EFFECT OF COPTIS (*Coptis chinensis*, Franch.) EXTRACT ON CALLA LILY (*Zantedeschia* sp.) SOFT ROT, GROWTH, YIELD AND QUALITY

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A Thesis Submitted to the Graduate School in Partial Fulfilment for the Requirements of the Master of Science Degree in Horticulture of Egerton University

EGERTON UNIVERSITY
FEBRUARY, 2015

DECLARATION AND RECOMMENDATION

DECLARATION

This thesis is my original work and has not wholly or in parts been previously presented for the award of a degree in this or any university.

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DEDICATION

To my beloved wife Medrine, our lovely daughter Mitchell and all those who garden for food, peace and pleasure instead of data.

ACKNOWLEDGEMENT

Three years ago, I began an academic journey not knowing what the future held or what would become of the pursuit. The only thing that kept me going was hope and the fact that many more had taken a similar path and met the light "at the end of the tunnel." This, coupled with dreams of a brilliant future kept me on the track. As with most of life's endeavours, successful completion of this degree would not have been realised without the support, guidance, assistance and encouragement of many great persons. First and foremost, my sincere gratitude to God for the strength and focus in finishing this work. To Him alone be all the glory. I owe a great deal of appreciation to my supervisors, Dr Samuel Nyalala and Prof Liu Gaoqiong whose insight and deep knowledge in Horticulture has made me the successful Horticulturalist that i am today. The two have steadily walked with me from the time of conception to the completion of this work. Many thanks to Dahwa Agrochemical Co. Ltd for providing the *Coptis chinensis* formulated product and logistical support for this work.

This work would not have proceeded without the very crucial input of Agriflora (Sian) Kenya Ltd who provided the planting material, planting space and other agricultural inputs. Special thanks to the Agriflora (K) Ltd team led by Mr Laban Koima, Kenneth Mbae, George Kiarie, Simon Kamau and all the other support staff who offered the much-needed assistance in plot maintenance. The great meals at your boardroom coupled with numerous laughters gave a soft touch to the otherwise seemingly unending journey. Professional input and pieces of advice offered by staff from Horticulture Section and Biological Sciences Department are highly appreciated. Brotherly relationship that I enjoyed from colleagues in the department is highly appreciated. Your contribution to this work is immense.

On a personal note, I would like to thank my parents, Gad and Rose Githeng'u, who from an early age instilled in me a sense of hard work and encouraged me to "spread my wings and fly". I never stopped flying and never intend to. Surely, your love, concern and tolerance cannot be matched. The support and great love of my brothers Peter, James, Patrick and Sisters Salome and Margret meant so much to me. To Medrine and Mitchell; your support, love and contribution to this work cannot be matched.

To all, I can finally reply "I'm done!!"

ABSTRACT

Calla lily (*Zantedeschia* sp.) is cultivated as a cut flower, pot plant or garden ornamental throughout the world and contributes to horticultural revenue for many countries including America, Holland, New Zealand and Kenya. However, bacterial soft rot caused by *Pectobacterium carotovorum* subsp. *carotovorum* affects the commercial viability of this crop in Kenya. The disease infects calla lily at all stages of growth resulting in death of plants and decay of tubers. Bactericidal formulations and antibiotics currently used to reduce *P. carotovorum* proliferation are ineffective, phytotoxic and may promote bacterial resistance. *Coptis chinensis* Franch, a traditional Chinese medicinal herb contains alkaloids which include berberine and palmatine. The two have strong antibacterial activities. *C. chinensis* obtained by supercritical fluid extraction is marketed in Kenya as foliar feed (Dumiza 0.5 % SL) has label claims of boosting crop growth and development, improve crop immunity, vigour and recovery after bacterial disease attack. This study sought to determine the antibacterial activity of *C. chinensis* extract against *Pectobacterium carotovorum* subsp. *carotovorum* (*in vitro*) and the effect of the extract on calla lily growth, yield and quality (*in vivo*). In the *in vitro* study, sterilized petri dishes containing nutrient agar arranged in a in CRD design were inoculated with the bacterium and a drop of *C. chinensis* extract at different dilution levels added onto each plate individually. Inhibition zones were measured after 24 hours of incubation. Results showed that only the undiluted level (100%) of the extract inhibited the development of the bacterium. *In vivo* studies included pre-plant tuber spraying and crop media drenching with *C. chinensis* extract either immediately from planting or after full-foliage stage set up in RCBD. Data on plant growth, yield and quality of flowers and tubers, disease incidence and severity for each field study were collected over a period of two growing seasons and analysed using JMP 9 statistical software. Results indicated that application of *C. chinensis* extract increased plant height and leaf numbers and suppressed soft rot disease. The *C. chinensis* extract product can be used for management of calla lily soft rot.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
BA	Berberine Alkaloids
CFU	Colony Forming Units
CRD	Completely Randomized Design
DAP	Days after planting
Ecc	*Erwinia carotovora* subsp. *carotovora*
LPDE	Low Density Polyethylene
MIC	Minimum inhibition concentration
Pcc	*Pectobacterium carotovorum* subsp. *Carotovorum*
PDI	Percent disease index
PG	Polygalacturonase
PL	Pectatelyase
RCBD	Randomized Complete Block Design
RH	Relative Humidity
SAS	Statistical Analysis System
TCM	Traditional Chinese Medicine
THSD	Tukey's Honestly Significant Difference

CHAPTER ONE
INTRODUCTION

1.1 Background information

On the global map, Kenya is ranked among the top cut flower exporters. In 2013, Kenya commanded 41.4% of all flower sales at the Dutch auction market. Rose was the leading cut flower imported in Holland while calla lily was number 15 among the top 25 cut flowers based on volume and value (Flora Holland, 2014).

Calla lily (*Zantedeschia* sp.) is cultivated as a cut flower, pot or garden ornamental plant throughout the world and contributes to horticultural revenue for many countries including America, Holland, New Zealand and Kenya. It is a popular ornamental crop in the international market and ranked within the top 20 cut flowers at Dutch flower auctions (Flora Holland, 2014), with more than 70 million stems being sold per year (Flower council of Holland, 2005; Chen 2011). The popularity of calla lily in the international flower market is partially because of its distinct and stylish inflorescence and its wide selection of colours (Singh, 1996). Calla lily inflorescence consists of coloured spathe subtending a spadix that contains true female and male flowers. In horticultural context, this combination of spathe, spadix and peduncle is what is harvested and marketed as a cut flower. Extensive interspecific breeding has resulted in a wide array of colours in calla lily including white, red, orange, pink, cream, yellow, purple and dark purple (Chen, 2011).

The genus *Zantedeschia* belongs to the Araceae family and comprises eight species, which are all endemic to South Africa i.e. Z. *aethiopica* (L) Spreng, Z. *albomaculata* (Hook) Basil, Z. *elliotiana* (Watson) Engl., Z. *jucunda* Letty, Z. *rehmannii* Engl., Z. *pentalandii* (Watson) Wittm., Z. *odorata* P.L. Perry and Z. *valida.* When grown in wet marshy ground in their native habitat, calla lily plants often reach 180 cm in height. Some varieties are hardy and can tolerate freezing conditions. Even when leaves are damaged by frost, the rhizomes usually survive. Calla lily is a tuberous day-neutral plant in which flowering is not induced by environmental signals (Naor *et al*., 2005).

Calla lily is usually propagated by using seeds, division of underground organs, or tissue culture. If grown from seeds, the plants of calla lily will naturally flower in three years, between 45 to 150 days if grown from two year old tubers, and two years if grown from tissue cultured plantlets. Bacterial soft rot caused by *Pectobacterium carotovorum* subsp. *carotovorum* is the most important disease of calla lily in Kenya, and is a major factor affecting the commercial viability of this crop (Snijder, 2004). The disease results in the death of plants and decay of tubers. Infection can occur at all stages of the calla life cycle

from young plants to tubers in store (Wright, 1998). The severity of soft rot depends mainly on soil and weather conditions, with warm, wet weather and poor-draining soils favouring infection.

Foliar fertilizers have been shown to enhance plant properties such as growth and disease immunity (Fernández &. Eichert, 2009). These fertilizers being water soluble are directly applied to the foliage for uptake by the plants. Foliar application of nutrients is an important crop management strategy for maximizing crop yields and can supplement soil fertilization. When nutrients are applied to soils, they are absorbed by plant roots and translocated to aerial parts. In case of foliar application, the nutrients penetrate the cuticle of the leaf or the stomata and then enter the cells. Hence, crop response occurs in shorter time in foliar application compared to soil application (Fernández &. Eichert, 2009). As a result, foliar fertilization provides more rapid utilization of nutrients and permits the correction of observed deficiencies in less time than would be required by soil application. Crops respond to soil applied fertilizers in five to six days if climatic conditions are favourable but when foliar fertilizers are applied, the response is within 3 to 4 days (Fageria *et al*., 2009).

The supercritical fluid extracted product of *Coptis chinensis* Franch (Dumiza 0.5 SL) is marketed in Kenya by Dahwa Agrochemical Co Ltd as a foliar feed. The product is claimed to boost crop growth and development, improve crop immunity and enhance plant vigour recovery after bacterial disease attack. Under field conditions, *Coptis chinensis* extract has been observed to suppress the symptoms of bacterial wilt *(Ralstonia solanacearum*) infection in tomatoes and potatoes (Personal observations). However, there is no information regarding the use of this product in calla lily production especially in regard to its main production constraint; tuber soft rot caused by *Pectobacterium carotovorum* subsp. *carotovorum*. This study was therefore conducted to determine the effects of pre-plant tuber spray and media drenching at different times on the growth, yield and quality of calla lily.

1.2 Statement of the Problem

Calla lily flower and tuber yields are usually low due to soft rot caused by pathogenic bacterium *Pectobacterium carotovorum* subsp. *carotovorum*, previously included in the genus *Erwinia*. Calla lily tubers may get contaminated by *P. carotovorum* either in the field or during storage. This leads to yearly loss of income for the grower for up to 100 percent if the disease is not managed. For a long time, copper-based fungicides have been used in the management of fungal pathogens which cause entry routes for the bacteria. These copper-

based bactericides used in the management of calla lily soft rot are usually ineffective and often, with continued use, are environmental pollutants and cause bacterial resistance. As a result, there is no known effective method for the management of the calla soft rot disease. Preliminary field observations showed that *Ralstonia solanacearum*-infected tomatoes recover after *Coptis chinensis* extract application. However, the antibacterial activity of the product against *P. carotovorum* and its effects on growth, yield and quality on calla lily has not been established. Furthermore, there are increased negative public perceptions about the use of synthetic pesticides, resistance of pest and diseases to pesticides and high cost of developing products with new active ingredients. These circumstances call for exploration of alternative control methods that are safe to applicants and benign to the environment.

1.3 Objectives

1.3.1 General objective

The general objective of this study was to contribute to increased yield and quality of calla lily cut flowers and tubers through management of soft rot using plant-based extract that is eco-friendly and safe to the applicant.

1.3.2 Specific objectives

1) To evaluate the antibacterial activity of *Coptis chinensis* Franch extract against *Pectobacterium carotovorum* subsp. *carotovorum.*
2) To determine the effects of pre-plant tuber spraying with *Coptis chinensis* Franch extract on calla lily soft rot, growth, yield and quality.
3) To determine the effects of media drenching with *Coptis chinensis* Franch extract on calla lily soft rot, growth, yield and quality.

1.4 Hypotheses

1) *Coptis chinensis* Franch extract has no antibacterial activity against *Pectobacterium carotovorum* subsp.*carotovorum.*
2) Pre-plant tuber spraying with *Coptis chinensis* extract has no effect on calla lily soft rot, growth, yield and quality of cut flowers and tubers.
3) Application of *Coptis chinensis* extract as media drenching has no effect on calla lily soft rot, growth, yield and quality of cut flowers and tubers.

1.5 Justification

Calla lily is a high potential plant for cut flower, pot and landscape production. Due to the losses from *P. carotovorum*, production of high quality disease-free calla lily cut flowers and tubers has been a major challenge to the growers. The use of the common bactericidal formulations such as copper-based compounds to reduce *P. carotovorum* proliferation is relatively ineffective and is often phytotoxic. Other measures, such as antibiotics, in addition to their high cost, may promote bacterial resistance within a few generations (Gracia-Garza *et al*., 2002). Plant-derived compounds have been used in human medicine from antiquity but very little has been done in relation to phytopathogenic bacteria. These compounds unlike the commonly used copper-based bactericides have low persistence hence safe to the environment. Constituents of *Coptis chinensis* Franch have been shown to have antibacterial activity against *Escherichia coli,* and *Staphylococcus aureus*. Berberine, one of the major constituent of *Coptis chinensis* Franch has extensively been employed to kill both gram-positive and gram-negative bacteria. The product (Dumiza 0.5% SL) is already being sold in Kenya as a foliar feed.

CHAPTER TWO
LITERATURE REVIEW

2.1 Overview of calla lily production

Calla lilies (*Zantedeschia sp.*) have been grown for cut flower production and as outdoor garden plants for many years. Calla lily is ranked among the top 20 cut flowers at Dutch flower auctions (Flora Holland, 2014), with more than 70 million stems being sold per year (Flower council of Holland, 2005; Chen 2011). New Zealand is the world's leader in calla lily production trading 3.2 ($ million, fob) worth of both cut flowers and tubers in 2011. In 2011, calla lilies accounted for 20% of the cut flower export earnings in New Zealand, second only to orchid export (Fresh facts, 2011). The genus *Zantedeschia* Sprengel (Araceae), also called 'calla lily' or 'arum lily', is a genus of about eight species distributed in two sections, *Zantedeschia* and *Aestivae*, all from southern Africa. Section *Zantedeschia* consists of *Z. aethiopica* L. and *Z. odorata* Perry, while section *Aestivae*, also known as 'colored callas', consists of *Z. rehmannii* Engl., *Z. jucunda* Letty, *Z. elliotiana* (Watson) Engl., *Z. pentlandii* (Watson) Whittm., *Z. albomaculata* (Hook) Baill. and *Z. valida* (Letty) Y. Singh. *Z. albomaculata* includes two subspecies *albomaculata* and *macrocarpa* (Brown *et al*., 2005).

In *Zantedeschia*, as in other Araceae, the inflorescence consists of many reduced unisexual flowers on a spadix enclosed by a spathe. The spadix and spathe are together referred to as the flower (Plummer & Welsh, 1990). Critical factors for *Zantedeschia* as a potted floricultural crop include flower number, plant height, and shoot and leaf number (Corr & Widmer, 1991); whereas if used as a cut flower, of greatest importance is the stem length and absence of blemishes on the spathe. *Zantedeschia* sp. has tuberous (section *Aestivae*) or rhizomatomous (section *Zantedeschia*) storage organ, is frost tender and, except for *Z. aethiopica*, requires a period of dormancy (Naor *et al*., 2005). After the growing season storage organs of all *Zantedeschia* sp. are lifted after onset of dormancy and stored for a period of about three months in order to break their dormancy.

Zantedeschia hybrids with coloured flowers are developed from crosses of species from section *Aestivae*, mainly *Z. rehmannii*, *Z. elliotiana* and *Z. albomaculata* (Chen, 2011). All *Zantedeschia* sp. are diploid with 32 chromosomes (Snijder, 2004). Cultivars have been bred for ornamental value of either the flowers or the entire plants mainly from *Z. rehmannii*, *Z. albomaculata*, *Z. elliotiana* and *Z. pentlandii*. Calla lily is usually propagated by use of seeds, division of tubers, or tissue culture. If grown from seeds, calla lily plants will naturally flower in three years, between 45 to 150 days if grown from two year old tubers, and two

years if grown from tissue cultured plantlets (Chen, 2011). Calla lily is a day-neutral plant in which flowering is not induced by environmental signals (Naor *et al*., 2005).

For production, clean water with an added residual bacteriostat is a proven component for achieving optimum calla production and controlling plant mortality/soft rot. Actual water volume depends on soil/media drainage capacity and evapotranspiration (Chen, 2011). Plant stress may occur from too much or too little water. Regular watering ensures nutrient availability and reduces salt build-up. Irrigation is usually done in the morning, prior to the heat of the day or as required in late afternoon. In a semi-hydroponic regime, water and nutrient may be applied 6-8 times per day. Larger callas (6cm) tend to create their own shade whereas small stock can become stressed on hot days. Mulching is usually done to help ensure moisture retention and reflect excess heat. Calla lily requires 50 to 120 L/ m^2 of water per day for optimal growth (Naor *et al*., 2005). Flower harvesting is done by pulling rather than cutting to ensure the longest possible stem length. The flowers are immediately placed in a bucket of clean water and transferred to a cold store and pre-cooled at 6-8^o C prior to grading. After pre-cooling, they are graded and bunched into either 5 or 10 stems depending on the market specifications. They may later be stored at the cold store at 6-8^{o}C and 80% relative humidity.

Tuber lifting is done when the leaves turn yellow and die down, roots regress and tuber surface becomes tougher. Early harvesting reduces tuber multiplication and maturation. The tubers cleaned to remove adhering media and spread on a single layer on mesh-bottomed trays to cure (Snijder, 2004). Curing is done for 2-5 days at 20-25^o C immediately after lifting ensuring good air movement. After initial curing, temperature should be held constant at 12-15° C for a further 3-5 weeks until tubers are stabilised before transferring them to longer term cool storage for further handling. Calla lily requires at least 10-12 week to break tuber dormancy after lifting. Long-term cool storage, (6-10 months) at 8-10^o C and 65-70% RH, enhances faster germination, consistent shoot emergence and better flower production (Brown *et al*., 2005). Maintenance of uniform temperature and good air circulation is the key to good storage with optimum relative humidity between 65-70 percent.

2.2 *Pectobacterium carotovorum* subsp. *carotovorum* and calla lily soft rot

Soft rots caused by *Pectobacterium carotovorum* subsp. *carotovorum* (formery *Erwinia carotovora* subsp. *carotovora)* (Snijder, 2004) are responsible for considerable losses in the floriculture industry. Diseases caused by erwinia soft-rot are the manifestation of

the activity of a number of plant cell-wall and membrane degrading enzymes produced by the bacteria (Mikicinski *et al*., 2010). Calla lily (*Zantedeschia* sp.) suffers serious soft rot problems that cause substantial losses of plants in the field through flower infections and of tubers in storage. All cultivars with coloured flowers, belonging to the section *Aestivae* of the genus, are very susceptible to soft rot. The disease attacks the crop and rapidly spreads causing heavy damages that may lead to 100% loss if unchecked. The disease not only causes erratic emergence and collapse of the shoot, but also tuber decay and post-harvest collapse of the peduncle.

This soil-borne facultative anaerobic bacterium causes maceration and rotting of parenchymatous tissue of all plant organs, resulting in the loss of the entire plant. During the infection process in plants, successive steps occur before soft rot symptoms appear. The bacteria present in the intercellular spaces at the infection sites start multiplying and, upon reaching a critical number, they produce and secrete extracellular enzymes such as pectatelyase (PL), polygalacturonase (PG), cellulose and protease (Mikicinski *et al*., 2010). These enzymes have been correlated with virulence, as they degrade the plant cell wall and membrane components, enabling penetration and colonization of plant tissue. Thereafter, generated digestion products activate regulatory mechanisms in the bacteria that further stimulate the production of additional extracellular enzymes, resulting in tissue maceration and cell death (Mikicinski *et al*., 2010; Ni *et al*., 2010).. Although the role and regulation of individual extracellular enzymes in pathogenicity vary with different pathogens and hosts, many studies have shown that PL plays an important role in soft rot disease development, as it cleaves structural pectic polymers. Mutants of pectatelyase genes show decreased pathogenicity, while addition of these genes enables *Escherichia coli* to macerate plant tissue. Purified PL can macerate plant tissue by itself (Ni *et al*., 2010).

