BIOLOGICAL CONTROL OF ROOT-KNOT NEMATODES (*Meloidogyne* spp.) IN COMMON BEAN USING *Bacillus* spp.

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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This thesis is submitted with our approval as university supervisors.

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DEDICATION

To my parents, Mrs. Milcah Njeri Macharia and the late Mzee Joseph Macharia

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ABSTRACT

Bacillus spp. were isolated from the surface of healthy bean roots. Greenhouse experiments were conducted to determine the effect of *Bacillus* spp. on root-knot nematodes and plant growth. Bean plants were treated with *Bacillus* isolates and nematodes and data on galling, egg mass and biomas were recorded. The isolates tested had varying (P=0.05) effects, with the majority of them reducing galling in beans. Out of 250 isolates evaluated, 93% reduced galling when compared to the control (water) with 12% of them being were more effective than carbofuran. Twenty percent of the isolates were found to promote plant growth. A laboratory experiment was conducted to assess the effect of culture filtrates *Bacillus* spp on mobility of second-stage *Meloidogyne* juveniles. Culture filtrates of the twenty *Bacillus* isolates tested immobilized more than 80% of the juveniles treated.

Twenty *Bacillus* isolates were selected due to their high activity against root-knot nematodes. The isolates were tested under sterile and non-sterile soil conditions. Galling and egg mass indices were lower in sterile soil than in non-sterile soil conditions. Fewer juveniles were recovered from sterile soil than in non-sterile soil.

The effect of selected *Bacillus* isolates on nodulation in beans was determined using nitrogen-free sterile sand in the greenhouse. Nodulation was variably (P=0.05) affected by treatment of bean plants with *Bacillus* spp. Four out of 20 selected isolates promoted nodulation, eleven isolates had no effect while six isolates suppressed nodulation. Growth promotion was confirmed in 17 out of the 20 *Bacillus* isolates selected from the preliminary trial. A greenhouse test to assess the effect of combining *Bacillus* and *Rhizobium* spp. on root-knot nematode showed varied reactions. Galling index was relatively lower in plant treated with some *Bacillus* and the rhizobia inoculants. The lowest galling was recorded in

plant treated with *Bacillus* isolate K194 with a mixture of *Rhizobium leguminosarum biovar phaseoli* and *R. tropici.*

Selected carriers (ondiri peat, filter mud, cow manure, charcoal dust, and compost) and adhesives (methylcellulose, sugar and gum arabic) were evaluated on their ability to support high *Bacillus* populations during their application in nematode control. Charcoal dust as a carrier mixed with *Bacillus* isolates was the most effective in nematode control, while cow manure was the least effective. Application of *Bacillus* isolates using carriers resulted significant (P=0.05) increased shoot and root weight as compared with the controls. Galling index was low in plants treated with *Bacillus* isolates using gum arabic and sugar as adhesives.

The potential *Bacillus* spp. as biocontrol agents of root-knot nematode, *Meloidogyne* spp. and its ability to promote plant growth was demonstrated under greenhouse conditions. These results show that *Bacillus* spp. can be incorporated in nematode management package. Application of *Bacillus* spp. in root-knot nematode management would therefore have an added advantage of enhancing nitrogen supply. Further work to determine the effect of these isolates under field condition should be carried out.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Bean Production in Kenya

Common bean (*Phaseolus vulgaris* L.) is the most important legume, second only to maize as a food crop in Kenya (Stroetzer, 1981; Wortmann and Allen, 1994; Gethi *et al.*, 1997). The crop is grown under a wide range of environmental conditions mainly between 900-2700 metres above sea level (Acland, 1971). Beans do not grow well below 600 metres above sea level due to high temperatures which affect the pod filling process (Stoetzer, 1981). Beans are primarily grown by small-hold growers, mainly intercropped with other crops such as maize, coffee, bananas, sorghum, millet, potatoes and cassava (Anon, 1990; Wortmann *et al.*, 1998). The crop is cultivated on an estimated 700,000 ha mainly in Eastern, Rift valley, Western, Central and Nyanza provinces as shown in table 1 (Anon, 1996).

Table 1. Acreage and yield of common bean in five major producing provinces in Kenya.

Province	Area (ha)	Output (tones)	Yield (ton/ha)
Eastern	205,700	110,000	0.5
Rift valley	188,270	144,790	0.8
Western	108,900	60,830	0.6
Central	89,600	43,820	0.5
Nyanza	72,740	64,570	0.9

Source: Anonymous, 1996.

1.2 Major constraints to bean production

Although there has been an increase in bean production, resulting from an extension of cultivation into marginal areas, productivity per unit area has continued to decline (Nderitu *et al.*, 1997). In Kenya, bean yields are low (750 kg/ha) under monocrop and even lower (375kg/ha) in mixed cropping systems against a potential of 1500-2000kg/ha (Rheenen *et al.*, 1981; Wortmann *et al.*, 1998). The principal constraints to higher bean yields include diseases, low soil fertility, insect pests and low and erratic rainfall (Allen, 1983; Nderitu *et al.*, 1997; Otsyula *et al.*, 1998).

The most important bean diseases in Kenya include anthracnose (*Colletotrichum lindemuthianum,* Corda), angular leaf spot (*Phaeoisariopsis griseola,* Ferraris), bean rust (*Uromyces appendiculatus,* Link & Unger), halo blight (*Pseudomonas syringae pv. Phaseolicola,* (Burkholder) Young, Dye & Wilke), common bacterial blight (*Xanthomonas campestris pv. phaseoli*, Smith), bean common mosaic virus (BCMV), root rots (caused by *Fusarium* spp., *Pythium* spp. and *Rhizoctonia* spp.) and root-knot nematode, *Meloidogyne* spp. (Ngundo and Taylor, 1974; Mukunya and Keya, 1975; Allen *et al.*, 1996; Nderitu *et al.*, 1997).

Common bean is attacked by a wide range of plant parasitic nematodes but only *Meloidogyne* spp. are of economic importance (Ngundo and Taylor, 1974, Kimenju *et al.*, 1999). Yield losses of up to 60% have been recorded in beans on fields heavily infested with root-knot nematodes (Ngundo and Taylor, 1974). Apart from being plant pathogenic, plant parasitic nematodes also act as wounding agents and break host resistance to other plant pathogens particularly soilborne (Hussey and McGuire, 1987; Mai and Abawi, 1987; France and Abawi, 1994). Nodulation potential of leguminous plants is adversely affected by root-knot nematode

fection, interfering with biological nitrogen fixation (Mani and Seth, 1987; Karanja, 1988; harma and Khurana, 1991; Abd-El-Samie *et al.,* 1993; Siddiqui and Mahmood, 1994).

.3 Control of Nematodes in Beans

'arious strategies including nematicides, cultural practices, organic amendments and esistant varieties have been developed for the management of root-knot nematode Rodriquez–Kabana, 1986; Sikora, 1992; Oka *et al.*, 1993; Sharma *et al.*, 1994; Bridge, 996). However, their adoption by small-hold bean growers is limited. Nematicides for nstance are too expensive and hence not affordable by small-scale bean producers (Hague and Gowen, 1987; Becker *et al.*, 1988; Oka *et al.*, 1993). Cultural practices such as fallowing and crop rotation are not practical due to scarcity of arable land and are ineffective due to the broad host range of root-knot nematodes. Although use of resistant cultivars is recognized as the cheapest and the most practical strategy of managing nematodes on low value crops such as beans, the strategy is limited due to unavailability of resistant bean germplasm. Organic amendments have successfully been used in the control of nematodes (Rodriquez-Kabana, 1986; Sikora, 1992). Despite the efficacy of organic amendments in nematode control, wide spread use of this strategy is limited by the large quantities needed for effective control (Luc *et al.*, 1990; Oka *et al.*, 1993). There is urgent need to develop other methods of managing root-knot nematode. Biological control is a viable alternative strategy in nematode management (Becker *et al.*, 1988; Bridge, 1996; Sikora, 1997; Hallmann *et al.*, 1998).

1.4 Biological control of nematodes

Control of soil borne pathogens especially nematodes has been achieved by use of their natural enemies residing in the soil (Stirling *et al.*, 1979; Rovira and Wildermith, 1981). Several fungi, bacteria and nematophagus nematodes have been used in the control of plant

arasitic nematode (Mankau, 1995). Specific bacteria referred to as plant health promoting izobacteria (PHPR) which reduce plant infection and stimulate plant growth have been idely investigated for practical use (Kloepper and Schroth, 1981). The ability of izobacteria especially *Bacillus* spp. and flourescent *Pseudomonas* to improve plant growth nd/or root health have been demonstrated (Becker *et al.*, 1988; Weller, 1988; Oostendorp nd Sikora, 1990; Bowmann *et al.*, 1993). This may result from improved nutrient uptake, nhanced atmospheric nitrogen fixation, induced disease resistance, competition for nutrient nd/or niches, parasitism or alteration of chemical components of root exudates (Frommel *et l.*, 1991; Tuzun and Ku, 1991; Wei *et al.*, 1991; Sikora, 1992; Sikora and Hoffmann-Hergarten, 1992). *Bacillus* spp. have been reported to affect nodulation in leguminous plants (Bauer, 1981; Holl *et al.*, 1988; Chanway *et al.*, 1991; Srinivasan *et al.*, 1996; Srinivasan *et al.*, 1997). Their use in nematode control could therefore be an added advantage as would lead to increased nodulation.

Oka *et al.*, (1993) demonstrated that integrated control of root-knot nematodes could be achieved through a combination of proteinaceous amendments and *Bacillus cereus* (Frankland and Frankland). Rhizobacteria are particularly desirable biocontrol agents because they are able to colonise plant roots and can be applied as seed treatment making them cost effective (Schroth and Hancock, 1981; Weller, 1988; Sikora, 1991; Sikora, 1995). A lot of investigation on PHPR on deleterious bacteria or fungal pathogens have been carried out, but their utilization for the control of plant parasitic nematodes has received little attention (Schippers, 1988; Racke and Sikora, 1992). There is therefore need to study their effect on root-knot nematodes especially in bean and their effect on nodulation.

5 Objectives

he main objective of the study was to develop a biological strategy based on *Bacillus* spp. r the control of root-knot (*Meloidogyne* spp.) nematodes. The specific objectives of the udy were;

i. To isolate and to screen *Bacillus* spp. for growth promotion and antagonistic effects against root-knot nematodes

ii. To determine the effect of *Bacillus* spp. on nodulation in beans

iii. To evaluate selected *Bacillus* application methods using locally available materials as carriers and adhesives

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History and origin of beans (*Phaseolus vulgaris L.*)

Common beans are thought to have originated from Mexico, Central America, and northern America (Wilsei, 1962; Kaplan, 1965). Spanish travelers are associated with the introduction of beans to Europe from where Portuguese sailors introduced them to eastern Africa in the sixteenth century (Greenway, 1945; Anon, 1975/76). Beans have been grown in Kenya for the last 300 years (Acland, 1971)

2.2 Bean classification

Common beans are also referred to as french beans, kidney beans, haricot beans, salad beans, string bean or frijoles in different areas (Wortmann *et al.*, 1998). Beans are classified under the division Spermatophyta, sub-division Angiospermae, class Dicotylidonae, sub-class Rosidae, family Fabaceae, or Papilionaceae, and genus *Phaseolus* (Holmes, 1986). The genus *Phaseolus* includes about 31 species of which about 10 are cultivated species (Martin, 1984). *Phaseolus vulgaris* is the best known and most widely cultivated species of the genus *Phaseolus* (Allen, 1983). Common beans are annual or perennial, twinning or bushy herbs with large trifoliate leaves, stipules and stipels, typically papillionaous flower in auxillary or terminal racemes (Purseglove, 1987).

2.3 Importance of beans

Beans are a good source of proteins, calcium, iron, thiamin and riboflavin especially for people who cannot afford expensive animal proteins (Walker, 1982; Jalil, 1977). Total carbohydrates, proteins, fibre and dietary energy are high in beans (Walker, 1982). Beans

also serve as a source of income, with bean crop residues being as livestock feeds and green manure (Nwokolo and Smart, 1996). The crop also improves soil fertility through biological nitrogen fixation (BNF) by rhizobia bacteria (Walker, 1982; Nwokolo and Smart, 1996). Beans are usually consumed with such other high carbohydrate foods like maize, rice, cassava, and bananas which lack some essential amino acids (Wortmann *et al.*, 1998). They are eaten as immature pods, green shelled beans, dry beans and young tender leaves are used as vegetable (Goode, 1987; Purseglove, 1987)

2.4 Bean production constraints

In Kenya, bean yields are low, 750 kg/ha, under monocrop and even lower, 375kg/ha, under mixed cropping systems against a potential of 1500-2000kg/ha (Rheenen *et al.*, 1981). The Low bean yields are attributed to a number of constraints which include poor agronomic practices, low soil fertility, lack of improved cultivars, moisture stress, weed competition and damage caused by insect pest and diseases (Allen *et al.*, 1989; Allen and Edje, 1990; Nderitu *et al.*, 1997). Diseases, low soil fertility and insect pests have been reported to be the main constraints to bean production (Otsyula and Ajanga, 1995; Otsyula *et al.*, 1998)

The most important bean diseases include anthracnose (*Colletotrichum lindemuthianum*), angular leaf spot (*Phaeoisariopsis griseola*), rust (*Uromyces appendiculatus*), halo blight (*Pseudomonas syringae pv. phaseolicola*), common bacterial blight (*Xanthomonas campestris pv. phaseoli*), bean common mosaic virus (BCMV), root rots (*Fusarium* spp, *Pythium* spp., and *Rhizoctonia solani)* and plant parasitic nematodes (Ngundo and Taylor, 1974; Mukunya and Keya, 1975; Allen *et al.*, 1996; Nderitu *et al.*, 1997). Root-knot nematodes cause yield losses of up to 60% on beans grown in fields that were heavily infested (Hollis, 1962; Ngundo and Taylor, 1974).

.5 Plant-parasitic nematodes affecting beans

Many plant parasitic nematodes have been associated with leguminous crops (Goodey *et al.*, 1965; Bridge, 1981; Mani *et al.*, 1982). Root-knot nematodes (*Meloidogyne* spp.), lesion nematode (*Pratylenchus* spp.), *Scutellonema, Helicotylenchus,* stunt nematode (*Tylenchorhynchus* spp.), *Tylenchus* spp., *Criconemella* spp., *Aphelenchus* spp., sheath nematode (*Hemicyclophora* spp.), stubby root nematode (*Trichodorus* spp.) and others are associated with beans (Kimenju *et al.*, 1999). Members of the genera *Meloidogyne, Pratylenchus, Scutellonema* and *Helicotylenchus* are widely distributed in bean growing areas in Kenya (Kimenju *et al.*, 1999). *Meloidogyne* spp. are of considerable importance due to their wide distribution especially in the warm region of the world coupled with their polyphagous nature (Luc *et al.*, 1990).

Root-knot nematodes have been reported to cause considerable beans losses. Hainsworth (1962) reported that 10% of the agricultural produce in Kenya was lost each year through nematode damage, principally from *Meloidogyne* species. Hollis (1962) estimated that in Kenya's small-holding, nematodes cause 50-100% yield loss of food crops including beans, while Ngundo and Taylor (1974) reported up to 60% yield losses in bean fields heavily infested with *Meloidogyne* spp.

Plant parasitic nematodes especially *Meloidogyne* spp. have been found to adversely affect the nodulation potential of leguminous plants (Mani and Seth, 1987; Karanja, 1988; Sharma and Khuran, 1991; Abd-El-Samie *et al.*, 1993; Siddiqui and Mahmood, 1994; Kimenju *et al.*, 1999; Sharma *et al.*, 2000)). They also break plant resistance by creating wounds for other pathogens leading to formation of disease complexes especially with fungal pathogens (Hussey and McGuire, 1987; Mai and Abawi 1987; France and Abawi, 1994). For example, damage by *Macrophomina phaseolina* Petr and *Fusarium solani* f.sp. *phaseoli* Link was

igher when beans were attacked by *M. arenaria* and *M. incognita* Kofoid & White (Al-azmi, 1985; Mani and Seth 1987; Sikora and Greco 1990; Dwivedi *et al.*, 1992).

2.6 Root-knot nematodes (*Meloidogyne* spp.)

2.6.1 Classification of root-knot nematodes (*Meloidogyne spp.*)

Root-knot nematodes belong to the kingdom Animalia, phylum Nematoda; class Nemata; sub-class Sercenentea; order Tylenchida; sub-order Tylenchina; family Heteroderidae and genus *Meloidogyne* (Chitwood, 1956). There are 51 species of *Meloidogyne* (Jepson, 1987), of which *M. incognita* (Kofoid & White) Chitwood, *M. javanica* (Treub) Chitwood, *M. arenaria* and *M. hapla* (Chitwood) are of economic importance in bean production across the world (Luc *et al.*, 1990).

Root-knot nematode populations consist of male and female which are easily distinguished morphologically. The males are worm-like and about 1.2-1.5 mm long by 30-60 µm in diameter, while mature females are pear shaped and about 0.40-1.30mm long by 0.27-0.75 mm in diameter (Sherf and Macnab, 1986; Agrios 1988). Second-stage juveniles are vermiform in shape while third and four stage juveniles are sausage-shaped and microscopic in size (Sherf and Macnab, 1986). *Meloidogyne* spp. are distinguished by use of distinct patterns in mature females which resemble finger prints of human which are referred to as perineal patterns (Williams, 1974; Machon and Hooper 1991).

2.6.2 Symptoms caused by root-knot nematodes

Root-knot nematodes cause both aboveground and underground symptoms. The aboveground symptoms include stunted growth and small, pale green or yellowish leaves which tend to wilt in warm weather (Dropkin, 1980). Flower and pods are usually of poor quality. These

symptoms occur in small patches in the field and are similar to those produced by other soilborne diseases or environmental factor (Agrios, 1988).

