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# EFFECT OF SOIL FERTILITY MANAGEMENT PRACTICES AND *BACILLUS SUBTILIS* ON PLANT PARASITIC NEMATODES ASSOCIATED WITH COMMON BEAN, *Phaseolus vulgaris*

# [EFECTO DE PRÁCTICAS DE MANEJO DE LA FERTILIDAD DEL SUELO Y BACILLUS SUBTILIS SOBRE NEMATODOS PARÁSITOS ASOCIADOS AL FRIJOL COMÚN, *Phaseolus vulgaris*]

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## SUMMARY

On-farm and on-station field experiments were carried out to determine the potential of combining *Bacillus subtilis* with soil fertility management practices for controlling plant parasitic nematodes associated with common bean, (*Phaseolus vulgaris*). The treatments were *Bacillus subtilis* (isolate K194), *B. subtilis* plus cow manure, *B. subtilis* plus mavuno, *Bacillus subtilis* plus calcium ammonium nitrate + tripple super phosphate, manure alone, mavuno alone with calcium ammonium nitrate + tripple super phosphate as the control. The recommended farmers' practice entailed application of tripple super phosphate and calcium ammoniun nitrate at the rate of 1000 and 890 kg/ha, respectively. Manure and mavuno were applied at the rate of 10 tons and 890 kg/ha, respectively. The on-farm trial was carried out in 12 different farms. The combination of *Bacillus subtilis* inoculum and cow manure led to a 54% reduction in numbers of plant parasitic nematodes, compared to the untreated control. Consequently, damage by root-knot nematodes produced galls with galling indices1.6 and 4.5 respectively in plots treated with the combination (*B. subtilis* and cow manure) and the untreated control, respectively. Compared to the other treatments, combining *B. subtilis* and organic amendments resulted in the highest nematode diversity. It can therefore be concluded that the plant parasitic nematodes associated with common bean can be maintained at levels below economic threshold using *B. subtilis* combined with cow manure, an integration which also demonstrated conservation of the nematode diversity.

**Key words**: Diversity; inorganic fertilizers; organic amendments; biological control

## INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is the most widely grown and consumed legume in Kenya. The crop is mainly interplanted with a wide range of crops, adding the benefit of biological nitrogen fixation thereby improving the overall yields (Karanja *et al.*, 2007). A steady decline in productivity of the crop has been recorded especially in the small scale sector. The major factors limiting bean production include, pests and diseases, erratic rainfall, declining soil fertility which have resulted in low yields of 750 kg, against a potential of 1500–2000 kg/ha (Perez and Lewis, 2004). More than 80% of these losses are attributed to pests and diseases which range from plant parasitic nematodes, foliar and soil-borne fungal diseases, bacterial and viral diseases. The causal agents are known to act individually but they often form synergistic complexes that lead to severe symptoms and yield loss. Among the biotic constraints, plant parasitic nematodes are a major threat to bean production causing up to 60% yield losses (Kimenju *et al.*, 1999).

Several strategies, including chemical nematicides, organic soil amendments, crop rotation, cover crops, resistant cultivars and biological control, have been developed for the management of plant parasitic nematodes (Wang *et al.*, 2004: Kimenju *et al.*, 2008). While, environmental concerns and increased regulations have phased out the use of chemical fumigants, (Nico *et al.*, 2004) crop rotation and cover cropping are limited by scarcity of arable land. Evidence has been provided that integrating biological control using microbial antagonists with other feasible methods is amongst the most pragmatic strategies of managing the nematodes (Mostafa, 2001; Kiewnick and Sikora, 2005). Biological control agents that have been assessed include egg-parasitic fungi, nematode-trapping fungi, bacteria, and polyphagous predatory nematodes (Kerry and Hidalgo-Diaz, 2004; Kiewnick and Sikora, 2005). Plant-growth promoting rhizobacteria (PGPR) especially belonging to the genera *Pseudomonas* and *Bacillus* have demonstrated potential for disease suppression without negative effects on the user, consumer or the environment (Li *et al.*, 1998). Some strains of *Bacillus subtilis* have exhibited enormous potential as biocontrol agents in the management of root-knot nematodes (Karanja *et al.*, 2007).

