PROPAGATION AND EVALUATION OF EFFECTIVENESS OF *COMMIPHORA SWYNNERTONII* (Burtt.) AND *SYNADENIUM GLAUCESCENS* (Pax.) AGAINST TOMATO FUSARIUM WILT

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CROP SCIENCE OF SOKOINE UNIVERSITY OF AGRICULTURE.

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EXTENDED ABSTRACT

Introduction

Over-exploitation and habitat destruction have become a major limitation to production, marketing and usage of botanical pesticides. In Tanzania, *Commiphora swynnertonii* and *Synadenium glaucescens* have been reported to be disappearing very fast. There is a need

to develop a technique that will ensure sustainable availability of these plants. The current study, therefore aimed at enhancing mass propagation and fungicidal effectiveness of *C. swynnertonii* and *S. glaucescens* against tomato fusarium wilt. Specifically, the study sought to: (1) To evaluate propagation potential of *C. swynnertonii* and *S. glaucescens*, (2) To determine field establishment of *C. swynnertonii* and *S. glaucescens* and (3) To determine effectiveness of *C. swynnertonii* and *S. glaucescens* in managing tomato fusarium wilt disease. The second, third and fourth chapter in the dissertation comprise manuscripts in the form of publishable papers which cover the first, second and third specific objectives.

Methods

With respect to specific objective 1, screen house and field experiments were carried at Sokoine University of Agriculture. Morogoro, Tanzania. In the screen house, two separate trials were conducted. The first trial evaluated the influence of pre-sowing seed treatments on germination. The second trial evaluated the influence of cutting types and growth regulators on rooting and sprouting of stem cuttings. Pre-sowing seed treatments involved soaking seeds in water at room temperature (25°C), hot water (60°C), Gibberellin (GA_3) solution and Potassium nitrate (KNO_3) at different concentrations. The experiment was set in a randomized complete block design (RCBD) with four replications. On the evaluation of the influence of cutting types and growth regulators, there were nine treatment combinations comprising of three types of cuttings (softwood, semi-hardwood and hardwood), two rooting hormones (Indole-3-Acetic Acid (IAA) and Naphthalene Acetic Acid (NAA)) and control. The experiment was set in a 3 x 3 factorial in RCBD with four replications. Survived plantlets from screen house were planted in the field as per specific objective 2.

Laboratory and screen house experiments were carried as per specific objective 3. In the laboratory experiment, there were sixteen treatment combinations comprising of four crude plant extracts obtained from resin of *C. swynnertonii*, latex, fresh and dry leaves of *S. glaucescens* and four extract concentrations (0.01 g/ml, 0.05 g/ml, 0.1 g/ml and 0.15 g/ml). Sterile distilled water and Linkmil 72 WP (Mancozeb 64% + Metalaxyl 8%) were used as a negative and positive control, respectively. The experiment was set in 4 x 4 factorial in a completely randomized design (CRD) with three replications. In the screen house experiment, there were four treatments; resinous extracts of *C. swynnertonii*, extract from latex and fresh leaves of *S. glaucescens* and dried leaves powder of *S. glaucescens*. Untreated soil and soil treated with Linkmil 72 WP were used as a negative and positive control, respectively. The experiment was set in RCBD with four replications.

Findings

The results revealed that seed germination of the two plant species was poor but was significantly affected by seed treatments. Better germination was recorded when *C. swynnertonii* and *S. glaucescens* seeds were treated with either KNO_3 at 10 ppm or soaked in water (25°C). Semi-hardwood cuttings of *C. swynnertonii* and softwood cuttings of *S. glaucescens* dipped in 2 000 ppm NAA solution led to higher rooting of 52.50% and 97.50%, respectively. In the field experiment, higher survival ability was recorded when *C. swynnertonii* and *S. glaucescens* plants were previously treated with either KNO_3 at 10 ppm or GA_3 at 250 ppm. Plants from hardwood cuttings of *C. swynnertonii* and semi-hardwood cuttings of *S. glaucescens* previously dipped in 2 000 ppm NAA solution survived better compared to the other treatments and control.

Laboratory experiment revealed that dry leaves extract of *S. glaucescens* and resinous extracts of *C. swynnertonii* at 0.15 g/ml had the highest inhibitory effect against mycelia

growth of *F. oxysporum* f. sp. *lycopersici.* In the screen house experiment, the results revealed that application of dried leaves powder of *S. glaucescens* exhibited the least disease severity and showed a significant effect on plant growth.

Conclusions

Based on the findings, *C. swynnertonii* and *S. glaucescens* can be propagated successful through stem cuttings. Cutting types and growth regulators had significantly enhance rooting and survival ability. Semi-hardwood and softwood cuttings treated with NAA 2 000 ppm was found to be the best for mass propagation of *C. swynnertonii* and *S. glaucescens*, respectively. Plants from hardwood and semi-hardwood cuttings previously treated with NAA 2 000 ppm were found to be the best for field establishment of *C. swynnertonii* and *S. glaucescens*, respectively. Dry leaves extract of *S. glaucescens* and resinous extracts of *C. swynnertonii* at 0.15 g/ml had the highest inhibitory effect against mycelia growth of *F. oxysporum* f. sp. *lycopersici.* The application of dried leaves powder of *S. glaucescens* exhibited the least disease severity. Tomato plants treated with dried leaves powder showed a significant stimulatory effect on plant growth.

Recommendations

The findings suggest that semi-hardwood cuttings and softwood cuttings dipped in 2 000 ppm NAA solution can be used for mass propagation of *C. swynnertonii* and *S. glaucescens*. The dried leaves powder and extracts of *S. glaucescens* can be used in management of tomato fusarium wilt disease. Further studies to determine the mechanisms of botanicals involved in the inhibition of mycerial growth of *F. oxysporum* f. sp. *lycopersici* is recommended. This will help to determine the mode and rates of the application without a significant reduction in plant growth.

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LIST OF PAPER CHAPTERS

DECLARATION

I, Saidi Babu, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my original work done within period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

Saidi Babu (MSc. Candidate)	Date

The above declaration confirmed by;

Dr. Hosea D. Mtui (Supervisor)	Date
Dr. Abdul B. Kudra (Supervisor)	Date
Dr. Faith P. Mabiki (Supervisor)	Date

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DEDICATION

This work is dedicated to my late father Abdulrahman A. Babu, my mother Hanifa S. Msangawenga and my beloved wife Vumilia M. Nandonde, my sister Husna, my brothers Seleman, Mussa, Ally, Hassan and Idd.

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ANOVA	Analysis of Variance
c. v.	Cultivar
CRD	Completely Randomized Design
CV	Coefficient of Variation
DMRT	Duncan´s Multiple Range Test
Fig.	Figure
FYM	Farm Yard Manure
GA_3	Gibberellic acid
HIV	Human Immunodeficiency Virus
IAA	Indole Acetic Acid
IBA	Indole-3-Butyric Acid
IUCN	International Union for Conservation of Nature
KNO_3	Potassium Nitrate
NAA	Naphthalene Acetic Acid
NaOCl	Sodium Hypochlorite
NC	Negative Control
PC	Positive Control
PDA	Potato Dextrose Agar
Ppm	Parts per million
RCBD	Randomized Complete Block Design
TB	Tuberculosis
WP	Wettable Powder

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background Information

Pesticidal plants play an important role in the control of plant diseases and have been used since time immemorial (Cueto-Wong *et al.,* 2010). The use of botanical pesticides has increased dramatically in recent years particularly in developing countries (Mishra *et al.,* 2014). The demand for botanical pesticides is set to rise due to increases in organic farming, consumers demanding safe food and environmentalists promoting for eco-friendly pesticides (Sola *et al.,* 2014). Botanical pesticides are safe and environmentally friendly than synthetic pesticides (Kumar *et al*., 2014).

In addition, they are cheap and easily available due to their natural occurrence (Ramaiah and Garampalli, 2015). Plants produce bioactive compounds that function as defence substances against insects attack, pathogens and herbivorous mammals. Several groups of phytobiocides such as alkaloids, carotenoids, coumarins, tannins, triterpenoids, anthocyanins, volatile oil and phenolic compounds from more than 2 000 plant species have been described for their pesticidal properties (Ghosh *et al.,* 2012).

The demands of botanical pesticides can only be met by formalizing production and usage of pesticidal plants (Sola *et al.,* 2014). Pesticidal plants can either be propagated from seeds, cuttings or modified plant organs such as suckers, rhizomes, bulbs and corms. The propagation through seeds is constrained by the presence of non-viable seeds, seed parasitism and some seeds are difficult to germinate (Lal and Kasera, 2014; Maduka *et al.,* 2017). The accessibility of modified plant organs as propagules is limited due to their over-exploitation (Amoo, 2014). An understanding of the best methods of

propagating pesticidal plants is essential to their successful cultivation, henceforward conservation.

Fusarium wilt diseases caused by members of the genus *Fusarium* have been reported to hinder crop production worldwide (Gullino *et al.*, 2015). Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* is the most important disease of tomato affecting tomato production worldwide (Amini and Sidovich, 2010). The pathogen is a soil-borne and can live saprophytically in the soil without a host (Arici *et al.*, 2018). The pathogen is found everywhere in the world where tomato is grown (Moretti *et al.*, 2008). This fungus enters the host plant through the roots and multiplies in the vascular tissue, impeding the transportation of water and causing necrosis of the leaves, huge wilting, stunted seedlings and rapid death of the plant (Horinouchi *et al.*, 2011; Abdel-Monaim, 2012). The crop losses between 10 to 90% caused by tomato fusarium wilt have been reported (Singh and Kamal, 2012).

Several methods have been tried for controlling the tomato fusarium wilt. These include the use of fungicides, fumigation, soil solarization, appropriate cultural and biological practices. However, none of these approaches can permanently control tomato fusarium wilt (Adedeji and Aduramigba, 2016). The use of resistant genotype is the most trustworthy method of disease control (Nirmaladevi *et al.*, 2016). However, they are limited (Minja *et al.*, 2011) and the presence of novel pathogens appear to overwhelm resistance genes in presently grown genotypes (Kutama *et al.*, 2013). Soil solarization depends on the climate and it is reported to be ineffective in managing soil-borne pathogens which are heat-tolerant (Barakat and Al-Masri, 2011). The use of sulphur as a fumigant is focussed only on suppression of the disease (Adedeji and Aduramigba, 2016). This leaves farmers with few options for managing tomato fusarium wilt and therefore at

the mercy of this disease. Several biological control agents such as *Pseudomonas fluorescens* (Asha *et al.,* 2011), *Pseudomonas aeruginosa* (Paramanandham *et al*., 2017) and *Trichoderma harzianum* (Karkachi *et al.,* 2010) have been successfully tested against tomato fusarium wilt, however their application and or uses have not been reported abundantly in Africa.

Pesticide application is a major pest management strategy of tomato pests and is usually applied on a weekly basis in Tanzania (Maerere *et al.,* 2010; Mamiro *et al.,* 2015; Mtui *et al.,* 2015). This aggravates both the production expenses, risks for human health and ecological risks presented by synthetic pesticides (Maerere *et al.,* 2010; Meya *et al.,* 2014). Synthetic pesticides are detrimental to natural enemies, pollinators, crop producers and consumers (Mishra *et al.,* 2014). There are limited market chances for conventionally produced crops especially for the export market due to heavy spraying (Mtui *et al.,* 2015). Therefore, there is a need to introduce control measures that will reduce reliance on synthetic pesticides. Botanical pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides (Ramaiah and Garampalli, 2015). Botanical pesticides are cheap, biodegradable and have no toxic effect on non-target organisms (Kumar *et al*., 2014). Therefore, pesticidal plants could be used as a raw material for formulating safer, affordable and environmentally friendly fungicides.

1.2 The Genus *Commiphora*

The family *Burseraceae* contain about 700 species comprised in 18 genera including *Commiphora* (Rüdiger *et al.,* 2007). The genus *Commiphora* contains more than 200 species of shrubs and trees, which are distributed throughout the tropical regions of Africa, Arabia and India (Priyanka *et al.,* 2014). In Tanzania, the *Commiphora* species is

abundantly available in Manyara, Kilimanjaro, Dodoma, Singida, Mwanza and Tanga (Bakari, 2013). *Commiphora* plants are known by different names in local languages of Tanzanians like *Mturituri* (Swahili), *Oltemwai* (Maasai), *Mguta* (Sukuma), *Dumbechanda* (Taturu) and *Mzilanzi* (Gogo) (Sambuta and Masola, 2006). The Afrikaans name for *Commiphora* is '*kanniedood* (meaning 'cannot die') refers to the fact that some *Commiphora* species cuttings grow very easily when planted (Priyanka *et al.*, 2014).

1.2.1 Features of *Commiphora* species

Commiphora species are readily distinguished from one another by their characteristics appearances such as spine, the colour of barks, fruits, the scent of sap and weather tree or shrubs (Plate 1.1). In many cases, their appearance together with the habitat in which they occur gives a fairly good indication of species. *Commiphora* species are small trees or shrubs with short, thorny branches (Moshi *et al.*, 2010). The plants grow between 3.5 to 4.0 m tall (Paraskeva *et al.*, 2008). The bark of most *Commiphora* species is papery and peels off into papery flakes, revealing a green bark underneath. When the bark is damaged exudate a watery milky sap which later becomes reddish-brown resinous exudates. The leaves are mostly compound, with only a few species bearing simple leaves (Priyanka *et al.*, 2014). Most of *Commiphora* species have small, usually yellow or white unisexual flowers. The fruit is drupe, red, ovate or acuminate in shape depending on species and when ripe splits into halves revealing a brightly coloured pseudo-aril (Swanepoel, 2014; Soni *et al.*, 2013). The fruit of *Commiphora* plant greatly enhances the identification of the species.

Plate 1.1: *C. swynnertonii* plant [A]; an incision on the stem showing resin [B].

1.2.2 Phytochemistry of *C. swynnertonii*

Species of the genus *Commiphora* contain various bioactive metabolites with their concentrations vary widely depending on species, season and geographical location. Diverse secondary metabolites including phenolic compounds, terpenoids, steroids, sugars and lignans have been discovered in the genus *Commiphora* (Bakari, 2013). Phenolic compounds are produced by plants in response to pests, ultraviolet radiation and wounding (Napal *et al.*, 2010). The capacity of the phenolic group to be deprotonated and oxidised explains their biologically important antioxidant properties (Polya, 2003). Terpenoids are produced for plant growth and development. However, a large amount of terpenoid is used for protection in the abiotic and biotic environment (Tholl, 2015). Phytochemical screening of *C. swynnertonii* is as shown in Table 1.1.

Table 1.1: Phytochemicals in root back and resins of *C. swynnertonii*

Phytochemical	Root bark	Resin
Tannins	++	+

Flavonoids	+++	+++
Terpenoids	++	+++
Anthraquinones	+++	-
Steroids	+++	++
Cardiac glycosides	+++	+++
Saponins	+	+++

Key: - = absence of a compound; + = Low amount; ++ = Moderate; +++ = Abundant

Source (Bakari, 2013).

1.2.3 Uses of *C. swynnertonii*

1.2.3.1 Pesticidal uses of *C. swynnertonii*

A number of studies suggest that *Commiphora* species elicits significant antifungal activity. El-Nagerabi *et al.* (2016) reported antifungal activity of resin of *Commiphora myrrha* against *Aspergillus niger,* the causal agent of black mould disease of onion. Alcoholic leaf extract of *Commiphora stoksiana* at 10% completely inhibited the growth of *Fusarium oxysporum* f. sp. *spinaciae* (Bhale *et al.,* 2005). Bhosale and Jadhav (2015) showed that *Commiphora mukul* resin contains antimicrobial activities against *Verticillium lecanii,* the causal agent of verticillium wilt disease of soybean.

The insecticidal, acaricidal, bacterial and antiviral activity of *Commiphora species* has been evaluated by different researchers. Matendo (2017) assessed the insecticidal effectiveness of *Commiphora swynnertonii* against tomato leaf miner (*Tuta absoluta*). Results indicated that ethanolic extract of *C. swynnertonii* resin caused significant mortality to larvae and adults *T. absoluta*. Shonouda *et al.* (2000) evaluated the effect of the *Commiphora molmol* against *Spodoptera littoralis.* The results showed that resinous extract of *C. molmol* at 10 000 ppm induced the highest mortality of 44.4%. The resin extract of *C. swynertonii* has proved to be potential in the management of ticks, fleas and tsetse flies (Kalala *et al.,* 2014). Resin and root bark extracts of *C. swynnertonii* showed significant activities against *Streptococcus pyogenes, Escherichia coli, Bacillus subtilis* and Newcastle disease virus (Bakari, 2013).

1.2.3.2 Other uses of *C. swynnertonii*

Species of the genus *Commiphora* comprise of very attractive commercial botanical source of odorants. The resinous exudates of the genus *Commiphora* are commonly used as glues, perfume, dentifrices, embalming ointment and in religious ceremonies as incense (Soni *et al.*, 2013). Extracts from the gum of the bark are also used to produce lather for washing. Some *Commiphora* species are used as live fence for boundary marking (Schmidt and Mbora, 2008). The wood of *Commiphora* is used for construction purposes for houses and animal enclosures because it is termite resistant (Hines and Eckman, 1993). The leaves of *Commiphora* are used as animal feed (Schmidt and Mbora, 2008).

1.3 The Genus *Synadenium*

The *Euphorbiaceae* family consists of approximately 8 900 species from 322 genera including *Synadenium* (Hassan *et al.*, 2012). This family is largely found in tropical or arid habitats of Africa and America (Bittner *et al.*, 2001). The *Euphorbiaceae* family consist of herbs, shrubs and large trees (Balakrishnan and Chakrabarty, 2007). The genus *Synadenium* contains 24 species indigenous to eastern Africa (Dev and Koul, 1997). The plants belonging to the genus *Synadenium* produce white latex that is caustic and toxic (Melo-Reis *et al.*, 2010). The genus name *Synadenium* originates from the Greek words *Syn* (meaning ‘united’) and *aden* (meaning ‘gland’). This is because the plants belonging to the genus *Synadenium* has two glands that are united into a ring on the rim of the involucre (Burrows and Tyrl, 2013).

1.3.1 Features of *Synadenium* species

The *Synadenium* species are shrubs or trees with sub-fleshy cylindrical branches and copious milky latex (Dawidar *et al.*, 2011) (Plate 1.2). The plants grow up to 10 m tall

with an equal spread of crown (Nicholson, 2008). Most of *Synadenium* species are succulent and when their bark is damaged they exudate a white latex which is highly irritating. Their branches profusely near the base with stems that are covered in alternate large, tropical-looking foliage. The leaves alternate, sessile, fleshy and thin, obovate, obtuse at the apex with a short point. Most of *Synadenium* species are monoecious; flowers usually green, yellow or dark red (Hassan *et al.,* 2012; Costa *et al.,* 2012).

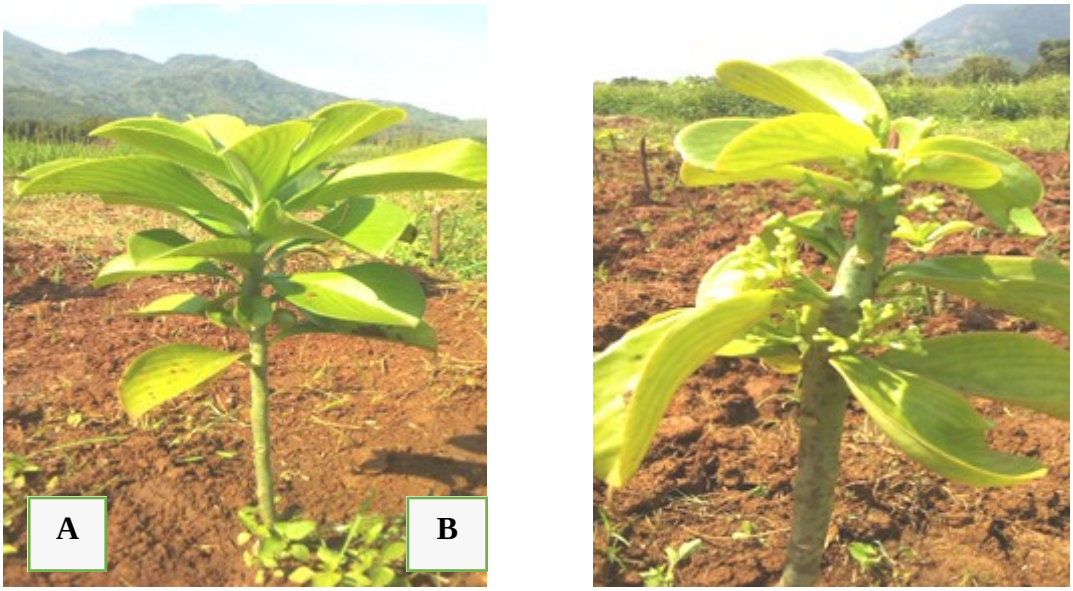

Plate 1.2: *S. glaucescens* plant (6 months old) [A]; cluster of flowers [B].

