EFFECTIVENESS OF ANTAGONISTIC BACTERIAL ISOLATES AGAINST CROWN GALL DISEASE ON ROSES IN KIAMBU COUNTY, KENYA

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DECLARATION

I, Aprodisia Kavutu Murero, declare that this thesis is my original work and has not been presented for the award of a degree in any other University or any other award.

Signature..... Date..26/10/2020.....

Aprodisia Kavutu Murero

Supervisors' Approval

We confirm that the work reported in this thesis was carried out by the candidate under our supervision and has been submitted with our approval as university supervisors

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DEDICATION

This work is heartily dedicated to my parents Mr. Nichasio Murero, Mrs. Caroline Ngai Murero and my sibling Popline Wanyaga for their encouragement in my education.

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ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
AST	Agricultural Science and Technology
CFU	Colony Forming Units
CIDP	County Integrated Development Plan
CMC	Carboxymethyl cellulose
CRD	Completely Randomized Design
DNA	Deoxyribonucleic acid
FPEAK	Fresh Produce Exporters Association of Kenya
GAP	Good Agricultural Practices
GD	Gall Diameter
GDP	Gross Domestic Product
HCD	Horticultural Crop Directorate
HCN	Hydrogen cyanide
IDM	Integrated Disease Management
IPM	Integrated Pest Management
KDLC	Kenya Development Learning Centre
KEPHIS	Kenya Plant Health Inspectorate Service
KFC	Kenya Flower Council
KU	Kenyatta University
LSD	Least Significant Difference
MoA	Ministry of Agriculture, Livestock and Fisheries
NACOSTI	National Commission for Science, Technology and Innovation
NPK	Nitrogen, Phosphorous and Potassium

PCR	Polymerase Chain Reaction
PDI	Percentage disease severity
SAED	School of Agriculture and Enterprise Development
SAS	Statistical Analysis Software
SPSS	Statistical Package for the Social Sciences
ST	Stem Diameter

ABSTRACT

Crown gall is one of the major constraint in rose flower production in Kenya. The study aimed at developing a bacterial based biopesticide to manage crown gall disease on roses. A baseline survey on status of crown gall disease was conducted from July to October, 2017 using a questionnaire administered to the production managers in randomly selected flower farms in Kiambu, Nakuru and Laikipia Counties. Pathogen and the biocontrol isolates were isolated from galls and soil samples, respectively at Kenyatta University. The isolates were screened to determine their effectiveness against *Agrobacterium tumefaciens*. The most effective four isolates were identified using biochemical, physiological and molecular tests and evaluated *in planta*. At KU, rose stems were inoculated with *A. tumefaciens* and the effective isolates at the same time before planting. In another set up, plants were inoculated with *A. tumefaciens* and the test isolates applied after galls formed. For experiments conducted at the flower farms, test isolates were smeared on galls and bruised stems of naturally infected plants. The test isolates included *Lactobacillus brevis* 2.28.11, *Micrococcus luteus* 2, *Micrococcus luteus* 1 and *Arthrobacter* sp 1; other treatments included funguran®, infected plants (with galls treated only with distilled water) and uninfected control (without galls). Treatments were replicated 7 times and arranged in a Complete Randomized Design. The number of galls, change of gall size and number of shoots were recorded at 7 days interval for 10 weeks. Heights of shoots were recorded for 6 weeks. Survey and field (*In vitro* and *in vivo*) data were analyzed using Statistical Package for the Social Sciences and Statistical Analysis Software (SAS), respectively. Field data was subjected to analysis of variance and the difference between the treatments means separated using the Fisher's least significant difference test at 5% probability level. Majority of the farms in the surveyed Counties recorded a disease incidence and severity above 50%. The largest inhibition zone *in vitro* resulted from *Arthrobacter* sp 1 with a mean of 7.8mm. *Lactobacillus brevis* 2.28.11 and *M. luteus* 2 reduced gall size by 25.5% and 26.1% on Tropical Amazon® variety, respectively. On Upper class® variety, *Lactobacillus brevis* 2.28.11 and *M. luteus* 2 reduced gall size by 21.0% and 20.3%, respectively. Plants treated with *L. brevis* 2.28.11 and *M. luteus* 2 had all galls completely dry by week 10 in all the sites. When isolates tested for preventive use, galls did not form on plants treated with *L. brevis* 2.28.11 and *M. luteus* 2. On number of shoots, *L. brevis* 2.28.11 and *M. luteus* 2 produced significantly more and taller shoots in all the sites. The study revealed that crown gall remains a threat in production of roses and local environments hold promising antagonistic bacteria against *A. tumefaciens*. *Lactobacillus brevis* 2.28.11 and *M. luteus* were effective against crown gall. The study recommends that awareness on greenhouse hygiene and its impact on crown gall spread be increased to rose flower growers. Further exploitation and screening of bacterial antagonists from the local environment against diseases should be encouraged. *Lactobacillus brevis* 2.28.11 and *M. luteus* can be advanced to commercialisation and promoted for use in managing crown gall disease.

CHAPTER ONE: INTRODUCTION

1.1 Background of the study

Agriculture accounts for 33% of the nation's Gross Domestic Product (GDP) while horticulture alone contributes to 24% to the GDP. Horticulture is among the top foreign exchange earners in Kenya contributing over 1 billion USD per year (KFC, 2019). According to Kenya Flower Council (KFC) (2019), cut flower industry, contributed 1.1% to the national Gross Domestic Product (GDP) in 2016.

The cut flower industry provides employment to over 500,000 people, with over 100,000 employees depending on floriculture which as a whole supports the livelihood of over two million people in Kenya (KFC, 2019). The sector has experienced growth in value and quantity of the flowers exported annually from 10,946 tons in 1988 to 133,658 tons in 2016 as reported by Fresh Produce Exporters Association of Kenya (FPEAK, 2019). In 2017, the sector earned Kenya 8.225 million USD and this was an increase of 11.6% from 2016 as stated by Horticultural Crop Directorate (HCD) (FPEAK, 2019). In Kenya, the overall sale of horticulture produce rose from 2.16 million USD in 2016 to 3.05 million USD in 2018 (FPEAK, 2019).

Flowers are grown in Nakuru, Murang’a, Laikipia, Nairobi, Kiambu, Trans Nzoia, Nyandarua and Uasin Gishu Counties (KFC, 2019). Roses, carnations and alstromeria are the main cut flowers produced in the country in small, medium and large scale farms (KFC, 2019). Production of cut flowers in

Kenya is faced by constraints such as high cost of pesticides, rejection of the flowers during export due to arthropod pests and diseases. Among the diseases, crown gall caused by *Agrobacterium tumefaciens* Smith and Townsend, is one of the main devastating disease (Maina *et al.*, 2011; Murugi, 2015). *Agrobacterium tumefaciens* is a soil-borne pathogen that causes tumorous growth at the crown on rose plant (Maina *et al*., 2011). Crown gall is widespread in rose farms and nurseries in most parts of Kenya (KDLC, 2011; Maina *et al*., 2011; Murugi, 2015).

Crown gall disease prevalence was noticed in Kenya in 1998 when the flower production decreased significantly (Real IPM, 2007; Maina *et al.*, 2011). The disease was introduced to Kenya from Israel through importation of infected stocks (Real IPM, 2007). Failure to observe strict nursery hygiene practices led to spread of the disease where Kenya rose flower growers reported 10-15% losses depending on the rose variety (Maina *et al*., 2011). Use of bacterial antogonists can be used in managing crown gall since no synthetic pesticide has been reported to manage the disease effectively (Singh *et al*., 2016).

1.2 Statement of the problem

Agrobacterium tumefaciens infects wounded plants and causes swellings at the stem and crown of a rose plant (Maina *et al*., 2011). The disease leads to losses between 5–60% on rose flower production in Kenya (Real IPM, 2007). In response to losses, farmers use synthetic pesticides due to their immediate

effects in overcoming various diseases (Campos *et al*., 2019). However, no pesticide has been reported to be effective against crown gall.

Local environment contains microorganisms that are useful in managing diseases. Biopesticides have been tried on various diseases but little has been done on crown gall. *Agrobacterium radiobacter* has been tested on crown gall disease on roses in Kenya but its effectiveness was temporal (Murugi, 2015). Results by Limanska *et al*. (2015) confirmed that *Lactobacillus plantarun,* Orla-Jensen suppressed crown gall (*A. tumefaciens*) on grapevines.

Application of synthetic pesticides does not always prove economical against soil-borne pathogens and it has also led to environmental pollution, low decomposition rates in the soil, high residue levels on the agricultural produce, pathogen resistance if they are not alternated with other methods, increased risk to human and animal health and creates an imbalance in the microbial community in soil (On *et al*., 2015; Itale *et al*., 2016; Abhiram *et al*., 2018; Campos *et al*., 2019). Use of copper based fungicides to manage crown gall leads to hazardous effects on the environment and sometimes they are phytotoxic to the plant (Maina *et al*., 2011; Campos *et al*., 2019). Use of fumigants like methyl bromide was effective against *A. tumefaciens* on grapevine plants; however it was banned in 2005 due to its adverse effects on the ozone layer (Ellena, 2016).

1.3 Justification of the study

In Kenya, horticulture provides job opportunities to more than 500,000 people and generates over US $ 1 billion per year (KFC, 2019). Apart from

being used as a symbol of love, rose oils are used in manufacture of perfumes and their petals too are used in washing eyes due to their antiseptic nature (Abdul *et al*., 2016).

Reducing use of synthetic pesticides and supplementing them with biopesticides in disease management cares for the environment and meets the consumers' needs (Abhiram *et al*., 2018). Biopesticides have been receiving much attention (Srijita, 2015; Chauhan *et al*., 2018) as potential supplements to synthetic pesticides because they decompose fast, are effective in the long term, have multiple modes of action on pathogens, target specificity, lack of residual effects and are less costly compared to synthetic pesticides especially if locally produced (Itale *et al*., 2016; Abhiram *et al*., 2018; Campos *et al*., 2019).

Many bacterial biocontrol agents found in the rhizosphere are capable of competing for nutrients required for growth and can suppress growth of phytopathogenic bacteria by producing enzymes and antibiotics. The rhizospheric bacterial biocontrol agents are also known to induce host resistance against plant pathogens (Rado *et al*., 2015).

1.4 Objectives of the study

1.4.1 General objective

To develop a bacterial based biopesticide for managing crown gall disease caused by *Agrobacterium tumefaciens* on roses.

1.4.2 Specific objectives

i. To determine the incidence and severity of crown gall disease on roses in Kiambu, Nakuru and Laikipia Counties.

ii. To isolate, screen and identify the bacterial isolates with activity against *A. tumefaciens in vitro.*

iii. To evaluate the effectiveness of the selected bacterial antagonists against crown gall *in vivo.*

1.5 Hypotheses

i. There is a difference in the incidence and severity of crown gall disease on roses in Kiambu, Nakuru and Laikipia Counties.

ii. Locally isolated antagonistic bacteria significantly suppress growth of *A. tumefaciens in vitro*.

iii. Bacterial antagonists with *in vitro* activity significantly suppress the development of crown gall on roses *in vivo.*

1.6 Conceptual framework

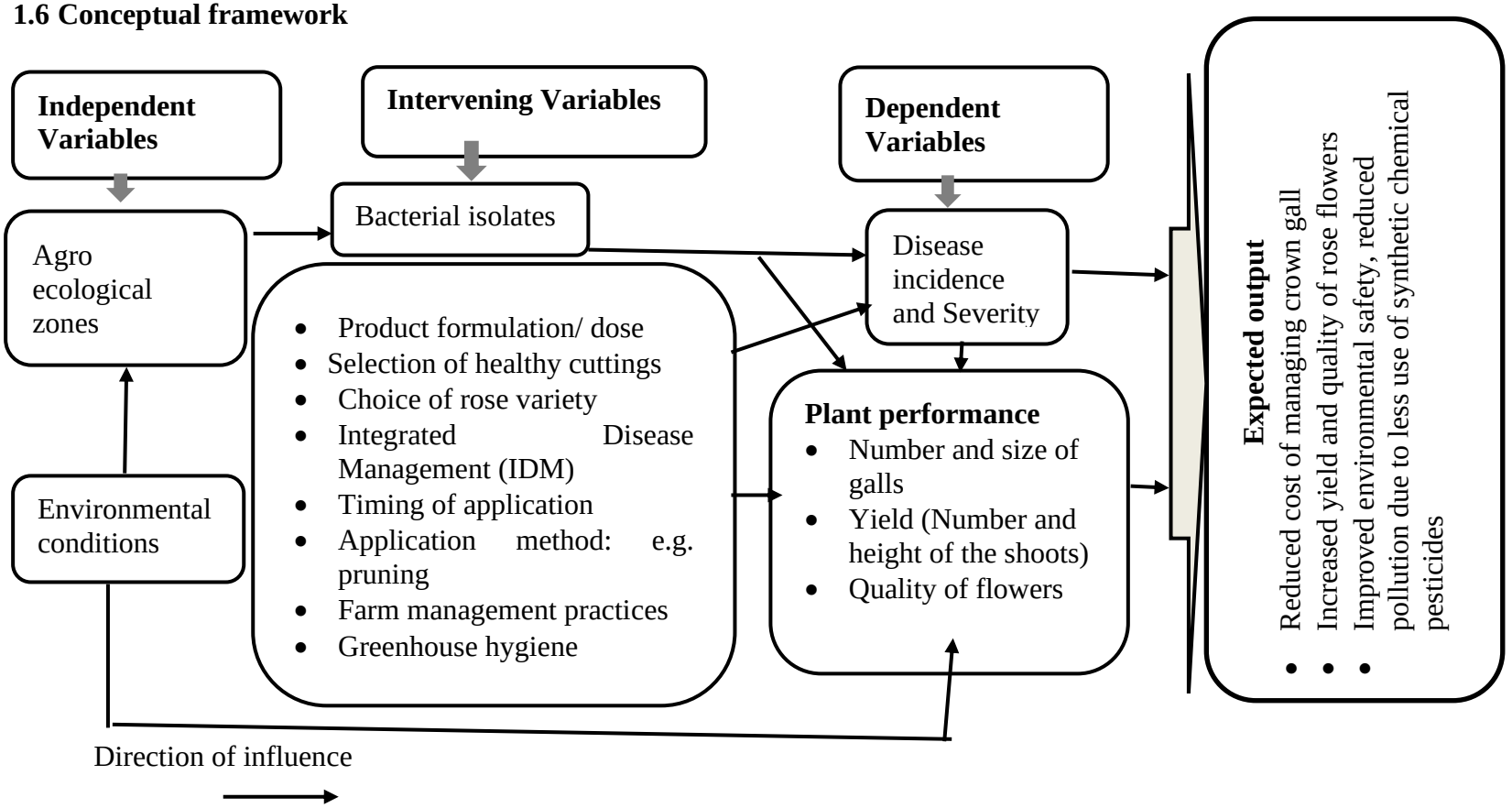

Figure 1.1 Conceptual framework

CHAPTER TWO: LITERATURE REVIEW

2.1 Horticultural sector in Kenya

Vegetables, flowers and fruits are some of the horticultural crops grown in Kenya. Being one of the top foreign exchange earners in Kenya, horticultural sector contributes to approximately US $ 1 billion per year, Kenya Flower Council (KFC, 2019). According to Ministry of Agriculture, Livestock and Fisheries (MoA) 2015, the industry contributed 1.45% to the national GDP with flower export contributing to 1.01% in 2015.

Growth of cut flower cluster started to pick up in the 1980's when the leading exporters started commercial rose cultivation. However, the industry was characterized by low value and simple open field flowers with limited assortment in the 1980's (Real IPM, 2007). In the 1990's the industry shifted to higher-value flowers grown in greenhouses. According to MoA (2015), Kenya was exporting over 200,000 tons of flowers by 2014. In 2013, the value of rose flowers was ksh.15.9 billion and rose by 7% to Ksh.59.8 billion in 2014 (MoA, 2015). The total value of horticultural produce exported in 2017 increased from ksh.101.5 billion to 115 billion (FPEAK, 2019).

2.2 Biology of roses

Roses belong to the genera *Rosa* (Mohammed, 2013). The family rosaceae contains shrubs, vines and herbs with the leaves arranged along the stems in alternating manner and may be simple or in division of three to seven leaflets with sharp margin. The plant appears bushy and shrubby with straight woody branches, which are covered with prickles (Irish, 2010). A layer of cork

separates the prickle from the stem. The flowers belonging to rose plants can be single or clusters which are comprised of stamens, sepals, pistils and petals inserted at the edges and inside a receptacle which contains ovaries. The sepals are positioned on the outer side while the petal is found inside. The flower contains five sepals and five petals which are fused together to form a cup like enlargement of the receptacle of a flower called hypanthium (Irish, 2010). Plants in this family are ornamentals where roses are grown for their beauty, scents and for decorations (Xue *et al*., 2017).

2.3 Agronomic practices and production requirements of roses in Kenya

Generally, rose plants perform well in areas of above 1500 m above sea level with temperature requirements of 15 – 28^0C. During planting, grafting is the common method used in production (Kroin *et al*., 2016; Hort, 2019). Other practices include defoliation, manuring, irrigation, mulching and pests and disease management. Nitrogen, Phosphorous and Potassium (NPK) is applied at 8.8.18g per plant (Hort, 2019). Farm yard manure is also applied at three months interval. Mulching in roses help to decrease loss of moisture from the soil through evaporation and may improve the systematic growth of roots (Gray and Ophardt, 2015). Pruning is also done to remove diseased plants branches (Gray and Ophardt, 2015). Roses require well drained loamy, sandy loam and silty loam soils with pH of 6 to 7.5 but can still do well under pH of 8 (Hort, 2019).

2.4 Rose production constraints

Rose production is faced by both abiotic and biotic constraints. Biotic factors that influence growth of roses are arthropod pests including aphids, mites, thrips, and whiteflies, and diseases such as crown gall, powdery mildew, downy mildew and Botrytis (MoA, 2015; Abdul *et al*., 2016; Liu *et al*., 2018). Abiotic factors that influence growth of roses include light, which provide energy required for photosynthesis (Gu *et al*., 2017; Bayat *et al*., 2018). Other factors include high cost of investment associated with the farm inputs (MoA, 2015).

2.5 Crown gall disease of roses

Crown gall is a bacterial disease in roses caused by *Agrobacterium tumefacien*s, a soil borne-pathogen that is capable of entering rose plant when wounded (Fuller *et al*., 2016). The bacteria can stay in the soil for over 10 years. The bacterium parasitizes plant tissue by integration of some of its own deoxyribonucleic acid (DNA) into the host genome resulting in tumours and changes in plant metabolism (Feist *et al*., 2016). Infection mostly starts from infected planting medium where a susceptible host is established. Other sources of inoculum are pruning equipment, irrigation water, cultivation equipment, infected planting materials, detached or disintegrated galls put back in the soil and rouged plants (Maina *et al*., 2011).

Agrobacterium tumefaciens enter the plants through wounds and the higher the inoculum the bigger the galls and the severe the infection (Feist *et al*., 2016). Infection can be harboured by the plant for a long time without

symptoms only to appear later when the plant is wounded. Once the bacteria enter the wounds into the plant it takes about two weeks for the galls to start appearing (Murugi, 2015). 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 (Murugi, 2015).

Crown gall manifests itself initially as small swellings on the crown of the rose plant near the soil line, and occasionally on aerial portions of the plant (Feist *et al*., 2016). Young tumours, which often resemble the callus tissue that results from wounding are soft, somewhat spherical and white to cream colored (Nitin and Prashant, 2017). The young tumours are used in further diagnosis of crown gall through isolating *A.tumefaciens* from those galls. As tumours become older, their shape becomes quite irregular, and they turn brown or black. Tumours may be connected to the host surface by only a narrow bit of tissue, or may appear as a swelling of the stem, not distinctly separate (Nitin and Prashant, 2017). Additional symptoms include stunting of mature plants by causing disruption of vascular flow in the stem, chlorotic leaves, and plants may be more susceptible to adverse environmental conditions and secondary infection (Fuller *et al*., 2016). It has been reported that crown call disease on roses in Kenya results in yield loss of up to 60% depending on variety (Maina *et al*., 2011).

2.6 Management of crown gall on roses

Various management practices of crown gall disease include use of synthetic chemicals such as copper based products, application of soil fumigants, cultural methods and soil amendments (UC IPM, 2017). Preventive management practices starting with clean field provide the basis of integrated management of grown gall disease (GAP, 2007; KDLC, 2011). Use of pathogen free soils and intercropping with a resistant variety or soil fumigation with Telone® reduces the number of *Agrobacterium* cells in the soil though the method is not effective (Agrios, 2005).

The selection of disease free cuttings as well as weeding is very important (KDLC, 2011). Proper inspection of planting materials and discarding the affected ones should be practiced. Production of clean planting materials can be done through monitoring, scouting and recording of diseases present. Other cultural considerations include avoiding damage of stems when handling the plants, use of clean tools or hands when pruning, disinfecting regularly and rouging all infected plants. It is also important to treat irrigation water, particularly recycled water to kill phytopathogens like *A. tumefaciens* (GAP, 2007; KDLC, 2011). Murugi (2015) reported that rose growers pluck galls as a management measure but the method was not effective.

Biopesticides obtained from plants and microorganisms manage diseases in a non-toxic manner (Dubois *et al*., 2016; Damalas and Koutroubas, 2018). They are effective against many diseases and reduce yield losses (Kumar, 2015; Mishra *et al*., 2015). The notable bacterial antagonists include *Pseudomonas* and *Bacillus* that are characterized as plant growth promoting

rhizobacteria (Rhitu *et al*., 2015; Singh *et al*., 2016). Growth of *A. tumefaciens* is also inhibited by *A. rhizogenes* as demonstrated by Murugi (2015) and Rhouma *et al.* (2008). The continued bioprospecting studies on potential bacterial antagonists suggest that the rhizosphere of plants is a rich source of useful biocontrol agents (Murugi, 2015; Singh *et al*., 2016).

Bacterial organisms such as *Bacillus* sp and fungal organisms such as *Beauvaria* sp have been confirmed to manage diseases (Dunham, 2015; Mishra *et al*., 2015; Punja *et al*., 2019). Awasthi *et al.* (2019) reported that *Bacillus subtilis* Cohn suppressed damping off disease in brinjal. Reduction in use of conventional pesticides can be achieved by flower growers through usage of biological agents in Kenya. Biocontrol products are safe to the environment, reduce pathogen resistance and improve agricultural productivity (Campos *et al*., 2019). Copper based compounds such as funguran® are the most commonly used chemicals against crown gall, but do not give adequate control because of pathogen resistance and phytotoxicity effects (Maina *et al*., 2011; Murugi, 2015). Fumigants like Metham sodium has also been used as a growth inhibitor among bacteria belonging to the genus *Agrobacterium* which also does give edequate management (Saika *et al*., 2018)

2.7 Mechanisms of action of bacterial antagonists

Antagonistic bacteria employ various mechanisms to inhibit and suppress growth, and multiplication of pyhtopathogens. The most common mechanisms of action of bacterial antagonists are competition, antibiosis, and promotion of plant growth and induced resistance (Premachandra *et al*., 2016). In

competition, microbes obtain nutrients from soils and living plant surfaces so as to colonize a certain environment. These nutrients deplete with time and this makes microbes to compete effectively for the limited nutrients (Jamalizadeh *et al*., 2011; Premachandra *et al.,* 2016). Both antagonists and the pathogens compete with one another for space, nutrients and for survival. This interaction between the antagonists and the pathogen excludes the pathogen by depleting food and space (Zeng *et al*., 2018).

Antagonists produce various types of antibiotics to suppress plant pathogens or diseases (Elbendary *et al*., 2018). The antibiotics include water soluble, nonpolar/volatile and polar/ non-volatile antibiotics. Among these, the nonpolar antibiotics are more effective as they can act at sites away from the site of production. *Bacillus cereus* strain UW85 Frankland produces multiple antibiotics (zwittermycin and kanosamine) which can suppress one or more pathogens (Singh *et al*., 2011; Das *et al*., 2019).

Bacteria in the genera *Bacillus* also secrete Beta-1, 3 glucanase and chitinase that directly degrade cell walls of other microorganisms (Singh *et al*., 2011; Asraoui *et al*., 2018). *Pseudomonas* and *Bacillus* competitively colonize plant roots and act as biofertilizers and /or antagonists thus promoting plant growth (Kalayu, 2019). The principle involved in this mechanism is production of phytohormones that stimulate plant growth, antibiosis and siderophore production (Ferjani *et al*., 2019). *Paenibacillus* and *Bacillus* species express antagonistic action by suppressing the pathogens both under *in vivo* and *in vitro* conditions (Arrebola *et al.,* 2010; Wang *et al*., 2019). Other antagonistic bacteria induce resistance against pathogens. *Bacillus* sp has been reported to

have plant growth-promoting and systemic resistance-inducing activities (Chung *et al*., 2015).

