MANAGEMENT OF POWDERY MILDEW (*Podosphaera pannosa*) ON ROSES (*Rosa hybrida*) USING *Bacillus* spp AND SODIUM NITROPHENOLATE

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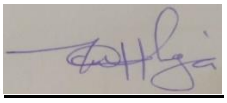
Chiaga Timon Oluoch …  Date 31/03/2022

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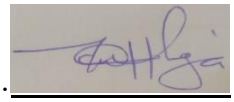
Signature … 

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To my beloved wife Dorothy Oniga for her constant prayers and material support; my sisters and brothers, friends and relatives for their moral and social support during the study period.

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ABBREVIATIONS

AFA	Agricultural Food Authority
AIPH	International Association of Horticultural Producers
ANOVA	Analysis of Variance
AUDPC	Area Under Disease Progress Curve
ASL	Above Sea Level
CO2	Carbon IV oxide
CV	Coeficient of Variation
EC	Electrical Conductivity
EU	European Union
GA	Gibberellic Acid
IAA	Indole Acetic Acid
ICM	Integrated Crop Management
ISR	Induced Systemic Resistance
JETRO	Japan External Trade Organization
KEPHIS	Kenya Plant Health Inspectorate Service
KFC	Kenya Flower Council
LSD	Least Significant Difference
PDS	Plant Disease Severity
PGPR	Plant Growth Promoting Rhizobacteria
pH	Potential of Hydrogen ions
UV-B	Ultra Violet – B
USDA	United States Department of Agriculture
VOC	Volatile Organic Compounds

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GENERAL ABSTRACT

Powdery mildew (*Podosphaera pannosa*) is a major disease of roses which reduces yield and quality through growth of mycelia on different parts of the rose plant which affects their salability. The disease is wide spread where roses are grown both in greenhouse and field. This study was carried out to evaluate the effects of *Bacillus* spp and sodium nitrophenolate on powdery mildew of roses in greenhouse conditions.

The trial was done in an already established bush of roses in the greenhouses at Kikuyu in Kiambu County and at Naivasha in Nakuru County both are in Kenya. The experiment was conducted on a variety known as A one which is grown in different farms in Kenya. Weekly foliar application of Real subtilis® (*Bacillus subtilis*) at the rate of 2ml/L, Hatake (*Bacillus amyloliquefaciens*) 3.0g/L, Atonik® (sodium nitrophenolate) 1ml/L and Meltatox® (*Dodemorph acetate*) 2.5ml/L. In the second experiment, foliar application of *Bacillus amyloliquefaciens* was done at different concentrations and at various intervals of application as follows 1.5g/L, 4 days, 3.0g/L, 4 days, 4.5g/L 4 days, 1.5g/L, 7 days, 3.0g/L, 7 days, 4.5g/L, 7 days, 1.5g/L, 10 days, 3.0g/L, 10 days, 4.5g/L, 10 days and no treatment as control. The experiment was laid out in a randomized complete block design with four replications for each treatment in plots with 44 plants. Data was collected on incidence and severity of powdery mildew on weekly basis while data on stem length, bud diameter, bud length and marketable grade were collected daily for twenty weeks and six weeks for the first and the second experiments respectively.

In the first experiment, *Bacillus subtilis*, *B. amyloliquefaciens*, sodium nitrophenolate and dodemorph acetate significantly reduced incidence of powdery mildew from 83% to 55% with dodemorph acetate posting the best results followed by *B. amyloliquefaciens*, sodium nitrophenolate and *Bacillus subtilis*. In the second experiment, at different rates and at various intervals *Bacillus amyloliquefaciens* reduced incidence of powdery mildew with the rate of 1.5g/L at four and seven day intervals gave better results than ten days interval. Disease severity was reduced significantly from 15.8% to 2.1% by application of dodemorph acetate and *B. amyloliquefaciens* while application of *B. subtilis* and sodium nitrophenolate did not significantly at $P \leq 0.05$ reduce severity of powdery mildew. Applications at the rate of 1.5g/L and 3.0g/L at four days and weekly applications had the highest reduction in severity in the second experiment. Dodemorph acetate had the highest area under disease progress curve (932) followed by *Bacillus*

amyloliquefaciens (988) in the first experiment while application of *Bacillus amyloliquefaciens* at the rate of 1.5g/L at the interval of seven days showed the highest AUDPC (799.8). Application of the test products did not significantly affect the yield and quality paramenters in the first experiment but in the second experiment, foliar application of *Bacillus amyloloquefaciens* at different rates and at various intervals improved the quality. Marketable stems significantly increased in the second experiment after the application of *Bacillus amyloliquefaciens* at different rates and at various intervals. Foliar application of *Bacillus Spp* and sodium nitrophenolate controlled powdery mildew of roses. Application of the same products did not have significant effects on quality and yield parameters of flowers such as flower bud length, stem length and bud diameter and number of stems produced in the first experiment, however, there was improvement in yield and quality in the second experiment.

Growers of roses should be encouraged to apply *Bacillus spp* and sodium nitrophenolate in managing powdery mildew. The optimal concentration of *Bacillus amyloliquefaciens* should be established and how sodium nitrophenolate induce resistance to plants against phytopathogens should be investigated.

Key words: *Rosa hybrida, Podosphaera pannosa, Bacillus subtilis, Bacillus amyloliquefaciens,* sodium nitrophenolate.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Roses (Rosa Spp.) are one of the most popular garden plants and the most economically important ornamental crop traded as cut flowers worldwide (Wen *et al*., 2006; Debener and Linde, 2009; Leus *et al*., 2018; United States Department of Agriculture-USDA, 2020). About 70% of the trade is done in the European Union markets. Large scale production is reported in countries such as Ecuador, Kenya and Colombia (Blom and Tsujita, 2003; International Association of Horticultural Producers-AIPH, 2019). Roses are grown for cut flower markets which are largely for its aesthetic value (Farooq and Kama, 2020; Tiwari *et al*., 2020). It is estimated that annual production for cut flowers ranges between 18 trillion stems, potted rose plants to be 80 million and garden roses to be 220 million (Pemberton *et al*., 2003; Agricultural and Food Authority-AFA, 2019).

In Kenya, roses is one of the major cut flowers grown and exported. Kenya is the leader in export of rose cut flower to the European Union (EU) with a market share of about 38% (Kenya trade.org, 2022). About half of the total export goes through the Dutch auction, although direct sales are also available. In some countries like United Kingdom, supermarkets are the main outlets for rose flowers (Kenya Flower Council, 2020). More markets are also coming up and they include Russia, Germany and Asian continent. About a quarter of the produced flowers are delivered directly to these markets which give opportunity for value addition through grading, sleeving, labelling and bouquet making (Adeola *et al*., 2018).

The main growing areas are Mt. Kenya, Nairobi, around Lake Naivasha, Kericho, Nakuru, Kitale, Athi River, Kiambu, Thika, Eastern Kenya, Uasin Gishu and Trans Nzoia. According to Kenya Flower Council, an estimate of 500,000 people including over 90,000 flower farms employees

depend on floriculture industry which in turn have got impact on livelihoods of over two million people. Production level comprises of small, medium and large scale farming (KFC 2020).

Roses grown in the greenhouse are affected by many fungal diseases including powdery mildew caused by *Podosphaera pannosa* (*Sphaerotheca pannosa)* which can easily be seen on the leaves, stems and upcoming shoots, floral parts and buds. The disease is associated with grayish or whitish patches on the affected parts of the plant (Eken, 2005; Matysiak, 2021). Powdery mildew causes leaf chlorosis, leaf curling, premature leaf drops and in severe cases death of affected plants (Shetty *et al.*, 2012; Scott, 2021), which leads to serious economic losses on productivity, quality and marketability of the produce (Suthaparan *et al.*, 2010; Lima *et al.*, 2019).

McGrath *et al.* (1996) pointed out that the most common method of disease control in roses is use of fungicides, however, their continued use may result in environmental pollution and development of resistant strains of the pathogen. Fungicides have also shown negative impacts on the beneficial microorganisms and insects, and this calls for softer alternatives to fungicides. Eken, (2005) and Khakimov *et al.*, (2020) contends that in the recent past, bio-fungicides have been used in the management of powdery mildew disease.

1.2 Problem statement

Powdery mildew is one of the major diseases of roses grown in greenhouses. The greenhouse environment is ideal for the growth and development of the disease all year round. The high level of repetitive fungicide application needed to lower the powdery mildew pressure normally result to faster fungicides resistance (Daughtrey and Benson, 2005; Kumar and Chandel, 2018; Wanasiri *et al.*, 2020). It is estimated that 40% of pesticide usage is directed in controlling powdery mildew in greenhouses which depicts high increased cost of production (Debener and Byrne, 2014;

Sambucci *et al.*, 2019). Continuous application of manmade fungicides has given rise to the growth of resistance of some of the most crucial pesticide molecules such as dimethyl inhibitors and sterner restrictions on usage of others (McGrath, 2001; Gao *et al.*, 2009; Ishii *et al.*, 2021). Growers continue to incur losses on production and quality of roses which in turn lowers their income due to powdery mildew infections. It is estimated that 30%-42% production losses may occur when infection levels are high (Sudheendra, 2014; Linde and Shishkof, 2014). Annual monetary losses amounting to approximately Kenya shillings 74.5 billion have been reported (AFA, 2019). Other monitary losses results from labour charges, acquisition and application of fungicides to treat the disease. Millions of stems are lost due to powdery mildew infections and loss to biodiversity (Debener and Byrne, 2014; Ribes *et al.*, 2018). There are also reports of losses due to rejection of flowers in the European Union markets as a result of non compliance to sanitary and phytosanitary standards (Pizano, 2019). Losses resulting from rejection due to excess residue on the products have been reported (Toumi *et al.*, 2016). Several strategies which includes biological control agents, such as *Tilletiopsis pallescens* (Ken and Leslie, 1997; Amin *et al.*, 2018; Tahir *et al.*, 2018; Verma *et al.*, 2020), anhydrous milk fat and soybean oil emulsion Chee *et al.*, (2011, 2018); Kamel and Afifi, 2020; Wurms *et al.*, 2021), synthetic fungicides (Scarito *et al.*, 2007), sodium bicarbonate (Salamone *et al.*, 2009; Shetty *et al.*, 2021) have been used in an effort to manage powdery mildew in greenhouse rose crop in Kenya and globally. However, the pathogen is still causing losses due to resistance to current molecules and inability of rose growers to fully explore other methods of disease management. Therefore, there is need for use of safer alternatives for disease control with no negative effects to both the environment, human beings and non target organisms.

1.3 Justification

Because of the losses incurred by growers and the negative effects of fungicides on workers, environment and other animals, it is important to explore other means of management of greenhouse powdery mildew on roses such as biological control agents like *Bacillus subtilis, Bacillus amyloliquafasciens* and sodium nitrophenolate. These microbial antagonists do not harm animals, human beings and they also help in the conservation of the environment and increases consumer acceptability (Tjosvoldo and Koike, 2001; Kumar and Chandel, 2018; Abhiram *et al.*, 2018). They are also easy to apply by the farmer as well as enhancing botanical progression and improve yield of the crop, have no residual effects, hydrolyses faster, have long term effects on the target organism and exhibit numerous modes of action (Whipps, 2001; Almoneafy *et al.*, 2012; Abhiram *et al.*, 2018; Campos *et al.*, 2019). Several reports have been fronted by various researchers indicating that *Bacillus* spp and sodium nitrophenolate have been used in managing various diseases of plants (Abbas *et al.*, 2019; Drobek *et al.*, 2019; Saxena *et al.*, 2019). An effective control will save rose growers from huge losses incurred in terms of money and labour and in return increase production level and income to workers. It is against this background that the evaluation of *Bacillus subtilis, Bacillus amyloliquefaciens* and sodium nitrophenolate is to be conducted to determine their effects on the control of the disease.

1.4 Objectives of the study

The broad objective was to improve the quality of roses through management of powdery mildew using *Bacillus Spp* and sodium nitrophenolate.

The specific objectives were

i. To evaluate potential of *Bacillus subtilis, Bacillus amyloliquefaciens* and sodium nitrophenolate in the management of powdery mildew on greenhouse roses.

ii. To evaluate the effect of *Bacillus subtilis, Bacillus amyloliquefaciens* and sodium nitrophenolate on quality of rose flowers.

1.5 Hypothesis

i. The antagonistic activities of *Bacillus subtilis, Bacillus amyloliquefaciens* and sodium nitrophenolate on powdery mildew of roses enhances management of the disease.

ii. The quality of rose flowers is improved through application of *Bacillus subtilis, Bacillus amyloliquefaciens* and sodium nitrophenolate due to enhanced production of growth hormones and improved tolerance to biotic and abiotic stress.

CHAPTER TWO: LITERATURE REVIEW

2.1 Taxonomy and morphology of rose plant

The term 'rose' is used to describe all members of the genus *Rosa*, subgenus *Rosa*. The *Rosa* genus belongs to the family Rosacea which is associated with pear, plum, apple, quince, blackberry, strawberry and cherry (Liu *et al.*, 2020). The genus is further divided into four subgenera of which genus *Rosa* includes nearly all the species (Nybom *et al.*, 2005; Khan *et al.*, 2020). Roses are one of the key, diverse and commonly grown ornamental crops with more than 150 species and over 20,000 varieties (Cai *et al.* 2005; Mercurio, 2007; Ahmad *et al.*, 2012; Elfina *et al.*, 2020; Khandaker *et al.*, 2020) which are in different colours such as white, yellow, pink, purple, orange and red. The subgenus Rosa is devided into sections, the actual number is still debatable (Suprun *et al.*, 2020). Generally, roses exhibit taxonomical challenges and nomenclatural complexities of which this genus have resulted in reports of about 100 to 300 *rosa* species, whereby uncountable number of varieties exist (Suprun *et al.*, 2020). These taxonomic challenges come as a result of high variations in the characteristics used in distinguishing the species and are aggravated by the simplicity by which the species naturally hybridize.

Rose stems can be erect, climbing or trailing (Garbez *et al.*, 2018). Perennial woody shrubs with erect stems that give new shoots continuously are the ones used as cut flowers for commercial purposes. A shoot is composed of 8-15 seconding units each made up of an auxiliary bud, a leaf, a node and internodes (Bloom and Tsujita, 2003). Roses have compound leaves arranged in odd-pinnate whereby the middle leaves in hybrid teas exhibit J-T leaflets, while asiastic may have up to 19 leaflets (Torre *et al.*, 2003; Irish, 2010; Shahrin *et al.*, 2015; Shamso *et al.*, 2019). Morphology of the roots differs based on whether the rose was rooted from seed, cutting (own rooted) or rootstock (grafted). Root morphology is also affected by nutrition and agronomic

activities such as pruning (Yim *et al.*, 2020). In most species of *Rosa*, flowers have five petals which may be solitary, panicled or corymbose (Leus *et al.*, 2018; Cheng *et al.*, 2021). Rose fruit is achene with pollen grains which are elliptical in shape with length: width ratio of 2:1 (Nybom and Werlemark, 2017).