Spread of bacteria takes place mainly through watering or handling during tuber lifting, storage and planting. Infections of *Pythium* and *Rhizoctonia* have also been reported to be followed by *Pectobacterium*. Plants are vulnerable in particular under low oxygen conditions, as they are less efficient in their wound healing and pathogen defence responses, while the bacteria are not affected due to their facultative anaerobic nature (Snijder, 2004). Environmental conditions can affect the enzymatic activity to macerate plant tissue by (i) regulating total extracellular enzyme production, which is the result of enzyme synthesis and secretion or (ii) influencing catalytic properties of the enzymes. For instance, PL catalysis capacities are different at different temperatures and pH. Soft rot symptoms therefore can develop at any time during the growing cycle when conditions favourable for soft rot, such as

high relative humidity (80% and above) , or when plants are under stress due to low soil aeration (high moisture content of soil) or high temperature, high nitrogen content of the soil (Snijder and vanTuyl, 2002; Ni *et al*., 2010).

2.3 Calla lily soft rot management approaches

Bacterial soft rot of calla lily is difficult to control. Chemical bactericides are not effective once plant or tuber infection has occurred. The antibiotic streptomycin could diminish disease incidence but there are dangers of development of resistance with continued use and also a concern over their safety (Snijder, 2004). As environmental factors affect both host susceptibility and parasite virulence, optimizing environmental conditions is recommended to reduce or eliminate the disease. Well balanced irrigation, mulching, and soil ventilation can improve soft rot control (Ni *et al*., 2010), the same as appropriate calcium or phosphorous fertilizations (Gracia-Garza *et al*., 2004). For instance, increased calcium uptake can reduce soft rot due to improved plant resistance, whereas excess phosphorous enhances the disease due to increased virulence of the pathogen (Snijder, 2004).

Gracia-Garza *et al*. (2002) evaluated the efficacy of various copper-based compounds, such as Phyton-27, Champ 2, fixed copper, and analytical copper, against *Erwinia carotovora* subsp. *carotovora* under both laboratory and greenhouse conditions. Their results indicated that copper-based compounds suppressed bacterial growth at various cupric ion concentrations in vitro, but the efficacy of some products was reduced by the presence of phosphate ions in fertilizer solutions used in commercial greenhouses. Under greenhouse conditions, the compounds were applied as a pre-plant or a post-plant treatment to tubers of calla lily. Post-plant applications were made either as an overhead drench or through sub-irrigation, and the applications were carried out at different stages of plant development. Pre-plant applications of Champ 2 or Phyton-27 did not reduce the number of diseased plants compared with the controls. In contrast, post-planting applications of Champ 2 or fixed copper were found to be effective in reducing soft rot; however, the two compounds also reduced overall plant growth.

In addition, Gracia-Garza *et al*. (2004) conducted experiments to test the effect of phosphorous added to soil-less mixes or to nutrient solutions used for irrigation on soft-rot caused by *Erwinia carotovora* subsp. *carotovora* (Ecc) in calla lily. The team found out that soft-rot incidence increased to 51% when soil-less mix was amended with superphosphate in comparison to regular soil-less mix (no superphosphate added) (31%). In contrast, addition of phosphorous in the nutrient solution did not enhance soft-rot. Plant height, fresh mass, and

number of flowers per plant were greater in calla lilies irrigated with nutrient solution containing phosphorous than treatments without phosphorous. In other experiments where tubers were sprayed with water, a bacterial cell suspension 1×10^2 cfu ml^{-1}, a solution of 0.5mM KH_2PO_4, or a suspension of bacteria in KH_2PO_4, there was a significant increase of soft-rot development in tubers treated with the suspension of Ecc prepared in a solution of KH_2PO_4 relative to other treatments. Further laboratory tests indicated that enzymatic activity (Polygalacturonase and pectatelyase) of Ecc increased when it is grown in the presence of phosphorous. These experiments indicate that there is increased soft-rot in the presence of phosphorous which is as a result of increased virulence of Ecc.

Blom and Brown (1999), conducted an experiment in Canada to determine the effectiveness of four bactericides–sterilants applied as a pre-plant dip, investigating whether physical damage through incisions of the rhizome influences the incidence of soft rot and lastly whether the level of calcium fertilization during forcing influences the severity of the bacterial soft rot. Four sterilants–bactericides (Physan-20, Fixed Copper, Phyton-27, and Virkon) were compared as pre-planting dips of *Zantedeschia elliottiana* Engl. W. Wats 'Yellow' (a susceptible cultivar) rhizomes to reduce plant losses due to latent field-infected *Erwinia carotovora* soft rot during greenhouse forcing as a flowering potted plant. They observed that more than 90% of the inoculated rhizomes collapsed within 5 weeks after potting due to bacterial soft rot. With the uninoculated rhizomes, the copper-based compounds (Fixed Copper or Phyton-27) provided better control of bacterial soft rot than either Physan-20 or Virkon only during the first 6weeks of forcing. During the remainder of the forcing period, there were no differences in weekly losses of rhizomes with the four sterilants. Incisions on the rhizome before planting or calcium nutrition during forcing did not have any significant effect on disease severity. Rhizomes treated with solutions of the copper-based compounds produced 0.5 flowers less per rhizome than either Physan-20 or Virkon. High calcium fertilization resulted in an increase of 0.5 flowers per plant compared to low calcium nutrition.

All *Aestivae* cultivars are susceptible to bacterial soft rot. Combining use of resistant cultivars with good agricultural practices can be a promising approach in overcoming the disease. *Z. aethiopica* is more tolerant to soft rot than *Aestivae* genotypes. However, incorporating resistance from *Z. aethiopica* into *Aestivae* genotypes is not possible due to major compatibility barriers (Snijder & van Tuyl, 2002). In any case, an effective method or an adequate combination of measures to control soft rot is yet to be found (Snijder, 2004).

2.4 Role of foliar feeds in plant growth and development

Plant nutrients affect growth, yield and physiological metabolism, and may also exert secondary, often unpredictable effects, by raising or lowering the resistance or tolerance of plants to pathogens and pests. Soil applications of fertilizers are the traditional means of providing adequate levels of most plant nutrients. These nutrients are, however, subject to chemical and biological processes such as denitrification, adsorption, leaching, and volatilization that may render them unavailable to the plant. Foliar applied N has been reported to be more efficient than soil application based on the increase in yield per unit of urea-N applied. Leaching, absorption, and fixation problems of soil nutrients are avoided with foliar applications (Bednarz *et al*., 1999).

The soil applied nutrient has long influence on plant growth. However, plant response to foliar application is often only temporary. This means that several foliar applications are necessary in case of severe nutrient deficiency. The foliar application is most successful for micronutrients, whereas soil application is effective for both macro and micronutrients. For some of the immobilized nutrients in the soils, such as iron, foliar application is more effective and economical compared to soil application. At early growth stage when plant roots are not well developed, foliar fertilization is more advantageous in absorption compared to soil application. However, for foliar application an adequate leaf area index (LAI) for maximizing spray interception is a primary requisite (Fageria *et al*., 2009).

Day timing of foliar fertilization is an important aspect for efficient nutrient absorption. For efficient absorption of foliar fertilization, leaf stomata should be open and temperature should not be too high to cause burning of plant foliage. Another factor that may affect foliar fertilization is a windy day, which can drift the spray solution. Therefore, windy days should be avoided for foliar spray. There should be at least 3 to 4 hours for the applied nutrient to be absorbed by plant foliage. Hence, there should not be rain for at least 3 to 4 hours after application of the nutrient solution. When applying a nutrient solution as a spray, some sticking material should be added to the solution to stick the spray drops to plant foliage. Crop responses to foliar fertilization have been mixed both positive, negative or no responses depending on species and nutrient applied. Foliar application of macronutrients is not very common because of the plants high requirement which if applied on the foliage would lead to leaf scorching. Application of micronutrients by foliar spray is more effective because of the small amounts required by the plant and therefore cases of foliar scorch are minimal (Amin *et al*., 2011)

2.5 Role of plant nutrition in soft rot management

Calcium plays an important role in the resistance of plants against bacterial pathogens. High calcium concentrations in plants have been related to the reduced disease severity. Calcium ions improve the structure and integrity of plant cell wall components, resulting in higher resistance to diseases involving tissue maceration. Calcium fertilization has been shown to reduce soft rot caused by *Pectobacterium* sp. in Chinese cabbage (Czajkowski *et al*., 2011). In a study conducted by Arvin *et al*. (2005) to evaluate the effects of calcium concentration in medium on microtuberization of potato (*Solanum tuberosum* L.), the response of cultivars 'Bintje' and 'Russet Burbank' to the Ca concentration in the media including 3 (control), 10, 15, 20, or 25 mM was found to be different for most parameters measured. Microtuber tissue Ca content was greatly increased by Ca treatment in both cultivars. Maximum increase was observed at 15 mM Ca (70%) and 10 mM Ca (61%) in 'Bintje' and 'Russet Burbank', respectively. Ten mM Ca also increased P (25%) and K (19%), in 'Bintje' whereas in 'Russet Burbank' only P was increased up to 20% at this level of Ca. The results indicated that supplemental Ca is important for tuber development and for the uptake of other nutrients such as P and K.

In another experiment whose main objective was to evaluate the effect of Ca and K rate, Ca placement and cultivar on tuber yield, tissue Ca and K concentrations, and tuber soft rot potential (*Erwinia carotovora*) on potatoes (*Solanum tuberosum* L.), application of Ca at 450 and 900 kg ha^{-1} from gypsum increased soil Ca, potato tissue Ca and tuber yields as compared to 0 Ca application. Bacterial soft rot severity among freshly harvested tubers (tested within 5 days of harvest) during the seasons averaged 45, 11 and 4 % for 0, 450 and 900 kg Ca ha^{-1} respectively. The severity of soft rot decreased as Ca rate increased. Tubers grown with 225 kg K ha^{-1} had a higher average rating than those with 450 kg K ha^{-1}. Therefore, interactions between potato cultivars, Ca and K levels had an impact on tuber yield and soft rot potential (Locascio *et al*., 1991).

2.6 Antimicrobial activities of *Coptis chinensis* Franch extract

Coptis chinensis Franch (Huanglian in Chinese) is a traditional Chinese medicinal (TCM) herb, and is officially listed in the Chinese Pharmacopoeia. According to Dan *et al*. (2009), the major active constituents in *C. chinensis* are alkaloids, including berberine, jatrorrhizine, coptisine, and palmatine. The berberine, present at about 10% in *C. chinensis* Franch, has strong antibacterial bioactivities on *Shigella dysenteriae*, staphylococci, and streptococci and has been used for the cure of dysentery. *Coptis chinensis* extract is being

sold in Kenya as Dumiza 0.5 SL. Palmatine has been shown to possess extensive pharmacological actions including antibacterial activity against *Escherichia coli,* and *Staphylococcus aureus* anti-inflammation, and anticancer effect (Yan *et al*., 2008).

Berberine alkaloids are also active components in a large number of plant-derived drugs such as antimicrobials from Berberidaceae and Rutaceae family. The first record for medical use of coptis has appeared in the Chinese medical literature of The Divine Farmer's Materia Medica (25 A.D. to 220 A.D). Coptis possess the following pharmacological properties: antimicrobial, antiviral, antifungal, and anti-inflammatory. It has a broad spectrum of antibiotic effects against a wide range of bacteria including *Bacillus dysenteriac, Mycobacterium tuberculosis, Salmonella typhi, E. coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus* (Sun *et al*., 2009).

Berberine is a plant alkaloid with a long history of medicinal use in both Ayurvedic and Chinese medicine. It is present in many plants, including *Hydrastis canadensis* (goldenseal), *Coptis chinensis* (Coptis or goldenthread), *Berberis aquifolium* (Oregon grape; *Mahonia aquifolium*), *Berberis vulgaris* (barberry), and *Berberis aristata* (tree turmeric). Berberine is found in the root, rhizome, and stem bark of the plants. Berberine extracts and decoctions demonstrate significant antimicrobial activity against a variety of organisms, including bacteria, viruses, fungi, protozoans, helminths, and Chlamydia (Kathleen, 2008). Elsewhere, *Coptis chinensis* extracts have been found to inhibit microbial growth on fresh strawberries.

Chung *et al.* (1998) found that low density polyethylene (LDPE) films (48 to 55 μm thick) impregnated with either 1.0% w/w *Rheum palmatum* and *Coptis chinensis* extracts or silver-substituted inorganic zirconium retarded the growth of total aerobic bacteria, lactic acid bacteria and yeast on fresh strawberries. Hou *et al*. (2010) evaluated the effect of *Coptis chinensis* Franch extract against *Monilinia fructicola* fungus, the cause of brown rot in peach. The ethanol extracts of *C. chinensis, Tripterygium wilfkrdii, Artemisia apiacea,* and *Melia toosendan* were chosen to investigate their inhibitory activities on the plant pathogenic fungi *M. fructicola, Botrytis cinerea* and *Alternaria solani*. They found out that *C. chinensis* extract showed 100 %, 74.4 % and 80.7 % inhibition rates against *M. fructicola*, *B. cinerea* and *A. solani,* respectively. Further studies showed that extract of *C. chinensis* exhibited a dose-dependent anti-proliferative effect, i.e. higher concentration of *C. chinensis* extract exhibited a higher inhibition rate. In addition, the EC_{50} s of *C. chinensis* extract against *M. fructicola, B. cinerea*, and *A. solani* were 0.91, 14.09 and 27.35 mg ml^{-1}, respectively. This is an indication that *Coptis chinensis* extracts contain active compound(s) with antimicrobial activity even

against phytopathogenic organisms. As a result therefore it is of interest to study the effects thereof on soft rot pathogen, *Pectobacterium carotovorum* subsp.*carotovorum*, growth and yield of calla lily.

CHAPTER THREE

Antibacterial Activity of *Coptis chinensis* Extract against *Pectobacterium carotovorum* subsp. *carotovorum*

Abstract

Soft rot of Zantedeschia, caused by *Pectobacterium carotovorum* subsp. *carotovorum* (Pcc), has caused a significant worldwide threat to calla lily production. In order to effectively manage this disease, an intensive management programme aimed at adequate suppression of the pathogen is necessary. An *in vitro* study was set up to investigate the antibacterial effect of a *Coptis chinensis* extract product on the soft rot- causing bacterium. The bacterial isolate was obtained from rotting calla lily tubers and maintained in Nutrient broth under refrigeration. Sterile petri plates containing 15 ml of Nutrient agar were prepared and aseptically inoculated with 0.1 ml of an overnight grown culture of a standardized *Pectobacterium carotovorum* subsp. *carotovorum* inoculum containing about 1×10^8 cfu/ml. The inoculum was spread evenly over the whole surface of the plates. After solidification, 1 ml of each of the following concentrations (1, 10, 25, 50 and 100 %) v/v of *C. chinensis* extract product (Dumiza 0.5 % SL) was individually placed at the centre of the inoculated petri dish and the resultant inhibition zones were measured. Streptomycin sulphate (100 ppm) and sterile distilled water were used as positive and negative checks respectively. Eight replicates were maintained for each treatment and the experiment was repeated thrice. Results indicated that concentrations of *C. chinensis* extract lower than 100 % did not cause any inhibition against Pcc. On the other hand, the undiluted treatment (100 % *C. chinensis* product) caused an inhibition zone that was statistically similar to that of streptomycin sulphate. The results demonstrate that *C. chinensis* extract has antibacterial activity and therefore feasible for use in crop protection against soft rots caused by *Pectobacterium carotovorum* subsp. *carotovorum.*

Key words: Zantedeschia, *in vitro*, Nutrient agar, inoculum, inhibition zone.

3.1 Introduction

Various plant based products have been shown to inhibit the growth of *Pectobacterium carotovorum* subsp. *carotovorum* either *in vitro* or *in vivo*. Bergey *et al*. (2003) reported *in vitro* inhibition of *Erwinia carotovor*a subsp. *carotovora* by Chilli (*Capsicum annum*) extracts. The ability to inhibit the bacterial growth was attributed to compounds such as *meta*-coumaric and *trans*-cinnamic acids that were present in the extract. *In vitro* potency of essential oils and crude extracts from various plants including *Psidium guajava*, *Thymus vulgaris*, *Rosmarinus officinalis*, *Coriandrum sativum*, *Cuminum syminum* and *Eucalyptus camuldulensis* to inhibit *Pectobacterium carotovorum* subsp. *carotovorum* has been reported by various researchers (Alamshahi *et al*., 2010; Nezhad *et al*., 2012; Sarah *et al*., 2012). Several reseachers have reported the effectiveness of plant extracts in controlling the development of many plant pathogens *in vitro* (An *et al*., 1998; Balestra *et al*., 2008; Quattrucci *et al*., 2013). Prakash & Karmegam, (2012) reported *in vitro* efficacy of four plant extracts namely *Aegle marmelos, Aristolochia indica*, *Ocimum canum* and *Plumbago zeylanica* in inhibiting five strains of pathogenic *Xanthomonas campestris* pv. *citri* *Pectobacterium carotovorum* subsp. *carotovotum* is the major cause of soft rot in plants including vegetables and ornamentals such as Zantedeschia. The soft rot disorder is characterized by loss of host tissue structural integrity which is mainly due to production of bacterial pectolytic and other macerating enzymes (Perombelon & Kelman, 1980; Buonaurio *et al*., 2002). Plants turn yellow when the disease has initiated, produce a foul smell and can become completely macerated within a few days (Snijder *et al*., 2004).

Coptis chinensis is a traditional Chinese medicinal herb that has strong antibacterial activity with extensive use in treating dysentry, cholera, leukemia, diabetes, allergies and inflammations (Yan *et al*., 2008). The antibacterial activity of *C. chinensis* has been attributed to one of its major consttuents, berberine, which also has been shown to possess antimicrobial activities in its pure form to both human and phytopathogenic agents (Hou *et al*., 2010; Leach, 2011; Gan, 2012). Berberine has been found to be effective against powdery mildew in cucumber grown under greenhouse conditions (Kuixian *et al*., 2009), tomato bacterial speckle caused by *Pseudomonas syringae* p.v tomato (Shen *et al*., 2010) and *Monilinia fruticola*, the causal agent of peach brown rot (Hou *et al*., 2010). The antibacterial mechanism of berberine has been shown to include inhibition of DNA duplication, RNA transcription and protein synthesis, damage to the bacterial cell surface structure which leads to leakage of Ca^{2+} and K^{+} from the cell (Jin *et al*., 2010). Hence it has been suggested that

there are little chances of the target organisms developing resistance against the compound since most of the target areas are essential for the functioning of a normal cell.

The aim of this study was to evaluate the antibacterial activity of a *C. chinensis* formulated product against *Pectobacterium carotovorum* subsp. *carotovorum*, the causal organism of calla lily soft rot.

3.2 Materials and Methods

3.2.1 Experimental site

The *in vitro* study was carried out in the Plant Pathology Laboratories at Egerton University. Egerton University lies at a latitude of 0º 23' South, longitudes 35º 35' East in the Lower Highland III Agro Ecological Zone (LH3) at an altitude of approximately 2,238 meters above sea level. Average maximum and minimum temperatures range from 19 ºC to 22 ºC and 5 ºC to 8 ºC, respectively (Egerton Metrological Station, 2009).

