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# Potential rotation crops and cropping cycles for root-knot (*Meloidogyne spp.*) nematode control in tomato

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**Abstract** Tomato is attacked by several plant parasitic nematodes but root-knot nematodes are the most devastating and cause considerable losses in Kenya. Studies were undertaken under greenhouse and field conditions to determine the suppressiveness of a wide range of plant species to root-knot (*Meloidogyne* spp.) nematodes. Potted plants were inoculated with 6000 eggs and /or juveniles while the field experiments were in nematode infested fields. Among the plants tested, *Tagetes patula, Gossypium hirsutum, Desmodium uncinatum, Chloris gayana, Zea mays, Alstroemeria* sp., *Capsicum annuum, Crotalaria juncea, Arachis hypogaea, Sorghum bicolor, Tithonia diversifolia and Pennisetum purpureum* were rated as poor hosts with galling and egg mass indices ranging from 0 to 3. High galling and egg mass indices ranging from 7-9 were recorded on *Lablab purpureus, Coriandum, sativum, Statice* sp., *Brassica oleracea* var. *gloria, Helianthus annuus, Vigna subterranea* while *Muguna pruriens, Lactuca sativa, Allium ampeloprasum, Sesamum indicum, Allium cepa, Onnis* sp., *Brassica Oleracea* Var. *chinensis, Asparagus* sp., *Brassica oleracea* var. *botrytis, Ornithogolum arabicum. Tuberose* sp. *and Chrysanthemum indicum*, were rated moderately resistant with galling and egg mass indices ranging from 3 to 6.

Damage by nematodes was significantly (P=0.05) reduced in tomato planted after sweetcorn or in sweetcorn with *Tagetes patula*, *Crotalaria juncea, Sorghum bicolor* and *Asparagus sp*. in the field. This study shows that despite the fact that *Meloidogyne* spp. have wide host ranges, there is a wide range of economically important plants from which suitable candidates can be selected for use as rotation or interplants in their management.

Key words**:** Antagonistic, *Crotalaria* sp., *Tagetes* spp., galling, egg masses, resistant.

## Introduction

Rotating crops in a sequential cropping system is widely regarded as a good agricultural practice in traditional and modern agriculture (Bridge, 1996). Crop rotation systems are particularly useful in maintaining soil fertility and reducing or preventing build up of pests and diseases especially in the soil (Netscher and Sikora, 1990). The principle that guides use of crop rotation in nematode management is to reduce populations of the damaging nematode species to levels that allow subsequent crops to complete early growth before being heavily attacked (Bridge, 1996). This can be achieved by alternating poor hosts, non-hosts or resistant crops with susceptible or tolerant crops (Swamy *et al*., 1995).

Although sequential cropping is recognized as a strategy in root-knot nematode control its adoption especially in the smallholder farms is restricted due to scarcity of arable land coupled with market-driven production of particular crops and/ or varieties (Bridge, 1996). In addition, a lot of skill is required to design and implement effective crop cycles for the control of such pathogens as root-knot nematode that have broad host ranges (Kerry, 1990, Yamada, 2002). Previous studies have been focused on plants such as *Tagetes* spp., *Crotalaria* spp., *Asparagu*s spp., sesame, and neem that are antagonistic to root-knot nematodes because they release root exudates that are toxic to the nematodes (Sukul, 1994, Varges-ayala *et al*., 2000). Low or lack of commercial value of the most intensively studied plants is however, a major hindrance to their adoption into cropping systems (Johnson *et al*., 1992). Suitability of a crop for incorporation into a rotation cycle is not only determined by its efficiency in nematode suppression but also by the economic returns. The challenge to research is therefore, to identify nematode suppressive crops that satisfy the economic considerations in crop production. This study was conducted with the aim of identifying potential rotation crops with food, forage or commercial value and incorporate them into cropping cycles for root-knot nematode management in tomato.

