florida, Gauteng (-26.157831 S, 27.903364 E) ka nako ya sehla sa 2017/2018 le 2018/2019 ka dinako tša go mela. Teko ye e ithekgile godimo ga dikokwana tše pedi – mabu (monola le mohlaba) le mabato a taolo ya go nošetša (tlhokego ya meetse ya lebato la fase, tlhokego ya meetse ye e lekanetšego le tlhokego ya meetse ya lebato la godimo). Diteko di be di beilwe ka mokgwa wo o sa rulaganywago gomme teko ye nngwe le ye nngwe e boeleditšwe ga senyane (9) bjalo ka ge e laeditšwe godimo. Dipharametha tšeo di lekantšwego di akaretša dikagare tša chlorophyll, stomatal conductance le bjalo ka tšhomišo ya meetse, palomoka ya dimela, dimela tše di bonagalago ka godimo, lenaneo la puno, botelele bja enywa, palo ya enywa le tšhomišo ya meetse ke dimela.

Dipoelo di tšweletša gore teko ya mo lebaleng le le bulegilego le meetse a a lekanetšego gammogo le monola di oketša palo ya dienywa. Teko ya mo lebaleng le le bulegilego go kopantšhwa le meetse ao a lekanetšego le monola, di laeditše tšhomišo ya meetse yeo e balelwago go (6.2 kg m^{-3}) ge go bapetšwa le diteko tše di ngwe. Go bohlokwa go lemoga gore ga ga go na diphapano magareng ga mohlaba le monola tšhomišong ya meetse (WUE). Efela, mohlaba o laeditše (WUE) ya godimo ge go bapetšwa le monola. Se se bolela gore, go ka tšewa sephetho sa gore teko ya dipeu lebaleng le le bulegilego, taolo ya go nošetša dimela (ye gabotse le ye e lekanetšego) le mohlaba ke didirišwa tšeo go eletšwago balemi gore ba di šomiše go humana (WUE) ya godimo le tšweletšo ye e atlegilego ya *C. metuliferus.*

Bontši bja phepo mo mabeleng bo hlohleletšwa ke dikokwana tša go swana le meetse, tikologo ya mo a melago gona, dihlase tša letšatši le mehuta ya mabu. Efela, dikokwana tše di huetšago bontši bja diphepo go dienywa tša *C. metuliferus* ga dišo di tsebjwa. Nepo ya thuto ye e be e le go nyakolla khuetšo yeo dikokwana tše di latelago; di nago le yona go bontši bja diphepo go enywa ya *C. Metuliferus*: mabato a meetse (tlhokego ya meetse ya lebato la fase, tlhokego ya meetse ye e lekanetšego le tlhokego ya godimo ya meetse), mehuta ya mabu (monola le mohlaba) le tikologo ya go mediša (mokhukhutšhireletšo, nnete ya moriti le lebala le le bulegilego). Diteko tša enywa yeo e omišitšwego ka setšidifatšing e ile ya šomišwa go tšweletša boleng bja β-carotene, vitamin C, vitamin E, total soluble sugars, crude proteins, total flavonoids, total phenols, le micro-nutrients (Cu, Fe, Mn le Zn).

Dipoelo di šupa gore dimela tše di godišitšwego ka fase ga nnete ya moriti, go akaretša le tlhokego ya meetse ya godimo le monola di nyološitše diswikiri tše di humanegago mo dimeleng (15.8 ˚Brix) ge go bapetšwa le diteko tše dingwe. Dimela tikologong ya nnete ya moriti go akaretša le tlhokego ya meetse ye e lekanetšego le monola di ile tša nyološa phroteine (6.31 ˚Brix). Teko go tlhokego ya meetse ya godimo go akaretša le monola ka tlase ga boemo bja mokhukhutšhireletšo go nyološitše diteng tša β-carotene (1.65 mg 100 g^{-1} DW) ge e bapetšwa le diteko tše dingwe. Teko go tlhokego ya meetse go akaretša monola ka fase ga nnete ya moriti go nyološitše Vitamin C (33.1 mg100 g^{-1} DW).

Teko go hlokego ya meetse ya godimo go akaretša mohlaba tikologong ya mokhukhutšhireletši go nyološitše diteng tša vitamin E (35.1 mg/100 g^{-1} DW) ge e bapetšwa le diteko tše dingwe. Teko ya go se hlokege ga meetse, go akaretša le monola tikologong ya lebala le le bulegilego e nyološitše palomoka ya diteng tša phenolic (6.4 mg GAE/g^{-1} DW) ge e bapetšwa le diteko tše dingwe. Teko lebala le le bulegilego ka fase ga hlokego ya meetse ye godimo go akaretša monola go okeditše diteng tša flavonoids (0.85 mg CE g^{-1} DW) mo dienyweng tša gona ge e bapetšwa le diteko tše dingwe. Teko go hlokego ya meetse ye e lekanetšego le mohlaba ka fase ga nnete ya moriti di laeditše go oketšega ga diteng tša Zn (12.7 μg g^{-1} DW) ge e bapetšwa le diteko tše dingwe.

Dipoelo di laetša gore semela se se thunya boleng bjo bo kgodišago bja dienywa ge go lebeletšwe bontši bja diphepo le dikokwana tša dikhemikhale ge di medišwa mo go sa hlokegago meetse go yela go mo go hlokegago meetse ka go lekanela, go šomišitšwe monola goba mohlaba mo nneteng ya moriti le mo lebaleng le le bulegilego. Dimetabolite tša motheo di tsebjwa bjalo ka motswako wa tlhago wo o lego bohlokwa go kgolo le tlhabollo ya dimela maphelong a tšona. Di na le khuetšothwii go dikokwana tša puno le khemikhale ya hlago ya dimela. Bontši bja dimetabolites tša motheo di humanwe ka go šomiša LC-MS-8040 triple quadrupole mass spectrometer (Shimadzu) ya go tšwa dienyweng tšeo di bunnwego ditekong tše di šetšego di boletšwe. Dipoelo di laeditše gore teko ya hlokego ya meetse ya lebato la fase go akaretša le mohlaba tikologong ya nnete ya moriti; e nyološitše asparagine content go tloga go 10×10^6 go ya go $80x10^6$ peak intensity ge e bapetšwa le diteko tše dingwe.

Tlhokego ya meetse ya lebato la fase e akaretša le monola tikologong ya lebala le le bulegilego ka nako ya sehla sa 2017/2018, 4-hydroxyproline go tšwa go 10×10^6 go ya go $90x10^6$ area under curve e ile ya nyušwa. Teko ya go swana le ye tikologong ya mokhukhutšhireletšo e ile ya oketša dikagare tša acetylcarnitine go tšwa go 3,761 go ya go 82, 841 peak intensity ka nako ya sehla sa 2018/2019. Teko go tlhokego ya meetse ye e lekanetšego go akaretšwa le monola tikologong ya lebala le le bulegilego e nyološitše dikagare tša norepinephrine go tloga go 71,577 go ya go 256,1045 peak intensity.

Diteko di šupa gore ge go kopantšwe taolo ya mabato a go nušetša (tlhokego ya meetse ya lebato la fase le tlhokego ya meetse ya lebato la godimo) le (monola le mohlaba) ka fase ga boemo bja mokhukhutšhireletšo le nnete ya moriti go ile gwa ama katološo ya dimetabolites tša motheo tša enywa ya *C. metuliferus* ge di bapetšwa le kokwana ye nngwe le nngwe.

DECLARATION

I, Mdungazi Knox Maluleke, declare that this thesis entitled,

‘Investigating the performance and quality of the *Cucumis metuliferus* (African horned cucumber) under different growing environments, for potential commercialisation’ Is my own work and all sources that I have used or quoted have been indicated and acknowldged by means of complete references.

Prior to the commencement of the research project, both the researcher and the Unisa library conducted a literature review, and ascertained that no other similar research had been conducted in South Africa/ or globally, prior to the registration of this project.

______________________ 18-12-2020

Signature Date

Student number 53376544

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Long time ago, people who sacrificed their sleep, family, food, laughter and other joys of life were called

SAINTS

Now, they are called

PhD students.

"Unknown"

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ABBREVIATIONS

AGB	Above-ground biomass
ARC	Agricultural Research Council
Cm	centimeter
CE	catechin equivalents
GAE	garlic acid equivelant
HPLC	high-performance liquid chromatography
NTC	non-treated certified
NTU	non-treated uncertified
TUC	treatred uncertified
TC	treated certified
VWC	volumetric soil water content
mg	milligram
mz	mass to charge
RWC	relative water content
TSS	total soluble sugars
µmol/m^2	mass per area of leaf surface/miligram per square meter
mmol m^{-2} s^{-1}	millimoles square meter per second
WUE	water use efficiency

GLOSSARY

βeta carotene	Organic oranged coloured pigment that forms part of vitamin A (Manthey & Perkins-Veazie, 2009).
Chlorophyll	The green substance that gives many plants their green colour and is for vital for photosynthesis (Abu-Zinada, 2015).
Commercialisation	The process of systemically managing a product for financial returns (Govereh, Jayne & Nyoro, 1999).
Crude protein	The amount of protein in plant material (Alomran & Luki, 2012; Wang, Shen & Zhu, 2018)..
Flavonoids	A large group of phyotochemical compunds found in plant pigment and have a base structure similar to flavone (Krogholm, 2011).
Germination	The metabolic proccess by which an embryo inside the seed develops into a seedling (Eifediyi & Remison, 2010).
Phenols	A group of phytochemical compunds found in plant tissues and normally derived from the aromatic amino acids (Hossain & Shah, 2015).
Stomatal conductance	The measurement of carbon dioxide entrance rate and water vapour exiting the leaf through the stomatal passage (Zhang et al., 2003) .
Total soluble sugars	The amount of soluble water carbohydrates, mainly in the form of soluble sugar content (Arrom & Munné-Bosch, 2012).
Macro-nutrients	Elements required by plants and animals in a larger quantity (Wang, Chen, Sciarappa & Camp, 2008).
Micro-nutrients	Elements required by plants and animals in a smaller quantity (Schauer & Fernie, 2006).

Metabolites	Biological compounds that are essential to growth and development of a plant during its life cycle (Abbey, Nwachoko & Ikiroma, 2017) .
Water use efficiency	The quantity of water used by crops during the growth and development process, normally calculated in kg over applied water (Mazahreh, Nejatian & Mousa, 2015).
Certified seeds	Seed certification, according to Penfield & MacGregor (2017), is a method of ensuring a crop's physical identification and genetic integrity by providing high-quality seeds and propagation material.

CHAPTER 1: INTRODUCTION

This chapter aims to introduce the research topic. Background information is provided on the different growing conditions required for plant production, performance and quality, for commercialisation. The purpose of the research and objectives of the study are also highlighted.

1.1 BACKGROUND

Cucumis metuliferus E. Mey. Ex Naudin (African horned cucumber) is one of the most important indigenous crops in the Central and Southern Africa regions; however this crop is under-utilised, due to lack of knowledge on its importance and uses as an agronomic crop (Reinten et al., 2011). Studies conducted suggested that it is commonly grown in Japan and New Zealand and has been named Kiwano fruit, and made readily available in their markets (Gumi, 2012). A study by Legwaila et al. (2011) suggest that there is an increase in the utilisation of indigenous crops. In addition, the *C. metuliferus* has the potential of being commercialised as a crop (Mutuku et al., 2013).

Davis et al. (2008) indicated that only non-bitter varieties are palatable, and have a similar blended taste of a mixture of banana and pineapple. The internal like-jelly content of *C. metuliferus* fruit can be scooped out and consumed as salad (Legwaila et al., 2011). The benefits of using the *C. metuliferus* fruits have been described and explained by several authors such as Dold and Cocks (2002), and Ajayi et al. (2017) as being a good source of vitamin B, helping to rehydrate the body and replenishes daily vitamins, particularly A, for boosting vision, the fruit have a higher water content, which is essential in eliminating body toxins and low calorie that is ideal diet for people who are aiming to lose weight (Tucker, 1999). Finally, Lin et al. (2015), reported that the fruit also has medicinal properties that has a role in human metabolism. Tona et al. (2004) and Ajayi et al. (2017), claimed that the fruit contains bioactive compunds that cure ulcers and are involved in regulation of blood pressure.

The genus *Cucumis* which this crop belongs to, originates from the Latin name of cucumber crops which were already cultivated worldwide, and has about 32 species found in different parts of the world, mainly in Africa, Asia, Australia and a few islands of the Pacific region (Weinberger, 2007; Davis et al., 2008; Hadid, 2016). The best-known crops of the *Cucumis* genus includes *Cucumis sativum* (Cucumber), *Cucumis anguria* (West Indian gherkin) from Asia, *Cucumis melon* (Muskmelon) and *Cucumis metuliferus* (African horned cucumber) from Africa. *Cucumis metuliferus* is natural and indigenous to the woodlands and grassland of tropical and subtropical regions of Africa (Van Wyk, 2005).

The species name *metuliferus* refers to the thorny spikes found on the fruit. In South Africa, it is also commonly found as a weed of abandoned fields. The crop's natural habitat include Limpopo, Mpumalanga, and the inland part of KwaZulu-Natal provinces (Markovskaya et al., 2007). The crop thrives well in loam soil that is well drained, and sometimes on river banks (Sigüenza et al., 2005). Optimum growing conditions, as well as other requirements such as water and temperature, are among some of the most prominent factors affecting the growth, development and bio-constituents of the crop, leading to either reduction or increase in yield (Benzioni et al., 1991). These were also identified as major factors affecting the growth, development, yield and quality of most agronomic crops in Southern Africa (Weng, 2010).

Commercialisation of crops is primarily based on its well-known uses, as well as its demand to fulfil a specific need such as nourishment or medicinal purposes (Lin et al., 2015). When there is adequate scientific information on the uses, value, growth and development of a crop, it is possible for farmers to start growing it on a larger commercial scale and make profitable returns (Pingali, 2001).

Cucurbitaceae crops are known to perform well under protected and open field environment. Some of the well-known crops include English cucumber and watermelon (Rahil & Qanadillo, 2015); however, there is limited information or findings about the performance of *C. metuliferus* growing under different conditions (Sigüenza et al., 2005). This has prompted this study to assess its performance under different growing environments, such as greenhouse, shade net and open field conditions (Benzioni et al., 1991).

Intensive research work has been carried out to study the biochemical constituents of *Cucumis sativus* (Cucumber) and *Citrullus lanatus* (Water melon) fruits exposed to different water levels for yield improvement, but very little on *C. metuliferus* (Gumi, 2012). In the present study, therefore, the fruits harvested from *C. metuliferus* plants which were grown under different growing environments, in terms of water levels and soil types, were analysed, in order to understand their biochemical constituents. The yield and other quality parameters were also measured to determine the productivity of the crop. In the work described in this study, environmental effects (growing conditions, soil type and irrigation water levels) on *C. metuliferus* fruit quality are the main factors that were under investigation. *Cucumis metuliferus* fruit quality components in this study related to total soluble sugars, individual sugars, crude protein and primary metabolites. Fruit weight was considered an important quality parameter that is influenced by different environmental factors and water stress levels (Nerson, 2009).

1.2 Problem statement

Indigenous crops are a source of food and posses some medical attributes for many people living in the rural areas of Southern Africa (Legwaila et al., 2011). *Cucumis metuliferus* is one of the indigenous crops that is suspected to have medicinal properties and can potentially contribute to continuous food security in the region. However, due to insufficient information on its production, it has limited usage. Research on the performance of such a crop under various environmental conditions, would assist in providing information that can be used to grow them for food security purposes and economic emancipation.

Cucumis metuliferus is believed to be tolerant of a wide range of environmental stress factors, but it has been established that the crop shows poor germination and seedling development under field conditions (Sigüenza et al., 2005). This problem has been associated with its hard seed coat which is presumed to prevent oxygen diffusion and moisture penetration, these are factors that help to accelerate germination of seeds (Gumi, 2012).

In addition, poor germination and seedling development were shown to be caused by variation of temperature levels, type of soil and moisture availability (Marta et al., 2016). Weinberger and Lumpkin (2007) suggested that different temperatures play a role on the morphological growth and development of crops, particularly in *C. metuliferus.* Besides the different temperature conditions, suitable growth mediums required for germination and water stress tolerance are some of the abiotic factors that were never studied for this crop. This study determined the performance, quality and yield of *C. metuliferus* under different growing media, growing environment and water stress levels.

1.3 Research questions

The research study addressed the following research questions below:

- What are the major factors that affect seed germination of *Cucumis metuliferus*?
- Does water stress improve the water use efficiency of *Cucumi*s *metuliferus*?
- What is the effect of different water stress levels on the biochemical concentration of *Cucumis metuliferus* fruit?
- What are the major factors that influence the primary metabolite profile of *Cucumis metuliferus fruit*?

1.4 Aims and objectives

1.4.1 Aim

The main aim of this study was to determine a suitable growth media and irrigation water levels of *Cucumis metuliferus* between the greenhouse, shade net and open field environment, so that a comparative yield and quality analysis can be done for the purpose of commercialisation of the crop.

1.4.2 Objectives

The objectives of the study were:

- To evaluate the effect of different growth media and seed type on the seed germination of *Cucumis metuliferus.*
- To determine the water use efficiency of *Cucumis metuliferus* under varying (irrigation water levels and soil types) grown in shade net, greenhouse and open field environment.
- To assess the biochemical constituents of *Cucumis metuliferus* grown in shade net, greenhouse and open field.

- To evaluate the effect of different treatments (varying substrates, irrigation water levels and growing environment) on the primary metabolites profile of *Cucumis metuliferus* fruit.

1.5 Reliability and validity

The degree of credibility in scientific research relies on the procedures and instruments applied to generate information and data analysis, in order to answer the research questions of interest. In this case, it was important to make use of reliable, valid, fair methods, manage experiments and, most importantly, record data with highest level of accuracy. Creswell (2014) defines reliability as the consistency and trustworthiness of instruments and methods applied in assessing the biological responses to plant growth, development and yield.

The randomised block design was adopted in this study, to evaluate the growth and development of the African horned cucumber crop under different environmental conditions, the yield performance, the biochemical constituents, water requirement associated with the use of irrigation levels, and primary metabolites profile. The resultant data were analysed using suitable statistical methods to draw conclusions.

1.6 Bias

When conducting an experimental work, it is very important to use practical strategies which will minimise biasness. Creswell (2014) defines bias as an error in design or execution of an experimental work, which is distorted in one direction because of non-random factors. In this study, bias was minimised by ensuring that the experimental error in each experiment was reduced through increased blocks, replications, randomisation and seasonal repetition of the experiments (Mouton, 2001).

1.7 Significance of the study

In investigating the potential for commercialisation of the crop under Gauteng growing conditions, it was important to anticipate variability in the performance and quality of the crop under the varying growing conditions. Interactions of these factors may affect the growth, development and yield quality of the crop positively or negatively. Factors such as extremely high temperature, water stress and different soil types, can either increase or reduce yield on several crops (Eifediyi & Remison, 2010).

With limited information on plant performance, most farmers may not grow the crop, because it may not be worth investing in it due to fear of risk of losses (Sigüenza et al., 2005). Providing scientifically proven information on the crop may, however, increase farmers' interest in adopting it for agronomic purposes.

1.8 Thesis overview

The thesis structure outline the research study process and activities, and report research outcomes in a systemic and comprehensive manner. The titles of different chapters and the summaries of their contents are explained below:

Chapter 2: Literature review

The literature review provides detailed background information on the importance of commercialisation of potential agronomic crops, factors which have a direct and indirect effect on the growth, development and yield of the crop, and the need to research the quality of the crop in the agricultural industry. This chapter concludes by identifying gaps in the literature and justifying the research.

Chapter 3: Materials and methods

Chapter 3 outlines the different research methodology processes applied to achieve the objective of the research study:

Experiment 1:

Focused on the effect of different growth mediums and seed treatment used on the seed germination of *C. metuliferus*.

Experiment 2:

Focused on effect of different water levels, soil types and growing environment on the growth, development, yield and water use effeciency of *C. metuliferus*.

Experiment 3:

Evaluated the biochemical constituants such as beta carotene, crude protein, total soluble sugars, total flavonoids, total phenols, vitamins (C and E), macro and micro-nutrients) of *C. metuliferus* fruit.

Eperiment 4:

Assessed the the treatment effect on primary metabolite profile of *C. metuliferus* fruit. Data gathered on harvested fruit subjected to different environmental conditions, were used to construct a primary metabolite profile.

Chapter 4: Results and discussions

This chapter includes the results, which have been gathered, presented and discussed. It includes different variables tested and method used to determine the outcome. The results gathered were analysed, discussed and interpreted.

Chapter 5: General conclusions and future work

This final chapter of the thesis summarises the main research findings, and articulates major conclusions arising from the research study. Recommendations on the potential commercialisation of *C. metuliferus* are made. Further research is suggested.

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CHAPTER 2: LITERATURE REVIEW

The focus of this literature review is to examine previous research work that was done on *C. metuliferus* and/ or related crops. It is also to assesses relevant research on other crops with regard to their performance under different growing environment such greenhouse, shade net and open field environment, as well as varying growth media. The various methodologies and parameters used to measure the performance, yield and quality of crops are important dynamics in the literature review.

In the 21st century, there is an urgent need to produce enough food crops for sustenance of the global food demand. Both developed and developing countries constantly demand high-quality food, which places food producers under severe pressure to grow crops in optimum conditions. To overcome the food security problems, a constant search, discovery and research of ‘‘new’’ crops that can add to the food basket and/ or for commercialisation is vital, to address the shortage of food and medicine globally (Mwangi & Kimathi, 2006). Growing crops under different growing conditions presents an opportunity for sustainable production and a future solution that will allow food producers to grow crops in the conditions that will suit their capital returns. Food crops require an environment that has balanced temperature, water availability, nutrients, and free from pests and diseases. Each particular crop species, however, requires a specific environmental condition for optimum growth, development and yield (Weinberger & Lumpkin, 2007).

2.1 Cultivation requirements

Van Wyk (2005) indicated that the non-bitter variety of kiwano is the one that has the potential to become a viable, agronomic, commercial crop, provided that appropriate scientific procedures are followed, that will help to develop suitable cultivation practices that will enable the crop’s growth, development and yield. The plant can be propagated through seeds in the same way as normal, well-known English cucumber, and it requires warm temperature for optimal growth and development (Eifediyi & Remison, 2010).

Authors such as Wahome & Masarirambi (2015) and Marta et al. (2016) suggested that certified seeds should be used in order to increase germination success compared to traditional seeds that are harvested from the plant ,and sawn without treatment. Penfield & MacGregor (2017) define seed certification as the method that ensures the physical identity and genetic purity of a crop by supplying high-quality seeds and propagation material. Other researchers have used propagules, instead of seed, for cucumber germination, and obtained different success rates (Markovskaya et al., 2007). The seed is recommended, because they can be stored for a longer period, and propagated when the temperature is suitable for germination (Mo et al., 2013).

Since dormancy accounts for approximately 40% of the germination success of *C. metuliferus* seeds (Atif et al., 2016), seed treatment, even with weak acid and warm water, can improve germination (Gvozdenac et al., 2011). This is particularly true, since the seed coat is hard and does not germinate easily (Penfield & MacGregor, 2017). Few studies, however, have addressed the possibility of seed treatment by various methods, with the possibility of increasing seed germination success of *C. metuliferus.* To the best of the researcher's knowledge, minimal information exists in the literature on any studies that have been conducted on the impact of certification, growth media and scarification on the seed germination of *C. metuliferus.*

The light availability, production system and trellising method will affect the exact planting space required for the plant growth, development and yield (Mercurio, 2002). Under good light conditions, 40 cm planting space should be sufficient. Tsirogiannis et al. (2010) state that there should be good air circulation and adequate light intensity for succesful fruit production. A well-ventilated greenhouse and shade net will lower the chances of disease development, and enable growers to have easier access to carry out day-to-day cultural practices such as removal of weeds, irrigation, pruning, and spraying of necessary pesticides for pest and disease control (Kara et al., 1996).

A great deal of work has been done on other *Cucurbitaceae* crops such as watermelon and English cucumber, with regard to comparing growth and development in the open field and protected environment (Rahil & Qanadillo, 2015). The sweet Armenian cultivar was trialled in the greenhouse and shade net under different temperatures. It was concluded that fruit yield increased in the greenhouse, compared to the shade net. The comparison varies with findings by Reinten et al (2011) and Alomran & Luki (2012), who suggested that growing conditions for research trials should be as varied as possible.

This is demonstrated by the research conducted by Arshad et al. (2014), on cucumbers grown under greenhouse and subjected to the same fertilizers. They found that the above-ground biomass was higher on the plants grown in the greenhouse conditions treated with optimum level of fertilizers, compared to those of shade net environment. However, the fruit yield of shade net grown plants was significantly higher than of those grown in the greenhouse.

The direct effect of soil properties and yield of cucumber has been shown by various researchers. Kultur et al. (2001) and Micheal et al (2017) are some of the researchers who have argued that soil properties are not the main factors responsible for plant growth and yield. Kassu et al. (2017) remarked that the combination of soil properties and water quality has a direct impact on the plant growth, development and yield of the cucumber crop; thus, water and soil type should be investigated extensively. Soil type, water quality and temperature contributes towards plant growth, development and yield quality of plants.

Water use efficiency should therefore take into consideration stomatal conductance and chlorophyll content, so that there is an understanding of their potential impact on the growth, development and yield of the crop. In the present study, therefore the impact of the different water stress levels on *C. metuliferus* cultivated under diferent growing environments and soil types filled a gap in the water use efficiency of the crop for future commercial production.The parameters that were measured for the most *Cucurbitaceae* crops have always been inclusive of growth rate and yield quality, which include sugar and water content (Abu-Zinada, 2015).

None of the research has investigated vitamins, total phenols, total flavonoids, total soluble sugars, crude protein, macro-nutrients, micro-nutrients and primary metabolite profile on *C. metuliferus* fruit grown under different environments. The metabolomic analysis is very important in the determination of quality, because it gives an indication of metabolites content as was described by several researchers such as Pratelli & Pilot (2018). Several researchers do not agree that primary metabolites are the only important determination of quality in food crops. For example, Samantaray et al. (1998) and Osuji et al (2015) suggested that sugars and protein are the main variants to be investigted for quality determination in fruit.

Flemotomou (2011) is one of those that subscribed to the notion that metabolites provide a snapshot quality of crops when they are exposed to various environmental conditions or treatment. *Cucumis metuliferus* fruit should be harvested when the fruits are at the desired stage of ripening (Yu et al., 2003), which is usually when the fruits have turned from green to yellow. Care should be taken when harvesting the fruits because they are spiky, and the use of gloves is highly recommended during harvest (Dris et al., 2003). Alimoradi et al. (2013) suggest that a good harvesting practice for African horned cucumber fruit is during the coolest time of the day, in order to minimise nutrient quality loss. However, Adachi et al (2000) harvested in the evening, and not during the hottest time of the day in order to minimise heat damage on fruits.

There are therefore differing views on the time of harvesting, which depends on the size of one's operation, as well as the crop type. In the present study, harvesting was done in the morning for practical purposes, as that was the only time there was access to the laboratories. El-Ramady et al. (2015) harvested fruits at different times of the day, and delayed storage by several hours after harvest. They discovered that physical damage such as bruising was higher on tomatoes, cucumbers and peaches, than on other fruits such avocadoes and mangoes.

2.2 Cultivation of *Cucumis metuliferus* L

In Europe (Germany), a study was conducted by Benzioni et al. (1991) to assess if *C. metuliferus* seed germination success is affected by the sowing dates. Results showed that the sowing dates, as well as the season, affect the seed germination success. Other studies did not have conclusive results on the sowing dates; for example, Mabundza et al. (2010) found no significant differences in sowing dates when they used gibberellic and sulphuric acid as pre-treatment for cucumber and watermelon seeds. None of the abovementioned studies was interested in the impact of certification, growth media and scarification on the seed germination success of *C. metuliferus*.

2.3 Environmental factors affecting growth, development and quality of crops

Pingali (2001) suggested that developing an understanding of the environment and crop adaptation plays a significant role on the successful cultivation of crops. Thus, environmental factors such as temperature, soil type and water directly affect crop production and yield (Sigüenza et al., 2005). It is therefore vital for growers to have a comprehesive understanding of environmental factors affecting growth, development and yield of crops, so that optimum growing conditions can be created for successful crop production and yield (Govereh et al., 1999; Parajuli, 2019).

2.3.1 Temperature

The direct effect of temperature on plant growth, development and yield has been investigated by various researchers (Adachi et al., 2000; Benzioni et al., 1991). Their findings reported that extreme temperature that ranges between 10 and 40˚C could lead to plant death. Some plants, however, are not directly affected by the extreme temperatures because of their natural habitat (Shvarts, Weiss & Borochov, 1997).

If the temperature range of a certain region prohibits the normal growth of certain crops, the use of plant growth structures such as greenhouses and shade net should be utilized as alternatives to overcome the challenges caused by unfavourable conditions (Hochmuth, 2001). *C. metuliferus* is not traditionally grown in greenhouses and shade net houses, unlike other cucumbers (Alaoui-Sossé et al., 2004).

The use of protected plant growth strucutures/facilities proved to be successful in maintaining optimum temperature required by plants for healthy growth, development and yield (Yasuor et al., 2013). Plant growth factors such as extreme temperature and transplant shock can be minimzed by transplanting seedlings, rather than direct seeding in the open field at the time when risk of frost or prolonged low temperature may kill the young plants (Markovskaya et al., 2007) . It implies that seeds should be sown in trays at protected environment until they are ready to be transplanted into the open field (Gama et al., 2015).

2.3.2 Irrigation management

Alomran and Luki (2012) indicate that plant water relation remains the most important factor that must be fulfilled, in order to sustain high crop yield quality. Plant organs such as roots, stems, leaves, fruits, and physiological aspects such as chlorophyll, stomatal conductance and height, are directly affected by water availability (Li et al., 2018). Plant water stress is described by De Pascale et al (2011) as a physiological and ecological requirement of a specific plant throughout its life cycle.

The energy used by plants for maintenance and repair of metabolomic pool is minimal; therefore, they are able to compensate for the stress (Maria et al., 2013). When stress factors such as water stress exceed the repair potential of a plant, its physiological and ecological performance will eventually diminish (Parajuli et al., 2019). The effect of different water stress levels in Cucurbitaceae crops has been investigated by Tsirogannis et al. (2010). They suggested that efficient water supply throughout the growing season is essential for higher yield and quality crops. Maintaining plant turgor pressure is important for photosynthesis, which plays a major role in energy build-up by the plant through respiration and regulation of stomatal activities, and is thus crucial to growth, development and yield of crops (Kassu et al., 2017).

Abdelraouf et al (2014) investigated the impact of water stress on watermelons, cucumbers and several other agricultural crops. They suggested that one has to ensure that the trial for both soil water plant relation and different water stress levels are crucial in order to have better irrigation management and improve yield. Rahil and Qanadillo (2015) stated that when water supply is balanced, the optimum performance of all plant organs will result in healthy growth. However, when either the balance of water is affected because there is insufficient available moisture in the soil, or the transpiration of water through the stomata of the leaves exceeds the plant's capacity to compensate for the internal loss, the plant comes under water stress (Zhao et al., 2017). Most crops have different critical growth periods, and if water stress occurs during critical stages of growth, development and yield is directly affected (De Pascale et al., 2011).

Dukes et al. (2010) reported that yield performance for most crops springs from vegetative parts of the plants, that possess high water content. The crop water requirement during the growing cycle is directly related to the length of time it takes a crop to reach a marketable stage and time of the year at which the crop is transplanted, until the completion of the growing cycle (Weinberger & Lumpkin, 2007). These authors concluded that for sustenance of the plant's steady growth, production of higher yield and quality produce requires supplementary watering through irrigation.

The nature of the soil and plants are the most challenging factors when regulating the water deficits on the control experiment (Kassu et al., 2017). In most cases, the researcher should then decide which method should be used to induce water stress on the experiment, whether by withholding water or through osmotic means. Direct measurement of soil water content using different instruments, or weighing of pots, has been the most practical and effective method used by De Pascale et al. (2011).

Water levels either reduced or increased moisture content in plant biomass and fruit (Li et al., 2018); however, little is known about fruit yield quality content caused by water deficit irrigation on *C.metuliferus* crop.The present study would therefore, investigate the effect of different water levels on the growth, development and yield quality of *C. metuliferus* cultivated in different soil types in diverse growing environments.

2.3.3 Water use efficiency

El-Mageed & Semida (2015) defined water use efficiency as the ratio of water used in plant metabolism to water loss by the plant through transpiration. Due to factors such as run-off, transpiration, evaporation and percolation, not all water in soil pores or growth media is accessible to plant roots, because the medium has limited ability to retain all irrigated water (Abdelraouf et al., 2014). These pores usually contain air that provides oxygen to the plant roots system for respiration (Mazahreh et al., 2015). Water is absorbed by roots and circulated through the vascular system to above-ground parts (De Pascale et al., 2011), and this water carries dissolved oxygen and mineral nutrients vital for plant growth, development and yield (Weinberger & Lumpkin, 2007).

Hirano et al. (1995) reported that during the photosynthesis process, only a small portion of the absorbed water is used by plants to create their own food. Most of the water is lost through the transpiration process via the stomata of the leaves (Zhang, et al., 2003). Stomatal pores are small holes that dominate mostly on the bottom side of the plant leaves, and control the plant water loss. In addition, they also control the movement of gases such as carbon dioxide and oxygen into plants during the photosynthesis process, and this ultimately impacts the general crop output (Kassu et al., 2017).

When the plant is subjected to water stress, it also affects the stomatal opening or closure, and ratio of photosynthesis that drives yield. Yildirim et al. (2008) found that, under water stress, crops lose water more quickly than carbon dioxide, which could lead to dieback of some plant organs or full plant death. In addition, excessive soil moisture may result in an anaerobic condition around the plant roots of crops. This implies that there is lack of oxygen in the media, which could lead to a severe decrease in root respiration, and influence the water and nutrient absorption from the soil by plant roots (Booker et al., 1992). There is a need to explore the influence of water use efficiency on water relation parameters of most indigenous crops of Southern Africa for commercialisation purposes (Abu-Zinada, 2015). Plant water status depends on the species, growth stages, duration and environmental factors affecting growth and evapotranspiration (Yu et al., 2003).