1.3.2 Phytochemistry of *S. glaucescens*

Members of the *Euphorbiaceae* family are well known for their diverse chemical ingredients and biological activities (Sabandar *et al.,* 2013). The genus *Synadenium* has been reported to have various classes of phytochemicals which include flavonoids, saponins, diterpenes and phorbol esters (Jassbi, 2006). Phytochemical screening of different morphological parts of the *S. grantii* has indicated the presence of diterpenoids, anthocynanin and terpenoids (Andersen *et al.,* 2010; Hassan *et al.,* 2012; Munhoz *et al.,* 2014). The leaves and stems extracts of *S. glaucescens* contains carotenoids, coumarins,

tannins, triterpenoids, anthocyanins, steroids, triterpenes, volatile oils and glucosides (Rukunga *et al.*, 1990; Neuwinger, 1996).

1.3.3 Uses of *S. glaucescens*

1.3.3.1 Pesticidal uses of *S. glaucescens*

Species of the *Synadenium* are used as botanical pesticides against storage pests (Nyigo *et al.*, 2016). Aqueous extracts from leaves, seeds and roots of *S. glaucescens* is sprayed to protect vegetables from caterpillars and seedlings from termites (Mabiki *et al.*, 2013). In Tanzania, it is planted surrounding the buildings to serve as a repellant for the ants. Stem branches and buds of *S. glaucescens* have insecticidal and repellant properties against aphids, grasshoppers and mosquitoes (Grainge and Ashmed, 1988). Gomes *et al.* (2019) assessed the nematicidal property of the latex of *Synadenium grantii* against *Meloidogyne incognita* and *Panagrellus redivivus*. The results show that the latex of *S. grantii* has a high effect against *M. incognita* and *P. redivivus* with a mortality of 100% and 72%, respectively.

1.3.3.2 Other uses of *S. glaucescens*

Plants from the family *Euphorbiaceae* are widely used for diverse purposes throughout the world (Mwine and Damme, 2011). The principal utilization of the genus *Synadenium* is for live fences (Nicholson, 2008). *Synadenium grantii* is used as an ornamental plant. The latex of *Synadenium* species can be fatal if ingested and is used as an illegal fish poison. In Tanzania, *Synadenium* species are used in traditional medicine for the cure of human and animal diseases (Mabiki, 2013). Boiled leaves and stem barks of *Synadenium* is used to control ticks (Nicholson, 2008). *Synadenium glaucescens* is traditionally claimed to be used for the treatment of Human Immunodeficiency Virus (HIV), Tuberculosis (TB), sores, wounds and worms (Mabiki, 2013).

1.4 Propagation of Pesticidal Plants

The art of propagation of plants through seeds and stem cuttings is a common practice in the field of agriculture. Propagation of some pesticidal plants is beset with the problems of poor seedling establishment and rooting of stem cuttings (Raina *et al.,* 2011; Diwakar *et al.,* 2011). Nitrogenous compounds, gibberellin solutions and water have been reported to improve seed germination (Lal and Kasera, 2014; Raji and Siril, 2018). Plant growth regulators have been reported to promote rooting of pesticidal plants which are difficult to root (Diwakar *et al.,* 2011). There is a limited report on propagation of *C. swynnertonii* and *S. glaucescens.* Kumar *et al.* (2002) reported that most of *Commiphora* species such as *Commiphora wightii* are propagated through seeds and stem cuttings. Gachathi *et al.* (2016) reported that *Synadenium pereskiifolium* is easily propagated from stem cuttings. According to Nicholson (2008) *Synadenium grantii* is propagated from seed, stem cuttings and root cuttings.

1.4.1 Seed germination

The seed remains inactive with low metabolic rate until it receives favourable environmental conditions that activate the growth of the embryo (Ruchala, 2002). Botsheleng *et al.* (2014) reported that most of the arid and semi-arid tree species their seeds cannot germinate on time when subjected to conditions favourable for germination due to hard seed coat that is impermeable to water. Understanding the seed germination is crucial for mass propagation of pesticidal plants. However, no information is available on the germination of *C. swynnertonii* and *S. glaucescens* seeds.

1.4.1.1 Effect of water soak on seed germination

Water is a medium for all plant physiological processes and plays a vital role in breaking seed dormancy by softening the seed coat and draining off chemical inhibitors (Olajide *et al.,* 2014). Pandey (2012) studied the effects of water (20 - 25°C) on germination of *Gymnema sylvestre* seeds. The results indicate that seeds soaked in water for 24 hours germinated better with germination percentage of 42.50%. The studies on the influence of soaking duration on seed germination and development of tomato showed that seeds soaked in water for 12 hours germinated earlier with the highest germination percent (Sabongari and Aliero, 2004). Olajide *et al.* (2014) observed that seeds of *Dialium guineense* soaked in cold water for 24 hours germinated earlier compared to other pre-germination treatments. A study on the effect of pre-sowing treatments on germination and seedling growth of *Tectona grandis* showed that soaking seeds in water for 24 hours improve seedling vigour (Offiong *et al.,* 2010).

1.4.1.2 Effect of hot water on seed germination

Hot water improves germination of hard-coated seeds by making the testa permeable to water and oxygen (Aydin and Uzun, 2001). It breaks chemical bonds in the seed coat responsible for triggering seed dormancy (Dewir *et al.,* 2011). Seeds of *Acacia melanoxylon* exposed to boiling water for 1 minute give higher germination percentages as compared with the other treatments and control (Burrows *et al.,* 2009). Tadros and Al-Mefleh (2011) observed that hot water (70°C) improves germination of *Leucaena leucocephala* seeds up to 68%. According to Zayed *et al.* (2012) soaking seeds in hot water (100°C) for 20 seconds promote early germination. Seeds of *Acacia origena* soaked in hot water for 2 and 4 minutes give significantly more germinated seeds (60.0%) as compared to acid-treated seeds and control (Aref *et al.,* 2011). Saberi *et al.* (2011)

obtained the highest germination percentage when *Citrullus colocynthis* seeds placed in hot water 90°C for 10 minutes. The studies done by Omokhua *et al.* (2015) on the influence of different pre-sowing treatments on germination and development of *Tetrapleura tetraptera* seeds showed that the seeds soaked in hot water for 1 minute had the least germination percentage. According to Amusa (2011) when seeds exposed to hot water for a long time can result in the death of the embryo.

1.4.1.3 Effect of gibberellins on seed germination

Gibberellin (GA) is an essential plant hormone that controls the growth and development of the plant (Gupta and Chakrabarty, 2013). Gibberellins control production of enzymes responsible for the hydrolysis of food reserves in seeds and hence stimulating germination (Hartmann *et al.,* 2010). Gibberellic acid (GA_3) is used to break the physiological dormancy of hard-coated seeds (Yao, 2015; Majidi *et al.,* 2016). Gibberellins enhance seed germination by inhibiting abscisic acid (ABA) activity (Miransari and Smith, 2014). Patel and Mankad (2014) observed the highest germination of 94% when *Tithonia rotundifolia* seeds treated with GA_3 at 500 ppm. *Sabal palmetto* seeds soaked in 500 ppm gibberellic acid (GA_3) for 24 hours germinated well with 95 percentage germination (Dewir *et al.,* 2011). Chetouani *et al.* (2017) reported the highest germination of 62% and 67% when *Thymus satureioides* L. and *Lavandula dentate* seeds treated with GA_3 at 50 ppm and 1 000 ppm, respectively.

1.4.1.4 Effect of potassium nitrate (KNO_3) on seed germination

A nitrogenous compound such as nitrate has been used to stimulate germination of seeds of various species (Hassan *et al.,* 2011; Gehlot and Kasera, 2011; Lal and Kasera, 2014). They play a major role in promoting the expansion of cells in the embryo and induces rupture of the seed coat, which accelerates imbibition (Toorop, 2015). Potassium nitrate

(KNO_3) enhance seed germination depending on concentrations and time of exposure (Lal and Kasera, 2014). Eremrena and Mensah (2016) reported that KNO_3 stimulates germination of *Capsicum frutescens* seeds when the concentration is low (1% – 4%) and inhibits when it is high (> 6%). Saberi *et al.* (2011) obtained high germination rate, velocity, root and shoot length of *Citrullus colocynthis* treated with KNO_3 (0.2%) for 72 hours. Karimmojeni *et al.* (2011) studied the effect of different pre-sowing treatments on germination of perennial pepper weed (*Lepidium latifolium*) and reported that KNO_3 could induce the seed germination of 61% in 0.02 M concentration.

1.4.2 Vegetative propagation

Vegetative propagation can be carried out naturally by using runners, suckers, rhizomes, tubers, corms or bulbs and artificially by using cuttings, grafting, layering, suckering or tissue culture. Propagation through stem cuttings is most widely used because it is cheap and easy (Hae and Funnah, 2011; Rafiri, 2010). The propagation by stem cuttings is done by harvesting shoot pieces containing nodes from a mother plant and planted in a suitable medium for the development of roots. Rooting and sprouting of cuttings depend on the differentiation of the plant cells. However, it can be affected by cutting types, growth regulators, rooting medium and season when the cuttings were made (Soundy *et al.*, 2008).

1.4.2.1 Effect of cutting types on rooting

Stem cuttings are categorised into softwood, semi-hardwood and hardwood depending on physiological age of the wood (Hae and Funnah, 2011). Softwood cuttings are taken from the soft, succulent new growth of woody plants. The semi-hardwood cuttings are usually prepared from partially mature wood of the current season’s growth while hardwood cuttings are prepared from dormant, mature stems of more than one year old (Agbo and

Obi, 2007). Rooting capability of the cuttings obtained from the different parts of the plant may vary due to differences in chemical composition (Rahbin *et al.,* 2012). Raup and Taylor (2015) suggested that the age of plant material determine the rooting success of *Cupressus cashmeriana*.

Softwood stem cuttings of night jessamine (*Cestrum nocturnum*) showed significantly better rooting and cutting percent than the cutting of lower part of the shoot (Rahbin *et al.,* 2012). The experiment carried by Soundy *et al.* (2008) on fever tea (*Lippia javanica* L.) reported that softwood cuttings rooted earlier than hardwood cuttings. The studies on the effect of size and type of cuttings on rooting of *Lavandula dentata* L. by Bona *et al*. (2012) showed better rooting in softwood cuttings than hardwood cuttings. Softwood cuttings contain many meristematic cells with fewer or none phenolic compounds (Hartmann *et al.,* 2002).

Semi-hardwood stem cuttings of *Argania spinosa* showed significantly better sprouting and rooting than softwood and hardwood stem cuttings (Benbya *et al.,* 2018). Semi-hardwood cuttings of *Moringa oleifera* induced the maximum number of shoots than softwood cuttings (Antwi-Boasiako and Enninful, 2011). Al-Zebari and Al-Brifkany (2015) studied the influence of the type of cutting and growth regulator (IBA) on rooting and development of Citron (*Citrus medica* L.). The results show that the semi-hardwood cuttings treated with IBA at 500 and 1 000 ppm had the highest rooting percentage. According to Benbya *et al.* (2018) semi-hardwood cuttings are lignified which increases capacity to withstand drought and other adverse conditions.

Hardwood stem cuttings of *Duranta repens* showed the highest rooting percent irrespective of the length of cutting (Okunlola, 2013). But the better result was obtained

with hardwood stem cuttings at 20 cm length. Hae and Funnah (2011) studied the influence of cutting types, growth media and growth hormones on rooting of Kei apple (*Dovyalis caffra*) stem cuttings. The results revealed that hardwood cuttings provide higher rooting percent while softwood cuttings did not produce any roots. Mahmood *et al.* (2017) carried an experiment on *Paulownia tomentosa* and reported that basal cutting gave the best results on most of the studied growth characters. According to Rolland *et al*. (2006) hardwood cuttings contain sufficient amount of carbohydrates, proteins and natural hormones that can be used for plant growth.

1.4.2.2 Effect of growth regulators on rooting

Growth regulators play essential roles in the growth and development of the plant (Pop *et al.*, 2011). They control many aspects of plant growth including division and enlargement of the cell (Hajam *et al.*, 2017). Auxins are plant growth regulators that promote shoot and root formation (Galavi *et al.*, 2013). Indole acetic acid (IAA) and Naphthalene acetic acid (NAA) are the most widely used auxins after Indolic-3-butyric acid (IBA) (Pop *et al.*, 2011). Malik *et al.* (2018) obtained the best shoot and root formation in terminal cuttings of Carnation (*Dianthus caryophyllus* L.) when treated with NAA at 500 mg/l.

However, the rooting percentage was found maximum under IAA 500 mg/l in terminal cuttings. Yan *et al.* (2014) working on *Hemarthria compressa* and observed that NAA at 200 mg/l rooted better compared to other concentrations. Shakouri *et al.* (2012) found that cuttings treated with NAA between 100 to 300 ppm rooted well with the maximum number of roots, root length and diameter of *Dracaena sanderiana*. Memon *et al.* (2013) observed that NAA at 6 000 mg/l promoted the growth of shoot and root of Bougainvillea stem cuttings. Abidin and Metali (2015) observed that leafy stem cuttings of *Dillenia suffruticosa* treated with NAA at 0.10 - 0.20% and IAA at 0.10% improved development

roots and shoots. Seran and Umadevi (2011) reported that IAA at 2 500 ppm improve root and shoot formations and promote the establishment of lemon stem cuttings.

1.4.2.3 Establishment of rooted seedlings

The production of vigorous plant stock is essential for plant survival and establishment (Mohamed, 2013). Seedling biomass characteristics determine the establishment of the plant in the field (Johansson *et al.*, 2012; Corpuz *et al.*, 2013). High-quality seedlings with vigorous root and shoot system have a chance to grow and develop healthy plants. Mehrabani *et al.* (2016) reported that the immediate formation and the subsequent growth of roots are the most influential factors affecting the survival of cuttings. Plant growth regulators are among the factor that influences the successful rooting and establishment of seedlings. Balestri *et al.* (2012) obtained the highest establishment percentage (90%) when *Ammophila arenaria* cuttings treated with NAA at 100 mg/l. Kamis *et al.* (2016) reported the highest establishment percentage when the stem cuttings of *Aidia racemosa* treated with clonex, containing 0.3% IBA as its active ingredient. Diwakar *et al.* (2011) recorded 100% establishment when *Commiphora wightii* stem cuttings treated with IBA and NAA both or singly.

1.5 Importance of Tomato in Tanzania

Tomato (*Solanum lycopersicum* L.) contributes 51% of total fruit and vegetable production in Tanzania (Mamiro *et al.*, 2015). It is produced by small-scale farmers for home consumption and as a cash crop. In 2017, area under tomato production was 39 251 ha with a production of 565 441 tons (FAOSTAT, 2017). The crop yield is estimated to be 14.4 t/ha. (FAOSTAT, 2017) which is low as compared with the world's average of 27.5 t/ha (Minja *et al.*, 2011). Insect pests and diseases have reported to cause low productivity of tomato in Tanzania (Minja *et al.*, 2011). Tomato is a fruit vegetable that can be used in salad, soup or processed into tomato sauce, paste and juice (Tasnia *et al.*, 2015; Bawa,

2016). Nutritionally, tomato provides vitamins A, C and K, potassium, folate, essential amino acids and dietary fibres. It is an excellent source of lycopene and beta-carotene compounds that protect cells against carcinogenic substances (Dagade *et al.*, 2015; Tasnia *et al.*, 2015).

1.6 Factors Affecting Tomato Production in Tanzania

Tomato production in the country is affected by both biotic and abiotic factors (Mbega *et al*., 2011; Minja *et al.*, 2011). Yield losses approaching 100% have been reported under heavy infestation of pests (Maerere *et al.*, 2010). Common arthropod pests occurring in Tanzania are spider mites (*Tetranychus spp.*), African bollworm (*Helicoverpa armigera* H.), cutworms (*Agrotis spp*.), thrips (*Thrips tabaci* L.), whiteflies (*Bemisia tabaci* G.) and tomato leafminer (*Tuta absoluta*) (Kariathi *et al.*, 2017). Tomatoes are also attacked by plant-parasitic nematodes such as root-knot nematodes (*Meloidogyne incognita*, *M. javanica* and *M. hapla*). The major diseases affecting tomatoes include tomato fusarium wilt (*Fusarium oxysporum* f. sp. *lycopersici* Sacc.), early blight (*Alternaria solani* Sorauer), late blight (*Phythophthora infestans* (Mont.) de Bary), septoria leaf spot (*Septoria lycopersici*), cladosporium leaf mould (*Mycovellosiella fulva* Cooke) and bacterial wilt (*Ralstonia solanacearum*) (Mamiro *et al.*, 2015). Abiotic stresses include salinity, drought, excessive heat and declining soil fertility (Minja *et al.*, 2011).

1.7 Tomato Fusarium Wilt Disease

Tomato fusarium wilt is a fungal disease caused by *Fusarium oxysporum* f. sp. *lycopersici.* The pathogen is a soil-borne and can survive saprophytically in the soil without a host (Rai *et al.*, 2011; Arici *et al.*, 2018). The pathogen has been grouped into three races (race 1, 2 and 3) based on their ability to cause disease (Van Dam *et al.*, 2016). Races 1 and 2 are found all over the world whereas race 3 has a limited geographical

distribution (Reis *et al.,* 2005). It is reported in countries such as California, Australia, Southwestern Georgia and Mexico.

1.7.1 Symptoms of tomato fusarium wilt disease

The initial symptoms of the tomato fusarium wilt is chlorosis of the lower leaves that often begins on one side of the plant followed by wilting of that foliage (Tistisgiannis *et al.,* 2008). As the disease progresses, growth is typically stunted with little or no fruit development (Bawa, 2016). Cutting a longitudinal section into the xylem at the base of the stem reveals a dark brown streak running lengthwise through the stem (Mishra *at al*., 2014). The dark brown streak of the xylem is a distinctive feature of the disease that can be used for its identification (Wong, 2003).

1.7.2 Disease epidemiology

Fusarium oxysporum f. sp. *lycopersici* transmitted from plant to plant within a field through irrigation water, infected transplants, contaminated farm equipment or soil and human movement around the infected field (Ajilogba *et al.,* 2013; Bawa, 2016). When healthy plants grow in contaminated soil, the mycelium penetrates root tips directly or enters the roots through wounds or at the point of formation of lateral roots (Mishra *et al*., 2014). The mycelium advances through the root cortex intercellularly and when it reaches the xylem vessels it enters them through the pits, branches and produces microconidia. The microconidia eventually germinate and the mycelium penetrates the upper wall of the vessels producing more microconidia in the next vessel. The characteristic wilt symptoms seem as a result of vessel blockage triggered by the gathering of hyphae of the fungus (Srinivas *et al*. (In press). The development of the disease is favoured by acidic soil conditions (pH of 5 to 5.6), poorly drained soil, dry weather, soil and air temperature around 28°C and root-knot nematodes (Harikrushana *et al.*, 2014;

McGovern, 2015; Arici *et al.*, 2018). *Fusarium oxysporum* f. sp. *lycopersici* survives well in sandy soil (Larkin and Fravel, 2002).

