THE POTENTIAL OF BRASSINOSTEROIDS TO ALLEVIATE THE EFFECT OF MESOTRIONE RESIDUE ON THREE LEGUME CROPS

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DECLARATION

I, Maipato Margaret Mota, declare that the Master's Degree research dissertation or interrelated, publishable manuscripts/published articles, or coursework Master's Degree mini-dissertation that I herewith submit for the Master's Degree qualification in Agronomy at the University of the Free State is my independent work, and that I have not previously submitted it for a qualification at another institution of higher education.

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DEDICATION

I dedicate this piece of work to my late brothers **Rethabile** (February 2007**) and Tumisang Mota** (August 2015), not a day passes by without thinking about you. I love you and miss you every minute of my life.

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LIST OF SYMBOLS AND ABBREVIATIONS

BRs - Brassinosteroids

HPPD - 4-Hydroxyphenyl pyruvate dioxygenase inhibitors

ANOVA - Analysis of varience

FWC - Field water capacity

SAS - Statistical analysis system

LSD - Least significant difference

ARC - Agricultural Research Council

SGI - Small grain institute

CEC - Cation exchange capacity

LAN – Lime ammonium nitrate

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ABSTRACT

Legume crops are warm climate crops with the potential of improving soil fertility, by fixing atmospheric nitrogen in the soil, utilizing the *Rhizobium* bacteria and after harvest the roots of the crop is left in the soil to decompose, which results in improved soil fertility. They are very important to both smallholder and commercial farmers, because they provide a source of income and food for both humans and animals. Due to their importance in increasing soil fertility it is economically viable to plant them in a crop rotation system. However, despite their importance, production can be low if weed competition is not eliminated and due to their sensitivity to certain herbicides. Mesotrione is a selective herbicide that is normally used to control annual broad weeds and grasses in maize production, and it can be used as a pre-emergence or post-emergence application in the field. However due to legume's sensitivity to this herbicide it can have a huge impact on the production in a rotation system.

To enable assessment of these impacts, the three legumes required testing with scenarios involving different levels of mesotrione residues in interaction with different application methods of brassinosteroids interactions. It is hypothesized that negative consequences of mesotrione on plant productivity would be mitigated by the application of brassinosteroids. This mitigation may be ascribed to BRs increasing both morphological, physiological and yield parameters and increased resistance or tolerance, which may buffer transient periods of herbicide stress. Under glasshouse conditions over two seasons, again BRs in combination with three concentrations of mesotrione residue have been shown to ameliorate damages caused by mesotrione residue on crops and all the morphological, physiological and yield parameters were significantly increased after treatment with BRs. The study confirmed the potential of

BRs to counteract the possible negative effects of the mesotrione residue on morphology, physiology and yield of the three legumes. Further studies need to explore the effect of residual activities using different herbicides and to determine the alleviating effect of brassinosteroids. This will enable more precise exploration of legume plant response to herbicide stress scenarios.

Key words: Mesotrione, brassinosteroids, physiological parameters, morphological parameters

CHAPTER 1

INTRODUCTION

1.1 Motivation and rationale

Agriculture is critical a sector of the world-wide economy contributing to the stability of the general global economy, therefore farmers are required to produce more food in the most efficient and sustainable manner. The world population is anticipated to increase over 8 billion people by 2030 and over 9 billion people by 2050 (Beaudreau, 2014). With this increase in the population more food is required, therefore agriculture is expected to play an important part in sustaining development, eliminating poverty and hunger as it is the main source of food production. As staple food and fodder, legumes are among the most important crops worldwide and are cultivated in most countries. As they rich in proteins thus they need to be produced in large amounts to contribute to food security. Legume crops are also important to farming systems and have been known for their soil improvement power, because they have the ability to fix atmospheric nitrogen thus enhancing soil fertility. They fix atmospheric nitrogen via Rhizobium bacteria through root nodules. Fixed nitrogen not only meets all the nitrogen needs of legume crops, but a sizable amount (30–60 kg/ha) is also left for the succeeding crops (Nieuwenhuis & Nieuwelink, 2005).

High production of legume crops or any crop is influenced by many factors, including weed control. However, weed control can be a constraint to production. Weeds negatively affect crop production, because they have the ability to grow faster, more vigorous and taller than cultivated crops. They develop canopies that shades crops, thus affecting plant growth. They are also normally resistant in nature, have vigorous growth with well-developed root systems, are productive, persistent, and more competitive than the crops. They compete with crops for nutrients, moisture and sunlight and they also act as host for insects, rodents and pathogens, which cause diseases (Lembi & Ross, 1999). All these factors may consequently decrease crop production. Weeds are reported to have greater root elongation and branching, which result in a root system that absorbs more nutrients and water from the soil at the expense of the crop. Some weed species increase competition by producing toxic allelopathic substances that affect growth and development of crops. These chemicals are released into the soil as root exudates of the living or dead plants (Qasem & Foy, 2001). It was reported that due to weeds in Africa, yield losses average 30%, but losses of 50% or more are reported in some parts of sub-Saharan

Africa (Sibuga, 1997). It was also indicated in a Nigerian experiment that one kilogram of weeds reduced the yield of rice by 500-900 g (Adeosun, 2008). In order to avoid such losses, weeds must be controlled effectively manually or by application of herbicides. Use of herbicides is more common than other methods of weed control due to its efficiency. When using herbicides, one must be very careful, because they can cause more harm than good if not applied (Allemann & Allemann, 2013). Herbicide activity or its residue can be affected by soil, climatic factors and herbicide properties (Rao, 2002; Hager & Nordby, 2007).

Soil factors include soil microbial activities and soil chemistry. High soil organic matter reduces activity by adsorbing the herbicide (Hager & Nordby, 2007; Yu, 2014) and soil microorganisms affect herbicide degradation (Rao, 2002; Yu, 2014). Climate factors involve temperature, soil moisture and radiation. High temperature and moisture result in higher herbicide degradation. Radiation is requiring to catalyse herbicide degradation and in the absence of sunlight herbicide activity is low. Herbicide properties is another factor that assist in herbicide activity and this include solubility, molecule's susceptibility to chemical and vapor pressure. Less soluble herbicides are strongly attracted to soil particles therefore less activity in the soil. High vapor pressure leads to low activity of the herbicide (Riddle, 2012). Herbicides are applied to control weeds during the growing season to eliminate competition between crops and weeds, but can be persistent if application rules are not followed. However, when a herbicide is not registered on the crop can cause damage to sensitive crops in a rotation system. The length of time herbicide remains persistent in the soil depends on the herbicide degradation, water solubility, rate of application, soil type, rainfall and temperature (Helling, 2005; Hager & Nordby, 2007).

Mesotrione is one of the herbicides that are intended to kill unwanted plants. Mesotrione is a triketone herbicide registered by Syngenta under the name *Callist*o for controlling annual broadleaf weeds in field of sweet corn, suger cane, cranberry, blueberry flax and pearl millet. It provides broad spectrum control of broadleaf weeds and some grasses supress certain weeds and providing residual control of later germinating weeds. The herbicide inhibits the essential plant enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD), which is found in the plant chloroplasts, protecting plant cells from photodegradation (Yu, 2014). The problem is that mesotrione is a very effective herbicide for weed control, however due to its residual activity, it can cause injury to sensitive crops grown in rotation system, even 24 months after application. In previous research, it was indicated that mesotrione was effective in controlling broadleaf weeds, such as smooth pigweed (*Amaranthus retroflexus L.*), flixweed (*Descurainia Sophia*), tumble mustard and lettuce (Affeldt, 2015). Visual phytotoxicity of mesotrione include bleaching of meristemic tissue that eventually leads to necrosis, chlorosis and followed by death of a plant (Soltani *et al.*, 2014). These symptoms appear three to five days after a post-emergent application with weed death occurring within two to three weeks (Yu, 2014). It is therefore vital to know the reaction of the plants if damaged by herbicides so that the appropriate measures can be taken to assist the plant to maintain its normal growth and to avoid negative effects that might be caused by herbicide injury.

The aim of this study was to evaluate phytotoxicity that might be caused by herbicides or herbicide residue and assess how the application of brassinosteroids could affect the three legumes and possibly be used to alleviate herbicide damage on crops. Studies by Vardhini *et al.* (2008) confirmed that use of brassinosteroids play an important role in improving physiological and metabolic processes in plants. Brassinosteroids can assist to relieve phytotoxicity of mesotrione since they can be directly absorbed by the crops and also, they increase herbicide degradation in the soil and help the plant to mantain its normal growth (Vardhini *et al.*, 2008). The objectives of this study were to 1) gain insight into the response of three legumes to three residual mesotrione concentrations, 2) to elucidate the effectivity of differences in application methods of brassinosteroids and 3) to determine if brassinosteroids combined with mesotrione can reduce phytotoxicity cause by mesotrione on the three legumes.

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CHAPTER 2

LITERATURE REVIEW

2.1 The importance of Legumes

Legumes are self-pollinated plants in the family Fabaceae that bear seed in pods. They were among the first plant species to be domesticated and were grown about 60 million years ago (Sprent, 2007). According to Stanley (2015) there are over 16,000 species of legumes that are planted universally. Well known grain legumes include crops such as peas, dry beans, groundnuts and soya beans, to mention only a few (Nieuwenhuis & Nieuwelink, 2005). Legumes are amongst the vital crops worldwide and cultivated extensively in most countries. They are very important to both smallholder and commercial farmers because they provide a source of income, food for humans, livestock feed and improve soil fertility (Nieuwenhuis & Nieuwelink, 2005). It was indicated by Nieuwenhuis & Nieuwelink (2005) that legume seeds can be consumed as split peas and also be crushed into flour. The well-known food products made from legumes are peanut butter and soymilk (Singh & Singh, 1992). They are highly nutritious, being 2-3 times richer in protein than cereal grains, contain oil (e.g in groundnut and soya beans), iron, potassium, magnesium, zinc, vitamin B and selenium (Stanley, 2015). Due to the high protein in legume crops they could be used as a good replacement for meat (Nunes *et al.*, 2007). Besides human consumption, these plants can also be used as livestock feed. It was highlighted by Nieuwenhuis & Nieuwelink (2005) that legume crops can be used as a source of animal feed after harvest and they are known to have high quality forages for livestock in cultivated pastures.

Legumes are one of the world's most important crops because of their capability to improve soil fertility by fixing atmospheric nitrogen in the soil, utilizing *Rhizobium* bacteria the root hairs of the plant to form nodules in which nitrogen is stored (Nieuwenhuis & Nieuwelink, 2005). The nitrogen absorbed from the atmosphere is used for the growth of the plant and is kept in the nodules of the root. A common cultivation practice is that the roots are left in the ground are harvested to decompose and release the nitrogen into the soil, making a rich source of nutrient-rich organic matter or mulches that supplies nitrogen for the following crops, especially during intercropping (Gutteridge & Shelton, 2015). For these reasons growing legumes as a rotating crop or in an intercropping system, is important and advisable because the next crop will always benefit from it. It reduces environmental pollution and eutrophication and can also help poor resource farmers, as they do not need to purchase large amounts of expensive chemical fertilizers and it reduces environment pollution. Among the legume crops that are commonly planted by farmers in South Africa are groundnut (A*rachis hypogaea L.*), soya bean (*Glycine max L.*) and dry bean (*Phaseolus vulgaris L*.).

Groundnut originated from South America (Southern Bolivia), and it is now grown throughout the world in the tropical and temperate areas (Chandraju *et al.*, 2011). It is believed that the crop was cultivated by native people of the New World and was later introduced to the Pacific Islands, Africa, Asia and Europe (Directorate plant production, 2010). Currently the leading countries in the production of groundnut include China and India, contributing approximately 70% to the world production (Chakraborty *et al*., 2013). The crop is grown on 19.3 million hectares across 82 countries in the world (Reddy *et al*., 2003). Groundnut is among the most vital legume crops in Sub-Saharan Africa and it is cultivated extensively in most countries. The use of groundnut as food has gradually increased from the mid-1970's to now with approximately 34% (Fletcher *et al*., 1992), and its popularity even extended to South Africa. In South Africa the western and northern Free State contribute 40%, North West 29% and Northern Cape 24% of the production while it's very low in Limpopo and Mpumalanga, (Mbonwa, 2013; Cilliers, 2014). Resource-poor farmers in the northern and eastern parts of South Africa grow groundnut mainly for home consumption (Cilliers, 2014).

Groundnut is one of the world's most important crops due to its high nutritive value containing about 44-56% oil and 22-33% protein (Dwivedi *et al*., 1996; Jauron, 1997). It was also indicated that groundnut, as a source of nutrition, especially in the northern KwaZulu-Natal and Mpumalanga areas, are utilized to fight malnutrition (Cilliers, 2014). Groundnut is also rich in minerals such as phosphorous, potassium, calcium and magnesium and vitamins E, K and B (Javaid *et al*., 2004). Groundnut is suitable for warm frost-free climatic conditions. Groundnuts are only nut that grows below the earth. Groundnut seed takes seven days to germinate and it grows up to 30 to 50 cm in height (Figure 2.1) (Grand, 2014). When plant maturity is reached after a month or older, it starts flowering and the flowers of the plant develop a stem (pegs), which enters into the soil, forming a pod containing usually one to five seeds. Groundnuts mature between 120- 150 days after which the leaves of the plant turn yellow and start to drop, the plant is then removed from the earth and allowed to dry (Crawford, 2014; Gober, 2014).

Figure 2. 1: Groundnut produced under field conditions.

Soya bean is indigenous to Manchuria, China and was one of the first planted crops used by the Chinese as food prior to 2500 BC (Directorate plant production, 2010). Worldwide production increased to more than 100 million metric tonnes in the last 30 years, where 51% is produced in the USA, 20% in Brazil, 10% in Argentina and 10% in China (Directorate plant production, 2010). Cultivation of the crop extended to South Africa. The leading provinces in soya bean production are Mpumalanga (42%), Free State (22%), Kwa-Zulu Natal (15%), Limpopo (8%), North West (5%) and Gauteng (2%) (Directorate plant production, 2010). In order for soya bean to grow well it needs warm temperatures. It’s a short-day grower, bushy, erect plant and branching annual crop (Figure 2.2). Soya bean emerges between five to seven days, and mature plants between 40 to 100 cm tall. It has an indeterminate growth habit. Flowering starts 50 to 60 days after sowing (20 to 35 cm height). The crop reaches physiological maturity 140 days after sowing (Directorate plant production, 2010).

Figure 2. 2: Soya bean under field conditions.

Dry bean is also an important legume for its subsistence and commercial farmers. It is regarded as one of the most essential field crops worldwide due to its high protein content and nutrition benefits. The crop originated in Central and South America over 7000 years ago and extended through Mexico and spread to most of the countries in the United States (Hardman & Meronuck, 1982) as well as other countries including South Africa. The major production areas in South Africa are Mpumalanga, Free State, North West, Gauteng and KwaZulu-Natal. Soya bean requires warm seasons to grow well and emerges between 3 to 7 days after planting (Directorate plant production, 2010). Its growth is upright and bushy (Figure 2.3). The crop bears pods that contain two to four seeds and has an indeterminate growth habit such as a few short and upright branches that grow after flowering. The crop matures 85 to 120 days after planting and when all the pods become yellow it can be harvested.

Figure 2. 3: Dry bean under field conditions.

2.2 Herbicides

In order to get high production, these legumes need good management practices such as weed and disease control (Directorate plant production, 2010). Weeds are considered as the main limiting factors in crop production and have detrimental effects on crop growth if not managed effectively (Pacanoski, 2007; El-Hadary & Chung, 2013). They compete with cultivated crops for soil moisture, nutrients, space and sunlight thus reducing dry matter accumulation in crops, resulting in a decrease in yield and quality. Weeds have a vigorous growth with well-developed root systems and are more productive, persistent, competitive and remove nutrients and water more efficiently and they are thus more resistant in nature than crops (Rao, 2002). The presence of weed debris reduce the marketability of grains during storage contaminating the grain. From this, it is important to control weeds, either manually or through the use of herbicides, before they cause any damage to crops (El-Hadary & Chung, 2013). Nowadays it is impossible to plant crops without herbicides because they require less labour than hand weeding, reduce the cost of farming and increase profitability compared to other methods of weed control. Herbicides have been used worldwide by farmers for a number of years, to control weeds and improve crop production (Pacanoski, 2007). Herbicides are the most effective, efficient and economical way to manage weeds if they are applied correctly (following recommended dosages) (Singh & Singh, 1992; Allemann & Allemann, 2013).

Herbicides can be defined as substances or synthetic chemicals that are toxic to plants and are used to kill, inhibit or destroy growth of unwanted vegetation (Meade, 1978). They are formulated and used to control certain plants without harming other plants (selective) or they can kill all plants (non-selective) (Allemann & Allemann, 2013). In order for the herbicide to kill plants it should be absorbed by the roots or foliage of the plant and be transported to the site of action without being deactivated (Gunsolus & Curran, 2002). When the herbicide reaches the site of action it interferes with plant processes such as photosynthesis, respiration and cell division, to mention only a few. Depending on its type of action, herbicides can be classified as systemic (herbicides are absorbed by the plant and is transported through the plant to the site of action to kill the plant) or non-systemic (herbicides which kill the plant parts they come into contact with) (Rao, 2002). Depending on time of application, herbicide can be divided in pre- and post emergence herbicide. Pre-emergence herbicides can be applied on the soil and is thus used before planting, prior to emergence of crops and/or weeds. Post emergence herbicides can be applied on foliage. Some herbicides may be used as pre- and post-emergence herbicide (Allemann & Allemann, 2013). Soil applied herbicides can be absorbed by the germinating seed, emerging roots and the shoots, while with foliar application it can be absorbed by the leaves of the plant after emergence. When pre-emergence herbicide reaches the soil, various processes occur that involve adsorption, movement, volatilization, leaching, runoff, plant uptake, microbial degradation, photodecomposition and chemical degradation (Figure 2.4) (Rao, 2002; Menalled & Dyer, 2004).

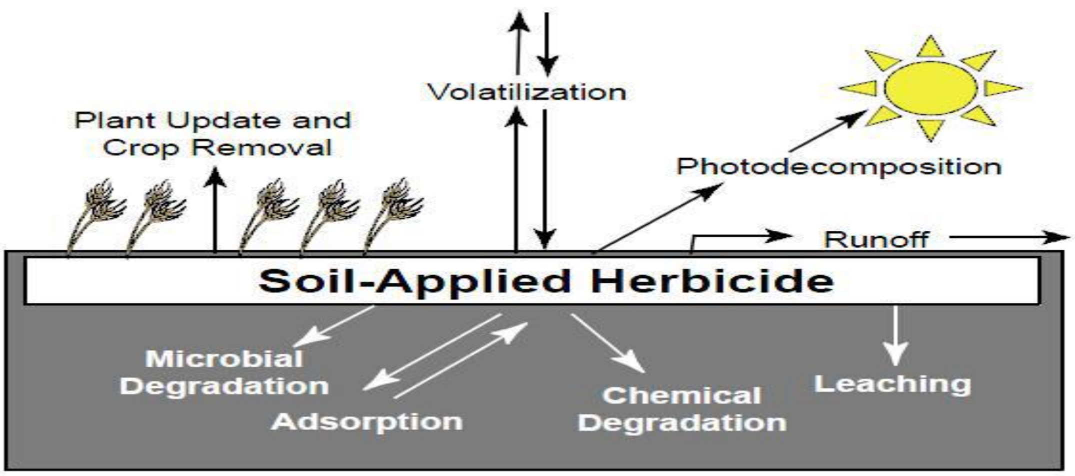


Figure 2. 4: Factors affecting the fate of soil-applied herbicides (Adapted from Menalled & Dyer, 2004).

When herbicides are applied to soil, it is adsorbed by the soil particles due to the attraction between the soil colloid surfaces and the herbicide molecule (Yu, 2014). Therefore, adsorption influences the efficacy of the herbicide in the soil and determines its availability and movement in the soil solution, determining the available amount for plant uptake.

It was also established that about 1% of the soil applied, pre-emergence herbicide is lost during volatilization (Yu, 2014). Volatilization is the process where a liquid or solid phase is changed into a gaseous phase (vapour). This depends on vapour pressure, soil or air temperature and wind speed (Heimann & Newman, 1997). During high temperatures and high wind velocity volatilization is high, this can lead to low weed control because most of the herbicide evaporates. For example, imazethapyr loss by volatilization is about 2% (Goetz *et al.,* 1990).

Herbicide movement by water involve runoff and leaching. Leaching involves the downward movement of the herbicide through the soil with the aid of water filtration (Zimdahl, 2007). Herbicide applied to the soil dissolves in water and moves from the site of application into the root area of the crop. High rainfall leaches some of the herbicide away from the target area. Loss of herbicide, due to runoff, is expected to be 2 to 3%, lowering the efficiency of the herbicide (Ross & Lembi 1999). After the herbicide is applied to the soil and it dissoves in water, it is absorbed by the plant. In order for the herbicide to be effective it must be in contact with the plant, either through the germinating seedlings, roots or stems.

Microbial degradation is another process that affects herbicide in the soil because soil micro-organisms, such as bacteria and fungi, change the molecular structure of the herbicide and this determines its fate in the soil (Yu, 2014). Photodecomposition also occurs when the herbicide is broken down by sunlight. The degree at which the herbicide will dissipate in the soil depends on the intensity of the sunlight (Yu, 2014). Chemical degradation involves hydrolysis, oxidation and reduction. Chemical degradation takes place when the herbicide is dissolved in the soil water solution (Yu, 2014).

2.2.1 Factors increasing herbicide damage on crops

Herbicide injury of plants is influenced by several factors including misuse or misapplication of herbicides, excessive or improper herbicide application rates, application of herbicides to stressed plants, spray drift, spraying of herbicides during a time when the crop is sensitive to the herbicides, volatilization as well as leaching (Heimann & Newman, 1997; Mathers, 2015).

Misapplication of herbicide occurs when the herbicide is applied accidentally to nontolerant crops, causing a decline in crop production (Mathers, 2015).

Herbicide injury of plants is influenced by several factors which can be grouped into three main categories namely improper application, spray drift and weather conditions and soil conditions (Heimann & Newman, 1997; Mathers, 2015).

Improper application lead to herbicide damage to crops. Misapplication of herbicide occur when the herbicide is applied accidentally to nontolerant crops, causing a decline in crop production (Mathers, 2015). According to Heimann & Newman (1997), herbicides should be applied at the recommended rates, as specified on the label. Over-application occurs when a higher than recommended rate of herbicide is applied. Faulty spary equipment can result in uneven pressure along the boom, causing under-dosing at the ends and overdosing along the middle of the boom. Overdosing can occur as a result of boom overlap, defective nozzles and fluctuating tractor speed. Insufficient mixing of water and herbicide can result in overdosing, then under-dosing as the tank empties. Field may contain variable soil texture. Incorrect assessment of varying soil texture in a field can result to overdosing on sandy and gravel areas. Impropser spray calibration is also one of the major contributing to herbicide over-application (Mathers, 2015).

Contamination can result from improperly cleaned spraying equipment. Damage becomes evident in areas which were sprayed first; and becomes less noticeable as spraying proceeds. Herbicides residue on previous sprayed stubble and other trash can damage emerging crop seedlings upon contact. If animals are given feed that contains certain herbicide residues, their manure can contain these residue (Yu, 2014).

The application of many herbicides is recommended within precisely defined stages of the crop's growth. It is always important to remember that younger plants are sensitive to herbicide applications and this can result in plant damage or the death of the plant. Failing to make the application at the proper stage may result in plant damage. Correct application timings are outlined on the label. Pre-harvest intervals, as listed on the label, give the required time after application to harvest the crop. Some crops cultivers are more sensitive than other culivers to particular herbicides (Gunsolus & Curran, 2002). Incompatable tank mixtures can be damaging to crop and not all herbicides can be mixed together. Label instructions should always be followed.

Spray drift and weather conditions are often the main reason for crop injury by herbicides. Spray or vapour drift can occur under windy conditions and can cause damage to neighbouring and/ or non-target crops (Heimann & Newman, 1997; Masabni, 2015). However, in still conditions temperature inversions may also cause off site droplets movement particularly when spaying is done at high pressure with small droplets (Heimann & Newman, 1997; Masabni, 2015). Droples size and nozzle height and wind velocity increase the distance that the wind can blow the herbicide, affecting non target crops when they come into contact with the herbicide ordamaging nearby plants. Spray or vapour drift is also possible when spot-applying or wiping herbicides that are applied selectively but can have advserse effect on the crop species. Damage from vapour drift is also possible when using volatile herbicide formulation (Dexter, 1993).

Weather conditions play a large role in herbicide damage. The occurance exceptionally warm weather soon after the application of pre-emergence soil-acting herbicide can lead to rapid crop growth and uptake of herbicide with subsequent crop damage. Large amounts of rain following an application of a soil-acting herbicide could wash the product down into the rooting area of the crop (Thomson, 1995). Leaching or runoff were already mentioned as possible cause of plant injury. Leaching can be explained as the movement of water or any other substance down through the soil, especially during heavy rainfall (Heimann & Newman, 1997; Mathers, 2015). Crops suffering from stress due to drought or infection by pests on the roots are more likely to be injured by soil herbicide residues than healthycrops. Drought stressed plants can also be injured by soil herbicide residues than healthy crops. Drought stressed plants can also be injured because under water deficit cinditions the microbial and hydrolyc breakdown of herbicides are reduced, decreasing its adsorption by soil particles (Dexter, 1993). Plants affected by early frost are more susceptable to damage from foliage-applied herbicide, although moderately low temperature may induce dormancy, making them more tolerant. Prolonged water stress can thicken leaves' waxy layer and increase leaf hairiness, resulting in less herbicide penetration. During extended period of cloudy, humid weather leaves develop less of a waxy and therefore more susceptable to damage from foliage-applied herbicide (Thomson, 1995). Wind damage to the leaf's waxy layer will also increase susceptable to herbicide damage. If drying conditions are poor following application, more herbicide may penetrate into the leaf causing damage. High relative humidity generally leads to more rapid penetration by the herbicide in to leaves followed by translocation within the palnt. Herbicide labels may contain weather restrictions during application and these limitations should be respected to avoid potential crop injury (Heimann & Newman, 1997).

Soil-applied herbicides are active in the soil, this means that they can kill susceptable plants whenever their roots come into contact with it. Soil types and organic matter influence the movement and availability of herbicides within the soil. For some soil applied herbicides the label will recommend different rates of application based on soi type. If inappropriate rates are applied (i.e high rates on sandy soil with low organic matter) crop injury may result. Some soil acting herbicides are persistant and residue can remain active for months (Mathers, 2015). In many cases, a minimum time period must pass before replanting susceptible crops, as outlined on the herbicide label. Planting before the end of this waithing period may result in crop damage. In exceptionally dry seasons, even moderately persistant herbicides may still be present in the soil beyond the ususal waiting period and affect next crop. Chemical breakdown can be influenced by soil pH. In low pH soils, some herbicides breakdown more readily. Soil compaction may also influence herbicide injury. When using acting herbicide, crop damage can occur if downward root development is restricted by soil compaction just below the seeding depth. Soil compaction results in a concentration of roots just below the surface of the soil, and excessive uptake of herbicide may occur. Seeding depth may also contribute to herbicide damage. Damage to a crop that has been deep seeded may occur due to shoot uptake of the herbicide. Volatilization of the herbicide is another factor that can lead to crop injury. This can occur when the herbicide evaporates in the air and the gasses or vapour blows over susceptable crops (Mathers, 2015).

All the above-mentioned factors lead to unintentional damage to crops that are economically important. This is why it is very necessary to use herbicides with proper care, making sure that the instructions on product labels are followed properly (O'sullivan *et al.*, 2002). All these factors can lead to visual damage that affects the morphological and physiological processes of vulnerable plants (Thomson, 1995). Herbicides are intented to kill unwanted vegetation in a field, but if not used properly or if they remain in the soil at the phytotoxic levels until planting of the next crop (herbicide residue) they kill or injure desirable plants (Mathers, 2015). Thus, it is very crusial to use herbicides according to the recommandations on the label (Allemann & Allemann, 2013).

2.2.2 Negative effects of herbicide injury on crops

In an attempt to timely control weeds and improve yield herbicides are normally used, but they can cause losses in crop yield if not applied correctly. Normally herbicides interfere with the physiological (photosynthesis, respiration, carbohydrates, membrane cell and metabolic changes) and morphological processes of the plant, which in turn affect its normal functioning. During photosynthesis plants absorb carbon dioxide through their leaves and water through their roots using light energy absorbed by chlorophyll converting carbon dioxide and water into sugar and oxygen i.e. $6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2$ (Ehlers & Goss, 2003). Absorbed herbicides affect the process of photosynthesis by interfering with the electron transfer system (PS II) by blocking the flow of electrons (Lehman, 2017). Consequently, leading to a build-up of energy, which in the end causes cell membranes to rupture and the nearby plant tissues to die. It was reported that without electron transfer, energy from the sun cannot be transformed into usable energy for the plants to generate new tissue and sustain life (Hess, 2000). For instance, in wheat, the photosynthesis activity was decreased during growth when bromphenol was applied, which lowered the chloroplast concentrations and 1/4 R, ½ R and R increased the activity (El-Hadary & Chung, 2013).

Besides photosynthesis, respiration can also be affected by the application of this herbicide (El-Hadary & Chung, 2013). Respiration takes place in the mitochondria of plants and it includes the synthesis of adenosine triphosphate (ATP) and the movement of electrons and protons. Herbicides interfere with mitochondrial activities by interrupting the electron transport, ATP synthesis and energy transmission. Consequently, it results in electron leakage and loss of energy (El-Hadary & Chung, 2013). Carbohydrate production is also affected. According to a study conducted by El-Hadary, (1988) it showed that mono-saccharides, di-saccharides, poly-saccharides and total carbohydrates were increased when low amounts of Brominal or Granstar were applied, but declined when high amounts of Brominal or Granstar were applied. Improper use of herbicides or herbicide residue has a negative impact on sensitive crops. It leads to stunted seedling growth, reduction of leave area, abortion of flowers and yellowing (necrosis) and wilting of leaves that will eventually lead to crop death and the delay of crop maturity (O'Callaghan, 2002).

2.3 Factors influencing herbicide residual activity in the soil

Herbicides are applied to control weeds during the growing season, and after application they are expected to be adsorbed, moved and degraded by the target plants and soil flora (Yu, 2014). Some of the herbicides persist and remain active in the soil longer than what is needed (Helling, 2005; Hager & Nordby, 2007). Herbicide residues in the soil are influenced by various factors such as soil factors, climate factors and herbicide properties to mention only a few.

2.3.1 Soil factors

Soil factors that contribute to herbicide residue include soil composition, soil microbial activities and soil chemistry (Hager & Nordby, 2007). Soil composition is measured by the proportion of sand, silt, clay particles and organic matter in the soil. It determines soil infiltration and the rate at which water drains through the soil (Havlin *et al.*, 2005). Soils with a high clay and organic matter content have high herbicide residues. This can injure susceptible crops in the future because they a have high number of binding sites and chemical reactivity (Hager & Nordby, 2007; Yu, 2014). Soils with a high clay and organic matter content have a higher cation exchange capacity and adsorb more cations, which can also increase herbicide adsorption (Hixon, 2008). Such soils also have low volatilization, low leaching, low runoff risks and high adsorption of herbicides that reduce herbicide uptake and activity. Soil texture influences herbicide residues for example, sandy soils (coarse textured soils) are porous and they have loose soil particles (no binding sites) resulting in the loss of herbicides by volatilization, which in turn lead to low herbicide persistence (Yu, 2014).

Soil microbes are normally responsible for the degradation of herbicides, but during unfavorable environmental conditions such as high temperatures, high soil pH, water logging and compacted soils, soil micro-organisms are not very effective, therefore this can increases the residual activity of herbicides (Hager & Nordby, 2007). Herbicide breakdown is higher during high temperatures and high soil moisture due to the higher activity of the soil micro-organisms (Hager & Nordby, 2007). However, under low soil moisture content and low temperatures herbicide degradation will lower, resulting in an increase in herbicide residues. The rate of herbicide degradation is also determined by the types of micro-organisms (fungi and bacteria) and the number of soil micro-organisms. Soil micro-organisms' activity and herbicide degradation are higher in warm, well-aerated soils (Yu, 2014).

Soil chemistry is another factor that influences herbicide residue, it includes soil pH, cation-exchange capacity (CEC) and nutrient status. Soil pH contributes to herbicide residue because it affects herbicide adsorption, plant uptake, degradation and leaching. As the pH decreases herbicide adsorption in the soil increases. When the pH is low there are more positive charges in the soil, which leads to better adsorption by the soil and low leaching of the herbicide, increasing residual activity (Zimdahl, 2007). Low soil pH can lead to a little dissipation of some herbicides such as imazethapyr, which can be small because of higher adsorption, leading to low availability of the herbicides for microbial degradation, resulting in herbicide persistence (Yu, 2014).

Nutrient status in the soil is another factor that can contribute to herbicide residue. Agricultural soils with high organic matter are considered fertile, but this can be a problem, since herbicide residue in the soil is normally high in soils with high organic matter. This is because more herbicide can be adsorbed in the soil colloids, leading to greater residual activity (Helling, 2005; Zimdahl, 2007).

2.3.2 Climate factors

The other factor that contributes to herbicide persistence is climate, which include temperature, moisture and radiation (photo decomposition). Herbicide breakdown is high during high temperature and high moisture in the soil, increasing the activity of soil micro-organisms (Hager & Nordby, 2007), this in turn lowers herbicide residues. While the opposite happens under low moisture in the soil and low temperature that causes low or no microbial activities (Van acker, 2005). Photo-degradation (breakdown of herbicides by light energy from the sun) is another factor that may contribute to herbicide carry-over (Menalled & Dyer, 2004). Sunlight act as a catalyst in the degradation of herbicide so it is clear that in the absence of sunlight there will be higher residual activity in the soil which will affect susceptible crops (Devlin, *et al*., 1992).

2.3.3 Herbicide properties

Herbicide properties such as water solubility, molecule's susceptibility to chemicals and vapor pressure contribute to herbicide residue (Hager & Nordby, 2007). If an herbicide is less soluble in water it will have high residues remaining in the soil because it will have a low leaching potential, remaining longer in the soil due to its strong adsorption and thus injuring crops. The vapour pressure of herbicides is another factor that contributes to its residual activity. The herbicide will have high volatilization in the air when it stays on the soil surface for too long (Riddle *et al.*, 2013). This will increase with higher temperatures and less air moisture, resulting in the loss of herbicides, reducing weed control and decreased residue injury to sensitive crops. It was indicated by Devlin, *et al*. (1992) that volatile herbicides have high vapour pressures, and therefore they dissipate more rapidly than herbicides with low vapour pressures. Mesotrione's vapour pressure is 5.69 x 10^{-3} mpa, this means that if this vapour pressure decreases it can cause residues, which will affect the crop planted in the next season. All these factors and other unmentioned factors cause herbicide persistence, which injure plants and lead to abnormal functioning of the plant, which may result in low production and death. Herbicides are classified according to the HRAC scale into different groups according to their action inside weeds. Mesotrione was used during this study and is classified as a HPPD inhibitor, a short discussion will follow on the importance of these herbicides and their effects in plants.

2.4 4-Hydroxyphenylpyruvate dioxygenase inhibitors (HPPD)

HPPD inhibitors are used to control broadleaf weeds and other grasses (Siehl *et al.*, 2014). They prevent plant growth by blocking the 4-Hydroxyphenyl pyruvate dioxygenase enzyme. The herbicides in this category include topramezone, tembotrione and mesotrione (Moran, 2014). These inhibitors interfere with photosynthesis by interrupting the conversion of tyrosine through 4-hydroxyphenyl pyruvate to homogentisate (Figure 2.5), this causes a failure to produce plastoquinone and tocopherol (Vitamin E). Therefore, resulting in the loss of carotenoid pigments and inhibited photosynthesis (Coats, 2009; Riddle, 2012; Moran, 2014). Plastoquinone is essential for electron transfer and also as a cofactor for phytoene desaturase in the carotenoid biosynthesis pathway (Coats, 2009). Inhibiting HPPD causes stunted growth due to excess tyrosine, and the plant suffers from oxidation damage due to the lack of tocopherol. Tocopherol is responsible for strengthening the membrane and scavenging for free radicals inside the chloroplast membrane (Riddle, 2012).

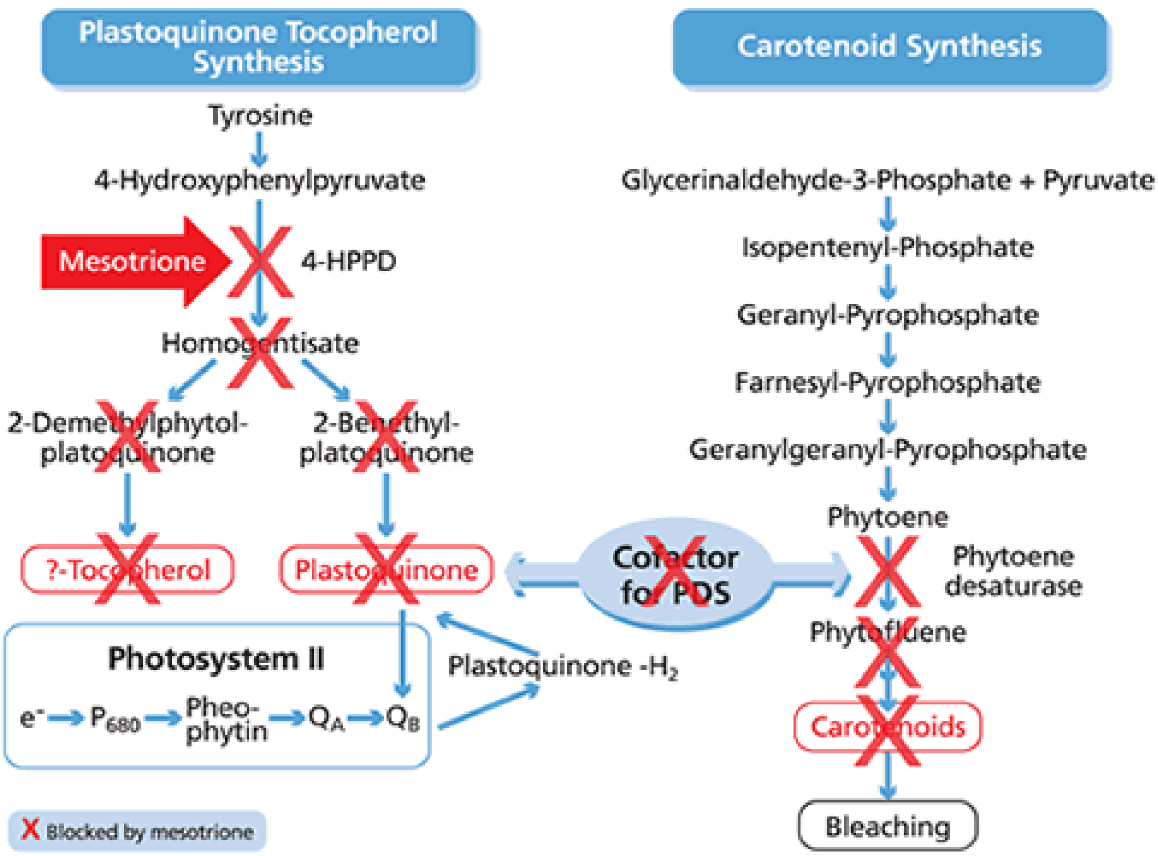


Figure 2. 5: Mesotrione inhibition of the enzyme HPPD and carotenoid biosynthesis (Adapted from Syngenta, 2008).

Topramezone are the HPPD inhibitor herbicides, controls more than 95% of redroot pigweed, ragweed and lambs' quarter at 50 g a/ha, barnyard grass and yellow foxtail at 67 g a/ha in maize, and more than 90% of 13 star-of-Bethlehem at 37 g a/ha when combined with the PSII inhibiting herbicide bromoxynil (Riddle, 2012). Tembotrione is another HPPD inhibitor herbicide and is formulated with isoxadifen-ethyl at concentrations of 44 g/L and 22 g/L respectively, as Laudis for broadleaf and grassy weed control in maize. Mesotrione was reported to control weeds such as creeping bent grass, nimble will and crabgrass (Siehl *et.*, *al* 2014).

HPPD is an enzyme that is found in almost all aerobic forms of life, and it uses two substrates while adding both atoms of diatomic oxygen into the product homogentisate (Moran, 2005). In plants the enzyme catalyses the conversion of tyrosine to plastoquionone and α-tocophyrol (Coats, 2009). It is Fe (II)dependent, containing non-hemo oxygenase, that breaks down the amino acids into compounds that are used by the plants to create other molecules that the plant needs (Moran, 2005; Coats, 2009). It was further indicated that the enzyme is also involved in the synthesis of carotene pigments, which contribute to photosynthesis by transferring some light energy to chlorophylls through resonance energy transfer, which then uses this energy to drive photosynthesis. Carotenoids are made from the central molecule isopentenyl pyrophosphate, which protects chlorophyll from being degraded by sunlight by harmlessly scattering excess light energy, which they absorb as heat (Riddle, 2012). Carotenoids are also responsible for collecting reactive oxygen species before the membranes are damaged, thus protecting the chloroplast. It was indicated that, without carotenoids, excess light energy destroys chlorophyll, proteins, membranes and other molecules and this results in bleaching, necrosis and subsequent death of the plant (Moran, 2005; Riddle, 2012). This also leads to plants not being able to protect themselves from photo-oxidation (Riddle, 2012). Mesotrione used during this study will be discussed shortly to show its importance and its effect on weeds.

2.5 Mesotrione

Mesotrione [2-(4-methylsulfonyl-2-nitrobenzoyl)-1, 3-cyclohexanedione] is derived from the natural phytotoxin produced by *Callistemon citrinus* and is a member of the triketone group of herbicides discovered in 1982 by Zeneca Agriculture products (Syngenta) (James *et al*., 2006). Triketones prevent the function of the enzyme 4-hydroxyphenylpyruvate dioxygenase. This in turn inhibits carotenoid development and without carotenoids light energy destroys chlorophyll and this leads to the death of the plant (James *et al*., 2006). This also leads to bleaching symptoms and necrosis of the meristematic tissue within 3 to 5 days after application (Wichert *et al.*, 1999). Mesotrione is a reduced risk herbicide with a good ecological and toxicological profile with low harmfulness to non-target plants, mammals, birds, underground water and sea species (Yu, 2014).

Mesotrione is one of the selective herbicides that are used to control annual broadleaf and grass weeds in maize and suger cane production, either as a pre-emergence (soil) or post-emergence (foliar) application in the field (Johnson *et al.*, 2002; Armel *et al*., 2003; Allemann & Allemann, 2013). In previous studies mesotrione was effective in controlling broadleaf weeds such as pigweed, flixweed, mustard and lettuce (Affeldt, 2015). It was also tested on bluegrass turf, rice, wheat, sorghum and maize (Armel *et al*., 2003). In most of the latter cases a pre-emergence application leads to chlorosis and bleaching of the developing shoots. Sometimes shoots emerge without apparent injury, while post-emergence treatment resulted in the death of all the unprotected leaves (James *et al*., 2006). Mesotrione inhibits photosynthesis because it interferes with the conversion of tyrosine (through HPPD) to homogentisate, resulting in the failure to produce plastoquinones essential for electron transport, consequently resulting in the loss of carotenoid pigments and ultimately photosynthesis failure and tocopherol production (Figure 2.5) (Coats, 2009; Moran, 2014). Some characteristics will be discussed shortly.

2.5.1 Chemical and physical properties of mesotrione

Herbicides have different chemical and physical properties that determines how they perform when applied to plants or soil. Herbicides from a similar group may have some characteristics in common but they each have particular properties, which will show their differences from other herbicides within the same group (Rao, 2002). Mesotrione is a weak acid, with a pH of 4.6 to 7.7. It contains organic matter of 0.6 to 3.6%. It has a half-life that ranges from 2 to 14 days with a mean of 9 days (Wichert *et al.,* 1999; Riddle, 2012). Table 2.1 shows the chemical and physical properties of mesotrione (adapted from Riddle, 2012).