2.2 Growing requirements and establishment

Seed propagation is generally used for breeding of new varieties and in the production of rootstock (Izadi *et al.*, 2012; Shivakumar *et al.*, 2018). Germination of rose seeds exhibit challenges and those progenies derived from the seed show variations in traits which also give different characteristics from parent plants (Zlesak, 2006; Khan *et al.*, 2020). Roses requires temperature of 28^{o}C daytime mean maximum and 15^{o}C night mean minimum, 10 hours day light, good air circulation, well aerated growing media, relative humidity of 75%, clean and abundant water (Xie *et al.*, 2019; Cola *et al.*, 2020). Root substrate pH should be around 6.0 (Valentine and Francis, 2009; Barbosa *et al.*, 2019) as this affects the solubility of nutrients (Smith *et al.*, 2004; Papafotiou *et al.*, 2007), uptake of nutrients and have influence on root formation (Harbage *et al.*, 1998; Noori and Muhammad, 2020) with implications on overall status of the plant. Rose crop is sensitive to salt levels therefore, the electron conductivity (E.C.) should be maintained between 1- 2. Salt level for instance of any value greater than or equal to 3.0 may result in reduced yield and quality of the crop (Bar-Yousaf *et al.*, 2009; Barbosa *et al.*, 2019; Khalaj and Noroozisharaf, 2020).

Rose crop may be established from propagated plantings through grafting or *in vitro* plantlets (Krasimira, 2015; Davoudi Pahnekolayi *et al.*, 2019). Rose crop may be grown in the soil systems whereby the soil should be rich in organic matter (about 30% on the top 40cm) (Blom and Tsujita 2003). Soilless media is gaining popularity among the rose growers depending on the resource

base of the company. Soilless system (hydroponics) is being used because the nutrients and the media can be recycled and it is environmentally friendly. Common materials used as growing media are pumice, vermiculite, perlite, peat, saw-dust and coconut fiber (Othman *et al.*, 2019). For soil systems, beds are raised and planting is done at 18cm to 22cm between plants and 30cm between the rows on raised beds. In the case of soilless media, planting is done at 17.5cm to 20cm between plants and 30cm between rows. Commercial cut-flowers production is done in the greenhouses covered with polythenes or glasshouse to protect the crop from rains which may gain entry into the flower buds thus giving room for fungal infections.

2.3 Constraints to production of roses

Commercial rose production is faced with a lot of constraints which include both biotic and abiotic factors. Abiotic factors which limits yield of roses are temperatures, relative humidity, light, salinity and mineral nutrients (Lorenzo *et al.*, 2000; Bayat *et al.*, 2018; Barbosa *et al.*, 2019). In greenhouse and hydroponic systems, roses are supplied with adequate nutrients which ensures proper growth and development of the plant (Cabrera, 2003; Bilal *et al.*, 2020). *Tetranichus spp, Planococcus citri, Francliniella occidentalis*, moths (caterpillars), aphids and whiteflies are some of the common insect pests which infest roses (Yao *et al.*, 2017; Rodriguez *et al.*, 2019; Hoogendoorn *et al.*, 2018; Singh and Kaur, 2020).

Roses just like any other crop are attacked by many pests and diseases which affect yield and quality. Some of the key pests which infest greenhouse rose crop includes Western flower thrips (*Frankliniella occidentalis*) which is the most common specie of thrips attacking greenhouse roses. This pest is polyphagous and has several host plants such as apricot, carnations, gladiolus, strawberry, watermelon, chrysanthemum, pepper (Gosh and Hasan, 2021). Thrips feed on the

flower petals causing injury to the tissues. They also leave speckles which act as entry point for pathogens. These damages renders stem unmarketable. Thrips undergo six development stages namely egg, two larval stages, pre-pupa, pupa and adult (Reitz, 2011; Hegde *et al.*, 2020; Tol *et al.*, 2021).

Another pest of importance in rose production is mealybugs (*Planococcus citri*). Mealybugs inflict injury to the plant by sucking sap resulting to dwarfed plants with short stems and small flower buds which does not meet export quality. In high levels of infestation, death of plants may result. Mealybugs also secret honey dew on the leaves where dark particles develops thus lowering surface area for photosynthesis in the leaves (Smith *et al.*, 1997; Mansour *et al.*, 2018; Cocco *et al.*, 2021). Physical presence of mealy bugs renders stem unsellable. Rose aphids is another key pest which damages the crop by sucking sap from the plant resulting to stunted growth and reduced yield. They also produce honey dew where dark particles develops which lowers surface area for biochemical reactions in botanicals (Legarrea *et al.*, 2012; Tun *et al.*, 2020). Two spotted spider mites is the most destructive pest of greenhouse rose crop. They feed on the under-side of the leaf by sucking sap from the plant. In high infestations, the plants become stunted, yellowish and of poor quality. They also form webs which restrict the growth of shoots and expansion of leaves (Ghosh and Hasan, 2021). Whiteflies are also major pests of greenhouse rose crop which inflict injury to the crop by sucking sap leaving the crop stunted with poor stems. Scales (young ones) presence on the underside of the leaf will definitely make such stems unsellable. White flies also secrets excess sugar in the form of honey dew where dark particles develops which lowers surface area for biochemical reactions in botanicals resulting to poor trapping of light which is necessary for normal growth of the plant. This in turn affects the overall growth of plants hence reduced yield and quality stems (Perring *et al.*, 2018).

Among the biotic factors, phytopathogens like *Peronospora sparsa* (Downy mildew), *Botrytis cinerea* (grey mould), *Podosphaera pannosa* (powdery mildew), *Diplocarpon rosae* (black spots of roses), *Phragmidium mucronatum* (rust of roses), *Agrobacterium tumefaciens* (crown gall), *Prunus necrotic virus* (ringspot virus of roses) are some of the serious pathogens which affect greenhouse production of roses (Anita and Shamim, 2014; Liu *et al.*, 2018; Salgado-Salazar *et al.*, 2018; Murero, 2020; Salcedo *et al.*, 2021) Another bacterial diseases which affects roses is *Ralstonia solanacearum* but has not been reported in Kenya (KEPHIS, 2015).

2.4 Powdery mildew of roses

2.4.1 Host range and distribution

Powdery mildew infects a variety of crops such as legumes where the causal agent is *Erisyphe poligony*, which has world wide distribution where legumes are grown. In okra, the disease is caused by *E. cichoracearum*, in the family *Curcubitaceae Podosphaera xanthii* (Castagne) U. Braun & Shishkoff is the causal organism and has worldwide distribution where curcubits are grown both in greenhouse and field conditions (Labo *et al.*, 2019; Mostafa *et al.*, 2021; Zhang *et al.*, 2021). In Brasicacea it is caused by *E. cruciferarum* and has worldwide distribution where the plant grown (Kumar *et al.*, 2015), *Podosphaera aphinas* is the causal agent of powdery mildew in strawberry, the disease has world wide distribution and affect plants grown both in the greenhouse and field (Sargent *et al.*, 2019; Carisse and Fall, 2021). In celery, powdery mildew is caused by *Erysiphe heraclei* DC and is found everywhere celery is grown (Ahmed *et al.*, 2021). Grapevine powdery mildew is caused by *Erysiphe necator* affecting cultivated and wild species is distributed world wide (Kunova *et al.*, 2021; Sanghavi *et al.*, 2021). In tomatoes and pepper grown both in

greenhouse and in the fields are affected by powdery mildew caused by *Leveillula taurica* (Jacob *et al.*, 2007). Cereal crops such as wheat, corns are affected by powdery mildew caused by *Blumeria graminis* (Pietrusinsk and Tratwal, 2020; Khan *et al.*, 2021; Luo *et al.*, 2021; Yuan *et al.*, 2021).

2.4.2 Causal agent of rose powdery mildew

Powdery mildew of roses is caused by *Podosphaera pannosa* which belongs to the phylum Ascomycota in the class Leotiomycetes, order Erysiphales, genus *Podosphaera* and the specie *P. pannosa* (CABI, 2021).

Research by Kumar and Chandel, (2017) found that shapes and sizes of structures of powdery mildew such as mycelium, conidia, and conidiophores were morphologically different among isolates collected from six different rose growing regions of India. Mycelia were found to be on the surface, hyaline, branched and septate while conidia were found to be cylindrical to ovoid, hyaline and aseptate. Upright stalks, hyaline, aseptate and unbranched conidiophores were found in some isolates. Similar reports were published by Gastelum *et al.*, (2014) and Faheem *et al.*, (2016). Genetic similarities were reported in research findings by Kumar and Chendal, (2017) through molercular characterization. A consistent report were made by Devi and Prakasan, (2015) working with different isolates of *Leveillula taurica* causing powdery mildew in chilli using RAPD markers. The similarity index was between 11 to 29 per cent. Leao *et al.*, (2019) reported that all samples collected, and sequencing indicated 100% similarity with those deposited in GenBank.

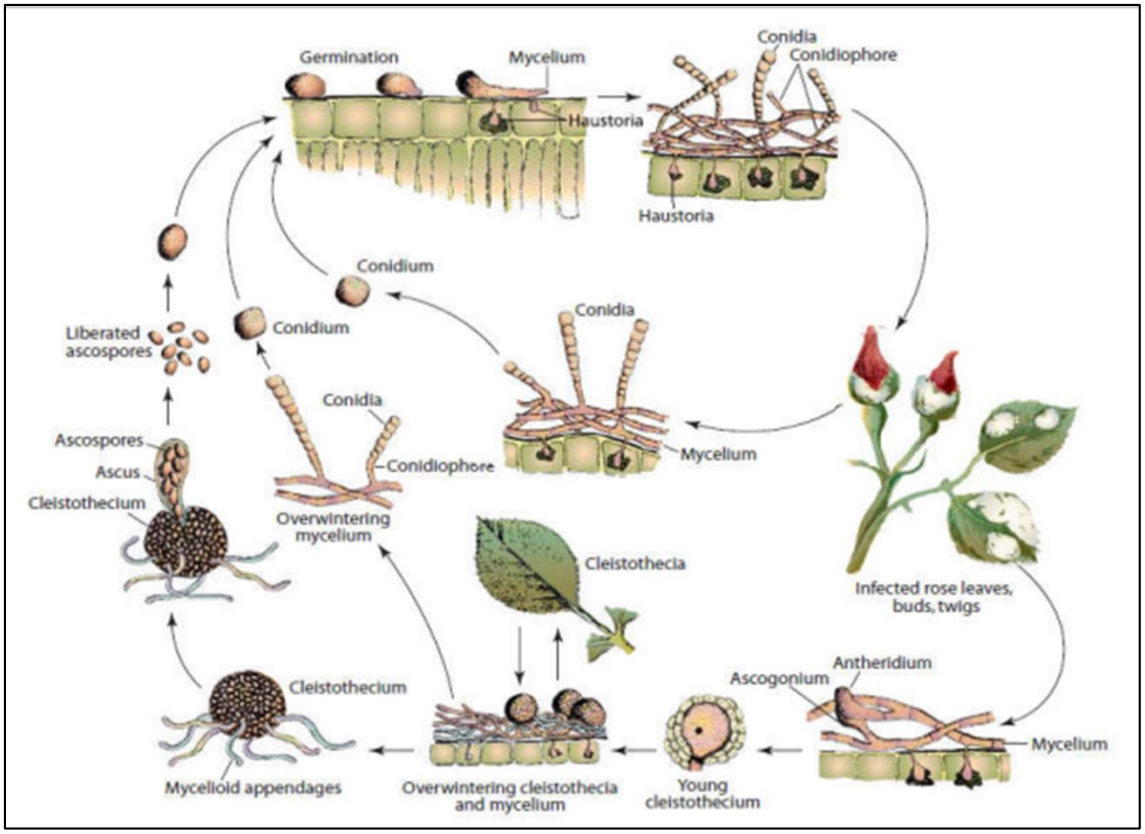

Figure 2. 1: Life cycle of *Sphaerotheca pannosa* causing powdery mildews on roses (Agrios, 2005)

2.4.3 Symptoms of powdery mildew

Symptoms of powdery mildew of roses is recognized in different parts of the plant as whitish grey powder. The initial signs begin as blister-like raised areas then followed by a dense thick mass of powdery mycelium, conidiophores and spores (Agrios, 2005). On older leaves, as the fungus grows, they appear as large white patches that cause little distortion which may eventually turn necrotic. In severe outbreaks, the whole crop can be rendered unmarketable as a result of damages such as yellowing of leaves and death of tissues, alteration of flowers, curling of foliage, defoliation, twisted new young shoots and stunted growth (Janice *et al.*, 2011; Sangani *et al.*, 2018).

When flower buds are attacked, they may fail to open or open improperly. All species and cultivars of roses are susceptible to powdery mildew infections. The growing tips of the crop may be malformed and killed, though the death of the entire plant is not common. Tissues of rose crop usually become resistant as the crop ages (Agrios, 2005). In some resistant varieties, they may show a hypersensitive reaction whereby the invaded dead cells appear as black-to-rusty on the leaf surface with minimal evidence of mildew growth. Other symptoms include scabies like lesions, 'witches brooms', twisted and distorted young shoots and premature leaf colouration (Agrios, 2005; Laoe *et al*., 2019; Yeluguri *et al*., 2021). At times symptoms often escape early detection where there are no systems in place for frequent monitoring of symptoms which can develop on foliage in lower or mid canopies. This kind of scenario has a bearing on reports of rapid spike of powdery mildew when the level of infections goes up to 70% from 10% in seven days (Yeluguri *et al*., 2021).

The disease causes reduced production of flowers and weakening of the plant by infecting the young leaves, stems and flower buds (Agrios, 2005). *Podosphaera rosae* is a biotrophic obligate parasite which attacks and survives in the tissues of living cells of specific host plant. Wrinkles on tender foliage are the initial evidence of the disease infections on the plant. There after the disease appear as whitish grey powder covering various parts of the plant as foliage, stems and flower buds (Gastelum *et al*., 2014; Yeluguri *et al*., 2021).

Yield losses resulting from powdery mildew infections depends on the favourable environmental conditions and ranges between 20% to 40% (Gastelum *et al*., 2014). According to Linde and Shishkoff (2003) the most serious and widespread fungal disease is powdery mildew (*Sphaerotheca pannosa* var. *rosae)*. It attacks plants grown both out doors and greenhouses for cut flower production (Leus *et al*., 2006). It causes severe yield and quality reductions due to the

formation of white powdery pustules that appear on above ground parts of the plant (Yan *et al.*, 2006; Laoe *et al.*, 2019). Flowers showing symptoms of diseases such as grey mould, powdery mildew, downy mildew, rust and black spots as well as those infested by various pests like spider mites, thrips, caterpillars, mealy bugs, aphids and scale insects are not marketable (Reid, 2009).

2.4.4 Epidemiology of powdery mildew of roses

Powdery mildew of roses affects the aerial parts of the plant, giving white to grey white patches of powdery fungus. The disease interferes with normal growth of the plant thus reducing quality and may destroy the plant (Shetty *et al.*, 2012). Powdery mildew is an obligate parasite and can only survive on a living host. It lives on the outer surface of host plants with whitish hyphae mycelium that form appresoria to penetrate epidermal cell walls and produce haustoria to absorb nutrients from leaf tissue (Linde and Shishkoff, 2003). Wind is main agent of dissemination of spores to other plants. Sporulation takes place on plant parts such as shoots, leaves and petals where a large number of microscopic spores (conidia) are formed. In cold periods, the fungus survives as cleistothecia which absorbs water and bursts open during warm humid weather to release small sac or ascus with 8 spores (ascospores) (Naik and Kulkarni, 2018). These structures are carried by air current to healthy plants and can cause infections. When conidia and ascospores land on the surface of host plant, they form appressorium, which at the bottom forms hypha which penetrates the epidermal cells forming a feeding structure known as haustoria. The fungus grows and forms a dense hyphae both on the surface and in the plant cells. The hyphae later form conidiophores with conidium at the end of the structure. Continous production of conidia make them easily carried away by wind to new site of infection. Vegetative hyphae produce conidia of

spores at their tips, giving a powdery appearance to the infected leaves. Sexual spores are occasionally produced in colonies (Linde and Shishkoff, 2003; Agrios, 2005).