In addition to nematode suppression, *B. subtilis* has also been shown to have beneficial effects on plant growth and nodulation in common bean. Other studies have established that application of organic amendments has wide ranging benefits to crops which include nutrient supply, improvement of the soil structure, and suppression of plant parasitic nematode populations (Kimenju *et al.*, 2004; Lang'at *et al.*, 2008). Information on the effect of combining biological agents with soil fertility management practices is not readily available. The present study was initiated to determine the potential of combining *B. subtilis* with soil fertility management practices on plant parasitic nematodes, and further investigate the impact of *B. subtilis* on the diversity of nematodes associated with common bean.

## MATERIALS AND METHODS

### Site description

The study area was located at Mt. Kenya Forest, near Irangi Market, Embu district. The area lies between latitudes $0^o\ 8^,$ and $0^o\ 35^,$ South and longitudes $37^o\ 19^,$ and $37^o\ 40^,$ East with an altitude ranging between 1500m and 4500 m above sea level. Embu was classified as humic nitisols (FAO, 1989) with bimodal rainfall pattern and a mean annual precipitation of 1495mm.

### On-station experimentation

The on-station experiment was conducted at the Embu Farmers' Training Centre on plots measuring 3 x 3 m. The test crop, common bean variety GLP 2 was intercropped with maize variety Hybrid 513 spaced at 30 × 90 cm. The beans were planted in between the rows of the maize crop to give a population of 100 plants per plot. The treatments were *Bacillus subtilis*, *Bacillus subtilis* + manure, *Bacillus subtilis* + Mavuno, *Bacillus subtilis* + calcium ammonium nitrate + triple super phosphate, manure, Mavuno, with calcium ammonium nitrate + triple super phosphate on its own included as the standard. The *B. subtilis* (isolate K194) used in this study was kindly supplied by the Microbial Resource Centre (MIRCEN), University of Nairobi. The isolate was selected based on the suppressive potential on root-knot nematodes and enhanced plant growth (Karanja *et al.*, 2007). The treatment calcium ammonium nitrate + tripple super phosphate consisted of triple super phosphate and calcium ammonium nitrate applied at the rate of 1000 and 890 kg/ha, respectively. Decomposed farmyard manure was applied at the rate of 10 ton/ha while Mavuno was applied at the rate of 890kg/ha. Untreated plots were included as negative controls.

### On-farm experimentation

Similar experiments were carried out on twelve randomly selected farms. In each farm, all the treatments with the exception of calcium ammonium nitrate + tripple super phosphate + *Bacillus subtilis* were arranged in completely randomized block design with five replications. After 9 weeks, the bean plants were uprooted and assessed for galling (Sharma *et al.*, 2001) and nematodes were extracted from 200 $cm^3$ soil using the Modified Baermann technique (Hooper *et al.*, 2005). Nematodes from each sample were fixed using the rapid Seinhorst technique and thereafter mounted on Cobb-type aluminum double cover glass slides that allow examination from either side (Siddiqui, 2000). Identification of the nematodes was based on morphological characteristics and pictorial keys under a microscope at high power magnification (Hunt *et al.*, 2005). In both experiments, *Bacillus* was re-isolated from soil by plating serial dilutions of 0.1 ml of $10^3$ to $10^6$ dilutions on nutrient agar and incubated at $27^0$ C for 48 hours. The colonies formed were identified using cultural and morphological characteristics as outlined by Claus and Berkeley (1986) and enumerated.

### Data analyses

Data on nematodes and colony forming units (CFU) counts were subjected to Log (x+1) transformation where necessary and then subjected to one way analysis of variance (ANOVA) and means separated using Fisher's LSD test (P=0.05). The analysis was performed by using PSWS Statistcis 18 (SPSS Inc.$^a$ 2009)

## RESULTS

### Effect of soil fertility practices on plant parasitic and non-parasitic nematodes

The various soil fertility management practices showed variable effects on the nematodes belonging to the genera *Meloidogyne* and *Pratylenchus* commonly associated with bean (Figure 1).