2.5.2 Mesotrione in the soil

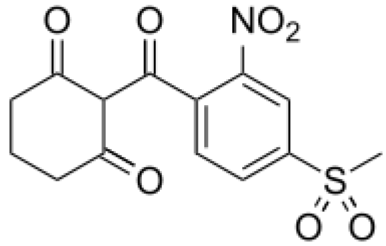
Mesotrione in the soil can be available for weed management or not, depending on its behaviour in the soil, and can be influenced by the soil organic matter and soil pH. Mesotrione is a weak acid (it does not dissociate completely in a water solution and does not give all its hydrogen ions into the water) (Mitchell *et al.,* 2001; Chaabane *et al.,* 2008). The soil pH and the amount of organic matter in the soil has an effect on the adsorption, movement and residual activity of mesotrione (Riddle, 2012). At a high soil pH, mesotrione changes to an anionic form and its adsorption declines, while at a low soil pH, its adsorption into the soil colloids is high and its degradation is low (Yu, 2014).

Mesotrione residue is also influenced by the amount of organic matter in the soil, rainfall, and the soil texture (Lehmann *et al*., 1992). The more organic matter in the soil, the greater the residual activity, due to the fact that the herbicide binds with the organic matter, which reduces its degradation (Lehmann *et al*., 1992; Rouchaud *et al.,* 2000).

The half-life of the herbicide in the soil also determines how long the herbicide will be in the soil, and that depends on the soil condition (e.g weak structure, poor water retention properties and high permeability), texture and degradation (Riddle *et al.,* 2012). Rouchaud *et al.* (2000) reported varying half-lives in the top 0-10 cm of different soil textures, for example loam soil was found to have the longest half-life recorded at 50 days, clay at 42 days and sandy-loam at

40 days, with sandy soils containing the shortest half-life at 34 days, sandy soils are fast-draining and they have little or no ability to transport water from deeper layers through capillary transport and has low organic matter. The amount of seasonal rainfall influences residues in the soil, for instance during high rain fall (after two months of herbicide application) there is more degradation and movement of mesotrione and therefore less herbicide in the soil (Riddle *et al.*, 2012).

Table 2. 1: Chemical and physical properties of mesotrione (Riddle, 2012).

Structure	
Formulation	Granular, water dispersible granules, can be emulsified, concentrate, soluble concentrate, solution-ready to use, pressurized liquid, and soluble concentrate/solid.
Common name	Mesotrione
Chemical name	2-(4-mesyl-2-nitrobenzoyl)-3-hydroxycyclohex-2-enone or 2-(4-methylsulfonyl-2-nitrobenzoyl)-1,3-cyclohexanedione
Chemical formula	$C_{14}H_{13}O_7NS$
Physical state	Opaque solid
Molecular weight	339.32 g/mole
Melting point	165 °C
Dissociation constant	3.12 (pKa@20 °C)
Vapour pressure:	4.27 x 10-8 mm Hg (20 °C)
Water solubility	2.2 g/l @ pH 4.8 (20 °C) 15 g/l @ pH 6.9 (20 °C) 22 g/l @ pH 9 (20 °C) 0.16 (in buffered H_2O)
Storage stability	0-6 °C
Odour	Faint pleasant odor
Stability	Stable to hydrolysis (pH 4-9).

2.6 Phytotoxicity of mesotrione residues on rotational crops

Herbicide residue can cause serious problems in sensitive crops (Greenland, 2003). A study conducted by Riddle *et al.* (2012) results revealed that mesotrione residues can cause damage and low yield to sensitive crops grown in the subsequent growing season (e.g. sugar beet, dry bean and green beans are sensitive to mesotrione residue). Similar results were obtained by Felix *et al.* (2007) who also found that mesotrione applied the year before was capable of causing injury to various vegetables like snap bean, cucumber; cabbage and tomatoes as well as red clover and this resulted in a decline in yield of these crops. It was further indicated that mesotrione residues, one year after application, can reduce biomass, yield and dry weight in wheat, broccoli, carrot, cucumber, and onions (Robinson, 2008; Soltani *et al*., 2014). However, this shows that mesotrione residues can potentially damage many crops planted during the next growing season. During herbicide residue stress certain critical stages of plant growth and physiology are severely affected and this leads to a reduction in crop production. This is why it is important to find solutions to alleviate these damages. One solution might be the application of bio-stimulants for example Brassinosteroids (BRs). In order to look at its potential to alleviate stress some background and its importance is supplied.

2.7 Brassinosteroids

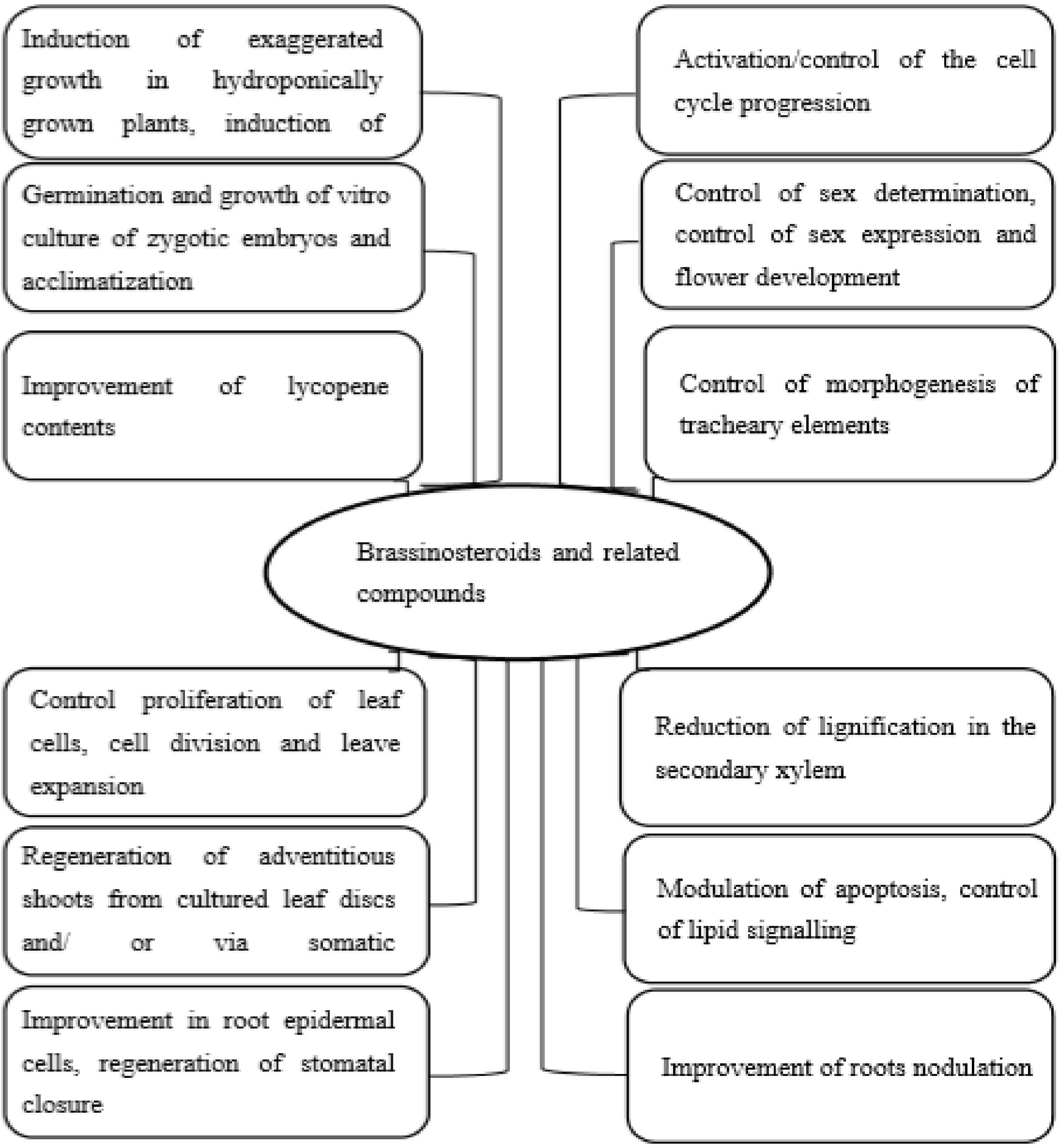


Figure 2. 6: Roles of brassinosteroids and related compounds reported in plants (Vardhini & Anjum, 2015).

Brassinosteroids are plant hormones or a group of polyhydroxy steroids that are accountable for many plant processes such as cell expansion, division, seed germination, xylem differentiation, reproductive development, pollen elongation, formation of the pollen tube, disease and abiotic stress resistance (Figure 2.6) (Vardhini & Anjum, 2015; Tang *et al.*, 2016). During cell elongation, brassinosteroids affect the mechanical properties of the cell walls through genomic and non-genomic pathways that allow turgor-driven cell enlargement to proceed (Shahbaz & Ashraf, 2007).

Brassinosteroids are extracted from *Brassica napus* pollen, which are growth promoter steroid hormones (Clouse & Sasse, 1998) and are categorised depending on the number of carbons present in their structures namely C_{27}, C_{28} and C_{29} (Vardhini & Anjum, 2015). To improve plant growth and metabolism BRs interact with plant hormones such as ethylene, auxins, cytokinins and gibberellins (Vardhini & Anjum, 2015). Brassinosteroids improve plant health and vigor in a number of ways and they consist of plant nutrients, phytohormones, vitamins, antioxidants and carbohydrates. These components are accountable for the many identified roles of BRs in plants.

Studies on brassinosteroids started 36 years ago and the top well-known brassinosteroids extracted from plants are brassinolide (BL) (Figure 2.7), castasterone (CS), teasterone (TE) and 6-deoxycastaterone (6-deoxy CS). They all belong to the C_{28}-BRs with a 24α-methyl group (Van der watt, 2005). This group of hormones have huge application potential in the agricultural industry and might play a large role in protecting plants or crops against herbicide stress. Brassinosteroid biosynthesis pathways start with campesterol, which is converted to campestenol through the reduction of the C-5 double bond in campesterol (Figure 2.7). The latter is then converted to castasterone via the early C6-oxidation pathway or the late C6-oxidation pathway and this results in brassinolide (Clouse, 2011).

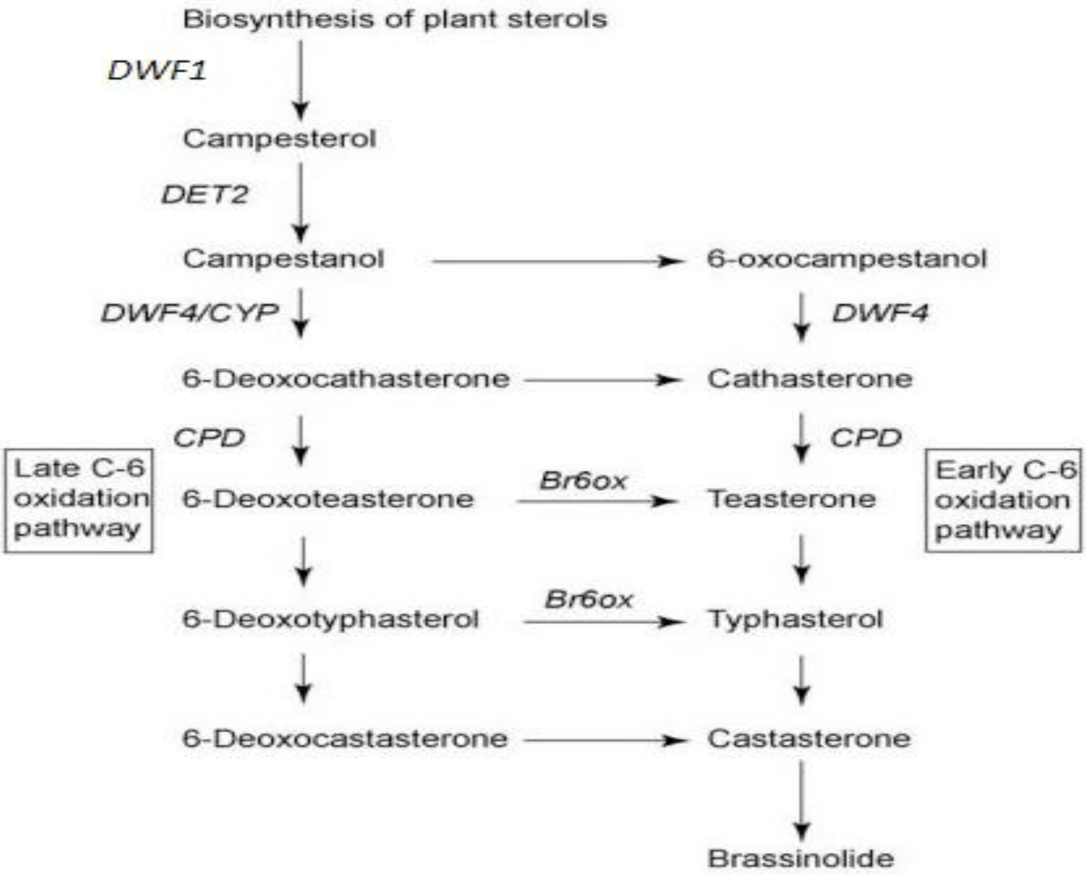


Figure 2. 7: Brassinosteroid biosynthesis pathway (Divi & Krishna, 2009).

2.7.1 The role of brassinosteroids in crops

Currently, avoiding crop losses and the production of more food, to meet the ever-increasing demands of the growing human population, have gained unprecedented importance. Therefore, it is important to find strategies that can be developed and deployed by plants to withstand abiotic stresses and maintain their growth and survival under harsh conditions. Crops are exposed to different abiotic stresses such as drought, water logging, high temperature, freezing and herbicide residue or injury to mention a few. All these stress factors affect the crops negatively, resulting in poor plant growth, development and productivity (Ogweno *et al.*, 2008; Sirhindi, 2013; Wu *et al*., 2014). It is therefore vital to aid crops by increasing their stress resistance and tolerance. This can be done by the application of BRs, which help the crop to survive such stresses by transforming physiological processes and developing defense mechanisms to improve tolerance to abiotic stress (Vardhini & Anjum, 2015). After a significant amount of research, these natural products were developed to address stress in crops. As was indicated by Tang *et al.* (2016) the brassinosteroids are environmentally friendly. They do not interfere with the environment because they are natural plant products and can be used as agrochemicals (plant growth regulators).

When a crop is exposed to stress it is important to have a comprehensive understanding of how this crop responds to specific stresses at its physiological and morphological levels. Abiotic stress causes changes in a crop's photosynthesis, this is one of the most vulnerable processes that gets severely affected during abiotic stress. Abiotic stress results in the over-reduction of the electron transport chain, which results in photo-oxidation (Vardhini & Anjum, 2015). So, in order for a plant to survive under these conditions the use of brassinosteroids is important, as it regulates the response of the photosynthetic apparatus to different abiotic stress conditions. During abiotic stress the antioxidants (from brassinosteroids) concentrate in the chloroplast, reviving the wilting or bleached leaves, and thus improving the photosynthetic processes. It was further indicated that brassinosteroids regulate the metabolism of plant oxidation radicals (ethylene synthesis) and this leads to the improvement of the adverse effects of different environmental stresses and produces resistance in crops against stresses (Wani *et al.*, 2016). Brassinosteroids are important for development and crop growth and show positive results in crop development (Shahbaz & Ashraf, 2007).

In a study conducted by Ogweno *et al.* (2008) it was found that brassinosteroids improved the recovery of carbon dioxide assimilation, stomatal conductance and the maximum carboxylation rate of Rubisco. The electron transport rate, relative quantum efficiency of photosystem 11 photochemistry, photochemical quenching and increased non-photochemical quenching also improved. Again, it was found that the application of brassinosteroids (28-homobrassinolide) to mustard seedlings caused an increase in the chlorophyll content and improved the net photosynthetic rate, resulting in increased yields (Hayat *et al.,* 2000: Hayat *et al.*, 2001). Brassinosteroids can be applied using different methods, which include foliar application at different stages of a crop, soil drenching and as a seed treatment. Agriculturally it was found that the application of brassinosteroids have caused increases in yield and improved stress resistance of many crops (Vardhini *et al.*, 2008). In a study that was conducted by Vardhini *et al.* (2008) it was reported that the foliar application of brassinosteroids improved growth yield in tomatoes and groundnut. This is in line with the findings of Vardhini & Rao, (2002), where spraying brassinosteroids have improved yields in crops such as wheat, rice, corn, melons, potatoes, oranges, grapes and pears.

2.8 Conclusion

Crop growth and production are exposed to different stresses such as biotic and abiotic stresses. Plants respond to stress in different ways, such as cellular homeostasis maintenance, detoxification of harmful toxins and recovery of growth. These responses are partially mediated by the modulation of gene expressions, which is mostly controlled by phytohormones and their crosstalk through cross-communicating signalling pathways. Brassinosteroids are the essential group of phytohormones that play a vital role in plant growth, development and stress management and and to increase yield. According to Van der Watt (2005) brassinosteroids are natural plant products that are environmentally friendly and can be used as agrochemicals. It should be recommended to farmers, as consumers are concerned that the amount of chemicals used in crop production could have a harmful impact on the whole ecosystem. As indicated earlier, brassinosteroids are organic products (natural) they do not pose any danger to plants and animals (including humans). This means that they have no harmful impact on the environment. Brassinosteroids conserve biodiversity and preserve ecosystems because they are natural and reduce the risk of soil pollution (Van der Watt, 2005). It is clear that, even though legume crops are important, they can be affected by herbicide residue if planted in rotation systems. In order to address future productivity, important improvements across a spectrum of technologies and the application of brassinosteroids will be necessary in order to solve the problem. The use of brassinosteroids must become important to farmers because they can help them to increase their production and avoid losses that might be caused by herbicide residue or any other kind of stress. This will enable the agricultural sector to produce enough food for the growing population and avoid unnecessary losses.

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CHAPTER 3

GENERAL MATERIALS AND METHODS

3.1 Experimental site and materials

A simulated carry-over study was conducted in pots over two growing seasons (2015/16 and 2016/17) to determine the effect of mesotrione residues on three legumes (Soya bean, groundnut and dry bean) in the glasshouses of the Department of Soil, Crop and Climate Sciences, University of the Free State Bloemfontein South Africa (29°07` S, 26°11`E). The trial was conducted under natural daylight conditions, with a day length of approximately 13 hours and day/night temperatures of 28°/18°C. Legume seeds, herbicide as well as standard fertilizer products were purchased locally from SENWES, Bloemfontein South Africa and Brassinosteroids from Agraforum GMBH, Germany. The experiment was laid out in a randomized complete block design in a factorial arrangement. The trial was conducted in two different ways using two polyethylene pot sizes. Small polyethylene pots (2.5 kg soil) were used for two weeks' interval destructive sampling, replicated eight times, each pot containing two plants for each crop. The big pots (6 kg soil) were allowed to grow up to maturity, used for non-destructive sampling and replicated four times, each pot containing four plants for each crop.

3.2 Soil

The pots were filled with reddish brown, fine sandy loam soil classified as Bainsvlei Amalia soil (Soil Classification Working Group, 1991) collected from Kenilworth Experimental Research Farm of the Department. Fertiliser was applied based on soil analysis and expected yield outcome according to the prescriptions of the Agricultural Research Council -SGI (ARC, 2015). Summarized details for soil nutrient analysis and fertilizer requirements for each crop are supplied below (Tables 3.1 and 3.2).

Table 3. 1: Soil nutrient analysis conducted by the Agricultural Research Council - SGI, Bethlehem, South Africa (2015).

N (mg/kg)	**P** (mg/kg)	**K** (mg/kg)	**Ca** (mg/kg)	**Mg** (mg/kg)	**Na** (mg/kg)	**pH** (KCl)	**Clay** (%)	**CEC** ($cmol_c kg^{-1}$)
-	1.4	88.2	469	234.0	6.3	5.0	8	4.52

CEC = Cation exchange capacity

Table 3. 2: Glasshouse trial specifics and fertilizer requirements for dry bean, soya bean and groundnut cultivated under irrigation conditions, based on expected yield outcome and soil analysis as recommended by the Agricultural Research Council - SGI, Bethlehem, South Africa (ARC Technical datasheet, 2015).

Crop	**Dry bean**	**Soya bean**	**Groundnut**
Cultivar	Kranskop	PAN 1522 R	Tufa choice
Plants/ha	± 90 000	± 90 000	± 90 000
Yield potential	2.0 ton/ha	2.0 ton/ha	2.0 ton/ha
Fertilizer	3:2:1 (25)	3:2:1 (25)	3:2:1 (25)
LAN	20.89 kg/ha	-	-
N	45 kg/ha	60 kg/ha	30 kg/ha
P	40 kg/ha	40 kg/ha	60 kg/ha
K	20 kg/ha	21 kg/ha	10 kg/ha

3.3 Measuring of soil field water capacity

To measure Field water capacity (FWC) in the pots, the mass of the oven dried soil in the pots were measured and then saturated with water and left to drain until a constant mass was observed. The difference between the mass of the drained soil and the dried soil was taken as the water content at FWC. It was determined gravimetrically to calculate the amount of water applied to bring the soil to 70% FWC for residual activity. Pots were weighed daily and distilled water was added when necessary to bring the water content of the pot back to 70% of the water available at field capacity.

3.4 Application of herbicide

The mesotrione concentrations used were calculated using the average half-life of mesotrione in the soil, which is given as nine days in the Herbicide Handbook (WSSA, 2007). Using this value the amount of mesotrione that would be available in the top 15 cm of a sandy loam soil with a bulk density of 1 625 kg m^{-3} every 45 days following application of 124.8 g ai ha^{-1} up to a period of 135 days was calculated using the following formula (Table 3.3):

SC = (Rate × Concentration)/ (Area × Depth × Density) (Allemann & Molomo, 2016)

Where SC = Soil concentration, Rate = recommended application rate of the herbicide for the particular soil, Concentration = concentration of the active ingredient in the herbicide, Area = 10 000 m^2 (area of 1 ha in m^2), Depth = depth of the soil layer in which the herbicide concentration must be calculated, and Density = bulk density of the soil being used (kg m^{-3}). The soil was weighed into polythene bags, after which the herbicide-water mixture was added in a polythene bags and thoroughly mixed through the soil. This was done by kneading the soil in the bag until it was completely moist and placed back into the pots with the bag. The planting was done on the same day for all the crops where two seeds were planted in small pots and four seeds were planted big pots at a depth of 2 cm in each pot.

Table 3. 3: Mesotrione concentrations (μg kg^{-1}) calculated to remain in the soil every 45 days following application rate of 124.8 g mesotrione ha^{-1} using 9-day half-life.

Control	Days after application		
	45	90	135
0	1.6	0.05	0.0016

3.5 Application of brassinosteroids (Seed treatment, soil drench and foliar application)

Both preventative and corrective treatments were done with the brassinosteroids. Concentration was determine during a previous screening study, thus only the most effective application concentrations will be tested. Because pure BRs are usually applied at ng quantities a carried was mixed with pure active compounds to ease application under glasshouse conditions. Three sets of legume seeds were treated with brassinosteroids; first as a seed treatment where seeds of three legume seeds were treated with 100g brassinosteroids /ha (5mg ai /5ml water /kg seed) (Preventative treatment) secondly as a foliar application where 100g (20g ai) brassinosteroids/ 40 000L water/ha was sprayed on the leaves one week after emergence (Corrective treatment) and thirdly using it as a drench where brassinosteroids at 100g (20 g ai) /40 000L/ha water was applied on soil (Preventative treatment) using a hand pump sprayer.

3.6 Experiment 1 - Destructive measurements

Small pots with dimensions 17.3 cm wide and 15 cm deep with volume of 2.5 kg were lined with polyethylene bags prior to filling with soil (2.5 kg) in order to prevent leaching and contamination from the sides of the pot. After two weeks of crop emergence, four replications were harvested and the remaining four replications harvested two weeks later. The following measurements were taken; chlorophyll content, carotenoids content, seed emergence, seedling fresh mass and dry mass, root fresh mass and dry mass.

3.6.1 Quantification of parameters

3.6.1.1 Physiological parameters

(a) Qualitative determination of chlorophyll content

A total of 0.2 g leaf material (third leaves from the top of each plant) from seedlings was cut into small pieces and 3 ml acetone added (1:15; m/v). The leave materials were crushed using a mortar and pestle until the supernatant had a dark green colour. The extract was then centrifuged at 6000 rpm and an aliquot of 1ml used to determine absorbance spectra using a Shimadzu UV spectrophotometer. The chlorophyll a, b, total chlorophyll and carotenoids content were read at three different wavelengths (661.6 nm, 644.8 nm and 470 nm) and the calculations were done as follows (Wellburn, 1994):

(1) Determination of the Chlorophyll a content

11.24 (constant) x Absorbance (661.6 nm) - 2.04 (constant) x Absorbance (644.8) = Chlorophyll a content as µg/ml plant extract.

(2) Determination of the Chlorophyll b content

20.13 (constant) x Absorbance (644.8 nm) – 4.19 (constant) x Absorbance (661.6) = Chlorophyll b content as µg/ml plant extract.

(3) Determination of the Chlorophyll a and b content

7.05 (constant) x Absorbance (661.6 nm) – 418.09 (constant) x Absorbance (644.8) = Total chlorophyll content as µg/ml plant extract.

(4) Determination of the total carotenoid content

$$\frac{1000 \times \text{absorbance } (470 \text{ nm}) - 1.90 \times \text{chlorophyll a content} - 63.14 \times \text{chlorophyll b content}}{214}$$

= Total carotene content in µg/ml plant extract.

3.7 Development or growth parameter

(a) Emergence

Counted the number of seeds that emerged above the soil within the first two weeks after planting and calculated it as percentage of the total.

3.8 Morphological parameters

Plant seedlings were harvested every two weeks and separated into roots and aerial parts of the crop. This was done by cutting the plant just above the soil surface to separate the aerial and root parts. Following is a list of parameters that were measured:

(a) Seedling fresh mass and dry mass

The seedlings were washed and dried using paper towel and fresh mass was determined with an electronic scale, after which the plant material were placed in an oven at 65°C until constant dry mass was obtained.

(b) Root fresh mass and dry mass

The roots were washed to remove all soil and dried using paper towel. Fresh mass was determined with an electronic scale, after which the materials were placed in an oven at 65°C until constant dry mass was obtained.

3.9 Experiment 2 - Non-destructive

Big pot 24.3 cm wide and 19.9 cm deep containing 6 kg soil were lined with polyethylene bags in order to prevent leaching and contamination from the sides of the pot. Crops were grown up to harvest and a few parameters measured including: photosynthesis rate, stem diameter, natural plant height (crop was measured from the soil surface to the highest point of the arch of the uppermost leaf whose tip is pointing down and extended plant height (the "highest point" on the plant), pod mass and number per pot, seed number per pot and mass of seeds per pot.

3.9.1 Quantification of parameters

3.9.1.1 Physiological parameters

(a) Photosynthesis rate

Photosynthesis was measured to determine physiological responses of gas exchange parameters such as the CO_2 assimilation rate, stomatal conductance, transpiration, and flourescence. Photosynthesis was determined using a LiCor 6400XT portable analysing meter, measuring the effect of the treatments after every two weeks under glasshouse conditions of every third leaf from the top of the plant. The system determines photosynthesis rate by establishing the rate at which CO_2 is used in every second for each sample measured. Photosynthesis rate was captured in the presence of white light (2000 *í*mol photons m^{-2} s^{-1}) in the glass house using a light source (LED light) and CO_2 supplied (400ppm) by the CO_2 tank of the system. Measurements were performed every two weeks between 11 h and 13 h under optimal growth conditions with a temperature of 25^0C and 6 cm^2 of exposed leaf surface.

3.10 Morphological parameters

(a) Stem diameter

Diameter was measured about 1 cm above the soil every two weeks up to week sixth using an electronic Vernier Calliper.

(b) Natural plant height

Plant measured from the soil surface to the highest point of the arch of the uppermost leaf whose tip is pointing down. The natural plant height was measured every two weeks before harvesting using a ruler as distance from soil surface excluding the first 2 cm which represented the planting depth.

(c) Extended plant height

The extended plant height (the highest point on the plant) was measured to the highest point every two weeks before harvesting using a ruler as distance from soil surface excluding the first 2 cm which represented the planting depth.

3.11 Yield parameters

(a) Pod mass and number per pot

All pods per pot were harvested after reaching physiological maturity, counted, weighed and the data recorded as average mass and number per pot.

(b) Seed number per pot and mass of seeds per pot

All pods were shelled and the number of seeds per pot counted and weighed and recorded as average seed number and seed mass per pot. The final yield was taken as seed mass per pot.

3.12 Data calculations and statistical analysis

Data from each crop was analyzed separately and data was subjected to analysis of variance using the statistical analysis system (SAS) program 9.2 package for Windows V8 (SAS Institute Inc, 1999 - 2010). Treatment means were compared using the least significant difference (LSD) at a 5% level of significance to determine significant differences between treatment means.

3.13 References

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CHAPTER 4

THE EFFECT OF BRASSINOSTEROIDS ON THE MORPHOLOGICAL, PHYSIOLOGICAL AND YIELD CHARACTERISTICS OF SOYA BEAN UNDER DIFFERENT MESOTRIONE RESIDUAL LEVELS

4.1 Introduction

Soya bean is one of the most important crops in many developing countries including South Africa. However, the successful production of soya bean is influenced by many factors and weed control is a major one. Worldwide, weeds affect the success of the crops by being very competitive and they sometimes produce allelopathic substances affecting crops (Rao, 1999). Keeping fields free of weeds during the season helps to improve on crop yields and it is recommended that weeds be controlled between 15-35 days after planting (Nieuwenhuis & Nieuwelink, 2005).

Weeds thrive better than crops because they have vigorous growth, well-developed root systems and persistent characteristics (Rao, 1999). Apart from hosting harmful insects, weeds normally grow faster and taller than crops and compete for sunlight, moisture, space and nutrients (Zimdahl, 2007). Research studies in Africa have reported 263 different weed species from 38 families in crop fields that comprise 72% of broadleaves, 24% grasses and 4% sedges (Chikoye & Ekeleme, 2001). In addition, a total of 100 to 300 million-weed seeds per hectare are buried in most African soils annually (Edossa, 2015). The yield losses caused by weeds in Africa range from 25% to total crop failure (100%), when farmers fail to control weeds (Vissoh *et al.*, 2004). It was also reported that one kilogram of weeds can decrease rice yield by 500-900g per ha in Nigeria (Adeosun, 2008). In a study conducted by Anonymous, (1979 b) it was indicated that soya bean dry matter accumulation was decreased from 10.0 g to 5.4 g/plant if weeds are not controlled. Another study also showed that weed competition in soya bean can decrease dry matter accumulation from 15.8 g to 8.3 g/ plant (Anonymous, 1979 a). Reported yield losses demonstrate the urgent need to control weeds on time before damages occur, which can be done either through physical or chemical methods. Chemical (herbicides) methods seem to be the best weed control option since it saves time, are cheaper, faster and more effective than physical methods that involve hiring of labour for manual weeding (Chikoye *et al.*, 2005). Herbicides can cause an overall reduction in production costs by reducing labour requirement for weeding from 39.2 to 1.3 person days^{-1} ha^{-1} (Overfield *et al.*, 2001). This production cost reduction associated with herbicides can lead to increased economic returns for a farmer, making it economical viable for the farmer.

Herbicides are chemicals that are used to kill unwanted green vegetation targeting either their germinating seeds or directly during the growing season. They can either be applied to the soil before planting or directly to emerging weeds (Overfield *et al.*, 2001). However, use of herbicides can cause plant injury to sensitive crops in a rotation system due to residues that remains in the soil. Mesotrione is one of the herbicides that can damage succeeding crops in a rotation system through its residual activity. Mesotrione is used to control weeds in maize and suger cane production in South Africa. It is a selective herbicide which can be applied either to soil or on leaves. When herbicide reaches the soil, it goes through different processes which include adsorption, movement, degradation and crop uptake to mention only a few. If these processes are too slow to reduce the concentration in the soil to levels below those that are biologically active, the herbicide stays longer than expected in the soil (Helling, 2005). This means that the un-degraded herbicides increase its residual activity in the soil and can cause plant injuries and yield decreases in sensitive crops, following the herbicide application (Helling, 2005).

Some vegetable crops especially cabbage and tomato are sensitive to residual activity of herbicides such as imazethapyr (O'Sullivan *et al.*, 2002), mesotrione (Felix *et al.*, 2007) and saflufenacil (Robinson & McNaughton, 2012). It was also indicated that imazethapyr residue caused damage and decrease in yield of crops like wheat, sorghum and maize one year after its application (Johnson *et al.*, 2002). All these problems caused by herbicide residue can be overcome by increasing the herbicide tollerance of the crop or by increasing the herbicide degradation in the soil. Increasing herbicide tollerance is easier than increasing soil degradation because it can be done through inducing depression of enzymes that inhibit photosynthesis or enhancing the photosynthetic rate of the stressed crop overcoming the damage caused by herbicide on photosynthesis (Taylor *et al.*, 2013). The use of brassinosteroids appear to be a possible solution that may relieve injury caused by herbicide residue on sensitive crops in a rotating system.

It has been found that when brassinosteroids are absorbed by plants, the compound improved growth, induced flowering, stimulated various metabolic processes and increased quality and crop production (Van der Watt, 2005). According to Vardhini & Anjum, (2015) brassinosteroids are responsible for improving physiological and morphological processes in crops such as plant growth, increase rate of photosynthesis, cell division and elongation. Another report by Vardhini *et al.* (2008) showed that foliar application of brassinosteroids improved growth and yield on tomato and groundnut. Again, it was indicated that the use of brassinosteroids assisted the plants to degrade herbicides faster and continue its normal activities, thus the crop became resistant and tolerant to herbicide damage (Sharma *et al.*, 2013). Additionally, application of brassinosteroids on winter rape improved energy absorption and increased electron transport by photosystem (PSII) reaction centres (Janeczko *et al.*, 2005).

Herbicide residue is one of the abiotic stresses causing plant injury to succeeding crops in a rotation system when herbicides are not applied correctly to control weeds. Soya bean is one of the leguminous crops commonly grown after maize, which use herbicides like mesotrione for weed control. However, the damaging effect of mesotrione residue to soya bean grown on field previously treated with mesotrione is not well understood. Therefore, the objectives of this study were to 1) determine the effect of three residual mesotrione concentrations (C) on soya bean. 2) determine the effect of three different application methods of brassinosteroids on soya bean and 3) determine the effect of brassinosteroids combined with mesotrione residues on soya bean.

4.2 Materials and methods

The procedure that is followed here is outlined in Chapter 3.

4.3 Results

4.3.1 The effect of three residual concentrations of mesotrione (C) on soya bean.

In both the growing seasons (2015/16 and 2016/17) the results showed the same trend so the results of one season were presented since there is no difference between the two seasons.

4.3.1.1 Effect on seedling emergence and morphological parameters

(a) Emergence percentage

All the seeds planted gave 100% emergence and therefore there was no significant difference between the different treatments.

(b) Visual symptoms of the crop injury

The response of soya bean growth to different residual concentrations of mesotrione are shown in Figure 4.1. Crop injury was evaluated by comparing three levels of herbicide residue concentration (1.6 µg ai kg^{-1} soil (equivalent to 45 days after application (DAA), 0.05 µg ai kg^{-1} soil (equivalent to 90 DAA) and 0.0016 µg ai kg^{-1} soil (equivalent to 135 DAA)) and the control (0). Figure 4.1 clearly demonstrated that soya bean is quite sensitive to the residue activity of different mesotrione concentrations as expected. The residual effects of mesotrione on growth parameters were more severe at the highest concentration compared to the control. The other two concentrations caused visible plant injury at early stages (two weeks after planting) such as thick, narrow and yellowing of leaves, stunted growth and a decrease in stem diameter. On the other hand, at the lower mesotrione residue concentration (0.0016 µg ai kg^{-1} soil) no phytotoxicity symptoms were visible. Plants also seem to recover during the season even at the higher concentration.

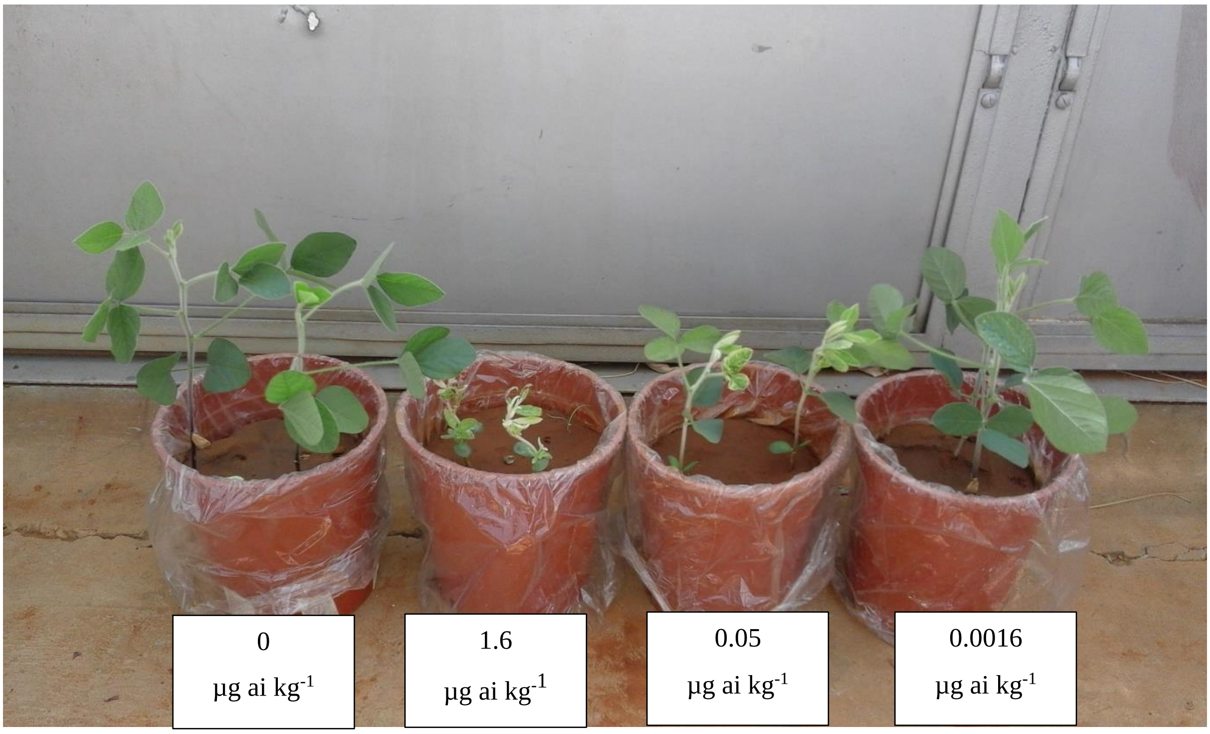

Figure 4. 1: Different visual phytotoxic symptoms of three mesotrione residual concentrations on soya bean two weeks after plant.

(c) Morphological parameters

1) Destructive parameters

The effect of three residual mesotrione concentrations on morphological parameters of soya bean was measured every two weeks is summarized in Figure 4.2. The three concentrations of mesotrione residue in the soil significantly decreased all the measured growth parameters compared to the control and the growth reduction increased with an increase in mesotrione residue concentration. The results showed that the interaction between concentrations and weeks were significant and also indicated that the parameters increased at the lowest residual concentration 0.0016 µg ai kg^{-1} followed by 0.05 µg ai kg^{-1} and 1.6 µg ai kg^{-1} respectively.This also confirming the significant negative effect of the highest concentration (1.6 µg ai kg^{-1}) on plant growth.

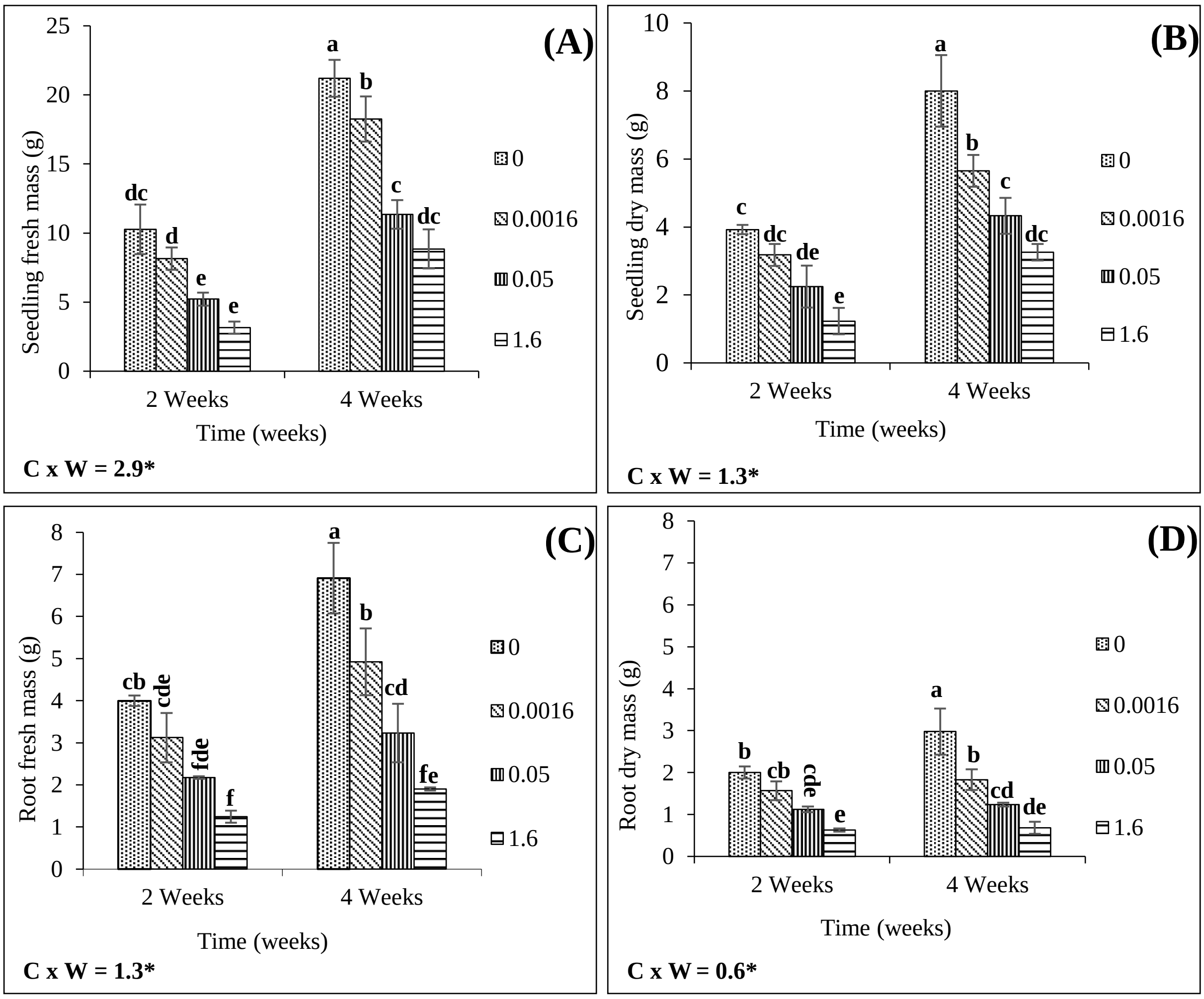

Figure 4. 2: The effect of three residual concentrations of mesotrione) (1.6 µg ai kg-1, 0.05 µg ai kg-1, 0.0016 µg ai kg-1 and the control 0) on destructive growth parameters including (A) fresh mass, (B) dry mass, (C) root fresh mass and (D) root dry mass measured every two weeks over a period of four weeks. Where C x W is the interaction between mesotrione concentrations and weeks. Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters and ns = not significant at 5% using Tukey's LSD test. Vertical bars with horizontal caps indicate standard deviation.

