SWISS CHARD (*BETA VULGARIS* VAR. FLAVESENS) SEEDLINGS RESISTANCE AND TOLERANCE TO SELECTED SOIL APPLIED HERBICIDES

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DECLARATION

I, Thabiso Setshwedi Malapane, hereby declare that the work obtainable in this study entitled, “**SWISS CHARD (BETA VULGARIS VAR. FLAVESENS) SEEDLINGS RESISTANCE AND TOLERANCE TO SELECTED SOIL APPLIED HERBICIDES”** is original work prepared by me under the mentorship of my supervisor. I further declare that the work presented herein has not been published or submitted at any institution as part of the requirements for any degree programme. Quoted writings in this dissertation from other persons or organizations have been recognized and listed in the reference section. I also confirm that I have complied with the rules, requirements, procedures, and policies of the University of South Africa.

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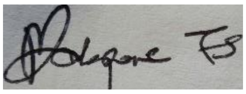
Signature : 

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DEDICATION

This thesis is dedicated to both my encouraging, ever faithful parents to me. And to Lord for granting me strength to keep going.

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I would like to acknowledge everyone who played a role in my academic accomplishments. First of all, my parents, who supported me with love and understanding. Without you, I could never have reached this current level of success.

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LIST OF ABBREVIATIONS AND ACRONYMS

CRD……...............completely randomized design

ANOVA……………...Analysis of Variance

BASAF………………….Bandische Anilin- und Soda-Fabrik

IUPAC……………………. International Union of Pure and Applied Chemistry

ABSTRACT

This study aimed to determine the effect of four herbicides; Alachlor, Metolachlor, Pendimethalin and Trifluralin; applied at five application rates and control, on selected Swiss chard growth parameters). The application rates of herbicides were 0.5, 1.0, 1.5, 2.0, and 2.5 l/ha plus control. The study was conducted in the greenhouse using a completely randomized design (CRD) with three replications. Thus, a total number of 21 treatments were replicated three times to give 63 pots for this study. The parameters measured were days of emergence, germination percentage, plant height, number of leaves, root length, and number of roots. A one-way analysis of variance was populated using SAS 15.0 and mean separation was done at $p<0.05$ using Tukeys' Multiple Comparison Test. Selected swish chard parameters were statistically influenced by the treatments except for the number of roots. The application of all herbicides at doses above 1.0 l/ha were generally found to delay seed emergence, germination %, crop height, number of leaves, and root length. Thus, this short-term study concludes that the application of all four herbicides can be applied in swish chards to control weeds at doses of 1.0 l/ha or less without negatively influencing swish chard growing parameters. However, future studies are recommended.

Keywords: Tolerance, resistance, Swiss chard, herbicides, parameters, weed management

CHAPTER 1: INTRODUCTION

1.1 Background of the Study

Swiss chard (*Beta vulgaris* var. flavesens), also known as stem chard is consumed principally for its palatable leaves (Pyo *et al*., 2004). It has broad green leaves which are harvested at different growth development stages (Dobbs, 2012). The crop is identified by numerous names such as silverbeet, spinach beet, unending spinach, brilliant lights, crab, beet, and Swiss chard beets (Ninfali and Angelino, 2013). In South Africa, it is called Spinach and is widely recognised for its soft green leaves. The crop is one of the most consumed vegetables that is supplied throughout the year and is preferred due to its dietary properties.

Swiss chard is routinely known for weight management plans all over the world when being consumed (Ninfali and Angelino, 2013). It has an amazing phytonutrient profile, phytonutrients comprise cell reinforcement, mitigating, and entire body benefits (Pyo *et al*., 2004). Furthermore, Swiss chard is a rich in dietary fibers, proteins and antioxidants such as alpha-lipoic acid, which is linked to lower glucose levels and increased insulin sensitivity (Ivanovic *et al*. 2019; Yang *et al*. 2014). For mineral substances, this vegetable has copper, sodium, potassium, manganese, and potassium which is considered the “acceptable salt” and has stood out as worthy of its function in aiding to lower the circulatory strain (Daiss *et al*., 2008a, b; Bozokalfa *et al*., 2011). Gil *et al*. (1998) revealed the flavonoid substance of chard leaves is generally within the weight range of 2.4-3.0 mg/g. The varieties (Bright Lights, Bright Yellow, Rhubarb Chard, Rhubarb Red, and Ruby) differ with the plant leaf colours which can be red or green (Nonnecke, 1989; Pyo *et al*., 2004). The red colour is from betacyanin, a compound firmly identified with anthocyanin which represents a large portion of the red hues in the plant family (Sarker and Karmoker, 2011). The leaves of this crop can be used in salads, to add colour, in soups, stews and it holds its shape well. Apart from the high energy value of cooked leaves, the leaves also contain relatively high levels of bioactive compounds such as Vitamin C and Vitamin A and also minerals, such as potassium, sodium, and iron (Maboko and Du Plooy, 2009).

Studies by (Harnis *et al*., 2012) and (Azadbakht *et al*., 2012) have demonstrated that the diet of mixed green vegetables has a significant effect on the advancement of the utilization of crops. Utilization of a green diet of mixed greens is useful in diminishing the danger of chronic and cardiovascular malady. Swiss chard served with mixed greens has been considered to be plentiful in nutrients and minerals (Longendra *et al*., 2012). Exposure to different chemicals, nutrients and cultivation techniques will probably affect the physiological response of the product. However, there is a relative scarcity of published research about the effect of organic production on quality of vegetables (Magkos *et al*., 2006). Thus, the need to guarantee its ceaseless inventory through the utilization of proper agronomic practices such as the application of herbicides. The crop needs to endure the expulsion of weeds in order to maximise its productivity.

The application of herbicides on plants might result in a change of leave colour (Shanmugasundaram and Kandasamy, 2003; Hatzinikolaou et al., 2004). The frequent use of agrochemicals causes danger to humans and animals. Herbicide application follows different procedures, depending on the precautions as directed on the chemical pamphlet i.e adsorption, herbicide change, and transportation. Herbicides effects are influenced by the amount and velocity of herbicide assimilation and translocation by the plant, just as the specific species' natural capacity to detoxify the herbicide. Any factor that impacts the measure of herbicide ingested, for example, the measure of herbicide accessible in the cultivar, temperature, soil dampness content, and other soil components would probably influence a plant's vulnerability to the herbicide (Allemann and Molomo, 2016). Presently the recommended herbicide of Swiss chard in South Africa is Chloridazon (Herbicides January 2019 by BASF SA).

Chloridazon is recommended for its main use for the control of annual broadleaf weeds. Pendimethalin as indicated by Hatzinikolaou (2004) is a dinitroaniline herbicide used in areas where yearly grasses and some yearly broadleaf weeds in maize (*Zea mays)*, cotton (*Gossypium hirsutum* L.), soybeans [*Glycine max* (L.) Merr.], and a few vegetable yields are a problem. All dinitroaniline herbicides prevent tubulin from polymerizing into microtubules and suppress mitosis. This hindrance causes a few morphological and anatomical variations in plants, of which root development restraint is the most self-evident (Hatzinikolaou, 2004). Pendimethalin is a selective herbicide belonging to dinithroaniline class and it is absorbed by both plant roots and leaves. It inhibits the formation of microtubules by which the cell division is ceased, after which plants dye immediately after germination (Janjić, 2005). This implies that the pendimethalin ingenuity in the soil is influenced by factors that modify microbial action, for example, organic matter content, soil temperature, soil moisture content, and aerobic conditions (Hatzinikolaou, 2004).

Four types of herbicides will be used in this study which are; Alachlor, Metalochlor, Pendimenthalin, and Trifluralin. Alachlor 2-chloro-N-(2, 6-diethyl phenyl)- N-(methoxymethyl) acetamide is an organochlorine herbicide. It is widely used to control grasses yearly and numerous wide weeds in cotton, brassicas, maize, soybean, and sugarcane. S-Metolachlor is a chloroacetamide herbicide enrolled for early pre-plant, PPI, or pre-emergence (PRE)-weed control in maize (*Zea mays* subsp. Mays.), soybean [*Glycine max* (L.) Merr.], and dry bean (*Phaseolus vulgaris* L.). S-Metolachlor can be utilized alone or mixed with other PRE-herbicides, to control yearly grasses, yellow nutsedge (*Cyperus esculentus* L.), and some little seeded broadleaf weeds. As of late, these herbicides were enlisted for post application in sugarbeet (Bollman *et al*., 2008).

Trifluralin belongs to dinitroanilines, having been broadly used to control weeds. The dinitroaniline herbicide prevents cells in roots and shoots from dividing and developing (Grover *et al*., 1997). The trifluralin herbicide is described as a microtubule-depolymerizing synthetic compound that connects to the tubulins by its radical NO_2 and compels, for the most part, the arrangement of mitotic fuse (Fernandes *et al*., 2007). The application of these herbicides is to ensure the survival of the Swiss chard crop and its continuous supply. Thus, the study aimed to determine the level of resistance and tolerance of Swiss Chard seedlings to selected herbicides.

1.2 Problem Statement

The presence of weeds is a significant field issue in commercial Swiss chard production areas of South Africa. Weeds are described as unwelcomed plants which interfere with the management of agricultural production systems. Weeds also compete with main crops for available nutrients, space and lead to a reduction in the growth, yield and quality of agricultural products up to a certain extent (Mustafa *et al*., 2019). Weeds compete relatively for short periods after transplanting or during crop emergence, thus, leading to significant yield losses in crops. As currently indicated by Badische Anilin und Soda Fabrik (BASF) in South Africa, there are not many herbicides (like Chloridozen) accessible for use in Swiss chard. Few weeds of economic importance are not managed by herbicides prescribed by BASF (Haggblade *et al*., 2017). As a result of the national wide difficulty managing weeds among Swiss chard ranch, it is therefore essential to manage weeds at the early stages of growth. Swiss chard is increasingly delicate to injury by explicit herbicides, in any event, even at suggested rates. To date, opposition and resistance of seedlings of Swiss chard to Alachlor, Metalochlor, Pendimenthalin, and Trifluralin herbicides has not been reported.