## Materials and methods

### Screening of potential rotation crops for root-knot nematode suppression

**Greenhouse experiment.** Thirty-six plant species (Table 1) were selected and evaluated to determine their reaction to root knot nematodes under greenhouse conditions. Tomato C.V. Moneymaker and *Tagetes minuta* were included as negative and positive controls, respectively. Pots measuring 21 cm in diameter were filled with 5 kg heat sterilized loam and sand, mixed in the ratio 2: 1(v/v). Three seeds of each test plant were sown in each pot but thinning was done after emergence to leave one seedling per pot. Ten days after seedling emergence, 6000 eggs and/ or juveniles, suspended in 10ml of water, were pipetted into indentations made around the base of the seedlings in each pot and soil pushed back to cover the roots. Treatments were arranged in a completely randomized design with ten replications. Plants were watered when necessary and fertilized on a biweekly basis by adding 5g of calcium ammonium nitrate (CAN) into each pot. The experiment was terminated eight weeks after inoculation. Plants were then gently uprooted and roots washed free of adhering soil using tap water. Galling was quantified using the scale of 0-10 as described by Bridge and Page (1980) where, 0 = healthy root system, 1 = very few galls only detected upon close examination, 2 = small galls easy to detect, 3 = numerous small galls, 4 = numerous small galls and a few big ones, 5 = 25% of the root system severely galled, 6 = 50% of the root system severely galled, 7 = 75% of the root system severely galled, 8 = no healthy root but plant still green, 9 = completely galled root system and plant dying, 10 = plants and roots dead. Plants with scores ranging from 0-3 were rated as resistant while those with scores ranging from 4-6 and from 7-10 were rated as moderately resistant and susceptible, respectively. Egg masses were stained using phloxine B (Holbrook *et al*., 1983) and quantified using a scale of 1-9 where 1=no egg masses, 2=1-5, 3=6-10, 4=11-20, 5=21-30, 6=31-50, 7=51-70, 8=71-100, 9= >100 egg masses per root system (Sharma *et al.,* 1994). Second-stage juveniles were extracted from 200cm$^3$ soil using the modified Baermann funnel technique and enumerated (Hopper, 1990). The experiment was repeated once.

**Field Experiment.** The plants selected for this test were *Tagetes patula*, *Crotalaria juncea*, *Sorghum bicolar*, *Desmodium* sp., sweetcorn and cotton which had been rated as resistant in the greenhouse experiment with tomato C.V. Moneymaker as a control. The plants were grown in nematode infested micro-plots measuring 1 x 1.8m. Each micro-plot had 4 rows with 5 plants/row planted in a spacing of 25 x 60cm. Fertilization was done by adding 5g of diamonium phosphate to each planting hole. Weeds were controlled regularly and plants irrigated when necessary. The experimental design was randomized complete block with three replications.

Initial nematode inoculum in the soil was determined by taking five soil samples at random following the procedure by Dropkin (1980) and second-stage *Meloidogyne* juveniles extracted using the modified Baerman funnel technique and enumerated (Hooper, 1990). After 90days, five plants were randomly selected from the middle rows of each micro-plot and carefully uprooted, and damage by nematodes assessed using the galling index scale developed by Bridge and page (1980). Soil samples were collected from ten different points in each micro-plot, composited and second-stage juveniles extracted as described above. The experiment was repeated once.

**Effect of growing tomato in rotation with antagonistic plants in combination with sweetcorn on *Meloidogyne* spp. in an infested field.** The effect of rotating tomato with sweetcorn or sweetcorn undersown with *Crotolaria juncea, Asparagus* spp., *Tagetes patula, Sorghum bicolor* or *Allium sativum* on root-knot nematodes was determined under field conditions. A nematode infested field was selected and the above plants sown in plots measuring 4 x 4m. The initial nematode density in each field was determined following the procedure described above.