2) Non-destructive growth parameters

The effect of three residual mesotrione concentrations on natural plant height, extended plant height and stem diameter of soya bean was measured every two weeks is summarized in Table 4.1. All three mesotrione residue concentrations caused a significant decrease in natural plant height, extended plant height and stem diameter compared to the control. The reduction in growth increased with an increase in residue concentration. This showed that the highest concentration (1.6 µg ai kg-^{1}) has the highest negative effect on plant growth.

Table 4. 1: The morphological response of soya bean to three residual mesotrione concentrations (1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1}, 0.0016 µg ai kg^{-1} and the control 0) over a period of 12 weeks. Measured parameter are natural plant height, extended plant height and stem diameter.

	Herbicide residue (µg ai kg^{-1})	**2 Weeks**	**4 Weeks**	**6 Weeks**	**8 Weeks**	**10 Weeks**	**12 Weeks**
Natural plant height (cm)	0	9.7 a	14.8 a	22.7 a	28.8 a	36.6 a	42.6 a
	1.6	5.2 d	10.9 d	16.8 d	21.2 d	28.9 d	35.2 d
	0.05	6.9 c	12.8 c	19.3 c	24.67 c	31.7 c	38.3 c
	0.0016	8.1 b	13.8 b	21.1 b	26.9 b	34.4 b	40.4 b
	LSD_T (5%)	**1.2***	**0.7***	**1.3***	**1.6***	**2.1***	**1.2***
Extended plant height (cm)	0	17.4 a	23.2 a	33.4 a	41.4 a	51.2 a	56.2 a
	1.6	10.7 d	16.2 d	22.8 d	29.2 d	35.7 d	41.6 d
	0.05	12.3 c	18.5 c	25.9 c	33.5 c	39.8 c	45.4 c
	0.0016	14.0 b	20.5 b	28.4 b	37.4 b	45.2 b	49.3 b
	LSD_T (5%)	**0.8***	**1.0**	**1.4***	**1.5***	**1.7***	**1.3***
Stem diameter (mm)	0	3.4 a	3.7 a	4.1 a	4.6 a	4.9 a	5.0 a
	1.6	3.1 d	3.4 d	3.5 d	3.6 d	3.8 d	4.1 d
	0.05	3.2 c	3.5 c	3.8 c	41 c	4.2 c	4.4 c
	0.0016	3.3 b	3.7 b	3.9 b	4.3 d	4.7 b	4.9 b
	LSD_T (5%)	**0.03***	**0.1***	**0.1***	**0.1***	**0.04***	**0.1***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

4.3.1.2 Physiological response of soya bean to three residual concentrations of mesotrione (C)

(a) Chlorophyll content

Figure 4.3 shows the effect of three concentrations of mesotrione on chlorophyll content measured every two weeks. The significant interaction between concentrations and weeks was observed in chlorophyll a while no significant interaction between concentrations and weeks was seen in chlorophyll b, total chlorophyll (a + b) and carotenoids. However significant differences were observed between the three concentrations with the highest chlorophyll content detected at the lowest mesotrione concentration (0.0016 µg ai kg^{-1}) as compared to the other two concentrations. In both weeks, herbicide residue affected the content of chlorophyll negatively since it increased with the decrease in mesotrione concentration.

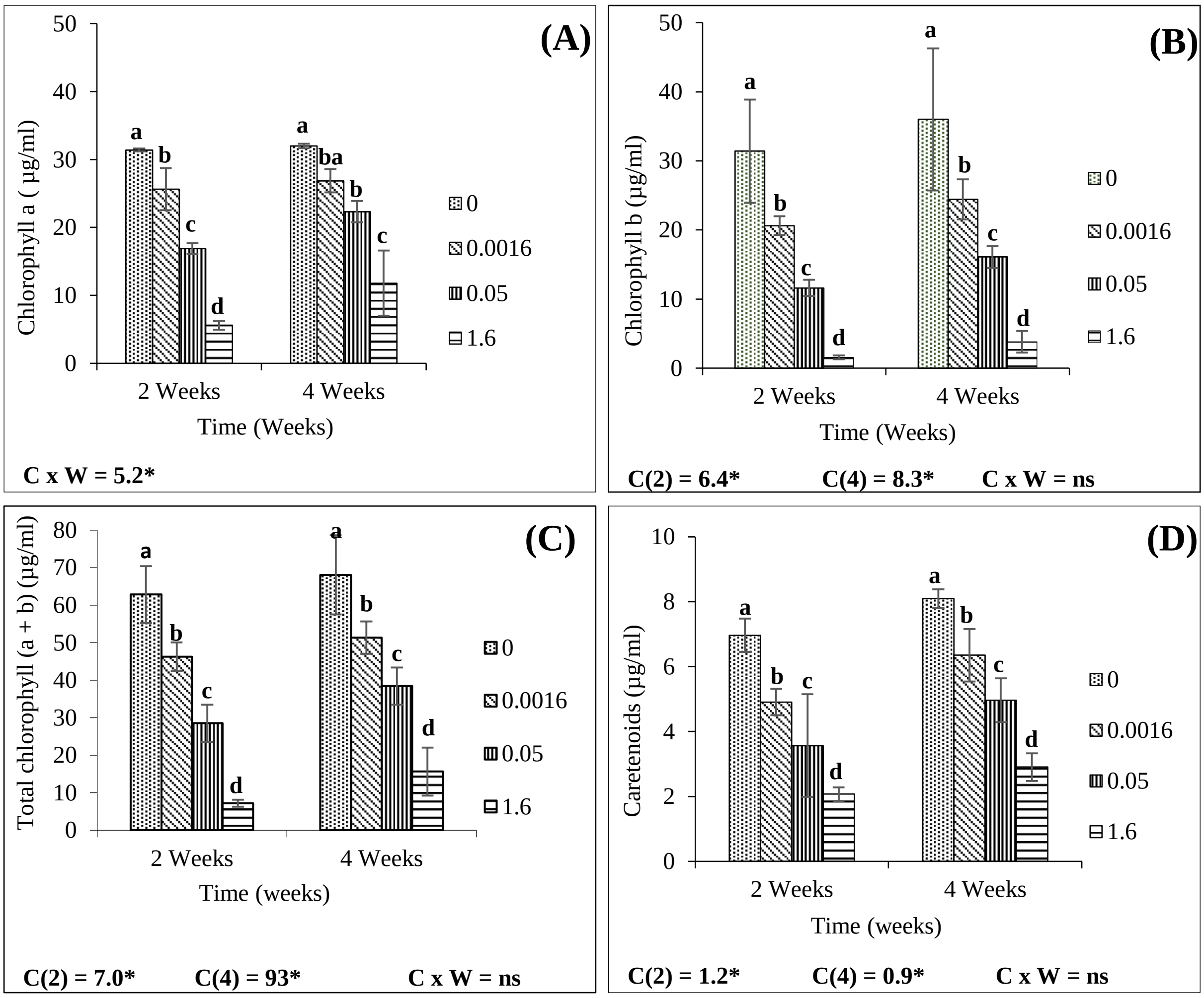

Figure 4. 3: The effect of three residual concentrations of mesotrione (1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1}, 0.0016 µg ai kg^{-1} and the control (0)) on (A) chlorophyll a, (B) chlorophyll b, (C) total chlorophyll and (D) carotenoids content measured every two weeks over a period of four weeks. Where C x W is the interaction between concentrations and weeks, C (2) = Effect of herbicide at two weeks after plant and C (4) = Effect of herbicide at 4 weeks after plant. Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test. Vertical bars with horizontal caps indicate standard deviation.

(b) Photosynthetic parameters

The effect of the three C on the rate of photosynthetic parameters (photosynthesis, stomatal conductance, transpiration rate and sub-stomatal CO_2 concentration) over a period 13 weeks were treated as absolute values in Figure 4.4. Significant interaction between C, over time, was observed in the transpiration rate and sub-stomatal CO_2 concentration. While the interactions showed no significant differences in the case of photosynthesis and stomatal conductance. However, the main effects C differed significantly compared to control. Photosynthesis, stomatal conductance and transpiration rate decreased with an increase in C, but with sub-stomatal CO_2 concentration the opposite happened. The parameters increased during the most active growth at 7-9 weeks during the season and then decrease as the plant reached physiological maturity. The same tendency was observed as previously mentioned that lower mesotrione concentration showed the least phytotoxicity but was still low compared to the control.

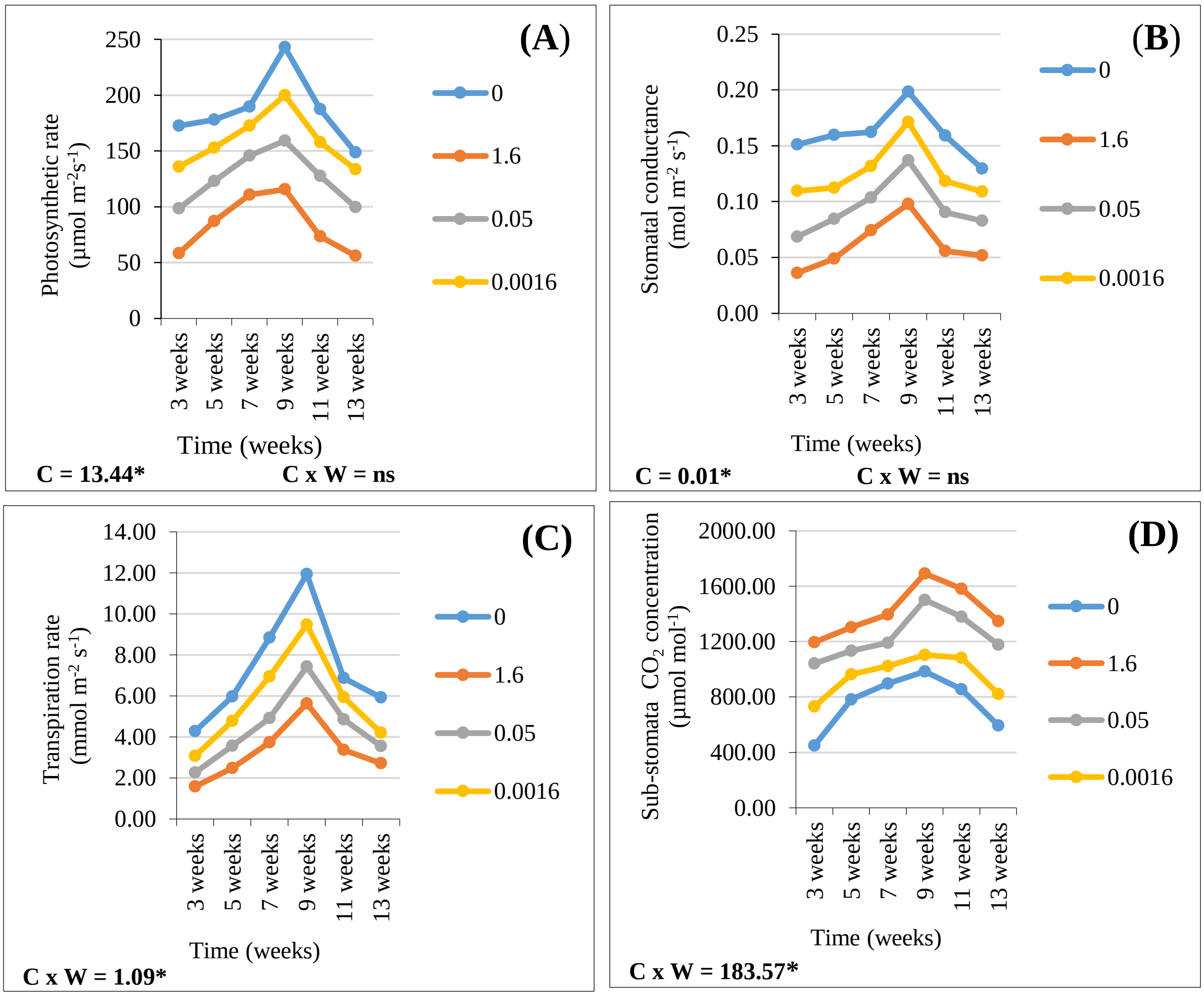

Figure 4. 4: The response of photosynthetic parameters of soya bean to three residual concentrations of mesotrione (1.6 μg ai kg^{-1}, 0.05 μg ai kg^{-1}, 0.0016 μg ai kg^{-1} and the control (0)) measured over a period of 12 weeks. Measured parameters include (A) Photosynthetic rate, (B) Stomatal conductance, (C) Transpiration and (D) Sub-stomatal CO_2 concentration. The interaction is indicated as C x W (Interaction between concentrations and weeks). Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and ns = not significant at 5% using Tukey's LSD test.

4.3.1.3 The effect of three residual concentrations of mesotrione on yield parameters

(a) Yield components

Table 4.2 provides the yield and yield components of soya bean. The results showed significant differences between the mesotrione residue concentrations. This illustrates that the reduction of yield and yield components is dose dependant when applying the herbicide. A huge decline in yield parameters can be observed at 1.6 µg ai kg^{-1} followed by 0.05 µg ai kg^{-1} and 0.0016 µg ai kg^{-1} respectively compared to the control.

Table 4. 2: The effect of three residual concentrations of mesotrione 1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1} and 0.0016 µg ai kg^{-1} and the control (0) on yield components. Measured parameters include number of pods per pot, mass of pods per pot, number of seeds per pot and mass of seeds per pot (final yield per pot).

Herbicide residue (µg ai kg^{-1})	**Number of pods per pot**	**Mass of pods per pot (g)**	**Number of seeds per pot**	**Mass of seeds per pot (g)**
0	45.8 a	21.8 a	89.3 a	15.9 a
1.6	29.5 d	7.3 d	48.0 d	4.8 d
0.05	34.5 c	12.1 c	63.5 c	9.2 c
0.0016	40.3 b	17.3 b	76.0 b	13.0 b
LSD_T (5%)	**4.1***	**4.3***	**11.1***	**2.9***

Statistical significance is indicated as LSD =* significant (P ≤ 0.05) and different letters, and ns = not significant at 5% using Tukey's LSD test.

4.3.2 The effect of three different application methods of brassinosteroids (T) on soya bean.

In both two growing seasons (2015/16 and 2016/17) the results show the same trend so the results of one season will be presented since there is no difference between the two seasons.

4.3.2.1 The effect on seedling emergence and morphological parameters

(a) Emergence percentage

All the seeds planted gave 100% emergence and therefore there was no significant difference between the different treatments.

(b) Symptoms of the crop injury

The brassinosteroids had no phytotoxic effects on the growth so there was no crop injury.

(c) Morphological parameters

1) Destructive parameters

The effect of three application methods of brassinosteroids (T) on the morphological parameters of soya bean measured every two weeks over four weeks (Figure 4.5). There was no significant interaction between brassinosteroids application methods and weeks, but there were significant differences between the brassinosteroids application methods compared to control. All the applied methods of brassinosteroids significantly increased plant growth compared to the control. However, no significant difference occurred between the seed treatment and the soil drench. The highest increase in plant growth was found in the foliar application, followed by the soil drench and seed treatment respectively.

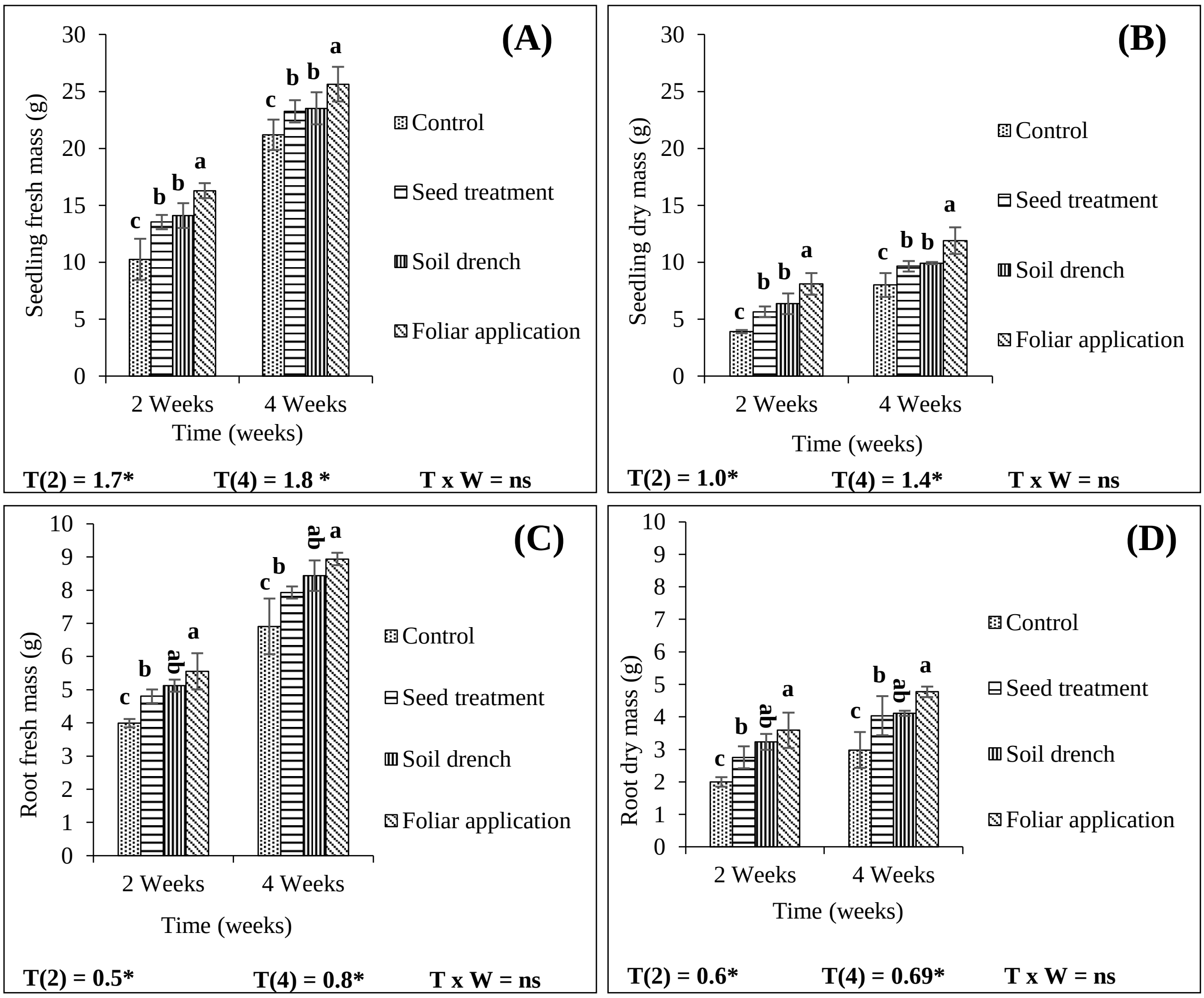

Figure 4. 5: The morphological response of soya bean to three brassinosteroids application methods (T), seed treatment, soil drench, foliar application and control, on destructive seedling growth parameters including (A) seedling fresh mass, (B) seedling dry mass, (C) root fresh mass and (D) root dry mass measured every two weeks over four weeks. Where T(2) = Effect of brassinosteroids at two weeks after plant, T(4) = Effect of brassinosteroids after 4 weeks and T x W is the interaction between brassinosteroids and weeks. Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test. Vertical bars with horizontal caps indicate standard deviation.

2) Non-destructive growth parameters

Table 4.3 shows the effect of three application methods of BRs on morphological parameters of soya bean measured over a period of 12 weeks. All the applied brassinosteroids caused an increase in plant growth as compared to control. From two weeks to twelve weeks the three treatments significantly increased all the measured morphological parameters as compared to the control. The highest increase in plant growth is found in foliar application, followed by soil drench and seed treatment respectively.

Table 4. 3: The effect of three brassinosteroids application methods, seed treatment, soil drench, foliar application and control on morphological parameter over a period of 12 weeks. Measured parameters included natural plant height, extended plant height and stem diameter.

	Treatment	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks	12 Weeks
Natural plant height (cm)	Control	9.7 d	14.8 d	22.7 d	28.8 d	36.6 d	42.6 d
	Seed treatment	11.0 c	18.0 c	25.0 c	34.6 c	42.6 c	48.4 c
	Soil drench	12.4 b	19.4 b	27.0 b	35.9 b	44.3 b	52. b
	Foliar application	14.0 a	21.9 a	29.1 a	38.5 a	47.6 a	55.4 a
	LSD_T (5%)	**1.2***	**0.9***	**1.8***	**1.2***	**1.1***	**1.1***
Extended plant height (cm)	Control	17.4 d	23.2 d	33.4 d	41.4 d	51.2 d	56.2 d
	Seed treatment	19.5 c	27.4 c	37.2 c	45.1 c	54.6 c	59.1 c
	Soil drench	21.9 b	30.8 b	39.5 b	47.3 b	58.3 b	65.1 b
	Foliar application	23.9 a	33.6 a	44.9 a	54.0 a	64.1 a	69.3 a
	LSD_T (5%)	**1.5***	**1.1***	**1.2***	**1.4***	**2.7***	**1.0***
Stem diameter (mm)	Control	3.4 d	3.7 d	4.1 d	4.6 d	4.9 d	5.0 d
	Seed treatment	3.7 c	3.8 c	4.7 c	4.9 c	5.5 c	5.7 c
	Soil drench	3.9 b	4.1 b	4.8 b	5.1 b	5.7 b	6.0 b
	Foliar application	4.3 a	4.4 a	4.9 a	5.3 a	5.9 a	6.1 a
	LSD_T (5%)	**0.1***	**0.1***	**0.1***	**0.1***	**0.1***	**0.1***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

4.3.2.2 The effects on physiological parameters

(a) Chlorophyll content

All application methods of the brassinosteroids caused an increase in the measured chlorophyll content as compared to the control (Figure 4.6). There was no significant interaction between brassinosteroids application methods and weeks, nonetheless the significant differences was observed between the brassinosteroids application methods compared to control. The results confirmed again that the foliar application significantly differed compared to seed treatment, soil drench and control respectively. While no differences could be seen between seed treatment and soil drench they still differ significantly compared to control.

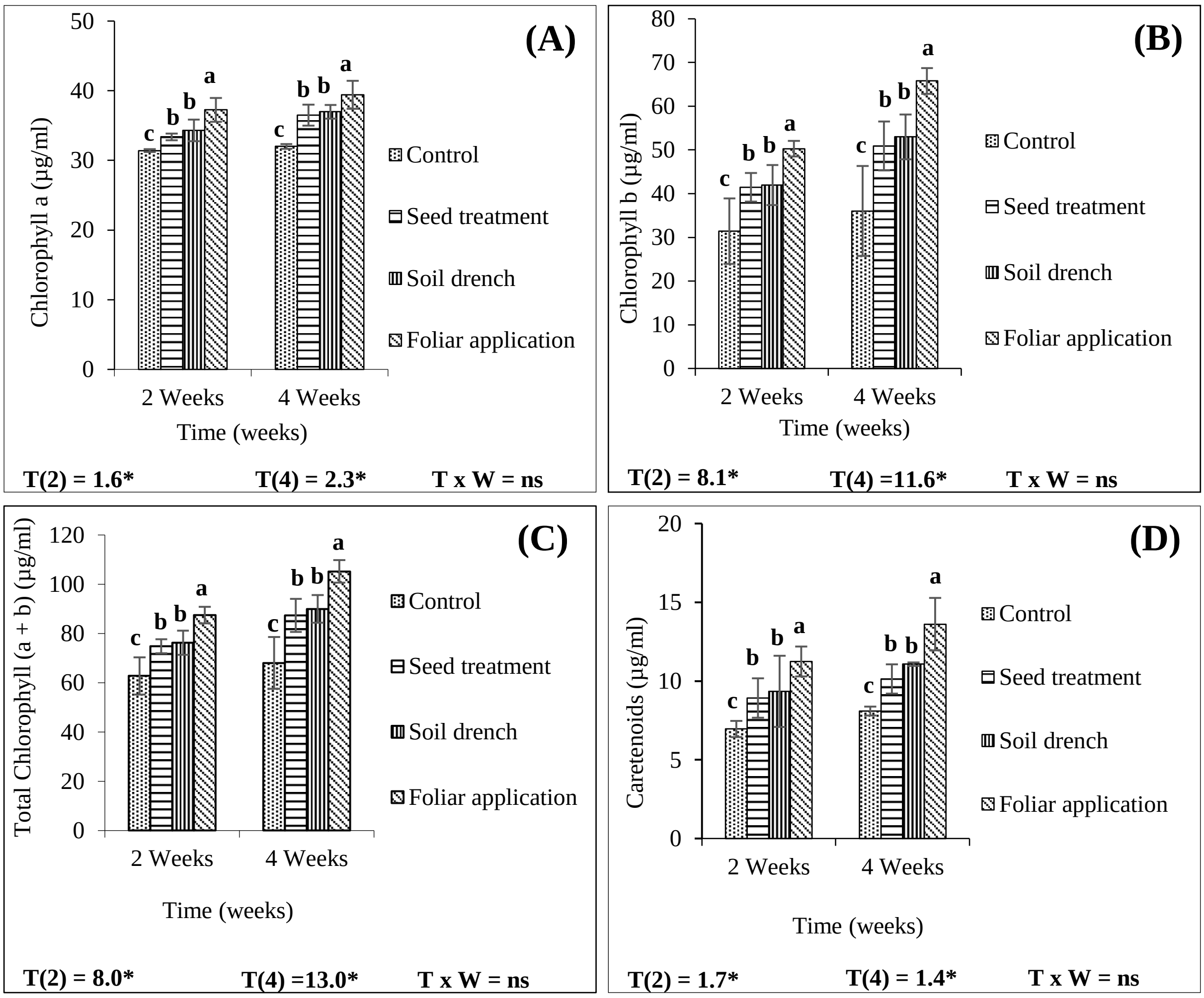

Figure 4. 6: The effect of three application methods of brassinosteroids (T), (seed treatment, soil drench foliar application and control) on (A) chlorophyll a, (B) chlorophyll b, (A) total chlorophyll and (D) carotenoids content measured every two weeks over a period of four weeks. Where T(2) = Effect of brassinosteroids at two weeks after plant, T(4) = Effect of brassinosteroids 4 weeks after plant and T x W is the interaction between brassinosteroids and weeks. Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test. Vertical bars with horizontal caps indicate standard deviation.

(b) Photosynthetic parameters

The effect of different application methods of brassinosteroids on photosynthetic rate, stomatal conductance, transpiration rate and sub-stomatal CO_2 concentration was measured over a period of 13 weeks (Figure 4.7). The interaction (T x W) between brassinosteroids and weeks showed significant differences on photosynthetic rate, transpiration rate and sub-stomatal CO_2 concentration. Only the main effects in the case of stomatal conductance showed significant difference between the three brassinosteroids application methods compared to control. All the applied methods of brassinosteroids caused an increase in all the measured parameters as compared to control. The highest measurement of parameters was observed at nine weeks which is the most active vegetative growth stage by all the three methods of application of brassinosteroids with foliar application as the highest, followed by soil drench and seed treatment respectively. At weeks 11 and 13 during physiological maturity a huge decline in physiological activity could be observed, nonetheless a significant difference between the application methods were observed with the foliar application outperforming the other methods.

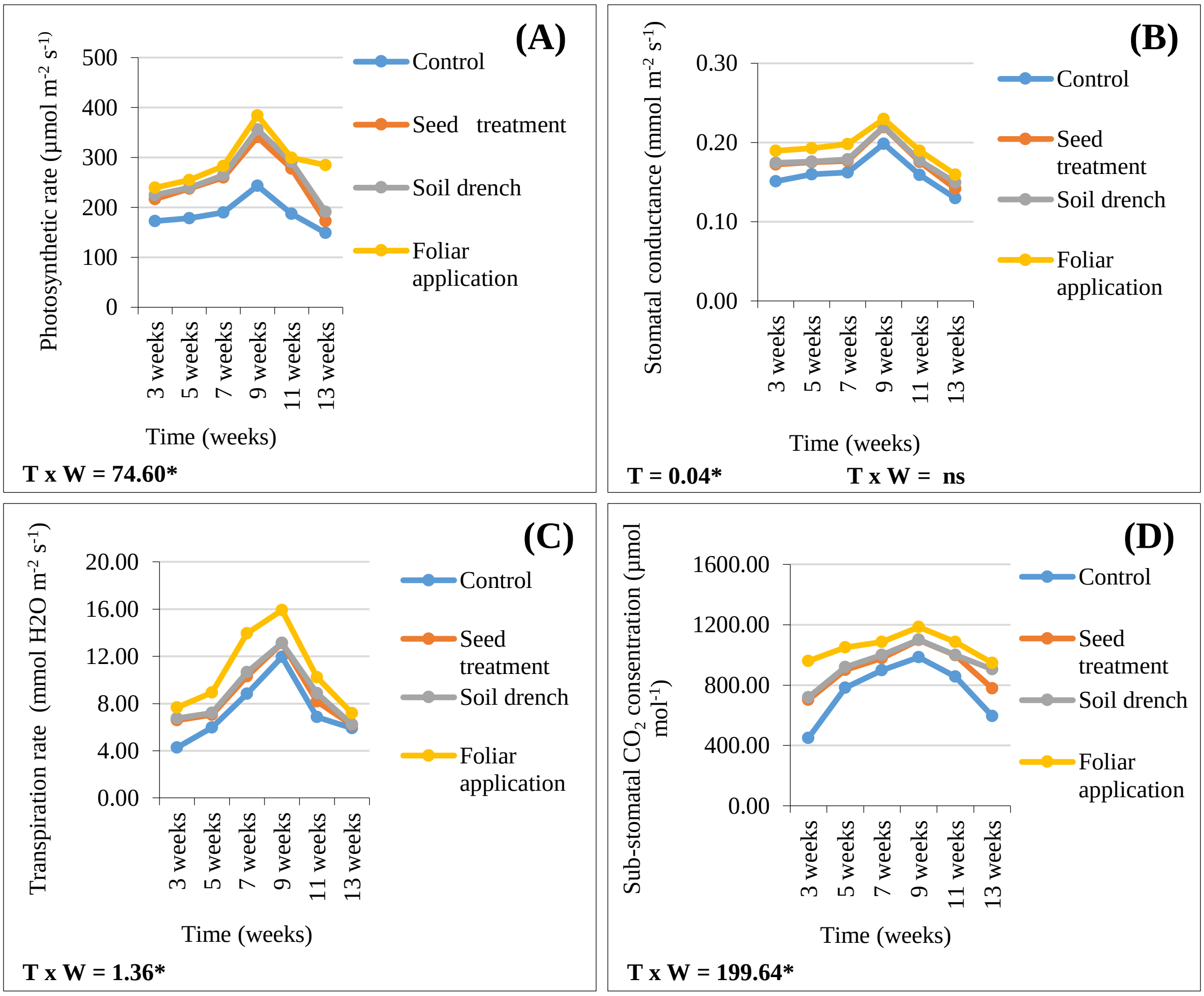

Figure 4. 7: The physiological response of soya bean to three application methods of brassinosteroids (T) (seed treatment, soil drench, foliar application and control) measured over 12 weeks. Measured parameters include (A) Photosynthetic rate, (B) Stomatal conductance, (C) Transpiration and (D) sub-stomatal CO_2 concentration. The interaction is indicated as T x W (Interaction between brassinosteroids and weeks). Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

4.3.2.3 The effect of three application methods of brassinosteroids on yield parameters

(a) Yield components

All of the applied brassinosteroids methods increased in the measured yield components and final yield compared to the control (Table 4.4). All the applied methods of brassinosteroids have caused significant increase in yield and its components. Seed treatment and soil drench did not differ significantly, but both were meaningful higher than the control.

Table 4. 4: The effect of three methods applications of brassinosteroids, soil drench, foliar application and control on yield components (number of pods per pot, mass of pods per pot, number of seeds per pot and mass of seeds per pot (final yield per pot**)**.

Treatment	Number of pods per pot	Mass of pods per pot (g)	Number of seeds per pot	Mass of seeds per pot (g)
Control	45.8 c	21.6 c	89.3 c	15.9 c
Seed treatment	56.5 b	29.5 b	114.0 b	31.0 b
Soil drench	56.8 b	30.0 b	118.0 b	31.2 b
Foliar application	78.5 a	38.1 a	134.8 a	41.0 a
LSDT (5%)	**10.1***	**7.4***	**14.6***	**7.7***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

4.3.3 The effect of brassinosteroids combined with mesotrione on soya bean.

In both two growing seasons (2015/16 and 2016/17) the results show the same trend so the results of one season will be presented since there is no difference between the two seasons.

4.3.3.1 The effect on seedling emergence and morphological parameters

(a) Emergence percentage

All the seeds planted gave 100% emergence and therefore there was no significant difference between the different treatments.

(b) Symptoms of the crop injury

The brassinosteroids in combination with the mesotrione had no phytotoxic effects on the growth.

(c) Morphological parameters

1) Destructive parameters

The effect of different brassinosteroids applications combined with residual mesotrione concentrations on different growth parameters is shown in Table 4.5. No significant interaction between the brassinosteroids application methods (T) and mesotrione concentrations (C) on week two was observed on seedling fresh mass, seedling dry mass, root fresh mass and root dry mass, however the main effects showed significance. At week four though the brassinosteroids and herbicide interaction was significant in all measured parameters except the root dry mass. All the applied brassinosteroids methods combined with the residual mesotrione concentrations increased the growth parameters alleviating the negative effect of mesotrione compared to the control. Even 0.05 µg ai kg^{-1} the foliar application almost nullified the negative effect of the herbicide at four weeks after application.

Table 4. 5: The effect of different brassinosteroids application methods (T) combined with various mesotrione residual concentrations (C) (1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1} and 0.0016 µg ai kg^{-1}) after application and its effect on seedling fresh mass, seedling dry mass, root fresh mass and root dry mass over 4 weeks.

		Seedling fresh mass (g)		**Seedling dry mass (g)**		**Root fresh mass (g)**		**Root dry mass (g)**	
Treatment	**Herbicide residue (µg ai kg^{-1})**	**2 Weeks**	**4 Weeks**	**2 Weeks**	**4 Weeks**	**2 Weeks**	**4 Weeks**	**2 Weeks**	**4 Weeks**
Control	0	10.3	21.2 dc	3.9	8.0 fecd	4.0	6.9 dc	2.0	3.0
	1.6	3.2	8.8 h	1.2	3.3 j	1.3	1.9 g	0.6	0.7
	0.05	5.2	11.4 gh	2.3	4.3 ij	2.2	3.2 fg	1.1	1.2
	0.0016	8.2	18.3 ed	3.2	5.7 ih	3.1	4.9 e	1.6	1.8
Seed treatment	0	13.5	23.3 bac	5.7	9.7 bcd	4.8	7.9 bdac	2.8	4.0
	1.6	6.7	11.5 gfh	3.1	5.7 ih	2.3	3.5 fe	1.5	1.7
	0.05	9.3	15.1 ef	3.9	7.6 fegh	3.6	6.5 d	2.1	2.6
	0.0016	12.6	21.6 bdc	5.0	9.0 ecd	4.1	7.5 bdac	2.1	3.6
Soil drench	0	14.1	23.5 bac	6.4	9.9 bc	5.1	8.4 ba	3.2	4.1
	1.6	7.01	12.0 gfh	3.4	6.0 igh	2.9	4.0 fe	1.8	2.2
	0.05	10.2	17.2 e	4.7	7.84 fegd	4.0	7.2 bdc	2.4	3.5
	0.0016	13.9	23.1 bac	6.0	9.5 becd	4.9	7.9 bdac	3.0	3.9
Foliar application	0	16.3	25.6 a	8.1	11.9 a	5.6	8.9 a	3.6	4.8
	1.6	9.1	13.1 gf	4.2	6.2 figh	3.3	4.4 fe	2.2	2.8
	0.05	11.8	23.3 bac	5.4	9.8 bc	4.2	7.8 bdac	2.9	4.1
	0.0016	15.7	24.9 ba	7.8	11.0 ba	5.1	8.1 bac	3.6	4.6
C x T: LSD_T (5%)		**ns**	**3.5***	**ns**	**2.0***	**ns**	**1.48***	**ns**	**ns**

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test. C x T indicates the interaction between brassinosteroids (treatments) and concentrations.

Table 4.6 shows the difference between main effects of brassinosteroids application methods on the morphological parameters of soya bean measured every two weeks over four weeks. All the applied methods of brassinosteroids significantly increased plant growth after four weeks. The highest increase in plant growth was found in the foliar application, followed by the soil drench and seed treatment respectively. There was no significant difference between the seed treatment and the soil drench during week two and four on seedling fresh mass, but the significant difference was seen between foliar application and soil drench also there was significant difference between foliar application and seed treatment.

Table 4. 6: The differences between brassinosteroids application methods (seed treatment, soil drench, foliar application and control) on seedling fresh mass, seedling dry mass, root fresh mass and root dry mass over 4 weeks.

	Seedling fresh mass (g)		Seedling dry mass (g)		Root fresh mass (g)		Root dry mass (g)	
Treatment	**2 weeks**	**4 weeks**	**2 weeks**	**4 weeks**	**2 weeks**	**4 weeks**	**2 weeks**	**4 weeks**
Control	6.7 c	14.9 c	2.7 d	5.3 c	2.6 c	4.2 c	1.3 d	1.7 d
Seed treatment	10.5 b	17.9 b	4.4 c	8.0 b	3.7 b	6.4 b	2.1 c	3.0 c
Soil drench	11.3 b	18.9 b	5.1 b	8.3 b	4.2 a	6.9 ba	2.6 b	3.4 b
Foliar application	13.2 a	21.7 a	6.4 a	9.7 a	4.5 a	7.3 a	3.1 a	4.1 a
LSD_T (0.05)	**1.0***	**1.3***	**0.5***	**0.7***	**0.4***	**0.5***	**0.3***	**0.4***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

The differences between the three main effects the residual mesotrione concentrations on morphological parameters of soya bean measured every two weeks is summarized in Table 4.7. From the table it can be seen that all three concentrations caused a significant decrease in all measured growth parameters compared to the control as confirmed previously.

Table 4. 7: The differences between three residual concentrations of mesotrione (1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1}, 0.0016 µg ai kg^{-1} and the 0) on seedling fresh mass, seedling dry mass, root fresh mass and root dry mass over 4 weeks.

	Seedling fresh mass (g)		Seedling dry mass (g)		Root fresh mass (g)		Root dry mass (g)	
Herbicide residue (µg ai kg^{-1})	**2 weeks**	**4 weeks**	**2 weeks**	**4 weeks**	**2 weeks**	**4 weeks**	**2 weeks**	**4 weeks**
0	13.6 a	23.4 a	6.0 a	9.9 a	4.9 a	8.1 a	2.9 a	4.0 a
1.6	6.5 c	11.4 d	3.0 c	5.3 d	2.4 d	3.5 d	1.5 d	1.8 d
0.05	9.1 b	16.7 c	4.1 b	7.4 c	3.5 c	6.2 c	2.1 c	2.9 c
0.0016	12.6 a	22.0 b	5.5 a	8.8 b	4.3 b	7.1 b	2.6 b	3.5 b
LSD_T (0.05)	**1.0***	**1.3***	**0.5***	**0.7***	**0.4***	**0.5***	**0.3***	**0.4***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

2) Non-destructive growth parameters

The interaction between brassinosteroids application methods and mesotrione concentrations was not significant except at week 10 (Table 4.8), but the rest of the time significant differences was observed between the main effects. All the applied brassinosteroids methods increase the growth parameters compared to the normal control with foliar application as the best method. Combined with residual mesotrione concentrations all the application methods increased the growth significantly compared to the control at 10 weeks. At 0.0016 µg ai kg^{-1} all BRs applications recovered all growth parameters showing no significant difference compared to the control. This confirms that brassinosteroids have a positive impact on the plant irrespective of the presence of herbicide residue in the soil.

Table 4. 8: The effect of different brassinosteroids applications (T) combined with various mesotrione concentrations (C) (0, 1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1} and 0.0016 µg ai kg^{-1}) remaining in the soil on natural plant height (cm) of soya beans over a period of 12 weeks.

Treatment	Herbicide residue (µg ai kg^{-1})	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks	12 Weeks
Control	0	9.7	14.8	22.7	28.8	36.6 fg	42.6
	1.6	5.2	10.9	16.8	21.2	28.9 i	35.2
	0.05	6.9	12.8	19.3	24.7	31.7 h	38.3
	0.0016	8.1	13.8	21.1	26.9	34.4 hg	40.4
Seed treatment	0	11.0	18.0	25.0	34.6	42.6 dc	48.4
	1.6	7.1	13.5	20.9	27.1	33.3 h	39.2
	0.05	9.3	15.7	22.9	29.6	36.3 fg	42.7
	0.0016	10.1	17.8	24.9	33.6	41.5 dc	47.4
Soil drench	0	12.4	19.4	27.0	35.9	44.3 bc	52.0
	1.6	7.6	14.1	22.9	30.5	38.9 fe	44.1
	0.05	9.8	16.6	24.1	32.3	41.2 de	47.4
	0.0016	11.6	18.5	26.3	35.6	43.5 dc	50.4
Foliar application	0	14.0	21.9	29.1	38.5	47.6 a	55.4
	1.6	8.6	15.8	24.5	33.9	41.0 de	45.9
	0.05	11.6	18.3	26.7	35.5	43.7 dc	49.9
	0.0016	12.9	20.9	28.2	37.3	46.9 ba	54.6
C x T: LSD_T (5%)		**ns**	**ns**	**ns**	**ns**	**2.7***	**ns**

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test. C x T indicates the interaction between brassinosteroids (treatments) and concentrations.

Table 4.9 shows the significant differences between the main effects brassinosteroids application methods on natural plant height over 12 weeks. The same tendency was confirmed again with the foliar application showing the highest growth followed by soil drench and seed treatment respectively.

Table 4.9: The differences between brassinosteroids application methods (seed treatment, soil drench, foliar application and control) over a period of 12 weeks on natural plant height (cm).

Treatment	**2 Weeks**	**4 Weeks**	**6 Weeks**	**8 Weeks**	**10 Weeks**	**12 Weeks**
Control	7.5 d	13.1 d	20.0 d	25.4 d	32.9 d	39.1 d
Seed treatment	9.4 c	16.2 c	23.4 c	31.2 c	38.4 c	44.4 c
Soil drench	10.3 b	17.1 b	25.1 b	33.6 b	42.0 b	48.4 b
Foliar application	11.8 a	19.2 a	27.1 a	36.3 a	44.8 a	51.4 a
LSD_T (0.05)	**0.7***	**0.8***	**0.8***	**1.1***	**1.0***	**1.0***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

According to Table 4.10 the main effects of the three concentrations of mesotrione again just confirmed its significant negative effect compared to the control. The highest concentration (1.6 µg ai kg^{-1}) affected the plan growth negatively followed by 0.05 µg ai kg^{-1} and 0.0016 µg ai kg^{-1} respectively.

Table 4. 10: The differences between three residual concentrations of mesotrione (1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1}, 0.0016 µg ai kg^{-1} and the control) over a period of 12 weeks on natural plant height (cm).

Herbicide residue (µg ai kg^{-1})	**2 Weeks**	**4 Weeks**	**6 Weeks**	**8 Weeks**	**10 Weeks**	**12 Weeks**
0	11.8 a	18.5 a	25.9 a	34.4 a	42.8 a	49.59 a
1.6	7.1 d	13.6 c	21.3 c	28.2 d	35.5 d	41.10 d
0.05	9.4 c	15.8 b	23.3 b	30.5 c	38.2 c	44.57 c
0.0016	10.7 b	17.7 a	25.1 a	33.4 b	41.6 b	48.18 b
LSD_T (0.05)	**0.7***	**0.8***	**0.8***	**1.1***	**1.0***	**1.01***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

The interaction (C x T) on extended plant height differed significantly except at week 8 (Table 4.11). The highest plant growth is noticed with the foliar application combination followed by soil drench and seed treatment respectively compared to the control. All the brassinosteroids combinations showed an increase in plant height compared to the control.

Table 4. 11: The effect of different brassinosteroids application methods combined with various mesotrione concentrations remaining in the soil, using three different application on extended plant height (cm) of soya beans over a period of 12 weeks.