The underground symptoms are characterized by reduced and malformed roots with galls (Bird, 1974; Sherf and Macnab, 1986; Agrios, 1988). The malformation of roots leads to decreased efficiency in the absorption of water and nutrients (Melakeberhan *et al.*, 1985; Wilcox and Loria, 1986). Four species *M. incognita, M. javanica, M. hapla* and *M. arenaria* attack common beans (*Phaseolus vulgaris),* lima bean (P. *lunatus*) and black-eyed beans *(Vigna unguinculata*) with lima and black eyed beans being most susceptible (Thomason and Omwega, 1985)

2.6.3 Disease development

The second stage juvenile is the only infective stage (Agrios, 1988). The juvenile enters a root at the region just behind the root tip and move intercellulary or extracellulary to the zone of cell differentiation (Agrios, 1988). The juvenile settle and starts feeding from the cells next to the head by secreting saliva-containing enzyme which dissolve the cell content. Two to three days latter, the cells around the head enlarge, the nuclei start dividing, but no new cell wall is laid down. The wall existing between some cell breaks down and disappears, and the protoplasmic content of the cells coalesce, giving rise to giant cells (Sherf and Macnab, 1986; Agrios, 1988). Each gall contains 3-6 giant cells which are maintained by a continuos stimulus from the nematode but collapses when it ceases to feed (Agrios, 1988). Xylem elements are affected due to mechanical pressure from enlarging cells. Swelling of the root result from hypertrophy and hyperplasia of the vascular parenchyma, pericycle and endodermis cell surrounding the giant cells (Sherf and Macnab, 1986).

Temperature determines the distribution and development of root-knot nematodes. The optimum temperature for *M. incognita*, *M. javanica* and *M. arenaria* which are prevalent in the tropic regions ranges from 25 – 35°C (Agrios, 1988). Distribution of root-knot nematodes is also determined by such soil factors as soil texture and structure (Luc *et al.*, 1990). Soil texture and structure are directly related to water holding capacity and aeration, influencing nematode survival, movement and disease severity (Sikora, 1989). Root-knot nematodes are able to survive and reproduce under a wide range of pH ranging from acid to alkaline (Ferris and Van Gundy, 1979).

2.7 Control of root-knot nematodes

Control strategies should be preventive rather than curative in nature and this is aimed at preventing build up of high population densities (Netcher and Sikora, 1990). Such strategies as crop rotation, soil amendment, soil solarization, host resistance, nematicides and use of natural enemies have been employed with varying degrees of success in nematode control (Katan, 1981; Rodriquez–Kabana, 1986; Mc Kerry, 1987; Sikora, 1992; Oka *et al.*, 1993; Sharma *et al.*, 1994; Bridge, 1996).

2.7.1 Crop rotation

Crop rotation is the most commonly used strategy of controlling nematodes in low-input agricultural systems (McSorley *et al.*, 1994; Bridge, 1996). This could be achieved by alternating non-host, tolerant or resistant crops with a susceptible one (Bridge, 1996). To enhance the efficacy of crop rotation, weeds should be controlled to reduce nematode multiplication in susceptible weeds (Netcher and Sikora, 1990). Crop rotation is, however, limited by the polyphagous nature of the root-knot nematodes (Overman, 1985; Thomason and Caswell, 1987; Noling and Becker, 1994).

2.7.2 Soil amendments

Incorporation of organic amendments into the soil has been shown to reduce densities of root-knot nematodes (Muller and Gooch, 1982; Rodrique-Kabana and Morgan–Jones, 1987; Sikora, 1992; Oka *et al.*, 1993). High organic matter stimulates the activity of indigenous soil micro-organisms some of which are antagonistic to nematodes and their decomposition results in accumulation of compounds with nematicidal effects (Rodriquez–Kabana, 1986; Rodrique-Kabana and Morgan–Jones, 1987; Sayre and Starr, 1988). Apart from suppressing nematode populations, organic amendments also releases nutrient and improve water-holding capacity of soil thereby improving plant growth thus enhancing tolerance to nematode attack (Bridge, 1996). Use of organic amendments in nematode control is limited by the large quantities required for effective management (Rodriquez–Kabana, 1986; Kerry, 1990; Netscher and Sikora, 1990; Oka *et al.*, 1993). In addition, use of organic amendments is largely dependent on availability, as well as the costs incurred in collection and incorporation into the soil (Bridge, 1996).

2.7.3 Soil solarization

Soil solarization has been used with success in the control of nematodes and other soilborne pathogens (Katan, 1981; Gaur and Perry, 1991). Incorporation of solar heated water by drip irrigation has been found to increase its efficiency (Saleh *et al.,* 1988). This is restricted in application because the polythene used is expensive, solar radiation periods in some places are too short and it is not feasible on large scale farms planted with low value crops (Netscher and Sikora, 1990; Gaur and Perry, 1991; Oka *et al.*, 1993).

2.7.4 Host resistance

Use of cultivars that are resistant to nematode infection is thought to be the most practical and cheapest means of nematode control especially in smallholder farming systems (Bridge, 1996). Cultivars with resistance to different species and races of *Meloidogyne* have been selected in beans (*Phaseolus vulgaris*), soya beans (*Glycine max*), peas (*Pisum sativum* L.), pepper (*Capsicum* spp.), tomatoes (*Lycopersicon esculatum*), sweet potatoes (*Ipomea batata*) and cowpeas (*Vigna unguilata*) (Sasser and Kirby, 1979; Netscher and Sikora, 1990). Resistance to *M. incognita* in common beans has been identified and incorporated in bean varieties through interspecific hybridisation (Omwega *et al.*, 1987). Ngundo (1977) reported that Kahuti, Red haricot, Rono saginaw and Kiburu are resistant to *M .incognita* and *M. javanica.* Widespread adoption of this strategy is limited by unavailability of these resistant materials to farmers, resistance breakdown after a few years and low acceptance of some of the resistant cultivars by farmers (Sharma *et al.*, 1994)

2.8 Biological agents in nematode control

Biological control may be defined as the reduction of inoculum or disease producing capacity of a pathogen accomplished through one or more organism other than man (Baker and Cook, 1974). Biological control is achieved through natural enemies of phytonematodes which act through such mechanisms as parasitism, predation, competition, and antibiosis (Stirling, 1991; Sikora, 1992).

Organisms that are antagonistic to nematodes include nematode trapping fungi, pathogenic fungi, parasitic of nematode eggs and cysts, fungi which produce metabolites that are toxic to nematodes, predacious nematodes and parasitic bacteria (Mankau, 1980a; Stirling, 1991; Sikora, 1992). Several symbiotic organisms such as fungi endophytes, verscular- arbuscular

mycorrhizal (VAM) fungi and rhizobacteria are know to protect their host against nematode attack (Hussey and Roncadori, 1982; Sikora, 1992).

Two antagonistic fungi namely, *Verticillium chlamydosporium* (De Leij *et al.*, 1992) and *Peacilomyces lilacinus* (Jatala, 1986), as well as the obligate bacterial *Pasteuria penetrans* (Sayre and Starr, 1988; De Leij *et al.*, 1992) have been intensively studied and have shown promising results as biocontrol agents of nematodes (Oka *et al.*, 1993)

2.8.1 Nematode trapping fungi

Many fungal species have been reported to predate on nematodes in the soil (Sayre, 1971; Mankau, 1980a; Jatala, 1986). The fungi capture nematodes using such structures as adhesive hyphal network, knobs, and branches or constricting rings (Jatala, 1986). Fungistasis in the soil and slow growth makes nematophagous fungi limited in nematode control (Kerry, 1984; Stirling *et al.*, 1979; Mankau, 1980a)

2.8.2 Nematode pathogenic fungi

Unlike the nematode trapping fungi which colonise the soil, these fungi produce spores which remain dormant until they adhere onto a passing nematode after which they germinate, penetrate the cuticle and colonise the host (Barron, 1977). Also some produce spores which have to be ingested by nematodes before infection starts (Barron, 1970). Most reports on this group of fungi show that the group has little potential in nematode control (Boosalis and Mankau, 1965).

2.8.3 Parasites of nematode eggs

Several fungi parasitise eggs of plant parasitic nematodes particularly those of sedentary endoparasitic nematodes whose eggs are aggregated within gelatinous matrix (egg masses) or cysts (Kerry, 1995). *Verticillium chlamydosporium* Goddard and *Peacilomyces lilacinus* have shown promising potential as parasites of the nematode eggs (De Leij and Kerry 1991; Kerry 1995; Al Raddad, 1995). De Leij *et al.* (1992) reported that V. *chlamydosporium* was more effective than aldicarb (nematicide) in the control of *M. incognita*. The egg-parasitizing fungi are, however, limited due to their inability to colonize eggs laid within the root tissue and those in the large egg masses which they seem to be unable to fully colonize (Stirling *et al.*, 1979; De Leij and Kerry, 1991).

2.8.4 *Pasteuria penetrans* (Thorne) Sayre and Starr

Pasteuria penetrans is an obligate bacterial parasite of some plant parasitic nematodes (Mankau, 1980b; Sayre and Starr, 1988; De Leij *et al.*, 1992; De-Channer, 1997). Their spores adhere onto the nematode and germinate by forming germ tubes which penetrate the cuticle where they proliferate, fill the body cavity thus killing the nematode (Sayre and Wergin, 1977; Mankau, 1980b; Kerry, 1987). The main obstacle to widespread use of *P. penetrans* is that being an obligate parasite mass production of inoculum is difficult (Jatala, 1986; Kerry, 1987; Becker *et al.*, 1988).

2.8.5 Rhizobacteria

Bacteria that inhabit the plant rhizospere have been reported to improve plant growth and/or root health (Schroth and Hancock, 1981; Schipper *et al.*, 1987; Sikora, 1988; Weller, 1988; Kloepper *et al.*, 1989). Rhizobacteria have been divided into two groups according to their mode of action; plant growth promoting rhizobacteria (PGPR) and plant health promoting

rhizobacteria (PHPR) (Sikora, 1988, 1990). PGPR represent all beneficial rhizobacteria while PHPR are those bacteria that stimulate plant growth by limiting plant pathogens or parasites (Kloepper and Schroth, 1981; Ordentlich *et al.*, 1987). The delicate nature of the nematode-host relationship as indicated by complex cellular syncytium, hypersensitivity mechanism in resistant cultivars and sex reversal due to adverse environmental conditions favours the possible use of rhizobacteria-induced mechanism for nematode control (Sikora and Hoffmann-Hergarten, 1992).

Antagonistic activity towards cyst and root-knot nematodes has been demonstrated in isolates of rhizobacteria obtained from rhizosphere of different crops (Oostendorp and Sikora, 1986; Becker *et al.*, 1988). Sikora (1988, 1990) reported that approximately 9% of rhizobacteria isolated from sugarbeet roots had plant health promotion activity against plant parasitic nematodes. The level of biological control varies with species present and is influenced by the degrees of microbial competitors, plant species, cultivars and abiotic environmental factors (Sikora and Hoffmann-Hergarten, 1992).

2.8.5.1 Mode of action

The major modes of action include alteration of root exudates, production of toxic metabolites and by reducing the activity of egg hatching factors (Sikora and Hoffmann-Hergarten, 1992). Some bacteria including *Bacillus* spp., flourescent *Pseudomonas* and *Telluria chitinolytica* have been shown to inhibit penetration of nematodes into the roots thereby reducing root galling (Becker *et al.*, 1988; Oostendorp and Sikora, 1990; Bowman *et al.*, 1993). These bacteria may interfere with host identification through receptor blockage on the roots or by modifying root exudates of the host plant, thus hindering the attraction,

hatching or penetration behaviour of the nematodes (Becker *et al.*, 1988; Oostendorp and Sikora 1990; Spiegel *et al.*, 1991).

Several *Bacillus* spp. have been shown to produce toxic or inhibitory metabolites that reduce infection and/or suppress development of plant parasitic nematodes (Sikora and Hoffmann-Hergarten, 1992; Mankau, 1995). The metabolite produced by *Bacillus thuringiensis* Berliner and *B. subtilis* Ehrnberg are known to be toxic to *Meloidogyne* spp. (Walker, 1971: Spiegel *et al.*, 1991; Devidas and Rehberger, 1992). The metabolites produced by *Bacillus* spp. include bacitracin, circulins, polymyxins, tyrocidins and surfactin (Brandbury, 1986). Most of the toxins are produced at the on set of sporulation (Baker and Cook, 1974; Mankau, 1995; Berger *et al.*, 1996).

Presence of *Bacillus* spp. in the rhizosphere is known to modify root exudates thus affecting nematode attraction to or recognition of the host (Oostendorp and Sikora, 1990). Franken *et al.* (1990) demonstrated that attraction of the cyst nematode, *Globodera pallida* to sugar beet tubers was reduced following tuber treatment with *Agrobacterium radiobacter*. Host recognition is controlled by the interaction between root surface lectin and nematode cuticular carbohydrate (Zuckerman, 1983). PHPR may induce biological control by binding lectins which are required in host recognition (Sikora, 1992). Bacterial metabolites are known to reduce or delay hatching of the nematodes eggs (Sikora and Hoffmann-Hergarten, 1992; Lumsder, 1993). *Bacillus sphaericus* Meyer & Neide and *Agrobacterium radiobacter* have been shown to reduce hatching of two species of cyst nematodes (Oostendorp and Sikora, 1989; Racke and Sikora, 1992).

2.8.5.2 Mode of application

Rhizobacteria and especially *Bacillus* have unique advantage as biocontrol agent since they can be pelleted onto seed, applied through drip irrigation systems or directly to transplants (Sikora and Hoffmann-Hergarten, 1992). Oostendorp and Sikora (1989) demonstrated that rhizobacteria could be applied as a seed dress to sugarbeet in the control of cyst nematode. Reduction in root infection ranging from 24-41 % was reported when *Bacillus sphaericus* and *Agrobacterium radiobacter* were used in the control of potato cyst nematode, *Globodera pallida* (Racke and Sikora, 1992). Becker *et al.* (1988) showed that seed treatment with either *Pseudomonas fluorescens* or *Bacillus* spp. reduced nematode infection in cucumber. Seed treatment of carrots, oat and peanut with *Bacillus subtilis* led to increased yield (Broadbent *et al.*, 1987; Turner, 1987).

2.8.5.2.1 Adhesive and Carriers used in *Bacillus* formulation

Several materials such as sucrose, polyvinyl alcohol, and methylcellulose have been used for bacteria adhesion onto seed (Becker *et al.*, 1988; Racke and Sikora, 1992). Methylcellulose applied at the rate of 0.2 percent was found to increase the number of bacteria on seed thus increasing antagonistic activity of the biocontrol agent against nematodes (Becker *et al.*, 1988; Racke and Sikora 1988; Racke and Sikora 1992). Gum arabic was found to be the best protector of cells against biotic and abiotic stress hence increasing survival of bacterial cells on the seed (Rodriguez-Navorro *et al.*, 1991; Undayasuriyan *et al.*, 1996). Daza *et al.* (2000) reported that sucrose as an adhesive and pertite as a carrier, enhanced survival of bacterial cells on seeds.

Other microorganisms such as *Bacillus* spp., *Agrobacterium radiobacter* K-84, *Trichoderma* spp., *Pythium oligandrum,* and *Pseudomonas* spp. were found to be more effect when applied

as seed treatment or root treatment rather than when they were incorporated in the soil by drenching (Baker and Cook, 973; Papavizas and Lumsden, 1981). Direct application of biocontrol agent to seeds or other plant parts gives it competitive advantage over the pathogen (Schroth and Hancock, 1981; Becker *et al.*, 1988; Sikora, 1988; Oostendorp and Sikora, 1989; Racke and Sikora 1992), reduces production cost, and simplified formulation and application (Sikora, 1997)

Integrated management approaches which include use of organic amendments in conjunction with inoculants of antagonistic organism have been found to enhance activity of boicontrol agents (Kokalis-Burelle *et al.*, 1992; Kokalis-Burelle and Rodriguez-Kabana, 1994). Oka *et al.* (1993) showed that integrated control of root-knot nematodes could be achieved through a combination of proteinaceous amendment and *Bacillus cereus*. Soil amendments such as peat, cotton seed cake, bacto peptone, clandosan, polymer gel (xanthan gum or alginate) have been used but with varying effects as carriers for rhizobacteria on nematode control (Kloepper and Schroth, 1981; Oka et al., 1993). Although peptone and clandosan were found to be effective as carrier for *Bacillus* spp., their use is limited due to their unavailability and high cost. Peat and other locally available carrier material tested for use with *rhizobia* have been found to be useful in rhizobacteria formulation (Thompson 1980; Chao and Alexander, 1984; Fravel *et al.*, 1985). Peat was found to considerably increase bacteria population on seed than those in granular formulation (Xi K Stephens and Verma 1996). Charcoal has been used as a better alternative to peat in *Rhizobium* inoculant preparation (Beck, 1991; Somasegaran, 1994). In a different experiment, charcoal-lignite and charcoal-soil mixtures favoured higher plant growth and survival of *Rhizobium* (Jauhri *et al.*, 1989; Pandher *et al.*, 1993). Use of locally available materials as carriers would lead to reduced cost of applying biocontrol agents.

