The effect of a non-ionic surfactant on the growth and development of tomato and wheat plants under deficit irrigation.

by

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A project submitted in partial fulfilment of the requirements for the degree MSc (Agric): Horticultural Sciences

in the

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Declaration

I hereby certify that this Dissertation is my own work, except where duly acknowledged. I also certify that no plagiarism was committed in writing this dissertation.

Signed______________________

(Full names)

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Abbreviation	**Definition**
ARC	Agricultural Research Council
C1/Control 1	Control at irrigation rate of 100% with no added surfactant
C2/Control 2	Control at irrigation rate of 75% with no added surfactant
C2/Control 3	Control at irrigation rate of 50% with no added surfactant
DD	Tomato and wheat seed receiving 10 ml water mixed with NIS at 0.48%
ERI	Emergence rate index
G	Final germination percentage
Germ Synch	Germination synchronicity
GS	Germination speed
HD	Tomato and wheat seed receiving 10 ml water mixed with NIS at 0.06%
IRRI	Irrigation
LSD	Least significant difference
MGR	Mean germination rate
MGT	Mean germination time
NIS	Non-ionic surfactant
RD1	Tomato and wheat seed receiving 10 ml water mixed with NIS at 0.12%
RD2	Tomato and wheat seed receiving 10 ml water mixed with NIS at 0.24%.
T50	Median germination time
TGI	Timson germination index
TMT	Treatment
T1/Treatment 1	Treatment at irrigation rate of 100% with the recommended application rate of non- ionic surfactant
T2/Treatment 2	Treatment at irrigation rate of 75% with the recommended application rate of non- ionic surfactant
T3/Treatment 3	Treatment at irrigation rate of 50% with the recommended application rate of non- ionic surfactant

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ABSTRACT

The effect of a non-ionic surfactant on the growth and development of tomato and wheat plants under deficit irrigation.

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Degree: Master of Science (Agric) Horticulture

The use of surfactants in plant production is becoming increasingly popular in the agricultural sector. Non-ionic surfactants (NIS) are being used for water conservation as well as the improvement of plant growth factors, such as biomass accumulation, plant development and economic yield. The effect that plant surfactants have on crop physiology is not well understood, with little research outside of commercial trial work. This may be due to the focus being on the effects of NIS on soil and water qualities, rather than the crop itself.

The trial work sought to identify if a NIS may have a noticeable effect on measurable parameters such as germination, chlorophyll content, physiological development, growth cycle and economic yield. The parameters were compared to control at various irrigation rates to establish if any key differences exist. To cover the full growth cycle of two types of crops, namely wheat as a monocot and tomato as a dicot, the trial work was separated into three distinct methodologies. The germination parameters were measured under laboratory conditions within a germination incubator to control temperature, light, and moisture. The wheat parameters were determined in a greenhouse pot trial where temperature, moisture and space were regulated. The tomato parameters were, as with the wheat measured within a greenhouse under temperature, moisture, and space control.

The germination data of the trial showed a lack of consistency between the two crops. Both crops were subject to increasing levels of NIS (0.06, 0.12, 0.24 and 0.48%) and compared to a control where distilled water was used. The results seem to indicate that mild germination inhibition may occur on wheat seeds as NIS dosage is increased. The tomato germination showed an improvement of germination at the half (0.06%) and double (0.48%) NIS dosage which contrasts both the literature and the results of the wheat.

The wheat and tomato greenhouse pot trial both showed deviation from the hypothesis for several growth parameters. Both crops were subjected to control (100% irrigation) and water stressed (75 and 50% irrigation) with or without the addition of a NIS. Several of the wheat parameters were similar between the control and NIS treatments, where others did not demonstrate a clearly defined trend. Effects of the NIS were more clearly observed in the once-off cumulative data parameters rather than the weekly readings. The tomato trial shows the first season of production having a greater correlation with the available literature, where the second season showed a greater degree of variation.

It is important to note that during both the first and second seasons of the greenhouse trial, there were technical challenges faced in the form of the greenhouse where the temperature and humidity may have been altered. It is also possible that the different dates on which the

trials took place may have affected the outcomes as well as the advent of problems arising from the Covid-19 pandemic during the strict lockdown measures in March and April 2020.The trial work indicate that non-ionic surfactants may have measurable effects on plant physiology, although further research is needed to better understand this aspect of the technology

INTRODUCTION

Water stresses in the agricultural sector is of great concern in South African production systems. The southern African region is historically a water-limited section of the continent (Pitman, 2011), with water scarcity being a hot topic of discussion within South Africa. According to Donnenfeld et al. (2018), the water resources of South Africa is currently classified as overexploited. The overexploitation of water in South Africa is due to the rapidly expanding sectors of industry, municipal, and agricultural usage (Donnenfeld et al. 2018). Agriculture is estimated to account for 62,5% of water withdrawal and use in South Africa (Dugmore et al. 2016). The state of disrepair of water infrastructure further exacerbates the scarcity of water during drought conditions (Donnenfeld et al. 2018).

South Africa has undergone several drought events in recent years, namely between 2014 to 2016 as the most current, which lead to water shortages in the Western Cape province (Donnenfeld et al. 2018). The average rainfall in South Africa is 464 mm per annum, making it lower than the global average value of around 786 mm (Mkhwanazi et al. 2018). South Africa receives freshwater from rivers such as the Zambezi and Congo River, but it contributes a comparatively small amount of overall water input (Pitman, 2011). According to Pitman (2011), water availability and usage are better understood using various GIS and computer models, allowing for an accurate gauge of water in South Africa. By better understanding water availability, it can be better weighed against the agricultural needs of the country.

An advent of new technology in the agricultural sector holds the key for the improvement of water usage. A rapidly developing field within agriculture is the use of surfactants in plant production and water conservation. The use of surfactants holds the promise of a drastic effect on agriculture and thus sustainable food production.

In terms of water conservation, non-ionic surfactant (NIS) and NIS blends are becoming increasingly popular in crop production. The use of NIS and NIS blends is claimed to improve crop water efficacy, yield and productivity (Nachshon and Arnon). The research on surfactants is often limited to the alteration of soil characteristics and water availability, rather than its effect on the crop (Song et al. 2018). The effects of the surfactant blends are often only measured within the context of commercial research, rather than the possible underlying physiological effects it might have on seed and plants.

Research on the effects that surfactants may have on the physiological development of crops is limited. This study investigated a popular surfactant blend in terms of alterations to a crop's physiological development. The study will assess the effect of a surfactant blend on several growth parameters of a monocot, wheat (*Triticum aestivum*) and a dicot tomato (*Solanum Lycopersicon*).

Problem statement and research question:

The effects of the non-ionic surfactants on crop physiology is a topic which has not undergone extensive research, regardless of its growing popularity amongst agricultural producers. The research the question was: What is the effect of adding a non-ionic surfactant to two crops, differing in physiology, under variable water stress?

Hypotheses:

1. The addition of a NIS to the culture solution will lead to an accelerated rate of germination in both wheat and tomato compared to untreated seeds.
2. The addition of NIS in combination with different levels of water stress, will increase all the growth and development parameters in both treated tomato and wheat crops compared to the control of the same water stress level.
3. The addition of NIS in combination with different levels of water stress will result in a significant increase in above ground and root mass as well as economic yield of the treated tomato and wheat crop compared to the control of the same water stress level.

Aim:

During the trial, the aim was to test the efficacy of a NIS product as a water reduction agent on wheat and tomato by measuring physiological parameters

Objectives:

- To determine if the addition of a non-ionic water surfactant to water stressed wheat and tomato crops can relief the effects of water stress.
- To determine if wheat and tomato crops will show different physiological reactions due to the addition of a non-ionic surfactant to water stressed crops.

CHAPTER 1 : LITERATURE REVIEW

1.1 Water stress in South Africa

Water stress in the agricultural sector is a major concern in South African production systems. The southern African region is historically a water-limited section of the continent (Pitman, 2011). According to Donnenfeld et al. (2018): the water resources of South Africa are currently classified as overexploited. The overexploitation of water in South Africa is due to rapidly expanding industrial, municipal, and agricultural usage (Donnenfeld et al. 2018). Agriculture is estimated to account for 62.5% of water withdrawal and use in South Africa (Dugmore et al. 2016).

The water shortages experienced in South Africa are exacerbated by poorly maintained infrastructure within the country as well as climatic factors such as frequent drought events (Donnenfeld et al. 2018). South Africa has undergone several drought events in recent years, with the most recently being between 2014 to 2016, which led to severe water shortages in the Western Cape Province (Donnenfeld et al. 2018). The average rainfall in South Africa is 464 millimetres per annum, making it lower than the global average value of around 786 millimetres (Mkhwanazi et al. 2018). South Africa receives freshwater from rivers such as the Zambezi and Congo Rivers, however, it is a comparatively small contribution to the overall water input (Pitman 2011).

According to Pitman (2011), water availability and usage are better understood using various GIS and computer models, allowing an accurate understanding of water in South Africa. It is essential to understand water availability, which can then be weighed against the agricultural Needs of the country.

According to Bonthuys (2018), approximately 1.3 million hectares of agricultural land are under irrigation in South Africa. The area of irrigated land will continue to increase as the population and demand for crops increase. Agriculture is a fundamental part of a nation as it plays a significant role in economic, food and social security. Due to agriculture forming such an essential part of a country, it has a considerable impact on resource use and availability. The pressure which agriculture places on water usage in South Africa is counterbalanced by its contribution to the Gross domestic profit at 4 to 5 % (Dugmore et al. 2016). A possible indirect added value of 14% to as high as 30% related to the Agricultural sector is in the form of employment, the informal hawking sector and value chain additions (Bonthuys 2018). The contribution of Agricultural production on both a social and economic level is indisputable but needs to be weighed against water availability in South Africa. It is estimated that as of 2016, the revenue generated through Agricultural production was R247 billion, with around R64 billion of this being made up through vegetable production (Bekker 2017).

There are approximately 349 000 farms or farming operations focusing specifically on vegetable production within South Africa (Nyambo et al. 2015). Vegetable production has a high-water demand. Dryland vegetable production is not as typical as grain production as water stress of vegetables leads to a severe reduction in yield (Allemann and Young, 2008).

Due to diverse agroclimatic conditions, there are several popular vegetable crops produced within South Africa. The crops which take up the highest area of production are potatoes, carrots, leafy green vegetables, pumpkins, sweet potatoes, onions, maize and tomatoes (Bekker 2017).

1.2 Tomato Overview

Tomato is a popular vegetable crop worldwide, originating from Central America in Mexico, from where it was introduced to Europe in the mid-14th century (Kelley et al. 2017). The tomato is a dicotyledonous plant from the Solanaceae family, with the scientific name *Solanum Lycopersicon.* The Solanaceae family includes other popular crops such as potatoes, peppers, eggplant, and tobacco (Kelley et al. 2017). The tomato is classified as a perennial plant but is treated as an annual, as the plants are unlikely to survive through the colder months of the year as it is sensitive to both temperature and frost (Malherbe and Marais 2015, Singh et al. 2016). Therefore, most tomato production systems start each growing season with seeds or seedlings.

For the germination of tomatoes, the optimum temperature is 18℃ to 28℃ which correlates well with optimum production (Singh et al. 2016). The optimum temperature for tomato growth is between 26℃ and 29℃ (Anon. 2019). A variation from the optimum temperature can lead to a decrease in plant productivity, negatively affecting fruit set and quality (Kelley et al. 2017). The plants typically grow to a height of between one to three meters. The plant is herbaceous, weak stemmed and exhibits trailing stems (Kelley et al. 2017). The tomato produces medium-sized leaves, with both the leaves and the stem covered with micro hairs. The plant can sprout many small yellow flowers, which becomes fleshy fruit (Singh et al. 2016). The fruit is most often red at maturity, but due to cultivar variations, it can be green, yellow, black, orange, or purple (Singh et al. 2016). The fruit is a berry with several cavities that are filled with seeds. The gelatine-like substance that surrounds each of the ovules called the mucilage, controls the germination of the seeds (Kelley et al., Boyhan, et al. 2017). The seeds produced are small, covered in hairs, brown and with an average length of two millimetres (Singh et al. 2016).

For tomatoes, two growth types are available, and the choice of growth type will depend on the specific operation: The most common commercial varieties have indeterminate growth (Kelley et al., Boyhan, et al. 2017), which is characterized by continuous flowering and vegetative growth (Kelley et al. 2017). Most of the speciality, heirloom and, greenhouse tomatoes are indeterminate varieties (Singh et al. 2016). The second type is determinate varieties which share the characteristic of a bushy growth pattern and a finite life cycle (Figure 1) of defined vegetative, flowering, and fruiting stages of growth (Kelley et al., Boyhan et al. 2017). The use and growing conditions will determine the array that a producer will use. Tomato varieties are selected according to fruit characteristics, growth form, disease resistance, water, and fertilizer efficacy (Singh et al. 2016).

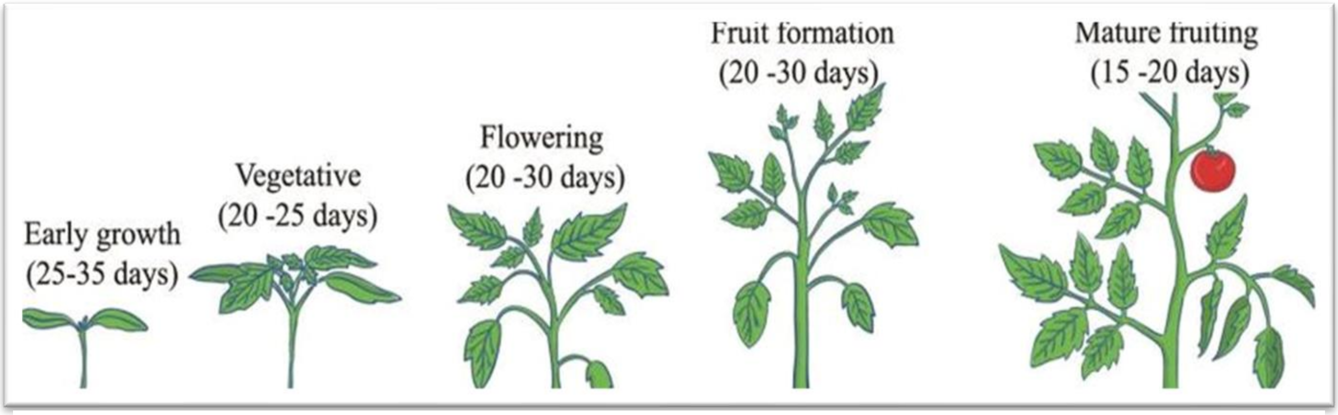


Figure 1.1: *Tomato lifecycle from seed to full maturity (Shamshiri 2014)*

Tomatoes are produced primarily for their fruit (Kelley et al. 2017). The fruit is used for various purposes, including as a fresh produce product, sold on the fresh produce markets, while processing tomato varieties are explicitly produced for processed products like tomato sauce and paste. The use of a specific tomato variety is determined by growth period, fruit size, shelf life, colour, and flavour (Singh et al. 2016). Tomato is one of the most popular produced vegetables globally, with an estimated worldwide production of 70 million tonnes (Costa and Heuyelink 2007, Kelley et al. 2017). South Africa produces an estimated 600 000 metric tonnes, making it the second-largest grown high-intensity vegetable crop in South Africa (Allemann and Young, 2008, Malherbe and Marais 2015). It is estimated that 6 000 hectares are annually planted to tomatoes in South Africa. In terms of vegetable production in the country, tomatoes compose 24% of the total hectarage (Kelley et al. 2017, Anon. 2020e).

South Africa is the largest producer of tomatoes in Sub-Saharan Africa, by default, making South Africa a net exporter of tomatoes to the rest of Southern Africa. The South African tomato export market has been growing fast with exports to especially Angola, Zimbabwe, and Mozambique as those country's local production cannot meet their demand (Malherbe and Marais 2015). South Africa has been able to become a dominant producer in the region due to several contributing factors: improved breeding and cultivar selection, the availability and use of agrochemicals such as fertilizers and pesticides, improved land-use, mechanization, development of infrastructure, and skills development (Malherbe and Marais 2015).

The production of tomatoes in South Africa varies in both complexity and scale. Available resources dictate the size and complexity of the farming enterprise (Bonthuys 2018). Within South Africa, it is possible to see tomato production units of below a single hectare (Bonthuys 2018) to over several hectares in size (Malherbe and Marais 2015). Tomato production occurs in both open field and undercover systems, depending on the capital availability of the farmer (Bekker 2017). Tomato production occurs under various production systems, either in greenhouses, under shade netting or open-field conditions (Kelley et al. 2017, Boyhan et al. 2017)

The soil and growth medium of tomato is an important factor to consider. Tomatoes are a versatile crop that can be produced on a variety of soil types, although it has been shown to grow optimally in soils classified as deep well-drained, loamy sandy or loam soil (Kelley et al. 2017). It is advised that for field production of tomatoes should be rotated with non-Solanaceous crops that are suitable to the soil type (Singh et al. 2016). The soil or growth medium will play a role in both the fertilization and irrigation management of the crop.

Most cultivated tomatoes require around 75 days from transplanting to the first harvest and can be harvested for several weeks before production declines (Kelley et al., Boyhan, et al. 2017). Ideal temperatures for tomato growth are 21-30℃ during the day and 18-21℃ at night. Significantly higher or lower temperatures can harm fruit set and quality (Singh et al. 2016). The tomato is a self-pollinating plant and, outdoors it can effectively be pollinated by wind currents (DAFF 2019).

Water is the single most crucial factor in plant production, making irrigation an essential element (Bonthuys 2018). Rainfall in South Africa is often erratic during the growing season, making the use of irrigation necessary (Mkhwanazi 2018, 2019). Tomatoes are known to root deeply, but this depends on water availability (Singh et al. 2016). Tomatoes require 30 to 40 millimetres of water per week, depending on the stage of growth (DAFF 2019).

The correct fertilization of tomatoes will allow for the best opportunity to produce an optimal yield. It is implausible to make a universal recommendation on the production of tomatoes, as it will lack efficacy and practicality for the producer (Kelley et al., Boyhan, et al. 2017). When making a nutrient recommendation for tomatoes, it is essential to consider soil pH, soil texture, water quality, climate, plant physiology, infrastructure, and cost (Kelley et al. 2017). The tomato plant is a heavy feeder, meaning that the plants require a medium to a high application rate of macronutrients (DAFF 2019). The production of tomatoes often requires multiple applications of especially nitrogen and potassium, as well as micronutrients (Kelley et al. 2017). According to The Department of Agriculture, Forest and Fisheries (DAFF) general guidelines, they recommend around 250 kg N and 100 kg K per ha (Table 1.1).

Table 1.1: *Recommended nutrient application rates for tomato production in South Africa (Daff 2019)*

Nutrient	**Per hectare**
Nitrogen	250 kg
Phosphorous	60 kg
Potassium	100 kg
Micronutrients (Foliar)	2.5-8 l

1.3 Wheat overview

In the category of agronomic crops, an important crop in South Africa is wheat. Wheat is considered a staple food crop, as it is used to make bread and other basic foodstuffs (Paulsen et al. 1997). The origin of the wheat plant is believed to be in the fertile crescent of the Middle Emonoast, and it has extensively been used throughout human history (Paulsen et al. 1997). Wheat is a monocotyledonous (monocot) plant with the scientific name *Triticum aestivum (*Acevedo et al. 2006) from the family Gramineae, which is also known as Poaceae. The Gramineae are grasses, which includes wheat, maize, sorghum, and bamboo, all of which have a long history of human use (Acevedo et al. 2006). Wheat is an annual grass grown during the winter months under dryland in the winter rainfall areas of the Western Cape as well as under dryland and irrigation in the summer rainfall areas of South Africa. Wheat has a hollow erect stem, growing to an average of 1.2 meters in height (Anon. 2018). The leaves of the wheat plant are narrow and flat, extending up to 40 centimetres long (2018). The wheat plant will form multiple tillers, with each terminating in an inflorescence, creating the wheat ear, which holds the crop's economic yield (Acevedo et al. 2006). Wheat can be produced in both the summer and winter seasons, with an optimal temperature of between 20°C to 30°C in the summer and 5°C and 25°C for the winter varieties (Acevedo et al. 2006).

The wheat growth stages can be described by various growth scales (Matthews and McCaffery 2007). The two most popular wheat growth scales are the Feekes scale and the Zadoks scale (Paulsen et al. 1997). The most relevant scale, Feekes, is presented in Figure 2. In general, the growth cycle follows the pattern; germination, tillering, stem extension, heading and finally ripening (Acevedo et al. 2006). The various stages of wheat growth can be affected by multiple biotic and abiotic stress factors (Acevedo et al. 2006).

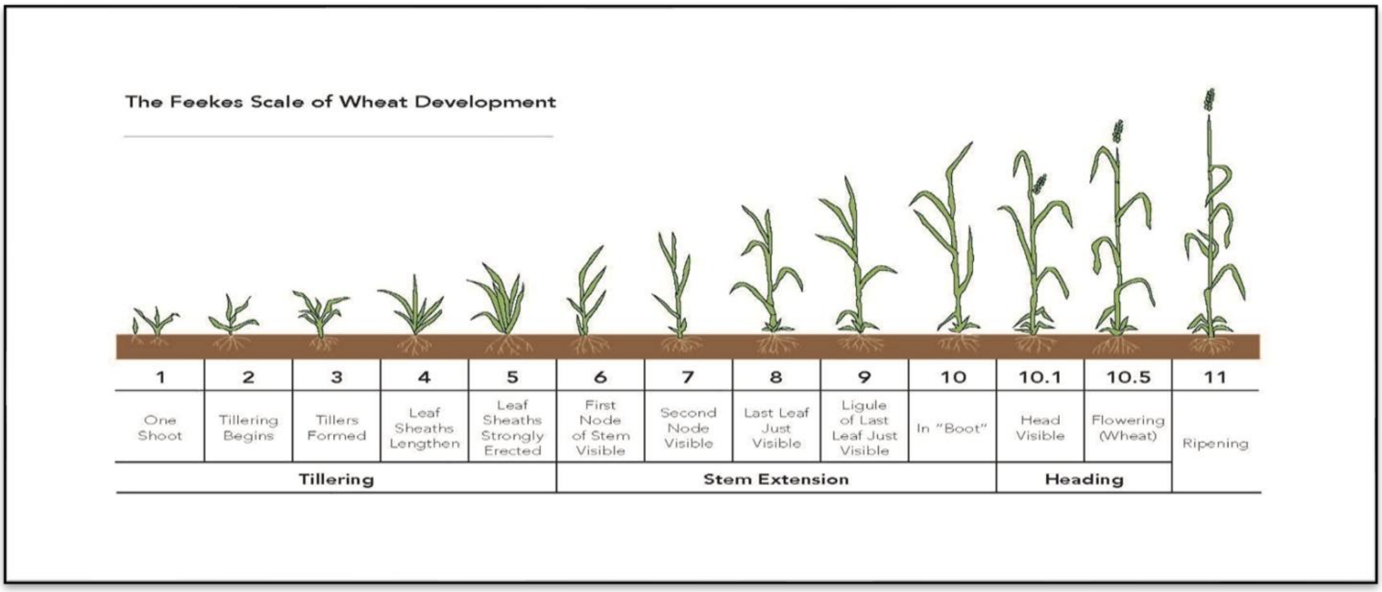


Figure 1.2: Feekes scale of wheat development (Large 1954)

Wheat is the second most-produced crop in South Africa, second only to the production of maize (Anon. 2018). Wheat is a popular rotation crop in South Africa with alternations with soybean and maize, meaning the planted hectarage will vary depending on the time of the year and region (Acevedo et al. 2006).

In South Africa, it is estimated that between 1.5 to 3 million tonnes of wheat is produced per annum Galal (2019). Wheat production is carried out across South Africa in most provinces where space and resources are available (Anon. 2010). It is important to note that South Africa is a nett importer of wheat, importing approximately 2 million tons per annum (Lyddon 2020).

Wheat is primarily grown as a grain crop. The seeds are high in starch, carbohydrates, fibre, vitamins, and several minerals (Siebert 2018). The grains are harvested, milled, and then used to produce a variety of products. The products that are created are dependent on the type that is cultivated (Paulsen et al. 1997). There are three cultivated varieties of wheat, the first and most popular, are hexaploid, while the second type is tetraploid species used in making flour with a nutty flavour. The third varieties are the least common; the diploid type (Acevedo et al. 2006).

Hexaploid varieties are the most popular because it is used primarily for bread flour production, making this variety the most cultivated, due to bread being a global staple food. Wheat is used in the manufacturing of couscous, a component of several types of noodles, wheat porridge, tortillas, pretzels, and roasted wheat (Acevedo et al. 2006). The products produced are dependent on the specific requirements and tastes of the region.

Although small scale wheat production does occur, most are on a large commercial scale due to viability (Anon. 2010). Much like tomato, South Africa has become a dominant producer of wheat in the region due to several contributing factors: improved breeding and cultivar selection, the availability and use of agrochemicals such as fertilizers and pesticides, improved land-use, mechanization, development of infrastructure, and skills development (Anon. 2010). Due to the economies of scale, wheat is only grown in open fields. The general soil requirements are loamy or sandy loam soil that is well-drained, and the soil pH should be between the values of 6 to 7.5 (Bonthuys 2018). It is vital to have a correct pH as wheat is negatively affected by low soil pH (Anon. 2010). According to Matthews and McCaffery (2007), the optimal soil temperature for germination is between 5℃ and 25℃.

Wheat is grown primarily from seeds; correct preparation of the soil needs to be carried out to allow an optimal chance for germination (Acevedo et al. 2006). Mechanized planting, fertilization and harvesting are used due to the large scale hectarage associated with wheat production (Acevedo et al. 2006). The irrigation of wheat is dependent on both rainfall and water resources (Matthews and McCaffery 2007). There is a large proportion of wheat produced under dryland conditions (Anon. 2010). In summer rainfall areas or under extreme dry conditions, wheat production is done under irrigation, most often using centre or parallel pivot irrigation systems. The water requirement for wheat will vary depending on the growth stage (Matthews and McCaffery 2007). The general water requirement for wheat is approximately 500 to 600 mm per hectare (Matthews and McCaffery 2007).