1.3 Overall Aim of Study

The study seeks to investigate the level of resistance and tolerance of Swiss chard seedlings to selected herbicides.

1.4 Objectives

Objective 1: To determine the effect of Alachlor, Metolachlor, Trifluralin and Pendimethalin herbicides on Plant height of Swiss chard seedlings.

Objective 2: To determine the effect of Alachlor, Metolachlor, Trifluralin and Pendimethalin herbicides on Number of days to emergence of Swiss chard seedlings.

Objective 3: To determine the effect of Alachlor, Metolachlor, Trifluralin and Pendimethalin herbicides on germination percentage and Number of leaves on Swiss chard seedlings.

Objective 4: To determine the influence of Alachlor, Metolachlor, Trifluralin and Pendimethalin on Root length and Number of Swiss chard seedlings.

1.5 Research Hypothesis

Hypothesis 1: Seedlings of Swiss chard are not resistant to Alachlor herbicide.

Hypothesis 2: Swiss chard seedlings will display symptoms of intolerance to Metolachlor herbicide.

Hypothesis 3: Pendimethalin herbicide will delay the growth and development of Swiss chard seedlings when applied as a pre-emergence or pre-planting incorporated.

Hypothesis 4: Trifluralin herbicide have negative effects on vegetative and generative growth among seedlings of Swiss chard

1.6 Thesis Organization

Chapter one covered the background, problem statement, objectives, and hypothesis of the study. Chapter two examined the relevant literature. Chapter three covered the materials and methodology applied in achieving the aims and objectives. Chapter four covered results and discussion. Conclusion and recommendations were covered in chapter five.

CHAPTER 2: LITERATURE REVIEW

2.1 Brief Taxonomy, distribution, and uses of Swiss chard

Swiss chard belongs to the genus *Beta* which has a morphology and a genetic variable group composed of wild, weedy, and domesticated forms that are used for sugar production or as vegetables (Bartsch and Ellstrand, 1999). The crop has been cultivated successfully for many years all over South Africa and neighbouring countries. Swiss chard alongside Beets (*B. Vulgaris* var. crassa) belongs to the goosefoot family (Chenopodiaceae) (James, 2015). These crops are believed to have developed from seabeet (*Beta Maritima*) that originates from southern Europe and have been grown since the third century A.D (Wayne and Keith, 2003). Swiss chard was recognised as a full agronomic crop in the nineteenth century by German and French breeders (Wayne and Keith, 2003). The leaves are consumed cooked or raw as salad. One of the major challenges of Swiss chard growth on the farm is weeds. Due to the economic importance of this crop, there is a need to control nearby growing weeds and examine the crop's ability to withstand the herbicides used in the eradication of the weeds.

2.2 Weeds

Weeds are plants that grow in places where they are not wanted. They compete with desired plants for different growth factors and add significantly to the cost of farm operations (Qasem, 2003; Zaragoza, 2003). Optimum crop production depends on successful weeds control, and a delay in weeds control early in the season can reduce yields of Swiss chard crops and create difficulties during harvesting. Weeds are different from pests, insects, and diseases that pose problems in crop production areas because of their difficulties to control (Gianessi and Sankula, 2003). The presence of weeds causes significant loss of yields as a results of direct competition for resources such as water, light, and nutrients (Bhadu and Kaswan, 2022). Both antagonistic processes, parasitism, and allelopathy take place because of weeds. Apart from these quantitative damages, weeds can cause qualitative indirect damages evidenced in cereal yield reduction, contamination of seeds slowing of tillage, and harvesting practices (Tsetkov *et al*., 2017)). Clark *et al.* (1998) compared corn production in California and concluded that weeds damage can cause a major loss than pests, insects, and diseases.

2.2.1 Weeds affecting Swiss chard (*Beta vulgaris* var. flavesens*)*

According to Brandenberger and Dainello (2017), weeds can be managed by inhibiting their germination or by eradication. New and perennial weeds can be prevented by hindering their sprouting. Germination inhibition is the preferred method for ceasing weed growth. The authors further indicated that limiting weeds infestation can provide less competition to Swiss chard, thus, enhancing productivity. However, achieving this level of control can increase the labour cost and some herbicides may cause damage to crops (Beckie, 2006). The example of weed infestation in Swiss chard is shown in Figure 1.1 and the list of common weeds known to grow and compete with Swiss chard are listed in Table 1.1.

Figure 1. 1 Swiss chard field infested by weeds

Table 1.1: List of weeds affecting Swiss chard.

Common names	Scientific names	References
Wild Radish	*Raphanus raphanistrum*	*Norsworth, 2009*
Redroot Amaranth	*Amaranthus spp.*	*Osman and Mutwali, 2022*
Hogweed / Wireweed	*Polygonum aviculare*	*DeCauwer, 2021*
BlackBerry Nightshade	*Solanum nigrum*	*Cakovic et al.2016*
Pigweed	*Amaranthus spp.*	*Osman and Mutwali, 2022*
Sowthistle / Milk Thistle	*Sonchus spp*	*Hyatt, 2006*
Fat Hen	*Chenopodium album*	*Hyatt, 2006*
Wild Turnip	*Brassica spp.*	*Hyatt, 2006*
Annual Nettle / Stinging Nettle	*Urtica urens*	*Coleman et al., 2018*
Chickweed	*Stellaria media*	*Bond and Turner, 2004*
Annual ryegrass	*Lolium rigidum*	*Bajwa et al., 2021*
Winter grass	*Poa annua*	*Tei and Pannacci, 2017*
Barnyard grass	*Echinochloa spp.*	*Bryson and Reddy, 2012*
Summer grass	*Digitaria spp.*	*Tei and Pannacci, 2017*

2.3 Methods of Controlling Weeds

Weed control is necessary in all countries including South Africa, due to the damages caused by weeds on crops and landscapes. The methods of weed control include mechanical, cultural, and chemical methods (Qasem and Hill, 2003; Zaragoza, 2003).

2.3.1 Cultural method

Weeds are an important obstacle to spinach productivity, as they reduce its commercial biomass and affect the quality of the harvested product (De Cauwer *et al*., 2021; Lati *et al*., 2015; Wallace *et al*., 2007). The impact of weed competition on spinach yield is likely to be higher in fields infested with low-diversity weed communities. Such weed communities are dominated by a small number of weed species that are highly competitive and well adapted to the soil and climatic conditions of a given agricultural area (Storkey and Neve, 2018; Travlos *et al*., 2018). Storkey and Neve (2018) indicated that as the number of species in a given weed community increases, crop yield losses increase. Furthermore, herbicides are not a sustainable weed control option in agricultural areas production areas because the usage of selected herbicides increases the risk of selection of herbicide resistant weed populations (Neve *et al*., 2014). Therefore, weed management in agricultural production areas should first rely upon sustainable cultural practices and non-chemical weed control methods.

Cultural methods are employed to cause a reduction in the number of weeds present among the crops and to prevent more seed production (Fasil and Verkleij, 2007). Intercropping, transplanting, and crop rotation are examples of cultural methods applied to control weeds (Jamil *et al*., 2011). Crop rotation is accepted as the simplest solution to weed infestations of non-susceptible crop farms. This is attributed to the interruption of the weed growth by the non-susceptible crops. Fasil and Verkleij (2007) indicated that the selection of crop rotation as a control method should focus on the suitability to the local conditions as well as the potential as a trap crop. However, the combination of crop rotation, intercropping, weeding, and the use of resistant varieties is believed to provide effective control measures (Mahmoud *et al.*, 2013). Intercropping involves cropping various crop types on the same piece of land (Liebman and Dyck, 1993). Hooper *et al*. (2009) reported that intercropping is a low-cost and sustainable technology that addresses low soil fertility and enhances weeds reduction on the farm.

2.3.2 Mechanical method

A mechanical method such as hand weeding is adopted by farmers to prevent the development and seed dispersal of weeds. However, this method can be tiresome and results in no improvement in the germination productivity of the infected crops. Khan *et al*. (2008) suggested that hand-pulling should be done after 2-3 weeks of planting the crops to prevent further germination of weeds and seedlings in the field. Hand weeding using the hand hoe is used to impair seed reinfestation and germination of weeds, it is necessary before the full development of the plant to prevent weed damage (Sauerborn *et al*., 2007). Due to the labour intensity and poor efficiency of this method of weed control, there is a need to adopt other methods of control such as biological and chemical methods either in combination with the hand weeding method or separately. Studies by Alba *et al*. (2020) and Kanatas and Gazoulis (2021) have shown that increasing the number of mechanical weed control treatments is a recommended strategy to increase the efficacy of mechanical weed control.

2.3.3 Biological control method

The biological control method employs smoother plants, microorganisms especially fungi, and herbivorous insects to reduce the load of weeds to a level where economic damage is minimal (Atera *et al*., 2011; Ejeta, 2007). This control method is a cost-effective and safe method when reducing weed populations in crops, forests or rangelands (De Groote *et al*., 2010). Koua *et al*. (2011) observed that the effectiveness of some weevils (*Smicronyx guineanus* and *Smicronyx umbrinus*) reduces the proliferation of weeds. The use of fungicides in controlling the growth of weeds has become a promising control method because of their host specificity, aggressiveness, mass production potential, and genetic diversity (Rebeka *et al*., 2013). Work done by Sauerborn *et al*. (2007) has shown the high effectiveness of the fungus *Fusarium oxysporum* in the control of the weed *S. hermonthica*. The authors indicated that the fungus hindered the germination, growth, and development of the weeds.