2.9 Interaction between *Bacillus* spp. and *Rhizobium* spp.

Legume plants are known to form nodules on their root resulting from symbiotic association with *Bradyrhizobium*. This process involves a sequential exchange of chemical signals between bacteria and the host plants (Fisher and Long, 1992; Relic *et al.*, 1994; Fellay *et al.*, 1995). Nodulation is affected by microorganisms, which alter the composition and activity of microflora in the rhizosphere (Schroth and Ole Becker, 1990). Several of the microorganisms referred to as nodulation promoting rhizobacteria (NPR) have been identified (Kloepper *et al.*, 1988). They belong to the genera *Azospirillum* (Schmidt *et al.*, 1988), *Pseudomonas* (Bolton *et al.*, 1990), *Streptomyces* (Li and Alexander, 1990) and *Bacillus* (Halverson and Handelsman, 1990). The benefits of these bacteria have been attributed to their ability to produce various compounds, including phytohormones, especially auxin (Schmidt *et al.*, 1988), toxins (Knight and Langstonunkefer, 1988), and antibiotics (Li and Alexander 1990).

Srinivasan *et al.* (1996) demonstrated that co-inoculation of *Rhizobium etli* TAL 182 with *Bacillus* spp. induced root hair proliferation on *Phaseolus vulgaris* and enhanced nodulation due to production indole-acetic acid (IAA) from *Bacillus.* In another study Srinivasan *et al.*, (1997) showed that co-inoculation of *R. etli* TAL 182 with *B. megaterium* S49 resulted in early nodulation of *P. vulgaris* compared to single inoculation with *R. etli.*

2.10 Effect of root-knot nematode infection on nodulation

Several authors have reported an adverse effect of root-knot nematodes on rhizobial nodulation (Mani and Sethi, 1987; Karanja, 1988; Shahid and Chohan, 1993; Siddiqui *et al.*, 1993; Siddiqui and Mahmood, 1994; Nejad and Khan, 1997; Sankaranarayanan and Sundarababu, 1998; Kimenju *et al.*, 1999; Sharma *et al.*, 2000). A reduction in the number of nodules formed was observed in plants inoculated with *M. incognita* (Siddiqui *et al.*, 1993;

Nejad and Khan, 1997; Kimenju *et al.*, 1999). Several mechanism are involved. Root-knot nematodes establish a feeding site in the vascular bundle inside the nodule and induces formation of giant cell which lead to premature senescence of the nodule (Vovlas *et al.*, 1998). Root-knot nematodes also affect nodulation through competition for ecological niches and nutrient, and suppression of lateral root formation, thus reducing sites for nodule formation and early degradation of nodules due to their infection (Taha, 1993)

This shows clearly that *Bacillus* spp. have variable effects on nodulation. Studies on the effect of *Bacillus* spp. on both nodulation and nematode control have received little attention, hence need to address the possibility of using *Bacillus* spp. to promote nodulation and to improve plant health through nematode control.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Isolation, culture and identification of *Bacillus* spp.

Bacillus spp. were isolated from the roots of healthy bean plants grown in soil collected from Kiambu, Machakos and Thika districts. The soil was placed in 15-cm-diameter pots and sown with bean cv. GLP-2 seeds in the greenhouse. Isolation of *Bacillus* spp. from bean roots was done using the procedure by Racke and Sikora (1992). Healthy bean roots were carefully removed from pots and transferred to the laboratory in a cool box. The roots were washed free from adhering soil using tap water, rinsed five times with sterile distilled water and blotted dry using sterile paper towels. The roots were cut into 2-cm segments using a flamed scalpel blade. The root segments were thereafter rinsed in sterile 0.02M potassium phosphate buffer (pH 7.0) and crushed using a mortar and pestle. The homogenate was heat treated for 10 minutes at 80°C to eliminate non-spore forming bacteria as described by Tuitert *et al.*, (1998). Serial dilutions of the homogenate were made and 0.1ml of 10^{-3}, 10^{-4},10^{-5},and 10^{-6} dilutions were plated on nutrient agar (Becker *et al.*, 1988). *Bacillus* isolates were identified using cultural characteristics such as colony appearance, form, colour, and shape of the bacteria cell as outlined by Claus and Berkeley (1986). Colonies resembling those of *Bacillus* spp. were purified before being stored in sterile soil in universal bottles.

3.2 Characterization of *Bacillus* isolates

Characterisation and further identification of *Bacillus* isolate to genus level was done using methods outlined by Claus and Berkeley (1986). The tests done included production of catalyse, hydrolysis of gelatin, hydrolysis of starch, reduction of nitrate, growth in sodium chloride, acid production from carbohydrate and ability to form endospores.

3.2.1 Production of catalyse

Bacillus isolates were grown on nutrient agar slant for 48 hrs. The cultures were flooded with 0.5ml of 10% hydrogen peroxide. The lid was replaced immediately to avoid dispersal of aerosol and observed for gas production.

3.2.2 Gelatin hydrolysis

Tubes containing nutrient agar supplemented with 0.4% gelatin were stab inoculated with each isolate. Cultures were then incubated at 27°C and observated for gelatin liquefaction at 3 days intervals for a period of 4 weeks.

3.2.3 Starch hydrolysis

Duplicate plates containing starch agar medium were inoculated with each test isolate by streaking and incubated at 27°C for 5 days. The cultures were flooded with lugol's iodine and observed for starch hydrolysis.

3.2.4 Nitrate reduction test

Nutrient broth containing 0.1% KNO_3 was prepared and 15 ml of the media was dispensed into test tubes. After sterilization, the tubes were inoculated with a loopful of each isolate. The tubes were incubated for 7 days at 27°C. Three drops of iodine solution were added into the tubes followed by similar amounts of hydrochloric acid solution. The resulting colour changes were observed.

3.2.5 Growth in sodium chloride

Plates of nutrient agar containing 2%, 5%, 7% and 10% sodium chloride were inoculated with each of the test cultures. Cultures were then incubated at room temperature and observed for bacterial growth after 5 days.

3.2.6 Utilization of carbohydrates

The isolates were evaluated for their ability to utilize different carbohydrates using the method by Hayward (1964). Nutrient broth was used as the basal medium supplemented with 5% glucose, sucrose, maltose, arabinose, xylose, mannitol, cellobiose, dulcitol or sorbital. The pH of the medium was then adjusted to 7.0. Bromothymol Blue was added as an indicator. The medium was then dispensed into universal bottles before being autoclaved at 121°C for 15 minutes. A loopful of each test isolate was used to inoculate the media in universal bottles before incubation at 27°C and observated after 14 days. Utilization of a given carbohydrate was positive when the medium gradually turned yellow from the top downward. Control bottles not stub inoculated with *Bacillus* isolate remained olivaceous green in colour.

3.2.7 Endospore formation test

a) Heat tolerance test (Heat test for endospores)

The test was aimed at killing all the vegetative cells leaving the endospores which tolerate high temperatures (Tuitert *et al.*, 1998). Two to three ml of a turbid bacterial suspension was heated in a test tube at 80°C for 30minutes, in a water bath. The bacterial suspension was then streaked on nutrient agar and incubated at 27°C. Observations were made 24-48hr later. Growth indicated endospore formation.

b) Staining for endospore formation

A loopful of bacterial suspension was smeared onto a microscope slide and then air dry. The smear was fixed by passing the slide through a flame 20 times. The smear was stained for 10 minutes using saturated malachite green (10g/100ml). After gently washing with running tap water, the smear was counter stained with 0.25% safranin for 15-30 seconds. The slide was

then washed, blotted dry, and examined under a microscope. Endospores appear bright green within pale-pink cells

3.2.8 Gram stain reaction

Twenty-four hour old cultures of *Bacillus* species were grown on nutrient agar were used in this test. A diluted bacterial suspension was made in a drop of sterile distilled water. The suspension was spread thinly on a clean microscope slide and air-dried. The smear was fixed by passing the slide, 2-3 times, through a flame. The smears were stained following the procedure described by Bergy (1974) and examined under microscope, using 100 objective (oil immersion lens) for gram-reaction. Gram-negative bacteria stain pink-red while gram-positive bacteria stain blue-violet.

3.2.9 Potassium hydroxide solubility test (KOH solubility test)

A loopful of bacteria was mixed with a drop of 3% KOH aqueous solution for 10 seconds on a microscope slide. A wire loop was raised a few centimeters from the mixed solution. Gram-negative bacteria form a viscid strand while gram-positive dissolve and do not form any strand on raising the loop.

3.2.10. Identification of *Bacillus* isolates to species level

The *Bacillus* isolates were sent to the Institut fur Pflanzenkrankheiten der Universitat Bonn, Phytomedizin in Bodenokosystemen, Germany for identification to species level.

3.3 Multiplication of root-knot nematodes (*Meloidogyne* spp.)

Root-knot nematodes were multiplied on tomato cv. Moneymaker plants in the greenhouse. Thirty-centimeter diameter pots were filled with steam sterilized potting mixture and four-

week old tomato seedlings transplanted into them. Nematode inocolum was obtained from galled tomato root using the technique described by Husseys and Baker (1973). One week after transplanting, 10ml of nematode suspension containing 6000 eggs and juveniles were pipetted into the root zone of the tomato seedling. The nematodes were allowed to multiply in the plant for eight weeks.

3.4 Preparation of *Meloidogyne* juveniles

Second stage juveniles were obtained for galled tomato roots using the method described by Omwega *et al.*, (1988). The roots were washed free of soil using tap water and then rinsed using distilled water. The roots were immersed in sterile tap water and aerated using an aquarium pump. Second stage juveniles were obtained in about 5-10 days.

3.5 Effects of *Bacillus* spp. on root-knot nematodes and plant growth

A greenhouse experiment was conducted to determine the effect of *Bacillus* spp. on root galling and root-knot nematode population build up in beans. *Bacillus* isolates were grown on nutrient agar at 27°C. The bacteria were harvested by flooding the plates with sterile distilled water and scrapping off the bacterial colonies using a sterile glass rod. The concentration of the *Bacillus* suspension was adjusted to ca. 10^9cfu per ml.

Eggs and juveniles of *Meloidogyne* spp. were extracted from galled tomato roots following the method by Hussey and Barker (1973). The roots were washed free of soil and then cut into segments of about 1-cm in length. The segments were macerated using a warring blender for 15 seconds at high speed. The macerate was vigorously shaken in 0.5% sodium hypochlorite for 3 minutes and then sieved through a 2mm aperture sieve placed above a

45µm aperture sieve. The eggs and second stage juveniles were collected by back washing the residue on the 45µm aperture sieve into a beaker.

Clean sand was placed in Leonard jars (Appendix 1) and steam sterilized for 1 hour. Three bean seeds were sown in each jar but thinned at emergence to leave 1 seedlings per jar. The plants were inoculated by pipetting 2ml of the bacterial suspension adjusted to 10^9 cfu/ml and 10ml of a nematode suspension containing 500 eggs/ml into each jar at emergence. Control jars were treated with nematodes as above and carbofuran (nematicide) at the rate of 1g per jar or 2ml of sterile distilled water. The plants were raised using nitrogen free nutrient solution (Appendix 2). Due to large number of isolates to be screened, lack of greenhouse space and other equipments, evaluation of *Bacillus* isolates was carried out in three separate experiments. The treatments were arranged in a completely randomized design with eight replications. Plants were uprooted eight weeks after inoculation and root were washed free of sand using tap water. Galling and egg mass indices were rated using a scale of 1-9 where 1= no of gall, 2=1-5, 3=6-10, 4=11-20, 5=21-30, 6=31-50, 7= 51-70, 8=71-100, 9=>100 galls per plant (Sharma *et al.*, 1994). Damage index was the percentage of infected root which was converted using a scale of 1-10 described by Bridge and Sasser (1980). Second stage juvenile (J_2) were extracted from $200cm^3$ soil using the sieving and filtration technique (Hooper, 1990). Plant growth assessment was done using dry shoot and root weight. Dry weight was determine after drying the root and shoot in an oven at 80°C for three days. Atleast six of the most effective isolates were selected from each experiment according to their ability to suppress nematodes as compared to carbofuran. A total of 20 isolates were selected from the different experiments. The experiment was repeated once.

3.6 Evaluation of selected *Bacillus* isolates against root-knot nematodes, under sterile and non-sterile soil conditions

This experiment was conducted to compare the effect of *Bacillus* isolates on root-knot nematodes under sterile and non-sterile soil conditions. Twenty of the most effective *Bacillus* isolates were selected from the 250 isolates screened. Ten-cm-diameter pots were filled with sterile and non-sterile sandy loam soil. The soil was sown with three bean cv. GLP-2 seeds. Each pot was infected with 2ml of *Bacillus* suspension (ca. 10^9 cfu/ml) and 10ml of nematode egg suspension at the rate of 500-eggs/ml. Controls plant were treated with carbofuran or sterile distilled water. Treatments were arranged in a completely randomized design with eight replications. Effect of *Bacillus* isolate on root-knot nematodes was assessed by determining galling and egg mass indices and second stage juvenile (J_2) count as described in section 3.5.

3.7 Effect of culture filtrates (CF) of *Bacillus* spp. on mobility of *Meloidogyne* juveniles

The aim of this experiment was to determine whether the mode of action of the *Bacillus* spp. on root-knot nematodes is by production of toxic extracellular metabolites. Culture filtrates were produced by growing each bacterial isolate in shaken liquid culture. One hundred milimetres into 250ml Erlenmeyer flasks and autoclaved at 121°C (1.5 bar) for 15 minutes. The broth was allowed to cool to about 40°C before a loopful of a 24 hours-old bacteria growth was transferred into each flask and incubated at 27°C for 5 days. Control flasks were not inoculated with bacteria. Treatments were arranged in a complete randomized design on a rotary shaker set at 120rpm. Bacterial cells were separated from the medium by centrifugation at 5000g for 10 minutes. The supernatant was filtered through a cellulose triacetate filter (0.2μm) before use. One ml of nematode suspension containing 1000 *Meloidogyne* juveniles, was pipetted into 9ml of culture filtrate and incubated at room

temperature for 24 hours. After agitating the suspension to evenly distribute the nematodes 1 ml aliquot was drawn and pipetted into a counting slide. Nematode juveniles were observed under a compound microscope and those which showed no movement (immobilized) and lay straight or slightly curved were considered paralyzed. Toxicity of the culture filtrate was determined by counting the number of immobile juveniles out of 100 observed.

3.8 Effect of *Bacillus* spp. on nodulation in bean plants

This experiment was conducted to determine whether *Bacillus* isolates with antagonistic effect on nematodes had any effect on nodulation. Twenty *Bacillus* isolates, selected based on their ability to suppress nematodes were used in this experiment. *Rhizobium tropici* (CIAT 899) and *Rhizobium leguminosarum biovar phaseoli* (USDA 2674) were used individually and in mixtures. The rhizobia isolates were obtained from MIRCEN (Microbial Resource Center) laboratory in the department of Soil Science University of Nairobi.

Clean sand was placed in Leonard jars (Appendix 1) and steam sterilized for 1 hr. Three bean seeds were sown into each jar but thinned at emergence to leave one seedling per jar. The treatments included plants inoculated with 2ml of *Bacillus* isolates (10^9) together with 1ml individual *Rhizobium* strains (10^9) and plants inoculated with *Bacillus* together with *Rhizobium* mixtures. Control plants were inoculated with either *Bacillus* isolates alone, *Rhizobium* alone or sterile distilled water. Treatments were:-

1. *Rhizobium tropici* + selected *Bacillus* isolates
2. *Rhizobium leguminosarum biovar phaseoli* + selected *Bacillus* isolates
3. Mixture of *R. tropici* and *R. leguminosarum biovar phaseoli* + selected isolates of *Bacillus* spp.
4. *Rhizobium tropici* alone

5. *Rhizobium leguminosarum biovar phaseoli* alone
6. Mixture of *R. tropici* and *R. leguminosarum biovar phaseoli* only
7. Control 1 (carbofuran)
8. Control 2 (sterile distilled water)

Plants were fertilized with a nitrogen-free nutrient solution (Appendix 2). The treatments were arranged in a completely randomized design with five replications. The experiment was terminated 45 days after inoculation. Data on number of nodules, nodule weight and shoot dry weight were recorded.

3.9 Effect of combining *Bacillus* spp. and *Rhizobium* bean inoculant on root-knot nematodes

The aim of this experiment was to determine the effect of combining *Rhizobium* spp and *Bacillus* spp. on nodulation and protection of bean root against root-knot nematodes. Seven *Bacillus* isolates, selected from the experiment on nodulation in section 3.8 were used. Sand was sterilized in Leonard jars and sown with bean cv. GLP-2 seeds. At emergence, the seedlings were inoculated with nematodes as outlined in section 3.6. *Rhizobium* spp. and *Bacillus* spp. were applied at the same time but separately. Treatments were:-

1. *Bacillus* alone
2. *Bacillus* isolate + *Rhizobium tropici*
3. *Bacillus* isolate + *Rhizobium leguminosarum biovar phaseoli*
4. *Bacillus* isolate + mixture of the two *Rhizobium* strains
5. *Rhizobium tropici* alone
6. *Rhizobium leguminosarum biovar phaseoli* alone
7. Mixture of *R. tropici* and R. *leguminosarum biovar phaseoli* only
8. Control 1 (Carbofuran)

9. Control 2 (sterile distilled water).

The plants were fertilized with nitrogen-free nutrient solution (Appendix 2). The treatments were arranged in a completely randomized design with eight replications. The experiment was terminated 45 days after inoculation. Data on gall numbers, number of nodules, Juvenile counts and dry shoot weight was collected.