Among the most prominent effects of water stress are reduction of growth, carbon fixation, enzyme activities and leaf photosynthesis (Zhang et al., 2003). Given the pressing need to save water in regions experiencing elevated temperatures and low rainfall, water use by indigenous crops needs to be investigated, in order to develop ways to minimise losses and increase yields (Weinberger & Lumpkin, 2007). To the best of our knowledge, research on water use efficiency of *C. metuliferus* has not been paid attention to; instead, there is lot of work on other crops (*Cucumis sativus, Citrus lanatus, Cucurbita pepo*) and various leafy vegetables (Davis et al. 2008; Hashem, 2011; Rahil & Qanadillo, 2015; Kassu et al (2017).

There appears to be scanty knowledge on the agronomic or yield responses of this crop plant to various growth factors such as the substrate and/or suitable irrigation levels, for optimum productivity.

2.3.4 Soil

Rhodes (1995) defined soil as a shallow, degraded mantle of material covering the bedrock sheath of the earth, that has been modified and advocated by biological, chemical and physical agents, with its primary roles being anchoring plant roots, holding nutrients and moisture, and making them available for plants roots. The main characteristics of good agricultural soil have been reported by Tsirogiannis et al. (2010) and Gama et al. (2015), as that it is rich in organic matter content, adequate nutrient levels, balanced soil pH, good drainage and moisture retention. When conditions such as higher temperatures drive the water and air out of the soil, more dry air moves in, accelerating drying problems which may result in plant wilting (Kassu et al., 2017). In yield and quality of cucumber (Burpless), investigators such as Atif et al. (2016) evaluated the effect of organic compost on soil performance for cucumber yield. They determined that yield increases when loam soil is mixed with compost, as compared to loam without compost and other organic matter.

Clay particles are highly responsible for providing the controlling influences on plant growth and responses (Mabhaudhi et al., 2013). In their review, Booker et al. (1992) and Ebrahim (2004) concluded that clay-rich soil has a better reaction in releasing adequate ion exchange than any other soils. Furthermore, they suggested that soil fertility be analysed in terms of mineral content and other factors affecting soil condition, so that supplements/fertilizers could be added to boost plant growth, development and yield.

2.4 Physiological effects of plants cultivated under different growing conditions

Weinberger & Lumpkin (2007) defined plant growth as progressive increase in weight and size of the plant due to the development of new leaves, cells, flowers, fruits and seeds. The plant cell uses water, carbon dioxide, light and enzymes for maximum production during the photosynthesis process (Adachi et al., 2000). The physiological growth of a plant can be measured through analysis of chlorophyll content, stomatl conductance, and biomass of roots, stems, leaves and fruitd Ebrahim (2004).

2.4.1 Chlorophyll content

Adachi et al. (2000) explained the chlorophyll content as the amount of chlorophyll accumulated during the photosynthesis process by which plants convert energy from the sunlight into chemical energy. The chlorophylls are the most effective plant leaf part for the absorption of blue and red wavelength, which contributes to the greener part of the plant leaves (Alomran & Luki, 2012). The chlorophyll is also highly responsible for the conversion of light energy into chemical energy and allow the plant to perform its metabolic functions (Abu-Zinada, 2015). When plants are cultivated in different growing conditions with high and low temperature, chlorophyll content is affected (Tewari & Tripathy, 1998; Shu et al., 2013).

There are several reports that suggested soil types and water stress have the potential to reduce or increase chlorophyll content on plant leaves (Shu et al, 2013; Penfield & MacGregor, 2017). The physiological growth and development of a plant can be measured through analysis of chlorophyll content (Ibrahim, 2014). The present study used different growth environments, soil types and water stress levels to fill a void on the physiological effect of the chlorophyll content of *C. metuliferus* leaves.

2.4.2 Stomatal conductance

Water stress on plant roots has a direct effect on the plant's physiological activities, and thus has the ability to increase or decrease the rate of photosynthesis (Zhang et al., 2003). The direct effect of stomatal opening or closure due to water shortage on plants' photosynthetic ability has been studied by various researchers. Hirano et al. (1995) and Savvides et al. (2012) are some of the researchers who have argued that temperature is the only important factor regulating the stomatal opening and closure. They remarked that low air and soil temperature are capable of disrupting the stomatal conductance (mmol m^2 s^1) in some heat-loving plants.

Wang et al. (2012) reported that crop that are categorised as C3 plant (cucumbers, cotton, rice, sunflowers, potatoes and tobacco) have low to medium water use efficiency compared to C4 crops (sorghum, sugar cane, and maize) due to their rate of stomatal conductance which result from changes in turgor pressure within the guard cells, meaning that carbon dioxide is first transformed into a 3-carbon compound, and the Rubisco enzyme plays a vital role in carbon dioxide uptake (Ikkonen et al.,2012). The current study paid special attention to the effect of different growing environments, soil types and irrigation water levels on the stomatal response of *C. metuliferus* leaves.

2.5 Application of metabolomics and biochemical responses to agricultural research

Metabolites, which are mainly determined through nuclear magnetic resonance (NMR), gas chromatography or liquid chromatography (LC) coupled to mass spectroscopy (MS) analytical technologies, systematically provide qualitative and quantitative information on how low-molecule-mass endogenous compounds provide a direct snapshot of the physiological condition in biological samples (Osuji et al, 2015). As a complement to proteomics, metabolomics has played a vital role in agriculture and food science research in terms of determing bio-chemical composition in food products.

In this study, the metabolomic profile of *C. metuliferus* was constructed in order to understand the kind of primary metabolites found in African horned fruit. The knowledge on the kind of compounds present in the samples of the crop will encourage the potential use of this crop as a natural medicine or agricultural food crop, for the treatment of some aliments or a supplement for some nutrients in the human body, when consumed. In a study conducted by Zhao et al. (2017), which comprehensively analysed the metabolomic changes of cucumber fruits from plants under nano-Cu stress, using the ^{1}H NMR and GC-MS methods, it was confirmed that the cucumber fruit metabolite profile was influenced by exposure to nano-Cu level in soil.

Minimal knowledge exist about the effect of different conditions, soil types and irrigation on the metabolomic profile of African horned cucumber fruit. Researchers have different views on which method could be regarded as the most effective and accurate for metabolomic analysis. Samantaray et al. (1998) and Abbey et al. (2017), for example, considered LC-MS as the most effective and accurate method in analysis of primary metabolites of fruit, whereas Morreel et al. (2009) found that the GC-MS was the most preferred method because of its ability to identify most metabolites, compared to other methods. Other researchers, e.g. Girelli et al. (2018) and Tang et al. (2017), used the NMR, due to its advantage in determining non-targeted metabolites on biological samples.

This study adopted the application of metabolomics LC-MS approach to identify and quantify the metabolites found on *C. metuliferus* cultivated in differing loactions, water levels and different substrates, because of its accuracy in determining target metabolites in biological samples.

2.6 Biochemical constituents in plants

Kader (2005) stated that people have different reasons for the consumption of specific groups of fruit; however, the main traditional aim for consumption is the potential supply of energy and nutrients. Humans obtain nutrients such as sugars by consumption of plant products such as fruit and vegetables, so it is vital that the sugar content of specific fruits be evaluated before commercialisation (Ali & Abdelatif, 2001).

The influence of soil type and fertilizers on cucumber fruit quality has been investigated by (Segovia et al., (2016); (P. Maria & Elena, 2016)Maria & Elena, (2016); Arshad et al., 2014). Their discovery outlined that the sugar content was lower in fruits which were treated with organic fertilizers on loam soil, when compared to those that were treated with chemical fertilizers on composted loam soil.

2.6.1 Carbohydrates

Jamnadass et al. (2011) stated that the three main carbohydrates groups are monosaccrides, disaccharides and polysaccharides. Carbohydrates are the most dominant nutrients in plant structures, with 60-95% of the total dry weight (Wang et al., 2013). They provide the structural composition of plants, with their main purpose being to transfer and store energy (Wani et al., 2012). Extensive research work has been carried out on the quality analysis of plants grown under polyhouse and in open field, by, for example, Das et al. (2018), in cucumber. There is limited literature concerning the effect of different irrigation water regimes, soil types and location on the quality aspects such as carbohydrates and other bio-constituents of *C. metuliferus* fruit quality.

2.6.2 Total soluble sugars

Osuji et al. (2015) defined soluble sugars as one of the most abundant compounds, making about 80% of sugar found in plant fruits. It is one of the first products of photosynthesis, and is mobilised in plant cells to produce energy during respiration and glycolysis (Arrom & Munné-Bosch, 2012). Total soluble sugar is mainly found in fruit, and the body cells depend on it as a primary source of energy (Ali & Abdelatif, 2001; Bernaert et al., 2013).

Studies on the effect of the genotype on total soluble sugar concentration on muskmelon fruits was conducted by Kultur et al. (2001). They concluded that genotype has a direct effect on the soluble sugar content of the fruit. Variation in glucose level may be caused by different growing environments, presumably such as greenhouse, shade net, open field, soil type and irrigation water regimes.

2.6.3 Crude protein

López et al. (2013) defined crude protein as the amount of protein content found in plant organs. It is normally estimated by measuring the total nitrogen in plant tissue mainly in fruit dry matter (Osuji et al., 2015). The total percentage of crude protein in fruit dry matter varies in accordance with the plant genotype and growing conditions in which the plant were exposed to during cultivation (Mabhaudhi et al., 2013).

The crude protein content in watermelon fruit has been reported by various researchers (Alomran & Luki, 2012; Wang et al., 2018). They discovered that different growing conditions do not cause a significant difference of protein content on some plant species. However, the crude protein content could be affected by the different cultural practices that the crop is exposed to during cultivation (Legwaila et al., 2011).

2.6.4 β-carotene

Ismail (2014) suggested that β-carotene has lipids which are mainly responsible for yellowing colour in fruit. β-carotene plays a significant role in human health, such as in the improvement of vision, cell division and differentiation, development of bones, and reproduction (Moyo et al., 2018). Also, it has several other roles in plants, such interception of blue radiation which is transferred to photosynthetic centres (Manthey & Perkins-Veazie, 2009). Various environmental factors have different impacts on the beta carotene content of fruit and vegetables. For instance, Fenech et al. (2019) discovered that there were significant differences in beta carotene content among carrots that were grown in areas that had different pollution levels. In a study conducted by Legwaila et al. (2011) to determine nutritional content of several crops that are indigenous to Southern Africa, it was confirmed that most crops contain high nutritional content such as β-carotene and other vitamins. This would therefore suggest that quantitative analysis of β-carotene in several fruit crops, such as *C. metuliferus,* should be conducted, and findings should be useful for commercial purposes.

2.6.5 Vitamin C and E

Esch et al. (2010) proposed that vitamins are vital organic molecules which are needed in large quantities for normal growth and development. The content of vitamins in fruit makes a significant contribution to human health, as they are responsible for day-to-day normal body activities (Locato et al., 2013). Vitamin content of fruit varies in ratio, due to environmental factors that surround the plants. For example, in the study conducted by Sinha (2015) to determine the vitamin C content of citrus fruit harvested from different locations, it was shown that the vitamin content was lower in fruit harvested from soil sites that were never treated with fertilizer, compared to those that were harvested from fertilised soil. Uusiku et al. (2010) indicated that differences in vitamin E concentration could still occur within cultivar varieties that are grown under same conditions and location. Locato et al. (2013) suggested that fruit harvested from water-stressed cucumbers had a lower vitamin E concentration compared to those of well-watered crops, due to low osmotic pressure caused by insufficent water required for transportation of nutrients within the plant cells (Sinha, 2015).

2.6.6 Total phenols

Total phenols are explained by Hossain and Shah (2015) as groups of compounds which originate from aromatic amino acids. The main role of these compounds is to act as a defence mechanism against potential predators, as well as protection against UV-radiation, and can also cause pigmentation in the fruit and flowers of most crops (Bernaert et al., 2013). In food science and nutrition, fruit such as berries are known to be rich in phenolic compounds (Oliveira et al., 2008; Saeed et al., 2012). Previous research work has been undertaken to extract and analyse phenols in different fruits. *Cucumis metulifeus* fruit seems to be one of those fruits that have not been well studied for phenolic content.

There is actually little evidence of total phenols quantification in *Curcubitaceae* fruit crops. For example, Weng (2010) investigated NMR-based metabolomics in cucumber fruit, where he found that genotype and environmental conditions are primarily responsible for variation in secondary metabolomic content.

In that study, the phenolic content of fruit was not investigated but was considered for the present research.

2.6.7 Total flavonoids

Total flavonoids are considered as naturally occurring substances with phenolic structures, and are mainly found in berries fruit, grains, tree bark, tea and grapes (Hu et al., 2019). Such organic products are well known for their beneficial human health effects, such as prevention of illnesses like autism, asthma and obesity (Saeed et al.,2012). Fenech et al. (2019) outlineed that total flavonoids are part of the reasons why fruit and vegetables should form part of human daily diet. People whose survival depends on a careful selection of certain foods, are likely to follow the diet programme that would encompass not only nutritional efficacy of food, but also acceptability and source of benefial compounds from such food (Krogholm, 2011). Earlier work has been done on fruit total flavonoid content by several researchers such as Segovia et al. (2016) in avocado, and Kujala et al. (2000) in beetroot, but none in *C. metuliferus*.

2.6.8 Macro and micro-nutrients

Macro-nutrients are a group of elements required in a large quantity, while micro-nutrients are required in a small quantity (Barrett et al., 2010). Various investigations showed stability in terms of macro-nutrient availability in fruit (Wang et al., 2008); however, change in concentration occurs with respect to different treatments such as soil type, irrigation water regime and location (Olle et al., 2012).

Some researchers, such as Schauer and Fernie (2006), reported that macro-nutrients (phosphate, magnesium and sulphur) are significantly affected by environmental factors such as water availability, soil conditions and temperature. Sezen et al. (2010) indicated that water stress results in reduction of some macro-nutrient elements, while micro-nutrients (iron, zinc and molybdenum) were not affected, and also increased in other fruits.

Previous studies on the concentration of macro- and micro-nutrients have mainly concentrated on exotic crops such cucumbers, tomatoes and pumpkins, but not much in indigenous ones such as *C. metuliferus (*Kujala et al., 2000; Sezen et al., 2010). *Cucumis metuliferus* presents more advantages over other exotic cucumbers, because it can tolerate unfavourable conditions by adapting to local conditions such as higher temperatures, minimal water supply, pests and diseases, has a short production period, and it has the potential to generate reasonable income in some regions, therefore it can be commercialised. However, indigenous crops presents some disadvantages, since they have unreliable yield because their growing conditions has not been scientifically tested, and have a lower consumption rate linked to rural communities who consume them when there are no alternative sources of food (Uusiku et al., 2010).

The nutritional composition of these crops has not been widely investigated, despite their usefulness to the communities. There appears to be scanty knowledge or evidence about the nutritional content, particularly when grown under different growing conditions. This knowledge could aid in influencing policy-makers in the commercialisation of these crops. Nyathi et al. (2019) reports that most of these crops have the potential to supplement several nutrients needed by the human body, in both smaller and larger quantities.

Earlier, Legwaila et al. (2011) suggested that there is a need to promote the consumption of indigenous crops, and that can be achieved by investigation of their agronomical viability and qualities such as nutritional content.

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Chapter 3: Methodology

3.1 Introduction

The chapter explains the experiments that were conducted at Florida science campus in Johannesburg of the University of South Africa (-26.157831 S, 27.903364 E) under varrying experimental conditions to investigate how some variables influence the productivity of *C. metuliferus* crop. The experiment was undertaken for two consecutive seasons/years [2017/18 and 2018/19] from seedling to harvesting phase. The roles of the certification by supplier and seed treatment processes, water scarcity, growth mediums and growing conditions were investigated, and different nutritional concentration of the crop were also measured.

3.2 Impact of water treatment, growth medium and scarification on germination and growth of the *Cucumis metuliferus* E. Mey.Ex Naudin (African horned cucumber)

This study was conducted during 2017/18 growing season in a greenhouse. The greenhouse temperature (using digital thermometer) was kept between 15 and 25 ˚C, the optimal range for cucumber seeds in general, and the relative humidity (Anden Wall Mount Digital Humidistat) was maintained between 60 and 75% using automated aerial sprinklers. Certified seeds by supplier were sourced from commercial seed company (Seed for Africa, Cape Town). The experiment consisted of four seed types grown in three growth media. The seed types were (1) seeds treated with water for 20˚C for 15 minutes and (1) certified by the supplier (TC), (2) untreated and certified seeds (UC), (3) treated but uncertified seeds (TU) and (4) untreated and uncertified seeds (UU).

The three-growth media were (1) sand + vermiculite, (2) peat and (3) potting mix. Sand + vermiculite has a good water-holding capacity, drains well and has low bulk density; all these properties aid root growth and seedling emergence. Sand + vermiculite is popular in the agricultural sector for propagating several crops. Peat holds moisture reasonably well, has good aeration and is a light material, which are good for seed germination. The potting mix is a mixture of bark, topsoil and other components used for germination before seedlings are transplanted from trays to pots. For each of the four categories of seeds, 45 seeds were sown in 30×10×4 cm cells (seedling tray) in each of the three-growth media, resulting in 540 seeds overall. The cells in the seedling tray were irrigated daily in the greenhouse for four weeks. The cells were then monitored for emergence after germination, following which the height of the seedlings was measured with a ruler for four weeks. The experiment was thus a full factorial block design with two factors, seed type and growth medium. Each of the 12 combinations of seed type and medium had 45 replicates. The proportion test was used to evaluate differences in number of seeds germinated in all categories. The two sample Kolmogorov–Smirnov tests were used to evaluate differences in seed germination date as a function of seed type and medium. A two-way ANOVA with and without interaction was used to test the effect of the combination of seed type and medium on height. A one-way ANOVA followed with Tukey's HSD post-hoc test was conducted for each of seed type and medium effects on seedling height. Tests of normality of residuals, uniformity of main effects and homogeneity of variances were carried before running the ANOVAs. Akaike (1987), Akaike's information criterion (AIC) was used to test for model fit. It calculates the most parsimonious model through minimizing the number of parameters used the variation explained. All tests and visualization were undertaken in R base (R Core Team 2019).

3.3 Water use efficiency of C. metuliferus E. Mey.Ex Naudin (African horned cucumber) under three different growing environments.

This study was conducted during [2017/18 and 2018/19] in a (greenhouse-controlled average temperature 16-28˚C, shade net-not controlled average 15-27˚C and open space environment-not controlled average 16-30˚C) at the Florida science campus of the University of South Africa (26º 10’ 30’’S, 27º 55’ 22.8’’ E). In this study, sterilised growth media (loamy soil and sandy loam) were used. In addition, certified seeds of *C. metuliferus* were purchased from Seeds for Africa, Cape Town. A factorial experiment with two factors – soil (loamy soil and sandy loam soil) and different water stress levels (no water stress, moderate water stress and severe water stress) was conducted under three different growing environments (greenhouse, shade net and open field). The pot experiment was a completely randomised design with nine (9) replicates. The pots were spaced 1 m apart, and an up-rope vertical trellising was used to support the plants. On each site, pots were either filled with loamy soil or sandy loam. Each block comprised 18 plants in pots, resulting in 54 plants per site.

A total of 162 plants were used for the experiment (Figure 3.1). Each site had plants used as guard plants. Well established, uniform and healthy *C. metuliferus* seedlings, germinated from peat substrate, that were 30 days old, were transplanted into 30 cm deep planting pots, and the treatments imposed four (4) weeks later after establishment. Plants were well irrigated (for 14 days) prior to imposition of the treatments. Data on plant growth parameters was collected during different stages.

(a)

(b)

(c)

Figure 3.1: Experimental layout of African horned cucumber grown in different locations: **(a)** greenhouse experiment; **(b)** shade net experiment; and **(c)** open field experiment.

The pots were irrigated to field capacity for four (4) weeks before the different irrigation water level treatments were imposed. Treatments were based on the amount of water to be applied per irrigation water level of each soil type as described by De Pascale et al. (2011). The three water stress levels were the following: no water stress regime (3 L), moderate water stress regime (2 L) and severe water stress regime (1 L). The soil water content was evaluated using a three-way soil meter, made in China. No water stress regime provides an indication of field water capacity, while moderate water stress regime indicates moderate stress, whereas severe water stress regime provides an indication of minimal water or semi-dry point.

Table 3.1: Soil analysis for the experiment (mineral/chemical analysis).

	Chemical analysis (micro-minerals)					
	Fe	**Mn**	**Cu**	**Zn**	**Total N%**	**pH**
	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	%	
Sandy loam	30.3	59.4	1.24	9.36	0.105	7.69
Loamy soil	33.2	59.8	1.27	8.96	0.113	7.74

	Chemical analysis (macro-minerals)				
	P	**Ca**	**Mg**	**K**	**Na**
	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
Sandy loam	35.16	1900	141	243	35.5
Loamy soil	34.4	1810	133	217	28.7

Before plant cultivation, soil samples (loamy soil and sandy loam) were analysed for mineral and/or chemical content (Table 3.1), using the method followed by Rahil and Qanadillo (2015). The above analysis was done at the Agricultural Research Council, Institute for Soil, Climate and Water (ARC-ISWC) in Pretoria (25° 44′ 19.4″ S28° 12′ 26.4″ E).

Chlorophyll

Chlorophyll content was measured at different growth stages (pre-flowering, flowering and fruiting) during the experimental period. The leaf chlorophyll content (µmol/m^2) was measured in the morning using a leaf chlorophyll meter (OPTI-SCIENCES-CCM 200 PLUS, USA).

The instrument records four (4) replicate readings of the adaxial or upper leaf surface, since chlorophyll activities are more dominant on the upper leaf surface when compared to the lower surface (Shu et al., 2013), and gives the average value.

Stomatal conductance

Stomatal conductance (mmol m^{-2} s^{-1}) was measured at different growth stages (pre-flowering, flowering and fruiting) during the experimental period. The abaxial or lower leaf surface was measured, due to the fact that stomatal opening and conductance activities are more dominant on the lower leaf surface when compared to the upper surface (Savvides et al., 2012). The porometer (Delta-T Device, AP4 Leaf Porometer, United Kingdom) was used for the measurement of stomatal conductance.

Total biomass and above-ground plant biomass

Above-ground fresh biomass (stem, leaves and fruits) was weighed at the end of the experiment using an electronic scale (Uni-Bioc, China). The plant materials that had already been counted were weighed, placed in paper bags and in an oven for 72 hours at 80˚C before re-weighing to determine dry weight. Total biomass was determined using the formula below:

Total biomass= above-ground biomass (dry) + fruit biomass (dry) **Equation 1**

Fruit number and length

Number of fruits were visually counted, and fruit lengths were measured at the end of the experiment (12 weeks) after transplanting.

Harvest index

The *C. metuliferus* harvest index was determined by adopting the formula used by El-mageed & Semida, (2015) below:

$$\textbf{HI} = \frac{\text{fruit dry biomass (dry)}}{\text{total biomass (dry)}}$$ **Equation 2**

Water use efficiency (WUE)

The formula used by Rahil & Qanadillo, (2015) was used to determine the WUE for *C. metuliferus* crop.

$$\textbf{WUE} = \frac{\text{Yield (kg)}}{\text{Total water applied (m}^3\text{)}} \qquad \text{Equation 3}$$

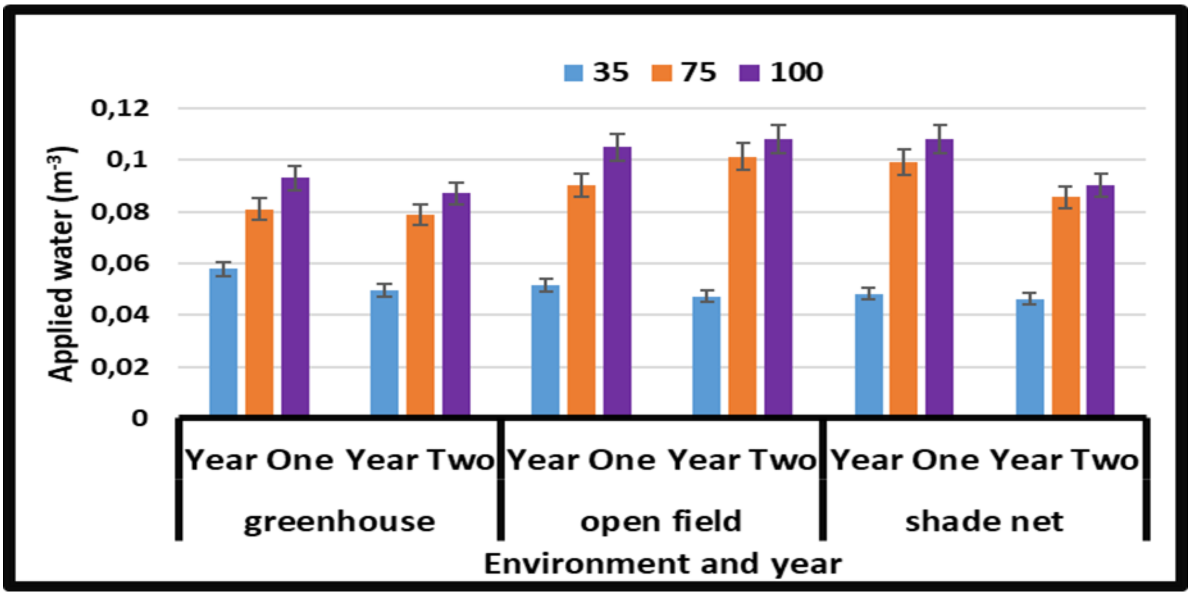

Figure 3.2: Total water applied for *C. metuliferus* grown in three different locations during two different seasons [2017/18 (year one) and 2018/19 (year two)]; 35 % means severe water stress, 75 % means moderate water stress, and 100 % means no water stress.

Figure 3.2 illustrate that total water applied was different across water stress levels. The observed trend was such that total applied water at no water stress was higher than other water stress levels (moderate water stress and severe water stress), showing that water treatments were successfully applied for both seasons (2017/18 and 2018/19). Total water applied for the greenhouse ranged from 0.05 to 0.09 m^3; for the open field, for shade net, it ranged from 0.05 to 0.11 m^3; and, for the shade net, total water applied ranged from 0.05 to 0.11 m^3.

3.4 Data analysis

Generalised linear mixed model procedures for GenStat (version 14, VSN, UK) were used for data analysis. The model was used to assess the fixed effects of (irrigation water levels, soil types, growing environment) during different seasons/years on the studied variables. In cases where there were no significant interactions between all three studied factors, significant differences for one or two factors were considered and reported under results section to determine the effects of all studied variables (water use efficiency, chlorophyll content, stomatal conductance, fruit length, above-ground biomass, total biomass = harvest and fruit dry biomass), harvest index, and overall harvested fruits. Shapiro Wilk's and Bartlett's test were used to check the normality and homogeneity of variance. All statistical analysis was done using GenStat (version 14, VSN, UK).

3.5 Nutritional concentration of *Cucumis metuliferus* E. Mey. Ex Naudin (African horned cucumber) fruit under different soil types, environments and varying water stress levels

The impact of soil, water and growing conditions on the nutrient composition of *C. metuliferus* fruit was evaluated at 12 weeks after planting during 2019. Prior to fruit analysis, optimisation analysis of fruits was carried out before the actual fruit analysis, whereby fully ripened fruit were harvested from each irrigation water level, soil type and growing conditions. The fruit colour chart developed by Nambi et al. (2015) was used to determine the ripeness of the fruit. The fruits were also analysed for crude protein and total soluble sugars. The goal for fruit optimisation analysis was to find the optimum value for one or more target variables among *C. metuliferus* fruit harvested under different treatments.

3.5.1 Determination of total soluble sugars

Cucumis metuliferus fruit harvested from greenhouse, shade net and open field irrigated with different water levels and soil types were analysed for total soluble sugars concentration (˚Brix) following the method by (Tavarini et al. 2008) . The fruit was cut into two portions, then juice was squeezed from a fruit portion by hand to release about 0.03 mL juice onto the aperture of the hand refractometer (HI 96801 Refractometer, USA) and readings were taken immediately. About 18 fruits were measured per treatment. The aperture was washed between different juice samples with distilled water and dried with a soft paper towel.

3.5.2 Determination of crude protein analysis

About 0.2 g of the freeze-dried fruit sample was weighed, duplicated and thereafter, analysed using a crude protein analyser (Trumac CN-Leco, Germany). The instrument uses the Dumas technique to quantify carbon and nitrogen percentage per 100 g. The universal protein factor 6.25 previously followed by López et al. (2013), was used to convert nitrogen to protein. Calibration of the Trumac CN analyser was done using Ethylenediaminetetra-acetic acid (EDTA). For quality control, glycine was used as a certified reference material.

3.5.3 Determination of *β-carotene*

The analysis of *β-carotene* was carried out on Prominence-in High Performance Liquid Chromatography-PDA model system equipped with sample cooler LC-2030C (Shimadzu, Japan), with slight modifications (triplicate) as described by Moyo et al. (2018). Approximately 0.1 g/mL of extracted sample with ice-cold hexane: acetone (1:1, v/v) was vortexed for two (2) minutes before being centrifuged at 2,000 rpm for two (2) minutes. The organic phase was decanted into a tube containing saturated sodium chloride solution and placed on ice cold. The remaining residue was similarly re-extracted until the extract was colourless. Each time, the extract separated organic phase was filtered through 0.45 µm syringe filtered before injection into the HPLC. Chromatographic separation was achieved using a C_{18} Luna® column (150 × 4.6 mm, 5µ) maintained at 35˚C.

An isocratic mobile phase which consisted of acetonitrile: dichloromethane: methanol (7:2:1) was used with a flow rate of 1 mL/min an injection volume of 20 µ1 and the detection was at 450 nm. Peak identification and quantification of the compound (*β*-carotene) were achieved based on authentic β-carotene standard which was used for plotting the calibration curves (Goulas & Manganaris, 2012).

3.4.5 Determination of vitamin C and E

The fruit samples were freeze-dried for 72 hours using a freeze drier (HARVEST-RIGHT, Barcelona). The freeze-dried fruit slices were rigorously homogenized using a sterilised food blender and mixed with dried powder before nutritional analysis. The method described by Moyo et al. (2018) was followed with slight modifications (triplicate). Individual samples were weighed (1g) into tube, followed by the addition of 5% metaphosphoric acid (10 ml). It was sonicated 15 minutes before centrifuging and filtration in the ice-cold water bath. The analysis was carried out on the model system described above, Prominence-i HLCP-PDA. A C18 Luna ® column (150/4.6 mm, 5 µl) held at 25 µC was used to achieve chromatographic separation. A water-based isocratic mobile phase: acetonitrile: formic acid (99:0.9:0.1) was used at a flow rate of 1 mL/min. The volume of injection was 20 µ1 and 245 nm of detection was set. Depending on the calibration curve plotted using L-ascorbic acid, sample quantification was achieved.

3.4.6 Determination of flavonoids

Cucumis metuliferus fruit samples were quantified using the aluminium chloride colorimetric method described by Baba & Malik (2018) with modification. The aluminium chloride colorimetric method was used to determine the total flavonoid content. In a nutshell, 50 mg of fruits powder (1 mg/mL ethanol) were dissolved in 1 mL methanol, combined with 4 mL distilled water, and then 0.3 mL of 5% NaNO2 solution; after 5 minutes of incubation, 0.3 mL of 10% AlCl3 solution was added, and the combination was left to stand for 6 minutes. The final volume of the combination was brought to 10 mL with double-distilled water after adding 2 mL of 1 mol/L NaOH solution. After allowing the mixture to sit for 15 minutes, the absorbance was measured at 510 nm. Catechin was used as a standard for calibration curve and total flavonoids content was expressed in mg catechin equivalents (CE) per dry weight.

3.4.7 Determination of total phenolic content

Total phenolic content determination of the fruit samples was carried out using Santos-Zea et al. (2011) and Moyo et al. (2018) with a slight modification (triplicate). Briefly, the total phenol concentration of freeze dried of *C. metuliferus* fruit was used for aw extraction of total phenolic content, which used gallic acid as a reference (Sigma, St. Louis, MO). In a 10:1 volume/volume ratio, Folin Ciocalteu reagent (2 N, Sigma, St. Louis, MO) was used to oxidize an aliquot of the extract. At room temperature, samples were incubated for 20 minutes in 96-well microplates, and absorbance was measured at 750 nm in a microplate reader (Synergy HT, Bio-Tek, Winooski, VT). Total phenolic content was expressed in mg garlic acid equivalents (GAE) per g dry weight (DW).

3.4.8 Determination of macro and micro-nutrients

Freeze dried fruit samples were digested in a diffused microwave system (MLS 1200 Mega; Milestone S.r. L, Sorisole, Italy) and samples further congelated-dried following the procedure described by Moyo et al. (2018) with minor modifications. The modifications were that samples were measured in three (3) replicates per treatment (around 15-25 mg) weighed into polytetrafluoroethylene vessels and 2 ml HNO_3 (67 %, analphur) and 1 ml H_20_2 (30 %, analytical grade) added in the vessels. Every solution was diluted to 15 ml in a deionized water test tube after digestion and analysed by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). An ICP-MS (Agilent 7,700; Agilent Technologies, Tokyo, Japan) based on quadrupole mass analyser and octapole reaction system (ORS 3), was used to conduct the analysis. Nutrient elements such Potassium **(K)**, phosphorus **(P)**, calcium **(Ca)**, magnesium **(Mg)**, sodium **(Na**), zinc (**Zn)**, iron **(Fe)**, molybdenum **(Mo)**, copper **(Cu)**, manganese **(Mn)**, and sulphur **(S)**, were analysed**.** The calibration solution was prepared by appropriate dilution of the single element certified reference material with 1.000 ± 0.002 g/L for each element (Analytika Ltd, Czech Republic) with deionised water (18.2 MΏ.cm, Direct-Q; Millipore, France). Measurement of accuracy was verified by using certified reference material of water TM-15.2 (National Water Research Institution, Ontario, Canada).