3.2.2 Experimental procedures

3.2.2.1 Collection, isolation and identification of the pathogen

Soft rot-infected calla lily tubers were obtained from Agriflora (K) Ltd farm. The tubers were cleaned by washing them under running water until all the dirt was washed off, surface-sterilized with 0.5 % sodium hypochlorite solution for 30 seconds, washed with sterile distilled water. Sections of about $1m^3$ bordering diseased portions were cut and ground in 1 ml of sterile water using sterile mortar under aseptic conditions. The resulting suspension was left undisturbed for a few minutes. A loopful of the suspension was streaked onto plates containing autoclaved nutrient agar (NA) (Bacto Agar 10g, NaCl 5g, K_2HPO_4 5g, KH_2PO_4 2g, Bacto-peptone 1g), and incubated at 28º C for 24 h. Individual colonies (transparent, circular, raised, shiny and creamy white) growing on NA were selected, re-suspended in 1 ml of sterile water, streaked on NA plates, and then incubated at 28º C for another 24 h. The procedure was repeated several times in order to obtain pure cultures. The isolate was subjected successfully to pathogenicity test by leaf disks immersion method. The pure cultures obtained were maintained at 4°C in the dark for subsequent use (Ni *et al*., 2010).

3.2.2.2 Pathogenicity test on calla lily leaf discs

Calla lily leaves were harvested from tissue culture- raised Black Magic plants. The plants were grown on soilless media (pumice) in an unheated polytunnel greenhouse. Harvested leaves were washed under tap water, air dried, dipped in 70% ethanol for 3 min,

rinsed in sterile water and air dried. Leaf discs measuring 20mm were prepared using a cork borer. A solution containing 125 ml of sterile distilled water and 50 µl of 10^8 cfu ml^{-1} bacterial suspensions was prepared and three leaf discs placed in each vessel (Fig 1 a). A control treatment was maintained in which the leaf discs were placed in a solution containing sterile distilled water only (Fig 1 b). Four replications of each were maintained. These were incubated at 24± 2°C and 80 ± 2% relative humidity (modified from Mikicinski *et al*., 2010).

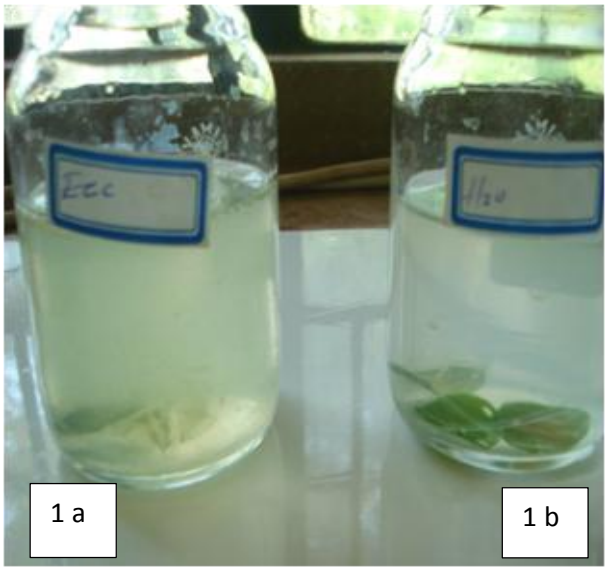

Figure 1: Calla lily leaf discs: (a) in bacterial suspension, (b) in sterile distilled water

3.2.2.3 *In vitro* antibacterial assay

The *in vitro* tests were carried out by direct inoculation method after the failure of disc diffusion method. Sterile 9 cm petri plates were prepared by pouring 15 ml of Nutrient Agar (NA) medium and allowed to cool. After solidification, the plates were seeded with a standardized inoculum containing 1×10^8 cfu/ml bacterial suspension. The suspension was uniformly spread with a sterile cotton swab on the media surface and allowed to dry for 4 minutes. In each of the test plate, 40 µl of each of the following concentration of *Coptis chinensis* extract 1%, 10%, 25%, 50%, and 100% v/v solution was applied. A standard reference antibiotic, streptomycin sulphate (100 ppm) and a negative control, sterile distilled water were also included. After incubation at 30° C ± 2 for 24 h, the resulting inhibition zone was measured in mm using a vernier calliper. The experiment was repeated thrice with eight replications in each.

3.2.2.4 Statistical analysis

The experiment was laid out in a completely randomized design.

Data on inhibition zone were subjected to analysis of variance (ANOVA) at 5% level of significance. Significant means were separated using Tukey's honestly significant difference test on JMP 9 (SAS Institute, Cary, NC.). The statistical model used for data analysis was

$Y_{ij}=\mu + \alpha_i + \beta_j + \varepsilon_{ij}$

Where: Y_{ij} is the inhibition zone,

μ is the overall mean,

α_i is the effect due to i^{th} treatment,

β_j is the effect due to j^{th} replicate and

ε_{ij} is the random error component.

3.3 Results

Exposure of *Pectobacterium carotovorum* subsp. *carotovorum* to undiluted (100%) *Coptis chinensis* Franch extract caused inhibition of the bacterial growth. However, there was no distinct inhibition zone for the diluted concentrations (1, 10, 25 and 50%) of *C. chinensis* extract (Table1). There was no significant difference in the size of the inhibition zone produced by the undiluted concentrations of *C. chinensis* extract (27.62 mm, Fig 2a) or that produced by Streptomycin sulphate (23.87mm, Fig 2 b, Table 1, $P< 0.0001$).

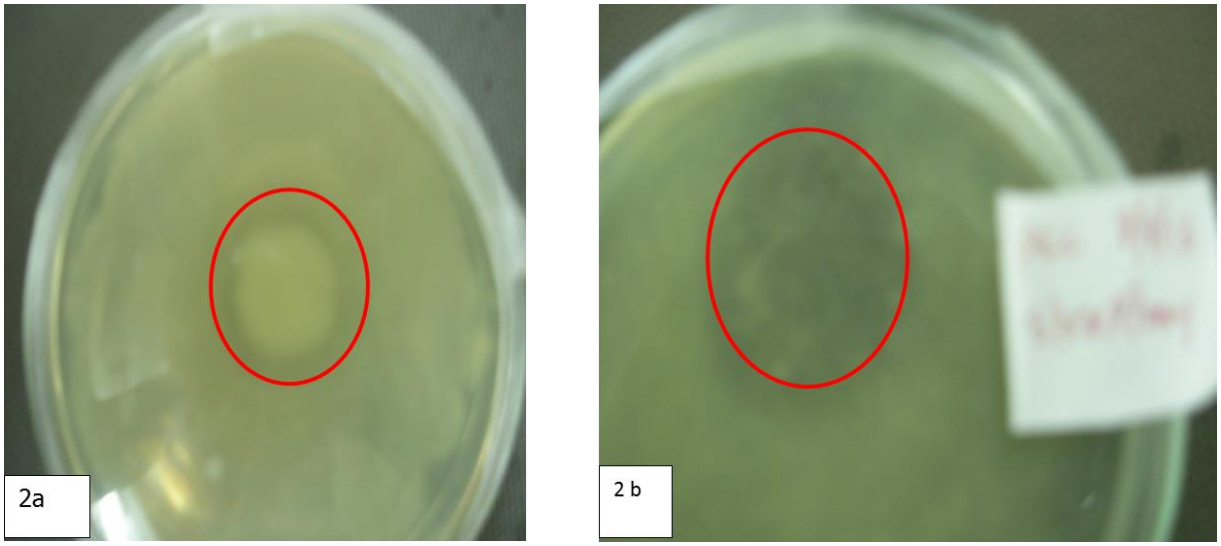

Figure 2: Inhibition zones caused by (a) undiluted *C. chinensis* extract, (b) streptomycin sulphate

Table 1: Diameter of inhibition zones (mm) caused by *C. chinensis*

Treatment	Inhibition zone(mm)
100 % *C. chinensis*	27.62 a
100 ppm streptomycin sulphate	23.88 a
1% *C. chinensis*	0.00 b
10% *C. chinensis*	0.00 b
25% *C. chinensis*	0.00 b
50% *C. chinensis*	0.00 b
water	0.00 b

P< 0.0001, n=8

3.4 Discussion

In our preliminary *in vitro* assays, the disc diffusion method was employed but was found to be ineffective and therefore direct inoculation method was utilised. Das *et al*. (2010) observed that standard anti-microbial susceptibility testing methods like the agar diffusion and Kirby-Bauer may result to misinterpretation of results especially for extracts with low anti-microbial activity or in cases where the active ingredient(s) may irreversibly bind to the paper discs. Therefore, direct inoculation was shown to overcome the challenges associated with the disc diffusion method.

From the results, apart from the undiluted concentration, the diluted concentrations of *Coptis chinensis* extract (Dumiza 0.5 % SL) did not show any inhibition zone against *Pectobacterium carotovorum* subsp. *carotovorum*. On the other hand, the undiluted concentration of the *C. chinensis* extract product produced an inhibition zone that was not significantly different from that produced by streptomycin sulphate (Table1).

Plant extracts have been reported to possess antibacterial activities against several phytopathogenic bacteria including *Pseudomonas syringae* pv. *syringae* (Balestra *et al*. 2008; Quattrucci *et al*. 2013), *Xanthomonas axonopodis* pv. *vesicatoria* (Kotan *et al*., (2007) and *Clavibacter michigenesis* (Pattnaik *et al*., (2012). There is no literature indicating the use of *Coptis chinensis* extract against the soft rot bacterium, *Pectobacterium carotovorum* subsp. *carotovorum*. However, *Coptis chinensis* has been shown to have antifungal activities by *in vitro* inhibition of spore-germination and mycelial-growth of *Colletotrichum gloeosporioides*, *Phytohpthora capsici*, *Pyricularia grisea*, *Rhizoctonia solani*, *Botryosphaeri dothidea* and *Glomerella cingulata* (Ahn *et al*., 2009). Many authors have correlated the antimicrobial

activity of *C. chinensis* to its major constituent, berberine. In their work, Hou e*t al*. (2010) reported the antimicrobial properties of berberine against phytopathogenic agents both *in vitro* and *in vivo.* The authors observed that berberine exerted its inhibitory effect against *Monilinia fructicola*, *Botrytis cinerea* and *Alternaria solani* in a dose-dependent manner. Other reseachers have found berberine to be ineffective against gram-negative bacteria such as *Serratia liquefaciens*, *Citrobacter* MFBF and *Providentia stuardii* (Nechepurenko *et al*., 2010), *Escherichia coli*, *Staphylococcus aureus* (An *et al*., 1998). Gram negative bacteria have been shown to be more resistant to antimicrobial agents than Gram-positive bacteria due to their additional outer membrane layer (Leach, 2011). This could partly explain why lower concentrations of *C. chinensis* extract used in this study showed no inhibition against the test bacterium.

A berberine-containing extract, *C. chinensis* rhizome, was reported to have inhibitory activity against sortase A and sortase B enzymes (Kim *et al*., 2004). Sortase is a bacterial surface protein anchoring transpeptidase. In *Staphylococcus aureus*, a Gram-positive bacteria, the inhibition of these enzymes has been shown to result in a marked reduction in its virulence and infection potential (Kim *et al*., 2004; Imanshahidi and Hosseinzadeh, 2008). Berberine has been shown to block the adherence of *Streptococcus pyogenes* and *E. coli* to erythrocytes and epithelial cells, and in effect, it is thought to exert an antibiotic effect even against organisms that do not exhibit *in vitro* sensitivity to the alkaloid (Timothy *et al*., 1997; Imanshahidi & Hosseinzadeh, 2008). Berberine and berberine-containing plant extracts also have bacteriostatic effects on streptococci, with a minimum inhibition concentration MIC of 30 mg ml^{-1} for *S. pyogenes*. Sub-MICs of berberine prevented the adherence of streptococci to host cells, immobilized fibronectin, and hexadecane. Concentrations of berberine below its MIC caused an eight fold increase in release of lipoteichoic acid from the streptococci. Higher concentrations of berberine directly interfered with the adherence of streptococci to host cells either by preventing the complexing of lipoteichoic acid with fibronectin or by dissolution of such complexes once they were formed. Thus, berberine interferes with the adherence of group A Streptococci by two distinct mechanisms: one by releasing the adhesin lipoteichoic acid from the streptococcal cell surface and another by directly preventing or dissolving lipoteichoic acid-fibronectin complexes (Sun *et al*., 1988). The same test concentrations of *C. chinensis* extract used for the *in vitro* assay in the current study were found to reduce disease incidence and severity in our field studies though they had showed no *in vitro* inhibition.

González-lamothe *et al*. (2009) suggested that some antimicrobial plant extracts when applied to the plants may trigger the accumulation of other defence compounds within the plant or become more potent in the presence of other components involved in the immune response. Similarly, other plant extracts that do not exhibit *in vitro* inhibition may undergo enzymatic processing to make them potent *in vivo*.

3.5 Conclusion

From the results, non-diluted concentration of *C. chinensis* extract inhibited the growth of *Pectobacterium carotovorum* subsp. *carotovorum.* It is therefore evident that extracts from *C. chinensis* have the capability for *in vitro* inhibition of the soft rot causing bacterium.

CHAPTER FOUR

Effects of Pre-plant Tuber Spraying with *Coptis chinensis* Extract on Plant Growth, Soft Rot Disease, Flower and Tuber Yield and Quality of Zantedeschia 'Black Magic'

Abstract

Two field experiments were conducted to determine the influence of pre-plant tuber spraying with a *Coptis chinensis* extract-formulated product on plant growth, soft rot disease, stem yield and quality, tuber yield and quality of Zantedeschia 'Black Magic'. The study was conducted in a commercial flower farm, Sian Agriflora that specializes in the production of cut flower roses and callas. Calla lily tubers, 3-4cm in diameter were spread in a single layer in a plastic crate and sprayed to run-off with either of the following chemicals: *Coptis chinensis* extract product (0.625, 1.25 and 2.5Lha^{-1}), copper hydroxide (2 Lha^{-1}), streptomycin sulphate (100 mg L^{-1}) and a control in which irrigation water was applied. Under a Randomized complete block design with three replicates of 20 tubers each, sixty tubers/ treatment were treated. The tubers were allowed to dry and planted 24 h later on raised beds measuring 1M^2 at a spacing of 20 × 20 cm. Conventional crop management practices were carried out on a need basis. Data on disease incidence and severity were collected, stem yield and stem length was measured at every harvest of the flower stems. Plant growth parameters including height and leaf numbers were measured fortnightly from emergence. Similarly, tuber yield and quality including size and presence and/ or absence of soft rot lesions were monitored at the time of lifting the tubers. Results indicated that pre-plant tuber spraying with *C. chinensis* extract had no significant effect on calla lily soft rot incidence but disease severity was low in plots treated with the extract. Similarly, its application resulted in long-stemmed flowers (>80 cm). Stem length increased with increasing concentration of the extract. Pre-plant tuber spraying with *C. chinensis* extract did not increase the total stem yield as compared to the control treatment in which water was applied. Pre-plant application of the *C. chinensis* extract also promoted calla lily plant growth with high leaf numbers noted in plants that were treated with the extract. Also, plants treated with the extract recorded a higher plant height which was significantly different from the control treatments. This was observed on a dose-dependent manner. Regarding the tuber quality, there was no significant difference in tuber soft rot among the treatments but spraying with the extract yielded large-sized tubers as compared to the control treatments. As a result, crop performance was enhanced when tubers were sprayed with the extract before

planting. From these results, it was demonstrated that pre-plant tuber spray application of *C. chinensis* extract had the potential to suppress calla lily soft rot severity alongside promotion of plant growth and flower quality improvement. The mechanism involved in promotion of plant growth and suppression of tuber soft rot by this extract is still unknown and therefore warrants further research.

Key words: Flower length, tubers, irrigation water.

4.1 Introduction

Despite calla lily's importance in the international trade, the plant is susceptible to several soil-borne pathogens which are capable of lowering production and the quality of the tubers. These include rots caused by *Pythium* and *Phytophthora* spp. as well as tuber soft rot caused by *Pectobacterium carotovorum* subsp. *carotovorum* (Gerik *et al*., 2006; Wright & Triggs, 2009). The disease can cause up to 100 percent loss to calla plants in the field and tubers in storage. Plants may be infected in the field or during storage but the rots may not occur due to latent infections. Infected plants turn yellow, emit a foul smell and can be completely macerated resulting in death within a few days (Snijder & Tuyl, 2002). The spread of the bacteria takes place mainly by watering or by handling during tuber lifting, storage and planting. Soft rot symptoms can develop at any time during the growing cycle when conditions favourable for soft rot, such as high relative humidity occur, or when plants are under stress due to low soil aeration or high temperature (Snijder & Tuyl, 2002; Wright & Clark, 2005). Control measures for calla soft rot include discarding diseased tubers before planting, growing callas in well-drained growing medium at moderate temperatures (<24°C), careful management of irrigation, and use of chemicals as dips before planting or as drenches during crop growth (Wright & Clark, 2005) and high calcium levels. However, no single method has been reported to give full control of the disease (Snijder & Tuyl, 2002). A number of chemicals including sodium hypochlorite, paracetic acid and copper-based bactericides have been shown to inhibit *Pcc* (Snijder, 2004). It has been shown that pre-plant application of copper-based bactericides provide an effective control of the disease (Blom & Brown, 1999) but concerns of environmental pollution and development of resistance of the bacteria come into play. These coupled with the growing demand of pesticide-free agricultural products has led to the search of novel, affordable and less toxic strategies for pest control. In this regard therefore, plant based products/ plant extracts have raised a great deal of interest in the recent past for the management of phytopathogens and in treating infectious diseases.

As a drug, *Coptis chinensis* is traditionally used for its antimicrobial and antiprotozoal properties in Ayurvedic, Chinese and Middle-Eastern folk medicine. Specifically, Ayurveda describes berberine extracts and decoctions to have significant antimicrobial activity against a variety of organisms including bacteria, virus, fungi, protozoa, helminthes and Chlamydia (Singh *et al*., 2010; Vuddanda *et al*., 2010). The potency of the extracts of this plant has been attributed to one of its major constituents, berberine. The compound has been shown to possess strong bacteriostatic activity against *Staphylococcus epidermis*, *Neisseria meningitides, Escheri chia coli* (Vuddanda *et al*., 2010).

On phytopathogens, Hou *et al*. (2010) showed that berberine and *Coptis chinensis* extracts could inhibit the development of peach brown rot disease caused by ascomycete fungus *Monilinia fructicola*. These authors also showed that berberine regulated cutinase expression with berbebrine –treated *M. fruticola* having lower levels of the enzyme at the end of the study period. Enzymes such as cutinases are inducible extracellular enzymes that are capable of degrading plant cell walls by catalysing the cleavage of ester bonds of cutin, which is the major component of plant cuticle. This enzyme in additional to other extracellular enzymes including pectin lyase, pectin methyl esterase, pectate lyase, polygalacturonase, cellulase and protease are important pathogenicity factors associated with *Pectobacterium carotovorum* subsp. *carotovorum* (Snijder, 2004). In our previous *in vitro* test, *C. chinensis* extract was found to inhibit development of the soft rot causing bacterium.

The antimicrobial activity of five protoberberine alkaloids against *Staphylococcus aureus* was demonstrated (Fan *et al*., 2008). Similarly, the ability of *C. chinensis* extracts to inhibit phytopathogenic fungi including *Colletotrichum gloeosporioides*, *Phytohpthora capsici*, *Pyricularia grisea*, *Rhizoctonia solani*, *Botryosphaeri dothidea* and *Glomerella cingulate* was reported by (Ahn *et al*., 2009). The authors observed that three times leaf spray of the extract promoted plant growth and increased fruit yield of red-pepper.

On this basis and on other proven antibacterial activity, we therefore conducted a guided experiment to evaluate if spray application of a *C. chinensis* formulated product on Zantedeschia tubers could suppress calla lily soft rot, promote plant growth and improve flower yield, tuber yield and quality.

