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Potential of green manure crops in suppressing root knot nematodes in French beans

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Abstract

Root knot nematode is one of the major pests on French bean (*Phaseolus vulgaris* L.) and the control is mainly through the use of synthetic nematicides. The high costs and increased safety concerns of nematicides has necessitated research for eco-friendly and sustainable methods. This study was conducted to evaluate the efficacy of green manure plants in suppressing root-knot nematode (RKN) in French beans. Field and greenhouse experiments were carried out in a Randomized Complete Block Design and Completely Randomized Designs, respectively. This was done at selected flower farms to evaluate the efficacy of four green manure crops namely Caliente mustard (*Sinapis alba*), Nemat (*Eruca sativa*), Sudan grass (*Sorghum sudanense*) and African Marigold (*Tagetes minuta*). These were compared with Metam sodium and Vydate® (Oxamyl) positive control. Damages on plants were assessed based on galling indices, crop biomass, J2 counts and tomato yield. There was a significant ($P \leq 0.05$) difference in RKN population densities, galling indices and yield between the green manure treatments and the control in both field and greenhouse trials. Root-knot nematode densities and galling indices were highest in the untreated control, however, no significant ($P \geq 0.05$) differences in the efficacy of green manure plants and Metam sodium in the greenhouse was observed. The green manure crops reduced the population densities of RKN by over 90% in the greenhouse condition and by over 67% under field conditions. The results of this study indicate that the green manure plants can be adopted to suppress RKN in French beans.

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Introduction

French bean is among the major horticultural crops produced in Kenya for export. Although the crop has mainly been grown for export purposes, there has been an increasing demand for domestic consumption (HCDA, 2010). Due to repeated planting of French beans on same parcels of land over the years with minimal rotation, there has been a lot of pressure on available nutrients in addition to build up of soil pests and diseases, most importantly *Fusarium* wilt and RKN (HCDA, 2005; HCD,A 2010). RKN has been noted to be a key pest in most of the French bean regions in Kenya, mainly because of improper crop rotation, lack of information on diagnosis and restriction on the approved nematicides for use. RKN generally reduces the rooting mass of the crop with malformation of the roots resulting to reduced uptake of water and nutrients and poor anchorage of the affected plants (Kimenju *et al*., 2008; Brand *et al*., 2010).

The only few nematicides approved for use on French beans against RKN are expensive and the mode of application is complex for the small scale farmers (Moens *et al*., 2009). Metam Sodium has been used with some level of success but it has been reported to eliminate useful micro-organisms. Many soil amendment practices aimed at increasing soil nutrition have been found to be having some effects in suppressing RKN as they act as biofumigants (TEAP, 1997; Kimenju *et al*., 2004).

Bio-fumigation from Brassicas is largely associated with high concentration of glucosinolates which are the precursors of isothiocyanates (ITCs) which have broad biocidal activity (Lord *et al*., 2011). For effectiveness of green manure crops in managing RKN, there is need for proper incorporation and proper timing with best timing being just at the onset of flowering (Hooks *et al*. 2010). This study was undertaken with the aim of assessing the effectiveness of various green manure crops in managing the RKN.

Materials and methods

Both greenhouse and field experiments were conducted at Dudutech Research and Development Station, Finlay's Kingfisher Farm, Naivasha, longitude 36.2^{o} E latitude 0.8.498^{o} S. Naivasha is situated approximately 100 kilometres northwest of Nairobi. Average regional temperatures range between 17.3 and 22.7 ^{o}C; annual rainfall ranges from 656 mm to 1134 mm per annum.

Field experiment

Bulking of root-knot nematode and growing of green manure crops

French bean crop was planted in rows in the experimental field previously cropped with French bean with a history of RKN infestation. The field was sampled prior to planting and it was ascertained to contain around 200 second stage juveniles (J2's) in 100 cm^{3} soil. The crop was grown for 8 weeks in order to build up the nematode population ahead of the trials. This was followed by planting of four green manure crops on the periphery of the blocks measuring 3m by 4m. The green manure crops used were Caliente mustard (*Sinapis alba*), Nemat (*Eruca Sativa*), African marigold (*Tagetes minuta)* and Sudan grass (*Sorghum sudanense*)