3.10 Effect of applying *Bacillus* spp using locally available material as carriers on root-knot nematode control

This experiment was carried out to determine the best locally available carrier for *Bacillus* spp. Five *Bacillus* isolates selected in experiment 3.5 above were used in the evaluation of locally available carriers. The carriers were expected to provide food and support for *Bacillus* isolates making them easy to handle and apply. The following materials were evaluated; Ondiri peat, filter mud, cow manure charcoal dust and compost. The carriers were dried, crushed and sieved through 2-mm-sieve. The C:N ratio of the carrier material was determined (Appendix 3) before they were sterilized at 121°C (1.5bars) for three hours and repeated once after a day. *Bacillus* isolates were grown on nutrient agar at 27°C for 48 hours. The *Bacillus* were harvested by flooding the plates with sterile distilled water and the bacterial colonies were scrapped off using a sterile glass rod. The sterilized carriers were impregnated with *Bacillus* isolates before being incorporated into the soil at the rate of 0.25ml per gram of the carrier by injecting using a syringe as described by Oka *et al.*, 1993.

Sterile sandy loam soil was infested with nematodes at the rate of 1000 eggs per 75 g soil. The soil was then were mixed thoroughly with *Bacillus* infested carriers. This mixture was placed into 10-cm-diameter pots and sown two bean seeds cv. GLP-2. The treatment were:-

1. Ondiri peat +*Bacillus* isolates
2. Filter mud +*Bacillus* isolates
3. Cow manure +*Bacillus* isolates
4. Charcoal dust +*Bacillus* isolates
5. Compost +*Bacillus* isolates
6. Drenching with *Bacillus*
7. Carbofuran (Control-1)
8. Sterile distilled water (Cotrol-2)

Treatments were arranged in a completely randomized design with ten replications. Data were collected as outlined in section 3.5.

3.11 Evaluation of different material as adhesive of *Bacillus* in root-knot nematode control

This experiment was carried out to determine the best adhesive which would support *Bacillus* adhesion on seeds. Five potential *Bacillus* isolates were used with three adhesives; sugar, gum arabic and methylcellulose. Sugar and gum arabic were used at the rate of 2% while methylcellulose was at 0.2% as described by Racke and Sikora, (1992). Bacteria suspensions were mixed with the adhesives at the ratio of 1:1 (v/v) before being applied onto seeds. The seeds were then dried overnight under sterile airflow and planted in 10-cm-diameter pots filled with sterile loam soil after 24hr. The treatments were;-

1. Sugar used as *Bacillus* adhesive
2. Gum arabic used as *Bacillus* adhesive

3. Methyl cellulose used as *Bacillus* adhesive
4. *Bacillus* in water through drenching
5. Control 1 (Carbofuran)
6. Control 2 (Sterile distilled water)

All plants were inoculated with root-knot nematodes as described in section 3.5. Treatments were arranged in a completely randomized design with ten replications. Data was collected as explained in section 3.5.

Data analysis

Data were subjected to analysis of variance using gentast statistical package and means were separated using the least significant difference test (LSD P=0.05).

CHAPTER FOUR

4.0 RESULTS

4.1 Isolation and characterization of *Bacillus* spp.

4.1.1 Cultural characteristics

The over 300 *Bacillus* strains that were isolated from the rhizosphere of healthy bean roots were heat stable, formed endospore with irregular margins and were varied in colour. Twenty *Bacillus* isolates reduced root-knot nematode populations. The twenty isolates had varying colony characteristics when grown on nutrient agar (Table 2). The colonies were dull or shiny in appearance while some were thick and opaque. Some *Bacillus* isolates attached strongly on the media but some did not. The colour of the colonies ranged from creamy to white. The bacterial cells were rod-shaped and straight.

4.1.2 Biochemical characteristics

Twenty *Bacillus* isolates, selected based on their effectiveness in controlling root-knot nematodes, tested positive to most of the biochemical tests indicated in table 3, except for isolate K34. All the *Bacillus* isolates tested positive for gram reaction, KOH solubility test, Mobility, endospore formation, gelatin hydrolysis, catalyse production, reduction of nitrate and NaCl tolerance (Table 3). All the *Bacillus* isolates hydrolysed starch with exception of isolate K34 while with the exception of K51 all isolates utilized carbohydrate.

Table 2: Colony characteristics of 48hr old cultures of *Bacillus* isolates grown on nutrient agar after at 27°C.

Bacillus isolates	Colour	Colony appearance	Margin	Form or shape	Elevation
K9	Creamy	Dull	Lobate	Irregular	Flat
K33	Creamy	Dull	Lobate	Irregular	Flat
K34	White	Shiny	Entire	Circular	Raised
K48	Creamy	Shiny	Filamentous	Filamentous	Flat
K51	White	Dull	Erose	Irregular	Flat
K66	Creamy	Shiny	Entire	Circular	Convex
K67	Creamy	Dull	Filamentous	Filamentous	Convex
K78	White	Dull	Filamentous	Irregular	Flat
K86	Creamy	Shiny	Lobate	Irregular	Raised
K89	Creamy	Shiny	Filamentous	Filamentous	Raised
K100	White	Dull	Filamentous	Irregular	Flat
K158	Creamy	Shiny	Undulate	Irregular	Convex
K194	White	Dull	Filamentous	Irregular	Flat
K227	White	Dull	Filamentous	Filamentous	Flat
K228	Creamy	Dull	Lobate	Irregular	Raised
K236	White	Dull	Filamentous	Filamentous	Flat
K269	White	Dull	Filamentous	Filamentous	Raised
K270	Creamy	Shiny	Undulate	Irregular	Raised
K273	Creamy	Dull	Erose	Irregular	Raised
CB4	White	Dull	Undulate	Irregular	Raised

Table 3: Biochemical characteristics of twenty *Bacillus* isolates

Bacillus isolates	Gram stain	KOH solubility test	Mobility	Endospore formation	Levan production	Gelatin hydrolysis	Catalyse production	Starch hydrolysis	Reduction of nitrate	NaCl tolerance	Utilization of CHO (Aerobic)	Utilization of CHO -anaerobic
K9	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
K33	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
K34	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve
K48	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve
K51	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve
K66	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve
K67	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve
K78	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
K86	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
K89	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
K100	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
K158	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve
K194	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
K227	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
K228	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve
K236	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
K269	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
K270	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
K273	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
CB4	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

Based on the above observation the isolates were identified as species belonging to the genus *Bacillus* and to the species *Bacillus subtilis* except isolate K34 which was identified as a member of *Salmonella cholerauesuis* (Table 4)

Table 4: Result of identification of *Bacillus* isolates

Bacillus isolates	*Bacillus* spp.	*Bacillus* group
K9	*Bacillus amyloliquefaciens*	*Bacillus subtilis* group
K33	*Bacillus amyloliquefaciens*	*Bacillus subtilis* group
K34	*Salmonella choleraauesuis*	*Salmonella*
K48	*Bacillus amyloliquefaciens*	*Bacillus subtilis* group
K51	*PaeniBacillus macerans*	*Bacillus subtilis* group
K66	*Bacillus amyloliquefaciens*	*Bacillus subtilis* group
K67	*Bacillus amyloliquefaciens*	*Bacillus subtilis* group
K78	*PaeniBacillus macerans*	*Bacillus subtilis* group
K86	*PaeniBacillus macerans*	*Bacillus subtilis group*
K89	*PaeniBacillus macerans*	*Bacillus subtilis* group
K100	*PaeniBacillus macerans*	*Bacillus subtilis group*
K158	*Bacillus amyloliquefaciens*	*Bacillus subtilis* group
K194	*PaeniBacillus macerans*	*Bacillus subtilis* group
K227	*PaeniBacillus macerans*	*Bacillus subtilis* group
K228	*Bacillus amyloliquefaciens*	*Bacillus subtilis* group
K236	*PaeniBacillus macerans*	*Bacillus subtilis* group
K269	*PaeniBacillus macerans*	*Bacillus subtilis* group
K270	*Bacillus amyloliquefaciens*	*Bacillus subtilis* group
K273	*Bacillus amyloliquefaciens*	*Bacillus subtilis* group

4.2 Effects of *Bacillus* spp. on root-knot nematodes

Out of the 250 *Bacillus* isolates that were tested 93% reduced galling when compared to the control (water) while only 30 (12%) of the isolates reduced galling and egg mass indices more than carbofuran. Fifty four percent of the isolates had no effect on damage by *Meloidogyne* spp. and reproductive capacity (egg masses) of the nematodes. Forty-six (18.4%) of the isolates reduced the number of root-knot nematode juveniles more than carbofuran while 185 (74%) of the isolates were as effective as carbofuran. The effect of *Bacillus* isolates on plant growth and root-knot nematode damage differed significantly (P=0.05) among the isolates (Tables 5-7).

Plants treated with 50 out of 250 isolates had higher shoot weight than the control (Tables 5-7). Root weight was significantly (P=0.05) higher in plants treated with ten of the isolates K6, K39, K41, K44, K91, K119, K128, K149, K151 and K281 when compared with the control (water). Based on the screening results, 20 *Bacillus* isolates that were most effective in reducing nematode damage were selected for further tests. These were isolates K9, K33, K34, K48, K51, K66, K67, K78, K86, K89, K100, K158, K194, K227, K228, K236, K269, K270, K273 and CB4.

Table 5. Galling index, egg mass index, numbers of *Meloidogyne* juveniles (J_2) and dry root and shoot weight of bean plants treated with *Bacillus* spp. 60 days after soil infestation with root-knot nematodes

Bacillus isolate	Galling index	Egg mass index	Damage index	J_2 count /200cm^3	Dry root weight (g)	Dry shoot weight (g)
K2	5.0	5.6	3.4	190	0.2	1.9
K4	4.3	4.5	4.0	124	0.1	1.3
K14	3.8	3.6	1.6	367	0.2	1.5
K15	6.6	5.6	2.4	804	0.3	2.6
K17	5.3	5.3	3.3	540	0.2	3.0
K20	5.0	4.8	2.4	649	0.1	2.7
K24	4.4	4.2	1.8	79	0.2	1.6
K25	4.8	4.6	1.8	747	0.2	2.4
K31	5.8	5.6	2.6	1104	0.2	2.9
K33	3.8	3.0	1.6	237	0.3	1.8
K37	4.0	4.0	1.6	194	0.1	2.2
K43	5.2	5.0	2.4	41	0.2	1.7
K46	5.6	5.6	2.8	151	0.1	2.1
K51	2.8	3.2	1.6	105	0.1	1.8
K58	5.2	5.4	2.8	133	0.1	1.3
K60	6.0	6.2	3.0	190	0.2	2.0
K66	1.8	2.0	0.6	76	0.2	1.9
K67	3.0	3.2	1.2	203	0.2	1.8
K75	5.6	5.4	2.4	1089	0.1	2.3
K83	6.4	6.0	2.6	199	0.2	2.7
K89	3.0	3.2	1.4	105	0.1	2.5
K95	3.4	3.2	1.4	625	0.1	2.1
K99	5.0	4.8	2.4	132	0.3	2.0
K113	3.8	4.0	2.0	126	0.1	2.4
K117	5.4	5.4	2.6	73	0.3	1.6
K120	4.6	4.2	2.4	80	0.2	1.6
K124	5.4	5.2	2.4	112	0.2	1.9
K127	3.4	3.4	1.4	130	0.3	2.5
K132	5.2	5.2	2.4	114	0.1	1.8
K134	5.8	6.0	2.2	395	0.2	1.7
K136	3.4	3.4	1.6	539	0.1	1.9
K147	4.2	4.6	1.8	529	0.2	2.3
K152	5.0	5.2	2.6	341	0.3	1.9
K157	6.0	6.0	3.0	96	0.1	1.5
K158	3.0	3.2	1.4	257	0.1	1.6
K160	6.0	6.0	2.8	935	0.2	1.7
K168	4.4	4.2	2.4	83	0.1	1.7
K184	6.0	5.8	2.6	258	0.2	3.0
K186	6.5	6.8	3.3	76	0.2	2.6
K194	1.6	1.8	0.6	93	0.1	2.4
K196	3.2	3.0	1.2	80	0.2	1.8
K204	3.4	3.2	1.4	42	0.1	2.7
K207	4.8	4.6	2.4	154	0.1	2.0
CA5	4.7	5.0	2.3	825	0.2	2.8

CA10	6.2	6.2	3.0	88	0.1	3.2
CB4	6.0	5.8	3.6	173	0.2	1.7
Furadan	3.4	3.6	1.8	173	0.1	2.3
Water	7.2	6.6	5.6	428	0.3	3.0
SE	1.0	1.1	0.5	51.7	0.1	0.6
CV(%)	21.6	23.3	22.7	17.3	37.5	29.6
LSD (P=O.O5)	1.2	1.3	0.7	65.0	0.1	0.8

Table 6. Galling index, egg mass index, numbers of *Meloidogyne* juveniles (J_2) and dry root and shoot weight of bean plants treated with *Bacillus* spp. 60 days after soil infestation with root-knot nematodes

Bacillus isolate	Galling index	Egg mass index	Damage index	J2 count /200cm^3	Dry root weight (g)	Dry shoot weight (g)
K1	5.0	4.5	2.5	48	0.47	2.5
K3	6.5	6.3	3.5	51	0.31	1.8
K11	6.8	7.0	3.0	166	0.36	2.4
K13	8.0	8.0	2.8	23	0.23	2.1
K16	7.5	8.3	3.5	9	0.30	2.3
K23	5.8	5.5	2.5	29	0.39	2.6
K26	6.5	6.8	2.5	40	0.24	1.9
K27	5.8	5.8	5.0	19	0.39	2.5
K28	6.8	8.3	3.5	186	0.29	3.6
K34	3.4	5.0	2.0	121	0.17	1.8
K40	5.8	6.0	3.0	176	0.26	2.2
K44	8.5	8.0	3.3	33	0.68	2.7
K49	6.3	5.8	3.5	33	0.36	3.1
K53	7.3	7.3	3.3	166	0.30	2.6
K55	5.8	6.0	3.8	83	0.41	3.1
K56	6.0	6.0	2.5	18	0.14	1.7
K61	4.5	4.5	2.8	16	0.19	1.7
K63	6.3	6.0	3.3	161	0.24	2.0
K65	6.3	6.3	4.3	61	0.32	2.6
K72	5.8	5.8	3.8	56	0.29	2.6
K76	5.8	5.8	2.8	30	0.38	2.1
K78	4.5	5.5	3.0	125	0.37	2.0
K80	5.5	5.3	2.3	85	0.31	2.1
K84	6.3	6.8	3.0	29	0.28	2.0
K86	4.0	4.3	2.0	175	0.47	4.1
K88	4.8	5.5	2.0	23	0.26	1.8
K96	4.8	4.8	2.5	4	0.26	1.9
K100	4.0	4.5	2.5	20	0.20	3.2
K101	7.0	7.8	3.5	49	0.27	1.8
K103	6.0	6.3	2.8	18	0.38	2.9
K106	7.8	8.0	3.0	31	0.28	3.2

K114	5.8	5.8	2.8	23	0.37	2.8
K116	8.5	8.3	4.3	85	0.31	1.6
K118	5.8	6.8	2.8	211	0.23	2.8
K122	5.8	6.3	3.0	31	0.49	2.4
K133	7.3	7.0	2.8	98	0.52	3.2
K135	7.0	6.8	2.8	28	0.41	2.9
K138	6.5	6.5	3.0	24	0.32	2.4
K140	7.0	6.8	2.8	18	0.26	1.4
K148	6.8	7.3	3.3	74	0.26	2.4
K155	8.0	8.5	3.5	233	0.33	2.9
K159	8.5	7.8	4.5	11	0.32	1.9
K161	4.8	3.8	2.8	30	0.32	1.9
K162	7.0	7.0	3.3	48	0.35	2.7
K164	7.8	8.0	3.5	79	0.35	2.8
K165	6.0	5.8	2.8	23	0.33	1.0
K167	7.0	6.8	3.3	23	0.31	2.7
K169	6.8	6.8	4.3	65	0.33	2.2
K170	7.3	6.5	3.5	23	0.28	1.7
K173	8.0	8.0	3.3	24	0.37	4.4
K175	6.0	6.0	2.3	18	0.31	1.5
K177	6.8	6.8	2.5	483	0.25	1.4
K178	5.8	5.8	3.5	115	0.35	1.9
K181	8.3	8.0	3.0	48	0.30	2.1
K182	7.8	7.8	2.5	20	0.31	2.1
K183	5.8	6.0	2.8	54	0.31	2.9
K184	6.5	6.8	2.8	50	0.29	2.3
K186	6.3	6.3	2.5	16	0.31	1.9
K188	6.0	5.8	2.5	23	0.33	2.7
K191	7.8	7.5	3.5	20	0.32	1.9
K195	6.8	6.5	2.8	39	0.33	2.9
K197	8.3	8.3	2.8	30	0.33	2.0
K199	5.8	6.0	2.3	9	0.26	2.1
K200	5.8	6.0	2.3	360	0.37	4.0
K202	5.8	5.8	2.5	51	0.39	3.3
K209	6.0	6.3	2.0	28	0.38	2.7
K212	6.5	6.8	2.8	290	0.41	3.3
K214	6.3	6.3	2.3	174	0.53	3.8
K216	7.0	7.3	2.5	14	0.44	3.1
K220	6.5	6.3	3.3	165	0.39	2.1
K225	6.5	6.8	3.0	8	0.45	4.3
K229	6.5	6.0	3.0	103	0.30	2.5
K233	5.8	5.8	3.0	90	0.39	2.4
K238	4.8	4.8	3.0	265	0.21	1.7
K240	6.5	5.8	2.8	106	0.43	1.9
K244	6.0	5.8	2.3	29	0.33	2.6
K245	6.5	6.0	2.3	26	0.42	2.8
K247	6.0	5.8	2.5	15	0.30	2.0
K251	5.0	5.5	2.5	33	0.34	2.2
K256	5.8	6.0	2.3	44	0.29	2.2
K258	5.5	6.0	2.5	61	0.41	2.9