2.8 Effectiveness of antagonistic bacteria as biopesticides

Various studies have been conducted to identify bacterial isolates from genera *Bacillus, Paenibacillus, Pseudomonas* and *Curtobacterium* with potential suppression against major plant pathogens. Tolba and Soliman (2013) reported that bacterial isolates from local environments in Egypt inhibited *A. tumefaciens in vitro* and also reduced incidence of crown gall on roses. Suppresiveness of *Rhizobacteria, Serratia plymuthica* Bizio alongside *Pseudomonas* sp. against crown gall caused by *A. tumefaciens* in tomatoes has also been reported (Dandurishvili *et al*., 2010).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Description of study sites

A survey to determine the incidence and severity of crown gall disease on roses was carried out from July to October 2017 in Kiambu, Laikipia and Nakuru Counties, Kenya. These Counties were selected based on their prominence in producing roses in Kenya and differed in terms of agro-ecological conditions (Murugi, 2015; KFC, 2019). The rainfall in the three Counties follows bimodal pattern where the long rains fall between March to May and short rains from October to November. In Kiambu County, a survey was conducted in Limuru and Kikuyu and Thika subcounties while in Laikipia County, the survey was conducted in Nanyuki subcounty which is on the leeward side of Mt Kenya and lies on a plateau zone. In Nakuru County, the survey was conducted in Naivasha subcounty (Kiambu CIDP, 2020; Laikipia CIDP, 2020; Nakuru CIDP, 2020)

Table 3.1 Location, rainfall, temperature and soil type of Nakuru, Kiambu and Laikipia Counties

Aspect	Nakuru	Laikipia	Kiambu
Coordinates	0°29'59.99"N, 36°00'0.00"E	0°01'0.01"N, 37°04'22.19"E	1°10'0.01"S, 36°49'59.99"E
Altitude	1,800 m asl	1,500-2,600 m asl	1,200-2,000 m asl
Rainfall per annum	500-1,000 mm	350-1,100 mm	962 mm
Temperatures (annual)	Low of 12 to a high of 30°C	Average of 22 - 26^0C	Low of 7 to a high of 34°C
Soil type	Volcanic, sandy	black cotton soil, dark reddish brown to red friable and rocky	clay, red and volcanic

Source: (Kiambu CIDP, 2018; Laikipia CIDP, 2018; Nakuru CIDP, 2018)

In vivo experiments were conducted at Magana Flowers farm, Valentine Flowers farm, Black petals Flower farm and Kenyatta University research farm. All the above farms are located in Kiambu County. Black petals flower farm is located at 1° 8' 29.07" S, 36° 44' 43.24" E and lies at 1,985m asl. Valentine Flowers is located at 1° 8' 42.63" S, 36° 45' 45.96" E and lies at 1,960m asl while Magana Flowers farm is located at 1° 15' 14.12" S, 36° 40' 33.56" E and it is found at 1,975m asl. Kenyatta University research farm is located at 1° 10' 36.12" S, 36° 56' 11.41" E and lies at 1,608 asl.

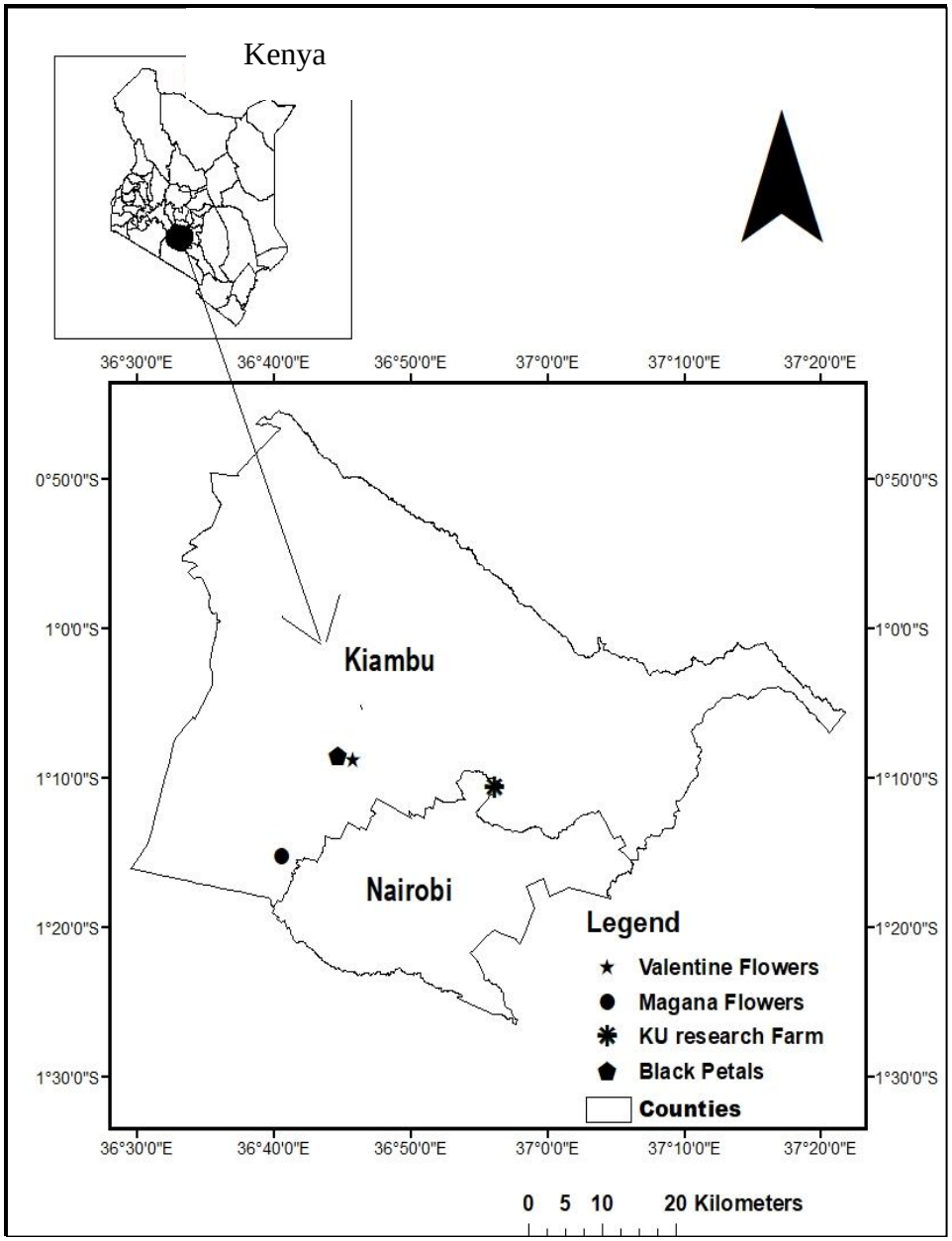

Figure 3.1 A map of Kenya showing the study sites (Magana Flowers farm, Black petals farm, Valentine Flowers farm and Kenyatta university research farm)

Source: Author

3.2 Determination of rose flower production practices under greenhouse conditions

Rose flower farms were randomly selected in Kiambu, Nakuru and Laikipia Counties. A total of 17 farms in the three counties were selected and a structured questionnaire was used to interview greenhouse production managers of each farm. The sample size was determined by Slovin's formula (n= N/ (1+ Ne2) (Almeda *et al*., 2010), where n= sample size N= total population and e= confidence level (error of tolerance). Information collected was on farm size, rose flower growth medium, area under rose flower production, years of rose flower production, production constraints, varieties grown and their susceptibility to crown gall, yield losses and disease management measures and their effectiveness.

3.3 Determination of incidence and severity of crown gall

The incidence and severity of crown gall on roses under greenhouse was determined visually in each farm. Seventeen greenhouses were sampled purposively and based on the most susceptible varieties as identified by the respondent. Plants were sampled in a zigzag method with a distance of two metres between plants and their number depended on the size of the greenhouse. Rose plants were counted and recorded as with or without galls. Disease incidence was calculated using the formula by Cao *et al.* (2011);

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

The diameter of galls and stem of the infected plants were measured using a Vernier calliper in centimetres to determine the disease severity of crown gall

based on the scale of 1-5 of Tao and Ming (2010), with modification (Table 3.2).

Table 3.2 Scale for scoring crown gall disease severity

Scale	Relevance
1	One gall formation on stem, ratio value of gall diameter (GD)/ stem diameter (SD) ≤ 1
2	One gall formation on the stem, with ratio value of GD/SD ≥ 1
3	Two galls on stem, with ratio value of GD/SD ≥1
4	More than two galls on the stem, with ratio value of GD/SD≥1
5	Gall formation on the whole stem, or plants dead

Source: (Tao and Ming, 2010).

The Percentage disease severity (PDI) was calculated using McKinney formula:

$$PDI = \frac{Total\ of\ numerical\ ratings}{Total\ number\ of\ plants\ observed} \times \frac{100}{Y}$$

where Y is maximum category value of the score chart.

3.4 Collection of galls and soil samples

Young and fresh galls were plucked from the crowns of the rose plants using sterilized blades. Soil sample (0.5 Kg) was extracted using a soil auger at a depth of 15-20 cm around the rhizosphere galled rose plants randomly selected in each greenhouse. The soil sample was mixed to obtain a composite sample from every greenhouse. The samples (galls and soil samples) were packed in Khaki bags, labelled and transported on the same day to Kenyatta University Agricultural laboratory in a cool box for isolation. The number of galls and soil samples depended on the size of greenhouse.

3.5 Isolation, purification and maintenance of *A. tumefaciens* from the galls

Agrobacterium tumefaciens isolates were obtained from young, fresh and tender galls of infected rose plants. The galls were chopped into small sizes using a sterile blade, surface sterilized in 1.3 % sodium hypochlorite for 3 minutes and rinsed in three exchanges of sterile distilled water to remove traces of sodium hypochlorite and dried by blotting using sterile paper towels. The chopped galls were crushed in one (1) millilitre of sterile distilled water using sterile pestle and mortar to form the suspension then kept undisturbed for ten minutes to allow the bacteria to be released from the tissue to the suspension. The suspension was streaked on Yeast Mannitol Agar supplemented with Congo red and incubated at 27°±2C at room temperature for 48 hours for bacterial growth (Bennali and Mohamed, 2013). After incubation, a single colony of *A. tumefaciens* was streaked on the same medium and incubated for 2-3 days to make pure cultures. The purified colonies were inoculated on Nutrient agar slants and then stored in the refrigerator at 4°C for use in subsequent experiments.

3.6 Isolation of antagonistic bacteria from the soil

Composite soil samples collected from the flower farms were air dried for 7 days at ambient room temperatures of 23±2°C. The samples were ground and serial dilutions made by diluting 1g of soil in 10ml sterile distilled water. One millilitre from the resultant suspension was added to 9 ml of sterile distilled water and repeated to obtain 10^{-2} and 10^{-3} dilutions, respectively.

One millilitre from each dilution was inoculated through pour plate method on Nutrient agar, King's Medium B base and Tryptic soy agar for isolation. This was done under aseptic conditions in the laminar flow hood. Incubation of bacterial cultures took 48 hours with petri plates in inverted position at temperature of 23±2°C. The cultures were purified and stored in slants. The isolation procedure was conducted according to Schaad *et al*. (2001)

3.7 Identification, mass multiplication and formulation of bacterial isolates

Morphological, biochemical and physiological tests were used to determine the identity of the bacterial isolates. The identification was conducted according to Schaad *et al.* (2001) and Bergey's manual of Determinative Bacteriology (Holt *et al.*, 1994). The size, colour, shapes and growth of antagonistic bacterial colonies were recorded after being incubated for 48 hours. Biochemical and physiological tests were performed for further identification of bacteria. Urease, catalase and oxidase are some of the biochemical tests performed to confirm the isolates. During gram staining, colonies of bacterial isolates were air dried and heat fixed by passing smeared slides over a Bunsen burner flame before viewing under oil immersion in a compound light microscope at 10 um. Further, the active bacterial isolates were subjected to molecular analysis by total sequence screening which involves genomic DNA extraction from cultured bacterial isolates and Polymerase Chain Reaction (PCR) amplification; this was conducted at Kenya Plant Health Inspectorate Service (KEPHIS).

Bacterial isolates were inoculated into nutrient ager broth and incubated at 25°C for 72 hours on a rotary shaker at 150 rpm. Rice bran was soaked in sterile distilled water overnight and excess water drained off and autoclaved at 121°C (15 Pa) for 20 minutes. Rice bran was allowed to cool to room temperature of 22 – 25°C prior to inoculation with each bacterial isolate. Two hundred millilitres of each bacterial isolates at cell density of 10^8 Colony Forming Unit (CFU) per ml in the broth were inoculated into polythene bags containing rice bran. The bag contents were then thorough mixed, and incubated at 25 ± 2°C for 14 days (Niranjana *et al*., 2009). After 14 days, talcum powder (carrier) was added to each of the growth medium in the ratio of 2:1 and placed in a plastic tray under aseptic conditions. Carboxymethyl cellulose (CMC) was then added at the rate of 1g/100 grams of the growth medium and mixed well.

Population density of bacterial isolate in the mixture was determined following serial dilution plate technique on nutrient agar medium. One gram of the mixture (rice bran, CMC, talcum powder and each of the isolate) was added to 9 milliliters of distilled water and allowed to mix well. One millilitre from the resultant suspension was added to 9 millilitres of sterile distilled water and repeated to obtain 10^{-3} dilutions. Cell density of each antagonistic bacteria was calculated using the formula:

$$\text{CFU/ml} = \frac{\text{Number of colonies per ml plated}}{\text{Total dilution factor}}$$

3.8 Screening of antagonistic bacteria against *A. tumefaciens*

In vitro antagonism to evaluate the effects of the 15 bacterial isolates was carried out using agar disk diffusion method (Habbadi *et al*., 2017).

Briefly, the bacterial suspension of *A. tumefaciens* with a cell density of 10^8 CFU/ml was prepared and spread on nutrient agar. The Petri dishes were dried aseptically for 15 mins. Three paper disks (6mm) containing fresh antagonistic bacteria were placed equidistantly on each petri dish and incubated at 25°C for 72 hours. Simultaneously, distilled water was added instead of antagonistic isolates to serve as control. Antagonistic activity was indicated by a clear zone of inhibition around the antagonistic bacteria. Diameters were measured using a ruler and expressed in millimetres to compare the performance of these antagonists. The best four bacteria that suppressed growth of *A. tumefaciens* with diameters of clear zones measuring above 5mm were selected for further studies. Figures 3.1, 3.2 and 3.3 shows the test pathogens, one of the active isolates and the method used during screening, respectively.

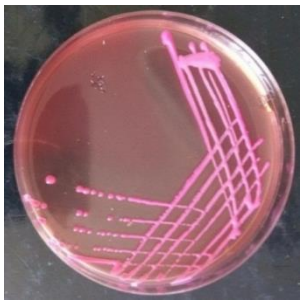

Plate 3.1 *Agrobacterium tumefaciens*

Plate 3.2 *Arthrobacter* sp. 1

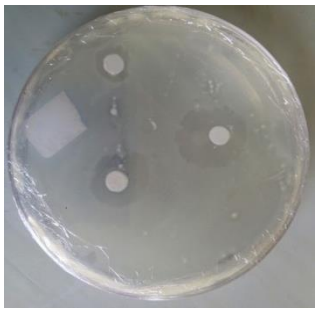

Plate 3.3 Agar disc method

Source: Author

3.9 Determination of antibacterial activity of the selected antagonistic bacteria against crown gall

The field trials were conducted from November 2018 to February 2019 at Kenyatta University and at three commercial flower farms (Magana Flowers farm, Black petals and Valentine Flowers) in Kiambu County. The management of the above named farms accepted the experiment to be conducted in their farms. The treatments included *Lactobacillus brevis* 2.28.11, *Micrococcus luteus* 1 Cohn, *Micrococcus luteus* 2 Cohn and *Arthrobacter* sp 1 (all isolated locally), Funguran® (Copper hydroxide) which is a synthetic product, control with galls and a control without galls) and were applied on two rose varieties susceptible to crown gall i.e. Tropical Amazon® and Upper Class®.

At Kenyatta University, soil, sand and cattle manure were autoclaved at 121°C for 20 min separately and allowed to cool under sterile conditions before mixing them at the ratio of 1:1:1 and filling in plastic pots measuring 20 cm in diameter and 20 cm in height. This was followed by planting rose stems obtained from certified commercial breeder. On the same day, plants were bruised using sterile blades at the crown and 10^8 CFU/ml of *A. tumefaciens* was inoculated on the bruised wounds. At the same time, each of the antagonistic bacteria (10^8 CFU/ml) was also introduced on the same wound inoculated with *A. tumefaciens*. The wounds were then wrapped using sterilized parafilm. Both *A. tumefaciens* and each of the antagonistic bacteria were applied by smearing using a brush and the plant left to grow. Presence of galls was checked and recorded after two weeks.

For the established plants, the stems were bruised using a sterile blade to introduce approximately 10^8 CFU/ml of *A. tumefaciens* which was followed by wrapping the wound with parafilm. The same amount (10^8 CFU/ml) of each bacterial isolate was introduced in the same way as *A. tumefaciens* to the wound after the galls had formed. The galls formed after two weeks. The galls were also smeared with each of the antagonistic bacterial isolates.

At Magana Flowers farm, Black petal farm and Valentine Flowers farm, plants selected in the greenhouses were already naturally infected with crown gall and were selected based on the presence of galls, number of galls, number of shoots, height of the shoots and size of the galls. Plants were selected with 2 galls and two shoots with heights of 1-10 cm. The sizes of the selected galls were between 2-5cm diameters. Further a plant without visible

galls was also considered for control. The treatments included *Lactobacillus brevis* 2.28.11, *Micrococcus luteus* 1, *Micrococcus luteus* 2 and *Arthrobacter* sp 1, Funguran® (Copper hydroxide) which is a synthetic product, control with galls and a control without galls. On farm Experiment was set in a Completely Randomized Design (CRD) with 7 replicates per treatment. Treatments were applied by smearing the bacterial isolates on to the galls using a brush and bruising the plant on the stem with a sharp sterile blade then smearing with each of bacterial isolates before wrapping the wound with a sterile parafilm.

Data from KU site and the three commercial flower farms were recorded on number of galls, size of galls, and number of shoots for 10 weeks at 7 days interval. Data on shoot height was recorded for six weeks (Harvesting time for commercial flower farms) apart from K.U site where the data on shoot height was recorded for 10 weeks. All the data was recorded at 7 days interval. The size of galls was measured using Vernier calliper while the height of the plant was taken using a string and a ruler. Height of shoots was expressed in centimetres while change in gall size was calculated and expressed as a percentage using the following formula:

Percentage growth of gall size = (Final-Initial)/Initial×100.

3.10 Data analysis

Data on incidence and severity of crown gall, farm size, main rose varieties grown, susceptible varieties, resistant rose varieties, education level, losses due to crown gall, variety preferred and reasons of preference management practices and their effectiveness was analyzed using Statistical

Package for the Social Sciences (SPSS) version 18. Diameters of inhibition zone (mm), number of shoots, height of shoots (cm), number of galls, and percentage change of gall size were subjected to analysis of variance (ANOVA) and the difference between the treatment means separated using the Fischer's least significant difference at 5% probability level using Statistical Analysis Software (SAS) version 9.2. Data was presented in tables and graphs.

CHAPTER FOUR: RESULTS

4.1 Survey results

A total of 17 questionnaires were administered in three Counties where rose production is carried out. All the respondents were production managers in the flower farm. Kiambu, Laikipia and Nakuru Counties had eight, four and five flower farms, respectively. The respondents had diverse work experience with over five years' experience being dominant across the three Counties. Among the respondents with over five years' experience, 80% were from Nakuru County, while 75% and 50% were from Kiambu and Laikipia Counties, respectively (Table 4.1).

Nearly all the respondents had attained post-secondary education across the counties (Table 4.1). In Laikipia and Nakuru Counties, all the respondents had attained post-secondary education whereas Kiambu County had 87.5% of the respondents with post-secondary education.

Table 4.1 Work experience and education level of respondents (%) on crown gall disease in Kiambu, Nakuru and Laikipia Counties

Respondents' (%) on work experience				Respondents' (%) on education level	
County	< one year	1-2 years	>5 years	Secondary	Post-secondary
Kiambu	12.5	12.5	75.0	12.5	87.5
Laikipia	25.0	25.0	50.0	0.0	100.0
Nakuru	0.0	20.0	80.0	0.0	100.0

Rose flower farms in Kiambu and Laikipia Counties were reported to have a farm size of 41-50 ha by 50% of the respondents, while in Nakuru

County, 60% of the respondent mentioned that their flower farms' size were over 50 ha (Figure 4.1). Surveyed flower farms had been engaging in rose production for more than 10 years as reported by all the respondents across the Counties. These roses were established on soil by 75%, 75% and 60% of the respondents in Kiambu, Laikipia and Nakuru Counties, respectively.

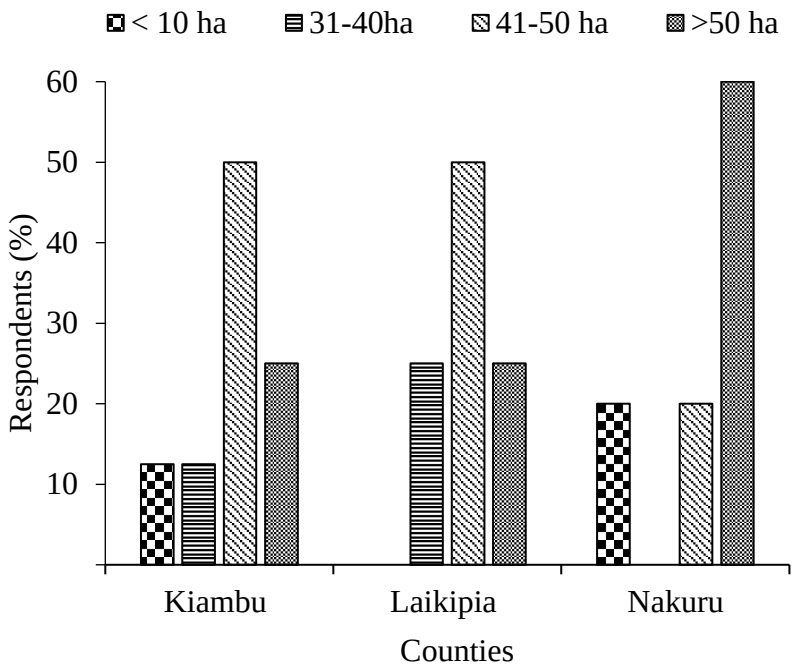

Figure 4.1 Farm size (ha) owned by rose flower growers (%) in Kiambu, Laikipia and Nakuru Counties.

4.2 Preference and reasons of choice of rose flower cultivars grown in Kiambu, Laikipia and Nakuru Counties

Results revealed that Cultivar Tropical Amazon® was the most widely grown variety across the three Counties (Table 4.2). In Kiambu County, 87.5% of the respondents mentioned Upper Class® as the leading variety and Intense as the least. Tropical Amazon® accounted for 50% in all the sampled flower

farms in Laikipia County. Intense®, Takazi® and Marosa®, were the least grown rose varieties in Lakipia County, each with a proportion of 20%. The latter two varieties, Takazi® and Marosa®, were only grown in Laikipia County. In Nakuru County, Red Kamala® was reported as the main variety grown by 60% of the respondents. In the same County, Tropical Amazon®, Upper Class®, Ace Pink®, Athena® and Madam Red® were grown by 40%, 40%, 20%, 20%, 20% of the respondents, respectively (Table 4.2).

Table 4.2 Preference of rose varieties by rose flower growers (%) in Kiambu, Laikipia and Nakuru Counties

	Respondents' (%) preference of rose varieties		
Variety	Kiambu	Laikipia	Nakuru
Tropical Amazon®	50.0	50.0	40.0
Intense®	12.5	25.0	20.0
Upper class®	87.5	0.0	40.0
Akito®	25.0	0.0	0.0
Ace pink®	12.5	0.0	20.0
Athena®	12.5	0.0	20.0
Sonrisa®	12.5	0.0	0.0
Takazi®	0.0	25.0	0.0
Marosa®	0.0	25.0	0.0
Red Kamala®	0.0	0.0	60.0
Madam Red®	0.0	0.0	20.0

The survey further sought to find out why Upper Class®, Tropical Amazon® and Red Kamala® were the most preferred. A unanimous finding in the three Counties by all the respondents was that the varieties were preferred due to customer demand (100%). However, the respondents also mentioned high yields, ability to resist diseases and pests and early maturity as reasons for their preference (Figure 4.2). High yields as a reason for varietal preference was mentioned by 50% of the respondents in Kiambu County while in Nakuru and Laikipia Counties, it was mentioned by 40% of the respondents. In Kiambu County, early maturity and resistance to pests and diseases as reasons for varietal preference were identified by 25% and 37.5% of the respondents, respectively (Figure 4.2).

In Laikipia County, early maturity and resistance to pests and diseases was identified as reasons for varietal preference by equal proportion of 25% of the respondents. In Nakuru County, early maturity and resistance to pests and diseases was identified by 20% and 40% of the respondents, respectively (Figure 4.2). Although one of the reasons for choice of preferred variety was resistance to pests and diseases, none of the cultivars preferred was resistant to crown gall disease. Apart from roses, some flower farms in Nakuru County produced other cut flower types like Ammivisnaga and Hypericums. However, crown gall was not reported on other flower types.