Environmental conditions influence the germination of spores. The optimal conditions are a temperature of 21°C and an average relative humidity of 97% (Xu, 1999; Kumar and Chandel, 2018). Under wet conditions, conidia can withstand a long period of low temperature. Both spores and mycelia are sensitive to extreme heat and direct sunlight. Some reports indicate that water might damage the viability of conidia and thus reduces infection by the pathogen (Wheeler 1973; Linde and Shishkoff, 2003; Rex and Deepika, 2020). However, leaf wetness in the first six hours after infection does not inhibit the germination of conidia (Linde and Shishkoff, 2003). Germination of mycelium takes place on plant tissue. Due to elongation process, conidiophores are formed which may burst to release conidia to the air which may cause reinfection of growing shoots. Some conidia may overwinter to form clestothecia on the stems or debris and survive between crops as hyphae or fungal strands in the living cells of the host or weed (Agrios, 2005; Kumar and Chandel, 2018).

2.5 Management of powdery mildew

There are many methods used in managing powdery mildew in roses worldwide. These methods may include conventional means such monitoring and sanitation, cultural methods, host resistance, biological means, plant extracts, natural products and chemical control.

Monitoring for disease presence or the weather pettern enhances better control of the greenhouse diseases (Traversari *et al*., 2021). Sanitation involves growing in a clean environment both inside and outside the greenhouse, removal of diseased plant parts, debris and weeds from the greenhouse

(Salgado-Salazar *et al.*, 2018). Diseased plant parts should be removed and placed in plastic bags to avoid dissemination of spores in the greenhouse.

Cultural methods such as aerations and ventillations are important aspects in the disease management in greenhouse environment. Increasing the carbon dioxide levels in the greenhouse also impede the presence and infection of the podery mildew (Bika *et al.*, 2021). Improved lighting due to polyfilm coverings in the greenhouse grately reduces the spread of the pathogen (Kumar and Chandel, 2018; Kruidhof *et al.*, 2020). Powdery mildew severity and formation of conidia are affected by supplemental ultra-violet-B (UV-B) radiation and lighting when the plants are exposed for a given time period during the growth of the plant (Suthaparan *et al.*, 2010; Michie *et al.*, 2013; Matysiak, 2021). Provision of adequate nutrition through organic and inorganic materials have resulted to management of the disease (Ribeiro *et al.*, 2015; Ramos *et al.*, 2020).

Host resistance has been used in managing powdery mildew of roses. This involves identification of genes and alleles which transfer resistance (Kusch and Panstruga 2017; Smulders *et al.*, 2019, 2020; Menz *et al.*, 2020; Fang *et al.*, 2021; Vieira *et al.*, 2021; Yuan *et al.*, 2021). Growing cultivars with resistance traits is desirable and effective in the management of greenhouse powdery mildew. Resistant cultivars are limited in their availability for commercial production. Host resistance can be achieved through breeding for resistance and should be both quantitative and qualitative. Report by Koh *et al* (2005) suggests that in susceptible cultivars, conidia will develop, penetrate the host epidermal cuticle and cell wall and establish haustoria within the epidermal cells. In non-susceptible cultivars, resistance is based on gene for gene resistance in which specific resistance gene in a host species confer resistance to specific genotypes or races of the pathogen. According to Dewitte *et al.*, (2006), Chandran *et al.*, (2020) roses with resistant genotypes are able

to withstand the infection of powdery mildew through morphological barriers, papillae formation and through formation of abnormal haustoria.

According to Singh *et al*., (2017) biological means heve been deployed in managing powdery mildew in greenhouse crops. This involves application of biocontrol agents to the diseased crop and lowers the population of pathogens in the greenhouse. Application of *Trichoderma harzianum* significantly reduced the disease severity and incidences of powdery mildew greenhouse (Marzani *et al*., (2021). Application of *Trichoderma* isolates reduced powdery mildew by 53.4% (Sawant *et al*., 2017). Similar results were posted by Manjunatha *et al*., (2020) who reported that mulberry powdery mildew were significantly reduced by application of potential *Trichoderma* isolate. Several species of *Bacillus* have been used in the management of powdery mildew in different crops (Li *et al*., 2015; Gao *et al*., 2017; Punja *et al*., 2019).

Anumber of plant extracts have been deployed in the management of powdery mildew in many crops. Garlic and ginger extracts showed high inhibitory effects on the germination of conidia of powdery mildew of roses *in vitro*. These extracts when applied to the greenhouse crops, they showed high reduction on disease severity index (Marzani *et al*., 2021). According to Manjunatha *et al*., (2020) ginger extracts at different concentrations reduced incidence and severity of powdery mildew in mulberry plants. Powdery mildew of roses and cucumber were controlled by compounds from garlic (Kumar and Chandel, 2018; Abd Elwahed *et al*., 2019). Germination of conidia of powdery mildew of okra caused by *Erysiphe cichoracearum* were inhibited by extracts of garlic (Jadav and Kadvani, 2019). Application of extracts from *Ocotea quixos* and *Piper carpunya* inhibited germination of conidia *in vitro* and controlled the development and growth spores of *Sphaerotheca pannosa* powdery mildew of roses (Cardenas *et al*., 2016).

Natural products such as sodium and potassium bicarbonates have been tested and proved to be effective in eliminating spores of the disease on roses (Salamone *et al.*, 2007). According to reports by Salamone *et al.*, (2007) essential oils from oregano and cloves and sodium bicarbonates controls rose powdery mildew. Potassium silicate when sprayed at interval of 12 days 5 times reduced severity of powdery mildew of tomato (Yanar *et al.*, 2011; Dallagnol *et al.*, 2020). Anhydrous milk fat and soybean oil emulsion control powdery mildew in greenhouse roses and tomatoes (Chee *et al.*, 2011). The use of anti-transpirants are effective in controlling powdery mildew in roses by forming a film of thin layer on the leaf surface which prevent the movement of moisture into and outside the leaf. This will also protect the leaf from fungus penetration (Hagiladi and Ziv, 1986). Some growers have used these anti-transpirants in combination with other fungicides where reports indicate that they have worked well. The effectiveness of the same relies on the fact that the physiological function of the plant is not interfered with and the plants needs to be watered thoroughly before application (Hagiladi and Ziv, 1986). Silicon has been tested and proved to be inducing resistance to plants against fungal pathogens in various species of crops, for example, *Blumeria graminis* f.sp. *tritici* in wheat and *Sphaerotheca fuligenea* in cucumber. Several reports confirm that roses treated with silicon had reduced levels of infection from powdery mildew (Menzies, 1991; Remus-Borel et al., 2005; Shetty *et al.*, 2011, 2021).

Grapefruit seed extract reduced the levels of rose powdery mildew in the field at 2000ppm (Toppe *et al.*, 2007). According to Hamza *et al.* (2013) and Stan (2014) many natural substances such as diatomite and bentocide have been used as pesticides. Diatomite is one of the naturally occurring sedimentary rocks basically comprising of fossilized remains of fresh water diatoms. Its composition is approximated to be as follows, 3% magnesium, 2% iron, 19% calcium, 33% silicon, and 5% sodium plus other rare minerals. Hamza *et al.* (2013) reported that bentocide which is also

a natural substance has been employed by many farmers against plant pathogens instead of using synthetic fungicides.

Biopesticides such as "compost tea" equally provides good control for powdery mildew of roses (Samin *et al.*, 2014; Seddigh and Kiani, 2018; Istifadah *et al.*, 2020). Fungicides plays an integral part in the management of powdery mildew both in the greenhouse and garden rose production. It is regarded as the weapon of the last resort where integrated pest management system is applied. The aim is to protect the crop from infection and to eradicate the already existing spores from the crop. The essence is to begin with less toxic compounds in the management programme. Vapourized sulphur and nano-sulphur (Gogoi *et al.*, 2013) indicates that sulphur can be used in controlling powdery mildew in many crops. Synthetic chemical products are used in control of powdery mildew in commercial rose production (Scarito *et al.*, 2007).

2.5.1 Management of plant diseases with antagonistic organisms

Biological control can be defined as a method in which living organism is used to control pest or disease by introducing or managing the natural enemy populations or reducing the reproductive rate of such organisms in a crop (Sterling, 2014). This method employs three strategies. Inundation is a strategy whereby commercially produced individual predators or antagonists to a pest are released in large numbers with a view to reducing population of the pest. Classical or inoculation is strategies in which exotic individuals are released into a new agro ecosystem with the hope of regenerating and establishing in populations which will have long-term effect on the pest population. Conservation biological control is a strategy in which the habitat for naturally occurring antagonists is enhanced to enable them work effectively and efficiently (Abrol and Shankar, 2012).

There is growing evidence that a number of biological control agents can be antagonistic to powdery mildew fungus and other pathogens causing plant diseases (Twamley *et al.*, 2019; Srivastava *et al.*, 2021). Such antagonists includes *Acremonium alternatum* Linc: Fr. (Malathrakis, 1985), *Ampelomyces quisqualis* Ces. (Sundheim, 1982; Menge and Makobe, 2016; Altin and Buku, 2018; Banupriya *et al.*, 2019), *Sporothrix flocculosa* Traquair, Shaw and Jarvis (Belanger *et al.*, 1994; Hajlaoui and Belanger, 1993), and *Trichoderma harzianum* (Kamal *et al.*, 2008; Ghorbanpour *et al.*, 2018), have been reported to attack mycelia and reproductive structures of the mildew fungi. *Tilletiopsis pallescence* (Hijwegen 1986; Kclecan *et al.*, 1990) is yeast like fungus which occur naturally on the leaves which affect powdery mildew in various species of crops. The ballistospore forming yeast acts on sporulation and hyphal growth of the mildew. Foliar application of yeast like isolates controlled *Xanthomonas axonopodis* pv. *vesicatoria* and viral diseases of pepper in the greenhouse (Lee *et al.*, 2016).

2.5.2 Management of plant diseases using *Bacillus* spp

Bacillus are gram-positive rod shaped bacteria capable of forming steady quiescent structures known as endospores in an environment of low nutrients. Such spores can stay for long period in unfavourable conditions (Niazi, 2014; Andric *et al.*, 2020). Today a lot of efforts are made in the field of research to come up with environmentally amiable substitute for managing phytopathogens and increasing plant performance which are an advocacy for integrated crop management (ICM) (Souza *et al.*, 2015).

Species of *Bacillus* are one of the highly researched as biocontrol agents which helps in suppressing plant disease causing organisms through competition or antagonistic actions (Khan *et al.*, 2018; Saxena *et al.*, 2019; Miljakovic *et al.*, 2020). *Bacillus* spp have been used due to their

ability to resist desiccation and good survival at increased temperatures because of their nature of forming endospores and promotion of plant growth (Zhang *et al.*, 2017; Abbas *et al.*, 2019; Afzal *et al.*, 2019; Elsisi, 2019).

Bacillus spp produces a number of antifungal agents, which has capacity to destroy cell walls of plant pathogenic fungi. These agents includes mycobacilin, subtilin, bacilysin, subtilosin, TasA and numerous enzymes produced by *Bacillus subtilis* making it a potential for biocontrol (Thangavelu and Mustaffa, 2012). A number of species of *Bacillus* have been reported to show positive impacts in various crops by reducing the population of disease causing organisms. There is a general knowledge that *B. subtilis*, *B. amyloliquefaciens* and *B. cereus* enhance vegetal development as well as protecting them against pathogens (Fedele *et al.*, 2020; Pan *et al.*, 2021). Antagonistic ability of *B. amyloliquefaciens* have been used to lower the population of *Ralstonia solanacearum* which had negative impact on the bacterial wilt and better growth of tomato plants. A strain of *Bacillus amyloliquefaciens* had antifungal activity against twelve different fungi causing plant diseases through dual culture. These fungi included *Alternaria panax*, *Botrytis cinerea*, *Colletotrichum acutatum*, *C. orbiculare*, *Corynespora cassicola*, *Fusarium oxysporum*, *Phytophthora capsici*, *Penicillium digitatum*, *Rhizoctonia solani*, *Stemphylium lycopersia*, *Pyricularia grisea* and *Sclerotinia sclerotiorum* (Seung *et al.*, 2013; Dadrasnia *et al.*, 2020).

Xu *et al*. (2017) selected an isolate from *Bacillus* spp as a potential biocontrol agent which is crucial in managing diseases of banana. They contended that NJN-6 a strain of *Bacillus amyloliquefaciens* was an important plant growth promoting rhizobacteria (PGPR) that has the capacity to produce secondary metabolites antagonistic to a number of disease causing organisms which resides in the soil. Hence, application of strain NJN-6 notably reduced incidence of *Fusarium* wilt and enhanced the growth of banana plants. The strain releases some viable

compounds that showed initial protection to disease causing organisms in the plants (Yuan *et al*., 2013; Fu *et al*., 2017; Roslan *et al*., 2020). HK34, which is a strain of *Bacillus amyloliquefaciens*, induced resistance to *Panax ginseng* against *Phytophthora cactorum* by native *Bacillus amyloliquefaciens* (Byung *et al*., 2015; Punja *et al*., 2019).

2.6 Management of plant diseases using nitrophenolates

.Sodium nitrophenolate is a phenolic compounds with the following as its active ingredients sodium para-nitrophenol, sodium nitro-guaiacol and sodium ortho-nitrophenol. These compounds induces plant activity without causing chemical poisoning and deformations to the plant and improves plasma streaming of the cells in the plant (Drobek *et al*., 2019). Nitrophenolates increases yield as a result of increased level of inherent auxins and heat tolerance from the effects of external spraying (Datta *et al*., 1986; Quintero-Calderon *et al*., 2021). Nitrophenolate mixtures have been used in various crops to increase crop yield, quality, bushing and root system (Arora *et al*., 1981; Purba *et al*., 2019). Wojdyla (2004) contend that nitrophenolates when applied at concentration of 0.1% against powdery mildew of roses, chrysanthemum rust, black spots of roses and rust of willow plants, there was significant level of reduction in infections by pathogens. These products work in the plant or plant rhizosphere through nutrient use efficiency, tolerance to abiotic stress and quality traits and availability of confined nutrients in the soil or rhizosphere. (EU, 2019).