During the first three months, sweetcorn was undersown with T. *patula, Sorghum bicolor, Asparagus* sp., *Crotalaria juncea*, sweetcorn and tomato C.V. Moneymaker as a control. Sweetcorn was planted at a spacing of 30x75cm and single rows of the antagonistic plants sown in between. Plants were fertilized by adding 5g of diammonium phosphate into each planting hole. Weeds were regularly controlled and the plants irrigated when necessary. The experimental design was randomized complete block design with five replications. After three months, ten sweetcorn plants were randomly selected from each plot, uprooted and their roots washed free of soil. Data on dry shoot and cob weights were taken. Soil samples were taken from ten different points in each plot for juvenile population assessment. Plots were tilled before transplanting one-month-old tomato C.V. Moneymaker seedlings at a spacing of 25 x 75cm.

The experiment was terminated 60 days after transplanting by gently uprooting 10 randomly selected tomato plants from each plot and roots washed free of soil before rating them for galling and egg masses following the procedures described above. Second-stage juveniles were extracted from soil samples and enumerated as described above. Dry shoot weight of the ten plants was also taken and the experiment repeated once following the same procedure.

## Results

**Greenhouse experiment.** There were significant ($P \leq 0.05$) differences in galling, egg masses and juvenile counts among the plants tested (Table 1). Galling and egg mass indices ranged from 6.6 – 9.0 in tomato, rapeseed, lablab, coriander, spring onion, and cabbage C.V. Gloria, sunflower, statice and bambara nuts. These plants were rated as susceptible. Ornithogolum, tuberose, onnis, leekswiss, chrysanthemum, garlic, velvetbean, chinese cabbage, asparagus, broccoli, lettuce, sesame and red onion were rated as moderately resistant with galling and egg mass indices ranging from 3 – 6. *Tagetes patula*, desmodium, rhodes grass, alstroemeria, cotton, crotalaria, napier, sorghum, peanut, sweetcorn, capsicum and tithonia were resistant with galling and egg mass indices ranging from 1-3.

No egg masses were observed on roots of desmodium, rhodes grass and alstroemeria. Few egg masses (<10 per root system) were observed on sweetcorn, cotton, capsicum and nappier grass roots. Tomato C.V. Moneymaker had the highest number of egg masses but was not significantly ($P=0.05$) different from cabbage C.V. Gloria, rapeseed, sunflower, lablab, bambara nuts and coriander (Table 1). The highest counts of *Meloidogyne* juveniles was recovered from soils grown with tomato whereas the lowest was from soils grown with peanut (Table 1).

**Field experiment.** Results of the microplot experiment were similar to those observed in the greenhouse. Significant ($P \leq 0.05$) differences in galling and egg mass indices were observed among the plants tested (Table 2). Galling indices ranged from 1.2 to 6.8 with rhodes grass having the lowest (1.2) and tomato the highest (6.8). All the tested plants had galling indices that ranged between 1.2 and 2.4 thus being rated as resistant compared to tomato (control) that was susceptible with a galling index of 6.8. The egg masses followed a trend similar to that of the galling index and ranged from 1.4 to 2.9 among the tested plants while tomato C.V. Moneymaker had the highest of 7.3. There were significant ($P \leq 0.05$) differences in juvenile ($J_2$) populations between treatments and the control (Table 2). *Meloidogyne* juvenile count was highest (1630) in plots where tomato was grown and lowest (373) in plots grown with *Tagetes* spp. (Table 2).

**Effect of growing tomato in rotation with sweetcorn undersown with antagonistic plants on root-knot nematodes.** This differed significantly ($P \leq 0.05$) among the treatments (Table 3). Galling was lowest (1.9) on tomato grown in rotation with sweetcorn undersown with *Tagetes patula* and highest (7.4) under continuous tomato (Table 3). Galling indices ranged from 1.9 to 3.0 on tomatoes grown in rotation with sweetcorn alone or in combination with plants antagonistic to nematodes. The egg masses on tomato grown in rotation with different rotational treatments followed a trend similar to that observed on galling index (Table 3). Tomato grown in rotation with sweetcorn undersown with *Tagetes patula* had the lowest (2.9) egg mass index.