K260	5.3	4.3	3.3	34	0.15	2.4
K263	5.8	6.5	4.5	104	0.29	2.2
K268	8.0	8.0	3.8	36	0.52	3.2
K271	6.0	6.3	3.0	41	0.32	2.9
K273	3.3	2.8	1.3	45	0.21	1.9
K279	5.3	4.8	2.5	138	0.26	2.7
K282	7.5	7.8	3.3	9	0.39	2.9
K284	7.0	6.8	3.5	13	0.36	2.7
CA6	6.5	6.0	2.3	16	0.45	2.9
CA12	8.5	8.5	3.3	8	0.35	2.3
CA48	6.5	6.5	2.8	5	0.24	1.3
CB3	6.3	6.0	3.8	40	0.29	1.3
CB8	8.5	8.3	3.8	63	0.38	2.9
CB22	6.0	6.3	3.0	46	0.45	3.2
Furadan	6.0	6.3	4.8	21	0.62	3.8
Control (H_2O)	8.5	8.5	5.3	84	0.45	2.4
SE	0.8	0.8	0.7	52.5	0.1	0.6
CV%	11.7	12.5	23.8	75.8	31.3	23.0
LSD (P=0.05)	1.0	1.1	1.0	73.1	0.2	0.8

Table 7. Galling index, egg mass index, numbers of *Meloidogyne* juveniles (J_2) and dry root and shoot weight of bean plants treated with *Bacillus* spp. 60 days after soil infestation with root-knot nematodes

Bacillus isolate	Galling index	Egg mass index	Damage index	J2 count /200cm^3	Dry root weight (g)	Dry shoot weight (g)
K5	8.3	7.5	2.5	847	0.5	3.0
K6	8.8	8.8	3.0	18	0.7	2.5
K9	4.0	4.3	1.8	129	0.5	1.9
K12	7.3	7.8	2.3	34	0.6	1.9
K18	7.8	7.8	4.5	283	0.5	1.9
K19	8.0	8.3	3.8	171	0.5	2.0
K21	7.3	7.0	2.5	125	0.6	1.7
K29	8.0	8.0	3.5	566	0.6	1.7
K30	7.0	6.8	3.5	224	0.5	1.1
K32	8.0	7.8	3.3	250	0.5	2.0
K35	6.8	7.3	2.0	933	0.4	1.5
K36	7.5	7.3	2.0	131	0.6	2.2
K38	7.8	7.8	3.5	179	0.5	1.7
K39	8.0	7.8	3.0	190	0.7	2.1
K41	7.0	7.5	2.0	233	0.7	2.3
K42	6.5	5.8	2.8	110	0.5	2.0
K45	8.5	8.5	3.8	106	0.6	2.4
K48	3.8	3.5	1.0	24	0.4	1.6
K50	7.5	7.8	2.8	168	0.6	2.4
K52	8.0	8.0	3.3	225	0.6	1.9

K54	8.3	8.8	2.8	325	0.6	1.9
K55	8.5	8.8	4.3	1603	0.5	1.5
K56	8.3	8.5	3.5	149	0.5	2.2
K59	6.8	7.0	3.0	168	0.6	1.6
K62	7.5	6.3	1.3	388	0.6	2.2
K64	8.3	8.5	2.5	60	0.5	2.1
K68	8.3	6.0	2.8	133	0.4	1.1
K69	7.8	8.3	3.3	234	0.5	2.0
K70	7.0	7.3	3.0	221	0.5	1.4
K71	7.0	7.0	2.0	2050	0.4	0.7
K73	8.3	8.5	3.5	188	0.6	1.8
K74	7.8	7.3	2.3	83	0.5	2.0
K77	6.0	6.3	2.5	55	0.4	1.6
K79	6.0	6.0	2.8	1419	0.4	1.9
K81	9.0	9.0	4.5	140	0.5	1.8
K87	8.3	8.3	2.8	128	0.5	2.0
K90	8.3	8.3	3.0	531	0.5	1.2
K91	9.0	9.0	3.3	743	0.7	2.8
K92	8.5	8.3	3.8	194	0.4	2.4
K94	9.0	9.0	3.8	371	0.6	1.8
K97	6.8	6.3	2.3	10	0.5	1.8
K98	9.0	9.0	3.3	123	0.5	1.8
K102	7.5	7.5	3.0	1738	0.4	2.1
K104	8.0	8.0	4.0	95	0.5	2.5
K105	8.3	8.8	4.5	35	0.6	1.6
K107	7.8	7.8	2.5	99	0.5	2.5
K115	8.8	8.5	4.3	235	0.6	1.8
K119	8.3	8.8	3.0	88	0.7	2.3
K121	8.8	8.8	4.0	704	0.5	2.0
K125	8.0	7.8	2.5	108	0.5	2.0
K126	6.5	6.5	2.0	130	0.5	1.7
K128	8.5	8.5	3.3	55	0.7	2.0
K129	7.8	7.8	3.5	108	0.5	2.2
K130	8.0	7.8	4.0	51	0.4	2.3
K131	8.5	8.5	3.5	93	0.5	2.0
K137	8.3	8.5	3.5	94	0.5	1.5
K139	8.8	8.8	3.5	103	0.3	1.6
K141	7.5	7.8	2.8	216	0.4	1.1
K142	7.3	7.8	3.0	196	0.5	1.2
K143	7.0	7.0	2.8	178	0.4	1.4
K145	8.3	8.8	3.3	470	0.4	0.7
K146	6.8	7.3	3.0	46	0.4	1.8
K149	8.8	8.8	3.5	95	0.7	1.7
K150	6.8	7.3	3.3	75	0.5	1.5
K151	8.5	9.0	3.5	63	0.7	2.4
K153	7.3	7.0	3.0	196	0.4	2.3
K154	8.8	8.5	4.3	528	0.5	2.2
K166	7.8	8.3	2.5	296	0.5	2.1
K171	8.5	7.8	3.0	34	0.5	2.2
K172	8.3	7.8	3.5	28	0.5	1.8

K174	8.0	8.0	2.3	214	0.5	1.2
K176	8.8	8.8	3.3	194	0.5	2.1
K179	7.8	7.5	3.0	1246	0.4	1.2
K180	6.0	6.5	4.3	285	0.3	1.2
K185	7.5	7.5	2.5	179	0.5	1.8
K187	8.5	8.5	3.3	93	0.4	1.8
K190	8.3	8.3	2.0	63	0.4	1.7
K192	8.5	8.5	3.0	181	0.5	2.3
K193	8.5	8.0	3.5	80	0.6	2.8
K198	7.5	7.5	3.3	30	0.5	2.4
K201	7.8	7.8	3.0	155	0.5	2.3
K203	7.5	7.0	2.0	95	0.4	2.2
K205	8.8	8.8	4.5	56	0.5	2.1
K206	7.5	7.5	2.5	113	0.4	2.6
K208	6.3	6.5	2.8	19	0.5	1.8
K210	7.0	7.3	3.3	111	0.4	2.2
K215	8.0	8.3	3.0	55	0.5	2.3
K218	7.3	7.0	4.0	60	0.4	2.3
K221	8.0	8.3	3.5	295	0.4	1.7
K224	7.8	7.8	4.0	84	0.5	1.8
K227	3.3	3.8	1.3	44	0.4	1.5
K228	3.7	4.8	1.8	96	0.5	2.3
K230	7.8	7.5	4.0	55	0.5	1.7
K232	8.3	7.5	3.8	80	0.5	2.0
K234	8.8	8.8	4.5	108	0.6	2.3
K236	4.5	4.5	2.3	84	0.5	1.5
K239	7.3	7.3	3.3	89	0.5	1.6
K241	7.3	7.3	2.5	44	0.5	2.1
K248	6.5	6.5	3.3	118	0.3	1.2
K250	6.8	6.8	3.0	115	0.6	2.2
K252	8.0	7.5	3.8	71	0.4	2.0
K259	8.8	8.3	4.5	170	0.6	1.9
K261	8.0	8.0	3.5	108	0.4	1.9
K269	2.8	3.8	1.3	40	0.3	1.8
K270	3.0	3.5	1.5	230	0.5	1.8
K275	7.0	6.3	2.8	81	0.4	2.2
K281	7.5	7.8	3.5	164	0.7	2.3
K283	7.5	7.0	3.3	55	0.5	2.0
CA5	8.5	8.3	3.3	115	0.4	2.3
CA10	6.0	6.0	1.8	69	0.4	1.6
CB4	4.5	5.3	1.8	91	0.4	1.8
Furadan	6.8	6.3	2.5	23	0.4	1.9
Water	8.8	8.8	4.3	309	0.5	2.0
SE	0.9	0.9	0.7	32.9	0.14	0.56
CV (%)	12.5	12.1	24.6	14.5	28.6	29.2
LSD (P=0.05)	1.3	1.3	1.0	45.8	0.20	0.77

4.3 Evaluation of selected *Bacillus* isolates against root-knot nematodes, under sterile and non-sterile soil conditions

Generally, the isolates were found to perform better in sterile than in non-sterile soil. Galling was lowest in bean plants treated with isolates K67, and K270 in sterile soil while in non-sterile soil K48 had the lowest galling. Similarly, egg mass index was lowest in plants treated with isolate K67 in sterile soil and K48 in non-sterile soil. The highest galling was recorded in plants treated with *Bacillus* isolates K78 and K236 in sterile and non-sterile soil, respectively. The effect of *Bacillus* isolates on root-knot nematodes in sterile and non-sterile soil was significant at P=0.05 (Table 8).

The number of *Meloidogyne* juvenile (J_2) was higher in non-sterile soil than in sterile soil (Table 8). Juvenile numbers were lowest in soil treated with *Bacillus* isolate K78 in sterile soil and in non-sterile soil treated with *Bacillus* isolates K194 and K227. The highest number of juveniles was recorded in soil treated with *Bacillus* isolates K227 and K100 under sterile and non-sterile soil conditions, respectively. The number of *Meloidogyne* juvenile (J_2) was significantly (P=0.05) different among treatments in sterile and non-sterile soil (Table 8)

Table 8: Galling index, egg mass index and J_2 count of bean plants inoculated with *Bacillus* isolates under sterile and non-sterile soil infested with root-knot nematodes 60 days after inoculation

Bacillus isolate	Galling index			Egg mass index			J_2 count/200 cm^3 soil		
	S[a]	NS[b]	Mean	S[a]	NS[b]	Mean	S[a]	NS[b]	Mean
K9	4.5	5.0	4.8	5.5	5.5	5.5	145	323	234
K33	4.5	6.3	5.4	5.8	6.8	6.3	173	193	183
K34	5.8	5.8	5.8	7.0	6.3	6.6	141	260	201
K48	4.0	3.5	3.8	4.3	4.3	4.3	81	271	176
K51	4.0	4.8	4.4	4.3	5.0	4.6	450	205	327
K66	2.8	4.8	3.8	3.3	5.0	4.1	160	223	191
K67	2.5	4.8	3.6	2.3	5.0	3.9	126	272	199
K78	7.0	5.5	6.3	7.5	6.0	6.8	42	190	116
K86	4.3	7.0	5.6	4.8	7.8	6.3	126	696	411
K89	4.3	5.5	4.9	4.3	6.3	5.3	109	157	133
K100	5.5	6.0	5.8	6.8	6.3	6.5	180	788	484
K158	3.5	2.8	4.1	4.3	5.3	4.8	90	182	136
K194	3.0	3.8	3.4	3.0	3.8	3.4	194	109	151
K227	4.3	3.8	4.0	5.0	3.8	4.4	465	132	299
K228	4.5	5.0	4.8	5.3	5.5	5.4	291	418	355
K236	3.3	7.3	5.3	4.5	7.0	5.8	141	531	336
K269	4.3	4.5	4.4	4.5	5.3	4.9	145	604	374
K270	2.5	5.5	4.0	3.0	6.0	4.5	165	404	284
K273	4.3	4.3	4.3	5.3	5.3	5.3	162	522	342
CB4	4.5	5.5	5.0	5.3	6.0	5.6	409	522	465
Water	8.3	9.0	8.6	9.0	9.0	9.0	525	596	561
Carbofuran	5.3	5.8	5.5	5.8	6.5	6.1	137	193	165
Mean	4.3	5.3		4.9	5.8		215	361	
SE	0.8			0.8			38.1		
CV (%)	16.7			15.7			13.2		
LSD (P=0.05)									
Bacillus	0.8			0.8			37.7		
Soil condition	0.2			0.2			11.1		
Bacillus vs soil condition	1.1			1.2			58.3		

[a] sterile sandy loam soil, [b] non-sterile sandy loam soil

4.4 Effect of culture filtrates (CF) of *Bacillus* spp. on mobility of *Meloidogyne* juveniles (J_2)

Culture filtrate of the *Bacillus* spp. tested had an immobilizing effect on *Meloidogyne* juvenile. Immobilized nematode lay straight or in a slightly curved position. All the selected *Bacillus* isolates produced culture filtrate which inactivated more than 80% of the *Meloidogyne* juveniles. Culture filtrate of *Bacillus* isolates K9 and K158 immobilized the highest (99.2%) and the lowest (89.2%) percentage of *Meloidogyne* juveniles (J_2), respectively (Table 9). In a repeat experiment, the culture filtrate of *Bacillus* isolates tested immobilized more than 80% of the juveniles (Table 9). The culture filtrate of *Bacillus* isolates K33 had the least effect while K194 and K100 had the highest effect on juvenile mobility. The culture medium, nutrient broth, had a significantly (P=0.05) higher activity against *Meloidogyne* juvenile than tap water. The number of immobilized juveniles were negatively correlated (r= -0.612) to galling index recorded under sterile and non-sterile experiment.

Dilution of the culture filtrate of *Bacillus subtilis* K194 reduced its activity against *Meloidogyne* juveniles (fig. 1). The difference in the percentage of juveniles immobilized by different concentrations of the culture filtrates was significant (P=0.05). Maximum activity was recorded on the undiluted culture filtrate.

Table 9: Percentage of immobilized second stage juvenile (J_2) of *Meloidogyne* spp. after treatment with to culture filtrate of *Bacillus* isolates

Bacillus isolate	Experiment I Immobile J_2 (%)[a]	Experiment II Immobile J_2 (%)[a]
K9	99.2 (9.96)	93.8 (9.68)
K33	96.2 (9.81)	88.4 (9.40)
K34	94.2 (9.71)	95.9 (9.80)
K48	99.0 (9.95)	97.1 (9.85)
K51	92.6 (9.62)	97.0 (9.85)
K61	95.4 9.76)	99.0 (9.95)
K66	97.7 (9.88)	96.9 (9.85)
K67	92.9 (9.64)	95.3 (9.76)
K78	92.9 (9.64)	96.9 (9.85)
K86	93.9 (9.69)	96.7 (9.83)
K89	94.4 (9.71)	96.3 (9.81)
K100	98.7 (9.94)	100.0 (10.00)
K158	89.2 (9.44)	97.2 (9.86)
K194	96.5 (9.82)	100.0 (10.00)
K227	98.7 (9.94)	98.6 (9.93)
K228	97.1 (9.85)	97.6 (9.89)
K236	98.7 (9.93)	92.4 (9.48)
K269	97.9 (9.90)	98.0 (9.90)
K270	93.9 (9.69)	96.7 (9.83)
K273	92.6 (9.62)	98.3 (9.91)
CB4	95.9 (9.80)	97.1 (9.85)
Nutrient broth	29.4 (3.46)	20.0 (4.45)
Water	5.0 (2.23	2.2 (1.44)
LSD (P=0.05)	0.204	0.128

[a]Number in blacket are square root transformed values used in statistical analysis.

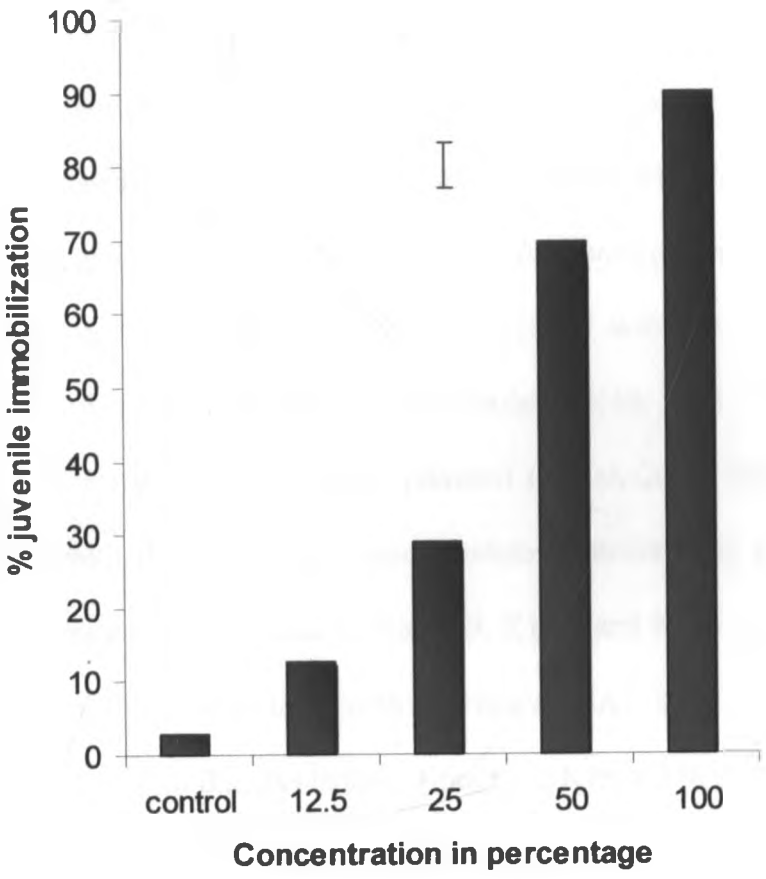

Fig 1. Effect of the concentration of culture filtrate of *Bacillus subtilis* strain K194 on its activity against *Meloidogyne* juvenile (J_2)

4.5 Effect of *Bacillus* spp. on nodulation in beans

The *Bacillus* isolates had variable (P=0.05) effects on nodulation in beans. Plants treated with some *Bacillus* isolates had more nodules while others had fewer nodules compared to the control (Plate 1; plate 2). Nodule numbers were significantly (P=0.05) higher in plant treated with 4 out of 20 *Bacillus* while six isolates suppressed nodulation (Table 10).