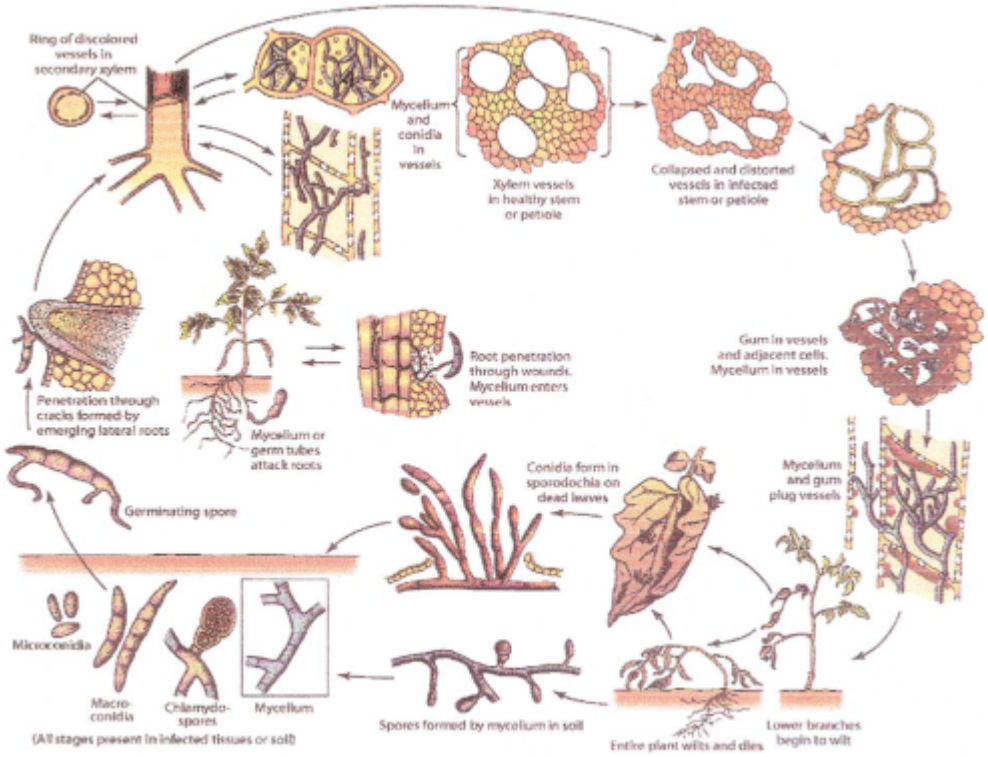

Figure 1.1: Disease cycle of tomato fusarium wilt caused by *F. oxysporum* f. sp. *lycopersici.* Source (Agrios, 2005).

1.7.3 Management of tomato fusarium wilt disease

Tomato fusarium wilt is mainly controlled through the use of resistant genotypes (Çolak and Biçici, 2013). Fungicides and soil solarisation fail to control the pathogen in the field (Nirmaladevi *et al.*, 2016). Disease resistant genotypes are limited and can be overwhelmed by novel pathogens and under higher disease pressure (Asha *et al.*, 2011). There is a need to introduce alternative methods of managing tomato fusarium wilt disease. Pesticidal plants such as *Ageratum conyzoides*, *Ageratum haustonianum*, *Clerodendrum inermae* and *Terminalia bellirica* have been claimed to have antifungal effect against *F. oxysporum* f. sp. *lycopersici* (Mishra *et al.*, 2014). Botanical pesticides

help to minimize the incidence of wilted plants and greatly increases marketable and total yields (Culver *et al.*, 2012; Thabet and Khalifa, 2018).

1.8 Justification

It is estimated that approximately 90% of pesticidal plants in use are collected from the wild in which 70% collection involves destructive harvesting (Ved *et al.*, 1998). Population growth rates in many developing countries have resulted in heavy exploitation of plant resources for their pesticidal values (Jäger and Van Staden, 2000). This increasing growth rate has also resulted in plant habitat destruction to allow for agricultural and settlement land. As a result, many plant species with pesticidal potential have become either extinct or are threatened with extinction. Initiating cultivation of pesticidal plants is viewed as the most viable long term alternative to ensure a sustainable supply of the raw material for the herbal industry (IUCN, 1993). Some species such as *Tephrosia vogelii* and *Tagetes minuta* are already cultivated and intercropped to take advantage of repellent properties (Anjarwalla *et al.*, 2016). Knowledge of propagation techniques for many species of pesticidal plants is scarce. Domestication and cultivation of most of pesticidal plants is hampered by lack of viable seeds, poor field establishment and low growth rates (Maduka *et al.*, 2017). The species, *C. swynnertonii* and *S. glaucescens* are available in Tanzania and have been reported to possess pesticidal properties and used as acaricide, antiviral, antifungal, antibacterial and insecticide in grain storage by local communities (Bakari *et al.*, 2012; Mabiki *et al.*, 2013). There are limited reports on propagation, field establishment and the efficacy of *C. swynnertonii* and *S. glaucescens* against tomato fusarium wilt disease. Therefore, this study is designed to assess propagation potential, field establishment and effectiveness of *C. swynnertonii* and *S. glaucescens* in control of tomato fusarium wilt.

1.9 Objectives

1.9.1 Overall objective

Enhancing mass propagation and fungicidal usage of *C. swynnertonii* and *S. glaucescens.*

1.9.2 Specific objectives

i. To evaluate propagation potential of *C. swynnertonii* and *S. glaucescens.*
ii. To determine field establishment of *C. swynnertonii* and *S. glaucescens.*
iii. To determine effectiveness of *C. swynnertonii* and *S. glaucescens* in managing tomato fusarium wilt disease.

1.10 Organization of the Dissertation

This dissertation is developed in the format of publishable manuscripts comprising of six main chapters. Chapter one is a general introduction, chapter two, three and four consist of manuscripts in the form of publishable papers. Chapter five is the general discussions and chapter six is the general conclusions and recommendations of the study.

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CHAPTER TWO

2.0 PROPAGATION POTENTIAL OF *COMMIPHORA SWYNNERTONII* (Burrt.) AND *SYNADENIUM GLAUCESCENS* (Pax.)

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2.1 Abstract

Plants provide pest control resources for many people worldwide. Nevertheless, harvesting is often destructive. The development of suitable propagation techniques will provide a strong base for the conservation of pesticidal plants. Screen house experiment was conducted to evaluate propagation potential of *Commiphora swynnertonii* and *Synadenium glaucescens*. Two separate trials were conducted. The first trial evaluated the influence of pre-sowing seed treatments on germination. The second trial evaluated the influence of cutting types and growth regulators on rooting and sprouting of stem cuttings. Pre-sowing treatments involved soaking seeds in water at room temperature (25°C), hot water (60°C), Potassium nitrate (KNO_3) and Gibberellin (GA3) solution at different concentrations. The experiment was set in a randomized complete block design (RCBD) with four replications. On the evaluation of the effect of type of cuttings and growth regulators, there were nine treatment combinations comprising of three types of cuttings (softwood, semi-hardwood and hardwood), two rooting hormones (Indole-3-Acetic Acid (IAA) and Naphthalene Acetic Acid (NAA)) and control. The experiment was set in a 3 x 3 factorial in RCBD with four replications. The study revealed that both plants have low seed germination potential. However, the seed germination was significantly affected by

pre-sowing treatments. In *C. swynnertonii*, early germination (9.75 days), high germination percentage (22.50%) and better survival percentage (20.00%) were recorded in seeds treated with KNO_3 at 10 ppm. While in *S. glaucescens*, seeds soaked in water (25°C) for 24 hours had the minimum number of days to germination (9.25 days), high germination percentage (25.00%) and better survival percentage (17.50%) compared to the other treatments and control. It was also found that semi-hardwood cuttings of *C. swynnertonii* and softwood cuttings of *S. glaucescens* dipped in 2 000 ppm NAA solution for 30 minutes led to higher rooting of 52.50% and 97.50%, respectively. The findings suggest that semi-hardwood cuttings and softwood cuttings dipped in 2 000 ppm NAA solution could be used for mass propagation of *C. swynnertonii* and *S. glaucescens*, respectively.

Keywords*: Conservation, germination, Indole-3-Acetic Acid (IAA) and Naphthalene Acetic Acid (NAA).*

2.2 Introduction

Over-exploitation, pressure from urbanization, mining, overgrazing and intensive agriculture have pushed more pesticidal plant species towards extinction (Bakari, 2013; Mabiki, 2013). There is a need to develop suitable conservation techniques that will provide a strong base for sustainable use of pesticidal plants. Among important pesticidal plants that are threatened with extinction include *C. swynnertonii* and *S. glaucescens.* The *C. swynnertonii* belongs to Burseraceae family and grows wild in northern regions of Tanzania particularly the Manyara region (Bakari *et al.*, 2012). Equally important; *S. glaucescens* is a succulent shrub or tree of several meters high belonging to the family Euphorbiaceae. It is endemic to eastern Africa regions and found in several regions of Tanzania such as Morogoro, Tanga, Njombe and Iringa (Mabiki *et al.*, 2013). Several investigations have focused on the validation of pesticidal activities of these plants. Matendo (2017) assessed the insecticidal effectiveness of these plants on the management of tomato leaf miner (*Tuta absoluta*). The results show that the ethanolic extract of *C. swynnertonii* resin caused significant mortality to larvae and adults of *T. absoluta.* The resin extract of *C. swynertonii* has been claimed to be potential in the management of ticks, fleas and tsetse flies (Kalala *et al.*, 2014). Latex of *S. glaucescens* is used as a seed dressing against vegetable plant-parasitic nematodes: *Tylenchorhynchus brassicae* and *Rotylenchus reniformis* (Matendo, 2017).

The availability of *C. swynnertonii* and *S. glaucescens* in natural forests is decreasing very fast. A survey conducted by Mabiki (2013) in Mufindi and Njombe region revealed the disappearance of the *S. glaucescens* in the wild. A total of 220 people were interviewed and 96% of the total respondents agreed that the plant is available, of them 80% agreed that the abundance of the plant is less compared to a few years ago. The survey conducted by Bakari (2013) in Manyara region revealed that there is over-exploitation of

C. swynertonii in Simanjiro district due to mining, overgrazing, urbanization and other agricultural activities.

The current demand of *C. swynnertonii* and *S. glaucescens* is mostly met from the wild collection in Tanzania (Bakari, 2013; Mabiki, 2013). Severe measures are needed for the conservation of these pesticidal plants before they are completely lost. One of the methods to meet the growing demand and decrease the pressure of wild collection is by mass propagation. However, propagation of some important pesticidal plants is beset with the problems of poor seedling establishment and rooting of stem cuttings (Diwakar *et al.,* 2011; Lal and Kasera, 2014). Several factors such as cutting types and size, growth regulators, growth medium and the environment affect rooting of the cuttings. Nitrogenous compounds, gibberellin solutions and water have been reported to improve seed germination (Stejskalová *et al.,* 2015; Eremrena and Mensah, 2016). This study tested stem cuttings and seeds as important potential propagation materials of *C. swynnertonii* and *S. glaucescens.*

2.3 Materials and Methods

2.3.1 Description of the study area

The study was conducted at the Sokoine University of Agriculture (SUA) Morogoro, Tanzania. The study area was located at 6° 05′ S, 35° 37′ E, at an elevation of 568 m above the sea level. The experiment was conducted in the screen house at Horticulture Section between November, 2018 and April, 2019.

2.3.2 Experimental materials

Seeds and stem cuttings of *C. swynnertonii* were harvested from Mererani ward in Simanjiro District of Manyara Region (3° 34.5′ S, 37° 0′ E at 1 009 m a.s.l) (Fig. 2.1). A

specialized botanist was involved for correct identification of the plants. The seeds and stem cuttings of *S. glaucescens* were obtained from the Department of Food Technology, Nutrition and Consumer Sciences premises at Sokoine University of Agriculture, Morogoro, Tanzania (6° 85′ S, 37° 65′ E at 556 m a.s.l) and Kola ward at Morogoro Municipal Council (6° 81′ S, 37° 69′ E at 531 m a.s.l), respectively. Growth regulators (NAA, IAA and GA_3), Potassium nitrate (KNO_3) and Sodium hypochlorite (NaOCl) were purchased from Jakovic General Supplies Ltd, Morogoro, Tanzania.

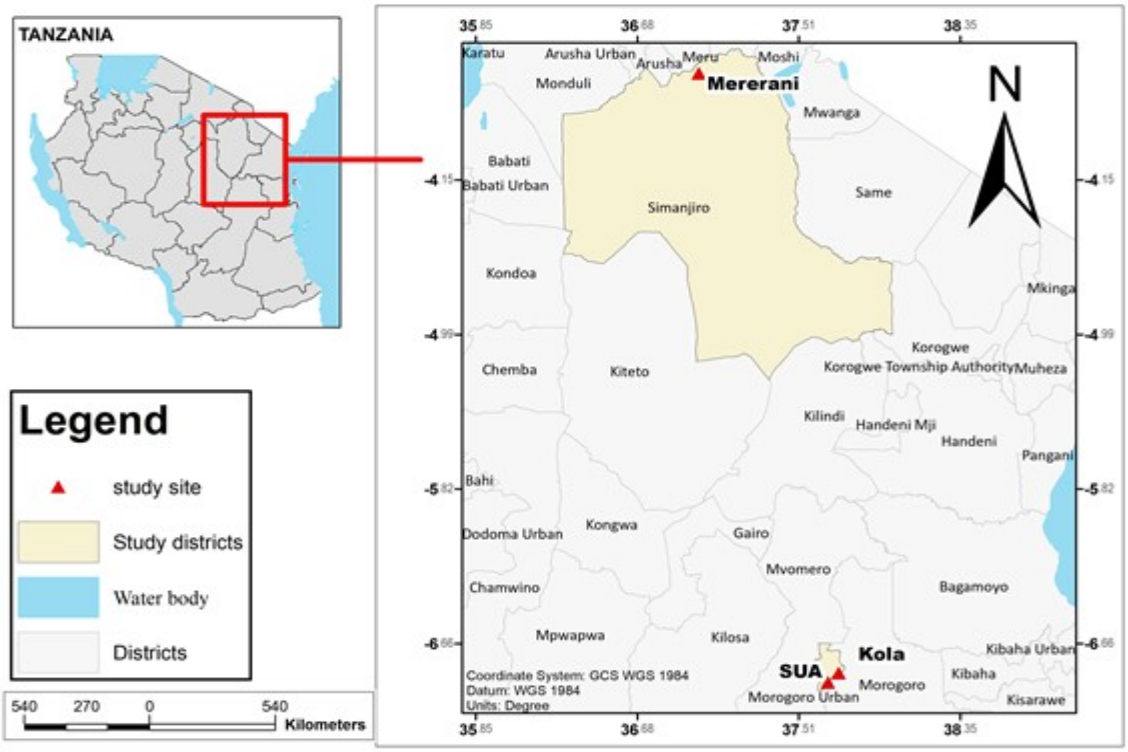

Figure 2.1: Map of Tanzania showing location of Mererani, SUA and Kola

2.4 Propagation Potential through Seeds

2.4.1 Treatments and experimental design

Mature seeds of *C. swynnertonii* and *S. glaucescens* were extracted from the fruits and shade dried for 3 days.

The seeds were disinfected with 2% sodium hypochlorite solution for 2 minutes and subjected to the following pretreatments: T_o: Control (no pretreatment given), T_1: Soaking seeds in water at room temperature (25°C) for 24 hours, T_2: Soaking seeds in hot water (60°C) for 10 minutes, T_3: Seeds treated with Potassium nitrate (KNO3) at different concentrations (10 ppm and 20 ppm) for 24 hours and T_4: Seeds treated with Gibberellin (GA_3) solution at different concentrations (GA_3 250 ppm, GA_3 500 ppm and GA_3 1000 ppm) for 72 hours. A total of 320 seeds were sown in 32 plastic pots (4-litre), each containing 10 seeds. Pots were filled with steam-sterilized forest soil, farmyard manure and rice husks at a ratio of 4:2:1. Seeds were sown approximately 1.0 cm deep. The pots were placed in the screen house and watered after every two days. Pots were inspected for weeds and removed when seen. The experiment was arranged in RCBD with four replications.

2.4.2 Data collection

Data were collected according to the method described by Sharma (2009) with some modifications. Seed pots were observed daily for seedling emergence and the number of days taken to seedling emergence was recorded. The total number of seedlings emerged in each treatment was recorded daily. Seedlings survival was recorded at the time of transplanting. The seed germination percentage and seedling survival percentage were computed as follows:-

$$\text{Germination (\%)} = \frac{\text{number of seedlings emerged}}{\text{number of seeds sown}} \text{ x } 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(i)}$$

Seed germination potential was categorized as High (>80%), Moderate (50 to 80%) and Low (<50%) germination (Butola and Badola, 2008).

$$\text{Seedling survival (\%)} = \frac{\text{number of seedlings survived}}{\text{number of seedling emerged}} \text{ x } 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(ii)}$$

2.5 Propagation Potential through Stem Cuttings

2.5.1 Treatments and experimental design

Evaluation of propagation potential using stem cuttings was conducted according to the method described by Pandey (2012) with some modifications. Softwood, semi-hardwood and hardwood cuttings of 25 - 30 cm length were harvested. Lower end of cuttings were individually dipped in two rooting hormones namely, NAA and IAA at 2 000 ppm. A total of 360 cuttings for each species were planted in 36 plastic pots (10-litre) each containing 10 cuttings. Pots were filled with steam-sterilized forest soil, farmyard manure and rice husks at a ratio of 4:2:1. The cuttings were planted 15 cm deep. Untreated cuttings were used as control. The experiment was arranged in a 3 x 3 factorial in RCBD with four replications. The pots were placed in the screen house and watered after every two days. The pots were inspected for weeds and removed when seen.

2.5.2 Data collection

All data were recorded after four months of planting according to the method described by Diwakar (2011) with some modifications. Data on number of days taken to sprout in each treatment was recorded by counting the number of days from planting. The total number of sprouts was counted and the number of sprouts per cutting was determined. The length of the longest sprout per cutting was measured from the point of sprout initiation to the growing point by using measuring tape. The total number of leaves of the longest sprout in each treatment was counted. Data on number of roots per cutting was obtained by counting the number of roots in each rooted cuttings and the average number of roots was determined. The length of the longest root per cutting was measured from the point of initiation of the root to the growing tip by using measuring tape. The total number of rooted and sprouted cuttings from each treatments was counted and their percentage were computed as follows:-

$$\text{Rooting (\%)} = \frac{\text{number of cuttings rooted}}{\text{total number of cuttings planted}} \text{ x } 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(iii)}$$

$$\text{Cutting survival (\%)} = \frac{\text{number of cuttings survived}}{\text{total number of cuttings planted}} \text{ x } 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(iv)}$$

2.6 Data Analysis

Data on number of days taken to seedling emergence, germination percentage and seedling survival percentage were square-root transformed $(X + 0.5)^{1/2}$ before analysis. All data were subjected to analysis of variance using GenStat software 15th Edition (VSN International Ltd. UK). Treatment means were separated by Duncan´s Multiple Range Test (DMRT) at $p \leq 0.05$.

2.7 Results

2.7.1 Effect of seed treatments on seed germination of *C. swynnertonii*

It was found that there was a significant difference between treatments in number of days taken to seedling emergence ($p < 0.001$), seed germination percentage ($p < 0.001$) and seedling survival percentage ($p = 0.002$). Seeds treated with KNO_3 at 10 ppm emerged earlier with higher germination and seedling survival percentage compared with the other treatments and control. No seed germination observed for seeds treated with GA_3 at any of the tested concentrations (Table 2.1).

2.7.2 Effect of seed treatments on seed germination of *S. glaucescens*

There was a significant difference between treatments in the number of days taken to seedling emergence ($p < 0.001$), seed germination percentage ($p < 0.001$) and seedling survival percentage ($p = 0.007$). Seeds soaked in water (25°C) for 24 hours had the lowest number of days taken to seedling emergence, higher germination and seedling survival

percentage compared with the other treatments and control. Seeds soaked in hot water (60°C) for 10 minutes and those treated with GA_3 solution at 500 and 1 000 ppm did not germinate (Table 2.2).

Table 2.1: Effect of seed treatments on germination and survival of C. swynnertonii

Treatments	Days to seedling emergence	Seed germination percentage	Seedling Survival percentage
Control	13.25b	12.50b	12.50bc
Water (25°C)	12.25b	17.50bc	10.00abc
Hot water (60°C)	12.75b	15.00bc	7.50ab
KNO_3 (10 ppm)	9.75b	22.50c	20.00c
KNO_3 (20 ppm)	13.75b	12.50b	7.50abc
GA_3 (250 ppm)	0.00a	0.00a	0.00a
GA_3 (500 ppm)	0.00a	0.00a	0.00a
GA_3 (1000 ppm)	0.00a	0.00a	0.00a
Mean	7.72	10.00	7.20
CV%	18.50	25.60	57.70
S.E	0.23	0.35	0.63
p-values	< 0.001	< 0.001	0.002

Means followed by the same letter in the same column are not significantly different at $p \leq 0.05$ according to DMRT.