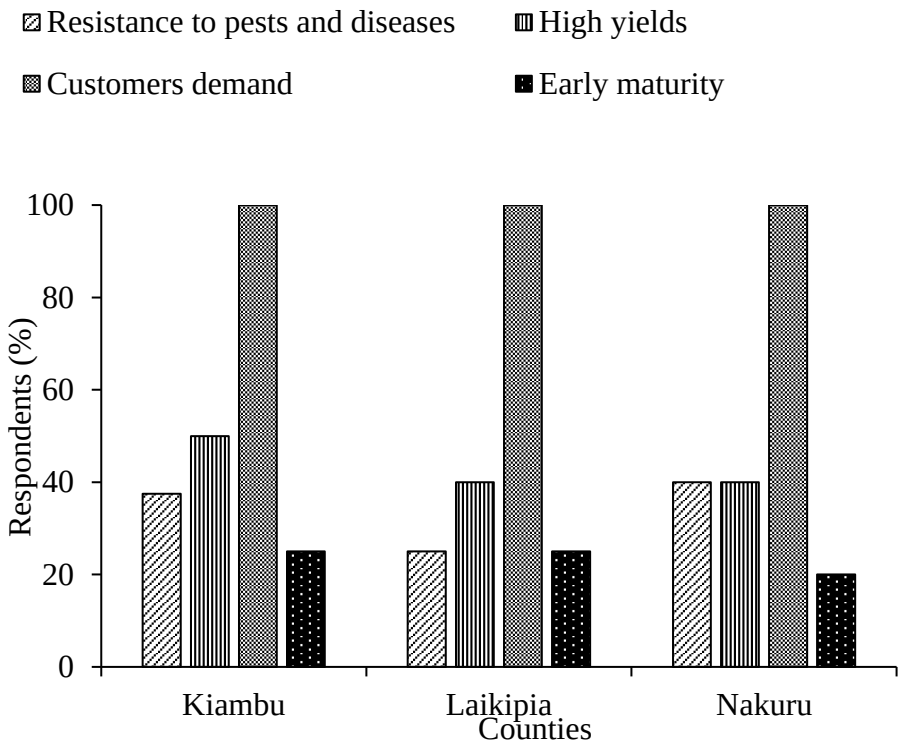

Figure 4.2 Reasons for choice of rose varieties by rose flower growers (%) in Kiambu, Laikipia and Nakuru Counties

Awareness of crown gall, susceptible and resistant rose varieties to crown gall in Kiambu, Laikipia and Nakuru Counties. All the respondents in the areas that were surveyed were aware of crown gall disease. Further, all the respondents indicated that crown gall on rose plants is diagnosed by presence of galls. In Nakuru County, 20% of the respondents also mentioned stunted growth of roses as a symptom of crown gall infection.

Susceptible rose varieties grown in the Counties surveyed varied across the Counties. Tropical Amazon® was the only susceptible variety that was found across the three Counties. Red Kamala® was the most susceptible variety reported by all the respondents in Nakuru County. Upper Class® in Kiambu County and Tropical Amazon® in Lakipia County were reported to be the most susceptible varieties grown by 62.5% and 75% of the respondents, respectively (Table 4.3). Additionally, Tropical Amazon® was reported to be susceptible to crown galls by 50% and 60% of the respondents in Kiambu and Nakuru Counties, respectively. Upper Class was also reported to be susceptible to crown gall in Nakuru County by 60% of the respondents (Table 4.3). Other susceptible varieties planted include Athena® and Madam Red®, Takazi® and Marosa®.

Table 4.3 Rose varieties susceptible to crown gall as reported by rose flower growers (%) in Kiambu, Laikipia and Nakuru Counties

	Respondents' (%) on varieties susceptible to crown gall		
Variety	Kiambu	Laikipia	Nakuru
Tropical Amazon®	50.0	75.0	60.0
Upper class®	62.5	0.0	60.0
Athena®	0.0	0.0	40.0
Takazi®	0.0	25.0	0.0
Marosa®	0.0	25.0	0.0
Red Kamala®	0.0	0.0	100.0
Madam Red®	0.0	0.0	40.0

Furiosa®, Red Calypso®, Shanty®, Ai® and Jupita®. Furiosa® and Red Calypso® rose varieties were reported to be resistant to crown gall across the Counties. In Kiambu County, 62.5 % of the respondents reported Furiosa® rose variety as the most resistant against crown gall disease, it was followed closely by Red Calypso® as reported by 50% of the respondents (Table 4.4). In Laikipia County, 50% of the respondents ranked first Jupita®, Red Calypso® and Furiosa® varieties in terms of resistance to crown gall disease. However, Jupita® variety was not reported to be grown in Kiambu and Nakuru Counties. In Nakuru County, Furiosa® and Red Calypso® were reported as resistant varieties to crown gall by 60% and 50% of the respondents, respectively (Table 4.4).

Table 4.4 Rose varieties resistant to crown gall as reported by rose flower growers (%) in Kiambu, Laikipia and Nakuru Counties

	Respondents (%) on varieties resistant to crown gall		
Variety	Kiambu	Laikipia	Nakuru
Furiosa®	62.5	50.0	60.0
Red Calypso®	50.0	50.0	50.0
Shanty®	12.5	0.0	0.0
Ai®	25.0	0.0	20.0
Jupita®	0.0	50.0	0.0

4.3 Factors contributing to the presence of crown gall in the surveyed areas

According to respondents, contaminated planting materials, infested soils and poor greenhouse hygiene were identified as main factors contributing to presence of crown gall disease in the surveyed areas. Overall poor greenhouse hygiene ranked first in contributing to the presence of crown gall across the Counties. However, poor greenhouse hygiene and contaminated planting materials tied at of 50% of the respondents in Laikipia County. In the same County, infested soils were reported by 25% of the respondents.

In Kiambu County, Poor greenhouse hygiene was the highest as reported by 75% of the respondents followed by contaminated planting materials and infested soils which were reported by 40% and 37.5% of the respondents, respectively. In Nakuru County poor greenhouse hygiene was reported by 60% of the respondents. In the same County, 40% and 20% of the respondents reported that contaminated planting materials and infested soils, respectively, led to presence of crown gall disease (Figure 4.3). The study went further to

find out the sources of planting materials for rose flower growers. These planting materials were from own cuttings, local commercial propagators and or both with means of 34.2%, 23.3% and 42.5%, respectively.

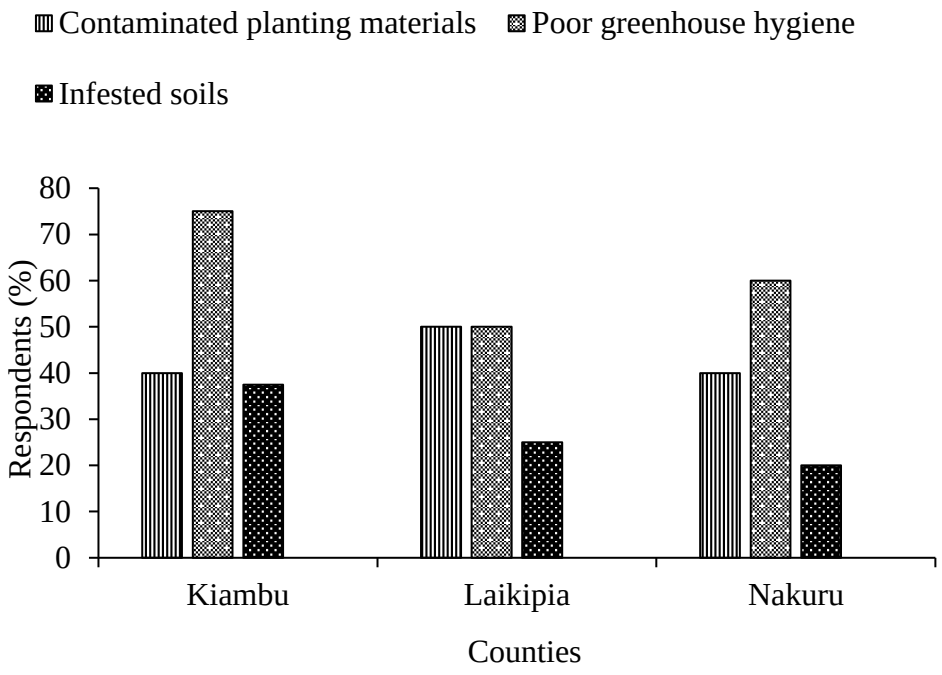

Figure 4.3 Factors contributing to presence of crown gall as reported by rose flower growers (%) in Kiambu, Laikipia and Nakuru Counties

4.4 Other diseases and constraints affecting rose production in Kiambu, Laikipia and Nakuru Counties

Other diseases that were found to be a threat to rose flower production are powdery mildew, downy mildew and *Botrytis.* Production of roses in the study areas is also constrained by other factors namely pests such as mites and thrips, price fluctuation, transport cost and high production cost. However, the leading factor was unanimously identified to be pests (Table 4.5).

Table 4.5 Other constraints affecting rose flower production as reported by fose flower growers (%) in Kiambu, Laikipia and Nakuru Counties

	Respondents (%) on other contraints affecting rose flower production		
Constraints	Kiambu	Laikipia	Nakuru
Pests (Thrips, mites)	100.0	100.0	100.0
Price fluctuation	50.0	25.0	20.0
Transport cost	37.5	50.0	62.5
High production cost	100.0	75.0	80.0

4.5 Incidence, severity and loses experienced due to crown gall

From the survey, it was noted that crown gall was present in the three counties. In Kiambu County, Magana flower farm had the highest disease incidence with a mean of 71.4%, followed by Lauren, Enkasiti, Branan, Simbi, Gatoka, Valentine, and Black petals flower farm with means of 65.3%, 56.3%, 56.1%, 52.6% 50%, 48%, and 32.3% respectively. All the flower farms in Nakuru County experienced crown gall, Van den berg farm had the highest incidence of 72% followed by Groove, Wildfire, Panda and De Ruiter with means 67.4, 60%, 57% and 18%, respectively. In Laikipia County, Equinox flower farm ranked first in disease incidence with a mean of 72.5%, followed by Everest, Kisima and Tambuzi with means of 72.4%, 63%, and 56.4%, respectively (Table 4.6).

Enkasiti flower farm in Kiambu County recorded the highest crown gall severity of 60%, followed by Valentine, Branan, Simbi roses, Lauren, Gatoka,

Magana, and Black petals with means of 58.8%, 57.6%, 57.4%, 57%, 56.4%, 53.2% and 39.2%, respectively. In Nakuru County, the highest level was recorded in Wildfire flower farm by a mean of 60.6%, followed by Panda, Van den berg, Groove and De Ruiter farm with means of 57.8%, 56.2%, 53.6% and 27.8%, respectively. In Laikipia County, Kisima flower farm had the highest disease severity of 57.4%, followed by Equinox, Tambuzi and Everest with means of 55.6%, 55.4% and 54.2%, respectively (Table 4.6).

Table 4.6 Incidence and severity (%) of crown gall disease in Kiambu, Laikipia and Nakuru Counties

County	Flower farm	Variety	Disease incidence (%)	Disease severity (%)
Kiambu	Enkasiti	Upper Class®	56.3	60.0
	Lauren	Tropical Amazon®	65.3	57.0
	Simbi	Upper Class®	52.5	57.4
	Branan	Upper Class®	56.1	57.6
	Gatoka	Upper Class®	50.0	56.4
	Valentine	Upper Class®	48.0	58.8
	Black petals	Tropical Amazon®	32.3	39.2
	Magana	Tropical Amazon®	71.4	53.2
Nakuru	Wildfire	Upper Class®	60.0	60.6
	Van den berg	Upper Class®	72.0	56.2
	De Ruiter	Red kamala®	18.0	27.8
	Panda	Upper class®	57.0	57.8
	Groove	Red kamala®	67.3	53.6
Laikipia	Kisima	Tropical Amazon®	63.0	57.4
	Everest	Takazi®	72.4	54.2
	Equinox	Tropical Amazon®	72.5	55.6
	Tambuzi	Marosa®	56.4	55.4

Majority of the respondents indicated that 41-50% of rose flower loss in Kiambu and Laikipia Counties was due to crown gall disease (Table 4.7). The loss differed by County. In Kiambu County, 62.5% of the respondents stated that the loss experienced due to crown gall disease was between 41 – 50%. In Laikipia and Nakuru Counties, the loss due to crown gall disease was reported to be 41-50% by 75% and 60% of the respondents, respectively (Table 4.7).

Table 4.7 Losses experienced due to presence of crown gall as reported by rose flower growers (%) in Kiambu, Laikipia and Nakuru Counties

	Respondents (%) on loss due to crown gall			
County	21-30%	31-40%	41-50%	> 50%
Kiambu	12.5	12.5	62.5	12.5
Laikipia	0.0	25.0	75.0	0.0
Nakuru	20.0	0.0	60.0	40.0

4.6 Management methods of crown gall disease in Nakuru, Laikipia and Kiambu Counties

Four strategies were used in managing crown gall on roses according to the baseline survey. These strategies included use of synthetic chemicals, specifically copper based products, cultural (plucking of galls, greenhouse hygiene), *Trichoderma* formulation and Integrated Disease Management (IDM) (synthetic chemicals, biological and cultural) (Figure 4.4). Majority of the production managers interviewed across the Counties indicated that their flower farms used synthetic chemicals for the management of crown gall disease. In Kiambu County, use of synthetic chemicals, IDM and cultural methods to manage crown gall was reported by 50%, 37.5%, and 12.5% of the respondents, respectively (Figure 4.4). In Laikipia County, use of synthetic chemicals and Integrated Disease Management (IDM) strategies ranked first and was reported by equal number of 50% of the respondents.

Use of cultural practices alone and biological methods were not reported in Laikipia County. In Nakuru County, use of synthetic chemicals and IDM methods were reported by 40% of the respondents. Use of biological method

(*Trichoderma* formulation) in Nakuru County was reported by 20% of the respondents (Figure 4.4).

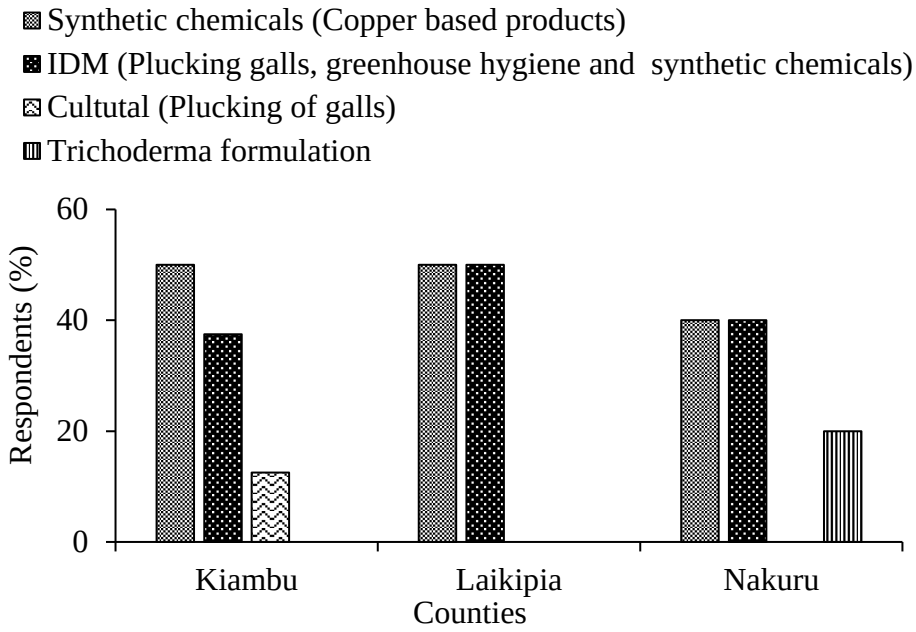

Figure 4.4 Crown gall management measures on roses as reported by rose flower growers (%) in Kiambu, Laikipia and Nakuru Counties

Use of synthetic chemicals and cultural method (plucking of galls) were ineffective as reported by all the respondents in Kiambu County. In the same county, integrated disease management (plucking of galls, greenhouse hygiene and synthetic chemicals) measures were reported to be effective by 33.3% of the respondents while 66.7% indicated that the method was not effective (Figure 4.5).

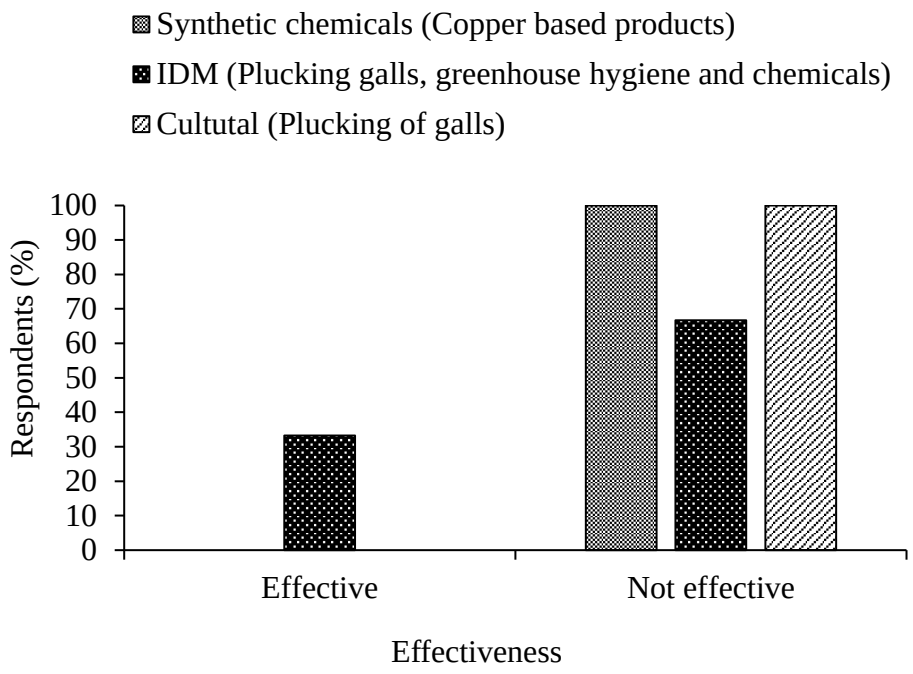

Figure 4.5 Effectiveness of management methods pf crown gall as reported by rose flower growers (%) in Kiambu County

In Laikipia County, all the respondents stated that synthetic chemicals were not effective. There was no agreement on effectiveness of IDM (plucking of galls, greenhouse hygiene and synthetic chemicals) use in Laikipia County (Figure 4.6).

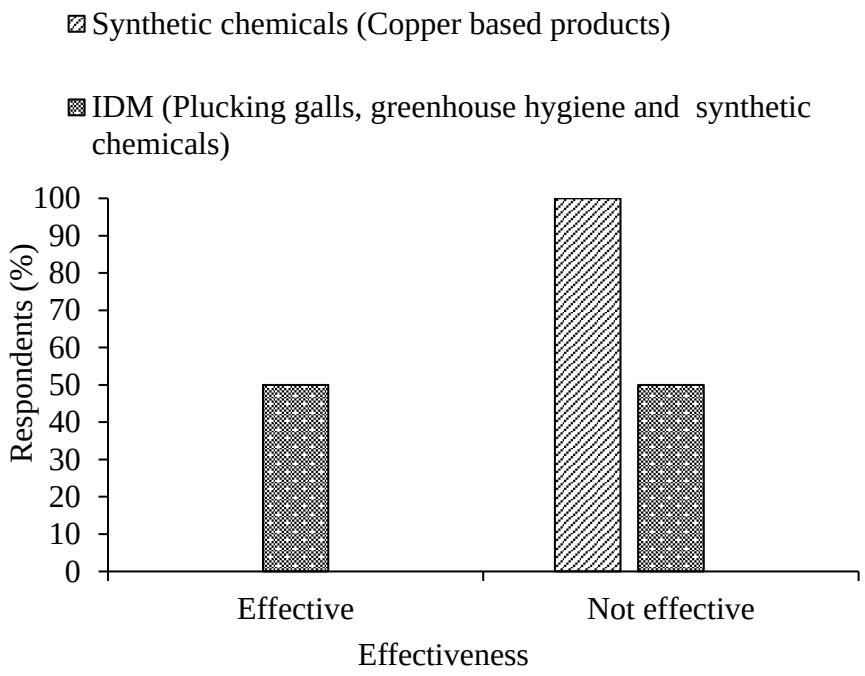

Figure 4.6 Effectiveness of management methods of crown gall as reported by rose flower growers (%) in Laikipia County

In Nakuru County, all the respondents reported that synthetic chemicals were ineffective (Figure 4.7). There was no agreement on effectiveness of IDM (plucking of galls, greenhouse hygiene, *Trichoderma* formulation and synthetic chemicals) in Nakuru County. In the same County, all those who used biological method, notably, *Trichoderma* formulation reported that it was effective.

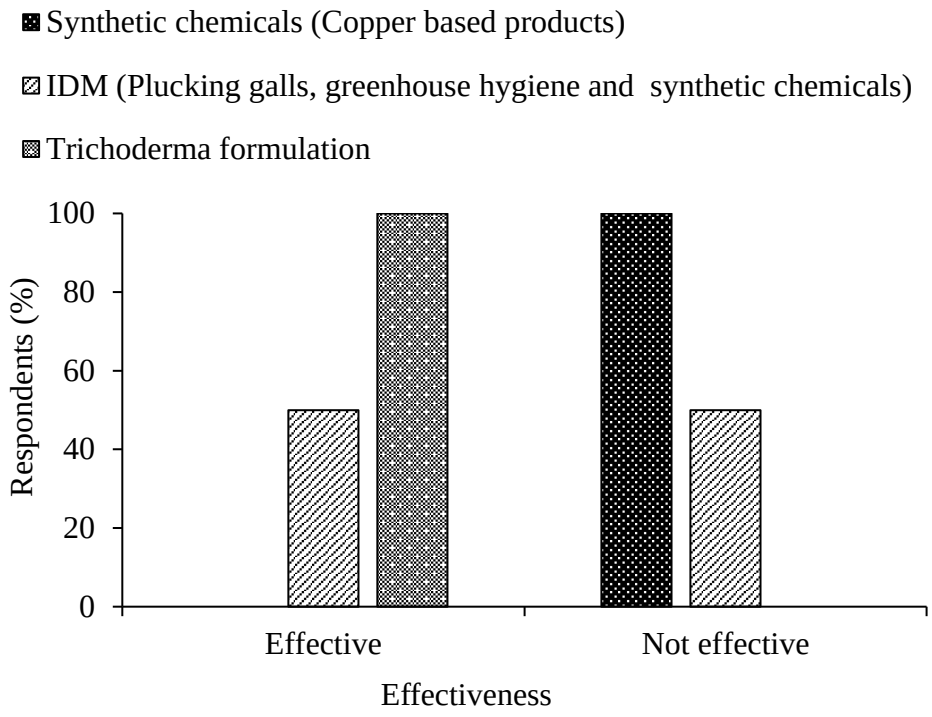

Figure 4.7 Effectiveness of management methods of crown gall as reported by rose flower growers (%) in Nakuru County

4.7 Antagonistic activity of bacterial isolates against *Agrobacterium tumefaciens in vitro*

The isolates showing clear zones of inhibition comprised of genera *Lactobacillus* sp, *Micrococcus* sp, and *Arthrobacter* sp. Out of the above mentioned isolates, four had diameters measuring over 5mm diameter of inhibition zone. The four included *Arthrobacter* sp 1, *Lactobacillus brevis 2.28.11*, *Micrococcus luteus 1* and *Micrococcus luteus 2*. The four mentioned isolates inhibited *A. tumefaciens in vitro* in variable degree and were considered for further trials in the field (Table 4.8).

The greatest inhibitory activity resulted from *Arthrobacter* sp with a mean of 7.8mm, followed by *Lactobacillus brevis* 2.28.11, *Micrococcus luteus* 1 and lastly *Micrococcus luteus 2* with means of 7.1mm, 5.4mm, and 5.1mm, respectively. The inhibitory activity of *Arthrobacter sp* 1 and *Lactobacillus brevis* 2.28.11 was not significantly different from each other. Additionally, the diameters due to *Lactobacillus brevis* 2.28.11 and *Arthrobacter* sp 1 were significantly (df = 15, 47; P = <.0001; F value = 60.2) higher than that of *Micrococcus luteus 2* and *Micrococcus luteus* 1 (Table 4.8). Diameters as a result of *Micrococcus luteus* 1 and *Micrococcus luteus* 2 did not differ significantly. Antagonistic bacterial isolates with clear zones with the diameters below 3 mm were considered to be less effective.