Hearne (2009) also observed an increase in sorghum biomass in the farm treated with *F. oxysporum,* with a biomass production rate of over 90%. Similarly, Teka (2014) observed that sorghum seed coated with the fungus enhanced host plant resistance with a 95% reduction in the weeds' proliferation. In Ethiopia, *F. oxysporum* in combination with the weeds' resistant sorghum genotype promoted effective control of weeds (Rebeka *et al*., 2013).

The biological control method of weeds is a technique that eliminates weeds and promotes high crop yield. However, the requirement of a long period for the actualization of the expected results in weeds control is a potential disadvantage of the method (De Groote *et al*., 2008).

2.3.4 Chemical method

The chemical control method involves spraying chemicals such as herbicides to control weeds in the field. Weeds primarily compete with crops for food which in turn affects crop production (Kabambe *et al*., 2008). The application of herbicides has the potential to prevent the development of weeds (Xie *et al*., 2010). The 2,4 D herbicide has been used most, this is attributed to its low cost of application. Ejeta and Gressel (2007) stated that the 2-methyl-4-chlorophenoxyacetic acid (MCPA), similar to 2,4-D, has also been found to be effective against weeds to a great extent when combined with bromoxynil. Recently, other types of herbicides have been produced in South Africa to enhance the fight against weeds and their devastating effects on crops (van Zyl, 2012).

Alachlor and metolachlor are chloroacetanilides herbicides widely used in the control of annual grasses and broadleaf weeds growing amongst cotton, soybean, rapeseed, rice, sunflower, and vegetables. These herbicides possess good water solubility and a low degree of mineralization, which facilitates the infiltration into the ground water (Huang *et al*., 2017). These properties enhanced their absorption by the weeds, impairing the synthesis of proteins needed for the growth of weeds. However, the application of these herbicides has the potential to cause damage to crops. Hence, the need to ascertain the effect of these herbicides on crops through the evaluation of the crop's resistance and tolerance to the applied herbicides.

2.4 Brief description and mechanism of action of selected herbicides

2.4.1 Alachlor

Alachlor is a herbicide with an International Union of Pure and Applied Chemistry (IUPAC) name 2-chloro-2',6'-diethyl-N-methoxymethylacetanilide and a molecular formula $C_{14}H_{20}ClNO_2$. The herbicide prevents the proliferation of weeds through the inhibition of protein synthesis in weeds. Physiologically, this herbicide prevents the synthesis of proteins through the inhibition of amino acid synthesis necessary for root elongation, thereby hindering weed growth (Kang *et al*., 2005). Alachlor inhibits the 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase enzyme necessary in the generation of weeds, their growth, and development (Herrmann and Weaver, 1999). Consequently, aromatic amino acids such as tryptophan, tyrosine, and phenylalanine levels are reduced leading to compromised biosynthetic metabolic pathways and ultimate weeds death (Maeda and Dudareva, 2012).

2.4.2 Metolachlor

Metolachlor is an herbicide with the IUPAC name RS-2-Chloro-N-(2-ethyl-6-methyl-phenyl)-N-(1-methoxypropan-2-yl) acetamide and a molecular formula $C_{15}H_{22}ClNO_2$. Sikkema *et al*. (2007) stated that pure metolachlor has an off-white to colourless and odourless liquid when stored at room temperature, with a molecular weight of 283.46. The herbicide prevents the growth of weeds by inhibiting cell division and elongation processes during protein synthesis (Buser, 2001). Upon its application, all germinating plants absorb it through their roots and translocate it to various parts of the plants (Miller, 2006). The plant roots display a compact appearance, respiration, lignification, anthocyanin production, protein, and fat synthesis are reduced (Al-Khatib *et al*., 1995). These physiological changes in the plant roots as a result of the herbicides could consequently lead to inhibition of root elongation and development (Kang *et al*., 2005). However, susceptible crops could also be prone to these physiological changes which may lead to poor yield. Hence, the need to ascertain the tolerance and resistance of crops to this herbicide before its recommendation.

2.4.3 Pendimenthalin

Pendimethalin is an herbicide with a generic name N-(1-ethylpropyl)-3, 4-dimethyl-2, 6-dinitrobenzenamine, and a molecular weight of 281.30. It is crystalline at room temperature and has a fruit-like odour. *Pendimethalin* is soluble in water (at 20^{o}C) and <0.50 ppm, aromatic and chlorinated hydrocarbon solvents. It is absorbed by both roots and shoots of emerging seedlings, but it is not readily translocated. It inhibits cell division and cell elongation (Peterson *et al*. 2001). *Pendimethalin* belongs to the dinitroaniline chemical family, herbicides belonging to this family target the microtubule of the root where they form an herbicide-tubulin complex inside the microtubule inhibiting the polymerization of microtubule during assembly (Wloga and Gaertig, 2010). This inhibition results in the deformation of the structure and function of the microtubule leading to the death of the cell (Wloga and Gaertig, 2010).

2.4.4 Trifluralin

Trifluralin (TFL) is a herbicide with a chemical composition α, α, α–trifluoro–2–6-dinitro–N–N– dipropyl–p–toluidine (Bellinaso *et al*., 2004). The herbicidal mechanism of TFL is to inhibit the polymerization of tubulin and tissue development, inhibit meristem cell division, destroy cells, and inhibit photosynthesis to make weeds die (Nyporko *et al*., 2002; Chen *et al*., 2021). According to Bansal (2011), most pesticides will get into the soil, especially for herbicides. TFL showed a long half-life in soil (Triantafyllidis *et al.*, 2010; Du *et al*., 2018; Lu *et al*., 2019), and that was potentially harmful to soil life (Bezchlebová *et al*., 2007; Merlini *et al*., 2012; Wang *et al*., 2016).

2.5 The tolerance or response of crops to different herbicides

Global development and advancement have created increasing pressure on available resources leading to increased demand for food for human sustainability and survival (Diao *et al*., 2003). This has also put pressure on agricultural practices because of the rising need for food for the people. Recent crop production depends mainly on the applications of herbicides to prevent the proliferation of weeds that compete with crops for available resources such as nutrients (Cserháti *et al*., 2004). Approximately 65% of all the pesticides applied in agricultural practices worldwide are mainly herbicides (Stevens and Summer, 1991). Herbicides have a profound impact on promoting the growth of food crops through the interruption of the weeds' proliferation and competition. However, the absorption and accumulation of these herbicides in food crops is inevitable and can lead to the death of the crops. Further potential consequences include the reduction of various growth parameters such as plant height and the transfer to humans and other animals through ingestion of the crops. Crops that exhibit high tolerance to these herbicides will have little or no interference in their growth.

Alachlor is taken up by growing shoots through the roots (Kang *et al*., 2005). The major disadvantage of using this herbicide is its action against non-targeted plants. Alachlor is a non-persistence herbicide with an 11.3 to 34.8 days half-life (Walker *et al*.,1992). In comparison to other forms of herbicides, the alachlor detection level in food crops is low. However, caution is needed when consuming vegetable crops containing alachlor because United State Environmental Protection Agency still classified it as a class B2 potential carcinogen (USEPA, 1991). Rahmana *et al*. (2013) determined the residue level of alachlor after 49 days of application in pepper and pepper leaf. The results showed that there were residues of alachlor in the pepper and pepper leaves although the levels were below the stipulated standard. However, the effect of this herbicides on the growth of the pepper was not determined in their work to ascertain the tolerance level of pepper to alachlor. The point to note is the presence of the residue in the pepper plant translocated to the leaf. The presence of this residue could have a negative impact on the growth of the plant. It was also observed that alachlor caused severe injury to snap beans (*Phaseolus vulgaris* L.) in cool and dry or warm and wet conditions.

Metolachlor, a chloroacetanilide herbicide, is applied as pre-emergence and early post-emergence weed control in *Arachis hypogaea*. This herbicide was recognised as the best amongst other herbicides in *Cyperus esculentus* (a major weed of *A. hypogaea*) control (Kanagam and Chinnamuthu, 2009). The herbicide is reported to effectively provide residual weed control of *A. palmeri* (Meyers *et al*., 2010; Whitaker *et al*., 2010), *Cyperus esculentus* L. (Dayton *et al*., 2017; Grichar *et al*., 2008), and annual grass species such as *Echinochloa crus-galli* (L.) P. Beauv., *Urochloa platyphylla* (Munro ex C. Wright) R.D. Webster, *Eleusine indica* (L.), and *Digitaria sanguinalis* (L.) Scop. (Clewis et al., 2008)

Pendimethalin is known as an herbicide that inhibits the growth of grasses that spreads its control potential on some annual broadleaves as well (Semidey *et al*., 1989; Skumriov and Boiadjiov, 1995). Its control potential has been reported successful in controlling weeds that affect the growth of tomatoes and chili peppers (Usoroh, 1988; Adigun *et al*., 1994). In all the fields where *pendimethalin* was used to treat weeds, its application was successful with no negative reaction from crops and soil residues (Bairambekov and Valeeva, 1996). Pepper (Sastre *et al*., 1998), cabbage (Al-Khatib *et al*., 1995), and lettuce (Henderson and Webber, 1993) were able to recover from initial growth reduction and tolerance of pendimethalin. However, more work is needed to ascertain the tolerance of other crops to pendimethalin. Hanson and Thill (2001) reported the impact of pendimethalin on lentil, pea, and winter wheat. In their work, it was discovered that the herbicide did not reduce lenticel or pea biomass and seed yield in comparison with the control. However, the biomass and seed yield of winter wheat was reduced. This implied that winter wheat was not able to tolerate the toxicity of pendimethalin. Miller *et al*. (2003) carried out both field and greenhouse experiments to evaluate the impact of pendimethalin on cabbage applied after transplant. The authors observed that the head number, yield, and dry weight of cabbage were reduced due to the herbicide application. Anatomical analysis was carried out to understand the structural damage done by the herbicide on cabbage. Stunted shoot apical meristem and the emerging leaves were observed as well as cell division, elongation and differentiation disruption at the vascular levels in the leaves and hypocotyls (Miller *et al*., 2003). These structural changes and disruptions in cell growth and development could be the reason behind the reduction in the head number, yield, and dry weight of cabbage.