CHAPTER THREE:
MANAGEMENT OF POWDERY MILDEW OF ROSES USING *BACILLUS SUBTILIS, BACILLUS AMYLOLIQUEFACIENS* AND SODIUM NITROPHENOLATE

3.1Abstract

Powdery mildew of roses is a serious disease of rose crop grown both in field and greenhouses. The disease results in heavy losses in quality and monetary value at farm level as well in the market. The disease is managed mainly by application of synthetic fungicides, which have negative impacts on environment, human beings and non-target organisms. In this study, the effect of *Bacillus* spp and sodium nitrophenolate on incidence and severity of powdery mildew of roses in greenhouse condition was investigated. *Bacillus subtilis* and *B. amyloliquefaciens* were applied at 2ml/L and 1.5g/L respectively while sodium nitrophenolate and dodemorph acetate were applied at 1ml/L and 2.5ml/L. In the second experiment, foliar application of *Bacillus amyloliquefaciens* was done at different concentrations and at various intervals of application as follows 1.5g/L, 4 days, 3.0g/L, 4 days, 4.5g/L 4 days, 1.5g/L, 7 days, 3.0g/L, 7 days, 4.5g/L, 7 days, 1.5g/L, 10 days, 3.0g/L, 10 days, 4.5g/L, 10 days and control. Data on incidence and severity of powdery mildew was collected on weekly basis by counting the number of leaves with powdery mildew symptoms for incidence and percentage of leaf area covered by powdery mildew for severity. Incidence and severity were reduced by application of *Bacillus subtilis*, *B. amyloliquefaciens*, sodium nitrophenolate and dodemorph acetate. Dodemorph acetate had the highest reduction in incidence (11.3%) followed by *B. amyloliquefaciens* (11.8%) while sodium nitrophenolate and *B. subtilis* had 15.3% and 17.3% respectively. Disease severity was reduced from 15.8% to 2.1% with dodemorph acetate having the highest reduction followed by *B. amyloliquefaciens*, sodium nitrophenolate and *B. subtilis*. In the second experiment, all rates at various intervals of *Bacillus amyloliquefaciens* reduced incidence and severity of powdery mildew with the rate rate of 1.5g/L,

4 days showing the highest reduction on incidences. Rates of 1.5g/L and 3.0g/L applied at intervals of four and seven days had the highest reduction on severity. The study revealed that foliar application of *Bacillus* spp and sodium nitrophenolate controls powdery mildew of roses, therefore, growers should embrace their usage for they will benefit due to reduced losses from disease infections. Establishment of the optimal rate of application of *Bacillus amyloliquefaciens* will save time for growers on conducting trials in the field.

Key words: Powdery mildew, *Bacillus Spp*, sodium nitrophenolate, management, roses

3.2 Introduction

Powdery mildew fungi (Erysiphales) are important group of phytopathogens affecting various plants consisting of over 500 species which infects over 1500 plant genera (Braun, 1987; Sulima and Zhukov, 2022). In roses, the disease is caused by obligate biotrophic fungi *Podosphaera pannosa* var.*rosae* and it has worldwide distribution where roses are grown. It is a problem for both greenhouse and field produced roses. The management of the disease relies haevily on repeated foliar application of synthetic fungicides which have worked well in controlling the pathogen (Ribeiro *et al.*, 2015; Kumar and Chandel, 2018). The application of fungicides has been continuous and disregarded safety to the environment, farm workers and non-targeted organisms. Seconded applications has also lead to the development of resistance to various molecules which were effective in the management of the disease (Perlin *et al.*, 2017; Belsky and Joshi, 2020; Lazaro *et al.*, 2021).

The negative effects of fungicides on human health, environment and other living organisms have called for exploration of safer alternatives (El-Baky and Amara, 2021). These alternatives includes microbial fungicides (Moreno-Gavira *et al.*, 2021), botanical fungicides (Yoom *et al.*, 2013), nanotechnologies (Atiq *et al.*, 2020). Microbial based and botanica fungicides have advantages of ease of handling, safer to the user, leave no residues to the environment, enhance plants growth and productivity as well induce resistance against phytopathogens (Campos *et al.*, 2019; Qiuntero-Calderon *et al.*, 2021).

This study was to evaluate the potential of *Bacillus subtilis*, *Bacillus amyloliquefaciens* and sodium nitrophenolate in the management of powdery mildew of roses in greenhouse conditions.

3.3 Materials and methods

3.3.1 Description of the experimental site and the crop

The first experiment was conducted at Magana Flowers Limited which is one of the flower farms in Kenya, located in Kiambu county. The farm is elevated at 1942 M above sea level (ASL), it lies on the latitude and the longitudes of -1.2543249 E and 36.6770272 S respectively. The second experiment was done at Rift Valley Roses which located in Nakuru County, Kenya. Elevation of the farm is at 2250 M ASL and it lies on latitude of 362816E and longitude of 003627 S (Google Map, 2020). The two experiments were conducted in greenhouses with already established bushes of roses. The size of the greenhouse was 0.56 ha (first experiment) with a single variety of roses known as A one. Flowers were planted in troughs and hydroponic system of feeding was practiced. The variety where the experiment was performed is called A one which is grown in Kenya. In the second experiment, roses are planted on soil in a greenhouse of 3.0 Ha. The same variety as in experiment one was used (A one). In the first experiment, age of the crop was three and half years old while in the second experiment, the crop was three years old. The greenhouse environment was maintained by application water to regulate temperature and relative humidity. The recommended agronomic practices such as fertigation involved dilution of stock solution which contained KNO_3, $CaNO_3$, $MgSO_4$, $MgNO_3$, KSO_4, M.K.P, NH_4SO_4, $ZnSO_4$, HNO_3, H_3PO_4. The nutrients were injected in the growing media through drip irrigation. Contest®, Evisect®, Arima®, Karate®, Match® were pesticides used for controlling insect pests during the period of experiment. Teldor®, Topguard®, Funginex® and Switch® were used for controlling grey mould while Ridomil Gold®, Milraz® and Afrizeb® were fungicides used for controlling downy mildew. Other agronomic practices such as weeding, pruning and dessuckering were applied to maintain the crop.

The first experiment was excuted between the months of March and July, 2019 while the second experiment was carried out in the months of September and October, 2020.

3.3.2 Experimental design and layout

The experiment involved application of five different treatments which included Real subtilis® (*Bacillus subtillis*) from Real IPM, Hatake (*Bacillus amyloliquefaciens*), Atonik® (sodium nitrophenolate) from Arista Life Science and Meltatox® (dodemorph acetate) from O- BASF kenya limited as the commercially used fungicide for roses and used as positive control for the experiment and water in the control plots. Foliar application of these products which contains *Bacillus subtilis, B. amyloliquafaciens*, sodium nitrophenolate and dodemorph acetate at the rate of 2ml/L, 3g/L, 1ml/L and 2.5ml/L respectively. The rates given are recommendations from the manufacturer. In the second experiment, foliar application of *Bacillus amyloliquefaciens* was done at different concentrations and at various intervals as follows 1.5g/L, 4 days, 3.0g/L, 4 days, 4.5g/L 4 days, 1.5g/L, 7 days, 3.0g/L, 7 days, 4.5g/L, 7 days, 1.5g/L, 10 days, 3.0g/L, 10 days, 4.5g/L, 10 days and negative control.

Rose bushes in the greenhouse were used for this experiment whereby four beds were randomly selected within the greenhouse and divided into plots. Each plot had 44 plants giving a total of 176 plants per treatment. The crop was planted at 20 cm X 30 cm with plant density of eight plants per square meter in both farms. Two meters space was left between plots and a polythene separator used during active spraying to prevent products from drifting to other plots. The treatments were laid on a randomized complete block design with four replications per treatment.

3.3.3 Application of treatments

Different stages of growth (shooting, bud formation, colour break and mature stems) with disease symptoms were treated with the test products. Four different crop cycles were monitored for this study. Monitoring was done for five months and 6 weeks for the first and second experiments respectively. Each plant was foliar sprayed for complete and thorough coverage of all appropriate plant parts using a motorized sprayer 16 L (Sea grow®) type in the application of test products. After application of each treatment, the knapsack sprayer was thoroughly rinsed with clean water to avoid mixing of different rates which may give wrong results. Clean water with a pH range of 6-8 was used as this ensured neutrality of water in the sprayer. Amino gold was used as a surfactant at the rate of 0.7ml/L of water for all treatments.

3.3.4 Assessment of incidence and severity of powdery mildew

Weekly assessment began after seven days from the first application of the treatments and continued for twenty-eight weeks in the first experiment. In the second experiment, weekly assessment was done over a period of six weeks. Assessment was done for six weeks since the effect of the treatments was observed within the first week of applications. Disease incidence was determined by counting the number of infected plants over the total number of plants assessed per treatment in each replicate. Plants showing whitish powdery on the leaves or red blotches under the leaves were tagged and followed for ease of monitoring. The tagged plants were maintained throughout the experiment period. Plant disease incidence was calculated using the formula below.

$$\text{Plant Disease Incidence} = \frac{\text{Total Number of Infected plants}}{\text{Total Number of plants Assessed}} \times 100$$

Disease severity was determined by the percent of photosynthetic area infected by powdery mildew using the scale of 1-5 by estimating as a percentage of the total leaf area of a single plant

which have powdery mildew symptoms where 1=0-10% (very low infection), 2=11-25% (low infection), 3=26-50% (moderate infection), 4=51-75% (high infection), 5≥75% (severe infection) (Awet *et al.*, 2016; Bem *et al.*, 2013). Thirty plants in each plot were randomly sampled and marked for study in this parameter. Stems of the same age were marked and followed from the time of marking until maturity. All compound leaves in every stem were assessed for disease progression. Plastic markers were used in the sampled plants for monitoring the progress. Plants with all stages of growth were critical for sampling to ensure that there were stems to monitor from the beginning to the end of the experiment. The following formula was used in calculating the disease severity:

$$\text{Plant Disease Severity (PDS)} = \frac{\text{Number of Individual Rating}}{\text{Number of Plants Assessed}} \text{ x } \frac{100}{\text{Maximum scale}}$$

Area under disease progress curve AUDPC measure the rate at which the pathogen spreads in the tissues of the plant. The AUDPC was calculated based on disease scores from severity using the formular below

$\text{AUDPC} = (\frac{yi+yi+1}{2})(ti + 1 - t1)$ where *yi* is the proportion of disease on the ith observation, *ti* is the time (days) of observation (Mirela *et al.*, 2013).

3.4 Data analysis

Data on disease incidence and severity was subjected to analysis of variance (ANOVA) using PROC ANOVA procedure of Genstat® (Lawes Agricultural Trust, Rothamsted Experimantal Station 2006, version 15) and differences among the treatment means compared using Fisher's least significant difference (LSD test). All analyses were conducted at significance value of $P \leq 0.05$.

3.5 Results

3.5.1 Efficacy of *Bacillus* spp and sodium nitrophenolate on incidence and severity of powdery mildew

Foliar application of *Bacillus subtilis*, *Bacillus amyloliquefaciens*, sodium nitrophenolate and dodemorph acetate reduced incidence and severity of powdery mildew. Incidence of powdery mildew started reducing steadily from week eight after treatments application to week 16 after treatments application (Table 3.1). Incidence of powdery mildew started to increase thereafter with all treatments applied. Application of *Bacillus amyloliquefaciens* and dodemorph acetate significantly $P \leq 0.05$ reduced the incidence of powdery mildew from 83% to 55%; with *Bacillus amyloliquefaciens* showing the highest reduction in powdery mildew incidence. Mean treatments indicate that application of *Bacillus amyloliquefaciens* and dodemorph acetate had overall better reduction on incidence of powdery mildew of roses.

Severity of powdery mildew differed significantly $P \leq 0.05$ among the five treatments tested (Table 3.2). However, there was no significant difference in application of *Bacillus subtilis* and sodium nitrophenolate in reducing the severity of powdery mildew of greenhouse roses. Plots treated with *Bacillus amyloliquefaciens* and dodemorph acetate were observed to have reduced severity of powdery mildew over time from 15.8% to 2.1%; with dodemorph acetate recording the highest level of reduction. Application of dodemorph acetate reduced disease incidence the most compared with control in area under disease progress curve (Table 3.3). This was followed by application of *Bacillus amyloliquefaciens*, sodium nitrophenolate and *Bacillus subtilis* in that order.

Table 3. 1: Incidence of powdery mildew on roses after treatments application of *Bacillus subtilis*, *B. amyloliquefaciens* and sodium nitrophenolate

Treatments	Weeks after treatment application					
	4	8	12	16	20	Mean
Bacillus subtillis	27.6^{a}	21.7^{ab}	10.9^{b}	8.8^{b}	17.3^{b}	17.3^{b}
Bacillus amyloliquefaciens	23.4^{a}	13.0^{b}	5.0^{c}	5.6^{c}	11.8^{c}	11.8^{c}
Sodium nitrophenolate	25.1^{a}	18.4^{ab}	10.4^{b}	7.4^{b}	15.3^{bc}	15.3^{bc}
Dodemorph acetate	21.4^{a}	12.3^{b}	5.9^{c}	5.3^{c}	11.3^{c}	11.3^{c}
Control	26.3^{a}	28.7^{a}	28.6^{a}	23.3^{a}	26.7^{a}	26.7^{a}
Mean	24.8	18.8	12.2	10.1	16.5	16.5
LSD ($P \leq 0.05$)	7.3	7.7	3.5	1.9	4.7	5.02
CV (%)	19.2	26.4	18.6	12	18.6	18.96

Means within column with different superscript indicates significant difference at $P \leq 0.05$; CV-coefficient of variation; LSD-Least significant difference at ($P \leq 0.05$)

Table 3. 2: Severity of powdery mildew expressed as percentage after application of *Bacillus subtilis*, *B. amyloliquefaciens* and sodium nitrophenolate

Treatments	Weeks after treatment application					
	4	8	12	16	20	Mean
Bacillus subtillis	21.49^{a}	27.29^{ab}	18.40^{bc}	12.08^{b}	10.63^{bc}	17.98^{b}
Bacillus amyloliquefaciens	16.21^{a}	15.76^{b}	7.43^{c}	5.35^{bc}	3.68^{c}	9.69^{b}
Sodium nitrophenolate	17.27^{a}	23.06^{b}	19.79^{b}	13.06^{b}	11.11^{b}	16.86^{b}
Dodemorph acetate	15.08^{a}	14.79^{b}	12.64^{bc}	3.12^{c}	2.08^{c}	9.54^{b}
Control	21.77^{a}	40.00^{a}	47.04^{a}	42.99^{a}	45.97^{a}	39.62^{a}
Mean	18.4	11.5	21.1	15.3	14.7	16.2
LSD ($P \leq 0.05$)	11.2	11.5	11.2	5.69	6.35	9.19
CV (%)	39.7	30.8	34.3	24.1	28	31.38

Means within column with different superscript indicates significant difference at $P \leq 0.05$; CV-coefficient of variation; LSD-Least significant difference at ($P \leq 0.05$)

Table 3.3: Effect of *Bacillus subtilis*, *B. amyloliquefaciens* and sodium nitrophenolate on area under disease progress curve (AUDPC)

Treatments	Rate	AUDPC
Bacillus subtilis	3.0ml/L of water	1426.0[ab]
Bacillus amyloliquefaciens	1.5g/L of water	998.0[b]
Sodium nitrophenolate	1.0ml/ l of water	1259.0[ab]
Dodemorph ecetate	2.5ml/L of water	932.0[b]
Control		1751.0[a]
Mean		1273.0
LSD ($P \leq 0.05$)		446.1
CV (%)		22.7

Means within column with different superscript indicates significant difference; LSD- least significant differences ($P \leq 0.05$); CV-coefficient of variation.