Fertilization of a crop is dependent on the region in which it is grown (Matthews and McCaffery 2007). Correct fertilization of wheat will allow the best opportunity to produce an optimal yield. Much like tomatoes, it is implausible to make a universal recommendation on the production as it will lack efficacy and practicality for the producer (Acevedo et al. 2006). When making a nutrient recommendation for wheat, it is essential to consider soil pH, soil texture, water quality, climate, plant factor, infrastructure, and cost. The fertilization of wheat can make up a large proportion of wheat production costs (Acevedo et al. 2006). The cost of wheat fertilization can be significantly reduced via practices such as no-till production systems and proper crop rotation (Matthews and McCaffery 2007) with especially a leguminous crop. In terms of fertilization guidelines, much more work has been done on this topic for wheat, and fine tuning of a fertilization programme suited to yield potential and soil nutrient status is possible (Table 1.2-1.5).

Table 1.2: *Recommended N application rate for wheat in South Africa, based on the target yield and soil P status (DAFF 2019)*

Target yield (t ha^{-1})	**Nitrogen (kg. ha^{-1})**
4-5	80-130
5-6	130-160
6-7	160-180
7-8	180-200
8+	200+

Table 1.3: Recommended P application rate for wheat in South Africa, based on the target yield (DAFF 2019)

Target yield (t ha^{-1})	**Soil phosphorus status (mg kg^{-1})**			
	>5	**5-18**	**19-30**	**>30**
4-5	36	28	18	12
5-6	44	34	22	15
6-7	52	40	26	18
7+	>56	>42	>28	21

Table 1.4: Recommended K application rate for wheat in South Africa, based on the target yield and soil K status (DAFF 2019)

Target yield (t ha^{-1})	**Soil potassium status (mg kg^{-1})**			
	>60	61-80	81-120	>120
4-5	50	25	25	0
5-6	60	30	30	0
6-7	70	35	35	0
7+	80	40	40	0

Table 1.5: Recommended micronutrient application rates for wheat in South Africa, based on concentration in the flag leaf (DAFF 2019)

Micronutrient (element) mg kg^{-1}	**Sufficient flag leaf concentration (%)**
S	>0.4
Cu	10
Zn	>70
Fe	>100
Mo	>180
B	>0.1

1.4 Water stress in plants

In crop production, water stress is a major concern and therefore an area with increased research interest. The effects of water stress are dependent on the plants' physiology. The water stress response in plants is regulated by the expression of Abscisic acid (ABA). The ABA phytohormone is associated with several functions within the plant: osmotic regulation, cuticular wax accumulation, bud dormancy, seed germination, leaf senescence, and growth inhibition and stomatal closure (Zeevaart, 2004).

Water deficit is an abiotic stress factor (Osakabe et al. 2014). Water stress on a physiological level generates a variety of responses in a plant. A primary response to water stress in a plant involves stomatal activity (Osakabe et al. 2014). Stomata are pore structures that are found on the epidermis of plant leaves (Pirasteh-Anosheh et al. 2016). Stomata are made up of two guard cells that are found encircling the stomatal pore (Osakabe et al. 2014). When the plant is well watered, the stomata are open, allowing water to be freely transpired as well as allowing the intake of CO_2. When the plant experiences water stress, the stomatal pore will be closed to reduce the loss of water (Daszkowska-Golec and Szarejko 2013). A sequence of events occurs to allow the stomatal closure; the two guard cells lose turgor pressure, a process that is mediated by ABA (Pirasteh-Anosheh et al. 2016).

When a plant experiences water deprivation, the water ion ratio within plant cells is altered, the variation causes a change in the turgor pressure of the guard cells which causes the plant's stomata to close (Osakabe et al. 2014). ABA is associated with stomatal reaction to water stress conditions. The biosynthesis of ABA is a relatively complex process, which can be sub-divided into three separate stages: the first is the assembly of phosphorylated intermediates to form carotenoid phytoene, stage two which are cleavage of the 9`-cis-neoxanthin group from the carotenoid phytoene and the formation of xanthoxal skeleton of Abscisic acid, the final stage which is not yet fully understood most likely involve xanthoxal oxidation and interaction with Aldehyde oxidase (Milborrow 2001, Chen et al. 2020). A rapid increase in ABA concentration correlates to an increase in hydraulic conductivity of the xylem and roots. An increase in conductivity allows the plant to acquire more water, allowing rapid recovery of the plant (Pirasteh-Anosheh et al. 2016)

Stomatal closure directly affects CO_2 intake, water and nutrient uptake and transpiration (Zeevaart 2004). Closing the stomata reduces water loss from the plant, but several important processes are restricted, such as photosynthesis, since CO_2 intake is disrupted. The process of photosynthesis is directly affected by water stress (Osakabe et al. 2014, Pirasteh-Anosheh et al. 2016), leading to a reduction in productivity (Tezara et al. 1999). The closure of stomata causes a drop in CO_2 concentration in the plant (Osakabe et al. 2014). The lack of both H_20 and CO_2 in the plants leads to a decrease in ATP and NADPH production (Osakabe et al. 2014). Photosynthesis and chlorophyll are directly proportional. According to Barraclough and Kyte (2002), chlorophyll and the absorption of nitrogen (N) are directly linked in all plants, this allows chlorophyll measurements to be used to diagnose and understand nitrogen update into a plant via SPAD and chlorophyll readings (Barraclough and Kyte 2002). The chlorophyll content is sensitive to water stress (Barraclough and Kyte 2002). The chlorophyll a/b ratio in leaves of stressed plants was found to be higher with an observed lag time; this demonstrates a reduction in chlorophyll b production during water stress (Barraclough and Kyte 2002). The effect of water stress on plants are often generalized. Various studies have shown the specific effects water deficit can have on tomatoes and wheat.

1.4.1 Water stress in wheat

According to Boutraa et al. (2010), depending on the growth stage, the height of a wheat plant can be affected by water stress. In a 2010 study, three cultivars were used and exposed to water stress at various growth stages. In the first week, low irrigation did not affect plant height, but by the 3^{rd} week, a substantial height variation was observed between 80%, 30% and 50% deficit irrigation rates respectively (Boutraa et al. 2010). The study also found that by week five, only the severely water-stressed plants exhibited height inhibition, where the height with 50% irrigation was not significantly different from 80% irrigation and the control plants. All these results were also affected by cultivar, leading to the conclusion that specific wheat cultivars will demonstrate height variation under severe water stress (Boutraa et al. 2010). An older study by Singh and Malik (1983) corroborates part of the finding of Boutraa et al. (2010) which linked the decrease in plant height to a reduction in straw yield under water stress at specific stages of development, but they did not consider variation between cultivars.

Wheat root biomass can be correlated with the water uptake of a plant. According to Fang et al. (2017), a significant correlation between root traits and wheat yield can be observed. That study demonstrated that when a wheat plant is under water-stressed the root density in the topsoil layer is higher, but the overall root length and density were lower (Fang et al. 2017). The implication is that there is a general reduction in root mass under water deficit conditions (Fang et al. 2017). The root density is however determined by the genotype of the specific cultivar, as newer cultivars will often produce greater root mass (Fang et al. 2017).

The overall wheat biomass can be correlated to water availability. If water stress is mild, the overall production of biomass is not significantly affected (Akram 2011). A study conducted in Bangladesh demonstrated by analysing that water stress, it can be correlated with the economic yield and yield components (Ye) of wheat plants (Akram 2011). The study measured stress as relative water content (RWC), leaf area index (LAI) and crop growth rate (CGR). Akram (2011) measured the mentioned parameters via leaf water potential (ψw), turgor pressure and osmatic potential (ψp) and biomass production. Reduction in yield was found to occur regardless of wheat cultivar under water-stressed conditions (Akram 2011). The Bangladeshi study further indicated that the irrigation rate reduced at specific critical growth stages, such as Feekes stage 3 and 10 had the greatest impact on biomass (Boutraa et al. 2010, Anon. 2018).

According to Abid et al. (2018): Tiller productivity is a difficult topic to study, as it is very dependent on environmental stresses. Tiller production of plants that is not under stress will accumulate biomass and progress naturally through the wheat production cycle until flowering and filling. When a wheat plant is stressed, the tiller production will develop at the same rate as the non-stressed plants, up until the point at which it experiences adverse conditions. After the removal of the water stress, the rate of tiller development increased exponentially (Abid et al. 2018).

The growth stages of a wheat plant can be directly affected by stress conditions caused by water deficit (Singh 1983, Ihsan et al. 2016). According to Ihsan et al. (2016) the adaptability of wheat genotypes to tolerate arid drought conditions shed light on crop water use efficacy within the crop. The study correlated specific crucial phenological growth stages with grain yield as linked to drought and high-temperature conditions (Ihsan, 2016). The trial concluded that there was a significant reduction in the number of days needed to complete a growth stage under adverse temperature and water-stressed conditions, emphasising a reduction of time in tiller development as shown in Feekes stage two and three (Ihsan et al. 2016). The alteration in the development of growth stages is dependent on the severity of the water stress conditions (Fang et al. 2017).

1.4.2 Water stress in tomatoes

Tomatoes like wheat have their unique response to water stress. The various effects of water stress can manifest in several ways. A study by Shinohara et al. (1995) found that tomato photosynthesis and transpiration are inhibited as soon as the water stress occurs in the plant. The study also revealed that the plants were able to slowly recover after stress as soon as re-watering took place, allowing the plant to return to a productive state (Shinohara et al. 1995). The study further found that when the tomato plant is exposed to water stress, there is a significant effect on the fruit, where yield was decreased with water stress but curiously saw an increase in aspects of fruit quality such as the Brix value. A more recent study by Nuruddin et al. (2003) found when a tomato plant is exposed to water stress during the flowering, fruiting, and ripening stages, there is a significant reduction in marketable yield and overall water use efficiency (WUE). The study did however find that when water stress is applied during the flowering stage, there is no significant effect on fruit quality, but yield and fruit number (Nuruddin et al. 2003). The study similar to Shinohara found evidence of increased fruit quality such as total dissolved solids (TDS) and colour for water-stressed plants when compared to the fully irrigated treated plants in the trial (Nuruddin et al. 2003). The study also found that while yield and WUE were reduced in the first trial when water stress was applied during flowering, the data was contradictory when carried out a second time (Nuruddin, 2003).

Water and water stress play an essential role in the root development of tomatoes (Senthilkumar et al. 2017). A study was conducted on how soil moisture content can affect a tomato plant root development (Senthilkumar et al. 2017). The study found that the root development in terms of root length, weight and volume of the tomato plant correlated with growth, yield, and overall fruit quality for specific cultivars (Senthilkumar et al. 2017).

Further research by Yang et al. (2016) corroborated that soil moisture has both direct and indirect effects on the growth and distribution of the tomato root system. The study found that under moderate to severe water stress, the above-ground parts showed inhibited growth and limited root system distribution within the soil (Yang et al. 2016). This was, however, not observed when the plants were placed under mild water restriction (Yang et al. 2016). It was observed that when severe stress was present, the overall development of the roots is inhibited as well as the extent of the root distribution in the soil (Shinohara et al. 1995). The study demonstrated that the moisture present in the soil was positively correlated with growth indices of the root as well as the above-ground parts such as the stem, leaves and flowers (Shinohara et al. 1995, Senthilkumar et al. 2017). The root density and moisture were measured at various depths for severe and moderate water stressed plants. At the first depth of 20 cm, water stress showed the highest correlation with root growth (Shinohara et al. 1995).

Gallardo et al. (2006) was able to correlate water stress with Solanaceae crop stem thickness. Gallardo et al. (2006) found that stem diameter variation (SDV) can be used as a water stress indicator in vegetable crops. Sibomana et al. (2020) found a statistically significant reduction in stem diameter of 18% when a tomato plant was placed under water-stressed conditions. The reduction in the stem diameter was linked to severe water stress rather than moderate water stress (Sibomana et al. 2020). The study also revealed statistically variation in plant height, with a reduction of 24% under severe water-stressed conditions (Sibomana, 2020).

Wudiri and Henderson (1985) conducted a study where flowering tomato plants at various levels of water stress was measured by measuring flower number, flower abortion and fruit set in two process tomato varieties (Wudiri and Henderson 1985). The study found no statistical differences in the average number of fruits and flowers produced between plants with no water stress and moderately stressed plants (Wudiri and Henderson 1985). The study did however find that there were significant differences between highly and severely stressed plants when compared to moderately and no water restricted plants (Wudiri and Henderson 1985). The 1985 study shows that water stress can affect the production cycle of tomatoes in specific phases, namely the reproductive phase as flowering and fruit formation. Wudiri is further corroborated by a 2003 study which also found that when a tomato plant is exposed to water stress during flowering, it had a greater rate of flower drop (Nuruddin et al. 2003).

The degrees Brix (°Bx) is the measure of the sugar content in the measured solution ((Barrett 2005). It is generally said that one degree Brix is equivalent to 1 gram of sugar in the form of sucrose in 100 grams of the measured solution, which is represented as a percentage value (Barrett 2005). According to Kleinhenz and Bumgarner (2013), Brix will not only vary between crops but even by the specific cultivar (Table 3). This is evident with tomatoes, as shown in table 3 where the reported Brix value across different cultivars and tomato types is shown. In terms of tomatoes, a high Brix reading is preferred, as it is an indication of high nutrient content, quality, and flavour (Rowley 2010). According to Takayama and Nishina (2007), it is possible to use the Brix value of the fruit of a tomato plant as early detection of water stress in the crop. The study found that an increase in the °Brix value of the tomato plant can be correlated to the physiological stress a tomato crop is experiencing, allowing the producer to adjust the irrigation accordingly. The addition of water stress under controlled conditions will promote the translocation of photosynthates into the fruit while improving the fruit quality (Ripoll et al. 2016).

Table 1.6: General Brix values expected from the different types of tomato varieties (Klienhenz and Bumgarner 2013)

Tomato type	Reported °Brix value
Processing	4.7–6.0
Greenhouse	3.8 –5.0
Fresh market	3.5–5.3
Cherry	5.0–8.0

1.5 Non-ionic soil surfactants

The use of soil surfactants is a relatively new technology to plant production and soil science. Soil surfactants are compounds that are defined as having the ability to lower the surface tension properties of water (Anon. 2020b). The properties of surfactants allow them to interact with substances like a variety of fats, oils, and grease compounds (Nakama 2017). Surfactants are used for a variety of purposes and industries such as lubricants, soaps, fabric softening detergents, printer and writing inks, anti-fogging agents, herbicides, adhesives, and emulsifiers (Anon. 2020b). Surfactants are added to cleaning products to allow the detergent agents to mix with the water (Van Ginkel 2007). According to Nakama (2017) without the addition of surfactants, the chemical cleaning agents will not be able to mix with the water but will act as a hydrophobic compound, reducing the effectiveness of cleaning.

Surfactants can be classified into the following general categories represented in Figure 3: anionic, non-ionic, amphoteric, and cationic (Castro et al. 2013). Anionic surfactants are defined by having a hydrophilic polar group that is negatively charged (Martins and Dias 2019). Anionic surfactants are used in soaps and detergents for both industrial and household use (Niraula et al. 2012). The cationic surfactant group are characterized by a positively charged functional group (Rhein 2007). The common usage of cationic surfactants is in acidic environments and high mineral content water, and they are used in cosmetics rather than detergents as their cleaning ability is weaker compared to anionic surfactants. Cationic surfactants are also used as antistatic agents and as a component in fabric softeners (Martins and Dias 2019, Rhein 2007). An amphoteric surfactant carries both an anionic and cationic hydrophilic group simultaneously within its structure (Van Ginkel 2007). The amphoteric surfactant is also known as a hermaphroditic ion as it can be in the form of a cation or anion according to changes such as pH (Van Ginkel 2007). Amphoteric surfactants, much like cationic surfactants are used in lotions and shampoos due to the mild nature of the chemical reactivity (Van Ginkel 2007).

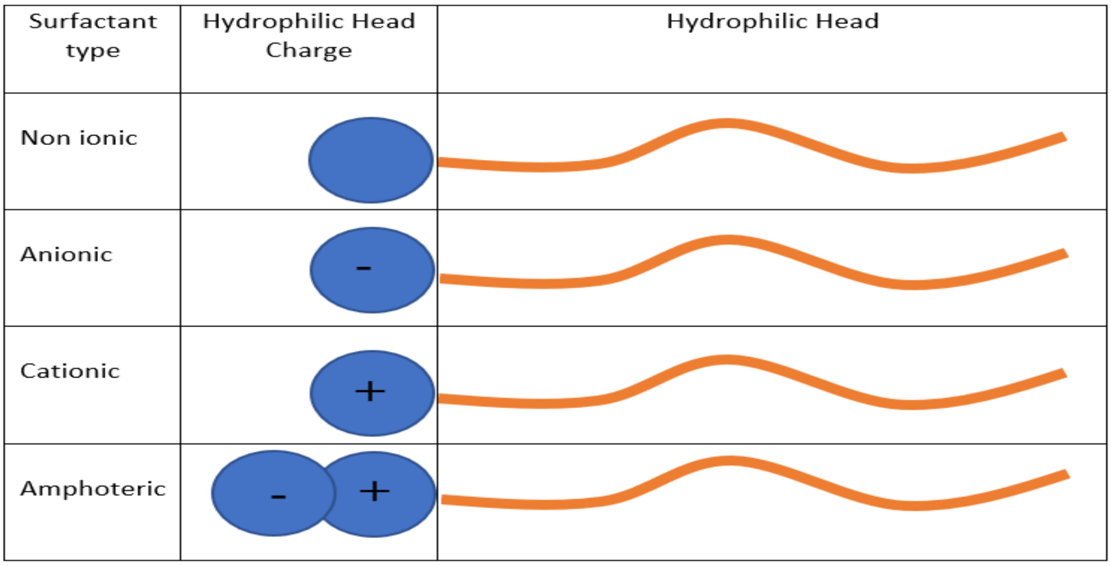


Figure 1.3: Various surfactant types according to the ionic charge

The surfactant group of focus for the trial is non-ionic surfactants. A non-ionic surfactant is a type of surfactant that does not carry a charge (Castro et al. 2013). The non-ionic surfactant carries a hydrophilic head group and is also reactively very mild (Nakama 2017). Non-ionic surfactants are long chain structures (Schick 1987). Many surfactants are produced via petroleum (Xiang et al. 2019). Non-ionic surfactant solutions often have the characteristic of making a cloudy solution when the temperature increases (Schick 1987). In solution, a water molecule will bond to the ether oxygen of a non-ionic surfactant's polyoxyethylene hydrophilic head group (Xiang et al. 2019). The effectiveness and reactivity of a non-ionic surfactant are dependent on the specific combination of compounds that are used (Castro 2013). The formulation that was utilized during the current study contained the following general chemicals: Ethylene oxide and Polyether compounds.

Ethylene oxide is a clear and colourless gas with an ether-like smell with a boiling point of 10.7°C (Anon. 2020c). The chemical formula for Ethylene Oxide is C_2H_4O (Anon. 2020c). Ethylene oxide is primarily used as an intermediate product in the formulation of polymers and other products (Schick, 1987). There is limited research into the effects of Ethylene oxide polymers on the soil as well as plant physiology. The research that has been conducted on Ethylene oxide polymers effectiveness as a pesticide in the control of insects and bacteria as a fumigant and sterilant (MacLachlan 2010).

When ethylene is metabolized, it results in the oxidation of ethylene, as well as into several by-products such as CO_2, ethylene glycol, ethylene oxide, and a glucose conjugate (Beyer, 1984). Metabolized ethylene can thus have several effects in the plant involving signalling pathways for stress response such as defence mechanisms, environmental resistance and resource seeking (Rodrigues et al. 2014). The various mechanisms manifested via ethylene can include abscission senescence, alterations in plant tissue elongation as well as shoot: root ratios (Rodrigues et al. 2014).

Polyether’s are compounds that are related to Ethylene glycol (Nakama 2017). This family of compounds are an effective soil release finisher as a characteristic that allows the easy dispersion of dyes by altering the characteristics of water, such as surface tension (Schick, 1987). Polyether's are a crucial component of many surfactant blends as it is linked to the ability to be used as an emulsifier, mixing oil compounds and water, which may allow easy absorption of fertilizers and pesticides (Holmberg 2003). One of the most important polyether's is Propylene glycol.

Propylene glycol or (PEG) is a synthetic substance that is known to absorb water (Anon. 2020d). Propylene glycol is used in the chemical, food, and pharmaceutical sectors including in fabric production, to create polyester compounds (Anon. 2020d). Propylene glycol is an antifreeze compound that is a clear, colourless substance, classified as being hygroscopic (Anon. 2020d). Propylene glycol, due to its chemical characteristics, is used as an organic solvent (Anon. 2020d). In terms of agricultural usage propylene glycol, it is considered a humectant as it helps to keep the soil moist (Fallourd 2009). A study was conducted on

polyethene glycols (PEG) as a decreasor of water potential in culture solutions. The study found that at high enough concentrations PEG was absorbed by the roots and could be isolated in the leaves of the plants in the culture solutions (Lawlor 1970). The Lawlor study suggested that the accumulation of PEG decreased the mobility of water within the plants, causing desiccation, leading to a decrease in overall biomass

1.5.1 The use of non-ionic surfactants in agriculture

The effectivity of an agricultural surfactant is improved when it is applied in combination with various compounds (Castro et al. 2013). According to Bially et al. (2005), the use of synergetic blends of polymers and surfactant compounds such as Alkyl Glucosides and Ethylene Oxide polymers has been shown to improve the infiltration of water into the soil as well as lower water repulsion in hydrophobic soil (Bially et al. 2005). It is also claimed that surfactants can enhance pesticide efficiency, as surfactants increase the foliar uptake of herbicides, defoliants, and growth regulators (Huggenberger et al. 1973).

Soil applied surfactants are used to alter the characteristics of the irrigation water as well as the soil environment, specifically unfavourable conditions and characteristics such as water infiltration rate and hydrophobicity (Mobbs et al. 2012, Song et al. 2018). Water repellence causes a reduction in both the rate and spreadability of water infiltration (Abu-Zreig et al. 2003). The hydrophobicity of soils is shown to cause low yield in various crops (Bially et al. 2005). Several studies into surfactants resulted in a reduction in the water repellent qualities of the soils, such as sandy soils. Hydrophobic soils can be due to several single or combined factors such as changes in molecular orientation of organic compounds, changes in ionic charge of functional groups in organic compounds, and an accumulation of hydrophobic compounds in the soil (Abu-Zreig et al. 2003).

Surfactants have also been used to improve crop production. For several surfactants, it is also claimed to affect plant factors such as physiology, morphology, economic yield, and water usage (Letey 1975). Surfactants and the related proprietary technology make claims related to the soil, water, and crop characteristics. Studies conducted with various types of crops show that the effects of surfactants on the crops are both direct and indirect, linked to water and soil. A survey was conducted on the commercial claims of agricultural surfactants, where they were applied at limited irrigation treatments. The products claimed to increase protein yield, reducing the digestive fibre and carbohydrate percentage. Many of the available studies of the effects of soil-applied surfactants have been conducted under commercial conditions, rather than those of an academic environment.

The soil characteristics that can be changed include water holding capacity, hydrophobicity, amount of plant-available water, electric conductivity (EC), pH and nutrient availability, (Bonthuy 2018). Soil is more than just a growing medium for plants. Instead, it is the dynamic environment that anchors the plant, provides both water and nutrients, and acts as a biosphere for beneficial organisms (Abu-Zreig et al. 2003).

Research has revealed the value of using a surfactant to improve soil conditions. Significant differences were seen in the change in capillary action, water tension, and soil penetration (Song et al. 2018). Petersen and McCaffery (2001) demonstrated how a mixture of surfactants can improve the characteristics of hydrophobic soils, allowing efficient water infiltration and reducing run-off. Figure 1.3 shows the improved movement of irrigation water through the soil profile by adding a non-ionic surfactant at 25% concentration to the soil profile. The non-ionic surfactant does not only increase the wetting zone width, but also slows down the water movement through the soil, increasing the opportunities of the roots to gain access to the water. A study conducted on a non-ionic surfactant explored the effects that the product had on the soil. The study concluded non-ionic surfactants most likely increase the water holding capacity of soil as a function of surface tension (σ), contact angle (α), gravity(g), and water density, allowing for an improved horizontal capillary movement of the water through the profile (Abu-Zreig et al. 2003).

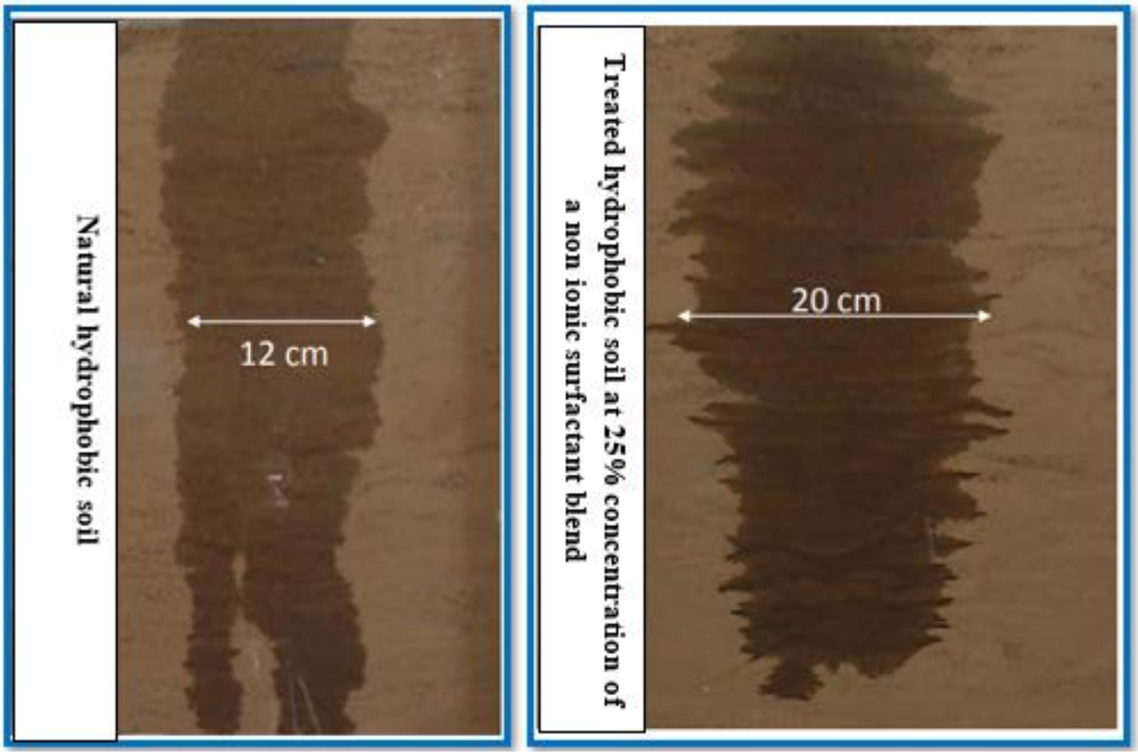


Figure 1.4: Demonstration of the spreading effect of a non-ionic surfactant on irrigation water in the soil profile (Nachshon and Arnon 2019)

According to Chaichi et al. (2017), tomatoes are sometimes grown in a high saline environment that placed them under water stress and ionic imbalance. The study found that both application rates of non-ionic surfactants had a significant improvement on plant height, dry leaf weight, dry stem mass and root dry mass (Chaichi et al. 2017). The improvement in tomato growth parameters was linked to greater nutrient uptake by the plants, and the reduction in sodium and boron uptake (Chaichi et al. 2017).