4.2 Materials and Methods

4.2.1 Experimental site

The current study was conducted at a commercial flower farm, Agriflora Ltd, Nakuru, Kenya. The farm lies at a latitude of 0º 17' South, longitudes 35º 54' East in the Lower Highland III Agro Ecological Zone (LH3) at an altitude of approximately 2,172 meters above sea level. Average maximum and minimum temperatures range from 19 ºC to 22 ºC and 5 ºC to 8 ºC, respectively, with a total annual rainfall ranging from 1200 to 1400 mm. Beds measuring 1m wide and raised to about 15cm high were prepared under a shaded structure that allowed about 50 % light, as is the common practice in the growing of calla lilies. The soils in the farm are predominantly loamy and are characterized with good drainage. Previously, the site had been cropped with maize-bean intercrop for about five seasons, a rotational programme aimed at controlling possible build-up of soft rot causing bacteria in the area. The study was done in two growing seasons running through December 2012 to May 2013 and May to December 2013.

4.2.2 Materials

Two-year old calla lily tubers 'Black Magic', 3-4 cm in diameter used for this experiment were provided by Agriflora Ltd. The variety was preferred because it is highly susceptible to bacterial soft rot. The *Coptis chinensis*-formulated product (Dumiza 0.5 % SL) was provided by Dahwa Agrochemical Co. Ltd packed in 100 ml plastic containers. Fertilizers, Osmocote, and other inputs including pesticides were purchased from Amiran K. Ltd.

4.2.3 Experimental design and treatment application

The experiment was laid out in a randomized complete block design with three replications. Raised plots each measuring $1m^2$ separated with a 0.5 m path were prepared under a shadenet-covered structure. Treatments included three application rates of a *Coptis chinensis* extract product (0.625, 1.25 and 2.5 L ha^{-1}), copper hydroxide (2 L ha^{-1}), streptomycin sulphate (100 mg L^{-1}) and a control in which irrigation water was applied. Zantedeschia tubers were evenly spread on a single layer in a produce plastic crate and each set sprayed uniformly with its corresponding treatment (Fig 3a). The tubers were allowed to dry overnight to allow proper sticking of the chemicals onto the tubers. Tubers were later planted at a spacing of 20cm by 20 cm giving a population of 20 tubers/ plot (Fig 3b). A slow release fertilizer, Osmocote® was added at the rate of 70g m^{-1}. Conventional crop

management practices including fertigation, weeding, pest management among others were applied uniformly across all the treatments as and when necessary.

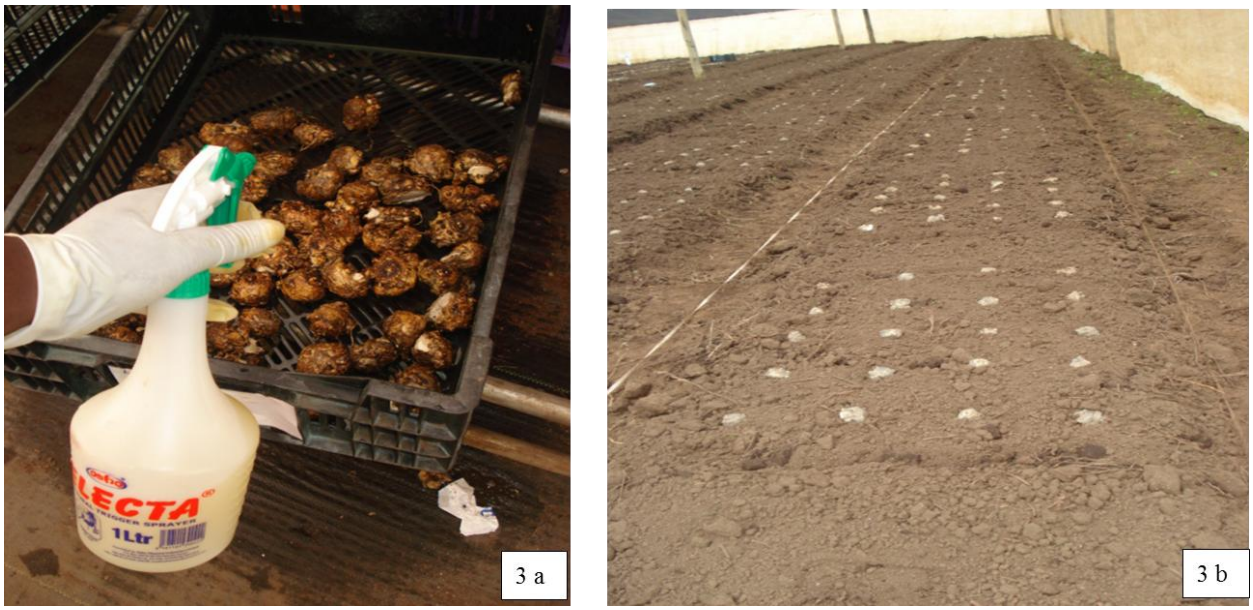

Figure 3: (a) Pre-plant tuber spraying, (b) Field establishment and layout

4.2.4 Data collection

Data on the following variables were collected:

Stem yield and quality:

Data on total number of stem harvested and stem length were collected during each of the harvesting period. Harvesting was done early in the morning by pulling the flower stalk in order to obtain the longest possible stem length. The stems were then placed in a bucket containing water to keep them hydrated. Before measuring the length, about 2 cm of the stem end was removed. Flower length measurement was done using a meter rule and obtaining the length from the end of the peduncle to the apex of the spathe.

Disease incidence and severity:

Disease incidence was evaluated by counting the number of diseased plants at 2-weeks interval. In assessing soft rot severity, ten diseased plants were randomly selected and the percent diseased area of the leaves estimated and rated on a four category scale according to the method described by Wright *et al*. (2005), where 0 = no soft rot symptoms; 1= 50 % of the plant has symptoms; 2 = 50 % of the plant has symptoms; and 3 = plant completely dead.

The ratings of disease severity were used in calculating percent disease index (PDI) per plot as described by Wright *et al*. (2005), Sudha & Lakshmanan (2009) and Anand *et al*. (2010):

$$\text{PDI} = \frac{\text{Sum of all individual ratings}}{\text{Total number of plants assessed} \times \text{Maximum disease category}} \times 100$$

Plant height and number of leaves:

Six plants per plot were selected for sampling in each of the plots. From the six plants, plant height (cm) was measured using a metre rule. This was done at a two-week interval starting from 65 DAP (days after planting) up to post-flowering period (121 DAP). Leaf numbers were monitored at the same interval and recorded.

Fresh and dry shoot weights:

Fresh weights of the above-ground portion of the sampling plants in each of the plots were taken using a digital balance model (SHANGPING JA12002). The shoots were later chopped to small pieces of about 5 cm in order to hasten the drying process. For dry weight, the chopped pieces, packed in brown khaki papers separately, were placed into the scientific drying oven (440W, 220V, 50Hz) where they were oven-dried (60°C) to a constant weight.

Tuber yield and quality:

After the aboveground portion had senesced (26 weeks from planting), the tubers were lifted by carefully digging them out using a forked hoe. Caution was taken not to injure the tubers during lifting. The tubers were graded according to size (diameter, cm) and presence/absence of soft rot lesions. The total number of tubers per plot was recorded.

4.2.5 Statistical Analysis

Data on tuber yield and quality, plant height, number of leaves, fresh and dry weight were collected over the two growing seasons. The data was then subjected to ANOVA using JMP 9 statistical software. The statistical model used was :$Y_{ij}=\mu + \beta_i + \alpha_j + \varepsilon_{ij}$ where; Y_{ij} is the calla lily response, μ is the overall mean, β_i is the *i*th blocking effect, α_j is the effect due to the *j*th treatment and ε_{ij} is the random error component. Significantly different means at $P \leq 0.05$ were separated using Tukey's HSD.

4.3 Results and Discussion

4.3.1 Effect on Zantedeschia plant growth

Pre-plant tuber spraying with *C. chinensis* extract had an effect on the general performance of the calla lily crop. Treating the tubers with the extract before planting had an effect on plant height. Earlier in the season in all the sampling periods, no significant difference in height was observed but during the last sampling session, 121 days after planting (DAP), height difference was observed. Spraying tubers with the 2.5 L ha^{-1} level of *C. chinensis* resulted in significantly taller plants than water and streptomycin treatments. However, there was no significant difference in plant height between the two lower *C. chinensis* rates tested. Similarly, the two lower tested rates were not different from copper, streptomycin and water treatments (Table 2). In both seasons, a similar trend was observed. This indicates the ability of *C. chinensis* extract in promoting calla lily plant height. Generally, plant height increased with an increasing concentration of the extract.

Table 2: Height of calla lily plant following pre-plant tuber spraying with *C. chinensis* Franch extract

Treatment	65 DAP	79 DAP	93 DAP	107 DAP	121 DAP
Season one					
Water	27.22	29.39	47.17	48.33	55.39 b
streptomycin100ppm	31.06	40.11	52.5	58.94	59.50 b
Copper hydroxide 2kg ha^{-1}	34.17	42.67	58.17	63.67	72.33 ab
C. chinensis 0.625 L ha^{-1}	31.61	43.89	51.78	61.06	70.78 ab
C. chinensis 1.25 L ha^{-1}	31.33	36.72	49.06	59.39	70.83 ab
C. chinensis 2.5 L ha^{-1}	36.28	47.28	62.56	63.89	82.00 a
P-value	0.3286	0.0821	0.2736	0.2599	0.0206
Season two					
Water	28.72	31.09	49.07	50.33	57.59 b
streptomycin100ppm	32.56	41.81	54.4	60.94	61.7 b
Copper hydroxide 2kg ha^{-1}	35.67	44.37	60.07	65.67	75.03 ab
C. chinensis 0.625 L ha^{-1}	33.11	45.59	53.68	63.06	72.98 ab
C. chinensis 1.25 L ha^{-1}	32.83	38.42	50.96	61.39	73.03 ab
C. chinensis 2.5 L ha^{-1}	37.78	48.98	64.46	65.89	84.2 a
P-value	0.435	0.298	0.376	0.599	0.0326

Means followed by the same letter within a column, a variable and a season are not significantly different according to Tukey's HSD at $p \leq 0.05$.

With regard to the influence on leaf numbers, at 79 DAP, there was a difference in the number of leaves in the treatments. Pre-plant tubers spraying with 2.5 L $ha^{-1}$of *C. chinensis* resulted in plants with significantly higher leaf numbers than water treatment There was no significant effect in leaf numbers when the tubers were treated with either the lower rates of *C. chinensis*, copper hydroxide, streptomycin sulphate or water (Table 3). The ability of *C. chinensis* extract to promote an early crop development could be very crucial in a number of ways. The earlier canopy development could enhance the photosynthetic mechanism resulting to a vigorous crop that can be able to withstand biotic and abiotic stresses. This is essential as a mode of evading stresses such as diseases.

Table 3 Leaf number of calla lily plant following pre-plant tuber spraying with *C. chinensis* Franch extract

Treatment	65 DAP	79 DAP	93 DAP	107 DAP	121 DAP
		Season one			
Water	2.68	4.02 b	7.02	7.78	7.83
streptomycin100ppm	3.13	5.53 ab	7.18	8.28	9.09
Copper hydroxide 2kg ha^{-1}	4.24	4.83 ab	7.02	7.78	8.83
C. chinensis 0.625 L ha^{-1}	3.69	5.06 ab	7.24	8.83	7.61
C. chinensis 1.25 L ha^{-1}	3.24	4.46 ab	5.44	7.19	8.44
C. chinensis 2.5 L ha^{-1}	4.14	6.28 a	7.61	9.56	10.17
P value	0.1220	0.0218	0.3238	0.2292	0.4720
		Season two			
Water	3.28	4.92 b	8.12	8.88	10.13
streptomycin100ppm	3.83	6.53 ab	8.18	9.38	10.69
Copper hydroxide 2kg ha^{-1}	3.94	5.53 ab	8.12	8.88	10.13
C. chinensis 0.625 L ha^{-1}	4.39	6.76 ab	8.34	9.93	10.91
C. chinensis 1.25 L ha^{-1}	4.44	5.26 ab	7.34	8.99	9.74
C. chinensis 2.5 L ha^{-1}	4.94	7.48 a	9.51	10.66	11.47
P value	0.3120	0.0128	0.642	0.337	0.624

Means followed by the same letter within a column, a variable and a season are not significantly different according to Tukey's HSD at $p \leq 0.05$.

4.3.2 Effect on Zantedeschia fresh and dry shoot weights

Pre-plant tuber spraying with *Coptis chinensis* extract had no significant effect on the above ground fresh and dry weights (Table 4). This was despite the fact that the observed weights were increasing with the increasing levels of the *Coptis chinensis* extract. The vigorous growth observed in plots treated with the extracts showed slightly higher number of leaves and plant height, which in turn influenced the biomass accumulated in the tissues.

Table 4: Fresh and dry calla lily plant shoot weights (g) following pre-plant tuber spraying with *Coptis chinensis* Franch extract

Treatment	Shoot fresh weigh (g)	Shoot dry weight (g)
	Season one	
Water	172.92	13.46
Streptomycin100 ppm	191.91	17.20
Copper hydroxide 2kg ha^{-1}	146.62	11.94
C. chinensis 0.625 L ha^{-1}	188.67	19.46
C. chinensis 1.25 L ha^{-1}	252.19	21.91
C. chinensis 2.5 L ha^{-1}	273.91	21.56
P value	0.0544	0.1039
	Season two	
Water	132.23	10.24
Streptomycin100ppm	141.22	16.21
Copper hydroxide 2kg ha^{-1}	150.32	12.32
C. chinensis 0.625 L ha^{-1}	165.45	17.81
C. chinensis 1.25 L ha^{-1}	230.33	20.83
C. chinensis 2.5 L ha^{-1}	264.71	21.12
P value	0.0632	0.2033

Means followed by the same letter within a column, a variable and a season are not significantly different according to Tukey's HSD at $p \leq 0.05$.

4.3.3 Effects on calla lily soft rot disease

Spraying calla lily tubers with *Coptis chinensis* extract before planting did not result in significant effect in soft rot disease incidence across the treatments (Table 5). However, though not significantly different, pre-plant tuber spray with the highest level of *C. chinensis* recorded lower disease incidence as compared to the water treatment. It is worth noting that as far as calla lily soft rot is concerned, a decrease in the number of diseased plants translates to lesser number of diseased tubers for subsequent growing seasons and for flower yield. With this in mind, though in a small magnitude, pre-plant tuber spray with *C. chinensis* extract contributed to the decrease in soft rot disease incidence, which would further lead to a reduction in losses associated with soft rot.

Table 5: Soft rot disease incidence of calla lily plants following pre-plant *C. chinensis* extract tuber application

Treatment	65 DAP	79 DAP	93 DAP	107 DAP	121 DAP	135 DAP
			Season one			
C. chinensis 0.625 L ha^{-1}	4.33	5.00	5.67	6.00	6.33	6.33
C. chinensis 1.25 L ha^{-1}	3.67	4.33	4.33	5.33	5.33	6.33
C. chinensis 2.5 L ha^{-1}	2.67	4.00	4.33	5.00	5.00	6.33
Copper 2kg ha^{-1}	2.33	2.67	2.67	3.67	4.00	5.00
streptomycin 100 ppm	2.67	4.00	4.00	5.00	5.00	6.00
Water	4.33	5.00	5.33	6.33	6.33	7.33
P value	0.5352	0.7648	0.4651	0.5900	0.5997	0.7571
			Season two			
C. chinensis 0.625 L ha^{-1}	6.33	7.00	7.33	8.33	8.33	9.33
C. chinensis 1.25 L ha^{-1}	5.67	6.33	6.33	7.33	7.33	8.33
C. chinensis 2.5 L ha^{-1}	4.67	6.00	6.33	7.00	7.00	8.33
Copper 2kg ha^{-1}	4.33	4.67	4.67	5.67	6.00	7.00
streptomycin 100 ppm	4.67	6.00	6.00	7.00	7.00	8.00
Water	6.33	7.00	7.67	8.00	8.33	8.33
P value	0.613	0.812	0.315	0.786	0.851	0.674

Means followed by the same letter or no letter within a column, a variable and a season are not significantly different according to Tukey's HSD at $p \leq 0.05$.

On the other hand, pre-plant tuber spraying with *C. chinensis* extracts effectively managed calla lily soft rot severity. During all the sampling periods starting from 65 days after planting (DAP) to 135 DAP (Fig 4), tuber spray with *C. chinensis* effectively reduced calla lily soft rot severity. Throughout the evaluation period, tuber spraying with the highest level of *C. chinensis* extract consistently recorded the lowest levels of soft rot severity that was significantly lower than water treatment. There was no significant difference in soft rot severity when tubers were sprayed with either copper hydroxide, streptomycin, or *C. chinensis* extract on most of the sampling dates. Though copper hydroxide is the compound

that is widely used in the control of calla soft rot either as pre-plant tuber sprays/ dip and plant drenches, the results indicate that this product can be substituted with *C. chinensis* in the management of calla lily soft rot.

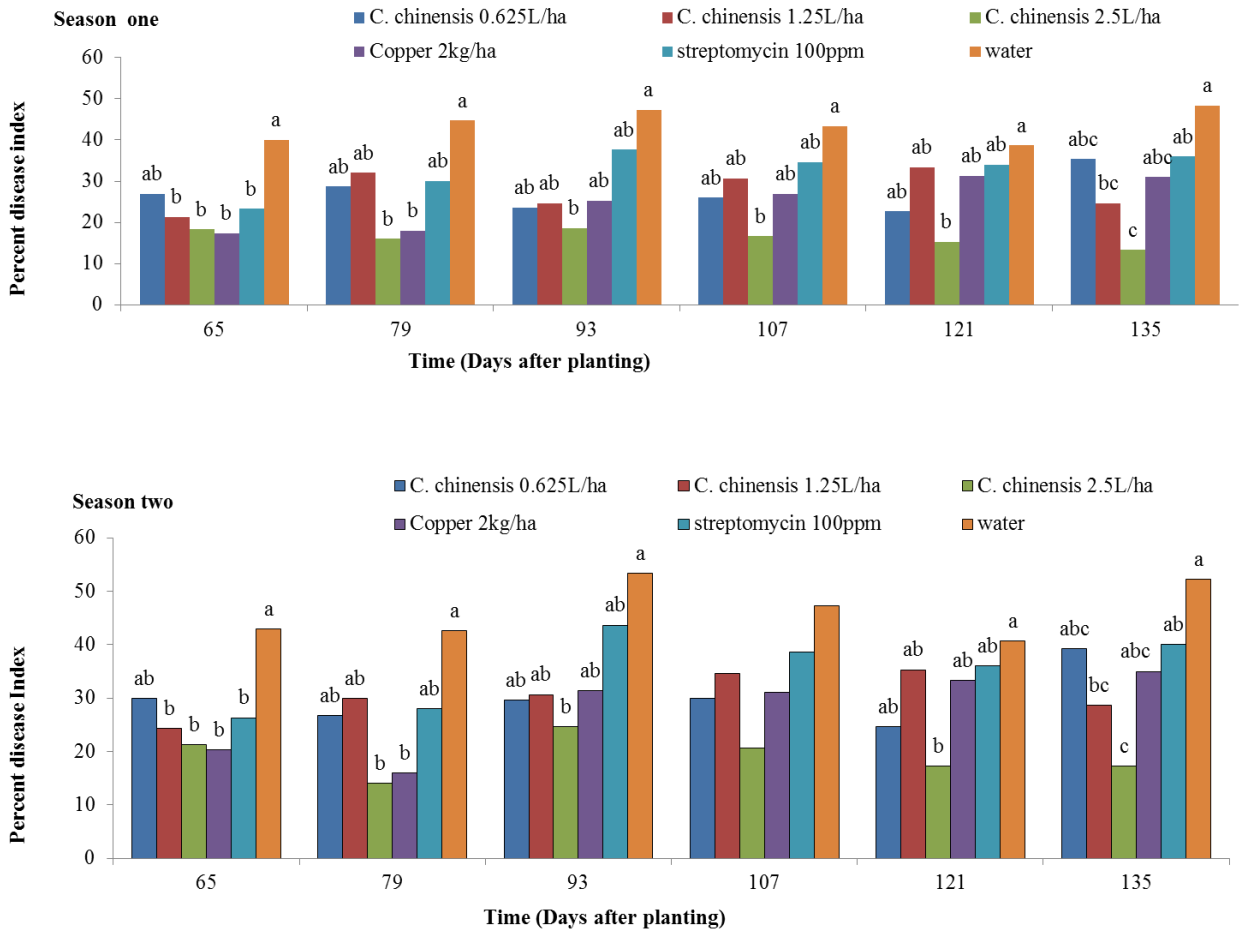

Figure 4: Soft rot disease severity of calla lily plants following pre-plant tuber spraying with *C. chinensis* extract

These results corroborate those reported by Satish *et al*. (1999) whose aqueous leaf extracts of eight plant species inhibited phytopathogenic *Xanthomonas campestris* pathovars *in vitro*. Similarly, methanolic extracts of twelve medicinal plants were found to inhibit growth of five bacterial strains including *Bacillus subtilis* ATCC6633, *Staphylococcus epidermidis* ATCC12228, *Pseudomonas pseudoalcaligenes* ATCC17440, *Proteus vulgaris* NCTC8313 and *Salmonella typhimurium* ATCC23564 *in vitro* (Parekh *et al*., 2005).