Table 4.8 Diameter (mm) of inhibition zone by bacterial isolates against *A.tumefaciens in vitro*

Source	Bacterial isolates	Zone of inhibition (mm) after three days (Means±S.E)
Kiambu	*Arthrobacter* sp 1	7.8±0.54^{a}
	Micrococcus luteus 1	5.4±0.16^{b}
	Arthrobacter sp 2	2.2±0.10^{c}
	Micrococcus sp 5	0.4±0.09^{ef}
Laikipia	*Micrococcus luteus* 2	5.1±0.20^{b}
	Lactobacillus brevis 2.28.11	7.1±0.06^{a}
	Micrococcus sp 3	0.8±0.11^{def}
	Micrococcus sp 6	0.7±0.15^{def}
	Lactobacillus sp 3	0.7±0.11^{def}
	Micrococcus sp 4	0.4±0.12^{ef}
Nakuru	*Micrococcus* sp 3	1.2±0.27^{de}
	Lactobacillus sp 1	1.1±0.49^{de}
	Lactobacillus sp 2	0.8±0.38^{def}
	Micrococcus sp 4	0.5±0.24^{ef}
	Arthrobacter sp 3	1.6±0.89^{c}
	Distilled water (Control)	0.0±0.00^{f}
	P value	<.0001
	LSD (P≤0.05)	0.9537
	F value	60.20

Means ± SE followed by similar letter(s) in the same column do not differ significantly at P≤0.05 (Fisher's protected L.S.D test).

4.8 Evaluation of antagonistic bacterial isolates on Rose Tropical Amazon® cultivar at Magana Flowers and Valentine Flowers farms

4.8.1 Number of galls formed on rose plants

At Magana Flowers farm, galls on plants treated with *Lactobacillus brevis* 2.28.11 and *Micrococcus luteus* 2 did not increase and their size remained contant throughout the monitoring period (Table 4.9). Contrary, the number of galls increased steadily over time in plants treated with *Arthrobacter* sp 1, *Micrococcus luteus* 1, funguran® and control; however they did not differ significantly. This was also confirmed at Valentine Flowers farm (Table 4.10). For the first three weeks of evaluation, the number of galls in all the treatments evaluated at Magana Flowers farm did not differ significantly (Table 4.9). Similar case was observed at Valentine Flowers farm in week 1 (Table 4.10). From week 4 to week 10, the number of galls on plants treated with *L. brevis* 2.28.11 and *M. luteus* 2 at Magana Flowers farm were significantly ($df = 5, 41$; $P<0.05$) lower than those produced by plants treated with *Arthrobacter* sp 1, *M. luteus* 1, funguran® and control (Table 4.9). Similar observation was made at Valentine Flowers farm from week 2 to week 10 (Table 4.10)

Table 4.9 Number of galls (Mean±S.E) on Rose Tropical Amazon® cultivar treated with bacterial isolates at Magana Flowers farm from November 2018 to February 2019

	Time (weeks) during treatment application (Mean±S.E)									
Treatments	1	2	3	4	5	6	7	8	9	10
Arthrobacter sp 1	2.3±0.18^{a}	2.3±0.18^{a}	2.6±0.20^{a}	2.9±0.14^{a}	3.1±0.14^{a}	3.3±0.29^{a}	3.4±0.30^{a}	3.6±0.30^{a}	4.0±0.31^{a}	4.1±0.26^{a}
Lactobacillus brevis 2.28.11	2.1±0.14^{a}	2.1±0.14^{a}	2.1±0.14^{a}	2.1±0.14^{c}	2.1±0.14^{b}	2.1±0.14^{b}	2.1±0.14^{b}	2.1±0.14^{b}	2.1±0.14^{b}	2.1±0.14^{b}
Micrococcus luteus 1	2.3±0.18^{a}	2.4±0.20^{a}	2.7±0.29^{a}	2.7±0.29^{ab}	3.0±0.22^{a}	3.0±0.22^{a}	3.3±0.29^{a}	3.4±0.30^{a}	3.7±0.36^{a}	3.7±0.36^{a}
Micrococcus luteus 2	2.3±0.18^{a}	2.3±0.18^{a}	2.3±0.18^{a}	2.3±0.18^{bc}	2.3±0.18^{b}	2.3±0.18^{b}	2.3±0.18^{b}	2.3±0.18^{b}	2.3±0.18^{b}	2.3±0.18^{b}
Funguran®	2.4±0.20^{a}	2.3±0.18^{a}	2.6±0.20^{a}	2.9±0.14^{a}	3.3±0.18^{a}	3.3±0.18^{a}	3.6±0.37^{a}	3.7±0.36^{a}	3.9±0.34^{a}	4.0±0.44^{a}
Control	2.3±0.18^{a}	2.3±0.18^{a}	2.6±0.20^{a}	2.7±0.18^{ab}	3.0±0.22^{a}	3.0±0.22^{a}	3.1±0.26^{a}	3.3±0.29^{a}	3.6±0.37^{a}	3.9±0.40^{a}
P value	0.938	0.938	0.391	0.038	0.001	0.001	0.001	0.001	0.001	0.001
L.S.D (P≤0.05)	0.520	0.520	0.595	0.538	0.529	0.603	0.767	0.779	0.853	0.911
F value	0.25	0.25	1.07	2.65	6.64	5.62	5.19	6.29	7.70	7.99

Means ± SE followed by similar letter(s) in the same column do not differ significantly at P≤0.05 (Fisher's protected L.S.D test).

Table 4.10 Number of galls (Mean±S.E) on Rose Tropical Amazon® cultivar treated with bacterial isolates at Valentine Flowers farm from November 2018 to February 2019

	Time (weeks) during treatment application (Mean±S.E)									
Treatments	1	2	3	4	5	6	7	8	9	10
Arthrobacter sp 1	2.1±0.14^{a}	2.4±0.20^{abc}	3.1±0.26^{a}	3.3±0.29^{ab}	3.3±0.29^{a}	3.6±0.37^{a}	3.7±0.36^{a}	3.9±0.26^{a}	4.1±0.40^{a}	4.1±0.40^{a}
Lactobacillus brevis 2.28.11	2.1±0.14^{a}	2.3±0.18^{bc}	2.3±0.18^{b}	2.3±0.18^{c}	2.3±0.18^{b}	2.3±0.18^{b}	2.3±0.18^{b}	2.3±0.18^{b}	2.3±0.18^{b}	2.3±0.18^{b}
Micrococcus luteus 1	2.4±0.20^{a}	2.9±0.14^{a}	3.0±0.00^{a}	3.3±0.18^{ab}	3.6±0.30^{a}	3.7±0.29^{a}	4.0±0.31^{a}	4.1±0.34^{a}	4.3±0.36^{a}	4.4±0.43^{a}
Micrococcus luteus 2	2.1±0.14^{a}	2.1±0.14^{c}	2.1±0.14^{b}	2.1±0.14^{c}	2.1±0.14^{b}	2.1±0.14^{b}	2.1±0.14^{b}	2.1±0.14^{b}	2.1±0.14^{b}	2.1±0.14^{b}
Funguran®	2.4±0.20^{a}	2.7±0.18^{ab}	2.9±0.14^{a}	2.9±0.14^{b}	3.3±0.29^{a}	3.3±0.29^{a}	3.7±0.36^{a}	3.7±0.36^{a}	3.9±0.26^{a}	4.0±0.38^{a}
Control	2.6±0.30^{a}	2.9±0.26^{a}	3.3±0.18^{a}	3.6±0.20^{a}	3.7±0.29^{a}	3.7±0.29^{a}	3.7±0.29^{a}	4.1±0.34^{a}	4.1±0.34^{a}	4.1±0.34^{a}
P value	0.450	0.044	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
L.S.D (P≤0.05)	0.563	0.546	0.492	0.563	0.729	0.773	0.820	0.814	0.853	0.951
F value	0.94	2.57	7.41	8.82	6.91	7.13	8.24	10.5	11.1	9.55

Means ± SE followed by similar letter(s) in the same column do not differ significantly at P≤0.05 (Fisher's protected L.S.D test).

4.8.2 Change of gall size on rose plants

Results at Magana Flowers and Valentine Flowers farms revealed that plants treated with *L. brevis* 2.28.11 and *M. luteus* 2 recorded a decrease in gall size throughout the monitoring period. Contrary, plants treated with *Arthrobacter* sp 1, *M. luteus* 1, funguran® and control recorded a steady increase in gall size which was comparable throughout the period. This was observed throughout the monitoring period at both sites. Figure 4.8 shows dry galls after treatment application while figures 4.9 and 4.10 show fresh galls after treatment application.

Figure 4.8 A-Dried gall on a rose plant treated with *Lactobacillus brevis* 2.28.11; B- Dried gall on a rose plant treated with *Micrococcus luteus* 2

Figure 4.9 A-Expanding gall on a rose plant treated with *Arthrobacter* sp 1; B-Expanding gall on a rose plant treated with *Micrococcus luteus* 1

Figure 4.10 A-Expanding gall on a rose plant treated with funguran®; B-Expanding gall on a rose plant treated with distilled water (control)

Effects of *L. brevis* 2.28.11 and *M. luteus* 2 on percentage change in gall size at Magana Flowers farm differed significantly (df = 5,41; P<0.05) with those of *M. luteus* 1, *Arthrobacter* sp 1, funguran® and control (Figure 4.11). A confirmation of what was observed at Valentine Flowers farm. This was observed throughout the monitoring period. At Magana Flowers farm, plants treated with *L. brevis* 2.28.11 and *M. luteus* 2 reduced gall sizes from -0.19±0.63% and -0.16±0.04% to -23.03±1.91% and -24.19±2.48%, respectively (Figure 4.11). At Valentine Flowers farm, *L. brevis* 2.28.11 and *M. luteus* 2 reduced gall size from -0.10±0.05% and -0.03±0.02% to -17.71±1.83% and -18.49±2.2%, respectively (Figure 4.12).

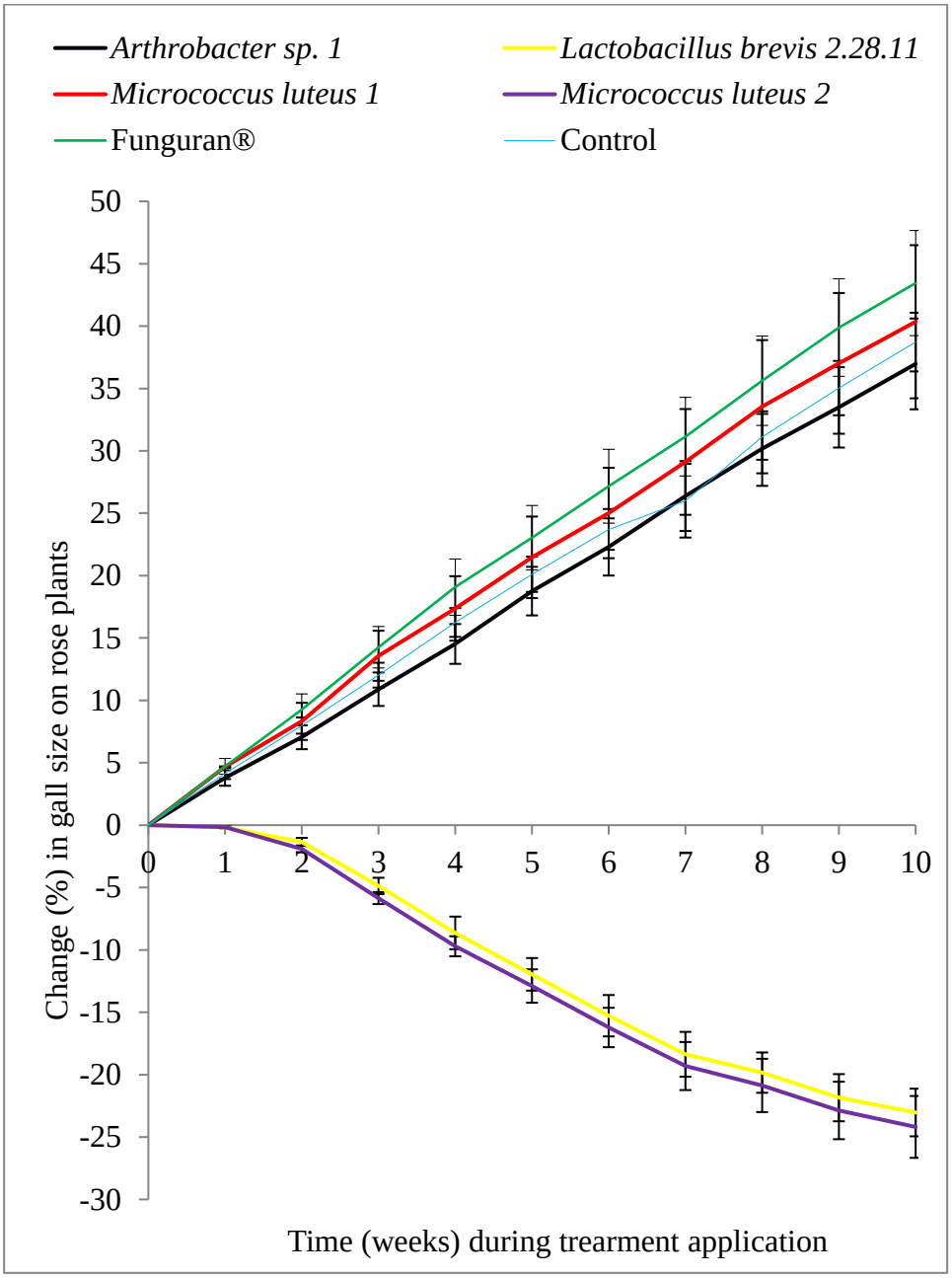

Figure 4.11 Change (%) in gall size on Rose Tropical Amazon® cultivar treated with bacterial isolates at Magana Flowers farm from November 2018 to February 2019

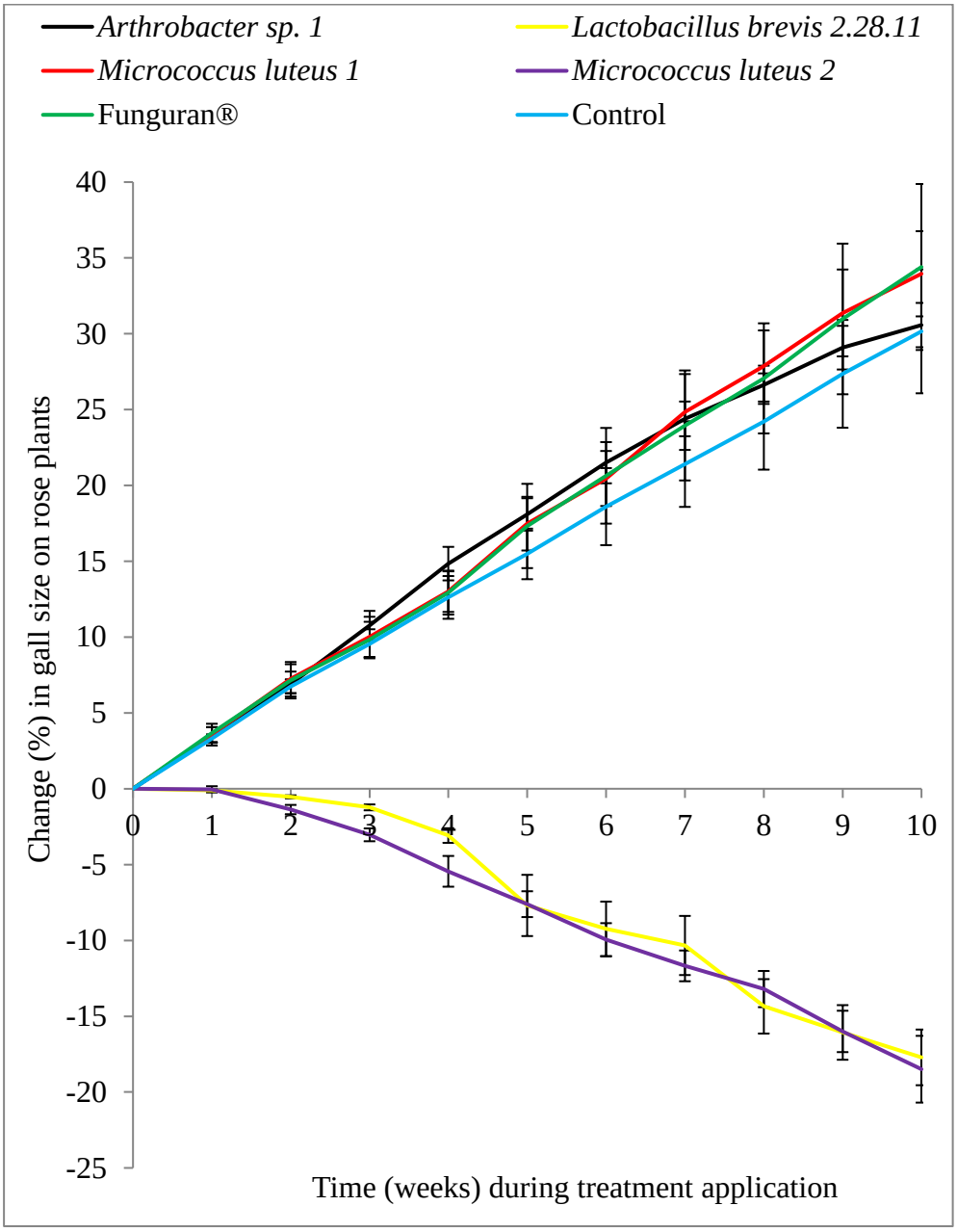

Figure 4.12 Change (%) in gall size on Rose Tropical Amazon® cultivar treated with bacterial isolates at Valentine Flowers farm from November 2018 to February 2019

4.8.3 Number of shoots on rose plants

From week 1 until week 10, plants in the control without galls produced significantly higher number of shoots (df = 6, 48; P<0.05) compared to plants under other treatments evaluated at Magana Flowers farm (Table 4.11). Similar case was observed at Valentine Flowers farm from week 2 to week 10 (Table 4.12). Between week 1 and 5, plants treated with *L. brevis* 2.28.11, *M. luteus* 2, *M. luteus* 1, funguran® and control with galls at Magana Flowers farm produced shoots which did not differ significantly. This was also confirmed at Valentine Flowers from week 2 to week 4 (Table 4.12). From week 6 to 10, the number of shoots in plants treated with *L. brevis* 2.28.11 and *M. luteus* 2 at Magana Flowers farm were similar and significantly (df = 6,48; P<0.05) higher than those produced by plants treated with *M. luteus* 1 and *Arthrobacter* sp 1 as well as funguran® and control with galls (Table 4.11). A confirmation of what was observed at Valentine Flowers farm from week 5 to week 10 (Table 4.12). Number of shoots in plants treated with *Arthrobacter* sp 1, *Micrococcus luteus* 1, funguran® and the control with galls did not differ significantly throughout the monitoring period at Magana Flowers and Valentine Flowers farms.

Table 4.11 Number of shoots (Mean±S.E) on Rose Tropical Amazon® cultivar treated with bacterial isolates at Magana Flowers farm from November 2018 to February 2019

	Time (weeks) during treatment application (Mean±S.E)									
Treatments	1	2	3	4	5	6	7	8	9	10
Arthrobacter sp 1	3.7±0.36^{b}	5.3±0.42^{b}	6.4±0.53^{b}	8.6±0.53^{b}	11.3±0.68^{b}	13.7±0.42^{cd}	15.1±0.70^{cd}	16.7±0.92^{c}	18.7±1.17^{c}	19.7±1.06^{c}
Lactobacillus brevis 2.28.11	3.7±0.29^{b}	5.6±0.37^{b}	6.6±0.43^{b}	8.0±0.53^{b}	11.9±0.34^{b}	16.1±0.46^{b}	18.6±0.81^{b}	20.6±1.31^{b}	23.4±1.04^{b}	25.7±1.04^{b}
Micrococcus luteus 1	3.3±0.29^{b}	5.7±0.42^{b}	5.9±0.34^{b}	8.6±0.37^{b}	10.6±0.43^{b}	12.4±0.37^{d}	13.9±0.51^{d}	16.0±0.53^{c}	18.4±0.61^{c}	19.9±0.59^{c}
Micrococcus luteus 2	3.0±0.31^{b}	5.4±0.57^{b}	6.4±0.69^{b}	8.6±0.57^{b}	11.0±0.31^{b}	15.1±0.26^{bc}	17.1±0.51^{bc}	19.4±0.69^{b}	22.0±0.98^{b}	24.9±1.18^{b}
Funguran®	3.9±0.55^{b}	5.1±0.40^{b}	6.3±0.36^{b}	8.9±0.34^{b}	10.6±0.37^{b}	12.4±0.61^{d}	14.6±0.65^{d}	16.1±0.63^{c}	17.9±0.70^{c}	19.1±0.74^{c}
Control with galls	3.9±0.40^{b}	5.0±0.31^{b}	6.6±0.37^{b}	8.7±0.68^{b}	10.4±0.95^{b}	12.1±0.99^{d}	13.3±1.08^{d}	15.6±1.11^{c}	17.7±0.92^{c}	20.6±0.84^{c}
Control without galls	5.4±0.57^{a}	8.1±0.26^{a}	10.4±0.48^{a}	12.9±0.59^{a}	15.3±0.71^{a}	20.0±0.87^{a}	22.9±0.80^{a}	25.1±0.86^{a}	27.9±1.01^{a}	31.1±1.26^{a}
P value	0.007	0.001	<0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
LSD (P≤0.05)	1.173	1.153	1.341	1.510	1.672	1.773	2.133	2.569	2.674	2.815
F value	3.52	7.15	11	9.73	8.55	20.77	20.28	14.98	16.39	20.31

Means ± SE followed by similar letter(s) in the same column do not differ significantly at P≤0.05 (Fisher's protected L.S.D test).

Table 4.12 Number of shoots (Mean±S.E) on Rose Tropical Amazon® cultivar treated with bacterial isolates at Magana Flowers farm from November 2018 to February 2019

	Time (weeks) during treatment application (Mean±S.E)									
Treatments	1	2	3	4	5	6	7	8	9	10
Arthrobacter sp 1	4.4±0.30^{ab}	5.4±0.37^{b}	7.6±0.43^{b}	9.1±0.40^{c}	10.7±0.36^{c}	12.1±0.46^{c}	14.4±0.37^{c}	16.7±0.52^{c}	18.7±0.61^{c}	21.1±0.59^{c}
Lactobacillus brevis 2.28.11	4.0±0.53^{b}	5.3±0.57^{b}	7.7±0.36^{b}	10.0±0.44^{bc}	13.9±0.40^{b}	17.3±0.42^{b}	20.6±0.48^{b}	22.4±0.65^{b}	24.4±0.81^{b}	27.3±0.87^{b}
Micrococcus luteus 1	4.6±0.48^{ab}	5.9±0.40^{b}	7.7±0.42^{b}	9.9±0.51^{bc}	11.6±0.43^{c}	12.9±0.40^{c}	14.9±0.55^{c}	16.7±0.42^{c}	18.6±0.48^{c}	20.9±0.67^{c}
Micrococcus luteus 2	4.3±0.57^{b}	5.7±0.42^{b}	7.3±0.42^{b}	10.6±0.48^{b}	13.6±0.30^{b}	16.9±0.34^{b}	20.2±0.64^{b}	22.0±0.62^{b}	24.1±0.67^{b}	26.6±0.65^{b}
Funguran®	4.3±0.52^{b}	5.3±0.47^{b}	7.9±0.40^{b}	9.6±0.37b^{c}	11.3±0.47^{c}	12.6±0.37^{c}	15.0±0.22^{c}	16.6±0.30^{c}	18.3±0.42^{c}	21.0±0.58^{c}
Control with galls	4.4±0.43^{ab}	5.0±0.69^{b}	8.0±0.49^{b}	9.9±0.34b^{c}	11.1±0.34^{c}	13.0±0.31^{c}	15.1±0.46^{c}	16.3±0.52^{c}	18.4±0.48^{c}	21.7±0.61^{c}
Control without galls	5.7±0.42^{a}	9.0±0.22^{a}	12.6±0.37^{a}	15.3±0.42^{a}	17.9±0.34^{a}	22.1±0.67^{a}	24.9±0.59^{a}	26.1±0.59^{a}	29.0±0.82^{a}	31.7±0.78^{a}
P value	0.250	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
LSD (P≤0.05)	1.346	1.341	1.184	1.217	1.091	1.252	1.407	1.513	1.799	1.953
F value	1.37	8.63	20.08	24.4	46.63	70.90	67.9	55.4	45.18	38.58

Means ± SE followed by similar letter(s) in the same column do not differ significantly at P≤0.05 (Fisher's protected L.S.D test).

4.8.4 Shoot heights of rose plants

In week 1, shoots produced by plants treated with *Arthrobacte*r sp 1, *M. luteus* 1, *M. luteus* 2, *L. brevis* 2.28.11, funguran® and control with galls had similar heights, although they were significantly shorter (df = 6,48; P<0.05) than those produced by plants in the control without galls at Magana Flowers faem (Figure 4.13). Similar case was recorded at Valentine Flowers farm in week 1 and week 2 (Figure 4.14). In week 2, shoots produced by plants treated with *L.brevis* 2.28.11at Magana Flowers farm had comparable heights with those produced by plants treated with *M. luteus* 2. Additionally, shoots heights produced by plants in the control without galls were similar to those produced by plants treated with *L. brevis* 2.28.11 (Figure 4.13).

