Case Study

PLANT PARASITIC NEMATODES ASSOCIATED WITH COMMON BEAN (*PHASEOLUS VULGARIS L.*) AND INTEGRATED MANAGEMENT APPROACHES

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ABSTRACT

A field study to determine the distribution and population densities of plant parasitic nematodes associated with beans was undertaken in Kakamega, Kiambu, Machakos and Siaya districts of Kenya. *Meloidogyne spp*. and *Pratylenchus spp.* were the most predominant endoparasites, occurring in 86 and 61% of the root samples, respectively. Ectoparasitic nematodes in the genera *Scutellonema* and *Helicotylenchus* were recovered in 86 and 59% of the soil samples, respectively. Field experiments were conducted to determine the efficacy of organic amendments (chicken manure, compost, neem leaves, baobab remains and farm yard manure) in the control of root-knot nematodes. The amendments showed varying levels of nematodes suppression with chicken manure being rated as the most effective with galling index of 2.4 while sisal wastes were least effective with galling index of 5.1. Another study was undertaken to determine the reaction of 35 bean genotypes to *Meloidogyne incognita*. Ten genotype were rated as susceptible while 3 and 22 genotypes were rated as resistant and moderately resistant, respectively. The potential of different *Bacillus* isolates to suppress galling by root knot nematodes in beans was investigated using sterile sand in Leonard jars under greenhouse conditions. The isolates had varying effect with the majority (93%) of the isolates causing a reduction in root galling when compared to the control (water). Twelve percent of the isolates were more effective than carbofuran (nematicide). In another greenhouse experiment investigating the interaction between *Bacillus spp.* and *Rhizobium* strains inoculations using N-free sterile sand, 4 out of the 20 *Bacillus* isolates significantly promoted nodulation in bean plants.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is the most important legume crop in Kenya and is cultivated on an estimated 700,000 ha. A low average yield of 750 kg/ha is realized, against a potential of 1500 kg/ha (Rheenen *et al.*, 1981). The major constraints to bean production are diseases, soil fertility, insect pest and low erratic rainfall (Otsyula *et al.*, 1998). Common bean is plagued by a wide range of plant parasitic nematode, but only

Meloidogyne spp. are of economic importance, causing up to 60% losses in yield (Ngundo and Taylor, 1974, Kimenju *et al.*, 1999). Apart from the direct losses resulting from root deformation, nematode infection is also known to break host resistance to other pathogens and to suppress nodulation. Several strategies have been developed for the control of root-knot nematode but their adoption level by smallhold farmers is limited (Table 1).

Table 1. Strategies of nematode control and their limitations

Strategies	***Major limitations***	***Reference***
Crop rotation	Wide host range of *Meloidogyne* spp.	Thomason and Caswell (1987)
Organic amendments	Too large quantities required	Rodriquez-Kabana (1986)
Resistant cultivars	Unavailable to farmers	Ngundo (1977)
Chemical Control	Uneconomical on common bean	Hague and Gowen (1987)
Biological control	A viable alternative to chemicals	Sikora (1992)

This study was undertaken with ultimate aim of developing an integrated strategy of managing root-knot nematodes on beans.

MATERIALS AND METHODS

- **Sample collection and nematode extraction**. A survey of nematodes associated with common bean (*Phaseolus vulgari*s L.) was carried out in Kakamega, Kiambu, Machakos and Siaya districts. Twenty soil and bean root samples were collected from each of the 25 randomly selected farms in each district. Plants were gently uprooted and a trowel was used to dig out soil from the bean rhizosphere to an average depth of 25cm. All the roots obtained from each farm were placed in a polythene bag but only about 3kg of the composite soil sample from each farm was transported to the laboratory. Nematodes were extracted from soil and roots using the sieving/filtration and maceration/filtration techniques as described by Hooper, (1990). Identification of nematodes to genus level was done using an identification key and descriptions by Mai and Lyon (1975). Nematode population levels were determined from a counting slide under a compound microscope and expressed either as number per 200cm^3 soil or 5g roots.
- **Isolation and screening of *Bacillus* spp. against root knot nematodes**. *Bacillus* spp. were isolated from the surface of healthy bean roots grown on soil collected from Machakos, Kiambu and Thika districts of Kenya and used in a greenhouse experiment where effect of *Bacillus* spp. on root-knot nematode populations and galling in beans was assessed. Two hundred and fifty *Bacillus* isolates were evaluated in three batches to determine their effect on nematodes and plant growth. *Bacillus* isolates were grown on nutrient agar at 27^{o}C

for 48 h, harvested and inoculum concetration adjusted to ca 10^9 colony forming units (cfu)/ml. Clean sand was placed in Leonard jars and steam sterilized for 1 h. Three bean seeds were sown in each jar but thinning was done at emergence to leave one seedling 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 pots were treated with carbofuran (nematicide) or sterile distilled water. Treatments were arranged in a completely randomized design with eight replications. Eight weeks after emergence, plants were gently uprooted, and washed free of sand. Galling and egg masses were rated using a scale of 1-9 (Sharma *et al*., 1994). Second- stage juveniles (J_2) were extracted from $200cm^3$ soil using the sieving and filtration technique and enumerated (Hooper, 1990). Twenty *Bacillus* isolate were selected from this experiment and further tested using sterile and non sterile soils.