Compared to the control, all the treatments significantly ($P \leq 0.05$) suppressed the numbers of the two plant parasitic nematode groups in bean. Among the treatments, manure and *Bacillus* were most effective, suppressing *Meloidogyne* spp. by 64 and 60%, respectively. *Bacillus subtilis* + Mavuno, *Bacillus subtilis* + manure and Mavuno, reduced *Meloidogyne* numbers in bean by 54%>51%>28% in decreasing order, respectively. The highest suppression of nematodes belonging to *Pratylenchus* genera were observed in Mavuno (62%) followed by *Bacillus* and *Bacillus* + manure, equally reducing the numbers by 40%. With the exception of combining

Mavuno and *Bacillus subtilis*, all the treatments suppressed *Pratylenchus* numbers on bean plants.

Application of selected soil fertility management practices led to significant (P≤0.05) differences in the numbers of non-parasitic nematodes in the soil (Figure 2). The highest increase (31%) in non-parasitic nematode numbers was recorded in plots amended with manure. Numbers of non-parasitic nematodes in plots amended with *Bacillus subtilis* +manure and *Bacilllus subtilis* + Mavuno were comparable to the control. However, a 21% and 15%, reduction in the numbers of non-parasitic nematodes was recorded in plots treated with *Bacillus subtilis* and Mavuno, respectively.

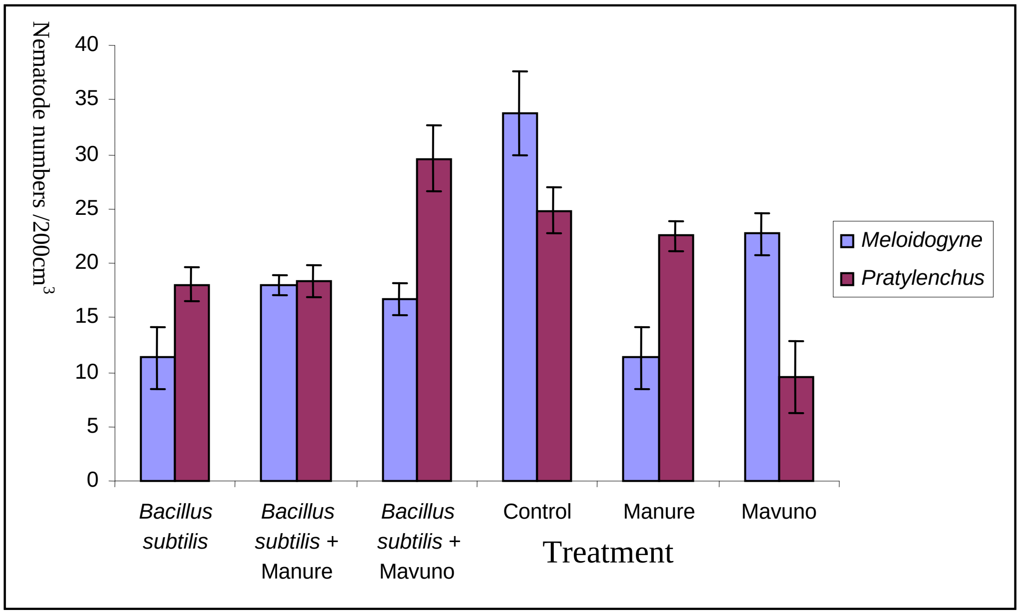


Figure 1. Response of *Meloidogyne* spp. and *Pratylenchus* spp. to soil fertility management practices

