USE OF AMINO OLIGOSACCHARINS AND ALTERNARIA ACTIVATED PROTEIN IN MANAGEMENT OF CROWN GALL AND ENHANCEMENT OF GROWTH IN ROSES

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DECLARATION

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This thesis is dedicated to:

My late mother Mary Musindalo Keya who walked me through this journey of education and ensured I become the best in whatever I do. Continue dancing with the angels mum.

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ACRONYMS AND ABBREVIATIONS

BLIS	Bacteriocins and bacteriocins-like inhibitory substances
DNA	Deoxyribonucleic acid
EU	European Union
GDP	Gross Domestic Product
HCDA	Horticultural Crops Development Authority
KFC	Kenya Flower Council
PAMPS	Pathogen Associated Molecular Patterns
PTI	PAMP-Triggered Immunity
RCBD	Randomized Complete Block Design
SAR	Systemic Acquired Resistance
WP	Wettable Powder

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ABSTRACT

Rose is cultivated for its beautiful flowers and is considered as the most prized flowers in the world because of its high ornamental and commercial value. The demand for cut flowers has risen in recent years, however, production of cut flowers face many challenges and the most important amongst the challenges is environmental conditions and diseases that hinders production. Crown gall disease caused by *Agrobacterium tumefaciens* causes a significant damage to roses in Kenya. The study was carried out to determine the effects of a mixture of amino oligosaccharins and alternaria fine proteins on the growth and quality of rose and crown gall disease on roses. The experiment was laid out in a Randomized Complete Block Design (RCBD) with four replications and five treatments comprising of three levels each of amino oligosaccharins and alternaria fine protein (0.5, 1, 1.5g/litre) and copper based fungicide (2ml/ litre) along with control (water spray) in the greenhouse. Crown gall tissues were gathered from four different roses per treatment in the two sites, counted and the size determined and recorded fortnightly. Bacteria were isolated from eight different galls gathered from four different plants. Representative colony types identified via their growth on selective media (MacConkey agar) and growing on nutrient agar media were chosen from each gall sample and sub cultured by streaking on new nutrient agar media. Biochemical test of different isolates was done to identify the bacterial isolates. Data was collected on rose height, number of shoots, disease incidence and severity, flowering duration, stalk diameter, flower head diameter at cut stage, colony types, and on biochemical analysis and was subjected to analysis of variance.

There were significant differences in the treatments applied, application of a mixture of amino oligosaccharins and alternaria fine protein at the rate of 1.5g/litre had significant ($P \leq 0.05$) effect on number of shoots, plant height, flowering duration, stalk diameter as well as flower head

diameter at cut stage. Maximum number of shoots after six weeks 13.6 and 13.9 was recorded in plots treated with amino oligosaccharins at the rate of 1.5g/litre in Winchester farm (Nairobi) and Bahati farm (Nakuru) respectively. However, maximum height (61.3cm) in Winchester and (59.6cm) in Bahati was recorded in plots treated with amino oligosaccharins at the rate of 1g/litre. Plants receiving amino oligosaccharins sprays at the rate of 1.5g per litre recorded significantly ($P \leq 0.05$) increased stalk length (27.7cm) in Winchester, (29.6cm) in Bahati and stalk diameter (0.48cm) in Winchester (0.40cm) in Bahati, had shortened flowering duration (19.2 days) in Karen, (11.6 days) in Bahati farm. Application of amino oligosaccharins at 1.5g/litre had significant ($P \leq 0.05$) effect on galling formation and reduced the numbers from 2.49 to 1.4 in the eight week and 1.08 in the tenth week in Winchester while in Bahati, the number reduced from 1.54 to 1.03 ten weeks after treatment application. The gram reaction indicates that the selected isolates were gram negative and were positive for catalase, motility, lactose, oxidase, salt tolerance and mannitol tests.

Exogenous applications of amino oligosaccharins and alternaria fine proteins in the greenhouse influenced positively the growth of roses and brought about significant decrease in the number of galls and size as well as improvement in plant growth. The ability of the treatments to manage the disease can be attributed to enhanced defense mechanism attributable to amino oligosaccharins and alternaria fine proteins.

Key words: *Agrobacterium tumefaciens*, chitosan, crown gall, oligosaccharins, proteins roses

CHAPTER ONE

INTRODUCTION

1.1 Background

Horticulture is an important sub-sector of Kenya's economy and a key index of achieving vision 2030. The floriculture industry contributes vastly to the country's gross domestic product (foreign exchange) as well as income and employment generation. In Kenya, the horticulture sub-sector contributes 33% of agriculture's share of Gross Domestic Product (GDP) (Maina *et al*, 2011). According to the statistics by Ministry of Agriculture (2010), the domestic value of horticultural produce was estimated to be Ksh. 153 Billion with earnings from flower exports being Ksh. 35.5 Billion. It was also noted that the value of Kenyan horticultural exports grew exponentially at an average rate of 15.9% between 2001 and 2010. Kenyan flowers account for 38% of flowers that are auctioned in Europe and roses make up 74% of Kenya's flower exports (KFC, 2016).

Roses are utilized in the export market for aesthetic gratification among other purposes and thus need to monitor the quality for customer satisfaction. The increased flower production and improved quality are the most important objectives to be recognized in cut-flower production as these are the key parameters that customers consider. The profit obtained in flower investment is also a function of flower yield and quality (Sardar, 2007). Among the factors that compromises the yield and quality of roses includes *Agrobacterium tumefaciens*. It is an aerobic gram negative that is rod in shape and a motile non-sporing bacteria. It is also soil inhabiting bacterium known for its disease inducing ability on numerous crops**,** for example apple, cherries, pear, grapes, peaches, apricots and ornamentals like chrysanthemums and roses (Kado, 2002). Vegetables such as sweet peppers and tomatoes are also infected by this bacterium. It causes an increase in

the size of the cells on parts of the susceptible plants especially at the roots and crown region that results to gall formation on the affected parts. The cause of the swellings is the presence of plasmids that have the ability to induce tumors when taken up by a susceptible host (Deacon, 2000).

The disease causes losses of economic importance on susceptible crops and this is as a result of its capabilities to move systemically all through the plant; from the roots to various parts of the plant and can completely kill the plant. Gall development is as a result of interference by growth hormones, the auxins and cytokinins produced by the infective *A. tumefaciens* once inside the host cell (Jochen and Rosalia, 2014). The infected host cell proliferates uncontrollably at the expense of the host. The bacterium uses the host systems to produce compounds for their growth thus keeping them active in the host (Maina *et al,* .2011). Once the bacteria enter the wounds into the plant, it takes about two weeks for the galls to start appearing (Kado, 2002).

It is difficult to control the bacteria as the effective chemicals against the bacteria are antibiotics that are shared in between human beings and animals. Plants possess an immune system which allows them to defend themselves against microorganisms such as fungi and bacteria (Boller and Felix, 2009). A key step of the microorganism detection is the activation of a defense reaction by the highly conserved molecular patterns called Pathogen Associated Molecular Patterns (PAMPS) which are secreted by microorganisms during interaction with the plant (Chistoholm *et al*. 2006).

According to Jones and Dangl, (2006), the perception or engagement during pathogen infection process, triggers defense reactions termed PAMP-triggered immunity (PTI) and so they are considered as compounds that are able to induce plant defenses. The general elicitors of plant

defenses can be from classes of compounds such as carbohydrates, (glyco) peptides, lipids and (glyco) proteins (Sophie *et al*., 2014).

The roles of sugars as elicitors of plant defense are an area of interest to many at the moment as fungicides remain largely as the only method of disease control. The fungicides have environmental related concerns, have effect on human health and therefore there is a need to research on a new mechanism to control plant diseases. Plant immunity inducers have been safely used on vegetable crops, fruits and tobacco and have been shown to trigger the regular plant defense system resulting in disease management (Perazolli *et al*., 2011).

1.2 Statement of the problem

Among the major factors affecting rose productivity, *A. tumefaciens* is a serious constrain that results in the affected rose plants becoming stunted, generally lack the vigor of healthy roses and produce fewer marketable stems as the bacterium affects production through reduced number of good quality stems, reduced length of harvestable stems and production lifespan. Despite the losses being incurred by growers in Kenya and around the world, there are limited options to control the disease and the common practices have been consistently unable to solve the problem. The current control measures involve the use of copper products that do not offer a long term management measure, are expensive and unfriendly to the environment as they cause pollution. In addition, there is also scanty information availed to farmers on management options as a result of limited research on the issue.

Eco-friendly and sustainable management measures against *A. tumefaciens* are therefore important so as to be able to produce roses of high quality; longer stems more marketable stems and vigorous crop that is able to survive the disease. Production lifespan would also be improved

as a result of improved plants immunity. The development of plant immunity is therefore important so as to maintain a healthy crop growth and reduce utilization of chemicals.

1.3 Justification

Kenya floriculture industry has always been a profitable venture with average revenue for cut flower increasing by 12% as compared to last year (Flora data, 2017). The subsector is also the fastest growing (Gitari, 2010). Roses are high value crops with a high production per metre square and based on above provided revenue estimates; Kenya has grown to become the major cut flower producer in Africa apart from being the third biggest grower and exporter to the European Union (EU). Kenya produces nearly 200 million stems per year in terms of cut flower and from these, roses account for 150 million stems. This therefore places roses at a premium as it controls a larger market share of the country's export i.e. 70% of all exported trees, foliage and buds (Muhammad, 2009).

Despite all the attributes, Kenya is still faced with myriad of challenges to supply good quality roses. Pests and diseases have been on the increase and among the diseases infecting roses, crown gall caused by *A. tumefaciens* causes a significant damage with no reliable management mechanism. The pathogen can survive for at least two years in soils and galls without a host and is recognised by round tumours in the crown region, on stems and on leaves. The tumours are white, black to brown in colour appearance and will mostly prevent shoot emergence from cut point. Rose plants that are infected will manifest a slower growth, chlorotic leaves, stunting as well as a failure to produce healthy flowers. Because of such deficiencies, infected plants tend to develop sensitivity to environmental stresses (Jochen and Rosalia, 2014).

Adjacent healthy tissues are destroyed and supply of water and mineral salts are compromised. Despite the losses being incurred by growers in Kenya and around the world, there is limited scope of the mechanisms to control the disease. In addition, there is also scanty information availed to farmers on management options as a result of limited research on the issue. The new plant immune elicitor containing amino oligosaccharins (3%) and *Alternaria* fine protein (3%) has been used to induce plants own immune system, promote plant growth, improve growth as well as to increase production in tomato and tobacco. The results in roses however, have not been documented. The success of this research will therefore be of great significance considering the larger market share that roses influence in the international market as quality and quantity will be improved. Growers of fresh cut flowers will benefit from use of environmentally friendly product in their farms that also increases the yield potential. As a result, the environment will be safer and more income will be realized through the use of this product. The products are also natural and have a high degree of acceptance by consumers (Perazolli *et al*., 2011).

1.4 Objectives

The general objective of this study was to contribute towards management of crown gall on roses using amino oligosaccharins and Alternaria fine protein.

The specific objectives of the study were;

1. To evaluate the effects of amino oligosaccharins and Alternaria fine protein on crown gall disease.
2. To determine the effects of amino oligosaccharins and Alternaria fine protein on growth and quality of rose plant.