3.4.9 Statistical analysis

Data on the interaction effect of irrigation water levels, soil types and growing environment on nutritional composition of *Cucumis metuliferus* fruit on the study variables (crude protein, total soluble sugars, *Beta carotene*, vitamin C, vitamin E, total phenols, total flavonoids, macro- and micro-nutrients) were analysed using a three-way ANOVA analysis. All study variables were tested at ($P \leq 0.05$) significance level and Duncan multiple range test was used for separation between treatment means at $P \leq 0.05$ (95% confidence level) significant test. For all statistical analysis, Statistica v. 10, StatSoft (USA) was used.

3.5 Experiment 4: Metabolite profile of *C. metuliferus* E. Mey. Ex Naudin (African horned cucumber) fruit grown under differing irrigation water levels, soil type and differing environmental conditions

3.5.1 Material and methods

This study was conducted during [2017/18 and 2018/19] in a (greenhouse-controlled average temperature 16-28˚C, shade net-not controlled average 15-27˚C and open space environment-not controlled average 16-30˚C) at the Florida science campus of the University of South Africa (26º 10’ 30’’S, 27º 55’ 22.8’’ E). Soil analysis was done at the Agricultural Research Council, Institute for Soil, Climate and Water (ARC-ISWC) in Pretoria (25° 44′ 19.4″ S28° 12′ 26.4″ E). Sterilized growth media (loamy soil and sandy loam) were used. In addition, certified seeds of African horned cucumber were purchased from Seeds for Africa, Cape Town. A factorial experiment with two factors: soil (loamy soil and sandy loam soil) and irrigation water levels (3L-filled capacity-no water stress, 2L-moderate water stress and 1L-severe water stress) was determined. The pot experiment was a completely randomised design with nine (9) replicates per treatment and the factorial design as indicated above. The pots were spaced 1 m apart, and an up-rope vertical trellising was used to support the plants. On each site, plant pots were either filled with loamy soil or sandy loam. Each block comprised 18 plants in pots, resulting in 54 plants per site. A total of 162 plants were used for the experiment. Plants were well irrigated prior to imposition of the treatments. Irrigation water level (treatments) was imposed four weeks after seedlings establishment.

3.5.2 LC-MS sample preparation an metabolites analysis

Primary metabolites were extracted following the protocol of Kim and Verpoorte (2010). Briefly, 1.5 mL of MeOH (75 % MEOH/25 % water) was added on 0.5 g of freeze-dried ground fruit powder material and mixed using a vortex mixture. The mixture was sonicated for five (5) minutes using the BRANDSON 1800 (Germany). The sonicated supernatant concentrate was then filtered through 0.2-micron syringe filters with 1 mL pipette. The supernatant filtered concentrate was then centrifuged in an Eppendorf tube (Centrifuge 5424, South Africa) at 10 000 revolutions per minute (rpm). The supernatant concentrate of 72 samples was then transferred into the HLPC vials for LCMS-8040 triple quadrupole mass spectrometer (Shimadzu) for analysis. The LCMS settings were as follows: total flow=0.4 ml/min, injection volume=1uL, oven temperature=40°C/max 85°C, nebulising gas flow=3L, drying gas flow= 15 L/min, and the mobile process was 50%/50% acetonitrile/water. To compare the treatments, the quantities of metabolites were plotted in Excel. The extracts were analysed by reverse phase LC-MS for their metabolomic contents. The MS analysis were carried out in electron spray (ESI) negative mode. A method followed by Djami-Tchatchou et al. (2018) was adopted, whereby peak intensity showing LCMS-8040 triple quadrupole mass spectrometer intensities represented the quantity of metabolites varying with treatments.

3.5.3 Statistical analysis

Data gathered in this research was statistically analysed using three-way ANOVA, and the Multiple-Range Duncan Test was used to separate treatment means between two growing seasons/years/planting period at $P \leq 0.05$ (95% confidence level) significance test. For all statistical analysis, Statistica v. 10, StatSoft (USA) was used. The final statistical analysis was carried out using (Past 4) version 3 of 2013. This software employs data normalization with functions such as data manipulation, univariate and multivariant statistics.

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Chapter 4: Results and discussions

4.1 Impact of certification, growth medium and scarification on germination and growth of the *Cucumis metuliferus* E. Mey. Ex Naudin (African horned cucumber) seeds

4.1.1 The germination period of *C. metuliferus* seeds

Table 4.1: Treatment interaction of three growth media sand-vermiculite, peat and standard potting mix and four seed treatments untreated uncertified (UU), treated uncertified (TU), untreated certified (UC) and treated certified (TC) on germination in the *Cucumis metuliferus* E. Mey. ex naudin seeds. [c,a,b] designate significant differences between scarification, growth medium and seed certification at α = 0.05.

	Germination rate (%)	
Treatment	**2017/2018**	**2018/2019**
M1NTC	72.8b	78.5c
M1TC	82.8a	91.3a
M1TUC	64.83c	76.8c
M1UTU	51.7d	51.5de
M2NTC	78.3b	83.6b
M2TC	83.3a	93.6a
M2TUC	33.3fg	45.2ef
M2UTU	45.6e	52.9d
M3NTC	78.5b	46.2f
M3TC	80.2a	88.8ab
M3TUC	11.2i	44.2e
M3UTU	10.6gh	37.2f
LSD0.05	7,34	7,34
P_{value}	**0,001**	**0,001**

[c,a,b] designate significant difference of growth medium and seed type at the 5% level.

Table 4.1 presents the interaction between different growth media and seed type on the germination success rate of *C. metuliferus* seeds. Of the 540 certified and uncertified seeds sown in the three-growth media, 80% germinated, significantly more that those that failed ($\chi^2 = 372$, df=1, $p<<0.001$). During 2017/18 season, germination success rate ranged from 11.2 to 82%, whereas 2018/19 season ranged from 37.2 to 91.3%. During 2017/18 season, treatment combination of treated certified seeds (CT) and sand + vermiculite had the highest germination success rate of 82%, followed by the treatment of non-treated certified seeds (NTC) and peat with an 80.2% success rate (Table 4.1). The treatment combination of untreated uncertified seeds had the lowest germination success of 10.6%. For 2018/19 season, results depicted that there was significant ($P \leq 0.05$) difference between interaction of different growth media and seed on the germination rate of *C. metuliferus* seeds. Seeds germination rate ranged from 37.2 to 93.6%. The treatment combination of treated certified seeds (TC) and peat demonstrated high germination success rate of 93.6%, followed by the treatment combination of treated certified seeds (TC) and sand+ vermiculite with germination success rate of 91.3%. The treatment combination of uncertified untreated (UTU) seeds and potting mix illustrated low germination success rate at 37.2% (Table 4.1). Therefore, the best predictor of germination rate was seed certification from supplier combined with peat, which was superior when compared to other treatments by a highly significant margin. The least effective combination on germination success rate was uncertified untreated seeds and potting mix with 10.6% success rate during 2017/18 season. In general, the study results revealed that certified seeds scarified with warm water combined had a higher germination rate that unscarified seeds, irrespective of the growth media (Table 4.1).

Table 4.2: Designated effect of of three growth media: (1) sand+vermiculite: (2) peat; and (3) standard potting mix; and four seed treatments: (utu) untreated uncertified, (tuc) treated uncertified, (ntc) non-treated certified and (tc) treated certified on germination day of *C. metuliferus* seeds:

Treatment	**Day 0-10**	**Day 10-15**	**Day 15-25**	**Day 25-30**
sand-vermiculite[a]	0.47	0.07	0.16	0.30
peat[b]	0.14	0.39	0.23	0.25
potting mix[c]	0.14	0.43	0.43	0.00
ntc[a]	0.00	0.91	0.09	0.00
tc[a]	1.00	0.00	0.00	0.00
tuc[c]	0.00	0.00	0.62	0.36
utu[c]	0.00	0.00	0.43	0.58

[c,a,b] designate significant difference of growth medium and seed type at the 5 % level.

Table 4.2 show the germination percentage success by day of seeds.The study results showed that there was no significant ($P>0.05$) difference between the interaction of growth media and seed. However, there was significant ($P\leq0.05$) difference on the growth types. As shown in Table 4.2, the seeds grown in sand-vermiculite mostly germinated in the first 7 days (47%), 7% in day 15, 16% in day 25 and 30% in between day 25-30. In peat, 14% of seeds germinated in the first 7 days, 40 % in day 15, while day 25 and 30 had 23%, and 25% seeds germinating, respectively. In the potting mix, none of the seeds emerged in the last week, while 43% emerged in day 15 and 25, and 14% germinated in the day 7. Using the Kolmogorov-Smirnov tests for sand versus peat, the statistics were $D=0.32$ ($p \ll 0.001$), for sand versus potting mix $D=0.4$ ($p \ll 0.005$), and for peat against the potting mix $D=0.25$ ($p= 0.004$).

For seed types, the results showed that germination of treated scarified seeds (TC) was almost complete within ten days (90%), whereas others emerged between day 15 and 25. The non-scarified seeds (NTC) germinated between ten to fifteen days (Table 4.2). Scarified but uncertified seeds (TUC) all germinated after 15 days. About 60% of TUC seeds germinated from day 15 to day 25, and 40% in the last 5 days. Similarly, 42% of untreated and uncertified (UTU) seeds germinated in day 15 and 25, while 57% emerged in day 25-30 (Table 4.2). Finally, all scarified certified seeds germinated after day 7, making this category the best performing seed type. There were highly $P \leq 0.05$ significant differences in the distribution of germination by day, since sowing according to a series of two sample Kolmogorov-Smirnov tests. There were significant differences between all paired combinations of seed types, in each case D=1 ($p << 0.001$) except TUC versus UTU seeds, where D was 0.24 ($p = 0.02$).

4.1.2 Seedling height

Table 4.3: Designated effect of different growth media; and seed type on the seedling height (cm) of *C. metuliferus.*

medium/ seed type	**Week 1**	**Week 2**	**Week 3**	**Week 4**
Sand	3.28 $\pm$ 0.03	4.7 $\pm$ 0.13	6.73 $\pm$ 0.24	8.13 $\pm$ 0.33[a]
Peat	3.22 $\pm$ 0.03	4.57 $\pm$ 0.13	8.96 $\pm$ 0.04	8.62 $\pm$ 0.33[a]
Potting	3.42 $\pm$ 0.02	4.66 $\pm$ 0.13	8.3 $\pm$ 0.14	8.78 $\pm$ 0.34[a]
NTC	0	3.35 $\pm$ 0.01	7.22 $\pm$ 0.16	11.2 $\pm$ 0.05[b]
TC	3.31 $\pm$ 0.01	5.73 $\pm$ 0.03	9.07 $\pm$ 0.04	12 $\pm$ 0.03[b]
TUC	0	0	3.02 $\pm$ 0.07	3.48 $\pm$ 0.03[c]
UTU	0	3.44 $\pm$ 0.02	3.05 $\pm$ 0.05	3.33 $\pm$ 0.02[c]

[c,a,b] designate significant differences between treatments, growth medium and seed certification.

Table 4.3 presents the effect of growth media and seed treatment on the seedling height by weeks, of *C. metuliferus* E. Mey. ex naudin seedlings. Results showed that there was no significant ($P>0.05$) difference between interaction of growth media an soil types. However, there was significant ($P\leq0.05$) difference on the different growth media types on seedling height of *C. metuliferus* (Table 4.3). The results showed that the average seedling height in sand+vermiculite by the end of week 1 was 3.28 cm (Table 4.3). A peak height of 8.13 cm was reached in the fourth week. For peat, the first week had seedlings averaging 3.22 cm, while in the last week, seedling height were 8.62 (Table 4.3). In the potting mix, seedling height reached 3.42 cm in the first week and 8.7 cm in the last week (Table 4.3). There were no significant differences in seedling height in the fourth week, according to a one-way ANOVA ($F_{1,143} = 0.22$, p = 0.64).

For seed types, Table 4.3 results showed that there was significant ($P \leq 0.05$) difference. NTC seedlings were 3.35 cm, while UTU seedlings were 3.44 cm in the second week, respectively. The scarified, uncertified (TUC) seeds only germinated in the third week and had a mean height of 3.02 cm. The maximum seedling height recorded for TUC was 3.48 cm (Table 4.3). The maximum seedling height observed for scarified certified seeds (TC) in week 4 was 12 cm (Table 4.3). The TC seeds were the only ones which germinated in the first week,and had an average height of 3.31 cm. Unscarified, uncertified (UTU) seedlings reached an average height of 3.44 cm in the fourth week (Figure 4.3). A one-way was highly significant for the fourth week height by seed type ($F_{1,143} = 4.2$, $p << 0.05$). There were clearly two groups of height categories (Table 4.3): those around 3.0 cm, representing uncertified seeds, and those about 12 cm, which all germinated from certified seeds.

Table 4.4. Results of one-way ANOVAs of the mean height in the fourth week by medium[1] and seed type[2], and two-way ANOVA of height by medium and seed type with[3] and without interaction[4].

Source of variation	dF	SS	MS	F	P
Medium	2	33	16	1	0.36
Error	426	6899	16		
Total[1]	428	6932			
Seed type	3	6851	2284	12016	<<0.001
Error	425	81	0		
Total[2]	428	6932			
Medium	2	33	16	108	<<0.001
Seed type	3	6835	2278	15020	<<0.001
Error	423	64	0		
Total[3]	428	6932			
Medium	2	33	16	110	<<0.001
Seed type	3	6838	2278	166683	<<0.001
Medium: Seed type	6	7	1	9	<<0.061
Error	417	57	0		
Total[4]	428	6935			

Results in Table 4.4 showed that all growth mediums are ideal for seedling growth (height), whereas for best germination rate was any certified seeds, scarified or not. For germination, this was 100% almost always while heights were persistently over 10 cm and averaged 11.5 cm. Uncertified seeds recorded the lower germination rate and seeddling height, scarified or not.

Unlike in the certified set, there was more variation as a function of medium, for germination, the % success was between 42% (treated in sand-vermiculite) and 64% (treated in peat). In the height comparison, the span was between 1.4 cm (scarified in sand-vermiculite) and 2.2 cm (scarified in peat). Thus peat planted seedlings were better performing when the seeds were certified, and, scarification also helped. A two-way ANOVA of medium and seed types for mean height in the fourth week was not significant ($F_{2, 3, 423}$ = 108, 15020, p << 0.061 for both factors) and with no interaction ($F_{2, 3, 6, 417}$ = 120, 16683, 8, p << 0.061 for all factors) (Table 4.4). The model best explaining the variation in fourth week height was the two-way ANOVA with interaction, albeit with 13 parameters compared with seven for the model without interaction. The best model had an AIC weight of 100%. The TukeyHSD post-hoc test for the model had 48 of the 66 media: seed type combinations did not show significant difference, with 17 (Table 4.4). Fourteen of those 17 were characterised by uncertified seeds whether treated (TU) or untreated (UU) growth in all three media (Table 4.4). The final model merely emphasises that uncertified seeds yield dwarf plants. Scarifying the seeds makes no difference, and growing them in different media also makes no difference.

4.1.3 Discussion

Recently, there has been great interest in several indigenous crops of Southern Africa, specifically targeting their cosmetic, nutritional and medicinal values (Legwaila et al., 2011). However, most of these crops have not been scientifically explored to develop progressive and reliable propagation, cultivation and post-harvest practices that will promote their commercialization. The development of reliable agronomic procedures will improve the livelihoods of rural communities which rely on these crops that may one day deliver their economic emancipation.

This study investigated the effect of seed type and different growth media on the germination of *C. metuliferus*. The study findings suggested that certification as the more significant parameter for germination and optimal growth compared to growth medium or scarification. Certification ensures that seeds are disease free, thus giving the plant a greater chance to thrive (Atif et al., 2016; Marta et al., 2016). This study also demonstrated that scarification with an affordable agent, warm water, was also important in aiding plant vigour, as was reported by Piri et al. (2009) and Fang (2011)., who found that pre-treatment increased germination of English cucumbers. Others have shown that sugared water is also a good scarification agent (Benzioni et al.,1991; Markovskaya et al., 2007; Mabundza et al., 2010; Mo et al., 2013). This study found warm water to be just as effective, and an affordable alternative.

Based on the data gathered, this study has show that seed certification is much more important than scarification or substrate type, for seeds to germinate successfully, rapidly and grow effectively. Certified seeds by supplier, regardless of growth medium, all germinated. This makes sense, because certified seeds are clean and free from fungal disease and other storage-related challenges (Gvozdenac et al., 2011). By contrast, uncertified seeds had much lower rates of germination, which varied according to growth medium. When sown in the potting mix, uncertified seeds had peak germination 11%, but when scarified. The least germinating uncertified seeds were in sand-vermiculite and were, paradoxically, unscarified. This suggests that scarification does not have a meaningful impact on germination success.

Similarly for height, uncertified seeds sown in all growth media reached a maximum mean height of 1.9 cm in week 4. For certified seeds, the mean height, regardless of growth medium, was 11.4 cm. Hence, both for height and for germination success, the most essential condition for best growth was seed certification.

4.1.4 Conclusion

The best performing combination of seed type and medium were scarified and certified seeds grown in sand and vermiculite and peat. The second best performing combination were scarified and certified seeds growing in potting mix. Based on the interaction with farmers and other users, the optimum conditions for successful propagation of *C. metuliferus* seeds are unknown. This study demonstrated that a combination of water soaked and certified seeds grown in either sand mixed with vermiculite, peat or potting mix are suitable germination practices for *C. metuliferus* seeds. The medium has a secondary role in germination success, the primary determinant is whether the seeds are certified and treated prior to sowing. Therefore, growers are encouraged to source certified seeds despite pricing and soak them in warm water to promote germination. Scarification of seeds in warm water is more practical, affordable and reliable treatment. Since the seedling-rootball integrity is vital for eventual transplant survival, this study suggests peat as the best medium because peaty soils have the most stable seedling root-ball when compared to other substrates.

4.2 Water use efficiency of *C. metuliferus* E. Mey. Ex Naudin (African horned cucumber) under different water levels, soil types and growing environments

4.2.1 Plant growth parameters

4.2.1.1 Chlorophyll content

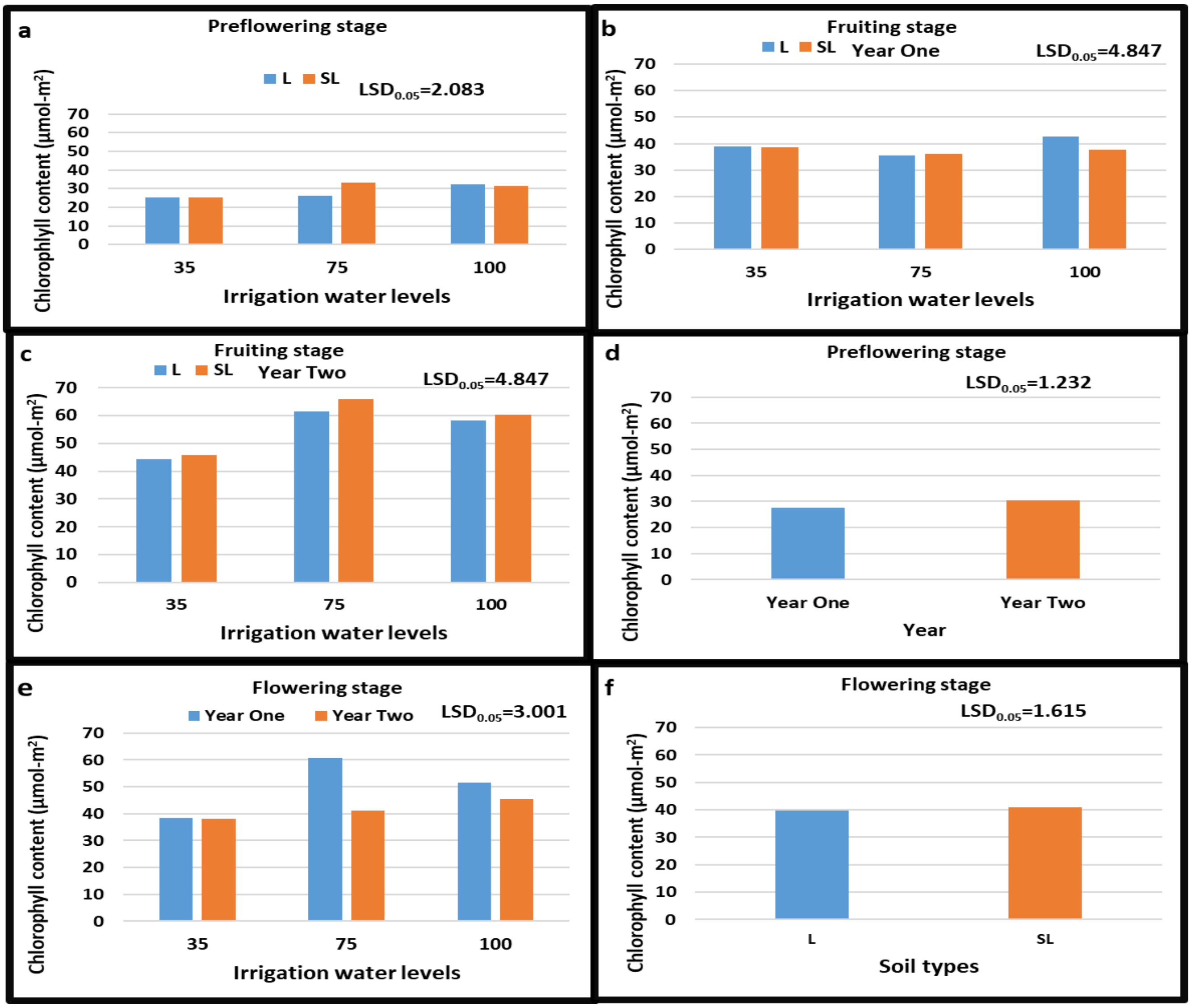

Figure 4.1: The effect of irrigation water levels and soil types on the chlorophyll content of *C. metuliferus* grown in the **greenhouse environment** at different growth stages; **(a)** means the interaction effect of irrigation water levels and different soil types (L = loamy soil and SL = sandy loam soil); **(b and c)** means the interaction effect of irrigation water levels; **(d)** means seasons; **(e)** irrigation water levels in different seasons [2017/18 (year one) and 2018/19 (year two)]; **(f)** means different soil types: soil types in different seasons [2017/18 (year one) and 2018/19 (year two)]; 35 % means severe water stress, 75 % means moderate water stress, and 100 % means no water stress. $LSD_{0.05}$ is the least significant difference of means.

Figure 4.1 presents the chlorophyll content of *C. metuliferus* grown under a greenhouse at different growth stages (pre-flowering stage, flowering stage and fruiting stage). The results delineated that there was a significant ($P \leq 0.05$) interaction between different water stress levels and soil types at different seasons (Figure 4.1 a, b and c) and different water stress levels at difference seasons (Figure 4.1e). The observed trend illustrates that chlorophyll content was higher during fruiting stage. During the 2017/18 season, it ranged from 36 to 43 µmol.m^{-2}, whereas during the 2018/19 season, it ranged from 44 to 66 µmol.m-^{2} when compared to pre-flowering, whereby it ranged from 25 to 33 µmol.m-^{2} during 2017/18, whereas during the 2018/19 season, it ranged from 27 to 31 µmol.m-^{2}. Figure 4.1a reveal that treatment of moderate water stress combined with sandy loam soil increased chlorophyll content from 25 to 33 µmol.m^{-2}, whereas treatment of severe water stress combined with both soils (loamy soil and sandy loam) decreased chlorophyll content from 33 to 25 µmol.m-^{2}.

During fruiting stage (Figure 4.1c) chlorophyll content from treatment of moderate water stress combined with sandy loam soil (2018/19 season) indicated a significant increase in chlorophyll content from 45 to 66 µmol.m-^{2}, whereas the treatment of severe water stress combined with both soil types during the 2017/18 season decreased chlorophyll content from 66 to 45 µmol-m-^{2} (Figure 4.1b).

In terms of water stress levels, results in Figure 4.1e illustrated that during the 2017/18 season, treatment of moderate water stress increased chlorophyll content from 38 to 60 µmol.m-^{2}, whereas treatment of severe water stress during both seasons [2017/18 and 2018/19] decreased chlorophyll content from 60 to 38 µmol.m-^{2}. It is worthwhile to note that soil types did not have a significant difference in the chlorophyll content of the crop (Figure 4.1f).

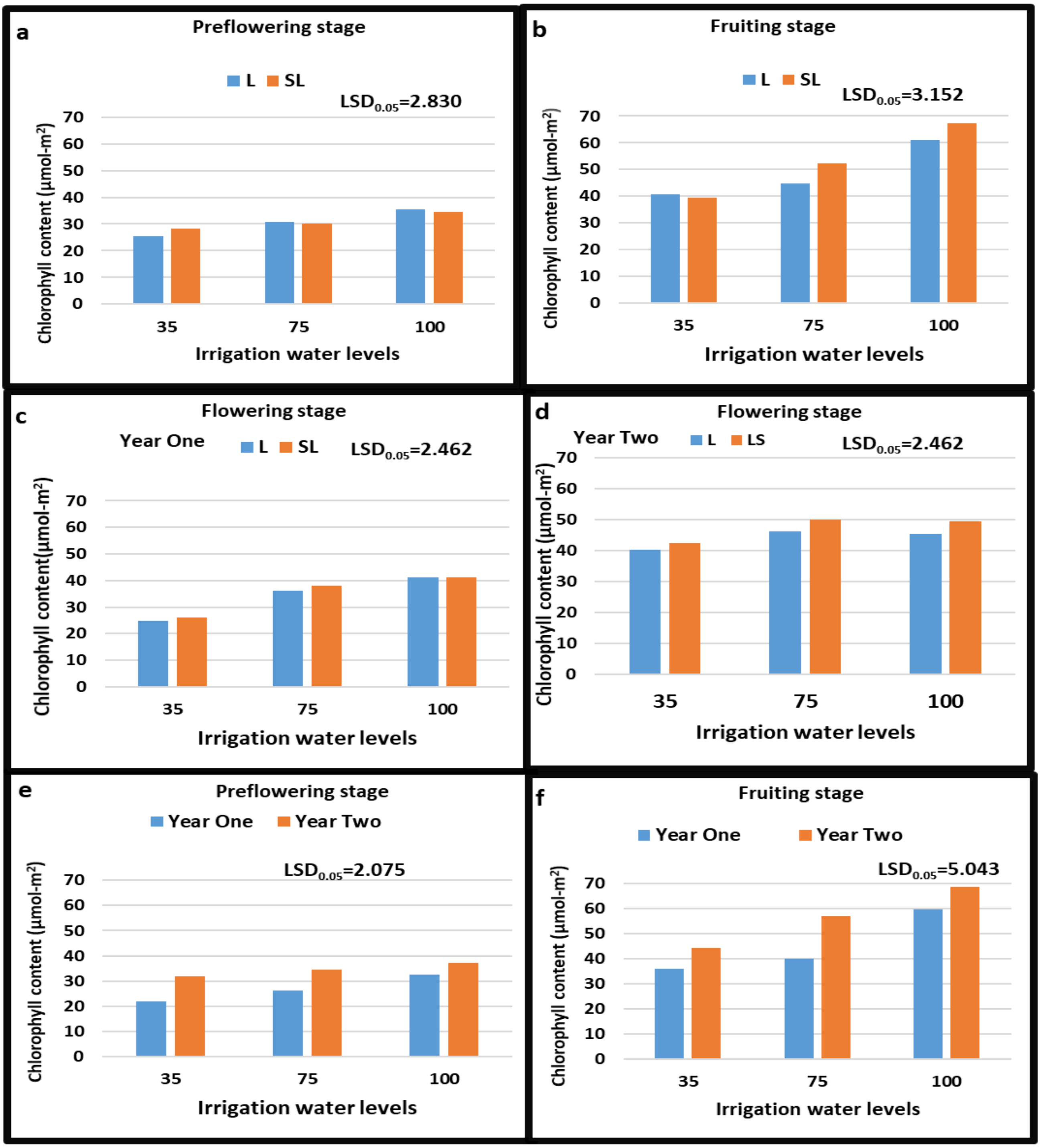

Figure 4.2: The effect of irrigation water levels and soil types on the chlorophyll content of *C. metuliferus* grown in **shade net environment** at different growth stages; **a, b, c and d** means the interaction effect of irrigation water regimes and soil types (L = loamy soil and SL = sandy loam during the 2017/18 (a and c) and 2018/19 seasons (**b** and **d**); **e and f** means the interaction effect of irrigation water regimes and seasons [2017/18 (year one) and 2018/19 (year two)]; 35 % means severe water stress, 75 % means moderate water stress and 100 % means no water stress. $LSD_{0.05}$ is the least significant difference of means.

Figure 4.2 presents the chlorophyll content of African horned cucumber grown under the shade net at different growth stages (pre-flowering stage, flowering stage and fruiting stage). The results outlined that there was significant ($P \leq 0.05$) interaction between different water stress levels and soil types (Figures a, b, c and d), and water stress levels and seasons (Figures 4.2e and f). In addition, the results revealed that chlorophyll content increased during fruiting stage when compared to other stages (pre-flowering and flowering stage). For example, chlorophyll content during pre-flowering stage ranged from 22 to 37 µmol-m^{2}, whereas during fruiting stage chlorophyll content ranged from 36 to 69 µmol-m^{2}. During flowering stage, results in Figure 4.2c and d demonstrated that the treatment of severe water stress combined with loamy soil decreased chlorophyll content from 50 to 25 µmol.m-^{2}; whereas fruiting stage presented a contradictory trend, whereby treatment of no water stress irrigation combined with sandy loam significantly increased chlorophyll content from 25 to 67 µmol.m-^{2} (Figure 4.2b).

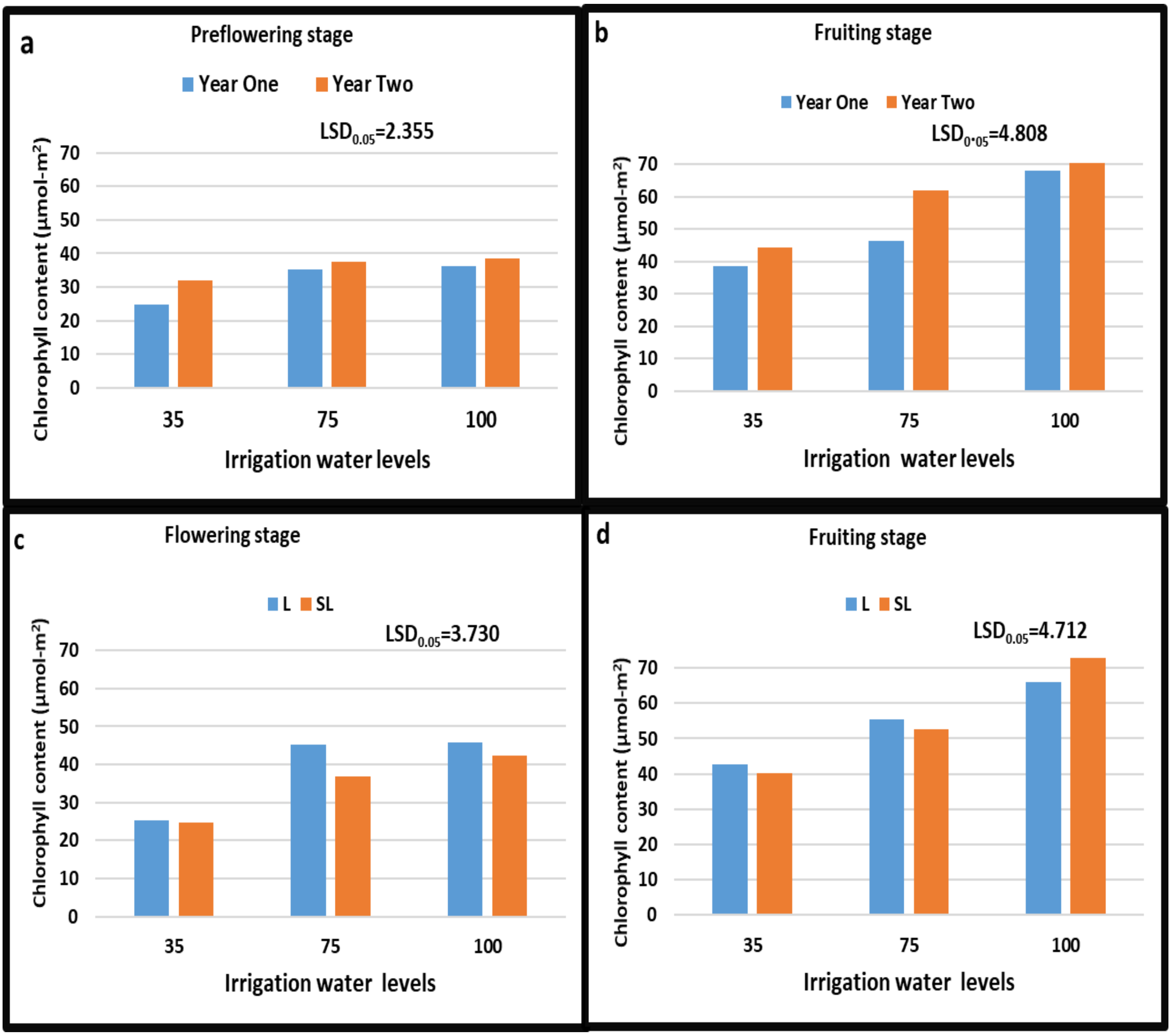

Figure 4.3: The effect of irrigation water levels and soil types on the chlorophyll content of *C. metuliferus* grown in **open space environment** at different growth stages; **a and b** means the interaction effect of irrigation water levels and seasons [2017/18 and 2018/19]; **c and d** means the treatment interaction effect of irrigation water levels and soil types (L = loamy soil and SL = sandy loam soil); 35 % means severe water stress, 75 % means moderate water stress and 100 % means no water stress. $LSD_{0.05}$ is the least significant difference of means.