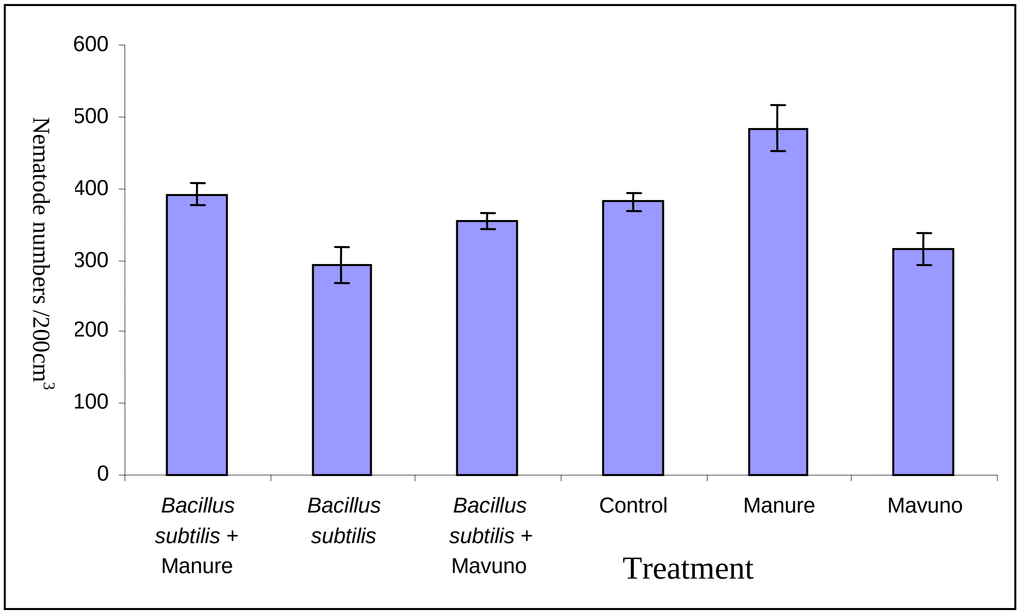


Figure .2. Influence of soil fertility management practices on non-parasitic nematodes

## Effect of soil fertility management practices on damage caused by nematodes on bean

Galling varied significantly among the bean plants grown under different soil fertility management practices (Fig. 1.3a). All the soil fertility management practices led to a significant reduction in galling. The highest reduction in galling (1.6) occurred when *Bacillus subtilis* inoculum was applied in combination with calcium ammonium nitrate + tripple super phosphate. Among the fertility management practices, manure, *Bacillus subtilis* and Mavuno recorded the highest galling indices of 3.2, 3.1 and 3.0, respectively. Moderate galling was observed under *Bacillus subtilis* + manure, *Bacillus subtilis* + Mavuno and *Bacillus subtilis* + calcium ammonium nitrate + tripple super phosphate. Similarly, all the treatments tested significantly reduced galling on beans in the farmers' fields (Fig 3b)

## Spatial distribution of nematodes

The spatial distribution of nematodes in soil varied with both depth of soil and the soil fertility management practices (Figure 4). Among the soil fertility practices, the highest nematode numbers occurred in the topsoils amended with manure while the lowest numbers were found in the subsoils treated with Mavuno. Compared to the control, incorporation of Manure + *Bacillus subtilis* in soil reduced nematode numbers in the subsoil by 22%. Similarly, a 17 and 24% decline in nematode numbers in the top soil was associated with *Bacillus subtilis* and Mavuno, respectively. While nematode numbers in the subsoils were not variable, there was a 72% increase in nematode numbers in the topsoils treated with manure (Fig. 4)

The evenness profiles of nematodes in the plots under soil management practices showed that *Bacillus subtilis* + manure exhibited the highest evenness followed by *Bacillus subtilis* + mavuno and manure (Figure 5). Nematode diversity was lowest in the plots treated with *Bacillus subtilis* alone. Detection of nematode species increased with increase in number of soil samples taken (Figure 6). However, as the curve indicates, all possible species were recovered from soil in 10 samples, implying that processing of additional samples would not have yielded more species.