From week 3 to week 6, shoots with similar heights were produced by plants treated with *L. brevis* 2.28.11, *M. luteus* 2 and control without galls, which were significantly taller (df = 6,48; P<0.05) than those produced by plants with other treatments evaluated at Magana Flowers farm (Figure 4.13). Similar case was observed at Valentine Flowers farm (Figure 4.14). Shoot heights of plants treated with *M. luteus* 1, *Arthrobacter* sp 1, as well as funguran® and control did not differ significantly throughout the monitoring period. Shoot heights in plants treated with *L. brevis 2.28.11* and *M. luteus* 2 at Magana Flowers farm increased steadily over time from a low of 5.7±1.00cm and 4.5±0.62cm to a high of 59.8±2.44cm and 58.2±2.37cm, respectively (Figure 4.13). Shoot heights in plants treated with *L. brevis* 2.28.11 and *M. luteus* 2 at Valentine Flowers farm increased from 4.9±1.02cm and 4.5±0.48cm to 52.0±3.30cm and 50.5±1.93cm respectively (Figure 4.14).

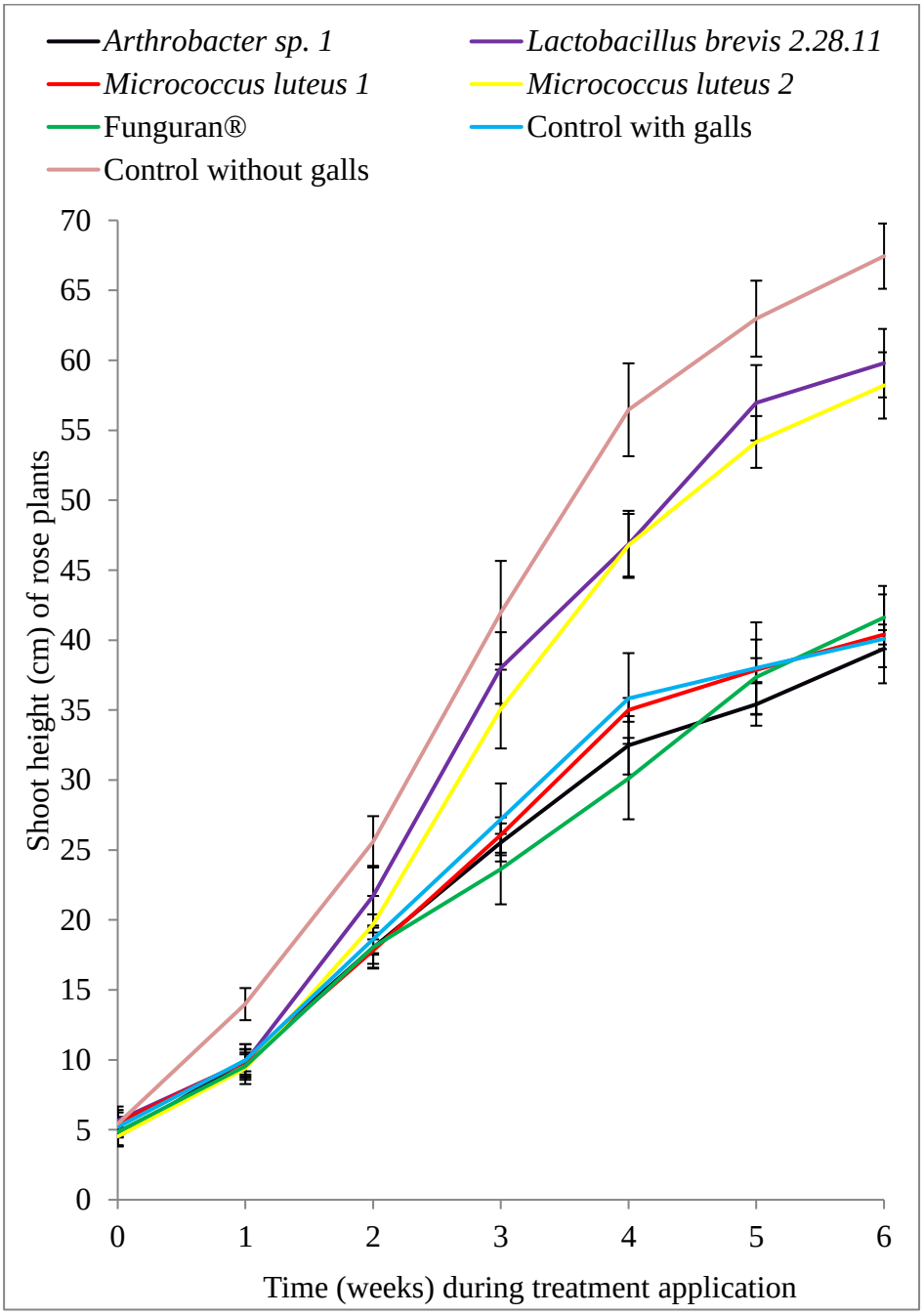

Figure 4.13 Mean shoot heights (cm) on Rose Tropical Amazon® cultivar treated with bacterial isolates at Magana Flowers farm from November 2018 to February 2019

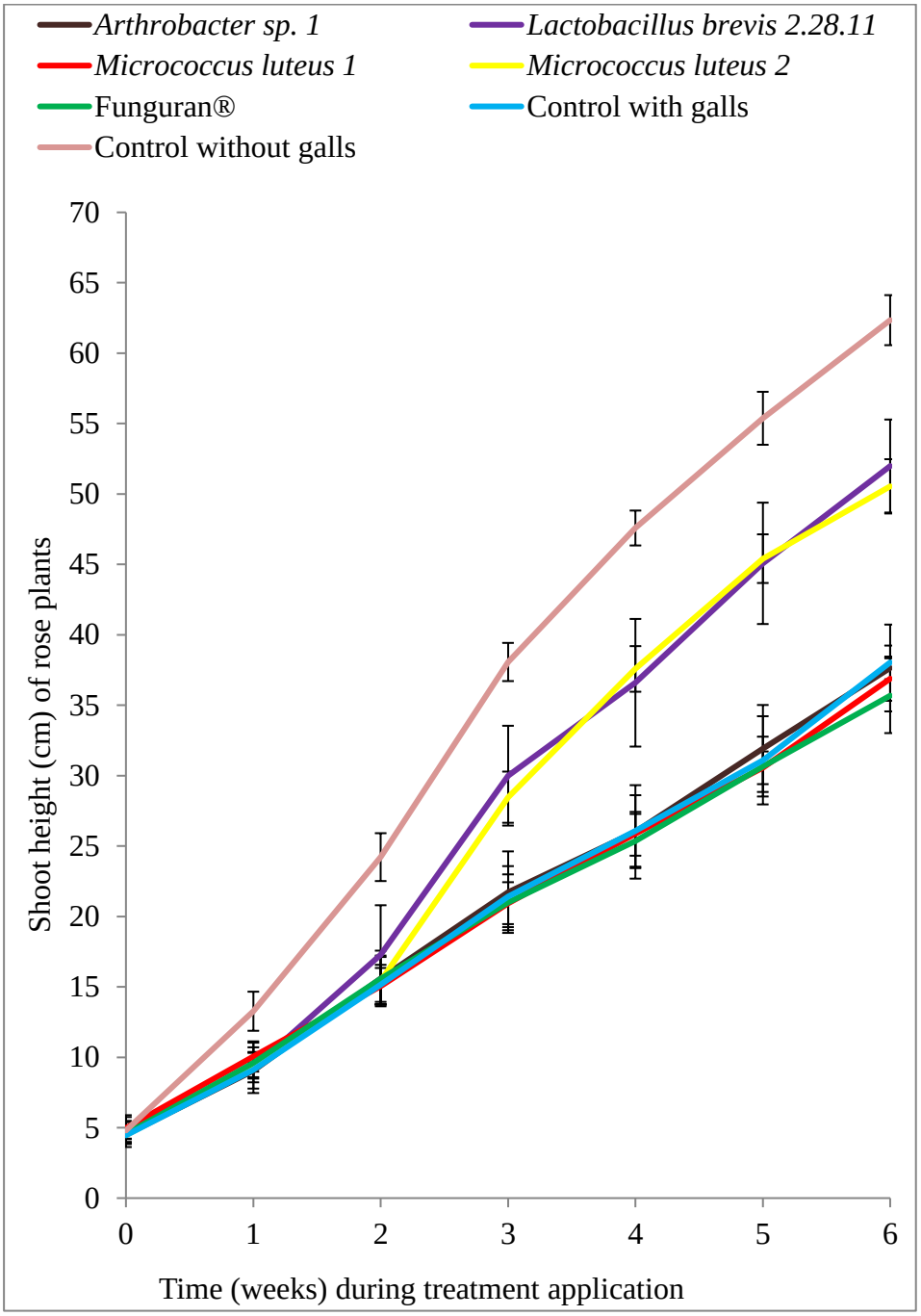

Figure 4.14 Mean shoot height (cm) on Rose Tropical Amazon® cultivar treated with bacterial isolates at Valentine Flowers farm from November 2018 to February 2019

4.9 Evaluation of antagonistic bacterial isolates on Rose Upper Class® cultivar at Magana Flowers and Black Petals farms

4.9.1 Number of galls formed on rose plants

Field results revealed that there was an increase in number of galls in plants treated with *Micrococcus luteus* 1, *Arthrobacter* sp 1, funguran® and control at Magana Flowers farm and the numbers did not differ significantly throughout the monitoring period (Table 4.13). Contrary, number of galls in plants treated with *Lactobacillus brevis* 2.28.11 and *Micrococcus luteus* 2 remained the same throughout the monitoring period. This observation was also confirmed at Black Petals farm (Table 4.14). From week 1 until week 4, the number of galls in plants treated with *L. brevis* 2.28.11, *M. luteus* 1, *M. luteus* 2, *Arthrobacter* sp 1, funguran® and control at Black Petals farm did not differ significantly (Table 4.14) confirming what had been observed at Magana Flowers farm (Table 4.13). From week 5 to 10, the number of galls on plants treated with *L. brevis 2.28.11* and *M. luteus* 2 at Magana Flowers farm (Table 4.13) and Black Petals farm (Table 4.14) were significantly ($df = 5, 41$; $P<0.05$) lower than those produced by plants treated with *M. luteus* 1 and *Arthrobacter* sp 1 as well as funguran® and control.

Table 4.13 Number of galls (Mean±S.E) on Rose Upper Class® cultivar treated with bacterial isolates at Magana Flowers farm from November 2018 to February 2019

	Time (weeks) during treatment application (Mean±S.E)									
Treatments	1	2	3	4	5	6	7	8	9	10
Arthrobacter sp 1	2.3±0.18a	2.3±0.18a	2.4±0.20abc	2.6±0.20ab	2.9±0.18abc	2.9±0.14ab	2.9±0.14a	3.1±0.26a	3.4±0.20a	3.7±0.18a
Lactobacillus brevis 2.28.11	2.1±0.14a	2.1±0.14b	2.1±0.14c	2.1±0.14b	2.1±0.14c	2.1±0.14c	2.1±0.14b	2.1±0.14b	2.1±0.14b	2.1±0.14b
Micrococcus luteus 1	2.0±0.00a	2.3±0.18b	2.6±0.20abc	2.6±0.20ab	2.6±0.20abc	2.9±0.26ab	3.0±0.22a	3.3±0.18a	3.3±0.18a	3.4±0.20a
M icrococcus luteus 2	2.0±0.00a	2.3±0.18b	2.3±0.18bc	2.3±0.18ab	2.3±0.18bc	2.3±0.18bc	2.3±0.18b	2.3±0.18b	2.3±0.18b	2.3±0.18b
Funguran®	2.3±0.18a	2.9±0.14a	2.9±0.14a	2.9±0.34a	2.9±0.14ab	2.9±0.14ab	2.9±0.14a	3.0±0.22a	3.1±0.26a	3.3±0.29a
Control	2.0±0.00a	2.4±0.20ab	2.7±0.29ab	2.9±0.34a	3.0±0.31a	3.0±0.31a	3.0±0.31a	3.3±0.36a	3.4±0.30a	3.6±0.30a
P value	0.270	0.096	0.138	0.123	0.037	0.024	0.001	0.002	0.001	0.001
LSD (P≤0.05)	0.348	0.502	0.571	0.611	0.581	0.595	0.571	0.67	0.626	0.641
F value	1.34	2.04	1.8	1.87	2.68	2.97	3.58	4.7	7.14	9.29

Means ± SE followed by similar letter(s) in the same column do not differ significantly at P≤0.05 (Fisher's protected L.S.D test).

Table 4.14 Number of galls (Mean±S.E) on Rose Upper Class® cultivar treated with bacterial isolates at Black Petals farm from November 2018 to February 2019

	Time (weeks) during treatment application (Mean±S.E)									
Treatments	1	2	3	4	5	6	7	8	9	10
Arthrobacter sp 1	2.1±0.14^{a}	2.4±0.20^{a}	2.6±0.20^{a}	2.6±0.20^{a}	2.6±0.20^{ab}	2.7±0.18^{ab}	2.9±0.26^{a}	3.0±0.10^{a}	3.0±0.22^{a}	3.1±0.14^{a}
Lactobacillus brevis 2.28.11	2.1±0.14^{a}	2.1±0.14^{a}	2.1±0.14^{a}	2.1±0.14^{a}	2.1±0.14^{b}	2.1±0.14^{c}	2.1±0.14^{b}	2.1±0.14^{b}	2.1±0.14^{b}	2.1±0.14^{b}
Micrococcus luteus 1	2.1±0.14^{a}	2.3±0.18^{a}	2.4±0.20^{a}	2.6±0.20^{a}	2.7±0.18^{a}	2.9±0.14^{a}	3.0±0.22^{a}	3.1±0.26^{a}	3.1±0.26^{a}	3.3±0.18^{a}
Micrococcus luteus 2	2.3±0.18^{a}	2.3±0.18^{a}	2.3±0.18^{a}	2.3±0.18^{a}	2.3±0.18^{ab}	2.3±0.18^{bc}	2.3±0.18^{b}	2.3±0.18^{b}	2.3±0.18^{b}	2.3±0.18^{b}
Funguran®	2.1±0.14^{a}	2.3±0.18^{a}	2.3±0.18^{a}	2.3±0.18^{a}	2.3±0.18^{ab}	2.4±0.20^{abc}	2.9±0.26^{a}	3.0±0.22^{a}	3.1±0.26^{a}	3.3±0.34^{a}
Control	2.1±0.14^{a}	2.3±0.18^{a}	2.4±0.20^{a}	2.4±0.20^{a}	2.6±0.20^{ab}	2.7±0.18^{ab}	3.0±0.00^{a}	3.3±0.18^{a}	3.1±0.14^{a}	3.4±0.20^{a}
P value	0.979	0.938	0.672	0.338	0.234	0.043	0.001	0.001	0.001	0.001
LSD (P≤0.05)	0.432	0.520	0.538	0.520	0.529	0.502	0.571	0.587	0.595	0.618
F value	0.15	0.25	0.64	1.18	1.44	2.58	3.58	5.38	5.05	6.8

Means ± SE followed by similar letter(s) in the same column do not differ significantly at P≤0.05 (Fisher's protected L.S.D test).

4.9.2 Change in gall size on rose plants

Application of bacterial isolates to rose plants at Magana Flowers farm provided varied degree of percentage change of gall size. Plants treated with *L. brevis* 2.28.11 and *M. luteus* 2 recorded a comparable percentage decrease in gall size throughout the monitoring period. By contrast, plants treated with *Arthrobacter* sp 1, *M. luteus* 1, funguran® and control recorded a similar percentage increase in gall size in the entire monitoring period. This was observed at Magana Flowers farm (Figure 4.15) and Black Petals farm (Figure 4.16). Percentage change of gall size in plants treated with *L. brevis* 2.28.11 and *M. luteus* 2 at Magana Flowers farm differed significantly ($df = 5, 41$; $P<0.05$) with that in plants treated with *Arthrobacter* sp 1, *M. luteus* 1, funguran® and control (Figure 4.15). This was observed from week 1 until week 10. Similar case was observed at Black Petals farm (Figure 4.16). The percentage reduction of gall size of *L. brevis* 2.28.11 and *M. lutes* 2 at Magana Flowers farm increased steadily over time from -0.27±0.06% and -0.11±0.04% to -18.73±2.49% and -17.41±1.71%, respectively (Figure 4.15). The percentage reduction of gall size of *L. braves 2.28.11* and *M. lutes* 2 at Black Petals farm the change increased from -0.60±0.20% and -0.85±0.29% to -21.03±0.61% and -20.25±2.74%, respectively (Figure 4.16).

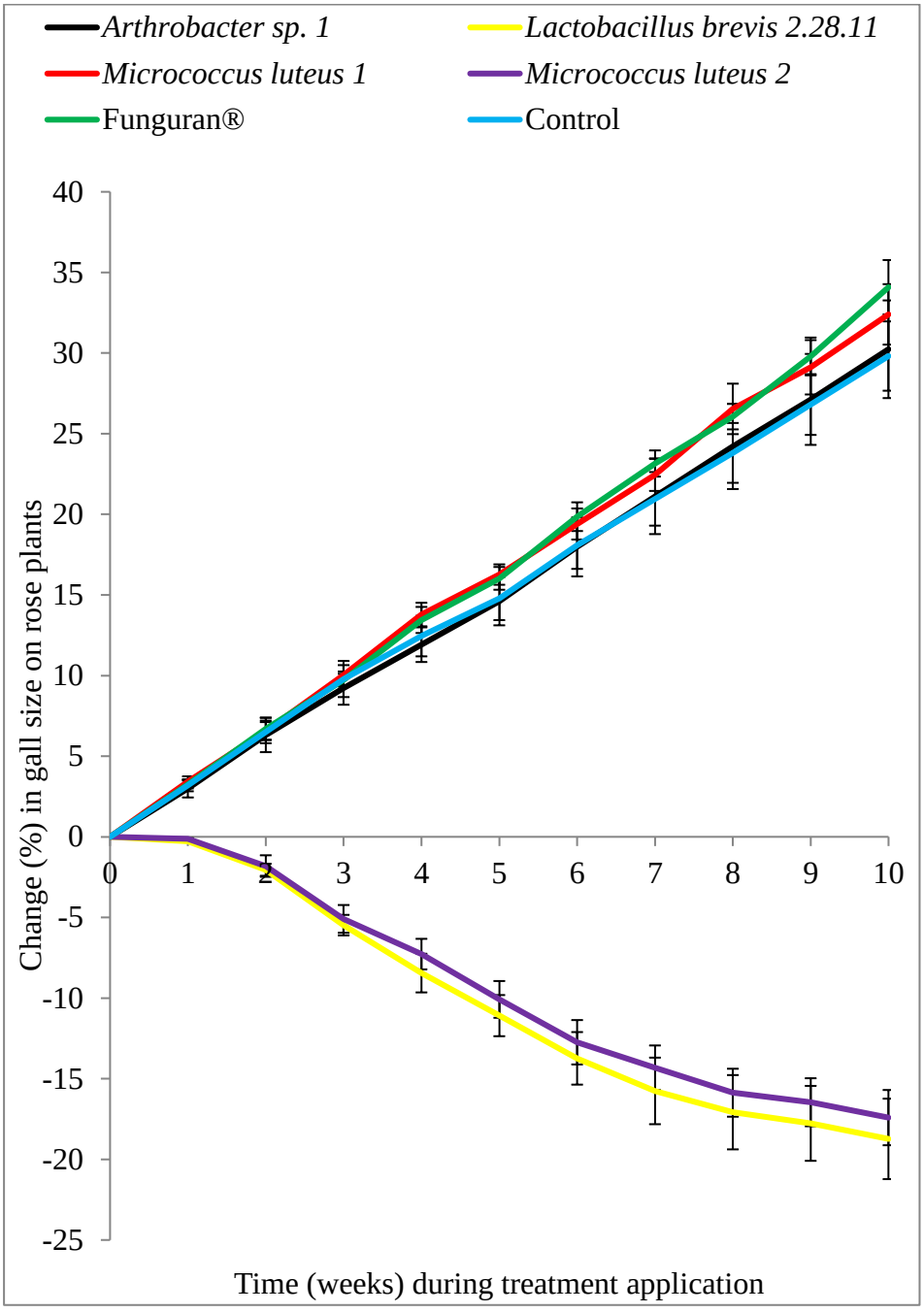

Figure 4.15 Change (%) in gall size on Rose Upper Class® cultivar as affected by bacterial isolates at Magana Flowers farm from November 2018 to February 2019

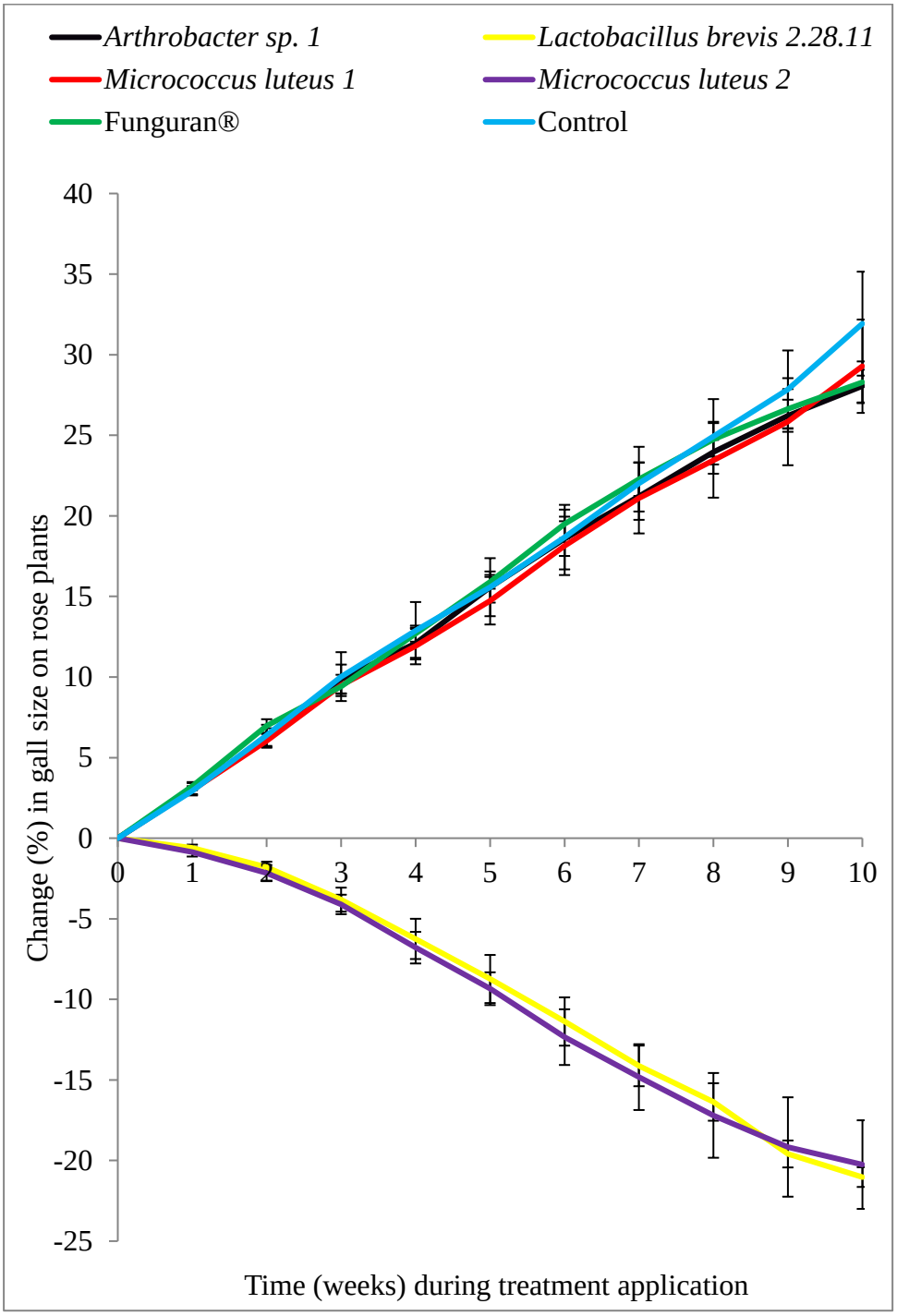

Figure 4.16 Change (%) in gall size on Upper Class® cultivar as affected by bacterial isolates at Black Petals farm from November 2018 to February 2019

4.9.3 Number of shoots on rose plants

At Magana Flowers farm, Statistical analysis demonstrated that plants under control without galls had significantly (df = 6, 48; $P<0.05$) higher number of shoots compared with plants under other treatments evaluated. Additionally, plants treated with *Arthrobacter* sp 1, *M. luteus* 1, funguran® and control with galls produced comparable number of galls. This was observed throughout the monitoring period (Table 4.15). Similar observations were recorded at Black Petals farm (Table 4.16). From week 1 to 3, number of shoots in plants treated with *L. brevis* 2.28.11, *M. luteus* 2, *M. luteus* 1, *Arthrobacter* sp, funguran® and control with galls at Black Petals farm were comparable (Table 4.16) confirming what had been observed at Magana Flowers farm from week 1 to 2 (Table 4.15). From week 4 to 10, plants treated with *L. brevis* 2.28.11 and *M. luteus* 2 produced significantly ($P<0.05$) higher number of shoots than the ones produced by plants treated with *Arthrobacter* sp 1, *M. luteus* 1, funguran® and control with galls (Table 4.15). Similar observation was made at Black Petals farm from week 3 to 10 (Table 4.16).