There was significant (P=0.05) effect on nodulation when *Bacillus* isolates and *Rhizobium* strains were inoculated together (Table 10). Plant inoculated with *Bacillus* isolates K9, K33, K67, K194, K270, and K273 in combination with *R. leguminosarium biovar phaseoli* (USDA 2674) had higher nodule numbers than those inoculated with the *Rhizobium* strain alone (Table 9). Bean plants treated with *Bacillus* isolates K48, K78, K86, K236 and CB4 combined with *R. leguminosarium biovar phaseoli* (USDA 2674) had fewer nodules than those inoculated with the *Rhizobium* alone. Nodule numbers were significantly (P=0.05) higher in plants treated with *Bacillus* isolates K9, K194, and K273 together with *R. tropici* (CIAT 899) than in plants inoculated with *R. tropici* (CIAT 899) *alone* (Table 10). Plants grown on soil infested with *Bacillus* isolates K66, K67, K78, K236 and CB4 combine with *R. tropici* (CIAT 899) had significantly (P=0.05) fewer nodules compared to plants inoculated with *R. tropici* (CIAT 899) alone.

Nodule numbers were lowest in plants treated with *Bacillus* isolates K9, K34, K51, K61, K67, K89 and CB4 combined with *Rhizobium* strain mixture (CIAT 899 & USDA 2674) as compared to the control (Table 10). With regard to the *Rhizobium* strains and their mixture, plants treated with *Bacillus* isolates K194 and K273 had significantly (P=0.05) more nodules. Differences in nodule numbers on plants inoculated with different *Rhizobium* strains were significant (P=0.05). Plants treated with *Bacillus* isolates K33, K48, K194, K269 and K273

had heavier nodules than the control (Table 11). Differences in nodule numbers on plants inoculated with different *Rhizobium* strains were significant (P=0.05).

Seventeen isolates were found to promote plant growth while 3 isolates (K33, K51, and K236) had no effect on growth (Table 11). Shoot weights were significantly (P=0.05) higher in plants treated with *Bacillus* isolates K48, K100, K227, K269 and K273 together with *R. leguminosarum biovar phaseoli* (USDA 2674) than in plants treated with the *Rhizobium* alone (Table 12). Plants treated with *Bacillus* isolates K34, K48, K194, K227 and K273 together with *R. tropici* (CIAT 899) had higher shoot dry weight than those treated with *Rhizobium* alone (Plate 3). Only *Bacillus* isolate K89 promoted shoot weight when inoculated together with a mixture of the two *Rhizobium* strains (Table 12). Shoot weight and nodule numbers were positively correlated (r = 0.343).

Table 10: Nodule numbers on bean plants treated with *Bacillus* isolates and *Rhizobium* strains

Bacillus isolates	Control	USDA 2674	CIAT 899	Mixture	Mean
K9	0	259.3	348.7	172.3	195.1
K33	0	294.0	264.0	197.0	188.8
K34	0	213.3	213.3	134.0	140.2
K48	0	155.0	278.0	229.7	165.7
K51	0	180.7	244.0	147.3	143.0
K66	0	203.3	188.3	238.0	157.4
K67	0	260.7	187.7	164.0	153.1
K78	0	108.0	170.7	201.3	120.0
K86	0	161.7	204.3	210.3	144.1
K89	0	243.7	247.0	127.3	154.3
K100	0	252.3	297.7	243.7	198.4
K158	0	235.3	210.0	224.7	167.5
K194	0	300.7	329.7	313.3	235.9
K227	0	185.7	201.3	232.7	154.9
K228	0	214.3	291.7	193.3	174.8
K236	0	153.3	197.7	194.7	136.4
K269	0	225.7	218.7	221.0	166.3
K270	0	262.3	225.7	235.0	180.8
K273	0	303.0	333.0	338.7	243.7
CB4	0	115.7	102.7	179.0	99.3
Control	0	209.7	244.0	226.0	169.9
Mean	0	216.8	239.6	207.1	
SE	28.1				
CV (%)	16.9				
LSD (P=0.05)					
Bacillus	22.6				
Rhizobium	9.7				
Bacillus vs *Rhizobium*	45.3				

CIAT 899= *Rhizobium tropici*

USDA 2674= *Rhizobium leguminosarum biovar phaseoli*

Mixture= Combination of CIAT 899 + USDA 2674

Table 11: Dry weight of nodules obtained from bean plants treated with *Bacillus* isolates and *Rhizobium* strains

Bacillus isolates	Control	USDA 2674	CIAT 899	Mixture	Mean
K9	0	0.24	0.26	0.23	0.18
K33	0	0.36	0.30	0.26	0.23
K34	0	0.29	0.33	0.23	0.21
K48	0	0.26	0.48	0.24	0.25
K51	0	0.26	0.29	0.20	0.19
K66	0	0.22	0.31	0.22	0.19
K67	0	0.26	0.30	0.22	0.19
K78	0	0.24	0.34	0.23	0.20
K86	0	0.23	0.23	0.22	0.18
K89	0	0.25	0.26	0.20	0.18
K100	0	0.25	0.31	0.28	0.21
K158	0	0.24	0.28	0.27	0.20
K194	0	0.25	0.34	0.29	0.22
K227	0	0.23	0.30	0.32	0.21
K228	0	0.23	0.36	0.25	0.21
K236	0	0.19	0.28	0.25	0.18
K269	0	0.26	0.33	0.28	0.22
K270	0	0.24	0.26	0.27	0.19
K273	0	0.26	0.34	0.27	0.22
CB4	0	0.19	0.20	0.26	0.16
Control	0	0.21	0.27	0.29	0.19
Mean	0	0.25	0.30	0.25	
SE	0.04				
CV (%)	20.0				
LSD (P=0.05)					
Bacillus	0.03				
Rhizobium	0.01				
Bacillus Vs *Rhizobium*	0.06				

CIAT 899= *Rhizobium tropici*

USDA 2674= *Rhizobium leguminosarum biovar phaseoli*

Mixture= Combination of CIAT 899 + USDA 2674

Plate 1. Bean plants:- A) not inoculated (control), B) Inoculated with *Bacillus* isolates K273 and *Rhizobium* strain CIAT 899

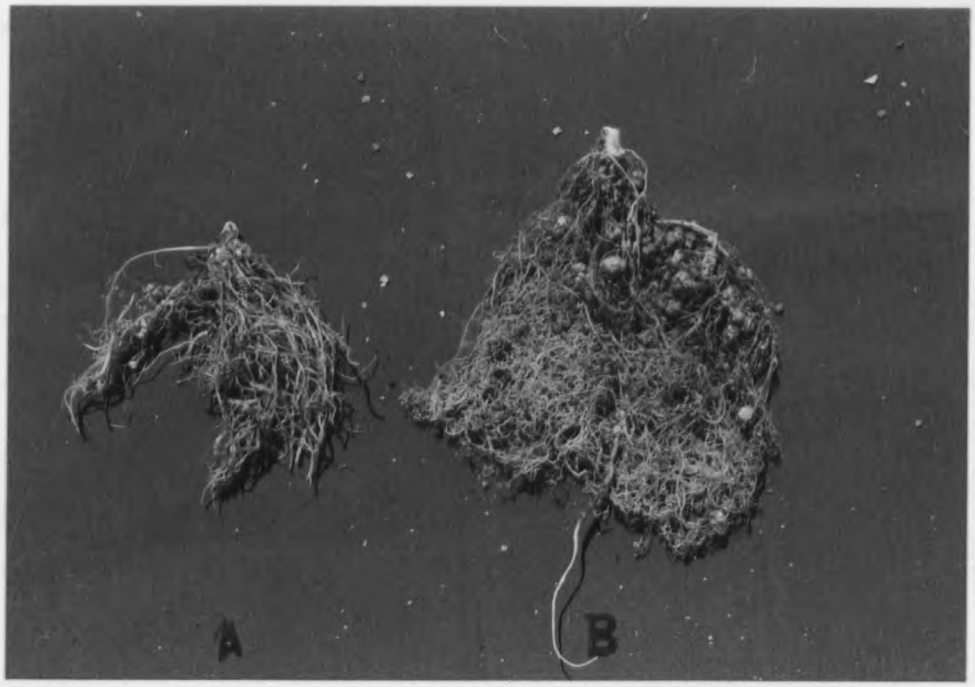

Plate 2. Bean plants:- A) control, B) Inoculated with *Bacillus* isolates K273 and *Rhizobium* strain CIAT 899

Table 12: Dry shoot weight of bean plants treated with *Bacillus* isolates and *Rhizobium* strains

Bacillus isolates	Control	USDA 2674	CIAT 899	Mixture	Mean
K9	1.9	3.1	3.6	2.8	2.9
K33	1.8	2.8	2.6	2.8	2.5
K34	1.8	2.3	4.4	3.7	3.1
K48	1.7	3.6	4.0	3.2	3.1
K51	1.8	3.0	2.9	2.4	2.5
K66	1.9	2.9	3.1	3.1	2.8
K67	1.8	2.8	2.8	2.9	2.6
K78	2.1	2.5	2.9	3.0	2.6
K86	4.1	2.5	2.4	3.1	3.0
K89	2.5	3.5	2.9	4.0	3.2
K100	3.0	3.6	3.7	3.1	3.3
K158	1.6	2.9	3.3	3.5	2.8
K194	2.4	3.5	3.8	3.6	3.3
K227	1.5	4.0	4.4	3.6	3.4
K228	2.3	3.0	2.8	3.1	2.8
K236	1.5	2.4	2.4	2.5	2.2
K269	1.8	3.6	3.4	3.5	3.1
K270	1.8	2.8	2.4	3.3	2.6
K273	1.9	3.6	4.3	3.7	3.4
CB4	1.8	3.1	3.0	2.7	2.6
Control	0.5	2.5	3.0	3.4	2.3
Mean	2.0	3.0	3.2	3.2	
SE	0.3				
CV (%)	12.1				
LSD (P=0.05)					
Bacillus	0.3				
Rhizobium	0.1				
Bacillus Vs *Rhizobium*	0.6				

CIAT 899= *Rhizobium tropici*

USDA 2674= *Rhizobium leguminosarum biovar phaseoli*

Mixture= Combination of CIAT 899 + USDA 2674

Plate 3. Bean plants treated with:-A) Sterile distilled water (control), B) *Rhizobium* strain CIAT 899 alone and C) *Bacillus* isolate K273 and *Rhizobium* strain CIAT 899.

4.6 Effect of combining *Bacillus* isolates with *Rhizobium* strains on root-knot nematodes

Treatment of beans with a combination of *Bacillus* pp. and *Rhizobium* strain reduced nematode infection as indicated by galling index and *Meloidogyne* juvenile count. Galling index was significantly (P=0.05) different between *Bacillus* isolates combined with different *Rhizobium* strains (Table 13). Galling was lowest in plants treated with *Bacillus* isolate K194 combined with a mixture of two *Rhizobium* strains and those treated with isolate K269 combined with *Rhizobium* (USDA 2674) strain. The highest galling was observed in plants inoculated with *Bacillus* isolate K66 combined with *Rhizobium* strain (CIAT 899). There were significant (P=0.05) differences in juvenile populations among plants treated with the various *Bacillus* and *Rhizobium* combinations (Table 14). The lowest number J_2 number was recovered from soil treated with *Bacillus* isolate K269 combined with *Rhizobium* strain CIAT 899. Juvenile number was highest in soil treated with *Bacillus* isolate K66 combined with *Rhizobium* strain USDA 2674 (Table 14).

There were significant (P=0.05) differences in nodule numbers on plants inoculated with *Bacillus* isolates and *R. leguminosarum biovar phaseoli* (USDA 2674), *R. tropici* (CIAT 899), or their mixture (Table 15). The highest nodule number was recorded on plants inoculated with *Bacillus* isolate K273 combined with a mixture of the two *Rhizobium* strains while the lowest nodule number was recorded on plants inoculated with *Bacillus* isolate K273 combined with *R. leguminosarum biovar phaseoli* (USDA 2674).

Shoot weights were significantly (P=0.05) different in plants inoculated with different *Bacillus* isolates combined with individual *Rhizobium* strains and their mixture (Tables 16; plate 4). The highest shoot weight was recorded in plants treated with *Bacillus* isolates K194 and K273 combined with *R. tropici* (CIAT899). Plant treated with *Bacillus* isolate K89 combined with *R. leguminosarum biovar phaseoli* (USDA 2674) had the lowest shoot weight. Galling index and shoot weight were negatively correlated at r = -0.26.

Table 13: Galling index of bean plants treated with *Bacillus* isolates combined with *Rhizobium* strain 45 days after inoculation

Bacillus isolate	*Bacillus* alone	USDA 2674	CIAT 899	Mixture	Mean
K66	5.0	5.4	7.0	5.0	5.6
K89	3.3	6.3	6.8	6.5	5.7
K100	5.3	4.5	5.8	6.0	5.4
K194	4.5	5.3	5.4	4.4	4.9
K269	5.0	4.4	5.6	5.0	5.0
K270	4.4	5.5	5.8	5.5	5.3
K273	5.0	5.0	5.4	5.6	5.3
Carbofuran	6.2	—	—	—	—
Control*	0	—	—	—	—
Control**	8.7	6.8	7.6	6.8	7.5
Mean	5.1	5.4	6.2	5.6	
SE	0.7				
CV (%)	12.2				
LSD (P=0.05)					
Bacillus	0.4				
Rhizobium	0.3				
Bacillus vs *Rhizobium*	0.8				

Control*= No *Bacillus*, *Rhizobium* and nematodes
Control**=*Rhizobium* and nematodes

Table 14: Number of second-stage juveniles (per 200 cm^3 soil) associated with bean plants treated with *Bacillus* isolates combined with *Rhizobium* strains 45 days after inoculation

Bacillus isolate	*Bacillus* alone	USDA 2674	CIAT 899	Mixture	Mean
K66	59	445	202	93	200
K89	37	101	78	131	87
K100	100	182	70	136	122
K194	137	104	226	88	139
K269	188	29	68	153	109
K270	71	140	36	221	117
K273	21	83	108	49	65
Carbofuran	100	—	—	—	—
Control*	0	—	—	—	—
Control	260	76	176	177	172
Mean	109	145	121	131	
SE	14.2				
CV (%)	11.2				
LSD (P=0.05)					
Bacillus	8.1				
Rhizobium	5.7				
Bacillus vs. *Rhizobium*	16.2				

Control=*Rhizobium* and nematodes
Control*= No *Bacillus*, *Rhizobium* and nematodes

Table 15: Nodule numbers on bean plants treated with *Bacillus* isolates combined with *Rhizobium* strains 45 days after inoculation

Bacillus isolate	*Bacillus* alone	USDA 2674	CIAT 899	Mixture	Mean
K66	20.0	88.7	152.5	186.4	111.9
K89	9.2	71.3	141.7	109.3	82.9
K100	9.0	125.7	86.2	91.5	78.1
K194	11.0	97.8	93.4	158.2	90.1
K269	10.6	91.8	55.0	105.2	65.6
K270	11.8	105.0	111.0	125.8	88.4
K273	9.3	45.4	130.2	200.2	96.3
Carbofuran	14.3	—	—	—	—
Control*	0	—	—	—	—
Control	10.3	131.7	166.8	168.0	119.2
Mean	11.4	94.7	117.1	143.1	
SE	18.4				
CV (%)	20.1				
LSD (P=0.05)					
Bacillus	10.5				
Rhizobium	7.4				
Bacillus vs *Rhizobium*	20.9				

Control=*Rhizobium* and nematodes
Control*= No *Bacillus*, *Rhizobium* and nematodes

Table 16: Dry shoot weight of bean plants inoculated with *Bacillus* isolates combined with *Rhizobium* strains 45 days after inoculation

Bacillus isolate	*Bacillus* alone	USDA 2674	CIAT 899	Mixture	Mean
K66	1.1	0.8	1.5	1.7	1.3
K89	0.4	0.5	1.0	1.6	0.9
K100	1.3	1.9	1.3	1.1	1.4
K194	1.1	0.8	2.1	1.3	1.3
K269	0.6	1.3	1.7	1.5	1.3
K270	0.7	0.6	1.9	1.7	1.2
K273	0.9	0.7	2.1	1.5	1.3
Carbofuran	1.0	—	—	—	—
Control*	0.6	—	—	—	—
Control**	0.3	1.5	2.4	1.5	1.4
Mean	0.8	1.0	1.8	1.5	
SE	0.3				
CV (%)	21.9				
LSD (P=0.05)					
Bacillus	0.2				
Rhizobium	0.1				
Bacillus vs *Rhizobium*	0.3				

Control*= No *Bacillus*, *Rhizobium* and nematodes
Control**=*Rhizobium* and nematodes

Plate 4. Bean plant treated with: A) a combination of *Bacillus* isolate K269, *Rhizobium* strain 2674 and nematodes. B) nematodes alone

4.7 Effect of applying *Bacillus* spp. using locally available material as carrier on root-knot nematode control

Application of *Bacillus* isolates using carriers resulted in varied responses ranging from reduction to increase in root-knot nematode galling. The *Bacillus* isolates were more suppressive on root-knot nematodes when mixed with carriers than when applied alone (Table 17). Charcoal dust followed by filter mud were the most effective carrier for the *Bacillus* isolate in nematode control (Plates 5 & 6). Use of carriers in application of *Bacillus* isolate had a significant (P=0.05) effect on galling caused by root-knot nematode infection.