Figure 4.3 presents the chlorophyll content of *C. metuliferus* grown in an open space environment at different stages (pre-flowering stage, flowering stage and fruiting stage). The results demonstrated there was a significant ($P \leq 0.05$) interaction between water stress levels and seasons (Figures 4.3 a and b) and water stress levels and soil types (Figures 4.3 c and d). The results in Figure 4.3b showed that the treatment of no water stress during the 2018/19 season at fruiting stage indicated the highest chlorophyll content (70 µmol-m^2) compared to other treatments, whereas the severe water stress treatment during the 2017/18 season at pre-flowering stage significantly decreased chlorophyll content from 39 to 25 µmol.m-^{2}. In terms of stress water levels and soil types, during fruiting stage, the results showed that the treatment of no water stress combined with sandy loam soil resulted in higher chlorophyll content (72 µmol.m-^{2}) compared to other treatments. Generally, severe water stress levels combined with both soil types during all seasons decreased chlorophyll content (Figure 4.3 a, b, c and d).

4.2.1.2 Stomatal conductance

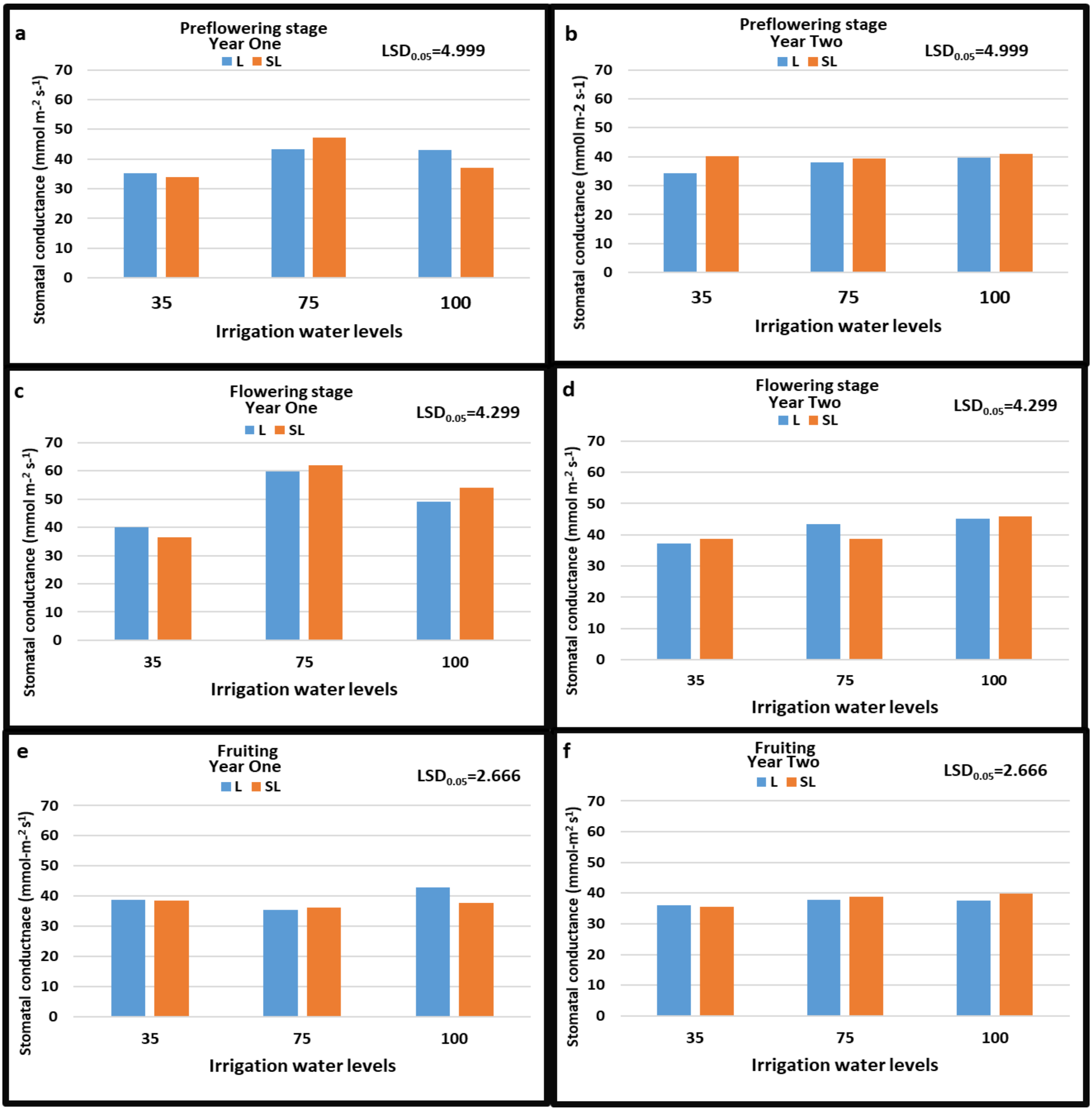

Figure 4.4: The effect of irrigation water levels and soil types on the stomatal conductance of *C. metuliferus* grown in **greenhouse environment** at different growth stages; the interaction of irrigation water levels and soil types (LS = loamy soil an SL = sandy loam) during different seasons (2017/18 and 2018/19). Figures **a, c and e** represent season one, and figures **b, d and f** represents season two)]; 35 % means severe water stress, 75 % means moderate water stress and 100 % means no water stress. $LSD_{0.05}$ is the least significant difference of means.

Figure 4.4 presents stomatal conductance of *C. metuliferus* grown under a greenhouse environment at different growth stages (pre-flowering stage, flowering stage and fruiting stage). The results showed a significant ($P \leq 0.05$) interaction between different water stress levels and soil types during different growth stages. During the 2017/18 season, stomatal conductance ranged from 34 to 47 $mmol.m^{-2}$ for pre-flowering stage, 36 to 62 $mmol.m^{-2}$ for flowering stage and 36 to 42 $mmol.m^{-2}$ for fruiting stage (figures a, c and e). During the 2018/19 season, stomatal conductance ranged from 34 to 41 $mmol.m^{-2}$ for pre-flowering stage, 37 to 46 $mmol.m^{-2}$ for flowering stage and 36 to 40 $mmol.m^{-2}$ for fruiting stage. During pre-flowering stage, treatment of severe water stress combined with loamy soil decreased stomatal conductance from 47 $mmol.m^{-2}$ to 34 $mmol.m^{-2}$ during 2018/19 season (Figure 4.4b), whereas treatment of moderate water stress combined with sandy loam soil during 2017/18 season increased stomatal conductance from 34 to 47 $mmol.m^{-2}$ (Figure 4.4a).

The treatment of severe water stress combined with sandy loam at flowering stage decreased stomatal conductance from 62 to 36 $mmol.m^{-2}$ during the 2017/18 season, whereas treatment of moderate water stress combined with sandy loam presented a contradictory trend, whereby stomatal conductance increased from 36 to 62 $mmol.m^{-2}$ (Figure 4.4c). During fruiting stage, severe water stress treatment combined with sandy loam soil decreased stomatal conductance from 43 to 35 $mmol.m^{-2}$ (Figure 4.4f), whereas treatment of no water stress combined with loamy soil increased stomatal conductance from 35 to 43 $mmol.m^{-2}$ during the 2018/19 season (Figure 4.4e).

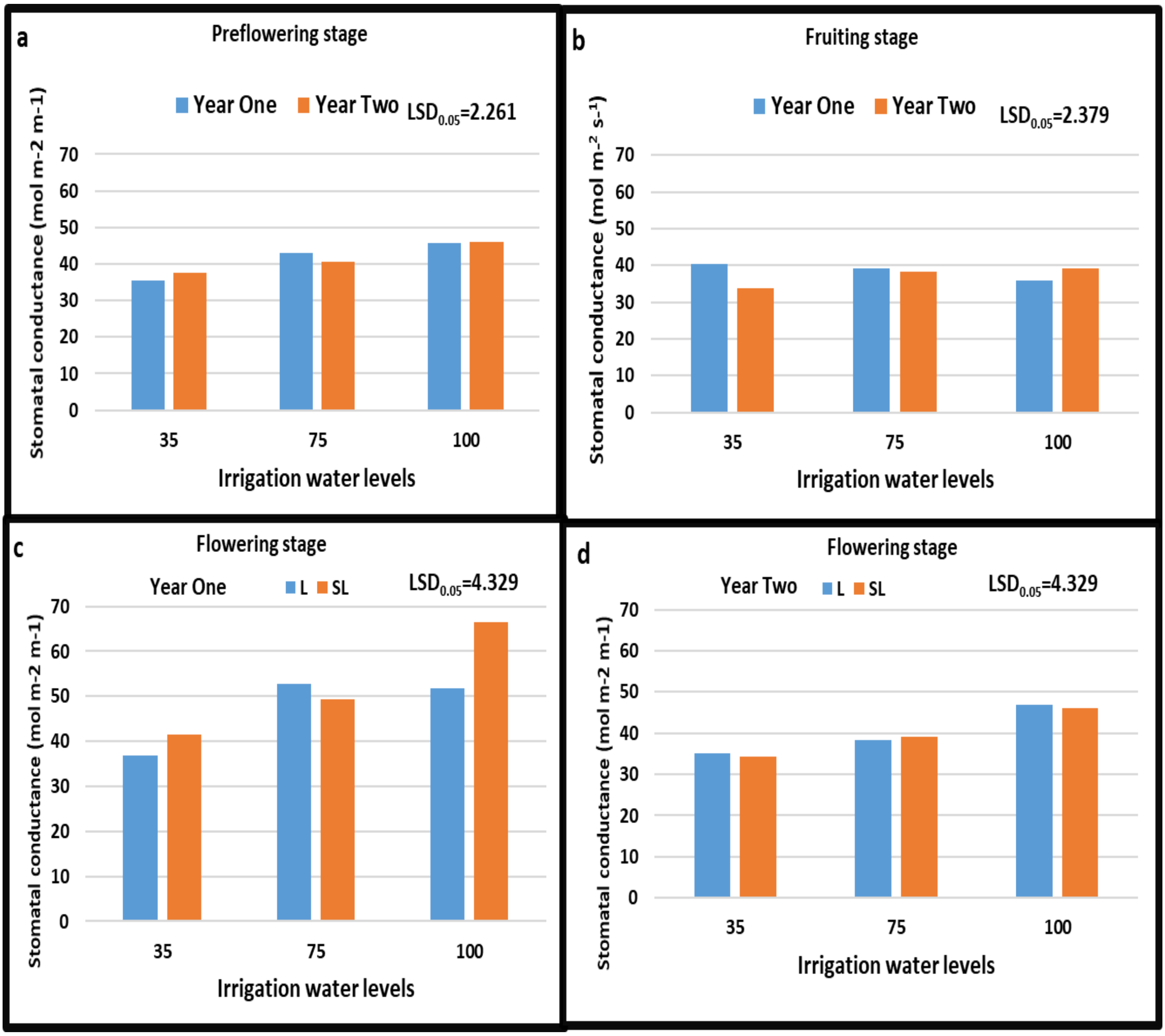

Figure 4.5: The effect of irrigation water levels and soil types on the chlorophyll content of *C. metuliferus* grown in **shade net environment** at different growth stages (pre-flowering stage, flowering stage and fruiting stage); **a and b** means the interaction effect of irrigation water regimes in different seasons [2017/18 (season 1) and 2018/19 (season 2)]; **c and d** means the interaction effect of irrigation water levels and soil types (L = loamy soil and SL = sandy loam in different seasons (year one 2017/18 and year two 2018/19); 35 % means severe water stress, 75 % means moderate water stress and 100 % means no water stress. $LSD_{0.05}$ is the least significant difference of means.

Figure 4.5 presents the stomatal conductance of *C. metuliferus* grown under a shade net environment at different growth stages (pre-flowering stage, flowering stage and fruiting stage). There was significant (P≤0.05) interaction of different water stress levels in different seasons (figures a and b) and soil types (Figure 4.5 c and d). During the 2017/18 season, stomatal conductance ranged from 36 to 46 mmol.m-^{2} for pre-flowering stage 37 to 66 mmol.m^{-2}. For fruiting stage, it ranged from 3 mmol.m-^{2} 6 to 40 mmol.m-^{2}. During the 2018/19 season, stomatal conductance ranged from 37 to 46 mmol-m^{-2} for pre-flowering stage, 34 to 47 mmol.m^{-2} for flowering stage, and 34 to 39 mmol.m^{-2} for fruiting stage.

In terms of different water stress, the results showed that during fruiting stage the severe water stress treatment decreased stomatal conductance from 46 to 33 mmol.m-^{2} (2018/19 season), whereas the treatment of no water stress during pre-flowering stage increased stomatal conductance from 33 46 mmol.m-^{2} in both seasons (Figure 4.5a). It is worthwhile to note that severe water stress combined with sandy loam soil during the 2018/19 season decreased stomatal conductance from 66 to 34 mmol.m-^{2} (Figure 4.5d), whereas the 2018/19 season results in Figure 4.5c presented a contradictory trend, whereby stomatal conductance increased (34 to 66 mmol.m-^{2}) from no water stress treatment combined with sandy soil.

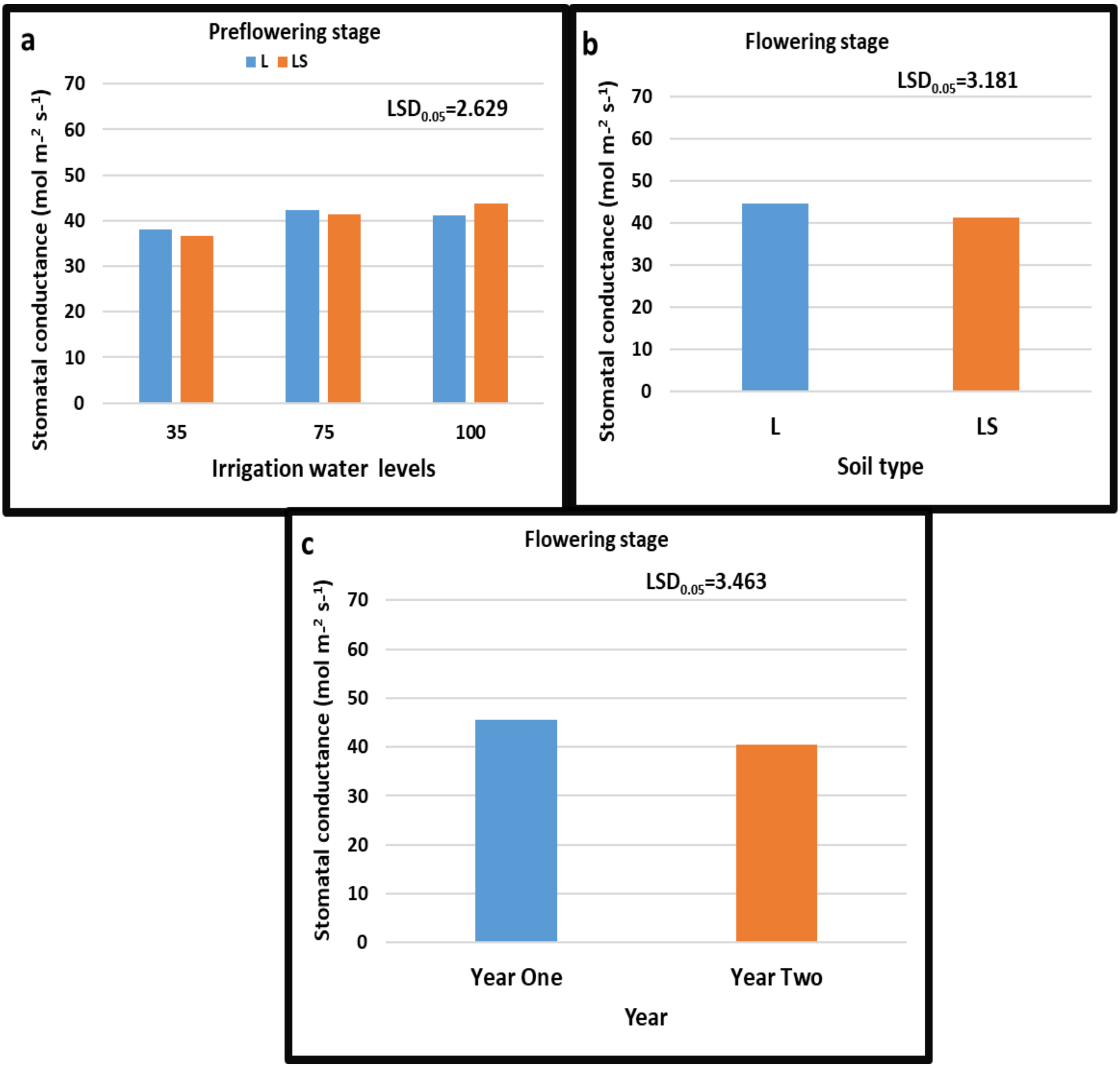

Figure 4.6: The effect of irrigation and soil types on the stomatal conductance of *C. metuliferus* grown in **open space environment** at different growth stages (pre-flowering stage, flowering stage and fruiting stage); **a** means the interaction effect of irrigation water regimes and soil types (L = loam soil and SL = sandy loam soil); **b** means the effect of soil types and **c** means the seasonal effect (year one 2017/18 and year two 2018/19); 35 % means severe water stress, 75 % means moderate water stress and 100 % means no water stress. $LSD_{0.05}$ is the least significant difference of means.

Figure 4.6 presents the stomatal conductance of *C. metuliferus* grown in an open space environment at different growth stages (pre-flowering stage and flowering stage). The results demonstrated that there was significant ($P \leq 0.05$) interaction between irrigation water levels and soil types (Figure 4.6a), soil types (4.6b) and seasons (4.6c). During pre-flowering stage, severe water stress treatment combined with sandy loam soil decreased stomatal conductance from 44 to 37 mmol.m-^{2}, whereas treatment of no water stress combined with sandy loam soil during presented contradictory results, whereby there was an increase in stomatal conductance from 37 to 44 mmol.m^{-2} was observed (Figure 4.6a). It is worthwhile to note that loamy soil stomatal conductance 45 mmol-m^{-2} was higher than sandy loam stomatal conductance 41 mmol.m^{-2} (Figure 4.6b). In addition, results in Figure 4.6c revealed that the 2017/18 season stomatal conductance at 45 mmol.m^{-2} was higher than the 2018/19 season stomatal conductance 41 mmol.m^{-2}.

4.2.1.3 Fruit length and number

Table 4.4 Treatment effect of different water levels, soil types and environment on the fruit number and length of *C. metuliferus* during different seasons.

	Total Fruit number		Fruits length	
Treatment	**2017/18**	**2018/19**	**2017/18**	**2018/19**
W1S1L1	6(2)	8(3)	78(9.6)	110(24.1)
W1S1L2	5(3)	13(2)	59(22.4)	99(11.8))
W1S1L3	20(4)	22(6)	208(18.3)	102(66.4)
W1S2L1	10(1)	11(4)	177(40.2)	175(29.8)
W1S2L2	11(2)	24(2)	128(14.2)	134(18.5)
W1S2L3	25(3)	39(9)	249(30.4)	144(15.4)
W2S1L1	7(3)	11(4)	94(71.2)	113(12.6)
W2S1L2	5(4)	13(2)	49(45.1)	99(51.4)
W2S2L3	16(10)	24(7)	154(121.3)	111(42.2)
W2S2L1	13(3)	22(3)	200(18.7)	202(35.2)
W2S2L2	10(5)	24(4)	120(9.3)	140(29.5)
W2S2L3	22(6)	41(8)	181(68.1)	149(16.5)
W3S1L1	5(3)	8(3)	58(61.2)	96(26.6)
W3S1L2	5(4)	9(4)	55(18.2)	67(48.3)
W3S1L3	15(7)	26(9)	136(21.2)	109(44.8)
W3S2L1	7(3)	16(3)	87(45.7)	131(23.2)
W3S2L2	12(2)	20(4)	127(27.5)	112(48.5)
W3S2L3	18(6)	40(8)	200(28.9)	129(26.4)
LSD0.05	3.5	3.5	30.55	30.55
P_{value}	0.39	0.39	0.955	0.955

W1 means no water stress; **W2** means moderate water stress; **W3** means severe water stress. **S1** means loamy soil and **S2** means sandy loam. **L1** means greenhouse. **L2** means shade net. **L3** mean open field. Numbers in brackets represent the standard deviations of the mean. $LSD_{0.05}$ is the least significant difference of means. Years (seasons one – 2017/18 and season two – 2018/2019). P values in bold are lower than 0.05. $LSD_{0.05}$ is the least significant difference of means.

Fruit number

Table 4.4 presents the treatment interaction effect on the fruit number of *C. metuliferus*. The results showed that there was no significant (P>0.05) interaction in fruit number between different water stress levels, soil types and growing environments. During 2017/18 season, fruit number ranged from 5 to 20, whereas 2018/19 season fruit number ranged from 8 to 41. In addition, results showed that no water stress treatment and loamy soil under greenhouse environment reduced fruit number from 41 to 5, whereas treatment of moderate water stress and sandy loam under open field environment increased it from 5 to 41.

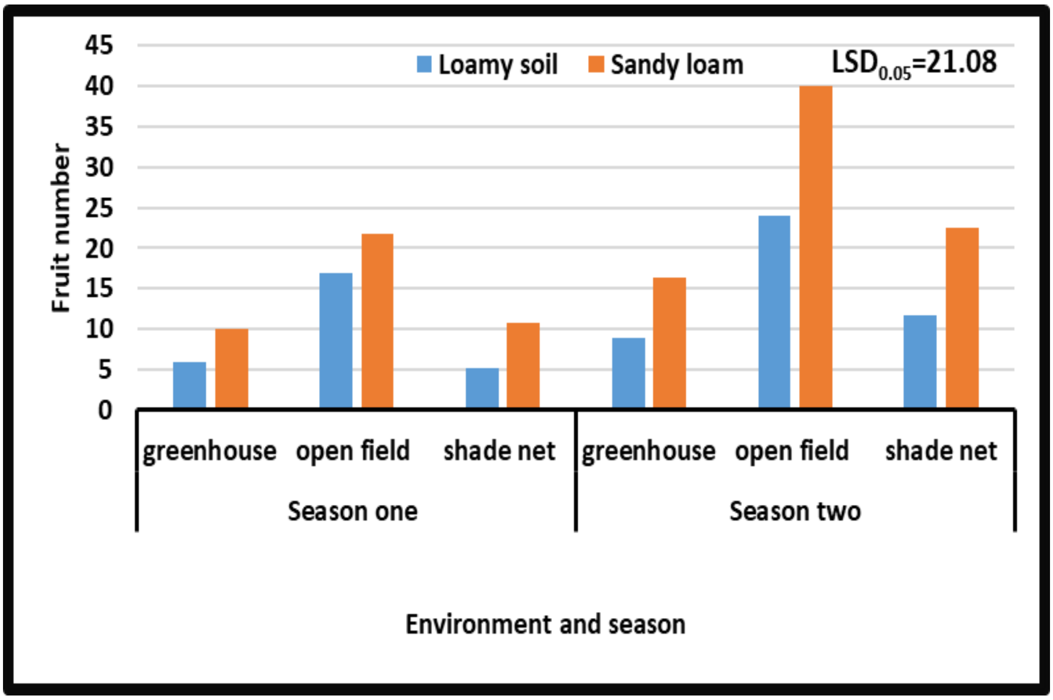

Figure 4.7: The effect of different soil types and environment on the fruit number *C. metuliferus* during different seasons. Seasons [2017.18 season one; 2018/19 season two]. LSD0.05 is the least significant difference of means.

Figure 4.7 results presents the interaction effect between growing environments and soil types on the fruit number of *C. metuliferus*. Results outlined that there was significant (P≤0.05) interaction between growing environment and soil types during different seasons. Treatment combination of open field environment and sandy loam during 2018/19 season illustrated higher fruit number (40), whereas the lowest fruit (6), was observed under treatment combination of severe water stress and greenhouse during 2017/18 season (Figure 4.7).

Fruit length

Table 4.4 presents the treatment effect on the fruit length of *C. metuliferus*. The results delineated that there was no significant (P<0.05) interaction on the fruit length of *C. metuliferus* between irrigation water levels, soil types and growing environment during different seasons. During 2017/18 season, fruit length ranged from 55 to 249 com, whereas 2018/19 season ranged from 96 to 202 cm. Moreover, results unveiled that severe water stress treatment combined with loamy soil under shade net during 2017/18 season indicated decreased in fruit length from 249 to 55 cm, while treatment combination of no water stress and sandy loam under open field environment indicated an increase from 55 to 249 during 2017/18 season.

4.2.2 Total biomass, aboveground biomass, harvest index and water use efficiency

Table 4.5: Treatment effect on the total biomass, above-ground biomass, harvest index, and water use efficiency of *C. metuliferus*.

	Total biomass		**AGB**		**Harvest index**		**WUE**	
Treatment	**2017/18**	**2018/19**	**2017/18**	**2018/19**	**2017/18**	**2018/19**	**2017/18**	**2018/19**
W1S1L1	0.25(0.0)	0.20(0.1)	0.18(0.1)	0.10(0.1)	0.27(0.1)	0.51(0.1)	2.6(0.4)	2.3(0.2)
W1S1L2	0.18(0.1)	0.17(0.0)	0.10(0.1)	0.09(0.1)	0.20(0.1)	0.51(0.1)	2.3(0.7)	1.9(1.2)
W1S1L3	0.29(0.0)	0.23(0.0)	0.12(0.2)	0.14(0.2)	0.62(0.0)	0.46(0.1)	5.1(0.2)	2.2(0.8)
W1S2L1	0.25(0.1)	0.30(0.1)	0.14(0.1)	0.12(0.2)	0.45(0.0)	0.52(0.0)	2.7(0.1)	3.5(0.2)
W1S2L2	0.23(0.1)	0.20(0.1)	0.16(0.2)	0.08(0.1)	0.29(0.1)	0.60(0.1)	2.8(0.1)	2.2(0.7)
W1S2L3	0.36(0.0)	0.33(0.1)	0.13(0.1)	0.15(0.0)	0.61(0.1)	0.57(0.1)	6.2(0.8)	3.1(0.4)
W2S1L1	0.20(0.1)	0.22(0.0)	0.13(0.1)	0.12(0.1)	0.32(0.1)	0.46(0.2)	1.8(0.5)	2.8(0.1)
W2S1L2	0.16(0.1)	0.16(0.1)	0.13(0.1)	0.07(0.1)	0.21(0.2)	0.58(0.1)	1.7(0.8)	1.9(0.2)
W2S2L3	0.24(0.0)	0.20(0.1)	0.08(0.1)	0.09(0.1)	0.67(0.0)	0.53(0.1)	4.7(0.3)	2.0(0.1)
W2S2L1	0.29(0.1)	0.27(0.1)	0.18(0.1)	0.11(0.0)	0.37(0.2)	0.60(0.1)	2.6(0.8)	3.4(0.5)
W2S2L2	0.18(0.0)	0.20(0.1)	0.12(0.1)	0.06(0.1)	0.34(0.1)	0.68(0.2)	1.9(0.6)	2.3(0.4)
W2S2L3	0.30(0.0)	0.27(0.1)	0.08(0.0)	0.10(0.1)	0.72(0.2)	0.60(0.1)	6.0(1.3)	2.7(0.6)
W3S1L1	0.15(0.0)	0.14(0.2)	0.12(0.1)	0.07(0.1)	0.23(0.1)	0.49(0.1)	1.4(0.4)	2.8(0.4)
W3S1L2	0.14(0.1)	0.13(0.1)	0.10(0.1)	0.07(0.2)	0.25(0.1)	0.43(0.2)	1.5(0.3)	2.7(0.6)
W3S1L3	0.27(0.0)	0.17(0.1)	0.12(0.2)	0.08(0.1)	0.58(0.1)	0.56(0.1)	5.4(0.5)	3.6(0.5)
W3S2L1	0.17(0.1)	0.18(0.1)	0.13(0.1)	0.06(0.0)	0.22(0.1)	0.52(0.1)	1.6(0.2)	3.7(0.1)
W3S2L2	0.19(0.0)	0.18(0.1)	0.12(0.1)	0.08(0.1)	0.39(0.2)	0.60(0.1)	2.1(0.1)	3.9(0.4)
W3S2L3	0.29(0.3)	0.21(0.2)	0.08(0.2)	0.10(0.0)	0.68(0.2)	0.57(0.2)	5.0(0.1)	4.5(1.2)
LSD0.05	0.06251	0.06251	0.04307	0.04307	0.09642	0.09642	0.903	0.903
P_{value}	0.380	0.38	**0.049**	**0.049**	**0.003**	**0.003**	0.466	0.466

W1 means no water stress; **W2** means moderate water stress; **W3** means severe water stress. **S1** means loamy soil and **S2** means sandy loam. **L1** means greenhouse. **L2** means shade net. **L3** mean open field. Numbers in brackets represent the standard deviations of the mean. $LSD_{0.05}$ is the least significant difference of means. Years (seasons one – 2017/18 and season two – 2018/2019). P values in bold are lower than 0.05. WUE means water use efficiency. $LSD_{0.05}$ is the least significant difference of means.

Total biomass

Table 4.5 presents the treatment effect on the total biomass, above-ground biomass, harvest index and water use efficiency of *C. metuliferus*. Results showed that there was no significant (P>0.05) different for the total biomass of *C. metuliferus* between interaction of different irrigation water levels, soil types and growing environments (Table 4.5). However, results in Figure 4.10 evinced that there was significant (P≤0.05) difference between interaction of (irrigation water levels and year) and (growing environment and irrigation water levels). During 2017/18 season, total biomass ranged from 0.14 to 0.30 kg, whereas 2018/19 season ranged from 0.13 to 0.33 kg (Table 4.5). in addition, the study revealed that severe water stress treatment combined with loamy soil and shade net environment decreased total biomass from 0.33 kg to 0.13 kg, while treatment of no water stress combined with sandy loam and open field environment increased it from 0.13 kg to 0.33 kg. It is worth to note that open field environment and no water stress recorded the highest total biomass when compared to other treatments (Figure 4.10b).

Aboveground biomass

Regarding aboveground biomass, the study results outlined that there was significant (P≤0.05) interaction between treatment combination of different irrigation water levels, soil types and growing environments (Table 4.5). The observed trend revealed that season one 2017/18 aboveground biomass was higher than season two 2018/19 aboveground biomass. During 2017/18 season, above ground biomass ranged from 0.08 to 0.18 kg, whereas 2018/19 season ranged from 0.06 to 0.15 kg. In addition, results illustrated that treatment of moderate water stress level combined with sandy loam and shade net decreased aboveground biomass from 018 to 0.06 kg, whereas no water stress treatment combined with loamy soil and greenhouse increased it from 0.06 to 0.18 kg (Table 4.5).

Harvest index

Table 4.5 presents the treatment interaction of different irrigation water levels, soil types and growing environment of *C. metuliferus*. The study results showed that there was significant (P≤0.05) interaction in harvest index between combination of different irrigation water levels, soil types and growing environment. During 2017/18 season, harvest index ranged from 0.20 to 0.72, whereas 2018/19 season ranged from 0.43 to 0.68. Moreover, results depicted that no water stress combined with loamy soil and shade net reduced harvested index from 0.72 to 0.20, whereas combination of moderate water stress and open field indicated increase in harvested from 0.20 to 0.72 (Table 4.5).

4.2.3 Water use efficiency (WUE)

The study results in Table 4.5 and present the treatment interaction effect of different irrigation water levels, soil types and growing environment on the WUE of *C. metuliferus*. The study results delineated that there was no significant (P>0.05) difference between interaction of different irrigation water levels, soil types and growing environment on the WUE of *C. metuliferus*. The study results showed that WUE ranged from 1.4 to 6.2 kg m^{-3}, during 2017/18 season, whereas 2018/19 season ranged from 1.9 to 4.5 kg m^{-3}. Furthermore, results illustrated that treatment of moderate water stress combined with loamy soil and shade net decreased WUE from 6.2 to 1.4 kg m^{-3}, whereas treatment combination of no water stress combined with sandy loam and open field environment demonstrated increase in WUE from 1.4 to 6.2 kg m^{-3} (Table 4.5).

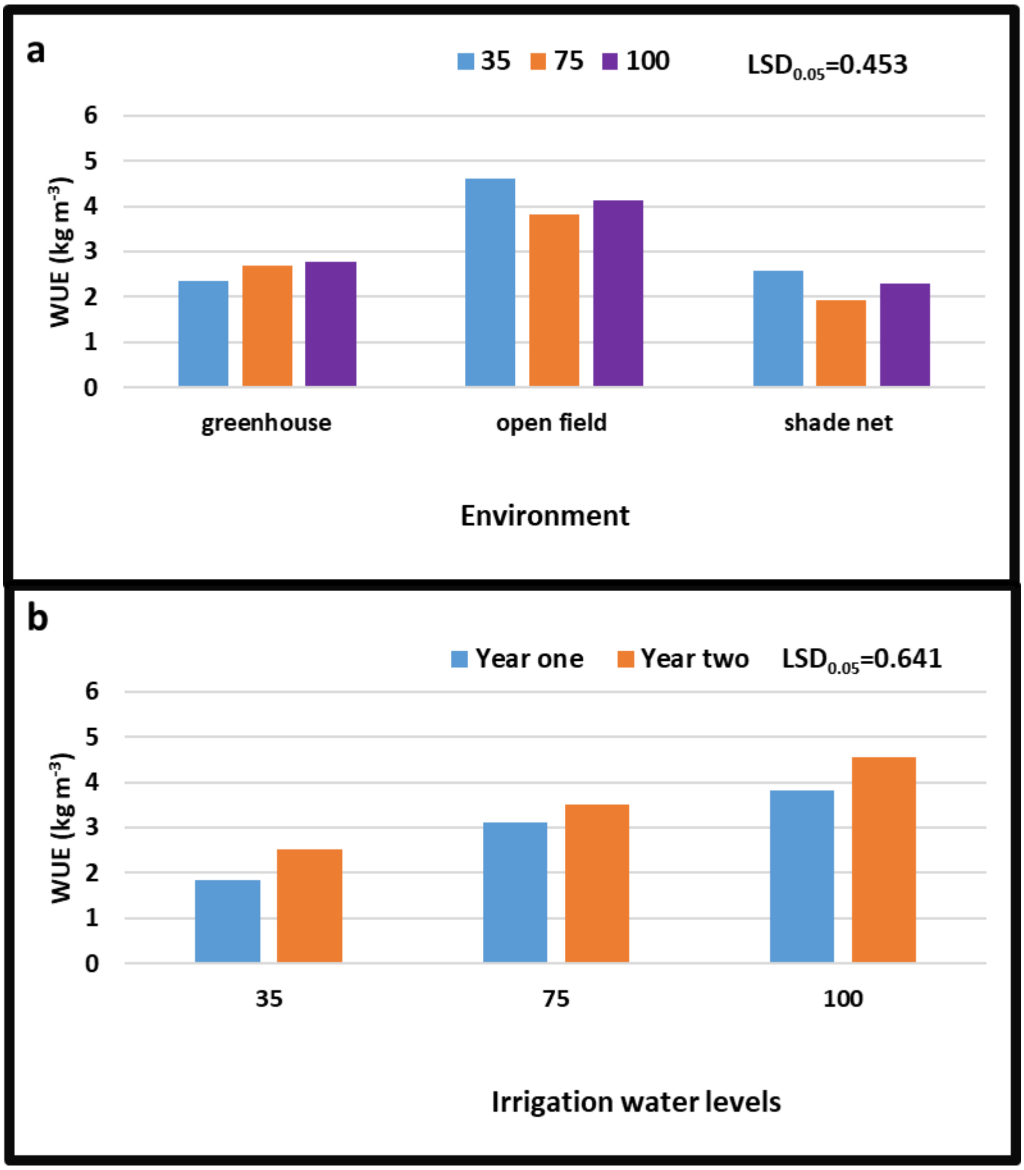

Figure 4.8: Designated water use efficiency of *C. metuliferus*. **(a)** means treatment interaction of irrigation water levels and environment. **(b)** means treatment interaction of irrigation water levels and season, year one [2017/18] and year two [2018/2019. (c) means treatment interaction of environment and seasons [year one 2017/18, and year two 2018/19].