Treatment	Herbicide residue (μg ai kg^{-1})	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks	12 Weeks
Control	0	17.4 d	23.2 fe	33.4 f	41.4 d	51.2 cfed	56.2 dfe
	1.6	10.7 i	16.2 j	22.8 j	29.2 g	35.7 k	41.6 k
	0.05	12.3 ih	18.5 i	25.9 i	33.5 f	39.8 jk	45.4 j
	0.0016	14.0 fgh	20.5 ihg	28.4 hi	37.4 e	45.2 ghi	49.3 ih
Seed treatment	0	19.5 c	27.4 c	37.2 dc	45.1 cb	54.5 cb	59.1 dc
	1.6	13.5 gh	20.4 ih	27.1 i	33.6 f	41.6 ji	47.2 ij
	0.05	14.7 fg	22.6 feg	30.2 hg	37.3 e	44.6 ghi	50.7 gh
	0.0016	17.4 d	23.6 e	32.3 fg	41.4 d	49.2 gfed	54.4 fe
Soil drench	0	21.9 b	30.8 b	39.5 bc	47.3 b	58.3 b	65.2 b
	1.6	13.4 fg	21.5 fhg	30.5 hg	37.1 e	43.3 jhi	49.3 ih
	0.05	15.4 fe	24.2 de	33.8 f	40.0 ed	47.9 gfeh	53.8 gf
	0.0016	18.3 dc	25.9 dc	36.3 de	42.6 cd	52.2 ced	59.4 dc
Foliar application	0	23.9 a	33.6 a	44.9 a	54.0 a	64.1 a	69.3 a
	1.6	16.9 de	25.8 dc	33.1 fg	43.0 cd	47.1 gfh	57.6 dce
	0.05	18.6 dc	27.5 c	37.8 dc	46.8 b	53.7 cbd	60.6 c
	0.0016	22.63 ba	32.2 ba	42.1 ba	51.5 a	63.3 a	68.1 ba
C x T: LSD_T (5%)		**3.9***	**2.1***	**2.9***	**ns**	**4.8***	**3.4***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test. C x T indicates the interaction between brassinosteroids (treatments) and concentrations.

Since the interaction did not show significant differences on extended growth at 8 weeks the main effect of brassinosteroids applications however significantly increased the extended plant height, confirming again the foliar application to be the best (Table 4.12).

Table 4. 12: The differences between three brassinosteroids application methods (seed treatment, soil drench, foliar application and control) over a period of 12 weeks on extended plant height (cm).

Treatment	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks	12 Weeks
Control	13.6 d	19.6 d	27.6 d	35.3 d	43.0 d	48.1 d
Seed treatment	16.3 c	23.5 c	31.7 c	39.4 c	47.5 c	52.8 c
Soil drench	17.2 b	25.6 b	35.0 b	41.7 b	50.4 b	56.9 b
Foliar application	20.6 a	29.8 a	39.4 a	48.8 a	57.1 a	63.9 a
LSD_T (0.05)	**0.7***	**0.8***	**1.1***	**1.2***	**1.8***	**1.3***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

The main effect residual acidity also significantly decreased the extended plant height over 12 weeks (Table 4.13). From the table it can be seen that all three concentrations yet again caused a significant decrease in plant growth compared to the control.

Table 4. 13: The differences between three residual concentrations of mesotrione (1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1}, 0.0016 µg ai kg^{-1} and the control) over a period of 12 weeks on extended plant height (cm).

Herbicide residue (µg ai kg^{-1})	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks	12 Weeks
0	20.7 a	28.7 a	38.7 a	46.9 a	57.0 a	62.4 a
1.6	13.6 d	21.0 d	28.3 d	35.7 d	41.9 d	48.9 d
0.05	15.0 c	23.2 c	31.9 c	39.4 c	46.5 c	52.6 c
0.0016	18.1 b	25.5 b	34.6 b	43.2 b	52.5 b	57.8 b
LSD_T (0.05)	**0.7***	**0.8***	**1.1***	**1.2***	**1.8***	**1.3***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

Table 4. 14: The effect of different three brassinosteroids applications methods combined with various mesotrione concentrations remaining in the soil on stem diameter (mm) of soya beans over a period of 12 weeks.

Treatments	Herbicide residue (μg ai kg^{-1})	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks	12 Weeks
Control	0	3.4 gfe	3.7 edf	4.1 egf	4.6 e	4.9 f	5.0 d
	1.6	3.1 g	3.4 i	3.5 h	3.6 h	3.8 h	4.1 g
	0.05	3.2 gf	3.5 h	3.8 hgf	4.1 g	4.2 g	4.4 f
	0.0016	3.3 gf	3.7 ghf	3.9 gf	4.3 f	4.7 f	4.9 d
Seed treatment	0	3.7 dc	3.8 ed	4.7 ba	4.9 c	5.5 dc	5.7 a
	1.6	3.1 g	3.6 gh	3.7 hg	4.1 g	4.4 g	4.7 e
	0.05	3.3 gf	3.7 eghf	4.2 edf	4.3 f	4.7 f	4.9 d
	0.0016	3.5 dfg	3.80 edf	4.6 bac	4.8 dc	5.51 dc	5.7 b
Soil drench	0	3.9 bc	4.1 b	4.8 ba	5.1 b	5.7 bc	6.0 a
	1.6	3.3 gf	3.7 ghf	4.1 ef	4.7 de	4.8 f	4.9 d
	0.05	3.4 dfe	3.9 cd	4.3 edc	4.8 dc	5.4 d	5.7 cb
	0.0016	3.8 bc	4.0 cb	4.8 ba	5.1 b	5.6 dc	6.0 a
Foliar application	0	4.3 a	4.4a	4.9 a	5.3 a	5.9 a	6.1 a
	1.6	3.3 gf	3.7 eghf	4.2 edf	4.8 dc	5.2 e	5.5 c
	0.05	3.7 dce	4.0 b	4.5 bdc	5.1 b	5.5 d	5.8 b
	0.0016	4.1 ba	4.3 a	4.9 a	5.3 a	5.9 ba	6.0 a
C x T: LSD_T (5%)		**0.3***	**0.2***	**0.4***	**0.1***	**0.2***	**0.2***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test. C x T indicates the interaction between brassinosteroids (treatments) and concentrations.

Once more the interaction significantly increased stem diameter over 12 weeks (Table 4.14). Regarding the main effects in both Tables 4.15 and 4.16 significant changes were observed for a second time.

Table 4. 15: The differences between brassinosteroids application methods (seed treatment, soil drench, foliar application and control) over a period of 12 weeks on stem diameter (mm).

Treatment	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks	12 Weeks
Control	3.9 d	3.6 d	3.8 d	4.2 d	4.4 d	4.6 d
Seed treatment	3.4 c	3.7 c	4.3 c	4.5 c	5.0 c	5.3 c
Soil drench	3.6 b	3.9 b	4.5 b	4.9 b	5.4 b	5.7 b
Foliar application	3.8 a	4.1 a	4.6 a	5.1 a	5.6 a	5.9 a
LSD_T (0.05)	**0.1***	**0.1***	**0.1***	**0.1***	**0.1***	**0.1***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

Table 4. 16: The differences between three residual concentrations of mesotrione (1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1}, 0.0016 µg ai kg^{-1} and the control) over a period of 12 weeks on stem diameter (mm).

Herbicide residue (µg ai kg^{-1})	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks	12 Weeks
0	3.8 a	4.0 a	4.6 a	5.0 a	5.5 a	5.7 a
1.6	3.2 c	3.6 d	3.9 c	4.3 d	4.5 c	4.8 c
0.05	3.4 b	3.8 c	4.2 b	4.6 c	5.0 b	5.2 b
0.0016	3.7 a	3.9 b	4.6 a	4.9 b	5.4 a	5.8 a
LSD_T (0.05)	**0.1***	**0.1***	**0.1***	**0.1***	**0.1***	**0.1***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

4.3.3.2 Physiological response of soya bean treated with the combination of brassinosteroids and mesotrione

(a) Chlorophyll content

The effect of different brassinosteroids applications on chlorophyll content was measured every two weeks (Table 4.17 to Table 4.20). The main effects and its interaction have significantly influenced chlorophyll a, while for chlorophyll b, total chlorophyll and carotenoids no significant interaction was observed, but the main effects exhibited significant results on the measured parameters. All the mesotrione concentrations significantly decrease the chlorophyll and carotenoid content compared to the control, while the brassinosteroids, alone and in combination with the mesotrione, meaningfully increased the content. The same trend was observed as previously mentioned.

Table 4. 17: The effect of different brassinosteroids applications combined with various mesotrione concentrations on chlorophyll a and chlorophyll b content over 4 weeks.

		Chlorophyll a (μg/ml)		Chlorophyll b (μg/ml)	
Treatment	**Herbicide residue (μg ai kg^{-1})**	**2 Weeks**	**4 Weeks**	**2 Weeks**	**4 Weeks**
Control	0	31.4 bdac	32.0 bdc	31.4	36.0
	1.6	5.6 h	11.8 g	1.6	3.8
	0.05	16.9 fg	22.3 ef	11.6	16.1
	0.0016	25.6 de	26.9 ed	20.6	24.4
Seed treatment	0	33.4 ba	36.5 ba	41.5	50.9
	1.6	13.2 g	19.0 f	11.9	15.6
	0.05	20.8 fe	26.3 ed	21.1	25.6
	0.0016	31.7 bac	32.0 bdc	39.7	49.8
Soil drench	0	34.3 ba	37.0 ba	42.0	53.0
	1.6	21.6 fe	24.7 ef	13.5	19.0
	0.05	29.6 bdc	31.6 bdc	25.2	35.4
	0.0016	34.2 ba	36.4 ba	41.9	51.6
Foliar application	0	37.3 a	39.4 a	50.3	65.8
	1.6	26.4 dec	28.8 edc	20.7	28.6
	0.05	31.5 bdac	33.5 bac	32.9	42.5
	0.0016	36.2 a	38.8 a	47.7	62.4
C x T: LSD$_T$ (5%)		**6.0***	**6.5***	**ns**	**ns**

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test. C x T indicates the interaction between brassinosteroids (treatments) and concentrations.

All application methods of the brassinosteroids caused an increase in the measured chlorophyll content as compared to the control (Table 4.18). In chlorophyll a, the results confirmed that the foliar application significantly differed compared to seed treatment, soil drench and control respectively. While in chlorophyll b no real differences could be seen between seed treatment and soil drench, but the foliar application differ significantly from the other treatments.

Table 4. 18: The differences between three brassinosteroids application methods (seed treatment, soil drench, foliar application and control) on chlorophyll a and chlorophyll b over 4 weeks.

	Chlorophyll a (µg/ml)		Chlorophyll b (µg/ml)	
Treatment	**2 Weeks**	**4 Weeks**	**2 Weeks**	**4 Weeks**
Control	19.9 d	23.3 d	16.3 c	20.0 c
Seed treatment	24.8 c	28.4 c	28.6 b	35.5 b
Soil drench	29.9 b	32.4 b	30.6 b	39.8 b
Foliar application	32.8 a	35.2 a	37.9 a	49.8 a
LSD_T (0.05)	**2.2***	**2.4***	**4.4***	**6.2***

Statistical significance is indicated as LSD =* significant (P ≤ 0.05) and different letters, and ns = not significant at 5% using Tukey's LSD test.

The main effect of mesotrione on chlorophyll differed significantly over the 4 weeks with almost no effect at the lowest concentration (Table 4.19).

Table 4. 19: The differences between three residual concentrations of mesotrione mesotrione (1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1}, 0.0016 µg ai kg^{-1} and the control) on chlorophyll a and chlorophyll b over 4 weeks.

	Chlorophyll a (µg/ml)		Chlorophyll b (µg/ml)	
Herbicide residue (µg ai kg^{-1})	**2 Weeks**	**4 Weeks**	**2 Weeks**	**4 Weeks**
0	34.1 a	36.2 a	41.3 a	51.4 a
1.6	16.7 c	21.1 d	11.9 c	16.8 c
0.05	24.7 b	28.4 c	22.7 b	29.9 b
0.0016	32.0 a	33.5 b	37.5 a	47.1 a
LSD_T (0.05)	**2.2***	**2.4***	**4.4***	**6.2***

Statistical significance is indicated as LSD =* significant (P ≤ 0.05) and different letters, and ns = not significant at 5% using Tukey's LSD test.

Yet again the same trend was observed however no outstanding difference in the interaction effect on total chlorophyll and carotenoid content was observed (Table 4.20).

Table 4. 20: The effect of different brassinosteroids (T) applications combined with various mesotrione concentrations (C) remaining in the soil on total chlorophyll (a + b) and carotenoids content over 4 weeks.

		Total chlorophyll (a +b) (µg/ml)		**Carotenoids content (µg/ml)**	
Treatment	**Herbicide residue (µg ai kg^{-1})**	**2 Weeks**	**4 Weeks**	**2 Weeks**	**4 Weeks**
Control	0	62.8	68.0	7.0	8.1
	1.6	7.2	15.6	2.1	2.9
	0.05	28.5	38.4	3.6	5.0
	0.0016	46.3	51.3	4.9	6.4
Seed treatment	0	74.8	87.4	8.9	10.1
	1.6	25.2	34.6	3.2	4.1
	0.05	41.9	51.9	5.5	6.9
	0.0016	71.5	81.8	8.2	9.8
Soil drench	0	76.3	90.0	9.4	11.1
	1.6	35.2	43.7	3.9	5.9
	0.05	54.8	67.0	6.5	8.4
	0.0016	76.1	88.1	9.2	10.9
Foliar application	0	87.5	105.2	11.3	13.6
	1.6	47.0	57.4	4.9	5.7
	0.05	64.3	76.1	7.9	9.1
	0.0016	83.9	101.2	11.0	13.2
C x T: LSD_T (5%)		**ns**	**ns**	**ns**	**ns**

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test. C x T indicates the interaction between brassinosteroids (treatments) and concentrations.

Since the interaction did not differ significantly, the main effects will be discussed in Table 4.21 and Table 4.22. Once more, the brassinosteroids increased all the measured chlorophyll and carotenoid content with the foliar application still being the best method. And the higher herbicide concentrations lowered the content but almost nulified at the lower concentration.

Table 4. 21: The differences between three brassinosteroids application methods (seed treatment, soil drench, foliar application and control) on total chlorophyll (a +b) and carotenoids content over 4 weeks.

Treatment	Total chlorophyll (a +b) (µg/ml)		Carotenoids content (µg/ml)	
	2 Weeks	**4 Weeks**	**2 Weeks**	**4 Weeks**
Control	36.2 d	43.3 d	4.4 c	5.6 d
Seed treatment	53.3 c	63.9 c	6.5 b	7.7 c
Soil drench	60.5 b	72.2 b	7.2 b	9.1 b
Foliar application	70.7 a	85.0 a	8.8 a	10.4 a
LSD_T (0.05)	**5.4***	**6.7***	**1.3***	**1.2***

Statistical significance is indicated as LSD =* significant (P ≤ 0.05) and different letters, and ns = not significant at 5% using Tukey's LSD test.

Table 4. 22: The differences between three residual concentrations of mesotrione (1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1}, 0.0016 µg ai kg^{-1} and the control) on total chlorophyll (a +b) and carotenoids content over 4 weeks.

Herbicide residue (µg ai kg^{-1})	Total chlorophyll (a +b) (µg/ml)		Carotenoids content (µg/ml)	
	2 Weeks	**4 Weeks**	**2 Weeks**	**4 Weeks**
0	75.4 a	87.7 a	9.1 a	10.7 a
1.6	28.6 d	37.8 d	3.5 c	10.1 c
0.05	47.4 c	58.3 c	5.9 b	7.3 b
0.0016	69.4 b	80.6 b	8.3 a	4.7 a
LSD_T (0.05)	**5.4***	**6.7***	**1.3***	**1.2***

Statistical significance is indicated as LSD =* significant (P ≤ 0.05) and different letters, and ns = not significant at 5% using Tukey's LSD test.

(b) Photosynthetic parameters

The effect of different brassinosteroids applications combined with mesotrione on photosynthetic parameter (photosynthetic rate (Table 4.23) and stomatal conductance (Table 4.26) were measured over a period of 13 weeks. The interaction between the brassinosteroids and mesotrione is not significant, except at week 11 and 13 on the photosynthetic rate, however the main effects showed significant differences. On the other hand, stomatal conductance showed a significant interaction between the brassinosteroids and mesotrine concentrations. All the applied brassinosteroids methods combined with mesotrione resulted in an improvement of plant performance while no real differences could be observed between 0 and 0.0016 µg ai kg^{-1}. The highest photosynthetic rate was seen in 0 and 0.0016 µg ai kg^{-1} followed by 0.05 µg ai kg^{-1} and 1.6 µg ai kg^{-1} respectively. The foliar application showed the best results followed by the soil drench and then the seed treatment, all improving photosynthesis when combined with mesotrione.

Table 4. 23: The effect of different brassinosteroids applications (T) combined with various mesotrione concentrations (C) remaining in the soil on photosynthetic rate calculated as percentage difference from the control.

Treatment	Herbicide residue (µg ai kg^{-1})	3 Weeks	5 Weeks	7 Weeks	9 Weeks	11 Weeks	13 Weeks
Control	0	100.0	103.3	110.0	140.7	108.7 cb	86.3 cefd
	1.6	33.8	50.6	64.1	67.1	42.6 f	32.7 i
	0.05	57.2	71.6	84.6	92.3	73.7 ed	57.8 gh
	0.0016	78.6	88.9	100.1	116.0	91.3 cbd	77.4 gefd
Seed treatment	0	125.7	138.3	150.8	198.2	161.1 a	100.0 cb
	1.6	48.1	66.7	67.4	70.0	54.8 fe	45.4 ih
	0.05	80.2	94.1	98.8	106.0	89.4 cbd	76.0 gefd
	0.0016	97.7	113.1	141.9	173.3	151.1 a	98.1 cbd
Soil drench	0	130.5	138.9	153.1	206.5	169.1 a	110.7 b
	1.6	53.2	70.3	76.6	81.7	76.5 ed	65.8 gfh
	0.05	84.1	97.6	108.6	131.7	100.4 cbd	88.3 cebd
	0.0016	101.8	113.7	149.1	181.3	162.1 a	108.2 cb
Foliar application	0	139.0	148.1	164.0	222.7	173.6 a	165.3 a
	1.6	62.7	77.7	83.7	105.6	82.0 ced	74.9 gef
	0.05	92.6	110.7	120.8	142.9	110.8 b	106.6 cb
	0.0016	117.8	144.9	162.0	205.3	160.2 a	158.1 a
C x T: LSD_T (5%)		**Ns**	**ns**	**ns**	**Ns**	**27.3***	**22.5***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test. C x T indicates the interaction between brassinosteroids (treatments) and concentrations.

Again, the main effects brassinosteroids (treatments) (Table 4.24) significantly increase the photosynthetic rate compared to the control. Identical results were reached with the mesotrione (Table 4.25) but the plants only managed to recover at the lowest concentration at week 7 and 13, but the rest of the time even the lower concentration differed significantly from the control.

Table 4. 24: The differences between brassinosteroids application methods (seed treatment, soil drench, foliar application and control) on photosynthetic rate calculated as percentage difference from the control.

Treatment	**3 weeks**	**5 Weeks**	**7 Weeks**	**9 Weeks**	**11 Weeks**	**13 Weeks**
Control	67.4 b	78.6 c	89.7 c	104.0 c	79.1 c	63.5 d
Seed treatment	87.9 a	103.0 b	114.7 b	136.9 b	114.1 b	79.9 c
Soil drench	92.4 a	105.1 b	121.9 ba	150.3 b	127.0 a	93.2 b
Foliar application	103.0 a	120.4 a	132.6 a	169.1 a	131.6 a	126.2 a
LSD_T (0.05)	**15.3***	**13.5***	**14.0***	**17.5***	**10.0***	**8.3***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

Table 4. 25: The differences between three residual concentrations of mesotrione (1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1}, 0.0016 µg ai kg^{-1} and 0) on photosynthetic rate calculated as percentage difference from the control.

Herbicide residue (µg ai kg^{-1})	**3 Weeks**	**5 Weeks**	**7 Weeks**	**9 Weeks**	**11 Weeks**	**13 Weeks**
0	123.8 a	132.1 a	144.5 a	192.0 a	153.1 a	115.6 a
1.6	49.5 d	66.3 d	73.0 c	81.1 d	64.0 d	54.7 c
0.05	78.5 c	93.5 c	103.2 b	118.2 c	93.6 c	82.2 b
0.0016	100.0 b	115.2 b	138.3 a	168.8 b	141.2 b	110.5 a
LSD_T (0.05)	**15.3***	**13.5**	**14.0***	**17.5***	**10.0***	**8.3***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

In the control and herbicide interaction all three concentrations lowered stomatal conductance and when combined with any of the brassinosteroids application methods there were an increase compared to the control combination (Table 4.26).

Table 4. 26: The effect of different brassinosteroids applications (T) combined with various mesotrione concentrations (C) remaining in the soil on stomatal conductance.

Treatment	Herbicide residue (µg ai kg^{-1})	3 weeks	5 Weeks	7 Weeks	9 Weeks	11 Weeks	13 Weeks
Control	0	0.15 bdc	0.16 bdc	0.16 bc	0.20	0.16 bcd	0.13de
	1.6	0.04 i	0.05 h	0.07 f	0.10	0.06 h	0.05 h
	0.05	0.07 hg	0.08 g	0.10 e	0.14	0.09 g	0.08 g
	0.0016	0.11 ef	0.11gf	0.13 de	0.17	0.12 fe	0.11 fe
Seed treatment	0	0.17 bac	0.18 ba	0.18 ba	0.22	0.18 a	0.14 bdac
	1.6	0.05 ih	0.11 egf	0.13 d	0.14	0.10 fg	0.09 fg
	0.05	0.10 efg	0.14 edf	0.14 dc	0.17	0.14 d	0.10 fg
	0.0016	0.17 bac	0.17 bac	0.18 ba	0.22	0.17 ba	0.14 bdc
Soil drench	0	0.17 bac	0.18 ba	0.18 ba	0.22	0.18 a	0.15 ba
	1.6	0.09 fg	0.11 egf	0.14 dc	0.14	0.11 fe	0.10 fg
	0.05	0.13 ed	0.14 edf	0.16 bc	0.17	0.14 cd	0.13 dc
	0.0016	0.17 bac	0.17 ba	0.18 ba	0.22	0.18 a	0.15 bac
Foliar application	0	0.19 a	0.19 a	0.20 a	0.23	0.19 a	0.16 a
	1.6	0.10 efg	0.14 edc	0.14 dc	0.15	0.12 e	0.10 fg
	0.05	0.14 dc	0.15 bdc	0.17 b	0.19	0.16 bc	0.13 dc
	0.0016	0.18 ba	0.19 a	0.20 a	0.23	0.19 a	0.15 ba
C x T: LSD_T (5%)		**0.03***	**0.03***	**0.02***	**ns**	**0.02***	**0.02***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test. C x T indicates the interaction between brassinosteroids (treatments) and concentrations.

Since all interactions was significant, the main effects were analysed at week 9 and once more comparable results were obtained as mentioned previously (Table 4.27 and Table 4.28).

Table 4. 27: The differences between three brassinosteroids application methods (seed treatment, soil drench, foliar application and control) on stomatal conductance.

Treatment	**3 weeks**	**5 Weeks**	**7 Weeks**	**9 Weeks**	**11 Weeks**	**13 Weeks**
Control	0.09 d	0.10 c	0.12 c	0.15 b	0.11 c	0.09 c
Seed treatment	0.12 c	0.15 b	0.16 b	0.19 a	0.15 b	0.12 b
Soil drench	0.14 b	0.15 b	0.16 b	0.19 a	0.15 b	0.13 a
Foliar application	0.15 a	0.17 a	0.18 a	0.20 a	0.16 a	0.14 a
LSD_T (0.05)	**0.01***	**0.01***	**0.01***	**0.01***	**0.01***	**0.01***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

Table 4.28: The differences between three residual concentrations of mesotrione (1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1}, 0.0016 µg ai kg^{-1} and 0) on stomatal conductance.

Herbicide residue (µg ai kg^{-1})	**3 weeks**	**5 Weeks**	**7 Weeks**	**9 Weeks**	**11 Weeks**	**13 Weeks**
0	0.17 a	0.18 a	0.18 a	0.22 a	0.18 a	0.15 a
1.6	0.07 d	0.10 d	0.11 c	0.13 c	0.10 d	0.08 d
0.05	0.11 c	0.13 c	0.14 b	0.17 b	0.13 c	0.11 c
0.0016	0.16 b	0.16 b	0.17 a	0.21 a	0.16 b	0.14 b
LSD_T (0.05)	**0.01***	**0.01***	**0.01***	**0.01***	**0.01***	**0.01***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

Exactly the same trend with the transpiration rate and sub-stomatal CO_2 concentration were detected so results are not shown.

4.3.3.3 The effect of brassinosteroids combined with mesotrione on soya bean yield parameters

(a) Yield components

The interaction significantly influenced the number and mass of the seeds per plant. Again, the herbicide decreased the seeds and pods. The brassinosteroids alone and in combination with mesotrione tended to or significantly increased all the yield components (Table 4.29).

The main effects, again like previously shown, increased the yield parameters with the brassinosteroids and decreased with the different mesotrione concentrations (Results not shown).

Table 4. 29: The effect of three different brassinosteroids applications (T) combined with various mesotrione concentrations (C) on soya bean yield and yield components. Measured parameters include number of pods per pot, mass of pods per pot, number of seeds per pot and mass of seeds per pot (final yield per pot).

Treatment	**Herbicide residue (µg ai kg^{-1})**	**Number of pods per pot**	**Mass of pods per pot (g)**	**Number of seeds per pot**	**Mass of seeds per pot (g)**
Control	0	45.8	21.6	89.3 ef	15.9 dc
	1.6	29.5	7.3	48.0 j	4.8 f
	0.05	34.5	12.1	63.5 ij	9.2 dfe
	0.0016	40.3	17.3	76.0 ihfg	13.0 dce
Seed treatment	0	56.5	29.5	114.0 dc	31.0 b
	1.6	36.0	11.6	66.8 ih	8.4 fe
	0.05	45.3	18.9	79.3 ihfg	14.1 dce
	0.0016	54.3	28.7	112.8 dc	29.9 b
Soil drench	0	56.8	30.0	118.0 bac	31.2 b
	1.6	39.0	14.0	70.3 ihg	9.2 dfe
	0.05	46.8	19.9	85.5 efg	15.3 dce
	0.0016	55.3	28.9	116.3 bc	29.8 b
Foliar application	0	78.5	38.1	134.8 a	41.0 a
	1.6	51.3	19.0	82.3 ehfg	13.0 dce
	0.05	61.3	24.9	97.0 ed	17.8 c
	0.0016	76.8	36.9	133.0 bc	39.5 a
C x T: LSD_T (5%)		**ns**	**ns**	**17.3***	**7.0***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test. C x T indicates the interaction between brassinosteroids (treatments) and residual herbicide concentrations.

4.4 Discussion

Herbicide residue is a serious problem causing injury to sensitive crops grown in a crop rotation system, especially if the herbicide is not registered on the follow-up crops. It leads to morphological and physiological damages in plants consequently resulting in a decreased yield. During this study soya bean was planted in three different residual concentrations of mesotrione. Brassinosteroids were applied using three methods to determine the most effective application method of brassinosteroids and whether or not the brassinosteroids can counteract the negative effects of the herbicide. The data presented in this study support the hypotheses that herbicide residues can cause significant crop injury and decrease crop morphology, physiology and yield, depending on the residual concentration. Also, the results obtained indicate that brassinosteroids are capable of helping the crop to survive herbicide stress and that all three application methods had a positive effect on soya beans with foliar application identified as the best application method.

When comparing the control to the three different residual concentrations of mesotrione in soil, it showed phytotoxic symptoms (Figure 4.1) and a decrease in morphological (Figure 4.2 and Table 4.1), physiological parameters (Figure 4.3 and 4.4) and yield (Table 4.2). The crop showed bleaching, interveinal chlorosis, leaf crinkling and stunting. At the highest application rate of 1.6 µg ai kg^{-1}, it also reduced all of the measured parameters compared to the residual concentration of 0.05 µg ai kg^{-1} and 0.0016 µg ai kg^{-1}. These results are consistent with those found by Riddle, (2012) who found herbicide injury in different crops (sugar beet, peas, and cucumber and soya bean) and that injury severity increases with the increase in herbicide residue concentration. Similar results were obtained by Felix *et al.* (2007) who also found that mesotrione applied a year before was capable of causing injury to snap bean, cucumber and red clover and this resulted in low yield of the crops. It was further indicated that mesotrione residue caused reduction in biomass, yield and dry weight in broccoli, carrot, cucumber, and onion a year after application in the soil (Robinson, 2008; Soltani *et al*., 2014).

Photosynthetic parameters (photosynthetic rate, stomatal conductance, transpiration rate and intercellular CO_2 concentration) of the crop are very sensitive to abiotic and biotic stress including herbicide stress. The results further showed that mesotrione residue had a negative impact on chlorophyll content and photosynthesis parameters as compared to the control. Therefore, it is clear that herbicide residue can have negative effects on the plant as a whole, it affects its performance (physiology) and development (morphology). This might be due to the fact that mesotrione destroy pigment production in plants by impeding 4-hydroxyphenylpyruvate dioxygenase (HPPD) which is the enzyme responsible for catalysing the conversion of tyrosine to plastoquionone and α-tocophyrol (Yu, 2014). It is also responsible for the synthesis of carotene pigments, which contribute to photosynthesis by transferring the light energy they absorbed to chlorophylls through resonance energy transfer, which then utilize this energy to drive photosynthesis. When HPPD is blocked, it results in the loss of carotenoid pigments and without carotenoids, excess light energy destroy chlorophyll, proteins, membranes and other molecules and this result in bleaching, necrosis and subsequent death of the plant (Moran. 2005; Riddle. 2012). This also leads to plants not being able to protect themselves from photo-oxidation (Riddle. 2012). These results are in correspondence with those found by Vital *et* a*l.* (2017), who found that photosynthetic rate, stomatal conductance and transpiration rate decrease with an increase in the amount of herbicide. With the application of brassinosteroids, a soya bean responded positively by showing an improvement in its growth and physiological processes which consequently resulted to high yield.

The growth and performance of a crop can be affected by different stresses including herbicides but this can be rectified or prevented by use of brassinosteroids. When comparing the control and three application methods of brassinosteroids, soya bean has responded positively by showing more improvement in plant development as a whole. From the results in this study it appeared that there is an increase in all growth characteristics (Figure 4.5 and Table 4.3), the an increase in chlorophyll content and photosynthesis (Figure 4.6 and Figure 4.7) which contributed to an increase in yield (Table 4.4). Similar results were reported by Verma *et al*. (2011) who found out that application of brassinosteroids significantly improved plant growth and increase chlorophyll content which in turn positively affected photosynthetic rate. Additionally, these results were similar to that of Cevahir *et al.* (2008), who observed an increase of chlorophyll content in soya bean due to the application of brassinosteroids. This shows that generally brassinosteroids have a positive effect on plants as a whole. This might be due to the fact that brassinosteroids increase the plant's nutrient absorption capacity by enhancing root development and elongation (Vardhini & Anjum, 2015). Again, brassinosteroids assist to sensitize and increase the permeability of the cell membrane thus helping with the uptake of nutrients and their availability in the root zone. Therefore, enhance crop production (yield). It is further indicated that when brassinosteroids are applied in the soil, they improve mineral uptake by stimulating the activities of soil micro-organisms which are responsible for soil fertility and encourage more fibrous growth of roots enabling plants to absorb nutrients from the soil (Van der Watt, 2005). Brassinosteroids combined with auxins and cytokinins, which acts as chemical messengers can control plant growth. Auxins are responsible for cell elongation and root elongation and cytokinins promote cell division activities in plants, therefore this leads to plant growth (Van der Watt, 2005). In a study that was conducted by Balabanova *et al.* (2016), it was confirmed that use of an amino acid extract improved the performance of sunflower and also improved its photosynthetic parameters.

To further determine the effect of different brassinosteroid application methods combined with various mesotrione concentrations remaining in the soil, the growth parameters (Table 4.5, 4.8, 4.11 and 4.14 and main effects Table 4.6, 4.7, 4.9, 4.10, 4.12, 4.13, 4.15 and 4.16), physiological parameters (Table 4.17, 4.20, 4.23 and 4.26) and main effects (4.18, 4.19, 4.21, 4.22, 4.24, 4.25, 4.27 and 4.28) and yield (Table 4.29) were measured. Combined applications of mesotrione residuals and brassinosteroids counteract the negative effects of mesotrione residual activity. The results showed a decrease in plant injury in all three application methods of brassinosteroids and also showed an increase in all morphological and physiological parameters and yield with the foliar application as the best application method. Brassinosteroids played an important role in nutrient availability and in return resulted in an increase in plant growth and yield, regardless of the presence of herbicide residue in the soil. Brassinosteroids enhance the degradation of herbicide in the soil, by increasing the activity of soil microorganisms which are responsible for soil fertility resulting in healthy plant growth (Marschner, 2007). In conclusion, regardless of the damage that was caused by mesotrione residue on plants, brassinosteroids played an important role in helping the crops to recover from herbicide stress by neutralising the negative impact. The brassinosteroids increased the morphological and physiological processes of the crop and improved the tolerance to herbicide stress. Research recently carried out by Balabanova *et al.* (2016) found that the combination of amino acid and imazamox improved the growth and photosynthetic parameters of a sunflower.

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CHAPTER 5

THE EFFECT OF BRASSINOSTEROIDS ON YIELD, MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF GROUNDNUT UNDER DIFFERENT MESOTRIONE RESIDUAL LEVELS

5.1 Introduction

Groundnut (A*rachis hypogea L.*) is a self-pollinated legume crop important for both human and animal consumption. Originating in the South America (Southern Bolivia), groundnut is now grown throughout the world in the tropical and temperate areas (Chandraju *et al.,* 2011). It is believed that the crop was cultivated by native people of the New World at the period of European development in the sixteenth century and was later introduced to the Pacific Islands, Africa, Asia and Europe (Directorate plant production, 2010). Currently worldwide, the leading countries in the production of groundnut in the world include China and India, contributing approximately 70% of the world groundnut production (Charkraborty *et al*., 2013). The crop is grown on 19.3 million hectares across 82 countries in the world (Reddy *et al*., 2003). Groundnut as a leguminous plant is suitable for warm climatic conditions (Optimum temperatures for germination are between 20-30°C with a minimum of 18°C) and free from frost. It is the only nut that grows below the earth. It is capable of fixing atmospheric nitrogen, and can relieve resource-poor farmers from purchasing expensive chemical fertilizers (Nunes *et al.,* 2007; Chandraju *et al*., 2011).

Groundnut is among the most vital crops in Sub-Saharan Africa and it is cultivated extensively in most countries. The crop is very vital to smallholder agriculturalists because they produce it as their main source of income and food (Mbonwa, 2013). The use of groundnut as food has gradually increased by 34% from mid-1970 (Fletcher *et al*., 1992). Its popularity as source of food has extended even to South Africa. In South Africa groundnut is mostly planted in the Western areas, where Western and Northern Free State produce 40%, North West 29% and Northern Cape 24%, while in Limpopo and Mpumalanga, production is very low (Mbonwa, 2013). Resource-poor farmers in the Northern and Eastern parts of South Africa grow groundnuts mainly for home consumption (Cilliers, 2014).

Groundnut is one of the world’s most important crops due to its high nutritive value e.g. 44-56% oil and 22-33% protein (Dwivedi *et al*., 1996; Jauron, 1997). It was also indicated by

Cilliers (2014) that groundnut, as a source of nutrition, has been used in the Northern KwaZulu-Natal and Mpumalanga areas to fight malnutrition. Javaid *et al*. (2004) also showed that groundnut is rich in minerals (phosphorous, potassium, calcium and magnesium) and vitamins (E, K and B group). This crop could certainly be a good replacement for meat due to its high protein content (Javaid *et al*., 2004; Nunes *et al.*, 2007). Groundnut seeds can be eaten raw or processed to make soup and peanut butter (Ayoola *et al*., 2012). It can also be eaten raw, roasted, boiled or fried, allowing both the shell and nut to be eaten (Chandraju *et al.*, 2011). Besides human consumption, groundnuts can also be used for producing medicine, make-ups as well as textile materials. Janila *et al*. (2013) also showed that animal feed industries in many Asian countries use groundnut residues (after oil extraction) for producing animal feeds. Thus, owing to its benefits, groundnut can be considered as one of the important cash crops. Despite groundnut's benefits for humanbeings, animals and soil, there are many factors contributing towards yield decline of this crop and including biotic and abiotic stresses such as extreme temperature, salinity, low atmospheric humidity, drought, nutrient deficiency and herbicide residue to mention only a few. Among all these factors, herbicide residue has recently been identified as the most limiting factor not only on groundnuts, but crop production as a whole (Riddle, 2012). Herbicide residue is a resultant effect of herbicide, which delayed to decompose or degrade. Herbicide residue is a main problem that agriculturalists experience during cultivation. The ability of the plants to tolerate herbicide residue is economically very important. Knowledge of the reaction of the plant to herbicide residue is vital so that the appropriate measures can be taken to assist the plant to maintain normal plant growth and to avoid the negative effects caused by some herbicide residue. However, after a significant amount of research, brassinosteroids were identified as one of the few compounds that can address the negative effects of herbicide residue on groundnut. Brassinosteroids can play an important role because they improve morphological, physiological and metabolic processes in plants (Van der Watt, 2005). Therefore, the aim and objective of this study is to review present literature on the effects of herbicide residue during a rotation system on the groundnut plant's growth, physiology and yield response of groundnuts in the absence and presence of Brassinosteroids. This study will help to formulate possible solutions to solve the negative effect of herbicide residue stress and to integrate the techniques in solving future problems that might be caused by herbicide residue on groundnuts. This study will focus on the residual effects of mesotrine.

5.2 Materials and methods

The procedure that is followed here is outlined in chapter 3.

5.3 Results

5.3.1 The effect of three residual concentrations of mesotrione (C) on groundnut.

The results of both the growing seasons (2015/16 and 2016/17) showed similar trends so the results of one season will be presented since there is no difference between the two seasons.

5.3.1.1 Effect on seedling emergence and morphological parameters

(a) Emergence percentage

All the seeds planted gave 100% emergence, thus there was no significant difference on seedling emergence.

(b) Symptoms of crop injury

Mesotrione residue has negative effect on groundnut, the symptoms were noted and differs depending on the consentration of herbicide residue concentration in the soil as well as the phenological stage of the plant. Visual phytotoxity of mesotrione residue phytotoxicity was evaluated by comparing three levels of herbicide residue concentration (1.6 µg ai kg^{-1} soil (equivalent to 45 days after application (DAA), 0.05 µg ai kg^{-1} soil (equivalent to 90 DAA) and 0.0016 µg ai kg^{-1} soil (equivalent to 135 DAA)) and the control (0). The crop appeared short and with yellowing on leaves and stems (Figure 5.1) were observed. Visual symptoms appeared at the highest concentration (1.6 µg ai kg^{-1}), while at the 0.0016 µg ai kg^{-1}, there were no visual phytotoxicity symptoms even though the crop was shorter compared to the control.

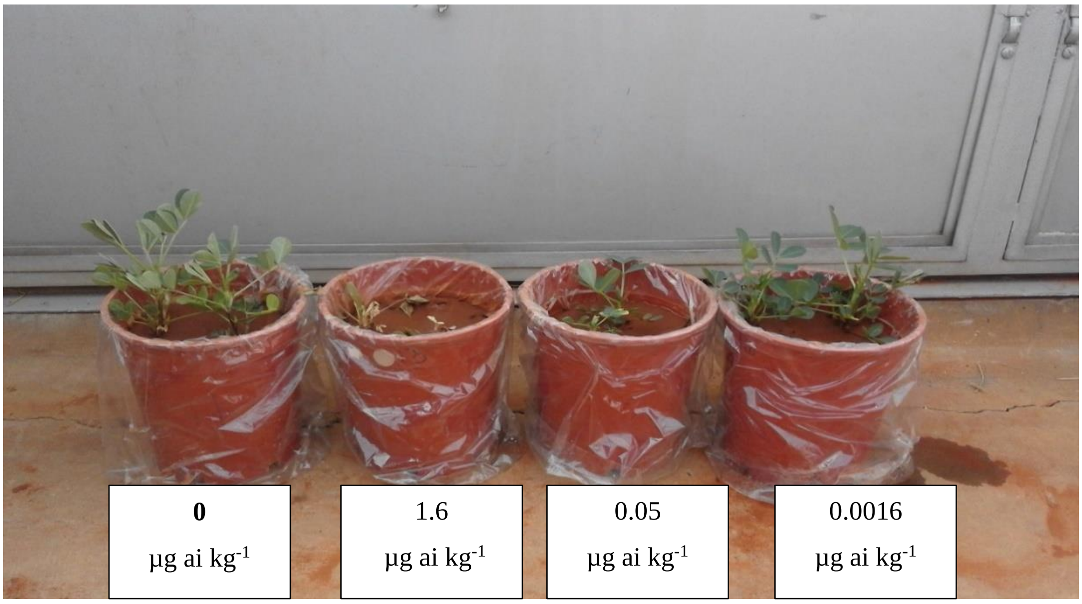

Figure 5. 1: Differences between phytotoxic symptoms of three mesotrione residual concentrations two weeks after plant on groundnut

(c) Morphological parameters

1) Destructive parameters

The effect of three residual mesotrione concentrations on morphological parameters on groundnut was measured every two weeks (Figure 5.2). Measure parameters included seedling fresh mass, seedling dry mass, root fresh mass and root dry mass. The results from the figure showed that all three concentrations caused a decrease compared to the control in all the measured plant growth parameters depending on the concentration in the soil. The decrease in plant growth increases with an increase in a concentration of mesotrione residue concentration. The ANOVA revealed that there was a significant interaction between the three herbicide residual concentrations and weeks in all the measured parameters. Also, it indicated that all the growth parameters were least affected at the lowest concentration (0.0016 µg ai kg^{-1}) as compared to other two concentrations. This showed that the highest concentration as expected has the biggest negative impact on the plant growth.