Galling index was lowest in plants inoculated with *Bacillus* isolates K270 mixed with charcoal dust. The highest galling index was observed in plants inoculated with *Bacillus* isolate K269 mixed with compost. Juvenile (J_2) population differed significantly (P=0.05) among the treatments (Table 17). The lowest juvenile population was recovered from soil infested with *Bacillus* isolates K273 carried in filter mud. Highest juvenile population was recovered in treatment where *Bacillus* isolates K66 was mixed with cow manure.

There was a significant (P=0.05) increase in root and shoot weight when bean plants were treated with some *Bacillus* isolates using some carriers (Tables 19 & 20). The highest root weight was recorded in plants inoculated with *Bacillus* isolate K66 using peat as a carrier (Table 19) while the highest shoot weight was recorded in plants treated with *Bacillus* isolate K194 with compost as a carrier. Charcoal dust, filter mud and compost mixed with *Bacillus* resulted in reduced root and shoot dry weight. Root and shoot weight were generally higher when *Bacillus* spp. were applied using peat and cow manure compared to the other carriers.

Table 17: Galling index of bean plants treated with *Bacillus* spp. mixed with different carriers, 60 days after inoculation

Bacillus isolate	*Bacillus* alone	Charcoal dust	Cow manure	Compost	Filter mud	Peat	Mean
K66	8.8	5.1	6.0	7.0	6.5	7.5	6.8
K194	4.1	4.7	5.8	5.7	4.2	4.7	4.9
K269	8.2	4.0	7.3	7.8	6.8	5.2	6.6
K270	8.7	3.6	6.0	4.7	5.5	5.8	5.7
K273	8.3	5.3	5.9	4.5	5.3	7.7	6.2
Carbofuran	6.6						
Carrier alone	9.0	6.6	6.7	6.0	4.6	6.5	6.6
Mean	7.9	4.9	6.3	5.9	5.5	6.2	
SE	0.6						
CV (%)	9.8						
LSD (P=0.05)							
Bacillus	0.2						
Carriers	0.2						
Bacillus vs. Carriers	0.5						

Table 18: Number of second stage juveniles (J_2) associated with bean plants treated with *Bacillus* spp. mixed with different carriers, 60 days after inoculation

Bacillus isolate	*Bacillus* alone	Charcoal dust	Cow manure	Compost	Filter mud	Peat	Mean
K66	501	193	978	178	70	443	396
K194	295	167	240	66	113	351	202
K269	406	86	650	178	333	474	355
K270	391	50	233	82	124	290	191
K273	187	59	175	148	49	529	187
Carbofuran	503						
Carrier alone	1189	402	978	263	269	644	633
Mean	495	159	542	152	160	455	
SE	35.1						
CV (%)	10.7						
LSD (P=0.05)							
Bacillus	11.7						
Carriers	12.6						
Bacillus vs. Carriers	30.9						

Table 19: Dry root weight of bean plants treated with *Bacillus* isolates mixed with different carriers 60 days after inoculation

Bacillus isolate	*Bacillus* alone	Charcoal dust	Cow manure	Compost	Filter mud	Peat	Mean
K66	0.7	0.4	0.6	0.3	0.7	0.8	0.6
K194	0.3	0.3	0.4	0.6	0.5	0.3	0.4
K269	0.6	0.3	0.4	0.6	0.4	0.5	0.5
K270	0.7	0.3	0.5	0.2	0.4	0.4	0.4
K273	0.6	0.2	0.6	0.3	0.3	0.5	0.4
Carbofuran	0.4						
Carrier alone	0.7	0.6	0.5	0.4	0.5	0.3	0.5
Mean	0.6	0.4	0.5	0.4	0.5	0.5	70.1
SE	0.11						
CV (%)	23.6						
LSD (P=0.05)							
Bacillus	0.04						
Carriers	0.04						
Bacillus vs. Carriers	0.10						

Table 20: Dry shoot weight of bean plants treated with *Bacillus* isolates mixed with different carriers 60 days after inoculation

Bacillus isolate	*Bacillus* alone	Charcoal dust	Cow manure	Compost	Filter mud	Peat	Mean
K66	1.9	1.7	2.1	1.6	2.0	2.1	1.9
K194	1.4	1.3	1.8	2.2	1.4	1.2	1.5
K269	1.8	1.7	1.6	1.6	1.2	1.2	1.5
K270	2.5	1.3	2.0	0.6	1.4	1.3	1.5
K273	1.4	1.4	1.7	1.4	1.6	2.0	1.6
Carbofuran	1.4						
Carrier alone Mean	1.6	1.8	1.7	1.7	1.5	1.6	1.6
	1.8	1.5	1.8	1.5	1.5	1.6	
SE							
CV (%)	0.25						
LSD (P=0.05)	15.6						
Bacillus							
Carriers	0.08						
Bacillus vs. Carriers	0.09						
	0.22						

Plate 5. Bean cv. GPL-2 root: - a) inoculated with nematode alone, b) treated with filter mud alone and c) treated with *Bacillus* isolates K194 in filter mud

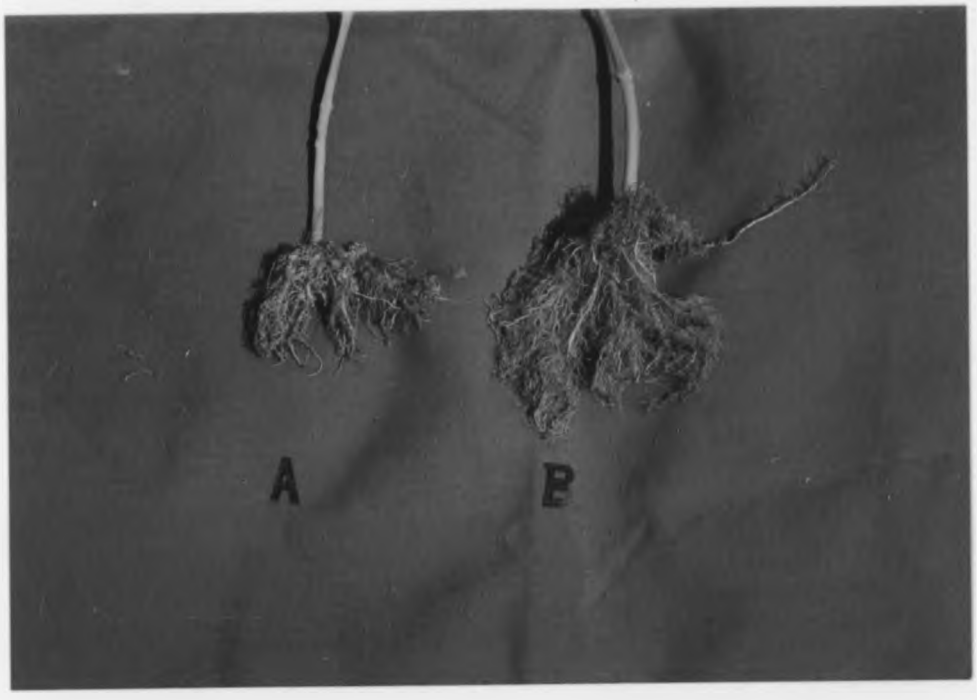

Plate 6. Bean cv. GPL-2 root: - A) treated with charcoal dust alone, B) treated with *Bacillus* isolate K270 and charcoal dust

4.8 Effect of seed treatment with *Bacillus* using different adhesives on root-knot nematodes

Sugar was the best adhesive for *Bacillus* followed by gum arabic while methylcellulose was the least effective adhesive. Galling index was highest in plants treated with *Bacillus* isolates K273 using methylcellulose as an adhesive (Table 21). Galling index was lowest on plants treated with *Bacillus* isolate K66 using sugar as an adhesive. Galling index was significantly (P=0.05) different among the treatments. The lowest J_2 count was obtained from soil planted with bean seeds treated with *Bacillus* isolate K273 using methylcellulose as an adhesive (Table 22). Juvenile numbers were highest in soil sown with seeds treated with *Bacillus* isolates K269, using sugar as an adhesive.

The highest shoot weight was recorded in plants treated with *Bacillus* isolate K273 using sugar as an adhesive (Table 23). The lowest shoot weight was recorded in plants treated with *Bacillus* isolate K270 using sugar as an adhesive. High root and shoot weight was recorded in plants treated with *Bacillus* isolates using sugar as an adhesive.

Table 21: Effect of seed treatment with *Bacillus* isolates, using different adhesives, on galling in bean plants inoculated with *Meloidogyne* spp.

Bacillus isolates	*Bacillus* alone	Gum arabic	Methylcellulose	Sugar	Mean
K66	6.2	6.9	7.9	6.1	6.8
K194	6.7	7.9	7.0	7.4	7.3
K269	6.9	6.6	7.4	7.4	7.1
K270	6.5	7.0	7.8	7.3	7.2
K273	7.2	6.8	8.3	6.3	7.2
Adhesive alone	8.8	7.8	7.7	7.2	7.9
Mean	7.1	7.2	7.7	7.0	
SE	0.9				
CV (%)	12.3				
LSD (P=0.05)					
Bacillus	0.20				
Adhesive	0.16				
Bacillus vs. adhesive	0.40				

Table 22: Effect of seed treatment with *Bacillus* isolates, using different adhesives, on *Meloidogyne* juveniles population 60 days after planting

Bacillus isolates	*Bacillus* alone	Gum arabic	Methylcellulose	Sugar	Mean
K66	203	1220	325	278	506
K194	427	467	639	526	515
K269	418	518	1326	1410	918
K270	314	358	477	556	426
K273	436	274	180	242	283
Adhesive alone	357	544	376	764	510
Mean	359	563	554	629	
SE	53.4				
CV (%)	10.1				
LSD (P=0.05)					
Bacillus	23.5				
Adhesive	19.2				
Bacillus vs. adhesive	47.1				

Table 23: Effect of seed treatment with *Bacillus* isolates, using different adhesives, on dry shoot weight of bean plants 60 days after planting

Bacillus isolates	*Bacillus* alone	Gum arabic	Methylcellulose	Sugar	Mean
K66	2.4	2.8	2.1	2.4	2.4
K194	1.9	2.9	2.7	3.3	2.7
K269	2.9	2.6	2.5	2.5	2.6
K270	2.4	2.6	2.2	2.0	2.3
K273	2.4	2.2	2.7	3.6	2.7
Adhesive alone	1.4	1.6	1.4	1.8	1.5
Mean	2.2	2.5	2.3	2.6	
SE	0.45				
CV (%)	18.9				
LSD (P=0.05)					
Bacillus	0.2				
Adhesive	0.16				
Bacillus vs. adhesive	0.40				

CHAPTER FIVE

5.0 DISCUSSION

5.1 Isolation and characterization of *Bacillus* isolates

The *Bacillus* spp. used were straight rod as described by Bergy manual of systematic bacteriology. Though the twenty *Bacillus* isolates were identified as *Bacillus subtilis*, they had varied colony characteristics and their effect on root-knot nematodes also differed. This indicates that they are different strains.

5.2 Effect of *Bacillus* spp. on root-knot nematodes

The ability of some of the *Bacillus* isolates (K194, K273, K269, K66, and K270) to suppress root-knot nematode induced galling and to promote plant growth is in agreement with finding by other authors (Becker *et al*, 1988; Oostendorp and Sikora, 1989; Stirling, 1991; Racke and Sikora, 1992; Bowmann *et al*., 1993; Oka *et al*., 1993; Siddique and Mahmood 1995; Hallmann *et al*., 1998). The 12% of the isolates that reduced root galling in this study was higher than that reported by Sikora (1988,1991), Oostenderp and Sikora, (1989) and Racke and Sikora, (1991). Zavaleta-Mejia and Van Gundy (1982) detected that 12% of the isolates were effective while Becker *et al*. (1988) reported 20% of bacteria reduced root-knot nematode damage

The ability of *Bacillus* spp. to suppress root-knot nematodes as observed in this study could be attributed to modification of root exudates which interferes with egg hatching, attraction and penetration of nematodes to the roots or through production of toxic metabolites which kill the nematodes (Becker *et al*., 1988; Oostendorp and Sikora, 1989; Sikora and Oostendorp, 1990; Spiegel *et al*., 1991; Sikora and Hoffman-Hergarten, 1992; Mankau, 1995; Hallmann *et al*., 1998). For instance, according to Oostenderp and Sikora, (1989), penetration

of *Heterodera schachtii* in sugar beet was inhibited through modification of root exudates after inoculation with *Bacillus sphaericus.* Other mechanisms include induced systemic resistance and improved plant nutrition (Hallmann *et al.*, 1998; Nagina-Parmar and Dadarwal, 1997). Presence of symbiotic microorganism and their interaction with plants could also have lead to nematode suppression through competition and/or antibiosis (Hussey and McGuire, 1987; Sikora, 1992). These possibilities, however, need to be tested.

Growth promotion in plants treated with rhizobacteria especially *Bacillus* and *Pseudomonas* has also been reported by Becker *et al.* (1988), El-sayed (1999), and Pal *et al.* (1999). Growth promotion has been associated with improved nutrient uptake, enhanced atmospheric nitrogen fixation, or induced disease resistance (Frommel *et al.*, 1991; Tuzun and Ku, 1991; Wei *et al.*, 1991; Sikora and Hoffmann-Hergarten, 1992; Sikora, 1992). According to Wei *et al.* (1991), increased root hair formation provided increased surfaces for nutrient uptake.

The higher suppressive potential of *Bacillus* on root-knot nematodes under sterile than in non-sterile soil could be as a result of competition and/or antagonism from other soil organisms present in non-sterile soil (Sikora 1992; Berggren *et al.*, 2001). Similarly lower juvenile number in sterile soil than in non-sterile soil conditions could be attributed to competition and antagonism. The growth promotion observed under non-sterile soil could be attributed to presence of beneficial rhizobacteria such as rhizobia, which enhance nutrient supply to the plant (Berggren *et al.*, 2001). These possibilities need to be tested.

5.3 Activity of culture filtrates of *Bacillus* spp. on mobility of *Meloidogyne* juveniles

Immobilization of *Meloidogyne* juvenile by culture filtrates obtained from some *Bacillus* isolates indicates that the filtrates had an inactivating effect on root-knot nematodes. Inactivation of nematodes by bacterial culture filtrate has been reported by Becker *et al.*

(1988), Spiegel *et al.* (1991), Sikora and Hoffmann-Hergarten (1992), Oka *et al.* (1993), Mankau (1995) and Hallmann *et al.* (1998). Oka *et al.* (1993) reported that *B. cereus* were able to produce filtrates that were toxic to *M. javanica* juveniles and also reduced egg hatching. The metabolites of *B. subtilis* were reported to be toxic to *Meloidogyne* spp. (Speigel *et al.*, 1991). The linear relationship observed between bacterial filtrate concentration and the percentage of juveniles immobilized by metabolites produced by *B. subtilis* K194 is consistent with previous reports by Mankau (1995) and Hallmann *et al.* (1998). Zaki (1994) reported that the proportion of *Meloidogyne* juveniles immobilized by culture filtrate from *Paecelomyces lilacinus* increased with increase in concentration of the culture filtrate. This shows that the practical use of bacterial filtrate in nematode management would be limited by sudden decline in toxicity with dilution. *Bacillus* isolates that reduced root galling also produced culture filtrates that inactivated root-knot nematodes.

5.4 Effect of *Bacillus* spp. on nodulation in bean plants

The increased nodulation when some *Bacillus* isolates were applied together with *Rhizobium* strains is consistent with earlier reports by Araujo *et al.* (1999), El-Sayed (1999) and Pal *et al.* (1999) in different crops. *Bacillus* isolates K273 and K194 consistently promoted nodulation and plant growth. Other studies have also demonstrated that *Bacillus* and other organisms in the rhizosphere enhance nodule formation in leguminous plants (Rolfe and Gresshoff, 1980; Grimes and Mount, 1984; Srinivasan and Holl, 1996). Increased nodulation could be attributed to increased root hair formation, production of phytohormone especially auxins and increased nitrogenase activity (Bauer, 1981; Holl *et al.*, 1988; Chanway *et al.*, 1991; Srinivasan *et al.*, 1996). Srinivasan *et al.* (1996) demonstrated that combining *Rhizobium etli* TAL182 and *Bacillus megaterium* induced root hair proliferation on *Phaseolus vulgaris* which led to increased infection site.

Some *Bacillus* isolates had no effect on nodulation in beans. This observation is in agreement with an earlier report by Srinivasan *et al.* (1997). Coinoculation of *Bacillus megaterium* and *Bradyrhizobium* strain TAL 644 neither induced root hair proliferation on *P. vulgaris* nor enhanced nodulation. An earlier report by Srinivasan *et al.* (1996) showed that co-inoculation with *Bacillus* spp. had no effect on nodulation in some treatments.

The increased plant growth was observed in plants that had higher numbers of nodules and is consistent with findings by Srinivasan *et al.*, 1997, Araujo *et al.* (1999), and El-Sayed (1999). Increased plant growth could be attributed to increased nutrient uptake due to increased root hair formation, influence of phytohormone especially auxin and nitrogen fixation due to increased nodulation (Bauer, 1981; Turner and Backman 1991; Srinivasan *et al.*, 1996).