Figure 4.8 present designated significant results of WUE of *C. metuliferus*. Results in (Figure 4.10) showed that there was significant (P≤0.05) difference between the interaction of irrigation water levels and growing environments (Figure 4.10a); and irrigation water levels and seasons (Figure 4.8b). The results evinced that combination of all irrigation water levels (no water stress, moderate water stress and severe water stress) and open field environment illustrated higher WUE when compared to other treatments (Figure 4.8a). For interaction between irrigation water levels and season, no water stress treatment during 2018/19 season demonstrated higher WUE when compared to the other treatments (Figure 4.8b).

4.2.4 Discussion

This study assessed water use efficiency of *C. metuliferus*, under three different environments (greenhouse, shade net and open space) and varying soil types (loamy soil and sandy loam). Previous studies evaluated water use and/ or productivity of various crops such as (English cucumber, sweet potatoes, leafy vegetables, sorghum, onions, wheat and maize). To the best of our knowledge, this is the first study to assess water use efficiency of *C. metuliferus*, therefore the findings of this study serve as a benchmark.

Total biomass

The difference in mean between the highest (0.33) and lowest (0.13 kg) treatments was 0.20 kg for total biomass. The means showed that productivity was higher in the open field environment (0.33 kg) under normal watering, whereas the lowest productivity was observed under shade net environment (0.13 kg) under severe water stress treatment. These observations suggest that treatments imposed on the *C. metuliferus.* Perhaps, total radiation intercepted by the plant in the open space environment played a crucial role demonstrating higher total biomass compared to shade net and the greenhouse. Interestingly, the mean results for both seasons also revealed that increase was more on the sandy loam soil, relatively to loamy soil treatments. These differences, though statistically, are not very substantial considering that Eifediyi & Remison (2010) and Ramadan (2014) found differences in the productivity of wheat due to varying soil types and deficit irrigation when the crop was subjected to water stress. Similar findings in terms of reduction in total biomass were observed from severe water stress treatment, relatively to normal watering on cucumbers and squash (Yu et al., 2003; Rahil & Qanadillo, 2015; El-Mageed & Semida, 2015;Suil et al., 2017; Tefera, 2017). The findings also revealed that the no water stress to moderate water stress treatment will exhibit higher biomass compare to the severe water stress. When plants are water stressed, their stomatal conductance is reduced, subsequently affect the photosynthesis process since carbon dioxide and light absorption by leaves is reduced due to stomatal closure as reported by (Ikkonen et al., 2012; Savvides et al.,2012; Abu-Zinada, 2015; Tawari & Tripathy, 2019).

The relationship between osmotic pressure, chloroplast and stomata is believed to be involved in the leaf stomatal conductance activities , hence there is a decrease when there is water stress, and an increase under normal watering (Hashem, 2011; Abdelraouf et al., 2014; Sui et al., 2017).

Harvest index

The harvest index varied from 0.20 in loamy soil and no water stress treatment under shade net to 72 in sandy loam soil and no water stress under open field environment. This means that soil type and growing environment have a great potential to increase productivity of *C. metuliferus*. The current study findings demonstrated reduction in harvest index was more on loamy soil in all growing environment, relatively to sandy loam treatment. Indistinguishable findings were reported on cucumbers, tomatoes and leafy vegetables by (Jasso-Chaverria et al., 2005; Davis et al., 2008; Banjaw et al., 2017; Nyathi et al.,2018). The superior harvest index on sandy loam treatments could be attributed to the higher macro-nutrient concentration (P, Ca, Mg, and K), when compared to the loamy soil (Figure 4.1). When the soil is rich in phosphorus, there is better water and nutrients movement within the plant; subsequently increase carbohydrates in plant tissue, better production of ATP, which regulate the rate of photosynthesis, whereas magnesium rich soil plays an important role in chlorophyll production during photosynthesis Alomran & Luki (2012). These results agree with studies conducted, which demonstrated that soil rich in major nutrients (N, P, and Mg) results in better plant growth, subsequently increase yield compare to soil with poor nutrients content as reported by (Yang, Wang, Wei, Hikosaka & Goto, 2010); He, Yu, Li, Du & Guo, 2018). The study showed that soil type, irrigation water levels and growing environment was the main contributor to the variation in harvest index of *C. metuliferus.*

Fruit number

The study findings revealed that there was no significant interaction between treatments on the fruit number of *C. metuliferus*. However, interaction of growing environments and soil types during different season revealed significant variation. Plants grown in sandy loam under open field environment exhibited higher fruit number, relative to those that were grown in the loamy soil regardless of the location. The fact that fruit number were higher in the open field, whose average values was about (41), relatively to the shade net (5), could mean that the soil type and growing environment play an important role in fruit productivity. Researcher such as and Kader, (2005) and Nerson (2009), reported comprehensively on the impact of pollination on fruit production of plants grown in the open field versus those that of protected structures such as shade net and greenhouse. They noted rapid increase in fruit number on open field grown crops when compared to those grown in protected structures. Ibarra-Jiménez et al. (2008) and Sezen et al. (2010) found that when the soil has good drainage, water holding capacity and nutrients, plant fruit number increases, but reduce under substrate with poor nutrients, and drainage. The fact that there was increase in fruit number under sandy loam grown plants could be associated with its higher nutrients content and good drainage compared to loamy soil substrate. In addition, the direct access to plant flowers by pollinating agents such as wind and bees as reported by Nerson (2009) could have been the secondary cause for increase in fruit number for open field space crops, when compared to those grown in protected structures. The current study affirms that pollination agents and soil conditions are major contributors to fruit number of *C. metuliferus* crop.

Fruit length

There was no significant interaction between treatments on the fruit length of *C. metuliferus*. The study findings show that open field environment bear longer fruit size (249 cm), whereas the shortest fruit size was observed under shade net environment with a mean fruit length of (55 cm). The difference between the mean length of the fruit were substantial at (194cm). The study findings also revealed that no water stress and sandy loam were the main contributor in increasing the fruit size, while severe water stress and loamy soil decreased it.

The fact that cucumber fruit comprises of more than 60 percent water content could be the reason for lower fruit length under water stress treatment compare no water stress. This variation could be of immense importance in the fresh market industry were fruit size play a pivotal role in sales. Similar findings were reported on bananas by (Maitlo, 2014; Mazahrih et al., 2015), who found that fruit length reduced under water stress treatment, but increased under normal watering. The present study affirms that that irrigation water levels, soil and growing environment are the major contributor on fruit length of *C. metuliferus* crop, subsequently affect yield.

Water use efficiency **(WUE)**

The goal of water use efficiency and/ or water productivity is to improve yield with less water supply (‘‘more crop per drop’’); however, these terms are used interchangeably (Kusangaya et al., 2013; World Bank Group, 2018; Nyathi et al., 2019). Van Halsema and Linden (2012) argued that these terms are not similar; the difference being the denominator. Water use efficiency uses total water applied as a denominator, whereas water productivity utilises crop evapotranspiration as a denominator. This study assessed water use efficiency of *C. metuliferus*.

Although, the interaction effect on WUE were not statistically significant, the mean were average low mean value on treatment combination of severe water stress and loamy soil under shade net environment (01.4 kg m^{-3}), whereas no water stress and sandy loam under open field environment exhibited higher WUE with mean value of (6.2 kg m^{-3}). The differences between the lowest mean value and higher mean value for WUE were once more substantial at (4.8 kg m^{-3}). In addition, the study findings revealed a significant interaction between growing environment, irrigation water levels and seasons. Once more, all irrigation water levels under open field environment demonstrated higher WUE when compared to the treatments. For interaction of irrigation water levels and season, the grand mean revealed that season tow WUE was superior that season. The highest WUE was noted under no water stress level during season two. Although stomatal opening and closing are the major factors affecting the rate of water use by plants, the radiation interception by plant in the open space environment could have been the main contributor for higher yield, subsequently increasing the WUE of open space crops compare to those of protected environment. Similar findings were observed on WUE from varying soil types on carrots, cucumbers, snap bean, wheat and watermelons by (Ramadan, 2014; Rahil & Qanadillo, 2015; El-Mageed & Semida, 2015; Banjaw, Megersa & Lemma 2017). When irrigation is applied to soil, water is then utilised by plants through capillary absorption by plant roots. Perhaps, WUE increased under sandy loam soil could be the fact that sandy loam soil good water holding and drainage ability had a direct impact compared to loamy soil as reported by Ramadan et al., (2014) and Rahil and Qanadillo (2015), who conclude that the water holding capacity status of a soil type has a direct impact on the crop's total WUE.

This attribute can be ascribed to the fact that soil needs to hold water and make it available to plant roots for a specific period, in order to enable the osmosis process to take place. However, other researchers such as El-Mageed & Semida (2015); Penfield & MacGregor, (2017); Abu-zinada, (2015) and Schapendonk, (2017), suggest that evapotranspiration is one of the major contributing factors for the overall crop's WUE. The study findings showed that growing environment and irrigation water levels were the major contributor for WUE of *C. metuliferus* compared to other factors.

4.2.5 Conclusion

Studies conducted by Shu et al. (2013) and Penfield & MacGregor, (2017) suggest that soil types and water stress have the potential to reduce or increase overall yield and WUE of crops. It was expected that plants grown in protected structures will have higher WUE since there is protection from the possible influence of meteorological parameters such as wind, rainwater and hail. However, open space environment presented unexpected data trends which exhibition higher WUE when compared to data from crops grown in protected structures. *Cucumis metuliferus* crop that was grown in the open field yielded increased number of fruits, which is important for fresh markets, where many fruits are required to meet the demand.

The study resolved that the best growing condition that enhance WUE of *C. metulifrus* crop was a combination of open space field environment with sandy loam soil under moderate water stress. These results are in harmony with those observed by Ramadan, 2014; Rahil & Qanadillo, 2015; El-Mageed & Semida, 2015; Banjaw et al. 2017, who found higher WUE from varying growing environments and soil types on carrots, cucumbers, snap bean, wheat and watermelons cultivated in the open field environment. The findings of this study close the information gap regarding the scanty knowledge on the agronomic or yield responses of this crop plant to various growth factors such as the substrate and/or suitable water stress levels, for optimum productivity. It can therefore be deduced from this study that for increased WUE of *C. metuliferus*, open field environment be considered, but also other growing environment (greenhouse and shade net) and the substrate sandy loam provide suitable combination – i.e. for optimum returns.

4.3 Nutritional concentration of *Cucumis metuliferus* E. Mey. Ex Naudin (African horned cucumber) fruit under different soil types, environments and varying water stress levels

4.3.1 Total soluble sugars and crude proteins

Table 4.6: Interaction effect of treatments on total soluble sugars and crude protein content of *C. metuliferus* fruit.

Treatment	Total soluble sugars (˚Brix)		Crude protein (%)	
Year	**2017/2018**	**2018/2019**	**2017/18**	**2018/19**
W1S1L1	8.0(3.1))	7.7(1.0)	6.2(0.0)	6.2(0.4)
W1S1L2	15.1(1.3)	13.2(1.2)	6.3(1.1	6.2(0.2)
W1S1L3	11.3(1.1)	12.6(2.1)	6.3(0.2)	6.2(1.1)
W1S2L1	8.1(0.4)	8.4(2.1)	6.2(1.1)	6.2(1.1)
W1S2L2	14.7(1.3)	13.8(1.3)	6.3(0.2)	6.2(0.1)
W1S2L3	11.0(1.1)	12.8(2.1)	6.3(2.1)	6.2(0.1)
W2S1L1	12.3(2.1)	11.3(1.1)	6.2(1.1)	6.2(1.3)
W2S1L2	14.7(11.2)	13.8(1.2)	6.3(1.1)	6.3(1.2)
W2S1L3	13.4(11.1)	13.7(2.2)	6.3(1.2)	6.3(1.1)
W2S2L1	13.0(1.0)	11.8(1.3)	6.2(1.2)	6.2(1.2)
W2S2L2	14.9(2.1)	13.5(2.4)	6.3(1.0)	6.3(1.0)
W2S2L3	13.1(1.1)	14.1(1.1)	6.3(1.2)	6.2(1.0)
W3S1L1	14.2(2.1)	12.6(1.0)	6.3(1.1)	6.3(1.0)
W3S1L2	15.8(1.1)	14.5(1.1)	6.3(1.2)	6.2(1.1)
W3S1L3	14.3(2.2)	15.2(2.4)	6.3(1.4)	6.2(0.2)
W3S2L1	14.7(1.1)	12.5(1.1)	6.2(1.1)	6.2(2.1)
W3S2L2	15.4(1.3)	14.6(1.3)	6.3(1.1)	6.3(0.4)
W3S2L3	14.6(1.1)	14.3(2.1)	6.3(1.3)	6.3(1.3)
LSD0,05	1.173	1.173	0.0	0.0
P_{value}	0.541	0.541	0.393	0.393

W1 means no water stress; **W2** means moderate water stress; **W3** means severe water stress. **S1** means loamy soil and **S2** means sandy loam. **L1** means greenhouse. **L2** means shade net. **L3** mean open field. Numbers in brackets represent the standard deviations of the mean. $LSD_{0.05}$ is the least significant difference of means. P values in bold are lower than 0.05. $LSD_{0.05}$ is the least significant difference of means. **Note that only season two results are presented, due to logistical costs, as analysis could not be done for season one treatments.**

Table 4.6 presents the treatment interaction effect on total soluble sugars content of *C. metuliferus* fruit grown at different environments (greenhouse, shade net and open field), soil types (loamy soil and sandy loam) and water stress levels (no water stress, moderate water stress and severe water stress). The results showed that there was no significant (P>0.05) interaction between location, different water stress levels and soil types on total soluble sugars content of *C. metuliferus* during both growing seasons. However, fruit total soluble sugars ranged from 8.0 to 15.8 ˚Brix. The observed trend showed that no water stress level treatment increased fruit total soluble sugars content when compared to other water stress levels during both seasons. In addition, the results illustrated that no water stress treatment combined with loamy soil under greenhouse conditions decreased total soluble sugars from 15.8 to 8 ˚Brix, whereas the treatment of severe water stress combined with loamy soil under shade net conditions increased it from 8 to 15.8 ˚Brix.

Table 4.6 presents the crude protein content of *C. metuliferus* fruit grown at different environments (greenhouse, shade net and open field), soil types (loamy soil and sandy loam) and different water stress levels (no water stress, moderate water stress and severe water stress). The results indicated that there was no significant (P>0.05) difference between interaction of different environments, water stress levels and soil types. However, the results illustrated that fruit crude protein ranged from 6.22 to 6.29%. In addition, the results of the study demonstrated two extremes: the treatment of no water stress and severe water stress combined with both soil types (loamy soil and sandy loam) at growing conditions (greenhouse and shade net) during both seasons decreased crude protein content from 6.29 to 6.22%. However, the treatment of severe water stress combined with loamy soil at shade net conditions increased crude protein content 6.22 to 6.29%.

4.3.2 ***β-carotene*, vitamin C, vitamin E, total flavonoids and total phenols**

Table 4.7: Treatment interaction effect on the *β-carotene,* Vitamin C, Vitamin E, total flavonoids and total phenols of *C. metuliferus* fruit

Treatment	**Beta carotene** (mg 100 g^{-1} DW)	**Vitamin C** (100 g^{-1} DW)	**Vitamin E** (100 g^{-1} DW)	**Total flavonoids** (CE g^{-1} DW)	**Total phenols** (GAE g^{-1} DW)
W1S1L1	1,60(0.0)	26,56(0.2)	11,74(1.1)	0.66 3(0.0)	3.13(0.1)
W1S1L2	1,52(0.0)	33,09(0.2)	18,05(04)	0.75(0.1)	4.16(0.2)
W1S1L3	1,59(0.0)	17,01(0.5)	10,65(16.4)	0.73(0.0)	6.14(0.1)
W1S2L1	1,52(0.0)	23,81(0.4)	9,33(3.7)	0.26(0.1)	4.39(0.1)
W1S2L2	1,52(0.0)	31,71(0.7)	10,00(0.5)	0.63 (0.1)	4.30(0.0)
W1S2L3	1,57(0.1)	18,66(0.4)	13,35(2.7)	0.42(0.0)	5.43(0.1)
W2S1L1	1,60(0.2)	24,28(0.5)	29,81(13.4)	0.56 (0.0)	5.19(0.1)
W2S1L2	1,58(0.1)	30,19(1.9)	16,92(5.5)	0.84(0.1)	5.26(0.1)
W2S1L3	1,50(0.5)	18,98(0.0)	11,81(5.9)	0.47(0.0)	4.12(0.0)
W2S2L1	1,58(0.1)	30,26(16.8)	31,67(3.4)	0.55(0.3)	5.75(0.1)
W2S2L2	1,55(0.2)	22,61(0.6)	12,51(3.0)	0.77 (1.1)	4.44(0.1)
W2S2L3	1,54(1.1)	27,45(0.6)	13,52(3.2)	0.41(0.2)	5.12(0.1)
W3S1L1	1,65(0.1)	23,54(0.9)	24,43(0.5)	0.25(0.0)	4.51(0.1)
W3S1L2	1,53(0.1)	28,16(0.0)	11,31(0.9)	0.54(0.1)	3.56(0.1)
W3S1L3	1,45(0.1)	16,57(0.1)	8,28(3.0)	0.85(0.1)	4.84(0.1)
W3S2L1	1,52(0.0)	23,15(0.1)	35,12(1.1)	0.21(0.0)	4.24(0.2)
W3S2L2	1,52(0.2)	27,20(0.9)	14,31(0.4)	0.49(0.3)	3.48(0.0)
W3S2L3	1,51(1.2)	15,51(0.7)	9,74(0.3)	0.65(0.0)	3.14(0.2)
LSD0,05	0,0	0,083	9,68	0.05075	0.2065
P_{value}	**0,001**	0,083	0.632	**0,001**	**0,001**

W1 means no water stress; **W2** means moderate water stress; **W3** means severe water stress. **S1** means loamy soil and **S2** means sandy loam. **L1** means greenhouse. **L2** means shade net. **L3** mean open field. Numbers in brackets represent the standard deviations of the mean. $LSD_{0.05}$ is the least significant difference of means. P values in bold are lower than 0.05. $LSD_{0.05}$ is the least significant difference of means. **Note that only season two results are presented, due to logistical costs, as analysis could not be done for season one treatments.**

Table 4.7 presents the treatment effect on *β-carotene* content of *C. metuliferus* fruit grown at different environments (greenhouse, shade net and open field), soil types (loamy soil and sandy loam) and water stress levels (no water stress, moderate water stress and severe water stress). The results showed that there was a significant ($P \leq 0.05$) difference in fruit *β-carotene* content between interaction of water stress levels and soil types at different growing locations. However, *β-carotene* content ranged from 1.45 to 1.65 mg 100 g^{-1} DW. The results illustrated that the treatment of severe water stress combined with loamy soil at open field environment decreased *β-carotene* content from 1.65 to 1.45 mg 100 g^{-1} DW, whereas treatment of severe water stress combined with loamy soil at greenhouse environment increased from 1.45 1.65 mg 100 g^{-1} DW.

Table 4.7 presents the treatment effect on vitamin C content of *C. metuliferus* fruit grown at different environments (greenhouse, shade net and open field), soil types (loamy soil and sandy loam) and water stress levels (no water stress, moderate water stress and severe water stress). The results showed that there was a significant ($P \leq 0.05$) difference in *C. metuliferus* fruit vitamin C content between interaction of water stress levels and soil types at different environments. Furthermore, results outlined that vitamin C content ranged from 15.51 to 33.09 100 g^{-1} DW. The results illustrated that treatment of severe water stress combined with sandy loam soil under open field environment decreased vitamin C content from 33.09 to 15.51 100 g^{-1} DW, whereas treatment of no water stress combined with loamy soil under shade net environment increased it from 15.51 to 33.09 100 g^{-1} DW.

Table 4.7 presents the treatment effect on Vitamin E content of *C. metuliferus* E. fruit grown at different environments (greenhouse, shade net and open field), soil types (loamy soil and sandy loam) and water stress levels (no water stress, moderate water stress and severe water stress). The results outlined that there was a significant ($P \leq 0.05$) difference in vitamin E content of *C. metuliferus* fruit that ranged from 8.28 to 35.12 100 g^{-1} DW. Also, results revealed that the treatment of severe water stress combined with loamy soil under open field conditions decreased vitamin E content from 35.12 to 8.28 100 g^{-1} DW (Figure 4.13a), whereas severe water stress treatment combined with sandy loam soil under greenhouse conditions increased vitamin E from 8.28 to 35.12 100 g^{-1} DW.

Table 4.7 presents the treatment interaction effect on the total flavonoid content of *C. metuliferus* fruit grown at different environments (greenhouse, shade net and open field), soil types (loamy soil and sandy loam) and different water stress levels (no water stress, moderate water stress and severe water stress). The results showed that there was a significant (P≤0.05) difference between different water stress levels and soil types in the total flavonoid content of fruit grown at different environments. The results showed that fruit total flavonoids ranged from 0.21 to 0.85 mg CE g^{-1} DW. The study results suggest that the severe water stress treatment combined with sandy loam soil under greenhouse environment decreased total flavonoids at 0.85 0.21 mg CE g^{-1} DW, whereas severe water stress combined with loamy soil under open field conditions increased them from 0.21 to 0.85 mg CE g^{-1} DW.

Table 4.7 presents the treatment interaction effect on the total phenolic content of *C. metuliferus* fruit grown at different environments (greenhouse, shade net and open field), soil types (loamy soil and sandy loam) and water stress levels (no water stress, moderate water stress and severe water stress). The results showed that there was a significant (P≤0.05) difference between different water stress levels and soil types in the total phenols content of fruit grown at different environments The results revealed that fruit total phenolic content ranged from 3.13 to 6.41 mg CE g^{-1} DW. The results indicated that the treatment of no water stress levels combined with loamy soil under greenhouse decreased fruit total phenolic content from 6.41 to 3.13 mg CE g^{-1} DW (Figure 4.15a), whereas no water stress treatment combined with loamy soil under open field conditions increased it from 3.13 to 6.41 mg CE g^{-1} DW.

4.3.3 Macro-nutrients

Table 4.8: Treatment interaction effect of different water levels, soil types and environment on macro-nutrients (µg g^{-1} DW) of *C. metuliferus* fruit.

Treatment	Calcium	Magnesium	Phosphorus	Potassium	Sodium	Sulfur
W1S1L1	227(67.6)	309 (3.1)	503 (7.6)	1599 (29.5)	18 (0.7)	138 (3.5)
W1S1L2	213 (16.8)	325 (4.6)	549 (4.2)	1601 (25.3)	44 (5.9)	154 (6.6)
W1S1L3	380(291.1)	279 (11.0)	469 (8.1)	1815 (576.3)	39 (5.6)	137 (7.8)
W1S2L1	363 (24.3)	332 (3.1)	590 (24.3)	1657 (30.6)	21 (0.3)	150 (3.4)
W1S2L2	248 (59.0)	313 (23.9)	524 (42.6)	1631 (62.0)	22 (0.1)	145 (11.3)
W1S2L3	171(10.9)	340 (31.4)	577 (61.7)	1864 (205.6)	32 (4.4)	165 (14.7)
W2S1L1	288 (64.4)	315 (7.0)	546 (21.2)	1705 (25.2)	21 (1.9)	157(1.0)
W2S1L2	211(20.1)	326 (3.5)	531(13)	1678 (19.3)	19 (0.2)	148 (3.2)
W2S1L3	246 (21.4)	308 (21.1)	511(47.4)	1606 (147)	33 (1.2)	153 (16.3)
W2S2L1	280 (55.3)	315 (11.4)	533 (27.3)	1660 (76.0)	20 (0.6)	146 (3.2)
W2S2L2	327(13.3)	321 (1.2)	521 (9.0)	1693 (40.6)	20 (1.7)	145 (6.9)
W2S2L3	208 (46.5)	330 (27.8)	557 (47.5)	1801 (173.7)	36 (5.7)	161(8.4)
W3S1L1	217 (26.5)	311 (6.1)	564 (20.9)	1645 (27.3)	24 (2.0)	143 (2.1)
W3S1L2	207(12.4)	314 (5.3)	523 (16.2)	1665 (40.6)	20 (1.4)	138 (4.9)
W3S1L3	240 (79.4)	315 (3.1)	537(6.1)	1661(49.4)	26 (5.4)	152 (11.6)
W3S2L1	286 (32.1)	299 (14.5)	531 (31.0)	1593 (95.4)	21(2.9)	137 (7.9)
W3S2L2	199 (29.9)	319 (13.3)	525 (30.3)	1577 (111.5)	19 (0.4)	138 (14.2)
W3S2L3	239 (74.0)	317 (1.2)	532 (9.2)	1665 (52.2)	27 (8.4)	151 (7.4)
Grand mean	253.28	316.11	534.52	1673.04	25.68	147.51
$LSD_{0.05}$	135.6	24.8	50.3	256.0	6.3	14.2
P_{value}	0.09	**0.03**	**0.01**	0.907	**0.001**	0.097

W1 means no water stress; **W2** means moderate water stress; **W3** means severe water stress. **S1** means loamy soil and **S2** means sandy loam. **L1** means greenhouse. **L2** means shade net. **L3** mean open field. Values are average over treatments mentioned. Numbers in brackets represent the standard deviations of the mean. $LSD_{0.05}$ is the least significant difference of means. P values in bold are lower than 0.05. **Note that only season two results are presented, due to logistical costs, as analysis could not be done for season one treatments.**

Table 4.8 presents the treatment interaction effect on macro-nutrients of *C. metuliferus* fruit grown at different environments (greenhouse, shade net and open field), soil types (loamy soil and sandy loam) and water stress levels (no water stress, moderate water stress and severe water stress). The results depicted that there was significant ($P \leq 0.05$) difference between irrigation water regimes and soil types at different environments in Mg, P and Na content of the fruit. Fruit Mg content ranged from 279.3 to 340 µg g^{-1} DW. Results showed that the no water stress treatment combined with loamy soil under open field conditions decreased Mg content from (340 to 279.3 µg g^{-1} DW), whereas no water stress treatment combined with sandy loam and open field location increased the content from 279 to 340 µg g^{-1} DW.

The results showed that fruit P content ranged from 468.7 mg-100g to 564 µg g^{-1} DW. The results evinced that treatment of no water stress combined with loamy soil under open field location decreased the content from 564 to 468.7 µg g^{-1} DW. Interestingly, severe water stress treatment combined with loamy soil under greenhouse environment obtained the highest fruit P content at 564 µg g^{-1} DW compared to other treatments. For Na content, values ranged from 18 to 44 µg g^{-1} DW. The results illustrated that the treatment of no water stress regime combined with loamy soil under greenhouse conditions decreased Na content from 44 to 18 µg g^{-1} DW, whereas the no water stress treatment combined with loamy soil under shade net environment increased it from at 18 mg to 44 µg g^{-1} DW. It is worth noting that the Ca, K and S content in *C. metuliferus* fruit was not significantly affected by the treatments imposed.

4.3.4 Micro-nutrients

Table 4.9: Treatment interaction effect of different water levels, soil types and environment on micro-nutrients (µg g^{-1} DW) of *C. metuliferus*.

Treatment	**Copper**	**Iron**	**Manganese**	**Zinc**
W1S1L1	0.92(0.0)	1.75(0.1)	0.76(0.0)	7.67(1.2)
W1S1L2	0.70(0.2)	0.92(0.1)	0.78(0.0)	7.15(1.1)
W1S1L3	0.48(0.0)	2.44(0.8)	0.54(0.1)	5.07(0.9)
W1S2L1	0.67(0.1)	2.81(1.5)	1.11(0.1)	12.65(0.8)
W1S2L2	0.55(0.3)	1.42(0.3)	0.78(0.1)	8.75(2.2)
W1S2L3	0.73(0.1)	1.25(0.3)	0.76(0.2)	6.83(0.4)
W2S1L1	0.71(0.4)	2.02(0.2)	0.94(0.2)	9.25(1.8)
W2S1L2	0.82(0.1)	2.72(0.9)	0.83(0.1)	7.13(0.5)
W2S1L3	0.76(0.2)	2.65(1.3)	0.72(0.1)	7.85(0.2)
W2S2L1	0.48(0.4)	3.79(1.8)	0.95(0.1)	8.56(1.5)
W2S2L2	0.79(0.2)	1.75(0.6)	0.79(0.0)	12.71(0.0)
W2S2L3	0.64(0.5)	2.11(0.2)	0.74(0.1)	6.93(0.4)
W3S1L1	0.81(0.0)	1.59(0.2)	1.01(0.2)	10.12(2.0)
W3S1L2	0.64(0.3)	2.72(0.2)	0.90(0.1)	7.22(0.6)
W3S1L3	0.60(0.1)	1.76(0.8)	0.65(0.0)	7.65(0.1)
W3S2L1	0.54(0.4)	0.49(0.1)	0.89(0.1)	10.61(0.6)
W3S2L2	0.62(0.1)	1.68(0.5)	0.80(0.1)	6.44(0.8)
W3S2L3	0.74(0.1)	0.63(0.2)	0.65(0.1)	7.53(1.4)
Grand mean	0.677	1.916	0.809	8.341
LSD0.05	0.4058	1.288	0.1828	1.8241
P_{value}	0.766	0.088	0.139	**0.001**

W1 means no water stress; **W2** means moderate stress; **W3** means severe water stress. **S1** means loamy soil and **S2** means sandy loam. **L1** means greenhouse. **L2** means shade net. **L3** means open field. Values are average over treatments mentioned. Numbers in brackets represent the standard deviations of the mean. $LSD_{0.05}$ is the least significant difference of means. P values in bold are lower than 0.05. **Note that only season two results are presented, due to logistical costs, as analysis could not be done for season one treatments.**

Table 4.9 presents the treatment interaction effect on micro-nutrients content of *C. metuliferus* fruit grown at different environments (greenhouse, shade net and open field), soil types (loamy soil and sandy loam) and water stress levels (no water stress, moderate water stress and severe water stress). The results revealed that there was a significant ($P \leq 0.05$) difference in Zn content between interaction of water stress levels and soil types at different environments. The Zn content ranged from 5.1 to 12.7 µg g^{-1} DW. The results illustrated that the no water stress treatment combined with loamy soil under open field location decreased it from 12.7 to 5.1 µg g^{-1} DW, whereas no water stress treatment combined with sandy loam soil under shade net conditions presented an increased content from 5.1 to 12.7 µg g^{-1} DW. For Cu, Fe and Mn content in the fruit, results showed that there was no significant ($P > 0.05$) difference between interaction of irrigation water regimes and soil types at different growing environments. However, Cu ranged from 0.48 to 0.82 µg g^{-1} DW, while Fe ranged from 0.63 to 3.91 µg g^{-1} DW and Mn ranged from 0.54 to 1.11 µg g^{-1} DW.

4.3.5 Discussion

This study evaluated the effect of different water stress levels and varying substrates on the nutritional content of *C. metuliferus* fruit grown in the greenhouse, shade net and open space environment. To the best of our knowledge, there is scanty knowledge on the biochemical constituents of *C. metuliferus* fruit harvested from different water stress level under and soil types under varying growing environment, therefore the results of this study serve as a benchmark. Previous studies conducted by Backeberg (2013) and Maseko et al. (2017) have evaluated the nutrient content of leafy vegetables grown under different water stress levels. There is minimal knowledge on fruity indigenous crops of Southern Africa. In addition, studies conducted by Legwaila et al. (2011) and Nyathi et al. (2018) focused on several micro-nutrients such as Fe and Zn. Macro-nutrient elements such as Mg, P, K, Mg, S, and other biochemical constituents including crude protein, total soluble sugars, total flavonoids, total phenols and vitamins, were not considered. Therefore, to the best of our knowledge, this study is the first study to evaluate the effect of different water stress levels and varying substrates on the nutrient content of *C. metuliferus* fruit grown under different environments (greenhouse, shade net and open space). The findings of this study serve as a benchmark for the biochemical constituents of African horned cucumber fruit, and potentially contribute to human nutrition of households located in both rural and urban areas.

Total soluble sugars

The findings of this study demonstrated that the treatment affects the total soluble sugars of *C. metuliferus* fruit. Fruits harvested from plants grown in the shade net illustrated higher sugar content compared to the other growing environments. In this study, it was also found that when plants are subjected to water stress, total soluble sugar content increased, but reduce under moderate to no water stress during both seasons. Perhaps, the sunlight radiation interception and transpiration rate in the shade net environment played an important role in facilitating water and nutrients absorption by plant roots from the substrate compared to other growing environment (shade net and greenhouse).