There were significant ($P \leq 0.05$) differences in juvenile ($J_2$) populations among the treatments (Table 3). The lowest juvenile population was recovered from plots planted with sweetcorn undersown with *Tagetes patula* while the highest was recovered from plots under tomato monoculture. Shoot weights of tomato were significantly ($P \leq 0.05$) different among the treatments (Table 3). The lowest shoot weight (10.5g) was recorded under tomato monoculture and the highest (21.6) on tomato grown in rotation with *Tagetes patula*. There were significant ($P \leq 0.05$) differences in the yield of sweetcorn among different rotational treatments (Table 3). The lowest sweetcorn yield was recorded in plots where sweetcorn was underswon with *Tagetes patula* while the highest was observed in plots undersown with *Crotalaria juncea.* The dry weight of sweetcorn stalks followed a similar trend (Table 3).

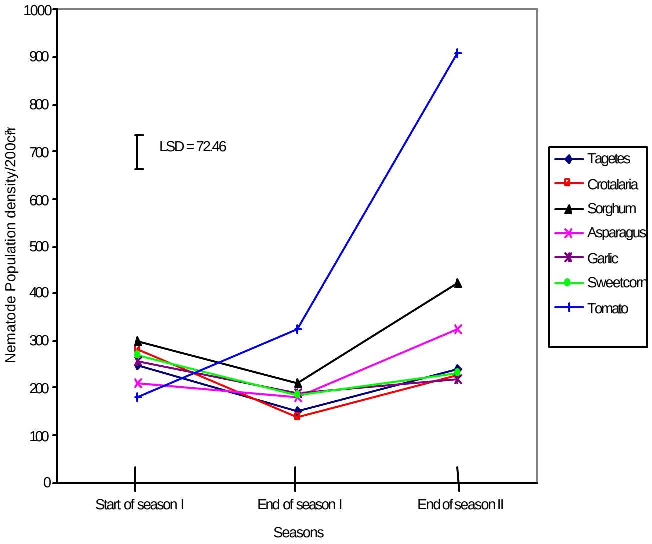
Generally nematode populations in plots planted with sweetcorn alone or sweetcorn undersown with *Tagetes spp.*, Crotalaria, sorghum, asparagus or garlic continued to decrease during season 1 compared to tomato monoculture (Fig. 1). However, at the harvest of the tomato crop, nematode population increases were observed in all the plots. The highest nematodes population increase was obtained from plots where tomato was rotated with sweetcorn and undersown with *Crotalaria juncea* while the lowest was in those plots of tomato rotated with sweetcorn undersown with *Tagetes patula* (fig.1). There was a continuous nematode population increase in tomato monoculture while the highest reduction in nematode population was noted in rotations using sweetcorn undersown with *Tagetes patula.*

## Discussion

This study demonstrated that marigold *(Tagetes patula* and *T. minuta),* sunnhemp *(Crotalaria juncea),* cotton (*Gossypium hirsutum*), desmodium, rhodes grass, sorghum, sweetcorn, alstroemeria, capsicum and peanuts were suppressive to root-knot nematodes under greenhouse and field conditions. Our findings are in agreement with previous studies (Mc Sorley, 1999; Kinloch and Rich 2001). However, little has been done on most of the above plants in Kenya. The explanation for effectiveness of marigold in the management of root-knot nematode is the toxic $\alpha$-terthienyl and derivatives of bithienyle that they produce that are toxic to nematodes (Uhlenbroek and Bijloo, 1957).