Preparation and application of green manures

After eight weeks of growing the green manure crops, these were chopped and incorporated into the soil directly. Chopping was timed to coincide with the onset of flowering. Two weeks later, French bean (Serengeti variety) was planted in the four plots previously planted with the four green manure plants (Caliente mustard, Nemat, African marigold, Sudan grass), (Fig 1) in addition two plots with no growth of green manure crops were added one with French beans and the positive control plot treated with Metam Sodium. Diammonium Phosphate fertilizer was added at a rate of 10g/plant at planting time and the treatments were arranged in a randomized complete block design (RCBD) replicated five times. Drip irrigation was used to irrigate the plants and calcium ammonium nitrate (CAN) fertilizer was applied at the rate of 5g/plant twice at second and sixth week after germination.

Assessment of French bean reaction to green manures

Assessment for the various parameters was done 50 days after planting and these parameters included; nematode densities, galling indices and dry biomass.

Five plants were randomly selected from each block uprooted and the soil washed off and the roots examined for the galling index. The plants were dried in an oven to a constant dry weight. Composite soil sample (200 cm^3) was obtained from each of the five treatments and nematodes extracted using Baermann technique as described by Hooper (1990). The galling indices were determined using a scale of 0-10 adopted from Bridge and Page (1980) where; 0 = No galls on roots, 1 = Few small galls difficult to find, 2 = Small galls clearly visible, main root clean, 3 = Some larger galls visible, main roots clean, 4 = Larger galls predominate but main root clean, 5 = 50% of roots infested, galling on parts of main roots, reduced root system, 6 = Galling on main roots, 7 = Majority of main roots galled, 8 = All main root galled, few clean roots visible, 9 = All roots severely galled, plant usually dying and 10 = All roots severely galled, no root system, plant already dead. The rest of the plants were left until maturity where other parameters and yields were recorded for each plot and later converted to equivalent ton/ha for each of the treatment.

Greenhouse experiments

Preparation and application of green manures

Eight weeks after planting the green manures, number of plants of each green manure plant species were randomly and carefully selected and uprooted at the start of flowering. Separation was done for the uprooted plants into shoots and roots. Roots were dipped in a bucket of water to soak and rinse off the soil. Roots and shoots were oven-dried at 70^oC to constant weight to determine dry matter of the various green manure plants.

Based on the dry matter of various green manure plants, the green manure plants were ground using motar and pestle and incorporated into the nematode-infested soil at the rate of 5% w/w so as to constitute a total of 5kg of medium per pot. The soil-green manure mixture was mixed thoroughly to homogeneity, sprinkled with water and left in the greenhouse for a period of 14 days after which French bean cultivar Serengeti was planted at a seed rate of 4 seeds per pot.

The four treatments (Caliente mustard, Nemat, African marigold and Sudan grass) and the two controls (untreated control and positive control treated with Metam Sodium) were arranged in a completely randomized design (CRD) replicated five times. Drip irrigation was used to irrigate the pots three times per week. Di-ammonium phosphate (DAP) fertilizer at a rate of 10g/pot was used at planting time and calcium ammonium nitrate (CAN) fertilizer at the rate of 5g/pot twice was applied twice, initially at second week followed by sixth week after germination.

Assessment of French bean growth and nematode infestation

The following parameters were assessed 50 days after sowing namely; nematode densities, galling indices and dry biomass. Galling index, dry matter and nematode determination was done as for the field experiment explained as above. For nematode analysis, a composite soil sample of 200 cm^3 was taken from each pot in the green house from a depth of 5-10 cm at the beginning, mid and end of the season. The nematode population density was done as explained above at Dudutech Laboratory.

Statistical analysis

Analysis of variance of each variable was carried out at 5% probability level using GENSTAT 7.2 programme and the means compared using the Least Significant Difference tests at 5% probability level.

Results

Effect of green manure plants on root-knot nematode (RKN) in French bean

Green manure plants suppressed RKN significantly compared to untreated control in both greenhouse and field experiments (Fig. 1). A significantly higher mean galling index was observed in French bean roots from untreated plots compared to those treated with green manures.