Yurdakul et al. (2016) found that the use of an anionic surfactant on wheat lead to a decrease in the dry weight of wheat, at a variety of different application rates. The study also found little significant variation in yield with the use of a non-ionic surfactant on wheat (Yurdakul et al. 2016). Trials on maize demonstrated that the application of a surfactant increased plant height, increased plant dry matter, and positively influenced leaf dry matter, dry stem matter, root dry matter, and leaf/stem ratio under deficit irrigation conditions (Petersen and McCaffery 2001). A further study showed that improved growth in potatoes was due to the decrease of nitrate leaching as a result of adding a surfactant (Anon. 2015). The study also showed a reduction in dry soil spots and increased availability of water around the active growing sites to such an extent that it became statistical relevant (Anon. 2015). A test using a soil probe showed the concentration of NO_3- below the point at which the plants could access was significantly decreased.

A study carried out by Endo et al. (1969) on the effects of non-ionic surfactants on monocots found that the surfactants studied demonstrated phytotoxicity when plants were grown in high surfactant concentration solution cultures when the temperatures were below 35°C. The study did, however, found that soil penetrants were slightly more toxic than the surfactant in the solution culture. The study further found that the addition of the surfactant may have stimulated plant growth when applied at the appropriate concentration to the soil (Endo, 1969). The study found an increase in induced shoot growth, but the physiological mechanism has not yet been explained. It is however insinuated that the increased growth might have been due to improved root growth leading to an increased nutrient or water uptake from the soil (Endo, 1969). The researcher noted that occasional stimulation of root growth was also seen in a dicot (*Nicotiana tabacum*) a close relative of tomato (Endo et al. 1969). It was noted in collaborative studies: non-ionic surfactants caused an alteration in the structure and permeability of specific membranes on Penicillin colonies (Endo et al. 1969). The Endo study further concluded that NIS could affect the germination of monocots, possible inhibiting or delaying germination.

The effects of the polyether: polyoxyethylene , containing NIS on both a monocot and a dicot weed seed germination was studied by Woodland and Hodgson (1987). In the study, *Echinochloa crus-galli* (monocot) germination was stimulated by the addition of the NIS across various controlled temperature ranges when compared to the control group. The dicot weed seeds tested during the trial reacted differently to the addition of the NIS, with *Amaranthus albus* L showing signs of germination inhibition and *Portulaca oleracea* L showing no significant reaction (Woodland, 1987).

Surfactants are becoming increasingly popular as an addition to seed coatings. Miyamoto and Bird (1978) investigated the effects of two soil wetting agents, evaluating the effects of surfactants on seed germination and seedling shoot elongation. The study concluded that the surfactants containing sulfonate and alkyl polyethylene glycol ether did affect germination and shoot growth. The study reported that both agents had a minor inhibitory effect on germination and elongation in hydrophobic soil and more significant inhibition in sandy soil. The germination of turfgrass exposed to non-ionic surfactants and water stress was studied (Madsen et al. 2016). The experiment concluded that a low concentration of a non-ionic, block co-polymer surfactant can influence plant physiological functions as well as plant growth. The surfactant was applied to the seed as part of a film coating. The treated grass seeds did show a greater rate of germination and root elongation under water deficit conditions as compared to the control group. During the trial, the researchers did not address the use of surfactants at various dosages and their possible effects.

Gálvez et al. (2019) tested various surfactants as a coating and their effects on seed germination as well as on root development. In the study the non-ionic surfactants had a positive effect on growth and development for onion (*Allium cepa*) and lettuce (*Lactuca sativa*) when applied as a seed coating at low concentrations (Gálvez et al. 2019). The conclusion of the study suggested that high concentrations of a non-ionic surfactant had a full inhibitory effect on the germination and growth of both onion and lettuce seeds and seedlings. The study, however, found that lower dosages of surfactants did benefit the germination rate in both crops. Similarly, a project was conducted using Pluronic F-68, a compound where the concentrations of the co-polymer polyoxypropylene are at 20% (Kumar et al. 1992). Kumar et al. (1992) found that *Solanum dulcamara* suffered root inhibition when a concentration of anything greater than 0,1% was used. When the solution was adjusted to 0,01%, a favourable increase in root mass was observed. Kumar et al. (1992) observed that plant cells, cultured in a non-ionic surfactant blend, had the feature of improved totipotency. Non-ionic surfactants also affected root length, fresh and dry weight of emerged plantlets (Kumar et al. 1992). Research conducted on germination and root growth is outdated with very little recent data dealing with its physiological effects on plants.

CHAPTER 2 : NON-IONIC SURFACTANT ON TOMATO AND WHEAT GERMINATION

2.1 Introduction

Germination of a crop is the initial stage of crop production. The addition of water to seed should if under suitable conditions (correct temperature and enough moisture), lead to a break in dormancy (Harrison 2019), resulting in a fast and high germination rate. Seed germination will occur when water is absorbed by the plant embryo, which causes hydration and the expansion of the cells (Harrison 2019).

Wheat and tomato belong to separate flowering plant classifications, namely monocotyledoneae and dicotyledoneae respectively. Monocots produce a single cotyledon on germination, where dicots produce two cotyledons (Grabowski 2015). The two crops can be further classified into the two main types of germination, either hypogeal or epigeal. The key difference between the two types is where the cotyledons are brought above ground (epigeal) or below ground (hypogeal). The tomato plant undergoes epigeal germination, where wheat is hypogeal (Kumar 2020).

Both types of germination undergo the same three phases of germination. The three phases are imbibition, respiration and other metabolic processes to mobilize reserves during seed germination and lastly development of embryo axis into a seedling (Kumar 2020). During phases one and two, the processes cannot be observed by the naked eye, while during the last phase the radicle can clearly be observed. Figure 2.1 shows the phases of germination as well as the two types of germination that can occur.

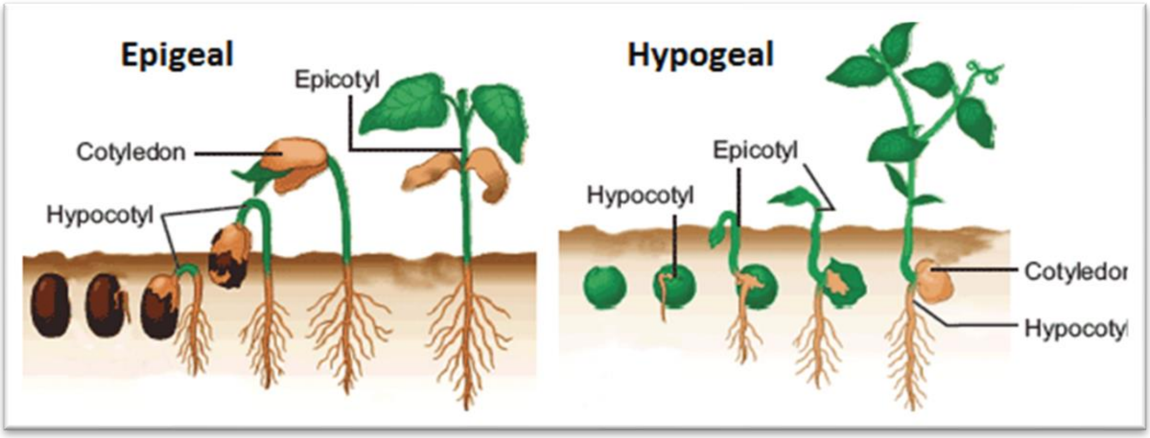


Figure 2.1: The three stages of germination in epigeal and hypogeal type plants (Anon. 2020a)

Since germination is the first stage of production, it can be affected by various factors, including the addition of chemicals, such as adjuvants, with soil-applied surfactants becoming a topic of greater interest. The use of NIS to improve crop production is an area of increasing interest in the agricultural world, holding the promise of improving water usage and decreasing input costs. The concern is the limited amount of academic research that has been invested into the effects that surfactants have on the life cycle of the applied crops. The germination of both monocots and dicots can be affected by the addition of NIS. NIS are used in crop production to enhance the plants' productivity from initial germination to harvest. The addition of surfactants may improve the germination rate, the germination percentage and the initiation shoot and root formation of the crop. The NIS may affect physiological mechanisms such as water uptake by the seeds, biochemical processes, or inhibition.

As mentioned in the literature review: although surfactants and their use within crop production are becoming increasingly popular, information on specific compounds and blends of NIS and their effects on germination is limited. The available literature indicates that there have been mixed results on how crops are affected by NIS, which can be no effect or to either enhance or inhibit germination of the seed. This study aimed to better understand the effects of a NIS on germination and root elongation. The study was carried out in highly controlled laboratory conditions in terms of temperature, light, water, space, and homogeny of the growth medium.

2.2 Methodology

Experimental site:

The experimentation for all the germination trials was carried out within a controlled environment at the University of Pretoria Experimental Farm (Phytotron D) Koedoespoort 456-Jr, Pretoria, Co-ordinates -25.7517732. 28.2586627. The site houses the incubators of the University of Pretoria.

Experimental information:

Crop:

Two different types of crops were used for the trials:

- A dicot: tomato (*Solanum lycopersicum* L.): The tomato cultivar used was Degas, a determinate fresh market tomato with fruits ranging between 160-180 g.
- A monocot: wheat (*Triticum aestivum* L.): The winter variety, PAN3497, with high vigour and adaptability to a variety of different growing conditions.

Treatments:

- Control (C): Tomato and wheat seed receiving 10 ml water.
- Half dosage (HD): Tomato and wheat seed receiving 10 ml water mixed with NIS at 0.06%.
- Recommended dosage 1 (RD1): Tomato and wheat seed receiving 10 ml water mixed with NIS at 0.12%.
- Recommended dosage 2 (RD2): Tomato and wheat seed receiving 10 ml water mixed with NIS at 0.24%.
- Double dosage (DD): Tomato and wheat seed receiving 10 ml water mixed with NIS at 0.48%.

2.2.1 Experimental Procedure

A total of five replicates were used during the trial to reduce experimental error and generate statistical significance. The procedure for both the tomato and wheat were the same. A single procedure is presented. Ten sterile Petri dishes were layered with sterile cotton wool. The Petri dishes were clearly marked and divided into two groups: the control and the NIS treated Petri dishes. In each of the Petri dishes, ten intact seeds of either tomato or wheat were added. The Petri dishes with seeds were then covered by another layer of cotton wool. In each of the control Petri dishes, 10 ml water was added, while the NIS treated Petri dishes received 10 ml water mixed with NIS at different concentrations. The incubator was set to 21℃ for the tomato and 19℃ for the wheat. Before the dishes were placed in the incubator, the Petri dish was statistically randomized using Research Randomizer. The trial was terminated when all the seeds in all the replicates were germinated.

Measured Parameters:

Germination:

Once a day until the conclusion of the trial, the Petri dishes were taken out of the incubator for a short time to make the necessary observations. A germinated seed count was done for each Petri dish. The readings are taken each day until all seeds were germinated.

Germination equations:

The collected data allowed several germination calculations to be carried out. The following parameters were calculated: Final germination percentage (G), Mean germination rate (MGR), Timson germination Index (TGI), Germination speed (GS), Emergence rate index (ERI), Median germination time (T50), Germination synchronicity (Germ synch) and Mean germination time (MGT)

The final germination percentage is an indicator of the number of seeds that germinate compared to those that were planted, which can indicate seed vigour, quality as well as possible inhibition (Al-Ansari et al. 2016). The G is calculated as TS/IS*100 where TS is the total germinated seeds at the end of the trial and IS, the initial number of seeds used for the trial (Aravind et al. 2021).

TGI is calculated ΣG/T, where G is the percentage value of seed germinated for the day, and T is the designated germination period (Aravind et al. 2021).

GS is calculated as Σnx/dx where n = number of germinated seeds, d = number of days (Aravind et al. 2021)

The ERI is germination speed at a given time interval. The ERI is calculated as Σk/Ni(k-i) where Ni is the number of seeds germinated within (i) days, with k as the total number of time intervals (Aravind et al. 2021).

The T50 value is defined as the average time for germination for the seeds during the trial.

MGR is a measure of the rate as well as the time frame of a given germination trial (Al-Ansari et al. 2016). The MGR value is calculated as = CV/100 = 1/T50, where T50 is the average germination time and CV.

The Germ synch also known as the synchronization index is a measure of the homogeny of the seed germination during a given period. Germ synch is calculated as $Z= \Sigma C_{Ni,2} /N$ where $C_{Ni,2}$ =Ni (Ni-1)/2 as the partial combination of two seeds from among the time N, for the time of (i) (Aravind et al. 2021).

The MGT is the average length of time required for maximum germination of a seed lot. $T = \Sigma\ k\ _{i=1}\ NiTi/\ k\ _{i=1}\ Ni$, where Ti is the time from the start of the experiment to the $(i)^{th}$ interval and k is the total number of time intervals.

Trial layout and Statistical analysis:

The trial was laid out as a completely randomised block design. The treatments and control were randomised using Research Randomizer to reduce the experimental error of plant placement and bias. The data collected was analysed by a statistician at the Agricultural Research Council (ARC) in Hatfield Pretoria. The analysis program GenStat© was used. The data underwent ANOVA analysis. The treatments (TMT) and specific dates (DATE) were analysed for significant variation. The least significant difference between means was calculated at a 5% level of probability. The ANOVA table can be found in the Appendix of the dissertation.

The treatment was carried out between crops within the same irrigation (IRRI) rates to further determine if there was an effect of the addition of NIS to the crop. The analysis sought to find significant variations that may occur between the NIS treated and control groups. The analysis also sought to find interactions between treatment and irrigation rate.

2.3 Results and Discussion

Figure 2.2 shows the wheat germination results at various NIS concentrations. The wheat seeds took a total of three days to germinate. All the treatments had a rapid germination rate, with all replicates having a 100% germination rate, within three days with no significant differenced detected for G. The most rapid germination was found under control conditions, which was followed sequentially with increasing NIS concentration. The control showed significantly better results than the NIS treated seed in terms of all the germination parameters (Table 2.1). The NIS treated seed at H(alf)D, R(ecommended)D1 and R(ecommended)D2 showed strong similarity in terms of MGT, TGI, GS, ERI and MGT, with the D(ouble)D show a strong variation in all germination parameters.

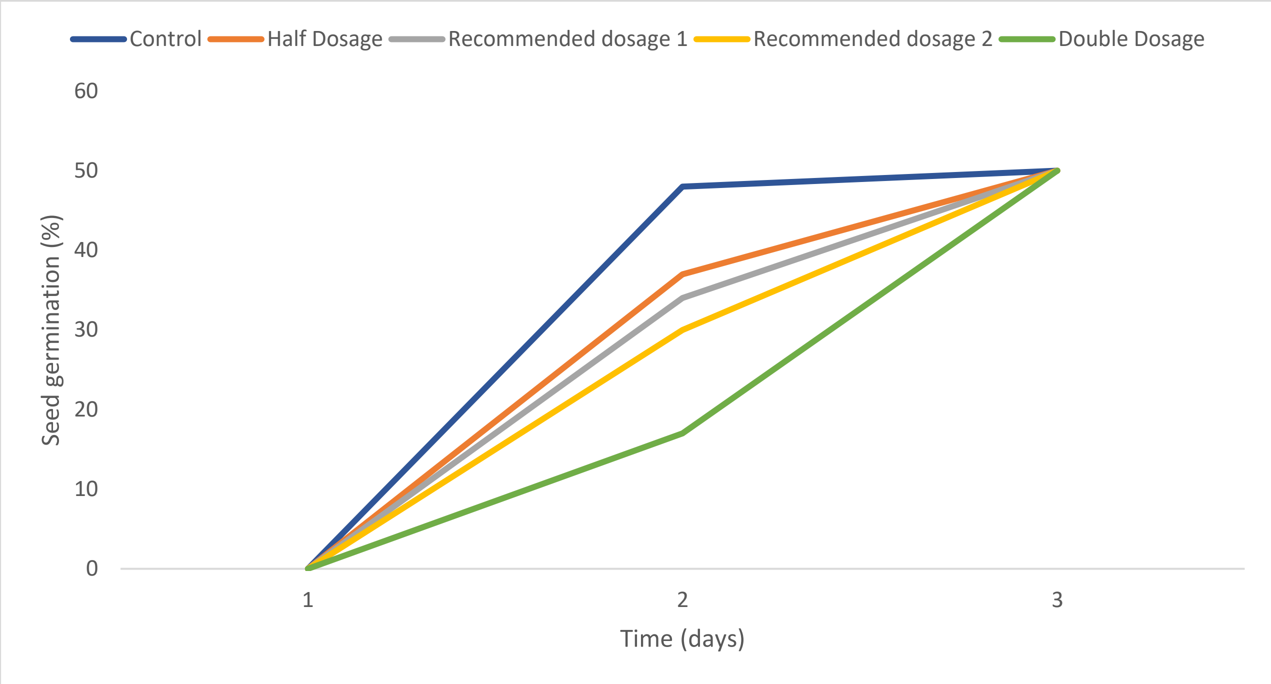


Figure 2.2: The germination percentage of wheat seeds with and without a non-ionic surfactant in solution

Table 2.1: Wheat seed germination parameters under various NIS dosages

Set	G	MGR	TGI	GS	ERI	T50	Germ synch	MGT
Control	100	0.4905[a*]	296.0[a]	4.933[a]	19.60[a]	1.574[c]	0.920[a]	2.040[c]
Half dose	100	0.4445[b]	274.0[b]	4.567[b]	17.40[b]	1.771[bc]	0.622[b]	2.260[b]
Recommended dose 1	100	0.4327[b]	268.0[b]	4.467[b]	16.80[b]	1.839[b]	0.564[b]	2.320[b]
Recommended dose 2	100	0.4181[b]	260.0[b]	4.333[b]	16.00[b]	1.948[b]	0.511[b]	2.400[b]
Double dose	100	0.3771[c]	234.0[c]	3.900[c]	13.40[c]	2.283[a]	0.551[b]	2.660[a]
LSD	NS	0.03493	19.66	0.3276	1.966	0.2393	0.1828	0.1966

G – Germination %, MGR - Mean Germination Rate, TGI -Timson germination Index, GS-Germination speed, ERI - Emergence rate index, T50- Median germination rate, Germ Synch - Germination synchronicity, MGT- Mean germination time. NS – no significant difference; * values in the same column with the same letters do not differ significantly from each other

The results of the trial did not demonstrate all the expected outcomes. It was predicted that the addition of the NIS to the growth solution would improve performance at, at least one of the dosages as compared to the control. A 100% germination rate was observed across all the treatments, which would suggest that no inhibition of the seeds occurred during the short germination period of the wheat. The control seeds germinated significantly faster, which would suggest that the addition of the NIS mixture may have slowed down the rate of germination in the monocot. The observation is further reinforced by the other calculated values, where the control performed significantly better.

The results of the trials seem to corroborate the findings of Gálvez et al. (2019) where high dosage of NIS in the seed coating of several vegetable crops was found to have delayed the germination and initial root development of the crops. The outcome of the trial may be due to the specific formulation and components used. The formulation used was Ethylene oxide polymers, polyether polymers such as Propylene glycol. At the highest dosage, DD, the Ethylene oxide polymers would be significantly higher. As mentioned in the literature, the effects of this compound on plant physiology are not yet well researched, but it is suggested that the compound's break down into other ethylene compounds may have a biochemical effect on the physiology of plants (Beyer 1984, MacLachlan 2010). The increase in the Propylene glycol may have improved water retention at the higher dose but acted as a minor inhibitor at lower dosages (Fallourd and Viscione 2009).

According to Miyamoto and Bird (1978), the use of sulfonate and alkyl polyethylene glycol did result in minor delay of both the germination time as well as elongation of the initial root. It was expected that the results of the current trial would correlate with the finding of Kumar et al. (1992) and Lowe et al. (1994) where concentrations of co-polymer containing surfactants with less than 0,1% and slightly more than 0,01% resulted in significant improvement in initial root development as well as the emergence of shoots. However, in the current trial, even at a concentration of 0,06% (Half dosage), the seeds did not perform better than the control. Older literature also concluded that the totipotency of plant tissue cultures could be improved with the addition of a NIS, but dosage as well as the purity of the chemicals added needed to be monitored carefully to avoid inhibition of growth (Woodland and Hodgson 1987).

Figure 2.3 demonstrates the results for the tomato germination at various NIS dosages. The tomato seeds took a total of nine days. During the first few days of the trial, little difference was seen between the treatments. From the third day onwards, the half dosage did significantly better than the control as well as the other dosages. From the fifth day to the conclusion of the trial the two paired highest and lowest values remaining the same, with the best performing sets found to be HD and DD, the control (C) was found to be the midpoint value, with the recommended dosages one and two coming in with the lowest values. The trend seen in Figure 2.3 for the germination percentage, is also reflected in the germination parameters represented in Table 2.2. In both cases the HD and DD treatments gave the best results followed by the control, and then the RD1 and RD2 with the poorest results. In terms of final germination value, the half and double dosage are both only significantly different from the recommended dosage 2. The germination parameters show no significant differences for MGR, T50 or Germ Synch for any treatments (Table 2.2). The HD treatment gave the best results in terms of TGI, GS, ERI and MGT.

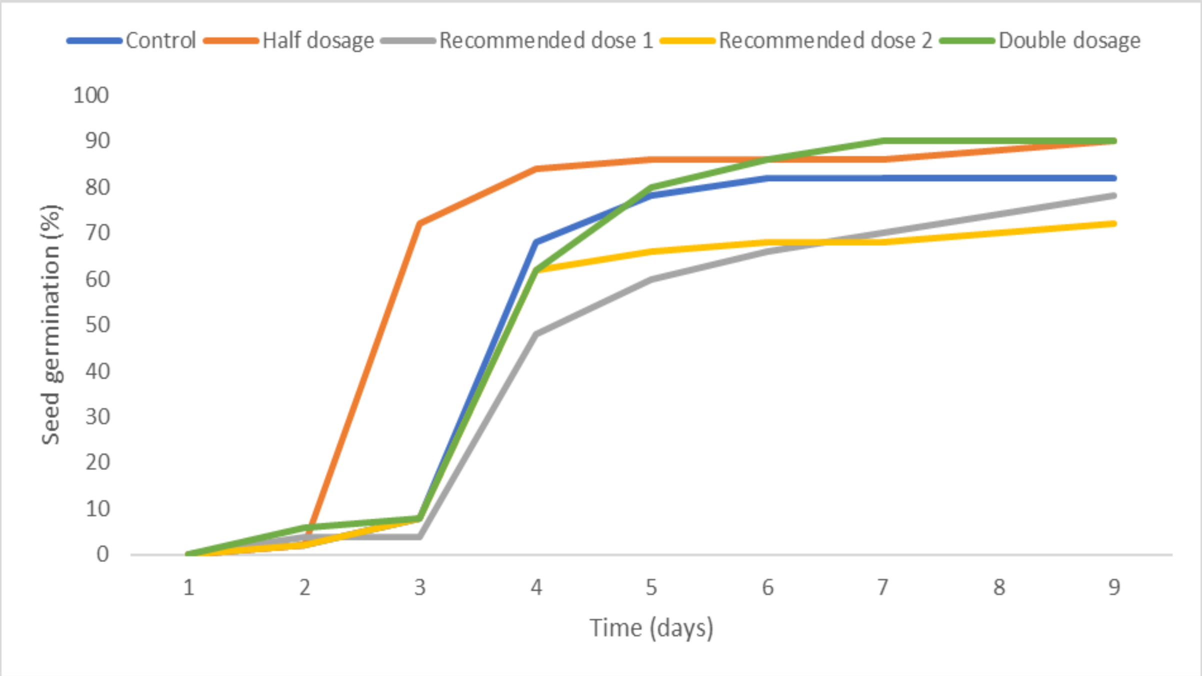


Figure 2.3: The cumulative germination of tomatoes sees at various NIS dosages

Table 2.2: Germination Parameters of Tomato seeds as affected by various NIS dosages

Set	G	MGR	TGI	GS	ERI	T50	Germ synch	MGT
Control	84^{ab}	0.2386	486^{ab}	2.088^{abc}	40.2^{abc}	3.44	0.508	4.207^{b}
Half dose	90^{a}	0.2463	534^{a}	2.504^{a}	44.4^{a}	3.42	0.384	4.087^{b}
Recommended dose 1	78^{ab}	0.2110	404^{b}	1.791^{c}	32.6^{c}	3.98	0.385	4.803^{a}
Recommended dose 2	72^{b}	0.2396	416^{b}	1.810^{bc}	34.4^{bc}	3.40	0.544	4.195^{b}
Double dose	90^{a}	0.2337	514^{a}	2.333^{ab}	42.4^{ab}	3.50	0.400	4.289^{b}
LSD	12.63	NS	93.8	0.5263	8.10	NS	NS	0.4355

G – Germination %, MGR - Mean Germination Rate, TGI -Timson germination Index, GS-Germination speed, ERI - Emergence rate index, T50- Median germination rate, Germ Synch - Germination synchronicity, MGT- Mean germination time. NS – no significant difference; * values in the same column with the same letters do not differ significantly from each other

The findings of the tomato trial differ from the results of the wheat germination trial. Where all the NIS treatments resulted in poorer results as compared to the control with wheat, some NIS dosages applied to tomato resulted in a positive reaction, while other dosages had an inhibitory effect. As previously stated by Gálvez et al. (2019) the dosage of NIS in the seed coating of several vegetable crops was found to have an inhibitory effect on germination, with increasing inhibition as the concentration was increased. Based on those results, the results in this trial deviated from the expected result, like the DD which was expected to have an inhibitory effect on tomato seeds germination gave better or similar results as the control. The findings of Kumar et al. (1992) and Lowe et al. (1994) as previously stated: that concentrations of co-polymer containing surfactants with concentrations less than 0,1% and slightly more than 0,01% would show a significant improvement for germination. The results of the trial found that both above 0,1% and around 0,01% had the best results.

In the older study conducted by Woodland and Hodgson (1987) seeds from two dicot weed species reacted differently, with one plant showing delay or inhibition, while the other demonstrated no significant variation from the control. The results of this older trial, as well as the observed results of the trial conducted, may demonstrate that the category of dicotyledoneae is too broad to make any discernible conclusion in terms of the effects of NIS on germination.