1.5 Hypotheses

1. Amino oligosaccharins and Alternaria fine protein have no effect on *Agrobacterium tumefaciens*

2. Amino oligosaccharins and Alternaria fine protein have no effect on growth and quality of rose plant.

CHAPTER TWO
LITERATURE REVIEW

2.1 Global cut flower industry

Worldwide market for cut flowers has grown tremendously since the mid-1980s with the total area apportioned to cut flower production overall being currently more than 200,000 ha and roses, chrysanthemums and carnations dominating (Van Rijswick, 2015). Netherlands remains the largest producer of cut flowers worldwide although developing countries such as Ecuador, Kenya, Zimbabwe and Colombia have turned out to be solid players in worldwide markets. Exports from the developing countries by 1998 accounted for 29% of the world total and provided employment to approximately 190,000 people (Van Rijswick, 2015). Flower industry in Kenya is the third largest exporter of flowers by volume and value behind the Netherlands and Colombia on a global level (Rikken, 2011). It is ranked second in foreign exchange earnings, with cut flowers dominating the sub-sector (Bolo *et al*., 2006). Statistics by (Van Rijswick, 2015), ranked Kenya as the top most supplier of cut flowers to the Dutch flower auction, representing 44.6% of total supplies.

2.2 Kenyan flower industry

The flower industry contributes approximately half of fresh horticultural exports with an estimated direct employment of over 90,000 and 500,000 in related industries (Arim, 2011). The industry is dominated by large-scale production which contributes 97% and small scale contributing a paltry 3%. The main focus of production in the country has been large scale and export market. Cut flower is one of the two commodities where large-scale production dominates in the country (Nyangito, 2008).

The industry is generally viewed as an incredible achievement and a model of the advantages of export oriented production due to its profitable nature (Arim, 2011). The production costs of growing flowers in Kenya is generally offset by the value attached to it as the production per hectare is incredibly high (Arim, 2011). According to (Hale, 2005), the industry is ranked the fastest developing agricultural sub-sector in the country and is also second largest agricultural source of foreign exchange. The yearly mean revenue generated from sale of cut-flowers is at 350 million US $ and this represents in general the extent of Kenya's economic growth according to Canadian Council, (2008).

The latest statistics by the Kenya Flower Council (KFC) shows that the flower exports in the country commands 32% of the European Union market. There are over one hundred and forty flower growers in Kenya and the major flower-growing areas are Thika, Athi River, Limuru, Nairobi, Mount Kenya region, Naivasha, Nakuru, Nanyuki, and Eldoret. The total value of horticultural exports represented by roses stands out at 35% according to (HCDA 2013). These makes roses to stand out as single most important export product, followed by carnations especially on non-romantic holidays. Most of the farms are fair trade certified making it easy for Kenya to be recognized as the largest supplier of Fair trade flowers to Europe, representing 50-60% of total volumes (Patton, 2008).

2.3 Abiotic and biotic factors influencing rose flower development

In commercial production of roses, yield is constrained by abiotic factors such as mineral nutrition, light, humidity, temperature and salinity (Lorenzo *et al*., 2000). In a controlled environment and a hydroponic system, they are optimally supplied with nutrients and are arguably oversupplied with nitrogen (Cabrera, 2003). Roses that are grown in a garden are

adaptable to a range of nutrient conditions although deficiencies may occur due to extreme conditions like low or high pH that compromises the availability of nutrients (Roxburgh, 2008).

Biotic factors common in cut-flower roses include pests and diseases Fungal endophytes occur in the vascular bundles of hybrid tea rose (*Rosa hybrida*) leaves (Salgado *et al*., 2007) and a fungal disease like stem canker result in dieback of the stem due to entry of spores through wounding of the crown (Botanic Gardens Trust, 2008). Other biotic factors include diseases like downy mildew (*Peronospora sparsa*), powdery mildew of roses (*Podosphaera pannosa*), grey mold (*Botrytis cinerea)* weeds, (Karlik, 2008), invertebrate pests such as thrips (*Thrip imaginis*) and nematodes (*Meloidodgyne* spp.) (Reid, 2005).

Amongest the major diseases affecting the industry is the crown gall disease of roses caused by *Agrobacterium tumefaciens*, a bacterium member of the family- *Rhizobiaceae* and is widely distributed (Furuya *et al*., 2004). The members of *Agrobacterium* spp. and *Rhizobium* spp. differ in some aspects of their chromosomal structure (Goodner *et al*. (2001). Farrand *et al*. (2003) reported that the data of Jumas-Bilak *et al*. (1998), on comparison of the genomic structures of *Agrobacterium* spp., *Rhizobium* spp. and *Sinorhizobium* spp. supports the differentiation of *Agrobacterium* spp. from *Rhizobium* Spp. Several studies have shown that the bacteria can effectively be isolated from stem, crown and leaves of roses (Aysan and Sahin, 2003).

2.4 Crown gall disease as a constraint to flower production in Kenya

There are many constraints to flower production in Kenya. Among them are pests and diseases and among the diseases, crown gall causes a significant damage to roses in Kenya. The bacteria are found in the soil and is responsible for the tumorous growth found in infected plants (Deacon, 2002). It is a widespread naturally-occurring bacterium which causes galling in many

plant species and has the potential to introduce new genetic material into the plant cell (Gelvin, 2003). They are also widely distributed in the nursery and even in non-nursery conditions and do affect a variety of other crops.

In Kenya, its incidence was first noticed in 1998, a time when many flower farms which had entered into commercial production of roses were severely affected by the outbreak of the disease. Research conducted by Smit (2011) indicated that the disease was introduced for the first time in Kenya through infected rose root stock that were imported from Israel. Smit further reveals that the disease is spread in production sites, nurseries as well as in uncultivated fields in Kenya. Despite losses incurred in flower exports due to effects of diseases and high residual effect of pesticides, empirical research especially focusing on management of *Agrobacterium tumefaciens* is limited. Therefore, there is paucity of information to farmers on the best practices to reap maximum profits (Maina *et al*., 2011).

Various remedial methods have been employed worldwide to control *Agrobacterium tumefaciens* and these include the use of a closely related strain of the bacteria *Agrobacterium radiobacter* (Riley and Wertz, 2002). The utilization of bacteriocins has been reported to be one of the safest methods to control the disease. Bacteriocins have been described as extracellular macro-molecular proteins or peptides antibiotics that are produced by certain bacteria that exert their lethal effects on bacteria of the same or related groups (Russel, 2002). This is in consideration of the risks in using antibiotics and broad- spectrum agro-chemicals to control the plant pathogens. Thus, bacteriocins are preferred due to their attributes that are considered desirable for microbial control. Riley and Wertz, (2002) reported inhibitory nature of bacteriocins against wide range of

bacteria. Bacteriocins and bacteriocins-like inhibitory substances (BLIS) are medically, industrially and agriculturally very important according to their studies.

Most of the phytopathogenic bacteria including members of the *Pseudomonas, Corynebacteria, Xanthomonas, Erwinia* and *Agrobacterium* produce proteinaceous bacteriocins (Heu *et al*., 2001). The bacteriocins produced are specific, safe for the users and the environment, cost effective and are effective for agricultural use in controlling plant diseases. Genetically modified *Agrobacterium radiobacter* releases a bacteriocin (agrocin) active against *Agrobacterium tumefaciens* which prevents the formation of crown gall tumors in the infected plants (Kado, 2002).

A study conducted in Australia by Ryder and Correll (1995) to investigate the effectiveness of using nopaline-producing strains of *Agrobacterium radiobacter* in controlling the disease found it to be a highly effective biological system of control. This method of control is now used world-wide though the practice is yet to be embraced in Africa and especially Kenya. In general, diseases of bacterial nature in plants are difficult to control due to lack of effective chemicals for their management. There is need therefore to explore *Agrobacterium radiobacter* isolates naturally occurring in Kenya which has potential effect on the management of *Agrobacterium tumefaciens*.

2.5 Crown gall infection process

The symptoms of crown gall are white masses of callus tissue that appear on roots, at the base of the stem and anywhere wounds occur. Formation of galls on the parts may be seen about 8-12 days after infection and once the bacterium enters its host, it will inject a section of its DNA called the T-DNA (tumor inducing) plasmid into its host (Moore *et al*., 1997). The injection is

followed by incorporation of the TDNA into the genome of the plant triggering the cells into making auxins and cytokinins that causes the cells to be irregular in shape and hence form a visible tumor. The bacterium can be moved by propagation tools between plants as well as wounds created during grafting create opening for the bacteria. It can also be moved by water splash although natural opening or wound is required for penetration into the plant tissues. Infection is also associated with infested substrate or contaminated irrigation water that finds its way through the plant root system (Dimitre, 2012).

2.6 Economic importance of crown gall disease

Crown gall is a common disease mostly affecting the dicot plants. Many of these includes woody shrubs, herbaceous plants, pome and stone fruit trees, grapevines and ornamental plants like roses (Rhouma *et al*., 2006). It also affects some monocots and gymnosperms and the galls produced provides a nutrient-rich growth environment for the bacterium that returns to the soil as they decompose (Pitzscke and Hirt, 2010). It produces crown gall disease in over 600 species of trees (Wang *et al*., 2000).

2.7 Pathogenesis

In the soil with the help of flagellum, *Agrobacterium spp*. swims towards photo assimilates found around the root regions of the plants. The bacterium typically infects plants with wounds where the release of plant saps attracts them to the wound by positive chemotaxis as the sap contains sugars, amino acids and organic acids. Once the bacterium reaches the wound, it gets attached to the plant surface by synthesizing cellulose fibers (Tzfira and Citovsky, 2007). *Agrobacteria* contains an extra chromosomal DNA that is designated as Ti (tumor inducing)

plasmid which carries two components: vir and T-DNA regions that are needed for genetic transformation (Tzfira *et al*., 2004).

The molecular apparatus that is needed for T-DNA generation and its transport into the host cell is generally comprised of proteins which are encoded by the bacterial chromosomal virulence (chv) genes and the Ti-plasmid virulence (vir) genes (Gelvin, 2003). In addition to these, phenolic compounds such as acetosyringone (AS) and coniferyl alcohol are produced by wounded plants (Lee and Gelvin, 2008) and triggers the bacteria to produce T-DNA, by a two component signaling system (virA/virG).

Krispin *et al*., (2007) noted that adhesion and induction of the bacterial virulence (vir) machinery is brought about by phenolic compounds that are released from wounded plant tissue. Acetosyringone activates virA, a membrane bond receptor, which activates the virG (transcription factor). The activated virG can then interact with activator elements found in the promoters of the virA, virB, virC, virD, virE and virG operons, resulting in elevation of their expression levels and ends with the expression of its T-DNA integrated in the host genome where the host facilitates it's in corporation into the genome, mediated by DNA repair system (Karami *et al*., 2009).

In cases of tumorigenic strains, it comprises of the fragment transfer of bacterial tumor-inducing plasmid into the plant cell and its consolidation into the genome of the plant. Ti plasmids are approximately 200 kilo bases (Kado, 2002). The T-DNA moreover carries qualities for the biosynthetic proteins for generation of unordinary amino acids, regularly octopine or nopaline. *Agrobacterium tumefaciens* too carries genes for the biosynthesis of the plant hormones (auxins and cytokinins) and for the biosynthesis of opines, giving carbon and nitrogen source for the

microbes that most other micro-organisms cannot utilize, this gives them a specific advantage (Andrea and Heribert, 2010). By modifying the balance of hormones within the plant cell, their cell division cannot be controlled by the plant, hence pronounced tumor formation. The proportion of auxins to cytokinins delivered by the tumor genes decides the morphology of the tumor.