The green manure Nemat had the lowest mean galling index (1.64) followed by *Tagetes* spp. (1.8). Reduction in galling was also observed in French bean roots from plants treated with Caliente and Sudan grass. The reduction was comparable to that of Nemat and Tagetes (Fig. 1).

Table 1. Effect of green manures on Root-knot nematode (RKN) population densities, biomass and yield of French bean plants under greenhouse conditions.

Green manure plants	RKN in 200 cc soil	Biomass of French bean (g)	Yield (Kg/ ha)
Caliente	22.0 ±4.47[a]	52.0[a]	9.4 [ab]
Nemat	12.0 ±5.70 [a]	65.7[a]	8.6 [ab]
Sudan grass	20.0 ±3.16 [a]	64.8[a]	9.2 [ab]
Tagetes	13.0 ±8.37 [a]	73.5[a]	12.2[b]
Control	1200 ±501.31 [b]	61.0[a]	7.5[a]

Values having same superscripts in a column are not significantly different at 95% confidence level.

Key: GHSE- Greenhouse; t ha^{-1}- Tonnes per hectare.

The nematode densities and galling indices at the end of the season in the untreated control were significantly higher ($P \leq 0.05$) compared to the RKN densities and galling indices in the green manure-treated plots (Table 1). Nematode suppression that occurred following treatment with all the green manures was not significantly different ($P \geq 0.05$) between the various treatments. There was no significant difference ($P \geq 0.05$) in biomass between the green manure-treated and the untreated control but the yields in the green manure treatments were relatively higher than the untreated control except for Tagetes which gave a significantly ($P \leq 0.05$) higher yield.

Furthermore, French bean plants treated with Tagetes yielded significantly higher ($P \leq 0.05$) than those treated with Caliente, Nemat and Sudan grass (Table 1).

Effect of different green manure plants and alternative fumigant Metam sodium on RKN in French bean

Greenhouse experiments

There were no significant differences ($P \geq 0.05$) observed in RKN second stage Juvenile (J_2) population densities, galling indices and biomass of French bean plants treated with different green manure plants and Metam sodium (Table 2).

Table 2. Effect of different green manure plants on root-knot nematode (RKN) infestation on French bean under greenhouse conditions.

Green manure plant / type of treatment	Mean RKN (in 200 cc soil)	Mean Galling index	Mean dry crop Biomass (g)
Caliente	1.72[a]	0.52[a]	1.72[a]
Nemat	1.63[a]	0.41[a]	1.82[a]
Sudan grass	1.70[a]	0.51[a]	1.82[a]
Tagetes	1.64[a]	0.45[a]	1.87[a]
Metam sodium	1.67[a]	0.46[a]	1.72[a]
Control	2.72[b]	0.82[b]	1.79[a]

Transformation by log (x+1). Values having the same superscripts along a column are not significantly different at 95% confidence level.

Higher ($P \leq 0.05$) RKN population densities and galling indices were observed in the control (520 J2s/200cc soil and galling index of 5.6, respectively) whereas the lowest nematode population densities were observed in the pots treated with Nemat (42 J2s/200cc soil and galling index of 1.6). High RKN densities and galling indices were observed in the untreated control which was significantly higher ($P \leq 0.05$) than other treatments. However, there were no significant differences ($P \geq 0.05$) in crop biomass among all the treatments (Table 2).

Population dynamics of root-knot nematodes (RKN) in the Field

A reduction of RKN in response to bio-fumigation was observed: Caliente and Metam sodium showed similar declining trends throughout the growing seasons. The reduction in RKN population density in the mid-season was followed by a further reduction at harvest (Fig. 2). Nemat, Sudan grass and Tagetes displayed a different trend, whereby a reduction in the population density of RKN in the mid-season was followed by an increase in the RKN density at the end of the experiment.

Fig. 1. (a) Green manure plants (Sudan grass) before incorporation into soil, and (b) Caliente and Marigold on the background.

The untreated control had the highest RKN population densities at the end of the season (Fig. 2).