Berberine, an isoquinoline alkaloid, is reported to possess excellent antibacterial properties. The previous in vitro study demonstrated that *Coptis chinensis* extract was able to inhibit the development of the soft rot pathogen. *Coptis chinensis* and stems of phellodendron

contain this antimicrobial agent. Several authors have reported the effectiveness of the *Coptis chinensis* extract to inhibit phytopathogenic agents and human and animal pathogenic agents. Sarkar *et al*. (2011), reported the efficacy of berberine chloride as a new antibacterial agent on textile substrates against *Staphylococcus aureus* and *Entorococcus faecalis*. Their results showed that berberine chloride is an effective antibacterial agent at a minimum inhibitory concentration of 0.2% in the solution. On textile substrates such as 100% polyester, 100% nylon and 50% cotton-50% polyester blend, there was a 62-76% reduction in bacterial counts. The effectiveness of antibacterial action was retained after laundering and exposure to light. Similarly, Yu *et al*. (2005) reported the antimicrobial activity of berberine alone and in combination with Ampicillin or Oxacillin against methicillin-resistant *Staphylococcus aureus.* The authors observed that there was an additive effect between berberine and ampicillin whereas a synergistic effect was found between berberine and oxacillin against methicillin-resistant *Staphylococcus aureus.* Jin *et al*. (2010) evaluated the mechanism of berberine and reasons for little resistance of bacteria to the compound. The authors observed that berberine could easily and tightly bind to DNA and RNA and hardly dis-band from DNA- and RNA- berberine complexes. Also berberine could easily bind to protein too. They also observed that berberine could damage bacterial cell structure especially for Gram-negative bacteria. Additionally, Ca^{2+} and K^{+} released from berberine-treated cells increased significantly compared to the control. Since bactericidal mechanism of berberine affected the most essential physiological functions of a living cell, it was observed that there were slim chances of ever obtaining a population of berberine resistant cells. By these mechanisms, it is probable that they influenced the reduction of soft rot in calla lily as a result of *C. chinensis* application.

4.3.4 Effects on calla lily stem quality

Application of *C. chinensis* extract as a pre-plant tuber spraying had an effect on calla lily stem yield and quality. Among all the flower grades evaluated, in season one, there was significant difference in 71-80 and > 81 grades. In 71-80 grade, copper hydroxide treatment had significantly higher number of flower stems than water treatment but was not different from all the other treatments. In the >80 grade, spraying the tubers with the highest level of *C. chinensis* extract gave the highest number of flower stems in this grade and was significantly higher than Streptomycin and water treatments. In the second season, on most of the flower grades evaluated there was no significant difference across the treatments (Fig 5).

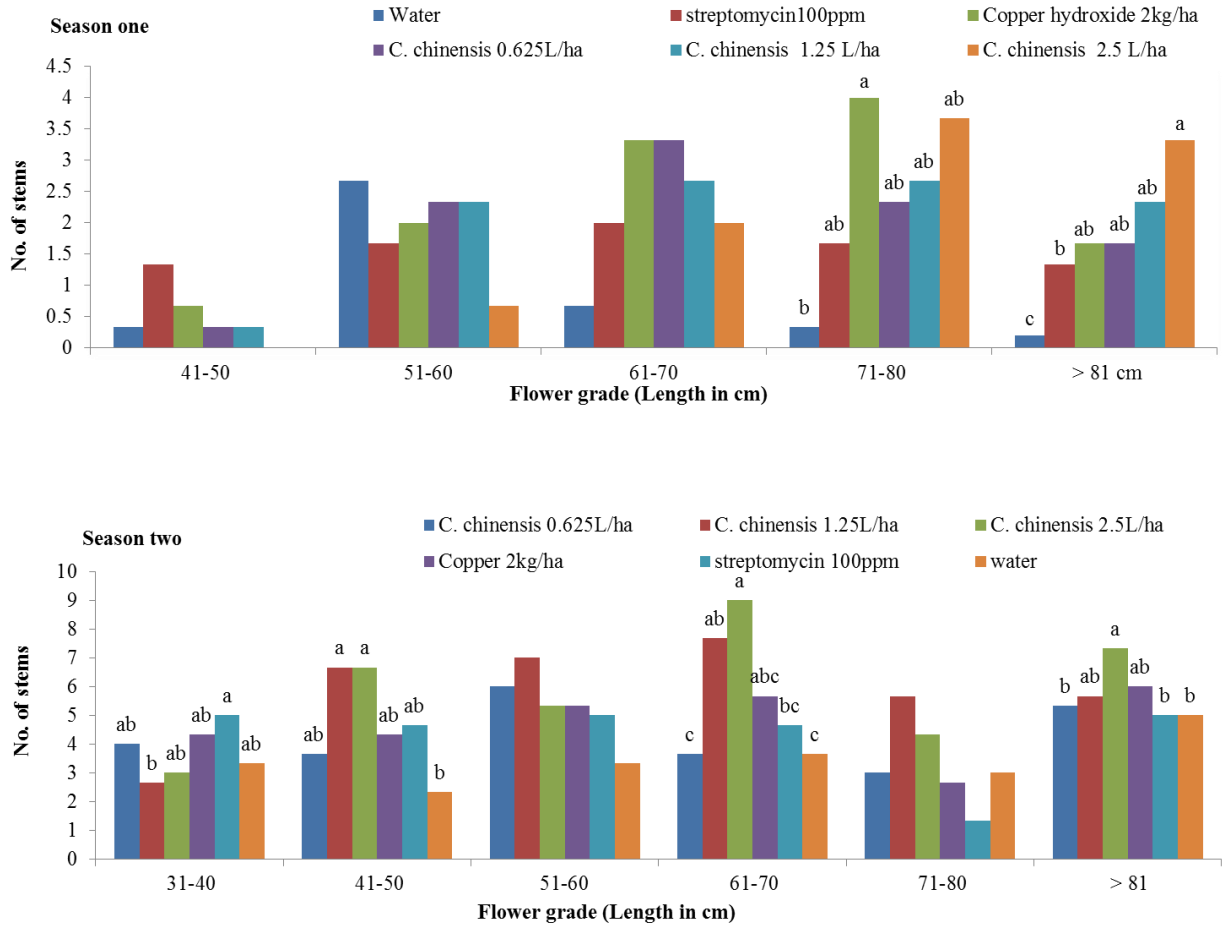

Figure 5: Flower grade of calla lily following pre-plant tuber spraying with *C. chinensis* extract

It is evident that *C. chinensis* extract has the ability to improve calla lily flower quality. The ability to improve flower quality could either be due to the ability of *C. chinensis* to suppress calla lily soft rot or availability of bioactives capable of promoting plant growth including stem height. On the other hand, tuber spraying with *C. chinensis* extract showed effects on total stem yield. Spraying the tubers with copper hydroxide gave the highest stem

yield which was significantly different from all the other treatments. Spray application of water on calla lily tubers gave the lowest number of marketable flower stems (Fig 6). There was no significant difference in stem yield when tubers were sprayed with the three rates of *C. chinensis* or Streptomycin sulphate. Water application on the tubers may have enhanced the activity of the soft rot pathogen leading to excessive damage and stem loss as a result of soft rot The above trend in promoting stem yield and quality by *C. chinensis* can be attributed to the ability of the extract to suppress calla lily soft rot. Being a crude extract of the *Coptis chinensis* plant, it is also probable that the extract could be containing growth promoting metabolites capable of promoting stem elongation and other calla lily growth parameters.

The current results agree well with those obtained by Ahn *et al*.(2009) who showed that 3-times leaf-spray of a hot water *C. chinensis* extract promoted red pepper growth alongside suppressing fungal pathogens in pepper. Several other medicinal plants have been evaluated for their potency to inhibit plant diseases and/or pathogens and their growth promoting ability. Pattnaik *et al*. (2012) showed that several medicinal plants exhibited the ability to suppress bacterial and fungal diseases in tomato as well as promote tomato growth and fruit yield.

Similarly, Ziosi *et al*. (2013) showed that two botanical extracts had biostimulant-like performances in promoting plant growth in in vitro and in vivo bioassays. Spraying Zinnia (*Zinnia elegans*) plants with plant based extracts was found to inhibit powdery mildew disease alongside promoting plant growth including plant height, branch number and leaf area (Hegazi & El-Kot, 2010). Garlic and onion extracts or their intercrops were shown to suppress damping-off and powdery mildew as well as promote growth and yield in cucumber (Morsy *et al*., 2009).

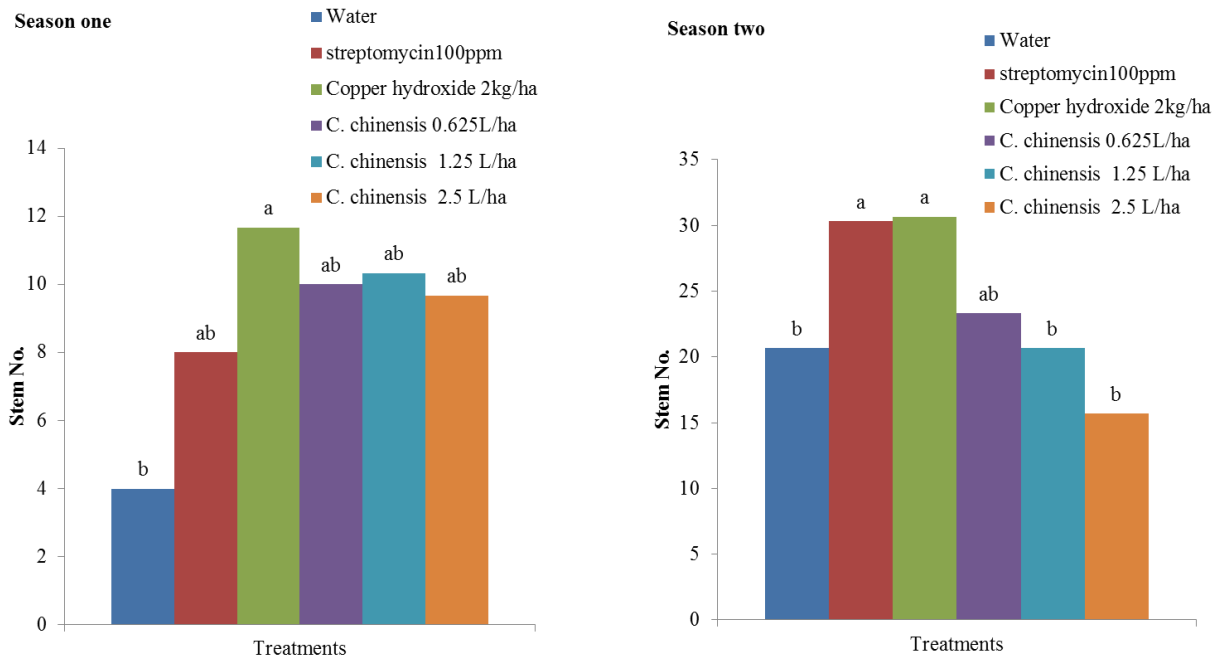

Figure 6: Calla lily flower yield following pre-plant tuber spraying with *C. chinensis* extract

These results show that application of *Coptis chinensis* extract on calla lily tubers prior to planting has a two-fold effect; ability to suppress calla lily soft rot and growth promotion effect . Being a plant based product, it is therefore benign on the environment, safe to the user and therefore offers a more sustainable way for the management of soft rots caused by *Pectobacterium carotovorum* subsp. *carotovora*.

4.3.5 Effect on Zantedeschia tuber yield and quality

Several parameters were evaluated while assessing the effect of pre-plant tuber spraying with the *C. chinensis* extract. On disease parameters, both the number of rotten tubers and the percentage rot, there was no significant difference across all the treatments. Though this was the case statistically, the levels of diseased lesions decreased with the rising concentration of the *C. chinensis* extract. This would therefore mean that a larger portion of the treated tubers could be sectioned out and separated from the diseased portion unlike the tubers treated with water whose lesions were large (60%). On tuber grade, tuber size, a significant difference was observed in the 5-6 cm wide tubers (Table 6). Tuber spraying with 2.5 L ha^{-1} *C. chinensis* extract level resulted in the highest number of tubers in this category which was significantly different from the two other lower rates and the control treatments. There was no statistical difference between the lower rate tested and water application. Similarly, there was no significant difference in the number of tubers in this grade between

copper hydroxide and streptomycin sulphate treatments. As shown, the increase in the number of tubers in this category was linearly related to the concentration of *C. chinensis* extract used. Tubers in this category are highly preferred by growers since they give more flowering shoots in their subsequent flowering periods as opposed to small-sized tubers which have to undergo bulking in order to obtain the right flowering sizes. Hence any amendments that would improve tuber quality are a plus in the Zantedeschia industry. On the other hand, very large tubers (Ɵ>8 cm) are not preferred due to their tendency of being highly susceptible to bacterial soft rot. Therefore, the treatments tested in this study were able to enhance tuber development to the most desired size, which would consequently result to better yields and indirect reduction of soft rot incidences.

Table 6: Tuber yield and quality of calla lily following pre-plant tuber spraying with *Coptis chinensis* Franch Extract

Treatment	Disease Parameters		Tuber grade					Yield
	No.rotten tubers	% Rot	1-2 cm	3-4 cm	5-6 cm	7-8 cm	9-10 cm	Tuber yield
Season one								
Water	4.00	60.00	3.33	4.67	1.67 c	1.33	0.67	22.00
streptomycin100ppm	4.00	40.00	2.00	6.00	3.67 b	3.33	0.33	19.67
Copper hydroxide 2kg ha^{-1}	6.33	36.67	6.67	4.33	3.00 b	2.00	0.33	23.67
C. chinensis 0.625 L ha^{-1}	7.00	23.33	3.33	6.67	2.00 c	2.67	1.67	23.33
C. chinensis 1.25 L ha^{-1}	4.33	26.67	1.00	4.33	3.33 b	4.33	0.33	18.00
C. chinensis 2.5 L ha^{-1}	5.00	18.33	6.00	5.67	6.00 a	3.00	0.50	21.00
P Value	0.8846	0.2088	0.4024	0.9747	0.0159	0.1468	0.5565	0.8854
Season two								
Water	6.00	73.00	2.33	5.33	2.22 c	0.33	1.22	18.00
streptomycin100ppm	7.00	54.00	3	5.22	3.45 b	2.32	2.11	18.43
Copper hydroxide 2kg ha^{-1}	6.54	42.00	7.2	4.52	2.80 c	2.00	0.32	22.46
C. chinensis 0.625 L ha^{-1}	8.2	32.00	2.6	7.22	2.00 c	2.12	1.82	19.06
C. chinensis 1.25 L ha^{-1}	3.33	30.00	2	4.32	4.12 b	3.82	1.64	15.33
C. chinensis 2.5 L ha^{-1}	4.00	20.00	7	3.21	5.62 a	3.00	0.56	22.34
P Value	0.5672	0.3210	0.4583	0.8745	0.0256	0.3241	0.4587	0.9276

Means followed by the same letter within a column, a variable and a season are not significantly different according to Tukey's HSD at $p \leq 0.05$.

Other researchers have reported effectiveness of plant extracts to promote growth alongside suppressing diseases in plants. Spinelli *et al*. (2010) showed the ability of a sea weed extract in enhancing vegetative and productive performances of strawberry plants grown on a lime inducing iron chlorosis substrate. They found out that the extract increased vegetative growth by 10 %, leaf chlorophyll content by 11 %, stomata density by 6.5 % in

addition to fruit production and berry weight. Similarly, Hegazi & El-Kot, (2010) showed that some biological control agents and plant extracts including miswak (*Salvadora persica*) and henna (*Lewsonia inermis*) could potentially control powdery mildew of Zinnia (*Zinnia elegans*) alongside promoting zinnia growth and yield.

Shen *et al*. (2010) reported the effectiveness of 0.5% berberine aqueous solution bactericides in controlling bacterial speckle disease (*Pseudomonas syringae* pv. *tomato*) in processing tomato. Additionally, three foliar applications of the *C. chinensis* extract was shown to promote plant growth including leaf, root and fruit of red pepper alongside *in vitro* antifungal activity against *Phytophthora capsici* (Ahn *et al*., 2009).

4.3.6 Conclusion

From the results above, the potential of *C. chinensis* Franch extract to suppress calla lily soft rot and promote growth and improve yields has been demonstrated. The highest level of *C. chinensis* extract was able to increase calla lily leaf number, reduce calla lily soft rot severity and promote production of average tuber sizes, which are preferred by the growers. Plots treated with the *C. chinensis* extract produced long stemmed flowers, which were significantly higher in number than those produced under water treated plots. Therefore, the *C chinensis* formulated product can be used effectively in the production of calla lilies.