Desmodium legume is a high quality forage crop that can be incorporated into cropping systems for soil fertility improvement and soil erosion control, with the added advantage of root-knot nematode suppression. Suppression

**Fig 1. Nematode population changes in plots where sweetcorn, was undersown with different antagonistic plants and rotated with Tomato**



of *Meloidogyne* by sorghum could be attributed to presence of glycosides in its cells that become exposed in its tissues if injured by nematodes leading to the release of highly toxic hydrogen cyanide (Meyer and Fry, 1978) that kill the nematodes. This cereal can be incorporated in diverse cropping systems particularly as a fallow crop during dry seasons in order to boost food security in these areas as it is well adapted to a wide range of environmental conditions.

These results also demonstrated that cotton suppressed root-knot nematode reproduction. Although *Meloidogyne* spp. are a serious pest of cotton, varieties that are highly resistant to the nematodes have been identified elsewhere but not in Kenya (Veech and McClure, 1977). This could be due to high concentrations of terpernoid aldehydes found in their roots that are toxic to the nematodes. This crop can be used in our country to revitalise our textile industry that has collapsed and alleviate poverty in cotton producing areas. Apart from production of nematicidal compounds, antagonistic plants may also reduce nematode populations by acting as trap crops (Bridge, 1996). Nematodes invade roots of such plants but their development and reproduction is inhibited. For instance, Dasaeger and Rao (1999) reported that juveniles of *Meloidogyne* species freely entered roots of resistant plants like sunhemp but failed to multiply. In addition, roots of some plants may not be a food source for certain nematodes, thereby reducing their numbers by starvation (Bridge, 1996).

There was moderate nematode damage on roots of garlic, velvetbean, lettuce, leekswiss, sesame, red onion, onnis, chinese cabbage, asparagus, broccoli, ornithogolum, tuberose and chrysanthemum. This indicates that these crops support root-knot nematode reproduction to a certain extent and should therefore be introduced into cropping systems with caution and particularly those farmers who constantly interplant these crops with the susceptible ones. Farmers should be encouraged to rotate them with resistant ones to reduce the nematode inoculum level in the soils.

Damage by root-knot nematodes on mustard, statice, spring onion, rapeseed, cabbage C.V. Gloria, sunflower, lablab, coriander and bambara nuts was similar to that observed on tomato. This is proof that these crops are susceptible and should be avoided in cropping systems particularly in soils known to heavily infested by *Meloidogyne*. These findings are very important since very little work has been done in our country concerning the reaction of root knot nematodes on these plants.

Undersowing sweetcorn with nematode antagonistic plants suppressed galling by root-knot on subsequent tomato nematodes resulting in vigorous growth of tomato crop. These findings agree with previous reports (Sikora, 1992; Korthals *et al*., 2000) Increase in nematode population density was slowest under tomato plants grown in plots previously under sweetcorn and *Tagetes patula.* indicating that there was continued nematode suppression after removal of marigold. Tomato plants grown in rotation with sweetcorn undersown with *Tagetes patula* had higher shoot weight than tomato grown after tomato.

Undersowing sweetcorn with *Crotalaria juncea* resulted in reduced nematode populations and minimal damage on the succeeding tomato crop. It also resulted into increase in yield of sweetcorn implying that nitrogen fixation was taking place that might have lead to improved plant growth. Therefore farmers should be encouraged to use crotalaria as a rotational crop as it both reduces rootknot nematode population density and increase the yield of a companion or succeeding crop.

## Conclusion

Observations made from this study were based on greenhouse, microplot and on-station field experiments. On-farm studies are required to verify these findings and establish the acceptability of selected crops as rotational or interplants for root-knot nematode management. Studies should be undertaken to explore the mechanisms of resistance involved in these plants such as, physical barriers like xylem bundle sheaths, production of toxic substances and post- infectional substances that these plants produce when attacked by nematodes. Use of antagonistic plants should be evaluated in combination with other control strategies like organic amendments to establish their effect on biodiversity that is necessary for sustainable management of nematodes in agro ecosystems.

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