In summary, the control of weeds has been a serious challenge globally and as such, various control measures which cut across cultural, mechanical, biological, and chemical methods have been adopted by many countries. Some of the methods have been proven effective. The chemical control method which is widely used has also proven to be effective. However, the impact of the residual chemical in the soil and on crops has resulted in poor crop yields. As a result, examining the impact of the chemicals (herbicides) on crops before applying them to controlling weeds is of paramount importance. This present study investigated the impact of four herbicides used in controlling weeds on Swiss chard farms.

CHAPTER 3: MATERIALS AND METHODS

3.1 Experimental Site

The herbicide trial to determine the level of resistance and tolerance of Swiss chard seedlings to selected herbicides (Alachlor, Metolachlor, Pendimethalin, Trifluralin) was conducted in a fully automated greenhouse. The concentrations of 0.5 l/ha to 2.5 l/ha derived from all the herbicides (Alachlor, Metolachlor, Pendimethalin, Trifluralin) used in this study. The greenhouse is located at Florida Campus Horticultural Centre, UNISA Science Campus, South Africa. The Global Positioning System (GPS) coordinates for the location is Latitude: 26,09501S and Longitude: 27,054113E. The temperature in the greenhouse was programmed between 18 and 30°C. The trials were conducted under natural daylight conditions.

3.2 Experimental Design, Variables, and Treatments

3.2.1 Experimental design

The experiment was laid out using a completely randomized design with five treatment levels for each herbicide and control replicated three times. The experimental trial consisted of three blocks with sixty-three pots spaced at 30 cm inter rows and 15 cm intra rows to encourage proper growth of the plants. Different herbicide application rates were used to evaluate the levels of resistance and tolerance to selected herbicides by Swiss chard seedlings. The greenhouse used consisted of fans, ventilation, heating, and cooling systems which were automated in stages corresponding to the researcher’s environmental selection and Swiss chard growing requirements. In order to reduce risks of experimental errors, the researcher employed experimental homogeneity factors that were round plant pods 2kg, loam soil, and irrigation was applied when needed to avoid the plants to experience water stress. The experiment lasted from 14 June to 14 August 2018, with regular monitoring performed to avoid bias during data collection.

3.2.2 Experimental variable

The known variables employed in this study are herbicide treatment rates having different treatment levels of Alachlor, Metolachlor, Pendimethalin, and Trifluralin.

Different levels of herbicide treatments were assigned to the pots. The five application rates including the control per active ingredient were applied according to the recommended application rates for herbicide weed control in non-minimum till (Felix *et al*., 2007).

Table 3.1: Summary of the different herbicide concentrations in litres/ hectare.

	Application rates l/ha				
Alachlor	0.5	1.0	1.5	2.0	2.5
Metolachlor	0.5	1.0	1.5	2.0	2.5
Trifluralin	0.5	1.0	1.5	2.0	2.5
Pendimethalin	0.5	1.0	1.5	2.0	2.5
Control	0				

3.2.3 Treatments

The trial had a total number of 5 treatment levels per herbicide plus the control (no herbicide). Treatments displayed in Table 3.1 were randomly assigned to pots and were replicated 3 times in the greenhouse.

3.3 Parameters Measured and Data Collection

3.3.1 Parameters measured

The following parameters were measured, days of emergence, germination percentage, plant height, number of leaves, root length, and number of roots.

Table 3.2: Parameters that were measured in the trial

	SEEDLING STAGE (≤ 21)	DURATION
1	Days of emergence	7 Days
2	Germination percentage	1-3 Days
3	Plant height	4 Weeks from the emergence
4	Number of leaves	4 Weeks from the emergence
5	Root Length	At maturity
6	Number of roots	At maturity

3.4 Statistical Model

Statistical Analysis

The data for each parameter measured was first arranged using Excel spreadsheets, followed by statistical analyses using one-way analysis of variance (ANOVA). The ANOVA was applied using the statistical package SAS (2015) to detect significant differences.

- Separation of Means

  In order to have a clear understanding of the data, it was preferred that means of Swiss chard response parameters, be separated using Tukeys' Multiple Comparison Test to compare the mean separation value $p \leq 0.05$.

- Determination of Coefficient of Variation

  The coefficient of Variation (CV) was tested for comparison between treatments.

3.5 Ethical Considerations

Ethics clearance was received before the commencement of this study under Ethics Approval number 2017/CAES/190 from the UNISA Ethics Committee of the College of Agriculture and Environmental Sciences (CAES).

CHAPTER 4: RESULTS AND DISCUSSION

This present study evaluated the ability of Swiss chard to tolerate the impact of four herbicides (Alachlor, Metolachlor, Pendimethalin, and Trifluralin).

4.1 Days to Emergence

Number of days to emergence of Swiss chard were measured as parameter to study the ability of Swiss chard to tolerate the herbicides at early growth stages. This was done in line with many studies which want to establish the ability of Swiss chard to tolerate herbicides application at different doses (Kang *et al*., 2005; Walker *et al*., 1992). The effects of the four herbicides on number of days to emergence are presented in Figure 4.1 and Appendix A. The application of the herbicides statistically influenced number of days to emergence of Swiss chard (Appendix A). In the current study, the application of all herbicides at 0.5 l/ha and 1.0 l/ha had the similar number of days to emergence as the control (Figure 4.1). This suggests that the application of these herbicide at low application rates of both 0.5 l/ha and 1.0 l/ha application rates do not suppress Swiss chard emergence. This support previous studies by Bairambekov and Valeeva, (1996), Sastre *et al*. (1998), Al-Khatib *et al*. (1995) and Henderson and Webber, (1993) who found that the application of herbicides like Pendimethalin does not surprises emergence of many crops. While on the other hand, the increase in application rate above 1.0 l/ha to 2.5 l/ha results in a delay to Swiss chard emergence (Figure 4.1). Skroach and Sheets (1979), Swann (1988) and Cardina and Swann (1998), found that the metolachlor herbicide delayed the emergence of *Arachis hypogaea* at increased doses. This suggest that high doses might interfere with chemical reactions during germination, thus, leading to delay in emergence.