Tumors hinder physiological capacities of the plant such as transport of water and nutrients and when expansive tumors are formed, many plants show a reduction in growth and appear stunted. In addition, breakdown of tumours inflict wounds on roots which become entry points for other pathogens. (Bliss *et al*., 1999). Infected plants appear unfit for the market. The most noteworthy losses are recorded in young plants, those still within the nursery. Infected plant materials can be disposed of but healthy looking infected plants from the same nursery can be obtained and planted by producers thus spreading the disease (Dimitre, 2012).

2.8 Disease cycle

Infection starts from infected soil where a susceptible host is planted. Other sources of inoculum are irrigation water, infected planting materials, pruning equipment, and cultivation equipment and rogued plants and detached or disintegrated galls put back in the soil. Lack of disinfection of pruning tools by field workers is a common means of bacteria dissemination (Agrios, 2005). Epiphytic growth phase is eminent prior to disease development and the pathogen will attach itself to the surface of the plant and proliferates until an entry site is obtained so as to overcome the structural defense barriers provided by cuticular or cell wall (Agrios, 2005).

Once the bacteria enter the wound into the plant, it takes about two weeks for the galls to start appearing. The gall cells are not protected by an outer epidermal layer and with time they start

cracking and become brittle and start disintegrating. Old galls darken and look rugged and sometimes become infested with insects that feed on the cells. Eventually they fall off back into the soil and are released to start the infection cycle once a host is replanted and the conducive environment of wounds occur (Montesinos *et al*., 2002).

2.9 Population diversity of *Agrobacterium tumefaciens*

They are broadly spread in soils and other natural surroundings and can be isolated from untilled soils (D'Hondt et al. (2004). A few soils are found to be tolerant to the bacteria and these soils permit the long term perseverance of pathogenic *Agrobacteria* to proceed for a few decades (Rhouma et al., 2008). Permissive soils are those most favorable to any Agrobacteria with more than 104 cfu/g of soil amid warm seasons (Krimi et al., 2002). Apart from diseased foci, the incredible larger part of soil *Agrobacteria* is, in any case, not pathogenic (Sobiczewski et al., 2005). Other species of the genus *Agrobacterium* include; *Agrobacterium rhizogenes* which induces root tumors and carries the distinct Ri (root inducing) plasmid, *Agrobacterium vitis* causes gall in grapevines, *Agrobacterium rubi* (cane gall) and *Agrobacterium radiobacter* (non-pathogenic). There are numerous other species of the genus *Agrobacterium* either within or out of the *Agrobacterium tumefaciens* complex such as unnamed species that includes strains NCPPB 1650, or the novel species that got the epithet skierniewicense (Pulawaska *et al*., 2011). *Agrobacterium* more often form ecological guilds in soil that consists of several genomovars with few strains. There are more other few strains, within a single tumor (Costechareyre et al., 2010).

2.10 Management of *Agrobacterium tumefaciens*

Crown gall disease development occurs when the ideal environment, the virulent pathogen and the susceptible plant host all interact at the same time to cause the disease (Agrios, 2005).

Treatments designed to eliminate *Agrobacterium* spp. directly must necessarily be exercised before infection because disease development will progress independently of the causal agent following the initial transformation event. Various biological, chemical and cultural ways are used to minimize spread of the disease.

A-tailing is the mixture formulation using the active ingredient (A.I) Fine Alternaria activated protein and amino oligosaccharins. The content of each active ingredient is three (3%) and the product is available in formulation specification as wettable powder (WP). The activator protein has gotten registration approval from China and is currently used in rice, cotton, vegetable, citrus and cash crops to protect the crop and aid in disease resistance. It has been marketed in China with annual sales of about Hundred (100) CNY and it can promote the syntheses of chlorophyll in the plant and keep the crop healthy. The mixture is diluted using the recommended rate and sprayed into the target crop to protect it and help resist bacterial diseases.

2.10.1 Chemical control

In chemical control, use of conventional pesticides has been unsuccessful and the commonly used copper hydroxide shows inadequate efficacy (Agrios, 2005). Copper compounds produce the best known results against crown gall, but rarely provides satisfactory control because of the pathogen resistance and the phytotoxicity it causes in some plant species (Agrios, 2005). In situations in which causation of a wound is inevitable, copper or bleached-based bactericides can be used to reduce *Agrobacterium tumefaciens* populations on plant surfaces, minimizing the disease re-infection (Burr, 2004). Intercropping with a resistant variety and the abandonment of highly infested soils can temporarily reduce soil populations of *Agrobacterium* (Agrios, 2005).

2.10.2 Biological control

Biological control in *Agrobacteria* involves the application of bio-control agent, mainly an antagonizing organism, with capabilities of suppressing pathogen development (Wilson, 1997). The cost of mass production of bio-control agents is slightly low-priced as compared to chemical synthesis of pesticides (Shoda, 2000). They can easily be applied by conventional techniques like spraying or drenching and therefore their ability to proliferate and establish stable populations reduces application cost to a minimum (Montesinos, 2003).

The impact of a bio-control agent on the environment and other organisms in the ecosystem is less severe as compared to broad-spectrum pesticides. Also, the control organism can easily interact with a pathogen in many ways and provide long term positive solution to the disease potency as the risk of resistance development is reduced. *Agrobacterium radiobacter* is used worldwide as a commercial agent for bio-control of crown gall disease caused by tumorigenic *Agrobacterium* strains (Farrand, 2003). One of the latest discoveries on bio-control mechanisms is quorum sensing silencing. Many virulence determinants are regulated in a cell-density dependent manner. In gram-negative bacteria the quorum sensing signals are N-acyl homoserine lactones (AHLs). K84 produces Agrocin 434 and Antibiotic-like (ALS) range of control beyond nopaline type *Agrobacterium tumefaciens* (Penyalver *et al*., 2002).

Agrocin-84 (produced artificially in laboratories) mimics agrocinopine A and is taken up by the same transport system used up by *Agrobacterium tumefaciens* to utilize agrocinopine A. Inside *Agrobacterium tumefaciens* cell, the antibiotic agrocin-84 inhibits DNA replication and cellular growth. Another improved method was by use of genetic modification by deletion of the gene responsible for exchange of DNA material among bacteria which resulted to the development of

a new strain, *Agrobacterium radiobacter*; strain K1026 in Australia (Manual of Bio-control agents, 2004). This strain is not capable of conferring resistance to other *Agrobacterium* that are sensitive to the agrocin antibiotic.

2.10.3 Cultural control

Agrobacterium tumefaciens can also be controlled by use of agronomic practices that prevents unnecessary plant wound that would significantly reduce crown gall by denying the pathogen an opportunity to introduce T-DNA into plant cells (Agrios, 2005).

Field hygiene is basic in each action meant to minimize rates of *Agrobacteria*. The bacteria can be culturally managed by avoiding introduction of contaminated nursery root stocks. Exercises that make wounds on plants ought to be avoided as this offers entry point for attachment of the bacteria. It is subsequently critical to avoid injuring the plant amid the normal field agronomic practices such as weeding and pruning. Pruning and harvesting apparatuses such as secateurs ought to be cleaned from one plant to another (Dimitre, 2005).

2.10.4 Use of plant immune inducers in control of plant diseases

Immunity inducers are a class of immune-active compounds that can induce systemic acquired resistance (SAR) in plants. Plant immune inducers include chitosan oligosaccharides, plant immunity-inducing proteins and microbial inducers. These compounds and micro-organisms can trigger defense responses and confer disease resistance in plants (Qiu *et al*., 2017). The biological active molecules will be active in small molecules produced during the interaction between a pathogen and its host and includes the metabolites such as; glycoproteins, glycopeptides, oligosaccharides, proteins as well as lipids and polypeptides. They trigger defense responses resulting in systemic resistance by the plants (Heese *et al*. 2007).

In their studies on resistance by plants, (Wei *et al*., 1992) noted that plant resistance was induced by a protein in the fire blight bacterium named the hypersensitive protein (Harpin). Oligosaccharides have been widely used in China as plant immune inducers (Qiu *et al*., 2017). The use of sea weed extract, chitosan oligosaccharides and trehalose as immune inducers have shown to confer disease resistance in a variety of crops (Tsutsui *et al*., 2015). Besides oligosaccharins, Harman *et al*., (2004) worked on micro-organisms (*Trichoderma sp*.) and found out their vast ability to induce plant immune response and trigger plant resistance to subsequent pathogen attacks. Such studies of micro-organisms as inducers of plant immune and conferring of resistance were further enhanced by (Zhang and Zou, 2010) by their activities on a bacterium (*Bacillus subtilis*) and a fungus (*Trichoderma sp*.) which can induce a microbe triggered immunity response in plants.

2.10.5 Plant immunity proteins

Recent studies conducted by (Zhang *et al*., 2011b) depicted the activity of the Fungus *Alternaria alternata* as an enhancer of plant disease resistance. The protein obtained in the fungus (PeaT1) improved plant resistance against viruses and therefore reduced viral diseases by 80% as well as increased the yields by 70%. A plant immunity-inducing agent (ATaiLing) was registered as a bio-pesticide in 2014 and has been utilized by agriculturists and in field trials and the result has been a lessening in viral and bacterial diseases by more than 70% and increment in yields of vegetables, fruits and tea crops by more than 10% (Qiu et al., 2017).

Through research conducted by (Djonovic *et al*., 2006), proteins from *Trichoderma* virens has been used to induce immune responses in cotton cotyledons, the synthesis of hydrogen peroxide, autoflourescence and an increased local and systemic transcription of defense genes. Through

Agrobacterium-mediated transient expression, the cerato-platinin from *Magnaporthe grisea* has been shown to induce a systemic defense response (Yang *et al*., 2009). It also results in the accumulation of salicylic acid (SA) throughout the plant apart from conferring resistance to two different plant pathogens. The utilization of such agents improves plant resistance so that crops are less vulnerable to diseases, reduced damages by pests and therefore avoids problems of environmental pollution (Zhang *et al*., 20011b).

CHAPTER THREE

EFFECTS OF AMINO OLIGOSACHARINS AND ALTERNARIA FINE PROTEIN ON CROWN GALL DISEASE OF ROSES

3.1 Abstract

Rose production is limited by a variety of factors such as poor mineral nutrition and high salinity, pests and diseases. Crown gall disease caused by *Agrobacterium tumefaciens* causes a significant damage to roses in Kenya. The study was carried out in Winchester farm (Nairobi) and Bahati farm (Nakuru) to determine the effects of a mixture of amino oligosaccharins and alternaria fine proteins on crown gall disease on roses. The experiment was carried out on rose variety Mariyo in a Randomized Complete Block Design (RCBD) with four replications. The treatments comprised of different rates 0.5, 1, 1.5g per litre of water of oligosaccharins and fine Alternaria protein at 3% concentration applied as foliar spray and a commonly used product in the market a copper based fungicide (Mastercop) applied at 2ml/ litre as the standard and water as a negative control. Crown gall tissues were collected from four different roses per treatment in the two sites, counted and the size determined and recorded fortnightly. Bacteria were isolated from eight different galls collected from four different rose plants. Representative colony types growing on nutrient agar media were then selected from each gall sample and sub cultured by successive streaking on nutrient agar media. Biochemical test for the different isolates was done to identify the bacterial isolates. Application of amino oligosaccharins at 1.5g/litre had greater effect on galling formation and reduced the numbers significantly from 2.49 to 1.4 in the eighth week and 1.08 in the tenth week in Winchester while in Bahati, the number reduced significantly from 1.54 to 1.03 ten weeks after treatment application. The gram reaction indicated that the selected isolates were gram negative and were positive for motility, catalase, oxidase, lactose,

mannitol, and salt tolerance tests. There was a significant reduction in the number of galls and size following application of amino oligosaccharins and alternaria fine proteins as well as improvement in plant growth. The ability of the treatments to manage the disease can be attributed to enhanced defense enzyme activity enhanced by amino oligosaccharins and alternaria fine proteins.