Apart from the direct effect of the NIS treatments, it is also important to note that the tomato seed had an inherent lower germination percentage as compared to that of the wheat used in this trial. This poorer initial quality resulted in RD2 having a germination percentage that was below the commercially acceptable 75% (Anon. 1998).

The trial results indicate that the two crops reacted differently due to fundamentally different physiological responses, or simply the genetic breeding or plant group. While treating wheat with NIS, regardless of the dosage, resulted in poor results, the dicot in this case tomato, reacted positively on H and DD.

2.4 Summary and Conclusions

The results of the trial were not consistent between the two crops. The trial may be a demonstration of the difference between the reactions of two completely different crop species to the addition of a NIS blend. From literature it was expected wheat seed to have improved germination with adding a NIS product. Instead, the control did do significantly better than the NIS treated seeds, regardless of dosage, in all but one parameter measured. This one parameter was Germination percentage, where there was no significant variation between the control and the NIS treated seeds. But despite this, it is not possible to accept the hypothesis that NIS would improve germination and germination related parameters.

On the other hand, from literature it was also anticipated that crop seeds subjected to higher dosages of NIS, would show lower germination. For tomato in this trial, it was however, not the case. The results show that the HD and DD performed the best, with the recommended dosage having the lowest values. Although the results of the tomato seeds do not correlate readily with any specific study, the unexpected result can be likened to that of the Woodland and Hodgson (1987) study, where seeds from two different dicots weed species did not demonstrate the same results, suggesting that the complex nature of dicots compared to monocots may contribute to the observed results of the trial. Yet again it was necessary to reject the hypothesis that increase dosages would result in poorer results.

It is also possible that the trial results might be due to the laboratory environment and applied dosage of the NIS. The environment in which the experiment was conducted can be classified as a closed one, where the lateral and vertical movement of the surfactant may be heavily restricted. The variation of germination between the Petri dish germination of the crops and the results found in field trials from literature further reinforces the concept that the experimental design rather than the surfactant may be at fault.

Apart from the crop and the trial environment, the results of the trial may also have been affected by chemical components in the NIS blend as compared to that used in the studies cited from literature. Therefore, the effect of NIS blends, concentrations and different crop species should be further investigated as not to make a blanket recommendation that may negatively affect some crop species.

CHAPTER 3 : EFFECT OF NON-IONIC SURFACTANTS ON WHEAT IN A POT TRIAL

3.1 Introduction

Wheat is an important agricultural crop in South Africa. Wheat is native to the Middle East, but due to its versatility as a crop and adaptation to several environments, it has become a widely produced cereal crop across the planet (Anon. 2010). Wheat is used in the production of the global staples: bread and pasta products, making wheat one of the essentially produced crops (Acevedo et al. 2006). The importance of wheat also makes it an important crop in agronomic research. In South Africa wheat is produced across the country, in both summer and winter rainfall areas (Anon. 2018). Wheat is the second most-produced cereal crop in South Africa by planted area, second only to maize (Anon. 2018). It is estimated that approximately 500 000 ha of wheat is planted annually across the country (Lyddon 2020). The demand for wheat in South Africa exceeds the supply, leading to imports from Argentina, the United States and the United Kingdom (Anon. 2018).

The increased interest in the use of soil surfactants in crop production may be an avenue to improve the production of monocots, such as wheat. In a South African context, this is especially of importance since the winter rainfall areas producing around 45% of South Africa's wheat have experienced severe drought conditions in the last 10 years. As previously mentioned in the literature review, surfactants can aid in the improvement of plant production systems by improving plant factors such as yield, water usage or stress tolerance or relieving adverse effects of the environment such as soil conditions, diseases, or water availability (Abu-Zreig et al. 2003, Peters et al. 2010, Trinchera and Baratella 2018). In this trial, a non-ionic surfactant (NIS) was used. Non-ionic surfactants are compounds that do not carry a charge (Castro et al. 2013). The effectiveness and reactivity of a NIS are dependent on the specific combination of compounds used (Castro et al. 2013). The effects of a NIS blend on yield, plant growth and water stress tolerance were the three factors of interest during the trial. Several studies conducted, both commercially and academically, have demonstrated the positive effects of using NIS (Petersen and Brumbaugh 2001, Yurdakul et al. 2016, Trinchera and Baratella 2018) in various crops. The trial aimed to explore the viability and effect of a NIS on monocots using wheat as a representative crop.

3.2 Methodology

Experimental site:

The trial was carried out in a controlled environment, where conditions such as temperature were regulated to eliminate error. The experiment was carried out within an A-Frame glass greenhouse at the University of Pretoria Experimental farm (Phytotron A) Koedoespoort 456-Jr, Pretoria, Co-ordinates -25.7517732. 28.2586627.

Experimental information:

Treatments:

A monocotyledon: wheat (Triticum aestivum L.) was used: The winter variety, PAN3497, with high vigour and adaptability to a variety of different growing conditions was used. Plastic pots with a ten-litre capacity were filled with 20 kg sand as the growth medium. There were six treatments and five replicates. The treatments were split into two groups: control and NIS treated. The pots were treated following the manufacturer's directions. The rate of NIS application was set at an initial application concentration of 0.24%, with monthly applications at a concentration of 0.12%. The irrigation rate was calculated according to the field capacity (θFC) of the soil. It was calculated that the full irrigation of the pot would be 1000 millilitres of water, 75% irrigation was thus 750 millimetres, and 50% irrigation was 500 millilitres. The control group consisted of C1- pots at 100% irrigation rate without the addition of NIS, C2- pots at 75% irrigation rate without the addition of NIS and C3-pots at 50% irrigation rate without the addition of NIS. The second group consists of NIS treated pots. The NIS pots were labelled as T1- pots at 100% irrigation rate with the addition of NIS at the recommended application rate. T2- pots at 75% irrigation rate with the addition of NIS at the recommended application rate and T3- pots at 50% irrigation rate with the addition of NIS at the recommended application rate.

3.2.1 Trial layout and Experimental Procedure

A total of 30 pots were filled where half of the pots were treated with the initial application of NIS. The pots were placed on a rotating table (Figure 3.1) after being randomised using Randomex. In each of the pots, two wheat seeds were placed 2 cm below the surface of the soil. It must be noted: once germination occurred, the better of the two germinated seeds were left in the pot, while the other was removed.

The plants were irrigated twice a week, for the duration of the trial to 100%, 75% or 50% field capacity. The pots could freely drain if excess water was present during the growing season. Fertilization of the pots was carried out every two to three weeks, following the DAFF wheat fertilization recommendations as presented in the Chapter 1. The fertilizer used during the trial was classified as water-soluble. A foliar application of macro and micronutrients (Sulphur, Calcium, Magnesium, Copper, Zinc, Iron, Molybdenum and Boron) were also applied every two to three weeks. The manufacturer of the soil-applied surfactant specifies that the product would work best when applied in conjunction with the fertilizer and synchronization with the fertilization schedule. The plants received irrigated until the final stages of wheat maturity. The irrigation was then discontinued, and harvesting commenced when the moisture content in the ears was at 10%. After harvesting the wheat ears, the rest of the plant, above ground and below ground, were harvested and separated into the different plant components. A total of five replicates per treatment were used to reduce experimental error. The trial was completed over approximately four months. The trial was carried out between the beginning of June to the end of September 2019. This is a similar time of year during which wheat is produced in the open field.

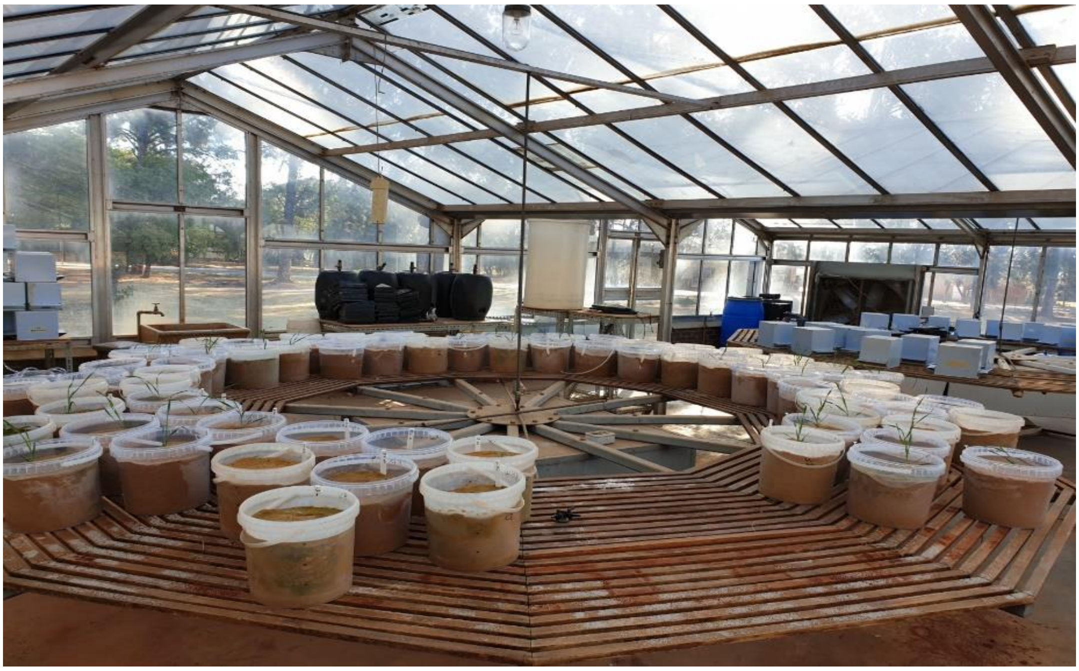


Figure 3.1: Rotating table with wheat plants growing in a sandy soil one week after germination (9th June 2019)

Measured parameters:

The measurements that were taken can be categorised into two groups: weekly readings and once-off readings. The weekly readings include chlorophyll readings, plant height, node number and tiller number. The once-off readings included biomass (root and top) and economic yield (Ye).

Weekly readings

The chlorophyll readings were carried out using a leaf chlorophyll meter. The readings were taken once a week during active growth. A total of two readings were taken per plant per week. The readings were taken from two new, fully unfolded leaves. The average of the two readings was recorded. Once a week during active growth readings were taken. The tiller number of each of the plants was recorded. Once a week during active growth of the crop a ruler was used to measure plant height. The measurements were taken from the base of the crop to the top of the primary wheat stem. Once a week during active growth readings were taken. The node number on the main stem was recorded.

Once-off readings

At termination of the trial, the number of wheat ears was recorded for each treatment. Once the wheat reached harvest maturity, in line with standard harvest practices recommended by the Department of Agriculture, Forestry and Fisheries (Department of Agriculture, 2019) the trial was terminated. Each plant was separated into three sections: Wheat head, above ground mass and roots. The three parts were analysed separately. All three of the sections that were harvested were placed in the drying oven at 60°C for 48 hours before weighing. The Economic yield was expressed as the wheat head. The wheat head or ear was separated into the spikelet, central stem, and grain. Economic Yield was calculated as $Y_e = G- (S+ Cs)$, where G is the seed grain, S is the spikelet and Cs are the Central stem as seen in the yield equation, the grain was the basis of the economically relevant component, not only for the trial by also commercially. The grain from each replicate was harvested and weighed separately. The above-ground mass was calculated by harvesting all biomass produced above the surface of the soil. The above ground mass includes the stem, leaves and hull, with the grains removed to determine the Economic yield (Ye). The root mass was calculated as all biomass produced below the surface of the soil. Each replicate pot was emptied into a fine sieve to separate the root matter. The separated root biomass was then washed using tap water to remove any excess soil. The roots were allowed to air dry for several hours before being placed in a brown paper bag for further drying in a drying oven over 48 hours at 60°C.

Trial layout and Statistical analysis

The trial was laid out as a completely randomised block design. The treatments and control were randomised using Research Randomizer to remove the experimental error of plant placement and bias. The data was analysed by a statistician at the agricultural research council (ARC) in Hatfield Pretoria. The data underwent ANOVA analysis using the GenStat © data analysis program. The data was analysed under Fisher's protected least significant difference test. The significance level for data was calculated at 90 or 95% confidence. The irrigation rates (IRRI), treatments (TMT) and specific date (DATE) were analysed for significant variation.

The analysis of IRRI will allow the differentiation between the effects of deficit irrigation, and the effects of the NIS on the crop. The DATE analysis allows the differentiation between environmental factors, such as temperature, solar radiation and experimental error and the effects of the non-ionic surfactant for specific measurement parameters such as chlorophyll, tiller, plant height and node number.

The TMT was carried out between crops within the same irrigation (IRRI) rates to further determine if there was an effect of the addition of NIS to the crop. The analysis sought to find significant variations that may occur between the NIS treated and control groups. The analysis also sought to find interactions between treatment and irrigation rate ((TMT)x(IRRI)).

3.3 Results and Discussion:

Figure 3.2 shows the leaf chlorophyll content readings of wheat throughout the trial between June and July 2019. The means of the overall chlorophyll readings did not yield a definitive trend and no significant difference for TMT.IRRI was generated during the trial. Initially (9 July) the NIS treated plants showed a higher chlorophyll content than the control plants, regardless of the irrigation level. As the season progressed these advantages could no longer be observed. Over time as the plants developed, the chlorophyll contents reached a high on 13 August after which it levels off as the plants starts to invest more and more N in the grain component.

When comparing plants receiving 100% irrigation, the T1 plants tended to have a lower chlorophyll content than the C1 plants. When the irrigation was decreased by 25%, the opposite was true with T2 plants tending to have a higher chlorophyll content than C2. By reducing the irrigation to 50%, the C3 and T3 plants tended to have similar chlorophyll contents. Treatment 1 had the lowest chlorophyll content over time of all the treatments. This is difficult to explain as all plants received equal amounts of N containing fertilizer and possible leaching from T1 (and C1) was only noted during the early growth stages when the plants were still young with few roots.

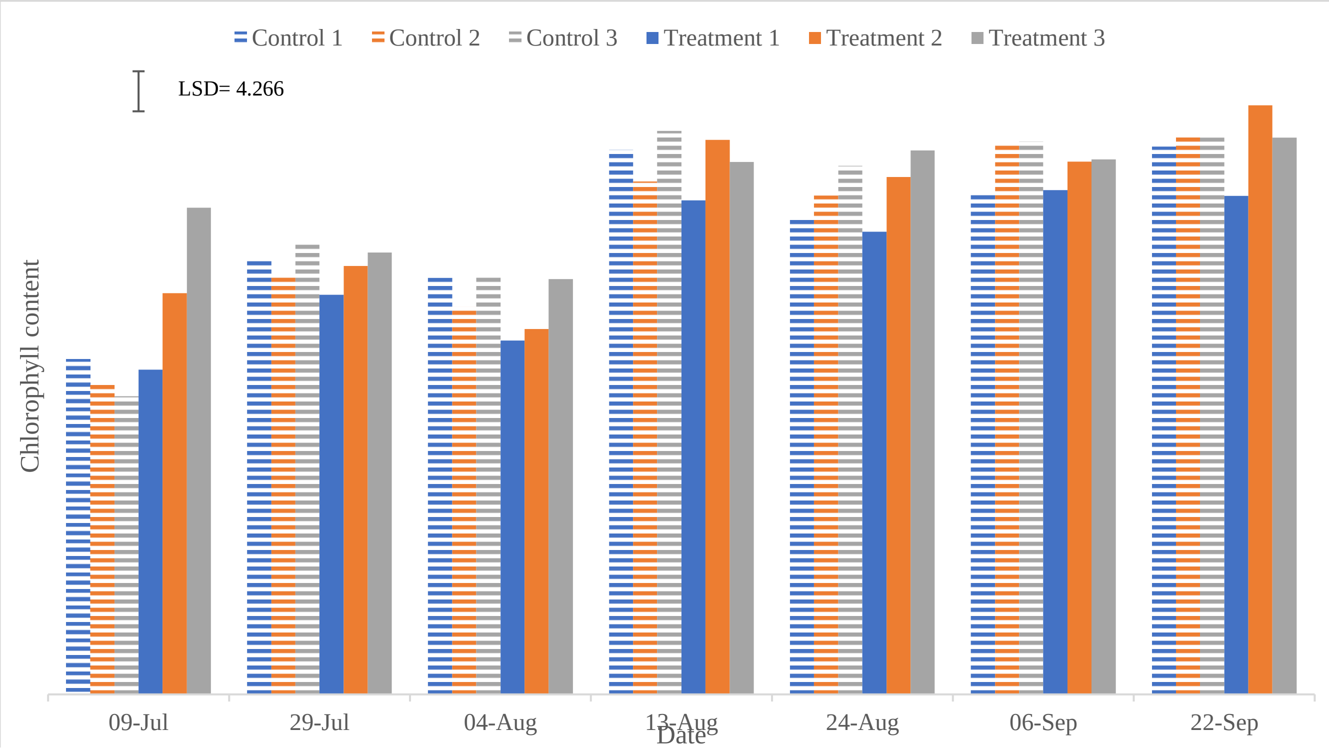


Figure 3.2: Wheat leaf chlorophyll content over time as affected by irrigation level and application of NIS over the course of the growing season

The results were expected to show a definite trend in which the surfactant treated crops would show a higher chlorophyll content across all the irrigation rates. As it was expected that the addition of a non-ionic surfactant would decrease plant water stress which would lead to higher chlorophyll readings (Khayatnezhad et al. 2012, Liwani et al. 2019). In the current trial, the relieving or prevention of water stress was expected with the application of NIS. The presence of NIS in the soil decreases negative water such as hydrophobicity and increases its lateral and horizontal flow allowing access to more plant available water (PAW).

The variation from the expected result may also be due to the natural physiological resistance which certain wheat varieties can have to water stress. The variety PAN 3497 is a drought-resistant variety that can be used in both dryland and irrigated conditions. According to Fang et al. (2017), the selection of drought-resistant wheat cultivars can improve biomass, plant height, photosynthesis, and economic yield. The use of the specific cultivar for this trial may have contributed to the recorded outcome.

Figure 3.3 shows the tiller number per plant over time for the wheat crop. The interaction between TMT.IRRI demonstrated a significant difference at the irrigation rate of 75% and 50% in favour of the NIS treated crop. A significant variation was not detected for the crops at 100% irrigation. The initial readings (up to 28 July) did not show variation as the wheat plant would still be in the seedling and seedling establishment growth phase during which tillers are not produced. After the initial homogenous readings, wheat plants entered the tillering phase with an increased number of tillers followed by the shooting stage (around 25 August) during which very little if any new tillers are produced. Water availability influenced the number of tillers produced with C2 and C3 both generally yielded the lowest values during the trial. By adding the surfactant, T3 plants generally recorded the next lowest values, but it was far better than the C2 and C3 values. The third-highest number of tillers was found to be T1, followed by T2, while the highest final tiller number was found to be for C1. As with the chlorophyll content, T2 plants tended to give similar results to the control plants receiving 100% irrigation.

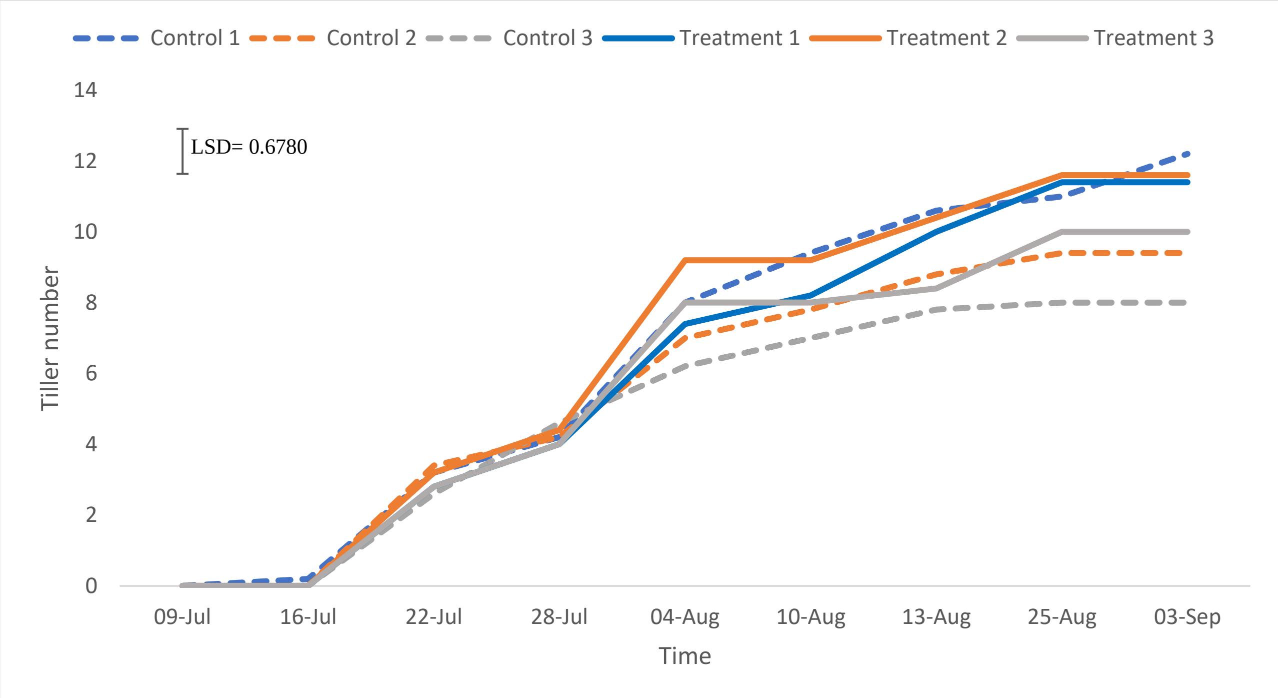


Figure 3.3 : The tiller number of the NIS treated and control wheat plants over the growing season

It was believed that the addition of the NIS would increase the number of tillers produced, regardless of the irrigation rate. The finding shows that there was a corroboration of the expected outcomes. The irrigation rate of 100%, C1 and T1, did not show a significant variation from each other, this result was in line with the expected results. The irrigation rates at both 75% and 50% did show a significant difference, where the surfactant treated crops (T2 and T3) had more tillers. The observations were in line with the findings of Abid et al. (2018). Tiller production that was not under stress conditions, will accumulate biomass and grow normally through the wheat growth cycle until flowering and grain filling. When a wheat plant is stressed, tiller production will continue until the point at which it experiences the adverse condition. In our case, water stress was constant over the growing season, but it was clear that under constant severe water stress conditions (T3 and C3) the plant could not develop as many tillers as under constant moderate water stress conditions (T2 and C2).

Figure 3.4 shows the node number on the main stems of the wheat plants taken at regular intervals. Since the node number is genetically pre-determined, the results were expected not to show much if any variation. Interesting to note is that the surfactant treated plants tended to have a smoother curve than the controls. There were not many differences between the 100% and 75% treatments, with the 75% treatments often giving higher values than the 100% irrigation levels, while the 50% irrigation often resulted in a lag in development. After 10 August, no increase in node number was observed, but in Figure 3.4 it can be observed that the internode lengths on the main stem now started to increase leading to increased plant height.

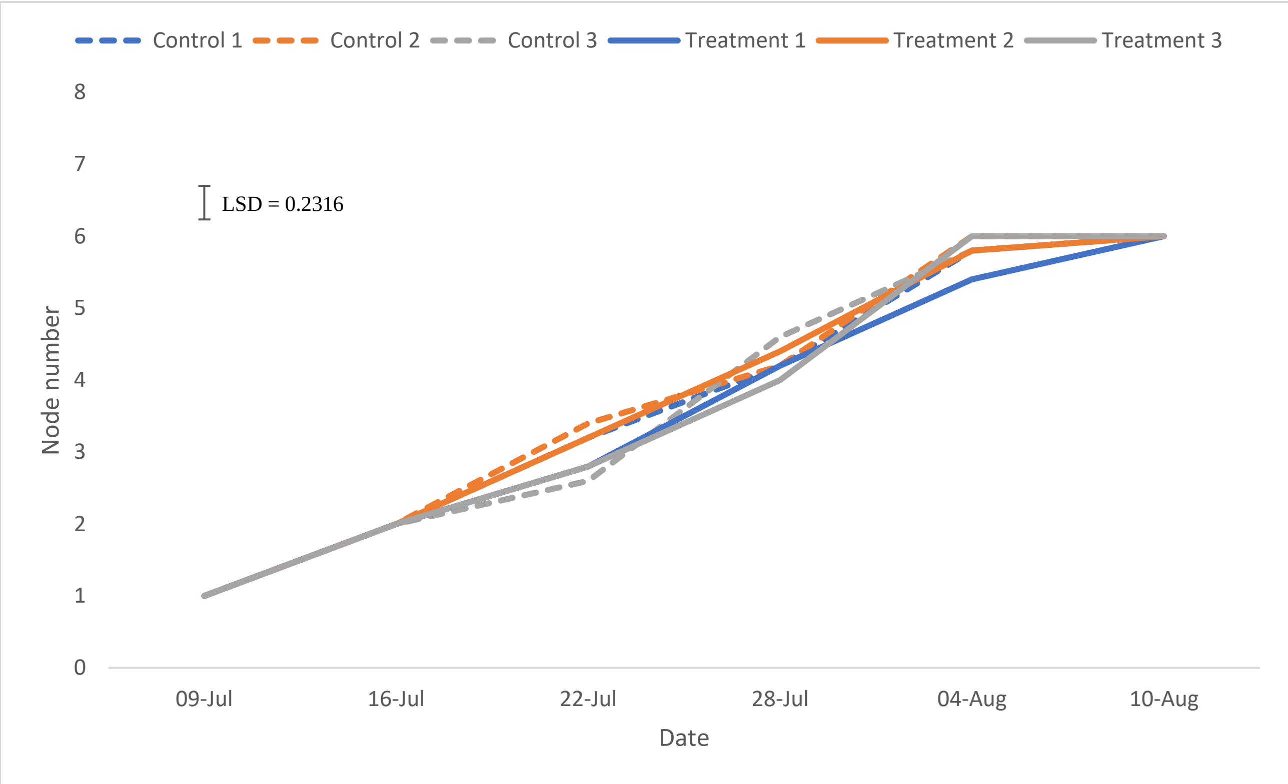
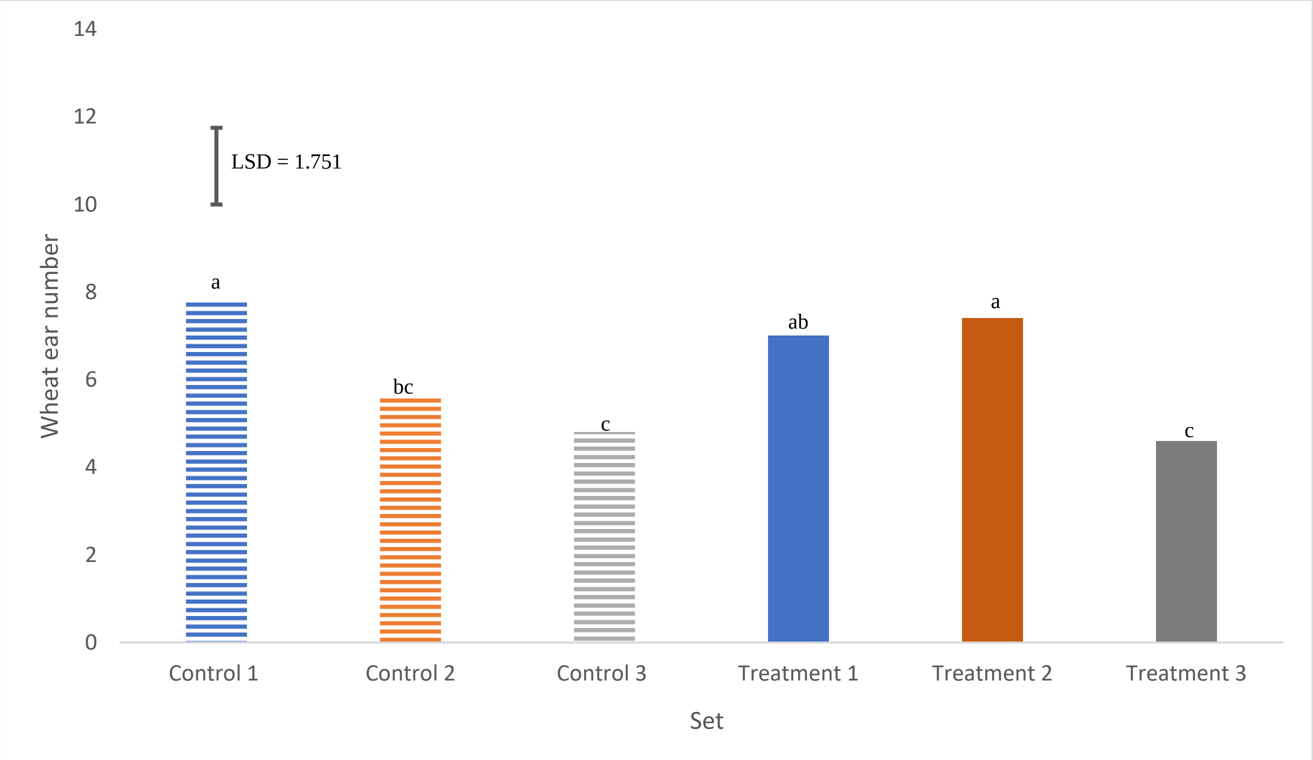


Figure 3.4 : The node number for NIS treated and control wheat plants for the growing season

The data collected correlates with the expected physiological development of the wheat plant, with variation (reading four to six) only observed during the trial as treatments affect the growth rate of the stems. The findings correlate with the findings of Ihsan et al. (2016) in which the phenological differences in development were attributed to the water stress to which the plants were exposed.