5.5 Effect of combining *Bacillus* isolates with *Rhizobium* bean strains on root-knot nematode

The reduced root damage by root-knot nematodes associated with double inoculations of bean plants with *Bacillus* isolates and rhizobia strains is consistent with earlier reports by Siddique and Mahmood (1995) which showed that use of *Bacillus subtilis* and *Bradyrhizobium japonica,* reduced nematode multiplication and wilting index. Nematode suppression in plants treated with *Bacillus* spp. could be due to production of toxic metabolites (Li and Alexander, 1991) and induced systemic resistance in plants (Nagina-Parmar and Dadarwal, 1997).

The suppressed nodulation that was observed when some *Bacillus* were applied into nematode infested soil is consistent with earlier reports by Srinivasan *et al.* (1997), and

Sharma *et al.* (2000). According to Sharma *et al.* (2000), rhizobial nodulation was adversely affected when pea plants were inoculated with *Meloidogyne javanica*. This could be due to nodule invasion by root-knot nematodes, inducing giant cells and hence disintegration of the nodules. The nematodes are able to complete their development and reproduction in the nodular tissue leading to premature death of the nodules (Vovlas *et al.*, 1998). Due to premature senescence, it is possible that most of the nodules disintegrated and fell off before termination of the experiment. Suppressed nodulation could also be due to competition for nutrient and suppression of lateral root formation as aresult of nematode infection (Taha, 1993).

5.6 Effect of applying *Bacillus* spp. using locally available materials as carriers on root-knot nematode control

Use of carriers was found to be an effective technique for the application of *Bacillus* spp. This finding is consistent with earlier reports by Kokalis-Burelle *et al.* (1992) and Oka *et al.* (1993). Use of some carriers in the application of *Bacillus* isolates was as effective as drenching. Application of *Bacillus* using carriers is cheap and the carrier offers protection and support to the bacterial cells before and after application. Increased antagonistic potential of biocontrol agents was recorded in organic carriers in which the organisms were able to multiply (Kokalis-Burelle *et al.*, 1992).

Charcoal dust was found to be suitable as a carrier in application of *Bacillus* spp. The dust has been used as an alternative to peat in *Rhizobium* inoculant preparation (Beck, 1991; Somasegaram and Hoben, 1994). Charcoal-lignite mixture and charcoal-soil mixtures were found to favour higher plant growth and increased survival of *Rhizobium* (Jauhri *et al.*, 1989; Pandher *et al.*, 1993). The effectiveness of the specified *Bacillus* isolate have been reported to

be related to the number of bacterial cells in the root rhizosphere (Racke and Sikora 1992). Therefore, use of a carrier that promotes multiplication of the biocontrol agent is important. Use of carriers in the delivery of *Bacillus* spp. resulted in increased root and shoot weight in beans. These findings are consistent with an earlier report by Pandher *et al.* (1993) where lignite and charcoal were found to favour higher plant growth and survival of *Rhizobium*. Increased shoot weight could be attributed to additional nutrients from the carriers, increased water holding capacity as well as increased microbial populations, which promote plant growth (Rodrquez-Kabana 1986).

5.7 Effect of seed treatment with *Bacillus* isolates, using different adhesives, on root-knot nematodes

Seed treatment with some *Bacillus* isolates using different adhesives was found to reduce root damage by root-knot nematodes. This is consistent with previous reports by Baker and Cook (1974), Papavisa and Lumsdem (1981), Oostendorp and Sikora (1989), Sikora and Hoffmann-Hergarten (1992), and Siddique and Mahmood (1995). Reduced root damage could be attributed to target placement of the antagonistic organism leading to early rhizosphere colonization and hence competitive advantage (Schroth and Hancock, 1982). Use of adhesives such as sugar and gum arabic also reduced the negative effects of biotic and abiotic factors on survival of bacteria thereby increasing their antagonistic activity (Racke and Sikora 1992).

Use of Gum arabic as an adhesive was found to enhance the activity of *Bacillus* isolates against *Meloidogyne* spp. Gum arabic has been shown to support high numbers of rhizobial cells on seeds (Undayasuriyan *et al.*, 1996). Similarly, gum arabic was found to be the best protector of cells against biotic and abiotic stresses hence increasing survival

of bacteria on seeds (Rodriguez- Navarro *et al.*, 1991). Use of sugar as an adhesive for *Bacillus* isolates was not significantly different from gum arabic. Daza *et al.* (2000) reported that sucrose, combined with perlite, enhanced survival of bacterial cells on seeds. The numbers of colony forming units that survive on the seed have been reported to be important as it influences the level of antagonistic activity (Oostendorp and Sikora, 1989; Racke and Sikora, 1992). Use of methylcellulose (0.02%) as an adhesive for *Bacillus* spp. on bean seed was less effective in nematode damage suppression when compared with sugar and gum arabic. These findings were inconsistent with previous observations by Becker *et al.* (1988), and Oka *et al.* (1993). This could be attributed to reduced number of bacterial cells that adhered onto bean seed when methylcellulose was used as compared to sugar and gum arabic.

CHAPTER 6

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The potential *Bacillus* spp. as biocontrol agents of root-knot nematode, *Meloidogyne* spp. and growth promotion in bean plants was demonstrated under greenhouse conditions. These results show that *Bacillus* spp. can be incorporated in nematode management package. The *Bacillus* spp. also produced culture filtrates that had nematostatic properties. Increased nodulation was observed in bean plants inoculated with some *Bacillus* isolates, which led to increased plant growth. Application of *Bacillus* spp. in root-knot nematodes management would therefore have an added advantage of enhancing nitrogen supply to the plant.

Seed treatment with *Bacillus* spp. was found to be as effective as drenching in nematode control. Seed treatment is a more practical technique of applying *Bacillus* spp. Locally available material as sugar and gum arabic should be used to boost the number of bacterial cells carried on individual seed. Use of locally available materials such as charcoal and filter mud as carriers of *Bacillus* spp. has proved to be a better alternative of applying the potential *Bacillus* spp.. Apart from providing nourishment, carriers also make *Bacillus* application easier.

6.2 Recommendations

1. The potential of *Bacillus* spp. as biocontrol agents of root-knot nematodes should be verified under field conditions.
2. The mechanism leading to nematode suppression in plants treated with *Bacillus* spp. should be determined.
3. The possibility of delivering potential *Bacillus* spp. and *Rhizobiun* spp. in one carrier should be explored in a bid to develop a cost effective mode of application.
4. Investigation should be initiated to determine the effect of *Bacillus* spp. on other soilborne pathogens that infect beans.

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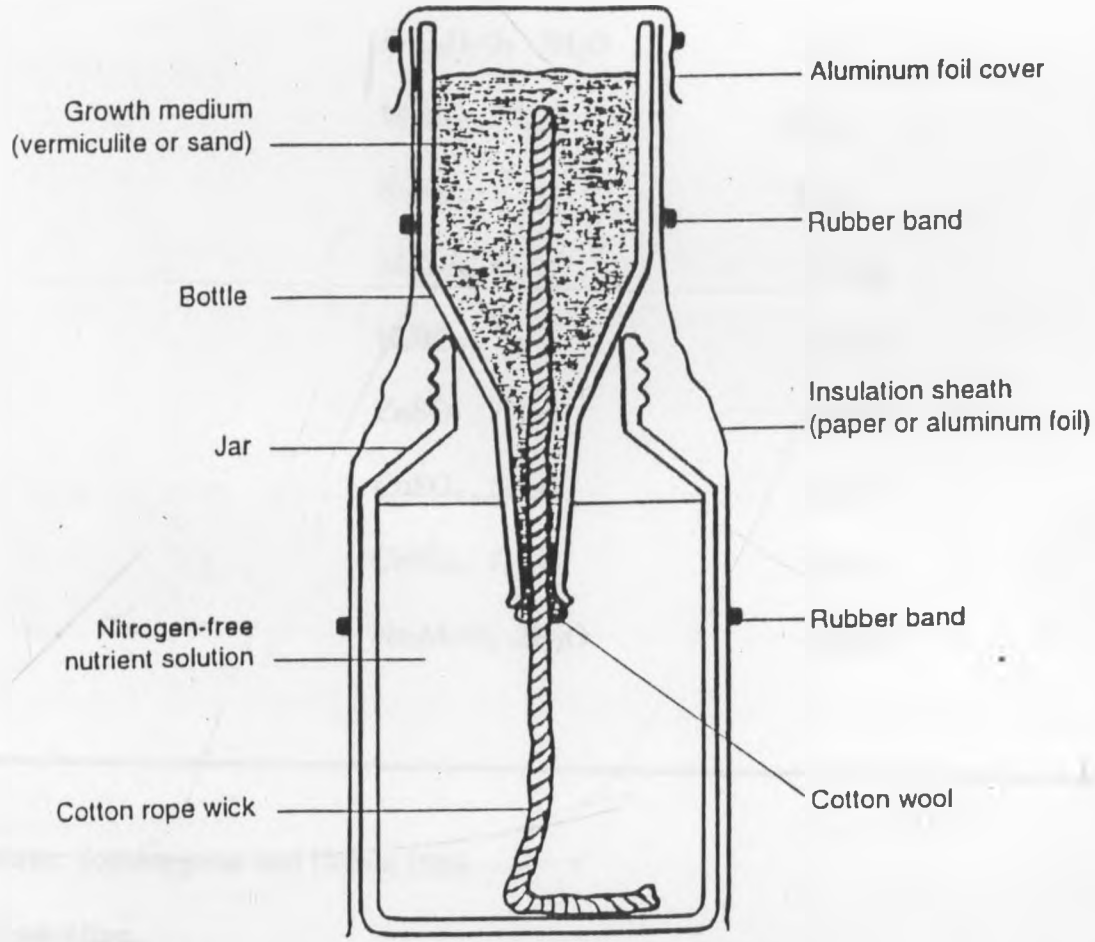
8.0 APPENDICES

Appendix 1. Leonard jar assembly



Appendix 2

N-free Nutrient solution (Broughton and Dilworth 1970)

Stock solution	Chemical	g/liter
1	$Cacl_2 \cdot 2H_2O$	294.1
2	KH_2PO_4	136.1
3	$FeC_6H_5O_7 \cdot 3H_2O$	6.7
	$MgSO_4 \cdot 7H_2O$	123.3
	K_2SO_4	87.0
	$MnSO_4 .H_2O$	0.338
4	H_3BO_3	0.247
	$ZnSO_4 . 7H_2O$	0.288
	$CuSO_4 . 5H_2O$	0.100
	$CoSO_4 . 7H_2O$	0.056
	$Na_2MoO_2 .2H_2O$	0.048

Source: Somasegaran and Hoben 1994

Preparation.

Stock solution was made using hot water to enable ferric-citrate to form solution. Five ml of each stock solution was added to 5 liters of water and diluted to 10 liters by adding 5 liters of water. The pH was adjusted to 6.6-6.8 with 1N NaOH.

Note: KNO_3 (0.05%) was added to give a concentration of 70ppm to supply nitrogen to the plants.

Appendix 3: The C: N ratio's of different materials used as carriers for *Bacillus* spp.

Carrier	C	N	C: N ratio
Cow manure	26.3	1.71	15.5
Filter mud	19.28	1.17	17
Peat	23.47	1.41	17
Compost	24.6	1.22	20.2
Charcoal	3.72	0.1	37

Appendix 4. ANOVA for galling index of nematode infested bean plants treated with *Bacillus* spp.

Source of variation	d.f.	SS	MS	F
Bacillus isolates	47	402.4	8.56	8.48**
Error	192	193.9	1.01	
Total	239	596.3		

Appendix 5. ANOVA for eggs mass index of nematode infested bean plants treated with *Bacillus* spp.

Source of variation	d.f.	SS	MS	F
Bacillus isolates	47	349.7	7.44	6.39**
Error	192	223.6	1.17	
Total	239	573.3		

Appendix 6. ANOVA for juvenile count of nematode infested soil planted with bean plants treated with *Bacillus* spp.

Source of variation	d.f.	SS	MS	F
Bacillus isolates	96	2458659.0	25611.0	9.31**
Error	291	800794.0	2752.0	
Total	387	3259453.0		

Appendix 7. ANOVA for shoot weight of nematode infested bean plants treated with *Bacillus* spp.

Source of variation	d.f.	SS	MS	F
Bacillus isolates	47	55.9	1.19	3.02**
Error	192	75.7	0.39	
Total	239	131.6		

Appendix 8. ANOVA for galling index of bean plants inoculated with *Bacillus* isolates under sterile and non-sterile soil infested with root-knot nematodes

Source of variation	d.f.	SS	MS	F
Bacillus isolates	22	245.7	11.06	17.36**
Soil type	1	45.0	45.00	69.98**
Bacillus vs soil type	22	68.1	3.10	4.81**
Error	138	88.8	0.64	
Total	183	447.6		

Appendix 9. ANOVA for egg mass index of bean plants inoculated with *Bacillus* isolates under sterile and non-sterile soil infested with root-knot nematodes.

Source of variation	d.f.	SS	MS	F
Bacillus isolates	22	275.8	12.53	18.07**
Soil type	1	30.6	30.57	44.06**
Bacillus vs soil type	22	71.3	3.24	4.67**
Error	138	95.8	0.69	
Total	183	473.4		

Appendix 10. ANOVA for number of juvenile's sterile and non-sterile soil inoculated with *Bacillus* isolates and infested with root-knot nematodes.

Source of variation	d.f.	SS	MS	F
Bacillus isolates	22	20737577	942617	285.47**
Soil type	1	27590014	27590014	8355.59**
Bacillus vs soil type	22	22082786	1003763	303.99**
Error	138	455673	3302	
Total	183	70866050		

Appendix 11. ANOVA for shoot dry weight of bean plants inoculated with *Bacillus* isolates under sterile and non-sterile soil infested with root-knot nematodes.

Source of variation	d.f.	SS	MS	F
Bacillus isolates	22	35.7	1.62	12.33**
Soil type	1	65.3	65.26	496.45**
Bacillus vs soil type	22	29.0	1.32	10.02**
Error	138	18.1	0.13	
Total	183	148.1		

Appendix 12. ANOVA for proportion of immobilized *Meloidogyne* juvenile exposed to culture filtrates of 21 *Bacillus* isolates

Source of variation	d.f.	SS	MS	F
Bacillus isolates	25	72173.71	2886.95	205.87**
Error	52	729.19	14.02	
Total	77	72902.90		

Appendix 13. ANOVA for nodule numbers of bean plants treated with *Bacillus* isolates and *Rhizobium* strains.

Source of variation	d.f.	SS	MS	F
Bacillus isolates	21	279644.1	13316.4	16.89**
Rhizobium strains	3	2457647.9	819216.0	103893**
Bacillus vs *Rhizobium*	63	311092.4	4938.0	6.26**
Error	176	138778.7	788.5	
Total	263	3187163.1		

Appendix 14. ANOVA for nodule dry weight of bean plants treated with *Bacillus* isolates and *Rhizobium* strains.

Source of variation	d.f.	SS	MS	F
Bacillus isolates	21	0.1	0.005	2.92**
Rhizobium strains	3	3.7	1.225	763.71**
Bacillus vs *Rhizobium*	63	0.2	0.004	2.31**
Error	176	0.3	0.001	
Total	263	4.3		

Appendix 15. ANOVA for shoot dry weight of bean plants treated with *Bacillus* isolates and *Rhizobium* strains

Source of variation	d.f.	SS	MS	F
Bacillus isolates	21	30.8	1.47	12.25**
Rhizobium strains	3	70.8	23.61	197.13**
Bacillus vs *Rhizobium*	63	48.2	0.77	6.39**
Error	176	21.1	0.12	
Total	263	1701.0		

Appendix 16. ANOVA for shoot dry weight of beans plants inoculated with *Meloidogyne* spp., *Bacillus* isolates combined with *Rhizobium* strains

Source of variation	d.f.	SS	MS	F
Bacillus isolates	7	4.7	0.67	8.82**
Rhizobium strains	3	28.0	9.31	121.63**
Bacillus vs *Rhizobium*	21	21.1	1.00	13.09**
Error	160	12.3	0.07	
Total	191	66.0		

Appendix 17 ANOVA for nodule dry weight of bean plants inoculated with *Meloidogyne* spp., *Bacillus* isolates and *Rhizobium* strains

Source of variation	d.f.	SS	MS	F
Bacillus isolates	7	0.1	0.01	5.88**
Rhizobium strains	3	0.5	0.18	91.94**
Bacillus vs *Rhizobium*	21	0.2	0.01	5.97**
Error	160	0.3	0.001	
Total	191	1.2		

Appendix 18. ANOVA for galling index of bean plants treated with *Bacillus* isolates in different carriers in *Meloidogyne* spp. infested soil

Source of variation	d.f.	SS	MS	F
Bacillus isolates	5	67.5	13.50	20.55
Carriers	6	21.5	3.58	5.44**
Bacillus vs carriers	30	123.7	4.12	6.27**
Error	378	248.4	0.66	
Total	419	461.0		

Appendix 19. ANOVA for galling index of bean plants treated with *Bacillus* isolates using different adhesives

Source of variation	d.f.	SS	MS	F
Bacillus isolates	5	26.1	5.21	6.54**
Carriers	3	18.6	6.21	7.78**
Bacillus vs carriers	15	59.6	3.97	4.98**
Error	216	172.4	0.80	
Total	239	276.7		

Appendix 20. ANOVA for egg mass index of bean plants treated with *Bacillus* isolates using different adhesives

Source of variation	d.f.	SS	MS	F
Bacillus isolates	5	12.4	2.47	3.59**
Carriers	3	17.7	5.91	8.59**
Bacillus vs carriers	15	46.5	3.10	4.50**
Error	216	148.7	0.69	
Total	239	225.3		