This implies that when plants are exposed to different water levels and growing environment, there is variation in fruit sugar content as reported by Tavarini et al. (2008) and Das et al. (2018), who found significant difference in total soluble sugars of kiwi fruit harvested from different sites, due to variation in temperatures and rainfall patterns. High total soluble sugar content was expected from open space and shade fruit under moderate water stress, as reported by Ali & Abdelatif (2001), Khattab et al. (2011) and Li et al. (2018) on cotton, cucumbers and pomegranate trees. These authors concluded that active osmoregulation caused by water stress was responsible for sugar variation in fruits, since there is imbalanced fluid movement within plant cells. Similarly, this study's findings agree with the fact that water stress and growing environments are the critical contributors of fruit total soluble sugars content compares to soil types since they directly affect the transpiration rate. Therefore, a relatively high total soluble sugar level in fruit is crucial for human nutrition, especial when ˚Brix level is above 5. However, the values obtained from this study are slightly higher, making it an important fruit for fresh and juice market. This suggest that the fruit is valuable and should be considered for commercialisation, as the fruit shows potential benefits for human nutrition.

Crude Proteins

Crude proteins are important in human nutrition because they aid in cell formation, nutrient storage, pH balance, and immune system improvement, and they serve as a messenger (Jamnadass et al., 2011; López et al., 2013). Previous studies have often reached conflicting findings regarding crude protein content of crops harvested from different treatments and growing conditions. For example, Bartova et al. (2009) presented their findings on crude protein of potatoes harvested under different regions that experience varying weather conditions and treated with varying level of fertilizers. They concluded that potatoes harvested from regions with moderate temperatures subjected to moderate nitrogen fertilizers resulted in higher significant crude protein content when compared to other treatments, due to high enzyme activities within cells, caused by different nitrogen content.

For this study, shade net conditions expressed high crude protein content compared to other locations. Perhaps the growing environment of shade net favoured higher crude proteins in moderate and no water stress treatments, compared to the water stressed treatment. When the surrounding conditions (adequate sunlight and water) are favourable, cells can carry out chemical reaction at optimum rate, but at lower rate under stress environment such as excessive radiation and water stress. These results agree with the fact that excessive temperature negatively affect protein activities (denature) and have other general destructive effects on plant cells as reported by Mart et al. (2016) and Lisiewska et al. (2018) who found higher crude protein content in fruits harvested from protected structures, but low in those harvested from open field conditions. This advocates that *C. metuliferus*, if grown under optimum environmental conditions may have several health benefits in human nutrition and may also be a potential solution for a hunger and health issues globally.

Bio-chemical constituents

The study assessed the effect of different water stress levels and soil types under three different conditions (greenhouse, shade net and open space). Previous study conducted by Moyo et al. (2018) determined the mineral constituents and phytochemicals of crops such as cabbage, swiss chard harvested from different locations.

β-carotene

The study findings showed that that loamy soil treatment increased *carotene* compared to sandy loam. The mean results also showed that reduction was higher on moderate to no water stress level treatment, relative to severe water stress treatment. This study also outlined increase of *β-carotene* in the greenhouse than other growing environments. Hurr et al. (2009) and Perkins-Veazie (2009) found that there was variation in *Β-carotene* among same plant varieties subjected to reduced water supply. The current study findings show that different in growing environment and water stress level will cause variation of *β-carotene* in plants. When plant is subjected to low light intensity and water stress, *β-carotene* increases, but decreases under normal light and watering as reported by (Santos-Zea et al., 2011; Nyathi et al., 2019).

The fact that carotene is responsible for radiation interception in plants could have been cause for variation, since there is control of light intensity in the greenhouse, due to cladding material used for protection, as compared to an open space environment. Flemotomou, (2011) found that there was variation among some plant varieties subjected to varying rainfall pattern. Their findings are in harmony with those of the current study, whereby varying water levels under different conditions significantly altered the *β-carotene* content of Cucumis metuliferus fruit. *β-carotene* promotes cell and tissue development, strengthens the immune system, and slows the aging process. Cucumis metuliferus fruit contains reasonable amount of *β-carotene,* which can be converted to vitamin A in the human body, to complement it. Therefore, optimum growing environment could serve as strong evidence for mass production and commercialisation globally.

Vitamin C

In the present study, vitamin C increased in plants subjected to no water stress under shade net environment but decreased under severe water stress under open field environment. Perhaps, high fluctuation in the vitamin C content, could be that there was unbalanced turgor pressure in plants caused by varying irrigation water levels and water holding capacity by a specific substrate, as reported by Esch et al. (2010) and Fenech et al. (2019), who mentioned that water and fertilizers stimulate the vitamin C content of cucumber and citrus fruit grown in open field and semi-protected structure.

Variation in vitamin C content exists among different varieties in the same plant species, due to genetic makeup and environmental conditions (Bernaert et al., 2013). They found that vitamin C content decreased in fruit subjected to water stress, but showed a significant increase than fruit harvested under normal watering treatment. This was authenticated by Wang et al. (2017), when they reported that plants respond to harsh environmental conditions such as excessive sunlight, heat and water stress by producing vitamin C as a defensive mechanism to protect themselves (Taylor et al., 2000; Bernaert et al., 2013). The mean results showed that the vitamin C reduction was more on plants subjected adverse conditions such as water stress level and open space compared to plants that were grown under protected environment (greenhouse and shade net).

The current study findings agree with findings by Ity and Canino (2006), who reported that plants can tolerate moderate water stress. However, such alteration has a negative impact on fruit vitamin C content of various fruit crops. Even though vitamin C deficiency is uncommon in today's world, dieticians prescribe vitamins C because it plays a critical role in the production of collagen, iron absorption, wound healing, bone and tooth health. Determination of optimal conditions that increase *C. metuliferus* vitamin C content could fill the void in human nutrition and increase its consumption.

Vitamin E

The current study findings exhibited that the treatment imposed caused significant variation in vitamin E content. The study findings showed that there was more increased under moderate to severe water stress under greenhouse, but significantly decreased under all water levels in the shade net and open field conditions. Perhaps, the evapotranspiration rate which regulates the osmoregulation could have played an important role in the vitamin E variation since there was a change in stomatal opening and closure due to alteration in turgor pressure within the guard cells. Carbon dioxide interception is higher when there is balance of solutes in movement within the open guard cells, but they close when the imbalance concentration due to high evapotranspiration rate caused by excessive conditions such as high wind and radiation, subsequently limiting the ability to synthesis Vitamin E there is limited activities in the chloroplast due to stomatal closure. Closing of stoma not only prevent water loss, but also prevent the plant's ability to vitamins and other biochemical compounds. The study findings affirm that growing water stress levels (moderate and no water stress) were the critical contributors of vitamin E content of *C. metuliferus* fruit compare to other factors. These findings agree with Chen et al. (2006); Manthey et al. (2009); Zhang et al. (2011) and Fenech et al. (2019) who found significant differences in vitamin E content of fruit such as chillies and peppers subjected to varying water stress, due to the balanced osmotic flow within plant organs. Vitamin E has a variety of functions in the human body, including preventing free radical damage and acting as antioxidant. In addition, the vitamin deficiency is associated with stunted growth development. The values obtain from this study serve as benchmark required by policy makers for commercialisation of *C. metuliferus*, since it has nutritional benefits to humans.

Total flavonoids and phenols

In this study, the results showed moderate to normal watering treatment under open field and shade net environment played a pivotal role exhibiting higher total flavonoids compared to other treatments (water stress and greenhouse). The study findings also remarked that the reduction was more on the severe water stress treatment, relative to moderate and no water stress treatment. The alteration in total flavonoids could have been caused positive turgor pressure within the plant's cells, which subsequently allow the plant to excess surrounding atmospheric elements through the epidermal cells. Thus, allowing the plant to absorb atmospheric elements needed by plants for cellular activities. When the stomata close, plant cellular activities get negatively affected, but function normally when there is good movement of water withing plant organs. However, contradictory findings were noticed by Santos-Zea et al., (2011) and Iglesias-Carres et al. (2019) on optunia and red grapes. They determined that total flavonoids significantly increased in fruit harvested from regions with a low rainfall pattern, but decreased in fruit harvested from regions experiencing higher rainfall patterns, due to varying active osmoregulation within plant organs, since plants were trying to cope with stress caused by the environmental conditions. Their findings are inconsistent with observations made in this current study; whereby normal stress fruit demonstrated a significant increase in total flavonoids when compared to stressed watered fruit.

Total flavonoids are well-known in human health for their function in controlling cellular activity, as well as fighting free radicals that cause oxidant stress. The total flavonoids values of *C. metuliferus* serve as benchmark information required by policy makers, therefore, the crop can be recommended for commercialisation, if grown under optimal conditions.

Phenolic content

In the present study, the total phenolic content of fruit harvested from normal watering on loamy soil under open field increased but decreased when subjected to water stress under a similar growing environment. Perhaps variation in water stress and soil types under different growing conditions could have been the major cause in variation of total phenolic content of *C. metuliferus* fruit. For example, Rimpapa et al. (2007) found significant differences in several edible fruits such as blackberry and cherry harvested from different locations experiencing varying rainfall patterns. Tavarini et al. (2008) also found a significant difference in total phenols of walnuts' green husks harvested during different periods. They found that fruits harvested earlier have a higher total phenolic content than those which were harvested late, after ripening, due to different metabolites released by plants at different stages of growth. The current study affirmed that different water stress levels are major triggers of metabolites responsible for this compound, since the plant has to adapt to variation in water levels, as reported by Lombardi et al. (2020), who found a significant total phenolic content in strawberries exposed to different environmental conditions such as water stress. Total phenols are known in human health for their antioxidant properties, which stop free radicals from reacting with other molecules in the body and prevent DNA damage, which is usually caused by a variety of health effects. Therefore, values in this study serve as concreate evidence needed by policymakers in order to consider this crop for commercialisation.

Macro and micro-nutrients

The present study findings delineated that concentration of Mg was higher on under the open space environment than other growing environments. The study results also showed that reduction was more under loamy soil treatment, relative to the sandy loam treatment. Perhaps the Mg, the total Mg concentration from the sandy loam substrate played an important role in increasing fruit Mg content compared to the loamy soil. Regarding P content, the study findings showed that there was higher concentration on sandy loam fruit under greenhouse environment compared to other treatments; the mean results outlined higher reduction on loamy soil fruit under open space environment. The fact that there was higher P content on sandy loam compare to loamy soil could be that the nutrient pathway from the epidermis to the endodermis of the plant root was much easier on sandy due to its ability to drain freely, subsequently promote nutrient absorption and good root system. For Na content, the study findings showed that Na content was higher in the shade net growing environment compare to the other growing environment. The mean results also demonstrated that the greenhouse environment reduced Na content compare to shade net and open space environment. Perhaps, the relationship between evapotranspiration rate and mineral absorption by plant roots played a crucial role in variation of Na content. Micronutrients deficiency, including of iron, copper, and zinc, may lead to decreased intellectual ability, development, bone mineralisation, and immune response, whereas deficiency in zinc may lead to poor digestion, metabolism, reproduction, and wound healing. Nutrient synthesis in plant is facilitated by the both environmental and physiological factors. The principal source of synthesis of nutrients are photosynthesis and substrates, which subsequently affect plant organs, particularly storage tissues. Most plant parts are unable to fully meet their nutritional needs when one process is interrupted due abnormal factors such as excessive temperature, which negatively affect the osmoregulation process due to abnormal evapotranspiration rate. Similar findings were reported by Barrett et al. (2010); Sezen et al. (2010); Sharma and Rao (2013) and Fenech et al. (2019), who found that factors such as varying substrate and temperature have direct impact on the nutrient content on apple, banana, pepper, grape, pear and strawberry. According to Uusiku et al. (2010), WHO recommended daily nutrients intake of zinc for children between four and six years should range from a minimum of 9.6 µg g^{-1} DW and above.

The study findings showed that Zn content from moderate water stress and sandy loam under greenhouse was superior (12.7 µg g^{-1} DW) than the other treatments. Moreover, the values were obtained from this study were 3.1 higher than those of recommended daily nutrients intake. Therefore, the Zn values of the fruit serve a great benchmark required by policy makers to consider this fruit for potential commercialisation since it has a potential to meet human nutritional needs. It worth noting that Cu, Fe and Mn were not significantly affected by treatment imposed. Barrett et al. (2010) observe that a nutrient element such as Mn depends on environmental factors such as adequate water supply, temperature and plant genotype.

4.3.6 Conclusion

Tavarini et al. (2008) and Legwaila et al. (2011) indicacted that quantification of quality parameters such total soluble sugars, crude proteins, macro-nutrients, total flavonids and vitamins contribute to the factors required by policy markers before commercilising a specific crop. Therefore, the outcome of this study has shown that *C. metuliferus* fruit contains vital biochemical constituents required by humans in both larger and smaller quantities. In addtion, this research has provided evidence that the *C. metuliferus* fruit quality content is significantly affected by treatments. This is useful information to farmers, as quality has become more significant to most consumers worldwide. When grown in the open field, total soluble sugars increased; this is important for the juice-manufacturing industry and for fresh markets, where many fruits are required to meet the demand. Quality parameters such as metabolites seem to increase under shade net. This is an important finding, as the metabolites influence the flavour of fruits. Where the market is geared towards organoleptic quality – in expensive markets, for example, it may be best to grow this crop under shade net. The other advantage is that the crop is protected from rainfall and extreme heat in summer.

4.4 Metabolite profile of *C. metuliferus* E. Mey. Ex Naudin (African horned cucumber) fruit grown under differing environment

4.4.1 Asparagine

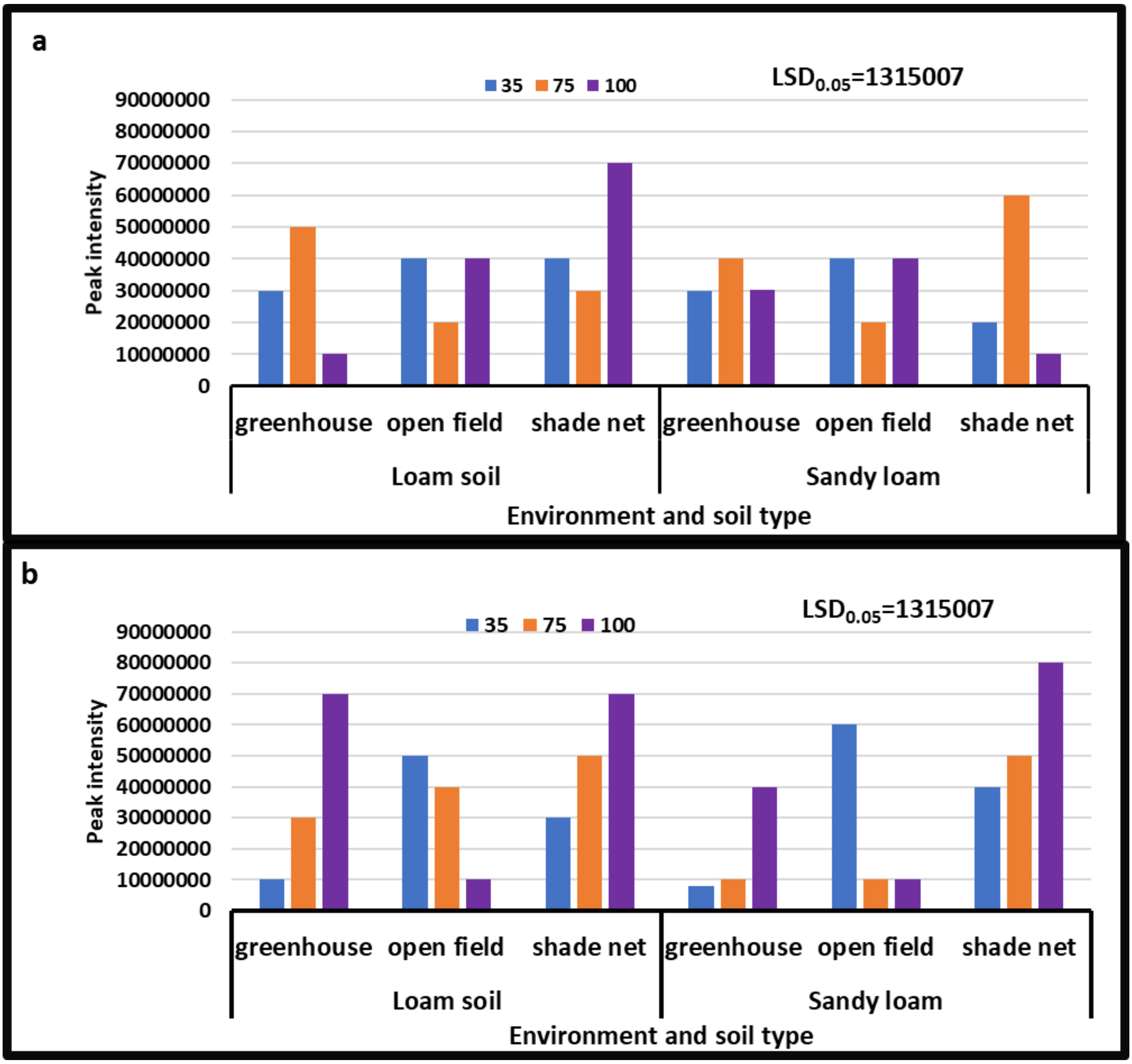

Figure 4.9: The treatment interaction effect on **asparagine** of *C. metuliferus* fruit; **(a)** means treatment interaction of irrigation water levels and soil types at different growing environments during the 2017/18 season; **(b)** means treatment interaction of water stress levels and soil types at different growing environments during the 2018/19 season. **35** means severe water stress; **75** means moderate water stress; **100** means no water stress. **Peak intensity showing LCMS-8040 triple quadrupole mass spectrometer intensities which represent the quantity of metabolites varying with treatments.** $LSD_{0.05}$ is the least significant difference of means.

Figure 4.9 presents the metabolite profile of asparagine of *C. metuliferus* fruit grown under different environments (greenhouse, shade net and open field), water stress levels (no water stress, moderate stress and severe water stress) and soil types (loamy soil and sandy loam) during both seasons, as previously explained above. The results showed that asparagine was significantly ($P \leq 0.05$) affected by treatment interaction between water stress levels and soil types under different environments. The observed trend revealed that the treatment of no water stress (control) under all growing environments during the year 2018/19, had higher asparagine content when compared to other treatments. During the year 2017/18, asparagine content ranged from 10×10^6 to 60×10^6 area under curve, while in the 2018/19 season it ranged from 10×10^6 to 80×10^6 area under curve. The severe water stress treatment combined with sandy loam at the greenhouse significantly decreased asparagine from 80×10^6 to 10×10^6 area under curve during the year [2018/19], but the no water stress treatment (control) combined with sandy loam under shade net environment increased it from 10×10^6 to to 80×10^6 area under curve (Figure 4.9).

4.4.2 Dopa

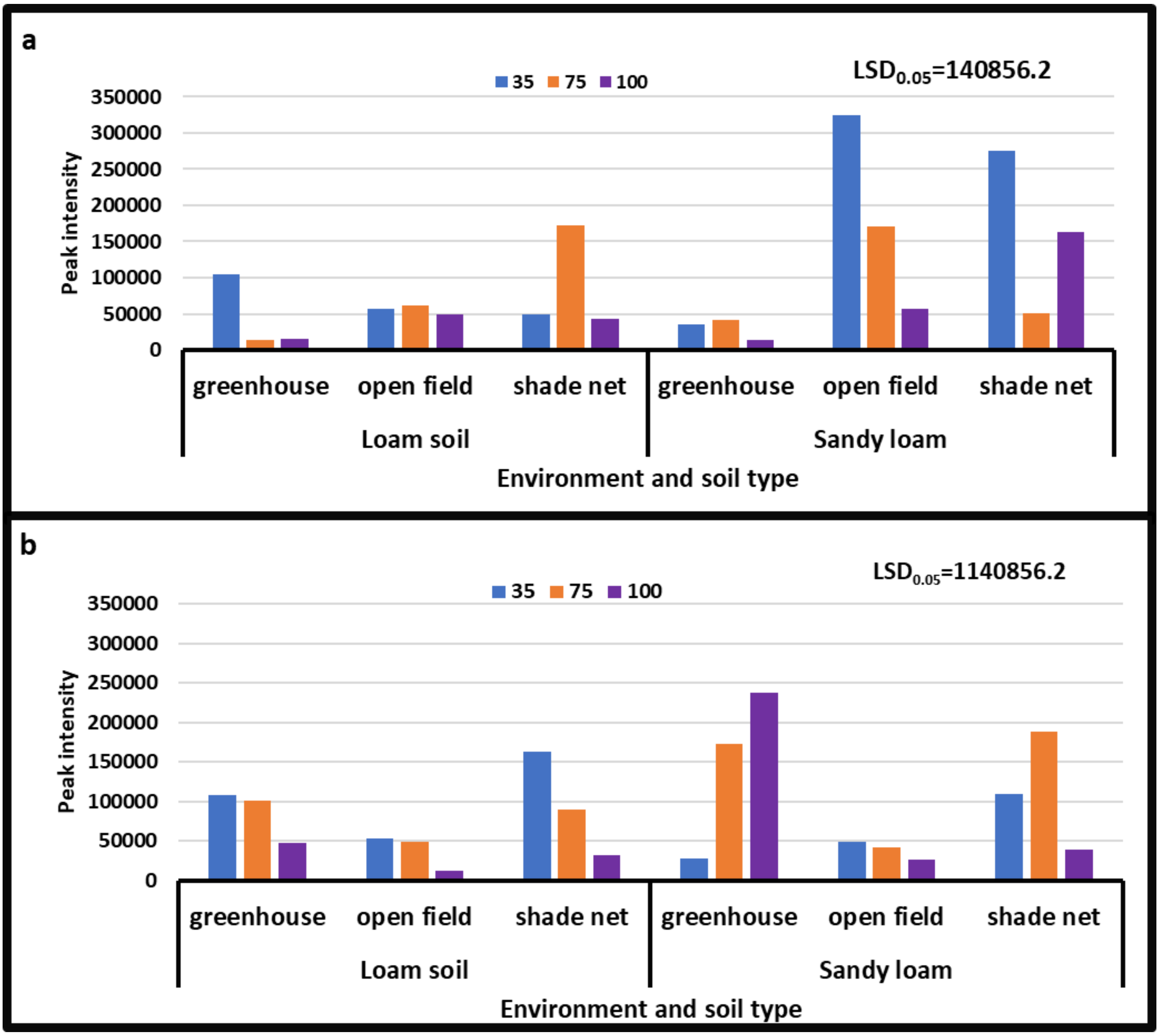

Figure 4.10: The treatment interaction effect on **Dopa** of *C. metuliferus* fruit during season one. Values are average over treatment; **35** means severe water stress; **75** means moderate water stress; **100** means no water stress; **(a)** means treatment interaction of water stress levels and soil types at different environments during the 2017/18 season; **(b)** means treatment interaction of irrigation water regimes and soil types at different environments during the 2018/19 season. **Peak intensity showing LCMS-8040 triple quadrupole mass spectrometer intensities which represent the quantity of metabolites varying with treatments.** $LSD_{0.05}$ is the least significant difference of means.

Figure 4.10 presents the metabolite profile dopa of *C. metuliferus* fruit grown under different environments (greenhouse, shade net and open field), water stress levels (no water stress, moderate stress and severe water stress) and soil types (loamy soil and sandy loam) during season one. The study results outlined that there was a significant ($P \leq 0.05$) difference between interaction of different water stress levels and soil types under different environments during both seasons. During the year 2017/18, dopa content ranged from 13,697 to 324, 240 peak intensity, whereas in the year 2018/19, it ranged from 12,030 to 47,611 peak intensity. The no water stress treatment (control) combined with loamy soil under open field conditions during the year [2018/19] decreased dopa content from 324,240 to 12,030 peak intensity, while treatment of severe water stress combined with sandy loam soil under open field conditions during the year [2017/18] increased it from 12,030 to 324,240 peak intensity (Figure 4.10).

4.4.3 4-Hydroxyproline

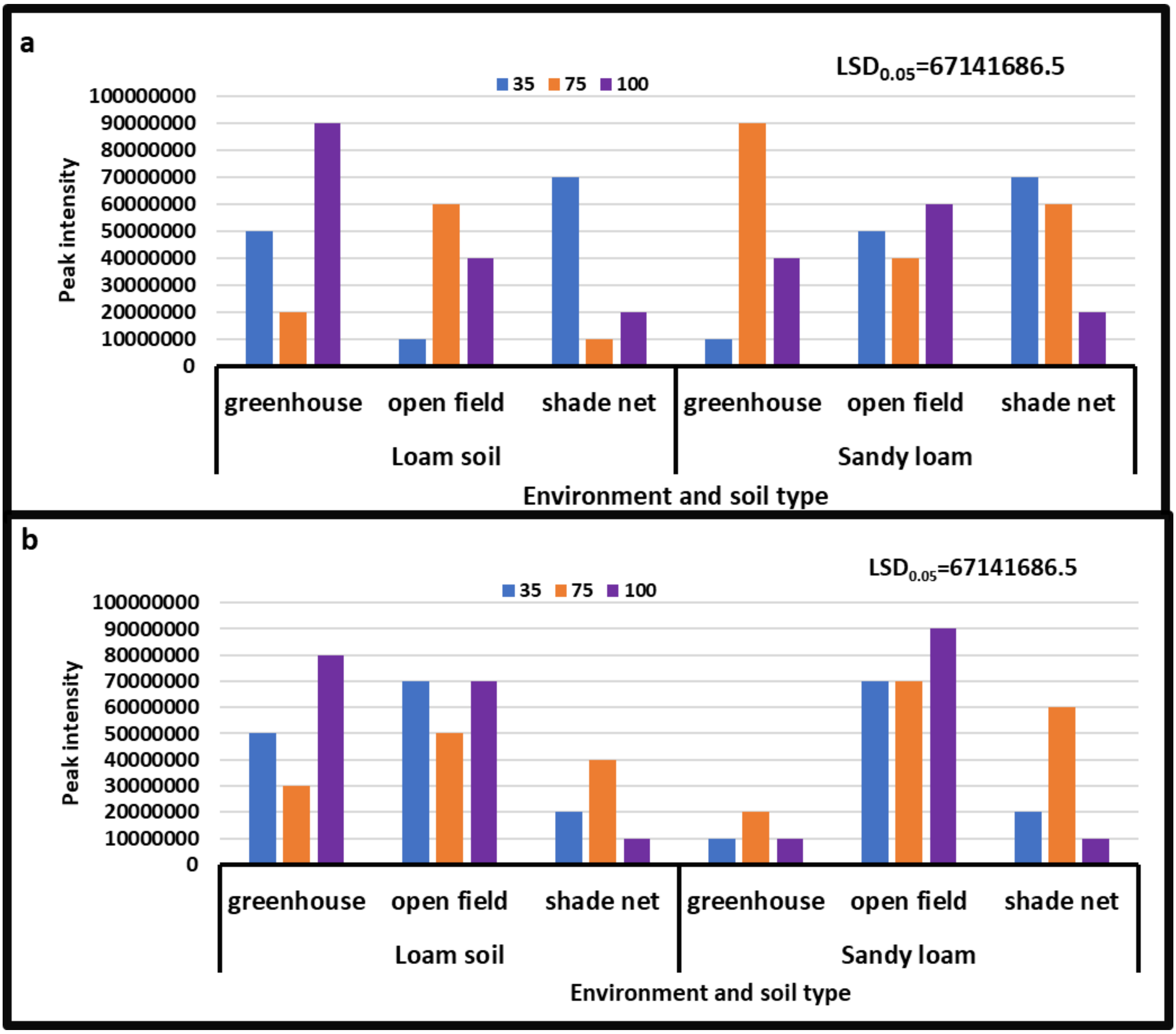

Figure 4.11: The treatment interaction effect on **4-hydroxyproline** of *C. metuliferus* fruit during season one; **35** means severe water stress; **75** means moderate water stress; **100** means no water stress. Values are average over treatment; **(a)** means treatment interaction of water stress levels and soil types at different environments during the 2017/18 season; **(b)** means treatment interaction of irrigation water regimes and soil types at different environments during season 2018/19. **Peak intensity showing LCMS-8040 triple quadrupole mass spectrometer intensities which represent the quantity of metabolites varying with treatments.** $LSD_{0.05}$ is the least significant difference of means.

Figure 4.11 presents the profile of metabolite 4-Hydroxyproline on *C. metuliferus* fruit grown under different environments (greenhouse, shade net and open field), water stress levels (no water stress, moderate stress and severe water stress) and soil types (loamy soil and sandy loam) during both seasons. The results showed that there was no significant ($P>0.05$) difference between interaction of different water stress levels and soil types at different growing environments during both seasons. However, there was a significant difference of 4-hydroxyproline content under different growing environments. During the year 2017/18, 4-hydroxyproline ranged from 10×10^6 to 90×10^6 peak intensity, whereas in the year 2018/19, it ranged from 10×10^6 to 90×10^6 peak intensity. The treatment of all irrigation water levels combined with both soil types under all growing conditions during the year 2017/18 and 2018/19, under shade net and greenhouse (Figure 4.11b) decreased 4-hydroxyproline content from 90×10^6 to 10×10^6 peak intensity respectively, whereas treatment of irrigation water levels combined with both soil types under greenhouse environment during the year 2017/18. Treatment of no water stress combined with sandy loam soil under open field conditions during the year 2018/19 significantly increased it from 10×10^6 to 90×10^6 peak intensity when compared to other treatments, respectively (Figure 4.11ab).

4.4.4 5-Glutamylcysteine

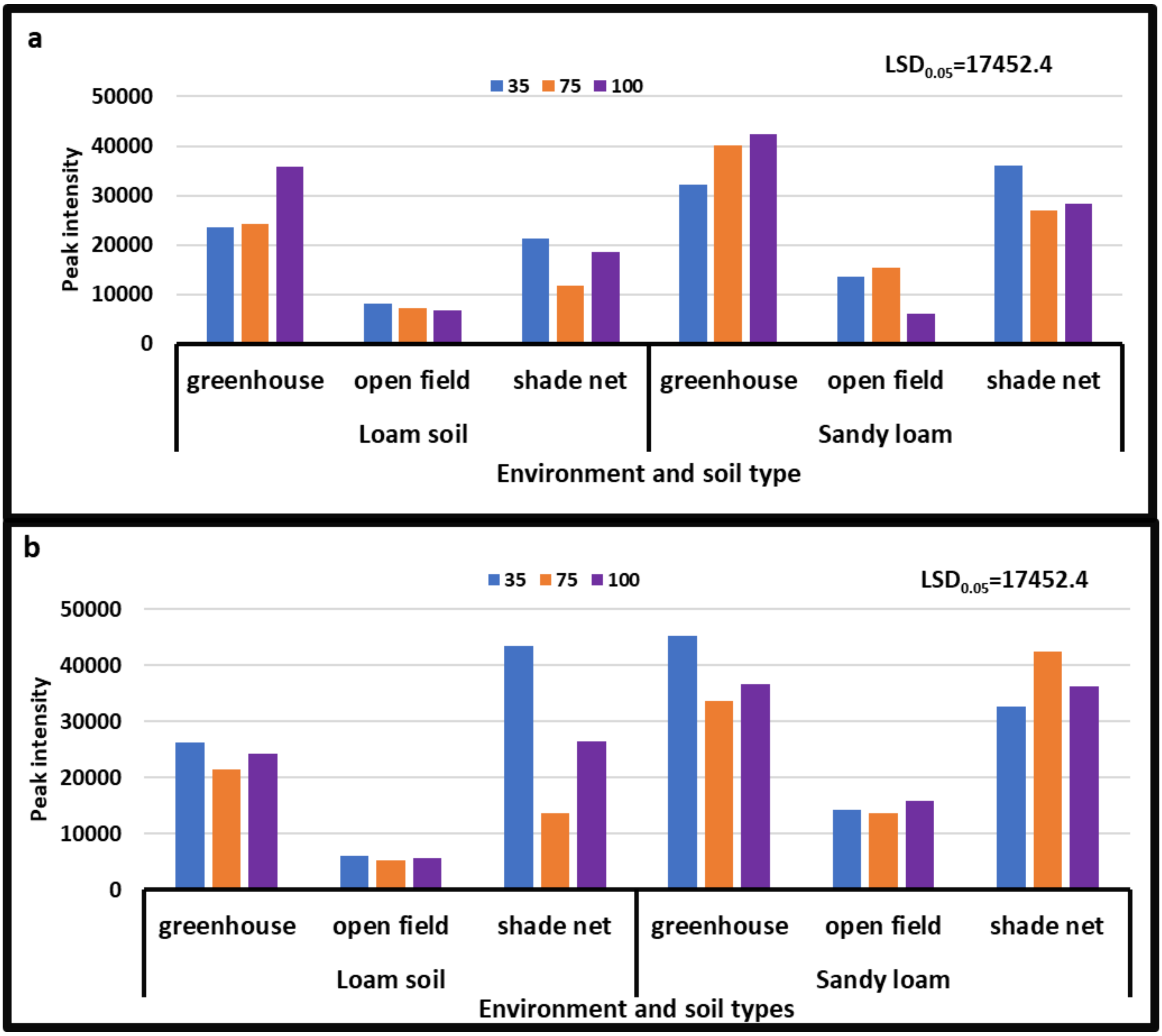

Figure 4.12: The treatment interaction effect on **5-Glutamylcystein** of *C. metuliferus* during season one; 35 means severe water stress; 75 means moderate water stress; 100 means no water stress; **(a)** means treatment interaction of water stress levels and soil types at different growing environments during the 2017/18 season one; **(b)** means treatment interaction of irrigation water levels and soil types at different growing environments during the 2018/19 season. **Peak intensity showing LCMS-8040 triple quadrupole mass spectrometer intensities which represent the quantity of metabolites varying with treatments.** $LSD_{0.05}$ is the least significant difference of means.