Figure 3.5 show the number of ears for the wheat crops. The highest number of ears were found to be from the control, C1, with the soil surfactant treated T2 found to have the second-highest value. The lowest values recorded were found to be the NIS treated T3 and control at 50% irrigation rate (C3). The final tiller number can be correlated with wheat ear number, allowing a percentage conversion to compare overall economic productivity. The control C1 had a conversion of 63.9% and T1 was found to be 61.4%. Control 2 was found to be 59,6%

were T2 was 63.8%. The lowest irrigation rates C3 and T3 were found to have a conversion rate of 60 and 46% respectively.



Bars with the same letter do not differ significantly from each other (P<0.05)

Figure 3.5 : The average ear number per plant of the NIS treated and control wheat plants at the conclusion of the growing season

The observations of the trial aligned well with the expected outcomes. No significant difference was expected between the control (C1) and the NIS treated crops receiving 25% less water (T2) while it was expected to differ from C2. According to Akram (2011), the overall wheat biomass can be correlated to water availability. If water stress was mild, the overall production of biomass was affected, but not significantly so. The outcome is further in line with the manufacturer recommendations that irrigation could be reduced when adding the NIS. The economic yield and yield components (Ye) were correlated with the relative water content (RWC) of the plants. Thus, by adding NIS, it should contribute to a decrease in water stress and an increase in PAW.

As each tiller terminates in an ear, the number of ears should show a close correlation with the stem number. Interesting is the continued good performance of the T2 treated plants with a conversion ratio almost equal to that of C1, which did not experience drought. The tiller to ear conversion ratio is directly linked to plant stress and allows a better understanding of the resource efficacy of each treatment. Abid et al. (2018) found that drought stress at a moderate to mild level could be reversible in most phases of growth for wheat, but severe stress led to damage that decreased tiller number and overall grain yield due to lack of available water. At the 75% irrigation rate, the NIS could have decreased the water stress, allowing for a high ear survival rate, while at the 50% irrigation rate the NIS could not prevent severe water stress. The observations made at 50% can be correlated to the findings of Boutraa et al. (2010)

where under severe water stress, the wheat yield, tiller number and ear formation were significantly affected when water stress was not alleviated.

Figure 3.6 shows the main stem height development of the wheat during the growing season. The growth followed the expected growth curve for crops. The first five measuring dates show very close clustering of the readings, with the final reading showing more discernable results. The lowest recorded height amongst the sets was that of the soil surfactant treated crop T2, with the highest recorded value that of the control C1. The soil surfactant treated T1 and control C2 were shown to have similar final values. The surfactant treated crop T3 and control C3 were found to be at very similar heights by the end of the trial.

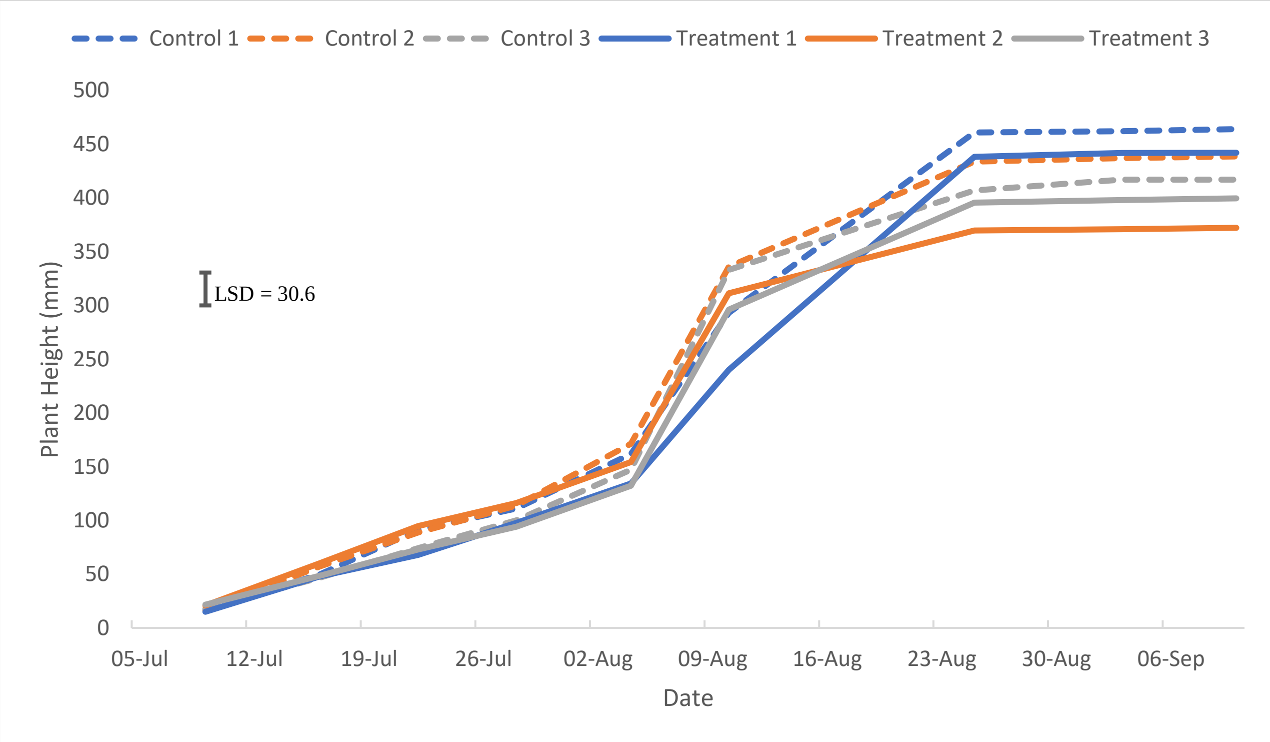
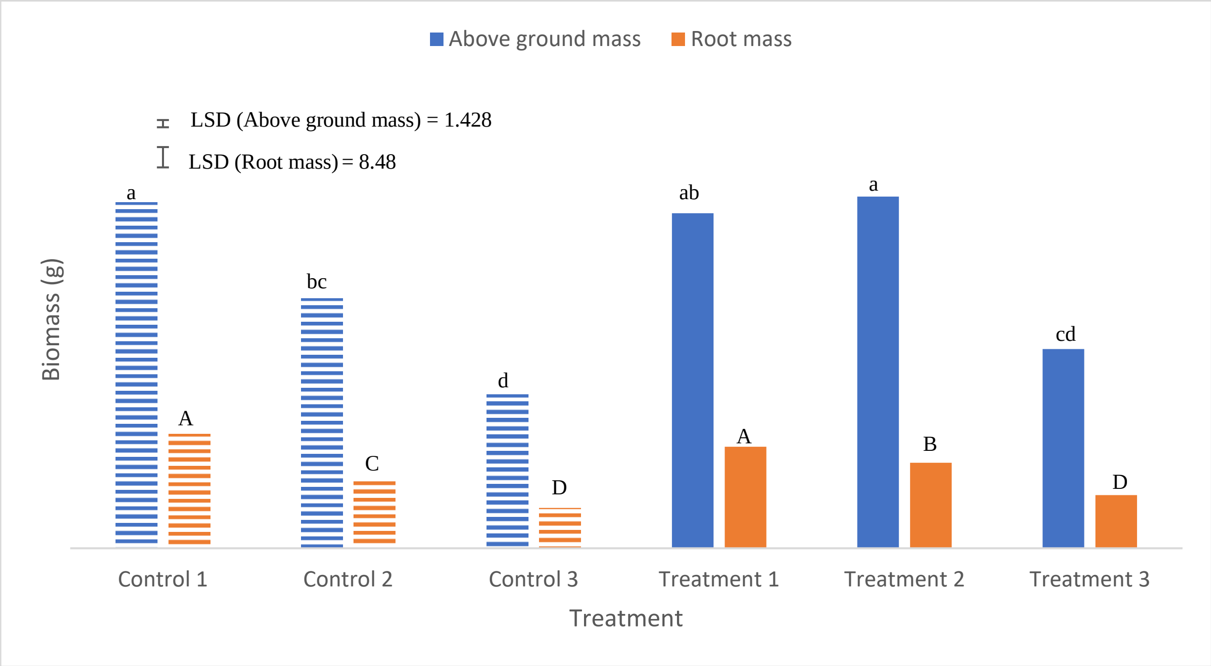


Figure 3.6: The plant height of the NIS treated and control wheat crop during the growing season

The plant height patterns correlate with the crop physiological cycle, with the transition between tillering (up to around 2 August), stem elongation (after around 2 August to about 23 August) and flowering (around 23 August). The results of the trial correlate to the findings of Boutraa et al. (2010) where the height of a wheat plant was affected by water stress at specific growth stages, concluding that in the absence of severe water deprivation, a variation in wheat height was cultivar dependant. It can thus be concluded that water stress during the stem elongation phase will have a more significant impact on plant height than near the end of the growing season of the wheat, which correlates to the transition to the reproductive stage. The decrease of crop height may not necessarily be a negative production factor as shorter wheat varieties are preferred under irrigation conditions due to standability issues.

Figure 3.7 shows the biomass yield per plant at the conclusion of the trial. The above ground mass of plants receiving 75% irrigation plus the surfactant (T2) gave the best results, closely followed by the mass of the C1 plants. The lowest above-ground mass was recorded for the C3 plants, experiencing the highest level of water stress. By adding a surfactant, the T3 plants yielded a higher above ground mass than C3, but it was not significantly so.

The root mass (Figure 3.7) was affected by the irrigation level, with both control and surfactant treatments, being reduced with increased water stress. The root data shows that the highest value was recorded for the C1 plants, closely followed by the surfactant treated plants T1. The surfactant treated crop at T2 showed the third-highest value with the lowest average belonging to the control C3.



Bars of the same colour with the same letter do not differ significantly from each other (P<0.05)

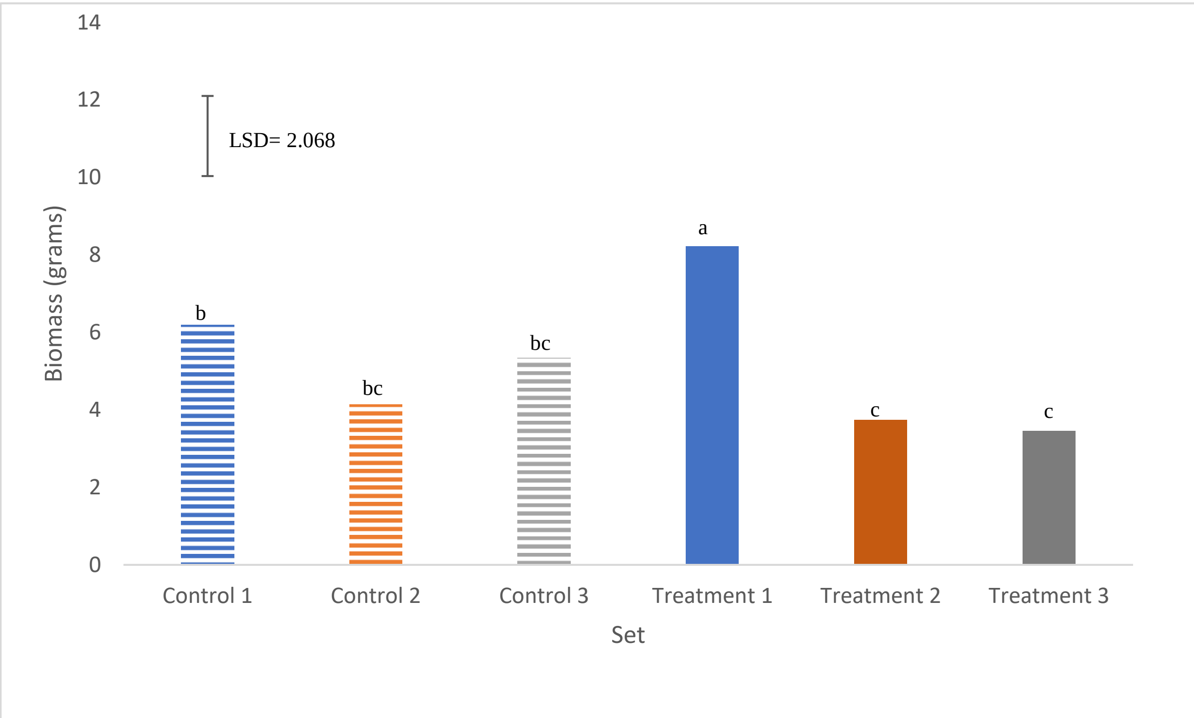
Figure 3.7: The harvested above ground and root mass of the NIS treated and control wheat crops at the conclusion of the trial

As compared to the wheat growth parameters presented earlier, the biomass readings for both the above ground and root mass showed clearer trends. It was hypothesised that all surfactant treated plants would have a higher above ground biomass mass when compared to the control. However, it was only with 100% irrigation where it was not true. In the case of T2, it was around 10 g more than C2 and for T3 it was around 4 g more than C3. Although not always significantly different, the mild (75% irrigation) and severe water stressed (50% irrigation) plants did benefit from the added NIS in terms of above ground mass. The increase of above-ground biomass and the use of non-ionic surfactant biomass was implied in the literature, but not guaranteed.

The observation that T2 did better than C1 and T1, were not expected, but it may have been due to experimental error. During the experiment, the watering of the crop was based on field capacity (θF). Therefore, the water provided to the crop at the 100% irrigation level might have exceeded the needs of the crop, resulting in inefficient water use as compared to T2.

From both the product claims and hypothesis it was expected that the root mass of the surfactant treated plants would be significantly higher compared to the control. The results of the trial correlate with the expected results of the increased root mass, but only under water-stressed conditions (T2 and T3). The reason for this may be because of the ability of the soil surfactants to alter the unfavourable conditions and characteristics of the soil. Soil characteristics that can be changed include water holding capacity, hydrophobicity, plant available water, electron conductivity, pH, and nutrient availability (Bonthuys 2018). The improved soil characteristics allow the spread of water, as well as the spread of wheat roots within the soil profile. The increased spread of the roots was then correlated to an increase in root mass.

Figure 3.8 shows the economic yield (Ye) of the crops at the conclusion of the trial. The highest economic yield was found to be from the surfactant treated plants T1. The second highest value was found to be that of the control C1. The third highest value was found to be the control C3, with the lowest value being found to be the surfactant treated crop T3. The results of the trial demonstrated partial variation from the predicted outcome. It was expected that the addition of the NIS would correlate directly with an increase in economic yield (Ye). The results did not demonstrate the expected yield increase with an increase in water stress (T2 and T3), while under full irrigation the surfactant (T1) resulted in a significant yield improvement. Similar findings were reported by Petersen and Brumbaugh (2001) where the addition of the surfactant allowed a substantial economic yield increase at lower water stress.



Bars with the same letter do not differ significantly from each other ($P<0.05$)

Figure 3.8 : The economic yield of NIS treated and control wheat crop at the conclusion of the trial

3.4 Summary and Conclusions

The results of the trial did not corroborate all the expected outcomes. It was expected that all measured parameters would be significantly improved by the addition of the NIS. However, the trial gave mixed results. One such a result is that the final node number on the main stem did not show any variation with the addition of the NIS or level of water stress but rather aligned with the genetic makeup of the crop.

The chlorophyll and plant height data from the trial did not show clear trend in terms of the applied NIS. It is generally seen that the crops responded to the irrigation rates, rather than to the NIS application. In terms of wheat tiller and ear number the crop demonstrated a possible reaction to the NIS. The irrigation rates of 50 and 75% show a significant improvement in tiller number with the application of NIS, while the tillers at an irrigation rate of 100% did not differ between C1 and T1. The wheat ear number demonstrated a significant variation in favour of the NIS treated crops at the 75% irrigation rate. At 100% and 50% irrigation, no clear effects could be observed. Several of the biomass readings show a correlation with the addition of the NIS: the economic yield at 100% irrigation, and top mass at 75%, while for the root mass, the addition of the NIS was shown to improve biomass. It can also be noted that non-significant improvements to yield with the addition of the NIS were recorded for economic yield at 75% and top mass at 50%.

As previously mentioned by Akram (2011), the overall wheat biomass can be correlated to water availability. If water stress was mild, the overall production of biomass was not significantly affected, this was found to be partially true in terms of the root mass.The economic yield was expected to correlate with the tiller, ear number and biomass results. The recorded results for Ye were found to be contradictory. The results may have been due to experimental error linked to unfavourable temperatures experienced by the crops. At this stage, the data seems to reflect that the effects of the NIS are more easily observed in the once-off or cumulative data points, rather than in weekly readings on wheat. Where there was an effect of NIS on the general growth of the wheat plants, T2 (75% irrigation level plus NIS) seemed to had the most advantages, while for economic yield T1 (100% irrigation plus NIS) gave the best results.

CHAPTER 4 : EFFECT OF NON-IONIC SURFACTANTS ON TOMATO IN A POT TRIAL

4.1 Introduction

Tomato is an important vegetable crop, not only in South Africa but globally. Tomato is grown primarily for its fruit (Costa and Heuvelink 2007). The fruit of the tomato plant is used for various applications that include fresh produce, secondary culinary products such as soups, pastes, and additives and for its chemical components such as lycopene (Kelley et al., Boyhan, et al. 2017).Tomato production generally forms part of an intensive system, in which the labour, start-up cost and maintenance costs are high, giving tomato's a high commercial value, but also leading to an increase in managing technical and practical aspects.

It is important to see how various inputs may affect the crop. The tomato plant is sensitive to environmental changes. The tomato plant has a specific physiological cycle that influences how it is produced. The physiological cycle is as follows: the early stage which includes germination and initial root and leaf formation, the vegetative phase where the bulk of biomass accumulation and plant development occur, the flowering, which is the initial stage of reproduction, fruit formation and finally fruit maturation (Singh et al. 2016). Water stress can affect the tomato physiological at several developmental stages.

Water is the single most important element in plant development. When planting a tomato crop, it is generally necessary to irrigate 25 to 50 mm of water per week in the initial stages of growth (Singh et al. 2016). When the rate of irrigation is decreased below the minimum, there can be direct physiological impacts on the tomato plant. As mentioned by Nuruddin et al. (2003): if a tomato plant is exposed to a significant amount of water deprivation during specific physiological stages, then there will be a noticeable effect. When there is a decrease in irrigation during the initial growth stages of growth, there is little evidence of significant physiological effect, as the plantlet requires little water after germination and initial sprouting (Shinohara, Akiba, et al. 1995). Several studies have shown that when water stress occurs during the flowering, fruit formation and maturation, there is a significant effect on the crop's overall productivity (Wudiri and Henderson 1985, Nuruddin et al. 2003).

Exposure to water stress has several physiological effects, this in turn can be linked to specific metrics that can be measured through the growing season. Several of the metrics that can be measured include chlorophyll content, root mass, top mass, fruit mass (Ye), fruit number, and plant height. The chlorophyll reading allows observations on the photosynthetic activity of the plant, revealing the overall productivity of the plant. The root mass reveals the dual purpose of biomass accumulation as well as the water stress status of the plant. The top mass would allow observation of biomass accumulation. Fruit mass can reveal the economic yield and biomass accumulation to be calculated. The plant height will allow observations into the developmental rate and reaction to water deficit.

The use of surfactants within the agricultural sector is growing, more so in higher-value crops such as vegetables. As previously mentioned, a non-ionic surfactant (NIS) is a compound that does not carry a charge (Castro et al. 2013). The effectiveness and reactivity of a NIS are dependent on the specific combination of compounds that is used (Castro et al. 2013). The formulation that was utilized during the trial contained the following: Ethylene oxide polymers, Polyether compounds.

The tomato crop is a suitable plant to better understand the effects that a NIS may have on not only vegetable crops but also Dicotyledons. Dicotyledons, also known as angiosperms, are defined as flowering plants that has a pair of leaves (cotyledons), in the embryo of the seed (Anon. 2019). Dicotyledons make up approximately 75 000 plants that can be found across the world. Dicots include several important food crops which include most vegetables used within intensive agriculture such as legumes, brassicas, and the aster plant family. The trial aimed to explore the viability and effect of a NIS on tomato as a representative crop for dicotyledonous vegetables.

4.2 Methodology

Experimental site:

The trial was carried out in a controlled environment, where conditions such as temperature were regulated. The pot trial was conducted at the University of Pretoria, Experimental Farm, Phytotron A (-25.7510859/ 28.2566342). The greenhouse is an A-frame structure with an automatic wet-wall system set to maintain an average temperature of 20℃ - 25℃. It is important to mention that on several dates, technical problems did occur within the greenhouse causing temperature and humidity variations, which could have affected the results. The greenhouse is equipped with four automatic rotating tables. One of the rotating tables were used during the trial (Figure 4.1). It must be noted that due to the Covid-19 pandemic, to gain access and better control over the pots during the lockdown period, all plants were moved to a secondary greenhouse which can be seen in Figure 4.2. This greenhouse did not have a rotating table, but treatments were re-randomised within this secondary greenhouse.

Figure 4.1: The tomato crop placed on the rotating table during the first trial at various NIS dosages and irrigation rates, within a climatic controlled greenhouse

Figure 4.2: Secondary Greenhouse (Palram essence) used during the second season (2020) due to the Covid-19 pandemic.

Experimental design:

Crop:

The dicotyledon, Tomato (*Solanum lycopersicum*), cultivar, Degas with a determinate growth from was used. A determinant variety was used due to the well-defined life cycle stages such varieties exhibit. Degas was bred as a fresh market type of tomato, producing medium-sized tomato fruit (160-180g). In each of the pots, two tomato seeds were placed one centimetre below the surface of the soil. It must be noted: As with the wheat trial, once germination has occurred, the better of the two germinated seeds were left in the pot, the other was removed. The first tomato trial ran simultaneously with the wheat trial in the greenhouse from the beginning of June to the end of October 2019. The duration of the trial was a total of five months. The second tomato trial commenced in March and was terminated at the end of July 2020.

Treatments:

A light sandy soil was used during the trial as it is ideal for these types of NIS blends. For each pot, 20 kg of soil was weighed out and placed within the 10-litre pots. There were two distinct groups of treatments with three subgroups each. The first group is the controls with the subgroups: Control one (C1): an irrigation rate of 100% with no added surfactant, Control two (C2): an irrigation rate of 75% with no added surfactant and Control three (C3): an irrigation rate of 50% with no added surfactant. The second group is the surfactant treated group with the subgroups: Treatment one (T1): an irrigation rate of 100% with the recommended application rate of non- ionic surfactant (NIS), Treatment two (T2): irrigation rate of 75% with the recommended application rate of non- ionic surfactant and Treatment three (T3): an irrigation rate of 50% with the recommended application rate of a non- ionic surfactant. The plants were irrigated twice a week, while fertilizer was applied to each pot every two to three weeks, in accordance with the DAFF tomato fertilization recommendations (DAFF 2019). The fertilizer used during the trial was classified as water-soluble. A foliar application of micronutrients was also applied every two to three weeks. The manufacturer specifies that the product would work best when applied in conjunction with the fertilizer.

Measurements

The measurement that was taken can be categorised into two groups: weekly readings and once-off readings. The weekly readings included chlorophyll readings, plant height, flower number and fruit number. The once-off readings include biomass (root and above ground), economic yield (Ye), fruit Brix readings and stem thickness.

Weekly readings:

Chlorophyll readings:

An Atleaf™ chlorophyll meter was used for the readings. Readings were taken once a week during active growth. A total of two readings were taken per plant. The readings were taken from two new and fully mature leaves. The average of the two readings was recorded.