Discussion

Effect of green manure plants on root-knot nematode (RKN) in French bean

Green manures were found to suppress nematodes as also reported by Wang and Chao (1995), Morris and Walker (2002), Wang *et al.* (2003) and Kimenju *et al.* (2008). Green manure crops have the ability to suppress nematode diseases by changing the soil physical and chemical properties and by enriching the soil with beneficial microflora. The suppression of RKN may also result from a combination of processes such as the decomposition of organic matter by many soil microbes (fungi, actinomycetes, and bacteria) and release of nematicidal compounds by the plants. The decomposing matter increases the populations of the natural enemies of PPN leading to an enhanced natural nematode control.

In addition to the build-up of microbial antagonists of nematodes, the presence of the nitrogenous compound released by green manures plays an important role in the reduction of nematode populations in treated soils (Lazarovits *et al*., 2000). Anhydrous and aqueous ammonia, urea and other ammonium compounds have been used for nematode control (Rodriquez-Kabana *et al*., 1987).

Root-knot nematode reduction was highest following treatment of French bean plots with Nemat (*Eruca sativa*) (79% reduction) followed by *Tagetes erecta* and Metam sodium in the greenhouse. The two brassicas; Nemat (*Eruca sativa*) and Caliente (*Sinapis alba*) have demonstrated their ability to suppress RKN as also reported by Lord *et al.* (2011) due to production of glucosinolates which when hydrolysed in the soil in the presence of moisture forms isothiocyanates (ITC). Halbrendt (1999) also reported reduction of *Xiphinema americanum* populations in temperate orchards following incorporation of brassicas in the soil.

In a recent study by Pakeerathan *et al.* (2009), incorporation of the fresh foliage of wild-grown Chrysanthemum (*Chrysanthemum coronarium* L.) into a *M. javanica* infested soil at rates ≥40g/kg reduced root galls of tomato plants and increased plant weights. Green manures tested in this study produced significantly high French bean biomass and pod weight which is due to decomposition of green manures releasing minerals such as N, P and K which alters the soil physical and chemical properties. The amount of nitrogen that is available to the succeeding crop from green manure crops is usually in the range of 40-60% of the total amount of nitrogen that is contained within the green manure crop (Sullivan *et al*., 2003).

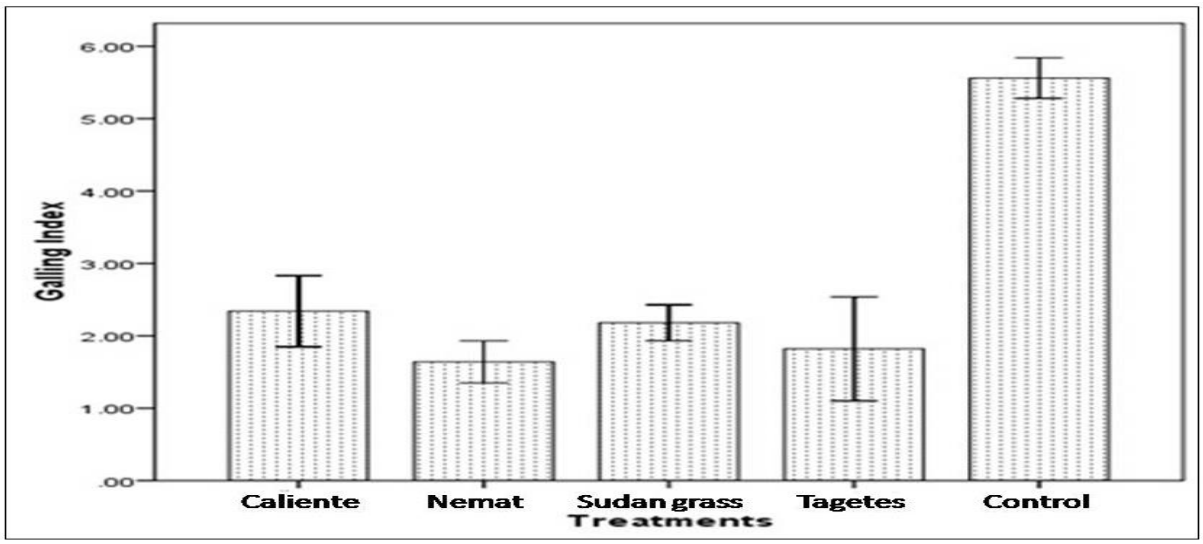

Fig. 2. Galling index in French bean roots in different green manure treatment.