(-) = no seed germination, CV% = Coefficient of variation, S.E = Standard errors of means.

Table 2.2: Effect of seed treatments on germination and survival of S. glaucescens

Treatments	Days to seedling emergence	Seed germination percentage	Seedling survival percentage
Control	20.50b	12.50bc	7.50ab
Water (25°C)	9.25b	25.00d	17.50b
Hot water (60°C)	0.00a	0.00a	0.00a
KNO_3 (10 ppm)	12.00bc	10.00b	10.00b
KNO_3 (20 ppm)	15.00c	20.00cd	15.00b
GA_3 (250 ppm)	11.25bc	10.00b	5.00ab
GA_3 (500 ppm)	0.00a	0.00a	0.00a
GA_3 (1000 ppm)	0.00a	0.00a	0.00a
Mean	8.50	9.69	6.88

CV%	36.10	21.00	63.60
S.E	1.54	0.28	0.67
p-values	< 0.001	< 0.001	0.007

Means followed by the same letter in the same column are not significantly different at $p \leq 0.05$ according to DMRT.
(-) = no seed germination, CV% = Coefficient of variation, S.E = Standard errors of means.

2.7.3 Effect of cutting types on shoot parameters of *C. swynnertonii*

Type of cuttings had a significant difference on number of days taken to sprout ($p = 0.005$), a number of sprouts per cutting ($p < 0.001$) and length of the longest sprout per cutting ($p < 0.001$) (Table 2.3). Softwood cuttings sprouted earlier compared to semi-hardwood and hardwood cuttings. Hardwood cuttings had the highest number of sprouts per cutting and length of the longest sprout per cutting. The type of cuttings did not have significant ($p \leq 0.05$) effect on the number of leaves of the longest sprout per cutting (Table 2.3). However, the semi-hardwood cuttings had the highest number of leaves of the longest sprout per cutting followed by hardwood and softwood cuttings.

2.7.4 Effect of growth regulators on shoot parameters of *C. swynnertonii*

Growth regulators had a significant difference on number of days taken to sprout ($p = 0.022$), length of the longest sprout per cutting ($p < 0.001$) and a number of leaves of the longest sprout per cutting ($p = 0.019$) (Table 2.3). The stem cuttings treated with IAA sprouted earlier compared to NAA and control. The stem cuttings treated with NAA had the highest length of the longest sprout per cutting and number of leaves of the longest sprout per cutting. The growth regulators did not differ significantly ($p \leq 0.05$) on a number of sprouts per cutting (Table 2.3). However, the highest and lowest number of sprouts per cutting was observed in stem cuttings treated with IAA and control, respectively.

2.7.5 Interaction effect of cuttings type and growth regulators on shoot parameters of *C. swynnertonii*

Interactions between type of cuttings and growth regulators were significant differences in the number of sprouts per cutting ($p = 0.001$) and length of the longest sprout per cutting ($p = 0.025$) (Table 2.3). Hardwood cuttings treated with IAA had higher number of sprouts per cutting compared to the other treatments and controls. Semi-hardwood cuttings treated with NAA had the highest length of the longest sprout per cutting followed by hardwood cuttings treated with NAA and softwood cuttings treated with NAA. The interactions between type of cuttings and growth regulators did not differ significantly ($p \leq 0.05$) on a number of days taken to sprout and the number of leaves of the longest sprout per cutting (Table 2.3). However, the lowest and highest number of days taken to sprout were observed in softwood cuttings treated with IAA and untreated hardwood cuttings (control), respectively. Semi-hardwood cuttings treated with NAA and untreated softwood cuttings (control) had the highest and lowest number of leaves of the longest sprout per cutting, respectively.

Table 2.3: Effect of cutting types and growth regulators on shoot parameters of *C. swynnertonii*

Treatments	Number of days taken to sprout	Number of sprouts per cutting	Length of the longest sprout per cutting (cm)	Number of leaves of the longest sprout per cutting
Factor A (Cutting types)				
Softwood	12.08a	3.90a	47.92a	53.25
Semi-hardwood	13.75a	4.46a	87.50b	68.17
Hardwood	17.42b	5.33b	87.54b	67.00
Mean	14.42	4.57	74.30	62.80
C.V%	25.10	16.10	25.20	64.30
S.E	1.05	0.21	5.41	11.66
p-values	0.005	< 0.001	< 0.001	0.609^{NS}
Factor B (Growth regulators)				
IAA	12.25a	4.68	63.50b	52.33a
NAA	14.33ab	4.55	111.75c	91.58b
Control	16.67b	4.47	47.71a	44.50a
Mean	14.42	4.57	74.30	62.80
C.V%	25.10	16.10	25.20	64.30
S.E	1.05	0.21	5.41	11.66

p-values	0.022	0.786^{NS}	< 0.001	0.019
Interaction (Factor A x B)				
S x IAA	11.00	3.88a	22.00a	51.50
S x NAA	12.00	3.81a	105.25cde	73.50
S x Control	13.25	4.03a	16.50a	34.75
SH x IAA	11.50	4.00a	77.50bc	49.00
SH x NAA	14.50	5.55b	122.75e	109.50
SH x Control	15.25	3.83a	62.38b	46.00
H x IAA	14.25	6.15b	91.00bcd	56.50
H x NAA	16.50	4.30a	107.25de	91.75
H x Control	21.50	5.55b	64.25b	52.75
Mean	14.42	4.57	74.30	62.80
C.V%	25.10	16.10	25.20	64.30
S.E	1.81	0.37	9.36	20.20
p-values	0.626^{NS}	0.001	0.025	0.899^{NS}

Means followed by the same letter in the same column for each factor and their interactions are not significantly different at $p \leq 0.05$ according to DMRT, C.V% = Coefficient of variation, S.E = Standard errors of means, NS = Non-significant.

2.7.6 Effect of cutting types on root parameters of *C. swynnertonii*

There were significant differences between type of cuttings on number of roots per cutting ($p = 0.004$), length of the longest root per cutting ($p = 0.037$), rooting percent ($p < 0.001$) and cutting survival percentage ($p < 0.001$) (Table 2.4). Hardwood cuttings had the highest number of roots per cutting followed by semi-hardwood and softwood cuttings. Semi-hardwood cuttings had higher length of the longest root per cutting, rooting and cutting survival percentage compared with the other cuttings.

2.7.7 Effect of growth regulators on root parameters of *C. swynnertonii*

Significant differences were observed among the growth regulators and control on number of roots per cutting ($p = 0.018$), rooting percent ($p = 0.014$) and cutting survival percentage ($p < 0.001$) (Table 2.4). The stem cuttings treated with NAA had the highest number of roots per cutting, rooting and cutting survival percentage compared with IAA and control. The growth regulators did not differ significantly ($p \leq 0.05$) on length of the longest root per cutting (Table 2.4). However, the highest and lowest length of the longest root per cutting was observed in stem cuttings treated with NAA and control, respectively.

2.7.8 Interaction effect of cuttings type and growth regulators on root parameters of *C. swynnertonii*

There were significant differences among the interactions between type of cuttings and growth regulators on rooting percent (p = 0.024) and cutting survival percentage (p = 0.003) (Table 2.4). Semi-hardwood cuttings treated with NAA had higher rooting and cutting survival percentage compared to the other treatments and control. The interactions between type of cuttings and growth regulators did not differ significantly ($p \leq 0.05$) on a number of roots per cutting and length of the longest root per cutting (Table 2.4). However, the semi-hardwood cuttings treated with NAA had higher number of roots per cutting and length of the longest root per cutting compared to other treatments and controls.

Table 2.4: Effect of cutting types and growth regulators on root parameters of *C. swynnertonii*

Treatments	Number of roots per cutting	Length of the longest root per cutting (cm)	Rooting Percent (%)	Cutting survival percentage (%)
Factor A (Cutting types)				
Softwood	0.59a	14.42a	7.50a	6.67a
Semi-hardwood	2.56b	44.42b	31.67c	29.17c
Hardwood	2.83b	34.17ab	22.00b	19.50b
Mean	1.99	31.00	20.40	18.40
C.V%	79.60	87.70	55.90	49.30
S.E	0.46	7.85	3.29	2.62
p-values	0.004	0.037	< 0.001	< 0.001
Factor B (Growth regulators)				
IAA	2.55b	33.67	18.33a	18.33b
NAA	2.59b	41.58	28.67b	27.00c
Control	0.83a	17.75	14.17a	10.00a
Mean	1.99	31.00	20.40	18.40
C.V%	79.60	87.70	55.90	49.30
S.E	0.46	7.85	3.29	2.62

p-values	0.018	0.113^{NS}	0.014	< 0.001
Interaction (Factor A x B)				
S x IAA	0.61	14.75	10.00abc	10.00a
S x NAA	0.75	18.00	7.50ab	5.00a
S x Control	0.40	10.50	5.00a	5.00a
SH x IAA	3.18	51.88	27.50c	27.50b
SH x NAA	3.95	56.75	52.50d	50.00c
SH x Control	0.55	24.62	15.00abc	10.00a
H x IAA	3.88	34.38	17.50abc	17.50ab
H x NAA	3.08	50.00	26.00bc	26.00b
H x Control	1.55	18.12	22.50abc	15.00ab
Mean	1.99	31.00	20.40	18.40
C.V%	79.60	87.70	55.90	49.30
S.E	0.79	13.59	5.70	4.54
p-values	0.317^{NS}	0.848^{NS}	0.024	0.003

Means followed by the same letter in the same column for each factor and their interactions are not significantly different at $p \leq 0.05$ according to DMRT, C.V% = Coefficient of variation, S.E = Standard errors of means, NS = Non-significant.

2.7.9 Effect of cuttings type on shoot parameters of *S. glaucescens*

Results indicate significant difference between the type of cuttings on a number of sprouts per cutting ($p < 0.001$), length of the longest sprout per cutting ($p = 0.026$) and number of leaves of the longest sprout per cutting ($p = 0.002$) (Table 2.5). Hardwood cuttings had the highest number of sprouts per cutting followed by semi-hardwood and softwood cuttings. Softwood cuttings had higher length of the longest sprout per cutting and number of leaves of the longest sprout per cutting compared with the other cuttings. The type of cuttings did not differ significantly ($p \leq 0.05$) on a number of days taken to sprout (Table 2.5). However, the lowest and highest number of days taken to sprout was observed in softwood cuttings and hardwood cuttings, respectively.

2.7.10 Effect of growth regulators on shoot parameters of *S. glaucescens*

Results show that there were significant differences between growth regulators and control on a number of leaves of the longest sprout per cutting ($p < 0.001$) (Table 2.5). The highest number of leaves of the longest sprout per cutting was observed in stem cuttings treated with NAA followed by IAA and control. The growth regulators did not differ significantly

($p \leq 0.05$) on the number of days taken to sprout, the number of sprouts per cutting and length of the longest sprout per cutting (Table 2.5). However, stem cuttings treated with NAA had lower number of days taken to sprout and higher length of the longest sprout per cutting compared to IAA and control. Control had the higher number of sprouts per cutting followed by NAA and IAA.

2.7.11 Interaction effect of cutting types and growth regulators on shoot parameters of *S. glaucescens*

Interactions between type of cuttings and growth regulators did not differ significantly ($p \leq 0.05$) on a number of days taken to sprout, number of sprouts per cutting, length of the longest sprout per cutting and number of leaves of the longest sprout per cutting (Table 2.5). There were significant difference between interaction of cutting types and growth regulators on a number of roots per cutting ($p = 0.015$) (Table 2.5). Hardwood cuttings treated with NAA had the highest number of roots per cuttings.

Table 2.5: Effect of cutting types and growth regulators on shoot parameters of S. glaucescens

Treatments	Number of days taken to sprout	Number of sprouts per cutting	Length of the longest sprout per cutting (cm)	Number of leaves of the longest sprout per cutting
Factor A (Cutting types)				
Softwood	10.42	2.49a	36.92b	27.83b
Semi-hardwood	10.58	3.54b	32.83ab	24.83ab
Hardwood	12.08	5.34c	27.00a	22.08a
Mean	11.03	3.79	32.20	24.92
C.V%	16.40	24.40	26.00	13.70
S.E	0.52	0.27	2.42	0.99
p-values	0.064^{NS}	< 0.001	0.026	0.002
Factor B (Growth regulators)				
IAA	10.83	3.65	31.17	24.08a
NAA	10.33	3.69	36.33	28.42b
Control	11.92	4.03	29.25	22.25a
Mean	11.03	3.79	32.20	24.92

C.V%	16.40	24.40	26.00	13.70
S.E	0.52	0.27	2.42	0.99
p-values	0.112^{NS}	0.556^{NS}	0.123^{NS}	< 0.001
Interaction (Factor A x B)				
S x IAA	10.00	2.85	34.00	26.00
S x NAA	10.00	2.44	43.25	32.00
S x Control	11.25	2.18	33.50	25.50
SH x IAA	10.25	3.75	33.00	25.25
SH x NAA	10.00	3.26	34.50	28.00
SH x Control	11.50	3.60	31.00	21.25
H x IAA	12.25	4.35	26.50	21.00
H x NAA	11.00	5.36	31.25	25.25
H x Control	13.00	6.30	23.25	20.00
Mean	11.03	3.79	32.2	24.92
C.V%	16.40	24.40	26.0	13.70
S.E	0.90	0.46	4.19	1.71
p-values	0.967^{NS}	0.083^{NS}	0.901^{NS}	0.812^{NS}

Means followed by the same letter in the same column for each factor and their interactions are not significantly different at $p \leq 0.05$ according to DMRT, C.V% = Coefficient of variation, S.E = Standard errors of means, NS = Non-significant.

2.7.12 Effect of cuttings type on root parameters of *S. glaucescens*

There were significant differences between type of cuttings on number of roots per cutting ($p < 0.001$), length of the longest root per cutting ($p = 0.002$), rooting percent ($p < 0.001$) and cutting survival percentage ($p < 0.001$) (Table 2.6). Hardwood cuttings had the highest number of roots per cutting followed by semi-hardwood and softwood cuttings. Softwood cuttings had higher length of the longest root per cutting, rooting and cutting survival percentage as compared with the other cuttings.

2.7.13 Effect of growth regulators on root parameters of *S. glaucescens*

Growth regulators significantly influenced the number of roots per cutting ($p = 0.002$), rooting percent ($p = 0.030$) and cutting survival percentage ($p = 0.030$) (Table 2.6). The stem cuttings treated with NAA had higher number of roots per cutting, rooting and cutting survival percentage compared with IAA and control. The growth regulators and control did not differ significantly ($p \leq 0.05$) on the length of the longest root per cutting

(Table 2.6). However, the highest and lowest length of the longest root per cutting was observed in stem cuttings treated with NAA and control, respectively.

2.7.14 Interaction effect of cutting types and growth regulators on root parameters of *S. glaucescens*

Interactions between cutting types and growth regulators had non-significant effect ($p \leq 0.05$) on length of the longest root per cutting, rooting percent and cutting survival percentage (Table 2.6). However, softwood cuttings treated with NAA had higher length of the longest root per cutting, rooting percent and cutting survival percentage compared to the other treatments and controls.

Table 2.6: Effect of cutting types and growth regulators on root parameters of S. glaucescens

Treatments	Number of roots per cutting	Length of the longest root per cutting (cm)	Rooting percent (%)	Cutting survival percentage (%)
Factor A (Cutting types)				
Softwood	26.16a	29.67b	90.83c	90.83c
Semi-hard wood	26.50a	21.58a	70.83b	65.83b
Hardwood	45.63b	19.33a	35.83a	31.67a
Mean	32.80	23.50	65.80	62.80
C.V%	25.60	27.50	20.70	22.90
S.E	2.42	1.87	3.94	4.15
p-values	< 0.001	0.002	< 0.001	< 0.001
Factor B (Growth regulators)				
IAA	26.16a	22.54	70.00b	65.83b
NAA	39.93b	27.12	70.83b	69.17b
Control	32.20a	20.92	56.67a	53.33a
Mean	32.80	23.50	65.80	62.80
C.V%	25.60	27.50	20.70	22.90
S.E	2.42	1.87	3.94	4.15
p-values	0.002	0.071^{NS}	0.030	0.030
Interaction (Factor A x B)				
S x IAA	20.07a	29.12b	90.00	90.00
S x NAA	28.92a	33.38	97.50	97.50
S x Control	29.47a	26.50	85.00	85.00

SH x IAA	27.22a	19.00	80.00	72.50
SH x NAA	28.70a	28.25	72.50	70.00
SH x Control	23.57a	17.50	60.00	55.00
H x IAA	31.18a	19.50	40.00	35.00
H x NAA	62.16c	19.75	42.50	40.00
H x Control	43.56b	18.75	25.00	20.00
Mean	32.8	23.5	65.8	62.8
C.V%	25.6	27.5	20.7	22.9
S.E	4.20	3.24	6.82	7.18
p-values	0.015	0.586^{NS}	0.773^{NS}	0.891^{NS}

Means followed by the same letter in the same column for each factor and their interactions are not significantly different at $p \leq 0.05$ according to DMRT, C.V% = Coefficient of variation, S.E = Standard errors of means, NS = Non-significant.

2.8 Discussion

Seed germination of both *C. swynnertonii* and *S. glaucescens* was generally poor but it was significantly affected by pre-sowing treatments. In *C. swynnertonii,* early germination, high germination and survival percentage were recorded in seeds soaked in KNO_3 solution at 10 ppm. This could be due to the role of KNO_3 in breaking seed dormancy by removing germination inhibitors like abscisic acid (Farajollahi *et al.,* 2014). Lal and Kasera (2014) observed that KNO_3 at low concentrations promote seed germination and seedling growth of *Commiphora wightii,* while at higher ones retarded them. Suppression of germination by higher concentrations of KNO_3 has also reported in *Lepidium latifolium* (Karimmojeni *et al.,* 2011), *Sorbus pohuashanensis* (Bian *et al.,* 2013) and *Capsicum frutescens* (Eremrena and Mensah, 2016). Similar results were observed in this study, as lower concentrations of KNO_3 (10 ppm) increased germination percentages, but higher concentrations (20 ppm) retarded germination. KNO_3 increase physiological efficacy and improve seed germination because of change in water relationship (Lal and Kasera, 2014). It has low water potential that enable water to enter seed slowly that lets steady seed imbibition and initiation of germination (Lutts *et al.,* 2016).

Seeds of *S. glaucescens* soaked in water (25°C) emerged earlier with the highest germination and survival percentages. Pandey (2012) observed that seeds of *Gymnema*

sylvestre give the highest germination percentage when soaked in water at room temperature. Water play an essential role in breaking seed dormancy by softening the testa and removal of germination inhibitors (Olajide *et al.,* 2014). This observation concurs with other studies (Sabongari and Aliero, 2004; Offiong *et al.,* 2010). Vegetative propagation of *C. swynnertonii* and *S. glaucescens* by stem cutting is achievable. The results of this study revealed that stem cuttings influenced the shoot and root development of *C. swynnertonii* and *S. glaucescens*. In *C. swynnertonii*, hardwood cutting has shown the best shoot performance particularly in the number and length of sprouts per cutting. According to Rolland *et al.* (2006) hardwood cuttings contain sufficient amount of carbohydrates, proteins and natural hormones that can be used for plant growth. Ayan *et al.* (2006) observed that basal cutting of *Alnus glutinosa* gave the highest sprout length compared with tip cutting. Semi-hardwood cuttings have shown the best root performance particularly in root length, rooting percent and cutting survival percentage. The reason may be due to the early differentiation of root cells and enhanced cell elongation by the effect of the hormone. Yeshiwas *et al.* (2015) found that semi-hardwood stem cuttings of rose provide higher root length compare to hard and softwood cutting. In *S. glaucescens*, softwood cuttings have shown the best shoot and root performance particularly in the number of days taken to sprout, sprout length, number of leaves, root length, rooting percent and cutting survival percentage. This is due to the higher concentration of shoot and root promoting substances forming in the apical shoots, which are translocated to the base of shoot and more available carbohydrates, which aid in rooting. It is however contrary to findings by Ayan *et al.* (2006).