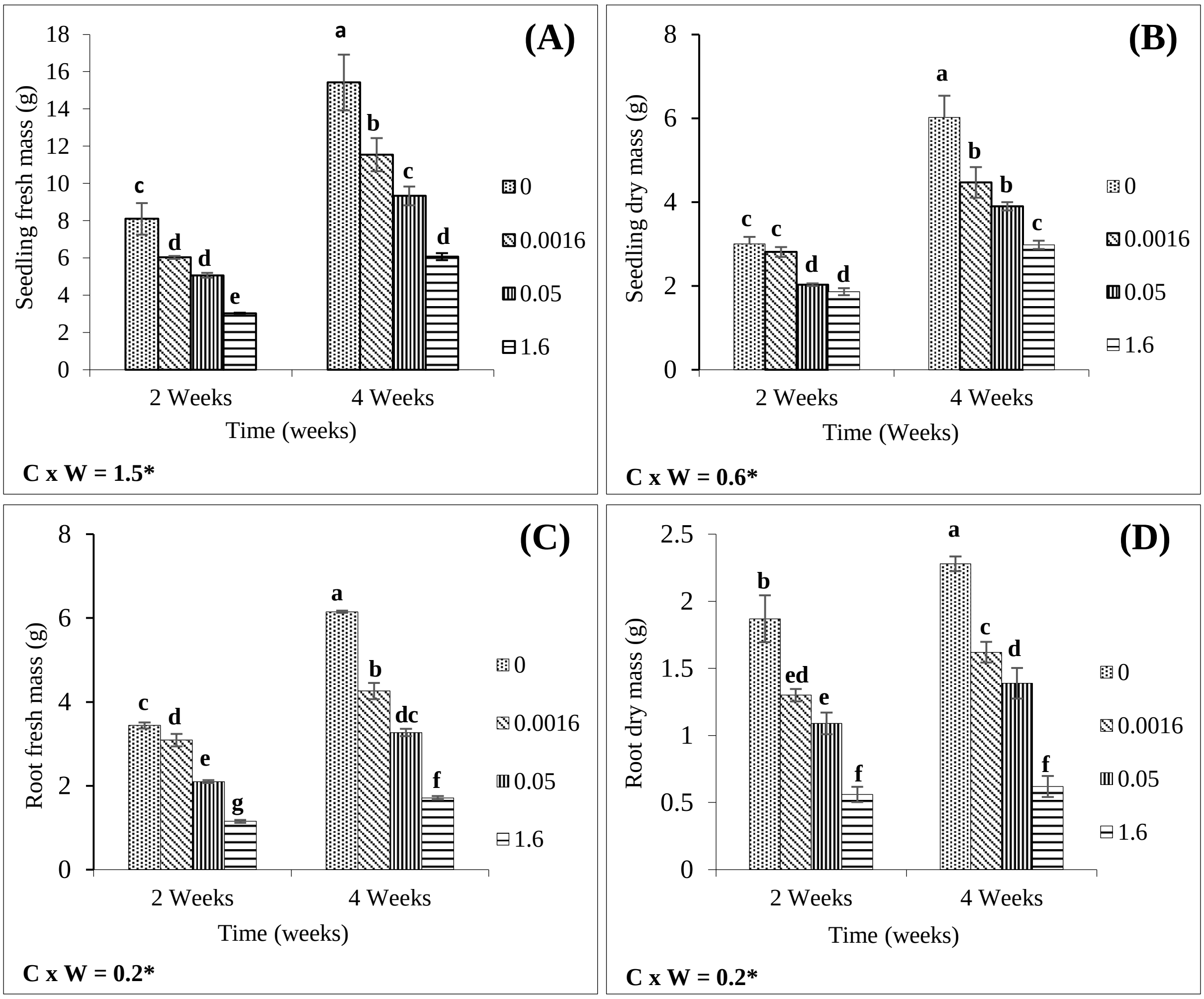

Figure 5. 2: The effect of three residual concentrations of mesotrione (1.6 µg ai kg-^{1}, 0.05 µg ai kg^{-1}, 0.0016 µg ai kg^{-1} and the control (0)) on plant growth parameters including seedling fresh mass (A), seedling dry mass(B), root fresh mass (C) and root dry mass (D) measured every two weeks over a period of four weeks. Where C x W is the interaction between concentrations and weeks. Statistical significance is indicated as LSD=* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test. Vertical bars with horizontal caps indicate standard deviation.

2) Non-destructive growth parameters

The effect of three residual mesotrione concentrations (1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1}, 0.0016 µg ai kg^{-1} and the control) on non-destructive morphological parameters on groundnut was measured every two weeks over a period of 12 weeks (Table 5.1). Over time all three concentrations caused a decrease in all the measured growth parameters (natural plant height, extended plant height and stem diameter) compared to control over time. This shows the significant differences between the three concentrations. The highest concentration (equivaleny to 45 DAA) showed more negative impact on the plant growth than the other two concentrations.

Table 5. 1: The morphological response of groundnut to three residual mesotrione concentrations (1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1}, 0.0016 µg ai kg^{-1} and the control 0) over a period of 12 weeks. Measured parameters include natural plant height, extended plant height and stem diameter.

	Herbicide residue (µg ai kg^{-1})	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks	12 Weeks
Natural plant height (cm)	0	8.9 a	14.9 a	20.8 a	27.0 a	32.8 a	37.5 a
	1.6	4.7 d	8.1 d	14.1 d	19.7 d	25.2 d	28.1 d
	0.05	6.2 c	10.1 c	16.2 c	22.3 c	27.1 c	30.3 c
	0.0016	7.9 b	12.6 b	18.2 b	24.5 b	29.4 b	33.9 b
	LSD_T (5%)	**0.9***	**1.6***	**1.8***	**1.3***	**1.5***	**2.1***
Extended plant height (cm)	0	12.2 a	19.1 a	25.8 a	32.1 a	37.1 a	41.0 a
	1.6	7.9 d	13.0 d	19.7 d	24.9 d	30.5 d	34.5 d
	0.05	9.3 c	14.8 c	20.9 c	26.6 c	32.0 c	37.1 c
	0.0016	10.5 b	17.1 b	23.3 b	29.1 b	34.8b	39.6 b
	LSD_T (5%)	**1.03**	**0.4***	**1.0***	**0.8***	**0.8***	**0.8***
Stem diameter (mm)	0	3.4 a	3.7 a	3.9 a	4.4 a	4.7 a	4.8 a
	1.6	3.0 d	3.3 d	3.4 d	3.6 d	3.8 d	4.0 d
	0.05	3.2 c	3.4 c	3.6 c	3.9 c	4.2 c	4.3 c
	0.0016	3.2 b	3.6 b	3.8 b	4.1 b	4.4 b	4.6 b
	LSD_T (5%)	**0.1***	**0.1***	**0.1***	**0.1***	**0.1***	**0.2***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

5.3.1.2 Physiological response of groundnut to three residual concentrations of mesotrione

(a) Chlorophyll content

The effect of three concentrations of mesotrione residue on chlorophyll content was measured every two weeks. During the whole growing period the results (Figure 5.3) showed no significant interaction between concentrations and weeks in chlorophyll a, chlorophyll b and total chlorophyll (a + b), while carotenoids content showed a significant interaction between herbicide residual concentrations and weeks. On the other hand, the main effects (herbicide residual concentrations) showed the significant differences in all the measured chlorophyll contents between the three residual herbicide concentrations. The highest chlorophyll content was observed in herbicide residual of 0.0016 µg ai kg^{-1} as compared to other two concentrations. In both weeks mesotrione residue affected the content of chlorophyll negatively. The highest residual herbicide concentration caused a decrease in all the measured chlorophyll content. Carotenoid content was the only parameter that significantly increase over the time measured. Caretenoid, however compared to control, still decrease with an increase in the concentration herbicide residuals.

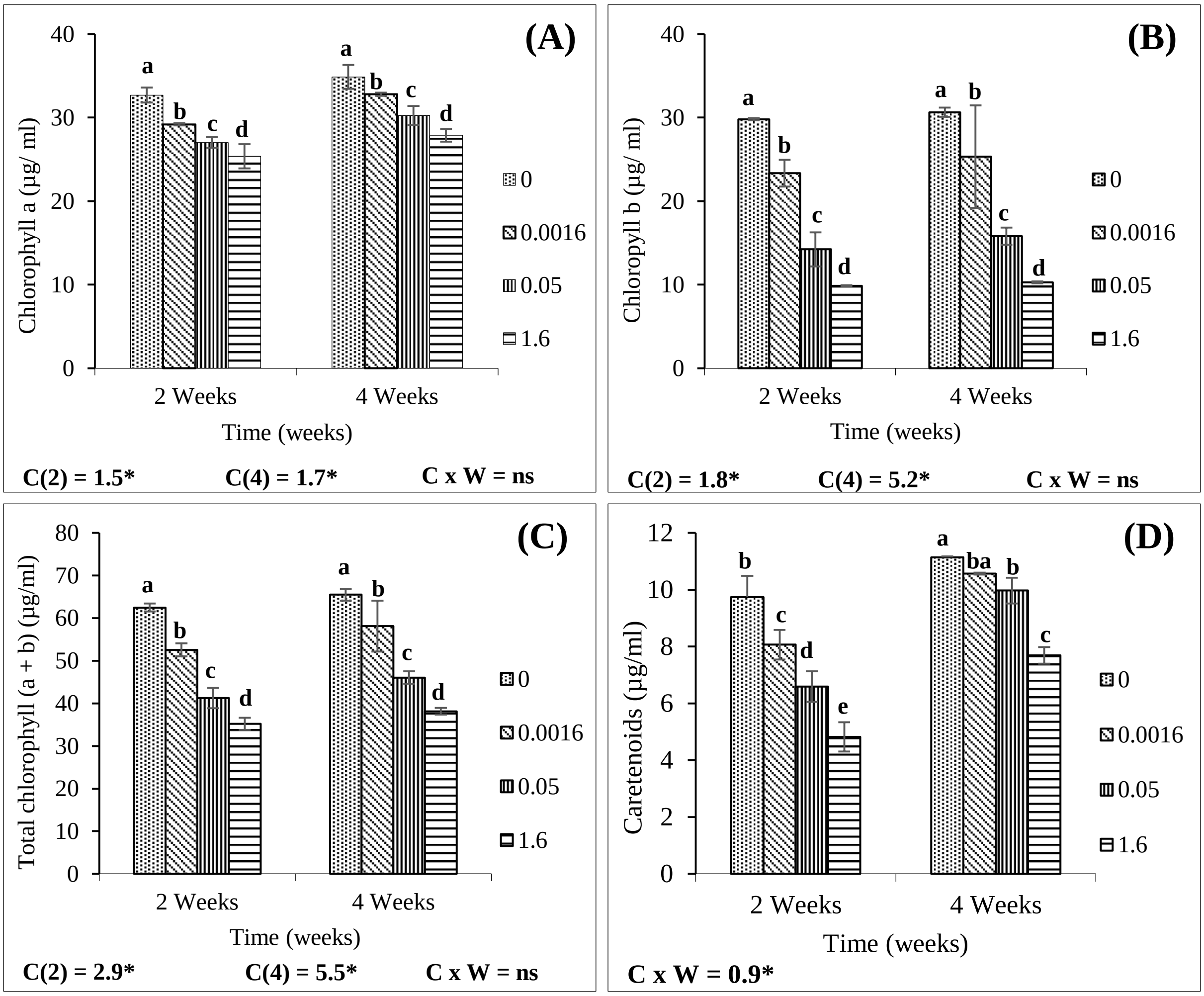

Figure 5. 3: The effect of three residual concentrations of mesotrione (1.6 µg ai kg-1, 0.05 µg ai kg-1, 0.0016 µg ai kg-1 and the control 0) on chlorophyll a (A) and chlorophyll b (B), total chlorophyll (C) and carotenoids content (D) of groundnut measured two times at two weeks interval. Where C (2) = Effect of herbicide at two weeks after plant, C (4) = Effect of herbicide after 4 weeks and C x W is the interaction between concentrations and weeks. Statistical significance is indicated as LSD =* significant (P ≤ 0.05) and different letters, and ns = not significant at 5% using Tukey's LSD test. Vertical bars with horizontal caps indicate standard deviation.

(b) Photosynthetic parameters

The effect of three concentrations of mesotrione residue on the photosynthetic parameters (photosynthetic rate (A), stomatal conductance (B), transpiration rate (C) and sub-stomatal CO_2 concentration (D)) was measured every two weeks and the photosynthetic rate were treated as absolute values (Figure 5.4). The results showed significant interaction between treatments and weeks on photosynthetic rate, but there was no significant interaction between treatments and weeks in stomatal conductance, transpiration rate and intercellular CO_2 concentrations. Significant differences were seen on main effects and the highest photosynthetic rate as well as all other measure parameters was observed at 9 weeks during the most active vegetative growth stage in all herbicide residual concentrations with the lowest concentration (0.0016 µg ai kg^{-1}) as the highest, followed by 0.05 µg ai kg^{-1} and 1.6 µg ai kg^{-1} respectively.

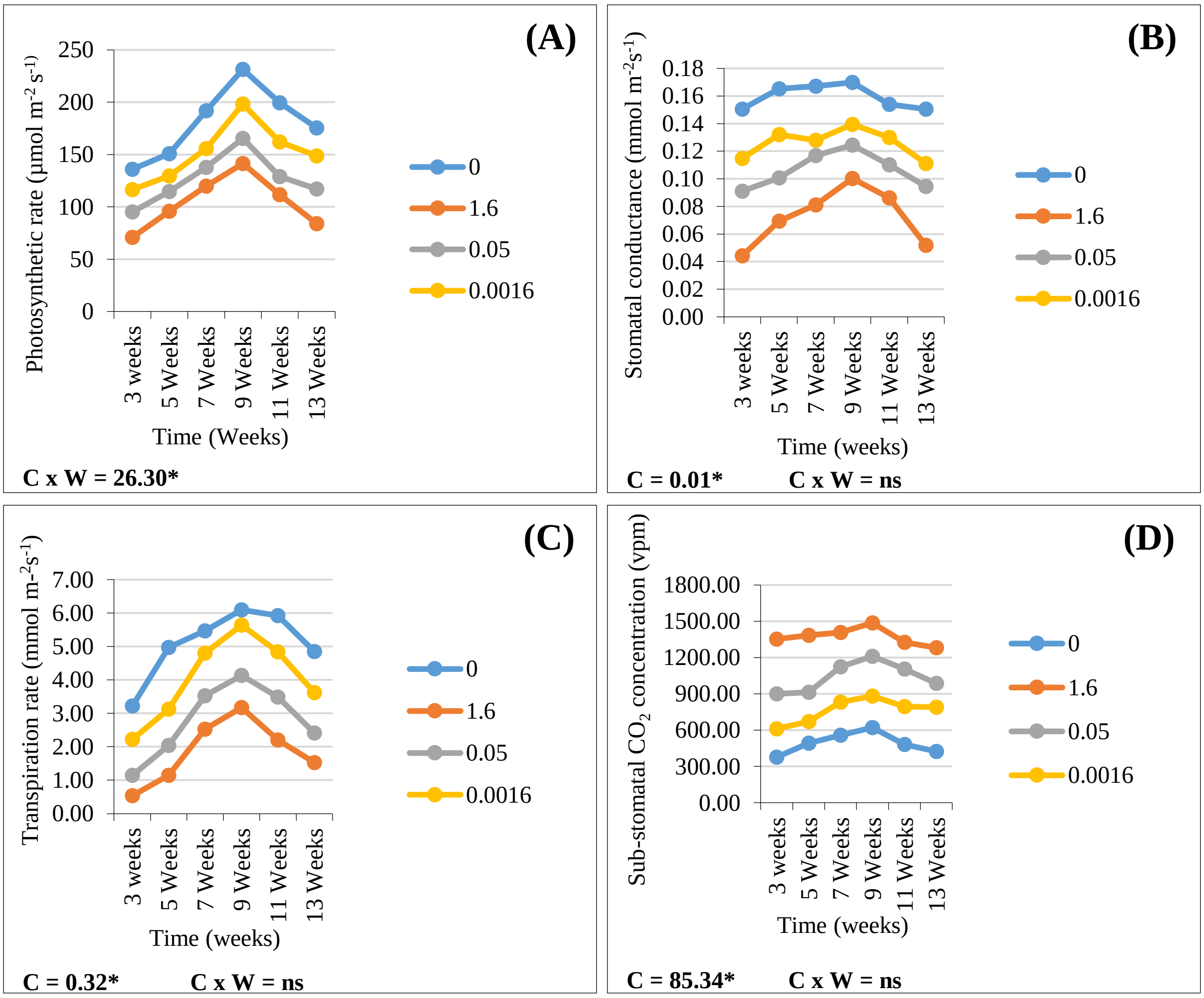

Figure 5. 4: The response of photosynthetic parameters on groundnut to three residual concentrations of mesotrione (1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1}, 0.0016 µg ai kg^{-1} and the control 0) measured over a period of 12 week. Measured parameters include (A) photosynthetic rate, (B) Stomatal conductance, (C) transpiration, (D) Sub-stomatal CO_2 concentration. Where C = Effect of herbicide and the interaction is indicated as C x W (Interaction between concentrations and weeks). Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and ns = not significant at 5% using Tukey's LSD test.

5.3.1.3 The effect of three residual concentrations of mesotrione on yield parameters

(a) Yield components

The table below provides yield components and yield of groundnut (Table 5.2). The results showed differences between the three concentrations (1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1} and 0.0016 µg ai kg^{-1}) and this illustrates that all the higher level of mesotrione residue has negatively affected the yield of groundnut and the reduction of yield differs with the amount of the concentrations in the soil. An enormous decline in yield was observed in 1.6 µg ai kg^{-1} followed by 0.05 µg ai kg^{-1} and 0.0016 µg ai kg^{-1} respectively.

Table 5. 2: The effect of three residual concentrations of mesotrione 1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1}, 0.0016 µg ai kg^{-1} and the control (0) on groundnut yield components. Measured parameters include number of pods per pot, mass of pods per pot, number of seeds per pot and mass of seeds per pot (final yield per pot).

Herbicide residue (µg ai kg^{-1})	Number of pods per pot	Mass of pods per pot (g)	Number of seeds per pot	Mass of seeds per plant (g) (yield per pot)
0	47.5 a	22.6 a	72.8 a	18.4 a
1.6	16.0 d	14.3 d	29.5 d	8.7 d
0.05	28.3 c	17.2 c	44.8 c	11.7 c
0.0016	38.5 b	19.7 b	58.3 b	16.2 b
LSD_T (5%)	**8.3***	**2.2***	**13.0***	**2.0***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

5.3.2 The effect of three different application methods of brassinosteroids (T) on Groundnut

In both the growing seasons (2015/16 and 2016/17) the results show the same trend. From this the results of one season will be presented since there is no difference between the two seasons.

5.3.2.1 The effect on seedling emergence and morphological parameter

(a) Emergence percentage

All the seeds planted gave 100% emergence and therefore there was no significant difference between the different treatments.

(b) Symptoms of the crop injury

The brassinosteroids had no phytotoxic effects on the growth. There was thus no damage on the crop.

(c) Morphological parameters

1) Destructive parameters

All the applied methods of brassinosteroids, caused significant increase in seedling fresh mass, seedling dry mass, root fresh mass and root dry mass compared to the control (Figure 5.5). The highest increase in all plant growth was obtained with the foliar application, followed by soil drench and seed treatment respectively. There was no significant interaction between treatments (brassinosteroids) and weeks (T x W) on seedling fresh mass and seedling dry mass, but the main effects (T) showed significant differences. Foliar application showed the highest significant increase in all parameters, followed by a no significant differences between seed treatment and soil drench. However, all treatments substantially increase the growth compared to the control. On the other hand, a significant interaction was observed between brassinosteroids and weeks on the root fresh mass and dry mass.

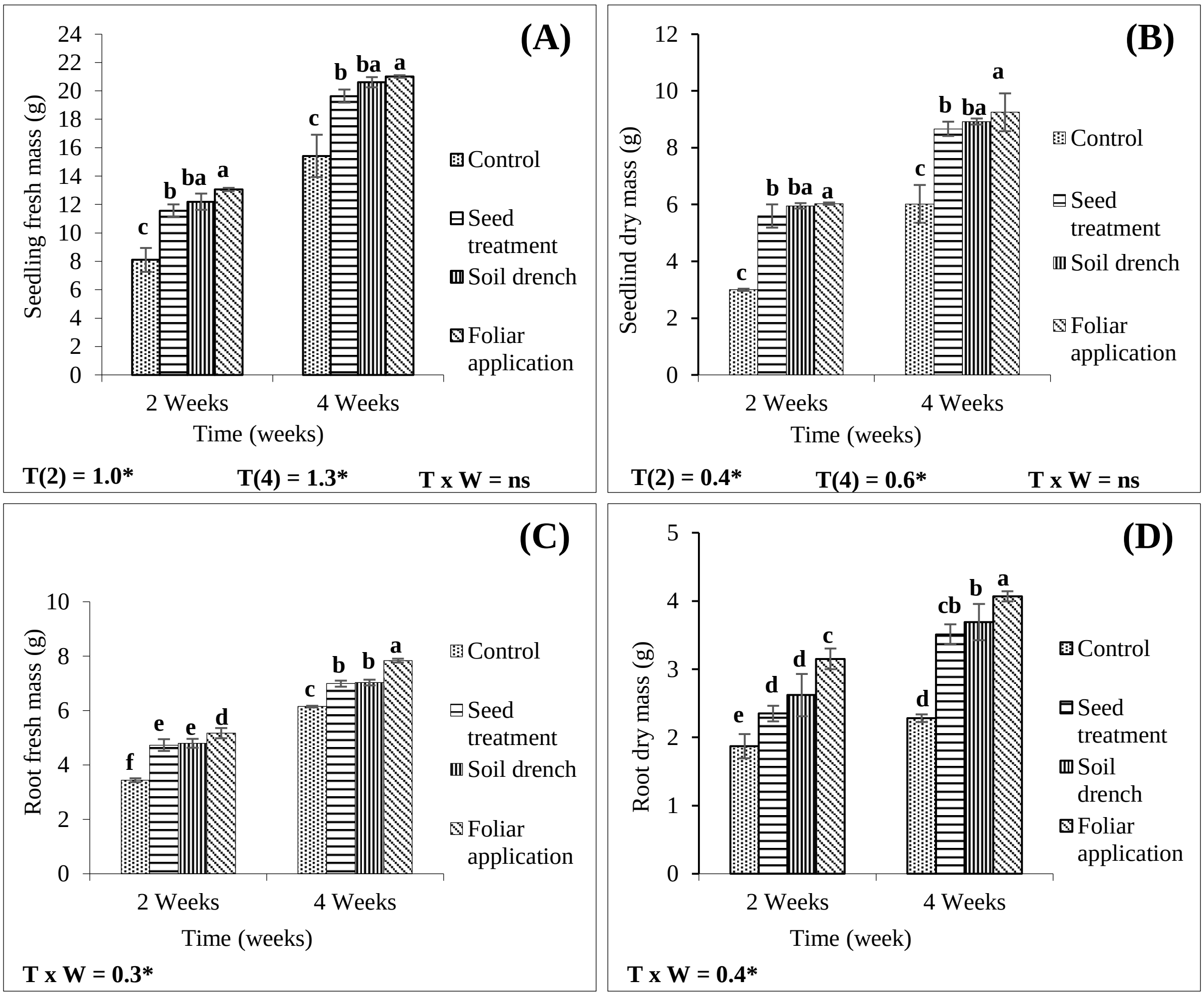

Figure 5. 5: The morphological response of groundnut to three brassinosteroids application methods, seed treatment, soil drench, foliar application and control on destructive seedling growth parameters including seedling fresh mass (A), seedling dry mass (B), root fresh mass (C) and root dry mass (D) measured every two weeks over a period of four weeks. Where T (2) = Effect of brassinosteroids at two weeks after plant, T (4) = Effect of brassinosteroids after 4 weeks and T x W is the interaction between brassinosteroids and weeks. Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test. Vertical bars with horizontal caps indicate standard deviation.

2) Non-destructive growth parameters

The effect of three application methods of brassinosteroids on groundnut natural plant height, extended plant height and stem diameter was measured on two weekly bases until week twelve (Table 5.3) The tables indicated that all the applied methods of brassinosteroids caused an increase in plant growth as compared to control. Foliar application showed the highest plant growth followed by soil drench and seed treatment respectively. In natural plant height during the first two weeks there was significant differences between the treatments but afterwards during the fourth week seed treatment and soil drench showed no significant differences while foliar application showed significant differences as compare to seed treatment, soil drench and control up to 12 weeks. Extended plant height showed no significant differences between soil drench and seed treatment while on the other hand showed significant difference between foliar application, soil drench and seed treatment as compared to control. No significant differences was observed between seed treatment and soil drench in stem diameter but the significant differences was observed between foliar application and seed treatment and soil drench as compared to control.

Table 5. 3: The effect of three brassinosteroids application methods, seed treatment, soil drench, foliar application and control on morphological parameter over a period of 12 weeks. Measured parameters consist of natural plant height, extended plant height and stem diameter.

	Treatment	**2 Weeks**	**4 Weeks**	**6 Weeks**	**8 Weeks**	**10 Weeks**	**12 Weeks**
Natural plant height (cm)	Control	8.9 d	14.9 c	20.8 c	27.0 c	32.8 c	37.5 c
	Seed treatment	9.5 c	16.9 b	23.4 b	28.9 b	35.8 b	39.5 b
	Soil drench	10.0 b	17.1 b	24.6 b	30.7 b	36.3 b	40.6 b
	Foliar application	11.0 a	18.8 a	27.2 a	34.2 a	39.0 a	45.6 a
	LSD_T (5%)	**0.4***	**1.4***	**1.3***	**1.9***	**1.9***	**1.3***
Extended plant height (cm)	Control	12.2 d	19.1 c	25.8 c	32.1 c	37.1 c	41.0 c
	Seed treatment	13.9 c	21.4 b	28.0 b	33.8 b	39.0 b	43.0 b
	Soil drench	16.3 b	22.3 b	28.2 b	34.1 b	39.3 b	44.1 b
	Foliar application	18.8 a	24.7 a	32.0 a	38.0 a	42.5 a	47.4 a
	LSD_T (5%)	**1.2***	**1.2***	**0.7***	**0.8**	**0.8***	**1.4***
Stem diameter (mm)	Control	3.4 c	3.7 c	3.9 c	4.4 c	4.7 c	4.8 c
	Seed treatment	3.5 b	3.8 b	4.2 b	4.6 b	4.9 b	5.0 b
	Soil drench	3.5 b	3.8 b	4.3 b	4.7 b	4.9 b	5.1 b
	Foliar application	3.8 a	3.9 a	4.4 a	4.8 a	5.1 a	5.2 a
	LSD_T (5%)	**0.1***	**0.1***	**0.1***	**0.04***	**0.1***	**0.2***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

5.3.2.2 The effects on physiological parameters

(a) Chlorophyll content

All the applied methods of brassinosteroids caused an increase in chlorophyll a, chlorophyll b, total chlorophyll and carotenoids content compared to the control (Figure 5.6). The results showed that there was a significant interaction between brassinosteroids and weeks on total chlorophyll and carotenoids and not in the case of chlorophyll a and b. Although all applications caused a noteworthy increase in all factors compared to the control, no differences between seed treatment and soil drench was observed with the ultimate increase caused by the foliar application.

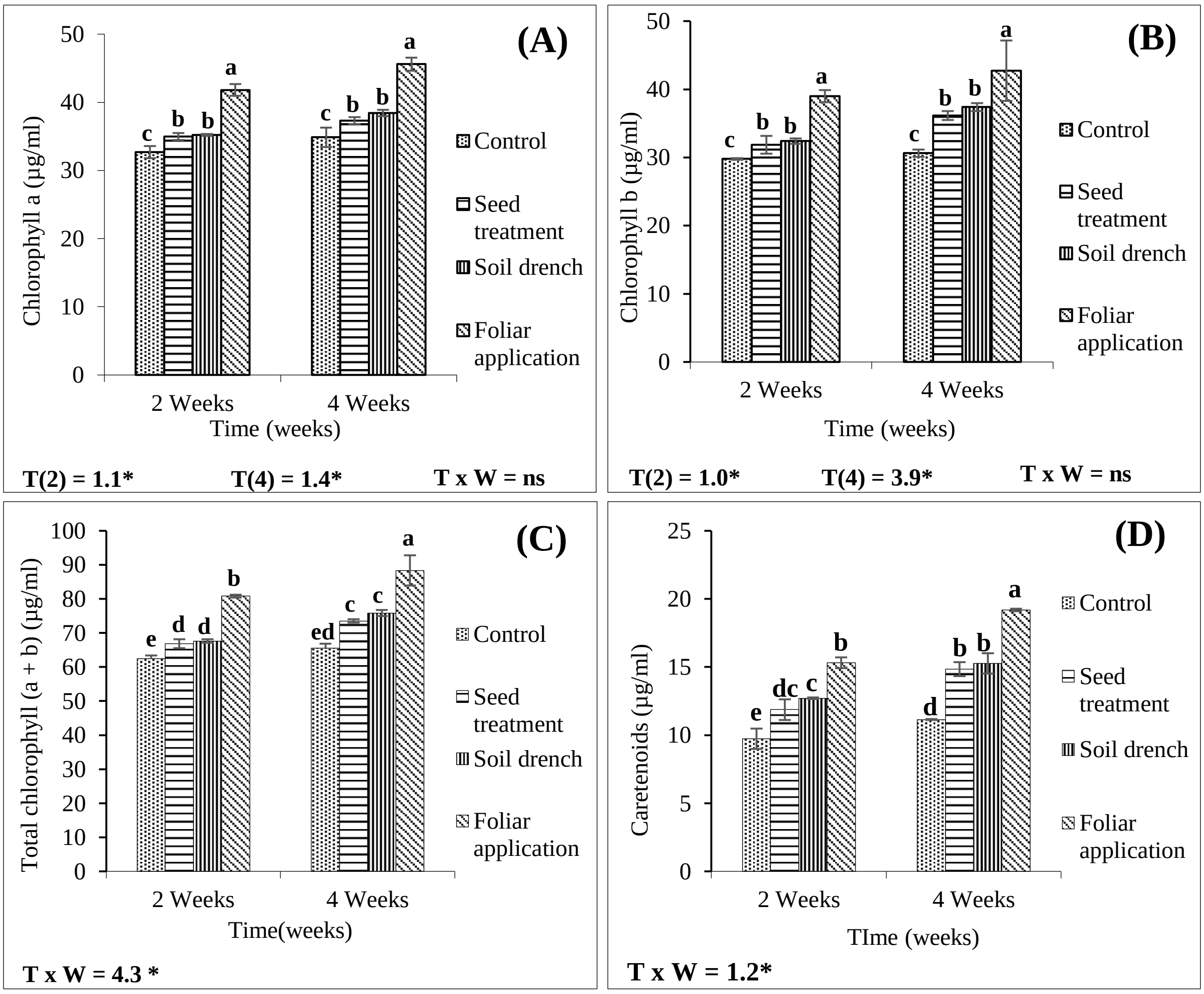

Figure 5. 6: The effect of three application methods of brassinosteroids seed treatment, soil drench foliar application and control on chlorophyll content (chlorophyll a (A), chlorophyll b (B), total chlorophyll (C) and carotenoids content (D)) measured after every two weeks over a period of four weeks on groundnut. Where T (2) = Effect of brassinosteroids at two weeks after plant, T (4) = Effect of brassinosteroids after 4 weeks and T x W is the interaction between brassinosteroids and weeks. Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test. Vertical bars with horizontal caps indicate standard deviation.

(b) Photosynthetic parameters

The effect of different application methods of brassinosteroids on photosynthetic parameters (photosynthetic rate (A), stomatal conductance (B), transpiration rate (C) and sub-stomatal CO_2 concentration (D)) was measured on a two-weekly basis until the twelfth week (Figure 5.7). It can be seen from the results that all the applied methods of brassinosteroids caused an increase in photosynthetic parameters as compared to control. The highest rates were observed at nine weeks which is the most active vegetative growth stage by all the three methods of application of brassinosteroids, with foliar application as the highest, followed by soil drench and seed treatment respectively. No significant interaction could be observed between treatments and weeks, but the main effects differed significantly. In photosynthetic rate there is significant differences between all the treatments, but in stomatal conductance, transpiration rate and intercellular CO_2 concentrations the significant differences were observed between foliar application and the other treatments no significant differences between seed treatment and soil drench.

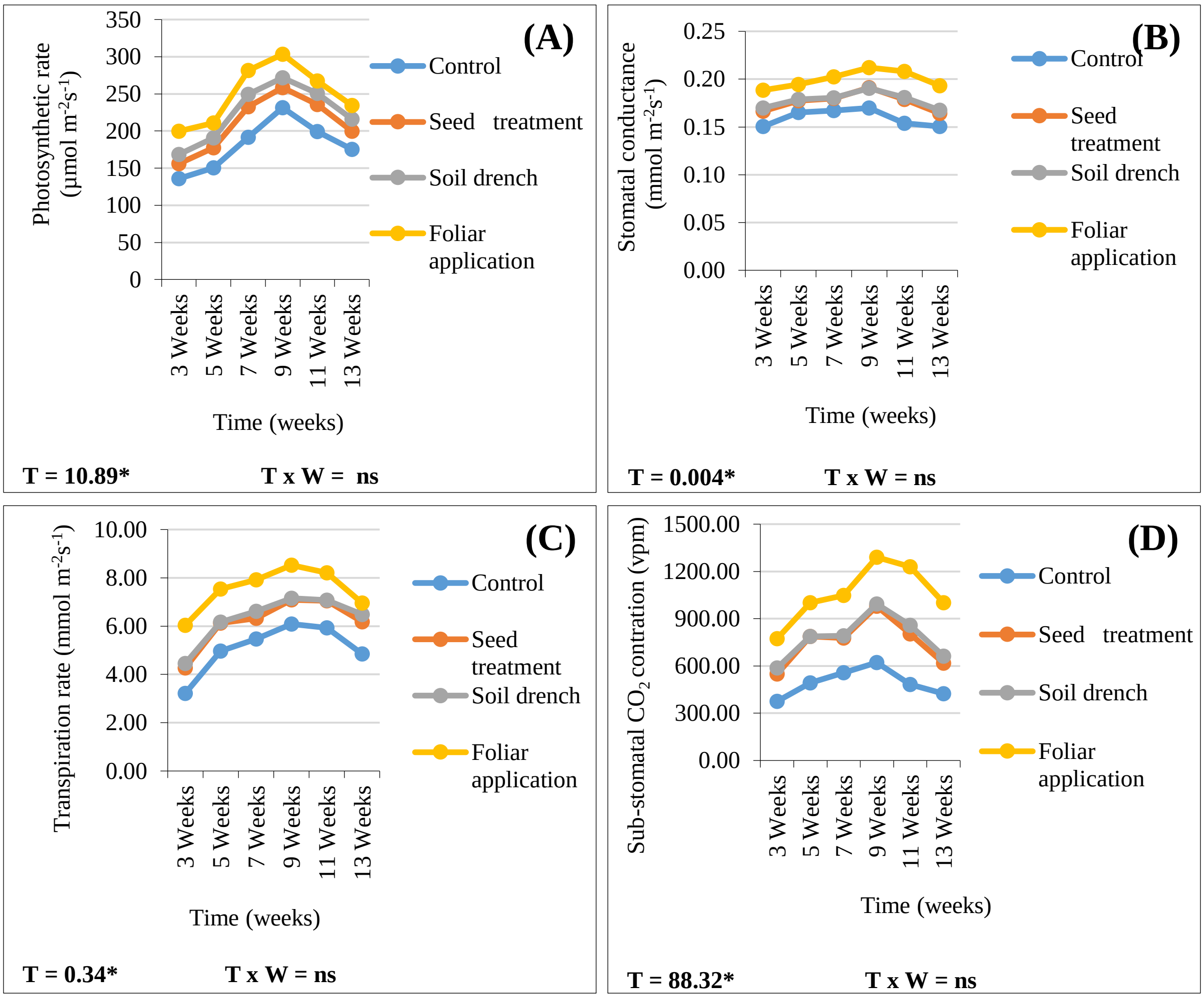

Figure 5. 7: The physiological response of groundnut to three application methods of brassinosteroids seed treatment, soil drench, foliar application and control measured over a period of 12 weeks. Measured parameters include (A) photosynthetic rate, (B) Stomatal conductance, (C) transpiration, (D) sub-stomatal CO_2 concentration. Where T is the effect of brassinosteroids at two weeks after plant and the interaction is indicated as T x W (Interaction between brassinosteroids and weeks). Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

5.3.2.3 The effect of application methods of brassinosteroids on yield parameters

(a) Yield components

The three methods applications of brassinosteroids caused a significant increase in the measured yield and yield components compared to control (Table 5.4). In all the yield components the foliar application differed significantly from the control but was high higher than the seed treatment and soil drench respectively. Seed treatment and soil drench did not show any significant differences between each other but they differ significantly compared to control.

Table 5. 4: The effect of three application methods of brassinosteroids seed treatment, soil drench, foliar application and control on groundnut yield components (number of pods per pot, mass of pods per pot, number of seeds per pot and mass of seeds per pot (final yield per pot).

Treatment	Number of pods per pot	Mass of pods per pot (g)	Number of seeds per pot	Mass of seeds per pot (g) (yield per pot)
Control	47.5 c	22.6 c	72.8 c	18.4 c
Seed treatment	55.8 b	27.8 b	115.8 b	24.5 b
Soil drench	56.8 b	28.2 b	119.0 b	24.8 b
Folia application	65.3 a	31.3 a	128.0 a	27.4 a
LSD_T (5%)	**8.1***	**2.2***	**7.3***	**2.1***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

5.3.3 The effect of brassinosteroids combined with mesotrione on groundnut.

In both the growing seasons (2015/16 and 2016/17) the results show the same trend so the results of one season will be presented since there is no difference between the two seasons.

5.3.3.1 The effect on seedling emergence and morphological parameters

(a) Emergence percentage

All the seeds planted gave 100% emergence and therefore there was no significant difference between the different treatments.

(b) Symptoms of the crop injury

The brassinosteroids in combination with mesotrione had no phytotoxic effects on the crop growth.

(c) Morphological parameters

1) Destructive parameters

All the methods (T) combined with mesotrione caused a significant increase in seedling fresh mass, seedling dry mass, root fresh mass and root dry mass of groundnut compared to control (Table 5.5). This imply that brassinosteroids have a positive impact on the overall plant growth and it is able to counteract the negative effect of herbicide residue in the soil. All the measured parameters showed significant interaction between the herbicide and brassinosteroids application methods except root fresh mass and root dry mass in week two. Even though these difference on root parameters were not significant the effect was still higher than the control. Since the interaction were not significant main effects were tested and showed significant differences respectively.

Table 5. 5: The effect of different brassinosteroids application methods combined with various mesotrione concentrations remaining in the soil on groundnut seedling fresh mass, seedling dry mass, root fresh mass and root dry mass.

		Seedling fresh mass (g)		**Seedling dry mass (g)**		**Root fresh mass (g)**		**Root dry mass (g)**	
Treatment	**Herbicide residue (µg ai kg^{-1})**	**2 Weeks**	**4 Weeks**	**2 Weeks**	**4 Weeks**	**2 Weeks**	**4 Weeks**	**2 Weeks**	**4 Weeks**
Control	0	8.1 fg	15.4 dc	3.0 g	6.0 d	3.4	6.2 dc	1.9	2.3 gf
	1.6	3.0 k	6.1 h	1.9 h	3.0 h	1.2	1.7 h	0.6	0.6 k
	0.05	5.1 ji	9.3 f	2.0 h	3.9 g	2.1	3.3 gf	1.1	1.4 ij
	0.0016	6.0 hi	11.54 e	2.81 g	4.47 gf	3.09	4.26 e	1.30	1.62 ih
Seed treatment	0	11.6 bc	19.6 ba	5.6 a	8.7 ba	4.7	7.0 b	2.4	3.5 dc
	1.6	4.6 j	7.0. gh	3.1 g	4.1 g	2.2	2.6 g	1.0	1.2 j
	0.05	7.1 hg	14.0 d	3.8 fe	6.2 d	3.1	4.3 e	1.6	1.9 gh
	0.0016	9.5 e	16.9 c	4.4 cbd	7.0 c	3.9	5.6 d	1.9	3.2 ed
Soil drench	0	12.2 ba	20.6 a	6.0 a	9.0 ba	4.8	7.0 b	2.6	3.7 bac
	1.6	5.8 i	8.5 gf	3.3 fg	4.9 ef	2.3	2.9 gf	1.5	2.1 gf
	0.05	9.0 fe	16.6 c	3.9 fed	6.94 c	3.3	5.5 d	2.0	2.4 f
	0.0016	10.9 dc	18.8 b	4.88 b	8.36 b	4.4	6.8 bc	2.3	3.6 bdc
Foliar application	0	13.1 a	21.0 a	6.0 a	9.3 a	5.2	7.8 a	3.2	4.2 a
	1.6	7.7 g	12.5 e	4.1 ced	5.5 ed	2.9	3.5 f	2.1	2.5 f
	0.05	9.9 de	18.8 b	4.6 cb	7.1 c	3.9	5.8 d	2.5	3.0 e
	0.0016	12.9 a	20.8 a	5.9 a	9.0 ba	5.0	7.4 ba	3.1	4.0 ba
C x T: LSD$_T$ (5%)		**1.1***	**1.5***	**0.6***	**0.7***	**ns**	**0.7***	**ns**	**0.4***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test. C x T indicates the interaction between brassinosteroids (treatments) and concentrations.

The difference between brassinosteroids application methods on the morphological parameters was measured every two weeks over four weeks (Table 5.6) on groundnut. All the applied methods of brassinosteroids significantly increased plant growth compared to the control. The highest increase in plant growth was found in the foliar application, followed by the soil drench and seed treatment respectively. This showed that brassinosteroids had a positive impact on all the measure parameters.

Table 5. 6: The differences between brassinosteroids application methods (seed treatment, soil drench, foliar application and control) on seedling fresh mass, seedling dry mass, root fresh mass and root dry mass over 4 weeks.

	Seedling fresh mass (g)		**Seedling dry mass (g)**		**Root fresh mass (g)**		**Root dry mass (g)**	
Treatment	**2 Weeks**	**4 Weeks**	**2 Weeks**	**4 Weeks**	**2 Weeks**	**4 Weeks**	**2 Weeks**	**4 Weeks**
Control	5.6 d	10.6 d	2.4 d	4.3 d	2.4 d	3.9 d	1.2 d	1.5 d
Seed treatment	8.2 c	14.4 c	4.2 c	6.5 c	3.5 c	4.9 c	1.7 c	2.5 c
Soil drench	9.5 b	16.1 b	4.5 b	7.3 b	3.7 b	5.6 b	2.1 b	3.0 b
Foliar application	10.9 a	18.3 a	5.2 a	7.7 a	4.2 a	6.1 a	2.7 a	3.4 a
LSD_T (0.05)	**0.4***	**0.6***	**0.2***	**0.3***	**0.2***	**0.3***	**0.15***	**0.1***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

Table 5.7 shows the differences between three residual mesotrione concentrations on morphological parameters of groundnut measured every two weeks. It can be seen that all three concentrations caused a significant decrease in all measured growth parameters compared to the control. The reduction in growth increased with an increase in residue concentration and the results showed significant differences between the three residual herbicide concentrations and also indicated that the parameters increased at the lowest residual concentration (0.0016 µg ai kg^{-1}) compared to the other two.

Table 5. 7: The differences between three residual concentrations of mesotrione (1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1}, 0.0016 µg ai kg^{-1} and the control (0)) on seedling fresh mass, seedling dry mass, root fresh mass and root dry mass over 4 weeks.

	Seedling fresh mass (g)		**Seedling dry mass (g)**		**Root fresh mass (g)**		**Root dry mass (g)**	
Herbicide residue (µg ai kg^{-1})	**2 Weeks**	**4 Weeks**	**2 Weeks**	**4 Weeks**	**2 Weeks**	**4 Weeks**	**2 Weeks**	**4 Weeks**
0	11.2 a	19.2 a	5.1 a	8.2 a	4.5 a	6.1 a	2.5 a	3.4 a
1.6	5.3 d	8.5 d	3.8 d	4.4 d	2.1 d	2.7 d	1.3 d	1.6 d
0.05	7.8 c	14.7 c	3.5 c	6.0 c	3.1 c	4.7 c	1.8 c	2.2 c
0.0016	9.8 b	17.0 b	4.5 b	7.2 b	4.1 b	6.0 b	2.1 b	3.1 b
LSD_T (0.05)	**0.4***	**0.56***	**0.2***	**0.3***	**0.2***	**0.3***	**0.2***	**0.1***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

2) Non-destructive

(i)Natural plant height

All the applied methods of brassinosteroids combined with residual mesotrione concentrations caused an increase in natural plant height compared to control (Table 5.8). This shows that brassinosteroids have a positive impact on the plant growth irrespective of the presence of herbicide residue in the soil. The results showed a significant interaction between herbicide and brassinosteroids in all weeks except during week 2, 8 and 10, but still increasing growth compared to the control that only received the herbicide residue. During these weeks the main effects showed significant differences (Table 5.9 and 5.10).

Table 5. 8: The effect of different brassinosteroids applications combined with mesotrione residue in the soil on natural plant height of groundnut over a period of 12 weeks.