Table 4.15 Number of shoots (Mean±S.E) on Rose Upper Class® cultivar treated with bacterial isolates at Magana Flowers farm from November 2018 to February 2019

	Time (weeks) during treatment application (Mean±S.E)									
Treatments	1	2	3	4	5	6	7	8	9	10
Arthrobacter sp 1	3.4±0.20^{b}	4.9±0.26^{b}	6.7±0.42^{cd}	8.6±0.30^{d}	10.6±0.53^{c}	13.1±0.40^{c}	14.6±0.78^{c}	16.6±0.84^{c}	17.9±1.20^{c}	19.4±1.54^{c}
Lactobacillus brevis 2.28.11	3.3±0.36^{b}	4.9±0.51^{b}	7.9±0.51^{bc}	10.3±0.61^{bc}	13.3±0.99^{b}	16.9±1.24^{b}	19.6±1.11^{b}	22.1±1.08^{b}	24.4±0.92^{b}	26.6±0.75^{b}
Micrococcus luteus 1	3.1±0.26^{b}	4.3±0.42^{b}	6.4±0.57^{cd}	8.0±0.44^{d}	10.4±0.72^{c}	12.3±0.52^{c}	14.4±0.87^{c}	17.1±0.91^{c}	18.7±1.19^{c}	20.1±1.50^{c}
Micrococcus luteus 2	3.3±0.29^{b}	5.6±0.48^{b}	8.7±0.52^{b}	11.3±0.64^{b}	13.4±0.65^{b}	16.0±0.76^{b}	19.3±0.99^{b}	22.3±1.06^{b}	24.6±1.25^{b}	26.3±1.51^{b}
Funguran®	3.6±0.20^{b}	5.0±0.44^{b}	6.0±0.72^{cd}	8.9±0.74^{cd}	11.0±0.93^{c}	13.3±0.52^{c}	15.0±0.62^{c}	17.0±0.38^{c}	18.9±0.74^{c}	20.3±1.23^{c}
Control with galls	3.4±0.43^{b}	4.9±0.74^{b}	5.9±0.99^{d}	8.1±0.59^{d}	10.1±0.94^{c}	12.4±1.13^{c}	14.4±1.21^{c}	16.4±1.46^{c}	18.3±1.54^{c}	20.1±1.49^{c}
Control without galls	6.3±0.42^{a}	10.6±0.61^{a}	14.7±0.81^{a}	17.9±0.63^{a}	21.4±0.65^{a}	24.1±0.40^{a}	26.7±0.52^{a}	29.4±0.72^{a}	32.0±1.00^{a}	34.9±1.06^{a}
P value	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
LSD (P≤0.05)	0.916	1.465	1.923	1.657	2.253	2.228	2.577	2.777	3.270	3.784
F value	12.08	17.95	21.37	36.07	25.66	29.27	25.71	24.69	20.83	18.41

Means ± SE followed by similar letter(s) in the same column do not differ significantly at P≤0.05 (Fisher's protected L.S.D test).

Table 4.16 Number of shoots (Mean±S.E) on Rose Upper Class® cultivar treated with bacterial isolates at Black Petals farm from November 2018 to February 2019

	Time (weeks) during treatment application (Mean±S.E)									
Treatments	1	2	3	4	5	6	7	8	9	10
Arthrobacter sp 1	3.7±0.29^{b}	5.6±0.37^{b}	7.3±0.36^{b}	9.4±0.37^{cd}	11.4±0.48^{bc}	13.7±0.37^{bc}	15.1±0.67^{c}	17.0±0.69^{c}	18.4±0.97^{c}	20.0±0.88^{c}
Lactobacillus brevis 2.28.11	3.6±0.30^{b}	5.4±0.20^{b}	7.7±0.29^{b}	10.6±0.30^{bc}	12.4±0.30^{b}	15.1±0.26^{b}	18.3±0.29^{b}	20.9±0.40^{b}	23.7±0.29^{b}	26.0±0.31^{b}
Micrococcus luteus 1	3.9±0.34^{b}	5.3±0.61^{b}	7.9±0.34^{b}	8.9±0.59^{d}	10.1±0.74^{c}	13.0±0.69^{c}	15.0±0.62^{c}	17.1±0.67^{c}	18.7±0.89^{c}	20.9±0.70^{c}
Micrococcus luteus 2	3.3±0.29^{b}	5.7±0.29^{b}	7.4±0.37^{b}	10.9±0.40^{b}	12.9±0.34^{b}	15.0±0.44^{b}	17.9±0.34^{b}	20.4±0.78^{b}	24.0±0.53^{b}	26.4±0.61^{b}
Funguran®	3.7±0.42^{b}	5.4±0.57^{b}	7.3±0.64^{b}	8.7±0.64^{d}	10.4±0.53^{c}	13.1±0.51^{c}	15.3±0.42^{c}	17.7±0.36^{c}	18.9±0.34^{c}	21.0±0.22^{c}
Control with galls	3.4±0.53^{b}	5.4±0.53^{b}	7.6±0.48^{b}	8.1±0.46^{d}	10.1±0.46^{c}	13.3±0.42^{c}	15.1±0.51^{c}	17.6±0.53^{c}	18.6±0.37^{c}	20.3±0.29^{c}
Control without galls	6.1±0.26^{a}	8.4±0.30^{a}	11.4±0.48^{a}	14.3±0.61^{a}	16.9±0.74^{a}	19.6±0.95^{a}	22.1±0.86^{a}	25.3±0.89^{a}	28.0±1.05^{a}	31.6±1.38^{a}
P value	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
LSD (P≤0.05)	1.018	1.239	1.249	1.421	1.531	1.599	1.599	1.839	2.005	2.096
F value	7.57	6.7	11.61	17.53	19.81	17.15	22.66	22.03	29.08	36

Means ± SE followed by similar letter(s) in the same column do not differ significantly at P≤0.05 (Fisher's protected L.S.D test).).

4.9.4 Shoot heights of rose plants

From week 1 to 6, plants in the control without galls at Magana Flowers farm had significantly taller shoots (df = 6, 48; P<0.05) than those under other treatments evaluated (Figure 4.17). Similar case was observed at Black Petal farm (Figure 4.18). From week 1 to 2, plants treated with *Arthrobacter* sp 1, *M. luteus* 1, funguran®, *L. brevis* 2.28.11 and control with galls at Black Petals farm produced shoots of comparable heights (Figure 4.18) confirming what had been observed at Magana Flowers farm in week 1 (Figure 4.17). From week 2 to 6, shoots in plants treated with *L. brevis 2.28.11* and *M. luteus* 2 exerted similar heights and significantly taller (P<0.05) than those in plants treated with *M. luteus* 1, *Arthrobacter sp* 1, funguran® and control with galls (Figure 4.17). Similar case was observed at Black Petals farm from week 3 to week 6 (Figure 4.18). Heights of shoots in plants treated with *M. luteus* 1, *Arthrobacter* sp 1, funguran® and control with galls were comparable at both sites The shoot heights in plants treated with *L. brevis 2.28.11* and *M. luteus* 2 at Magana Flowers farm increased from 6.0±0.83cm and 6.5±1.09cm to 56.7±2.09cm and 54.4±1.96cm, respectively (Figure 4.17). The height of shoots under *L. brevis* and *M. luteus* 2 at Black Petals farm increased from 4.4±0.53cm and 4.8±0.51cm to 58.5±1.07cm and 56.7±2.09cm, respectively (Figure 4.18).

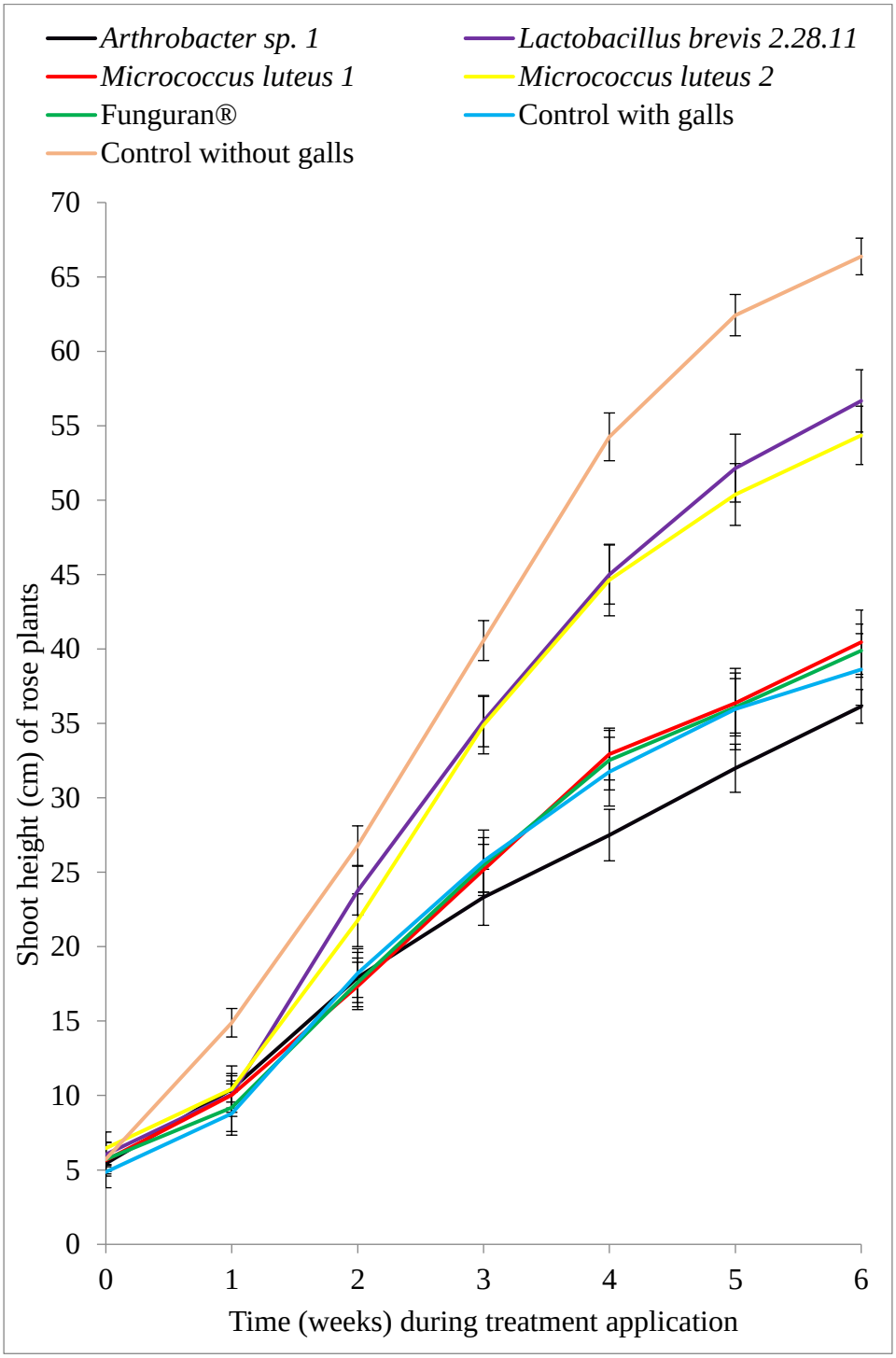

Figure 4.17 Mean shoot heights (cm) on Rose Upper Class® cultivar as affected by bacterial isolates at Magana Flowers farm from November 2018 to February 2019

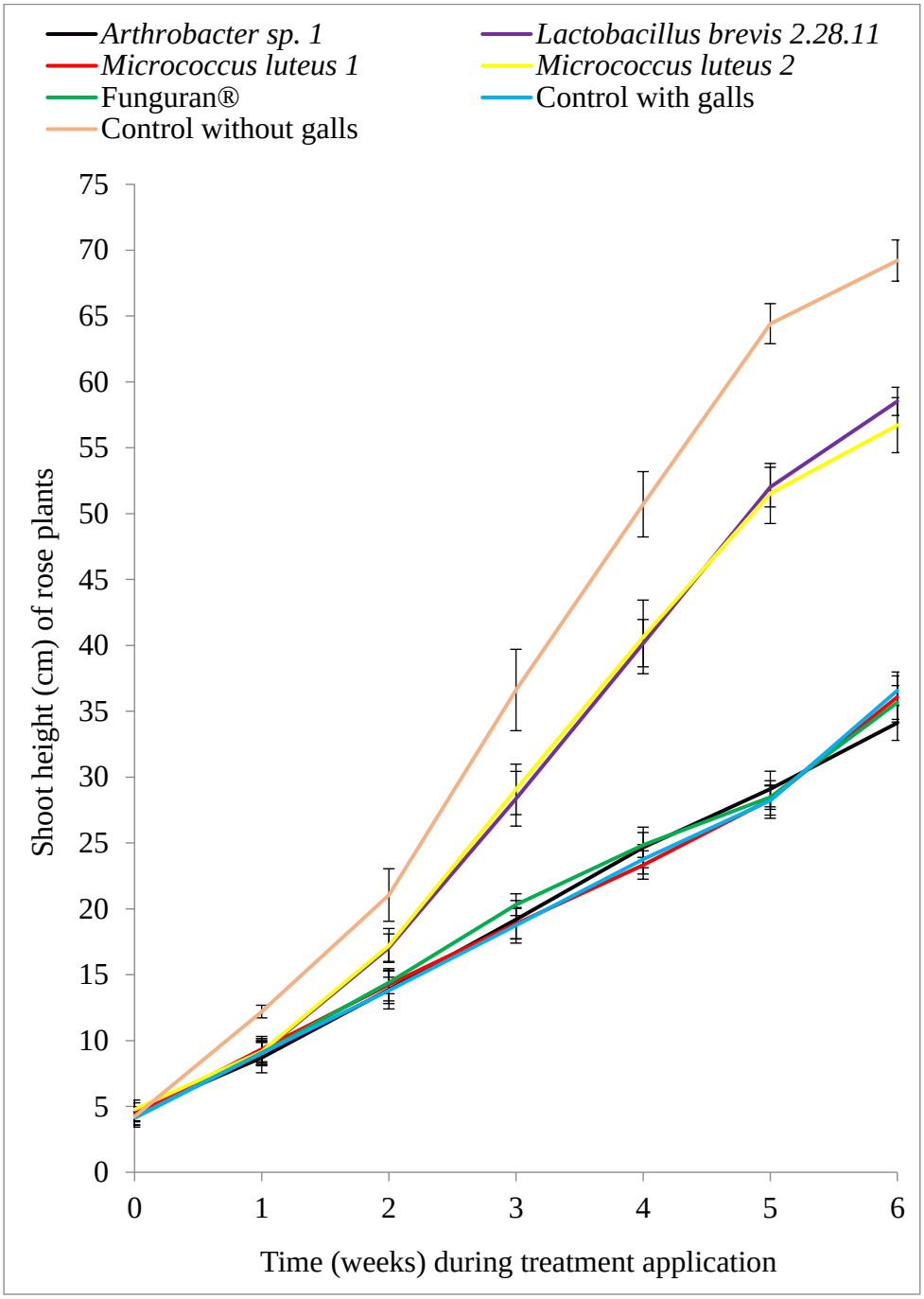

Figure 4.18 Mean shoot heights (cm) on Rose Upper Class® cultivar as affected by bacterial isolates at Black Petals from November 2018 to February 2019

4.10 Evaluation of antagonistic bacterial isolates on Rose Tropical Amazon® cultivar at Kenyatta University Research farm (Curative and preventive approaches)

4.10.1 Number of galls formed on rose plants

In curative approach, plants treated with *L. brevis* 2.28.11 and *M. luteus* 2, resulted to similar number of galls which remained the same from the start until the end of experiment. Contary, plants treated with *Arthrobacter* sp 1, *M. luteus* 1, funguran® and control had steady increase in the number of galls increased over time until the end of experiment (Table 4.17). The number of galls in plants treated with all the bacterial isolates, funguran® and control did not differ significantly ($5, 41; P>0.05$) until the end of the experiment (Table 4.17).

In preventive approach, plants treated with *M. luteus* 2 and *L. brevis* 2.28.11 did not form galls throughout the experimental period (Table 4.18). Contrary, plants treated with *Arthrobacter* sp 1, *M. luteus* 1, funguran® and control formed galls with a steady comparable increase in number that was observed throughout the period. The number of galls in plants treated with *L. brevis* 2.28.11 and *M. luteus* did not differ significantly but were significantly ($5, 41; P< 0.05$) lower than the number in plants treated with *Arthrobacte*r sp 1, *M. luteus* 1, funguran® and control (Table 4.18).

Table 4.17 Number of galls (Mean±S.E) on Rose Tropical Amazon® cultivar treated with bacterial isolates at Kenyatta University research farm (Curative approach) from November 2018 to February 2019

	Time (weeks) during treatment application (Mean±S.E)									
Treatments	1	2	3	4	5	6	7	8	9	10
Arthrobacter sp 1	1.1±0.14[a]	1.3±0.18[a]	1.3±0.18[a]	1.3±0.18[a]	1.3±0.18[a]	1.3±0.18[a]	1.4±0.30[a]	1.4±0.30[a]	1.4±0.30[a]	1.4±0.30[a]
Lactobacillus brevis 2.28.11	1.0±0.00[a]	1.0±0.00[a]	1.0±0.00[a]	1.0±0.00[a]	1.0±0.00[a]	1.0±0.00[a]	1.0±0.00[a]	1.0±0.00[a]	1.0±0.00[a]	1.0±0.00[a]
Micrococcus luteus 1	1.4±0.20[a]	1.4±0.20[a]	1.4±0.20[a]	1.4±0.20[a]	1.6±0.30[a]	1.6±0.30[a]	1.6±0.30[a]	1.6±0.30[a]	1.6±0.30[a]	1.6±0.30[a]
Micrococcus luteus 2	1.0±0.00[a]	1.0±0.00[a]	1.0±0.00[a]	1.0±0.00[a]	1.0±0.00[a]	1.0±0.00[a]	1.0±0.00[a]	1.0±0.00[a]	1.0±0.00[a]	1.0±0.00[a]
Funguran®	1.3±0.18[a]	1.4±0.30[a]	1.4±0.30[a]	1.4±0.30[a]	1.4±0.30[a]	1.4±0.30[a]	1.4±0.30[a]	1.4±0.30[a]	1.4±0.30[a]	1.4±0.30[a]
Control	1.3±0.29[a]	1.4±0.30[a]	1.4±0.30[a]	1.4±0.30[a]	1.4±0.30[a]	1.6±0.37[a]	1.6±0.37[a]	1.6±0.37[a]	1.6±0.37[a]	1.6±0.37[a]
P value	0.423	0.402	0.402	0.402	0.355	0.334	0.400	0.400	0.400	0.400
LSD (P≤0.05)	0.492	0.587	0.587	0.587	0.641	0.690	0.742	0.742	0.742	0.742
F value	1.02	1.05	1.05	1.05	1.15	1.19	1.06	1.06	1.06	1.06

Means ± SE did not differ significantly at P≤0.05 (Fisher's protected L.S.D test).

Table 4.18 Number of galls (Mean±S.E) on Rose Tropical Amazon® cultivar treated with bacterial isolates at Kenyatta University research farm (Preventive approach) from November 2018 to February 2019

	Time (weeks) during treatment application (Mean±S.E)									
Treatments	1	2	3	4	5	6	7	8	9	10
Arthrobacter sp 1	0.4±0.20^{ab}	1.0±0.22^{a}	1.3±0.18^{a}	1.3±0.18^{a}	1.4±0.30^{a}	1.4±0.30^{a}	1.4±0.30^{a}	1.4±0.30^{a}	1.4±0.30^{a}	1.4±0.30^{a}
Lactobacillus brevis 2.28.11	0.0±0.00^{b}	0.0±0.00^{b}	0.0±0.00^{b}	0.0±0.00^{b}	0.0±0.00^{b}	0.0±0.00^{b}	0.0±0.00^{b}	0.0±0.00^{b}	0.0±0.00^{b}	0.0±0.00^{b}
Micrococcus luteus 1	0.6±0.20^{a}	1.1±0.34^{a}	1.1±0.34^{a}	1.1±0.34^{a}	1.1±0.34^{a}	1.1±0.34^{a}	1.1±0.34^{a}	1.1±0.34^{a}	1.1±0.34^{a}	1.1±0.34^{a}
Micrococcus luteus 2	0.0±0.00^{b}	0.0±0.00^{b}	0.0±0.00^{b}	0.0±0.00^{b}	0.0±0.00^{b}	0.0±0.00^{b}	0.0±0.00^{b}	0.0±0.00^{b}	0.0±0.00^{b}	0.0±0.00^{b}
Funguran®	0.7±0.18^{a}	1.1±0.14^{a}	1.3±0.29^{a}	1.3±0.29^{a}	1.3±0.29^{a}	1.3±0.29^{a}	1.3±0.29^{a}	1.3±0.29^{a}	1.3±0.29^{a}	1.3±0.29^{a}
Control	0.4±0.20^{ab}	1.0±0.00^{a}	1.4±0.20^{a}	1.1±0.14^{a}	1.1±0.14^{a}	1.1±0.14^{a}	1.1±0.14^{a}	1.1±0.14^{a}	1.1±0.14^{a}	1.1±0.14^{a}
P value	0.012	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
LSD (P≤0.05)	0.463	0.502	0.611	0.587	0.648	0.648	0.648	0.648	0.648	0.611
F value	3.37	10.13	9.9	9.47	8.39	8.39	8.39	8.39	8.39	9.9

Means ± SE followed by similar letter(s) in the same column do not differ significantly at P≤0.05 (Fisher's protected L.S.D test)..

4.10.2 Change in gall size on rose plants

In curative approach, there were variations in the effectiveness of the bacterial isolates in (%) change of gall size over the monitoring period (Figure 4.19). In plants treated with *M. luteus* 1, *Arthrobacter* sp 1 funguran® and control, percentage increase in gall size was observed. Contrary, percentage decrease in gall size was observed in plants treated with *L. brevis* 2.28.11 and *M. luteus* 2 (Figure 4.19). Percentage change of gall size in plants treated with *L. brevis* 2.28.11 and *M. luteus* 2 did not differ significantly but were significantly ($df = 5, 41$; $P<0.05$) different with that in plants treated with *Arthrobacter* sp 1, *M. luteus* 1, funguran® and control throughout the monitoring period. However, percentage change in gall size in plants treated with *Arthrobacter* sp 1, *M. luteus* 2, funguran® and the control did not differ significantly. *Lactobacillus brevis* 2.28.11 and *M.luteus* 2 reduced the gall size by 25.5±2.51% and 26.1±0.95%, respectively (Figure 4.19). In preventive approach, plants treated with *Arthrobacter* sp 1, *M. luteus* 1, funguran® and control recorded similar percentage increase in gall size throughout the experiment (Figure 4.20).