On the other hand, the results shown a significant effect of growth hormones on the shoot and root parameters of *C. swynnertonii* and *S. glaucescens.* The cuttings treated with NAA at 2 000 ppm was found to be the best in both plants. NAA play a vital role in hydrolysis

and translocation of stored food substances and caused cell elongation and division (Hartmann *et al.*, 2007). The superiority of NAA was also observed in *Lawsonia inermis* by Quainoo *et al.* (2014) who reported that NAA affected the number of leaves, roots and root length per cutting. The findings indicated that there were significant interaction effects of cutting types and growth regulators on the shoot and root parameters of *C. swynnertonii* and *S. glaucescens.* In *C. swynnertonii*, semi-hardwood cuttings treated with NAA has shown the best performance while in *S. glaucescens*, softwood cuttings treated with NAA has shown to be superior. Ullah *et al.* (2005) reported that semi-hardwood and softwood stem cuttings of guava treated with 1 000 ppm NAA sprouted early and had the maximum root length.

2.9 Conclusion

It is evident from the current study that, *C. swynnertonii* and *S. glaucescens* can be propagated through stem cuttings. The cuttings type and growth regulators had a notable effect in improving the rooting and sprouting percentage. Among the different growth regulators, NAA at 2 000 ppm was found to be the best for mass propagation of *C. swynnertonii* by semi-hardwood cuttings and *S. glaucescens* by softwood cuttings. Pre-sowing treatments have only marginally improved the seed germination of both plants. Among the two plants, *S. glaucescens* was superior to *C. swynnertonii*. Further study on *in vitro* propagation of these plants is recommended. Seeds and clonal gene banks should be established to conserve the genetic diversity of these plants.

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CHAPTER THREE

3.0 FIELD ESTABLISHMENT OF *COMMIPHORA SWYNNERTONII* (Burrt.) AND *SYNADENIUM GLAUCESCENS* (Pax.)

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3.1 Abstract

Plants are among the most common source of bio-pesticides which are used as an alternative to synthetic pesticides in many parts of the world. Major drawbacks facing pesticidal plants include destructive harvesting, poor seed set, low seed viability, habitat destruction due to pressure from urbanization, mining, overgrazing and other agricultural activities. Field trials were conducted at the Crop Museum, Department of Crop Science and Horticulture of Sokoine University of Agriculture, Morogoro, Tanzania. The purpose of the study was to determine field establishment of *C. swynnertonii* and *S. glaucescens.* The experimental field was ploughed and levelled properly. Plots measuring 3 m x 2 m were prepared. The planting holes of 30 m x 30 m x 30 cm size were dug at a distance of 1 m between the rows and 1 m between plants giving 6 plants per plot. Each planting hole was filled with 5 kg of well decomposed Farm Yard Manure. Survived plantlets grown in the screen house for four months were planted in the field. The experiment was arranged in RCBD for seedlings and in a 3 x 3 factorial in RCBD for rooted cuttings with three replications. The study showed that both plants can be established by seeds as well as by rooted cuttings. It was found that KNO_3 at 10 ppm improves the survival ability of *C. swynnertonii* plants. While in *S. glaucescens* plants previously treated with GA_3 at 250

ppm were found to have high survival ability. It was also found that plants from hardwood cuttings of *C. swynnertonii* and semi-hardwood cuttings of *S. glaucescens* previously treated with NAA at 2 000 ppm had the longest branch, the highest number of branches, number of leaves, leaf area, fresh and dry weight of leaves per plant. The results suggest that hardwood cuttings and semi-hardwood cuttings dipped in 2 000 ppm NAA solution are proper for field establishment of *C. swynnertonii* and *S. glaucescens*, respectively. The results from this study can potentially be used as basic information on the conservation of *C. swynnertonii* and *S. glaucescens*.

Keywords*:* *Pesticidal plants, cuttings and growth regulators*

3.2 Introduction

The species *Commiphora swynnertonii* (Burrt) and *Synadenium glaucescens* (Pax) are currently drawing global attention as the plants reported to possess a wide range of activities (Bakari, 2013; Mabiki, 2013). The products of these plants have been reported to be useful to animal scientists, pathologists, entomologists and practitioners of natural medicine (Bakari *et al.,* 2012; Mabiki *et al.,* 2013; Nyigo *et al.,* 2016). Because of diverse use, there is a continuous demand for these plants which has made them highly vulnerable in nature. Though these plants are available in Tanzania not much has been done to enhance their large scale production and to ensure sustained availability. Domestication and cultivation of some pesticidal plants are beset with the problems of poor seedling establishment and rooting of cuttings (Diwakar *et al.,* 2011; Lal and Kasera, 2014). In addition, failure to adapt to the field environment, inability to recover after transplanting and attack by different micro-organisms after rooting causes seedlings of many plant species including *C. swynnertonii* and *S. glaucescens* not to survive for a long time after establishment (Araya, 2005; Grossnickle and MacDonald, 2018).

The successful establishment of seedlings and rooted cuttings depends on the quality of the planting materials and the environmental conditions of the planting site (Pinto *et al.,* 2018). The plantlet is said to be of good quality when it has a high shoots to roots proportion, large root size, volume and biomass and long thick stem (Grossnickle and MacDonald, 2017). The environmental conditions influencing seedlings establishment includes poor soil nutrients, insufficient soil moisture contents and extreme temperature regimes (Mohamed, 2013). Plantlet with well-developed root and shoot system has high chance to survival field stresses (Santoso and Parwata, 2014). The success of seedlings and rooted cuttings of *Commiphora swynnertonii* and *Synadenium glaucescens* after field establishment is not known. There is a need to determine the survival ability of

Commiphora swynnertonii and *Synadenium glaucescens* in the field. Therefore this study was aimed to determine field establishment of *Commiphora swynnertonii* and *Synadenium glaucescens.*

3.3 Materials and Methods

3.3.1 Description of the study area

The study was conducted at the Crop Museum, Department of Crop Science and Horticulture of Sokoine University of Agriculture (SUA) Morogoro, Tanzania between April and July, 2019. The study area was located at 6° 05′ S, 35° 37′ E, at 543 m a.s.l. The study area has bimodal pattern of the rainfall. The short rains begin in November to January while the long rains start in February to May. The annual rainfall ranges between 800 and 950 mm (Kisetu and Teveli, 2013). Data on weather conditions during the experiment period are presented in Fig. 3.1

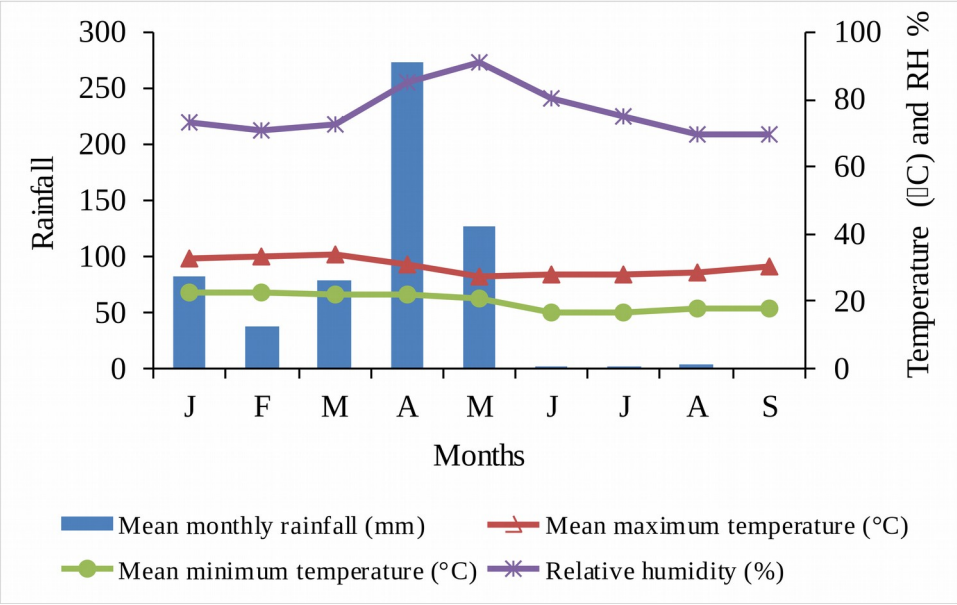

Figure 3.1: Weather parameters for the period of January to September 2019. (Source: TMA Morogoro station)

3.3.2 Experimental materials

Seeds and stem cuttings of *C. swynnertonii* were collected from Mererani ward in Simanjiro District, Manyara Region (3° 34.5′ S, 37° 0′ E; 1 009 m a.s.l). The seeds and stem cuttings of *S. glaucescens* were obtained from the Department of Food Technology, Nutrition and Consumer Sciences premises at Sokoine University of Agriculture, Morogoro, Tanzania (6° 85′ S, 37° 65′ E; 556 m a.s.l) and Kola ward in Morogoro Municipal Council (6° 81′ S, 37° 69′ E; 531 m a.s.l), respectively. Growth regulators (NAA, IAA and GA_3), Potassium nitrate (KNO_3) and Sodium hypochlorite (NaOCl) were purchased from Jakovic General Supplies Ltd, Morogoro, Tanzania.

3.3.3 Treatments and experimental design

Seeds were sterilized with 2% NaOCl solution for 2 minutes and exposed into the following treatments; soaked in water (25°C) for 24 hours, hot water (60°C) for 10 minutes, treated with different concentrations of KNO_3 (10 ppm and 20 ppm) for 24 hours and treated with GA_3 solution at different concentrations (250 ppm, 500 ppm and 1000 ppm) for 72 hours. The seeds were sown in the pots containing steam sterilized forest soil, farmyard manure and rice husks at a ratio of 4:2:1 respectively. The stem cuttings were grouped into softwood, semi-hardwood and hardwood. The bottom parts of the cuttings were individually dipped into NAA and IAA at 2 000 ppm and planted in pots containing steam sterilized forest soil, farmyard manure and rice husks at a ratio of 4:2:1 respectively. The seeds and stem cuttings were grown in the screen house for four months then transplanted in the field. The experimental field was ploughed and levelled properly. Plots measuring 3 m x 2 m were prepared. The planting holes of 30 cm x 30 cm x 30 cm size were dug at a distance of 1 m between the rows and 1 m between plants giving 6 plants per plot. Each planting hole was filled with 5 kg of well decomposed Farm Yard Manure. Survived plantlets from screen house were planted.

The experiment was arranged in RCBD for seedlings and in a 3 x 3 factorial in RCBD for rooted cuttings with three replications. No synthetic fertilizers, pesticides or herbicides were applied during field management. Weeds were removed when seen.

3.3.4 Data collection

All data were recorded after three months of field establishment. Data were collected from two sampled plants in each plot and the mean was determined. The height of the plant was measured from the base to the highest point branch using a measuring tape. The length of the longest branch was measured from the point of branch initiation to the highest point using measuring tape. The total number of branches and leaves was counted from the sampled plants. The length and middle width of the four leaves each from two sampled plants in each treatment were measured using a ruler and the values were multiplied according to Awal *et al.* (2004). The leaves were harvested and their fresh weight was measured using electronic balance. The harvested leaves were then oven-dried for 72 hours at 70°C. The weight of dried leaves was measured using electronic balance. The number of plants survived in each treatment was recorded and the percentage establishment was computed using the following formula.

$$\text{Percentage establishment} = \frac{\text{number of plants survived}}{\text{total number of plants planted}} \text{ x } 100 \ldots\ldots\ldots\ldots\ldots\ldots \text{(i)}$$

3.3.5 Data analysis

Data collected were subjected to analysis of variance using GenStat software 15th Edition (VSN International Ltd. UK). Treatment means were separated by Duncan´s Multiple Range Test (DMRT) at $p \leq 0.05$.

3.4 Results

3.4.1 Effect of seed treatments on seedling establishment of *C. swynnertonii*

The findings have shown that there were significant difference between treatments in number of branches per plant ($p = 0.006$), number of leaves per plant ($p = 0.007$), leaf area ($p = 0.007$) and leaves fresh weight ($p = 0.016$) (Table 3.1). Plants from seeds previously treated with KNO_3 at 10 ppm had the highest number of branches per plant, number of leaves per plant, leaf area and leaves fresh weight. Seed treatments did not differ significantly ($p \leq 0.05$) in plant height, leaf dry weight and establishment percentage (Table 3.1). However, plants from seeds previously treated with KNO_3 at 10 ppm had the highest height and leaf dry weight. While plants from seeds previously treated with KNO_3 at 20 ppm and plants from seeds previously soaking in hot water had the highest establishment percentage.

3.4.2 Effect of seed treatments on seedling establishment of *S. glaucescens*

Significant difference between treatments were observed in plant height ($p = 0.005$), leaf area ($p = 0.022$), leaves fresh weight ($p = 0.022$), leaves dry weight ($p = 0.020$) and establishment percentage ($p = 0.002$) (Table 3.2). Plants from seeds previously treated with GA_3 at 250 ppm had the highest plant height, leaf area, leaves fresh weight, leaves dry weight and establishment percentage. Seed treatments did not have significant ($p \leq 0.05$) difference in the number of branches and number of leaves per plant (Table 3.2). However, plants from seeds previously treated with GA_3 at 250 ppm had the highest leaves per plant and number of branches per plant which is similar to control.

Table 3.1: Effect of seed treatments on growth and establishment of **C. swynnertonii**

Treatments	Plant height (cm)	Number of branches	Number of leaves	Leaf area (cm^2)	Fresh weigh t (g)	Dry weight (g)	Establishm ent (%)
Control	28.80	3.00a	26.87a	2.97a	2.90a	0.57	66.67
Water (25°C)	32.23	5.67a	43.53ab	3.53ab	3.53a	1.27	50.00
Hot water (60°C)	33.07	4.33a	25.33a	4.60abc	2.90a	0.83	100.00
KNO_3 (10 ppm)	42.80	9.33b	64.87c	6.93bc	6.33b	2.70	77.78
KNO_3 (20 ppm)	33.70	5.67a	48.07bc	8.37c	3.67a	1.83	100.00
Mean	34.10	5.60	41.70	5.28	3.87	1.44	78.90
C.V%	20.80	25.50	24.00	36.80	26.20	53.50	25.60
S.E	4.09	0.82	5.78	1.12	0.59	0.45	11.65
p-values	0.260^{NS}	0.006	0.007	0.040	0.016	0.056^{NS}	0.064^{NS}

Means followed by the same letter in the same column are not significantly different at $p \leq 0.05$ according to DMRT.
CV% = Coefficient of variation, S.E = Standard errors of means, NS = Non-significant.

Table 3.2: Effect of seed treatments on growth and establishment of *S. glaucescens*

Treatments	Plant height (cm)	Number of branches	Number of leaves	Leaf area (cm^2)	Fresh weight (g)	Dry weight (g)	Establish ment (%)
Control	11.00ab	1.67	12.33	22.67a	23.40ab	1.38a	100.00b
Water (25°C)	13.50b	1.00	11.33	31.83a	29.77bc	1.59a	38.89a
GA_3 (250 ppm)	19.00c	1.67	13.67	53.47b	40.67c	3.73b	100.00b
KNO_3 (10 ppm)	14.33bc	1.00	10.67	31.20a	29.03abc	1.43a	83.33b
KNO_3 (20 ppm)	6.50a	1.00	7.00	13.47a	16.00a	0.73a	50.00a
Mean	12.87	1.27	11.00	30.50	27.80	1.78	74.40
C.V%	21.00	30.60	38.30	36.50	24.70	47.50	19.60
S.E	1.56	0.22	2.43	6.42	3.96	0.49	8.43
p-values	0.005	0.111^{NS}	0.436^{NS}	0.022	0.022	0.020	0.002

Means followed by the same letter in the same column are not significantly different at $p \leq 0.05$ according to DMRT.
CV% = Coefficient of variation, S.E = Standard errors of means, NS = Non-significant.

3.4.3 Effect of cuttings type on field establishment of *C. swynnertonii*

Type of cuttings had significant difference on length of the longest branch per plant ($p < 0.001$), number of branches per plant ($p = 0.003$), leaf area ($p < 0.001$), leaf fresh weight ($p = 0.008$), leaf dry weight ($p = 0.005$) and establishment percentage ($p = 0.007$) (Table 3.3). Plants from hardwood cuttings had the highest length of the longest branch per plant, the number of branches per plant, leaf area, leaf fresh and dry weight. Plants from semi-hardwood cuttings had higher establishment percentage compared to others.

There was no significant ($p \leq 0.05$) effect among cutting types in the number of leaves per plant (Table 3.3). However, plants from softwood cuttings had higher number of leaves per plant compared to others.

3.4.4 Effect of growth regulators on field establishment of *C. swynnertonii*

Growth regulators differ significantly on length of the longest branch per plant ($p < 0.001$), number of branches per plant ($p = 0.041$), leaf fresh weight ($p = 0.046$) and leaf dry weight ($p = 0.046$) (Table 3.3). Plants from stem cuttings previously treated with NAA had the highest length of the longest branch per plant, leaf fresh and dry weight. Plants from stem cuttings previously treated with IAA had higher number of branches compared to others. No significant ($p \leq 0.05$) effects were found among growth regulators in the number of leaves per plant, leaf area and establishment percentage (Table 3.3). However, plants from stem cuttings previously treated with NAA had the highest number of leaves per plant and leaf area. While control had the highest establishment percentage.

3.4.5 Interaction effect of cuttings type and growth regulators on field establishment of *C. swynnertonii*

Interactions between type of cuttings and growth regulators were significantly different on the length of the longest branch per plant ($p = 0.045$) (Table 3.3). Plants from hardwood cuttings previously treated with NAA had higher length of the longest branch per plant compared to the other treatments and controls. There were no significant ($p \leq 0.05$) difference among interactions between type of cuttings and growth regulators on the number of branches per plant, the number of leaves per plant, leaf area, leaf fresh and dry weight and establishment percentage (Table 3.3). However, plants from hardwood cuttings previously treated with NAA had the highest number of branches per plant, the number of leaves per plant, leaf fresh and dry weight. Plants from hardwood cuttings previously

treated with IAA had the highest number of branches per plant. Plants from semi-hardwood cuttings previously treated with IAA had the highest establishment percentage which is similar to untreated semi-hardwood cuttings. Plants from untreated hardwood cuttings (control) had higher leaf area compared to the other treatments and controls.

Table 3.3: Effect of cutting types and growth regulators on growth and establishment of *C. swynnertonii*

Treatments	Length of branch (cm)	Number of branches	Number of leaves	Leaf area (cm^2)	Fresh weight (g)	Dry weight (g)	Establishment (%)
Factor A (Cutting types)							
Softwood	78.50a	3.56a	100.33	4.84a	4.38a	1.66a	50.18a
Semi-hardwood	108.90b	3.67a	95.67	8.22a	5.12a	2.20a	68.89b
Hardwood	136.80c	4.56b	93.78	15.44b	7.73b	3.09b	60.37ab
Mean	108.10	3.93	97.00	9.50	5.74	2.31	59.80
C.V%	23.40	21.20	60.50	41.30	35.90	34.60	17.80
S.E	8.43	0.39	19.50	1.31	0.69	0.27	3.55
p-values	< 0.001	0.003	0.970^{NS}	< 0.001	0.008	0.005	0.007
Factor B (Growth regulators)							
IAA	109.90b	4.44b	85.11	8.84	6.01ab	2.36ab	56.85
NAA	136.00c	4.33b	138.00	11.22	6.92b	2.81b	61.11
Control	78.30a	3.00a	66.67	8.44	4.30a	1.78a	61.48
Mean	108.10	3.93	97.00	9.50	5.74	2.31	59.80
C.V%	23.40	21.20	60.50	41.30	35.9	34.60	17.80
S.E	8.43	0.39	19.50	1.31	0.68	0.27	3.55
p-values	< 0.001	0.041	0.723^{NS}	0.295^{NS}	0.046	0.046	0.602^{NS}
Interaction (Factor A x B)							
S x IAA	64.70a	3.67	78.33	3.20	4.200	1.47	52.78
S x NAA	88.90ab	4.67	147.67	8.07	5.13	1.87	50.00
S x Control	82.00ab	2.33	75.00	3.27	3.80	1.63	47.78
SH x IAA	123.70bcd	4.33	100.67	8.00	5.40	2.43	70.00
SH x NAA	147.70de	4.33	107.67	10.93	6.07	2.50	66.67
SH x Control	55.30a	2.33	78.67	5.73	3.90	1.67	70.00
H x IAA	141.30cde	5.33	76.33	15.33	8.43	3.17	47.78
H x NAA	171.30e	4.00	158.67	14.67	9.567	4.067	66.67
H x Control	97.70abc	4.33	46.33	16.33	5.20	2.03	66.67
Mean	108.10	3.93	97.00	9.50	5.74	2.31	59.80
C.V%	23.40	21.20	60.50	41.30	35.90	34.60	17.80
S.E	14.60	0.68	33.70	2.27	1.19	0.46	6.15
p-values	0.045	0.067^{NS}	0.723^{NS}	0.525^{NS}	0.711^{NS}	0.372^{NS}	0.267^{NS}

Means followed by the same letter in the same column for each factor and their interactions are not significantly different at $p \leq 0.05$ according to DMRT, C.V% = Coefficient of variation, S.E = Standard errors of means, NS = Non-significant.