Plant height:

Once a week during active growth, readings were taken using a ruler. The measurements were taken from the base of the crop to the top of the primary tomato stem.

Flower number:

Once a week for all the treatments, flower number was counter per pot and recorded. The flower number can fluctuate weekly as either abortion or the formation of fruit occurred.

Fruit number:

Once a week, once fruit formation was initiated in the crop, a count was done. The fruit number for each of the treatments was counted. The fruit value may also fluctuate due to new fruit forming while ripe fruit was harvested. Flower to fruit conversion percentage was calculated after the data collection. The flower to fruit ratio is used to determine the conversion efficacy of each treatment.

Once-off Readings:

Stem thickness:

The diameter of the main stem of each of the plants was measured after the trial. The measurement was done with a ruler to measure the main stem cross-section.

Tomato biomass:

Once the tomatoes reached the end of the season, in line with standard harvest practices recommended by the Department of Agriculture, Forestry and Fisheries (DAFF 2019), the plants were harvested and divided into two separate sections: tomato above ground mass and root mass. The material was kept separate for further analysis.

Above ground and root biomass:

The above ground material was placed in brown paper bags and dried in an oven set at 60°C for 48 hours. After drying the dry mass was determined and recorded. The roots were removed from the pot by using a fine sieve as well as washing away the potting soil. The collected roots were placed in a brown paper bag, dried at 60°C for 48 hours after which the dry mass was recorded.

Tomato fruit readings:

Tomato fruits were picked continuously throughout the growing season when it fully turned red. The fruit was immediately weighed, and juice extracted to carry out Brix readings. The procedure was carried out until most of the fruit was collected from the plants. At the conclusion of the trial, the total fruit number per plant was calculated and recorded. The irrigation was discontinued when most of the fruit was harvested

Trial layout and Statistical analysis

The Experimental design used for the trials is a completely randomised design (CRD). A total of five replicates were used per treatment combination. The pots were placed on the experimental rotating table after being randomised using Randomex. The collected data was analysed by a statistician at the agricultural research council (ARC) in Hatfield Pretoria. The data underwent ANOVA analysis. The Irrigation rates (IRRI), treatments (TMT) and specific dates (DATE) were analysed for significant variation. The least significant difference of means was calculated at a 5% level.

4.3 Results and discussion

Table 4.1 illustrates the chlorophyll readings for the first season of the tomato crop, between July to September 2019. A date-by-date analysis of the chlorophyll content illustrates that in general throughout the trial, the irrigation rate at 50% (C3 and T3) yielded the highest values, with the 100% irrigation rate (C1) having the lowest values. It is also generally observed that the crops at the two irrigation rates of 75 and 100% often had statistically the same and the lowest values, with a few exceptions during the season. A comparison between sets at the same irrigation rate shows the NIS crop (T1) generally yielding a higher value for 100% irrigation, with the control (C3) yielding higher at 50% irrigation and little to no variation in readings at 75% irrigation (C2 and T2). There was, however, no significant differences between the sets (control versus NIS) at the same irrigation level.

Table 4.1: Leaf chlorophyll readings for the first season of Tomato production at various irrigation rates and NIS dosages

Date	16 Jul	29 Jul	4 Aug	13 Aug	24 Aug	6 Sep	22 Sep
C1	36.06^{kl}	36.06^{kl}	35.5^{kl}	51.54^{fghi}	52.86^{efg}	51.4^{fghi}	48.36^{i}
C2	36.1^{kl}	36.24^{kl}	35.88^{kl}	55.48^{bcde}	53.74^{cdef}	56.58^{bcd}	56.82^{bc}
C3	40.3^{j}	40.32^{j}	38.16^{jk}	57.92^{ab}	55.98^{bcde}	58.22^{ab}	60.36^{a}
T1	37.0^{jkl}	37.06^{jkl}	37.78^{jkl}	49.48^{ghi}	51.9^{fgh}	53.62^{cdef}	48.86^{hi}
T2	36.4^{kl}	36.42^{kl}	34.36^{l}	55.68^{bcde}	50.84^{fghi}	53.62^{cdef}	53.2^{def}
T3	37.8^{jkl}	37.86^{jkl}	37.86^{jkl}	58.26^{ab}	53.53^{cdef}	56.98^{bcd}	58.08^{ab}
LSD	3.530						

The letter present in the table are indicative of significant differences between values. If a letter is shared between values, then there is no significant difference between those values.

For the second season of the tomato crop, recordings were made between March and June 2020 (Table 4.2). The second season did not show the same trends when compared to the first season. The first season showed significant increases or variations between several of the dates, where the second season shows a gentler slope when an increase occurred. The highest readings for the second season were generally found to be the control at 100% (C1) for the initial four readings up to the 26th of April, with the lowest reading being with NIS treated at 100 (T1) and 75% (T2). For the second half of the trial from the 5th of May, the control at 75% (C2) was shown to have the highest values with the lowest values being interchangeable with little discernible trend to be observed. The second half of the season showed greater homogeny of readings between the irrigation rates, with non-significant variations between the values.

Table 4.2: Leaf chlorophyll readings for the second season of Tomato production at various irrigation rates and NIS dosages

Date	10 Mar	18 Mar	18 Apr	26 Apr	6 May	18 May	26 May	8 Jun	27 Jun
C1	44^{stuvw}	45^{qrstuv}	$52^{abcdefghi}$	55^{ab}	$49^{hijklmnopqr}$	54^{abcdef}	$51^{abcdefghijk}$	$49^{efghijklmnopqr}$	42^{tuvw}
C2	42^{tuvw}	46^{pqrstu}	$51^{abcdefghijk}$	$53^{abcdefgh}$	$53^{abcdefg}$	55^{a}	54^{abcde}	54^{abcd}	$49^{fghijklmnopqr}$
C3	41^{uvw}	46^{opqrst}	$49^{ghijklmnopqr}$	$49^{ghijklmnopqr}$	$51^{abcdefghijk}$	54^{abc}	$53^{abcdefg}$	54^{abcdef}	$51^{abcdefghij}$
T1	41.3^{w}	$46^{lnopqrst}$	45^{rstuv}	$50^{cdefghijklmnop}$	$47^{klmnopqrs}$	$52^{abcdefghi}$	$52^{abcdefghi}$	$50^{abcdefghijklm}$	42^{tuvw}
T2	33^{x}	44^{stuvw}	$47^{jklmnopqrs}$	$52^{abcdefghi}$	$50^{bcdefghijklmno}$	54^{abc}	$52^{abcdefghi}$	$53^{abcdefgh}$	$50^{abcdefghijkl}$
T3	35^{vw}	45.8^{rstu}	$48^{ijklmnopqrs}$	$50^{abcdefghijklmn}$	$50^{abcdefghijkl}$	$51^{abcdefghijk}$	$50^{defghijklmnopq}$	$51^{abcdefghijk}$	$49^{defghijklmnopqr}$
LSD	4.517								

The letter present in the table are indicative of significant differences between values. If a letter is shared between values, then there is no significant difference between those values.

Note: Decimals have been removed from the table to accommodate the values in the table

The analysis of the chlorophyll data showed a partial variation from the hypothesis of the experiment, in terms of each season individually, as well as a comparison between the two. For the trials to fully corroborate the stated hypothesis 2, it was expected that across all the irrigation rates the NIS treated crops would yield a higher chlorophyll reading. An expected variation to be noted in the trial would be the second set of readings from the second season of production due to technical errors and environmental changes.

The first season trend showed the lowest irrigation stressed crops, without NIS treatment consistently yield the highest or second-highest readings. The same trend was observed in the wheat crop. As previously stated, according to Farooq et al. (2009) generally water stress is associated with a decrease in chlorophyll content in plants due to physiological alterations to survive high-stress events such as drought by closing stomata and down regulating photosynthesis. As stated, in the wheat chapter, the results observed in the first season of tomato production may further corroborate the finding of Khayatnezhad et al. (2011), which found that in experimentation with maize (*Zea mays)* several cultivars with specifically bred genotypes had an increase in chlorophyll content under water-stressed conditions.

For the tomato, a partial rejection of the hypothesis is evident when comparisons between sets in the same irrigation rate are carried out. It is only at 100% that the NIS treated crop (T1) often performed nominally better when compared to the control (C1).

The initial readings of the second season of production did not yield the same result as the first. For the second season, the results first show contrarian readings with each of the controls generally performing better, than the NIS counterpart. The second season saw a change in environmental conditions to which the crops were exposed. The results of the second season, although not in line with the first may still be a possible indication of the NIS effect.

As with the wheat trial, the tomato plants were expected to demonstrate the same outcome, where the addition of a NIS would produce a strong correlation with a decrease in plant water stress which would lead to higher chlorophyll readings (Khayatnezhad 2012, Liwani, Magwaza et al. 2019). The variation from the expected result for the tomato would suggest that the results are linked to underlying factors.

The first and second seasons both saw abiotic stress factors in the form of temperature, time of year the trial was conducted, physical damage, and temperature. As stated in the methodology: technical issues with the greenhouse and the outbreak of Covid-19 led to factors that could not be accounted for. The results do suggest that NIS may interact with stress factors that could not be accounted for between the two trials, leading to the observed results. This explanation is further corroborated by the observations of the wheat trial.

Tomato plant height as affected by water stress and NIS application in the first season (between July and October 2019) is illustrated in Figure 4.3 . The first readings of the season demonstrate no variation in any of the irrigation rates or treatments. This is to be expected since, after germination, tomato seedlings of similar height were chosen to remain in the pots. As the season continued, variation was observed between the treatments and the irrigation rates of the crops. The treatment T1 receiving an irrigation rate of 100% in addition to the NIS, initially lagged as compared to the control C1 treated crop but eventually overtook the control by the conclusion of the trial. At the irrigation rate of 75%, the opposite was observed, where the control and the NIS treated crop had initially similar readings, but with the control C2 ending with a higher value after the trial. The 50% irrigation rate counterparts had a similar trend to those at the 100% irrigation rate, but where the NIS treated crops (T3) were already far taller than the control C3 plants from about the middle of the growing cycle till the conclusion of the trial.

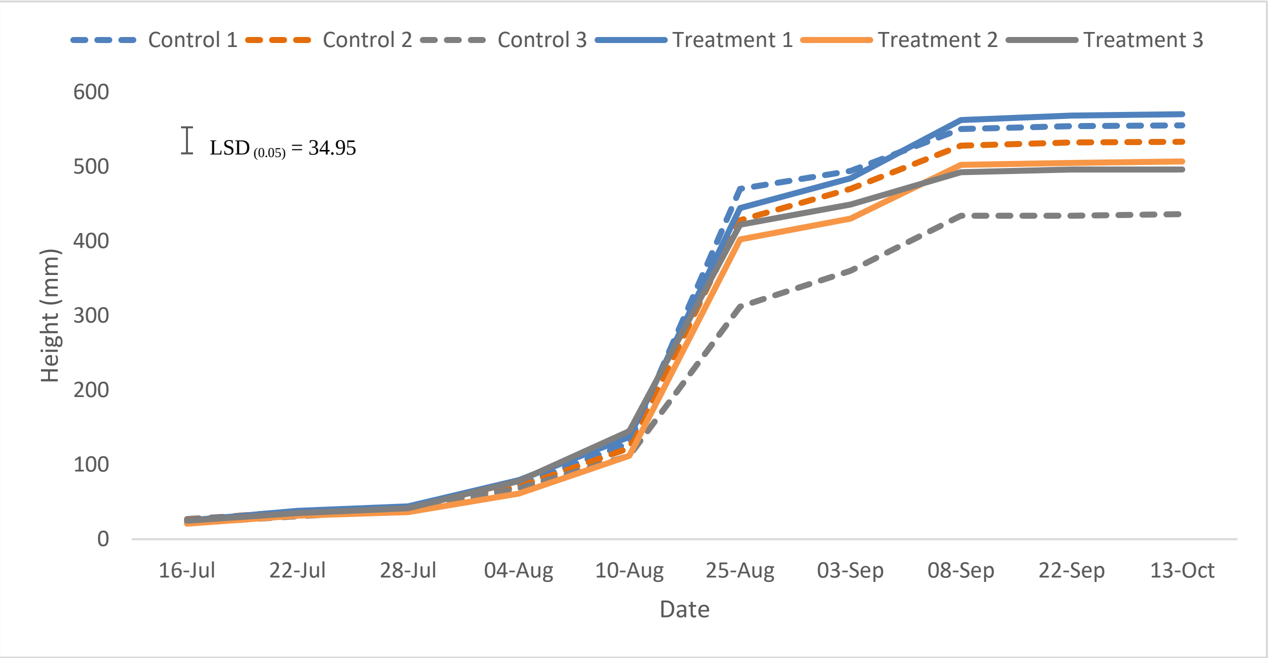


Figure 4.3: Tomato plant height of the first season's trial at various NIS dosages and irrigation rates

In Figure 4.4 the second season of the tomato production of the crop at various NIS dosages and irrigation rates between March and June 2020 is illustrated. Since the trial was started earlier in the season, the temperatures were higher which led to tomato plants being taller in the second than the first season. The tomato plant height in the second season shows a greater level of clustering than that of the first season, much like the second season of the chlorophyll readings. The most noticeable variation of the second season's data is that all the irrigation rates of the trial show the NIS treated crop having a higher value when compared to the control group. The data also shows that the control groups across all the irrigation rates have smaller variations when compared to the first season. It should be noted that the differences between the NIS treated, and control groups were not generally statistically significant except for a single date (7 May). The plant height data did not fully support the stated hypothesis of the trial. As previously seen in the chlorophyll data, the two seasons did not correlate with each other. The first season showed a greater spread when compared to the second. The first season did partially correlate with the hypothesis, as two of the irrigation rates did show results that favoured the NIS treated crop. The unexpected variation is seen at 75%, as a 25% reduction in irrigation is the general recommendation from the surfactant producer. The most statistically significant variation is seen at 50%. This result does correlate with the first season of tomato's chlorophyll data, although it did not align with the findings of the wheat. The second season demonstrated a greater level of overlap between the sets. The first and second seasons both show that both seasons had the same high and low values. The overlap of the results would suggest that if any interaction were present, it was not significant enough to be observed.

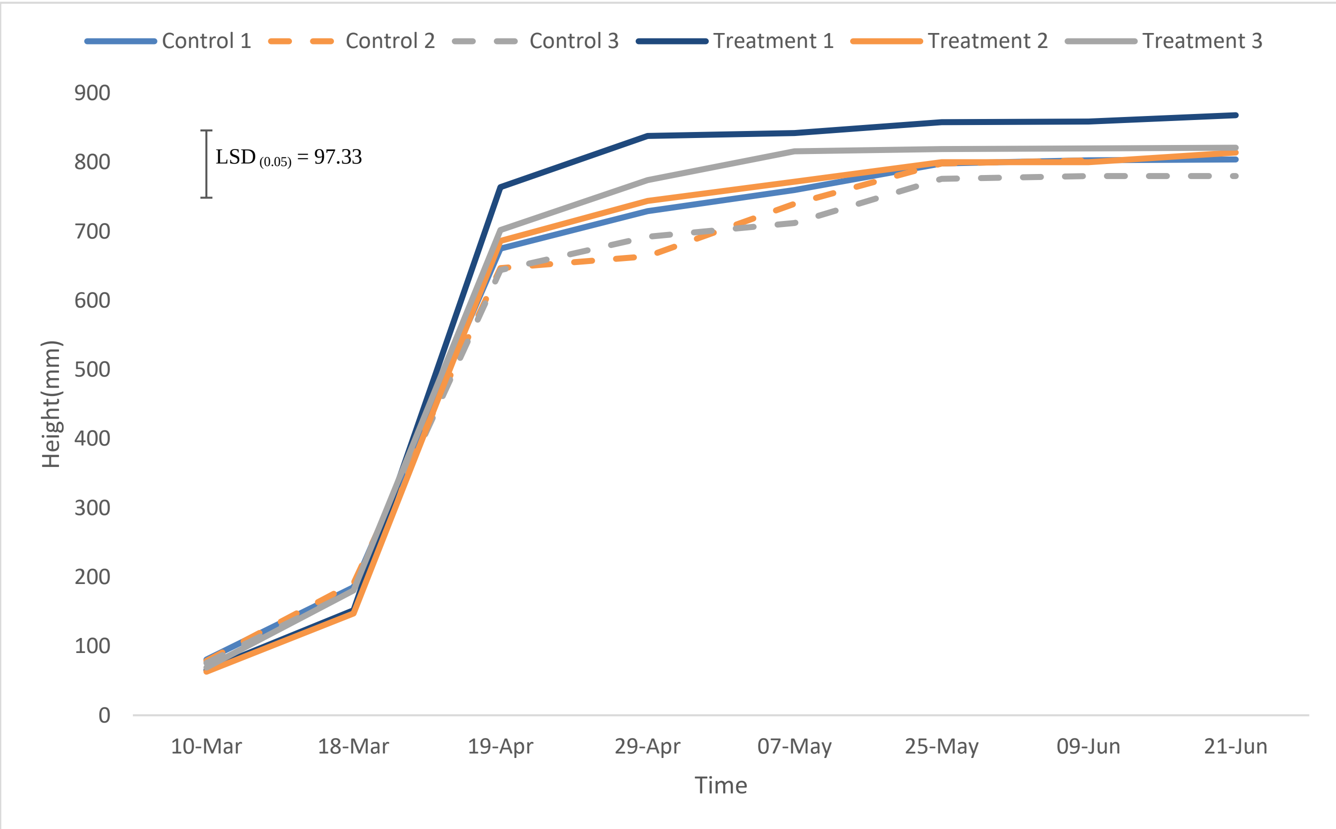


Figure 4.4: Tomato plant height of the second season at various NIS dosages and irrigation rates

As previously stated, the second season of production experienced several technical challenges not seen in the first season. The technical problems of shifting the crop may have added additional stress to the plants, leading to an outcome that is different from that seen in the first season of production. It must be noted that although the NIS treated crops for both the first and second season did perform better as compared to the controls, it was generally not significant, more so in the second season. The second season of the tomato production, although not showing much statistical significance, did align more with the expected outcome of the trial.

As stated in the literature; the moisture present in the soil is positively correlated with growth indices of the root as well as the above-ground parts such as the stem, leaves and flowers (Senthilkumar et al. 2017, Shinohara et al. 1995). The literature also stated that a tomato exposed to a high level of consistent water stress is found to be significantly shorter when compared to plants with a greater level of irrigation (Sibomana, Aguyoh, et al. 2020).

In Figure 4.5 the tomato flower number recorded during the first season of production at various NIS dosages and irrigation rates is illustrated. All the sets began flowering at the same time, with the first flowers observed on 4 October. The flower number of the control group (C1, C2 and C3) correlated with the applied irrigation rates, with the plants receiving more water tending to have the highest number of flowers and those receiving the least amount of water producing the least number of flowers. By adding NIS, there was no clear trend by adding less water, and in most cases, it recorded fewer flowers than the control counterparts. The maximum flower number for C1 is 35, with C2 as 29.6 and C3 as 24.4. For the NIS treated crops the maximum values were T1 as 26.4, T2 as 27.8 and T3 as 23.

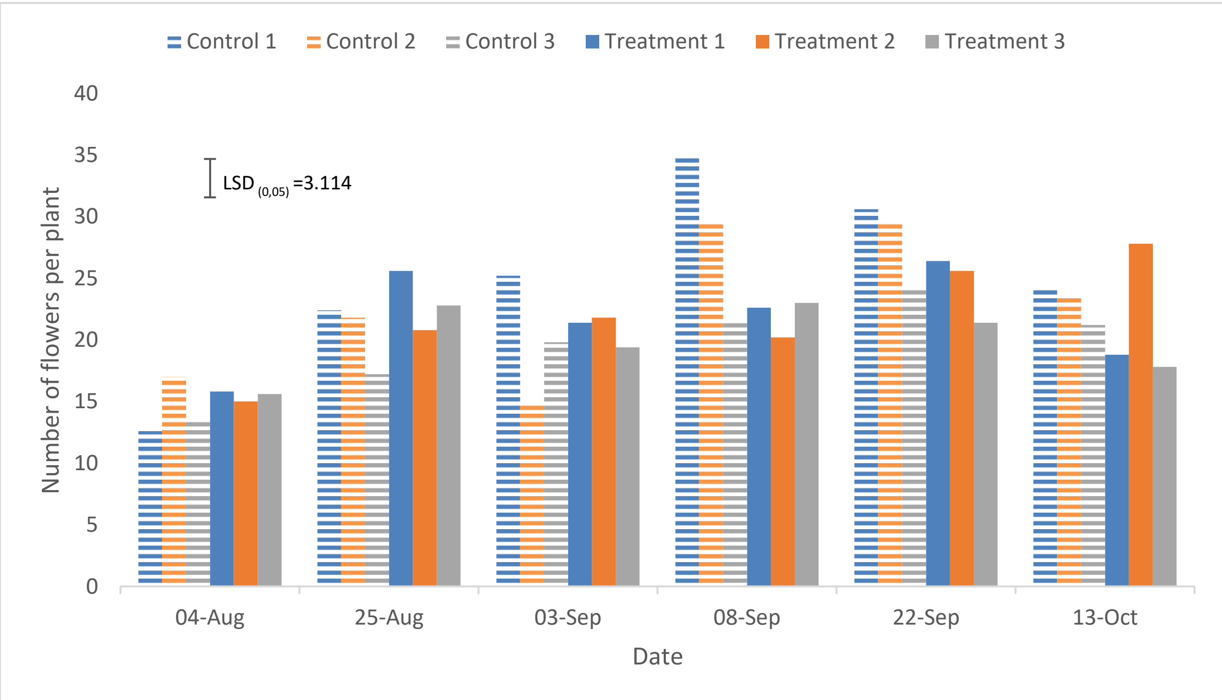


Figure 4.5: Tomato flower number of the first season recorded during the trial at various NIS dosages and irrigation rates.

The flower number of the second season (Figure 4.6) does not reflect what happened in the first season. During the second season, the highest values were recorded for the NIS treated T1 plants, followed by the control C2 plants. The main variation was that the NIS treated plants generally produced more flowers as compared to the control at the same irrigation rate. In addition, the plants produced on average around 5 to 10 flowers less in the second than in the first season.

This might be explained by the differences in temperatures experienced by the plants in the two seasons. It is important to note that, unlike the first season, an accurate indication of flowering initiation was not observed due to technical complications that arose during the growing season. The maximum flower number for the control was seen as C1 as 22.9, C2 as 27.2 and C3 as 19.4. The maximum flower number for the treatment is T1 was 29, T2 was 21.4 and T3 was 22.2.

The results of both trials, as with the previously discussed data did not show comparable results, with mixed results in terms of the stated hypothesis. The flower data for the first and second seasons demonstrates that the addition of the NIS did not influence the initiation of flowering. Thus, the NIS, did not affect the plants' transition between the vegetative and reproductive phases.

The first season does suggest a possible interaction of the crops with the addition of the NIS, although, not as expected. The erratic trend seen amongst NIS treated plants when compared to the control crops would suggest that the NIS did affect the plants. It is not possible to say that the addition of the NIS had a positive or negative effect on overall production.

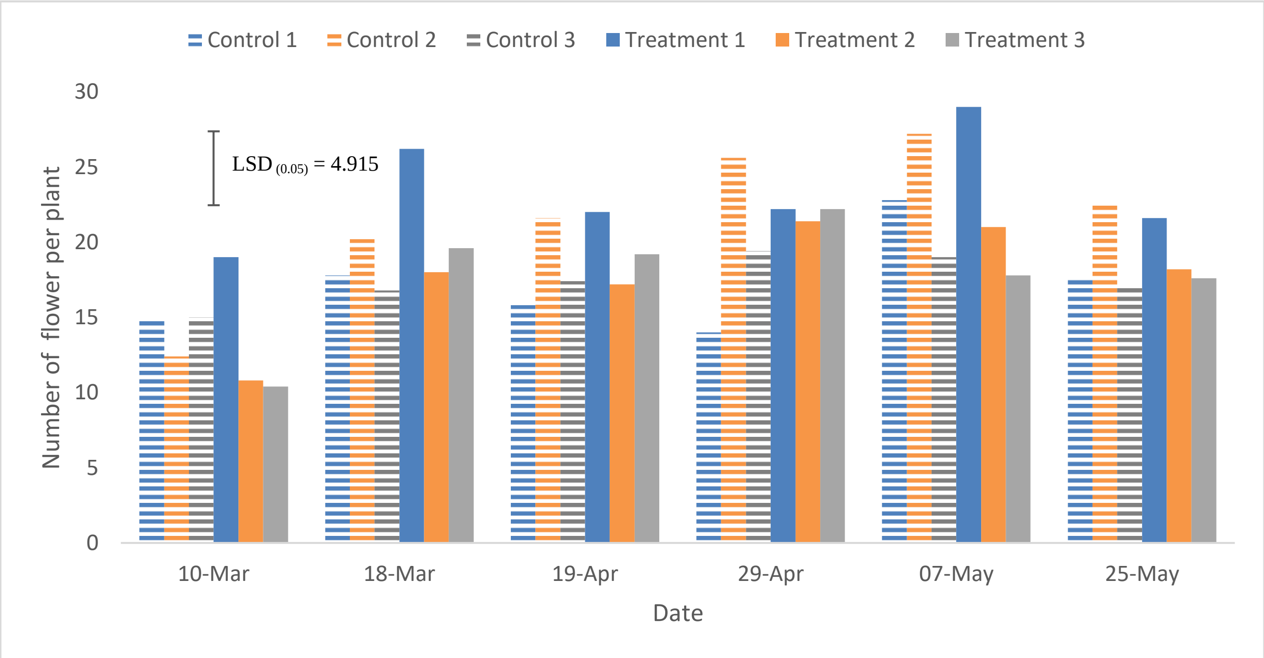


Figure 4.6: The average tomato flower number of the second season recorded during the trial at various NIS dosages and irrigation rates

The second season of production showed the same erratic pattern for both the control and the NIS treated sets, comparable to that of the NIS treated crop from the first season. Between the irrigation rates, in the second season, the plants not exposed to water stress (T1) tended to outperform the controls, which may be indicative of a possible NIS effect.As the results for both seasons of the trial show strongly mixed results, the flower data, generally did not align with the stated hypothesis. It was believed that the addition of the NIS would lead to a decrease in plant water stress leading to a greater recorded flower number, lower flower abortion of tomato flowers as well as overall fruit number increase (Birhanu and Zeleke 2010). It is important to note that flower number needs to be analysed in the context and flower abortion and fruit number to conclude if the NIS has a positive or negative effect on overall production.