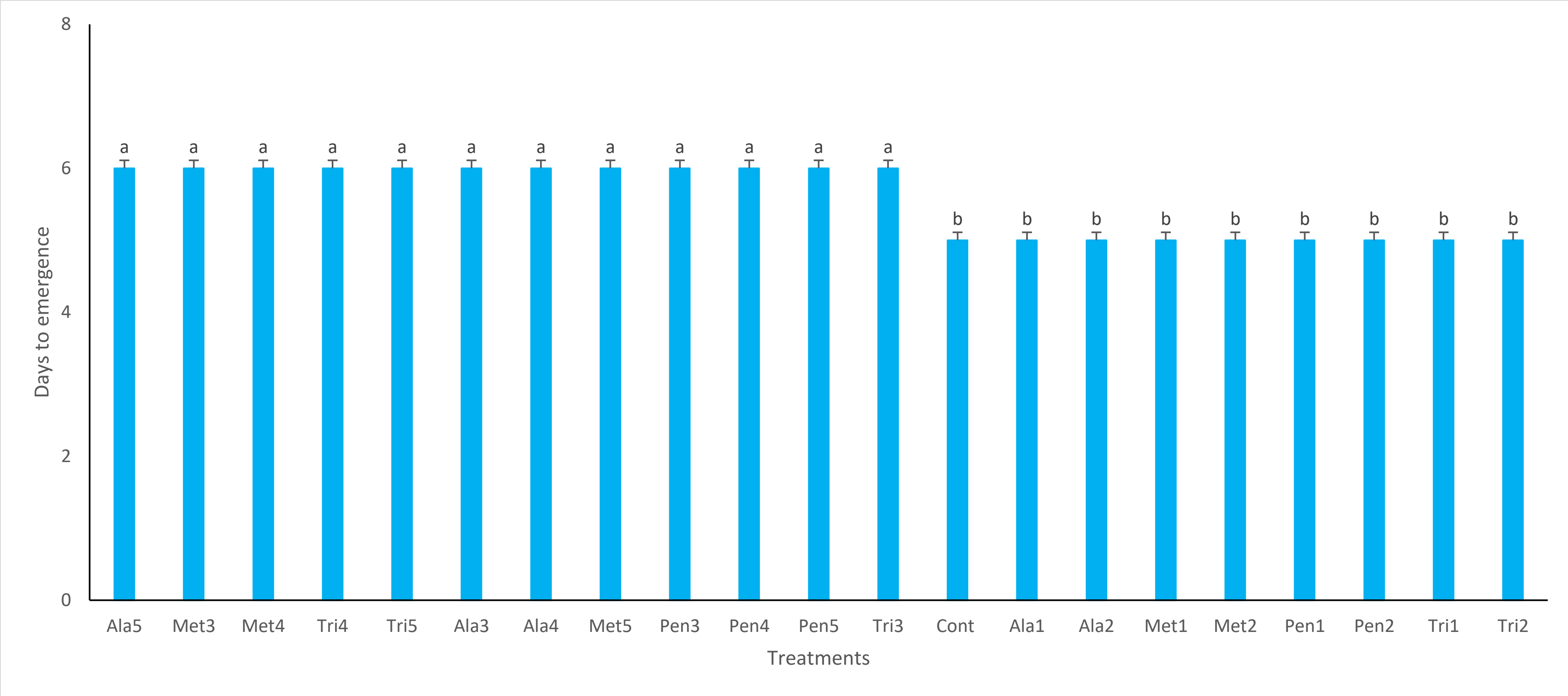


Figure 4. 1 Mean separation of Alachlor, Metolachlor, Trifluralin and Pendimethalin herbicides on Days to emergence of seedlings of Swiss chard. Means with the same letters showed no significantly differences. Cont: control, Ala1: Alachlor 0.5 l/ha, Ala2: Alachlor 1.0 1/ha, Ala3; Alachlor 1.5 1/ha, Ala4: Alachlor 2.0 1/ha, Ala5 Alachlor 2.5 1/ha, Met1: Metolachlor 0.5 1/ha, Met2: Metolachlor 1.0 1/ha, Met3: Metolachlor 1.5 1/ha, Met4: Metolachlor 2.0 1/ha, Met5: Metolachlor 2.5 1/ha, Tri1: Trifluralin 0.5 l/ha, Tri2: Trifluralin 1.0 l/ha, Tri3: Trifluralin 1.5 l/ha, Tri4: Trifluralin 2.0 l/ha, Tri5: Trifluralin 2.5 l/ha, Pen1: Pendimethalin 0.5 1/ha, Pen2: Pendimethalin 1.0 1/ha, Pen3: Pendimethalin 1.5 1/ha, Pen4: Pendimethalin 2.0 1/ha, and Pen5: Pendimethalin 2.5 1/ha.

4.2 Germination Percentage

The effects of the four herbicides germination percentage are presented in (Figure 4.2 and Appendix B). Statistically Swiss chard germination percentage was found to be similar between control and doses up to 2.0/ha germination percentage, while when increased to 2.5 l/ha led to a decline in germination percentage (Figure 4.2 Appendix B). The results suggest that Swiss chard can germinate within a wide range of 0.5 l/ha to 2.0 1/ha herbicides doses before any statistical decline. The results from this study are agreement with what was obtained by Kumar and Jagannath (2015). The authors reported germination percentage of soyabeans only declined when the herbicide was applied at higher concentrations of 2.5 l/ha. In addition, Rajashekar *et al*., (2012) examined the effect of pendimethalin on *Zea mays* L., the author observed drastically decrease in the germination percentage of the crop with respect to the increase in the pendimethalin concentration when compared to the control. Fathi *et al*. (2011), application of trifluralin at high doses reduces the percentage germination of the wheat crop when compared to the control. Thus, in agreement with the finding in the current study. As reported by Shanmugasundaram and Kandasamy (2003) and Hatzinikolaou et al. (2004) this could be an attribute of the herbicide’s impact on the degradation and mobilization of seed reserves.

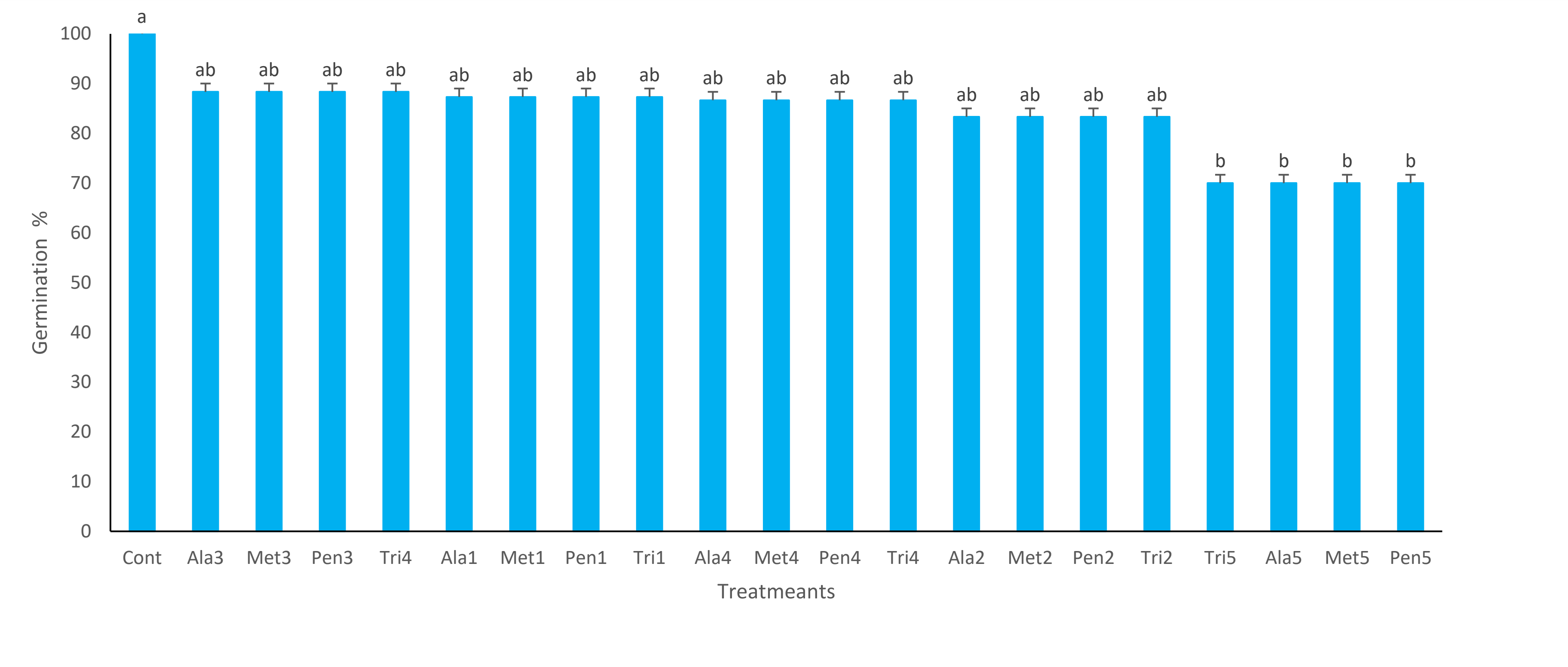
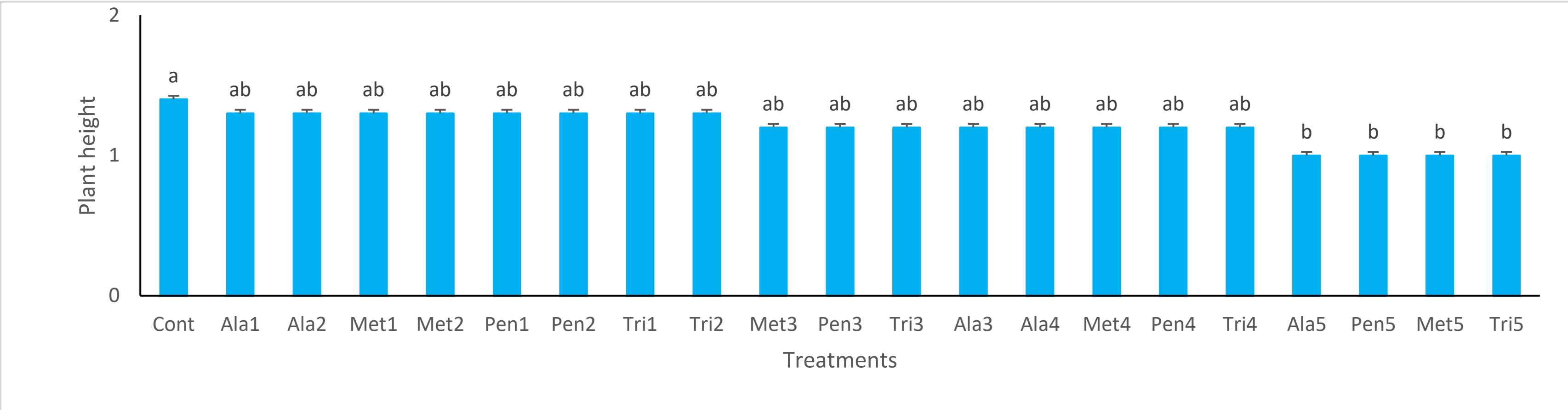


Figure 4.1: Mean separation of Alachlor, Metolachlor, Trifluralin and Pendimethalin herbicides on Germination percentage of seedlings of Swiss chard. Means with the same letters showed no significant differences. Cont: control, Ala1: Alachlor 0.5 l/ha, Ala2: Alachlor 1.0 1/ha, Ala3; Alachlor 1.5 1/ha, Ala4: Alachlor 2.0 1/ha, Ala5 Alachlor 2.5 1/ha, Met1: Metolachlor 0.5 1/ha, Met2: Metolachlor 1.0 1/ha, Met3: Metolachlor 1.5 1/ha, Met4: Metolachlor 2.0 1/ha, Met5: Metolachlor 2.5 1/ha, Tri1: Trifluralin 0.5 l/ha, Tri2: Trifluralin 1.0 l/ha, Tri3: Trifluralin 1.5 l/ha, Tri4: Trifluralin 2.0 l/ha, Tri5: Trifluralin 2.5 l/ha, Pen1: Pendimethalin 0.5 1/ha, Pen2: Pendimethalin 1.0 1/ha, Pen3: Pendimethalin 1.5 1/ha, Pen4: Pendimethalin 2.0 1/ha, and Pen5: Pendimethalin 2.5 1/ha.