3.4.6 Effect of cuttings type on field establishment of *S. glaucescens*

Type of cuttings had significant difference on length of the longest branch per plant (p = 0.009), number of branches per plant (p < 0.001), number of leaves per plant (p = 0.004) and leaf dry weight (p = 0.004) (Table 3.4). Plants from semi-hardwood cuttings had the highest length of the longest branch per plant, number of leaves per plant and leaf dry weight. Plants from hardwood cuttings had higher number of leaves per plant compared to others. Type of cuttings did not have significant ($p \leq 0.05$) difference on leaf area and leaf fresh weight (Table 3.4). However, plants from semi-hardwood cuttings had higher leaf area and leaf fresh weight compared to others. All tested treatments had 100 establishment percentage.

3.4.7 Effect of growth regulators on field establishment of *S. glaucescens*

Growth regulators did not have significant ($p \leq 0.05$) difference on length of the longest branch per plant, number of branches per plant, number of leaves per plant, leaf area, leaf fresh weight, leaf dry weight and establishment percentage (Table 3.4). However, plants from stem cuttings previously treated with NAA had higher length of the longest branch per plant, the number of branches per plants and leaf fresh weight compared to IAA and control. Plants from stem cuttings previously treated with IAA had the highest leaf area and leaf dry weight while plants from untreated cuttings (control) had the highest number of leaf per plant. All tested treatments had 100 establishment percentage.

3.4.8 Interaction effect of cuttings type and growth regulators on field establishment of *S. glaucescens*

There were significant difference between type of cuttings and growth regulators in length of the longest branch per plant (p = 0.045), number of leaf per plant (p = 0.001), leaf fresh (p = 0.022) and dry weight (p = 0.005) (Table 3.4). Plants from semi-hardwood cuttings

previously treated with NAA had higher length of the longest branch per plant, the number of leaves per plant, leaf fresh and dry weight compared to IAA and control. The interactions between the type of cuttings and growth regulators did not differ significantly ($p \leq 0.05$) on the number of branches per plant and leaf area (Table 3.4).

Table 3.4: Effect of cutting types and growth regulators on growth and establishment of *S. glaucescens*

Treatments	Length of branch (cm)	Number of branches	Number of leaves	Leaf area (cm^2)	Fresh weight (g)	Dry weight (g)	Establishment (%)
Factor A (Cutting types)							
Softwood	38.68b	1.61a	29.50a	97.46	106.10	9.88a	100
Semi-hardwood	39.74b	2.50a	41.06b	98.18	149.70	15.62b	100
Hardwood	31.66a	4.17b	34.44a	90.51	103.60	9.22a	100
Mean	36.70	2.76	35.00	95.40	119.80	11.57	-
C.V%	14.20	36.20	17.80	26.50	35.00	32.20	-
S.E	1.74	0.33	2.08	8.42	13.99	1.24	-
p-values	0.009	< 0.001	0.004	0.779^{NS}	0.058^{NS}	0.004	-
Factor B (Growth regulators)							
IAA	34.45	2.67	31.78	98.11	119.40	12.41	100
NAA	39.18	3.17	36.50	95.93	123.60	12.22	100
Control	36.44	2.44	36.72	92.09	116.30	10.09	100
Mean	36.70	2.76	35.00	95.40	119.80	11.57	-
C.V%	14.20	36.20	17.80	26.50	35.00	32.20	-
S.E	1.74	0.33	2.08	8.42	13.99	1.24	-
p-values	0.188^{NS}	0.317^{NS}	0.196^{NS}	0.878^{NS}	0.935^{NS}	0.363^{NS}	-
Interaction (Factor A x B)							
S x IAA	38.30bc	1.50	36.33bc	106.46	158.70c	16.03bc	100
S x NAA	34.48abc	2.00	22.67a	81.29	70.20ab	5.97a	100
S x Control	43.25c	1.33	29.50ab	104.61	89.40abc	7.65a	100
SH x IAA	37.97bc	2.67	38.83bc	94.91	150.20bc	16.38bc	100
SH x NAA	44.65c	2.50	45.67c	106.75	160.50c	18.78c	100
SH x Control	36.62abc	2.33	38.67bc	92.88	138.30bc	11.68ab	100
H x IAA	27.08a	3.83	20.17a	92.97	49.40a	4.82a	100
H x NAA	38.42bc	5.00	41.17bc	99.76	140.10bc	11.92abc	100
H x Control	29.47ab	3.67	42.00c	78.79	121.30abc	10.93ab	100
Mean	36.70	2.76	35.00	95.40	119.80	11.57	-
C.V%	14.20	36.20	17.80	26.50	35.00	32.20	-
S.E	3.02	0.58	3.60	14.58	24.23	2.15	-
p-values	0.045	0.806^{NS}	0.001	0.543^{NS}	0.022	0.005	-

Means followed by the same letter in the same column for each factor and their interactions are not significantly different at $p \leq 0.05$ according to DMRT, C.V% = Coefficient of variation, S.E = Standard errors of means, NS = Non-significant.

However, plants from hardwood cuttings previously treated with NAA had the highest number of branches per plant while plants from semi-hardwood cuttings previously treated

with NAA had the highest leaf area compared to other treatments and control. All tested treatments had 100 establishment percentage.

3.5 Discussion

The establishment of the plants in the field is dependent on the quality of the seedlings at the time of transplanting and the environmental conditions of the planting area. Survival ability of *C. swynnertonii* and *S. glaucescens* plants were affected by different pre-sowing treatments. The *C. swynnertonii* plants from seeds previously soaked in hot water at 60°C and plants from seeds previously treated with KNO_3 at 20 ppm recorded better establishment percentage (100%) than the other treatments and control. However, plants from seeds previously treated with KNO_3 at 10 ppm recorded a high number of branches and leaves, large leaf area and leaf fresh weight. KNO_3 serve as a nutrient and helps in translocation of the sugar in the plant. It also provide turgidity of the plant cells (Hegazi *et al.*, 2011). KNO_3 encourages the establishment and branching of a root system that better absorbs water from the soil. The superiority of KNO_3 was also observed in *Helianthus annuus* L. and *Carthamus tinctorius* L. (Jabeen and Ahmad, 2011), *Gossypium hirsutum* L. (Waraich *et al.*, 2011) and *Coriander sativum* L. (Elhindi *et al.*, 2016).

Synadenium glaucescens plants from seeds previously treated with GA_3 at 250 ppm recorded the maximum value of all growth parameters and had better establishment percentage than the other treatments and control. This is because GA_3 enhance hydrolysis of carbohydrates in the plant and increases somatic absorption of nutrients, triggering cell elongation (Harsha *et al*., 2017). Zang *et al.* (2016) found that GA_3 increased leaf size, leaf biomass and chlorophyll content of rabbiteye blueberry. Jholgiker *et al*. (2017) observed

the maximum plant height, root length and biomass of guava c.v. SR - 4 seedlings treated with 250 ppm GA_3 solution.

Survival ability of *C. swynnertonii* and *S. glaucescens* plants was affected by cuttings type. In *C. swynnertonii,* plants from hardwood cuttings recorded the maximum length of the longest branch per plant, a number of branches per plant, leaf area, leaf fresh and dry weight and better establishment percentage. This is because hardwood cuttings contain sufficient stored food such as hydrocarbons, nucleic acids, proteins and natural hormones such as IAA that can be used for plant growth and development (Rolland *et al*., 2006). Similar results were observed by Yeshiwas *et al.* (2015) who reported that hardwood cuttings showed a significant positive effect on growth and development of rose. Mahmood *et al.* (2017) observed that basal cuttings of *Paulownia tomentosa* gave the best results on most of the studied growth characters. In *S. glaucescens*, all plants recorded better establishment percentage (100%). However, plants from semi-hardwood cuttings recorded the maximum length of the longest branch per plant, number of leaf per plant and leaf dry weight. This could be due to their lignification which increases their ability to withstand dry or other adverse conditions (Benbya *et al.,* 2018). This result is in agreement with the findings of Antwi-Boasiako and Enninful (2011) who reported that semi-hardwood cuttings of *Moringa oleifera* performed better than softwood cuttings. They observed that plants from semi-hardwood cuttings produced the highest number of shoots and the longest shoots than softwood cuttings.

Application of hormone affected the establishment of *C. swynnertonii* plants. Plants from stem cuttings previously treated with NAA recorded the maximum length of the longest branch per plant, leaf fresh and dry weight. This is because of the action of the auxin (NAA) which might have promoted growth of stems, leaf formation and enlargement

(Hajam *et al.*, 2017). The application of auxin would have induced the endogenous synthesis of native auxin resulting in early active growth. An increase in the length of branches, number and length of the leaves per plant due to NAA was also reported by Memon *et al.* (2013) in Bougainvillea. Tamilselvi and Vijayaraghavan (2014) reported similar results in *Capsicum annuum* L. Spraying with 25 mg/L NAA enhanced the plant growth and development of Mokara Chark Kuan orchid (Khandaker *et al.,* 2017). Growth regulators did not have a significant influence on the establishment of *S. glaucescens.* The survival ability of the rooted cuttings was found 100% in all treatments reflected that it is an easily cultivated species even without hormone treatments. This also indicates the influence of growth regulators on rooting and root and shoot parameters but not on the establishment.

The results of the analysis of variance showed that there were significant interaction effects of growth regulators and cutting types on plant height of *C. swynnertonii.* Plants from hardwood cutting previously treated with NAA recorded the maximum plant height. Increased plant height by NAA application could be due to better cell division and cell elongation. In *S. glaucescens*, plants from semi-hardwood cuttings previously treated with NAA has shown to be the best in growth performance.

3.6 Conclusion

Pesticidal plants propagation and cultivation is a major thrust area for expansion of botanical pesticides market to meet the growing raw material demand. The present study indicate that *C. swynnertonii* and *S. glaucescens* can be established by seeds as well as by rooted stem cuttings. Among pre-sowing treatments, KNO_3 at 10 ppm and GA_3 at 250 ppm was found to be the best for the establishment of *C. swynnertonii* and *S. glaucescens* seedlings, respectively. Naphthalene Acetic Acid (NAA) performed better in most of the

studied parameters than indole acetic acid (IAA). Hardwood cuttings and semi-hardwood cutting treated with NAA was found to be suitable for field establishment of *C. swynnertonii* and *S. glaucescens*, respectively. Further study on the propagation of these plants in different locations is recommended. Evaluation of pesticidal activities between cultivated and wild-harvested pesticidal plants is encouraged.

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CHAPTER FOUR

4.0 EFFECTIVENESS OF *COMMIPHORA SWYNNERTONII* (Burrt.) AND *SYNADENIUM GLAUCESCENS* (Pax.) IN MANAGING TOMATO FUSARIUM WILT DISEASE

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4.1 Abstract

Tomato fusarium wilt disease caused by *Fusarium oxysporum* f. sp. *lycopersici* is an important fungal disease, causing significant reduction in tomato yield worldwide. The pathogen is a soil-borne and can cause a yield loss of about 90%. In the present study, extracts from two plants, namely *C. swynnertonii* and *S. glaucescens* were evaluated against *F. oxysporum* f. sp. *lycopersici* in a laboratory and screen house experiments. In the laboratory experiment, there were sixteen treatment combinations comprising of four crude extracts obtained from resin of *C. swynnertonii*, latex, fresh and dry leaves of *S. glaucescens* and four crude extract concentrations (0.01 g/ml, 0.05 g/ml, 0.1 g/ml and 0.15 g/ml). Sterile distilled water was used as a negative control and Linkmil 72 WP (Mancozeb 64% + Metalaxyl 8%) was used as positive control. The experiment was set in 4 x 4 factorial in a CRD with three replications. It was found that using poisoned food technique of the tested crude plant extracts, the extracts caused significant inhibition of radial growth of *F. oxysporum* f. sp. *lycopersici.* Both *C. swynnertonii* and *S. glaucescens* extract at 0.15 g/ml showed over 65% inhibition of mycelia growth compared to Linkmil 72 WP (23.58%) and negative control (0%) after eight days of incubation. In the screen

house experiment, there were four treatments namely resinous extracts of *C. swynnertonii*, extract from latex and fresh leaves of *S. glaucescens* and dried leaves powder of *S. glaucescens.* Untreated soil was used as a negative control and soil treated with Linkmil 72 WP was used as positive control. The experiment was set in RCBD with four replications. The results revealed that 72.92% of disease reduction was in plants treated with dried leaves powder followed by latex of *S. glaucescens* (68.75%) and resin (56.25%) of *C. swynnertonii.* Plants treated with dried leaves powder of *S. glaucescens* had high value of all measured growth parameters followed by plants treated with latex and fresh leaves. The crude extracts of *C. swynnertonii* and *S. glaucescens* used in this study had shown a high fungicidal potential against *F. oxysporum* f. sp. *lycopersici* and they can be recommended as part of integrated management of tomato fusarium wilt disease.

Keywords: *Fusarium oxysporum* f. sp. *lycopersici, crude extracts, resin and latex.*

4.2 Introduction

Tomato fusarium wilt is a devastating disease of tomato reducing yields worldwide (Ramaiah and Garampalli, 2015). It is a soil-borne fungal disease caused by *Fusarium oxysporum* f. sp. *lycopersici* (Ohunakin and Bolanle, 2017). The pathogen belongs to the Kingdom Fungi, Phylum Ascomycota and the Genus Fusarium. Tomato fusarium wilt reduces tomato yield of up to 90% (Singh and Kamal, 2012). The pathogen survives in soils of all types, but sandy soils provide the most favourable conditions for growth and development (Larkin and Fravel, 2002). Soil and air temperatures of 28°C are optimum for disease development (Arici *et al.*, 2018). Reports show that in the field the fungus is dispersed through irrigation water, infected transplants, contaminated farm equipment or soil and human movement around the infected field (Ajilogba and Babalola, 2013; Bawa, 2016).

The initial symptom of the tomato fusarium wilt is chlorosis of the older leaves that often begins on one side of the plant followed by wilting of that foliage (Tistisgiannis *et al.*, 2008). Wilt symptoms are more commonly observed during the hottest part of the day. As the disease progresses the entire plant turns yellow and wilts resulting in the death of the plant. The infected plants can be severely stunted. Cutting a longitudinal section into the xylem at the base of the stem reveals a dark-brown to red discolouration (Mishra *et al.*, 2014).

Management of tomato fusarium wilt diseases depends mainly on resistance cultivars and fungicides (Nirmaladevi *et al.*, 2016; Cueto-Wong *et al.*, 2010). However, none of these methods can permanently control tomato fusarium wilt. The longevity of resistance of many resistant cultivars is shortened by the high pathogenic variability (Kutama *et al.*, 2013). Moreover, synthetic fungicides have a negative effect on the environment and

human health and few fungi have developed resistance (Dias, 2012). Sustainable and effective tomato fusarium wilt control can be achieved through the use of botanical pesticides. Botanical pesticides have minimal environmental impact and health risks to consumers in contrast to synthetic pesticides (Ramaiah and Garampalli, 2015).

The fungicidal activity of pesticidal plants has been recognized for many years. Foristance leaf extracts of *Azadirachta indica*, *Ageratum conyzoides* and *Datura metel* have been reported to have significant fungicidal activities against *F. oxysporum* f. sp. *spinaciae* (Hadian, 2012: Mishra, 2014 and Rinez *et al.,* 2013). *Commiphora* species such as *Commiphora stoksiana* have been reported to inhibit radial growth *F. oxysporum* f. sp. *spinaciae* (Bhale *et al.,* 2005). However, there are limited reports on the use of botanical pesticides in managing tomato fusarium wilt in Tanzania. Therefore, there was a need to determine the efficacy of *C. swynnertonii* and *S. glaucescens* against *F. oxysporum* f. sp. *lycopersici.* Thus, the current study reports on the effectiveness of *C. swynnertonii* and *S. glaucescens* in managing tomato fusarium wilt disease.

4.3 Materials and Methods

4.3.1 Study area

Laboratory and screen house experiments were conducted at the Sokoine University of Agriculture (SUA) Morogoro, Tanzania. The study area is located at 6º05’S, 35º37’E, at an elevation of 568 m above the sea level. A laboratory experiment was conducted in the African Seed Health Centre Laboratories between March and April, 2019. The screen house experiment was conducted at the Horticulture Section between June and September, 2019.

4.3.2 Experimental materials

Resins of *Commiphora swynnertonii* were collected from Mererani ward in Simanjiro District of Manyara Region (4° 0′ 0 S, 36° 30′ 0 E: 1 009 m a.s.l) (Fig. 4.1). Latex and leaves of *Synadenium glaucescens* were collected from the Department of Food Technology, Nutrition and Consumer Sciences premises at SUA, Morogoro, Tanzania (6° 85′ S, 37° 65′ E; 556 m a.s.l). Linkmil 72 WP and tomato seeds c.v. Cal J were purchased from local agro-dealer in Morogoro town. Tween 20, Sodium hypochlorite and Potato Dextrose Agar (PDA) were purchased from Jakovic General Supplies Ltd, Morogoro, Tanzania.

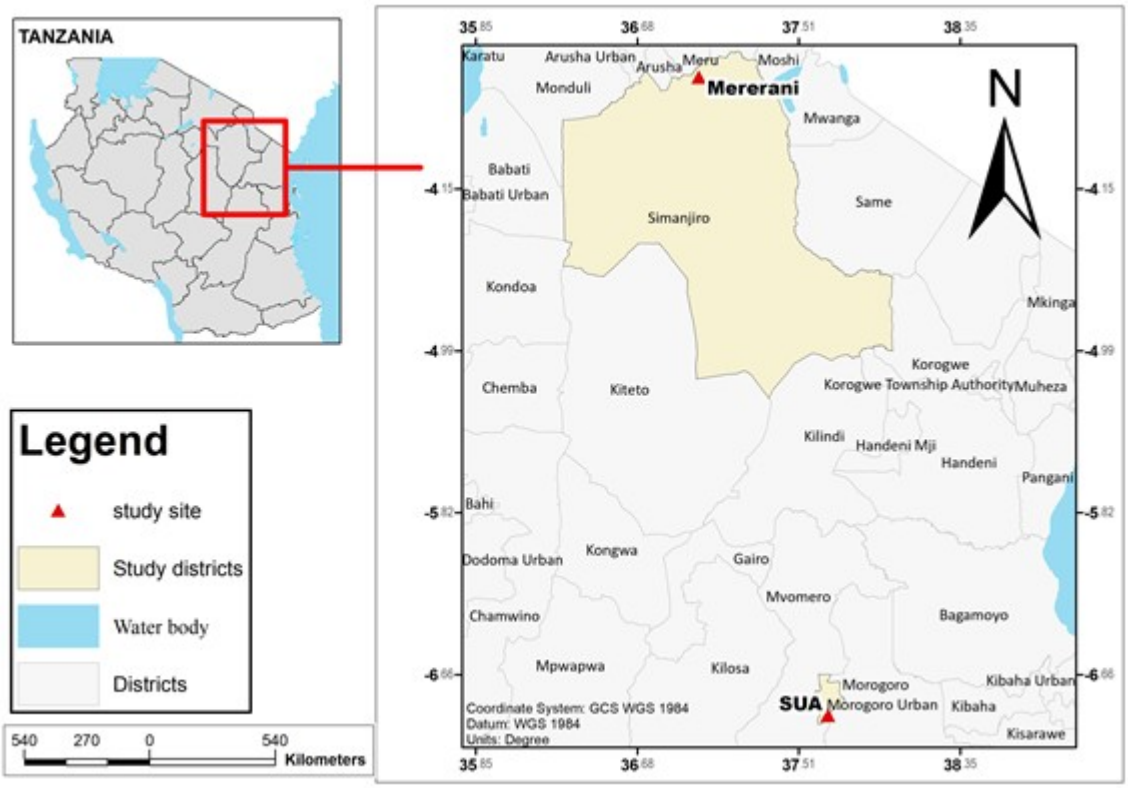

Figure 4.1: Map of Tanzania showing location of Mererani and SUA.