3.5.2 Frequency and rate of application of *Bacillus amyloliquefaciens* on incidence and severity of powdery mildew

The results showed that application of *Bacillus amyloliquefaciens* at all rates and at various intervals had significantly $P \leq 0.05$ reduced incidences of powdery mildew of roses over time. Week five and six had the lowest incidences of powdery mildew as the bacteria had well established in the plants. Application of dodemorph acetate significantly $P \leq 0.05$ reduced incidence of powdery mildew over time compared with the negative control. Four days and weekly application intervals of *Bacillus amyloliquefaciens* at different rates had better reduction on incidence compared to the ten days interval. Disease incidence significantly increased at $P \leq 0.05$ in areas of negative control over the weeks (Table 3.4). Concentration means indicates that applications at the rate of 1.5g/ L of water had better reduction in incidence of powdery mildew as compared to other rates at various intervals. Applications at 4.5g/L of water had the highest level of incidence as shown by the treatment means (Table 3.5).

Table 3. 4: Incidence of powdery mildew of roses at different rates and intervals of application of *Bacillus amyloliquefaciens*

Treatment	Weeks after treatment application						
	1	2	3	4	5	6	Mean
B. amylol 1.5g/L, 4 days	12.0^{c}	12.3^{a}	8.0^{d}	9.7^{c}	4.7^{e}	3.0^{d}	8.2^{b}
B. amylol 3.0g/L, 4 days	14.0^{abc}	13.3^{a}	10.3^{cd}	9.7^{c}	6.0^{de}	4.0^{d}	9.6^{b}
B. amylol 4.5g/L, 4 days	17.0^{a}	12.7^{a}	11.0^{bcd}	10.7^{bc}	6.0^{de}	3.7^{d}	10.2^{b}
B. amylol 1.5g/L, 7 days	13.0^{bc}	12.7^{a}	10.0^{cd}	9.7^{c}	6.0^{de}	3.7^{d}	9.2^{b}
B. amylol 3.0g/L, 7 days	11.0^{c}	14.0^{a}	10.0^{cd}	8.7^{c}	7.0^{cd}	4.3^{d}	9.2^{b}
B. amylol 4.5g/L, 7 days	11.7^{c}	15.0^{a}	10.3^{cd}	10.3^{bc}	7.3^{cd}	5.3^{cd}	10.0^{b}
B. amylol 1.5g/L, 10 days	16.3^{ab}	14.0^{a}	12.7^{abc}	11.7^{bc}	9.0^{bc}	7.3^{c}	11.8^{b}
B. amylol 3.0g/L, 10 days	12.7^{c}	12.7^{a}	15.0^{a}	12.3^{bc}	7.3^{cd}	5.7^{cd}	11.0^{b}
B. amylol 4.5g/L, 10 days	14.3^{abc}	13.0^{a}	15.3^{a}	13.7^{b}	10.0^{bc}	8.3^{bc}	12.4^{b}
D. acetate 2.5ml/L, 7 days	12.0^{c}	14.0^{a}	12.7^{abc}	11.7^{bc}	13.0^{b}	10.7^{b}	12.4^{b}
Control	14.0^{abc}	11.7^{a}	12.0^{abc}	20.7^{a}	33.0^{a}	39.3^{a}	21.8^{a}
Mean	13.5	13.2	11.6	11.7	9.94	8.67	11.44
LSD (P ≤0.05)	3.52	4.94	4.04	3.7	4.46	3.3	3.99
CV (%)	15.4	21.9	20.5	18.6	26.4	20.5	20.55

Means within column with different superscript indicates significant difference at P≤0.05; CV-coefficient of variation; LSD-Least significant difference at (P ≤0.05)

Table 3. 5: Incidence of powdery mildew of roses after application of different concentrations of *Bacillus amyloliquefaciens*

Concentration	Weeks after application of different concentrations						
	1	2	3	4	5	6	Mean
1.5g/L	13.8^{a}	13.0^{a}	12.0^{a}	10.3^{b}	6.6^{c}	4.7^{c}	10.07^{b}
3.0g/L	12.6^{a}	13.3^{a}	11.8^{a}	10.2^{b}	6.8^{c}	4.7^{c}	9.9^{b}
4.5g/L	14.3^{a}	13.6^{a}	12.2^{a}	11.6^{b}	7.8^{c}	5.8^{c}	10.88^{b}
2.5ml/L	12.0^{a}	14.0^{a}	12.7^{a}	11.7^{b}	13.0^{b}	10.7^{b}	12.35^{b}
Control	14.0^{a}	11.7^{a}	12.0^{a}	20.7^{a}	33.0^{a}	39.3^{a}	21.78^{a}
Mean	13.5	13.2	11.6	11.7	9.9	8.7	11.43
LSD (P ≤0.05) Con. Weeks	3.5	3.7	4.1	3.2	3.8	3.1	3.56
CV (%)	19.1	20.2	26.1	20.1	27.5	26.2	23.20

Means within column with different superscript indicates significant difference at P ≤0.05; CV-coefficient of variation; LSD-Least significant difference at (P ≤0.05)

Severity of powdery mildew was significantly at P ≤0.05 reduced by *Bacillus amyloliquefaciens* applications at different rates and intervals. Reduction was gradual over the weeks with week six recording the least severity. Disease severity was low in plots treated at four days and weekly applications compared to ten days with weekly applications giving better reduction. Applications at the rate of 1.5g/L of water and 3.0g/L of water showed higher levels of reduction in severity according to the treatment means. Untreated plots had the highest severity level going up to 77.1%. Application of dodemorph acetate also significantly at P ≤0.05 reduced severity of powdery mildew over the weeks of experiment (Table 3.6 and Table 3.7).

Table 3. 6: Severity of powdery mildew of roses at different rates and intervals of application of *Bacillus amyloliquefaciens*

Treatment	Weeks after treatment application						
	1	2	3	4	5	6	Mean
B. amylol 1.5g/L, 4 days	13.5^{a}	12.9^{a}	6.4^{e}	4.5^{d}	1.3^{b}	0.8^{b}	6.6^{b}
B. amylol 3.0g/L, 4 days	13.7^{a}	10.8^{a}	6.1^{e}	5.1^{cd}	2.4^{b}	0.9^{b}	6.5^{b}
B. amylol 4.5g/L, 4 days	15.9^{a}	9.2^{a}	7.6^{cde}	7.1^{bcd}	1.5^{b}	1.1^{b}	7.1^{b}
B. amylol 1.5g/L, 7 days	12.8^{a}	9.6^{a}	7.9^{cde}	5.6^{cd}	1.9^{b}	0.5^{b}	6.4^{b}
B. amylol 3.0g/L, 7 days	11.5^{a}	11.8^{a}	7.3^{de}	5.9^{cd}	2.5^{b}	0.7^{b}	6.6^{b}
B. amylol 4.5g/L, 7 days	10.4^{a}	10.6^{a}	7.6^{cde}	5.5^{cd}	1.9^{b}	0.8^{b}	6.1^{b}
B. amylol 1.5g/L, 10 days	11.9^{a}	10.3^{a}	9.9^{bcd}	7.9^{bcd}	4.1^{b}	1.6^{b}	7.6^{b}
B. amylol 3.0g/L, 10 days	14.4^{a}	12.9^{a}	12.8^{ab}	10.5^{b}	3.7^{b}	1.3^{b}	9.3^{b}
B. amylol 4.5g/L, 10 days	13.7^{a}	10.9^{a}	11.9^{abcd}	8.6^{bc}	5.5^{b}	2.01^{b}	8.8^{b}
D. acetate 2.5g/L, 7 days	12.1^{a}	7.9^{a}	11.1^{abcd}	10.2^{b}	9.1^{b}	9.7^{b}	10.0^{b}
Control	10.6^{a}	10.5^{a}	15.0^{a}	30.1^{a}	59.9^{a}	77.1^{a}	33.9^{a}
Mean	12.8	10.7	9.44	9.17	8.54	8.78	9.91
LSD (P ≤0.05)	6.11	5.23	4.51	3.76	8.69	9.39	6.28
CV (%)	28.2	28.8	28.1	24.1	59.8	62.8	38.63

Means within column with different superscript indicates significant difference at P≤0.05; CV-coefficient of variation; LSD-Least significant difference at (P ≤0.05)

Table 3. 7: Severity of powdery mildew of roses after application of different concentrations of *Bacillus amyloliquefaciens*

Concentration	Weeks after application of different concentrations						
	1	2	3	4	5	6	Mean
1.5g/L	12.7^{a}	10.9^{a}	8.1^{b}	5.9^{c}	2.4^{c}	0.96^{c}	6.8^{b}
3.0g/L	13.2^{a}	11.8^{a}	8.7^{b}	7.2^{c}	2.9^{bc}	0.98^{c}	7.46^{b}
4.5g/L	13.3^{a}	10.2^{a}	9.1^{b}	7.1^{c}	3.0^{bc}	1.3^{c}	7.33^{b}
2.5ml/L	12.1^{a}	7.9^{a}	11.1^{ab}	10.2^{b}	9.1^{b}	9.7^{b}	10.02^{b}
Control	10.6^{a}	10.5^{a}	15.0^{a}	30.1^{a}	59.1^{a}	77.0^{a}	33.72^{a}
Mean	12.8	10.7	9.4	9.2	8.5	8.8	9.9
LSD ($P \leq 0.05$)	4.9	3.9	4.4	3.6	6.4	6.7	4.98
CV (%)	27.7	27.1	34.3	28.8	54.7	55.3	37.98

Means within column with different superscript indicates significant difference at $P \leq 0.05$; CV-coefficient of variation; LSD-Least significant difference at ($P \leq 0.05$)

Application of *Bacillus amyloliquefaciens* at different rates and intervals controlled powdery mildew better than dodemorph acetate. Plots treated weekly at the rate of 1.5g/L controlled greenhouse powdery mildew of roses on higher level compared with other rates and intervals of applications. Areas with negative control registered highest level of powdery infection followed by dodemorph acetate. Applications at different rates and intervals of ten days showed high levels of AUDPC when compared to applications at different rates and at various intervals. In general, application at the rate of 1.5g/L proved to have high effect on powdery mildew at different intervals (Table 3.8).

Table 3. 8: Area under disease progress curve of powdery mildew of roses at different rates and intervals of application of *Bacillus amyloliquefaciens*

TREATMENT	**Rates and Interval**	AUDPC
Bacillus amyloliquefaciens	1.5g/L of water, 4 days	825[c]
Bacillus amyloliquefaciens	3.0g/L of water, 4 days	845.3[c]
Bacillus amyloliquefaciens	4.5g/L of water, 4 days	878.9[c]
Bacillus amyloliquefaciens	1.5g/L of water, 7days	799.8[c]
Bacillus amyloliquefaciens	3.0g/L of water, 7 days	835.1[c]
Bacillus amyloliquefaciens	4.5g/L of water, 7 days	803.3[c]
Bacillus amyloliquefaciens	1.5g/L of water, 10 days	964.3[c]
Bacillus amyloliquefaciens	3.0g/L of water, 10 days	1194.1[c]
Bacillus amyloliquefaciens	4.5g/L of water, 10 days	1123.8[c]
Dodemorph acetate	2.5g/L of water, 7 days	1749.4[b]
Control		3647.5[a]
Mean		1242
LSD (P ≤0.05)		424
CV (%)		41.2

Means within column with different superscript indicates significant difference; LSD- least significant differences; CV-coefficient of variation.

Figure 3. 2: T6 P. M before *Bacillus amyloliquefaciens* (3.0g/L, 4days application

Figure 3. 1: T6 P. M after *Bacillus amyloliquefaciens* application

Figure 3. 4: T4 P. M before *Bacillus amyloliquefaciens* application

Figure 3. 3: T4 P.M weeks after *Bacillus amyloliquefaciens* (1.5g/L) application

3.4 Discussion

Results of this experiment show that *Bacillus* spp has effect on powdery mildew of roses under greenhouse conditions in different agro-ecological zones. This is evident by reduction in the disease incidence and severity. *Bacillus* Spp has been used in managing various diseases of plants across the world. Results posted here concurs with those posted by (Lim *et al.*, 2017) where a strain of *Bacillus velezensis* G341 controlled various phytopathogenic fungi by preventing mycelial growth of *Alternaria panax*, *Botritys cinerea*, *Colletotricum coccodes*, *Fusarium oxysporium, Magnaporthe oryzea*, *Phytophthora capsici*, *Pythium ultimum, Rhizoctonia solani,* and *Sclerotinia scletiorum.*

Bacilomycin D which was extracted from *Bacillus velezensis* HN-2 inhibited mycelia growth of *Colletotricum gloesporioides* (Penz) by secretion of iturin which is a member of the lipopeptins (Jin *et al.*, 2019). Results by Roslan *et al.* (2020) confirmed that *Bacillus amyloliquefaciens* PEP3 inhibited growth of spores of *Phytophthora cacisi* with 2.00±0.1 cm inhibition zone. Kumari *et al.* (2021) isolated five different strains of *Bacillus* species and found that all the species had

antifungal activity on *Sclerotium rolfsii* through release of phenolic acids and hydrolytic enzymes which inhibited the growth of the mycelia of the fungi. Elsisi (2019) found out that squash sprayed with bio-agents *B. subtilis* being one of them reduced severity and incidence of powdery mildew of squash grown in greenhouses. Application of *B. subtilis* on cucumber plants under greenhouse conditions reduced powdery mildew incidence and severity (Punja *et al.*, 2019). Research conducted by Rotich *et al.* (2019) indicated that *Bacillus thuringiensis* reduced powdery mildew by parasitism on the hyphal cells and spores of powdery mildew on *Cornus florida*. It was established that LJ02 a strain of *Bacillus amyloliquefaciens* controlled powdery mildew by inducing systemic acquired resistance on cucurbits (Li *et al.*, 2015).

Results presented in this experiment shows that all the test products *Bacillus* spp, sodium nitrohenolate and dodemorph acetate decreased the area under disease progress curve (AUDPC) compared to control. Dodemorph acetate and *Bacillus amyloliquefaciens* showed higher reduction compared to *Bacillus subtilis* and sodium nitrophenolate. Weekly application of Bacillus amyloliquefaciens at the rate of 1.5g/L of water posted better results compared to the other rates at various intervals. This results are in agreement with the findings by Tanaka *et al.* (2017); Sawant *et al.* (2017); Punja *et al.* (2019) which indicated that application of *Bacillus* spp and *Trichoderma* spp reduced severity and AUDPC of powdery mildew.

Antifungal lipopeptides was shown to be the principal compound enhancing antagonism roles on fungal cells (Cawoy *et al.*, 2015). Some of the antifungal components released by species of *Bacillus* includes fengycins, iturins, surfactins, mycobacillins, mycosubtilins, subsporins and bacillomycins (Ortiz and Sansinenea, 2019). Tsegaye *et al.* (2019) postulates that *Bacillus* species also release hydrolytic enzymes such as amylase, cellulose, protease, lipase and chitinase which enable them to work against fungal infections. Species of *Bacillus* also produce volatile organic

compounds-VOC (Vinodkumar *et al*., 2017; Gotor-Vila *et al*., 2019; Roslan *et al*., 2020) which possess antifungal properties to phytopathogenic fungi such as *Alternaria alternata, A. solani, Botrytis cinerea, Cladosporium oxysporum, Fusarium oxysporum, Moniliophtora perniciosa, Paecilomyces lilacinus, P. variotii,* and the oomycete *Pythium afertile* (Gao *et al*., 2017). Findings by Guevara-Avendano *et al*. (2019) showed that isolates (HA, SJ, SO, SX, HB and SJJ) from the avocado rhizobacteria emitted VOCs which inhibited mycelial growth of *Fusarium solani* on avocado plants. Effectiveness of VOCs components is different among various strains of *Bacillus*. *Bacillus velezensis* strain ZSY-1 released four main VOCs as pyrzine017, benzothiazole, 4-chloro-3 methyl and phenol-2, 4-bis (1,1-dimethylethyl) (Gao *et al*., 2017).