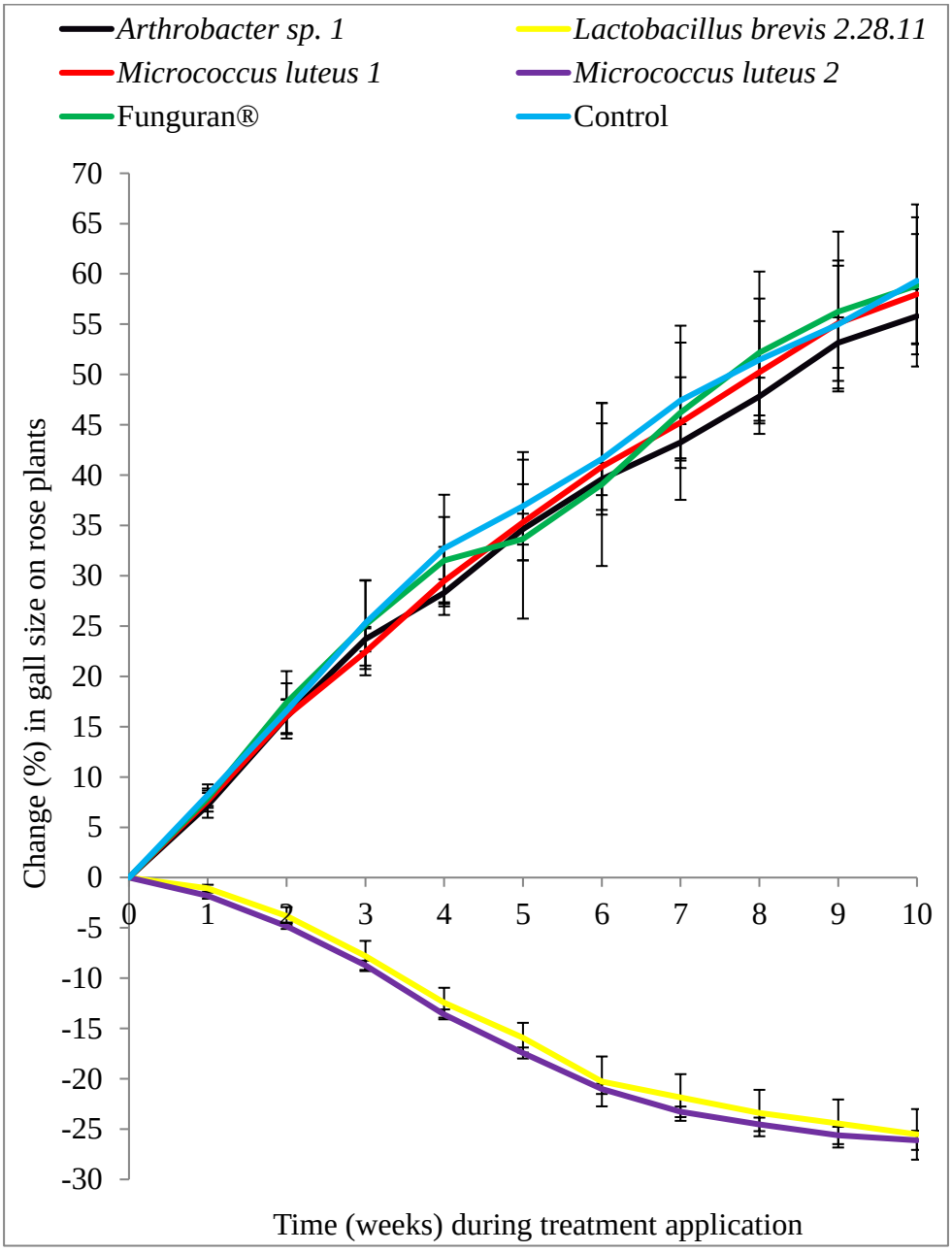

Figure 4.19 Change (%) in gall size on Rose Tropical Amazon® cultivar as affected by bacterial isolates at Kenyatta University research farm (Curative approach) from November 2018 to February 2019

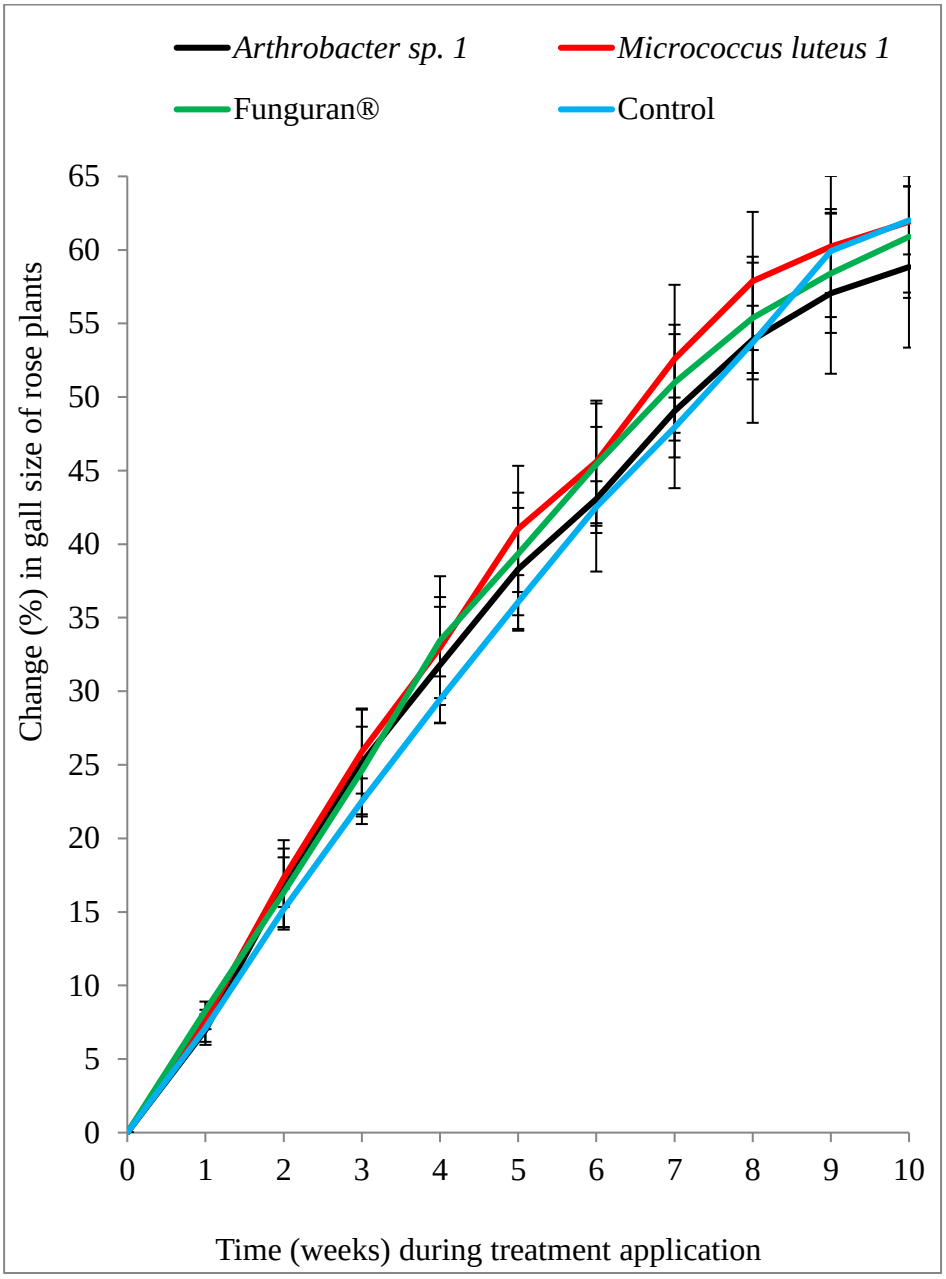

Figure 4.20 Change (%) in gall size on Rose Tropical Amazon® cultivar as affected by bacterial isolates at Kenyatta University research farm (Preventive approach) from November 2018 to February 2019

4.10.3 Number of shoots on rose plants

In curative approach, plants treated with *L. brevis* 2.28.11, *M. luteus* 2, *M. luteus* 1, *Arthtobacter* sp 1, funguran® and the control with galls recorded similar number of shoots which were significantly ($df = 6,48$; $P<0.05$) lower than the ones produced by plants in the control without galls from week 2 until the end of the monitoring period (Table 4.19). From week 5 until the end of experiment, the number of shoots produced by plants treated with *L. brevis* 2.28.11 and *M. luteus* 2 was similar but significantly ($P<0.05$) higher than the ones produced by plants treated with *Arthrobacter* sp 1, *M. luteus* 1, funguran® and control with galls. However, the number of shoots in plants treated with *Arthrobacter* sp 1, *M. luteus*, funguran® and control with galls did not differ significantly (Table 4.19).

In preventive approach, number of shoots in plants treated with *L. brevis* 2.28.11, *M. luteus* 2, and those in the control without galls were similar and significantly ($df = 6,48$; $P<0.05$) higher than those produced in plants treated with *Arthrobacter* sp 1, *M. luteus* 1, funguran® and the control with galls. This was observed from week 4 to week 10 (Table 4.20). Number of shoots produced by plants treated with *Arthrobacter* sp 1, *M. luteus* 1, funguran® and the control with galls were comparable from week 1 until week 10 (Table 4.20).

Table 4.19 Number of shoots (Mean±S.E) on Rose Tropical Amazon® cultivar treated with bacterial isolates at Kenyatta University research farm (Curative approach) from November 2018 to February 2019

	Time (weeks) during treatment application (Mean±S.E)									
Treatments	1	2	3	4	5	6	7	8	9	10
Arthrobacter sp 1	2.4±0.20^{a}	3.1±0.26^{b}	4.1±0.26^{b}	5.1±0.40^{b}	6.4±0.43^{c}	7.1±0.50^{d}	8.1±0.34^{c}	8.9±0.14^{c}	9.4±0.30c	9.6±0.20^{c}
Lactobacillus brevis 2.28.11	2.3±0.18^{a}	2.7±0.18^{b}	4.0±0.31^{b}	6.3±0.64^{b}	8.1±0.63^{b}	10.1±0.74^{b}	11.6±0.81^{b}	12.7±0.87b	13.4±0.81b	14.0±0.72^{b}
Micrococcus luteus 1	2.3±0.18^{a}	2.7±0.29^{b}	3.9±0.26^{b}	5.1±0.40^{b}	6.0±0.49^{c}	6.9±0.55^{d}	7.6±0.37^{c}	8.6±0.37^{c}	8.9±0.34^{c}	9.3±0.29^{c}
Micrococcus luteus 2	2.4±0.20^{a}	2.7±0.18^{b}	4.3±0.36^{b}	6.0±0.44^{b}	7.6±0.61^{bc}	8.7±0.57^{bc}	10.1±0.67^{b}	11.3±0.52^{b}	13.0±0.53^{b}	14.4±0.48^{b}
Funguran®	2.3±0.18^{a}	2.7±0.36^{b}	3.9±0.34^{b}	5.0±0.44^{b}	6.4±0.72^{c}	6.9±0.67^{d}	7.7±0.47^{c}	8.7±0.29^{c}	9.4±0.30^{c}	9.6±0.20^{c}
Control with galls	2.3±0.18^{a}	3.0±0.22^{b}	4.4±0.30^{b}	5.4±0.48^{b}	6.6±0.37^{bc}	7.4±0.48^{c}	8.4±0.43^{c}	8.86±0.51^{c}	9.6± 0.48^{c}	9.7±0.47^{c}
Control without galls	2.7±0.23^{a}	4.7±0.29^{a}	6.6±0.43^{a}	8.9±0.40^{a}	10.8±0.59^{a}	12.7±0.61^{a}	14.7±0.61^{a}	16.0±0.72^{a}	17.1±0.74^{a}	18.3±0.71^{a}
P value	0.736	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
LSD (P≤0.05)	0.590	0.744	0.933	1.329	1.602	1.697	1.577	1.541	1.531	1.387
F value	0.59	7.87	8.62	8.49	9.03	13.6	22.58	27.3	33.07	52.01

Means ± SE followed by similar letter(s) in the same column do not differ significantly at P≤0.05 (Fisher's protected L.S.D test).

Table 4.20 Number of shoots (Mean±S.E) on Rose Tropical Amazon® cultivar treated with bacterial isolates at Kenyatta University research farm (Preventive approach) from November 2018 to February 2019

	Time (weeks) during treatment application (Mean±S.E)									
Treatments	1	2	3	4	5	6	7	8	9	10
Arthrobacter sp 1	2.4±0.20^{a}	3.4±0.37^{a}	5.3±0.47^{a}	6.1±0.51^{c}	6.4±0.48^{c}	7.0±0.44^{b}	7.9±0.26^{c}	8.4±0.30^{c}	9.0±0.31^{c}	9.6±0.20^{c}
Lactobacillus brevis 2.28.11	2.3±0.18^{a}	3.9±0.46^{a}	5.7±0.47^{a}	8.3±0.36^{a}	11.4±0.30^{ab}	13.1±0.46^{a}	15.1±0.67^{ab}	16.9±0.63^{ab}	18.7±0.68^{a}	19.9±0.77^{a}
Micrococcus luteus 1	2.3±0.18^{a}	3.6±0.30^{a}	5.6±0.37^{a}	6.3±0.29^{bc}	6.6±0.37^{c}	7.1±0.46^{b}	7.6± 0.30^{c}	8.7±0.36^{c}	9.0±0.31^{c}	9.9±0.26^{c}
Micrococcus luteus 2	2.1±0.15^{a}	3.9±0.34^{a}	5.4±0.37^{a}	8.4±0.20^{a}	11.7±0.36^{a}	14.0±0.44^{a}	16.0±0.53^{a}	17.6±0.57^{a}	19.0±0.53^{a}	20.1±0.51^{a}
Funguran®	2.4±0.20^{a}	4.3±0.47^{a}	5.4±0.57^{a}	6.3±0.71^{bc}	6.4±0.72^{c}	6.9± 0.67^{b}	7.7±0.47^{c}	8.7±0.29^{c}	9.4±0.30^{c}	9.6±0.20^{c}
Control with galls	2.3±0.18^{a}	4.0±0.38^{a}	6.1±0.46^{a}	6.4±0.57^{bc}	6.6±0.48^{c}	7.1±0.34^{b}	8.1±0.34^{c}	8.9±0.14^{c}	9.6±0.43^{c}	9.6±0.43^{c}
Control without galls	2.4±0.30^{a}	4.1±0.51^{a}	5.6±0.65^{a}	7.6±0.57^{ab}	10.1±0.59^{b}	12.7±0.57^{a}	14.6±0.65^{b}	15.9±0.83^{b}	17.1±0.74^{b}	18.4±0.65^{b}
P value	0.944	0.772	0.921	0.002	0.001	0.001	0.001	0.001	0.001	0.001
LSD (P≤0.05)	0.583	1.170	1.398	1.395	1.401	1.401	1.387	1.421	1.429	1.367
F value	0.28	0.54	0.32	4.24	26.02	46.94	67.41	76.40	94.54	121.73

Means ± SE followed by similar letter(s) in the same column do not differ significantly at P≤0.05 (Fisher's protected L.S.D test).

4.10.4 Height (cm) of shoots on rose plants

In curative approach, shoot heights in plants treated with *Arthrobacter* sp 1, *Micrococcus luteus* 1, funguran® and control with galls were comparable but differed significantly (df = 6, 48; P<0.05) with those of plants in the control without galls and those treated with *L. brevis* 2.28.11 *M. luteus* 2 from week 2 until week 10. In the same week, plants treated with *L. brevis* 2.28.11 *M. luteus* 2 produced significantly (df = 6,48; P<0.05) shorter shoots than those in the control without galls. This trend was observed until the end of experiment (Figure 4.21).

In preventive approach, shoots heights in plants treated with *L. brevis* 2.28.11 *M. luteus* 2 and those in the control without galls did not differ significantly (P≥0.05). However, they were significantly (df = 6, 48; P≤0.05) taller than those in plants treated with *Arthrobacter* sp 1, *M. luteus* 1, funguran®, and control with galls. This difference was recorded from week 2 to week 10. Shoot heights in plants treated with *Arthrobacter* sp 1, *M. luteus* 1, funguran®, and control with galls were comparable throughout the monitoring period (Figure 4.22).

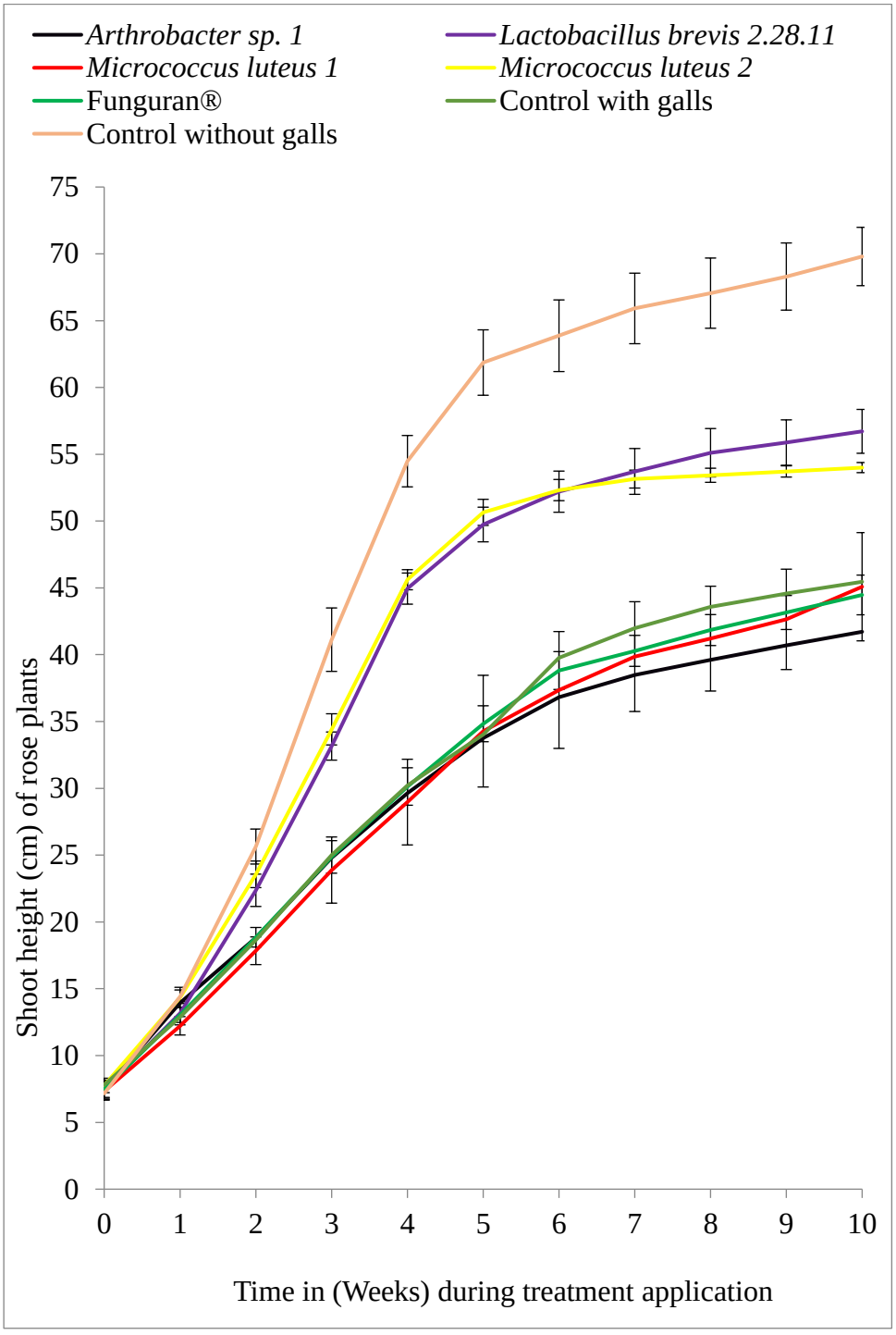

Figure 4.21 Mean shoot heights (cm) on Rose Tropical Amazon® cultivar as affected by bacterial isolates at Kenyatta University reseach farm (Curative approach) from November 2018 to February 2019

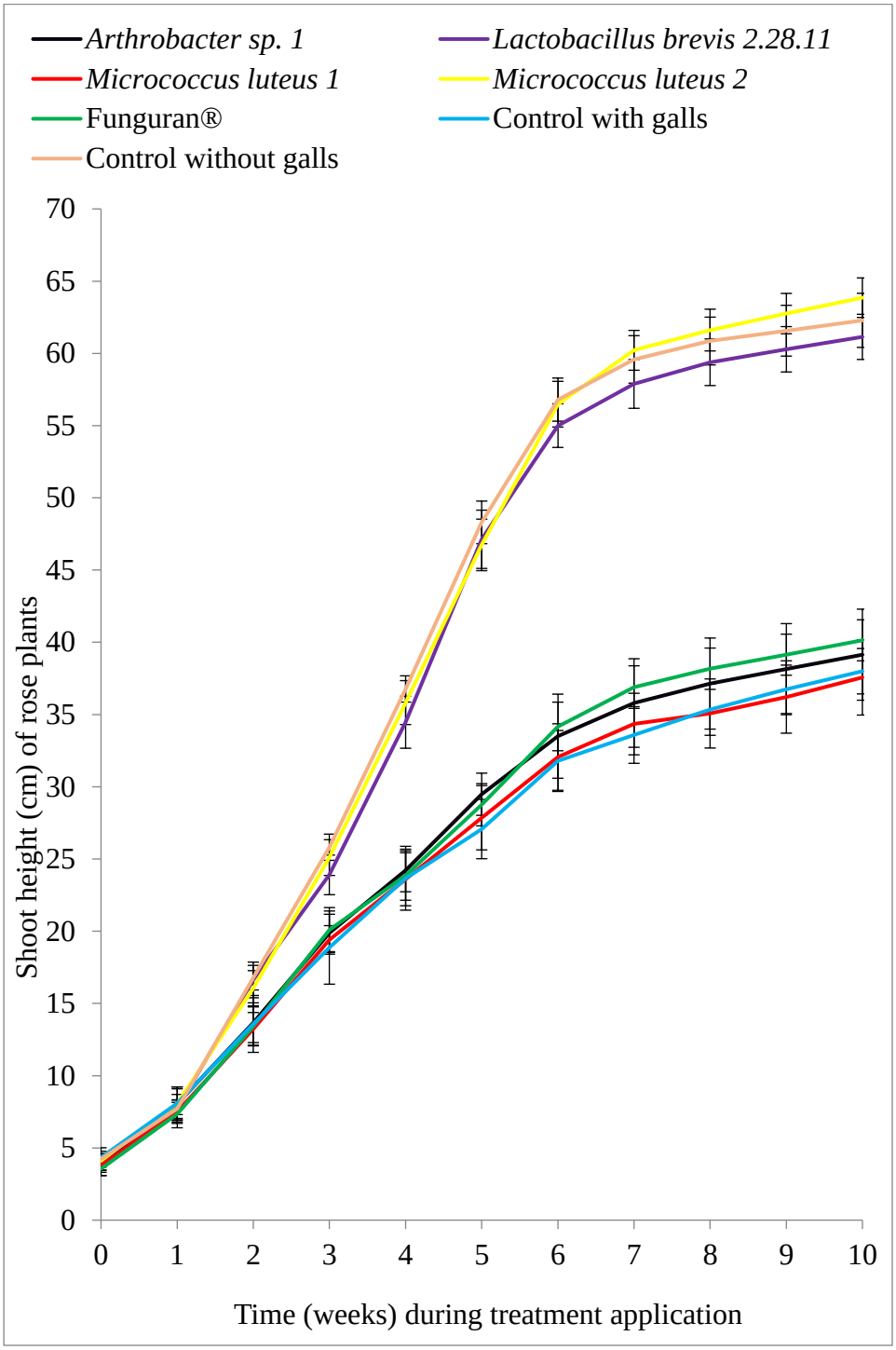

Figure 4.22 Mean shoot heights (cm) on Rose Tropical Amazon cultivar as affected by bacterial isolates at Kenyatta University research farm (Preventive approach) from November 2018 to February 2019

CHAPTER FIVE: DISCUSSION

5.1 Status of crown gall disease in roses

The baseline study focused on determining the knowledge and experience of rose flower production managers on crown gall disease and its management. The critical parameters were varieties of roses, constraints faced in rose production, incidence and severity of crown gall disease, management practices and effects on yield. Rose flower production is an important venture in Kenya (KFC, 2019). The size of farms shows that production of roses is specifically for commercial purposes. Long periods of farm existence coupled with more years of work experience of production managers means greater exposure to crown gall which can lead to informed response to crown gall infections and spread from the respondents. Education level of most of the respondents was tertiary and this suggests that the production managers are adequately trained on production practices of roses, diagnosis of crown gall disease and its management.

According to the findings of this study, the main rose flower varieties grown differed across the Counties. The multiple varieties of roses grown are intended to capitalize on an export strategy termed as product diversification to enable farms to remain competitive (Kamau, 2011). Product diversification has been implemented by Sian Roses, a group of five rose flower farms in Kenya, that plant superior multiple rose varieties to meet market demand (Kamau, 2011). In terms of varietal preference Upper Class®, Tropical Amazon® and Red Kamala® were the most preferred.

The reasons for varietal preference were found to be customer demand, high yields, early maturity, and resistance to pests and diseases such as thrips and downy mildew, respectively. Upper Class®, Tropical Amazon® and Red Kamala® are hybrid varieties (FPEAK, 2019). Hybrid varieties have improved yields, resist diseases and are of good quality. However, the above named varieties do not resist crown gall.

Crown gall is a severe constraint in rose flower production in Kenya as all surveyed flower farms were affected. The disease was more pronounced in greenhouses that had susceptible rose varieties (Tropical Amazon® and Upper Class®) as evidenced by presence of galls. The key factors leading to presence of crown gall in the study areas include use of contaminated planting materials, poor farm hygiene and infested soils. Similar results were reported by Maina *et al*. (2011) and Murugi (2015). Majority of flower growers reported that they propagate their own cuttings for planting. They also stated that they acquire scions from the already planted roses.

Agrobacterium tumefaciens is a soil borne disease and can still be in the plant system only to manifest when conditions are favourable. This therefore suggests that the scions could be infected by *A. tumefaciens* before grafting in readiness for planting. Also farm hygiene was not well observed on the pruning materials. Disinfecting of harvesting scissors was done after cutting a number of stems on different rose plants. This therefore suggests that *A. tumefaciens* inoculum could easily spread from one plant to the other in the greenhouse before the pruning material is disinfected.

Infested soils can also lead to presence of crown gall since no respondent mentioned use of sterilized soils or changing the soils. Water can also facilitate subsequent infections of the host plant or other plants in the area (Horst, 2007). A similar case was reported by Murugi (2015) showing that rose flower stocks raised on different growth medium were infected with *A. tumefaciens.*

The above reasons could also explain the high incidence and severity of crown gall. Some farms, example Equinox and Wildfire farms had higher disease incidence and severity than others. This could be attributed to differences in the level of greenhouse hygiene between farms. This problem could also be exacerbated by agronomic practices like use of unsterilized pruning knives and gall plucking that inflicts wounds (Feist *et al*., 2016). The frequency of disinfecting pruning scissors could also bring difference in disease and severity levels.

The current approaches of managing crown gall disease included cultural methods, biological methods, use of synthetic pesticides (copper based products) and IDM (cultural, biological and use of synthetic chemicals). According to the respondents cultural methods included plucking of galls, sanitation and at least removal of the whole crop. This is in agreement with what Murugi (2015) reported in similar study in Meru and Murang'a Counties.