4.3 Plant Height

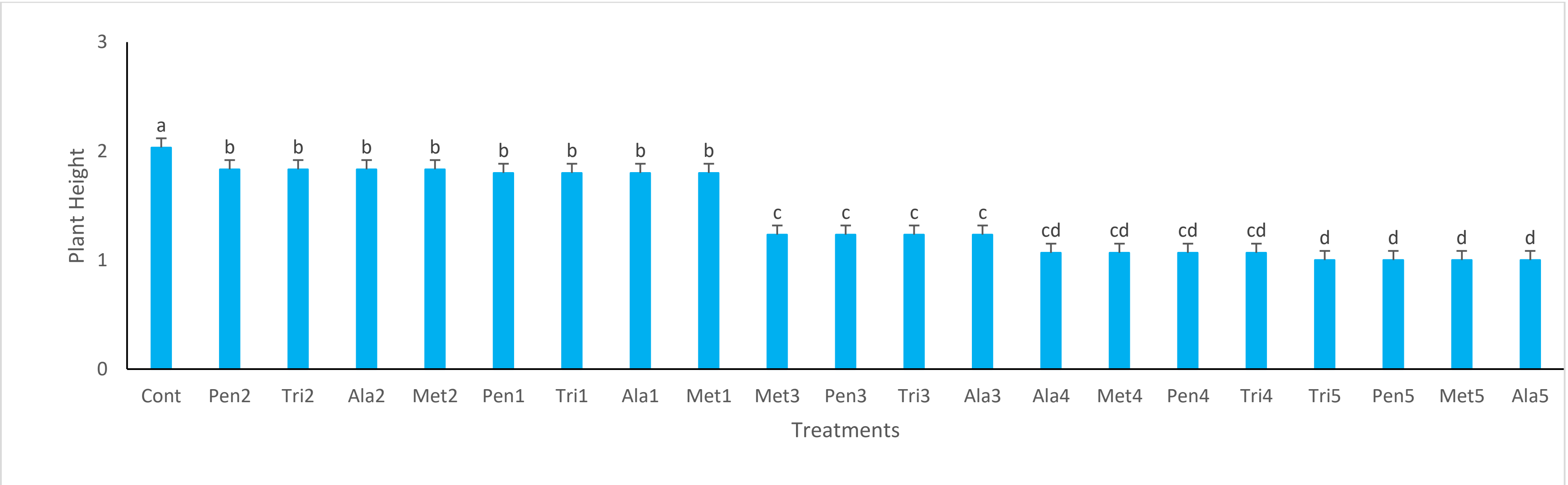
The application of herbicides at different doses were found to significant influence Swish chard height during the period of the study (Figure 4.3 and appendix C). In the first week after emergence, the application of all herbicides at a dose up to 2.0 l/ha had no statistical influence on crop height compared to control (Figure 4.3A). The application of 2.5 l/ha dose for all herbicides resulted in reduction in crop height (Figure 4.3A). The application of all doses of herbicides statistically reduced crop height on week two and three compared to control (Figure 4.3 B and C). The general trend found was that the application of 0.5 l/ha and 1.0 l/ha doses resulted in similar crop height while the increase in dose above 1.0 l/ha had different supressing to crop height (Figure 4.3 B and C). Crop height was generally at the lowest at the highest application dose, 2.5 l/ha, followed by 2.0 l/ha with 0.5 l/ha and 1.0 l/ha being close to the height in control treatment (Figure 4.3). Thus, the general take is that the increase in application rates of all the herbicides lead to supressing in crop height compared to control treatment.. The work by Rahmana *et al.* (2013) on the residue level of alachlor in pepper and pepper leaf support the current findings. The authors reported that the application of Alachlor at low concentration does not affect crops growth height especially at the yearly growth stages. Furthermore, El-Nahhal and Hamdona, (2017) also observed that alachlor showed less phytoxicity to melon *(Cucumis melo)*, molokhia *(Nalta jute)* and wheat *(Triticum)* when applied in the field at lower concentration. The different in the results from these studies might be because of different in the crop and site-ecific conditions. Thus, before recommendations can be made, crop and site-specific conditions must be considered. This is also supported by Fathi *et al*. (2011), who found that trifluralin had no significant effects on the wheat crop when applied at lower concentration. That was not the same case in the current study where application of trifluralin influenced crop height even at lower concentration especially from week two after emergence (Figure 4.3).

A



2
1
0
Plant height
a
ab
b
Cont
Ala1
Ala2
Met1
Met2
Pen1
Pen2
Tri1
Tri2
Met3
Pen3
Tri3
Ala3
Ala4
Met4
Pen4
Tri4
Ala5
Pen5
Met5
Tri5
Treatments

B



3
2
1
0
Plant Height
a
b
c
cd
d
Cont
Pen2
Tri2
Ala2
Met2
Pen1
Tri1
Ala1
Met1
Met3
Pen3
Tri3
Ala3
Ala4
Met4
Pen4
Tri4
Tri5
Pen5
Met5
Ala5
Treatments

C

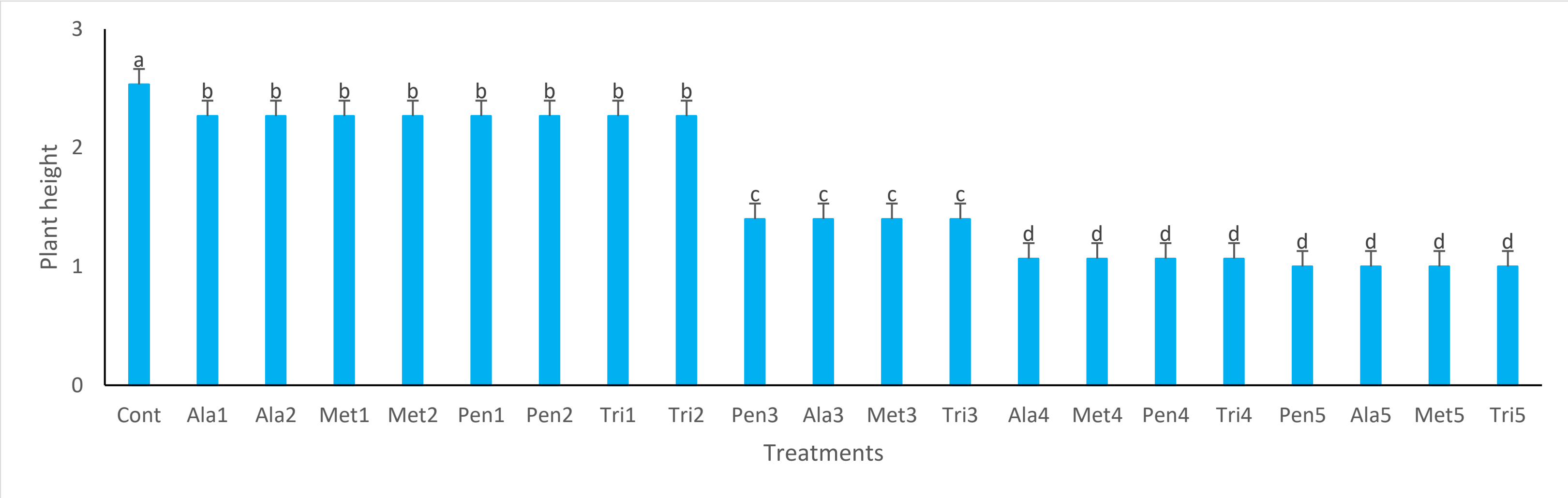
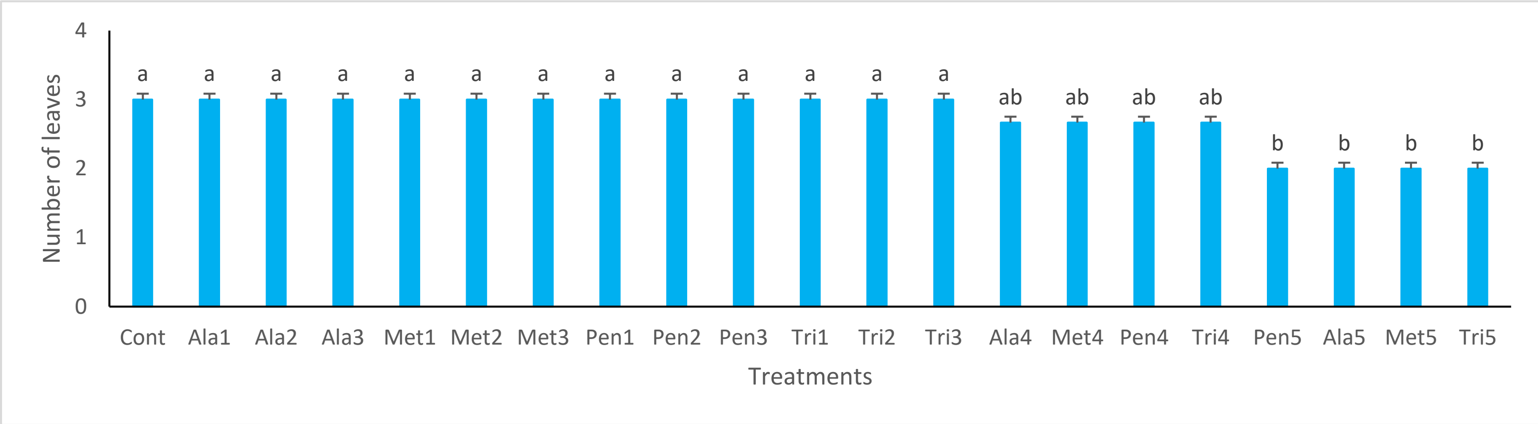


Figure 4.2: Mean separation of Alachlor, Metolachlor, Trifluralin and Pendimethalin herbicides on Plant height of seedlings of Swiss chard. Means with the same letters showed no significant differences. Cont: control, Ala1: Alachlor 0.5 l/ha, Ala2: Alachlor 1.0 1/ha, Ala3; Alachlor 1.5 1/ha, Ala4: Alachlor 2.0 1/ha, Ala5 Alachlor 2.5 1/ha, Met1: Metolachlor 0.5 1/ha, Met2: Metolachlor 1.0 1/ha, Met3: Metolachlor 1.5 1/ha, Met4: Metolachlor 2.0 1/ha, Met5: Metolachlor 2.5 1/ha, Tri1: Trifluralin 0.5 l/ha, Tri2: Trifluralin 1.0 l/ha, Tri3: Trifluralin 1.5 l/ha, Tri4: Trifluralin 2.0 l/ha, Tri5: Trifluralin 2.5 l/ha, Pen1: Pendimethalin 0.5 1/ha, Pen2: Pendimethalin 1.0 1/ha, Pen3: Pendimethalin 1.5 1/ha, Pen4: Pendimethalin 2.0 1/ha, and Pen5: Pendimethalin 2.5 1/ha. A: plant height on week one, B: plant height on week two and C: plant height on week three.