## Impact of soil fertility management practices on *Bacillus subtilis*

The influence of the selected soil fertility management practices on the populations of *Bacillus subtilis* colony forming units (CFU) was investigated. Soil fertility practices had variable effects on *Bacillus subtilis*. The highest *Bacillus subtilis* CFU (5) were re-isolated from *Bacillus subtilis* mixed with manure followed by *Bacillus subtilis* alone with a value of 4.9 (Figure 7). Calcium ammonium nitrate + tripple super phosphate + *Bacillus subtilis* had the lowest CFU value of 3.41 followed by *Bacillus subtilis* + Mavuno, but both were significantly higher than control. It was observed that calcium ammonium nitrate + tripple super phosphate and Mavuno tended to reduce the abundance of *Bacillus subtilis* in the soil whereas manure boosted it.

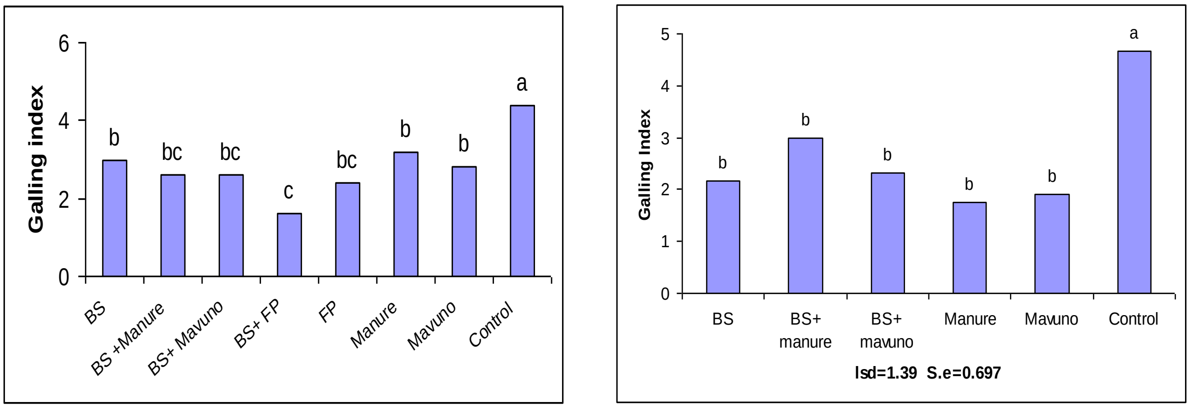
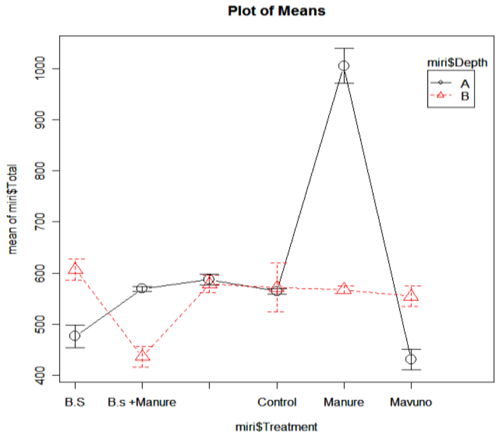
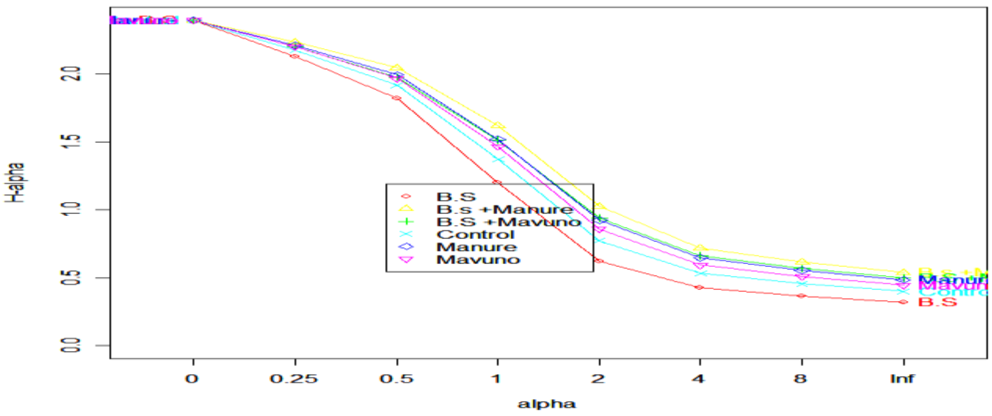