Sodium nitrophenolate, a growth regulator have been proved to work against phytopathogens in varying degrees. Research by Wojdyla (2004), Sharma *et al*. (2014), Bulgari *et al*. (2015) pointed out that growth promotors and regulators induce plant resistance to abiotic and biotic stresses. A combination of sodium nitrophenolate compound with foliar fertilizers has been reported to lower the duration of infection of *Phytophthora infestans* in potato production and also promoted growth of the crop (Sawicka, 2003). According to Sawicka and Skiba (2009*) in vitro* treatment of potato with nitrophenolates delayed incidence of *P. infestans* by 3-7 days while according to Glosek-Sobieraj *et al*. (2018) foliar application of sodium nitrophenolate on potatoes reduced severity of late blight. Study by Cwalina-Ambroziak *et al*. (2015) pointed out that application of sodium nitrophenolate reduced severity of early blight of potatoes crop and incidences of dry rots on potato tubers.

Synthetic growth regulators when applied to the plants help in strengthening of the cell walls and inducing resistance to unfavourable environmental conditions and phytopathogens (Mikos-Bielak, 2005).

3.5 Conclusion

Foliar application of *Bacillus* spp and sodium nitrophenolate significantly reduced the symptoms of powdery mildew of roses in all the experimental plots compared with the negative control plots. Both incidence and severity were reduced by foliar application of the test products in both first and second experiments. Weekly application of *Bacillus amyloliquefaciens* at the rate of 1.5g/L of water reduced the incidence and severity the most compared to other rates and at different intervals in the second experiment. Positive control which involved the application of dodemorph acetate also controlled powdery mildew. The results shows that the test products have the potential in controlling powdery mildew of roses, therefore, their use should be adopted by rose growers in managing powdery mildew in the crop.

CHAPTER FOUR:
EFFECT OF *BACILLUS SUBTILIS, BACILLUS AMYLOLIQUEFACIENS* AND SODIUM NITROPHENOLATE ON QUALITY OF ROSE FLOWERS

4.1 Abstract

Quality of rose stem is an importance aspect for acceptability in the market. Longer stem length, larger and longer flower buds are usually achieved through proper nutrition and modification of the growing condition. Sometimes, quality of rose stems cannot be achieved due to high cost of fertilizers, harsh weather conditions that make modifications difficult. This experiment was done to evaluate the effect of *Bacillus subtilis*, *B. amyloliquefaciens* and sodium nitrophenolate on quality of roses in greenhouse conditions. In the first experiment, foliar application of *Bacillus subtilis* at the rate of 2ml/L, *B. amyloliquefaciens* at 3.0g/L, sodium nitrophenolate at 1ml/L and dodemorph acetate at 2.5ml/L. In the second experiment, foliar application of *Bacillus amyloliquefaciens* was done at different rates and at various intervals as follows 1.5g/L, 4 days, 3.0g/L, 4 days, 4.5g/L 4 days, 1.5g/L 7 days, 3.0g/L, 7 days, 4.5g/L, 7 days, 1.5g/L, 10 days, 3.0g/L, 10 days, 4.5g/L, 10 days and no treatment as control. Data was collected on stem length, bud diameter, bud length and marketable grade of harvested stems on daily basis. There was significant difference among the treatments on bud length, bud diameter, stem length and marketable grade. Foliar application of *Bacillus subtilis*, *B. amyloliquefaciens* and sodium nitrophenolate improved the quality of rose plants compared with plants in area of negative control. Effects of *Bacillus* spp and sodium nitrophenolate on stem elongation, flower sizes and flower grade requires further investigation.

Key words: Quality, *Bacillus Spp*, sodium nitrophenolate, roses, marketable grade.

4.2 Introduction

Rose (*Rosa hybrida*) is one of the most top ranking ornamental cut flowers in the world due to its beauty, fragrance and other uses (Hummer and Janick, 2009; Ulaszewski et al., 2021). Appearance of the rose stem in terms of size of the flower, the colour, shape of the bud, stem length and form have been generally used in highlighting the quality parameters (Reid, 2004; JETRO, 2011).

According to Fanourakis *et al*. (2013), interaction between genotype with growing conditions such as relative humidity, amount of light in the greenhouse will affect the morphological and physiological aspects of the plant thus quality of such crop. Plant spacing which results to higher population has shown to give higher production and quality blooms of the crop (Sujatha and Singh, 2003). Yield and quality in rose crop is also affected by the number of branches per plant and the stalk length. More branches reflects high production while longer lengths are for quality (Subiya *et al*., 2017). Adequate lighting and improved levels of CO_2 in the greenhouse will lead to better quality and increased production in rose crop. This will facilitate more branching, longer stems and reduced number of blind shoots (Naing *et al*., 2016; Fanourakis *et al*., 2019). The age and amount of bent shoots determines the extent of growth and development of the stem in the plant. Plants with younger bent shoots performs photosynthesis better than older shoots (Zhang *et al*., 2020). Plant growth, yield of plants and quality aspects of rose is improved by bending of shoots at the junction and the age of the plant (Vasudevan and Kannan, 2014; Tsanakas *et al*., 2017; Matloobi *et al*., 2018).

Greenhouse temperatures during both day and night with small range encourages better growth of plants thus increased yield and quality (Zou et al., 2020). Well-planned irrigation system and frequency of nutrient application through drip lines ensures good crop growth which results to quality production of flowers (Katsoulas *et al*., 2006; Nikolaou *et al*., 2019). The type and quality

of poly film cover affects the amount and quality of light that penetrates in the crop. The cover influences the growth and development of the crop besides protecting it from environmental dangers as well as providing conducive condition for enhanced production and quality of flowers (Oloo-Abucheli, 2018). Fungicides of biological origins enhances shoot elongation, flowering and sprouting in plants (Sitinjak, 2017; Szparaga *et al.*, 2018; Anguiano *et al.*, 2019; Lara-Capistran *et al.*, 2020). This experiment was conducted to evaluate the effects of *Bacillus subtilis*, *Bacillus amyloliquefaciens* and sodium nitrophenolate on quality of roses in greenhouse conditions. *Bacillus* spp and sodium nitrophenolate improves the quality of roses through enhanced production growth hormones which relieves plants from abiotic and biotic stresses.

4.3 Material and methods

4.3.1 Description of the experimental site and the crop

The experimental site and the crop is as described in the previous chapter.

4.3.2 Experimental design and layout

The experimental design and layout is as described in chapter three.

4.3 Data collection

Stems were harvested daily and those without symptoms of powdery mildew were counted and recorded. This was achieved by visual inspection or observation during harvesting for symptoms on parts of the stem such as leaves, stem, sepals and flower. Total number of harvested stems for the whole bed was recorded for the study. Stems which were showing symptoms of powdery

mildew on the leaves and the 'neck' were discarded since they do not meet export quality and those with deformities such as bent neck, bull heads and bent stems were equally discarded. Harvested stems which met the market quality were analyzed on length and bud size. Stem length was measured using tape measure while head sizes were measured using Vanier caliper for each stem and data was recorded for every stem harvested.

4.3 Data analysis

Data collected on stem length, bud diameter, bud length and marketable grade was subjected to analysis of variance (ANOVA) using PROC ANOVA procedure of Genstat® (Lawes Agricultural Trust, Rothamsted Experimantal Station 2006, version 15) and differences among the treatment means compared using Fisher's least significant difference (LSD test). All analyses were conducted at significance value of $P \leq 0.05$.

4.5 Results

4.5.1 Efficacy of *Bacillus* spp. and sodium nitrophenolate on quality and marketable grade

Stem length improved significantly at $P \leq 0.05$ with application of all treatments in week sixteen compared with harvested stems from the negative control but there was no significant differences at $P \leq 0.05$ in stem length in other weeks in the first experiment. Mean treatment indicates that application of *Dodemorph acetate* and sodium nitrophenolate had better stem length followed by *Bacillus subtilis* and *Bacillus amyloliquefaciens* (Table 4.1). There was no significant difference at $P \leq 0.05$ in bud diameter among the treatments applied during the period. There were no significant difference in bud length and diameter after treatments application in weeks four, eight and sixteen. However, week twelve of treatments applications showed significant $P \leq 0.05$

improvement in bud length in plots treated with *Bacillus subtilis* and sodium nitrophenolate. Generally, after 12 to 20 weeks of treatments application, control had the lowest values for bud length (Tables 4.2 and 4.3). There were no significant difference among treatments on marketable grade of harvested stems, however, significant difference at $P \leq 0.05$ were noted in week 20 after treatments application compared to control plots which did not give any marketable stems due to high level of powdery mildew infections (Table 4.4).

Table 4. 1: Stem length of roses after application of *Bacillus subtilis, B. amyloliquefaciens* and sodium nitrophenolate

Treatments	Weeks after treatment application					
	4	8	12	16	20	Mean
Bacillus subtillis	64.9[a]	67.28[a]	69.41[a]	66.11[b]	65.05[a]	66.54[a]
Bacillus amyloliquefaciens	67.3[a]	68.70[a]	68.78[a]	61.70[c]	64.71[a]	66.20[a]
Sodium nitrophenolate	67.9[a]	69.09[a]	67.78[a]	66.04[b]	65.41[a]	67.24[a]
Dodemorph acetate	67.1[a]	67.34[a]	67.12[a]	69.90[a]	65.72[a]	67.44[a]
Control	68.0[a]	65.98[a]	63.71[b]	61.64[c]	63.94[a]	64.65[a]
Mean	67	67.7	67.4	65.1	65	66.44
LSD ($P \leq 0.05$)	9.27	4.41	4.6	3.2	1.78	4.65
CV (%)	9	4.2	4.4	3.2	1.8	4.52

Means within column with different superscript indicates significant difference at $P \leq 0.05$; CV-coefficient of variation; LSD-Least significant difference at ($P \leq 0.05$)

Table 4. 2: Bud diameter of roses after application of *Bacillus subtilis, B. amyloliquefaciens* and sodium nitrophenolate

Treatments	Weeks after treatment application					
	4	8	12	16	20	Mean
Bacillus subtillis	2.85[a]	3.11[a]	2.99[a]	2.77[a]	3.08[a]	2.96[a]
Bacillus amyloliquefaciens	3.09[a]	3.23[a]	2.96[a]	2.79[a]	3.13[a]	3.04[a]
Sodium nitrophenolate	3.03[a]	3.1[a]	3.01[a]	2.84[a]	2.99[a]	2.99[a]
Dodemorph acetate	3.04[a]	3.11[a]	3.04[a]	2.81[a]	3.14[a]	3.03[a]
Control	3.03[a]	3.04[a]	3.06[a]	2.75[a]	2.46[b]	2.87[a]
Mean	3	3.1	3	2.8	3	2.98
LSD ($P \leq 0.05$)	0.3	0.4	0.2	0.2	0.3	0.28
CV (%)	7	7.4	3.4	5.2	5.4	5.68

Means within column with different superscript indicates significant difference at P ≤0.05; CV-coefficient of variation; LSD-Least significant difference at (P ≤0.05)

Table 4.3: Bud length of roses after application of *Bacillus subtilis, B. amyloliquefaciens* and sodium nitrophenolate

Treatments	Weeks after treatment application					
	4	8	12	16	20	Mean
Bacillus subtillis	4.93[a]	5.26[a]	5.21[ab]	4.12[a]	5.50[a]	5.00[a]
Bacillus amyloliquefaciens	5.31[a]	5.29[a]	5.40[a]	4.16[a]	5.25[a]	5.08[a]
Sodium nitrophenolate	5.08[a]	5.44[a]	5.30[a]	4.14[a]	5.29[a]	5.05[a]
Dodemorph acetate	5.24[a]	5.31[a]	5.23[ab]	4.09[a]	5.27[a]	5.03[a]
Control	5.24[a]	5.12[a]	4.89[b]	4.04[a]	4.34[b]	4.73[b]
Mean	5.2	5.3	5.2	4.1	5.1	4.98
LSD (P ≤0.05)	0.65	0.32	0.25	0.28	0.2	0.34
CV (%)	8.2	4	3.1	4.5	2.5	4.46

Means within column with different superscript indicates significant difference at P ≤0.05; CV-coefficient of variation; LSD-Least significant difference at (P ≤0.05)

Table 4.4: Marketable grade of roses after application of *Bacillus subtilis, B. amyloliquefaciens* and sodium nitrophenolate

Treatments	Weeks after treatment application					
	4	8	12	16	20	Mean
Bacillus subtillis	3.13[a]	4.19[a]	3.25[a]	6.06[a]	6.25[ab]	4.58[a]
Bacillus amyloliquefaciens	2.19[a]	3.88[a]	3.69[a]	5.56[a]	7.44[a]	4.55[a]
Sodium nitrophenolate	2.94[a]	3.94[a]	4.00[a]	4.81[a]	6.00[b]	4.34[a]
Dodemorph acetate	3.13[a]	4.13[a]	3.25[a]	6.81[a]	6.44[ab]	4.75[a]
Control	1.00[a]	0.00[b]	0.00[b]	0.00[b]	0.00[c]	0.20[b]
Mean	2.5	3.2	2.8	4.7	5.2	3.68
LSD (P ≤0.05)	1.66	0.73	0.83	2.07	1.15	1.29
CV %	43.4	14.7	19.1	28.9	14.3	24.08

Means within column with different superscript indicates significant difference at P ≤0.05; Means of treatment is separated with LSD treatment; CV-coefficient of variation; LSD-Least significant difference at (P ≤0.05)

4.5.2 Effect of rate and interval of application of *Bacillus amyloliquefaciens* on quality and marketable grade of harvested stems

Stem length differed significantly at P ≤0.05 among treatments over the weeks with different application rates and at various intervals of *Bacillus amyloliquefaciens* during the period of the experiment. However, application of *Bacillus amyloliquefaciens* at the interval of 10 days in weeks

four and five had better average stem length compared to applications at intervals of four days and weekly. Application at the rates of 1.5g/L of water and 3.0g/L of water had longer stem length compared to applications at the rate of 4.5g/L of water. Application of dodemorph acetate significantly $P \leq 0.05$ improved stem length over the weeks (Table 4.4). However, the untreated plots showed that stem length was reducing as over the weeks.

Bud length and diameter over the weeks differed significantly at $P \leq 0.05$ with application of *Bacillus amyloliquefaciens* at different rates and intervals. Bud length with concentrations of 3.0g/L and 4.5g/L differed significantly at $P \leq 0.05$ particularly in weeks five and six (Table 4.9). However, average weekly means indicate that week four and five had better bud diameter compared to other weeks during the period of study (Table 4.6 and Table 4.7). Weekly application of dodemorph acetate significantly at $P \leq 0.05$ improved the bud length and diameter over the weeks. Control plots had the lowest values on both bud length and diameter.