3.2 Introduction

Globally, the Kenyan flower industry is the third major flower exporter by value and volume just behind the Netherlands and Colombia on a global level (Rikken, 2011) while in Africa, it is the largest producer and the leading supplier of Fair trade flowers to Europe accounting for 50-60% of total volumes (Patton, 2008). The flower production in the country is mainly large scale and export market oriented (Murigi, 2010). Cut flower is one of the two commodities where large-scale production dominates in the country and roses make up over 70% of all buds, trees, roots, flowers and foliage that is exported from Kenya (Muhammad, 2009). Roses alone account for about 35% of the total value of horticultural exports and therefore form the most important export product (HCDA, 2010).

In commercial production of roses, yield is restricted by an assortment of variables such as light, humidity, mineral nutrition, temperature and salinity (Lorenzo *et al*., 2000) and biotic factors. The common biotic factors in cut-flower roses include pests and diseases. Crown gall caused by *Agrobacterium tumefaciens* causes a significant damage to roses in Kenya. The bacterium is found in the soil and is responsible for the tumorous growth found in infected plants (Deacon, 2002). The pathogen is widespread, naturally occurring soil bacterium that causes crown gall in numerous plant species in nurseries, commercial production and uncultivated areas and has the capacity to introduce new genetic material into the plant cell. Significant annual losses of

between 5-6% are faced by the flower growers worldwide due to the disease in the form of lack of vigor, reduction in foliage, low quality rose flower, low productivity and increased susceptibility of infected plants to pathogens and environmental stress (Rouhrazi and Rahimian, 2014). In other instances, losses of between 10-30% in nursery stalk have been reported in fruit trees. Both yield loss and stunting of growth may occur when seedlings or young cuttings are infected in the early stages of plant growth (Rouhrazi and Rahimian, 2014). Many strategies have been used in the management of crown gall disease including the use of chemicals, pre-plant application of soil sterilants, soil solarization, use of herbicides and soil amendments (Tolba and Soliman, 2013). Despite losses incurred in flower exports due to effects of the disease and high residual effect of pesticides, research especially focusing on management of *A. tumefaciens* is limited. The use of oligosaccharins and alternaria fine protein has the potential to be one of the safest means to manage the plant disease. Oligosaccharins induce responses that may help the plant to resist disease and have positive effects on growth and development while the alternaria fine protein accelerates plant growth vigor, increases proline content and cellulase strength. Therefore, this study sought to investigate effects of amino oligosaccharins and alternaria fine protein on crown gall disease of roses.

3.3 Materials and methods

3.3.1 Experimental Materials

The experimental material used was rose variety Mariyo both at Winchester and Bahati farms. The variety was chosen because it's high yielding and has a continuous market as compared to other varieties whose market is seasonal. It is also susceptible to *Agrobacteria* and hence more appropriate to conduct research on them. An existing infected crop was used for the research.

3.3.2 Experimental design and treatment application

The experimental design used was randomized complete block design. An existing infected crop was used for the research. Two-year-old rose of variety Mariyo planted in pumice was pruned and sprayed at two weeks' intervals with oligosaccharins and fine Alternaria protein at various concentrations. The treatment concentrations comprised of a mixture of oligosaccharins and fine Alternaria protein at 3% concentration applied as foliar spray at 0.5, 1, 1.5g per litre of water. Commonly used product in the market copper based fungicide (Mastercop) was applied at 2ml/ litre and was used as the standard and a negative control. Re-application of the treatments was done at fortnight intervals. Ten plants from each plot were randomly selected from each treatment and used for data evaluation.

3.3.3 Collection of crown gall samples

Ten galls of diameter 3-10cm were collected and put in sterile bags from two different rose farms (Winchester farm and Bahati roses) located in Nairobi and Nakuru respectively. The samples were labeled and transferred under a cool box to the laboratory in Kabete for isolation.

3.3.4 Determination of size of galls

Size of *Agrobacteria* galls formed on rose plants were measured and recorded fortnightly. This was used for disease severity determination.

3.3.5 Isolation of bacteria from the gall

Agrobacteria gall samples from treated and untreated plots having young tumors were transferred to the laboratory. Bacteria were isolated from eight different galls collected from four different plants in the greenhouses, in Bahati and Winchester farms. Small fragments of tumor

tissue were chopped and crushed with sterile scalpel blade in a few drops of sterile distilled water. The suspension was then left to stand for approximately 15 min and 100µl was spread with a loop onto the nutrient agar (NA) and incubated at 28°C. Representative colony types growing on nutrient agar media were selected from each gall sample by use of a sterilized wire loop and sub cultured by successive streaking on new nutrient agar media.

3.3.6 Disease assessment

Flower plants were randomly inspected for the assessment of crown gall disease using disease incidence and severity levels. Disease incidence was determined using the formula by Ali *et al*., (2010).

Disease incidence = $\frac{Number\ of\ infected\ plants}{total\ number\ of\ plants} \times 100$

While severity was assessed based on the size of the infection where mild – 1-3cm, moderate -2-6cm and severe – above 6cm.

3.3.7 Characterization of *Agrobacterium tumefaciens*

3.3.7.1 Biochemical test

Biochemical test for the distinctive isolates was carried out following the Bergey's manual of Determinative Bacteriology (Holt *et al*., 1994). Motility, gram stain, catalase, oxidase production, utilization of lactose and mannitol and salt tolerance (2%) tests were conducted.

3.3.7.2 Gram staining

Gram staining was done by making bacterial smears from two day old cultures on sterile microscope slides. The smears were air-dried and then after, heat fixed by passing the slides over a flame and then Gram stained as described by Beck *et al*. (1993). The slides were observed under a compound microscope at magnification of × 1000.

3.3.7.3 Utilization of lactose

Phenol red mannitol broth with 1% lactose was used. After media preparation, each test tube was aseptically inoculated using an inoculating loop and incubated at 37^{o}C for 18-24 hours. A positive result was indicated by gas production (bubbles) on inverted durham tube.

3.3.7.4 Motility test

A semi solid agar medium was prepared in a test tube and inoculation done by use of a wire loop to make a single stab at the centre of the tube. Incubation at 37^{o} C (conditions favoring motility) were carried out and examined at intervals (6hours, 24 hours and 48hours).

3.3.7.5 Growth on MacConkey agar

Isolates were introduced into agar plates containing MacConkey agar by use of a sterile wire loop. Pink colonies on the MacConkey agar confirmed the bacteria to be gram negative.

3.3.7.6 Salt tolerance

The nutrient broth was prepared by use of sodium chloride (65g) (1%, 2%, 3%, 4%, and 5%) and dextrose (1.0g) and allowed to settle at room temperature. Two to three colonies of the isolates were picked and inoculated into the broth and incubated. Turbidity was observed after 12 and 24 hours.

3.3.7.7 Oxidase production

A filter paper was soaked with the substrate (tetramethyl-p-phenylenediamine dihydrochloride) and moistened with sterile distilled water. The bacterial colony was then picked using a wooden loop and smeared on the filter paper. Appearance of deep-purple colour was observed after 15 seconds.

3.3.7.8 Catalase test

Slide method test was used for catalase test. Small amount of bacterial colony was transferred to the surface of a clean, dry glass slide by use of a sterile wire loop and a drop of 3% hydrogen

peroxide added onto the slide and mixed. The rapid elaboration of oxygen bubbles after five (5) seconds confirmed the bacterium catalyzes the breakdown of oxygen from hydrogen peroxide.

3.3.7 Pathogenicity test

Isolates suspected to be *Agrobacterium tumefaciens* were inoculated onto an indicator carrot disc bioassay and potato disc bioassay. The same was inoculated onto healthy rose plants and observed.

3.3.8.1 Carrot disc assay

Carrots were obtained from a local market in Rongai. The collected carrots were then sterilized with 1-2% sodium hypochlorite and rinsed in three changes of sterile distilled water. Aseptically, the discs were prepared by chopping into smaller discs using a sterile surgical blade. The discs were placed in sterile petri dishes lined with sterile filter paper. Soriful *et al*., (2010) procedures were followed and one hundred microliters of the inoculum was overlaid on each disc and sealed with parafilm and incubated in growth chamber at 28^0C for 3 weeks with continuous observation for tumor formation from meristematic tissues around the vascular system.

3.3.8.2 Potato disc assay

Potatoes were obtained from local market in Rongai. The collected potatoes were sterilized with 1-2% sodium hypochlorite and rinsed in three changes of sterile distilled water. Aseptically, the discs were prepared by chopping into smaller discs using a sterile surgical blade. The discs were placed in petri dishes containing water agar. According to Munsina *et al*., (2010) recommendations, each disc was overlaid with 100µl of the inoculum and sealed with parafilm and incubated in growth chamber at 28^0C for three weeks with continuous observation. The discs were then stained with 5% Lugols Iodine for thirty (30) minutes and examined under microscope for tumor formation, where there was tumor formation, the starch was utilized.

3.3.9 Data analysis

The data was analyzed using statistical analysis software Genstat Discovery Edition 15 (VSN International Ltd. 2015). Treatment means were compared using revised LSD test at the 0.05 level of probability using Fisher's protected LSD.

3.4 Results

3.4.1 Effect of amino oligosaccharins and alternaria fine protein on the incidence and severity of crown gall

Table 3.1 shows severity of crown gall as affected by amino oligosaccharins and alternaria fine protein treatments. Disease severity was categorized into mild, moderate and severe. Flower plants treated with various rates of amino oligosaccharins and alternaria fine protein had mild severity rates compared with the control plots in both sites. The incidence of the disease was assessed fortnightly for ten weeks. In Winchester, there was no significance differences in plots applied with amino oligosaccharin and alternaria fine protein at the rate of 1.5g/litre and plots applied with copper based fungicide. These plots showed a pattern of crown galls reducing tremendously after the tenth week. In Bahati however, plots treated with amino oligosaccharin at 1.5g/litre showed reduced number of galls on the tenth week after application compared with other treatments. The least incidence was observed in control plots in both farms.