The present study shows that biological methods (*Trichoderma* sp formulation) has only been adopted by a limited number of flower farms and it was found to be effective in Nakuru County confirming previous results by Koppert (2012). The low adoption of *Trichoderma* formulation despite its reported effectiveness could be attributed to lack of adequate information on

the biological products. Murugi (2015) reported that lack of proper information on management of crown gall affects its management.

Use of copper based products such as funguran® as synthetic chemicals was the major strategy that growers employ but it was found to be ineffective in suppressing crown gall in all the flower farms surveyed. High failure of synthetic pesticides to manage crown gall disease could be attributed to overuse of one type of the products and the pathogen may have developed resistance. Itale *et al.* (2016) reported that a pathogen may develop resistance when the products used to manage the target disease are not alternated with other management methods. The active ingredients used in managing crown gall are copper hydroxide and copper oxychloride both of which are contact fungicides. Additionally, high usage of synthetic chemicals especially copper based products over other methods suggests that farmers have adequate information on synthetic chemicals which disadvantages other methods.

Cultural methods alone were reported to be ineffective; how they practice the method could lead to its ineffectiveness. An observation was made that flower growers drop galls on the ground after plucking for collection later. This can lead to spread of the disease. When plucking galls, they may disintegrate and spread the inoculum to the plant and the soil. Broken galls on the ground or in the soil are primary inoculum sources of *A. tumefaciens* (Paret *et al*., 2011). As tumours break and degrade, infected tissue that falls off into the soil releases bacteria (Horst, 2007).

Integrated disease management (plucking galls, farm hygiene and use of synthetic chemicals) was found to be practiced in all the three Counties though

at a lower proportion than synthetic chemicals. Use of IDM as a viable approach to combat crown gall menace increases agricultural productivity; it is cost-effective and not harmful to the environment (Sithanantham, 2017).

Although IDM was mentioned across the three Counties, there was no agreement on its effectiveness as reported by majority of the respondents. Lack of agreement on effectiveness of this method could be attributed to method of application used and timing of application.

Although use of resistant varieties as an option in managing crown gall was reported in the study areas, none of the rose varieties with high market demand falls under this category since variety preference depends on market. Resistant varieties reported included Furiosa®, Red Calypso®, Shanty®, Ai® and Jupita® which were reported to be inferior in terms of customer demand, yields and early maturity.

Production of crops involves being aware of possible biotic threats so as to be able to employ effective management strategies to avoid loss of the product. Apart from crown gall disease, other constraints were fungal diseases, insect pests, price fluctuation, transport cost and high production cost. Multiple infections of roses by powdery mildew, downy mildew and *Botrytis* were reported across all the areas surveyed.

Chewing insects create wounds on rose plants which become entry point of pathogens and lowers both quantity and quality of roses (Ali *et al.,* 2010). Rose flowers like any other price-elastic good experience price fluctuation as a result of market forces. High production cost can be attributed to cost of

various farm inputs including pesticides, high power cost and cost of labour (Klonsky, 2012).

5.2 Evaluation of biocontrol agents *in vitro* and *in vivo*

Evaluation of antimicrobial efficacy of microorganisms in *in vitro* bioassays is a necessary step in developing products for crop protection. Microorganisms isolated from local environments included bacteria in the genera *Lactobacillus, Micrococcus* and *Arthrobacter*. A considerable variation was observed in effect of bacterial isolates against *A. tumefaciens* based on the inhibition on the culture media. Generally, antagonistic bacteria are known and have been reported to produce enzymes which degrade the cells of the pathogen and inhibit its growth (García-Fraile *et al*., 2015). Reddy *et al*. (2014) reported that differences in levels of hydrolytic enzymes produced by each species of microorganism when they attack pathogens influences antagonism activity. Suppression of *A. tumefaciens* by the antagonistic bacteria tested could also be attributed to the production of substances such as cyclic dipeptides, proteinaceous compounds, organic acids, fatty acids and reuterin (García-Fraile *et al*., 2015). However, these should be investigated in future studies in regard to the isolates evaluated in this study.

Arthrobacter sp 1 was particularly effective in inhibiting growth of *A. tumefaciens in vitro.* Arseneault *et al*. (2013) reported that *Arthrobacter* produces enzymes that degrade the cells of pathogens and inhibits its growth. Munaganti *et al.* (2016) also reported that *Arthrobacter kerguelensis* Gupta had suppressed fungal and bacterial diseases. Donmez *et al.* (2015) reported that

Arthrobacter sp was effective against root rot disease of potato and beans *in vitro*. The above mentioned studies suggest that *Arthrobacter* sp inhibits pathogens *in vitro*.

Lactobacillus brevis 2.28.11 also inhibited growth of *A. tumefaciens in vitro*. *Lactobacillus brevis* produces bacteriocins which disrupt the integrity of the target pathogen's cell membrane leading to cell death (Mechoud *et al.*, 2017). According to Gajbhiye and Kapadnis (2016), bacteria in the genera *lactobacillus* produces organic and fatty acids, proteinaceous compounds, cyclic dipeptides and phenolic compounds with antimicrobial activities. The antagonistic activity against *Agrobacterium tumefaciens, in vitro*, could also be attributed to the low pH of organic acids produced by the microorganisms in the genera *Lactobacillus* which can influence growth of *A. tumefaciens.* A *Lactobacillus plantarun* produced organic acids that suppressed *A. tumefaciens in vitro* (Limanska *et al*., 2015).

Dranas *et al*. (2019) reported that *Lactobacillus plantarum* CC10 inhibited growth of *Xanthomonas fragariae* Kennedy and King *in vitro.* Limansnka *et al.* (2015) also worked with *Lactobacillus plantarum* B4496, *Lactobacillus brevis* 207 and *Lactobacillus sanfranciscensis* BB12 Weis and Schillinger and found that they inhibited growth of *A. tumefaciens in vitro*. Further, Kato *et al.* (2016) reported that *Lactobacillus* sp inhibited growth of *Escherichia coli* Migula *in vitro*. Lynch *et al.* (2016) also reported that *Lactobacillus brevis* JJ2P Orla-Jensen and *Lactobacillus reuteri* R2 Kandler suppressed *Zymoseptoria tritici* Fuckel causal agent of *Septoria tritici* Desm blotch of

wheat *in vitro*. The above studies demonstrate the significant potential of *Lactobacillus* species in managing crop diseases.

Micrococcus luteus 1 and *Micrococcus luteus* 2 also inhibited growth of *A. tumefaciens in vitro* but to a lesser extent. Sharma and Saharan. (2016) reported that *Micrococcus luteus* SNSr7 strain NH54PC02 Cohn produced enzymes like cellulose, protease and chitin that degrade the cells of *Rhizoctonia solani* Kuhn and inhibits its growth.

In the field, *Lactobacillus brevis* 2.28.11 and *Micrococcus luteus* 2suppressed crown gall while *Micrococcus luteus* 1 and *Arthrobacter* sp 1 did not suppress the disease on both Tropical Amazon® and Upper Class® rose varieties. The difference observed in the isolates could be because *A. tumefaciens* could be more aggressive by establishing faster than *Arthrobacter* sp 1 and *Micrococcus luteus* 1. Some pathogens are fast growers which make them flourish before the effect of the biocontrol agent is established (Rodino *et al*., 2014). Kumar *et al.* (2018) reported that bacteria in the genera *Agrobacterium* are fast growers.

The effectiveness of *Lactobacillus brevis* 2.28.11 and *Micrococcus luteus* 2 *in vivo* varied over time which could be attributed to the fact that microorganisms take time before they colonize and establish prior to effecting action on the pathogen (Ríos-Moreno *et al*., 2016). *Lactobacillus brevis* 2.28.11 increased the number and height of shoots steadily over time in all the sites and in all the varieties. Bacteria in the genera *Lactobacillus* are mostly regarded as biofertilizers and biostimulants. They are known to improve nutrient availability, alleviate biotic and abiotic stresses, and directly stimulate

plant growth (Lamont *et al.*, 2017). *Lactobacillus brevis* 2.28.11 reduced the number of galls as well as percentage growth of galls and made them dry.

For preventive approach in the greenhouse, *Lactobacillus brevis* 2.28.11 resulted to no gall formation on the plants. Genera *Lactobacillus* produces bacteriocins which protects plants against pathogens (Salazar *et al*., 2017). Bacteriocins are part of the inherent defense system of bacteria and play other roles such as niche colonization, direct killing of competing strains (Varez-Sieiro *et al*., 2017). Further, the genus *Lactobacillus* produces acetic and lactic acid which diffuse through the membrane of the target organisms in their hydrophobic undissociated form, dissociating within the cell and thus reducing the cytoplasmic pH which interferes with metabolic activities of the pathogen. Dranas *et al*. (2019) reported that *Lactobacillus plantarum* CC10 suppressed *Xanthomonas fragariae* in strawberry in *vivo* while Limanska *et al*. (2015) reported that *Lactobacillus plantarun* suppressed crown gall (*A. tumefaciens*) on grapevines.

Micrococcus luteus 2 also suppressed crown gall *in vivo*. The isolate led to a decrease in both the number and size of galls. In preventive approach, galls did not form in plants treated with *Micrococcus luteus* 2. The genus *Micrococcus* is a well-known phosphate-solubilizing bacterial strain which is used as biofertilizers and bio-control agents in agriculture (Kalayu, 2019). Also, *Micrococcus luteus* SNSr7 strain NH54PC02 is a phyllospheric bacterium having plant growth promoting ability through production of siderophore (ion) (Sharma and Saharan, 2016). Further, Sharma and Saharan (2016) reported that *M. luteus* SNSr7 strain NH54PC02 acts as biocontrol

agent by production of certain compounds like Hydrogen Cyanide (HCN), inhibitory enzymes like chitinase, protease, and cellulase and secondary metabolites that may protect plants from pathogenic microorganisms.

Hydrogen cyanide (HCN) gas plays defensive role against phytopathogens (Attar *et al.,* 2015). Shang *et al.* (2016) stated that bacterial antagonists compete for nutrients with pathogens which tend to exclude the pathogen. Sangthong *et al*. (2016) found that application of *Micrococcus* sp. TISTR2221 improved maize growth. The modes of action *of Micrococcus luteus 2* can be determined further in future studies.

The overall results have revealed that crown gall is a great threat to rose flower production. Currently, there is no effective management of crown gall, however, local environments have potential antagonistic bacteria that can be used to effectively manage crown gall. The isolates *Lactobacillus brevis* 2.28.11 and *Micrococcus luteus* 2 from local environment suppressed crown gall and increased yields and quality of flowers by increasing the number of shoots, increasing their heights and reducing the number of galls.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

I. Crown gall remains a significant threat to rose flower production in Kenya which necessitates investment in research to develop effective, affordable and environment friendly management measures.

II. The *in vitro* results demonstrated that local environment is a potential source of *Lactobacillus brevis* 2.28.11, *Micrococcus luteus* 1, *Micrococcus luteus* 2 and *Arthrobacter* sp 1 that suppressed *A. tumefaciens*.

III. Use of *L. brevis* 2.28.11 and *M. luteus* 2 for management of crown gall was effective under *in vivo* conditions. These isolates can be used for effective management of crown gall.

6.2 Recommendations

I. Awareness should be increased among flower growers on propagation of clean cuttings, greenhouse hygiene and importance of using biocontrol products.

II. The isolates *Lactobacillus brevis* 2.28.11 and *Micrococcus luteus* 2 that were effective *in vivo* can be used by flower growers to manage crown gall disease upon formulation, registration and commercialisation.

III. Further studies should be conducted on:

a. Full identification of the active isolates and determination of their modes of action.

b. Development of galls beyond week 10 after treatment with effective isolates.

c. Synergistic effects between and among the antagonistic bacteria.

d. Increasing intervals between treatment applications.

e. Determining why *Arthrobacter* .sp 1 and *M. luteus* 1 were not effective in the greenhouse.

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APPENDICES

Appendix I. Questionnaire

A field survey on incidence and severity of crown gall disease of roses and management practices in Kiambu, Laikipia and Nakuru Counties

Questionnaire No:.……Date of data collection:……

Name of the flower farm.....

Sub-county: …… Ward: …… Location…… Village: ……. GPS coordinates: Longitude:……. Latitude:……. Altitude……Agro-Ecological Zone……

Type of ownership

Local Foreign

Position of the respondent…….

Cell phone No:…….

Period worked at the farm in your current position

< 1 yr. 1-2 yrs. 2-4yrs >5yrs

Level of education:

None Primary Secondary Post-secondary

Respondent:

Owner Manager Employee Other specify……

Farm details and crown gall related questions

1. What is the size of your farm in hectares?

< 10 ha 2. 10-50ha 3. 50-100ha 4. >100 ha

2. What is the size of the area under roses?

< 10 ha 10-50ha 50-100ha >100ha

3. For how long have you been growing roses?

<2yrs 2. 2-5yrs 3. 5-10yrs 4. >10yrs

4. Are you aware of crown gall disease?

Yes No

If yes how do you identify it?

Presence of galls stunting of plants chlorotic leaves

Multiple Others specify……

5. At what stage of plant growth does crown gall occur?

Young stage vegetative stage maturity stage

all stages

6. Which planting materials do you use?

Soil Pumice Coco peat

Compost

7. What are some of the factors leading to presence of crown gall?
Contaminated planting material poor greenhouse hygiene

infested soils

8. What are source of planting materials?
Own cuttings local commercial propagators

importedothers specify

9. Which parts of the plants do you sell?
Flower Cuttings

10. How do you sell your flowers/cuttings?
Local markets Export

11. Where do you obtain water used in growing flowers?
Rain water Rivers Boreholes Dams

12. Do you treat water before use?

Yes No

13. What type of irrigation do you use

Drip irrigation Sprinkler irrigation Others

	Reasons for preference				
Variety	Early maturity	Customers demand	Resistant to pests and diseases	High yielding	Others
Furiosa					
Ace pink					
Akito					
Tropical Amazon					
Upper Class					
Others					

14. What is the main rose flower variety (ies) grown in this farm?

15. Which varieties are susceptible to crown gall disease in order of susceptibility

Varieties	1	2	3	4	5
Impact					
Furiosa					
Ace pink					
Akito					
Tropical Amazon					
Upper Class					
Others					

- One (1) represent the most susceptible while 5 is the least

16. Are there varieties resistant to crown gall? If yes, are they resistant to crown gall?

17. What is the incidence of crown gall disease in your farm?

1-10% 11-20% 21-30% 31-40% 41-50% over 50%

18. What is the incidence of crown gall disease?

1-10% 11-20% 21-30% 31-40% 41-50% over 50%

19. What is the yield loss of flowers due to crown gall disease?

1-10% 11-20% 21-30% 31-40% 41-50% over 50%

20. Are there other types of flowers grown in this farm? If yes, mention them and state whether they are affected by crown gall disease

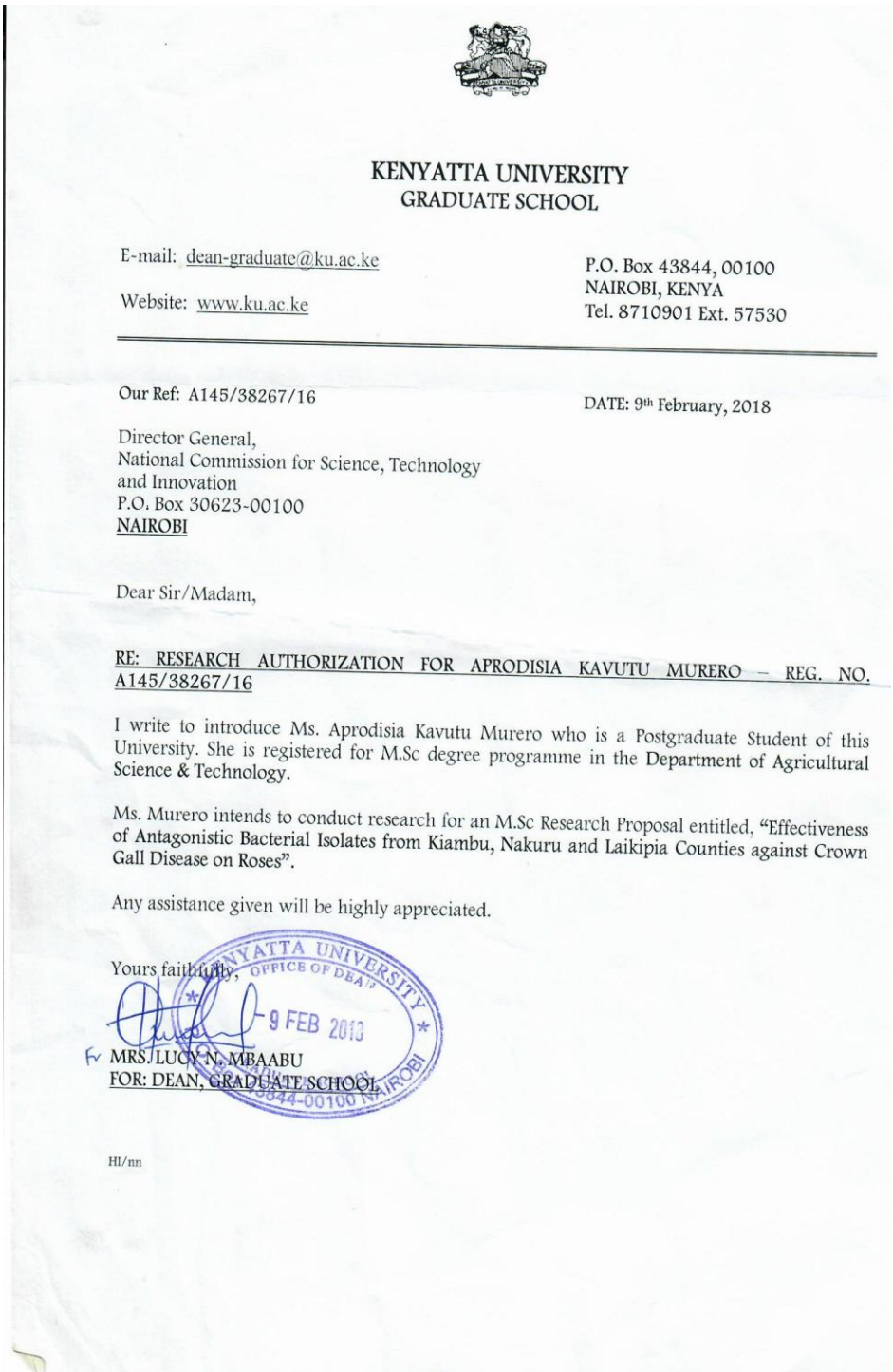
21. Which other disease attack your farm?

Management methods of crown gall disease

Control methods	Mention the specific practice or technology	Where obtained (1. Imported 2.Local markets 3. Multiple 4. No idea 5. Others specify)	Accessibility (1.readily available 2.Not readily available 3. No idea)	Effectiveness (1.Very effective 2. Effective 3. Not effective 4. No idea)
Use of resistant varieties				
Cultural methods				
Biological methods				
Chemical methods				
Integrated disease management				

Thank you for your attention

Appendix II. Research authorization letter from Kenyatta university



KENYATTA UNIVERSITY
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P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 8710901 Ext. 57530

Our Ref: A145/38267/16

DATE: 9th February, 2018

Director General,
National Commission for Science, Technology
and Innovation
P.O. Box 30623-00100
NAIROBI

Dear Sir/Madam,

RE: RESEARCH AUTHORIZATION FOR APRODISIA KAVUTU MURERO – REG. NO. A145/38267/16

I write to introduce Ms. Aprodisia Kavutu Murero who is a Postgraduate Student of this University. She is registered for M.Sc degree programme in the Department of Agricultural Science & Technology.

Ms. Murero intends to conduct research for an M.Sc Research Proposal entitled, "Effectiveness of Antagonistic Bacterial Isolates from Kiambu, Nakuru and Laikipia Counties against Crown Gall Disease on Roses".

Any assistance given will be highly appreciated.

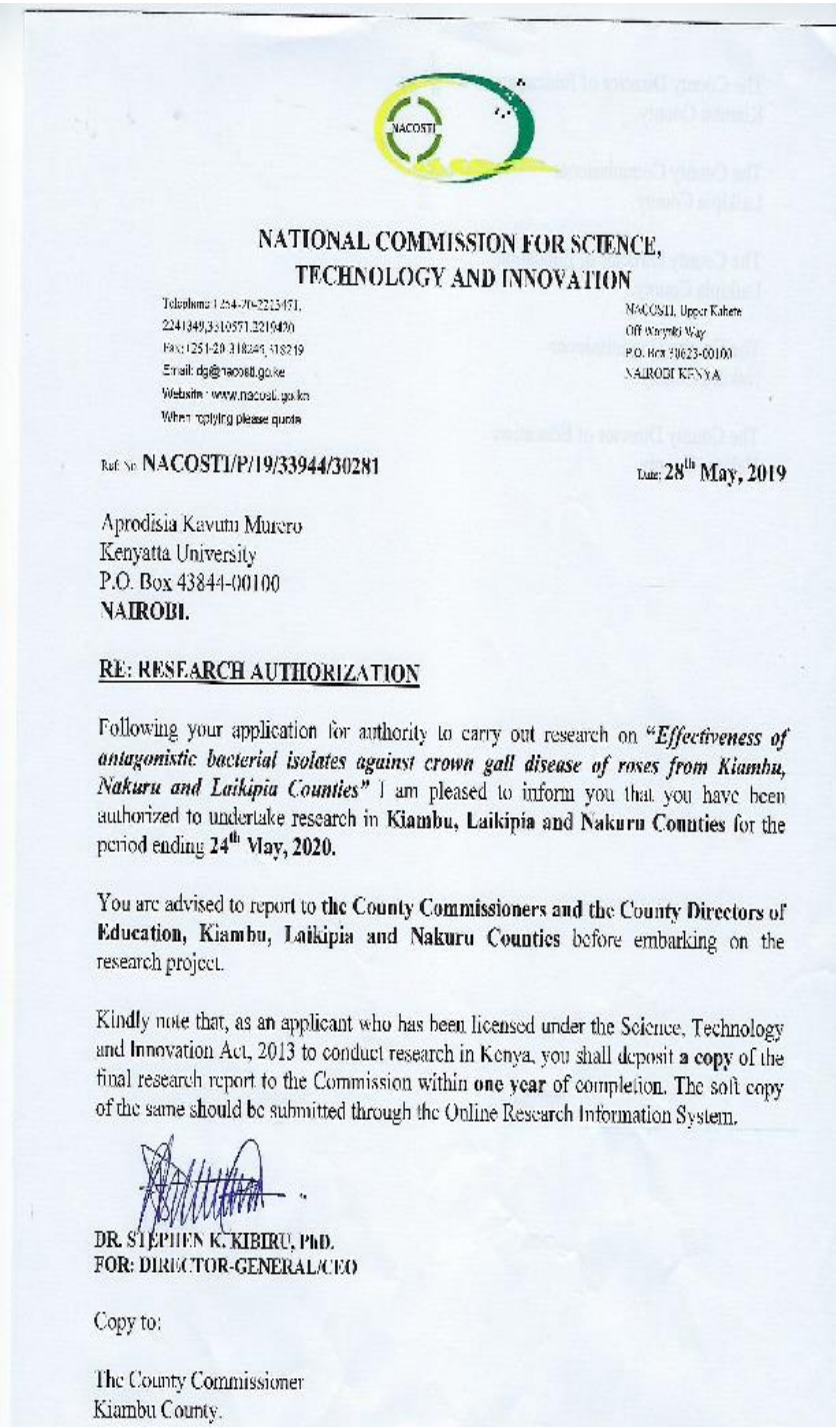
Yours faithfully,

KENYATTA UNIVERSITY OFFICE OF DEAN 9 FEB 2018 P.O. Box 43844-00100 NAIROBI

MRS. LUCY N. MBAABU
FOR: DEAN, GRADUATE SCHOOL

HI/nn

Appendix III. Research permit from National Commission for Science, Technology and Innovation (NACOSTI)



NATIONAL COMMISSION FOR SCIENCE,
TECHNOLOGY AND INNOVATION

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NACOSTI, Upper Kabete
Off Waiyaki Way
P.O. Box 30623-00100
NAIROBI KENYA

Ref No: **NACOSTI/P/19/33944/30281**

Date: **28th May, 2019**

Aprodisia Kavutu Murero
Kenyatta University
P.O. Box 43844-00100
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Effectiveness of antagonistic bacterial isolates against crown gall disease of roses from Kiambu, Nakuru and Laikipia Counties"*** I am pleased to inform you that you have been authorized to undertake research in **Kiambu, Laikipia and Nakuru Counties** for the period ending **24th May, 2020.**

You are advised to report to **the County Commissioners and the County Directors of Education, Kiambu, Laikipia and Nakuru Counties** before embarking on the research project.

Kindly note that, as an applicant who has been licensed under the Science, Technology and Innovation Act, 2013 to conduct research in Kenya, you shall deposit **a copy** of the final research report to the Commission within **one year** of completion. The soft copy of the same should be submitted through the Online Research Information System.

DR. STEPHEN K. KIBIRU, PhD.
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioner
Kiambu County.