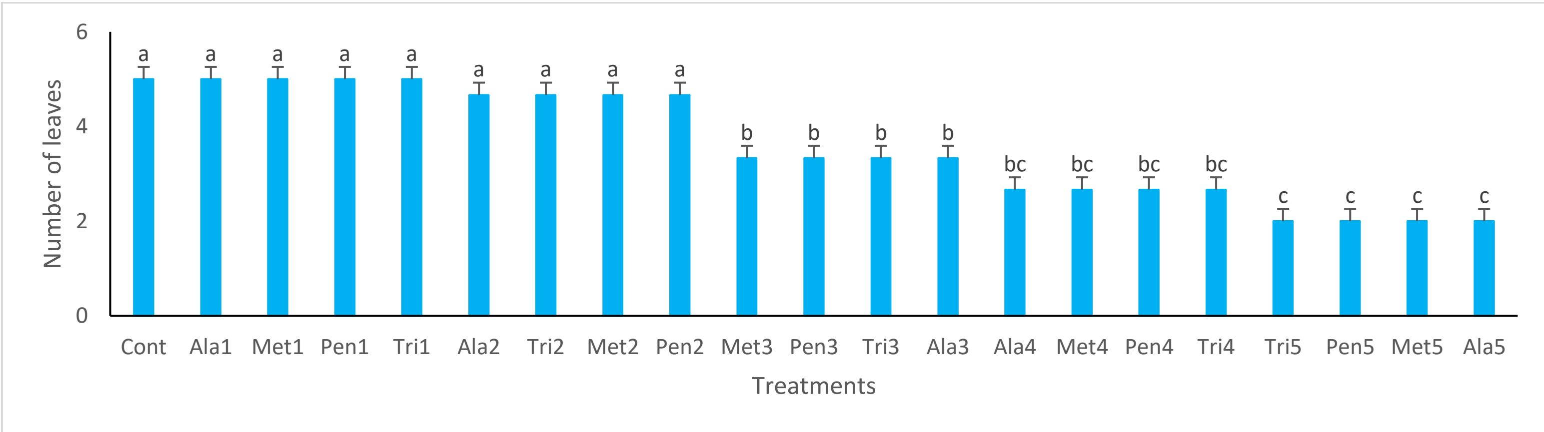
4.4 Number of Leaves

The general trend found was that during week one and two after emergence, the application of all herbicides at 0.5 /ha and 1.0 l/ha statistically did not influence number of leaves compared to control while application above 1.0 l/ha generally resulted with fewer leaves compared to control (Figure 4.4 A and B). On week three after the application of herbicides results resulted in fewer leaves compared to control (Figure 4.4 C). The increase application rate for all the herbicides led to a fewer number of leaves (Figure 4.4 C). The reduction in number of Swiss chard leaves translate to decline in crop yield. A similar number of leaves during the early growth stages of the crop might be due to the crop morphological characteristics under optimum conditions while the increase in herbicide application might be compromising the soil conditions and thus, affecting the crop basic morphology. In addition, the similar number of leaves show that Swiss chard is less susceptible to the applied herbicides at low concentration especially in the early growth stages(Rahmana *et al*., 2013; El-Nahhal and Hamdona, 2017). The literature revealed the reduction of chlorophyll and sugar content in wheat leaves treated with herbicides such as isoproturon which could have led to fewer leaves as photosynthesis was compromised (Sharma, 2002). This might be even under the current study, that the increase in application rate could have influenced crop chlorophyll and thus, the crop's ability to generate more energy to develop new leaves.

A


Number of leaves
4
3
2
1
0
a a a a a a a a a a a a a ab ab ab ab b b b b
Cont Ala1 Ala2 Ala3 Met1 Met2 Met3 Pen1 Pen2 Pen3 Tri1 Tri2 Tri3 Ala4 Met4 Pen4 Tri4 Pen5 Ala5 Met5 Tri5
Treatments

B


Number of leaves
6
4
2
0
a a a a a a a a a b b b b bc bc bc bc c c c c
Cont Ala1 Met1 Pen1 Tri1 Ala2 Tri2 Met2 Pen2 Met3 Pen3 Tri3 Ala3 Ala4 Met4 Pen4 Tri4 Tri5 Pen5 Met5 Ala5
Treatments

C

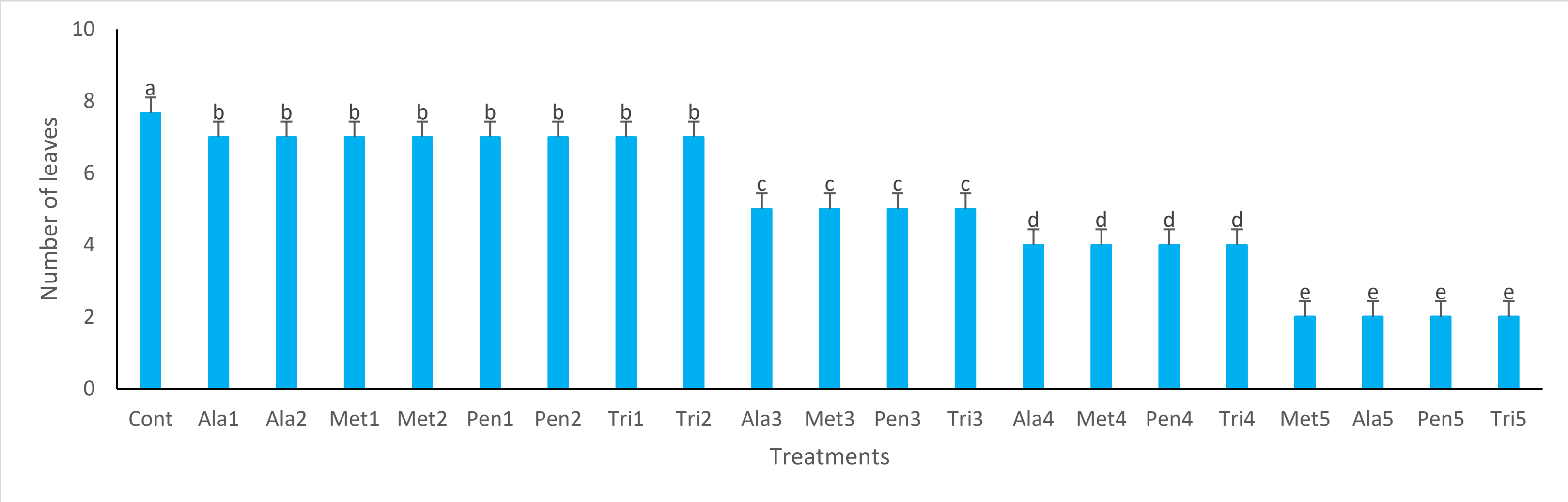


Figure 4.4: Mean separation of Alachlor, Metolachlor, Trifluralin and Pendimethalin herbicides on Plant height of seedlings of Swiss chard. Means with the same letters showed no significant differences. Cont: control, Ala1: Alachlor 0.5 l/ha, Ala2: Alachlor 1.0 1/ha, Ala3; Alachlor 1.5 1/ha, Ala4: Alachlor 2.0 1/ha, Ala5 Alachlor 2.5 1/ha, Met1: Metolachlor 0.5 1/ha, Met2: Metolachlor 1.0 1/ha, Met3: Metolachlor 1.5 1/ha, Met4: Metolachlor 2.0 1/ha, Met5: Metolachlor 2.5 1/ha, Tri1: Trifluralin 0.5 l/ha, Tri2: Trifluralin 1.0 l/ha, Tri3: Trifluralin 1.5 l/ha, Tri4: Trifluralin 2.0 l/ha, Tri5: Trifluralin 2.5 l/ha, Pen1: Pendimethalin 0.5 1/ha, Pen2: Pendimethalin 1.0 1/ha, Pen3: Pendimethalin 1.5 1/ha, Pen4: Pendimethalin 2.0 1/ha, and Pen5: Pendimethalin 2.5 1/ha. A: number of leaves on week one, B: number of leaves on week two and C: number of leaves on week three.