Table 3. 1: Severity of crown gall affected by various treatment applications

Treatment	Disease Severity		
	Mild	Moderate	Severe
Winchester			
Amino oligosaccharins 0.5g/litre	69.0bc	14.8b	14.8cd
Amino oligosaccharins 1g/litre	59.1c	19.7a	19.7cd
Amino oligosaccharins 1.5g/litre	83.7a	9.9c	4.9d
Copper at 2ml/litre	93.6a	4.9d	4.9d
Control	9.9e	14.8b	73.9a
Bahati			
Amino oligosaccharins 0.5g/litre	57.1c	19.7a	19.7cd
Amino oligosaccharins 1g/litre	64.0bc	9.9c	24.6c
Amino oligosaccharins 1.5g/litre	78.8ab	4.9d	14.8cd
Copper at 2ml/litre	83.7a	14.8b	4.9d
Control	29.6d	14.8b	54.2b
Mean	63.1	12.8	23.6
LSD (P≤0.05)	18.6	3.8	16.3
CV (%)	25.9	5.3	22.8

Means followed by the same letters along the columns are not significant at 5% level of probability

Table 3. 2: Disease Incidence Progress as affected by various treatment application

Treatments	Average number of galls				
	Pre- Treatment	2 weeks	4 weeks	6 weeks	10 weeks
Winchester					
Amino oligosaccharins 0.5g/litre	1.4f	1.5e	1.4g	2.1bc	3.3a
Amino oligosaccharins 1g/litre	2.1de	1.9d	1.5fg	1.3d	1.2bc
Amino oligosaccharins 1.5g/litre	3.4a	1.9d	1.7ef	1.3d	1.1c
Copper at 2ml/litre	2.5cd	2.0d	2.0cd	2.0c	1.9b
Control	1.5f	1.5e	1.9de	2.4ab	3.6a
Bahati					
Amino oligosaccharins 0.5g/litre	2.0e	2.6ab	2.3ab	2.1bc	1.9b
Amino oligosaccharins 1g/litre	2.3de	2.1cd	2.2bc	1.5d	1.0c
Amino oligosaccharins 1.5/litre	2.9bc	2.2cd	2.0cd	1.5d	1.0c
Copper at 2ml/litre	3.3ab	2.8a	2.0cd	1.6d	1.4bc
Control	2.0e	2.4bc	2.5a	2.5a	3.3a
Mean	2.34	2.09	1.95	1.83	1.97
LSD (P≤0.05)	0.49	0.31	0.24	0.31	0.74
CV (%)	0.4	0.16	0.1	0.17	0.97

Means followed by the same letters along the columns are not significant at 5% level of probability

3.4.2 Effect of amino oligosaccharins and alternaria fine protein on the galling and size of galls

There were significant differences in number of galls in each treatment and in the two sites (P≤0.05) (Table 3.2). Application of the different rates of amino oligosaccharins had greater effect on the number of galls in Bahati than in Winchester. There was increase in the number of galls in all the treatments in the second and fourth week after treatment application. However, decline in the number of galls was noticed sixth week after treatment application and it was least in plots treated with amino oligosaccharins at the rate of 1.5g/litre. Application of amino oligosaccharins at 1.5g/litre had greater effect on galling formation reducing the number significantly from 2.49 in the fourth week to 1.40 in the eight week and to 1.08 in the tenth week in Winchester while in Bahati, the number reduced significantly from 1.54 to 1.03 ten weeks after treatment application. The same can be reported for plots treated with copper at 2ml per liter. Plot with no treatment application had the number of galls increasing every week.

Table 3. 3: Effect of amino oligosaccharins at different rates on the number of galls induced by *A. tumefaciens* in Winchester and Bahati

Treatment	Weeks after treatment application				
	2	4	6	8	10
Winchester					
Amino oligosaccharins 0.5g/litre	1.99abcd	1.92de	2.05d	2.48b	2.56ab
Amino oligosaccharins 1g/litre	2.38ab	2.78ab	2.58bc	2.48b	2.29bc
Amino oligosaccharins 1.5g/litre	2.11abc	2.49bc	2.18cd	1.40ef	1.08d
Copper at 2ml/litre	2.12abc	2.42bc	2.21cd	1.50def	1.38cd
Control	2.46a	3.12a	3.12a	3.24a	3.30a
Bahati					
Amino oligosaccharins 0.5g/litre	1.54de	1.58e	1.54e	2.17bcd	2.67ab
Amino oligosaccharins 1g/litre	1.87bcde	2.04cd	2.00d	2.00bcde	2.43ab
Amino oligosaccharins 1.5g/litre	1.37e	1.54e	1.46e	1.25f	1.03dcd
Copper at 2ml/litre	1.67cde	1.50e	1.46e	1.75cdef	2.56ab
Control	1.92bcd	1.83de	2.79ab	2.42bc	3.17ab
Mean	1.67	1.70	1.85	1.92	2.43
LSD (P≤0.05) (Trt × Site)	0.51	0.47	0.44	0.71	1.00
CV (%)	18.00	15.1	15.60	22.30	25.00

Means followed by the same letters along the columns are not significant at 5% level of probability

There were significant differences in the size of galls before and after treatment applications (Table 3.3). There was significant reduction in sizes of galls after treatment application with the highest percentage reduction observed in plots treated with amino oligosaccharins at the rate of 1.5g/litre and amino oligosaccharins at the rate of 1g/litre in both sites. Application of copper fungicide as the standard also resulted in reduced gall sizes in both sites. There was significant increase in gall sizes in control plots.

Table 3. 4: Effect of amino oligosaccharins at different rates on the size of galls induced by *A. tumefaciens* in Winchester and Bahati

Treatment	Gall size before treatment application (mm)	Gall size 10 wks after treatment application (mm)	% Reduction in size
Winchester			
Amino oligosaccharins 0.5g/litre	10.0	9.3bc	7.0c
Amino oligosaccharins 1g/litre	12.2	8.6bcd	29.5abc
Amino oligosaccharins 1.5g/litre	14.0	7.6cd	45.7a
Copper at 2ml/litre	7.9	6.2d	21.5bc
Control	12.0	16.4a	-36.7d
Bahati			
Amino oligosaccharins 0.5g/litre	11.3	10.2b	9.7bc
Amino oligosaccharins 1g/litre	13.2	10.3b	22.0bc
Amino oligosaccharins 1.5g/litre	12.6	8.7bcd	31.0ab
Copper at 2ml/litre	8.9	7.4cd	16.9b
Control	9.3	14.6a	-57.0d
Mean	11.1	9.7	8.9
LSD (P≤0.05)	1.4	2.5	22.7
CV (%)	4.1	12.4	33.8

Means followed by the same letters along the columns are not significant at 5% level of probability

3.4.2 Biochemical tests

The biochemical tests for the different isolates of *A. tumefaciens* are presented in Table 3.4. The gram reaction indicates that the selected isolates were gram negative. The isolates were gram positive for motility, catalase, oxidase, lactose, mannitol, and salt tolerance tests. In terms of shape, the colonies were circular and slightly raised and were cream white in colour with smooth margins (Table 3.5)

Table 3. 5: Characteristics of the selected strains of *A. tumefaciens*

Biochemical tests	Bahati				Winchester			
	Bht 1	Bht 2	Bht 3	Bht 4	Krn 1	Krn 2	Krn 3	Krn 4
Gram stain	-	-	-	-	-	-	-	-
Motility Test	+	+	+	+	+	+	+	+
Catalase test	+	+	+	+	+	+	+	+
Oxidase test	+	+	+	+	+	+	+	+
Utilization of Carbohydrates								
Lactose	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+
Salt tolerance	+	+	+	+	+	+	+	+

+: Positive, –: Negative

Table 3. 6: Morphological characteristics of *A. tumefaciens*

Character	**Nutrient Agar**
Shape	Circular, slightly raised
Color	Cream white,
Surface margin	Smooth

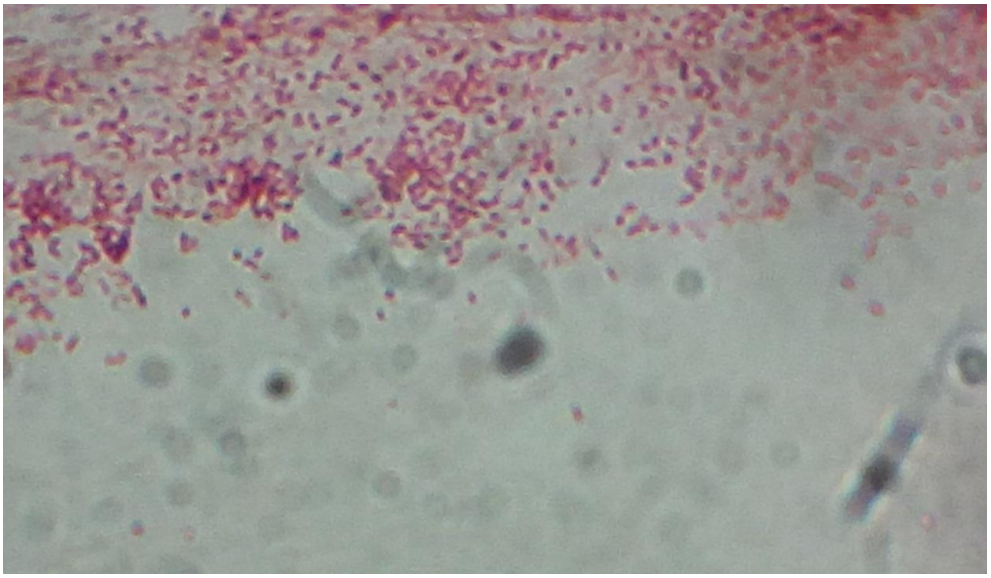

Figure 3. 1: Gram staining of *Agrobacteria* isolates

Figure 3. 2: Incubation the liquid in the tube turned yellow

Figure 3. 3: Gas production on inverted Durham tubes during lactose utilization

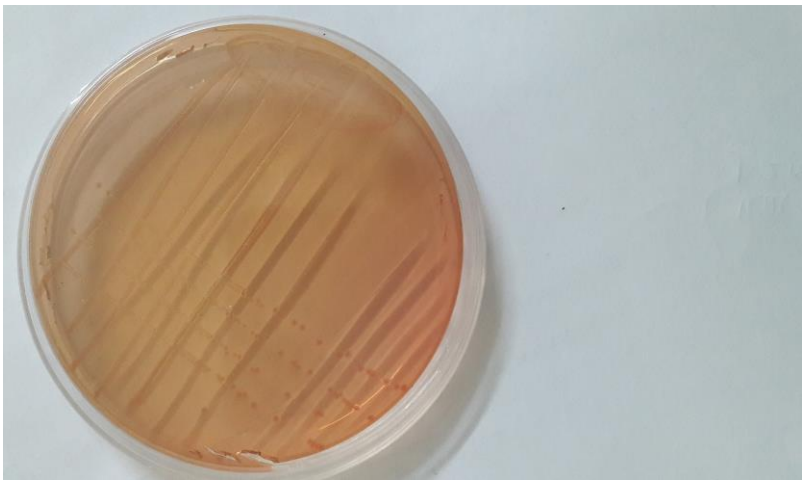

Figure 3. 4: Pink colonies on MacKonkey agar

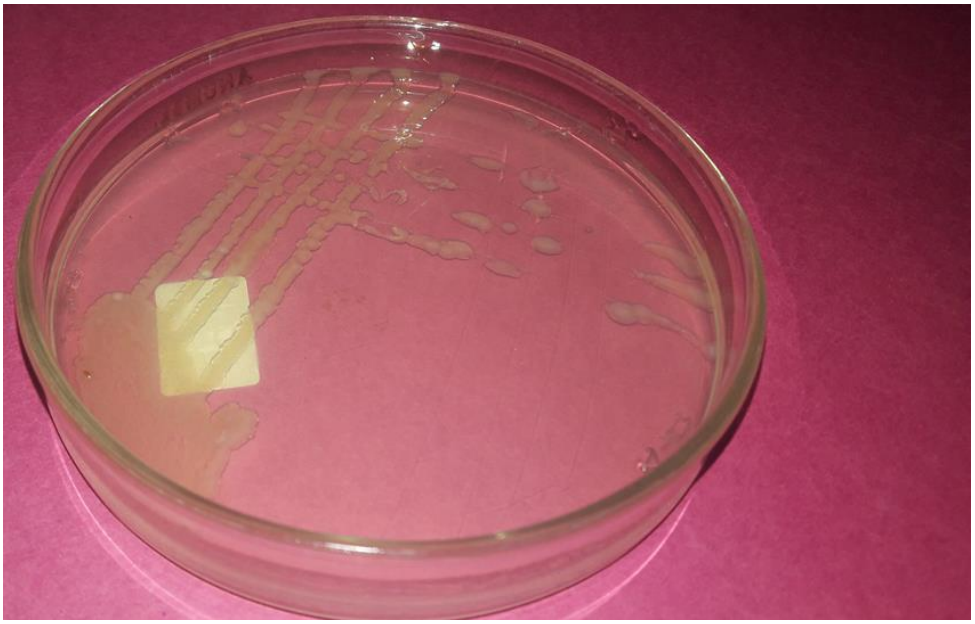

Figure 3. 5: Pure culture of *Agrobacterium tumefaciens* in nutrient agar

3.4.3 Pathogenicity test for *Agrobacterium tumefaciens* isolates

Agrobacterium tumefaciens isolates from Winchester, Nairobi produced pronounced tumors when inoculated on carrot and potato discs compared to isolates from Bahati, Nakuru. Young galls (tumors) were observed developing at the central part of the carrot and potato discs two weeks after inoculation. However, no symptoms were observed in control discs