Figure 4.12 presents the profile of metabolite 5-glutamylcysteine on *C. metuliferus* fruit grown under different environments (greenhouse, shade net and open field), water stress levels (no water stress, moderate stress and severe water stress) and soil types (loamy soil and sandy loam) during both seasons. The study results showed that was a significant (P≤0.05) difference of 5-glutamylcysteine content between interaction of water stress levels and soil types under different growing environments during all seasons. During the year 2017/18, 5-glutamylcysteine content ranged from 6,115 to 35,818 peak intensity, whereas in the year 2018/19, it ranged from 5,345 to 45, 214 peak intensity. The treatment of moderate water stress combined with loam soil under open field environment indicated a significant decrease in 5-glutamylcysteine content from 45,214 to 6,115 peak intensity during the year 2018/19 (Figure 4.12b). The severe water stress treatment combined with sandy loam under greenhouse conditions during the year [2018/19] indicated a significant increase in fruit 5-glutamylcysteine content from 6, 115 to 45,214 peak intensity (Figure 4.12b).

4.4.5 Acetylcarnitine

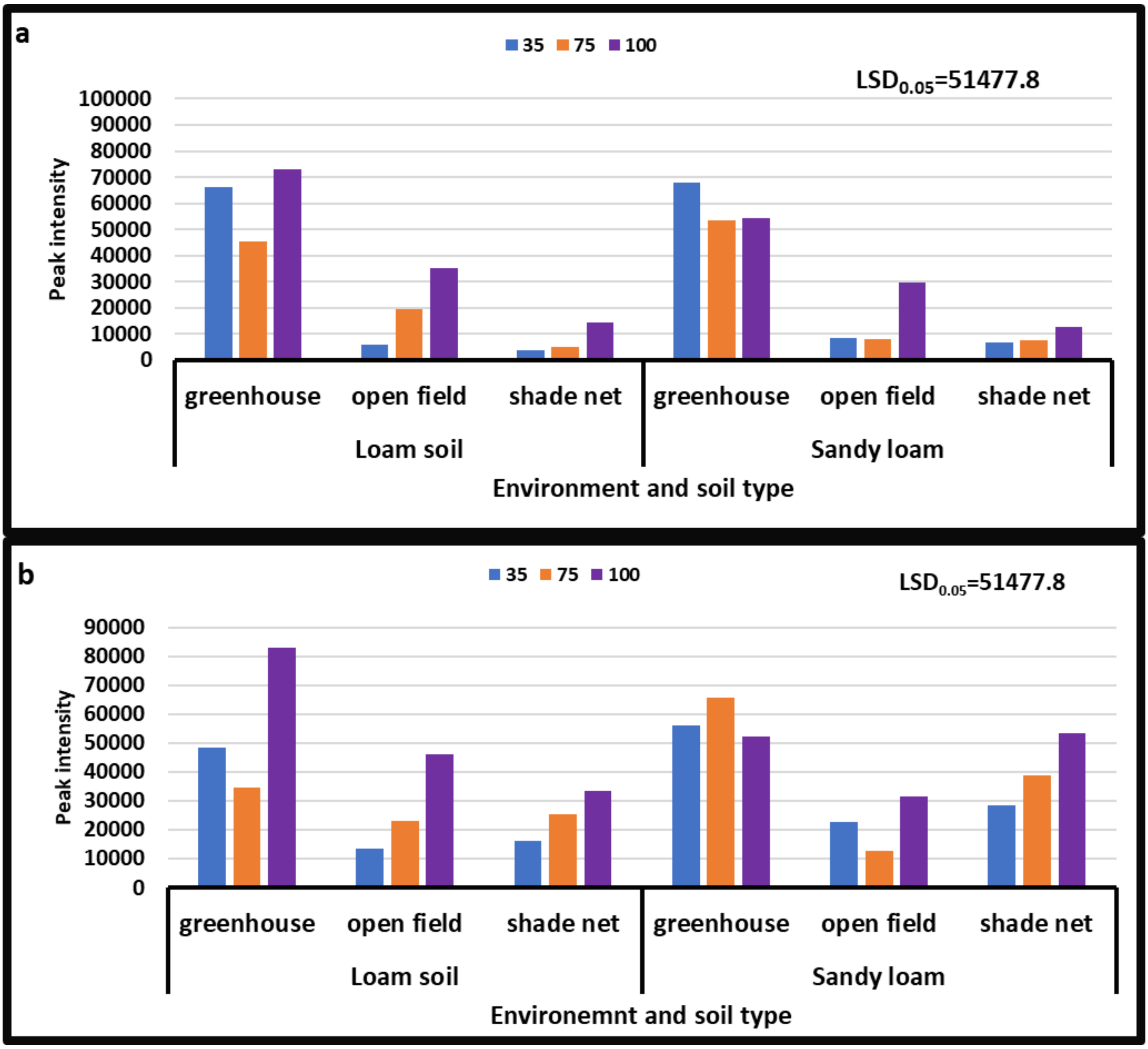

Figure 4.13: The treatment interaction effect on **Acetylcarnitine** of *C. metuliferus* during season one; **35** means severe water stress; **75** means moderate water stress; **100** means no water stress; **(a)** means treatment interaction of water stress levels and soil types at different growing environments during the 2017/18 season one; **(b)** means treatment interaction of irrigation water levels and soil types at different growing environments during the 2018/19 season. **Peak intensity showing LCMS-8040 triple quadrupole mass spectrometer intensities which represent the quantity of metabolites varying with treatments.** $LSD_{0.05}$ is the least significant difference of means.

Figure 4.13 presents the profile of metabolite acetylcarnitine on *C. metuliferus* fruit grown under different environments (greenhouse, shade net and open field), water stress levels (no water stress, moderate stress and severe water stress) and soil types (loamy soil and sandy loam) during both seasons. The study results showed that there was a significant ($P \leq 0.05$) difference between different water stress levels and soil types at different locations during both seasons. The observed trend showed that during the year 2017/18, acetylcarnitine content was higher than that of year 2018/19. During the year 2017/18, acetylcarnitine content ranged from 3,761 to 72, 841 peak intensity, whereas year 2018/19 ranged from 12,514 to 82, 841 peak intensity. The results also demonstrated that treatment of severe water stress combined with loamy soil under shade net environment significantly decreased acetylcarnitine content from 82,841 to 3,761 peak intensity during the 2017/18 (Figure 4.13a), while the treatment of no water stress (control) combined with loamy soil under greenhouse environment significantly increased its content from 3,761 to 82,841 peak intensity during year 2018/19 (Figure 4.13b).

4.4.6 Kynurenine

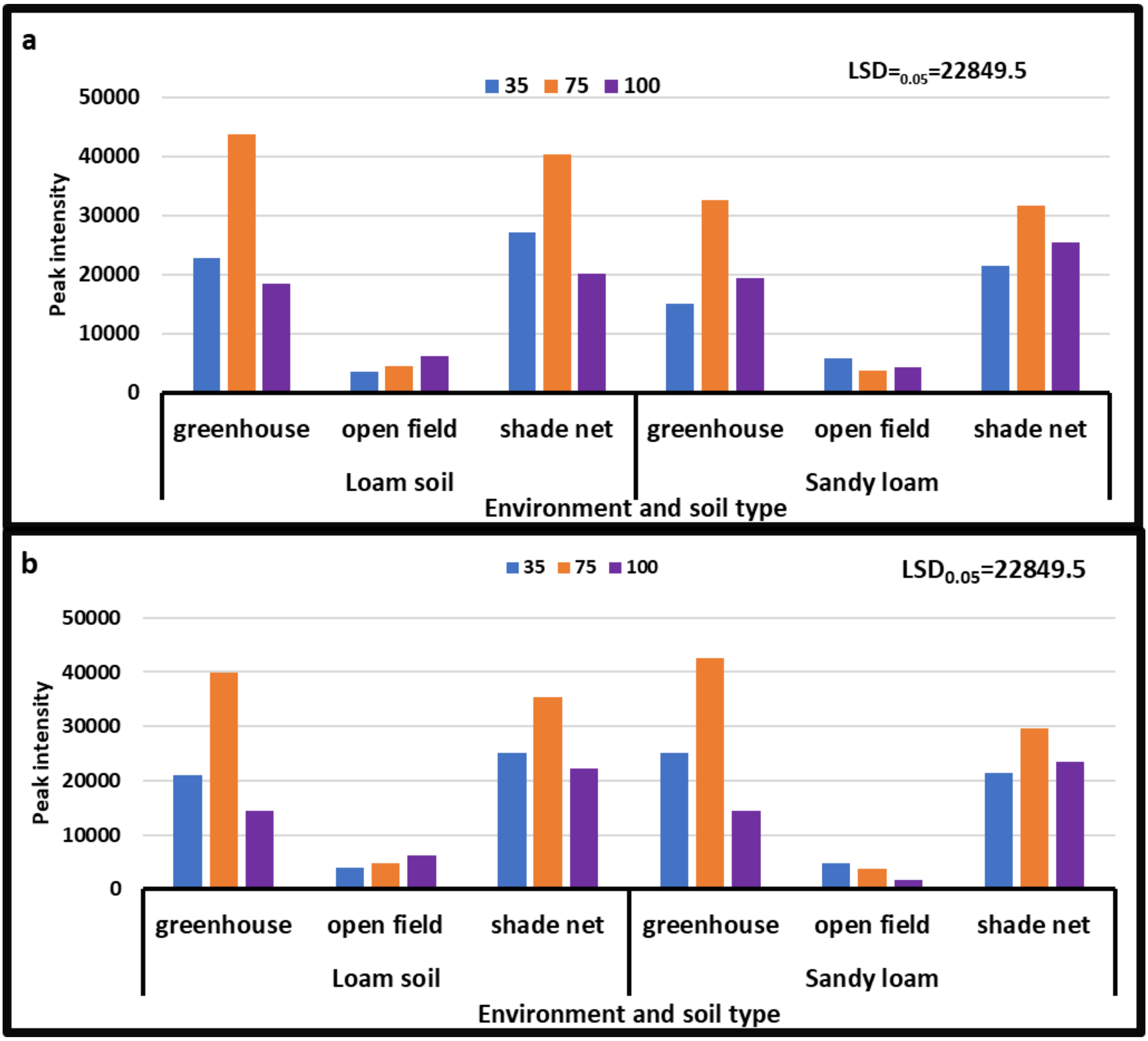

Figure 4.14: The treatment interaction effect on **Kynurenine** of *C. metuliferus* fruit during season one; 35 means severe water stress; 75 means moderate water stress; 100 means no water stress; (a) means treatment interaction of irrigation water levels and soil types at different growing environments during the 2017/18 season; (b) means treatment interaction of irrigation water levels and soil types at different growing environments during the 2018/19 season. **Peak intensity showing LCMS-8040 triple quadrupole mass spectrometer intensities which represent the quantity of metabolites varying with treatments.** $LSD_{0.05}$ is the least significant difference of means.

Figure 4.21 presents the profile of metabolite kynurenine on *C. metuliferus* fruit grown under different environments (greenhouse, shade net and open field), water stress levels (no water stress, moderate stress and severe water stress) and soil types (loamy soil and sandy loam) during both seasons. The study results showed that there was a significant ($P \leq 0.05$) difference of kynurenine content between interaction of irrigation water levels and soil types under different growing environments during both seasons. The observed trend depicted that the underwater stress treatment, kynurenine content was higher when compared to other irrigation water levels. During the year 2017/18, it ranged from 3,602 to 43,808 peak intensity, while in the year 2018/19, it ranged from 1,678 to 42,555 peak intensity. The no water stress treatment (control) combined with sandy loam soil under open field environment during the year 2018/19, indicated a significant decrease in kynurenine content from 43,808 to 1,678 area peak intensity (Figure 4.14b), whereas the treatment of moderate water stress combined with loamy soil at greenhouse environment during the year 2017/18, increased from 1,678 to 43,808 peak intensity (Figure 4.14a).

4.4.7 Norepinephrine

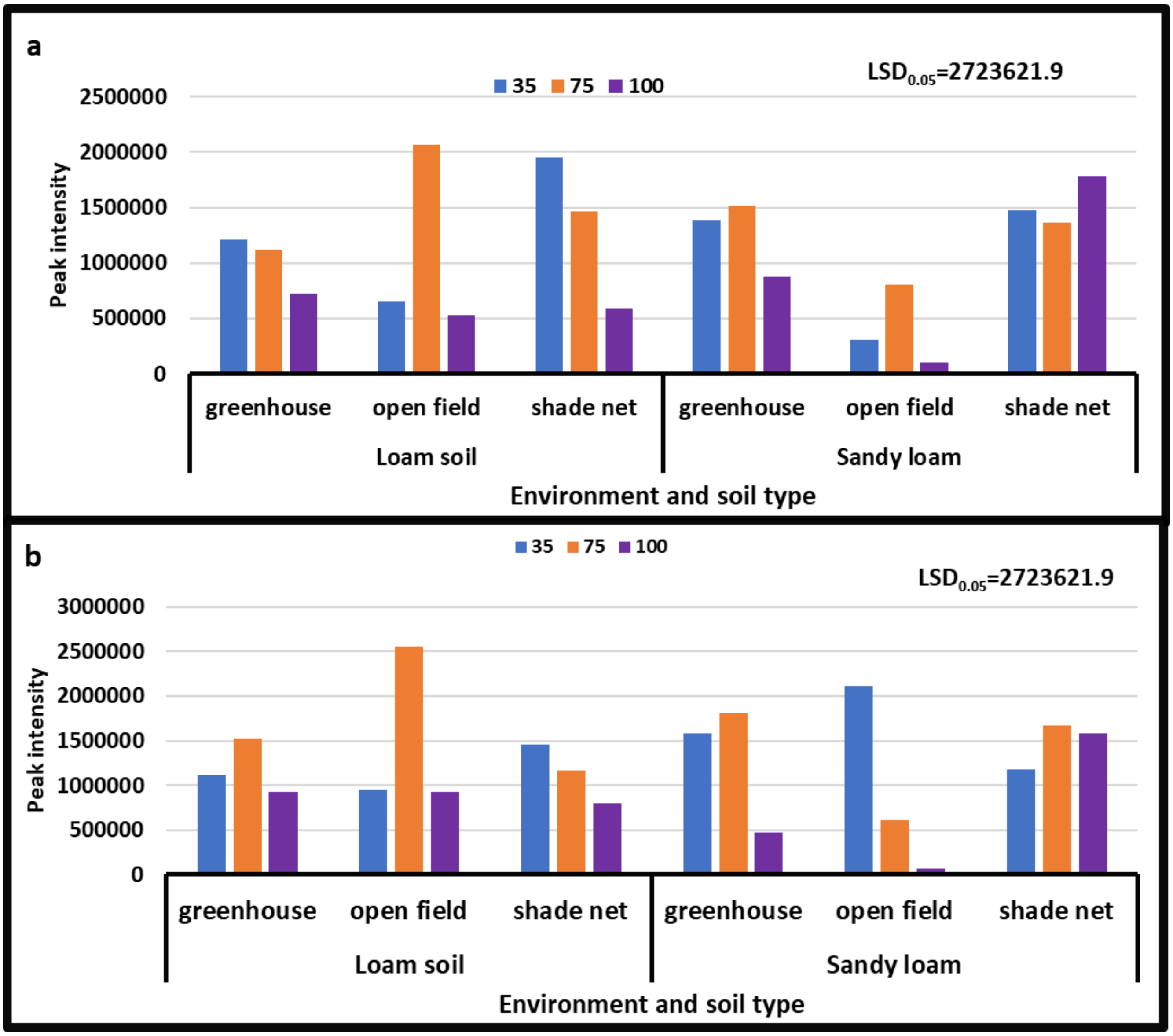

Figure 4.15: The treatment interaction effect on **Norepinephrine** of *C. metuliferus* fruit during season one; 35 means severe water stress; 75 means moderate water stress; 100 means no water stress; **(a)** means treatment interaction of water stress levels and soil types at different growing environments during the 2017/18 season; **(b)** means treatment interaction of irrigation water levels and soil types at different growing environments during the 2018/19 season. **Peak intensity showing LCMS-8040 triple quadrupole mass spectrometer intensities which represent the quantity of metabolites varying with treatments.** $LSD_{0.05}$ is the least significant difference of means.

Figure 4.15 presents the profile of metabolite norepinephrine on *C. metuliferus* fruit grown under different environments (greenhouse, shade net and open field), water stress levels (no water stress, moderate stress and severe water stress) and soil types (loamy soil and sandy loam) during both seasons. The results showed that there was no significant ($P<0.05$) difference of norepinephrine content between interaction of irrigation water levels and soil types at different growing conditions. However, there was a significant ($P\leq0.05$) difference of norepinephrine content under different growing environments. During the year 2017/18, norepinephrine ranged from 99,577 to 206, 1 peak intensity, and in [2018/19] year, it ranged from 71,577 to 256,1 peak intensity. The no water stress treatment (control) combined with sandy loam soil under open field environment decreased norepinephrine content from 256,10 to 71,577 peak intensity (Figure 4.15b), while the treatment of moderate water stress combined with loamy soil at open field environment significantly increased it from 71,577 to 256,1 peak intensity (Figure 4.15b).

4.4.8 Niacinamide

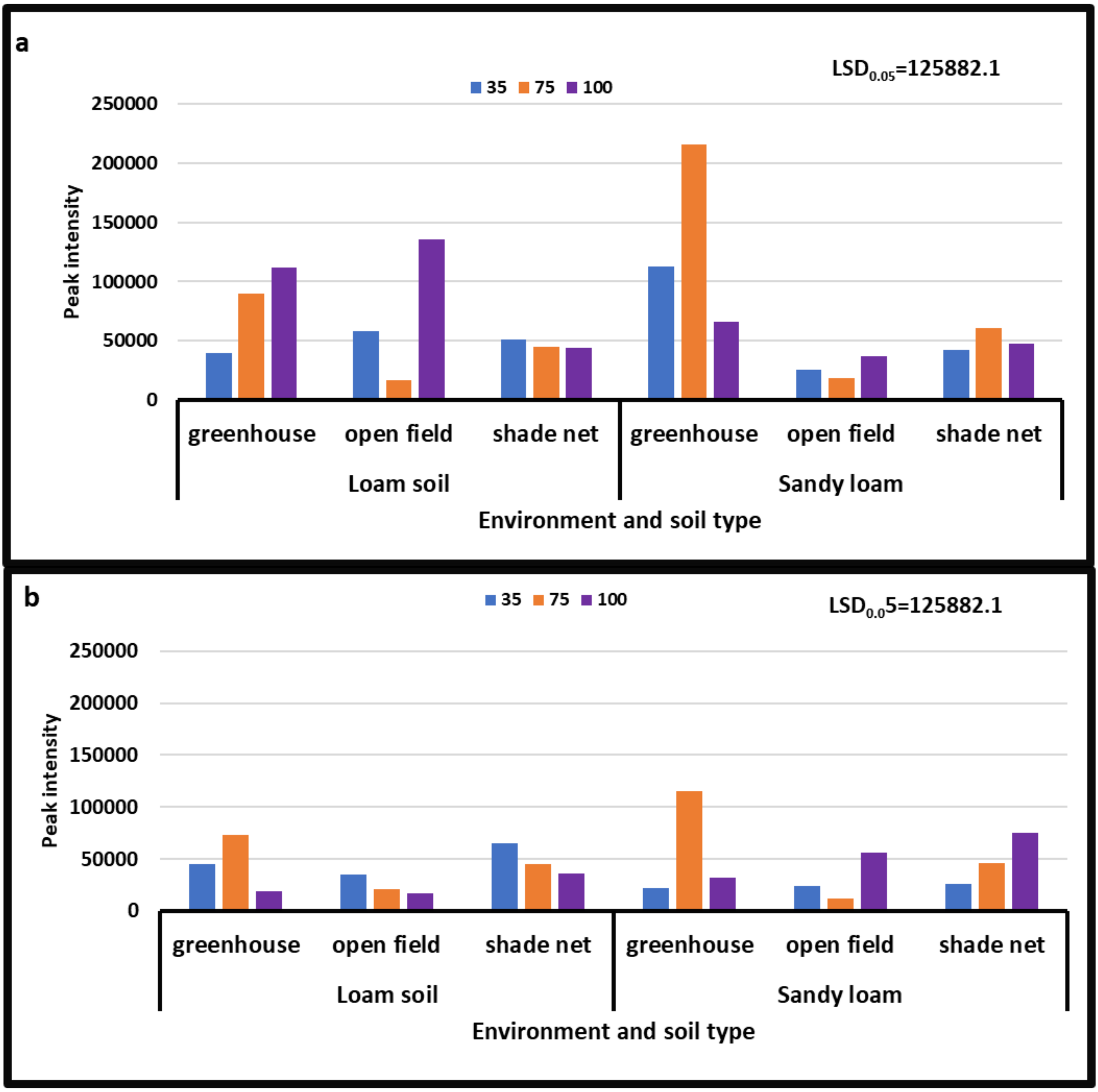

Figure 4.16: The treatment interaction effect on **Niacinamide** of *C. metuliferus* fruit during season one; 35 means severe water stress; 75 means moderate water stress; 100 means no water stress; **(a)** means treatment interaction of irrigation water regimes and soil types at different growing environments during the 2017/18 season; **(b)** means treatment interaction of water stress levels and soil types at different growing environments during the 2018/19 season. **Peak intensity showing LCMS-8040 triple quadrupole mass spectrometer intensities which represent the quantity of metabolites varying with treatments.** LSD$_{0.05}$ is the least significant difference of means.

Figure 4.16 unveils the profile of metabolite niacinamide on *C. metuliferus* fruit grown in different environments (greenhouse, shade net and open field), water stress levels (no water stress, moderate stress and severe water stress) and soil types (loamy soil and sandy loam) during both seasons. The results outlined that there was a significant ($P \leq 0.05$) difference between interaction of different water stress levels and soil types under different growing environments during both seasons. During the year 2017/18, niacinamide content ranged from 16,318 to 216,137 peak intensity, and in the year 2018/19, it ranged from 12,521 to 115, 147 peak intensity. Results illustrated that the treatment of moderate water stress combined with sandy loam under open field environment during the year [2018/19] decreased niacinamide content from 216,137 to 12,521 peak intensity (Figure 4.16b). The moderate water stress treatment combined with sandy loam under greenhouse environment during the year [2017/18] significantly increased this compound from 12,521 to 216,137 peak intensity during season one (Figure 4.16a).

4.4.9 LC-MS primary metabolites analysis using PCA analysis

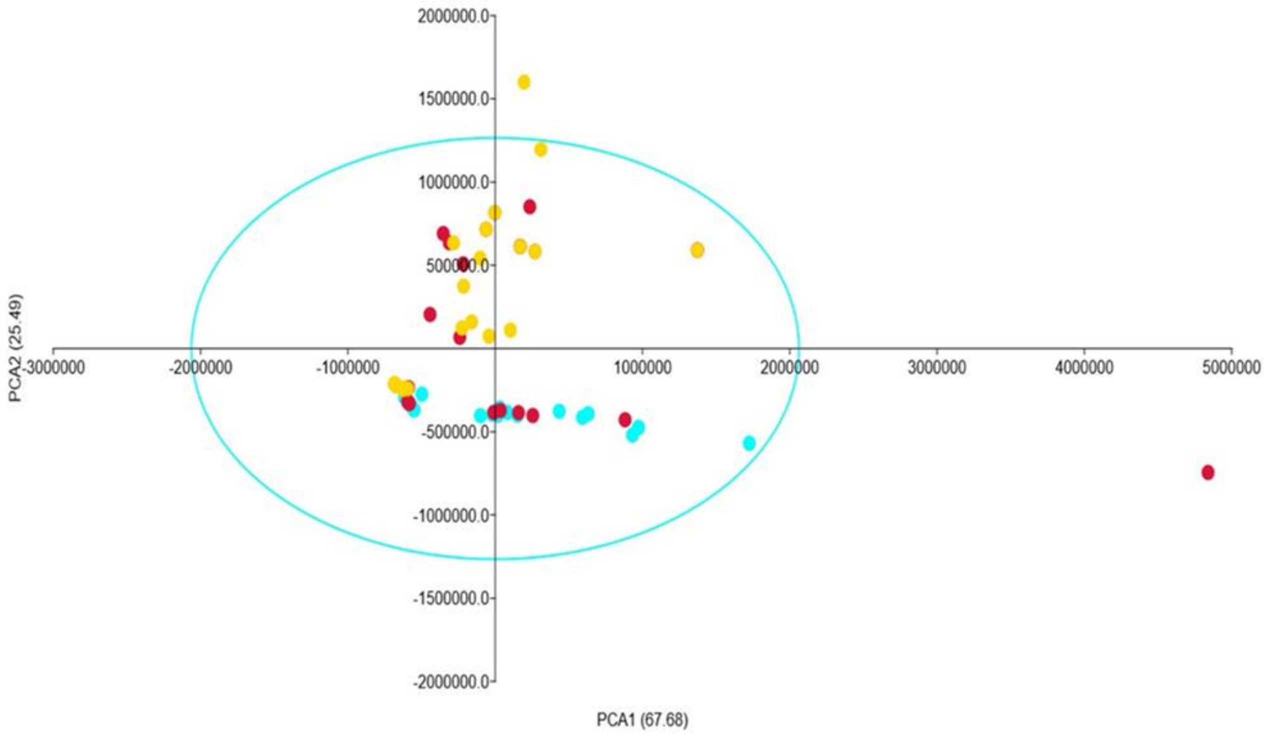

Figure 4.17: Principal component analysis of primary metabolites profile of *C. metuliferus* fruit harvested from different treatments. The explained variables are shown in different colours **(Blue = Greenhouse, Red = shade net, and Yellow = open field).**

Although different metabolites were detected by LC-MS chromatograms in the samples from different locations, data was then analysed more holistically using Principal Component Analysis (PCA) to explore the relative variability in the samples from different sites. Data from all 152 fruit samples were analysed, and clear separation was achieved (Figure 4.17). The samples from the open field environment presented the greatest differences, in that they were far apart from the other samples collected in the greenhouse and shade net environment (Figure 4.17). When contributing plots were constructed, it was clear that the metabolites abundance in greenhouse and shade net fruit was not significantly separated by the growing environment as compared to the open field growing environment.

Overall, the metabolites in the greenhouse and shade net did not show a distinct separation when compared to those of fruit harvested from the open field environment. Visual inspection of the contribution plot confirms that metabolites from open field clearly separated when compared to those of greenhouse and shade net (Figure 4.17).

4.4.10 Discussion

This study assessed the primary metabolite shifts of *C. metuliferus* fruit, under three different water stress levels (no water stress, moderate water stress and severe water stress) and environments (greenhouse, shade net and open space). To the best of our knowledge, this is the first study to assess metabolites shifts of *C. metuliferus*, therefore the findings serve as a benchmark.

The results obtained from the present study showed that interaction between water stress levels (no water stress, moderate water stress and severe water stress) and soil types (loamy soil and sandy loam soil) at different growing environments (greenhouse, shade net and open field) presented a significant difference on some primary metabolites content of *C. metuliferus* fruit, whereas some primary metabolites were significantly affected by either water stress levels or soil types under different environments. Previous study conducted by Zhang et al. (2011) and Bernillon et al. (2013) found significant differences in metabolites content of fruits harvested from different conditions such as temperature and differing growth locations. Asparagine has been reported as one of the most vital metabolites that play a central role such as nitrogen circulation and recycling in plant vegetative organs (Maco et al., 2006; Kim & Verpoorte, (2010); Weng et al., 2010; Fang, 2011) . It is also responsible for filling of nitrogen in fruit seeds (De vos et al., 2007; Gumi, 2012). Perhaps, asparagine was higher in fruit harvested from protected structures compared to open space could be because of low atmospheric nitrogen content in protected structures, as reported by (Dold & Cocks, 2002; Friedman (2006). They found that when atmospheric and soil nitrogen is low, the asparagine concentration tends to fluctuate in cucumber fruit. This argument was taken further by Hadid (2016) and Sarker et al. (2020) in cucumbers and melons, where they found varying asparagine content due to lower levels of nitrogen in the soil and greenhouse atmosphere.

Dopa is known to be a compound released by plants, primarily to inhibit neighbouring plants' growth and dominance (Guo et al., 2010; Zhao et al., 2016; Moing et al., 2011). Lisiewska et al. (2018) reported that abundance of dopa was prominent in crops grown under greenhouse conditions subjected to acidic soil compared to those of open field conditions. The actual cause for high content of this compound on fruit harvested from open space could be that higher fruit load, or competition within individual plants; therefore, dopa is synthesised as a response against the competition. This suggests that this metabolite will be altered when there is higher fruit number in a plant, since it is associated with inhibiting dominance from neighbouring fruit, as reported by Zhang et al. (2011). Their findings further confirm that when there is a higher fruit load in plants, there is an increase in dopa content in strawberry fruit.

Mukherjee et al. (2013) and Ahmad (2015) reported that dopa content often shifts immediately when there is limited space due to high fruit load in cucumbers. Similar findings were observed by Vinson et al. (2001), who concluded that metabolite dopa was high in oranges harvested from crops that were planted at lesser spacing, but lower on crops that were planted at longer spacing. In contrast, Ity and Canino (2006) noted that water stress and acidic level in soil were major causes of shift of this compound in apricots. When water and acid level is low, dopa increases in apricot fruit. This indicated that dopa content in fruit seems to respond to water stress and soil type used. These findings agree with those of the current study that water stress and soil types under different growing environments are directly responsible for dopa fluctuation in *C. metuliferus* fruit.

In this study, 4-hydroxyproline increase may be a response to normal watering under protected structures. Shu et al. (2013) identified the role of this compound as a vital constituent of plant cells, while researchers such as t'Kindt et al. (2008) explained that 4-hydroxyproline is a significant component in the cell membrane, since it contains glycoprotein that protects the cell wall.

The findings that when water content is high, 4-hydroxyproline significantly increased in fruit, but significantly decreased at low water levels, means that it is possible that plant cell membrane is affected by water availability within plant organs such as xylem and phloem. The current study results clearly demonstrate that 4-hydroxyproline fluctuation in fruit is directly linked to water availability and the growing environment.

Flemotomou (2011) showed significant variation in metabolites content of crops cultivated under protected structures and subjected to different water stress levels when compared to the control. Plants develop defensive mechanisms against excessive stress caused by environmental stresses (Sanchez et al., 2008; Savvides et al., 2012). In the present study, 5-glutamylcysteine abundance was higher in protected structures (greenhouse and shade net) when compared to open space environment; it is probably due to environmental factors in protected structures (greenhouse and shade net), since the crop naturally grows in the wild (Backeberg & Water, 2013). Biochemical activities such as enzymes are triggered by the differing environment conditions such as high humidity and excess water.

Regarding acetylcarnitine, the study findings revealed that a significant difference between treatment interactions of water stress levels and soil types under different growing conditions during all seasons. Perhaps, the major caused of alteration of this compound in fruit could be water stress, reduce transportation of solutes via xylem and phloem due to stomatal closure. Bernillon et al. (2013) mention that this compound serves as a carrier for transporting fatty acids into mitochondria for cell activities, subsequently improving plant health. Weng (2010) discovered that low temperature caused significant decrease in acetylcarnitine, but warm temperature increase its content in melons. Guo et al. (2010) found variation in acetylcarnitine content of cucumber fruit harvested from plants of different genetic traits subjected to different water levels. At normal water levels, acetylcarnitine increased significantly, but decreased under water stress. In the present study, acetylcarnitine significantly increased due to no water stress treatment and use of loam soil in the greenhouse environment.

Perhaps the actual cause for this significantly in greenhouse environment could be that the treatments imposed reduced evapotranspiration rate, subsequently resulting in improved osmotic pressure affecting dissolved mineral movement within xylem as reported by (El-Mageed & Semida, 2015).

Kynurenine significantly increased due to the moderate water stress treatment and in loamy soil, during the 2017/18 season one. Similar findings was reported by Gumi (2012), who found that this compound effectively inhibit ethylene in plant root tissue, consequently reducing fruit growth and development. The significant increase in abundance of this compound could be that moderate water stress combined with loamy soil created ideal conditions for anaerobic respiration of plant roots, as it was discovered by Kassu et al. (2017), who discovered that lower ethylene causes delay in tuber development.

In this study, norepinephrine content was not significantly affected by interaction of water stress levels and soil types under different growing environments. However, there was significant abundance of this metabolite at open space environment. Singh et al. (2017) explain the function of this compound as being involved in regulating physiological functions such as carbohydrate metabolism and stress tolerance. Variation in abundance of norepinephrine due to growing conditions could be linked with sunlight intensity. Under open space environment, norepinephrine significantly increased, but decreased under greenhouse and shade net conditions. Similar findings were observed by Savvides et al., (2012), who found that stomatal opening and closure caused by radiation has a direct influence on the abundance of norepinephrine in cucumber.

Zhang et al. (2011) explained the function of niacinamide as being responsible for protection of cell leakage and DNA damage caused by environmental stress. Evidence from the present study reveals that treatment of moderate stress combined with sandy loam under greenhouse environment significantly caused an increase in abundance of this compound when compared to other treatments. Variation in niacinamide content could be that greenhouse temperatures have negatively affected the enzymic activities, consequently affecting stomatal opening and closure, since the crop grows naturally in the wild, according to Osuji et al. (2015), who discovered that greenhouse-grown fruit has a higher metabolites content compared to open field crops. The findings of the current study demonstrated that growing conditions are the major cause of fluctuation in primary metabolites, thus affecting their abundance in fruit.

4.4.11 Conclusion

Minimal knowledge exist about the effect of different environment, soil types and irrigation on the metabolomic profile of *C. metuliferus* fruit. Researchers have different views on which method could be regarded as the most effective and accurate for metabolomic analysis. Abbey et al. (2017), for example, considered LC-MS as the most effective and accurate method in analysis of primary metabolites of fruit, whereas Morreel et al. (2009) found that the GC-MS was the most preferred method because of its ability to identify most metabolites, compared to other methods. This study adopted the (application of metabolomics) LC-MS approach to identify and quantify the metabolites found on *C. metuliferus* cultivated in differing locations, water levels and different substrates, because of its accuracy in determining target metabolites in biological samples.