- **Effect of organic amendments on damage by root-knot nematodes on beans.** A greenhouse experiment was conducted using chicken, cow manure, leaves of *Mucuna pruriens, Azadirachta indica* (neem) and *Tagetes minuta* (marigold) as organic amendments. The amendments were applied at the rate of 5% (w/w) to soil which was held in 5kg pots. The pots were sown with beans (GLP-2) at the rate of 3 seeds/pot which ere later thinned to two plants per pot. A nematicide (carbofuran), applied at the rate of 1g/kg soil, and soil alone were included as controls. The pots were infested with eggs and juveniles of *Meloidogyne* at the rate of 6000 eggs/pot. A non-inoculated control was included. The experimental design was completely randomized with 10 replicates. Sixty days after soil infestation with nematodes, the plants were uprooted, washed free of soil using tap water. Galling, egg mass indices were and juvenile numbers were assesses using the methods described above. Plant growth was assessed using dry shoot and root weight. The experiment was repeated once following the procedure described above but with 8 replicates instead of 10 to confirm repeatability of the experiment.

RESULTS

Endoparasitic nematodes of the genera *Meloidogyne* and *Pratylenchus* and ectoparasitic species belonging to the genera *Scutellonema* and *Helicotylenchus* were frequently extracted from soil or bean roots (Table 2). The endoparasitic nematodes, *Meloidogyne* and *Pratylenchus* spp., were present in 86 and 61% of the root samples, respectively. Eighty and 59% of the soil samples haboured *Scuttellonema* and *Helicotylenchus* spp., respectively.

- **Effect of *Bacillus* spp on root-knot nematodes and plant growth**

The effects of *Bacillus* spp. on plant growth and root-knot nematode infection differed significantly (P=0.05) among the isolates. Out of the 250 *Bacillus* isolates that were tested against root-knot nematodes, 93% reduced galling when compared to the control (water) while 12% were more effective in reducing galling and egg mass indices than carbofuran. One hundred and thirty five (54%) isolates were found to be as effective as carbofuran. Fifty (20%) isolates were found to promote plant growth. One hundred and fifty six isolates had no effect on plant growth while 44 isolates suppressed growth. In the repeat experiment, the isolates showed a pattern that was consistent with the first experiment.

Table 2. Diversity and occurrence of plant parasitic nematodes in soil and bean roots collected from four districts in Kenya

Nematode genus	***% frequency of nematode occurrence per district***				***Overall***
	Kakamega	***Kiambu***	***Machakos***	***Siaya***	
Soil					
Meloidogyne	96	42	84	72	74
Pratylenchus	96	48	80	48	68
Scutellonema	100	80	64	76	80
Helicotylenchus	76	80	24	56	59
Tylenchorhynchus	12	36	52	32	33
Tylenchus	4	8	12	0	6
Criconemella	0	0	8	0	2
Aphelenchus	0	8	4	0	3
Hemicycliophora	0	44	0	0	11
Trichodorus	0	8	0	0	2
Roots					
Meloidogyne	96	80	88	80	86
Pratylenchus	76	32	76	60	61

The effect of *Bacillus* isolates on root-knot nematodes was assessed using selected strains in sterile and non-sterile soils was as shown in Table 4. Generally, the isolates were found to perform better in sterile than in non-sterile soil. Galling index was lowest in bean plants treated with K61 and K67 in sterile soil while in non-sterile soil K48 had the lowest galling. Similarly, egg mass index was lowest in plants treated with isolates 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 soil and non-sterile soil, respectively. The number of *Meloidogyne* juveniles was significantly (P=0.05) different among treatments in sterile and non-sterile soil (Table 4). Numbers of second-stage juveniles (J_2) were higher in non-sterile soil than in sterile soil. Juvenile numbers were lowest in sterile soil treated with *Bacillus* isolate K78 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 K61 and K100 under sterile and non-sterile soil conditions, respectively.

Table 3. Galling index, egg mass index and J_2 count of bean plants inoculated with *Bacillus* isolates

Bacillus isolate	Galling index			Egg mass index			J_2 count/200 cm^3 soil		
	S	NS	Mean	S	NS	Mean	S	NS	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
K61	2.5	4.0	3.3	2.8	5.0	3.9	488	505	496
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 $_{0.05}$									
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	

S = Sterile soil, NS = Non-sterile soil

Application of organic amendments resulted in reduced galling, egg masses, juveniles and improved growth of bean plants (Table 5). Chicken manure had significantly (P=0.05) different effects in all the parameters measured compared to the other amendments.