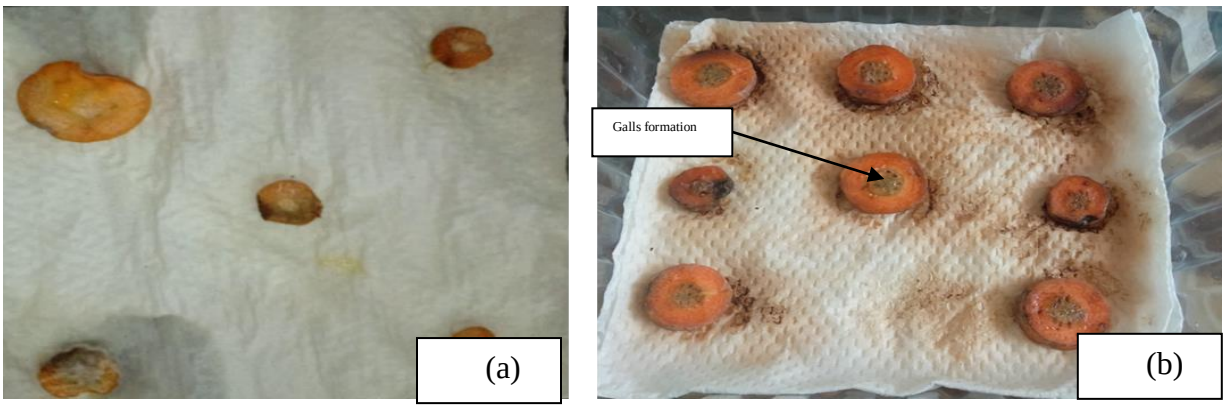

Figure 3. 6:(a) Control and (b) inoculated carrot discs after fourteen days

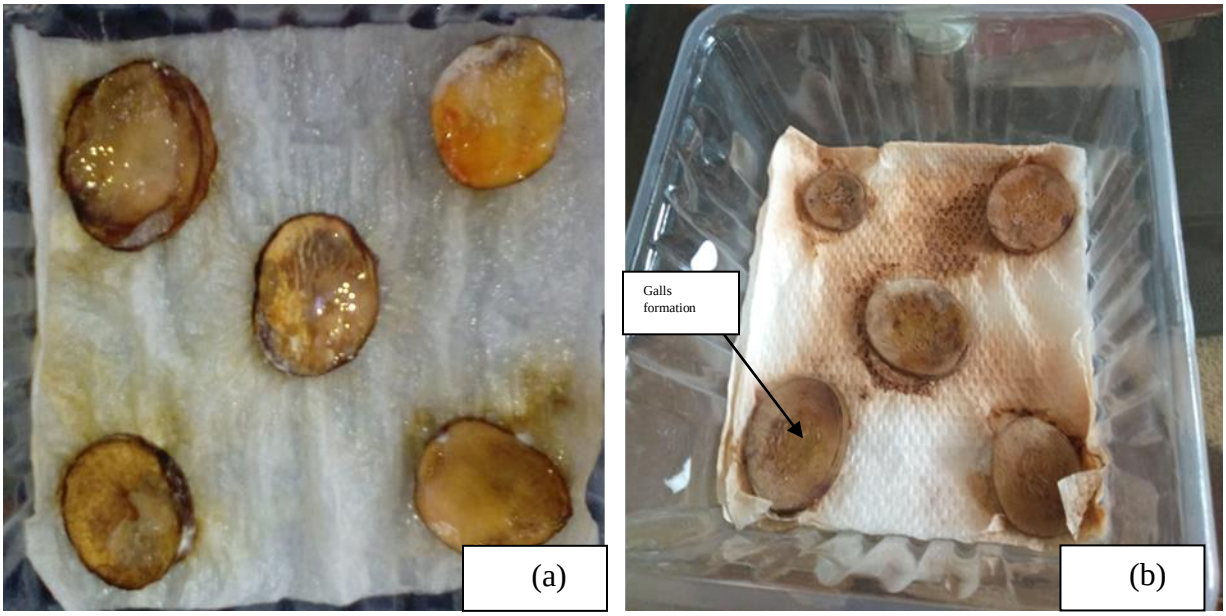

Figure 3. 7:(a) Control and (b) inoculated potato discs after fourteen days

Figure 3. 8: Control plants treated in water

Figure 3. 9: Control plants treated with water and re-planted back in pots after inoculation

Figure 3. 10: Control plants after six weeks

Figure 3. 11: Rose plants inoculated with *Agrobacteria* and re-planted back in pots

Figure 3. 12: *Agrobacterium* inoculated plants after six weeks

3.4.4 Relationship among flower growth parameters and crown gall galls

From correlation analysis, significant positive correlation was displayed between total number of galls and the size of gall and shoot length (0.6416, -0.695, P≤ 0.05).

Table 3. 7: Correlation coefficient among flower growth parameters and crown gall galls

	Shoot length	Size of galls	Stem length	Total Galls
Shoot length	-			
Size of galls	0.0193	-		
Stem length	0.4628	-0.1858	-	
Total Galls	-0.695*	0.6416*	-0.0809	-

* Significantly correlated

3.5 Discussion

Application of amino oligosaccharins had greater effect on the number of galls; however, the effect depended on the rate of application. There was increase in the number of galls in all the

treatments in the second and fourth week after treatment application. However, reduction in the number of galls was noted in the sixth week after treatment application and it was highest in plots treated with amino oligosaccharins at the rate of 1.5g/litre. Amino oligosaccharins and alternaria fine proteins can constitute an important control agent for crown gall in roses. Similar results were reported by Rabea and Steubaut, (2010). In their findings, Rabea and Steubaut, (2010) found chitosan and its derivatives to inhibit the growth of *Agrobacterium tumefaciens* and *Erwinia* spp and displayed highest antibacterial activity against *Agrobacterium tumefaciens*, *Erwinia* spp with MIC 500 mg/l and 480 mg/l, respectively. Similarly, when oligosaccharin derivatives chitosan were used in controlling *Xanthomonas vesicatoria*, infections were greatly reduced by above 60% (Ramkisson *et al*., (2016). Ramikson *et al*., (2016) reported significant reduction of the disease incidence and delayed symptoms development and the size of the lesions were smaller in comparison with other treatments.

The reduction of number of galls was due to oligosaccharides derivatives unique elicitation of physiological and biochemical changes in roses resulting to induced resistance (Cabrera *et al*., 2013). There is an enhanced activity of enzymes that are linked to defense against plant diseases in response to application of oligosaccharins. Enzymes such as Chi, β-1,3-Glucanase(GLU), peroxidase (PO), phenylalanine ammonia-lyase (PAL) and polyphenol oxidase (PPO) are activated and are able to eliminate infectious agents such as *Agrobacterium tumefaciens* (Ramkisson *et al*., 2016). Increase in Phenylalanine ammonia-lyase (PAL) enzyme activity is important as it manages pathogenic infection. Mandal*et al*., (2013) working on tomatoes reported enhanced activities in response to *Ralstonia solanacearum* inoculation in tomato pre-treated with chitosan.

According to El-Haldramani *et al*., (2010) and Ramkisson *et al*., (2016) oligosaccharins act by inducing several activities of defense enzymes in roses that are involved in pathogen defense. Alternaria fine protein accelerates plant growth vitality, increase proline content, strengthen cellulase and promote the growth of plant cells, strengthen root activity, thereby managing plant diseases. Chitosan, a biopolymer amino polysaccharides has been reported to have strong antibacterial activities against plant pathogenic bacteria such as *Agrobacterium tumefaciens, Erwinia* spp (Rabea and Steubaut, 2010), *Streptomyces scabies* (Beausejour *et al*., 2003), *Xanthomonas* spp (Li *et al*., 2008), *Pseudomonas syringae* (Ferrante and Scortichini, 2010). However, the activity of chitosan oligosaccharins depends on the concentration (Rabea and Steubaut, 2010); Yang *et al*., 2014) and molecular weight (Liu *et al*., 2006) and the type of bacteria (Shanmugam *et al*., 2016).

The mechanism of antimicrobial activity of chitosan and its derivatives remains a mystery as it has not been understood well and it is therefore considered to be complex. However, according to Raafat *et al*. (2008), at the initial stages chitosan ties with negatively charged components on the bacterial surface by means of electrostatic interactions which changes the permeability of bacterial wall and permits chitosan to get to the internal cell targets subsequently closing down cell division causing death (Je and Kim, 2006).

In this study, bacteria were isolated using nutrient agar media which has been previously used for isolation of *Agrobacterium tumefaciens* from rose samples with crown gall. A series of biochemical tests such as motility, gram staining, oxidase production and catalase, utilization of lactose and mannitol, salt tolerance (2%) potato and carrot disc assay revealed that the isolated bacterium was gram negative and had the capacity to cause tumor in plant disc sample.

The biochemical approaches have been utilized in the past investigations for the recognizable proof of *A. tumefaciens* from crown gall samples from diverse plant species (Chen et al., 1999). Tumor forming ability of the isolates confirmed that they were virulent, however, according to Chen et al. (1999) virulence of *A. tumefaciens* was because of the nature of the host, internal physiology of the strains and environmental conditions. Crown gall disease can be severe in rose plantations if young trees are infected. *Agrobacterium tumefaciens* was found on root surfaces and was effectively isolated from galls. The bacterium was isolated and confirmed using different morphological and biochemical tests.

The incidence and severity of the disease was assessed in each plot and control plots had the greatest incidence. Based on the progress of the disease after ten weeks of assessment, the least incidence was reported on plots treated with amino oligosaccharin and alternaria fine protein at 1.5g/litre and were not significant different from the plots treated with copper based fungicides. The severity index shows infection being severe in control plots after ten weeks of assessment.