CHAPTER FIVE

Effect of Drenching *Coptis chinensis* Franch Extract on Plant Growth, Soft Rot Disease, Flower and Tuber Yield and Quality of Zantedeschia 'Black Magic'

Abstract

Calla lily (*Zantedeschia sp.*) is an important cut flower, pot plant or garden ornamental. However, bacterial soft rot (*Pectobacterium carotovorum* subsp. *carotovorum*) adversely affects the commercial viability of this crop. We conducted two field experiments to determine the effect of drench application of a formulated *Coptis chinensis* extract product (Dumiza 0.5 % SL) on plant growth, calla soft rot disease, tuber and flower yield and quality of Zantedeschia 'Black Magic'. Three application rates of *C. chinensis* extract product (0.625, 1.25 and 2.5 L ha^{-1}) were compared with copper hydroxide at 2kg ha^{-1}, streptomycin at 100 ppm and water as control treatments in a randomized complete block design experiment with 3 replications. Sprouting tubers of Zantedeschia were planted on $1m^2$ plots at a spacing of 20 cm × 20 cm and drenched four times with the treatments at 14 days interval from either planting or at full foliage stage. Plant growth parameters including height and leaf numbers were measured fortnightly and so was the soft rot incidence and severity. Flower yield and quality was monitored at each harvesting period. Tuber yield and quality including size and presence and/or absence of soft rot lesions were monitored at the time of lifting the tubers. Application of *C. chinensis* extract significantly affected plant growth, suppressed calla lily soft rot severity on both the shoots and tubers with the highest rate (2.5 L ha^{-1}) consistently recording a lower percent disease index (PDI). Plots drenched with *C. chinensis* extract immediately after planting yielded significantly more flower stems compared with those treated with water or streptomycin. On the other hand, drenching at full-foliage stage had no effect on total flower yield. Drenching *C. chinensis* extract also resulted in significantly higher number of long stemmed flowers and medium-sized tubers, 5-6 cm diameter, compared with the control treatments. There was no significant difference in total tuber yield when plots were drenched either immediately from planting or from full foliage stage. These results demonstrate the potential of *C. chinensis* extract to promote calla lily plant growth, suppress soft rot and promote yield and quality of Zantedeschia.

Key words: PDI, Drench, Calla lily.

5.1 Introduction

Calla lily is faced with numerous challenges among which is the soft rot disease. This disease is caused by *Pectobacterium carotovorum* subsp. *carotovorum*, and happens to be the most important disease of calla lily in Kenya affecting the commercial viability of this crop. The disease results in the death of plants and decay of tubers. Infection can occur at all stages of the calla life cycle from young plants to tubers in store (Wright, 1998). The severity of soft rot depends mainly on soil and weather conditions, with warm, wet weather and poor-draining soils favouring infection. The disease attacks the crop and rapidly spreads causing heavy damages that may lead to 100% crop loss if unchecked. It not only causes erratic emergence and collapse of the shoot, but also tuber decay and post-harvest collapse of the peduncle. All cultivars with coloured flowers, belonging to the section *Aestivae* of the genus, are very susceptible to soft rot.

Scientists have shown great interest on plant extracts that improve plant growth and tolerance to biotic and abiotic stresses (Ziosi *et al*., 2013; Colla *et al*., 2013) Several medicinal plants have been shown to possess secondary metabolites such as amino acids and other growth regulating hormones that are ideal in promoting plant growth and development (Nouman,*et al*., 2012). These plant extracts are able to do this by stimulating plant physiological processes caused by the existing metabolites within the extracts.

In our previous *in vitro* study, it was demonstrated that *C. chinensis* extract was able to inhibit the development of *Pectobacterium carotovorum* subsp. *carotovorum.* Also, pre-plant tuber spraying with *C. chinensis* extract was able toincrease calla lily plant height and suppress calla lily soft rot severity.

The aim of the present study was to determine the effect of drenching a formulated *C. chinensis* extract product on calla lily soft rot, growth, yield and quality of Zantedeschia flowers and tubers.

5.2 Materials and Methods

5.2.1 Experimental site

The experimental site was Agriflora Ltd, Nakuru, Kenya The plants were grown outdoors on soil media under shade net. The soils in the area are predominantly loamy soils characterized with good drainage. The site had previously been cropped with maize and bean intercrop for about 5 years, a common practice, to break a possible build-up of soft rot causing bacteria. The study was done in two growing seasons running through December 2012 to July 2013 and May to December 2013.

5.2.2 Plant material

Two-year old calla lily tubers 'Black Magic', 3-4 cm in diameter used for this experiment were provided by Agriflora Ltd.

5.2.3 Experimental design and treatment application

The experiment was laid out in a randomized complete block design with three replications. Raised plots each measuring $1m^2$ separated with a 0.5 m buffer area were prepared under a shade net and Zantedeschia tubers planted at a spacing of 20cm × 20 cm giving a density of 20 tubers/plot (Fig 7 a). A slow release fertilizer Osmocote® was added at the rate of $70g/m^2$. Treatments included three application rates of *Coptis chinensis* extract product (0.625, 1.25 and 2.5 L ha^{-1}), copper hydroxide (2 g L^{-1}), streptomycin sulphate (100 mg L^{-1}) and a control in which irrigation water was applied. Drenching was done four times at two weeks interval either immediately after planting or from full-foliage stage. Conventional crop management practices including irrigation, weeding and pest management were applied across the treatments as and when required (Fig 7 b).

Figure 7: (a) Field layout, (b) Overhead irrigation on Zantedeschia

5.2.4 Data collection

Plant height and number of leaves

Six plants per plot were selected for sampling. From the six plants, plant height (cm) was measured using a metre rule. Plant height was measured from the soil interface to the tallest part of the plant. This was done at a two-week interval starting from 65 DAP (Days after planting) up to post-flowering period (121 DAP). Leaf numbers were monitored at the same interval and recorded.

Fresh and dry shoot weights

Fresh weights of the above-ground portion of the sampling plants in each of the plots were taken using a digital balance (model SHANGPING JA12002). The shoots were later chopped to small pieces of about 5 cm in order to hasten the drying process. For dry weight, the chopped pieces, packed in brown khaki papers separately, were placed into the scientific drying oven (440W, 220V, 50Hz) and dried at 60°C to a constant weight.

Soft rot disease:

Disease incidence was evaluated by counting the number of diseased plants at 2-weeks interval where ten diseased plants were randomly selected and the percent diseased area estimated and rated on a four category scale according to the method described by Wright *et al*. (2005), where 0 = no soft rot symptoms; 1 = 50% of the plant has symptoms; 2 = 50% of the plant has symptoms; and 3= plant completely dead. The ratings of disease severity were used in calculating percent disease index (PDI) per plot as described by Wright *et al*. (2005), Sudha & Lakshmanan (2009) and Anand *et al*. (2010):

$$\text{PDI} = \frac{\text{Sum of all individual ratings}}{\text{Total number of plants assessed} \times \text{Maximum disease category}} \times 100$$

Flower yield and quality:

Data on total number of stem harvested and stem length were collected during each of the harvesting period. Harvesting was done early in the morning by pulling the flower stalk in order to obtain the longest possible stem length. The stems were placed in a bucket containing water to keep them hydrated. Before measuring the length, about 2 cm of the stem ends were removed. Flower length measurement was done using a meter rule and obtaining the length from the end of the peduncle to the apex of the spathe.

Tuber yield and quality

After 26 weeks from planting, when the above-ground portion had senesced, the tubers were lifted by carefully digging them out using a forked hoe (Fig 8 a). Caution was taken not to injure the tubers during lifting. The tubers were graded according to size (diameter, cm, using a digital vernier calliper), presence/absence of soft rot lesions and the total count of tubers per plot was made (Fig 8 b)

Figure 8: (a) Tuber lifting, (b) Tuber measurement using a digital vernier calliper

5.3 Statistical Analysis

Data on plant height, number of leaves, fresh and dry shoot weight, soft rot disease, tuber and flower yield and quality were collected over the two growing seasons and pooled. The data was then subjected to ANOVA using JMP 9 statistical software. The statistical model used was :$Y_{ij}=\mu + \beta_i + \alpha_j + \varepsilon_{ij}$ where;

Y_{ij} is the calla lily response, μ is the overall mean,

β_i is the *i*th blocking effect,

α_j is the effect due to the *j*th treatment and

ε_{ij} is the random error component

Significantly different means at $P \leq 0.05$ were separated using Tukey's HSD.

5.4 RESULTS AND DISCUSSION

5.4.1 Effect on Zantedeschia plant growth

Drenching *Coptis chinensis* extract immediately from planting had an effect on calla lily plant height (Table 7). At 121 days after planting, 2.5 L ha^{-1} of *Coptis chinensis* extract had the highest plant height that was significantly different from Streptomycin and water treatments.

Table 7: Height (cm) of calla lily plants following *Coptis chinensis* Franch extract drenching immediately from planting

Treatment	65 DAP	79 DAP	93 DAP	107 DAP	121 DAP
		Season one			
Water	30.61	43.17	59.22	58.72	61.78 b
streptomycin100 ppm	37.39	50.83	58.83	67.44	61.11 b
Copper hydroxide 2kg ha^{-1}	33.89	39.72	55.78	61.72	75.72 ab
C. chinensis 0.625 L ha^{-1}	38.17	43.44	55.94	59.11	74.11 ab
C. chinensis 1.25 L ha^{-1}	31.78	38.83	49.78	60.23	75.17 ab
C. chinensis 2.5 L ha^{-1}	33.56	41.56	58.02	64.50	77.33 a
P value	0.3014	0.0966	0.4757	0.6399	0.0077
		Season two			
Water	32.11	44.87	60.72	61.12	63.98 b
streptomycin100ppm	38.89	52.53	60.73	63.31	69.44 b
Copper hydroxide 2kg ha^{-1}	35.39	41.42	57.68	63.72	77.92 ab
C. chinensis 0.625 L ha^{-1}	39.67	45.14	57.84	61.11	76.31 ab
C. chinensis 1.25 L ha^{-1}	33.28	40.53	51.68	62.28	77.37 ab
C. chinensis 2.5 L ha^{-1}	35.06	43.26	59.96	66.5	79.53 a
P value	0.5123	0.1082	0.5278	0.8573	0.0234

Means followed by the same letter within a column, a variable and a season are not significantly different according to Tukey's HSD at $p \leq 0.05$.

A similar trend was observed in plant height when the plants were drenched with the *C. chinensis* extract product from full foliage stage Drenching the plants with *C. chinensis* extract product at 2.5 L ha^{-1} had a significant effect on plant height compared to water and streptomycin treatments (Table 8).

Table 8: Height (cm) of calla lily plants following *Coptis chinensis* Franch Extract drenching from full foliage stage

Treatment	65DAP	79 DAP	93 DAP	107DAP	121DAP
Season one					
Water	33.67	30.25	51.78	56.44	57.50 b
streptomycin100ppm	28.39	32.22	51.22	55.89	60.78 b
Copper hydroxide 2kg ha^{-1}	33.78	41.78	54.78	58.83	72.89 ab
C. chinensis 0.625 L ha^{-1}	30.94	38.83	51.67	56.94	74.44 ab
C. chinensis 1.25 L ha^{-1}	32.89	41.39	50.11	55.44	73.50 ab
C. chinensis 2.5 L ha^{-1}	35.28	46.06	52.78	64.06	86.22 a
P value	0.7834	0.4321	0.7893	0.7531	0.0121
Season two					
Water	35.17	31.95	53.68	58.44	59.7 b
streptomycin100ppm	29.89	33.92	53.12	57.89	62.98 b
Copper hydroxide 2kg ha^{-1}	35.28	43.48	56.68	60.83	75.09 ab
C. chinensis 0.625 L ha^{-1}	32.44	40.53	53.57	58.94	76.64 ab
C. chinensis 1.25 L ha^{-1}	34.39	43.09	52.01	57.44	75.7 ab
C. chinensis 2.5 L ha^{-1}	36.78	47.76	54.68	66.06	88.42 a
P value	0.5496	0.3519	0.9954	0.8639	0.0093

Means followed by the same letter within a column, a variable and a season are not significantly different according to Tukey's HSD at $p \leq 0.05$.

Drenching the plants with *Coptis chinensis* extract immediately from planting had an effect on the number of leaves of Zantedeschia 'Black Magic' (Table 9).

Table 9: Number of leaves of calla lily plants following *Coptis chinensis* Franch extract drenching immediately from planting

Treatment	65DAP	79 DAP	93 DAP	107 DAP	121 DAP
		Season one			
Water	2.22	3.06 c	7.56	7.17	9.17
streptomycin100ppm	3.06	4.33 b	8.67	10.22	9.50
Copper hydroxide 2kg ha^{-1}	2.78	3.19 c	5.61	6.39	8.17
C. chinensis 0.625 L ha^{-1}	2.28	3.89 c	7.11	7.50	10.06
C. chinensis 1.25 L ha^{-1}	3.22	4.94 b	5.78	6.72	8.44
C. chinensis 2.5 L ha^{-1}	3.50	6.44 a	7.00	8.17	8.44
P value	0.2532	0.0143	0.2531	0.0912	0.3141
		Season two			
Water	4.22	4.46 c	8.47	8.76	10.57
streptomycin100ppm	5.06	5.94 b	9.87	11.52	10.9
Copper hydroxide 2kg ha^{-1}	3.78	4.89 c	6.81	7.69	9.57
C. chinensis 0.625 L ha^{-1}	4.28	4.44 c	8.31	8.8	11.46
C. chinensis 1.25 L ha^{-1}	4.22	5.39 b	6.98	8.02	9.84
C. chinensis 2.5 L ha^{-1}	4.5	6.83 a	8.2	9.47	9.84
P value	0.1753	0.0288	0.1049	0.0744	0.1343

Means followed by the same letter within a column, a variable and a season are not significantly different according to Tukey's HSD at $p \leq 0.05$.

At 79 DAP, the number of leaves varied across the treatments. Drenching the plants with 2.5 L ha^{-1} *C. chinensis*, extract level resulted in plants with significantly higher leaf number than all the other treatments. 1.25 L ha^{-1} *C. chinensis* level had a similar number of leaves as streptomycin treatment. There was no significant difference in leaf number between the lower 0.625 L ha^{-1} *C. chinensis* level, copper hydroxide or water treatment. Higher leaf numbers in the early stages of crop development may establish a desired plant canopy which is capable of efficient photosynthetic mechanism thereby improving on plants health in the long run. Though this was observed early in the season, in the other sampling dates no significant difference in leaf number was recorded. On the other hand, application of the drenches after development of the full foliage did not have a significant effect on the number of plant leaves (Table 10).

Table 10: Number of leaves of calla lily plants following *Coptis chinensis* Franch extract drenching from full foliage stage

Treatment	65 DAP	79 DAP	93 DAP	107 DAP	121 DAP
Season one					
Water	4.11	4.78	7.11	7.72	8.17
streptomycin100ppm	3.11	4.00	6.78	7.89	7.33
Copper hydroxide 2kg ha^{-1}	4.44	6.28	7.94	9.11	8.00
C. chinensis 0.625 L ha^{-1}	2.78	3.67	4.72	5.33	8.06
C. chinensis 1.25 L ha^{-1}	3.50	4.72	5.94	6.33	7.89
C. chinensis 2.5 L ha^{-1}	3.72	5.89	6.67	8.11	8.94
P value	0.3212	0.2051	0.2132	0.0892	0.7842
Season two					
Water	4.61	5.48	8.01	8.82	9.47
streptomycin100ppm	3.61	4.70	7.68	8.63	8.99
Copper hydroxide 2kg ha^{-1}	4.94	6.98	8.84	9.30	10.21
C. chinensis 0.625 L ha^{-1}	3.28	4.37	5.62	6.43	9.36
C. chinensis 1.25 L ha^{-1}	4.00	5.42	6.84	7.43	9.19
C. chinensis 2.5 L ha^{-1}	4.22	6.59	7.57	9.21	10.24
P value	0.2155	0.1007	0.1562	0.0731	0.8785

Means followed by the same letter within a column, a variable and a season are not significantly different according to Tukey's HSD at $p \leq 0.05$.

Drenching calla lily plants with the *Coptis chinensis* extract during both periods, either immediately from planting or from full foliage stage had no significant effect on the shoot fresh and dry weights (Table 11). This result could be due to the fact that application of the extract had no significant effect on leaf number especially at the end of the sampling period which would have otherwise influenced biomass differences across the treatments. The effect of the *Coptis chinensis* extract on calla lily growth appears to be gibberellin-like as shown in its ability to increase plant height and the length of the peduncles.

Table 11: Fresh and dry shoot weights of calla lily plants following drenching with *Coptis chinensis* Franch extract

Treatment	From planting		From full-foliage stage	
	Fresh weight	Dry weight	Fresh weight	Dry weight
Season one				
Water	212.60	16.15	148.52	12.08
Streptomycin100ppm	219.91	15.00	217.52	16.33
Copper hydroxide 2kg ha^{-1}	222.94	16.53	149.54	12.16
C. chinensis 0.625 L ha^{-1}	182.86	12.79	173.23	17.94
C. chinensis 1.25 L ha^{-1}	196.40	12.58	156.43	13.30
C. chinensis 2.5 L ha^{-1}	223.94	19.89	170.73	12.84
P value	0.8554	0.1120	0.5552	0.3157
Season two				
Water	177.52	13.45	160.32	16.21
Streptomycin100ppm	182.13	13.00	180.33	15.72
Copper hydroxide 2kg ha^{-1}	205.12	13.21	130.67	19.10
C. chinensis 0.625 L ha^{-1}	193.65	11.78	163.29	17.32
C. chinensis 1.25 L ha^{-1}	213.45	12.62	141.33	14.69
C. chinensis 2.5 L ha^{-1}	215.67	14.32	205.98	15.32
P value	0.9234	0.5213	0.7893	0.2192

Means followed by the same letter within a column, a variable and a season are not significantly different according to Tukey's HSD at $p \leq 0.05$.

5.4.2 Effect on Zantedeschia soft rot

Drenching *Coptis chinensis* extract immediately from planting had a disease suppression effect against calla lily soft rot. Plots drenched with *C. chinensis* extract progressively recorded lower disease levels (incidence and severity) as compared to the control/water drenched plots. Generally, plots drenched with the highest level of *Coptis chinensis* extract recorded significantly lower number of diseased plants as compared to the two lower tested rates. There was no significant difference in disease incidence between copper and the highest rate of *C. chinensis* extract drenched (Table 12). Plots drenched with water recorded the highest number of diseased plants. Disease incidence increased progressively from earlier growing stage 65 Days after planting and reached maximum levels

at post-flowering stage, 135 days after planting. Calla lily soft rot has been associated with either new infections or latent infections in tubers which cause disease when the conditions become favourable (Snijder, 2004). This could partly explain the current results that show an upward trend in disease development as the growing season proceeds.

Table 12: Soft rot incidence of calla lily plants following *C. chinensis* extract drenching either from planting or from full foliage stage

Treatment	Diseased plants when drenched from planting	Diseased plants when drenched from full-foliage stage
	Season one	
water	7.94 a	4.83
C. chinensis 0.625 L ha^{-1}	6.67 ab	4.00
C. chinensis 1.25 L ha^{-1}	6.22 abc	3.61
streptomycin 100ppm	6.06 bc	4.06
Copper hydroxide 2kg ha^{-1}	4.78 c	3.61
C. chinensis 2.5 L ha^{-1}	4.61 c	3.56
P value	0.0212	0.1310
	Season two	
water	8.22 a	5.27
C. chinensis 0.625 L ha^{-1}	7.12 b	5.02
C. chinensis 1.25 L ha^{-1}	7.32 b	4.32
streptomycin 100ppm	7.04 b	4.20
Copper hydroxide 2kg ha^{-1}	4.18c	3.74
C. chinensis 2.5 L ha^{-1}	4.27 c	3.78
P value	0.0031	0.582

Means followed by the same letter within a column, a variable and a season are not significantly different according to Tukey's HSD at $p \leq 0.05$.

Drenching calla lily plants with *Coptis chinensis* extract immediately from planting significantly suppressed calla lily soft rot (Fig 9). There was significant difference in percent disease index of soft rot on most scouting days during the course of the study. Drenching calla lily plants with water consistently resulted in higher disease index throughout the study period as compared to the other treatments. Among the three tested rates of *C. chinensis*

extract, application at 2.5 L ha^{-1} consistently recorded a lower percent disease index but was not significantly different from the other two lower rates.