Treatment	Herbicide residue (µg ai kg^{-1})	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks	12 Weeks
Control	0	8.9	14.9 egdf	20.8 d	27.0	32.8	37.5 cfed
	1.6	4.7	8.1 k	14.1 g	19.7	25.2	28.1 j
	0.05	6.2	10.1 jk	16.2 gf	22.3	27.1	30.3 ji
	0.0016	7.9	12.6 h	18.2 ef	24.5	29.4	33.9 h
Seed treatment	0	9.5	16.9 ebdac	23.4 c	28.9	35.8	39.5 cbd
	1.6	5.9	10.4 ji	16.2 gf	22.1	26.6	31.1 i
	0.05	7.3	13.4 gh	19.0 ed	24.7	29.8	34.8 gh
	0.0016	9.3	16.2 edfc	23.0 c	27.3	34.8	37.6 ced
Soil drench	0	10.0	17.1 bac	24.6 bc	30.7	36.3	40.6 b
	1.6	6.9	12.3 ih	18.8 ed	24.0	29.1	34.8 gfh
	0.05	8.3	14.8 egf	20.8 d	25.6	31.4	36.9 gfed
	0.0016	9.3	16.3 ebdfc	24.0 c	30.0	35.6	40.0 cb
Foliar application	0	11.0	18.8 a	27.2 a	34.2	39.0	45.6 a
	1.6	7.9	14.3 gfh	20.3 d	26.1	31.4	36.7 gfeh
	0.05	9.6	16.9 bdac	24.0 c	28.2	34.5	39.9 cb
	0.0016	10.5	18.3 ba	26.2 ba	33.2	37.6	45.3 a
C x T: LSD$_T$ (5%)		**ns**	**2.1***	**2.1***	**ns**	**ns**	**2.8***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test. C x T indicates the interaction between brassinosteroids (treatments) and concentrations.

Table 5.9 shows the effect of three application methods of brassinosteroids on natural plant height over 12 weeks. All the applied methods of brassinosteroids improved plant growth and the results showed significant differences between the treatments with foliar application showing the highest growth followed by soil drench and seed treatment respectively.

Table 5.9: The differences between brassinosteroids application methods (seed treatment, soil drench, foliar application and control) over a period of 12 weeks on natural plant height (cm).

Treatment	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks	12 Weeks
Control	6.9 d	11.4 d	17.3 d	23.4 d	28.6 d	32.4 d
Seed treatment	8.0 c	14.2 c	20.4 c	25.8 c	31.7 c	35.7 c
Soil drench	8.6 b	15.1 b	22.0 b	27.6 b	33.1 b	38.1 b
Foliar application	9.7 a	17.0 a	24.4 a	30.4 a	35.6 a	41.8 a
LSD_T (0.05)	**0.5***	**0.8***	**0.8***	**0.8***	**1.0***	**1.0***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

Table 5.10 summarized the effect of three residual mesotrione concentrations on natural plant height of groundnut measured over 12 weeks. The three concentrations of mesotrione showed significant difference compared to control. The highest concentration (1.6 µg ai kg^{-1}) as expected significantly affected the plan growth negatively followed by 0.05 µg ai kg^{-1} and 0.0016 µg ai kg^{-1} respectively.

Table 5. 10: The differences between three residual concentrations of mesotrione (1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1}, 0.0016 µg ai kg^{-1} and the control (0)) over a period of 12 weeks on natural plant height (cm).

Herbicide residue (µg ai kg^{-1})	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks	12 Weeks
0	9.8 a	16.9 a	24.0 a	30.2 a	35.9 a	40.8 a
1.6	6.3 d	11.3 d	17.4 d	23.0 d	28.1 d	32.7 d
0.05	7.8 c	13.8 c	20.0 c	25.2 c	30.7 c	35.5 c
0.0016	9.2 b	15.8 b	22.8 b	28.7 b	34.3 b	39.2 b
LSD_T (0.05)	**0.53***	**0.8***	**0.8***	**0.8***	**1.0***	**1.0***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

(ii) Extended plant height

All the applied methods of brassinosteroids combined with mesotrione caused an increase to extended plant height compared to control (Table 5.11). This shows that brassinosteroids have positive impact on the plant growth irrespective of the presence of herbicide residue in the soil. There was significant interaction between herbicide and brassinosteroids in all weeks except week 12. In main effect the significant differences were observed in all the treatments, foliar application showed the highest growth followed by soil drench and seed treatment respectively.

Although no significant difference was observed at week 12 the combination of all application methods with the herbicides was still higher that the control which only received the herbicide.

Table 5. 11: The effect of different brassinosteroids applications combined with various mesotrione concentrations remaining in the soil using three different applications on extended plant height of groundnut over a period of 12 weeks.

Treatment	Herbicide residue (µg ai kg^{-1})	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks	12 Weeks
Control	0	12.2 e	19.1 e	25.8 fe	32.1 cd	37.1 ed	41.0
	1.6	7.9 i	13.0 j	19.7 h	24.9 i	30.5 i	34.5
	0.05	9.3 igh	14.8 i	20.9 h	26.6 h	32.0 h	37.1
	0.0016	10.5 fgh	17.1 g	23.3 g	29.1 fe	34.8 gf	39.6
Seed treatment	0	13.9 dc	21.4 cd	28.0 cb	33.8 b	39.0 b	43.0
	1.6	9.1 ih	15.7 hi	22.8 g	27.4 hg	32.6 h	36.6
	0.05	10.7 fg	17.5 fg	24.7 f	28.5 fg	34.7 g	39.5
	0.0016	11.8 fe	19.2 e	27.1 cbd	33.1 cb	38.5 cb	42.3
Soil drench	0	16.3 b	22.3 cb	28.2 cb	34.1 b	39.3 b	44.1
	1.6	11.8 fe	16.7 hg	24.7 f	29.9 e	35.2 gf	39.0
	0.05	12.9 de	18.4 fe	26.0 fed	31.6 d	36.1 ef	41.7
	0.0016	16.0 b	21.7 cbd	27.9 cb	33.8 b	39.1 b	43.6
Foliar application	0	18.8 a	24.7 a	32.0 a	38.0 a	42.5 a	47.4
	1.6	14.4 c	20.8 d	26.9 ced	31.6 d	37.5 cd	42.6
	0.05	16.3 b	22.6 b	28.3 b	33.7 b	39.2 b	44.7
	0.0016	18.0 a	24.0 a	31.7 a	37.3 a	42.0 a	46.7
C x T: LSD_T (5%)		**1.4***	**1.2***	**1.4***	**1.4***	**1.3***	**ns**

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test. C x T indicates the interaction between brassinosteroids (treatments) and concentrations.

The effect of three brassinosteroids application methods on extended plant height was measured over 12 weeks (Table 5.12). The results showed significant differences between the treatments and control with foliar application showing the highest growth followed by soil drench and seed treatment respectively.

Table 5. 12: The differences between brassinosteroids application methods (seed treatment, soil drench, foliar application and control) over a period of 12 weeks on extended plant height (cm).

Treatment	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks	12 Weeks
Control	10.0 d	16.0 d	22.4 d	28.1 d	33.6 d	38.1 d
Seed treatment	11.4 c	18.5 c	25.7 c	30.7 c	36.2 c	40.3 c
Soil drench	14.2 b	19.8 b	26.7 b	32.4 b	37.4 b	42.1 b
Foliar application	16.9 a	23.0 a	29.7 a	35.1 a	40.3 a	45.4 a
LSD_T (0.05)	**0.5***	**0.4***	**0.5***	**0.5***	**0.5***	**0.6***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

The effect of three residual mesotrione concentrations on extended plant height of groundnut measured over period of 12 weeks is summarized in Table 5.13. The results showed that all three herbicide residue concentrations caused a significant decrease in plant growth compared to the control. Growth decrease with an increase in residue concentration. The results showed significant differences between the three herbicide residue concentrations and also indicated that the parameters increased at the lowest residual concentration (0.0016 µg ai kg^{-1}) compared to the others.

Table 5. 13: The differences between three residual concentrations of mesotrione (1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1}, 0.0016 µg ai kg^{-1} and the control (0)) over a period of 12 weeks on extended plant height (cm).

Herbicide residue (µg ai kg^{-1})	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks	12 Weeks
0	15.3 a	21.9 a	28.5 a	34.5 a	39.5 a	43.9 a
1.6	10.8 d	16.6 d	23.5 d	28.4 d	34.9 d	38.2 d
0.05	12.3 c	18.3 c	25.0 c	30.1 c	35.5 c	40.8 c
0.0016	14.1 b	20.5 d	27.5 b	33.3 b	38.6 b	43.0 b
LSD_T (0.05)	**0.5***	**0.4***	**0.5***	**0.5***	**0.5***	**0.6***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

(iii) Stem diameter

The effect of different brassinosteroids application methods combined with mesotrione caused an increase to stem diameter compared to control (Table 5.14). This shows that brassinosteroids have a positive impact on the plant growth irrespective of the presence of herbicide residue in the soil. There was significant interaction between herbicide and brassinosteroids in all weeks, foliar application showed the highest growth followed by soil drench and seed treatment respectively.

Table 5. 14: The effect of different brassinosteroids applications combined with various mesotrione concentrations remaining in the soil using three different application on stem diameter of groundnut over a period of 12 weeks.

Treatment	**Herbicide residue (µg ai kg^{-1})**	**2 Weeks**	**4 Weeks**	**6 Weeks**	**8 Weeks**	**10 Weeks**	**12 Weeks**
Control	0	3.4 cd	3.7 dc	3.9 dc	4.4 d	4.7 c	4.8 de
	1.6	3.0 h	3.3 g	3.4 h	3.6 i	3.8 h	4.0 j
	0.05	3.2 fg	3.4 f	3.6 g	3.9 h	4.2 f	4.3 h
	0.0016	3.2 ef	3.6 e	3.8 dfe	4.1 f	4.4 ed	4.6 f
Seed treatment	0	3.5 cb	3.8 bc	4.2 ba	4.6 bc	4.9 b	5.0 bc
	1.6	3.1 hg	3.4 gf	3.7 gf	3.8 h	4.0 g	4.2 ij
	0.05	3.3 ed	3.5 ef	3.8 dfe	4.1 gf	4.2 f	4.4 gh
	0.0016	3.4 cb	3.7 c	4.2 b	4.6 c	4.8 cb	5.0 dc
Soil drench	0	3.5 b	3.8 bac	4.3 ba	4.7 bac	4.9 b	5.1 bc
	1.6	3.2 efg	3.5 ef	3.7 gfe	3.9 gf	4.2 f	4.3 ih
	0.05	3.4 cd	3.6 ed	4.0 c	4.2 ef	4.4 ed	4.5 gf
	0.0016	3.4 cb	3.8 bc	4.2 ba	4.6 bc	4.9 b	5.0 bc
Foliar application	0	3.8 a	3.9 a	4.4 a	4.8 a	5.1 a	5.2 a
	1.6	3.2 ef	3.6 e	3.8 dce	4.1 f	4.3 ef	4.5 gf
	0.05	3.5 b	3.8 bc	4.2 b	4.3 ed	4.5 d	4.8 e
	0.0016	3.8 a	3.9 ba	4.3 ba	4.8 ba	5.1 a	5.2 ba
C x T: LSD$_T$ (5%)		**0.1***	**0.1***	**0.2***	**0.1***	**0.1***	**0.2***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test. C x T indicates the interaction between brassinosteroids (treatments) and concentrations.

5.3.3.2 Physiological response of groundnut against brassinosteroids combined with mesotrione

(a) Chlorophyll content

The effect of different brassinosteroids application methods combined with mesotrione on chlorophyll content was measured over 4 weeks (Table 5.15 and 5.16). There was significant interaction between herbicide and brassinosteroids in all the measured parameters. All the application methods of brassinosteroids showed an increase in all chlorophyll and carotenoid content as compared to control since it's an early stage of plant growth. The highest chlorophyll content was observed in herbicide residue 0.0016 µg ai kg^{-1} by all the three brassinosteroids, with foliar application as the highest followed by soil drench and seed treatment respectively. The different methods of brassinosteroids all increased the pigment content when combined with the mesotrione residue, but it confirmed again that the most effective method still was the foliar application.

Table 5. 15: The effect of different brassinosteroids applications combined with various mesotrione concentrations remaining in the soil using three different application on groundnut chlorophyll a and chlorophyll b content.

		Chlorophyll a (µg/ml)		Chlorophyll b (µg/ml)	
Treatment	**Herbicide residue (μg ai kg^{-1})**	**2 Weeks**	**4 Weeks**	**2 Weeks**	**4 Weeks**
Control	0	32.7 cd	34.9 fde	29.8 cbd	30.6 e
	1.6	25.4 i	27.9 h	9.9 h	10.3 j
	0.05	27.0 hi	30.2 g	14.2 g	15.8 i
	0.0016	29.2 fg	32.8 f	23.4 f	25.3 fg
Seed treatment	0	35.0 b	37.3 cb	31.9 cb	36.2 dc
	1.6	28.4 hg	30.5 g	11.9 hg	15.4 ij
	0.05	30.6 fde	33.5 fe	24.7 fe	26.5 feg
	0.0016	33.7 cd	35.9 cd	27.4 fed	35.8 dc
Soil drench	0	35.2 b	38.4 b	32.4 b	37.4 bc
	1.6	30.5 fge	33.6 fe	12.2 hg	18.2 ih
	0.05	32.4 cde	35.3 cde	26.1 fed	29.5 fe
	0.0016	35.0 b	38.2 b	32.0 cb	36.9 bc
Foliar application	0	41.8 a	45.6 a	39.0 a	42.7 a
	1.6	32.5 cde	35.5 cde	15.1 g	22.1 hg
	0.05	35.1 b	39.0 b	28.1 ced	31.3 de
	0.0016	40.7 a	45.1 a	38.1 a	41.5 ba
C x T: LSD_T (5%)		**2.2***	**2.2***	**4.1***	**5.1***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test. C x T indicates the interaction between brassinosteroids (treatments) and concentrations.

Table 5. 16: The effect of different brassinosteroids applications combined with various mesotrione concentrations remaining in the soil using three different applications on total chlorophyll (a + b) content and carotenoids content.

		Total chlorophyll (a +b) (µg/ml)		Carotenoids content (µg/ml)	
Treatment	**Herbicide residue (µg ai kg^{-1})**	**2 Weeks**	**4 Weeks**	**2 Weeks**	**4 Weeks**
Control	0	62.5 ed	65.5 ed	9.7 d	11.1 ed
	1.6	35.2 j	38.1 j	4.8 h	7.7 g
	0.05	41.3 i	46.1 i	6.6 g	10.6 edf
	0.0016	52.6 g	58.1 g	8.1 fe	4.0 ef
Seed treatment	0	66.9 cb	73.5 cb	11.9 cb	14.8 c
	1.6	40.3 i	45.9 i	7.4 fg	9.4 f
	0.05	55.3 gf	60.0 gf	8.6 e	11.5 d
	0.0016	61.1 ed	71.7 cb	11.2 c	14.2 c
Soil drench	0	67.6 b	75.8 b	12.7 b	15.3 c
	1.6	42.7 i	51.9 h	9.8 d	11.9 d
	0.05	58.5 ef	64.8 ef	10.9 c	14.6 c
	0.0016	67.0 cb	75.2 cb	12.6 b	14.8 c
Foliar application	0	80.8 a	88.3 a	15.3 aa	19.2 a
	1.6	47.7 h	57.6 g	11.2 c	14.6 c
	0.05	63.1 cd	70.3 cd	12.8 b	16.8 b
	0.0016	78.9 a	86.6 a	15.2 a	18.9 a
C x T: LSD$_T$ (5%)		**4.0***	**5.4***	**1.1***	**1.5***

Statistical significance is indicated as LSD =* significant (P ≤ 0.05) and different letters, and ns = not significant at 5% using Tukey's LSD test. C x T indicates the interaction between brassinosteroids (treatments) and concentrations.

(b) Photosynthetic parameters

The effect of different methods of brassinosteroids applications combined with mesotrione on photosynthetic parameters (photosynthetic rate, stomatal conductance and transpiration rate) was measured over 13 weeks (Table 5.17, 5.20 and 5.23) and the photosynthetic rate numbers were treated as percentage differences from control. Although no significant interactions between brassinosteroids in combination with mesotrione was observed it still caused an increase in photosynthetic parameters. Only the main effects (Table 5.18 and 5.19) showed significant differences between the treatments. These tables again confirm the effectivity of the brassinosteroids in increasing photosynthesis and the negative effect of the herbicide.

Table 5. 17: The effect of different brassinosteroids applications combined with various mesotrione concentrations remaining in the soil using three different applications on groundnut photosynthetic rate calculated as percentage difference from the control.

Treatment	Herbicide residue (µg ai kg^{-1})	3 weeks	5 Weeks	7 Weeks	9 Weeks	11 Weeks	13 Weeks
Control	0	100.0	111.1	141.5	170.4	147.4	129.9
	1.6	52.5	70.8	88.2	104.7	82.6	61.7
	0.05	69.8	84.9	101.3	121.9	95.3	86.2
	0.0016	86.0	95.7	115.1	146.5	120.0	109.5
Seed treatment	0	115.1	131.6	172.0	190.3	174.3	147.4
	1.6	67.8	81.9	95.8	114.9	84.9	74.7
	0.05	88.1	102.7	130.3	147.0	122.8	97.0
	0.0016	106.9	126.4	169.7	180.7	164.7	129.2
Soil drench	0	124.6	140.5	184.2	200.7	185.0	159.0
	1.6	80.8	86.9	97.8	124.1	99.9	86.9
	0.05	98.2	111.5	134.5	162.9	149.3	137.2
	0.0016	118.3	136.9	177.7	193.7	175.4	148.0
Foliar application	0	147.7	155.5	208.3	224.4	197.9	173.5
	1.6	92.7	97.4	113.0	140.6	118.8	94.9
	0.05	109.6	122.7	139.9	172.8	160.0	141.9
	0.0016	136.7	141.2	197.5	209.9	178.6	169.4
C x T: LSD$_T$ (5%)		**ns**	**ns**	**ns**	**ns**	**ns**	**ns**

Statistical significance is indicated as LSD =* significant (P ≤ 0.05) and different letters, and ns = not significant at 5% using Tukey's LSD test. C x T indicates the interaction between brassinosteroids (treatments) and concentrations.

Table 5. 18: The differences between brassinosteroids application methods (seed treatment, soil drench, foliar application and control) on photosynthetic rate calculated as percentage difference from the control.

Treatment	3 weeks	5 Weeks	7 Weeks	9 Weeks	11 Weeks	13 Weeks
Control	77.1 d	90.6 c	111.5 c	135.9 c	111.4 c	96.9 c
Seed treatment	94.5 c	110.7 b	142.0 b	158.3 b	136.7 b	112.1 b
Soil drench	105.5 b	119.0 ba	148.5 ba	170.3 b	152.4 a	132.8 a
Foliar application	121.7 a	129.2 a	164.7 a	186.9 a	163.8 a	144.9 a
LSD$_T$ (0.05)	**8.0***	**11.0***	**17.5***	**12.6***	**15.6***	**13.3***

Statistical significance is indicated as LSD =* significant (P ≤ 0.05) and different letters, and ns = not significant at 5% using Tukey's LSD test.

The differences between three residual concentrations of mesotrione on photosynthetic rate was measured over a period of 13 weeks (Table 5.19). The results showed significant differences between the three residual concentrations of mesotrione. The highest photosynthetic rate again was observed at a concentration of 0.0016 µg ai kg^{-1} the lowest herbicide concentration compared to the other two concentrations.

Table 5. 19: The differences between three residual concentrations of mesotrione (1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1}, 0.0016 µg ai kg^{-1} and the control (0)) on photosynthetic rate calculated as percentage difference from the control.

Herbicide residue (µg ai kg^{-1})	**3 weeks**	**5 Weeks**	**7 Weeks**	**9 Weeks**	**11 Weeks**	**13 Weeks**
0	121.8 a	134.7 a	176.5 a	196.5 a	176.2 a	152.4 a
1.6	73.5 d	84.3 c	98.7 c	121.1 d	96.6 d	79.6 d
0.05	91.4 c	105.5 b	126.5 b	151.1 c	131.9 c	115.6 c
0.0016	112.0 b	125.1 a	165.0 a	182.7 b	159.7 b	139.1 b
LSD_T (0.05)	**8.0***	**11.0***	**17.5***	**12.6***	**15.6***	**13.3***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

Table 5. 20: The effect of different brassinosteroids applications combined with various mesotrione on stomatal conductance.

Treatment	Herbicide residue (µg ai kg^{-1})	3 weeks	5 Weeks	7 Weeks	9 Weeks	11 Weeks	13 Weeks
Control	0	0.15	0.17	0.17 bdc	0.17 dc	0.15 dc	0.15
	1.6	0.04	0.07	0.08 h	0.10 i	0.09 i	0.05
	0.05	0.09	0.10	0.12 hgf	0.12 hig	0.11 gh	0.09
	0.0016	0.11	0.13	0.13 egf	0.14 efg	0.13 fe	0.11
Seed treatment	0	0.17	0.18	0.18 bac	0.19 bc	0.18 c	0.16
	1.6	0.06	0.07	0.09 h	0.11 hi	0.10 ih	0.06
	0.05	0.11	0.12	0.12 egf	0.14 ef	0.13 feg	0.11
	0.0016	0.16	0.17	0.18 bac	0.19 bc	0.17 dc	0.16
Soil drench	0	0.17	0.18	0.18 ba	0.19 bac	0.18 bc	0.17
	1.6	0.06	0.09	0.11 hg	0.11 hi	0.10 ih	0.08
	0.05	0.12	0.13	0.14 edf	0.15 e	0.14 fe	0.12
	0.0016	0.17	0.18	0.18 bac	0.19 bc	0.18 c	0.17
Foliar application	0	0.19	0.19	0.20 a	0.21 a	0.21 a	0.19
	1.6	0.08	0.09	0.11 hgf	0.12 hfg	0.12 fgh	0.11
	0.05	0.13	0.14	0.15 edc	0.16 ed	0.14 fe	0.13
	0.0016	0.18	0.19	0.20 ba	0.21 ba	0.20 ba	0.19
C x T: LSD_T (5%)		**ns**	**ns**	**0.03***	**0.02***	**0.02***	**ns**

Statistical significance is indicated as LSD =* significant (P ≤ 0.05) and different letters, and ns = not significant at 5% using Tukey's LSD test. C x T indicates the interaction between brassinosteroids (treatments) and concentrations.

The effect of three brassinosteroids applications methods on stomatal conductance (Table 5.20) was measured over the period of 13 weeks. Only the main effects where the interactions weren't significant is indicated in Table 5.21 and 5.22. The results showed significant differences between the treatments in weeks 3, 5 and 13 where the interactions did not show significance. Foliar application of brassinosteroids again was the best followed by seed treatment and soil drench, with no difference between the last two respectively.

Table 5. 21: The differences between brassinosteroids application methods (seed treatment, soil drench, foliar application and control) on stomatal conductance.

Treatment	**3 weeks**	**5 Weeks**	**13 Weeks**
Control	0.10 c	0.12 c	0.10 c
Seed treatment	0.12 b	0.13 bc	0.13 b
Soil drench	0.13 b	0.14 ba	0.13 b
Foliar application	0.15 a	0.15 a	0.16 a
LSD_T (0.05)	**0.01***	**0.02***	**0.01***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

The differences between three residual concentrations of mesotrione on stomatal conductance was measured over a period of 13 weeks (Table 5.22). Only the weeks that did not show significant differences with the interaction is shown in the table. The results showed significant differences between the three residual concentrations of mesotrione. As expected, the highest stomatal conductance was observed in a mesotrione residue of 0.0016 µg ai kg^{-1} as compared to the other two concentrations.

Table 5. 22: The differences between three residual concentrations of mesotrione (1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1}, 0.0016 µg ai kg^{-1} and the control (0)) on stomatal conductance.

Herbicide residue (µg ai kg^{-1})	**3 weeks**	**5 Weeks**	**13 Weeks**
0	0.17 a	0.18 a	0.17 a
1.6	0.06 c	0.08 c	0.07 c
0.05	0.11 b	0.12 b	0.12 b
0.0016	0.16 a	0.17 a	0.16 a
LSD_T (0.05)	**0.01***	**0.02***	**0.01***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

Only during the first 5 weeks of the trial a significant interaction between brassinosteroids and herbicide on transpiration rate could be observed (Table 5.23). Table 5.24 show significant differences between the brassinosteroid applications in the week that showed no significance. All three applications methods significantly increased the transpiration rate compared to the control. The herbicide concentrations (Table 5.25) all significantly decreased transpiration rate.

Table 5. 23: The effect of different brassinosteroids applications combined with various mesotrione concentrations on transpiration rate.

Treatment	Herbicide residue (μg ai kg^{-1})	3 Weeks	5 Weeks	7 Weeks	9 Weeks	11 Weeks	13 Weeks
Control	0	3.2 c	5.0 dce	5.5	6.09	5.9	4.9
	1.6	0.5 f	1.2 h	2.5	3.18	2.2	1.5
	0.05	1.2 ef	2.0 gh	3.5	4.13	3.5	2.4
	0.0016	2.2 d	3.1 gf	4.8	5.64	4.8	3.6
Seed treatment	0	4.3 b	6.1 bc	6.3	7.09	7.1	6.2
	1.6	0.7 f	2.5 g	3.4	3.88	3.4	2.9
	0.05	1.9 ed	3.8 fe	4.8	4.8	4.9	4.2
	0.0016	4.2 b	6.0 dc	6.1	6.92	6.8	6
Soil drench	0	4.5 b	6.2 bc	6.6	7.16	7.1	6.5
	1.6	0.9 f	2.9 gf	3.8	4.78	4.2	3
	0.05	2.1 d	4.0 fe	4.9	5.94	5	4.6
	0.0016	4.3 b	6.0 dc	6.5	7.02	7	6.3
Foliar application	0	6.0 a	7.5 a	7.9	8.52	8.2	7
	1.6	1.2 ef	3.2 gf	4.1	4.82	4.3	3.8
	0.05	3.1 c	4.8 de	5.8	5.94	5.6	4.9
	0.0016	6.0 a	7.4 ba	7.7	7.95	7.9	6.7
C x T: LSD_T (5%)		**0.8***	**1.3***	**ns**	**ns**	**ns**	**ns**

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test. C x T indicates the interaction between brassinosteroids (treatments) and concentrations.

Table 5. 24: The differences between brassinosteroids application methods (seed treatment, soil drench, foliar application and control) on Transpiration rate.

Treatment	3 weeks	5 Weeks	7 Weeks	9 Weeks	11 Weeks	13 Weeks
Control	1.8 c	2.8 c	4.1 c	4.8 d	4.1 c	3.1 c
Seed treatment	2.8 b	4.6 b	5.2 b	5.7 c	5.5 b	4.8 b
Soil drench	2.9 b	4.8 b	5.5 b	6.2 b	5.8 b	3.1 ba
Foliar application	4.1 a	5.7 a	6.4 a	6.8 a	6.5 a	5.6 a
LSD_T (0.05)	**0.3***	**0.5***	**0.6***	**0.5***	**0.5***	**0.6***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

Table 5. 25: The differences between three residual concentrations of mesotrione (1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1}, 0.0016 µg ai kg^{-1} and the control (0)) on Transpiration rate.

Herbicide residue (µg ai kg^{-1})	**3 weeks**	**5 Weeks**	**7 Weeks**	**9 Weeks**	**11 Weeks**	**13 Weeks**
0	4.5 a	6.2 a	6.6 a	7.2 a	7.1 a	6.1 a
1.6	0.8 d	2.4 d	3.5 c	4.2 c	3.5 c	2.8 c
0.05	2.1 c	3.7 c	4.7 b	5.2 b	4.8 b	4.0 b
0.0016	4.2 b	5.6 b	6.3 a	6.9 a	6.6 a	5.7 a
LSD_T (0.05)	**0.3***	**0.5***	**0.6***	**0.5***	**0.5***	**0.6***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

Over 13 weeks no significant interactions between the two treatments brassinosteroids and herbicide concentrations were observed (results not shown) on sub-stomatal CO_2 concentration. Therefore, main effects in Table 5.26 and 5.27 was summarized displaying the same tendency as observed previously. The brassinosteroids have a positive effect while the herbicides all decreased the parameters.

Table 5. 26: The differences between brassinosteroids application methods (seed treatment, soil drench, foliar application and control) on sub-stomatal CO_2 concentration.

Treatment	**3 weeks**	**5 Weeks**	**7 Weeks**	**7 Weeks**	**11 Weeks**	**13 Weeks**
Control	810.1 c	864.7 c	979.4 c	1049.9 c	927.1 d	870.0 c
Seed treatment	889.9 cb	1036.5 b	1075.4 cb	1216.1 cb	1086.4 c	954.4 cb
Soil drench	981.4 b	1104.8 b	1160.3 b	1333.8 b	1239.8 b	1091.6 b
Foliar application	1094.4 a	1327.1 a	135.3 a	1589.6 a	1453.5 a	1290.6 a
LSD_T (0.05)	**112.2***	**149.2***	**144.3***	**170.6***	**140.6***	**138.1***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

Table 5. 27: The differences between three residual concentrations of mesotrione (1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1}, 0.0016 µg ai kg^{-1} and the control (0)) on sub-stomatal CO_2 concentration.

Herbicide residue (µg ai kg^{-1})	**3 weeks**	**5 Weeks**	**7 Weeks**	**9 Weeks**	**11 Weeks**	**13 Weeks**
0	571.4 c	767.0 c	793.4 c	970.9 c	843.3 c	676.1 c
1.6	1485.5 a	1574.840 a	1610.2 a	1769.1 a	1624.7 a	1537.1 a
0.05	1079.7 b	1167.9 b	1276.9 b	1040.3 b	1296.3 b	1199.5 b
0.0016	638.7 c	823.5 c	885.9 c	1409.1 c	942.4 c	793.8 c
LSD_T (0.05)	**112.2***	**149.2***	**144.3***	**170.6***	**140.6***	**138.1***

Statistical significance is indicated as LSD=* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

5.3.3.3 The effect of brassinosteroids combined with mesotrione on groundnut yield

(a) Yield

The effect of brassinosteroids combine with mesotrione residue on groundnut yield is summarized in Table 5.28. All the three applied brassinosteroids showed a positive impact on groundnut yield by showing a significant increase in all yield parameters except number of pods. Since the interaction between herbicide and brassinosteroids was significant in all yield components except in number of pods per pot only the effect of the two main effects brassinosteroids and herbicide will be summarized in Table 5.29 and 5.30.

Table 5. 28: The effect of different brassinosteroids applications combined with various mesotrione concentrations remaining in the soil on groundnut yield and yield components. Measured parameters include number of pods per pot, mass of pods per pot, number of seeds per pot and mass of seeds per pot (final yield per pot).

Treatment	Herbicide residue (µg ai kg^{-1})	Number of pods per pot	Mass of pods per pot (g)	Number of seeds per pot	Mass of seeds per pot (g) (yield per pot)
Control	0	47.5	22.6 dce	72.8 ed	18.4 ed
	1.6	16.0	14.3 i	29.5 h	8.7 j
	0.05	28.3	17.2 ghi	44.8 gfh	11.7 ih
	0.0016	38.5	19.7 gfe	58.3 ef	16.2 ef
Seed treatment	0	55.8	27.8 ba	115.8 bac	24.5 bc
	1.6	21.8	16.1 hi	41.0 gh	9.9 j
	0.05	34.8	19.2 ghfe	67.3 ed	13.1 gh
	0.0016	50.3	25.1 bc	108.5 bc	23.5 c
Soil drench	0	56.8	28.2 ba	119.0 ba	24.8 bac
	1.6	23.5	18.0 ghf	50.0 gf	11.3 ihj
	0.05	39.0	21.1 dfe	75.8 d	15.9 ef
	0.0016	55.5	27.9 ba	116.8 bac	23.9 bc
Foliar application	0	65.3	31.3 a	128.0 a	27.4 a
	1.6	38.8	19.0 ghf	79.8 d	15.5 gf
	0.05	49.5	23.9 dc	100.3 c	19.6 d
	0.0016	63.8	30.1 a	127.3 a	26.4 ba
C x T: LSD_T (5%)		**ns**	**3.6***	**16.9***	**2.8***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test. C x T indicates the interaction between brassinosteroids (treatments) and concentrations.

All of the applied brassinosteroids increased in the amount of pods produced significantly compared to the control (Table 5.29). Foliar application was significantly higher than the seed treatment, soil drench and control. Seed treatment and soil drench did not differ significantly on number of pods per pot but both were meaningful higher than the control in all the measure parameters.

Table 5. 29: The differences between brassinosteroids application methods (seed treatment, soil drench, foliar application and control) on number of pods per pot.

Treatment	**Number of pods per pot**
Control	32.6 c
Seed treatment	40.6 b
Soil drench	43.7 b
Foliar application	54.3 a
LSD_T (0.05)	**3.7***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

In Table 5.30 the results showed a reduction in the number of pods which is dose dependant. A huge decline in yield parameters can be observed in 1.6 µg ai kg^{-1} followed by 0.05 µg ai kg^{-1} and 0.0016 µg ai kg^{-1} respectively compared to the control.

Table 5. 30: The differences between three residual concentrations of mesotrione (1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1}, 0.0016 µg ai kg^{-1} and the control (0)) on number of pods per pot, mass of pods per pot, number of seeds per pot and mass of seeds per pot (final yield per pot).

Herbicide residue (µg ai kg^{-1})	**Number of pods per pot**
0	56.3 a
1.6	25.0 d
0.05	37.9 c
0.0016	52.0 b
LSD_T (0.05)	**3.7***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

5.4 Discussion

Groundnut is one of the major important oilseed crops grown worldwide. It is used as food for both human and livestock, it can improve soil fertility and the economic status of many people. One of the major constrains in groundnut production is its sensitivity to herbicide residues in the soil especially when planted in a crop rotation system. Previous studies confirmed the negative impact of herbicide residue on legumes which may result in some changes on the plants morphology and physiology. In this study, groundnut was planted in three different mesotrione concentrations (1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1} and 0.0016 µg ai kg^{-1}) and brassinosteroids was applied using in three methods (foliar application, soil drench and seed treatment) to determine whether or not the brassinosteroids can counteract the negative effects of the mesotrione. This study succeeded in showing that mesotrione residues has a negative impact on groundnut growth as a whole and application of brassinosteroids was able to improve both the morphology and physiology of the crop which consequently increased yield. When comparing a control treatment and three different mesotrione concentrations, results showed that mesotrione residue as expected had a negative impact on groundnut crop as a whole, especially at 1.6 µg ai kg^{-1} followed by 0.05 µg ai kg^{-1} and 0.0016 µg ai kg^{-1} respectively. The crop appeared stunted, bleaching of the leaves (Figure 5.1) and also overall reduced plant growth occurred (Figure 5.2 and Table 5.1). It was also evident from the results mesotrione residue caused a decline in physiological parameters (Figure 5.3 and 5.4) and yield (Table 5.2) and that differs with the dose of mesotrione residue. Similar results were found by Felix *et al.* (2007), who also found that mesotrione residue caused similar symptoms or injury to snap bean, cabbage, bell paper and cucumber one year after application. Again, research recently carried out by Riddle (2012), found that herbicide residue caused injury to different crops such as sugar beet, peas, and cucumber and soya bean and injury severity increases with the increase in herbicide residue concentration. Additionally, it was reported that that mesotrione residue caused reduction one year after application in biomass, yield and dry weight in broccoli, carrot, cucumber, and onion (Robinson, 2008; Soltani *et al*., 2014). Zobiole *et al.* (2010) found a decrease in photosynthetic parameters of soybean by glyphosate at different plant growth stages. Also, a study conducted by Vital *et al*. (2017), found that photosynthetic rate, stomatal conductance and transpiration rate decline with an increase in the quantity of herbicide and sub-stomatal CO_2 concentration increase with an increase in the concentration of the herbicide. This might be due to a decrease in stomatal conductance to CO_2 concentration intake, low chlorophyll content and electron transport.

All these negative effects on morphological, physiological parameters and decline of groundnut yield caused by mesotrione residue might be due to the fact that mesotrione herbicide is a 4-hydroxyphenylpyruvate dioxygenase inhibitor, it prevents plant growth by blocking 4-Hydroxyphenyl pyruvate dioxygenase which is responsible for catalysing the conversion of tyrosine to plastoquionone and α-tocophyrol (Coats, 2009). It was further indicated by Moran, (2005), that the enzyme is also involved in the synthesis of carotene pigments which contribute to photosynthesis by transferring some light energy they absorb to chlorophylls through resonance energy transfer, which then use this energy to drive photosynthesis. Therefore, in the absence of carotenoids, excess light energy destroy chlorophyll, proteins, membranes and other molecules and this result to bleaching, necrosis and subsequent death of the plant (Moran, 2005; Riddle, 2012). This also leads to plants not being able to protect themselves from photo-oxidation (Riddle, 2012) and consequently leading to overall reduced plant growth.

On the other hand, when comparing three different application methods of brassinosteroids on groundnut morphological, physiological parameters and yield, all the three applied methods of brassinosteroids enhanced plant growth as a whole but that differs with the method of application. From the results it clear that there is an increase in all growth characters (Figure 5.5 and Table 5.3) and the results further showed an increase in chlorophyll and photosynthetic parameters (Figure 5.6 and 5.7) which contributed to an increase in yield (Table 5.4). This results are in accordance with Verma *et al.* (2011) who reported that application of brassinosteroids improved groundnut growth, chlorophyll and shoot elongation and consequently improve photosynthetic rate of the crop. Additionally, the results are similar to that of Vardhini *et al.* (2008) who found that application of brassinosteroids have caused increase in yield and improved stress resistance of many crop plants. In the study by Wu *et al*. (2014), application of brassinosteroids increased biomass accumulation and chlorophyll content of eggplant seedlings which resulted in high photosynthetic rate. It was further indicated by Divi & Krishna, (2009); Peleg & Blumwald, (2011) that brassinosteroids have a positive effect on the overall plant growth because these plant hormones play an increasing role in seed germination, cell elongation and cell division. Again, brassinosteroids have been reported to increase resistance to many biotic and abiotic stress which include drought, water logging, high temperature, freezing and herbicide residue to mention a few. Brassinosteroids help the crop to survive stresses by transforming physiological processes of the crop and developing defensive mechanisms to improve tolerance to stress (Vardhini & Anjum, 2015).

Additionally, during this study, the effect of brassinosteroids combined with mesotrione residue on groundnut was investigated. The results showed that regardless of the presence of the herbicide residue in the soil, the three methods of brassinosteroids application counteract the negative effects of mesotrione residue. Brassinosteroids caused a decrease in plant injury in all the three applied methods stimulated all morphological (Table 5.5, 5.8, 5.11 and 5.14) and physiological parameters (Table 5.15, 5.16, 5.17, 5.20 and 5.23 and main effects Table 5.18, 5.19, 5.21, 5.22, 5.24, 5.25, 5.26 and 5.27) and yield parameters of the crop (Table 5.28) and main effects Table 5.29 and 5.30). According to Van der Watt, (2005), brassinosteroids improve mineral uptake by stimulating the activities of soil microorganisms which are responsible for soil fertility and encourage more fibrous growth of roots enhancing plant to absorb nutrients from the soil. This might be due to the fact that brassinosteroids enhance degradation of herbicide in the soil and it increases the activity of soil microorganisms in the soil which are responsible for soil fertility which lead to improved plant growth and health (Marschner, 2007).

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CHAPTER 6

THE EFFECT OF BRASSINOSTEROIDS ON MORPHOLOGICAL, PHYSIOLOGICAL AND YIELD CHARACTERISTICS OF DRY BEAN UNDER DIFFERENT LEVELS OF MESOTRIONE

6.1 Introduction

Dry bean *(Phaseolus vulgaris* L.*)* is an important legume in crop production throughout the world. Dry bean is used as a staple food in most countries like Latin American, Eastern and South African countries. In South Africa an average production of 58 000t on 56 000 ha has been documented for the past ten years and it is mostly planted commercially in areas such as Mpumalanga (56%), Free State (28%), North-west (7%), KwaZulu-Natal (5%) and Northern Cape (4%) provinces (Fourie, 2002). Dry beans such as the red speckled sugar (79%), small white (12%) and large white (4%) beans are popular in South Africa (Fourie, 2002). Dry bean nutritionally is taken as the second most important plant protein source after groundnuts for both human beings and animals (Frassinetti *et al*., 2015). The crop is also used to; improve soil fertility by fixing atmospheric nitrogen in the ground and as a source of income.

In most cases, dry bean is used as a rotating crop after harvesting maize, because it helps in breaking the cycles of pests. The successful production of the crop depends on many factors including weed control, which is the most important constraint during production. In previous studies it was found that weeds reduced dry bean yield and quality (Chikoye *et al.,* 1995; Burnside *et al*., 1998). So in order to control weeds and optimise yield, herbicides are normally used because they are the most effective method to control weeds but they can also cause losses in crop yield if not used properly (Heimann & Newman, 1997). The response of dry bean may differ depending on the residue concentration of the herbicide that was applied the previous year, especially if the herbicide is not registered for that crop (Riddle *et al.,* 2013; Soltani *et al.,* 2014). Herbicide residue can have a serious phytotoxic effect on sensitive rotating crops. The persistence of the herbicide is determined by the absorption, movement, plant uptake and rate of degradation (Helling, 2005). Mesotrione is a selective herbicide that is registered in South Africa for pre-emergence and post-emergence and it controls annual broadleaf weeds, grasses and suppresses other weeds in maize (Syngenta SA 2011; Riddle 2012). When mesotrione when it reaches the crop it is absorbed by an emerging root, shoot and foliar tissue, and is translocated via the xylem and phloem leading to the inhibition of the enzyme phydroxyphenylpyruvate dioxygenase (HPPD) which is responsible for the conversion of tyrosine to plastoquinone and it destroys the plant pigments biosynthesis and causes the plant to starve to death (Mitchell *et al.,* 2001).

Alternative methods that can help to alleviate herbicide damage on sensitive crops are increasing herbicide degradation in the soil, enhancing the photosynthetic rate in a crop and inducing herbicide tolerance. The use of brassinosteroids appears to be a possible option that can relieve herbicide injury caused by residue in sensitive crops. This study was conducted to 1) determine the effect of residual activity of mesotrione at three concentrations on dry bean. 2) Determine the effect of three different application methods of brassinosteroids on dry bean and 3) determine the effect of brassinosteroids combined with mesotrione residue on dry bean.

6.2 Materials and methods

The procedure that is followed here is outlined in Chapter 3.

6.3 Results

6.3.1 The effect of three residual concentrations of mesotrione (C) on dry bean.

The results of the two growing seasons (2015/16 and 2016/17) showed a similar trend so the results of one season will be presented since there is no difference between the two.

6.3.1.1 Effect on seedling emergence and morphological parameters

(a) Emergence percentage

All the seeds planted gave 100% emergence therefore no significant difference could be observed.

(b) Symptoms of the crop injury

Mesotrione residue phytotoxicity symptoms were assessed by comparing three levels of herbicide residue concentration in the soil (1.6 µg ai kg^{-1} soil (equivalent to 45 days after application (DAA), 0.05 µg ai kg^{-1} soil (equivalent to 90 DAA) and 0.0016 µg ai kg^{-1} soil (equivalent to 135 DAA)) and the control (0). Visual injury symptoms differed depending on the level of mesotrione in the soil (Figure 6.1). Crop injury was noted especially at the highest concentration of mesotrione (1.6 µg ai kg^{-1} soil) and plant appeared stunted, bleached and necrotic stripes occurred on the leaves, while at the residual concentration of 0.0016 µg ai kg^{-1} there were no phytotoxicity symptoms visible even though the crop was shorter compared to the control.