CHAPTER FOUR

EFFECTS OF AMINO OLIGOSACHARINS AND ALTERNARIA FINE PROTEIN ON GROWTH AND QUALITY OF ROSE PLANT

4.1 Abstract

Rose (*Rosa hybrida*) is considered as the most prized flower of the world because of its high ornamental and commercial value. The demand for cut flowers has risen in recent years; however, production is limited by majorly environmental factors, which limits production. Controlling these conditions is costly. However, these problems can be remedied by optimizing the production conditions and utilization of plant growth regulators such as amino oligosaccharins and alternaria fine protein. A study was conducted to determine the influence of amino oligosaccharins and alternaria fine protein on growth and quality of rose plant. The study was carried out in Winchester farm (Nairobi) and Bahati farm (Nakuru) regions between January and September 2018 in a greenhouse. The experiment was laid out in a randomized complete block design with four replications. The treatments comprised three levels each of amino oligosaccharins and alternaria fine protein (0.5, 1, 1.5 g/litre), a copper based fungicide (2 ml/ litre) and a water spray (control) in a greenhouse. Application of 1.5 g/litre amino oligosaccharins and alternaria fine protein had significantly higher shoot growth, average plant height, stalk diameter, flowering duration and flower diameter at cup shape than the control and other treatments. Maximum number of shoots after six weeks was recorded in Winchester (13.6) and in Bahati farm (13.9) in plots treated with 1.5 g/litre of amino oligosaccharins. However, maximum height (61.3 cm) in Winchester and (59.6 cm) in Bahati was recorded in plots treated with amino oligosaccharins and alternaria fine protein at the rate of 1g/litre. Plants receiving 1.5g/litre of amino oligosaccharins spray shortened flowering duration by eight days compared

to the control. The use of amino oligosaccharins at 1.5g/litre had greater effect on all the flower parameters as compared to the reference product (copper based fungicide) and this was significantly different from control which had the least effect. Exogenous applications of oligosaccharins and alternaria fine protein at the rate of 1.5g/litre in the greenhouse significantly influenced the growth of roses and therefore have the potential to improve the yield of roses.

4.2 Introduction

Horticulture is an important sub-sector of Kenya's economy and contributes tremendously to the country's gross domestic product (foreign exchange) as well as income and employment generation. In Kenya, the horticulture sub-sector contributes 33% of agriculture's share of the Gross Domestic Product (Maina *et al*., 2011). According to the Ministry of Agriculture (2010), the domestic value of horticultural produce was estimated to be Ksh. 153 Billion with earnings from flower exports being Ksh. 35.5 Billion. It was also noted that the value of Kenyan horticultural exports grew exponentially at an average rate of 15.9% between 2001 and 2010. Flowers from Kenya account for 38% of flowers that are auctioned in Europe and roses make up 74% of Kenya's flower exports (KFC, 2016).

Rose is cultivated for its beautiful flowers and is considered as the most prized flowers of the world because of their high ornamental and commercial value. In the flower industry, it occupies the top position in terms of acreage, production and consumption of more than $40 billion (Parmar *et al*., 2015). Roses are utilized in the export market for aesthetic gratification among other purposes and thus need to monitor the quality for customer satisfaction. The increased flower production and improved quality are the most important objectives to be recognized in cut-flower production as these are the key parameters that customers consider. The profit obtained in flower investment is also a function of flower yield and quality (Sardar, 2007). The

demand for cut flowers has risen in recent years, however, production of cut flowers face many problems and demand special care for it to bloom. The most important one being environmental conditions, that hinders production. Controlling this condition is costly and therefore does not encourage producers to produce cut flowers. These issues can be tended to by optimizing the production conditions and utilization of plant growth regulators (PGRs) (Hashemabadi and Mohammad, 2010).

These regulators work by changing nutritional and hormonal status of the plant, repressing, promoting and altering the physiological processes of the plant (Parmar et al., 2015). They improve the quality by modifying the behaviour of plant systems as well as increasing the flower yields. They help in synthesis of metabolites and translocation of nutrients and in assimilation of these into diverse plant parts, which eventually result into higher yields and flower quality improvement. Good quality production is accomplished by controlling growth variables such as light and temperature. These physical components are exceptionally difficult to control and costly (Parmar *et al*., 2015).

Oligosaccharins are natural polysaccharides and oligosaccharides that occur as part of plant and microorganisms cell wall, however, the main sources of raw materials for large scale production are by-products from agriculture and wasted crustacean exoskeletons from fishing industry. Polysaccharides and glycoproteic components from cell walls constitute a source of oligosaccharides; some of them have positive effects on plant growth and development at low concentrations. Exogenous application of oligosaccharins influences plant tissue growth and development. Oligosaccharides, therefore, could be a beneficial tool for plant growth promotion in agricultural systems since they are inexpensive natural products and can easily be integrated

into both organic and sustainable agriculture production systems. This experiment was carried out to evaluate different levels of amino oligosaccharins and alternaria fine protein on growth and quality of rose plant.

4.3 Materials and methods

4.3.1 Study Site

The study was carried out at Winchester farm limited (Nairobi) and Bahati Farm limited (Nakuru) in a greenhouse**.** The two areas lie at a latitude of 1º 19’59 South, longitudes 36º 42’58 East and an altitude of 1831 and 0º 16’59 North, longitudes 36º 04’0.01 East and an altitude of 1912 meters above sea level respectively. The areas receive an annual rainfall of approximately 908 to 1012 mm and annual mean temperature of between 15.6^{o}C to 23^{o}C.

4.3.2 Experimental Materials, Design and Treatment Application

The experimental materials, design and treatment application is as explained in section 3.3. This experiment was carried out in a greenhouse hydroponic system, 20-25^{o}C, 50-70% Relative Humidity (RH) and natural photoperiod. Fertigation was done using drip irrigation system. The experiment was laid out in Randomized Complete Block Design (RCBD) with four replications. Each plot measured 3m by 2m and contained twelve (12) plants. Six plants from each plot were randomly selected from each treatment, tagged and used for data evaluation.

4.3.3 Determination of different growth parameters in roses

4.3.3.1 Number of visible bud breaks/ shoots

The number of bottom breaks per plant appearing 7 days from the beginning of the flush were identified as visible bud breaks were counted and recorded.

4.3.3.2 Plant height

Plant height from base level to apex was measured weekly using a tape measure. The harvested stems were graded according to the specification given by Post (1952) with modifications as

follows: Shorts < 40 cm, Mediums 40-49 cm, Extras 50-59 cm, Fancy 60-69 cm, Specials 70- 79 cm and Extra specials > 80 cm.

4.3.3.3 Days to first flower development

Days to first flower development was recorded starting from the date of bending for the first flush. Time taken for flower development for subsequent flushes was determined starting from the date of harvesting till the stage at which buds showed colour (show-colour stage).

4.3.4 Data Analysis

The data collected was subjected to analysis of variance (ANOVA) using statistical analysis software Genstat Discovery Edition 15 (VSN International Ltd. 2015). Treatments means were compared using LSD test at the 0.05 level of probability using Fisher's protected LSD.

4.4 Results

4.4.1 Effect of different levels of amino oligosaccharins and alternaria fine protein on the number of shoots of rose plant

The application of amino oligosaccharins and alternaria fine protein resulted in significant ($P \leq 0.05$) influence on the rejuvenation of the shoot (Table 4.1). Shoot growth was determined by counting the number of shoots prior to treatment application and subsequent fortnight counts made on the number of shoots. Application of Amino oligosaccharins at the rate of 1.5g/litre had the significant effect on number of shoots while the minimum shoot growth was recorded in plots treated with amino oligosaccharins at the rate of 0.5g/litre. The trend was not significant different in Bahati; however, treatment applications in Winchester had better performance compared to treatment applications in Bahati. Maximum number of shoots after six weeks (13.6) in Winchester and (13.9) in Bahati was found in plots treated with amino oligosaccharins at the rate of 1.5g/litre while the minimum number of shoots (6.0) in Winchester and (7.9) in Bahati was reported in plots treated with amino oligosaccharins at the rate of 0.5g/litre.

Table 4. 1: Effect of different concentrations of amino oligosaccharins and alternaria fine protein on number of visible bud breaks/shoots at different times

Treatment	Weeks after treatment application		
	2	4	6
Winchester			
Amino oligosaccharins 0.5g/litre	2.7bc	3.9e	6.0g
Amino oligosaccharins 1g/litre	2.8bc	5.3cde	9.4def
Amino oligosaccharins 1.5g/litre	4.0a	8.4a	13.6ab
Copper at 2ml/litre	3.8a	8.2a	13.6ab
Control	3.3ab	6.8abc	12.6abcd
Bahati			
Amino oligosaccharins 0.5g/litre	2.7bc	4.6de	7.9f
Amino oligosaccharins 1g/litre	2.1cd	4.9de	8.8ef
Amino oligosaccharins 1.5g/litre	2.7bc	7.6ab	13.9a
Copper at 2ml/litre	2.5bc	5.8bcd	11.5bcd
Control	1.5d	7.2ab	10.9cde
Mean	2.81	5.6	10.8
LSD ($P \leq 0.05$)	0.96	1.7	2.1
CV (%)	23.5	21.1	13.5

Means followed by the same letters along the columns are not significant different at 5% level of probability

4.4.2 Effect of different levels of amino oligosaccharins and alternaria fine protein on the height of rose plant

The data in Table 4.2 shows that there were significant differences between the treatments and the sites ($P \leq 0.05$). However, there were no significant differences between the treatments in the initial assessment. Four, six and eight weeks after treatment applications, plots treated with amino oligosaccharins and alternaria fine protein at the rate of 1.5g/litre had the greatest effect on the height of rose plants while those treated with amino oligosaccharins 0.5g/litre had the

least effect on plant height. Maximum height after six weeks (55.6cm) in Winchester and (59.6cm) in Bahati was found in plots treated with amino oligosaccharins at the rate of 1.5g/litre while the minimum height 45.0cm in Winchester was observed in plots treated with copper based fungicide and 46.3cm in Bahati was observed in plots treated with amino oligosaccharins at the rate of 0.5g/litre.

Table 4. 2: Effect of different concentrations of amino oligosaccharins and alternaria fine protein on plant height at different times

Treatment	Weeks after treatment application		
	2	4	6
Winchester			
Amino Oligosaccharins 0.5g/Litre	20.1a	33.2d	47.2cd
Amino Oligosaccharins 1g/Litre	19.4b	32.5d	50.0bcd
Amino Oligosaccharins 1.5g/Litre	22.7ab	47.7a	55.6ab
Copper at 2ml/Litre	21.0ab	42.2ab	45.0d
Control	21.0ab	41.0bc	46.7cd
Bahati			
Amino oligosaccharins 0.5g/litre	23.7ab	35.1cd	46.3cd
Amino oligosaccharins 1g/litre	20.2ab	31.2d	52.0bc
Amino oligosaccharins 1.5g/litre	22.7ab	45.9ab	59.6a
Copper at 2ml/litre	21.7ab	42.4ab	52.0bc
Control	24.1a	47.7a	49.8bcd
Mean	21.7	39.4	54.2
LSD ($P \leq 0.05$)	4.5	6.6	6.9
CV (%)	14.4	11.6	8.8

Means followed by the same letters along the columns are not significant at 5% level of probability

4.4.3 Effect of amino oligosaccharins and alternaria fine protein on flowering parameters

There were significant variations among the treatments in the two sites (Table 4.3). Plants receiving amino oligosaccharins sprays at the rate of 1.5g per litre showed greater average height (44.0cm) in Winchester, (47.7cm) in Bahati and stalk diameter (0.48cm) in Winchester, and had

shortened flowering duration (35.2 days) in Winchester and (48.4 days) in Bahati. The average height achieved from the two sites were however not significantly different from the other treatments despite the greater average height observed. Flower diameter at cup shape stage was maximum (5.7cm) in Winchester and 5.6cm in Bahati in plots treated with amino oligosaccharins sprays at the rate of 1.5g per litre and were significantly different as compared to control(2.9cm) and (3.2cm) in the two sites respectively. Control plants exhibited the shorter stalk diameter in Winchester (0.28cm) and this was not significantly different from the results in Bahati (0.33cm).