Effect of different green manure plants and alternative fumigant Metam sodium on RKN

As reported by Rahman (2012) marigolds (*Tagetes patula*) and African marigolds (*Tagetes erecta*) release the chemical alpha-terthienyl which is nematicidal, insecticidal, antiviral and cytotoxic. The presence of alpha-terthienyl inhibits the hatching of nematode eggs (Siddiqui and Alam, 1987).

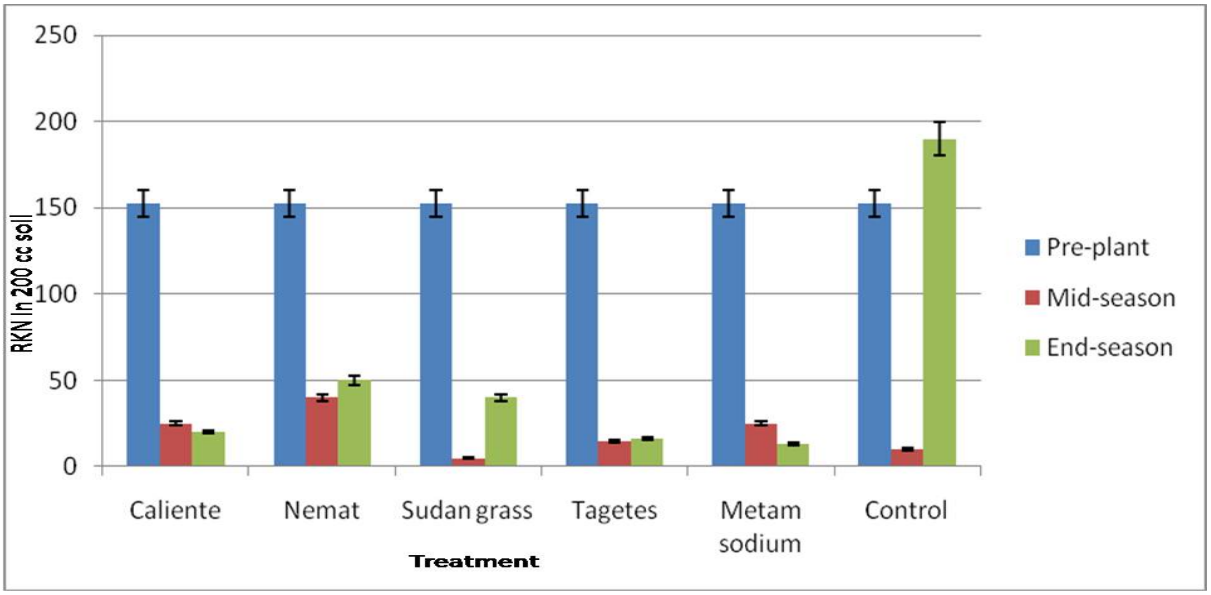

Fig. 3. Population trend of root-knot nematodes (RKN) through the growing season in the field.

According to Ploeg and Maris (1999), *Meloidogyne* spp. juveniles were unable to fully develop in the roots of *T. erecta*. The low juvenile counts observed following treatment with *Tagetes* spp. agrees with reports from Steiner (1941) who observed that only a few of the nematodes are able to penetrate marigold roots and reach maturity resulting to low juvenile counts. In this study, *T. erecta* gave consistent performance in the suppression of RKN both in the greenhouse and in the field.

Metam Sodium had a significant reduction in nematode counts as was also observed by Ingham *et al.* (2000) who reported reduction in RKN densities following treatment with metam sodium.

Conclusion

Green manure crops have a significant potential in the suppression of RKN (*Meloidogyne* spp.) under both greenhouse and field conditions.

Various types of green manures have variable effects on the general biological activities in soil and more so against RKN. This study showed evidence that green manures can be used for the management of RKN. The addition and maintenance of high levels of organic matter, especially the active fraction greatly improves the physical, chemical and biological properties of the soil, therefore increasing productivity. The introduction of green manures as an option in the management of plant parasitic nematodes and improvement of soil fertility should become a major component in the overall sustainable management of soil health and productivity. More studies should be done to evaluate the effect of the green manures against other plant pathogenic organisms.

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