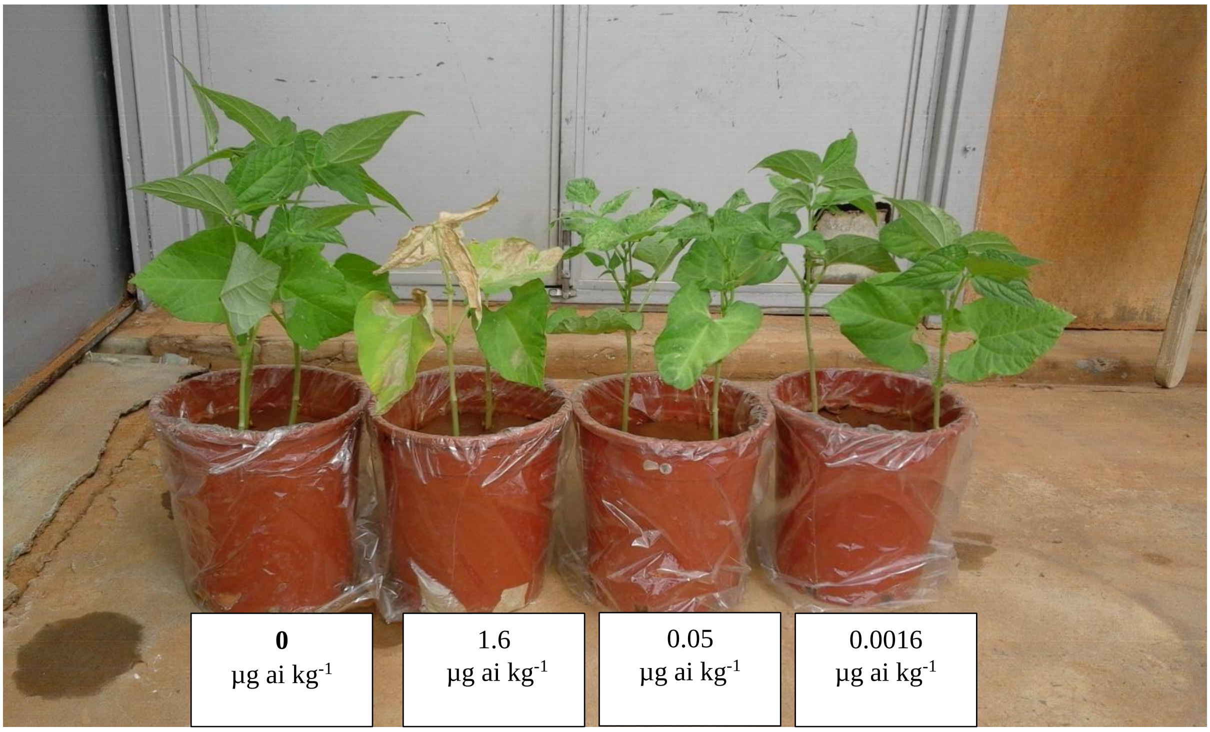

Figure 6. 1: Different phytotoxic symptoms of three mesotrione residual concentrations two weeks after emergence on dry bean.

(c) Morphological parameters

1) Destructive parameters

The effect of three residual mesotrione concentrations on growth of dry bean was measured every two weeks (Figure 6.2). From the figure below it is clear that the three concentrations of mesotrione caused a decrease compared to the control in all measured parameters, depending on the level of mesotrione. The plant growth decreased with an increase in the amount of mesotrione. The results indicated the significant interaction between concentrations (C) and weeks (W) on seedling fresh mass and seedling dry mass. All the measured crop parameters increased significantly at the lowest herbicide concentration (0.0016 µg ai kg^{-1}) compared to the other two concentrations, but still remained lower than the control even after 4 weeks.

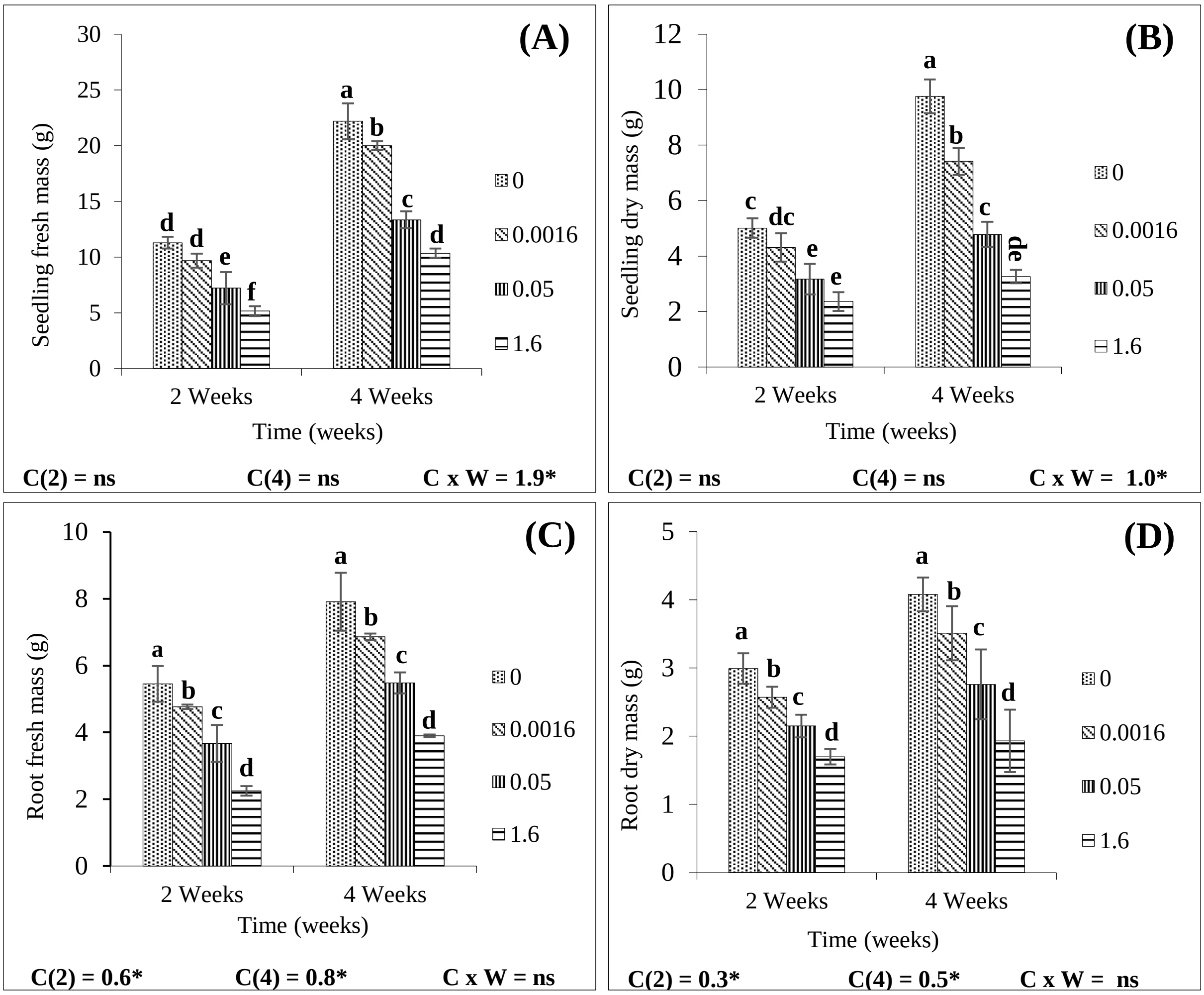

Figure 6. 2: The effect of three concentrations of mesotrione (1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1}, 0.0016 µg ai kg^{-1} and the control (0)) on dry bean growth parameters including (A) seedling fresh mass, (B) seedling dry mass, (C) root fresh mass and (D) root dry mass measured every two weeks over a period of four weeks. Where C (2) = Effect of herbicide two weeks after plant, C (4) = Effect of herbicide after 4 weeks and C x W is the interaction between concentrations and weeks. Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test. Vertical bars with horizontal caps indicate standard deviation.

2) Non-destructive growth parameters

Results presented in Table 6.1 show the effect of three herbicide concentrations (1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1}, 0.0016 µg ai kg^{-1} and the control (0)) of mesotrione on dry bean growth parameters (natural plant height, extended plant height and stem diameter). The results showed decrease in all the measured plant growth depending on the level of mesotrione residue in the soil and this indicated the significant differences between the three concentrations compared to control. The highest concentration (equivalent to 45 DAA) showed more reduction in all the plant growth parameters than the other two concentrations (equivalent to 90 DAA and equivalent to 135 DAA).

Table 6. 1: The morphological response of dry bean to three residual mesotrione concentrations (1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1}, 0.0016 µg ai kg^{-1} and the control (0)) over a period of 12 weeks. Measured parameter includes natural plant height, extended plant height and stem diameter.

	Herbicide residue (µg ai kg^{-1})	**2 Weeks**	**4 Weeks**	**6 Weeks**	**8 Weeks**	**10 Weeks**	**12 Weeks**
Natural plant height (cm)	0	12.9 a	17.9 a	23.9 a	30.3 a	34.4 a	38.8 a
	1.6	7.99 d	12.9 d	17.8 d	23.5 d	27.4 d	32.8 d
	0.05	9.4 c	14.2 c	19.9 c	25.5 c	29.6 c	34.4 c
	0.0016	11.2 b	16.4 b	21.8 b	27.6 b	32.4 b	36.5 b
	LSD_T (5%)	**1.1***	**0.6***	**0.8***	**0.6***	**0.9***	**0.6***
Extended plant height (cm)	0	18.1 a	25.2 a	32.7 a	39.8 a	47.78 a	55.8 a
	1.6	11.2 d	18.4 d	24.9 d	29.95 d	35.5 d	40.5 d
	0.05	14.8 c	20.3 c	26.7 c	32.80 c	38.5 c	44.8 c
	0.0016	16.7 b	23.7 b	29.5 b	35.40 b	40.7 b	47.6 b
	LSD_T (5%)	**0.6***	**0.5***	**0.6***	**0.70**	**0.6***	**0.4***
Stem diameter (mm)	0	3.4 a	3.7 a	4.2 a	4.6 a	4.77 a	5.0 a
	1.6	3.2 d	3.3 d	3.5 d	3.8 d	3.94 d	4.2 d
	0.05	3.2 c	3.4 c	3.8 c	3.9 c	4.19 c	4.4 c
	0.0016	3.4 b	3.5 b	3.9 b	4.3 b	4.42 b	4.7 b
	LSD_T (5%)	**0.03***	**0.1***	**0.1***	**0.1***	**0.07**	**0.1***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

6.3.1.2 Physiological response of dry bean to three residual concentrations of mesotrione (C)

(a) Chlorophyll content

The effect of three concentrations of mesotrione residue on chlorophyll content was measured every two weeks. Results in Figure 6.3 revealed that the interaction between the three concentrations of mesotrione over time signify

cantly affected chlorophyll a. While the main effects concentrations significantly affected all the measured chlorophyll content. The higher the concentration (1.6 µg ai kg^{-1}) in the soil, the lower the chlorophyll content compared to other two concentrations (0.05 µg ai kg^{-1} and 0.0016 µg ai kg^{-1}) respectively.

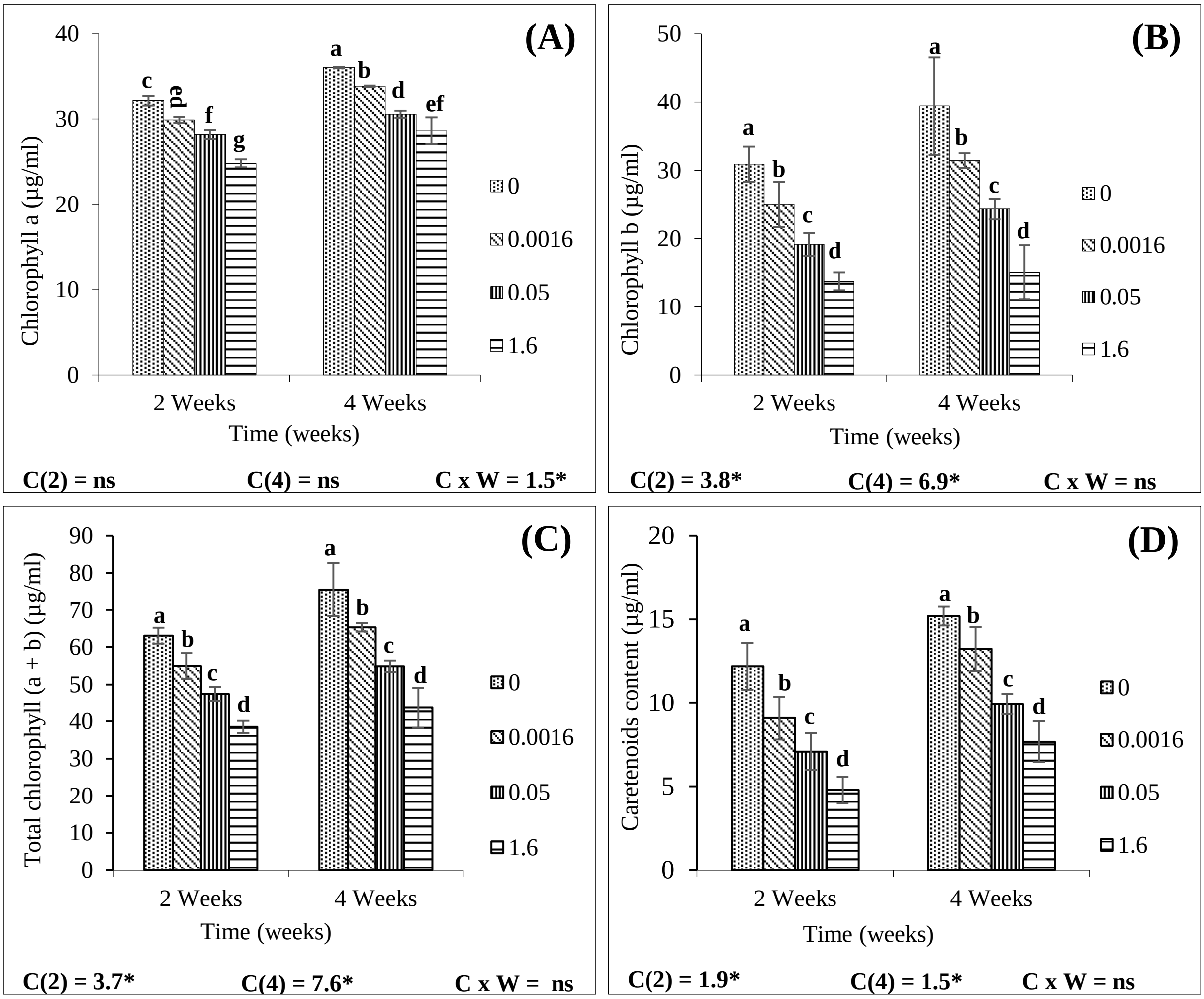

Figure 6. 3: The effect of three residual concentrations of mesotrione (1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1}, 0.0016 µg ai kg^{-1} and the control (0)) on (A) chlorophyll a and (B) chlorophyll b, (C) total chlorophyll and (D) carotenoids content of dry bean measured every two weeks over a period of four weeks. Where C (2) = Effect of herbicide at two weeks after plant, C (4) = Effect of herbicide after 4 weeks and C x W is the interaction between concentrations and weeks. Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test. Vertical bars with horizontal caps indicate standard deviation.

(b) Photosynthetic parameters

The effect of three concentrations of mesotrione on the photosynthetic parameters (photosynthetic rate, stomatal conductance, transpiration rate and sub-stomatal CO_2 concentration) was measured every two weeks and the numbers were treated as absolute values (Figure 6.4). All three concentrations caused a decrease on three of the photosynthetic parameters. However, an increase in sub-stomatal CO_2 concentration at the higher concentration occurred. The results showed a significant interaction only in the case of stomatal conductance. When comparing the main effects, the third concentration showed the highest photosynthetic rate, stomatal conductance and transpiration rate was observed at the low herbicide concentration (0.0016 µg ai kg^{-1}), followed by 0.05 µg ai kg^{-1} and 1.6 µg ai kg^{-1} respectively.

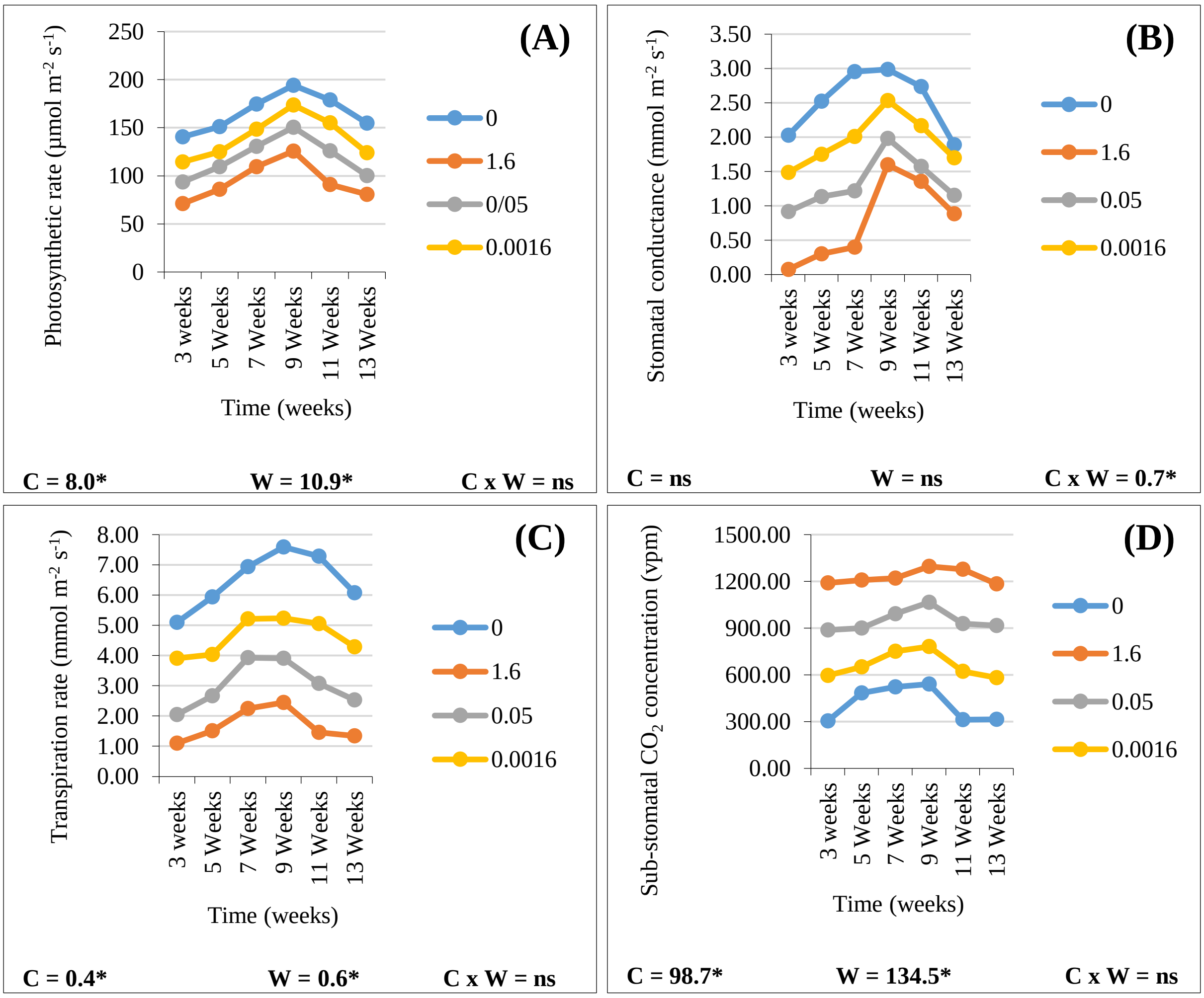

Figure 6. 4: The response of photosynthetic parameters of dry bean to three residual concentrations of mesotrione (1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1}, 0.0016 µg ai kg^{-1} and the control (0)) measured over a period of 12 week. Measured parameters include (A) Photosynthetic rate, (B) Stomatal conductance, (C) Transpiration and (D) Sub-stomatal CO_2 concentration. The interaction is indicated as C x W is interaction between herbicide concentrations and weeks. Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and ns = not significant at 5% using Tukey's LSD test.

6.3.1.3 The effect of three residual concentrations of mesotrione on dry bean yield

(a) Yield

The table below shows the yield response to three residual concentrations of mesotrione (Table 6.2). The results showed a significant reduction in yield and yield parameters depending on the amount of mesotrione in the soil and biggest decline was observed with 1.6 µg ai kg^{-1} followed by 0.05 µg ai kg^{-1} and 0.0016 µg ai kg^{-1} respectively.

Table 6. 2: The effect of three residual concentrations of mesotrione (1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1}, 0.0016 µg ai kg^{-1} and the control (0)) on dry bean yield components. Measured parameters include number of pods per pot, mass of pods per pot, number of seeds per pot and mass of seeds per pot (final yield per pot).

Herbicide residue (µg ai kg^{-1})	Number of pods per pot	Mass of pods per pot (g)	Number of seeds per pot	Mass of seeds per plant (g) (yield per pot)
0	20.0 a	15.6 a	30.0 a	13.5 a
1.6	6.8 d	4.9 d	13.8 d	3.9 d
0.05	12.5 c	8.9 c	19.8 c	7.5 c
0.0016	16.0 b	12.0 b	24.3 b	9.5 b
LSD_T (5%)	**3.2***	**3.1***	**2.9***	**1.5***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

6.3.2 The effect of three different application methods of brassinosteroids (T) on dry bean.

In both two growing seasons (2015/16 and 2016/17) the results show the same trend so the results of one season will be presented since there is no difference between the two seasons.

6.3.2.1 The effect on seedling emergence and morphological parameters

(a) Emergence percentage

All the seeds planted gave 100% emergence and therefore there was no significant difference between the different treatments.

(b) Symptoms of the crop injury

The brassinosteroids had no phytotoxic effects on the growth so no crop injury occurred.

(c) Morphological parameters

1) Destructive parameters

The figure below shows the effect of three application methods of brassinosteroids (T) on morphological parameters of dry bean and the parameters were measured every two weeks (Figure 6.5). The interaction between application method and weeks showed no significance. While all the main effects differed significantly from the control. All the applied brassinosteroids methods caused substantial increase in dry bean growth compared to the control. The highest increase was observed with the foliar application, followed by the soil drench and seed treatment respectively.

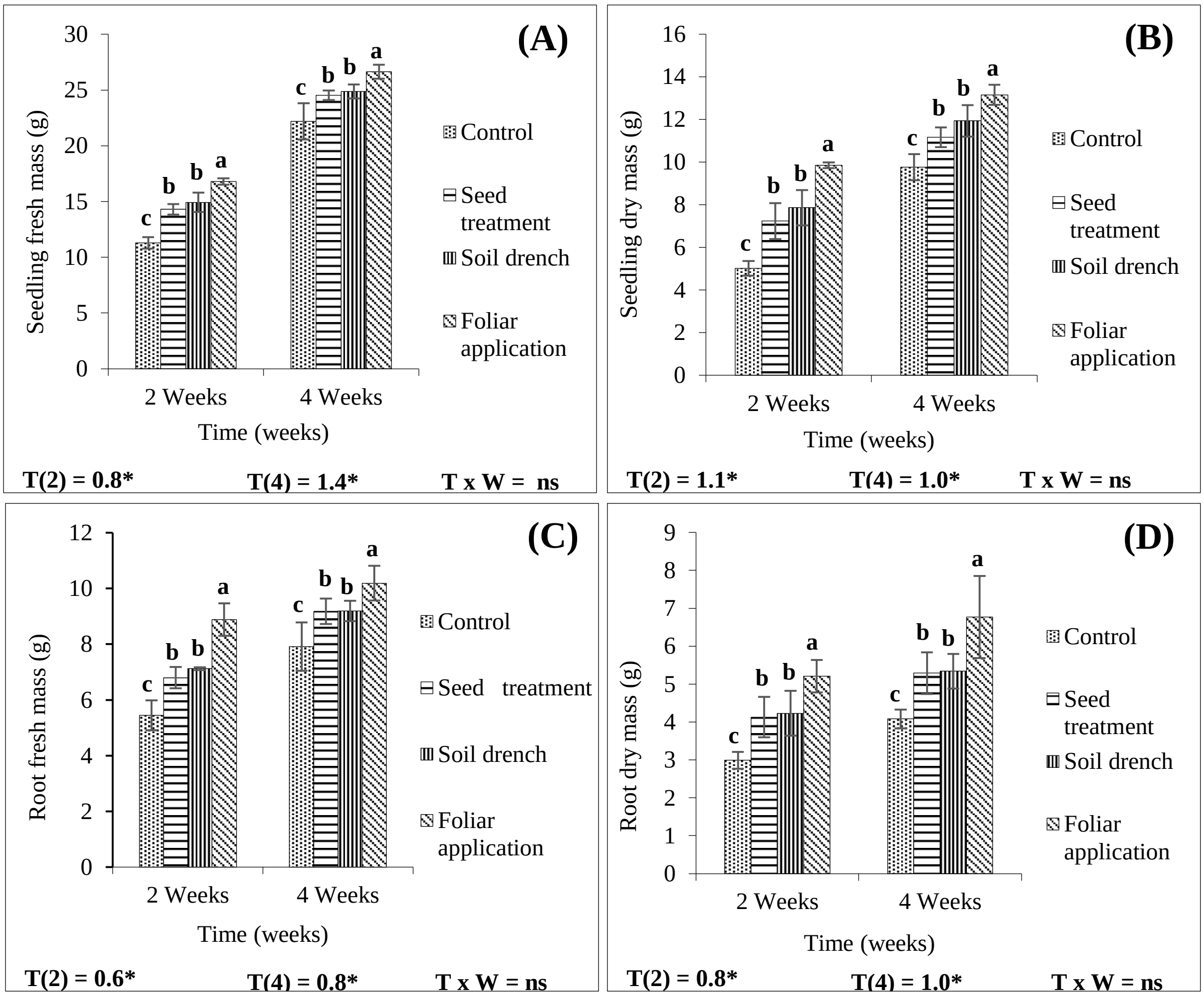

Figure 6. 5: The morphological response of dry bean to three brassinosteroids application methods (T), seed treatment, soil drench, foliar application and control on destructive seedling growth parameters including (A) seedling fresh mass, (B) seedling dry mass, (C) root fresh mass and (D) root dry mass measured every two weeks over a period of four weeks. Where T (2) = Effect of brassinosteroids at two weeks after plant, T (4) = Effect of brassinosteroids after 4 weeks and T x W is is the interaction between brassinosteroids and weeks. Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test. Vertical bars with horizontal caps indicate standard deviation.

2) Non-destructive parameters

All the applied methods of BRs had marked increase effect as compared to control in all the measured morphological parameters (natural plant height, extended plant height and stem diameter) (Table 6.3). Foliar application showed the highest plant growth followed by soil drench and seed treatment respectively. In natural plant height during the first two weeks there was significant differences between the treatments but afterwards seed treatment and soil drench showed no significant differences while foliar application showed significant differences as compare to seed treatment, soil drench and control. Extended plant height showed significant difference between the treatments during the second week and no significant differences was observed between soil drench and seed treatment during the fourth week up to 12 but there was significant differences between foliar application and both soil drench and seed treatment. No significant differences was observed between seed treatment and soil drench in stem diameter but the significant differences was observed between foliar application and both seed treatment and soil drench as compared to control.

Table 6. 3: The effect of three brassinosteroids application methods, seed treatment, soil drench, foliar application and control on morphological parameter of dry bean measured over a period of 12 weeks. Measured parameters being natural plant height, extended plant height and stem diameter.

	Treatment	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks	12 Weeks
Natural plant height (cm)	Control	12.9 d	17.9 d	23.9 c	30.3 c	34.4 c	38.8 c
	Seed treatment	14.6 c	18.8 c	26.9 b	32.9 b	38.8 b	43.9 b
	Soil drench	16.7 b	21.8 b	27.3 b	33.5 b	39.1 b	44.1 b
	Foliar application	18.9 a	24.8 a	30.5 a	36.7 a	41.5 a	47.2 a
	LSD_T (5%)	**0.7***	**0.5***	**0.7***	**0.7***	**0.8***	**0.6***
Extended plant height (cm)	Control	18.1 d	25.2 c	32.7 c	39.8 c	47.8 c	55.8 c
	Seed treatment	21.1 c	27.8 b	35.9 b	43.9 b	51.8 b	57.8 b
	Soil drench	22.1 b	28.1 b	36.0 b	44.2 b	52.1 b	58.1 b
	Foliar application	25.2 a	31.5 a	38.3 a	46.9 a	54. a	61.6 a
	LSD_T (5%)	**1.0***	**0.6***	**0.5***	**0.9***	**0.7***	**0.9***
Stem diameter (mm)	Control	3.4 c	3.7 c	4.2 c	4.6 c	4.8 c	5.0 c
	Seed treatment	3.7 b	3.9 b	4.4 b	4.8 b	5.0 b	5.2 b
	Soil drench	3.7 b	4.0 b	4.4 b	4.8 b	5.0 b	5.3 b
	Foliar application	4.0 a	4.4 a	4.8 a	5.3 a	5.4 a	5.7 a
	LSD_T (5%)	**0.1***	**0.1***	**0.1***	**0.04***	**0.04***	**0.1***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

6.3.2.2 The effects on physiological parameters

(a) Chlorophyll content

The results presented in Figure 6.6 show the effect of three application methods of brassinosteroids on chlorophyll content measured every two weeks. The results revealed that there is significant differences between the three main effects the applied methods of brassinosteroids compared to the control, but there is no significant differences between seed treatment and soil drench. Foliar application showed the highest chlorophyll and carotenoid content compared to the other applications. On the other hand, the significant interaction (T x W) between treatments (brassinosteroids) over weeks was observed only in the case of chlorophyll a.

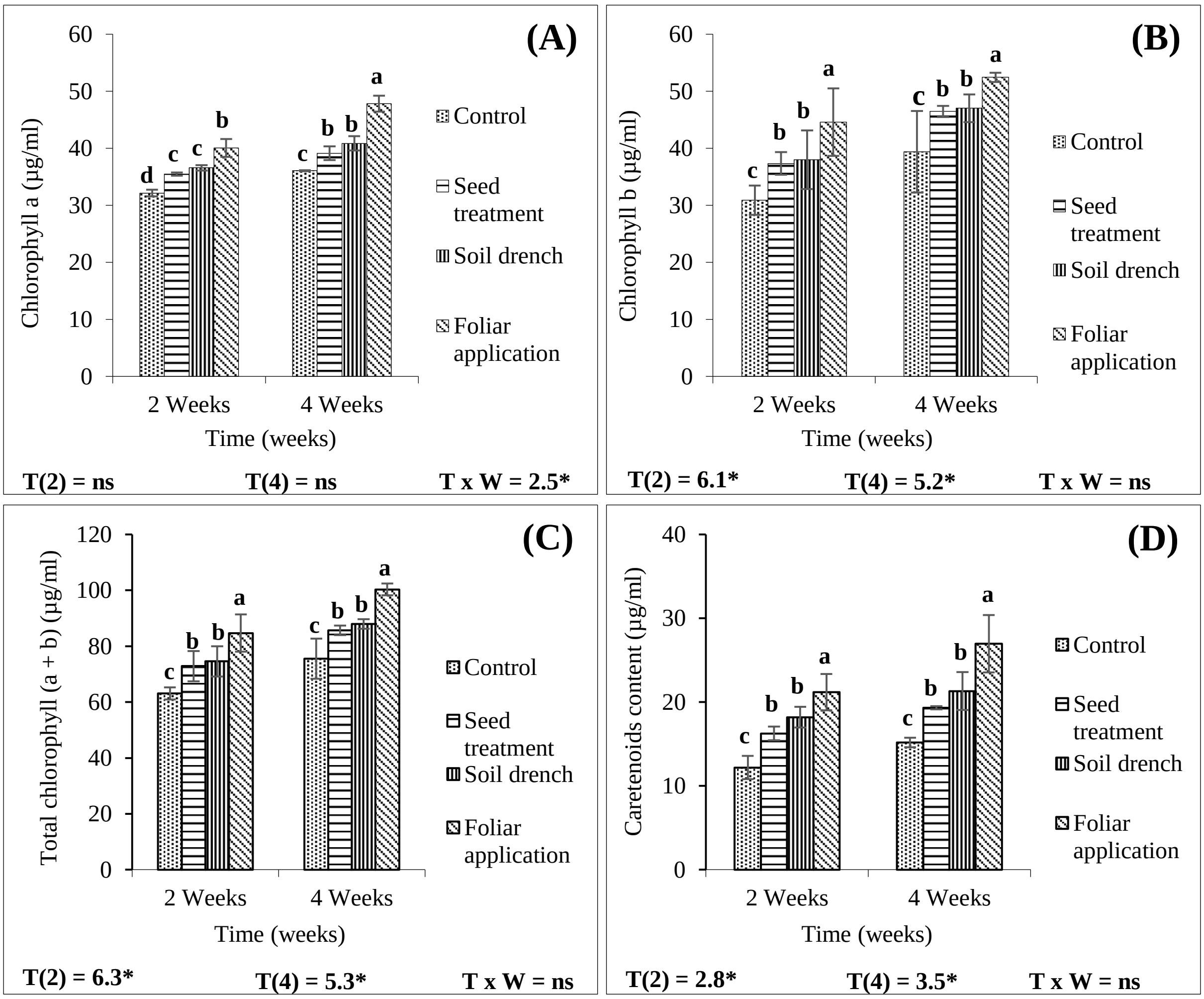

Figure 6. 6: The effect of three application methods of brassinosteroids seed treatment, soil drench, foliar application and control) on (A) chlorophyll a, (B) chlorophyll b, (C) total chlorophyll and (D) carotenoids content measured every two weeks every two weeks over a period of four weeks. Where T (2) = Effect of brassinosteroids at two weeks after plant, T (4) = Effect of brassinosteroids after 4 weeks and T x W is the interaction between brassinosteroids and weeks. Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test. Vertical bars with horizontal caps indicate standard deviation.

(b) Photosynthetic parameters

The effect of different application methods of brassinosteroids on photosynthetic parameters (photosynthetic rate, stomatal conductance, transpiration rate, sub-stomatal CO_2 concentration) was measured on a two-weekly basis until the twelfth week (Figure 6.7) and photosynthetic rate numbers were taken as absolute values. All the measured parameters showed significant interaction between treatments and weeks (T x W) except the photosynthetic rate. The results revealed that there were no significant differences between seed treatment and soil drench but the significant differences were observed between foliar application and both seed treatment and soil drench respectively. Foliar application showed the highest results followed by soil drench and seed treatment respectively.

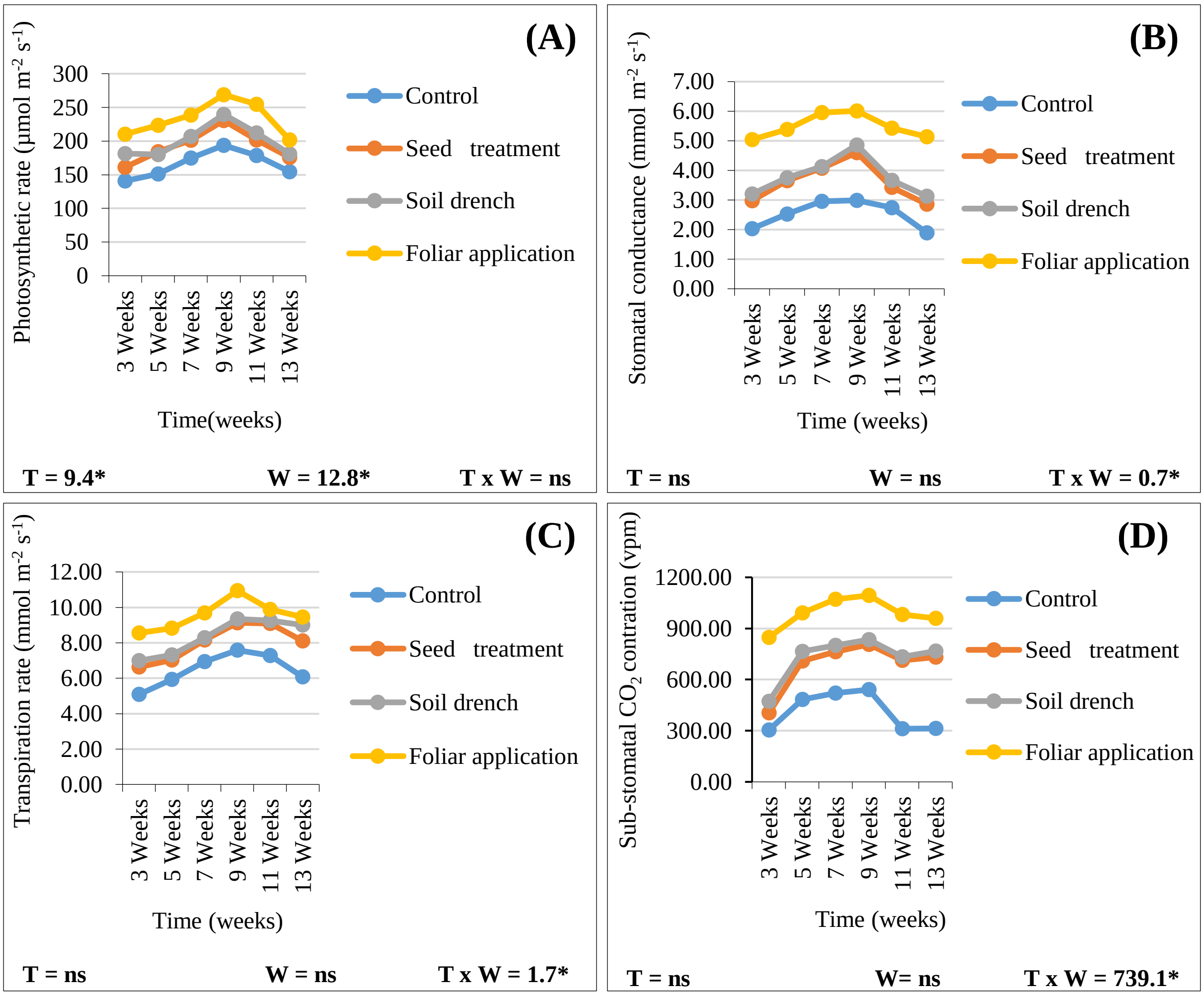

Figure 6. 7: The physiological response of dry bean to three application methods of BRs seed treatment, soil drench, foliar application and control measured over 12 weeks. Measured parameters include (A) Photosynthetic rate, (B) Stomatal conductance, (C) Transpiration rate, (D) Sub-stomatal CO_2 concentration. The interaction is indicated as T x W (Interaction between brassinosteroids and weeks). Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

6.3.2.3 The effect of three application methods of brassinosteroids on yield parameters

(a) Yield components

The three applications methods of brassinosteroids caused significant increase in the measured yield and yield components of dry bean compared to the control (Table 6.4). The results showed the highest yield with the foliar application, seed treatment and soil drench respectively compared to the control

Table 6. 4: The effect of three methods applications of brassinosteroids seed treatment, soil drench, foliar application and control on dry bean yield components (number of pods per pot, mass of pods per pot, number of seeds per pot and mass of seeds per pot (final yield per pot).

Treatment	Number of pods per pot	Mass of pods per pot (g)	Number of seeds per pot	Mass of seeds per pot (g) (yield per pot)
Control	20.0 c	15.6 c	30.0 c	13.5 c
Seed treatment	23.8 b	19.7 b	43.3 b	16.2 b
Soil drench	24.3 b	21.9 b	44.8 b	17.6 b
Folia application	30.0 a	28.3 a	48.5 a	23.0 a
LSDT (5%)	**2.4***	**2.8***	**3.4***	**2.5***

Statistical significance is indicated as LSD and different letters.

6.3.3 The effect of brassinosteroids combined with mesotrione on dry bean.

In both two growing seasons (2015/16 and 2016/17) the results show the same trend so the results of one season will be presented since there is no difference between the two seasons.

6.3.3.1 The effect on seedling emergence and morphological parameters

(a) Emergence percentage

All the seeds planted gave 100% emergence and therefore there was no significant difference between the different treatments.

(b) Symptoms of the crop injury

The brassinosteroids in combination with the mesotrione had no phytotoxic effects on the growth.

(c) Morphological parameters

1) Destructive parameters

The effect of different application methods of brassinosteroids combined with various mesotrione concentrations on seedling fresh mass, seedling dry mass, root fresh mass and root dry mass of dry bean is shown in Table 6.5. The significant interaction between herbicide and brassinosteroids (C) over weeks (T) was seen in all the measured parameters except seedling fresh mass at week 2 and root dry mass. All the applied methods of brassinosteroids in combination with the herbicide caused an increase in seedling fresh mass, seedling dry mass, root fresh mass and root dry mass compared to the control as see before the foliar application again showed the best results in enhancing the growth.

Table 6. 5: The effect of different brassinosteroids applications combined with various mesotrione concentrations remaining in the soil on dry bean seedling fresh mass, seedling dry mass, root fresh mass and root dry mass.

		Seedling fresh mass (g)		**Seedling dry mass (g)**		**Root fresh mass (g)**		**Root dry mass (g)**	
Treatment	**Herbicide residue (μg ai kg^{-1})**	**2 Weeks**	**4 Weeks**	**2 Weeks**	**4 Weeks**	**2 Weeks**	**4 Weeks**	**2 Weeks**	**4 Weeks**
Control	0	11.3	22.2 dc	5.0 gfe	9.8 cd	5.5 de	7.9 dc	3.0	4.1
	1.6	5.2	10.3 g	2.4 i	3.3 f	2.3 i	3.9 f	1.7	1.9
	0.05	7.2	13.4 f	3.3 ih	4.8 f	3.7 gh	5.5 e	2.2	2.8
	0.0016	9.7	20.0 ed	4.3 gh	7.4 e	4.8 gef	6.9 d	2.6	3.51
Seed treatment	0	14.3	24.5 bac	7.2 dc	11.2 bc	6.8 c	9.2 ba	4.1	5.3
	1.6	7.4	12.3 gf	4.3 gfh	7.0 e	3.1 ih	4.2 fe	2.2	2.7
	0.05	9.8	19.3 e	5.9 dfe	9.3 cd	4.8 dgef	7.8 d	3.0	3.6
	0.0016	14.1	24.1 bc	7.0 dc	11.0 bc	6.2 dc	9.1 bac	4.1	5.2
Soil drench	0	14.9	24.9 ba	7.9 bc	11.9 ba	7.1 bc	9.2 ba	4.2	5.3
	1.6	8.3	12.5 gf	4.5 gfh	7.3 e	3.4 ih	4.4 fe	2.3	2.9
	0.05	11.2	20.4 ed	6.4 dce	10.1 cd	5.1 def	8.0 bdc	3.2	4.0
	0.0016	14.4	24.6 ba	7.8 bc	11.8 ba	7.1 bc	9.2 bac	4.2	5.0
Foliar application	0	16.8	26.7 a	9.85 a	13.2 a	8.9 a	10.19 a	5.2	6.8
	1.6	10.1	14.4 f	5.2 gfe	7.7 e	3.9 ghf	5.0 fe	2.9	3.0
	0.05	12.3	24.0 bc	7.2 dc	10.8 bcd	5.1 def	8.1 bdc	3.9	4.9
	0.0016	16.3	26.1 ba	9.4 ba	13.0 a	8.3 ba	10.1 a	5.0	6.0
C x T: LSD$_T$ (5%)		**ns**	**2.4***	**1.6***	**1.6***	**1.4***	**1.3***	**ns**	**ns**

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test. C x T indicates the interaction between brassinosteroids (treatments) and concentrations.

The differences in the main effects brassinosteroids application methods on the morphological on seedling fresh mass at two weeks and the root dry mass is shown in Table 6.6. All the applied methods of brassinosteroids significantly increased the plant growth parameters compared to the control. Foliar application showed the highest growth followed by the soil drench and seed treatment respectively. There was no significant difference between the seed treatment and the soil drench.

Table 6. 6: The differences between brassinosteroids application methods (seed treatment, soil drench, foliar application and control) on seedling fresh mass and root dry mass over 4 weeks.

	Seedling fresh mass (g)	**Root dry mass (g)**	
Treatment	**2 Weeks**	**2 Weeks**	**4 Weeks**
Control	8.3 d	2.4 c	3.1 c
Seed treatment	11.4 c	3.4 b	4.2 b
Soil drench	12.2 b	3.5 b	4.4 b
Foliar application	13.9 a	4.3 a	5.2 a
LSD_T (0.05)	**0.7***	**0.3***	**0.5***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

The differences between three residual mesotrione concentrations on morphological parameters was summarized in Table 6.7. Only the main effects that showed no significant interactions in shown Two of the three concentrations caused a significant decrease in all measured growth parameters however the lowest concentration did not differ considerably from the control. The reduction in growth increased with an increase in concentration as expected.

Table 6. 7: The differences between three residual concentrations of mesotrione (1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1}, 0.0016 µg ai kg^{-1} and the control (0)) on seedling fresh mass, seedling dry mass, root fresh mass and root dry mass over 4 weeks.