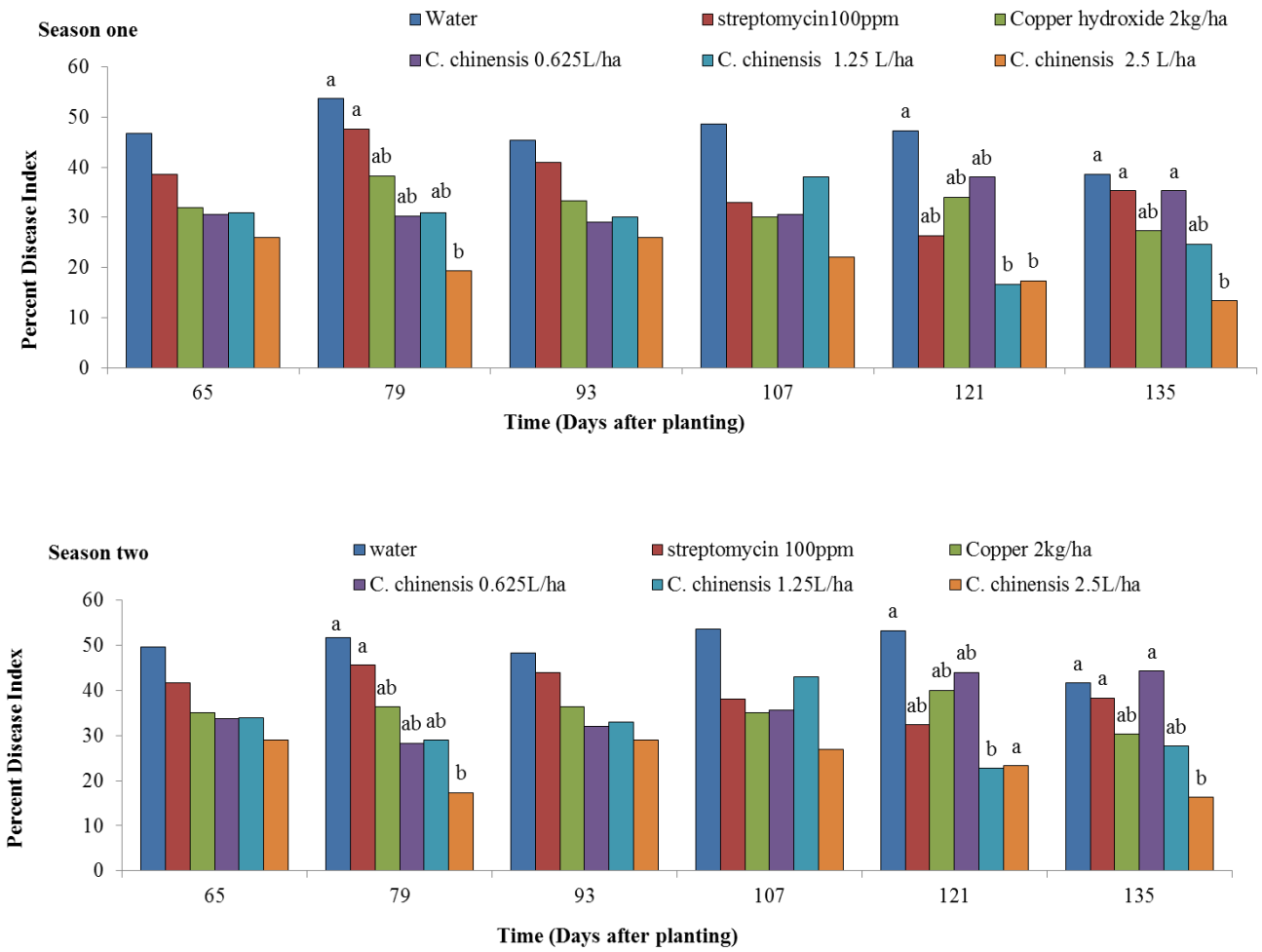

Figure 9: Percent Disease index of soft rot in calla lily plants following *C. chinensis* extract drenching immediately from planting.

Drenching calla lily plants with *Coptis chinensis* extract after full foliage stage did not have any significant difference on disease incidence. There was no significant difference in the number of infected plants across the treatments. Number of diseased plants increased with time hitting the highest level at post-flowering stage. However, drenching calla lily plants with *C. chinensis* extract had an effect on disease severity. Plants drenched with *C. chinensis* extract recorded significantly lower percent disease index compared to those drenched with water (Fig 10). Among the three tested rates, the highest rate of *C. chinensis* extract recorded significantly lower disease severity at all sampling dates. There was no significant difference in disease severity when plants were drenched with 2.5 L ha^{-1}*Coptis chinensis* extract or

copper hydroxide. Therefore, the *C. chinensis* extract can be used as an alternative to copper hydroxide.

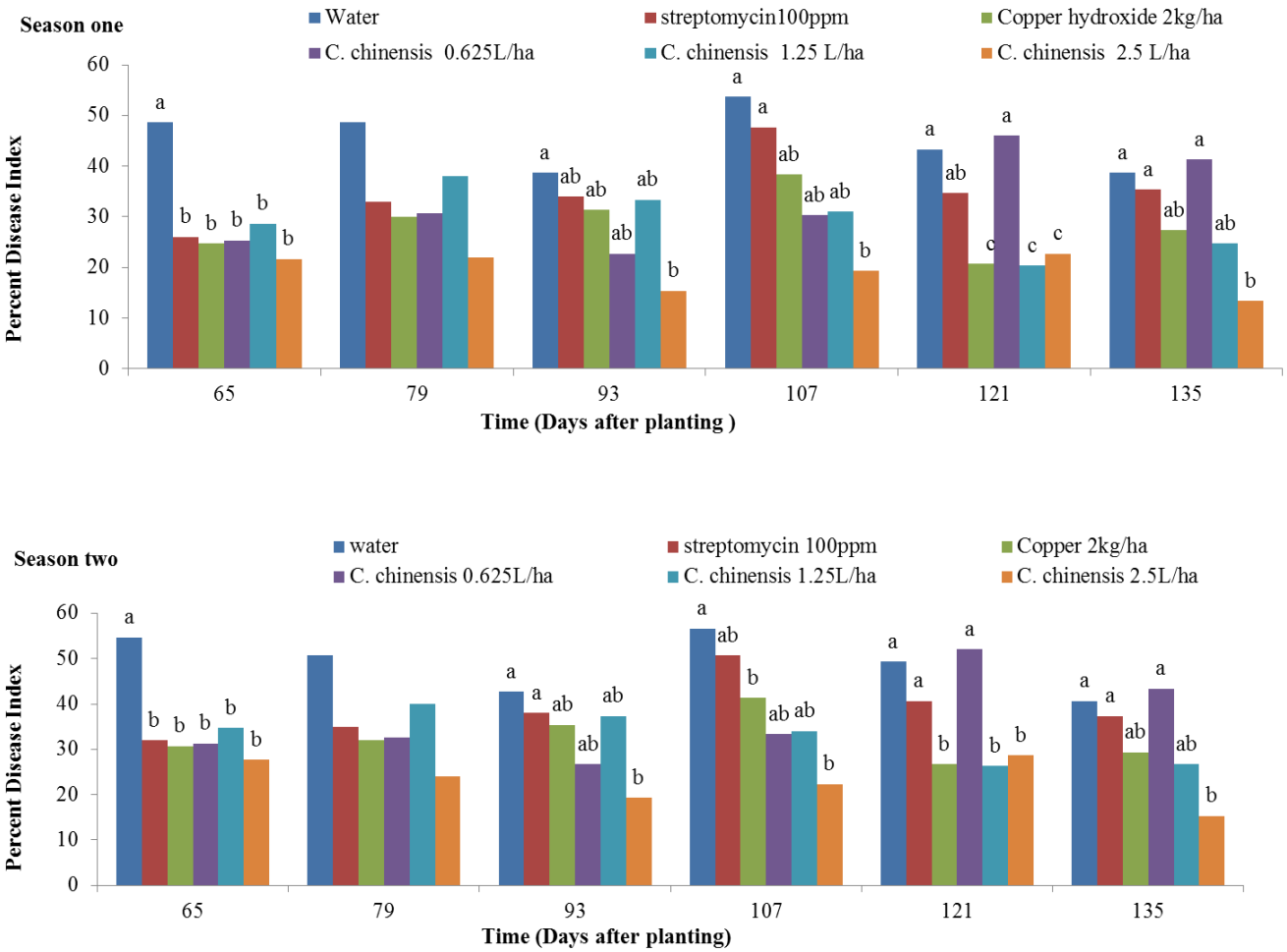

Figure 10: Percent Disease index of soft rot in calla lily plants following *C. chinensis* extract drenching from full-foliage stage.

These results demonstrate that *Coptis chinensis* extract has the ability to suppress calla lily soft rot caused by *Pectobacterium carotovorum* subsp. *carotovorum. C. chinensis* extract has been shown to exhibit strong antibacterial activity and has often been used traditionally to treat dysentery, cholera and other diseases. Its effectiveness to treat various diseases has been linked to one of its major component, Berberine (Yan *et al*., 2008; Dioum *et al*., 2010). Berberine alkaloids are active components in a large number of plant-derived drugs such as antimicrobials from Berberidaceae and Rutaceae family (Yan, *et al*., 2008; Dan *et al*., 2009). Our results agree with those obtained by Chung et al. (1998) who found out that low density polyethylene films (48 to 55 µm thick) impregnated with either 1.0% w/w *Rheum palmatum* and *C. chinensis* extracts or silver-substituted inorganic zirconium retarded the

growth of total aerobic bacteria, lactic acid bacteria and yeast on fresh strawberries. Similarly, *Dioum et al*. (2010) reported the effectiveness of the extract from *C. chinensis* to inhibit *Monilinia fruticola*, the fungal pathogen that causes brown rot in peach fruits. Plant extracts have been shown to control many plant diseases caused by various pathogens. They have been reported to effect this by inducing host resistance through increasing the activities of various enzymes such as peroxidise and polyphenol oxidase which play a defense role against invading pathogens (Hegazi & El-Kot, 2010).

5.4.3 Effect on Zantedeschia Flower Yield and Quality

Drenching Zantedeschia with *Coptis chinensis* extract immediately from planting had a significantly higher number of long stemmed flowers (>60 cm) compared to water and streptomycin treatments (Fig 11). Generally, the number of long-stemmed flowers increased with increase in *C. chinensis* concentration. The highest tested rate of *C. chinensis* extract (2.5 L ha^{-1}) consistently resulted in significantly longer stemmed flowers than streptomycin and water treatments. There was no significant difference in stem number in those grade between the *C. chinensis* extract treatments and copper hydroxide treatment.

Drenching with *Coptis chinensis* extract significantly increased total stem yield (Fig 12). Among the three tested rates of *C. chinensis*, 2.5 L ha^{-1} yielded higher than water and streptomycin treatments. However, there was no significant difference in yield between copper hydroxide and the *C. chinensis* extract treatments in the first season although in the second season, copper hydroxide yielded the highest number of flower stems (Fig 11).

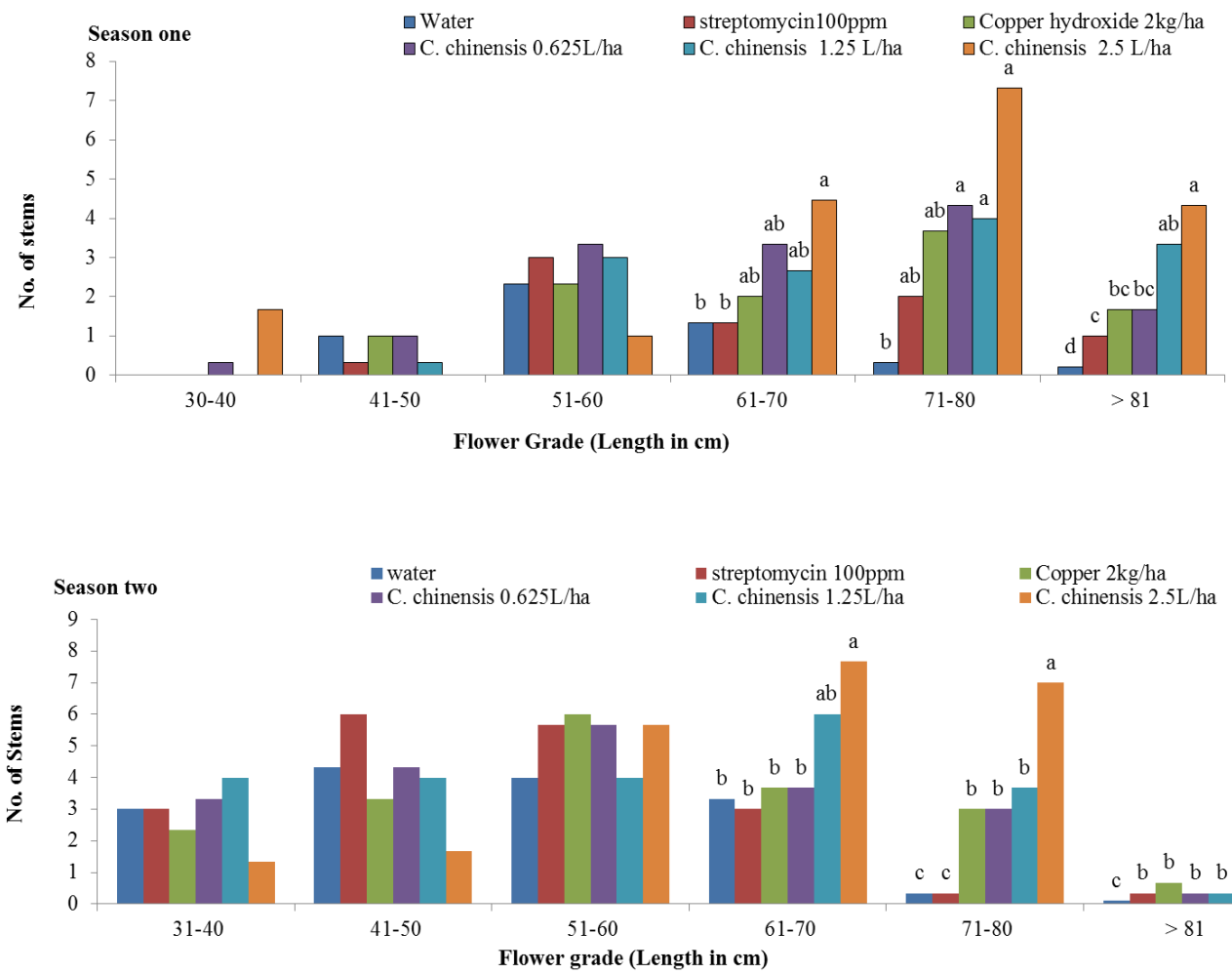

Figure 11: Flower grades of Zantedeschia 'Black Magic' following *Coptis chinensis* Franch extract drenching immediately from planting

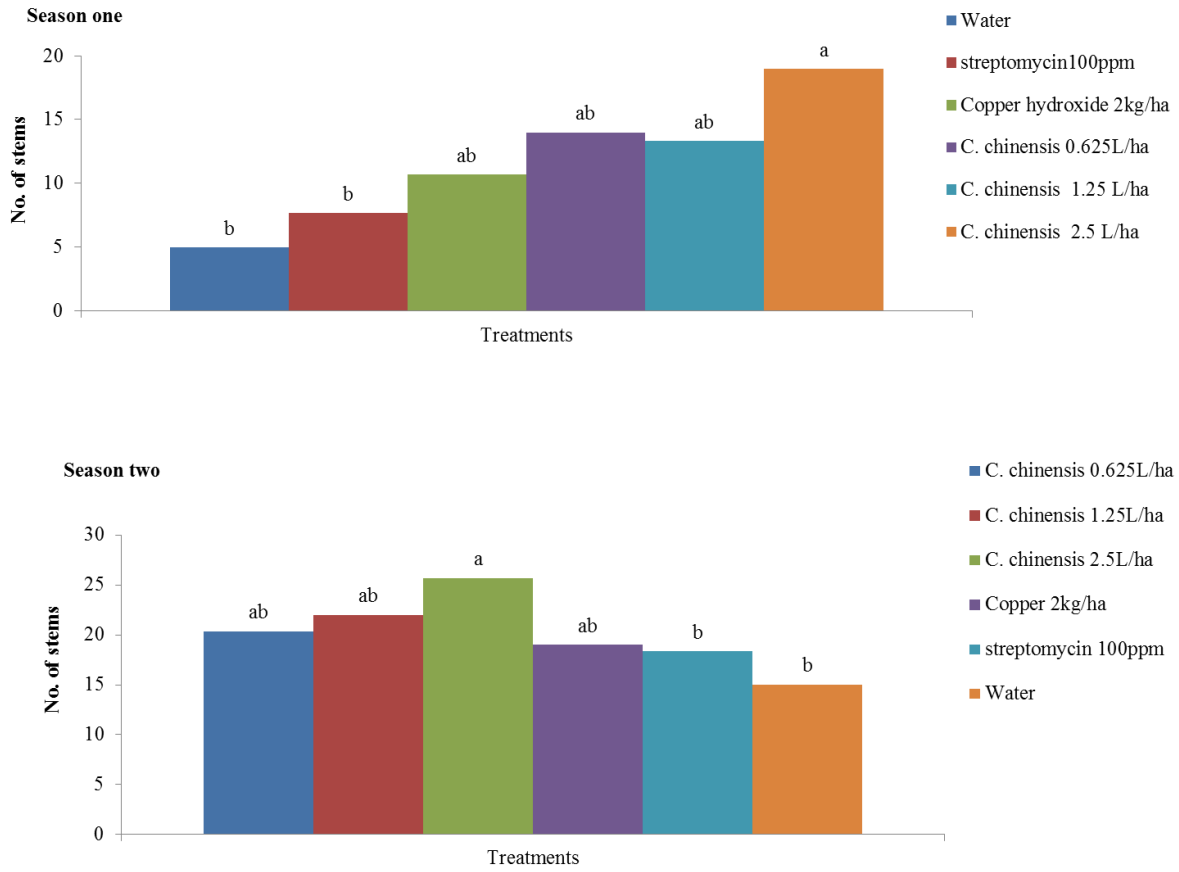

Figure 12: Flower yield of Zantedeschia 'Black Magic' following *Coptis chinensis* Franch extract drenching immediately from planting

Drenching with *Coptis chinensis* extract after full foliage stage had an effect on the number of long-stemmed flowers. The highest tested level of *C. chinensis* extract gave the highest number of long-stemmed (>71 cm) flowers which was significantly higher than streptomycin and water treatments (Fig 13).