4.3.3 Preparation and extraction of plant materials

Extraction of plant materials was done based on Anjarwalla *et al.* (2016) method with some modifications. Resin of *C. swynnertonii*, latex and leaves of *S. glaucescens* were harvested and transported to the Department of Physics and Chemistry laboratories of SUA. The resin and latex were kept in an airtight bottle and stored in the refrigerator at 4°C. The leaves were cleaned with tap water and rinsed in distilled water. The leaves were used both in fresh and dried form. The fresh leaves were finely blended using electric blender (Kenwood, Model BL 490, China). The dried form were prepared by drying leaves in the shade for a week before being ground to pass through a 1.5 mm sieve.

Aqueous extracts were prepared by adding 1 g, 5 g, 10 g and 15 g of resins, latex, blended fresh leaves and leaves powder individually into a beaker and adding sterile distilled water until the 100 ml mark to make concentrations of 0.01 g/ml, 0.05 g/ml, 0.1 g/ml and 0.15 g/ml. The contents in the beaker were thoroughly mixed and left to stand for 24 hours. Thereafter filtered with a Whatman No.1 filter paper and stored at 4°C in airtight bottles until used. Plate 4.1 below shows the preparation of crude plant extracts.

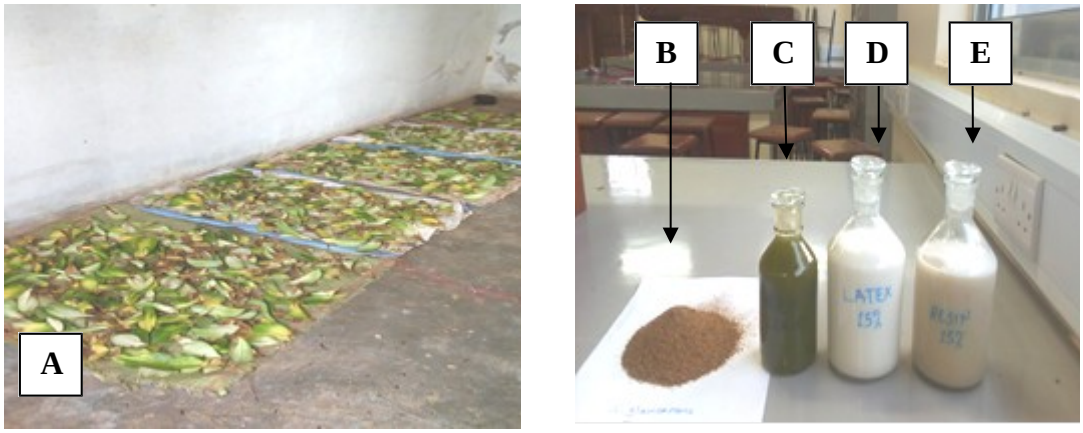

Plate 4.1: Shade drying of *S. glaucescens* leaves [A]; grounded dried leaves [B], blended fresh leaves [C] and latex [D] of *S. glaucescens* and resin of *C. swynnertonii* [E].

4.3.4 Inoculum collection and preparation

The stem samples of tomato plant showing symptoms of fusarium wilt disease were collected from Mlali village, Morogoro, Tanzania. Samples were packed in paper envelopes and transported to the African Seed Health Centre Laboratories, SUA, Tanzania, for isolation of *F. oxysporum* f. sp. *lycopersici*. The stem was cut into small pieces, rinsed with distilled water, disinfected with sodium hypochlorite (2%) for 2 min and rinsed again with sterile distilled water to remove traces of bleach water and then dried using sterile filter papers. The sterilized stem pieces were placed on PDA medium and incubated at 25ºC for 5 days. After incubation, fungal spore's colonies were observed and identified on the basis of morphological and reproductive characters of the pathogen. Single spore culture techniques was followed to obtain a pure culture. The pure cultures were kept on the PDA slant in the refrigerator at 4°C for further use.

4.3.5 Pathogenicity test

Different isolates obtained from the inoculum collected from different farms in Morogoro were inoculated on tomato seedlings c.v. Cal J planted in 4-L plastic pots. Inoculation was done following procedures described by Adedeji and Aduramigba (2016). The conidia of *F. oxysporum* f. sp. *lycopersici* were suspended in two drops of Tween 20 adjusted at 1 x 10^6 spores/ml and inoculated in 20 days old tomato seedlings by standard root dip inoculation method. The inoculated seedlings were then transplanted in the pots containing steam-sterilized soil, farmyard manure and rice husks at a ratio of 4:2:1. After 4 weeks of inoculation, yellowing and wilt symptoms that appeared on leaves of inoculated plants were recorded. The pathogen was re-isolated from the collar region of artificially inoculated plants to confirm Koch's postulates. A highly virulent isolate was selected for further tests. Plate 4.2 shows symptoms of tomato fusarium wilt.

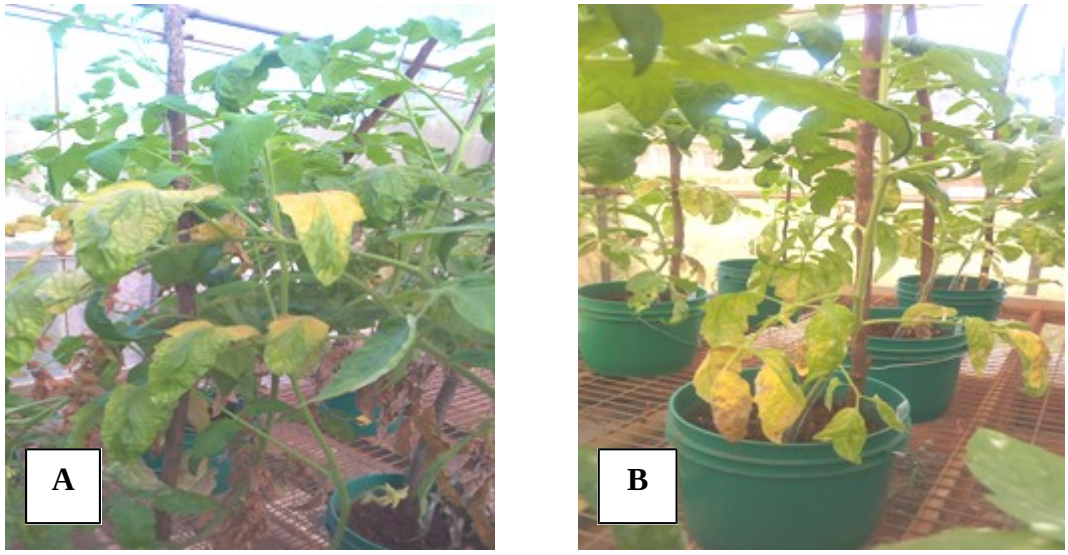

Plate 4.2: Chlorosis of one side of tomato plant [A]; Chlorosis of older leaves of tomato plant [B].

4.3.6 *In vitro* test of *C. swynnertonii* and *S. glaucescens* against growth of *F. oxysporum* f. sp. *lycopersici*

4.3.6.1 Treatments and experimental design

The fungicidal property of crude plant extracts against *F. oxysporum* f. sp. *lycopersici* were tested by poisoned food technique (Adedeji and Aduramigba, 2016). Two millilitre of plant extract at different concentrations 0.01 g/ml, 0.05 g/ml, 0.1 g/ml and 0.15 g/ml were added individually in 20 ml of sterilized PDA in petri plates. A 5 mm diameter of the actively growing mycelium disc of the pathogen from 7 days old culture was placed in the centre of the petri dish using sterile cork borer. The PDA petri dishes without plant extracts and PDA mixed with Linkmil 72 WP at 3 g/l was used as a negative and positive control, respectively. The experiment was carried out in 4 x 4 factorial (4 plant extracts x 4 plants extract concentrations) in a completely randomized design (CRD) with three replications. All plates were incubated for eight days at 24°C.

4.3.6.2 Data collection

Observations were made after 2, 4, 6 and 8 days of inoculation and colony radii were measured. The percent inhibition of fungal growth was estimated based on Ogbebor and Adekunle (2005) method as follows;-

$$\text{Inhibition } (\%) = \frac{\text{Radial growth in control} - \text{Radial growth in treatment}}{\text{Radial growth in control}} \text{ x } 100 \ldots \text{(i)}$$

4.3.7 *In vivo* evaluation of *C. swynnertonii* and *S. glaucescens* against *F. oxysporum* f. sp. *lycopersici*

4.3.7.1 Treatments and experimental design

In vivo evaluation of pesticidal plants against *F. oxysporum* f. sp. *lycopersici* was done following procedures described by Sharma *et al.* (2017) with some modifications. Tomato seeds c.v. Cal J were sown in seedling trays filled with compost in the screen house and watered daily. The most promising concentrations of extracts (0.15 g/ml of SDW) in *in vitro* trial were used in *in vivo* experiment. The pathogen was cultured in sterilized sorghum grains in the dark room at 25°C for 14 days. The grain was then mixed with steam-sterilized soil, FYM and rice husks (4:2:1) at the rate of 25 g inoculum per kg mix and incubated for 5 days. The soil mix was treated with aqueous crude plant extracts (5.0 ml/150 cm^3 soil). Dry leaves powder was applied at 20 g/kg soil. The treated soil mix was transferred in 4 L- plastic pots and incubated for 48 h. The pots having only fungus infested soil were considered as negative control and the pots treated with Linkmil 72 WP was used as a positive control. Tomato c.v. Cal J (3 – 5 leaf stage) were transplanted into the pots. The experiment was laid out in RCBD with four replications.

4.3.7.2 Disease severity

Disease severity was recorded weekly for four consecutive weeks. The first record was taken five weeks after inoculation. The disease severity were scored on a scale of 0 – 4 as described by Grattidge and O'Brien (1982). The disease severity index (DSI) and disease reduction (DR) were determined based on Sharma *et al.* (2017) as follows;-

$$\text{DSI (\%)} = \frac{\Sigma(\text{grade x number of plants in that grade})}{(\text{maximum grade x total number of assessed plants})} \text{x } 100 \ldots\ldots\ldots\ldots \text{(ii)}$$

$$\text{DR (\%)} = \frac{(\text{DSI of negative control} - \text{DSI of treatment})}{(\text{DSI of negative control})} \text{x } 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(iii)}$$

4.3.7.3 Growth parameters

All growth data were collected two months after inoculation. The height of plant was measured from the ground to the tip of the two sampled plant using a measuring tape. The number of branches was counted from two sampled plant in each treatment and the mean was determined. The average number of leaves from two sampled plant in each treatment were recorded. The length and middle width of the four leaves from two sampled plants in each treatment were measured using a ruler and the values were multiplied according to Awal *et al.* (2004). The average area was taken for further analysis.

4.3.7.4 Data analysis

Data collected were subjected to analysis of variance using GenStat software 15th Edition (VSN International Ltd. UK). Treatment means were separated by Duncan´s Multiple Range Test (DMRT) at $p \leq 0.05$.

4.4 Results

4.4.1 Effect of plant extracts on *F. oxysporum* f. sp. *lycopersici* mycelia growth

Plant extracts showed a highly significant effect ($p < 0.001$) in mycelia inhibition of *F. oxysporum* f. sp. *lycopersici* at all incubation days (Table 4.1). Latex of *S. glaucescens* had higher mycelial inhibition percentage at second and fourth days of incubation compared to other treatments and control. Dry leaves had the highest mycelial inhibition percentage at sixth day of incubation. Resin of *C. swynnertonii* had higher mycelial inhibition percentage eighth day of incubation compared to other treatments and control. The negative control showed the least mycelia inhibition percentage followed by positive control in all incubation days.

4.4.2 Effect of concentration of plant extracts on *F. oxysporum* f. sp. *lycopersici* mycelia growth

It was observed that there was highly significant difference ($p < 0.001$) between concentrations of plant extracts in mycelia inhibition of *F. oxysporum* f. sp. *lycopersici* at all incubation days (Table 4.1). There were varying levels of inhibition with a concentration of 0.15 g/ml having the highest inhibiting capacity against the tested pathogen followed by 0.1 g/ml and 0.05 g/ml while the concentration of 0.01 g/ml had the least overall inhibitory effect.

4.4.3 Interaction effect of plant extracts and concentrations on *F. oxysporum* f. sp. *lycopersici* mycelia growth

Interaction between plant extracts and concentrations had a significant difference on mycelial inhibition of *F. oxysporum* f. sp. *lycopersici* at second ($p = 0.022$), fourth ($p = 0.002$) and sixth ($p = 0.011$) day of incubation (Table 4.2). Latex of *S. glaucescens* at 0.15 g/ml had the highest mycelial inhibition percentage on the second day of incubation

followed by dry leaves at 0.15 g/ml while dry leaves at 0.01 g/ml had the least inhibitory effect. Dry leaves of *S. glaucescens* at 0.15 g/ml had the highest mycelial inhibition percentage on the fourth day of incubation followed by latex at 0.15 g/ ml while resin at 0.01 g/ml had the least inhibitory effect. The resin of *C. swynnertonii* at 0.15 g/ml had the highest mycelial inhibition percentage at sixth and eighth days of incubation followed by dry leaves at 0.15 g/ml and latex of *S. glaucescens* at 0.15 g/ml.

Table 4.1: Effect of crude plant extracts and concentrations on mycelia growth inhibition (%) of *F. oxysporum* f. sp. *lycopersici*

Treatments	Days after incubation			
	2	**4**	**6**	**8**
Factor A (Plant extracts)				
Resin	54.21d	51.08d	53.17cd	64.69d
Latex	68.56e	64.90f	52.21cd	64.53d
Fresh leaves	38.46c	41.28c	48.15c	50.90c
Dry leaves	52.40d	57.25e	56.22d	56.39c
Linkmil 72 WP	21.78b	15.63b	25.83b	23.58b
Control	0.00a	0.00a	0.00a	0.00a
Mean	39.20	38.36	39.26	43.35
C.V%	22.60	17.20	15.40	19.20
S.E	2.39	1.81	1.75	2.20
p-values	< 0.001	< 0.001	< 0.001	< 0.001
Factor B (Concentratons (g/ml))				
0.01	29.46a	26.76a	29.85a	32.79a
0.05	34.35a	33.67b	34.71b	39.34b
0.1	42.81b	42.13c	40.52c	45.41c
0.15	50.31c	50.87d	51.97d	55.84d
Mean	39.20	38.36	39.26	43.35
C.V%	22.60	17.20	15.40	19.20
S.E	1.95	1.48	1.43	1.80
p-values	< 0.001	< 0.001	< 0.001	< 0.001

Means followed by the same letter in the same column for each factor are not significantly different at $p \leq 0.05$ according to DMRT.
CV% = Coefficient of variation, S.E = Standard errors of means.

***Table 4.2: Interaction effect of crude plant extracts and their concentrations on mycelia growth inhibition (%) of F. oxysporum f. sp.* lycopersici**

Treatments	Concentrations (g/ml)	Days after incubation 2	4	6	8
Resin	0.01	33.48bc	24.71bc	31.45bc	49.52de
	0.05	49.28cde	44.90de	44.54def	59.95ef
	0.1	59.75efg	58.39fg	56.60gh	65.62fg
	0.15	74.32gh	76.32hi	80.10i	83.69h
Latex	0.01	60.89efg	51.65ef	43.51def	55.32def
	0.05	63.85defgh	59.54fg	44.38def	58.73def
	0.1	71.56gh	69.77gh	47.17defg	65.30efg
	0.15	77.93h	78.66hi	73.7i	78.77gh
Fresh leaves	0.01	32.25b	34.83cd	39.64cde	34.17bc
	0.05	33.68bc	38.16d	47.00defg	50.48def
	0.1	37.68bcd	39.35d	51.14efg	53.28def
	0.15	50.22def	52.80ef	54.80fgh	65.67fg
Dry leaves	0.01	28.35b	33.72cd	38.71cd	34.17bc
	0.05	37.48bcd	43.79de	46.50defg	43.31cd
	0.1	66.12fgh	69.66gh	62.38h	64.71efg
	0.15	77.63h	81.84i	77.32i	83.36h
Linkmil 72 WP	3 g/l	21.78b	15.63b	25.83b	23.58b
Control	0	0.00a	0.00a	0.00a	0.00a
Mean	-	39.20	38.36	39.26	43.35
C.V%	-	22.60	17.20	15.40	19.20
S.E	-	4.78	3.63	3.51	4.41
P-values	-	< 0.001	< 0.001	< 0.001	< 0.001

Means followed by the same letter in the same column are not significantly different at $p \leq 0.05$ according to DMRT. CV% = Coefficient of variation, S.E = Standard errors of means.

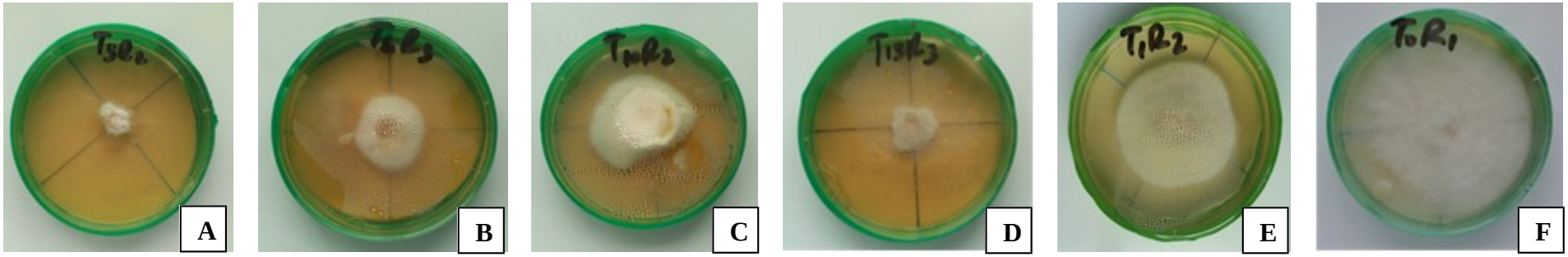

Plate 4.3: Effect of different treatments on mycelia radial growth inhibition of *F. oxysporum* f. sp. *lycopersici* after incubation for 8 days

(A = Resin, B = Latex, C = Fresh leaves, D = Dry leaves, E = Linkmil 72WP and F = negative control)

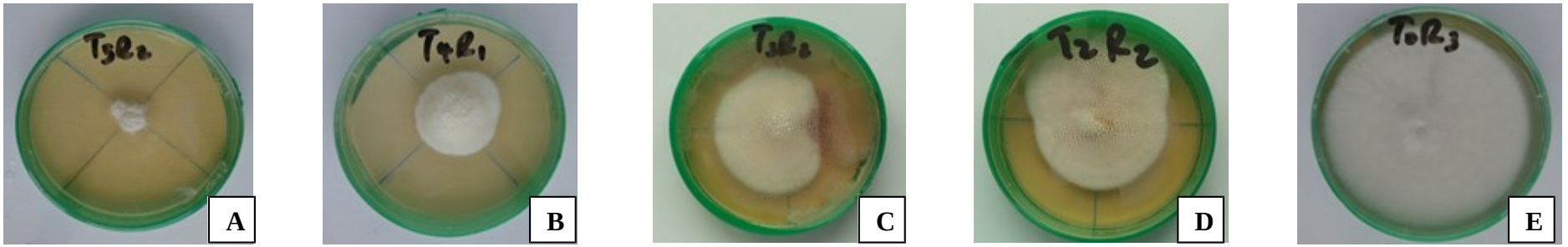

Plate 4.4: The effect of different concentrations of resin on mycelia radial growth inhibition of *F. oxysporum* f. sp. *lycopersici*

(A = 0.15 g/ml, B = 0.1 g/ml, C = 0.05 g/ml and D = 0.01 g/ml and E = negative control)

4.4.4 Effect of crude plant extracts on severity of tomato fusarium wilt disease

Plant extracts differed significantly on their effect to the severity of tomato fusarium wilt disease after fifth ($p = 0.038$), sixth ($p < 0.001$), seventh ($p < 0.001$) and eighth ($p < 0.001$) weeks of inoculation (Fig. 4.1). The least disease severity index (DSI) was recorded in plants treated with dry leaves powder of *S. glaucescens* followed by latex and then resin of *C. swynnertonii.* Untreated plants (negative control) had the highest disease severity index followed by plants treated with Linkmil 72WP (positive control). The disease reduction (DR) was significantly different after fifth ($p = 0.050$), sixth ($p < 0.001$), seventh ($p < 0.001$) and eighth ($p < 0.016$) weeks of inoculation (Fig. 4.2). The highest disease reduction (DR) was recorded in plants treated with dry leaves powder of *S. glaucescens*.