	Seedling fresh mass (g)	**Root dry mass (g)**	
Herbicide residue (µg ai kg^{-1})	**2 Weeks**	**2 Weeks**	**4 Weeks**
0	14.3 a	4 a	5.4 a
1.6	7.7 c	2 c	2.6 c
0.05	10.1 b	3 b	3.8 b
0.0016	13.6 a	4 a	5.0 a
LSD_T (0.05)	**0.7***	**0.3***	**0.5***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

2) Non-destructive

(i)Natural plant height

The effect of brassinosteroids combined with mesotrione on dry bean natural plant height is summarized in Table 6.8. The interaction between the applied methods of brassinosteroids combined with mesotrione had a substantial impact on the crop, causing an increase in the natural plant height. The foliar application showed the highest growth followed by soil drench and seed treatment respectively over the 12 weeks.

Table 6. 8: The effect of different brassinosteroids applications combined with mesotrione concentrations (1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1}, 0.0016 µg ai kg^{-1} and the control (0)) on natural plant height (cm) of dry beans over a period of 12 weeks.

Treatment	**Herbicide residue (µg ai kg-1)**	**2 Weeks**	**4 Weeks**	**6 Weeks**	**8 Weeks**	**10 Weeks**	**12 Weeks**
Control	0	12.9 d	17.9 de	23.9 ef	30.3 f	34.4 ed	38.8 ef
	1.6	7.9 h	12.9 i	17.8 i	23.5 k	27.4 h	32.8 i
	0.05	9.4 g	14.2 h	19.9 h	25.5 j	29.6 g	34.4 h
	0.0016	11.2 ef	16.4 fg	21.8 g	27.6 h	32.4 f	36.5 g
Seed treatment	0	14.6 c	18.8 dc	26.9 cb	32.9 dc	38.8 b	43.9 b
	1.6	8.5 hg	14.3 h	21.3 gh	26.5 i	32.8 f	38.2 f
	0.05	10.6 f	16.1 g	22.6 gf	27.5 h	35.6 d	40.4 d
	0.0016	14.2 c	18.3 de	26.0 cd	32.5 d	38.5 b	43.6 b
Soil drench	0	16.7 b	21.8 b	27.3 cb	33.5 c	39.1 b	44.1 b
	1.6	9.3 g	16.8 fg	22.0 g	28.2 h	33.5 ef	39.4 ed
	0.05	14.7 c	19.4 c	24.7 ed	31.5 e	36.9 c	41.7 c
	0.0016	16.2 b	21.5 b	26.9 cb	33.1 dc	38.5 b	44.1 b
Foliar application	0	18.9 a	24.8 a	30.5 a	36.7 a	41.5 a	47.2 a
	1.6	11.9 ed	17.3 fe	23.9 ef	29.2 g	35.1 d	40.1 d
	0.05	16.0 b	21.8 b	28.0 b	34.8 b	39.5 b	46.2 a
	0.0016	18.6 a	24.2 a	30.1 a	36.4 a	41.0 a	47.0 a
C x T: LSD_T (5%)		**1.2***	**1.1***	**1.5***	**0.9***	**1.2***	**1.0***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test. C x T indicates the interaction between brassinosteroids (treatments) and concentrations.

(ii) Extended plant height

The interaction between the different brassinosteroid application methods combined with mesotrione caused significant increase to extended plant height compared to the control (Table 6.9). The foliar application showing the highest growth followed by soil drench and seed treatment respectively. The brassinosteroids on its own also significantly increased the growth compared to the control without herbicide stress.

Table 6. 9: The effect of different brassinosteroids applications (T) combined with various mesotrione concentrations (C) remaining in the soil using three different applications on extended plant height (cm) of dry beans over a period of 12 weeks.

Treatment	Herbicide residue (μg ai kg^{-1})	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks	12 Weeks
Control	0	18.1 f	25.2 e	32.7 d	39.8 d	47.8 c	55.8 c
	1.6	11.2 i	18.4 i	24.9 g	30.0 i	35.5 h	40.5 h
	0.05	14.8 h	20.3 h	26.7 f	32.8 h	38.5 g	44.8 g
	0.0016	16.7 g	23.7 f	29.5 e	35.4 g	40.7 f	47.6 f
Seed treatment	0	21.1 cd	27.8 c	35.9 b	43.9 b	51.8 b	57.8 b
	1.6	14.8 h	21.6 g	27.1 f	32.9 h	39.6 gf	45.4 g
	0.05	17.2 gf	23.9 f	30.3 e	36.9 f	42.5 e	48.8 e
	0.0016	20.6 d	27.3 cd	35.3 cb	43.6 cb	51.5 b	57.5 b
Soil drench	0	22.1 cb	28.1 cb	36.0 b	44.2 b	52.1 b	58.1 b
	1.6	16.6 g	23.6 f	29.5 e	36.8 f	42.8 e	48.7 e
	0.05	20.6 d	25.8 e	34.3 c	39.3 ed	46.5 d	52.5 d
	0.0016	22.0 cb	28.0 c	35.9 b	43.8 cb	51.8 b	58.1 b
Foliar application	0	25.2 a	31.5 a	38.3 a	46.9 a	54.2 a	61.6 a
	1.6	19.2 e	26.1 ed	31.8 d	38.5 e	45.7	51.8 d
	0.05	22.9 b	29.3 b	35.6 b	42.8 c	48.6 c	55.7 c
	0.0016	25.0 a	30.9 a	38.1 a	46.3 a	54.1 a	61.1 a
C x T: LSD$_T$ (5%)		**1.0***	**1.3***	**1.2***	**1.1***	**1.2***	**1.0***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test. C x T indicates the interaction between brassinosteroids (treatments) and concentrations.

(iii) Stem diameter

The effect the interaction of different brassinosteroid application methods combined with mesotrione over time on the stem diameter caused significant increases compared to the control (Table 6.10). This shows that brassinosteroids have a positive impact on the plant growth regardless of the presence of mesotrione in the soil. The foliar application showed the highest growth followed by soil drench and seed treatment respectively. Even when the brassinosteroids were applied alone and in combination with the mesotrione concentrations it significantly increased the growth compared to the mesotrione on its own as well as to the control without the herbicide.

Table 6. 10: The effect of different brassinosteroids applications methods combined with mesotrione concentrations on stem diameter of dry beans (mm) over a period of 12 weeks.

Treatments	Herbicide residue (µg ai kg^{-1})	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks	12 Weeks
Control	0	3.4 ed	3.7 ed	4.2 d	4.6 d	4.8 d	5.0 d
	1.6	3.2 g	3.3 i	3.5 i	3.8 j	3.9 i	4.2 h
	0.05	3.2 gf	3.4 h	3.8 hg	3.9 i	4.2 h	4.4 g
	0.0016	3.4 e	3.5 gfh	3.9 f	4.3 f	4.4 fe	4.7 e
Seed treatment	0	3.7 cb	3.9 cb	4.4 b	4.8 cb	5.0 cb	5.2 b
	1.6	3.2 gf	3.5 gh	3.6 h	3.9 i	4.0 i	4.3 hg
	0.05	3.4 e	3.6 ef	4.0 fe	4.2 h	4.3 hg	4.5 f
	0.0016	3.6 c	3.9 c	4.4 cb	4.8 cb	5.0 cb	5.2 cb
Soil drench	0	3.7 cb	4.0 b	4.4 b	4.8 b	5.0 b	5.3 b
	1.6	3.3 f	3.5 gf	3.9 fg	4.2 gh	4.3 fg	4.5 f
	0.05	3.5 d	3.8 d	4.1 e	4.5 e	4.8 d	5.1 c
	0.0016	3.7 cb	4.0 cb	4.4 b	4.8 b	5.0 b	5.3 b
Foliar application	0	4.0 a	4.4 a	4.8 a	5.3 a	5.4 a	5.7 a
	1.6	3.4 e	3.7 ed	3.9 fe	4.3 gf	4.5 e	4.8 e
	0.05	3.7 b	3.9 cb	4.3 cd	4.7 c	4.9 c	5.6 a
	0.0016	3.9 a	4.4 a	4.8 a	5.3 a	5.4 a	5.6 a
C x T: LSD_T (5%)		**0.1***	**0.1***	**0.1***	**0.1***	**0.1***	**0.1***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test. C x T indicates the interaction between brassinosteroids (treatments) and concentrations.

6.3.3.2 Physiological response of dry bean against brassinosteroids combined with mesotrione residue

(a) Chlorophyll content

The effect of different brassinosteroids application methods combined with mesotrione over time on chlorophyll content was measured on a two-weekly basis until the fourth week (Table 6.11). The three application methods of brassinosteroids caused over time a significant increase in chlorophyll content in the presence of mesotrione res. More chlorophyll content was observed in 0.0016 µg ai kg^{-1} mesotrione by all the three brassinosteroids with foliar application as the highest followed by soil drench and seed treatment respectively. The interaction between herbicide and brassinosteroids are not significant to all the measured parameters. For example, in the case of chlorophyll b the interaction between the two was non-significant, but the main effects of brassinosteroids as well as the mesotrione concentrations significantly differed.

Table 6. 11: The effect of different brassinosteroids applications combined with various mesotrione concentrations on chlorophyll a and chlorophyll b content.

		Chlorophyll a (µg/ml)		Chlorophyll b (µg/ml)	
Treatment	**Herbicide residue (µg ai kg^{-1})**	**2 Weeks**	**4 Weeks**	**2 Weeks**	**4 Weeks**
Control	0	32.2 ed	36.1 fced	30.9	39.4
	1.6	24.8 h	28.6 h	13.7	15.1
	0.05	28.2 gf	30.6 hg	19.1	24.3
	0.0016	29.9 f	33.9 feg	25.0	31.4
Seed treatment	0	35.5 cb	39.2 cbd	37.4	46.5
	1.6	27.6 g	32.6 fhg	17.6	21.6
	0.05	30.2 ef	35.3 fegd	24.5	30.0
	0.0016	34.1 cd	38.8 cebd	37.0	42.8
Soil drench	0	36.6 b	40.9 cb	38.0	47.1
	1.6	30.2 ef	33.0 fhg	21.7	26.7
	0.05	33.9 cd	37.0 fcebd	28.7	33.9
	0.0016	35.7 cb	40.0 cbd	37.0	46.8
Foliar application	0	40.1 a	47.9 a	44.6	52.5
	1.6	34.0 cd	35.2 fegd	26.7	32.1
	0.05	37.4 b	41.3 b	35.4	37.7
	0.0016	40.3 a	47.3 a	44.0	50.1
C x T: LSD$_T$ (5%)		**2.2***	**5.0***	**ns**	**ns**

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test. C x T indicates the interaction between brassinosteroids (treatments) and concentrations.

All the application methods of the brassinosteroids caused an increase in the chlorophyll b content compared to the control (Figure 6.12). In chlorophyll b no real differences could be seen between seed treatment and soil drench, but they differ significantly compared to control. The most efficient treatment again was the foliar application and was significantly higher than the rest of the treatments.

Table 6. 12: The differences between brassinosteroids application methods (seed treatment, soil drench, foliar application and control) on chlorophyll a and chlorophyll b over 4 weeks.

	Chlorophyll b (µg/ml)	
Treatment	**2 Weeks**	**4 Weeks**
Control	22.2 c	27.6 c
Seed treatment	29.1 b	35.2 b
Soil drench	31.4 b	38.6 b
Foliar application	37.7 a	43.1 a
LSD_T (0.05)	**2.8***	**3.5***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

The effect of three residual concentrations of mesotrione on chlorophyll content was measured every two weeks (Table 6.13). During the measured period the results showed significant differences between the three, with the highest chlorophyll content observed in 0.0016 µg ai kg^{-1} residual herbicide compared to the other two concentrations.

Table 6. 13: The differences between three residual concentrations of mesotrione (1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1}, 0.0016 µg ai kg^{-1} and the control (0)) on chlorophyll a and chlorophyll b over 4 weeks.

	Chlorophyll b(µg/ml)	
Herbicide residue (µg ai kg^{-1})	**2 Weeks**	**4 Weeks**
0	37.72 a	46.4 a
1.6	20.0 c	23.9 d
0.05	27.0 b	31.5 c
0.0016	35.7 a	42.8 b
LSD_T (0.05)	**2.8***	**3.5***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

The interaction between brassinosteroids combined with mesotrione on the total chlorophyll and carotenoids was measured on a two weekly basis and showed no significant differences (Table 6.14). Thus, the main effects are summarized in Tables 6.15 and 6.16. Results indicated that, all the applied methods of brassinosteroids caused a significant increase in total chlorophyll and carotenoids content (Table 6.15). All three application methods significantly increased the total chlorophyll and carotenoid content compared to the control. In Table 6.16

the mesotrione concentrations also decreased the total chlorophyll and carotenoid content markedly compared to the control without herbicide. But at the lower mesotrione concentration (0.0016 μg ai kg^{-1}) no significant differences were observed compared to the treatment without the herbicide.

Table 6. 14: The effect of different brassinosteroids applications combined with various mesotrione concentrations remaining in the soil on total chlorophyll (a + b) and carotenoids content.

		Total chlorophyll (a +b) (μg/ml)		**Carotenoids content (μg/ml)**	
Treatment	**Herbicide residue (μg ai kg^{-1})**	**2 Weeks**	**4 Weeks**	**2 Weeks**	**4 Weeks**
Control	0	63.1	75.5	12.2	15.2
	1.6	38.6	43.7	4.8	7.7
	0.05	47.4	54.9	7.2	9.9
	0.0016	54.9	65.3	9.10	13.2
Seed treatment	0	72.9	85.6	16.3	19.3
	1.6	45.2	53.2	6.1	10.1
	0.05	54.6	65.3	10.4	14.3
	0.0016	71.1	81.6	14.8	17.6
Soil drench	0	74.6	87.9	18.2	21.3
	1.6	51.9	59.7	10.1	14.2
	0.05	62.5	70.9	13.5	17.9
	0.0016	72.7	86.8	17.0	20.8
Foliar application	0	84.7	100.3	21.2	27.0
	1.6	60.7	67.3	14.2	17.2
	0.05	72.8	79.0	17.9	22.0
	0.0016	84.3	97.4	20.9	26.5
C x T: LSD_T (5%)		**ns**	**ns**	**ns**	**ns**

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test. C x T indicates the interaction between brassinosteroids (treatments) and concentrations.

Table 6. 15: The differences between brassinosteroids application methods (seed treatment, soil drench, foliar application and control) on total chlorophyll (a +b) and carotenoids content over 4 weeks.

	Total chlorophyll (a +b) (µg/ml)		**Carotenoids content (µg/ml)**	
Treatment	**2 Weeks**	**4 Weeks**	**2 Weeks**	**4 Weeks**
Control	51.0 d	59.9 d	8.3 d	11.5 d
Seed treatment	60.9 c	71.4 c	11.9 c	15.3 c
Soil drench	65.4 b	76.4 b	14.7 b	18.5 b
Foliar application	75.6 a	86.0 a	18.5 a	23.2 a
LSD_T (0.05)	**3.0***	**4.3***	**1.6***	**1.6***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

Table 6. 16: The differences between three residual concentrations of mesotrione (1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1}, 0.0016 µg ai kg^{-1} and the control (0)) on total chlorophyll (a +b) and carotenoids content over 4 weeks.

	Total chlorophyll (a +b) (µg/ml)		**Carotenoids content (µg/ml)**	
Herbicide residue (µg ai kg^{-1})	**2 Weeks**	**4 Weeks**	**2 Weeks**	**4 Weeks**
0	73.8 a	87.3 a	17.0 a	20.7 a
1.6	49.1 d	56.0 d	8.8 c	12.3 c
0.05	59.3 c	67.5 c	12.2 b	16.0 b
0.0016	70.7 b	82.8 b	15.4 a	19.6 a
LSD_T (0.05)	**3.0***	**4.3***	**1.6***	**1.6***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

(b) Photosynthetic parameters

The effect of brassinosteroids combined with mesotrione on photosynthetic parameters (photosynthetic rate, stomatal conductance, transpiration rate, sub-stomatal CO_2 concentration) was measured every two weeks (Table 6.17, 6.20, 6.21 and 6.24). A significant interaction between herbicide and brassinosteroids was observed over time on the photosynthetic rate at week 5. All the applied methods of brassinosteroid combined with mesotrione caused an increase in photosynthetic parameters, although not significantly, but still showing that brassinosteroids can have a positive impact in the presence of herbicides.

Table 6. 17: The effect of different brassinosteroids applications combined with various mesotrione concentrations on dry bean photosynthetic rate calculated as percentage difference from the control.

Treatment	Herbicide residue (µg ai kg^{-1})	3 weeks	5 Weeks	7 Weeks	9 Weeks	11 Weeks	13 Weeks
Control	0	100.0	108.8 cd	125.3	138.9	128.0	110.8
	1.6	51.3	61.5 g	78.5	90.2	64.6	57.6
	0.05	67.3	78.3 gf	93.4	108.0	90.2	71.1
	0.0016	83.7	92.0 edf	109.2	127.8	114.0	90.9
Seed treatment	0	115.6	131.9 b	144.3	165.0	144.7	125.9
	1.6	63.7	86.1 ef	91.8	100.1	78.6	68.6
	0.05	85.9	99.8 ed	109.0	125.2	105.4	99.2
	0.0016	100.5	125.8 cb	138.4	154.1	137.9	114.2
Soil drench	0	129.9	128.9 b	148.2	171.5	152.8	129.5
	1.6	71.1	92.0 edf	101.6	116.1	86.6	78.0
	0.05	94.7	105.3 d	120.8	138.9	114.8	107.4
	0.0016	121.4	128.4 b	143.7	161.6	148.0	121.6
Foliar application	0	150.3	160.0 a	169.6	192.7	181.7	144.4
	1.6	88.6	100.5 ed	107.7	129.4	99.8	88.5
	0.05	115.1	131.4 b	139.5	164.7	159.0	116.0
	0.0016	146.0	154.1 a	170.1	189.2	171.7	142.5
C x T: LSD_T (5%)		**ns**	**18.2***	**ns**	**ns**	**ns**	**ns**

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test. C x T indicates the interaction between brassinosteroids (treatments) and concentrations.

All application methods of the brassinosteroids caused an increase in photosynthetic rate compared to the control (Table 6.18). The results showed that the foliar application significantly differed compared to seed treatment, soil drench and control respectively and no real difference could be seen between seed treatment and soil drench during week 7, 11 and 13.

Table 6. 18: The differences between brassinosteroids application methods (seed treatment, soil drench, foliar application and control) on photosynthetic rate calculated as percentage difference from the control.

Treatment	3 weeks	5 Weeks	7 Weeks	9 Weeks	11 Weeks	13 Weeks
Control	75.6 d	85.1 c	101.6 c	116.2 d	99.2 c	82.6 c
Seed treatment	91.4 c	110.9 b	120.9 b	136.1 c	116.7 b	102.0 b
Soil drench	104.3 b	113.7 b	128.6 b	147.0 b	125.6 b	109.1 b
Foliar application	125.0 a	136.5 a	146.7 a	169.0 a	153.1 a	122.8 a
LSD_T (0.05)	**10.0***	**6.7***	**10.9***	**10.3***	**12.2***	**11.8***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

The differences between three residual concentrations of mesotrione on photosynthetic rate was measured over a period of 13 weeks (Table 6.19). The results showed significant differences between the three residual concentrations of mesotrione. The highest photosynthetic rate was observed in 0.0016 µg ai kg^{-1} as compared to the other two concentrations. Although the herbicide still even at the lowest concentration lowered the photosynthesis compared to the control. The stomatal conductance (Table 6.20) significantly enhanced the interaction between the brassinosteroid and herbicide combination over time.

Table 6. 19: The differences between three residual concentrations of mesotrione (1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1}, 0.0016 µg ai kg^{-1} and the control (0)) on photosynthetic rate calculated as percentage difference from the control.

Herbicide residue (µg ai kg^{-1})	**3 Weeks**	**5 Weeks**	**7 Weeks**	**9 Weeks**	**11 Weeks**	**13 Weeks**
0	124.0 a	132.4 a	146.9 a	167.0 a	151.8 a	127.6 a
1.6	68.7 d	85.0 d	94.9 c	109.0 c	82.4 c	73.2 c
0.05	90.8 c	103.7 c	115.7 b	134.2 b	117.4 b	98.4 b
0.0016	112.9 b	125.1 b	140.4 a	158.2 a	142.9 a	117.3 a
LSD_T (0.05)	**10.0***	**6.7***	**10.9**	**10.3***	**12.2***	**11.8***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

Table 6. 20: The effect of different brassinosteroids applications combined with various mesotrione concentrations remaining in the soil using three different applications on dry bean stomatal conductance.

Treatment	Herbicide residue (µg ai kg^{-1})	3 weeks	5 Weeks	7 Weeks	9 Weeks	11 Weeks	13 Weeks
Control	0	2.03 cd	2.52 cd	2.96 cbd	2.99 fhig	2.74 cebd	1.89 gef
	1.6	0.08 g	0.30 h	0.40 h	1.60 j	1.36 g	0.89 h
	0.05	0.92 fge	1.14 eghf	1.22 fgh	1.98 jhi	1.57 fg	1.15 gfh
	0.0016	1.49 de	1.75 edf	2.01 fged	2.53 jhig	2.17 fegd	1.70 gfh
Seed treatment	0	2.98 b	3.66 b	4.08 b	4.60 bdec	3.43 cb	2.86 cbd
	1.6	0.38 g	0.74 gh	1.13 gh	1.86 ji	1.53 g	0.94 h
	0.05	1.27 fde	1.50 egf	1.80 fged	2.58 jhig	2.38 fcegd	1.98 gefd
	0.0016	2.35 cb	3.46 cb	3.87 cb	4.09 fdec	3.33 cb	2.64 cebd
Soil drench	0	3.21 b	3.75 b	4.13 b	4.86 bac	3.66 b	3.12 b
	1.6	0.54 fg	0.93 ghf	1.38 fgeh	2.10 jhig	1.83 feg	1.12 gh
	0.05	1.44 de	1.65 egdf	2.45 fed	3.27 fheg	2.64 fcebd	2.05 cefd
	0.0016	3.14 b	3.59 b	4.00 b	4.71 bdac	3.40 cb	2.95 cb
Foliar application	0	5.04 a	5.39 a	5.95 a	6.01 a	5.42 a	5.14 a
	1.6	0.70 fge	1.13 eghf	1.87 fged	2.35 jhig	2.05 feg	1.64 gfh
	0.05	1.53 cde	1.88 ed	2.57 ced	3.37 fdeg	3.16 cbd	3.09 b
	0.0016	4.97 a	5.09 a	5.62 a	5.84 ba	5.38 a	5.09 a
C x T: LSDT (5%)		**0.86***	**0.93***	**1.31***	**1.35***	**1.10***	**0.92***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test. C x T indicates the interaction between brassinosteroids (treatments) and concentrations.

At weeks 7, 9 and 13 the interaction between the two treatments over time significantly increased the transpiration rate (Table 6.21).

Table 6. 21: The effect of different brassinosteroids applications combined with various mesotrione concentrations on dry bean transpiration rate.

Treatment	Herbicide residue (µg ai kg^{-1})	3 Weeks	5 Weeks	7 Weeks	9 Weeks	11 Weeks	13 Weeks
Control	0	5.1	5.9	6.9 bc	7.6 bc	7.3	6.1 bc
	1.6	1.1	1.5	2.3 f	2.4 g	1.5	1.3 g
	0.05	2.1	2.7	3.9 dfe	3.9 dgef	3.1	2.5 gfe
	0.0016	3.9	4.0	5.2 dc	5.2 de	5.1	4.3 dce
Seed treatment	0	6.6	7.0	8.2 ba	9.1 ba	9.1	8.1 a
	1.6	1.4	1.7	2.5 f	3.1 gf	2.7	2.4 gf
	0.05	2.8	2.8	4.1 dfe	4.8 def	4.6	3.5 fe
	0.0016	6.5	6.9	8.0 ba	9.0 ba	8.4	7.9 ba
Soil drench	0	7.0	7.3	8.3 ba	9.4 ba	9.3	9.0 a
	1.6	2.5	2.7	3.0 fe	3.7 gef	3.2	2.8 gfe
	0.05	3.9	4.2	4.9 dce	5.8 dc	5.6	4.2 de
	0.0016	6.7	7.2	8.1 ba	9.2 ba	8.6	8.3 a
Foliar application	0	8.6	8.8	9.7 a	10.9 a	9.9	9.5 a
	1.6	2.7	2.9	3.6 dfe	3.9 dgef	3.6	3.4 fe
	0.05	4.1	4.4	4.9 dce	6.0 dc	5.8	5.6 dc
	0.0016	8.5	9.1	9.7 a	10.7 a	9.4	9.1 a
C x T: LSD_T (5%)		**ns**	**ns**	**2.1***	**2.1***	**ns**	**1.8***

Statistical significance is indicated as LSD =* significant (P ≤ 0.05) and different letters, and ns = not significant at 5% using Tukey's LSD test. C x T indicates the interaction between brassinosteroids (treatments) and concentrations.

The effect of three brassinosteroids applications methods on transpiration rate (Table 6.22) was measured over the period of 13 weeks. The results showed no significant differences between seed treatment and soil drench during week 3 and 11, but the real differences could be seen between foliar application and the other two treatments.

Table 6. 22: The differences between brassinosteroids application methods (seed treatment, soil drench, foliar application and control) on Transpiration rate.

Treatment	**3 Weeks**	**5 Weeks**	**11 Weeks**
Control	3.0 c	3.5 d	4.2 b
Seed treatment	4.3 b	4.6 c	6.2 a
Soil drench	5.0 b	5.3 b	6.7 a
Foliar application	5.9 a	6.3 a	7.2 a
LSD_T (0.05)	**0.8***	**0.7***	**1.0***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

The differences between three residual concentrations of mesotrione on transpiration rate was measured over a period of 13 weeks (Table 6.23). The results showed significant differences between the three residual concentrations of mesotrione compared to the control. The highest stomatal conductance was observed in 0.0016 µg ai kg^{-1} compared to the other two concentrations.

Table 6. 23: The differences between three residual concentrations of mesotrione (1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1}, 0.0016 µg ai kg^{-1} and the control (0)) on Transpiration rate.

Herbicide residue (µg ai kg^{-1})	**3 Weeks**	**5 Weeks**	**11 Weeks**
0	6.8 a	7.3 a	8.9 a
1.6	1.9 c	2.2 c	2.7 c
0.05	3.2 b	3.5 b	4.8 b
0.0016	6.4 a	6.8 a	7.9 a
LSD_T (0.05)	**0.8***	**0.7***	**1.0***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

The effect of three brassinosteroids applications methods (Table 6.24) was measured over the period of 13 weeks on sub-stomatal CO_2 concentration. The interaction showed no significant differences between the treatments over time. In Table 6.25 all the brassinosteroid treatments significantly improved the sub-stomatal CO_2 concentration, confirming again that the foliar application was the best application method. Interesting enough the sub-stomatal CO_2 concentration increased with an increase in the herbicide concentration (Table 6.26).

Table 6. 24: The effect of different brassinosteroids applications combined with various mesotrione concentrations on dry bean sub-stomatal CO_2 concentration.

Treatment	Herbicide residue (µg ai kg^{-1})	3 Weeks	5 Weeks	7 Weeks	9 Weeks	11 Weeks	13 Weeks
Control	0	303.5	483.3	521.1	540.8	311.6	313.1
	1.6	1189.2	1208.7	1221.1	1295.2	1277.7	1183.1
	0.05	887.5	899.9	991.1	1065.9	927.3	914.9
	0.0016	595.8	650.5	750.9	780.7	621.3	581.4
Seed treatment	0	405.4	708.2	763.7	805.4	713.5	731.3
	1.6	1330.4	1358.3	1371.6	1498.1	1488.2	1475.0
	0.05	991.7	1024.5	1091.8	1197.3	1095.0	1059.4
	0.0016	658.307	700.7	799.2	812.1	768.3	737.1
Soil drench	0	472.1	764.3	799.7	834.7	732.6	767.4
	1.6	1633.8	1719.9	1871. 2	1998.3	1878.6	1767.3
	0.05	1099.4	1145.1	1289.1	1398.8	1260.0	1208.4
	0.0016	674.7	732.7	811.6	842.6	788.0	771.8
Foliar application	0	847.8	990.8	1071.7	1094.2	981.0	959.1
	1.6	1710.4	1891.1	1991.2	2018.9	1925.6	1838.9
	0.05	1324.9	1416.7	1581.2	1637.3	1548.7	1422.8
	0.0016	890.0	1017.6	1094.7	1181.8	1075.3	1009.6
C x T: LSD$_T$ (5%)		**ns**	**ns**	**ns**	**ns**	**ns**	**ns**

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test. C x T indicates the interaction between brassinosteroids (treatments) and concentrations.

Table 6. 25: The differences between brassinosteroids application methods (seed treatment, soil drench, foliar application and control) on sub-stomatal CO_2 concentration.

Treatment	3 Weeks	5 Weeks	7 Weeks	9 Weeks	11 Weeks	13 Weeks
Control	744.0 c	810.6 c	871.1 c	920.7 c	784.5 c	748.1 c
Seed treatment	846.4 cb	947.9 cb	1006.6 cb	1078.2 c	1016.2 b	1000.7 b
Soil drench	970.0 b	1090.5 b	1192.9 b	1268.6 b	1164.8 b	1128.7 b
Foliar application	1193.3 a	1329.1 a	1434.7 a	1483.0 a	1382.6 a	1307.6 a
LSD$_T$ (0.05)	**193.5***	**148.8***	**194.3***	**182.6***	**194.6***	**152.4***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

Table 6. 26: The differences between three residual concentrations of mesotrione (1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1}, 0.0016 µg ai kg^{-1} and the control (0)) on sub-stomatal CO_2 concentration.

Herbicide residue (µg ai kg^{-1})	**3 Weeks**	**5 Weeks**	**7 Weeks**	**9 Weeks**	**11 Weeks**	**13 Weeks**
0	507.2 d	736.6 c	789.0 c	818.8 c	684.7 c	692.7 c
1.6	1465.9 a	1544.5 a	1613.8 a	1702.6 a	1642.5 a	1566.1 a
0.05	1075.9 b	1121.6 b	1238.3 b	1324.8 b	1207.7 b	1151.4 b
0.0016	704.7 c	775.4 c	864.1 c	904.3 c	813.2 c	775.0 c
LSD_T (0.05)	**193.5***	**148.8***	**194.3***	**182.6***	**194.6***	**152.4***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

6.3.3.3 The effect of brassinosteroids combined with mesotrione residue on dry bean yield

(a) Yield

The effect of brassinosteroids combined with mesotrione residue on dry bean yield, all the three applied methods of brassinosteroids showed a positive impact on dry bean yield by showing increase in yield (Table 6.27). Number of pods per pot and mass of pods per pot did not show a significant interaction between herbicide and brassinosteroids, but the number of seeds per pot and mass of seeds per pot showed a significant interaction.

Table 6. 27: The effect of different brassinosteroids applications combined with various mesotrione concentrations on dry bean yield and yield components. Measured parameters include number of pods per pot, mass of pods per pot, number of seeds per pot and mass of seeds per pot (final yield per pot).

Treatment	Herbicide residue (μg ai kg^{-1})	Number of pods per pot	Mass of pods per pot (g)	Number of seeds per pot	Mass of seeds per pot (g) (yield per pot)
Control	0	20.0	15.6	30.0 cb	13.5 dc
	1.6	6.8	4.9	13.8 e	3.9 f
	0.05	12.5	8.9	19.8 ed	7.5 fe
	0.0016	16.0	12.0	24.3 cd	9.5 de
Seed treatment	0	23.8	19.7	43.3 a	16.2 c
	1.6	9.0	7.1	20.3 ed	5.0 f
	0.05	14.5	13.1	33.3 b	8.2 fe
	0.0016	21.5	18.2	42.8 a	14.9 c
Soil drench	0	24.3	21.9	44.8 a	17.6 bc
	1.6	11.3	9.5	20.3 ed	7.3 fe
	0.05	18.0	14.3	25.5 cbd	10.4 de
	0.0016	21.8	20.2	43.3 a	16.0 c
Foliar application	0	30.0	28.3	48.5 a	23.0 a
	1.6	16.5	14.5	25.5 cbd	9.5 de
	0.05	22.5	19.0	32.5 b	13.2 dc
	0.0016	28.5	25.5	47.3 a	21.0 ba
C x T: LSD$_T$ (5%)		**ns**	**ns**	**8.1***	**4.5***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test. C x T indicates the interaction between brassinosteroids (treatments) and concentrations.

All of the applied brassinosteroids meaningfully increased the measured yield components number and mass of the pods compared to the control (Table 6.28). Foliar application was significantly higher than the seed treatment, soil drench and control. Seed treatment and soil drench did not differ significantly from each other in the case of the number and mass of pods per pot but was still meaningful higher than the control.

Table 6. 28: The differences between brassinosteroids application methods (seed treatment, soil drench, foliar application and control) on number of pods per pot, mass of pods per pot, number of seeds per pot and mass of seeds per pot (final yield per pot).

Treatment	**Number of pods per pot**	**Mass of pods per pot (g)**
Control	13.8 c	10.3 c
Seed treatment	17.2 b	14.5 b
Soil drench	18.8 b	16.5 b
Foliar application	24.4 a	21.8 a
LSD_T (0.05)	**2.1***	**2.0***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

Table 6.29 provides the differences between three residual concentrations of mesotrione on number and mass of pods. The results showed significant differences between the three mesotrione residual concentrations and the control. The results showed that the reduction of the yield components is dose dependant when applying the herbicide. A huge decline in yield parameters can be observed in herbicide residue of 1.6 µg ai kg^{-1} followed by 0.05 µg ai kg^{-1} and 0.0016 µg ai kg^{-1} respectively compared to the control.

Table 6. 29: The differences between three residual concentrations of mesotrione (1.6 µg ai kg^{-1}, 0.05 µg ai kg^{-1}, 0.0016 µg ai kg^{-1} and the control (0)) on number of pods per pot, mass of pods per pot, number of seeds per pot and mass of seeds per pot (final yield per pot).

Concentration (µg ai kg^{-1})	**Number of pods per pot**	**Mass of pods per pot (g)**
0	24.5 a	21.4 a
1.6	10.9 d	9.0 d
0.05	16.9 c	13.8 c
0.0016	21.9 b	19.0 b
LSD_T (0.05)	**2.1***	**2.0***

Statistical significance is indicated as LSD =* significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test.

6.4 Discussion

Agriculture is a sector of the world-wide economy that will remain critical to the lives of people and to the stability of the general global economy. Farmers will be required to become more efficient and more productive to produce sufficient food for a rising and starving world, but at the same time crops continue to be exposed to stress such as weeds, drought, herbicides, and diseases as well as other stresses that cause a decline in crop production. Weeds are a major problem in crop production because they have vigorous growth, well-developed root systems, prolific, and are more persistent than crops (Rao, 1999). They also compete with the cultivated crop for nutrients, moisture and sunlight and sometimes act as host for insects, rodents and pathogens, which cause disease and interfere with the normal growth of the crops, thus causing a decline in the yields (Rao, 1999). In an attempt to timely control weeds and improve crop yield, herbicides are normally used because they are more effective than other methods of weed control, but they also cause losses in crop yield due to its residual activity or if not used properly (Heimann & Newman, 1997).

This study succeeded in confirming that mesotrione residue have a negative impact on the growth of dry bean crop as a whole. From the results the phytotoxic symptoms in the crop differs depending on the level of mesotrione residue in the soil (Figure 6.1). In the previous studies it was indicated that the injury severity increases with the increase in mesotrione residue concentration in the soil (Felix *et al.*, 2007; Soltani *et al.*, 2007; Riddle *et al.*, 2013). From the results the crop appeared shorter, bleached and necrotic stripes occurred on the leaves in the highest application rate of 1.6 µg ai kg^{-1}. These results are consistent with those found by Riddle *et al.* (2013) where soya bean showed injuries like chlorosis and necrosis, sugar beet, cucumber and pea showed bleaching, chlorosis and necrosis of the tissue with herbicide application. Again, in a previous study it was reported that legume crops are sensitive to herbicide residue of a mixture of atrazine and mesotrione (Simarmata *et al.*, 2018). These results also revealed that all the measured parameters were affected negatively by mesotrione residue in the soil depending on the dose of mesotrione. There was a decrease in plant growth (Figure 6.2 and Table 6.1), physiology (Figure 6.3 and 6.4), and yield (Table 6.2) parameters with all the mesotrione concentrations. The decline was caused by mesotrione residue because mesotrione herbicide is a 4-hydroxyphenylpyruvate dioxygenase inhibitor. When it makes contact with a sensitive crop or weed it blocks 4-Hydroxyphenylpyruvate dioxygenase, which is responsible for catalysing the conversion of tyrosine to plastoquionone and α-tocophyrol

(Coats, 2009). This in turn leads to the destruction of pigment production in plants, which in turn results in bleaching and reduced plant growth, thus lowering yield (Moran, 2005; Riddle, 2012). These results are in line with those found by Simarmata *et al.* 2018 where legume seedlings (soybean, yard long bean, peanut, and mung bean) were injured by herbicide residue. In order to avoid the loss that might be caused by herbicide residue, the application of brassinosteroids could possibly be used to alleviate herbicide residue damage on crops. With the application of BRs, when comparing the control and three methods of brassinosteroids application, the crop responded positively by showing more improvement in its growth (Figure 6.5 and Table 6.3) and physiological processes (Figure 6.6 and 6.7) which consequently resulted in high yield (Table 6.4). This might be due to the fact that brassinosteroids are accountable for many plant processes such as cell expansion and division, xylem differentiation, reproductive development, disease resistance and abiotic stress (Tang *et al.*, 2016). In previous studies it was found that foliar spray and seed treatment methods of brassinosteroids improved the growth and yield of wheat, beetroot bulbs, lettuce heads and carrots (Alam, 2004; Van der Watt, 2005).

Furthermore, the effect of different brassinosteroids application methods combined with various mesotrione residue concentrations was determined and the growth, physiological and yield parameters were measured. The results showed that all three the application methods of the brassinosteroids had a positive impact on growth parameters (Table 6.5, 6.8, 6.9 and 6.10 and main effects Table 6.6 and 6.7), physiological parameters (Table 6.11, 6.14, 6.17, 6.20, 6.21 and 6.24 and main effects Table 6.12, 6.13, 6.15, 6.16, 6.18, 6.19, 6.22, 6.23, 6.25 and 6.26) and yield (Table 6.27 and main effects Table 6.28 and 6.29) alone and in combination with mesotrione. This might be due to the fact that brassinosteroids improve herbicide degradation in the soil and increase the activity of soil micro-organisms in the soil, which are responsible for soil fertility, which resulted in better plant growth and health (Krishna, 2003). Again, brassinosteroids regulate the metabolism of plant oxidation radicals, ethylene synthesis and this leads to the improvement of the adverse effects of different stresses and produces resistance and tolerance in crops (Wani *et al.,* 2016). During stress, the antioxidants concentrate in the chloroplast reviving the wilting or bleaching leaves and thus improving the photosynthetic processes and growth of the crop as a whole (Wani *et al.*, 2016). In conclusion it is clear from the results that mesotrione residue affect dry bean crop negatively and brassinosteroids succeeded in minimizing the negative effect caused by mesotrione.

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CHAPTER 7

GENERAL DISCUSSION AND CONCLUSION

It is a well-established fact that herbicide residue cause crop loss or has negative impact on the crop especially if the herbicide is not recommended for the rotating crop. In this study three legumes were planted to evaluate the effect of residual activity of mesotrione at three concentrations and to determine the effect of three different application methods of BRs, as well as to evaluate a combination of the two treatments. The study hypothesised that BRs would mitigate the negative consequences of mesotrione on three legumes. The findings of this study could assist in explaining how the legume crops responded under both stressed and non-stressed conditions with the application of BRs. With the three mesotrione residue concentrations, it was observed that mesotrione residue has the potential to cause damage to all three legume crops and the severity depend on the concentration. The morphological, physiological and yield parameters of the three legume crops were affected negatively. The first two concentrations (equivalent to 45 DAA and 90 DAA) caused noticeable crop injury such as thick, narrow and yellowing of leaves, stunted growth and a decrease in stem diameter in all the three legumes, while at the lower mesotrione residue concentration (equivalent to 135 DAA) there were no phytotoxicity symptoms visible. This might occur due to the fact that mesotrione herbicide blocks 4-Hydroxyphenylpyruvate dioxygenase, which is responsible for catalysing the conversion of tyrosine to plastoquionone and α-tocophyrol and that result in damaging pigment production in crops, leading to bleaching and reduced plant growth (Riddle, 2012). These findings are in agreement with those of Riddle, (2012) stating that herbicide residue caused injury in different crops such as sugar beet, peas, and cucumber and soya bean and also found that injury severity increases with the increase in herbicide residue concentration. Soltani *et al*., (2007) also found that mesotrione residue caused reduction in biomass, yield and dry weight in broccoli, carrot, cucumber, and onion. The results also showed that mesotrione residue had a negative effect on physiological parameters (chlorophyll content and photosynthesis parameters) of the three legumes. This occurred because photo-bleaching herbicides can produce singlet oxygen leading to oxidative stress and oxidation of carotenoid and chlorophyll concentrations, negatively impacting photosynthesis. This is in agreement with findings by Vital *et* a*l.*, (2017) who found that photosynthetic rate, stomatal conductance and transpiration rate decrease with an increase in the amount of herbicide and intercellular CO_2 concentration increase with an increase in the concentration of the herbicide.

Again, during this study BRs were applied as a seed treatment, soil drench and foliar application to determine the effect of three different application methods of BRs on morphology, physiology and yield of the three legumes. With the application of the BRs it was observed that all the three applied methods improved plant growth, physiology and yield, but that differs depending on the method of application. Foliar application seemed to perform better followed by soil drench and seed treatment respectively. This occurred for the reason that BRs help the crop to endure any stress by changing physiological processes of the crop and increase defensive mechanisms to increase tolerance to stress (Vardhini & Anjum, 2015). This finding concurs with that of Van der Watt, (2005) who found that foliar spray and seed treatments methods of BRs improved the growth and yield of wheat, beetroot bulb, lettuce head and carrots. It was also reported by Wu *et al*. (2014) that BRs application improved biomass accumulation and chlorophyll content of eggplant seedlings which resulted in high photosynthetic rate. This result are in accordance with Verma *et al.* (2011) who showed that BRs enhanced growth, chlorophyll and shoot elongation in groundnut, consequently improving the photosynthetic rate of the crop.

The combined effects of BRs and mesotrione residue on the three legumes were also measured. Combined applications of mesotrione and BRs neutralized the negative effects of mesotrione residual activity in all three legumes growth, physiological and yield parameters. The results showed a reduced damage in crops in all three application methods of BRs and also showed an increase in all morphological and physiological and yield parameters with the foliar application as the best application method, followed by soil drench and seed treatment respectively. This happened because the applied BRs played an important role by improving uptake of nutrients, which resulted in an increase in plant growth, physiology and yield. This finding was similar to those obtained by Marschner, (2007) who found that BRs improved the degradation of herbicide in the soil, by increasing the activity of soil microorganisms, which are responsible for soil fertility, resulting in healthy plant growth. BRs also control the metabolism of plant oxidation radicals and ethylene synthesis improving the adverse effects of different stresses and produces resistance in crops against biotic and abiotic stress.

In conclusion agriculture is the major sector of the economy worldwide, but crop production will continue to be subject to biotic and abiotic stress. During stress, certain critical growth stages and development of crops are badly affected, declining the quantity and quality of yield. It is therefore vital to aid crops to increase their stress resistance by using various method to alleviate stress of which BRs are one of. The overall observation made from this study was that, even though mesotrione residue has negative effects on morphology, physiology and yield in three legume crops as expected, the application of BRs significantly managed to improved growth and physiological processes in all of the three crops tested. Use of BRs will be very important to farmers, because they can help them to increase their production and avoid loses that might be caused by herbicide residue and other stress. BRs are cheap and are easily available in South Africa and can be used by farmers to improve their production.

7.1 References

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