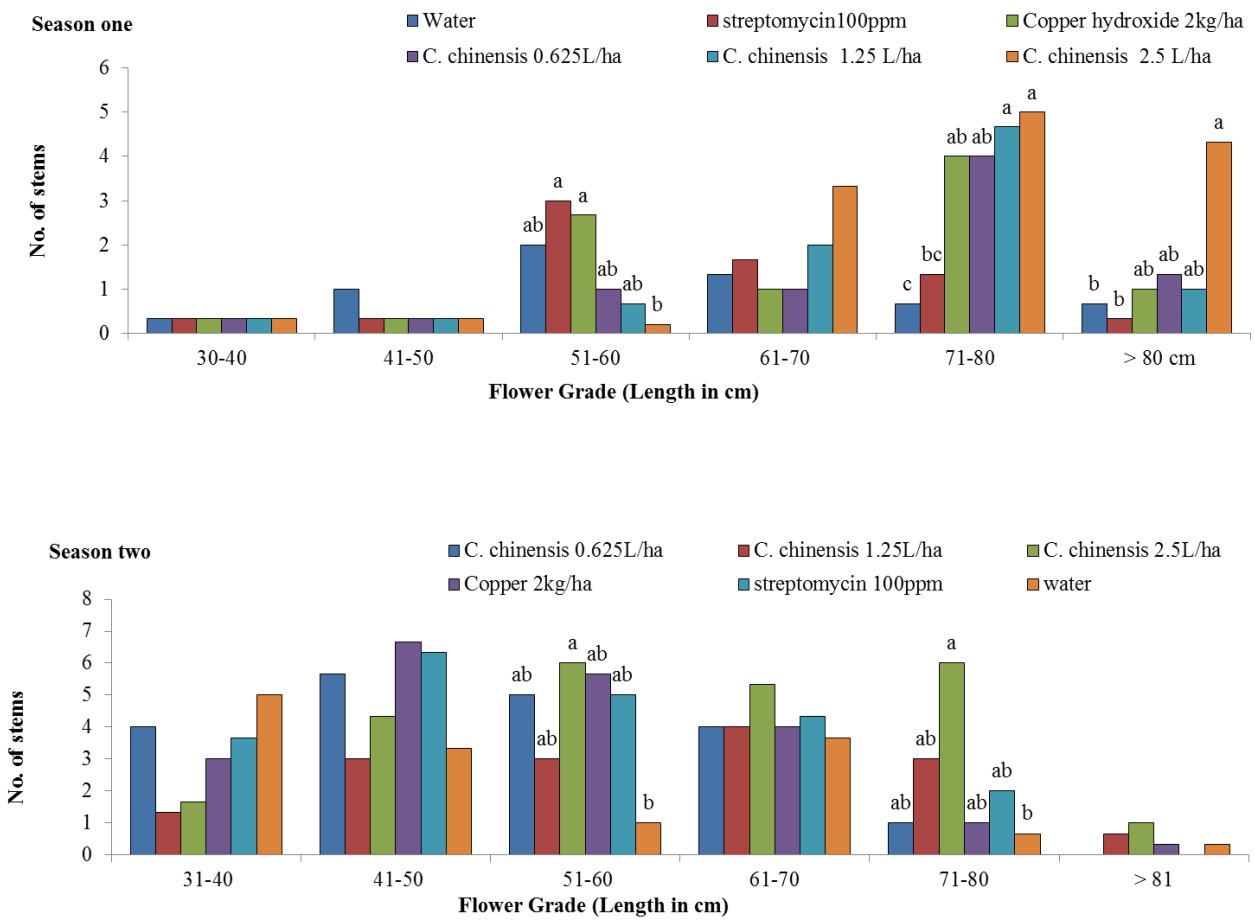

Figure 13: Flower grades of calla lily following Coptis chinensis Franch Extract Drenching from full-foliage stage

Plots that were drenched with *C. chinensis* extract yielded more flower stems as compared with the controls. Though this was observed, there was no significant difference in total yield across the treatments (Fig 14).

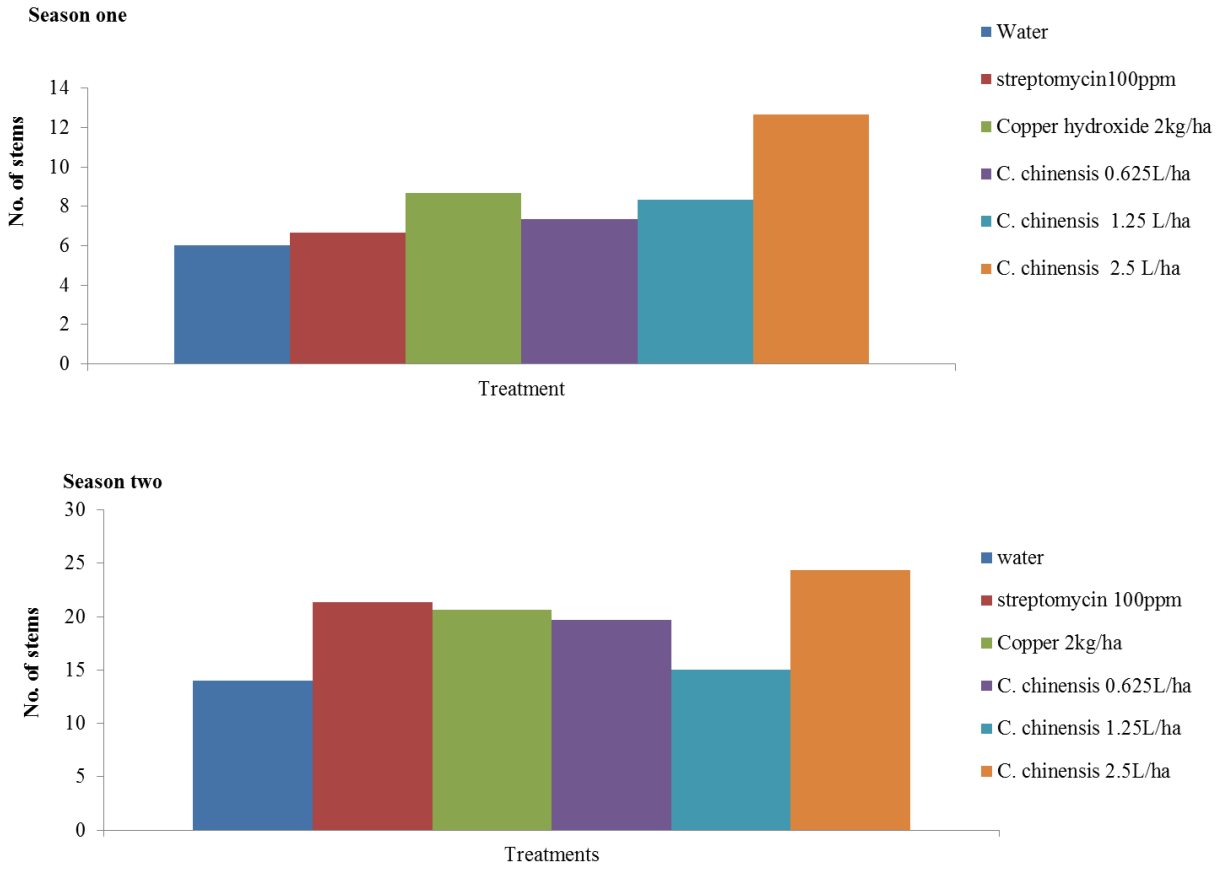

Figure 14: Flower grades of calla lily following *Coptis chinensis* Franch Extract drenching from full-foliage stage

These results compare positively with those reported by Ahn *et al*. (2009) whose foliar application of *Coptis chinensis* extract against *Phytophthora capscici* promoted red pepper plant growth including leaf, root and fruit. The average weight and rind of each fruit were increased by 119% and 117% in comparison to the control (blank) treatments. According to Pattnaik *et al*. (2012), a large number of the plant extracts significantly increase tomato growth parameters including shoot height, number of branches, number of leaves, number of flowers and yield along with reduction of plant diseases.

These results are also in line with those obtained by Hanafy *et al*. (2012) where drench application of natural extracts of garlic was significantly superior in promoting the growth of *Schefflera arboricola* especially the plant height, stem diameter, dry weight of leaves/plant, leaf area, total carbohydrates and N contents. Similar response was observed with application of 1% neem seed extract in okra (Moharam *et al*., 2012), and ginger, cinnamon or clove oils in *Zinnia elegans* (Hegazi., 2010). Flower length is an important

factor during the marketing of cut flowers. Long-stemmed flowers fetch premium prices compared to short-stemmed flowers.

5.4.4 Effect on Zantedeschia tuber yield and quality

Drenching calla lily plants with *C. chinensis* Franch extract had an effect on tuber grades and quality. Drenching *C. chinensis* extract immediately from planting reduced the percent rotten surface on the tubers, improved the average tuber diameter and resulted in large-sized tubers as compared to the control plots. The highest tested level of *C. chinensis* had tubers with significantly lower rotten surface (15.00, Table 15) as compared to streptomycin (40.00) and water (43.33) treatments. Drenching the plants with either of the three rates of *C. chinensis* extract had a similar effect as drenching the plants with the farmer's check, copper hydroxide in suppressing the tuber rots. Generally, regarding the tuber rots suppression ability, improved performance increased with increasing the *C. chinensis* extract level. Streptomycin, being an antibacterial agent did not perform better (40.00) than any of the three rates tested or even drenching with copper hydroxide. There was no significant difference in the total number of rotten tubers across the treatments. Drenching calla lily plants with *C. chinensis* extract immediately from planting had an effect on the size distribution of the resulting tubers as compared to the control plots (Table 15). Of all the grades evaluated, the only significant difference was in the 5-6 cm tuber grade. The 2.5 L ha^{-1} of *C. chinensis* extract had a significantly higher number of medium-sized tubers than copper, streptomycin and water treatments. The highest tested rate of *C. chinensis* had the highest number of tubers in this grade but was not significantly different from the other two rates. Drenching either copper hydroxide or streptomycin was not significantly different from the water treatment. The treatments did not result to any significant effect on the total tuber yield.

Table 13: Tuber yield and quality of calla lily following *Coptis chinensis* Franch extract drenching immediately from planting

Treatment	Disease Parameters		Tuber grade					Yield
	Rotten tubers	% Rot	1-2 cm	3-4 cm	5-6 cm	7-8 cm	9-10 cm	Tuber yield
Season one								
Water	5.00	43.33 a	4.67	3.67	2.00 b	3.33	1.00	21.67
streptomycin100ppm	3.00	40.00 ab	4.33	5.33	2.67 b	3.33	1.33	23.33
Copper hydroxide 2kg ha^{-1}	4.33	20.00 bc	4.33	2.33	4.33 b	2.33	2.00	22.33
C. chinensis 0.625 L ha^{-1}	6.00	23.33abc	3.33	5.33	4.67 ab	2.00	1.33	18.00
C. chinensis 1.25 L ha^{-1}	5.00	20.00 bc	2.33	3.00	4.67 ab	1.33	2.00	19.67
C. chinensis 2.5 L ha^{-1}	5.00	15.00 c	0.00	8.00	9.00 a	3.33	2.67	23.00
P value	0.7213	0.0070	0.5363	0.3330	0.0053	0.7269	0.8869	0.723
Season two								
Water	6.00	46.87 a	5.68	5.78	4.21 b	2.33	2.00	22.78
streptomycin100ppm	3.00	42.22 ab	5.34	8.33	4.12 b	2.00	1.33	25.67
Copper hydroxide 2kg ha^{-1}	5.33	30.21 b	5.78	4.55	5.22 ab	1.33	1.00	20.12
C. chinensis 0.625 L ha^{-1}	7.00	32.12 b	4.88	7.23	5.22 ab	1.00	1.33	21.43
C. chinensis 1.25 L ha^{-1}	4.00	30.10 c	4.32	6.23	4.67 b	1.33	2.00	17.45
C. chinensis 2.5 L ha^{-1}	4.00	21.55 c	2.11	4.21	6.23 a	2.00	2.67	25.56
P value	0.5344	0.0021	0.6781	0.4512	0.0231	0.3212	0.7653	0.8321

Means followed by the same letter within a column, a variable and a season are not significantly different according to Tukey's HSD at $p \leq 0.05$.

Similarly, drenching Zantedeschia with *C. chinensis* extract from full foliage stage had an effect on the performance of the tubers (Table 16). Though there was no significant effect on the number of rotten tubers across the treatments, there was significant difference in percentage rot on those tubers. Drenching the plants with 2.5 L ha^{-1} *C. chinensis* had a significantly lower percent rot as compared to all the other treatments. There was no

significantly difference in percent rot when plants were either drenched with the two lower rates of *C. chinensis* extract, copper hydroxide or streptomycin treatments. Water drench resulted in the highest rot percentage in the tubers.

On the tuber grade distribution, significant difference was on the 3-4 cm sized tubers. The highest tested rate of *C. chinensis* extract had the highest number of tubers in this grade which was significantly higher than streptomycin and water treatments. There was no significant difference among the two lower tested rates and copper hydroxide. Streptomycin and water drench recorded the lowest number of tubers in this grade. Although drenching the plants with the highest rate of *C. chinensis* resulted in fairly higher number of total tuber yield, there was no significant difference in yield among all the treatments.

Table 14: Tuber yield and quality of calla lily following *Coptis chinensis* Franch extract drenching from full foliage stage

Treatment	Disease Parameters		Tuber grade					Yield
	No. of rotten tubers	% Rot	1-2 cm	3-4 cm	5-6 cm	7-8 cm	9-10 cm	Tuber yield
Season one								
Water	8.33	53.33 a	3.33	3.00 b	2.33	5.00	0.33	20.33
streptomycin100ppm	7.67	33.33 ab	1.67	2.67 b	2.67	2.67	0.33	20.00
Copper hydroxide 2kg ha^{-1}	5.22	40.00 ab	1.33	4.00 ab	4.00	5.33	0.67	18.67
C. chinensis 0.625 L ha^{-1}	8.33	26.67 ab	2.67	4.00 ab	4.33	3.00	0.67	17.00
C. chinensis 1.25 L ha^{-1}	7.12	16.67 b	1.00	5.17 ab	1.00	0.67	0.33	13.00
C. chinensis 2.5 L ha^{-1}	4.22	13.33 c	0.50	8.29 a	2.00	1.33	1.67	24.33
P value	0.2880	0.0210	0.7212	0.0197	0.3872	0.1237	0.3062	0.4651
Season two								
Water	6.67	60.24	2.33	2.00 b	0.33	0.33	5.00	22.82
streptomycin100ppm	6.00	32.06	2.33	1.67 b	0.33	0.33	2.67	21.16
Copper hydroxide 2kg ha^{-1}	2.33	38.91	1.65	5.00 ab	0.67	0.67	5.33	19.03
C. chinensis 0.625 L ha^{-1}	9.00	30.01	2.55	4.00 ab	0.67	0.67	3.00	17.89
C. chinensis 1.25 L ha^{-1}	6.00	18.92	2.00	6.67 ab	0.33	0.33	0.67	14.06
C. chinensis 2.5 L ha^{-1}	5.00	12.89	2.00	9.67 a	1.67	1.67	1.33	26.01
P value	0.4598	0.0124	0.7651	0.0281	0.6451	0.4130	0.8101	0.5602

Means followed by the same letter within a column, a variable and a season are not significantly different according to Tukey's HSD at $p \leq 0.05$.

Regarding plant growth and disease suppression, similar trend has been reported elsewhere in recent literature where plant extracts have been shown to promote plant growth and development (Pattnaik *et al*., 2012). The authors reported the efficacy of various medicinal plant extracts in promoting tomato growth including number of branches, leaves, flowers and fruits alongside suppression of both fungal and bacterial diseases. The same was

noted in the current study whereby *C. chinensis* extract has been shown to both promote Zantedeschia plant growth alongside suppressing soft rot in tubers.

Various plant derived agents including jojoba oil, extract of *Reynoutria sachalinensis* and neem seed oil were reported to control powdery mildew of Okra caused by *Erysiphe cichoracearum.* Apart from disease control, spray application of the three extracts was shown to be effective in increasing okra growth parameters including plant height, number of branches, leaves and pods per plant and fresh pod yield/plant as compared to the negative and positive controls (Moharam & Obiadalla, 2012).

On disease suppression, the ability of *C. chinensis* extracts to suppress calla lily soft rot could be attributed to the presence of secondary metabolites found in the plant. *C. chinensis* herb, the source of the extract under study, contains berberine alkaloids such as berberine, coptisine, jatrorrhizine and palmatine. Individually or in combination, these alkaloids have been shown to possess antimicrobial activities on both human pathogenic agents (Dan, *et al*., 2009) and phytopathogens (Hou *et al*., 2010) and on pathogens associated with textiles especially bacterial pathogens *Staphylococcus aureus* and *Enterococcus faecalis* (Sarkar *et al*., 2011).

The current results agree with those reported by Morsy *et al*. (2009) who showed that intercrop and extracts of both onion and garlic could suppress cucumber damping-off, powdery mildew and promote its growth including plant height, fresh and dry weight of shoots and roots as well as number of flowers per plant as compared to untreated plants. Culver *et al*. (2012) showed that spray application of *Moringa oleifera* leaf extracts significantly increased tomato growth parameters as compared to the control. Similarly, Nouman *et al*. (2012) reported the ability of *Moringa oleifera* leaf extracts to promote range seed grass germination and growth after seed priming with the extracts. Plant derived agents based on plant extract or essential oils are a mixture of secondary metabolites and as such they can both inhibit the effect of the pathogen and also induce host resistance through the increase of activity of enzymes that are involved in defence mechanism against invading pathogens (Caruso *et al*., 2001) including peroxidase and polyphenol oxidase activities (Hegazi & El-Kot, 2010).

These results are in agreement with Hegazi & El-Kot, (2010) whose miswak (*Salvadora persica*) and henna (*Lawsonia inermis*) plant extracts not only suppressed Zinnia (*Zinnia elegans*) powdery mildew but also significantly improved the plant height, branch number and leaf area. Availability of secondary metabolites such as tanins, glycosides,

terpenes, flavonoids and alkaloids in medicinal plants has been linked to their ability to suppress both fungal and bacterial plant pathogens(Hou *et al*., 2010; Pattnaik *et al*., 2012)

In conclusion, the ability of the *C. chinensis* extract to promote calla lily plant growth could be attributed to their ability in the suppression of calla soft rot and/or presence of plant growth promoting entities within the extract. However, it would be prudent to investigate the specific entities involved in growth promotion and/ or the mechanism involved in disease suppression.

5.5 Conclusion

Besides improving plant growth, flower and tuber yield and quality attributes, *C. chinensis* extract had the potential to suppress calla lily soft rot caused by *Pectobacterium carotovorum* subsp. *carotovorum.* The product can provide an alternative means to chemical bactericides, which have been found to be unfriendly to the ecosystem in addition to being expensive.

CHAPTER SIX

Summary of Research Findings, Recommendations and Areas for Further Research

6.1 Research Findings

Based on observations made in this research work undiluted concentration of *Coptis chinensis* Franch extract product (Dumiza 0.5% SL) inhibit *in vitro* development of *Pectobacterium carotovorum* subsp. *carotovorum.* Pre-plant tuber spraying with *Coptis chinensis* Franch extract potentially suppresses calla lily soft rot severity, promotes leaf numbers, plant height, tuber sizes and production long-stemmed flower stems. The response was dose-dependent with highest concentration of *C. chinensis* recording lower percent disease incidence and improved quality of both flower stems and tubers.

Media drenching with *Coptis chinensis* Franch extract had a significant effect on calla lily soft rot disease, growth, yield and quality. Drenching plants with the extract immediately from planting resulted in increased number of the harvested stems, suppressed the soft rot disease and increased the stem length, and tuber size. When plants were drenched with the extract from the full-foliage stage, there was an increase in plant height and a decrease in soft rot disease severity. *Coptis chinensis* extract application performed in a similar manner as copper hydroxide for most of the parameters evaluated.

6.2 Recommendations

Before planting, calla lily growers should spray the tubers with a 2. L ha^{-1} *Coptis chinensis* extract in order to promote plant growth and quality and suppress calla soft rot. Drench application of the *Coptis chinensis* extract product is recommended at a concentration of 2.5 L ha^{-1} in order to promote plant growth, improve calla lily flower and tuber quality besides suppressing calla lily soft rot. Drenching the plants from immediately after planting at a two-week interval is the most preferred mode for maximum benefits on the above-mentioned traits.

6.3 Areas for Further Research

From the study, the variables measured responded in a dose-dependent manner and therefore other rates of the extract should be investigated to determine their performance. Also, the *Coptis chinensis* formulated product should be analysed for chemical constituents in order to quantify all the bioactives present in a given volume of the product.

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