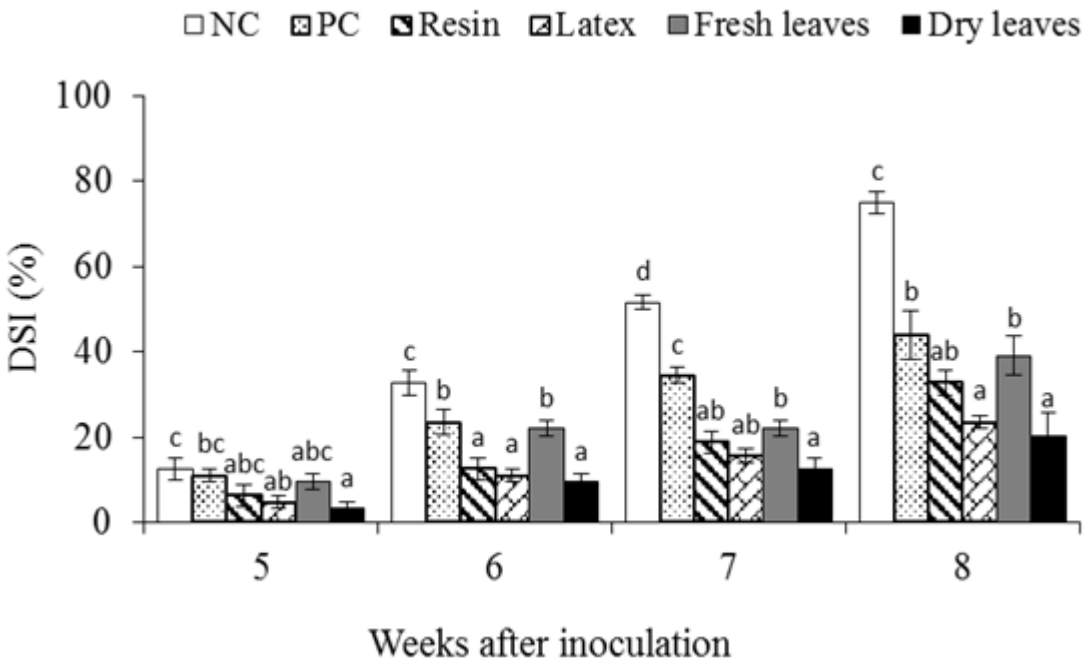

Figure 4.2: Effect of different treatments on disease severity index (DSI) of tomato fusarium wilt

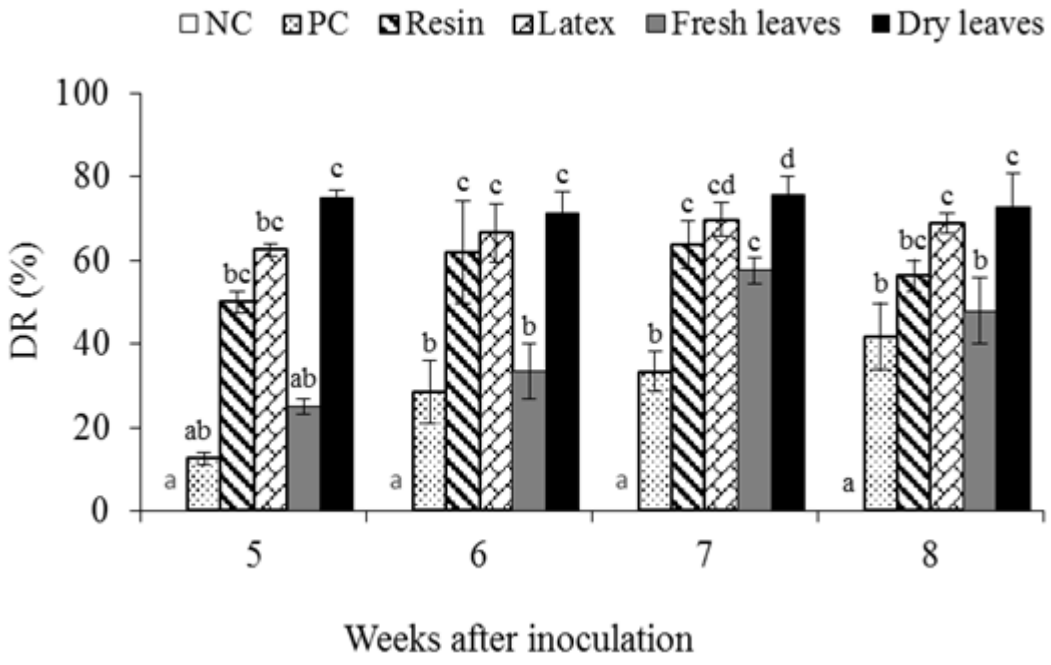

Figure 4.3: Effect of different treatments on disease reduction (DR) of tomato fusarium wilt

4.4.5 Effect of crude plant extracts on growth parameters of tomato

There was a significant difference (p = 0.001) between plant extracts in leaf surface area and highly significant difference (p < 0.001) in plant height, number of branches per plant and number of leaves per plant (Table 4.3). Plants treated with dried leaves powder of *S. glaucescens* had the highest value of all measured growth parameters followed by plants treated with latex and fresh leaves. The plants treated with the resin of *C. swynnertonii* had the least value of all measured growth parameters followed by untreated plants (negative control).

Table 4.3: Effect of crude plant extracts on growth of tomato plant

Treatments	Plant height (cm)	No. of branches/plant	No. of leaves/plant	Leaf area (cm^2)
Resin	38.22a	9.50a	46.50a	33.00a
Latex	77.65d	18.50c	86.25b	51.27cd
Fresh leaves	73.40cd	16.00bc	82.25b	45.53bc
Dry leaves	85.85e	19.25c	99.50c	59.39d
Linkimil 72 WP	71.25c	15.75bc	77.50b	41.18abc
Negative control	62.92b	12.25ab	76.75b	38.23ab
Mean	68.22	15.21	78.10	44.80
CV%	5.80	17.60	9.30	16.00
S.E	1.99	1.34	3.63	3.58
p-value	< 0.001	< 0.001	< 0.001	0.001

Means followed by the same letter in the same column are not significantly different at $p \leq 0.05$ according to DMRT.
CV% = Coefficient of variation, S.E = Standard errors of means.

4.5 Discussion

Plants contain various secondary metabolite that can be used as a source of botanical pesticides (Mengal *et al.*, 2015). Pesticidal plants have been used to control plant diseases caused by fungi, bacteria, nematodes and insect pests since time immemorial (Kumar *et al*., 2014; Din *et al.*, 2016; Dutta *et al.*, 2019; Khan *et al.*, 2017). Botanical pesticides are cheap, easy to develop, have less ecological impact and health risks to consumers compared to industrial pesticides (Mishra *at al*., 2014). They can be used as altenative to industrial pesticides for the control of plant pests.

The results of *in vitro* experiment revealed that all evaluated plant extracts inhibited the mycelial growth of *F. oxysporum* f. sp. *lycopersici.* The varied activity of plant extracts is dependent on the concentrations and parts of the plants used. Dry leaves extract of *S. glaucescens* and resinous extract of *C. swynnertonii* was found to be more effective in reducing the mycelial growth of the fungus followed by latex of *S. glaucescens* at their highest dose. The fungicidal activity exhibited by these plants is due to the presence of secondary metabolites that impair growth of the fungus. *Commiphora* species contain

terpenoids and phenolic compounds that serve as defensive compounds against biotic and abiotic stresses. *Synadenium* species contain flavonoids, saponins, diterpenes and phorbol esters. These compound interfere physiological activities of the pests. Hadian (2012) tested neem seed extracts against *F. oxysporum* f. sp. *lycopersici* and found 98% growth inhibition. Neem seeds contain secondary metabolite *azadiractin* that affect mycelia growth of the pathogen (El-Wakeil, 2013). Beg *et al.* (2011) reported that the aqueous extract of *Blumea lacera* had positive activity against *F. oxysporum* f. sp. *lycopersici*. The extract inhibited 78% mycela growth of the fungus. The findings attained in screen house experiment are in agreement with the findings attained under laboratory conditions. The dried leaves powder of *S. glaucescens* showed very strong inhibitory effect causing significant reduction of wilt disease in tomato plants. This treatment also had the highest value in all measured growth parameters. The phytochemical evaluation of *Synadenium* species indicated that the various activities shown by the extracts could possibly be due to their phenolic compounds and phorbol esters (Hassan *et al.*, 2012). Singha *et al.* (2011) found that crude plant extracts obtained from *Piper betle* leaves had a fungicidal effect against the *F. oxysporum* f. sp. *lycopersici.* Rinez *et al.* (2013) studied the fungicidal properties of aqueous extract of *Datura metel* in managing *F. oxysporum* f. sp. *lycopersici* and reported that plant extract inhibited mycelial growth by 69%.

However, a resinous extract of *C. swynnertonii* inhibited tomato plant growth and had lower disease reduction compared to dry leaves powder and latex of *S. glaucescens*. This could be due to the phytotoxic effect of resin on plant growth. Sharma *et al.* (2017) found that clove oil at high dose (10%) caused phytotoxic effects to the growth of tomato plants. The aqueous methanol extracts of *Ocimum tenuiflorum* plant at concentrations higher that 10 mg/ml showed inhibitory activity on the shoot and root growth of *Lactuca sativa*, *Lepidium sativum*, *Medicago sativa*, *Lolium multiflorum*, *Echinochloa crus-galli* and

Phleum pretense (Islam and Kato-Noguchi, 2014). Ibanez and Blazquez (2019) observed that extract from *Lavandula angustifolia* was phytotoxic against germination of tomato seeds.

4.6 Conclusion

The study showed that plant extracts have the potential to inhibit growth of fungal pathogens. Dry leaves extract of *S. glaucescens* and resinous extracts of *Commiphora swynnertonii* at 0.15 g/ml had the highest inhibitory effect against mycelia growth *F. oxysporum* f. sp. *lycopersici.* The application of dried leaves powder of *S. glaucescens* exhibited the least disease severity. Also, tomato plants treated with dried leaves powder showed a significant stimulatory effect on plant growth. Therefore, the dried leaves powder and extracts of *S. glaucescens* can be used in management of tomato fusarium wilt disease. These botanicals could be a good alternative to synthetic pesticides in managing *F. oxysporum* f. sp. *lycopersici.* More studies are needed to confirm the current findings and to determine the most effective formulation against *F. oxysporum* f. sp. *lycopersici* to avoid effects in plant growth. Studies on the phytotoxicity effect of these botanicals are encouraged. These botanicals could be further subjected to field trials to access their effectiveness under open field conditions.

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CHAPTER FIVE

5.0 GENERAL DISCUSSIONS

The development of suitable propagation method is an important step towards conservation of pesticidal plants. This current research which reports the propagation and effectiveness of *Commiphora swynnertonii* and *Synadenium glaucescens* against tomato fusarium wilt creates an important knowledge base as it registers some propagation techniques and pesticidal information of these plants. The fact that it is the first report on propagation and fungicidal usage of these plants in crop protection, it adds value to science and creates an important avenue for further studies on these plant species.

Seed germination potentials of *C. swynnertonii* and *S. glaucescens* was generally poor. However, it was positively influenced by pre-sowing seed treatments. The seeds of *C. swynnertonii* germinated well when treated with KNO_3 at 10 ppm. KNO_3 play an important role in breaking seed dormancy by removing germination inhibitors like abscisic acid and promotes cell expansion in the embryo resulting in the rupture of the testa, which accelerates water uptake (Toorop, 2015). Similar results were reported by Shim *et al.* (2008) in *Paspalum vaginatum*. The seeds of *S. glaucescens* germinated well when soaked in water at room temperature (25°C). Water play an essential role in breaking seed dormancy by softening the testa and washout germination inhibitors (Olajide *et al.*, 2014). Sabongari and Aliero (2004) reported that seeds soaked in water for 24 hours had the highest germination percentage.

Propagation of *C. swynnertonii* and *S. glaucescens* by stem cutting is attainable. The cutting types and growth regulators have influenced the shoot and root development of *C. swynnertonii* and *S. glaucescens.* Hardwood cuttings of *C. swynnertonii* have shown to be the best in shoot performance particularly in the number of sprouts per cutting and length of the longest sprout per cutting. Hardwood cuttings

contain higher amount of carbohydrates, proteins and natural hormones than semi-hardwood and softwood cutting (Rolland *et al*., 2006). These results have also been observed by Mahmood *et al.* (2017) when studying the influence of type of cuttings and plant growth regulator on rooting percentage and vegetative growth characteristics of *Paulownia tomentosa.* Semi-hardwood cuttings of *C. swynnertonii* have shown the best root performance particularly in length of the longest root per cutting, rooting percent and cutting survival percentage. Semi-hardwood cuttings have higher tissue sensitivity and greater meristematic activity (Saumitro and Jha, 2014). The superiority of semi-hardwood cuttings on root performance was also observed in *Argania spinosa* by Benbya *et al.* (2018). Softwood cuttings of *S. glaucescens* have shown the best shoot and root performance. According to Hartmann *et al.* (2002), apical parts of the stem cuttings contain numerous meristematic cells which are actively growing. Kouakou *et al.* (2016) reported that softwood cuttings of *Garcinia kola* had high capability to form new adventitious roots and shoots. The cuttings dipped in NAA at 2 000 ppm was found to be superior in both plant species. NAA play a vital role in hydrolysis and translocation of stored food substances and caused cell elongation and division (Hartmann *et al.,* 2007). According to Thakur *et al.* (2016) exogenous application of auxins promote formation of advitious roots and rooting uniformity.

Survival ability of *C. swynnertonii* and *S. glaucescens* plants was affected by pre-sowing treatments. Plants from seeds of *C. swynnertonii* previously soaked in hot water (60°C) and plants from seeds previously with KNO_3 at 20 ppm had the highest establishment percentage. Hot water breaks seed dormancy by disintegrate chemical bonds in the testa resulting into production of vigorous seedlings (Dewir *et al.*, 2011). Singh *et al.* (2019) found that hot water improves seed and seedling quality of bell pepper. KNO_3 enhances establishment and formation of adventitious roots that help

absorption of moisture from the soil (Hegazi *et al.*, 2011). Plants from seeds of *S. glaucescens* previously treated with GA_3 at 250 ppm recorded the maximum establishment percentage. GA_3 controls plant growth and development (Gupta and Chakrabarty, 2013). Hela *et al.* (2012) reported that GA_3 improve seedling growth of lettuce in the field.

Survival ability of *C. swynnertonii* and *S. glaucescens* plants were affected by cuttings type. Plant from hardwood cuttings of *C. swynnertonii* had the highest establishment percentage. Plant from semi-hardwood cuttings of *S. glaucescens* recorded the highest length of the longest branch per plant, number of leaves and leaf dry weight. High survival ability of plant from hardwood and semi-hardwood cuttings is due to their lignification which protects them from rapid drying and pests (Benbya *et al.*, 2018). Saumitro and Jha (2014) reported that plant from hardwood and semi-hardwood cuttings had higher survival ability than softwood cuttings. Growth regulators affect the survival ability of *C. swynnertonii.* Plant from cuttings previously dipped in NAA 2 000 ppm had the highest length of the longest branch per plant, leaves fresh and dry weight.

The application of auxins (NAA) would have induced the endogenous synthesis of native auxins resulting in early active growth. These findings have also been observed by several researchers when studying the influence of auxins on the establishment of the rooted cuttings (Reddy *et al.*, 2008; Memon *et al.*, 2013; Thakur *et al.*, 2016). Growth regulators did not have a significant influence on the establishment of *S. glaucescens.* Establishment of 100% was recorded in all rooted cuttings. These results indicate that the exogenous application of growth regulators is not a requirement for establishment of this plant species.

Laboratory experiment revealed that aqueous crude plant extracts had strong fungicidal properties against *F. oxysporum* f. sp. *lycopersici.* Dry leaves extract of *S. glaucescens* and resinous extract of *C. swynnertonii* was found more effective in reducing the mycelia growth of the fungus at 0.15 g/ml. In the screen house experiment, the dried leaves powder of *S. glaucescens* showed strong inhibitory effect causing significant reduction of wilt disease in tomato plants. This treatment also had the maximum value in all measured growth parameters. According to Mengal *et al.* (2015) plants contain various secondary metabolites that can be used as a source of botanical pesticides. Similar results have been reported by several researchers (Ramaiah and Garampalli, 2015; Akaeze and Aduramigba-Modupe, 2017; Sharma *et al.*, 2017).

CHAPTER SIX

6.0 GENERAL CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

An effective propagation technique was developed for *C. swynnertonii* and *S. glaucescens* plants. Based on the results from the present study, these plants can be propagated successful through stem cuttings. Cutting types and growth regulators had significantly enhance rooting and survival ability. Semi-hardwood and softwood cuttings treated with NAA 2 000 ppm was found to be the best for mass propagation of *C. swynnertonii* and *S. glaucescens*, respectively. Propagation potential of *C. swynnertonii* and *S. glaucescens* through seeds is very poor. Pre-sowing treatments have marginally improved the seed germination.

Field establishment of the rooted cuttings of *C. swynnertonii* and *S. glaucescens* is affected by cutting types and growth regulators. Plants from hardwood and semi-hardwood cuttings previously treated with NAA 2 000 ppm were found to be the best for field establishment of *C. swynnertonii* and *S. glaucescens*, respectively. Establishment of both plants is affected by pre-sowing treatments. Plants from seeds previously treated with KNO_3 at 10 ppm and plants from seeds previously treated with GA_3 at 250 ppm was found to be the best for the establishment of *C. swynnertonii* and *S. glaucescens*, respectively. Botanical pesticides have high potential to inhibit the growth of *F. oxysporum* f. sp. *lycopersici.* Dry leaves extract of *S. glaucescens* and resinous extracts of *C. swynnertonii* at 0.15 g/ml had the highest inhibitory effect against mycelia growth *F. oxysporum* f. sp. *lycopersici.* The application of dried leaves powder of *S. glaucescens*

exhibited the least disease severity. Tomato plants treated with dried leaves powder showed a significant stimulatory effect on plant growth.

6.2 Recommendations

Based on the findings from the current studies, the following are recommended:

i. Semi-hardwood and softwood cuttings could be used for mass propagation of *C. swynnertonii* and *S. glaucescens*, respectively.

ii. The exogenous application of NAA at 2 000 ppm is required for production of roots and shoots of *C. swynnertonii* and *S. glaucescens* plants.

iii. Development of a protocol for multiplication of *C. swynnertonii* and *S. glaucescens* through tissue culture is encouraged.

iv. The influence of a combination of growth regulators, rooting media and seasonal variation of harvesting of planting materials of these plants is needed to be studied.

v. Field gene bank and guideline for sustainable harvesting of pesticidal plants should be established.

vi. The present investigation was carried out in Morogoro region, the eastern zone of Tanzania, there is a need to conduct studies in different regions.

vii. Since the crude extracts of *S. glaucescens* exhibited the highest inhibitory effect against mycerial growth of *F. oxysporum* f. sp. *lycopersici in vitro* and in the screen house, it should be subjected to open field to see its effectiveness.

viii. Further studies to determine the mechanisms of botanicals involved in the inhibition of mycelial growth of *F. oxysporum* f. sp. *lycopersici* is recommended. This will help to determine the mode and rates of the application without a significant reduction in plant growth.

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