Table 4. Effect of organic amendments on root knot nematode damage on beans root and bean biomass production

Amendment	Galling index	Egg mass index	Juveniles/200cm^3 soil	Shoot dry weight	Root dry weight
Tagetes	3.5	4.8	133	5.6	1.0
Neem (A. indica)	3.5	5.4	174	7.4	1.1
Mucuna sp.	4.2	4.5	213	7.2	0.8
Chicken manure	2.1	3.3	83	11.1	1.4
Cow manure	4.6	7.8	521	5.4	0.7
Carbofuran	6.8	7.9	1192	3.0	0.5
Control	6.5	8.1	1112	2.2	0.2
L.S.D.(P=0.05)	0.7	0.8	86	1.2	0.3

Chicken manure followed by mean and Tagetes was the most effective amendment. Cow manure which is commonly used by farmers was least effective. The nematicide (carbofuran) that is widely used by farmers in the control of nematodes especially in vegetable production had no effect on nematodes.

DISCUSSION

Nematodes in the genera *Meloidogyne*, *Pratylenchus*, *Scutellonema* and *Helicotylenchus* are widely distributed in bean fields in Kenya. Diversity and frequency of occurrence of nematodes in the four genera were highest in Kakamega district. Warm and wet conditions prevailing in the district (Jaetzold and Schmidt, 1983), coupled with a high cropping intensity of *P. vulgaris* are ideal for plant parasitic nematode population build-up. Incidence and population densities of the predominant nematodes were, however, low in Kiambu district in spite of high cropping intensities in the district. This could be attributed to frequent use of cow manure for soil fertility improvement by most farmers in this district (Woomer *et al*., 1998). *Pratylenchus* spp. are common inhabitants of the rhizosphere of bean plants. Lesion nematodes, especially *P. zeae*, is a serious pest on maize in Kenya (Kimenju *et al*., 1998) where maize and beans are grown as intercrops by the small-holder farming communities in Kenya (Wortmann and Allen, 1994; Gethi *et al.*, 1997).

Locally isolated *Bacillus* strains showed potential for use as biocontrol agents of root-knot nematodes on beans. Out of 250 isolates that were tested, 12% of them caused a reduction in galling. This percentage was greater than that reported by Oostendorp and Sikora (1989) and Sikora (1988) on sugar beets and cotton, respectively. Zavaleta-Mejia and Van Gundy (1982) detected that 12% of the isolates were effective while Becker *et al*., 1988 reported that 20% of bacteria were antagonistic to root-knot nematodes in cucumber. The isolates were found to protect plants against plant parasitic nematodes as reported by Becker *et al*. (1988); Bowmann *et al*. (1993); and Oka *et al*. (1993). The ability of *Bacillus* isolates to suppress nematodes can be attributed to reduced egg hatching, modification of root exudates which interferes with the host finding processes of the nematodes or production of metabolites that are toxic to the nematodes (Sikora and Hoffman-Hergarten, 1992; Mankau 1995; Hallmann *et al*., 1998).

A remarkable reduction in activity and/or mobility was observed when second-stage *Meloidogyne* juveniles were treated with extracts from organic amendments suggesting that substances released by decomposing amendments had nematostatic effects (Miano, 1999). Extracts from chicken manure, *Tagetes*, *Mucuna* and Neem (*A. indica*) appear to have strong nematicidal properties. Several workers have reported this aspect (Padma *et al*., 1997; Kaplan and Noe, 1993).

The ability of chicken manure to reduce nematode damage and population in amended soils is well documented (Kaplan and Noe, 1993; Akhtar and Mahmood 1997; Miano,1999). Extracts from *Mucuna* spp. were found to reduce the activity of Meloidogyne juveniles (Marisa *et al*., 1996). This was attributed to aliphatic alcohol and esters released during decomposition. *Mucuna* has also been used as a soil amendment in the management of plant parasitic nematodes (Chavarria-Carvajal and Rodriguez- Kabana, 1998).

CONCLUSIONS

Root knot nematodes are widely distributed and cause substantial yield losses of common bean in Kenya. The potential of organic amendments to suppress root knot nematodes and to increase bean yield was demonstrated. This study further demonstrated that *Bacillus spp*. are a viable component of integrated nematode management packages.

Further work is however required to test the efficacy of the *Bacillus* isolates under field conditions and to develop a cost effective mode of application preferably leading to a method whereby *Bacillus spp*. and *Rhizobium* inoculant would be packaged together.

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