The weekly fruit number for season one is presented in Figure 4.7. The first fruit was recorded on 25 August. The irrigation rates of 75% and 50% (controls and NIS treated plants) were the first to bear fruit, with the 100% irrigation rate (C1 and T1) initiating fruit a week later on 3 September. Although C3 and T3 initiated fruits before C1 and T1, the weekly fruit number at the end of the season for the water-stressed plants (50% irrigation rate) was significantly lower than for any of the other four treatments. The highest final value was recorded for the 100% control C1, followed by the control at 75% C2 and NIS treatment at the 75% irrigation rate (T2). At the conclusion of the first trial, the highest fruit number was determined for each set. The highest average fruit number recorded produced for C1 was 6.2, C2 was 5.4 and C3 was 2.8. The highest fruit number for the NIS treated crop: T1 was 4.2, T2 was 5.2 and T3 was 2.8. The data collected from the flower and weekly fruit number allow the generation of a flower to fruit conversion percentage to understand flower and fruit abortion rate. For T1 it was 15.9%, T2 was 18.7% and T3 was 12.1. The control values were C1 at 17.71%, C2 at 18.2% and C3 at 11.5%.

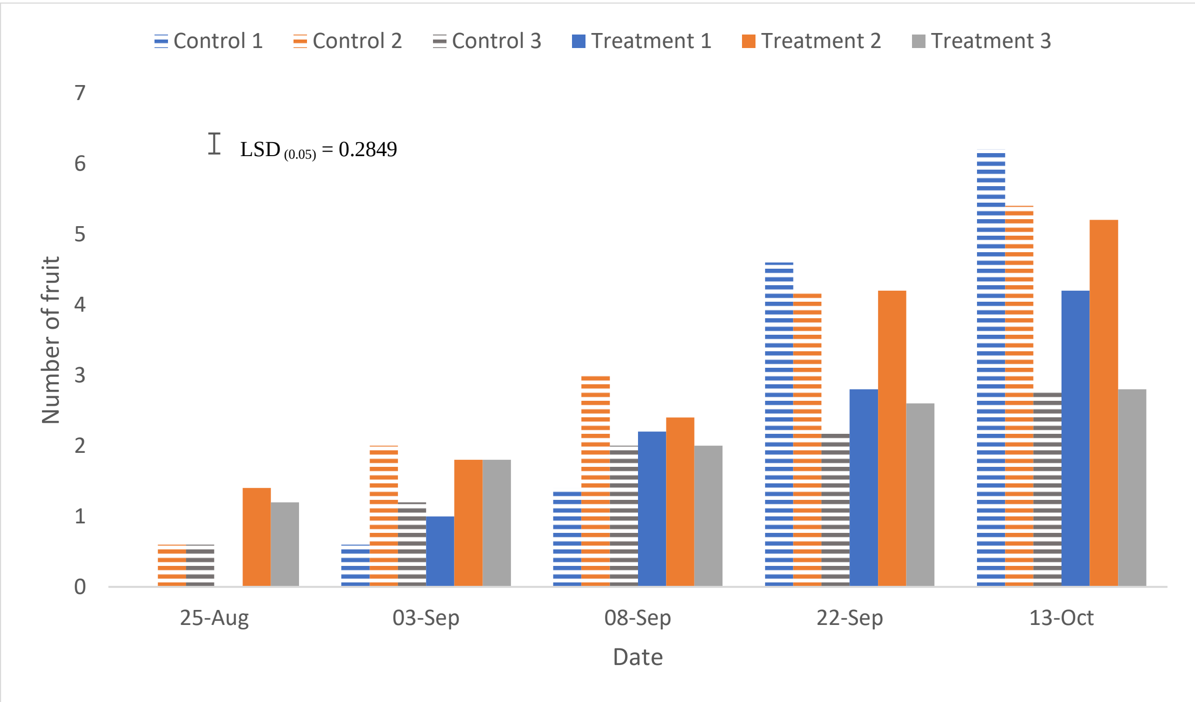


Figure 4.7 : The fruit number of tomato plants recorded weekly for the first season

Figure 4.8 shows the fruit number for the tomato plants during the second season of production at various dosages of NIS and irrigation rates between March and June 2020. As previously stated, an accurate recording of fruit initiation could not be carried out due to technical difficulties. The second season varies from the first, as the control group shows a strong correlation with the irrigation applied with decreased yield as water stress increased. The NIS treated crops for the second season followed a similar trend to the first, where they generally performed better than the control, especially T1 and T2. At the conclusion of the first trial, the final fruit total was determined. The total average fruit produced for C1 was 7.2, C2 was 10.2 and C3 was 6. The NIS treated crop values were: T1 was 12.4, T2 was 10 and T3 was 6.6. The flower to fruit conversion percentage was for T1 it was 44.1%, T2 was 60.7% and T3 was 30.6%. The control values were C1 at 36.8%, C2 at 29.4% and C3 at 38.1%. The flower to fruit conversion was around three times better in season 2 than in season 1.

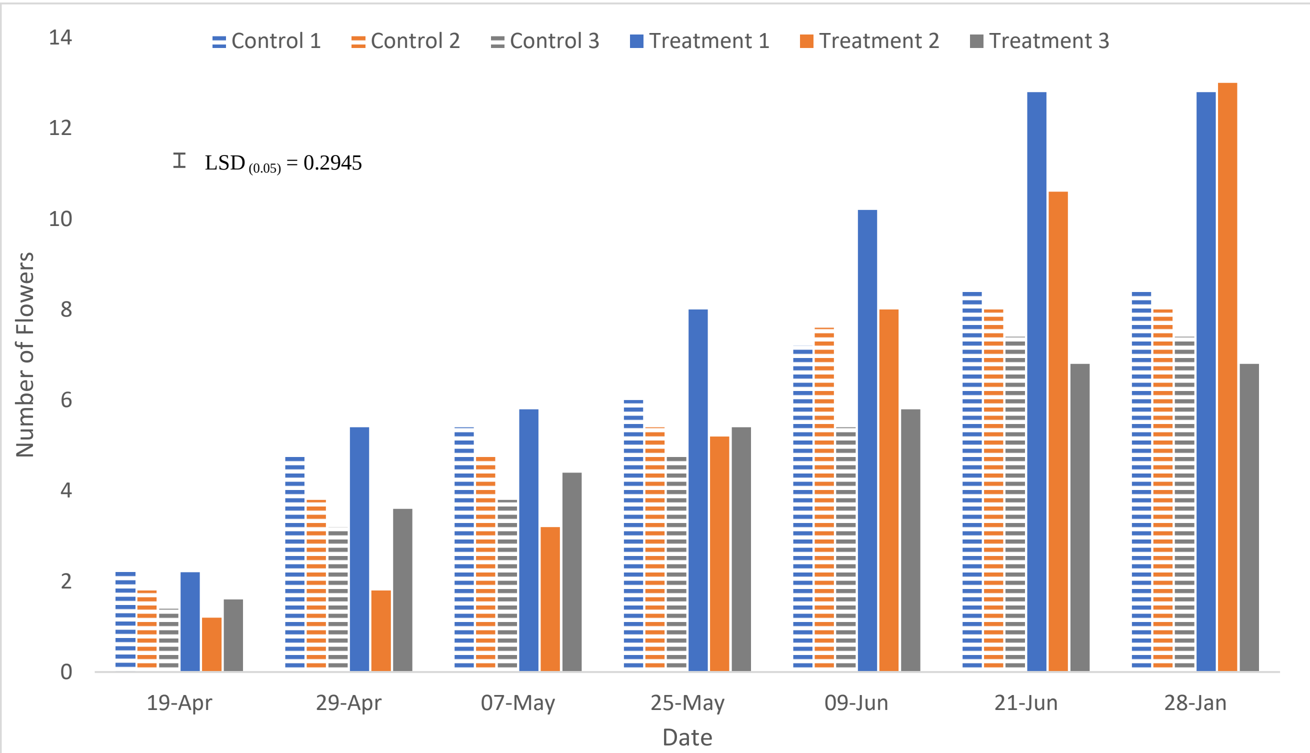


Figure 4.8: The fruit number of tomato plants recorded weekly for the second season

The results of the fruit number data did not vary to the extent of the plant height and chlorophyll data. The flower to fruit conversion values allows a better understanding of the economic productivity of the crops. A higher conversion value is indicative of a better yield as well as lower plant stress. A plant that is under lower stress, will have a lower rate of both flower and fruit abortion.

The first season of the tomato fruit number does not immediately show a positive correlation with adding NIS. But when the flower to fruit ratio was calculated, it shows that at two of the irrigation rates, 75% and 50%, the crops did better with the addition of the NIS, although it can be noted that the improvement is not significantly larger than the control. The percentage conversion could suggest a possible positive impact, where adding the NIS while reducing irrigation, the values were improved.

The second season of production, although facing technical difficulties showed a similar trend to the first, differing by the extent of the variation of the plants in each irrigation rate. All plants produced a better result, but the NIS treated plants did substantially better at 100% and 75%. The result of the second trial may further reinforce the possibility that the NIS application improved the tomatoes fruit number. With the positive correlation mentioned, it must be noted that the technical difficulties experienced during the second trial may have led to experimental error.

Birhanu and Zeleke (2010) illustrated a decrease in economic yield as well as overall biomass, where a tomato crop was exposed to high levels of water stress. A tomato plant that is exposed to both water stress and high temperature is shown to have a lower flower and fruit number when compared to the control (Wudiri and Henderson 1985). The addition of the surfactant is expected to aid in the relief of water stress, therefore relieving the physiological stress that the plant is under, allowing an increase in flower and fruit number (Trinchera and Baratella 2018).

Figure 4.9 shows the cumulative economic yield of the first and second season of tomato, under various NIS dosages and irrigation rates at the conclusion of the trial. The first season shows a similar trend between the control and the NIS treated crops, where the economic yield is correlated to the irrigation rate that was applied. The highest values were therefore for the crops at 100% irrigation for each treatment set, where the lowest yield was at the 50% irrigation rate. The first season did show that at the irrigation rate of 100%, the control (C1) yielded a better than T1, where the NIS treatments (T2 and T3) both performed better than C2 (75% irrigation) and C3 (50% irrigation). The second season showed a similar trend to the first, but with yields being substantially higher than in the first season. The control and the NIS treated crops both showed yield that again correlated with the irrigation rate applied, thus reduced yield with increased water stress. The difference between the first and second season was that the results were inverted, where now the irrigation rate of 100% was higher in the NIS treated (T1 versus C1) crop, and the controls (C2 and C3 respectively) performed better at the 75% and 50% irrigation level.

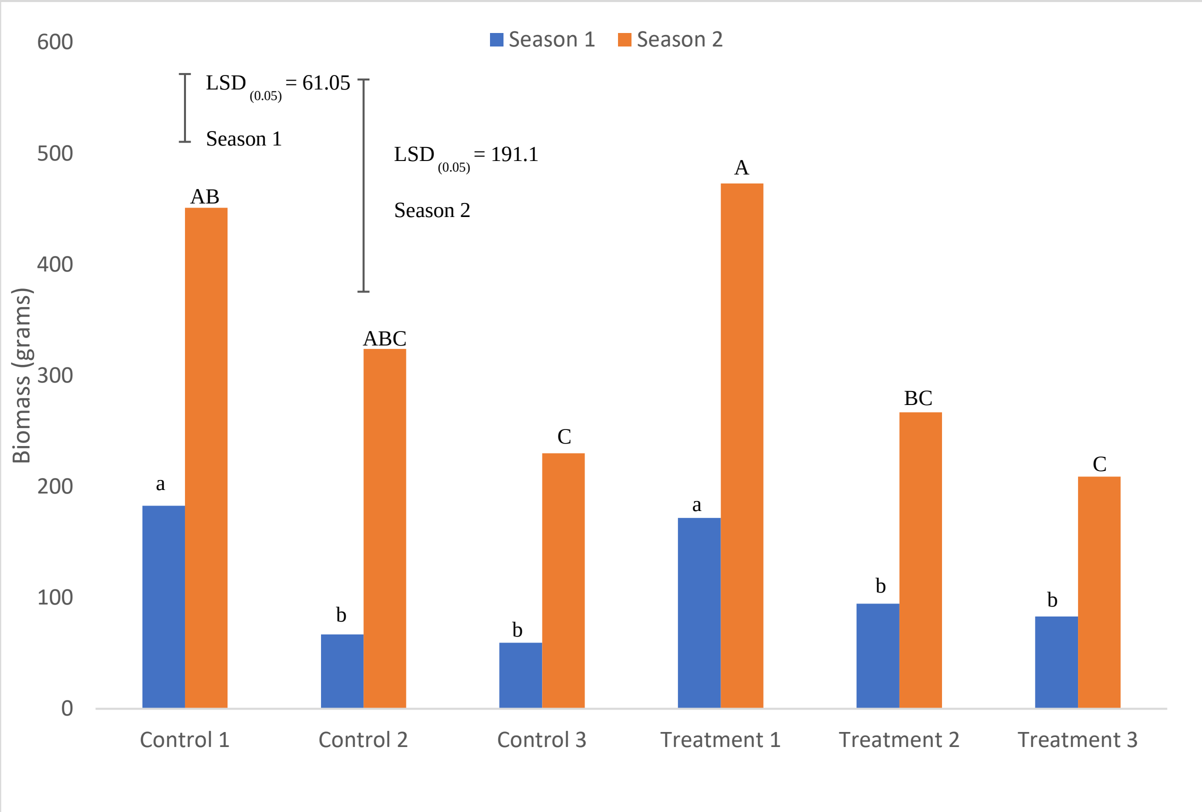


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Figure 4.9: Cumulative economic yield of the two tomato seasons, under various NIS dosages and irrigation rates at the conclusion of the trial

In the first season, there was a slight yield improvement by adding NIS to the water-stressed plants (T2 and T3 versus C2 and C3), but unfortunately, the yield was significantly lower than that of the 100% irrigated plants (C1 and T1). This resulted in the rejection of the stated hypothesis. From a fruit number context, the NIS treated crop at 100% (T1), had a lower number of fruits than C1, with 75% (T2) and 50% (T3) irrigation levels having similar values to their control counterparts (C2 and C3 respectively). This would suggest that although there was less fruit at T1, the fruit, were generally larger. The result may suggest the application of the NIS did, for the first season show improved fruiting in terms of individual fruit mass at T1.

The second season although yielding higher, show a slightly different trend than that observed in season 1. The results again may indicate an interaction. T1 was shown to have a higher flower number and a higher fruit mass, with the sets again showing variation from the control group to a greater degree when compared to season 1. This may then be possible to say that the results for the two seasons are not contradictory, but rather complementary, demonstrating the effect of the application of the NIS as a response to different environmental stress factors.

As previously stated, the addition of the NIS to the crop cycle should increase the plant available water (PAW), which should show improvement of factors which includes economic yield (Birhanu and Zeleke 2010). The Birhanu and Zeleke (2010) study also stated that fruit number and set may be negatively affected, although this may cultivar dependant.

A different study carried out by Ripoll et al. (2016) found that the effects of water stress on tomato fruit production is dependent on both the stage of development and the specific genetics of the cultivar. That study reported that water stress may increase the fruit mass as well as improve quality depending on the time of stress occurrence (Ripoll, Brunel, et al. 2016). The outcomes of the two trials may be correlated to the previously mentioned studies. The trial has a greater correlation to the Birhanu and Zeleke (2010) study in which the stress lead to more homogenous results between the two sets, with possible variation due to interaction with the NIS relieving some of the water stress that the crop was exposed to. The trial may also indicate, as two variable environments were used during the two trials that: the degree to which the NIS will affect the crop was possibly a product of other environmental factors excluding water availability.

In terms of the tomato fruit Brix readings in the first season, the controls (C1, 2 and 3) generally have higher readings when compared to the NIS treated counterparts, across all the irrigation rates (T1, 2 and 3) (Figure 4.10). The results do not show a linear increase with an increase in water stress but does show a similar trend between the control and the NIS treated crops. The highest reading was recorded to be at the irrigation rate of 75% (C2 and T2), with the lowest being that at the 100% irrigation rate (C1 and T1).

The second season of production showed generally lower readings when compared to the first season (Figure 4.10). In addition, the Brix readings from the NIS treated plants in this season were generally higher than the controls. The readings were shown to be inversely correlated to the irrigation rate, where the plants receiving the least amount of water, had the highest reading, this trend was seen for both the control and the NIS treated crops.

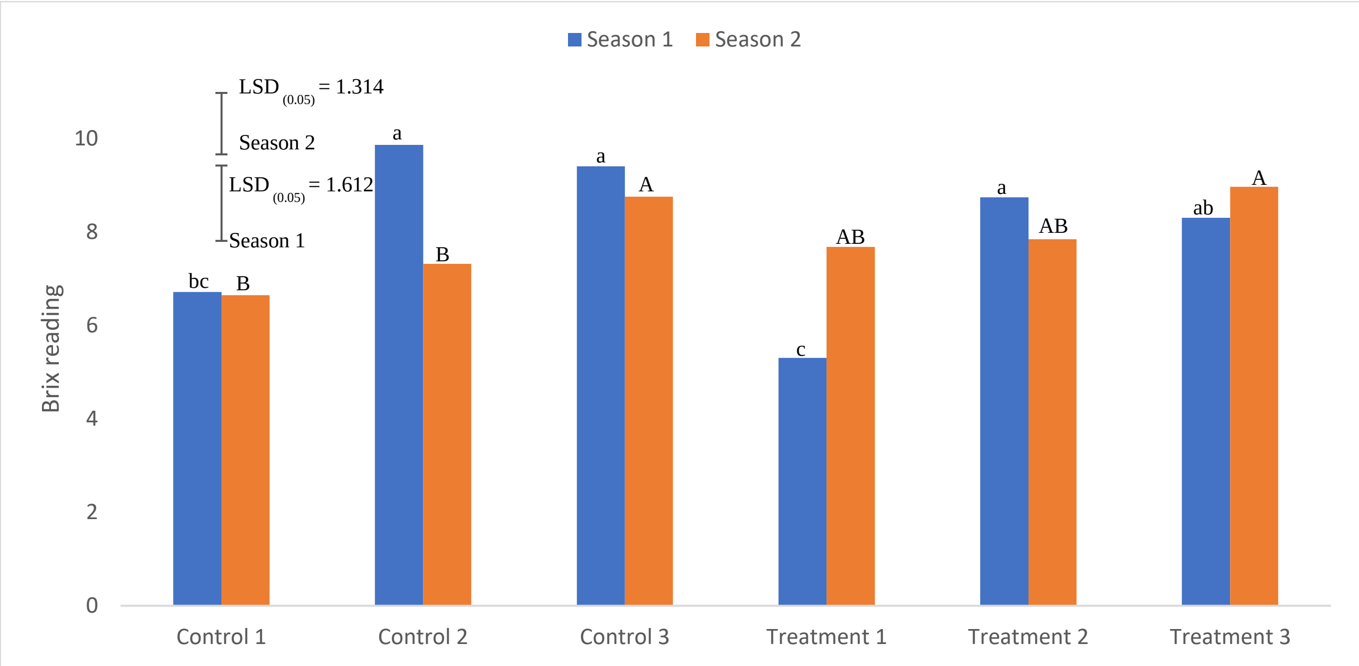

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Figure 4.10: *The brix reading from fruit at various irrigation rates and NIS dosages.*

As stated for the other data sets, the first and second seasons of production did not show a strong correlation in terms of outcome. It is important to keep in mind that the results of the first and second seasons may have been affected by the cooling issues in the glasshouse in the first season and the changing over to another glasshouse during the second season.

The first season showed the tomato crops which did not receive the application of the NIS, produced a higher Brix reading, at all the irrigation rates. Also, important to note is that the Brix did not decrease with increased irrigation but rather showed the mildly stressed crop having the highest reading.

In the second season, the control and the NIS treated crops showed a similar trend, with the water-stressed plants (C/T2 and C/T3) having higher Brix readings than without water stress (C1 and T2). The Brix result of the second season showed a similar general trend to the first season. The data for both seasons do seem to show an irrigation rate of 50% yielding a value that is higher than expected at the severely water-stressed level. According to Jintao Cui et al. (2020), a decrease in water content can increase the overall nutrient content of the tomato fruit in specific stages of tomato plant development

The similarities in the two seasons would suggest a possible trend, with a possible effect from the application of the NIS, although not the expected one. It was expected that the application of the NIS would improve the quality of the tomato, which will lead to a higher Brix reading, within the irrigation rates (Kleinhenz and Bumgarner 2013). According to Takayama and Nishina (2007), the early detection and treatment of water stress in tomatoes can increase the Brix value of closely monitored crops.

The largest recorded main stem diameter was found to be the NIS treated T2, followed by the control C1 (Figure 4.11) in the first season. The lowest recorded value was found to be the control C3. By adding NIS to water stressed plants, it did improve the stem growth, clearly to be seen as T3 having a significantly higher stem diameter than C3. The stem diameters between seasons tend to be similar, but in the second season, it did not vary as much as in the first season between treatment combinations. The lowest recorded value was found to be the NIS treated T2, with crops receiving 100% irrigation (C1 and T1) showing the same and highest readings. In this season there was no significant differences between the results of T3 and C3, but T3 still had the higher stem diameter reading.

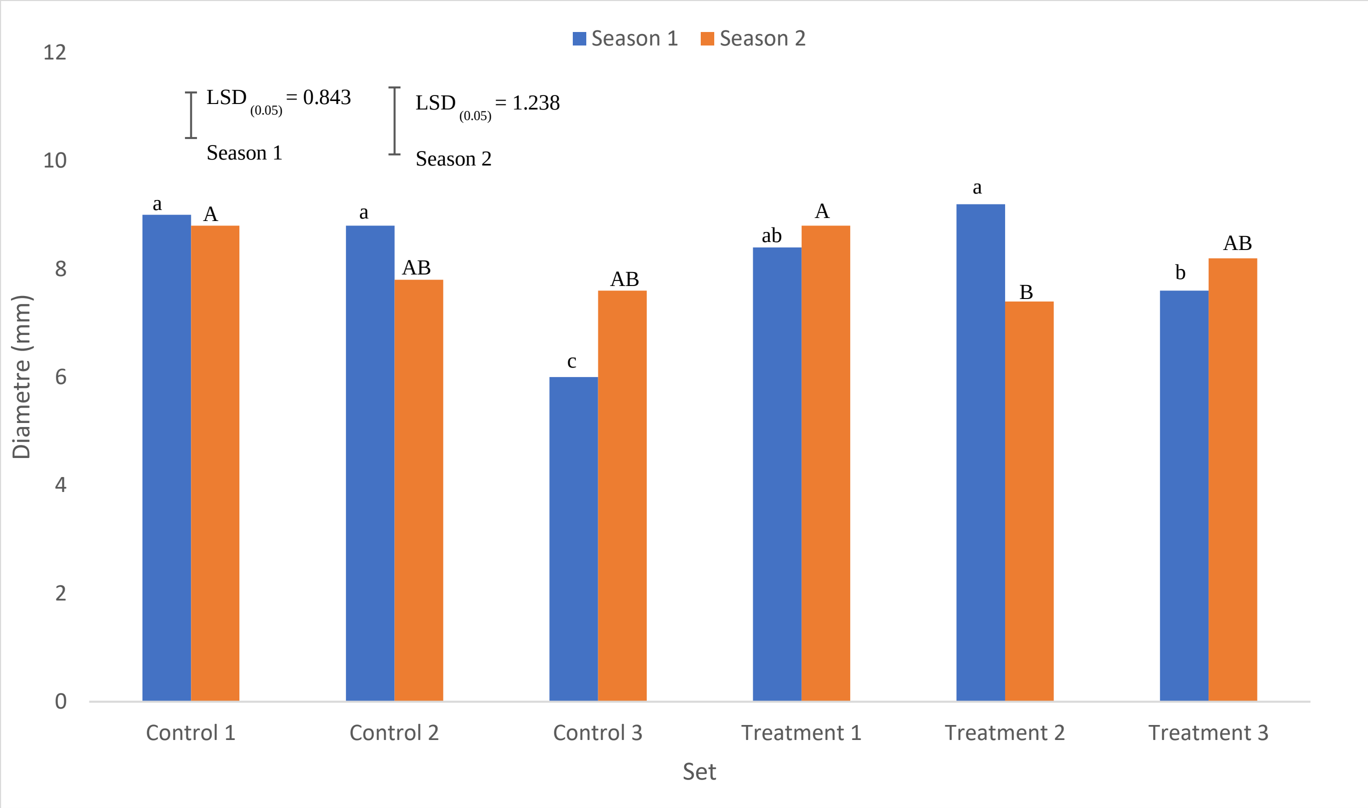

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Figure 4.11: The main stem diameter of tomato plants at various irrigation rates and NIS dosages

In the continuation of what has been observed in the other data sets, the two seasons off the tomato production did not correlate as expected. The first season shows a trend that was aligned with the hypothesis, where the second season showed partial contradictory results.

The first season shows a strong trend within the control crop where the reduction of water applied can be correlated to a direct decrease in stem thickness. The NIS treated crop does not demonstrate the same trend, which may be indicative of a possible effect of the applied surfactant. As previously stated, 75% irrigation was considered moderate stress, with 50% irrigation being severe water stress. The statistically significant variation between the plants of each of the irrigation rates is only observable in the severely water-stressed plants, where the NIS crops performed better, possibly due to relief of water-related stress on the plant due to the NIS.

The second season of production, various from the first, only in treatments where NIS was applied. The irrigation rate of 75% shows the NIS treated crop with the smallest stem diameter. The expected trend would lead to the expectation that the severe water stress would further reduce the stem diameter by 50%, but this did not occur. As previously stated in the literature, the stem thickness of a tomato plant can be correlated to the irrigation of the plant (Gallardo et al. 2006). The study did state that the variation in the stem thickness was only pronounced when the water stress that the tomato plant was exposed to was classified as severe.

Figure 4.12 shows the plant dry mass in the first season of the tomato crop at various irrigation rates and NIS dosages. The root biomass was negatively affected by increased water stress during season one. Although the root mass of the control plants did not differ from that of their corresponding NIS counterparts, the root mass of the NIS counterparts was slightly higher. The above ground mass results also correlate with the irrigation rates, with a reduction in mass as water stress increases. Despite this, the only significant differences can be reported on for the control values C1 and C2 which were significantly higher than that of the severely water-stressed crop T3.