The study findings showed that primary metabolites shift of *C. metuliferus* fruit are indeed affected by different treatment combinations, such as different water stress levels and soil types at varying growing conditions. The key findings of this study are as follows:

- Primary metabolites such as asparagine, dopa, 4-hydroxyproline, 5-glutamylcystiene, acetylcarnitine, kynurenine and niacinamide were higher in protected growth structures when compared to open field conditions, but norepinephrine was higher in open field conditions when compared to protected growth structures (greenhouse and shade net) conditions. This great shift in metabolites profile could be a possible explanation of yield variation between protected growth structures (greenhouse and shade net) compared to open field. This means that understanding of the optimum growing conditions of *C. metuliferus* will reduce the metabolites shifts, and consequently improve yield, fruit quality and returns.

Since the results of this study are the first in profiling primary metabolites shift of *C. metuliferus* fruit under different environmental conditions, the future studies should consider the following:

- ➢ Assess the role of metabolites in individual sugars content of *C. metuliferus* E. Mey. ex naudin fruit harvested under different treatments, since total soluble sugars are not the only indication of quality in fruits.
- ➢ Assess the impact of different breeding methods in fruit nutritional quality *of C. metuliferus* fruit for quality improvement.
- ➢ The effect of different fertilizers' level on metabolites shift of *C. metuliferus* fruit quality for better fruit quality.
- ➢ Analyse medicinal properties of African horned cucumber fruit harvested from different environmental conditions.

The abovementioned studies should be carried out, since individual sugars are not the only reflection on the overall quality of the crop.

4.4.12 References

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CHAPTER 5: SUMMARY AND FUTURE WORK

5.1 GENERAL

In this thesis, it has been shown that water scarification, certification and growth media influence the germination success of *C. metuliferus* seeds. The treatment interaction (growing environment, irrigation water regimes and soil types) have a direct impact on the growth, development and yield of *C. metuliferus* crop. Furthermore, results also revealed that treatment interaction also affects the nutritional quality and primary metabolite profile in the fruit. The summary of each theme and the associated future work are presented below.

5.1.1 Germination of *C. metuliferus* seeds

The optimum conditions for successful propagation of *C. metuliferus* seeds are unknown. This study demonstrated that a combination of water-soaked and certified seeds grown in either sand mixed with vermiculite, peat or potting mix, are suitable germination regimes for *C. metuliferus* seeds. The best performing combination of seed type and medium were scarified and certified seeds grown in sand mixed with vermiculite and the potting mix. The second best performing combination were scarified and certified seeds growing in peat. This study demonstrated that a combination of water-soaked and certified seeds grown in either sand plus vermiculite, peat or potting mix are suitable germination regimes for the seeds. The medium has a secondary role in germination success. The primary determinant is whether the seeds are certified and treated prior to sowing. Growers are therefore encouraged to source certified seeds despite pricing, and soak them in warm water to promote germination. Scarification of seeds in warm water is a more practical, affordable and reliable treatment. Since the seedling-rootball integrity is vital for eventual transplant survival, this study advocates for peat as the best medium, because peaty soils have the most stable seedling root ball.

5.1.2 Water use efficiency of *C. metuliferus*

This study showed that to maximise the yield of the *C. metuliferus*, it is important to understand the effect of water stress on measured parameters such as total biomass, above-ground biomass, harvest index, fruit number and length, since they affect water productivity of the crop.

The total biomass increased in the open space environment when combined with the no water stress treatment under sandy loam soil growing conditions. The shade net combined with severe water stress treatment and loamy soil decreased total biomass. The results further illustrated that above ground biomass from the greenhouse plants combined with the no water stress irrigation treatment on loamy soil, was higher when compared to other treatments; while the shade net combined with irrigation water regimes moderate water stress treatment on loamy soil, during decreased above-ground biomass.

Concerning harvest index, results showed that open field combined with moderate water stress irrigation and sandy loam soil was superior to other treatments in the harvest index parameter. The open field combined with the no water stress irrigation treatment on loamy soil, resulted in decreased harvest index.

In terms of water use efficiency (WUE), the results showed that the open field combined with no water stress irrigation and sandy loam gave the highest WUE when compared to other treatments.

The key findings on WUE were as follows:

- Severe stress negatively affects the biophysiological activities of the crop.
- Growing the crop in the open space environment, combined with no water or moderate water stress, increased yield components such as fruit number, fruit length, total biomass and harvest index.
- The WUE was higher on open field combined with the no water stress treatment on sandy loam soil.

5.1.3 Nutritional composition of *C. metuliferus* fruit

Irrigation water levels, treatment of severe water stress and moderate water stress regimes, total soluble sugars content increased, as opposed to the control. Fruit grown under shade net and open field environment had increased crude protein content when compared to greenhouse environment. It can also be reported that the severe water stress treatment combined with loamy soil under open field conditions showed a decrease in β-carotene content, but the severe water stress treatment combined with using loamy soil under greenhouse environment increased it.

The severe water stress treatment combined with sandy loam soil under open field environment resulted in decreased vitamin C content, but, on the contrary, the treatment of no water stress combined with sandy loam at shade net conditions increased it. However, an analysis of vitamin E when subjected to severe water stress combined with loamy soil at open field location, decreased the vitamin content concerned.

For fruit total flavonoids content, results illustrated that treatment of severe water stress combined with sandy loam soil under greenhouse environment decreased it, whereas treatment of severe water stress combined with loamy soil under open field indicated an increase. Total phenolic content under the treatment of no water stress combined with loamy soil at greenhouse environment decreased, whereas treatment of no water stress combined with loamy soil increased phenolic content.

Macro- and micro-nutrient content, Mg, P, Na and Zn, were significantly affected by treatments imposed, whereas Ca, K, S, Cu, Fu and Mn were not affected by treatments. The study findings have shown that growing conditions, irrigation water levels and soil types have a direct impact on the *C. metuliferus* E. Mey. ex naudin nutrient content.

5.1.4 Primary metabolites of *C. metuliferus* fruit

The study showed that primary metabolites shift of *C. metuliferus* fruit are mostly affected by different treatment combinations, such as different irrigation water levels and soil types at varying growing conditions. It was important to understand the influence of different growing environments on primary metabolites shift, since they affect plant growth, development and yield. The results were conclusive, in that there was a great shift in primary metabolites of *C. metuliferus* fruit from plants grown in protected structures compared to those under open field growing environment.

The key findings of this study were the following: Primary metabolites such as asparagine, dopa, 4-hydroxyproline, 5-glutamylcystiene, acetylcarnitine, kynurenine and niacinamide were significantly higher in crops grown under protected growth than in the open field environment, whereas metabolite norepinephrine was higher in open field environment when compared to protected growth structures (greenhouse and shade net environment).

5.2 Future work

Germination of *C. metuliferus* E. Mey. ex Naudin

The findings of the current study are the first in determining the effect of certification, growth media and scarification on germination of *C. metuliferus* seeds. It is suggested that future studies should consider the following:

- The effect of seeds' storage period on germination success of *C. metuliferus* seeds.
- The effect of different temperature and moisture levels on the germination success of *C. metuliferus* seeds.
- Investigate suitable breeding method to improve germination success and yield of *C. metuliferus* seeds for potential commercialisation.

Water use efficiency (WUE) of *C. metuliferus* E. Mey. ex Naudin

This research has provided evidence that the *C. metuliferus* can perform optimally under open space environment because the WUE of the plants improved, and there was an increase in total biomass, harvest index and fruit number.The crop's physiological performance is also noteworthy, judging from the increased photosynthetic and stomatal activity. When grown in the open space environment, the numbers of fruits can be increased, this is important for fresh markets, where many fruits are required to meet the demand. However, there is higher reduction of WUE on the protected structures under loamy soil and stress water level. It is recommended that future studies should consider the following:

- Investigate the WUE of *C. metuliferus* under open field conditions. It would be interesting to see what happen regarding the actual evapotranspiration and total yield.
- In addition, crop models are key in agronomy, it would be interesting to calibrate and validate crop models for *C. metuliferus*.

Nutritional composition of *C. metuliferus* E. Mey. ex Naudin fruit

The results of the current study are the first in determining the nutritional composition of *C. metuliferus* fruit under different environments, varying irrigation water levels and soil types. It is suggested that future studies consider the following:

- This study assessed the nutritional composition of *C. metuliferus* from different environments (greenhouse, shade net and open field), soil types (loamy soil and sandy loam) and irrigation water levels (no water stress, moderate stress and severe stress). A future study should be conducted to assess the effect of different fertilizers' level on the *C. metuliferus* fruit, and such study should also consider location and water stress levels.
- In this study, phytochemicals such as total flavonoids, total phenols, crude proteins, vitamins and β-carotene were quantified. It is suggested that further studies be conducted to determine potential products that could be developed and used in the food and pharmaceutical industry – that is, from this crop.

- It may also be worthwhile to assess what would be the optimum postharvest storage temperature and period on nutrient fruit quality of this crop plant.
- It may also be interesting to assess what would the nutrient concentration of *C. metuliferus* fruit cultivation in the open field conditions under different water stress levels.

Primary metabolite profile of *C. metuliferus* E. Mey. ex Naudin fruit

Since the results of this study are the first in profiling primary metabolites shift of *C. metuliferus* fruit under different environmental conditions, it is suggested that future studies consider the following:

- Assess the role of metabolites in individual sugars content of C. *metuliferus* fruit harvested under different treatments, for quality improvement.
- Investigate different breeding methods for quality improvement parameters such as shape and taste, for fresh market purposes.

5.3 Contribution to the agricultural field/or body of knowledge

This work has provided solid evidence that *C. metuliferus* can be manipulated to produce biomass and better-quality fruit under optimal growing conditions such as irrigation water levels (no water stress and moderate stress) and sandy loam soil under open field and shade net conditions. In addition, this research has provided evidence that the *C. metuliferus* fruit quality is significantly affected by treatments. This is useful information to farmers, as quality has become more significant to most consumers worldwide. When grown in the open field, total soluble sugars increased; this is important for the juice-manufacturing industry. The number of fruits can be increased under open field space compare to protected structures; this is important for fresh markets, where many fruits are required to meet the demand.

Furthermore, quality parameters such as metabolites seem to increase under protected structures (greenhouse and under shade net). This is an important finding, as metabolites influence the flavour of fruits. Where the market is geared towards organoleptic quality – in expensive markets, for example, it may be best to grow this crop under protected structures. The other advantage for protected strcures is that the crop is protected from rainfall and extreme heat in summer.

Based on the findings of the current study, there is a great potential to commercialise this crop. However, there is still a much that is not well understood of its growth habits and biological / biochemical constituents as a future alternative crop for food and medicine.

APPENDIX I: Treatment effect on the seed germination days of *C. metuliferus*.

	Germination by day			
Treatment	**Day 0-10**	**Day 10-15**	**Day 15-25**	**Day 25-30**
2017/18				
M1NTC	0.47(1.2)	0.07(1.1)	0.16(0.4)	0.30(0.2)
M1TC	0.14(1.1)	0.39(0.3)	0.23(0.1)	0.25(1.1)
M1TUC	0.14(0.2)	0.43(1.1)	0.43(1.1)	0.00(0.0)
M1UTU	0.00(0.0)	0.04(0.2)	0.09(1.1)	0,11(1.1)
M2NTC	1.00(1.1)	0.00(0.0)	0.00(0.3)	0.00(0.0)
M2TC	0.00(0.0)	0.00(0.0)	0.62(1.1)	0.36(1.1)
M2TUC	0.00(0.0)	0.00(0.0)	0.43(1.1)	0.58(1.1)
M2UTU	0.00(0.0)	0.00(0.0)	0.45(0.1)	0.11(0.2)
M3NTC	0.56(0.0)	0.12(1.1)	0.08(0.2)	0.00(0.0)
M3TC	0.72(1.2)	0.12(0.2)	0.10(0.1)	0.00(0.0)
M3TUC	0.17(1.2)	0.43(0.3)	0.4(0.1)	0.05(0.1)
M3UTU	0.00(0.1)	0.00(0.0)	0.24(0.2)	0.15(1.1)
LSD0.05	0.736	0.736	0.736	0.736
Pvalue	0.51	0.51	0.51	0.51
2018/19				
M1NTC	0.44(1.1)	0.21(0.2)	0.10(0.1)	0.00(1.1)
M1TC	0.52(1.0)	0.25(0.1)	0.12(0.1)	0.00(0.0)
M1TUC	0.00(0.0)	0.00(0.0)	0.42(0.0)	0.21(0.2)
M1UTU	0.00(0.0)	0.00(0.0)	0.35(1.1)	0.18(1.1)
M2NTC	0.51(1.1)	0.27(1.1)	0.10(1.1)	0.00(0.0)
M2TC	0.74(1.2)	0.12(1.1)	0.14(1.1)	0.00(1.1)
M2TUC	0.00(0.0)	0.00(0.0)	0.32(1.1)	0.24(1.1)
M2UTU	0.00(0.0)	0.00(0.0)	0.42(1.1)	0.19(0.1)
M3NTC	0.65(1.3)	0.32(1.1)	0.11(1.1)	0.00(0.0)
M3TC	0.72(1.1)	0.20(1.1)	0.08(0.1)	0.00(0.0)
M3TUC	0.00(0.0)	0.44(0.2)	0.12(1.1)	0.00(0.0)
M3UTU	0.00(0.0)	0.00(0.0)	0.32(1.1)	0.22(1.0)
LSD0.05	0.736	0.736	0.736	0.736
Pvalue	0.51	0.51	0.51	0.51

M1 means sand+vermiculite; **M2** means peat TS1; **M3** means potting mix. **NTC** means non-treated certified seeds; **TC** means treated certified seeds by supplier; **TUC** means treated uncertified seeds; UTU means untreated uncertified seeds. Numbers in brackets represent the standard deviations of the mean. $LSD_{0.05}$ is the least significant difference of means. Years (seasons one – 2017/18 and season two – 2018/2019). P values in bold are lower than 0.05.

APPENDIX II: Treatment interaction effer on chlorophyll content of *C. metuliferus*

	Chlorophyll content					
	2017/2018			2018/2019		
Treatment	Preflowering	Flowering	Fruiting	Preflowering	Flowering	Fruiting
W1S1L1	31,4(0.1)	37,38(1.3)	47,8(1.1)	32,9(1.1)	41,1(1.1)	58,1(1.0)
W1S1L2	32,9(0.0)	41,27(1.1)	55,8(1.1)	37,9(1.3)	45,5(1.2)	66,2(1.3)
W1S1L3	35,8(0.1)	42,42(0.1)	64,6(1.0)	37,43(0.3)	46,5(1.1)	67,1(1.1)
W1S2L1	29,2(0.2)	26,42(1.1)	46,7(1.1)	27,55(1.1)	49,4(1.2)	60,2(1.3)
W1S2L2	32,9(0.1)	41,27(0.1)	63,5(0.0)	36,8(2.3)	48,8(1.1)	70,5(1.2)
W1S2L3	36,8(0.0)	45,82(1.1)	71,7(0.1)	39,58(1.2)	43,9(1.2)	73,8(1.2)
W2S1L1	26,1(1.1)	37,17(0.1)	51,6(0.0)	34,4(0.2)	47,5(1.2)	61,4(1.2)
W2S1L2	27,1(0.1)	36,08(0.0)	37,2(1.1)	34,65(1.1)	46,2(1.2)	52,4(1.4)
W2S1L3	36,4(1.1)	44,72(1.1)	46,3(0.1)	39,58(1.3)	49,5(1.2)	64,7(1.1)
W2S2L1	31,9(1.0)	41,42(0.1)	50,3(1.1)	34,4(1.5)	49,4(1.1)	65,9(1.2)
W2S2L2	25,6(1.1)	37,95(0.1)	43,1(0.0)	34,65(1.1)	50,1(1.1)	61,5(1.2)
W2S2L3	34,1(0.0)	36,77(1.1)	46,4(1.1)	39,33(1.2)	53,1(1.1)	58,8(1.1)
W3S1L1	22,9(1.1)	25,73(0.1)	40,7(1.1)	27,9(1.4)	48,9(1.1)	44,4(1.2)
W3S1L2	20,6(0.1)	24,88(0.0)	35,3(0.1)	29,5(1.2)	40,2(1.0)	46,5(1.1)
W3S1L3	25,7(1.1)	25,15(1.1)	40,6(1.2)	39,33(1.4)	46,3(1.2)	44,8(1.1)
W3S2L1	23,3(0.1)	26,42(0.1)	36,6(1.2)	27,55(1.4)	47,6(1.1)	45,8(1.2)
W3S2L2	23,3(1.1)	24,88(1.1)	36,4(1.0)	29,5(1.2)	42,5(1.2)	42,2(1.2)
W3S2L3	23,8(0.0)	24,82(1.1)	36,6(1.3)	33,33(1.1)	54,2(1.4)	43,87(1.1)
Grand mean	**41,44**	**41,44**	**41,44**	**41,44**	**41,44**	**41,44**
LSD0,05	**4,134**	**4,134**	**4,134**	**4,134**	**4,134**	**4,134**
P_{value}	**0,995**	**0,995**	**0,995**	**0,995**	**0,995**	**0,995**

W1 means no water stress; **W2** means moderate water stress; **W3** means severe water stress. **S1** means loamy soil and **S2** means sandy loam. **L1** means greenhouse. **L2** means shade net. **L3** mean open field. Numbers in brackets represent the standard deviations of the mean. $LSD_{0.05}$ is the least significant difference of means. Years (seasons one – 2017/18 and season two – 2018/2019). P values in bold are lower than 0.05. $LSD_{0.05}$ is the least significant difference of means.

APPENDIX III: Treatment effect of different water levels and soil types during different seasons on the fruit length and number of *C. metuliferus*.

	Total Fruit number		Fruits length	
Treatment	**2017/18**	**2018/19**	**2017/18**	**2018/19**
W1S1L1	6(2)	8(3)	78(9.6)	110(24.1)
W1S1L2	5(3)	13(2)	59(22.4)	99(11.8))
W1S1L3	20(4)	22(6)	208(18.3)	102(66.4)
W1S2L1	10(1)	11(4)	177(40.2)	175(29.8)
W1S2L2	11(2)	24(2)	128(14.2)	134(18.5)
W1S2L3	25(3)	39(9)	249(30.4)	144(15.4)
W2S1L1	7(3)	11(4)	94(71.2)	113(12.6)
W2S1L2	5(4)	13(2)	49(45.1)	99(51.4)
W2S2L3	16(10)	24(7)	154(121.3)	111(42.2)
W2S2L1	13(3)	22(3)	200(18.7)	202(35.2)
W2S2L2	10(5)	24(4)	120(9.3)	140(29.5)
W2S2L3	22(6)	41(8)	181(68.1)	149(16.5)
W3S1L1	5(3)	8(3)	58(61.2)	96(26.6)
W3S1L2	5(4)	9(4)	55(18.2)	67(48.3)
W3S1L3	15(7)	26(9)	136(21.2)	109(44.8)
W3S2L1	7(3)	16(3)	87(45.7)	131(23.2)
W3S2L2	12(2)	20(4)	127(27.5)	112(48.5)
W3S2L3	18(6)	40(8)	200(28.9)	129(26.4)
LSD0.05	3.5	3.5	30.55	30.55
P_{value}	0.39	0.39	0.955	0.955

W1 means no water stress; **W2** means moderate water stress; **W3** means severe water stress. **S1** means loamy soil and **S2** means sandy loam. **L1** means greenhouse. **L2** means shade net. **L3** mean open field. Numbers in brackets represent the standard deviations of the mean. $LSD_{0.05}$ is the least significant difference of means. Years (seasons one – 2017/18 and season two – 2018/2019). P values in bold are lower than 0.05. $LSD_{0.05}$ is the least significant difference of means.

APPENDIX IV: Treatment interaction on stomatal conductance of *C. metuliferus*.

	Stomatal conductance					
	2017/2018			2018/2019		
Treatment	Preflowering stage	Flowering stage	Fruiting stage	Preflowering stage	Flowering stage	Fruiting stage
W1S1L1	41,2(1.1)	47,1(1.1)	40,2(1.1)	43,2(1.4)	46,5(1.2)	44,2(1.1)
W1S1L2	46,4(1.2)	49,3(0.0)	37,1(2.3)	42,2(0.2)	48,2(1.1)	35,1(1.4)
W1S1L3	41,2(0.5)	47,2(1.3)	36,9(1.4)	40,4(1.1)	45,7(0.4)	37,2(2.2)
W1S2L1	39,0(1.1)	50,0(1.2)	38,8(0.5)	36,2(1.3)	47,3(1.4)	36,3(1.4)
W1S2L2	45,3(1.1)	56,2(1.2)	38,1(1.1)	42,5(1.4)	51,0(1.1)	34,9(0.4)
W1S2L3	43,7(1.3)	46,0(1.4)	36,4(1.3)	40,4(2.3)	46,2(1.3)	35,1(2.1)
W2S1L1	40,7(1.2)	51,5(1.3)	36,7(1.1)	44,3(1.1)	50,4(0.7)	36,2(3.2)
W2S1L2	41,9(1.1)	45,6(1.3)	38,3(1.4)	43,6(1.4)	48,2(1.2)	37,3(1.1)
W2S2L3	40,7(1.3)	44,9(1.6)	38,4(1.7)	41,8(1.3)	42,6(1.1)	34,3(1.4)
W2S2L1	43,3(1.2)	50,3(1.1)	37,4(1.2)	39,4(1.4)	52,4(1.3)	37,4(1.2)
W2S2L2	41,6(1.1)	44,2(1.3)	39,3(1.6)	44,3(1.3)	45,3(1.1)	36,6(3.2)
W2S2L3	41,6(1.4)	43,0(1.1)	67,6(1.4)	38,4(1.1)	44,3(2.4)	59,7(2.1)
W3S1L1	37,1(1.5)	38,7(1.4)	37,4(1.0)	35,5(1.2)	39,4(1.6)	35,7(1.4)
W3S1L2	36,4(1.3)	35,9(1.1)	38,0(0.0)	37,6(2.3)	33,5(2.3)	36,7(2.2)
W3S1L3	36,5(1.3)	41,8(1.3)	35,5(1.4)	34,3(0.8)	39,7(2.4)	37,4(2.1)
W3S2L1	34,8(1.2)	37,6(1.4)	37(0.1)	39,3(1.2)	34,2(1.4)	36,4(1.1)
W3S2L2	36,9(1.3)	37,9(1.1)	36,0(1.2)	34,2(1.1)	34,2(1.5)	36,5(2.2)
W3S2L3	38,2(1.4)	35,2(1.3)	36,7	38,2(0.5)	37,6(2.2)	38,2(1.4)
Grand mean	**41,41**	**41,41**	**41,41**	**41,41**	**41,41**	**41,41**
LSD0,05	**11,674**	**11,674**	**11,674**	**11,674**	**11,674**	**11,674**
P_{value}	**0,58**	**0,58**	**0,58**	**0,58**	**0,58**	**0,58**

W1 means no water stress; **W2** means moderate water stress; **W3** means severe water stress. **S1** means loamy soil and **S2** means sandy loam. **L1** means greenhouse. **L2** means shade net. **L3** mean open field. Numbers in brackets represent the standard deviations of the mean. $LSD_{0.05}$ is the least significant difference of means. Years (seasons one – 2017/18 and season two – 2018/2019). P values in bold are lower than 0.05. WUE means water use efficiency. $LSD_{0.05}$ is the least significant difference of means.

APPENDIX V: ETHICS APPROAVAL

UNISA university of south africa

UNISA-CAES HEALTH RESEARCH ETHICS COMMITTEE

Date: 18/11/2019

Dear Mr Maluleke

NHREC Registration # : REC-170616-051
REC Reference # : 2017/CAES/127
Name : Mr MK Maluleke
Student #: 53376544

Decision: Ethics Approval Renewal after Second Review from 01/10/2019 to 30/09/2020

Researcher(s): Mr MK Maluleke
malulm@unisa.ac.za

Supervisor (s): Prof D Modise
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Prof SJ Moja
sjmoja@gmail.com; (012) 841-1485

Dr M Nyathi
mnnyathi@arc.agric.za; 072-683-3254

Working title of research:

Investigation of the performance and quality of the African horned cucumber (*Cucumis metuliferus*) under protected and field conditions, for potential commercialisation

Qualification: PhD Agriculture

Thank you for the submission of your progress report to the UNISA-CAES Health Research Ethics Committee for the above mentioned research. Ethics approval is renewed for a one-year period. After one year the researcher is required to submit a progress report, upon which the ethics clearance may be renewed for another year.

Due date for progress report: 30 September 2020

*The **low risk application** was **reviewed** by the UNISA-CAES Health Research Ethics Committee on 07 September 2017 in compliance with the Unisa Policy on Research Ethics and the Standard Operating Procedure on Research Ethics Risk Assessment*

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The proposed research may now commence with the provisions that:

1. The researcher(s) will ensure that the research project adheres to the values and principles expressed in the UNISA Policy on Research Ethics.
2. Any adverse circumstance arising in the undertaking of the research project that is relevant to the ethicality of the study should be communicated in writing to the Committee.
3. The researcher(s) will conduct the study according to the methods and procedures set out in the approved application.
4. The researcher will ensure that the research project adheres to any applicable national legislation, professional codes of conduct, institutional guidelines and scientific standards relevant to the specific field of study. Adherence to the following South African legislation is important, if applicable: Protection of Personal Information Act, no 4 of 2013; Children's act no 38 of 2005 and the National Health Act, no 61 of 2003.
5. Only de-identified research data may be used for secondary research purposes in future on condition that the research objectives are similar to those of the original research. Secondary use of identifiable human research data require additional ethics clearance.
6. No field work activities may continue after the expiry date. Submission of a completed research ethics progress report will constitute an application for renewal of Ethics Research Committee approval.

Note:

*The reference number **2017/CAES/127** should be clearly indicated on all forms of communication with the intended research participants, as well as with the Committee.*

Yours sincerely,

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APPENDIX VI: TURNITIN ORIGINALITY REPORT

Turnitin Originality Report

PhD Agriculture thesis by Mk Maluleke

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APPENDIX VII: PROOF OF LANGUAGE EDITING

2 December 2020

I, Marlette van der Merwe, hereby certify that both the text and list of references of the doctoral thesis titled "Investigation of the performance and quality of the *Cucumis metuliferus* L (African horned cucumber) under protected and field conditions, for potential commercialisation" have been edited by me, according to the APA referencing method as required by the College of Agriculture, University of South Africa.

Marlette van der Merwe

BA (English) HDipLib (UCT)

APPENDIX VIII: ARTICLE NUMBER 1 (SUBMITTED)

Title: Impact of certification, growth medium and seed treatment on germination and growth of *Cucumis metuliferus* (African horned cucumber) seeds

Authors: Mdungazi K Maluleke[1], Lesego Khomo[1], David M Modise[2], Shadung J Moja[3] and Melvin Nyathi[3]

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Corresponding author: 011 471 3838/ 076 101 2482 or malulm@unisa.ac.za/ knox2mdu@gmail.com

Journal: *Scientia Horticulturae*

Dear Dr lesego khomo:

Your above referenced manuscript, entitled "Impact of certification, growth medium and scarification on germination of the African horned cucumber" requires some further changes before it is ready for reviewing in South African Journal of Plant and Soil. Your submission has been returned to you and is located in your Author Centre as a draft, so that you can make the required changes to it and submit it again.

Ensure that the reference list is in line with the journal's requirements.
Please re-do Figure 1 or present the data in a different manner. The current graph is incomprehensible.

Your submission along with all files you submitted is now in your Author Centre, at https://mc.manuscriptcentral.com/sajps Please read the Quick Guide to Continuing your Submission, which shows how you can access your manuscript, and submit it back to the site. The Guide is located at http://mc.manuscriptcentral.com/societyimages/tandf_qs0/Continuning%20a%20Submission_screenshot.pdf

Your manuscript must be set out in our journal’s style before we will review it. Please revise it in line with our style, and submit an improved manuscript. See the Instructions for Authors for details, which can be reached via the “Instructions & Forms” link at the top right of this page.

You may contact the Editorial Office if you have further questions.

Sincerely,

South African Journal of Plant and Soil Editorial Office

APPENDIX IX: ARTICLE NUMBER 2 (PUBLISHED)

horticulturae

MDPI

Article

Nutrient Concentration of African Horned Cucumber (*Cucumis metuliferus* L) Fruit under Different Soil Types, Environments, and Varying Irrigation Water Levels

Mdungazi K Maluleke [1,*], Shadung J Moja [2], Melvin Nyathi [3] and David M Modise [4]

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Abstract: The nutrient concentration of most crops depends on factors such as amount of water, growing environment, sunlight, and soil types. However, the factors influencing nutrient concentration of African horned cucumber fruit are not yet known. The objective of the study was to determine the effect of different water stress levels, soil types, and growing environments on the nutrient concentration of African horned cucumber fruit. Freeze-dried fruit samples were used in the quantification of *β-carotene* and total soluble sugars. The results demonstrated that plants grown under the shade net, combined with severe water stress level and loamy soil, had increased total soluble sugars (from 8 to 16 °Brix). Under the shade-net environment, the combination of moderate water stress level and loamy soil resulted in increased crude protein content (from 6.22 to 6.34% °Brix). In addition, the severe water stress treatment combined with loamy soil, under greenhouse conditions, resulted in increased *β-carotene* content (from 1.5 to 1.7 mg 100 g^{-1} DW). The results showed that African horned cucumber fruits are nutrient-dense when grown under moderate water stress treatment on the loamy or sandy loam substrate in the shade-net and open-field environments.

Keywords: biochemical constituents; *β-carotene*; vitamins; micro-nutrients; growing environments

Citation: Maluleke, M.K.; Moja, S.J.; Nyathi, M.; Modise, D.M. Nutrient Concentration of African Horned Cucumber (*Cucumis metuliferus* L) Fruit under Different Soil Types, Environments, and Varying Irrigation Water Levels. *Horticulturae* **2021**, *7*, 76. https://doi.org/10.3390/horticulturae7040076

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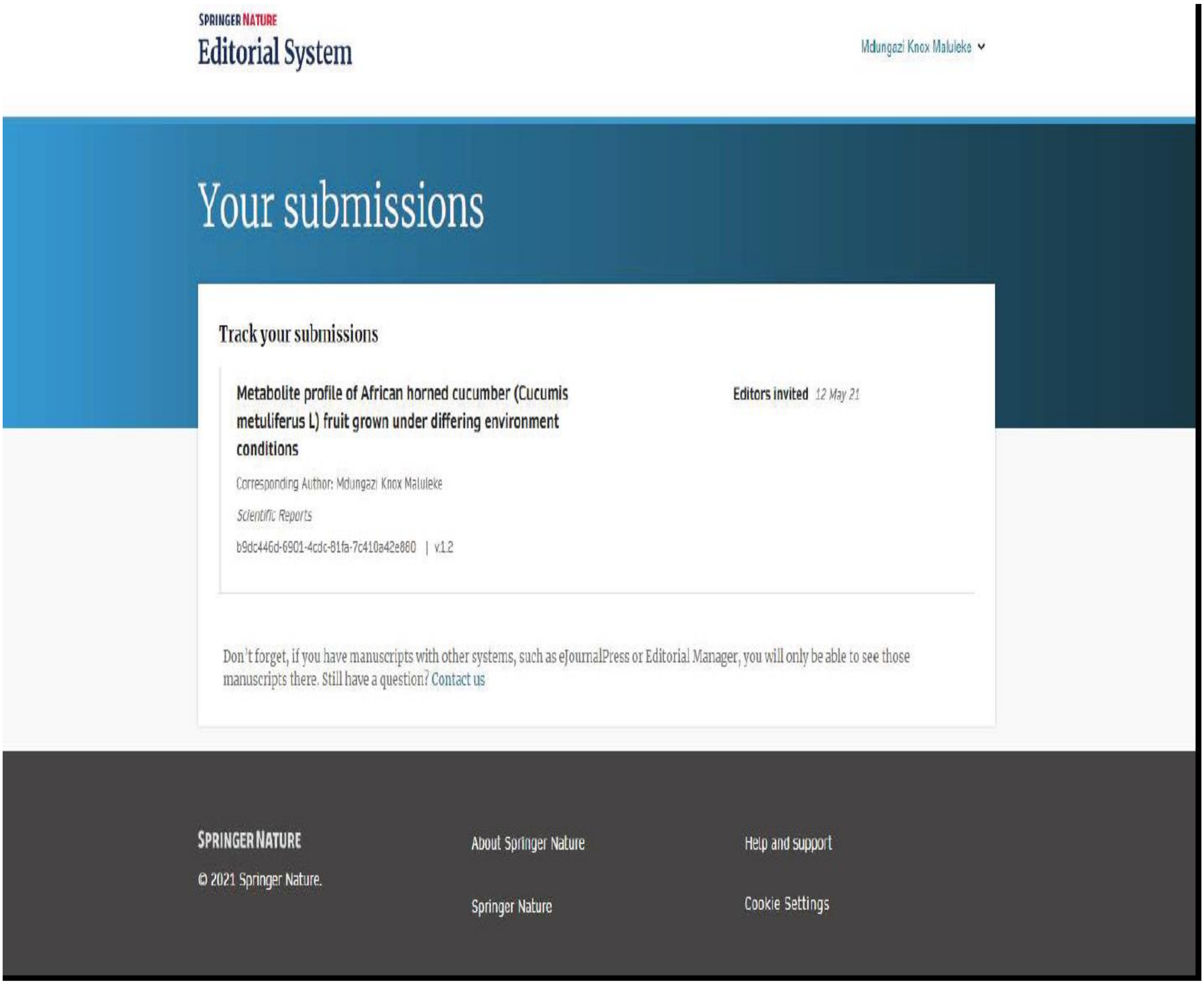
APPENDIX X: ARTICLE NUMBER 3 (SUBMITTED)



APPENDIX XI: ARTICLE NUMBER 4: (DRAFTED)

Article 4: Water use efficiency of *C. metuliferus* (African horned cucumber) under three different growing environments

Authors: Mdungazi K Maluleke[1]. Melvin Nyathi[2]. David M Modise[3]. Shadung J Moja[4].

Targeted Journal: *Agricultural Water Management*