Table 4. 3: Effect of different concentrations of amino oligosaccharins and alternaria fine protein on flower parameters of roses

Treatments	Average height (cm)	Stalk diameter (cm)	Flowering duration (Days)	Flower diameter at cup shape stage (cm)
Winchester				
Amino oligosaccharins 0.5g/litre	39.1bc	0.34c	41.3cde	2.9d
Amino oligosaccharins 1g/litre	36.7c	0.39b	39.4de	3.1cd
Amino oligosaccharins 1.5g/litre	44.0abc	0.48a	35.2e	5.7a
Copper at 2ml/litre	40.7abc	0.39b	44.4cd	3.4c
Control	41.9abc	0.28e	44.3cd	2.9d
Bahati				
Amino oligosaccharins 0.5g/litre	40.1abc	0.29de	59.8a	2.4e
Amino oligosaccharins 1g/litre	46.4ab	0.32cde	52.1b	2.8de
Amino oligosaccharins 1.5g/litre	47.7a	0.39b	48.4bc	5.6a
Copper at 2ml/litre	45.1ab	0.40b	63.5a	3.9b
Control	40.4abc	0.33cde	63.4a	3.2cd
Mean	42.3	0.36	49.2	3.5
LSD (P≤0.05)	8.2	0.04	7.2	0.4
CV (%)	13.5	54.5	10.2	0.3

Means followed by the same letters along the columns are not significant at 5% level of probability

4.5 Discussion

Different rates of amino oligosaccharins and alternaria fine protein resulted in significant (P≤0.05) influence on the rejuvenation of the rose shoot. Application of amino oligosaccharins at the rate of 1.5g per litre had the greatest effect on shoot growth. The results are comparable to those reported by Cabrera *et al.* (2013) in wheat. In their findings they reported that application of oligosaccharins on wheat crop as seed coating had positive effect on germination-related parameters. Thus, oligosaccharins can be used as seed inoculant to increase yields. Camejo *et al*. (2011) working on the effect of OGA on alfalfa seedlings reported increased root length and

growth promotion. Application of oligosaccharins influences plant tissue growth and development through induction of phytoalexin formation (Hans *et al*., 1981), stimulation of cell walls thickening and flower bud formation (Altamura *et al*., 1998), proliferation of cytokinin-induced formation of stems and stem growth segments stimulated by auxin (Falsaca *et al*., 2008). According to Larskaya and Gorshkova, (2015), oligosaccharides have effects on root and flower bud formation, stimulation of guard cell division and pericycle cell wall thickening while Alternaria fine protein accelerates plant growth vigor, increases proline content and cellulase strength while at the same time promote the growth of plant cells, root activity and increased production purposes (https://patents.google.com/patent/CN103828828A/en)

Rose height is an important quality factor that was influenced by different concentrations of oligosaccharins. According to the results from this study, application of amino oligosaccharins at the rate of 1.5g per litre had significant effect on rose height than any other treatment. The results are in line with those reported by Roberts *et al*. (1999) and Saffari *et al*. (2004). In their findings, oligosaccharins had positive effect and increased the internode length and therefore could have positive effect on rose stem height. Similar results reported by Cabrera *et al*., (2013) where chitosan and its oligomers (DP 15) were tested for their plant growth regulator properties and coating of bulb with this polysaccharide demonstrated clear stimulation of stem-length of cut flowers. Oligosaccharins are complex carbohydrates that are capable of modulating plant growth and development at low concentrations. For instance, according to LoSchiavo *et al*., (1991); Filippini *et al*., (1992); Creelman and Mullet, (1997) oligogalacturonides induce auxins stimulated rooting in leaf explants, and auxin-dependent somatic embryogenesis in carrot cultures and also induce flower formation in tobacco. Exogenous oligosaccharins can modulate

the elicitation of defense responses, morphogenesis in tissue culture, growth in excised stem segments, and induction of root nodules (Creelman and Mullet, 1997).

At the same time, different concentrations of amino oligosccharins had different effect on rose flower parameters. Plants receiving amino oligosaccharins sprays at the rate of 1.5ml per litre showed advanced average height (44.4cm) in Winchester, (47.7cm) in Bahati and stalk diameter (0.48cm) in Winchester and (0.40cm) in Bahati, and had shortened flowering duration (35.2 days) in Winchester and (48.4 days) in Bahati. Exogenous application of oligosaccharins influences plant tissue growth and development. This is evidenced by oligosaccharides derived from plant cell wall polymers, chitin and chitosan (Falcon *et al*., 2015). Both chitosan polymer and its smaller derivatives are plant growth regulators stimulating root, vegetative growth, shortening flowering period and improving flowering and fruiting (Utsunomiya *et al*., 1998; Ohta *et al*., 2004). Consistent improvement in quality of blueberry plant was demonstrated when chitosan was used during the transplanting phase (Romanazzi, 2010). In the study by Cabrera *et al*., (2013), pre-harvest foliar application of chitosan 2 weeks before harvest reportedly increased postharvest fruit quality and shelf-life of blueberry fruits. Therefore, applications of oligosaccharides have the potential to improve even the shelf life of flowers. The alternaria activated protein composition has been used to increase the plant chlorophyll content thereby keeping crops green, inhibiting plant senescence, promoting root development and effective absorption of fertilizers, assisting in good growth of weak parts of the crops, enhancing the absorption of water by plants and regulating the balance of water in the plants so as to improve resistance to drought and cold.

Exogenous applications of oligosaccharins in the greenhouse have shown that, oligosaccharins influence the growth of roses and therefore has the potential to improve the yield of roses. The

composition of alternaria activated protein promotes root development and effective absorption of fertilizers by the crops thereby assisting in good growth of the crops through enhanced absorption of water and fertilizers and regulating the balance of the water in the plants

CHAPTER FIVE
GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 General discussion

Application of amino oligosaccharins at the rate of 1.5g per litre had the greatest effect on shoot growth. Similar results were reported by Camejo *et al*. (2011) working on the effect of OGA on alfalfa seedlings who reported increased root length and growth promotion. Application of oligosaccharins influences plant tissue growth and development. According to Cabrera *et al.*, (2013), application of oligosaccharins as a seed coating has positive effect on germination and other growth parameters on wheat. Therefore, they concluded that this substance could also be applied as seed inoculant. Alternaria fine protein accelerates plant growth vigor; increases proline content and cellulase strength while at the same time promote the growth of plant cells, strengthen root activity and increases production.

According to these results, application of amino oligosaccharins at the rate of 1.5g per litre had significant effect on rose height than any other treatment. The results correspond to those reported by Saffari *et al*. (2014). According to the researchers, plant growth regulators such as oligosaccharins have positive effect on plant height and increases plant internode length. Creelman and Mullet, (1997) concluded that oligogalacturonides induce auxins stimulated rooting in leaf explants, and auxin-dependent somatic embryogenesis in carrot cultures and also induce flower formation in tobacco.

Those plots treated with amino oligosaccharins at the rate of 1.5g per litre had increased stalk length and shortened flowering duration. Oligosaccharins derivatives such as chitosan stimulate

root, vegetative growth, and shortened flowering periods (Falcon *et al*., 2015). This was reported with blueberry where chitosan was applied at transplant stage. According to Cabrera *et al*. (2013), foliar application of chitosan just towards harvesting increases fruit post-harvest quality and shelf life. Studies have shown plant protection effect of chitosan on various crops. According to Jayaraj *et al*. (2009), there was reduced incidence of plant diseases when the plants were sprayed with chitosan oligosaccharins derivatives. Ramkinsoon *et al*. (2016) proposed that reduction in disease prevalence was as a result of chitosan elicitation of physiological and biochemical changes in plants that resulted in induced resistance in tomatoes. A combination of amino oligosaccharins and alternaria fine proteins may prove appropriate alternative to plant diseases such as *Agrobacterium tumefaciens* as alternaria fine protein has been reported to increase content of plant chlorophyll, inhibiting plant senescence, promoting root development and effective absorption of fertilizers and water by crops. Also it has been reported to strengthen weak parts of the crops, and regulating the balance of water in the plants so as to improve the drought resistance and the cold resistance of the plants

Application of amino oligosaccharins in combination with alternaria fine proteins considerably reduced the number of galls associated with *Agrobacterium tumefaciens*. The results show that amino oligosaccharins and alternaria fine proteins constitute an important control agent for crown gall in roses. Similar results were reported by Rabea and Steubaut, (2010). In his findings, MIC 500 mg/l and 480 mg/l displayed highest antibacterial activity on both *Agrobacterium tumefaciens* and *Erwinia* spp. Elicitation of physiological and biochemical changes in roses by oligosaccharides derivatives led to induced resistance thus reduction of number of galls in plots treated with amino oligosaccharins and alternaria fine proteins.

According to Ramkisson *et al*. (2016), there is an enhanced activity of enzymes such as Chi, Glu, PO, PAL and PPO linked to defense against plant diseases in response to application of oligosaccharins. These enzymes are activated and are able to eliminate infectious agents such as *Agrobacterium tumefaciens* the causal agent of crown gall in roses in this case. When alternaria fine proteins are used with amino oligosaccharins that induces elimination of bacterial pathogens their impact on management of plant diseases will be felt.

Alternaria fine protein is known to; accelerate plant growth vitality, increase in proline content, increase in cellulase and promote the growth of plant cells, strengthens root activity, increase production purposes and through this it's able to manage plant diseases The biochemical tests confirmed the causal agent of crown gall in roses.

5.2 Conclusion

The study demonstrates the ability of amino oligosaccharins and alternaria fine proteins to be used as an alternative to management of plant diseases. A significant reduction in the number of galls and size following application of amino oligosaccharins and alternaria fine proteins was observed as well as improvement in plant growth. The ability of the treatments to manage the disease can be attributed to enhanced defense enzyme activity. Therefore, this study provides good support for incorporation of amino oligosaccharins and alternaria fine proteins in the management and yield enhancement of roses.

The composition of alternaria activated protein promotes root development and effective absorption of fertilizers by the crops. This improves growth of the crops through enhanced absorption of water and fertilizers and by regulating the balance of the water in the plants.

Amino oligosaccharins and alternaria fine proteins have the ability to be used as an alternative to chemical management method of plant diseases.

Rose growers have now a safe way of managing *Agrobacterium tumefaciens* by use of an environmentally safe product.

5.3 Recommendations

i. Following successful use of a mixture of amino oligosaccharin and alternaria activated protein on roses in the two sites, its usage can be recommended at the rate of 1.5g/litre to aid in management of crown gall in roses. The positive response observed on flower parameters; plant height, stalk diameter and flower head diameter at cut stage means significant improvement in flower quality. Further, an increase in number of rejuvenating shoots after harvesting implies more revenue in the part of the grower. In this regard, the product can be used to further better the quality of roses implying a competitive advantage in the market and better revenue. A shorter harvest duration as a result of the use of the product will be helpful to rose growers in the world as harvests per year will be increased, increase in revenue will be experienced as a result.

ii. The ability to effectively reduce the number of galls in roses can be replicated in other crops infected with *Agrobacteria*.

iii. With an improvement in growth, yield and quality in roses, the use of amino oligosaccharin and alternaria activated protein can be tried in other crops.

iv. Tailor made oligosaccharides for specific purposes should be done in order to enhance flower production since oligosaccharides display different biological activities which may lead to increased flower production.

v. Future research should focus on establishing how oligosaccharides biological characteristics affect activities in both plant growth and crown gall occurrence

vi. Studies should be conducted to understand how oligosaccharides act as elicitors to evoke pathogen defense responses in management of crown gall of roses

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