Figure .3. Effect of selected soil fertility management practices on (a) galling of bean on station and, (b) on farmers fields



A - Top soil B - Subsoil

Figure 4. Effect of soil fertility management practices and soil depth on distribution of nematodes



B.s – *Bacillus subtilis*

Figure 5. Evenness of nematode populations isolated from soil under different soil management practices in Embu District, Kenya.

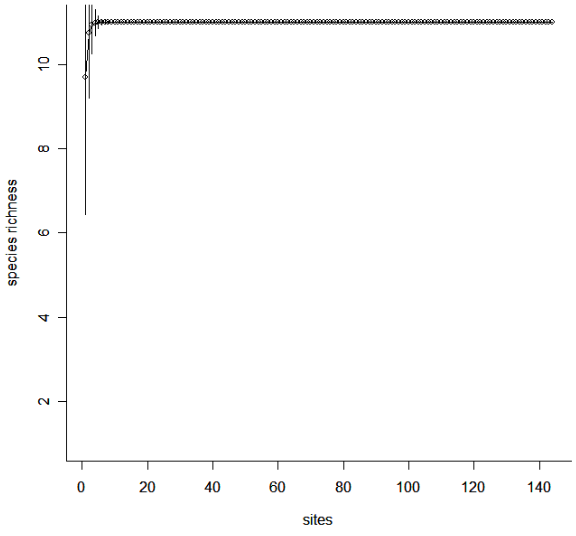
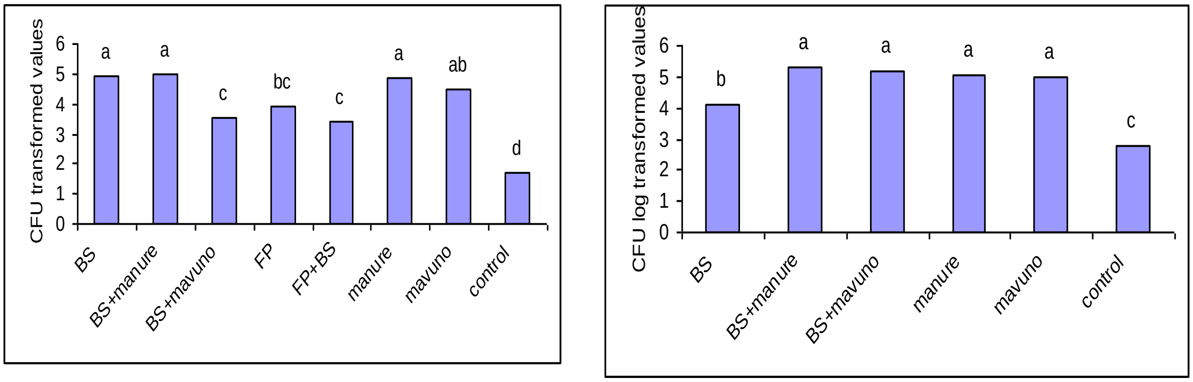


Figure 6 . Accumulation curve of nematodes isolated from Embu district, Kenya.