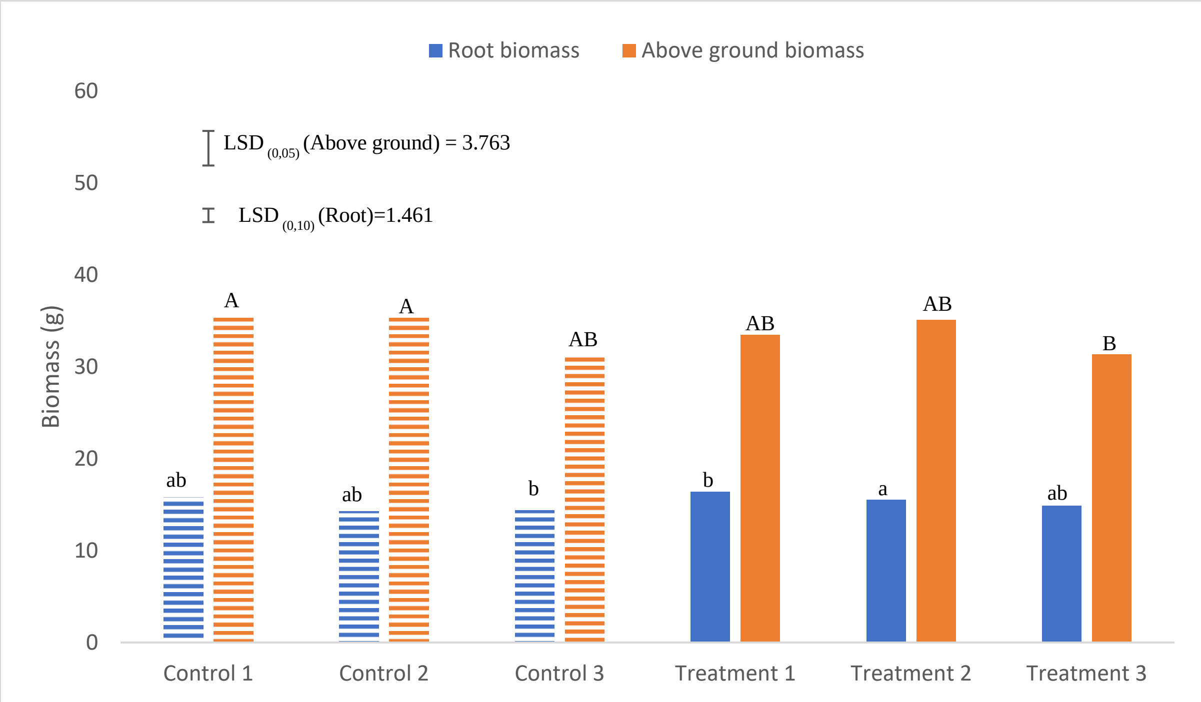

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Figure 4.12: The biomass of the first season of tomato crop at the conclusion of the trial at various NIS dosages and Irrigation rates

In the second season, the root and above ground biomasses (Figure 4.13) were higher than in the first season. The root mass shows that the NIS treated crop T2 had the highest value, followed by the Control C1. The results of the trial did not correlate with the irrigation rates applied as they did in the first season. In this season, none of the treatment combinations resulted insignificant differences between above ground mass means. Despite this, the above ground mass under severe water stress conditions did have the lowest values, which is similar to what happened in the first season. The above ground and root mass data allow insight into the growth of the tomato crops. The two seasons did demonstrate variable results. As previously stated, both the first and second season of production experienced stress events that may have occurred during crucial physiological stages of development, this would include excessive temperature increase and rapid loss of soil moisture that was not replenished in an adequate time frame.

The above ground mass for the two seasons showed variable results. The first season showed no improvement with the addition of the NIS to the crop, where the second season did show improvement for two of the sets. In terms of possible trends, the seasons showed an inverted result, where the mass of the controls can be correlated with the irrigation rate was seen in the second season in the NIS treated crop. A similar inverted pattern is seen for the NIS of the first season, where the peak in the irrigation at 75% was seen. The results of both trials do not support the stated hypothesis, where an expected increase in above-ground biomass with the addition of the NIS was expected. It is also important to state that the variation between treatments in all the irrigation rates for above ground mass was not significant, leading to a possible indication of an interaction with the irrigation rates, experimental error, or natural variation rather than the application of the NIS to the crop.

The root mass for the two seasons did not show the same results as seen in the above groundmass. The comparison between the two seasons for the root mass also demonstrated variable results. In both the first and second seasons for the control groups, the root dry mass showed the same trend, where the 100% and 50% marginally outperformed the 75% rate. The results do not fully corroborate the stated hypothesis as the NIS treated crop only performed significantly better in the second season at 75% irrigation. The finding does align with the available literature where a tomato crop exposed to water stress lead to a decrease of hydraulic and osmatic conductivity which in turn causes an overall decrease in carbon assimilation but did not show a significant decrease in overall root mass (Hernandez-Espinoza and Barrios-Masias 2020). A possible reason for the observed results in the second season was the movement of the plants, as well as the fluctuation in temperature that may have, may have increased the stress of the plants, which may have been mitigated by the addition of the NIS. T2 is the recommended rate of NIS application and concentration for the product, which is where the best result was expected to be observed.

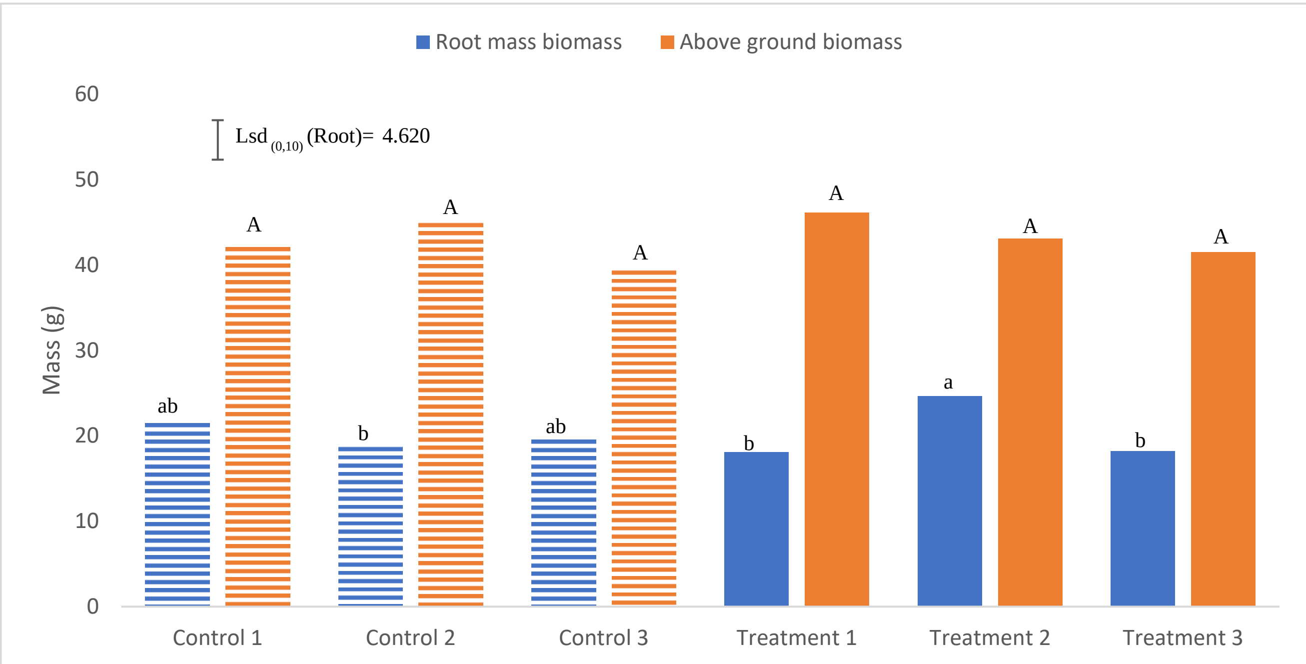

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Figure 4.13 : The biomass of the second season of tomato crop at the conclusion of the trial at various NIS dosages and Irrigation rates

4.4 Summary and Conclusion

The results of the tomato trial show both acceptance and rejection of the hypothesis as the data both correlated and contradicted expected outcomes. The first season's data showed a greater correlation with the literature, with the second season showing a greater degree of variation. It is important to note that the two seasons of production were carried out at different times of the year, season one was carried out from July to October 2019, where the second season was done from March to June, leading to climatic variations in terms of temperature and light. The variation in results between the two seasons may also be attributed to both technical challenges faced in the form of the greenhouse, where load-shedding occurred causing the wet-wall and fan systems to fail, leading to the fluctuation in the maximum and minimum temperature and humidity within the greenhouse. It is also possible that the different dates on which the second trial took place may have affected the outcomes as well as the advent of problems arising from the Covid-19 pandemic.

The chlorophyll data partially corroborated the hypothesis, where the first season seem to indicate a possible genotypical result for the outcome, as the highest readings were recorded for the most water-stressed plants, a trend not observed in the wheat. The second season demonstrated somewhat contrarian results with the control performing better amongst each irrigation rate.

The plant height data for the two seasons again did not fully support the stated hypothesis but contained rather mixed results, where the first season did show two of the NIS treatments performing better, the second season did not show much variation between the irrigation sets, or the treatments in general.

The data seems to indicate that the addition of the NIS did not have a significant effect on the growth cycle of the tomato, in terms of the conversion between the vegetative to reproductive phase. The strongly mixed nature of the flower results in isolation do not allow a conclusive acceptance or rejection of the hypothesis, rather it needed to be analysed in the context of fruit data. The fruit number and subsequence flower to fruit percentage show a lower variation between the two seasons of production. The two seasons both seem to support that the addition of the NIS with mild water stress (T2) had a positive effect on the fruit number and flower to fruit conversion.

The cumulative fruit mass or economic yield values can also be viewed in the context of fruit number, in terms of individual fruit size and quality. Although for the first season T1 may have generated a lower value, the individual fruit was larger, with minimal variation seen amongst the other irrigation rates. The second season due to several factors yielded higher values with possible complementary results to the first season.

The Brix data did not yield the expected outcome, as it did not correlate with the hypothesis or the cumulative fruit data that was collected. The stress that the plants were exposed to during crucial stages may have contributed to the observed outcome.

The tomato stem thickness in season one showed little variation between the irrigation rates, with a significant difference only being observed at the irrigation rate of 50%. The second season showed a similar trend to the first, except for no variation at the irrigation rate of 50%. The results may indicate that the first season demonstrate possible interaction with NIS, where the second season showed variation that may be due to previously mentioned technical difficulties.

The biomass data, which can be subdivided into the root and above ground mass allowed insight into the accumulation of matter during the seasons. It was shown for both seasons that the above ground mass did not demonstrate improvement from the addition of the NIS. A notable difference between the root mass and the above-ground mass data was observed in the second season, in which the addition of the NIS did show slight benefit at the irrigation rate of 75%. It must be noted that overall, the two seasons of production did not show a strong correlation.

CHAPTER 5 : CONCLUSIONS AND RECOMMENDATIONS

The trial was undertaken to understand how the addition of a NIS to the irrigation cycle of two important crops would affect several important aspects of growth and development. Commercial trial work has been extensively carried out by Agro-Chemical producers of NIS products, with less of a focus on the academic aspects of the product. The aim of the study was to contribute to a better understanding of how a crop will react to the addition of the NIS as part of the full production cycle, from germination to harvest.

The objective of the project was to determine if the addition of NIS compounds or blends may demonstrate measurable changes in crops exposed to these chemicals. This was determined in terms of physiological, growth and production-related factors such as germination parameters, chlorophyll content, biomass production, crop dimensions and economic yield.

The trials focused on two crops: tomato and wheat, not only due to their importance in food production but due to the variation in physiology. The crops are representatives of monocotyledoneae and dicotyledoneae, epigeal and hypogeal germination as well as intensive and extensively cultivated crop types.

The study was undertaken in two separate trial environments to give a full image of the plant production cycle exposed to the NIS blend. A germination trial was undertaken within a germination incubator, while the other plant growth factors were tested in a pot trial within a greenhouse. In both trial environments elements such as temperature, humidity, disease exposure and irrigation were controlled.

It is important to note that during both greenhouse seasons, technical difficulties occurred which may have affected the outcome of the trial or altered the data that was collected. During the first trial, several of the days during the growth of both the wheat and the tomato crops, the greenhouse's wet wall and fan were not operational. This caused an increase in the internal temperature of the greenhouse exceeding 34℃, placing all the plants under severe stress. The shutdown of the wet wall also led to a drop in humidity which is maintained between 60% to 80%. This was due to both mechanical issues and the advent of load-shedding in Pretoria. The second season faced difficulties due to the outbreak of the SARS-coV-2 global pandemic. The disease led to the enforcement of a nationwide lockdown, leading to the closure of the University of Pretoria Experimental farm. The closure forced the movement of the crop to a secondary location in which the crop could be maintained, and readings taken. The movement of the crop as well as the absence of a rotating table may have led to experimental variation that occurred during the second trial. The humidity and temperature could also not be regulated to the extent of what was possible in the University of Pretoria's experimental greenhouses. But despite these difficulties, the results of the trial suggest that the different crops may react differently to the application of the NIS.

5.1 Germination

Germination as the initial stage of production can be used as a predictor of the rest of the season for a crop. As previously stated, several parameters can be used to measure the effectiveness of the seed's germination. The trial did provide insight into the possible effects the addition of the NIS may have on tomato and wheat production. For this trial, the seeds of the two crops were exposed to different concentration of NIS namely: half the recommended dosage (0.06%), two recommended dosages (R1 = 0.12 and R2 = 0.24%) and double dosage of R2 (0.48%). The effects of these were compared to an untreated control that were only irrigated with distilled water.

The two crops did not demonstrate the same results or show the same trends. The wheat seeds as a monocotyledon and with hypogeal germination, demonstrated that by adding the NIS it negatively correlated to several germination parameters. Several of the parameters were significantly affected, with the control outperforming the NIS treatments.

The tomato seeds as the dicotyledon with epigeal germination, did not show the same trend. The results show that the highest and lowest concentration of NIS perform the best, with several germination parameters being significantly better as compared to the control.

The variation between the tomato and wheat presents an opportunity to understand how different crop types may react to the addition of NIS. The monocot, wheat, reacted adversely to the addition of the specific blend of NIS used during the trial. Several studies reported on in literature indicated that certain concentrations of the compounds present in NIS blends were inhibitory to both monocots and dicots, with several different plant types reacting differently during the trials. The tomato’s reaction as a dicot crop did correlate to studies that found the addition of the surfactant to be stimulating to growth. Due to the different reactions of the two crops, it emphasises the importance of further trial work in which guidelines specific to a variety of crops can be obtained.

5.2 Wheat results

To demonstrate the effect of the NIS on the remaining aspects of the production cycle of a crop, a pot trial in a greenhouse was conducted. Both wheat and tomato plants were exposed to three levels of water stress (100% irrigation, 75% irrigation and 50% irrigation) and two levels of NIS application (with or without NIS). Wheat is considered to have a higher stress resistance when compared to tomato. The higher resistances may contribute to the divergent results that were observed during the trial.

The chlorophyll and plant height data from the trial did not show clear trends in terms of the applied NIS. It is generally seen that the crops responded to the irrigation rates, rather than to the NIS application. In terms of wheat tiller and ear number, irrigation rates of 50 and 75% show a significant improvement in tiller number with the application of NIS, while the tillers at an irrigation rate of 100% did not differ between C1 and T1. The wheat ear number demonstrated a significant variation in favour of the NIS treated crops at the 75% irrigation rate.

The wheat crop did not fully corroborate all the expectations of the trial. In most cases the once of measured parameters such as final tiller and ear number, as well as root mass and economic yield did demonstrate a trend, while parameters measured throughout the season did not show consistent reactions or trends.

5.3 Tomato results

The tomato pot trial was carried out over two seasons, but still using the same treatments. Much like the wheat trial, the results were mixed, with both confirmation and rejection of the expected outcomes. The two seasons did not show strongly correlated results between the same parameters. As previously stated, the variation between the two seasons may be attributed to technical difficulties experienced during both trials. The variation may also be due to the different times of year in which the trial was carried out.

The results do however indicate, from both seasons that the growth cycle of the tomato was not significantly affected by the addition of the NIS blend. This is seen in the timing of the shift of the crop from vegetative to reproductive phase, as well as within the reproductive as the plant shift from flowering to fruiting.

Several of the parameters did not yield a definitive trend or a significant variation between the NIS treated crop and the control. The height data showed mixed results, with the first season indicating an interaction with the NIS, while the second season did not show much variation. The ºBrix readings did not allow a definitive rend to be observed nor did it correlate with the fruit data. Tomato stem thickness did not yield any significant results outside of suspected technical issues encountered during the trial.

The biomass readings did not indicate improvement for the above-ground mass during either of the seasons of production. The root mass did show a possible positive effect on the crop in the second season where the NIS was applied.

For the economic yield (fruit) values when analysed in the context of flower number, fruit number, flower abortion rate, flower to fruit ratio and individual fruit mass, the first season may indicate a positive effect of NIS at the highest irrigation rate. The second season did not yield significant variation between sets possibly due to previously mentioned technical problems.

With the two seasons of tomato trial data giving inconsistent results, it is not easy to identify which combinations of water stress and added NIS gave the best results. But the observation that some parameters in at least one of the seasons did react positively to the NIS under water stressed conditions, may warrant further investigation.

5.5 General recommendations

When conducting germination type studies with an NIS blend, one need to take in consideration that these products are recommended for application in a free-flow environment, namely, a soil profile. The germination trial was carried out in a laboratory environment in which the seeds were placed within a closed environment of a Petri dish. The water applied to the seed was restricted from moving laterally and vertically. Analysis of the divergent literature on the topic can, with relative certainty affirm that experimental error did not lead to the variation in the results of the trial, although the type of experiment may be problematic. For further studies, it is suggested that a deeper container filled with sand is used to allow for the NIS to work optimally.

The germination trial showed clear differences in terms of the reaction of different crop types on the applied NIS product as well as concentrations. In neither crop did the NIS result in reduced final germination count, showing it should be safe to use as seed treatment. On the other hand, the NIS also did not give any advantage in terms of germination and the different germination indices tested. Therefore, it is suggested: to run more tests with different crop types to clarify it there is a need to adjust the recommendations for use of this product during germination in different crops, and to verify if the NIS does have any advantages as seed treatment.

From the pot trial, the results suggest that a range of NIS dosage should be trialled. It may be possible to establish if yield at 50% irrigation is affected positively or negatively if the concentration of the NIS blend is applied above the recommended rate of application. The increase in the surfactant concentration may add additional humectant effect to the soil allowing a significant biomass variation.

Several of the wheat biomass readings showed a correlation with the addition of the NIS: the economic yield at 100% irrigation, and top mass at 75%, while for the root mass, the addition of the NIS was shown to improve biomass. It can also be noted that non-significant improvements to yield with the addition of the NIS were recorded for economic yield at 75% and top mass at 50%. Furthermore, it was also observed that the NIS seemed to have had the most advantage on the general growth of the wheat plants at T2 (75% irrigation level plus NIS), while for economic yield T1 (100% irrigation plus NIS) gave the best results. The companies selling these surfactants should maybe also investigate if a blanket application over the season should not be replaced by tailor made dosages to address the specific needs of the crop as affected by crop growth stages.

Both of the tomato pot trials experienced issues beyond our control. This might also have resulted in very little correlation between the two seasons and the lack of clear crop reaction to the applied NIS. The contrasting times of year could also have had an effect, but since tomatoes are produced throughout the year in various parts of South Africa, it illustrates that the interaction of temperature x water stress x NIS can be a complicated issue and should be addressed in trials.

Lastly, it would also suggest that these pot trials should be followed up with field trials and that an economic analysis on the use of these products should be conducted.

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APPENDIX 1: TOMATO

Chlorophyl								
Season 1					Season 2			
Significance level	0.05				Significance leve	0.05		
Source	reps	F pr	l.s.d		**Source**	reps	F pr	l.s.d
TMT	105	0.095	0.986		TMT	135	0.007	1.258
IRRI	70	<.001	1.207		IRRI	90	0.131	1.54
DATE	30	<.001	1.381		DATE	30	<.001	1.736
TMT.IRRI	35	0.139	1.707		TMT.IRRI	45	0.853	2.178
TMT.DATE	15	0.680	2.038		TMT.DATE	15	0.263	2.608
IRRI.DATE	10	<.001	2.496		IRRI.DATE	10	<.001	3.194
TMT.IRRI.DATE	5	0.626	3.530		TMT.IRRI.DAT	5	0.168	4.517

Plant Height							
S1	TMT	IRRI	DATE	TMT.IRRI	TMT.DATE	IRRI.DATE	TMT.IRRI.DATE
Source							
Significance level	0.05						
reps	150	100	30	50	15	10	5
d.f.	24	24	214	24	90.24	90.24	90.24
l.s.d	20.18	24.71	14.77	34.95	27.85	34.11	48.24
S2	TMT	IRRI	DATE	TMT.IRRI	TMT.DATE	IRRI.DATE	TMT.IRRI.DATE
Source							
Significance level	0.05						
reps	120	80	30	40	15	10	5
d.f.	24	24	166	24	45.14	45.14	45.14
l.s.d	56.19	68.82	25.25	97.33	64.56	79.07	111.82

Fruit Number								
S1					S2			
Significance level	0.05				Significance level	0.05		
Source	reps	F pr	l.s.d		**Source**	reps	F pr	l.s.d
TMT	150	0.786	0.1645		TMT	120	0.394	0.1700
IRRI	100	0.450	0.2015		IRRI	80	0.016	0.2082
DATE	30	<.001	0.1423		DATE	30	0.375	0.1964
TMT.IRRI	50	0.885	0.2849		TMT.IRRI	40	<.001	0.2945
TMT.DATE	15	0.629	0.2484		TMT.DATE	15	0.436	0.3067
IRRI.DATE	10	<.001	0.3042		IRRI.DATE	10	0.129	0.3756
TMT.IRRI.DATE	5	0.494	0.4302		TMT.IRRI.DATE	5	0.489	0.5311

Flower								
Season 1					Season 2			
Significance level	0.05				Significance level	0.05		
Source	reps	F pr	l.s.d		**Source**	reps	F pr	l.s.d
TMT	120	0.303	1.798		TMT	120	0.624	2.838
IRRI	80	0.051	2.202		IRRI	80	0.481	3.475
DATE	30	<.001	2.522		DATE	30	<.001	2.973
TMT.IRRI	40	0.438	3.114		TMT.IRRI	40	0.102	4.915
TMT.DATE	15	0.009	3.751		TMT.DATE	15	0.906	4.786
IRRI.DATE	10	0.098	4.594		IRRI.DATE	10	0.275	5.861
TMT.IRRI.DATE	5	0.072	6.497		TMT.IRRI.DATE	5	0.858	8.289

Economic Yield								
S1					S2			
Significance level	0.05				Significanc	0.05		
Source	reps	F pr	l.s.d		**Source**	reps	F pr	l.s.d
TMT	15	0.437	35.25		TMT	15	0.729	110.3
IRRI	10	<.001	43.17		IRRI	10	0.004	135.1
TMT.IRRI	5	0.603	61.05		TMT.IRR	5	0.838	191.1

Brix								
S1					S2			
Significance level	0.05				Significanc	0.05		
Source	reps	F pr	l.s.d		**Source**	reps	F pr	l.s.d
TMT	15	0.013	0.931		TMT	15	0.077	2.659
IRRI	10	<.001	1.140		IRRI	10	0.279	3.257
TMT.IRRI	5	0.952	1.612		TMT.IRR	5	0.591	4.606

Stem Diameter								
S1					S2			
Significance level	0.05				Significanc	0.05		
Source	reps	F pr	l.s.d		**Source**	reps	F pr	l.s.d
TMT	15	0.059	0.486		TMT		0.849	
IRRI	10	<.001	0.596		IRRI		0.025	
TMT.IRRI	5	0.003	0.843		TMT.IRRI		0.505	

Above ground mass								
S1					S2			
Significance level	0.05				Significance level	0.05		
Source	reps	F pr	l.s.d		**Source**	reps	F pr	l.s.d
TMT	15	0.324	2.173		TMT	15	0.559	
IRRI	10	0.024	2.661		IRRI	10	0.331	
TMT.IRRI	5	0.723	3.763		TMT.IRRI	5	0.570	

Root Mass								
S1					S2			
Significance level	0.05				Significanc	0.05		
Source	reps	F pr	l.s.d		**Source**	reps	F pr	l.s.d
TMT	15	0.129	0.844		TMT	15	0.851	2.668
IRRI	10	0.054	1.033		IRRI	10	0.355	3.267
TMT.IRRI	5	0.804	1.461		TMT.IRR	5	0.055	4.620

APPENDIX 2: WHEAT

Chlorophyl			
Season 1			
Significance level	0.05		
Source	reps	F pr	l.s.d
TMT	105	0.694	2.463
IRRI	70	0.020	3.017
TMT.IRRI	35	0.093	4.266
Date	30	<.001	3.159
TMT.DATE	15	0.001	4.759
IRRI.DATE	10	0.854	5.828
TMT.IRRI.DATE	5	0.275	8.242

Plant height			
Season 1			
Significance level	0.05		
Source	reps	F pr	l.s.d
TMT	135	<.001	8.77
IRRI	90	0.048	10.74
TMT.IRRI	45	0.296	15.19
Date	30	<.001	11.66
TMT.DATE	15	<.001	17.67
IRRI.DATE	10	<.001	21.64
TMT.IRRI.DATE	5	0.069	30.60

Tiller			
Season 1			
Significance level	0.05		
Source	reps	F pr	l.s.d
TMT	135	0.018	0.3914
IRRI	90	<.001	0.4794
TMT.IRRI	45	0.010	0.6780
Date	30	<.001	0.4732
TMT.DATE	15	<.001	0.7338
IRRI.DATE	10	<.001	0.8988
TMT.IRRI.DATE	5	0.110	1.2711

Wheat ear			
Season 1			
Significance level	0.05		
Source	reps	F pr	l.s.d
TMT	15	0.591	1.011
IRRI	10	<.001	1.238
TMT.IRRI	5	0.097	1.751

Root mass			
Season 1			
Significance level	0.10		
Source	reps	F pr	l.s.d
TMT	15	0.072	4.06
IRRI	10	<.001	4.97
TMT.IRRI	5	0.183	7.03

Above ground mass			
Season 1			
Significance level	0.05		
Source	reps	F pr	l.s.d
TMT	15	0.210	0.824
IRRI	10	<.001	1.010
TMT.IRRI	5	0.017	1.428

Economic Yield			
Season 1			
Significance level	0.05		
Source	reps	F pr	l.s.d
TMT	15	0.908	1.194
IRRI	10	<.001	1.462
TMT.IRRI	5	0.031	2.068