Table 4.4: Stem length of roses after application of different rates and at various intervals of *Bacillus amyloliquefaciens*

Treatment	Weeks after treatment application						
	1	2	3	4	5	6	Mean
B. amylol 1.5g/L, 4 days	59.0^{ab}	59.0^{ab}	66.3^{a}	59.0^{ab}	57.0^{abc}	58.3^{ab}	59.8^{a}
B. amylol 3.0g/L, 4 days	64.3^{a}	57.3^{ab}	55.7^{bc}	59.0^{ab}	52.0^{d}	59.7^{a}	58.0^{ab}
B. amylol 4.5g/L, 4 days	59.0^{ab}	58.0^{ab}	54.7^{c}	60.7^{ab}	53.0^{d}	52.3^{c}	56.3^{ab}
B. amylol 1.5g/L, 7 days	58.0^{b}	55.0^{b}	58.0^{bc}	56.3^{b}	58.0^{ab}	57.3^{ab}	57.1^{ab}
B. amylol 3.0g/L, 7 days	64.0^{a}	65.7^{a}	62.0^{a}	61.3^{ab}	54.7^{bcd}	58.7^{ab}	61.1^{a}
B. amylol 4.5g/L, 7 days	58.7^{b}	62.3^{ab}	56.0^{bc}	51.7^{bc}	54.7^{bcd}	56.0^{abc}	56.6^{ab}
B. amylol 1.5g/L, 10 days	58.0^{b}	64.7^{a}	58.7^{bc}	58.7^{ab}	59.3^{a}	59.3^{a}	59.8^{a}
B. amylol 3.0g/L, 10 days	55.3^{b}	57.3^{ab}	58.7^{bc}	65.3^{a}	54.3^{cd}	56.0^{abc}	57.8^{ab}
B. amylol 4.5g/L, 10 days	55.0^{b}	61.3^{ab}	59.0^{bc}	61.3^{ab}	56.0^{abc}	55.0^{bc}	57.9^{ab}
D. acetate 2.5ml/L, 7 days	59.0^{ab}	58.0^{ab}	59.0^{bc}	61.0^{ab}	51.3^{d}	55.0^{bc}	57.2^{ab}
Control	61.0^{ab}	60.0^{ab}	58.0^{bc}	47.3^{c}	41.3^{e}	43.7^{d}	51.9^{b}
Mean	59.2	59.9	58.7	58.3	53.8	55.6	57.58
LSD ($P \leq 0.05$)	5.33	9.28	7.04	7.98	3.51	4.67	6.3
C.V (%)	5.3	9.1	7	8	3.8	4.9	6.35

Means within column with different superscript indicates significant difference at $P \leq 0.05$; CV-coefficient of variation; LSD-Least significant difference at ($P \leq 0.05$)

Table 4.5: Stem length of roses after application of different concentrations of *Bacillus amyloliquefaciens*

Concetration	Weeks after application of different concentrations						
	1	2	3	4	5	6	Mean
1.5g/L	58.3^{a}	59.6^{a}	61.0^{a}	58.0^{a}	58.1^{a}	58.3^{a}	58.9^{a}
3.0g/L	61.2^{a}	60.1^{a}	58.8^{a}	61.9^{a}	53.7^{b}	58.1^{ab}	58.9^{a}
4.5g/L	57.6^{a}	60.6^{a}	56.6^{a}	57.9^{a}	54.6^{b}	54.4^{b}	56.9^{ab}
2.5ml/L	59.0^{a}	58.0^{a}	59.0^{a}	61.0^{a}	51.3^{c}	55.0^{b}	57.2^{ab}
Control	61.0^{a}	60.0^{a}	58.0^{a}	47.3^{b}	41.3^{d}	43.7^{c}	51.9^{b}
Mean	59.2	59.9	58.7	58.3	53.8	55.6	57.58
LSD ($P \leq 0.05$)	5.3	8.1	6.4	7.1	2.9	3.8	5.6
CV (%)	6.5	9.9	7.9	8.1	4	5	6.9

Means within column with different superscript indicates significant difference at $P \leq 0.05$; CV-coefficient of variation; LSD-Least significant difference at ($P \leq 0.05$)

Table 4. 6: Bud diameter of roses at different rates and intervals of application of *Bacillus amyloliquefaciens*

Treatment	Weeks after treatment application						
	1	2	3	4	5	6	Mean
B. amylol 1.5g/L, 4 days	2.2^{ab}	2.0^{a}	2.2^{a}	2.1^{ab}	2.2^{bc}	2.1^{bc}	2.1^{a}
B. amylol 3.0g/L, 4 days	2.0^{ab}	2.1^{a}	1.9^{bc}	2.1^{ab}	2.3^{b}	2.1^{bc}	2.1^{a}
B. amylol 4.5g/L, 4 days	2.1^{ab}	2.1^{a}	2.0^{ab}	2.3^{a}	2.0^{d}	2.2^{b}	2.1^{a}
B. amylol 1.5g/L, 7 days	1.9^{b}	2.1^{a}	2.2^{a}	2.1^{ab}	2.1^{cd}	2.1^{bc}	2.1^{a}
B. amylol 3.0g/L, 7 days	2.0^{ab}	2.1^{a}	2.0^{ab}	2.0^{b}	2.0^{d}	2.2^{b}	2.1^{a}
B. amylol 4.5g/L, 7 days	2.0^{ab}	2.1^{a}	2.0^{ab}	2.3^{a}	2.0^{d}	2.0^{c}	2.1^{a}
B. amylol 1.5g/L, 10 days	2.3^{a}	2.0^{a}	2.2^{a}	2.2^{ab}	2.7^{a}	2.6^{a}	2.3^{a}
B. amylol 3.0g/L, 10 days	2.0^{ab}	2.1^{a}	2.1^{ab}	2.2^{ab}	2.0^{d}	2.2^{b}	2.1^{a}
B. amylol 4.5g/L, 10 days	1.9^{b}	2.0^{a}	2.1^{ab}	2.1^{ab}	2.2^{bc}	2.0^{c}	2.1^{a}
D. acetate 2.5ml/L, 7 days	2.1^{ab}	2.1^{a}	2.1^{ab}	2.2^{ab}	2.1^{cd}	2.0^{c}	2.1^{a}
Control	2.2^{ab}	2.1^{a}	1.7^{c}	1.6^{c}	1.4^{e}	1.4^{d}	1.7^{b}
Mean	2.08	2.08	2.06	2.1	2.1	2.07	2.08
LSD ($P \leq 0.05$)	0.31	0.24	0.21	0.27	0.17	0.21	0.24
CV (%)	8.7	6.6	5.8	7.5	4.8	5.9	6.55

Means within column with different superscript indicates significant difference at $P \leq 0.05$; CV-coefficient of variation; LSD-Least significant difference at ($P \leq 0.05$)

Table 4. 7: Bud diameter of roses after application of different concentrations of *Bacillus amyloliquefaciens*

Concentration	Weeks after application of different concentrations						
	1	2	3	4	5	6	Mean
1.5g/L	2.1^{a}	2.1^{a}	2.2^{a}	2.1^{a}	2.4^{a}	2.2^{a}	2.2^{a}
3.0g/L	2.0^{a}	2.1^{a}	2.0^{a}	2.1^{a}	2.1^{a}	2.2^{a}	2.2^{a}
4.5g/L	2.0^{a}	2.1^{a}	2.1^{a}	2.2^{a}	2.1^{a}	2.1^{a}	2.1^{a}
2.5ml/L	2.1^{a}	2.1^{a}	2.1^{a}	2.2^{a}	2.1^{a}	2.0^{a}	2.1^{a}
Control	2.2^{a}	2.1^{a}	1.7^{b}	1.6^{b}	1.4^{b}	1.4^{b}	1.7^{b}
Mean	2.1	2.1	2.1	2.1	2.1	2.1	2.1
LSD (P ≤0.05)	0.2	0.2	0.2	0.2	0.3	0.3	0.23
CV (%)	8.5	6	5.8	7.3	9.2	9.8	7.77

Means within column with different superscript indicates significant difference at P≤0.05; CV-coefficient of variation; LSD-Least significant difference at (P ≤0.05)

Table 4. 8: Bud length of roses at different rates and intervals of application of *Bacillus amyloliquefaciens*

TREATMENT	Weeks after treatment application						
	1	2	3	4	5	6	Mean
B. amylol 1.5g/L, 4 days	5.4^{ab}	5.4^{ab}	5.2^{a}	5.2^{a}	5.6^{bc}	5.4^{bc}	5.4^{ab}
B. amylol 3.0g/L, 4 days	5.6^{a}	5.4^{ab}	5.3^{a}	5.4^{a}	5.2^{de}	5.1^{c}	5.3^{ab}
B. amylol 4.5g/L, 4 days	5.5^{ab}	5.6^{a}	5.2^{a}	5.4^{a}	5.4^{cd}	5.5^{b}	5.4^{ab}
B. amylol 1.5g/L, 7 days	5.2^{b}	5.3^{ab}	5.3^{a}	5.2^{a}	5.8^{ab}	5.5^{b}	5.4^{ab}
B. amylol 3.0g/L, 7 days	5.5^{ab}	5.4^{ab}	5.4^{a}	5.1^{a}	5.2^{de}	5.1^{c}	5.3^{ab}
B. amylol 4.5g/L, 7 days	5.4^{ab}	5.2^{b}	5.4^{a}	5.3^{a}	5.1^{d}	5.4^{bc}	5.3^{ab}
B. amylol 1.5g/L, 10 days	5.6^{a}	5.3^{ab}	5.4^{a}	5.5^{a}	6.0^{a}	5.9^{a}	5.6^{a}
B. amylol 3.0g/L, 10 days	5.2^{b}	5.3^{ab}	5.4^{a}	5.3^{a}	5.3^{cd}	5.4^{bc}	5.3^{ab}
B. amylol 4.5g/L, 10 days	5.4^{ab}	5.2^{b}	5.3^{ab}	5.3^{a}	5.2^{de}	5.1^{c}	5.3^{ab}
D. acetate 2.5ml/L, 7 days	5.2^{b}	5.4^{ab}	5.5^{a}	5.2^{a}	5.0^{e}	5.1^{c}	5.2^{b}
Control	5.5^{ab}	5.4^{ab}	4.7^{b}	4.4^{b}	4.2^{f}	4.4^{d}	4.8^{c}
Mean	5.39	5.37	5.29	5.21	5.28	5.25	5.29
LSD (P ≤0.05)	0.39	0.38	0.35	0.33	0.31	0.35	0.35
CV (%)	4.2	4.2	3.9	3.7	3.4	4.2	3.93

Means within column with different superscript indicates significant difference at P≤0.05; CV-coefficient of variation; LSD-Least significant difference at (P ≤0.05)

Table 4. 9: Bud length of roses after application of different concentrations of *Bacillus amyloliquefaciens*

Concentration	Weeks after application of different concentrations						
	1	2	3	4	5	6	Mean
1.5g/L	5.4[a]	5.4[a]	5.3[a]	5.3[a]	5.8[a]	5.6[a]	5.5[a]
3.0g/L	5.4[a]	5.4[a]	5.4[a]	5.3[a]	5.3[ab]	5.2[ab]	5.3[a]
4.5g/L	5.4[a]	5.4[a]	5.3[a]	5.3[a]	5.2[ab]	5.3[ab]	5.3[a]
2.5ml/L	5.2[a]	5.4[a]	5.5[a]	5.2[a]	5.0[b]	5.1[b]	5.2[a]
Control	5.5[a]	5.4[a]	4.7[b]	4.4[b]	4.2[c]	4.4[c]	4.8[b]
Mean	5.4	5.4	5.3	5.2	5.3	5.3	5.32
LSD (P≤0.05)	0.3	0.3	0.3	0.3	0.3	0.4	0.32
C.V. (%)	4.7	4.3	3.7	3.8	3.7	5.2	4.23

Means within column with different superscript indicates significant difference at P ≤0.05; CV-coefficient of variation; LSD- Least significant difference at (P ≤0.05)

Application of *Bacillus amyloliquefaciens* resulted to production of more sellable stems due to reduction and clearing of powdery mildew spores on the leaves, stems and sepals of the flowers. There was significant diffenece among treatments in week three to week five. Week six had the highest production with daily average production of 49 stems (Table 4.10). Treatment means indicates that applications at the interval of ten days at the rate of 1.5g/L gave more stems than applications at the interval of four days and weekly applications. The rate of 3.0g/L resulted in high production (Table 4.11). Application of dodemorph acetate had the highest production of stems while the control had lowest number of stems produced during that period of study.

Table 4.10: Marketable grade of harvested rose stems at different rates and interval of application of *Bacillus amyloliquefaciens*

TREATMENT	Weeks after treatment application						
	1	2	3	4	5	6	Mean
B. amylol 1.5g/L, 4 days	8.3^{e}	18.3^{a}	24.3^{bcd}	25.3^{b}	26.0^{c}	35.0^{a}	22.9^{a}
B. amylol 3.0g/L, 4 days	13.0^{abcd}	20.0^{a}	26.0^{abcd}	28.3^{ab}	33.3^{ab}	33.7^{a}	25.7^{a}
B. amylol 4.5g/L, 4 days	12.7^{bcd}	18.3^{a}	28.3^{ab}	27.7^{ab}	31.7^{abc}	31.3^{a}	25.0^{a}
B. amylol 1.5g/L, 7 days	11.0^{cd}	14.7^{a}	29.3^{a}	25.3^{b}	31.0^{bc}	34.3^{a}	24.3^{a}
B. amylol 3.0g/L, 7 days	17.0^{a}	17.0^{a}	24.0^{bcd}	29.3^{ab}	31.3^{abc}	34.0^{a}	25.4^{a}
B. amylol 4.5g/L, 7 days	10.7^{de}	18.0^{a}	23.7^{cd}	25.7^{b}	30.3^{bc}	30.7^{a}	23.2^{a}
B. amylol 1.5g/L, 10 days	13.3^{abcd}	19.3^{a}	25.7^{abcd}	30.0^{ab}	33.0^{ab}	35.0^{a}	26.1^{a}
B. amylol 3.0g/L, 10 days	15.3^{abc}	16.3^{a}	22.0^{d}	31.7^{a}	34.3^{ab}	35.3^{a}	25.8^{a}
B. amylol 4.5g/L, 10 days	13.0^{abcd}	17.7^{a}	22.0^{d}	32.0^{a}	26.3^{c}	34.7^{a}	24.3^{a}
D. acetate 2.5g/L, 7 days	15.3^{abc}	15.7^{a}	27.0^{abc}	30.0^{ab}	37.7^{a}	33.0^{a}	26.5^{a}
Control	16.0^{ab}	15.7^{a}	14.0^{e}	9.7^{c}	6.0^{d}	3.3^{b}	10.8^{b}
Mean	13.2	17.3	24.2	26.8	29.2	30.9	23.6
LSD ($P \leq 0.05$)	4.3	5.9	4.5	5.9	6.5	6.7	5.6
CV (%)	19.4	20.2	10.9	13.1	13.2	12.7	14.92

Means within column with different superscript indicates significant difference at $P \leq 0.05$; CV-coefficient of variation; LSD-Least significant difference at ($P \leq 0.05$)

Table 4. 11: Marketable grade of roses after application of different concentrations of *Bacillus amyloliquefaciens*

Concentration	Weeks after application of different concentrations						
	1	2	3	4	5	6	Mean
1.5g/L	10.9^{c}	17.3^{a}	26.4^{a}	26.9^{a}	30.0^{b}	34.8^{a}	24.4^{a}
3.0g/L	15.1^{ab}	17.8^{a}	24.0^{a}	29.8^{a}	33.0^{ab}	34.0^{a}	25.6^{a}
4.5g/L	12.1^{bc}	18.0^{a}	24.7^{a}	28.4^{a}	24.4^{b}	32.0^{a}	23.3^{a}
2.5ml/L	15.3^{ab}	15.7^{a}	27.0^{a}	30.0^{a}	37.7^{a}	33.0^{a}	26.5^{a}
Control	16.0^{a}	15.7^{a}	14.0^{b}	9.7^{b}	6.0^{c}	3.0^{b}	10.7^{b}
Mean	13.2	17.3	24.2	26.8	29.2	30.9	23.6
LSD (P ≤0.05)	3.8	4.7	4.4	5.2	5.6	4.9	4.77
CV (%)	21.1	19.7	13.3	14.1	14	11.7	15.65

Means within column with different superscript indicates significant difference at P≤0.05; CV-coefficient of variation; LSD-Least significant difference at (P ≤0.05)

4.6 Discussion

This experiment was conducted to evaluate the effect of *Bacillus* spp and sodium nitrophenolate in promoting growth by improving quality of roses flowers. Results of the data from harvested stems showed that the *Bacillus* spp and sodium nitrophenolate improved quality of the rose stem-stem length, bud diameter and bud length compared with stems harvested from the control plots.. These results are in line with findings by Tanaka *et al*. (2017), Punja *et al*. (2019), Sarhan *et al.* (2020) who pointed out that foliar application of *Bacillus* spp increased yields of cucumber under greenhouse conditions. Getahun *et al*. (2020) who pointed out that isolates strain BS 45- *Bacillus* sp improved the growth of *Acacia abyssinica* in terms of length.