*B.S-Bacillus subtilis*

Figure 7. Effect of selected soil fertility management practices on *Bacillus subtilis* on station (a) and on farmers fields (b)

## DISCUSSION

This study has demonstrated that soil fertility management practices including the use of manure integrated with a biological agent, *B. subtilis*, can be used in the management of plant parasitic nematodes associated with common bean. The successful management of plant parasitic nematodes, keeping the populations below economic threshold levels, may be attributed to the compatibility of the biological control agent, *B. subtilis* and organic amendments. The complex environment of soils has often hampered biological control. According to Walker (2004), there exists a direct relationship between biological agents and organic amendments. Where successful, biological control of nematodes has mainly been achieved by conservation of existing biological agents and building up of beneficial organisms through the use of various soil amendments (Wang *et al.,* 2003). The impact of organic amendments on nematodes has been documented by Langat *et al.* (2008). While incorporation of organic amendments has been shown to be detrimental to plant parasitic nematodes (Wang *et al.,* 2004) due to release of $NH_4^+$, formaldehyde, phenols, and volatile fatty acids, it also led to stimulation of the populations of free-living nematodes. According to Langat *et al.* (2008), addition of organic amendments accelerated the decomposition of organic matter and increase in mineralization, triggering a chain reaction that favours the increase of free living nematodes as our study has shown.

The introduction of beneficial soil organisms to the soil has only been attempted successfully in a few instances. *Pasteuria* spp which are bacterial parasites, nematode trapping fungi (Wang *et al*., 2004, *Pochonia chlamydosporia* (Hildiago-Diaz and Kerry, 2008) are examples of biological agents that have successfully been used in suppression of *M. incognita* and *M. javanica* on a wide variety of crops. *B. subtilis* was explored because the microbes inhabited the rhizosphere, which have shown a notable influence on plant health and soil quality (Karanja *et al.,* 2007). The suppression of root-knot nematode by *B. subtilis*, as found in our study, agrees with previous reports (Siddiqui *et al.,* 2001; Kokalis-Burelle *et al.,* 2006; Karanja *et al.,* 2007). Among the plant growth-promoting rhizobacteria (PGPR), *Pseudomonas* and *Bacillus* are the genera most commonly described as having PGPR but many other taxa also contain PGPR (Barea *et al.,* 2005). The reduction of nematode plant parasitic nematodes associated with *B. subtilis* may be attributed to diverse mechanisms which involve phytohormones production, mineral solubilisation, reduction of the activity of egg hatching factors, alteration of root exudates and inhibition of nematode penetration into the roots thereby interfering with host finding process and reducing galling as indicated in our results (Karanja *et al.*, 2007). Chemotaxis towards exudate components has also been regarded as an important trait for root colonization (De Weert *et al*., 2002).

The diversity of nematodes was highest in plots treated with a combination of *B. subtilis* and Manure. Incorporation of manure is thought to have improved the soil environment to aid the establishment of *B. subtilis*. Generally, plant-associated microbiota have been demonstrated to have a crucial role in maintaining the soil ecological balance and therefore the sustainability of either natural ecosystems or agroecosystems (Barea *et al.*, 2005). It is therefore this ecological balance that seems to have sustained the diversity of nematodes where *B. subtilis* was applied as a nematode antagonist (Siddiqui *et al.,* 2007). It is well established that agricultural intensification including the indiscriminate use of fertilizers and pesticides has resulted in deterioration of soil microbe diversity and health (Kar *et al.*, 2008). As a result, intermittent use of inorganic fertilizers impact on soil dwelling microorganisms and invertebrates and/or through the food chain by bio-accumulation through trophic levels with detrimental effects on soil fauna diversity and functional services (Ekschmitt and Korthals, 2006). Among the soil microorganisms, nematodes have been shown to be potentially affected since they have repeatedly been shown to respond differentially to xenobiotic substances (Jonker *et al.*, 2004). The diversity of soil nematode communities is often related to various aspects of soil status, and has to be responsive to inorganic nutrient enrichment and pollution (Ekschmitt *et al.,* 2001).

This study has demonstrated the ecological benefits on integrating a biological control agent, *B. subtilis* and organic amendments in the management of plant parasitic nematodes in common bean. It can be concluded that the plant parasitic nematodes associated with common bean can be maintained at levels below economic threshold using *B. subtilis* combined with cow manure.

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