4.5 Root Length and Number

The application of herbicides doses had a significant impact on root length (Figure 4.5 A) and no effect on the number of roots (Figure 4.5 B). Generally, the roots length was statistically the similar between control and lower application rate of 0.5 l/ha of all herbicides (Figure 4.5 A). It is also important to note that roots length under the application of doses between 0.5 l/ha and 2.0 l/ha were statistically similar to the control treatment. This is despite the control having longer roots. The application of 2.5 l/ha of all herbicides generally reduce root length compared to control treatment (Figure 4.5 A). The unclear pattern of the effects of application rates of herbicides on root length might be due to the fact that the root length was a function of the crop morphology, duration of the study and the size of the pots. Water was not a limiting condition which also could have led to less difference in root growth. The results in this study is in line with the study by J. Allemann & G.M. Ceronio (2009) who found that the application of Alachlor and Metolachlor on the growth of Peas (*Pisum sativum* L. 'Alaska') and oats (*Avena sativa* L. 'Victory') significantly reduced the root length of the crops at high application rates.. Dissanayake *et al*. (1998) also observed that pendimethalin application rates caused varying degrees of sugarcane roots length. This was also supported by the current study in Swiss chard. All the application rates of herbicides didn't influence number of roots compared to control (Figure 4.5 B). This show that in the current experimental setup, the number of roots was a function of the crop morphology than effects of the treatments.

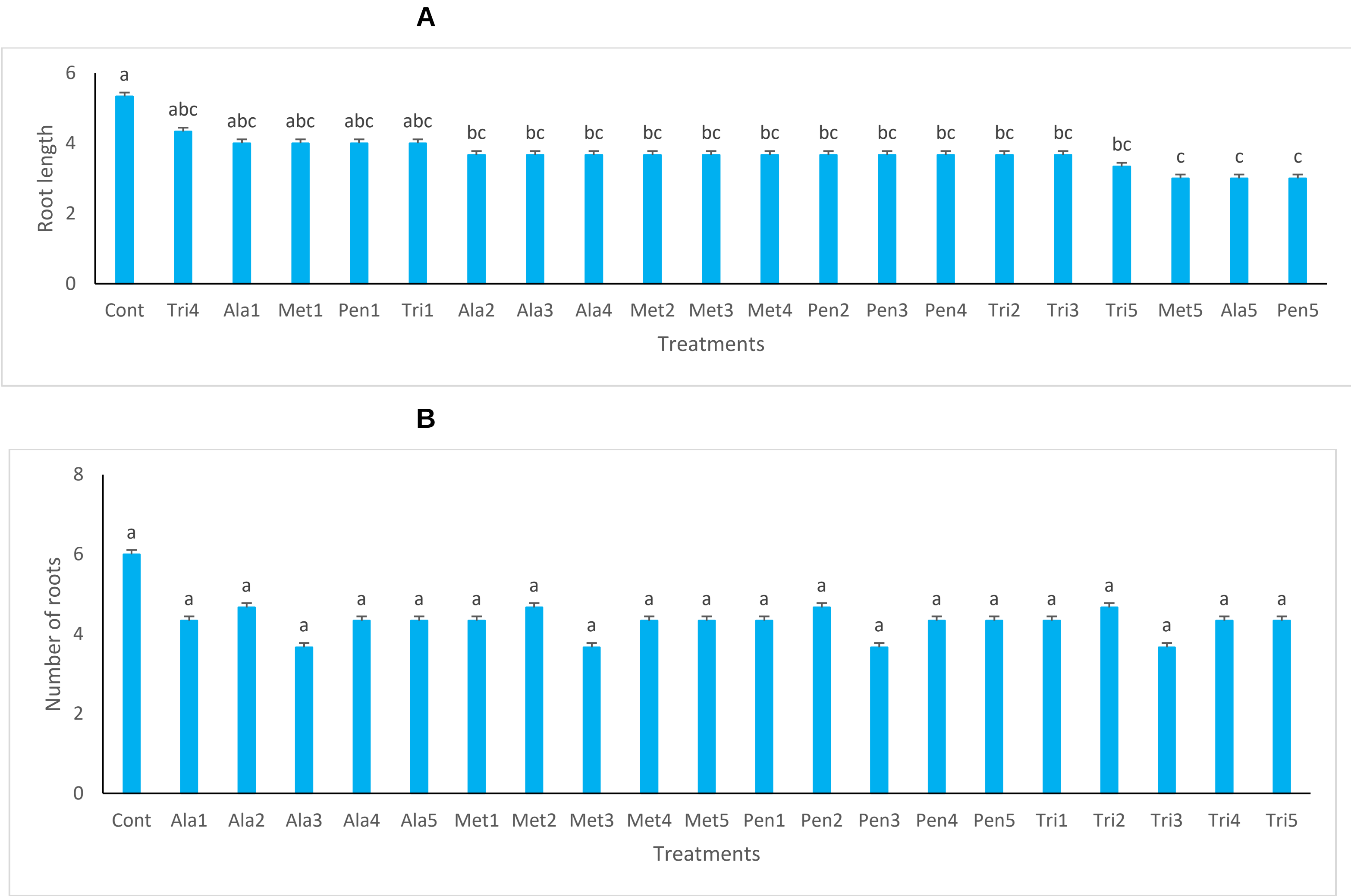


Figure 4. 2 : Mean separation of Alachlor, Metolachlor, Trifluralin and Pendimethalin herbicides on Root length and Number of roots of seedlings of Swiss chard. Means with the same letters showed no significant differences. Cont: control, Ala1: Alachlor 0.5 l/ha, Ala2: Alachlor 1.0 1/ha, Ala3; Alachlor 1.5 1/ha, Ala4: Alachlor 2.0 1/ha, Ala5 Alachlor 2.5 1/ha, Met1: Metolachlor 0.5 1/ha, Met2: Metolachlor 1.0 1/ha,

Met3: Metolachlor 1.5 1/ha, Met4: Metolachlor 2.0 1/ha, Met5: Metolachlor 2.5 1/ha, Tri1: Trifluralin 0.5 l/ha, Tri2: Trifluralin 1.0 l/ha, Tri3: Trifluralin 1.5 l/ha, Tri4: Trifluralin 2.0 l/ha, Tri5: Trifluralin 2.5 l/ha, Pen1: Pendimethalin 0.5 1/ha, Pen2: Pendimethalin 1.0 1/ha, Pen3: Pendimethalin 1.5 1/ha, Pen4: Pendimethalin 2.0 1/ha, and Pen5: Pendimethalin 2.5 1/ha. A: root length on week one, B: number of roots on week two.

CHAPTER 5: CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The application of four herbicides; Alachlor, Metolachlor, Pendimethalin and Trifluralin; at five application rates; 0.5, 1.0, 1.5, 2.0, and 2.5; provided an opportunity to study their effects on selected Swiss chard growth parameters compared to control; 0 l/ha of any herbicide. The application rates of all herbicides influenced all measured variables except the number of roots. This short-term study concludes that:

- Application of all herbicides at doses of 1.0 l/ha does not reduce Swiss chard's number of days to emergence.
- Application of all herbicides at doses of 2.0 l/ha does not reduce Swiss chard number of seed germination percentage.
- Application of all herbicides doses reduces Swiss chard height, especially three weeks after emergence.
- Application of all herbicides doses reduces Swiss chard number of leaves, especially three weeks after emergence.
- Application of all herbicides at doses of 2.0 l/ha does not reduce Swiss chard root length while all doses of herbicides do not influence the number of roots.

5.2 Recommendation

This study recommends that Alachlor, Metolachlor, Pendimethalin, and Trifluralin can be applied on Swiss chard generally at an application of 1.0 l/ha. However, more research still needs to be done to establish if it is true that the higher doses of the used herbicides might interfere with chemical reactions during germination, thus leading to delay in emergence on Swiss chard. And crop and site-specifications must be considered.

Future research should include:

- The effect of Alachlor, Metolachlor, Pendimethalin, and Trifluralin application rates on Swiss chard nutrients composition.

- The effect of Alachlor, Metolachlor, Pendimethalin, and Trifluralin application rates on Swiss chard biomass yields.
- The effect of Alachlor, Metolachlor, Pendimethalin, and Trifluralin application rates, and harvesting cycles on Swiss chard yield and nutrients composition.

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APPENDIX A: ANALYSIS OF VARIANCE FOR THE EFFECTS OF THE DIFFERENT HERBICIDES ON THE DAYS OF EMERGENCE ON SWISS CHARD (<,0001*)

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	20	20	18,720000	7,03e+16	<,0001*

APPENDIX B: ANALYSIS OF VARIANCE FOR THE EFFECTS OF THE DIFFERENT HERBICIDES ON THE GERMINATION PERCENTAGE ON SWISS CHARD (P<,0001*)

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	20	20	4171,4133	4,3510	<,0001*

APPENDIX C: ANALYSIS OF VARIANCE FOR THE EFFECTS OF THE DIFFERENT HERBICIDES ON THE PLANT HEIGHT ON SWISS CHARD (0,0010*)

WK1 plant height

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	20	20	2,6448000	2,8305	0,0010*

WK 2 plant height

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	20	20	10,419200	130,2400	<,0001*

WK 3 plant height

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	20	20	26,626667	156,9969	<,0001*

APPENDIX D: ANALYSIS OF VARIANCE FOR THE EFFECTS OF THE DIFFERENT HERBICIDES ON THE NUMBER OF LEAVES ON SWISS CHARD (<,0001*)

Wk 1 number of leaves

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	20	20	9,9200000	7,7500	<,0001*

Wk 2 number of leaves

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	20	20	104,74667	27,2778	<,0001*

Wk 3 number of leaves

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	20	20	271,68000	849,0000	<,0001*

APPENDIX E: ANALYSIS OF VARIANCE FOR THE EFFECTS OF THE DIFFERENT HERBICIDES ON THE ROOT LENGTH ON SWISS CHARD (0,0001*)

Root Length

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	20	20	22,053333	3,4458	0,0001*

APPENDIX F: ANALYSIS OF VARIANCE FOR THE EFFECTS OF THE DIFFERENT HERBICIDES ON THE NUMBER OF ROOTS ON SWISS CHARD (0,1620)

Number of roots

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	20	20	16,000000	1,3889	0,1620