Inoculation of bell pepper with *Bacillus subtilis* mixed with vermicompost improved morphological characteristics of the plant. The plant recorded increased height, stem diameter, leaf, buds, flower number, leaf area and production (Lara-Capistran *et al*., 2020). Research findings by Shi *et al*. (2010) pointed out that sugar beet plants treated with different strains of

Bacillus spp showed an improved plant height, plant dry weight and carbon assimilation and chlorophyll content and also stimulated maximum efficiency of Photosystem II. Results by Gowtham *et al.* (2017), Anguiano *et al.* (2019) showed that tomato plants inoculated with PGPB including *Bacillus* spp increased plants shoot and root length in greenhouse conditions but did not have significance differences in controlled conditions. Hashmi *et al.* (2019) indicated that a consortia of *Bacilli* when inoculated with oats seeds promoted growth of the plants by increasing plant biomass. However, Costa-Santos *et al.* (2021) found out that tomato plants treated with *Bacillus* spp did not significantly improve on shoot and root length as well as fresh biomass of the tomato seedlings. Cendales *et al.* (2017) reported that application of *Bacillus subtilis* strain GIBI 200 and *Bacillus pumilus* GIBI 206 did not significantly increase the biomass of tomato and plant height and roots.

Bacillus spp promote growth of plants through both direct and indirect ways which includes nitrogen fixation, production of phytohormone and siderophores, solubilization and mineralization of phosphorous, production of antimicrobial compounds and hydrolysis of enzymes, tolerance to abiotic stress and induced systemic resistance-ISR (Goswami *et al.*, 2016). Various species of *Bacillus* possess fixation capability for atmospheric nitrogen into the plant system. *Bacillus* with this capability has a specific gene which enables it to achieve this role. *Bacillus* spp is also capable of producing indole acetic acid (IAA) and auxin- promoting genes which in turn promote growth of plants (Shakeel *et al.*, 2015; Ambreetha *et al.*, 2018). Research by Howell *et al.* (2003), Xie *et al.* (2018), Wu *et al.* (2021) showed that cytokinins which are secreted by some *Bacillus* spp are essential in mitosis, development of young stems, radicles progression, callus formation which are employed in undifferentiated roots and stems tissues. Joo *et al.* (2004), pointed out that some

Bacillus spp may produce gibberelin acids (GA) which are vital in stem elongation, flowering, fruit formation and sex expression in plants.

Sodium nitrophenolate is a growth regulator with functions to stimulate roots, shoots, fruits and improve uptake of nutrients, increase flower buds, as well as improved quality of crops Nitrophenolates enhance active stimulation on all tissues which grow biochemically and invoke seepage into plant tissues thus accelerating metabolism (Francesca *et al.*, 2020). Sodium nitrophenolates has been used in improving quality and yield of different agricultural crops. This results shows that sodium nitrophenolate did not improve the quality of roses in comparission with stems from control plots. This result are consistent with findings by Bala and Sala, (2020) working on hibiscus noted an improvement on growth of the plant, Godlewsk and Ciepiela, (2021) with ryegrass indicated high yield in terms of plant biomass and quality of fodder. According to Pagar *et al.* (2011), Aksona and Unay (2019) nitrophenolates used in cotton improved the production of the crop. Szczepanek *et al.* (2017) showed that carrot plants had increased number of roots and desirable quality grade after treatment with nitrophenolate based biostimulant. However, Przybysz *et al.* (2014) showed that cucumber and Thaliana plants did not increase in height after application of nitrophenolate based biostimulant. Research findings by Poberezny *et al.* (2020) indicated that application of nitrophenolate did not improve yield and quality of carrots. Wierzbowska *et al.* (2017) contends that carrot plants treated with nitrophenolated had no significant difference in quality compared with those grown in ecological system.

Nitrophenolate have been used in increasing the number of tomato fruits (Djanaguiraman *et al.*, 2004). Research by Cerny *et al.* (2002) showed that sodium nitrophenolate compounds improved the production and quality of selected sugar beets. (Amin *et al.*, 2019) pointed out that growth and production of gladiolus improved significantly after application of nitrophenolate mixtures.

Growth of shoot cuttings and stem cuttings were improved significantly compared with the control in citrus (Sitinjak, 2017). Pacholczak *et al.* (2012) points out that application of nitrophenolate improved the growth of two different cultivars of dogwoods. Research findings by Szydlo and Pacholczak, (2010) showed that sodium nitrophenolate enhanced growth and development of hydrangeas, it also increased the vegetative growth of broad been (Tawfeeq, 2012). Potato plants sprayed with nitrophenolate to dripping resulted to high production compared to control (Majeed et al., 2019). Foliar application of sodium nitrophenolate improved the production of carrot roots by 20% compared to the control treatment (Kwiatkowski *et al.*, 2013), it also improved the yield of dry herb of both thyme and basil by increasing productivity (Kwiatkowski, 2011; Kwiatkowski and Juszczak, 2011). Research by Gulluoglu *et al.* (2006) showed that foliar application of nitrophenolates improved the production of soybean crop by increasing pod numbers and grain weight. Yield and quality (weight of 1000 grains) of beans was improved by foliar application of nitrophenolates through double application at higher rate (Szparaga *et al.*, 2019), also it helped in increasing the height of plants and pods, hull counts on the and the grain counts in the husks (Szparaga *et al.*, 2018).

Nitrophenolates are phenolic compounds which helps in metabolism processes such as increase in the indigenous level of auxins, improves growth and development, enhance uptake of nutrients, phototsynthetic, nitrate assimilation, hormonal and antioxidant activities in plants (Djanaguiraman *et al.*, 2010; Valero *et al.*, 2014; Francesca *et al.*, 2020). Application of nitrophenolates improves photosynthetic capability of the plant by lowering resistance of the stomata by enabling an effortless and increased flow of carbon dioxide to the chloroplast, maximizing photochemical efficiency and increasing chlorophyll biosynthesis or reducing its degradation (Przybysz *et al.*, 2014; Kazda *et al.*, 2015). These compounds are also involved in the capture of free radical by

phenolic compounds and reactive oxygen species ravaging through peroxidase activity (Djanaguiraman *et al*., 2010; Szparaga *et al*., 2018).

4.7 Conclusion

Quality of rose stem is a key factor in rose production and trade. Long stems, large flower buds are preferred in the market and fetch better prices. Production of stems with such quality parameters will guarantee sale and better revenue to the organization. Any product with the ability to improve quality of rose crop will be welcome by rose growers.

In the current study, the results obtained shows that application of bacillus *Bacillus* spp and sodium nitrophenolate applied to the rose bushes improved the quality of roses in terms of stem length, bud diameter and lengths over time. Results shows that the test products have effect on quality parameters and production of more stems on rose crop. Rose growers should incoperate *Bacillus subtilis, B. amyloliquefaciens* and sodium nitrophenolate in the crop production system to help in improving the quality of the crop.

CHAPTER FIVE:

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 General discussion

This study showed that powdery mildew of roses is controlled by foliar application of *Bacillus* spp and sodium nitrophenolate. It is also evident that application of *Bacillus* spp and sodium nitrophenolate improves quality of roses in relation to control. Powdery mildew of roses in greenhouse conditions is managed mainly through application of synthetic fungicides (Scarito *et al.*, 2007). Biological means of managing powdery mildew in greenhouses is gaining popularity in the current days (Singh *et al.*, 2017). According to Ntushelo *et al.* (2019) growth of *Fusarium graminearum* was inhibited by species of *Bacillus* by deploying different modes of action. Miljakovic *et al.* (2020) showed that *Bacillus* spp suppressed several plant pathogens and promoted growth of field and vegetable crops. *In vitro* experiment by Khan *et al.* (2018) showed that strains of *Bacillus* spp, *B. simplex* 30N-5 and *B. subtilis* 30VD-1 and *B. simplex* 11 and 237 inhibited dense hyphal growth of various species of *Fusarium* (*F. oxysporum* f. sp. *conglutinans*, *F. oxysporum* f. sp. *matthioli* and *F. solani*. A strain of B. amyloliquefaciens, CNU114001 inhibited growth of mycelia by 70% of different plant pathogens *Colletotrichum orbiculare, C. acutatum, Fusarium oxysporum, Alternaria panax, Rhizoctonia solani, Pyricularia grisea, Botrytis cinerea, Phytophthora capsici* and *Sclerotinia sclerotiorum* (Ji *et al.*, 2013). Roslan *et al.* (2020) pointed that *Bacillus amyloliquefaciens* strain PEP3 had inhitory effect on spores of *Phytophthora capsici*. Application of *Bacillus subtilis* and *B. amyloliquefaciens* reduced area under disease progress curve (AUDPC) of powdery mildew. Severity and AUDPC of powdery mildew were reduced after application of *Bacillus* spp and *Trichoderma* spp (Sawant *et al.*, 2017; Punja *et al.*, 2019).

Growth of mycelia of *Verticelium dahliea* and *Fusarium oxysporium* of strawberry were reduced *In vitro* by antifungal activity of strain of *Bacillus velezensis* CT32 (Li *et al*., 2020). Species of *Bacillus* achieve phytopathogen reduction through various processes including release of volatile organic compounds (Avendano *et al.*, 2019; Gotor-Vila *et al.*, 2019; Morita et al., 2019; Roslan *et al*., 2020; Li *et al*., 2020), production of antifungal components (Kulimushi *et al*., 2017; Alizadeh *et al*., 2020) release of hydrolytic enzymes and production of siderophore (Khan *et al*., 2018; Alizadeh *et al*., 2020; Andric *et al*., 2020; Roslan *et al*., 2020).

Sodium nitrophenolate has been used in controlling phytopathogens in different crops. Research findings by Glosek-Sobieraj *et al*. (2018) showed that foliar application of nitrophenolate controlled *Phytophthora infestans* in potato. Incidence and severity of early blight and dry rots in potato tubers were significantly reduced after application of sodium nitrohenolate (Cwalina-Ambroziak *et al*., 2015). Nitrophenolates work by inducing tolerance to both abiotic and biotic stress and enhancing nutrient use efficiency (EU, 2019).

From this study, foliar application *Bacillus* spp and sodium nitrophenolate resulted to better quality of flowers compared with control. Longer stem length, flower bud length and larger diameters in relation to shorter stem length and smaller bud sizes compared with the negative control. These findings are in agreement with those of (Saxena et al., 2019; Sarhan *et al*., 2020; Getahun *et al*., 2020; Miljakovic *et al*., 2020) who pointed out that application *Bacillus* spp improved growth and yield of different plants. Banana plants treated with *Bacillus* spp had improved production (Dadrasnia *et al*., 2020).

Bacillus spp promote growth of plants through nitrogen fixation, production of phytohormone and siderophores, solubilization and mineralization of phosphorous, production of antimicrobial

compounds and release of enzymes, inducing tolerance to abiotic and biotic stress (Goswami *et al.*, 2016; Andric *et al.*, 2020).

This study showed that foliar application of sodium nitrophenolate improved the quality of rose flowers. This results concur with those of (Poberezny *et al.*, 2020) who showed that carrot plants treated with nitrophenolates improved productivity and quality during harvest and in storage. Murawska *et al.* (2017) showed that winter wheat treated with nitrophenolate and other biostimulants had better stem length and root mass compared to the crops from the control. Foliar application of sodium nitrophenolate on rice crop showed an improved yield, taller plants and increased number of pannicles and production tillers (Banful and Attivor, 2017). Aksona and Unay (2019) pointed out that foliar application of sodium nitrophenolate on cotton plants showed improved yield in terms of seed index and boll weight. Root elongation in *Hibiscus* spp was experienced after treatment with nitrophenolate (Bala and Sala, 2020). *Calendula officinalis* plants sprayed with nitrophenolates showed better vegetative growth compared to plants from the control area (Swaefy and El-Ziaty, 2017). Nitrophenolates promote plants growth through induction of tolerance and nutrient use efficiency (Francesca *et al.*, 2020).

5.2 Conclusion

From this experiment, foliar application of *Bacillus subtilis*, *B. amyloliquefaciens* and sodium nitrophenolate reduced incidence and severity of powdery mildew in roses. Application of *Bacillus amyloliquefacens* registered highest level of reduction in the first experiment. In the second experiment, application of *Bacillus amyloliquefacins* at different rates and at various intervals also reduced incidence and severity of powdery mildew of roses. Foliar application these test products in both first and second experiments improved the quality of rose crop. This was indicated by longer stem length, bud length and bud diameter and increased number of sellable stems compared

to the negative control. From the results, rose growers should use *Bacillus* spp and sodium nitrophenolate in the management programme for powdery mildew of roses as this help in controlling the disease as well improve the quality of the crop.

5.3 Recommendations

i. Growers of roses should be encouraged to use *Bacillus amyloliquefaciens* and sodium nitrophenolate to manage powdery mildew in the crop.

ii. Further research should be done to determine the optimal rate of application of *Bacillus amyloliquefaciens* and sodium nitrophenolate for effective management of powdery mildew.

iii. Further research to be conducted to determine how exactly sodium nitrophenolate induce resistant to various crop against phytopathogens.

iv. Further research should be carried out to establish how *Bacillus* spp and sodium nitrophenolate increases the yield and improves quality of crops.

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