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# Effects of plant extracts on tomato (*Solanum lycopericum*) infection by root-knot nematodes in Kenya

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**Abstract**

Tomato (*Solanum lycopericum*) is an annual subtropical vegetable. Its production in Kenya has suffered a major blow due to the high disease incidences caused by parasitic nematodes, especially root-knot nematodes. The current method of nematode control involves the use of chemical nematicides. Unfortunately, significant concerns such as environmental pollution have been encountered as a result of their usage. This makes it important to continually look for new and effective methods of nematode management that pose less risk to humans and the environment. The objective of this study was to determine the effects of *Allium sativum*, *Azadirachta indica*, and *Lantana camara* extracts on the level of infection of tomatoes by root-knot nematode disease and the influence of tomato performance in Mwea-East, Kenya. Screen house experiments were done to assess the effect of the plant extracts in the control of the root-knot nematode; *Meloidogyne* spp. Treatments included; *Azadirachta indica*, *Allium sativum*, *Lantana camara* extracts, vydate (a commercialised nematicide) and untreated (negative) control. The experimental design was a completely randomised design including five treatments. Data on tomato growth parameters and *Meloidogyne* spp. infestation was collected. Results showed that all the three extracts significantly ($P \leq 0.05$) increased growth as indicated by the significantly higher shoot height, root length and dry weight when compared to the untreated plant. Galling index and egg mass index differed significantly among the treatments ($P \leq 0.05$). These results show that plant extracts are an alternative to chemical nematicides that cause pollution to the environment.

**Keywords** – Kenya – *Meloidogyne* spp. – Nematicide – Plant extracts

## Introduction

Tomato (*Solanum lycopericum*) is an annual subtropical vegetable. Its origin is in southern American Andes (Peralta & Spooner 2007). Tomatoes grow well in warm conditions and its varieties are now found growing in many regions of the world, sometimes in greenhouses in regions that have very cool climates (Naika et al. 2005). Uses of tomatoes may range from an ingredient in dishes, a salad, a drink, or a sauce (Da Silva et al. 2008). The tomato fruit contains lycopene, which is of importance to health. It is rich in calcium, magnesium, iron, Vitamin A, B6 and C (Raffo et al. 2002, Naika et al. 2005, Ravelo-Perez et al. 2008). It is important in improving the levels of antioxidants

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such as ascorbic acid, lycopene and phenols in the diet (George et al. 2004). Tomato production in Kenya is for domestic consumption and export even though most of the produce is sold in urban centers with less than 15% being exported (HCDA 2012).

Even though there is increasing demand for the crop, the major blow is its susceptibility to diseases. Many plant parasitic nematodes attack tomato plants growing in different regions. These nematodes include genus such as Helicotylenchus, Haplolaimus, Meloidogyne, Pratylenchus, Rotylenchulus and Tylenchus (Osei et al. 2012). Tomato plants are known to be the most favourable hosts for root-knot nematodes (RKN) (Dnyaneshwar et al. 2010). The RKN groups known to attack tomatoes include the four major species of Meloidogyne (*M. hapla*, *M. arenaria*, *M. incognita* and *M. javanica*) and their known races in both indoor and outdoor cultivations (Onkendi et al. 2014). The estimated global yield losses for arable crops associated with RKNs are estimated between 5-43% in the tropical and subtropical areas (Surendra et al. 2014). In Africa, *M. arenaria*, *M. incognita* and *M. javanica* are the most dominant species and their presence and damage on small holder tomato production in Mwea, Kenya has been documented (De Waele & Elsen 2007).

The use of synthetic nematicides has been a common practice in managing plant parasitic nematodes (PPN). The indiscriminate use of these pesticides in nematode control has resulted in environmental pollution, phytotoxicity and nematode resistance (Yudelman et al. 1998). Some synthetic pesticides are also costly, and some farmers cannot buy them (Koon-Hui et al. 2007). Due to the rise in pollution, there is a need to protect the environment due to the current pressure against synthetic chemicals (FAO 2006). This has necessitated more research for alternative ecologically and safe nematode management methods.

Therefore, the study was carried out to evaluate the possibility of *Allium sativum*, *Azadirachta indica*, and *Lantana camara* extracts being used as alternatives for the management of root-knot nematodes in tomatoes. The botanical extracts are considered less costly, readily available, and more ecologically friendly as they do not cause pollution.

## Materials & Methods

### Description of study area

The study was carried out in Mwea East, Kirinyaga County in Kenya. Mwea-East occurs on latitudes 0º 42’ 0’’S and longitude 37º22’ 0’’E. The area mainly has black cotton soils with some areas having vertisol (Wamari et al. 2016). Kirinyaga County is in central region of Kenya and covers an area of 1479.1 $km^2$ with a temperature range of 12°C – 26°C. Rainfall ranges between 1100-1250 mm. The altitude of the area is 1158 – 5380 metres above sea level. Agriculture is the major economic activity in the county due to its fairy good weather patterns. In this area tomato production is widely practiced and is done for both domestic and commercial purposes. The main economic activities in the area include horticulture, rice farming and commercial businesses (Kabutha & Mutero 2000).

### Tomato plant sample collection

Purposive sampling method was used in the collection of tomato plant samples from tomato farms. These farms had long history of root-knot nematode disease. Samples of diseased (galled) tomato plants were collected in polythene bags from the farmers’ fields. A composite sample was obtained by mixing the subsamples collected from the various farms. These composite samples were then transported to the plant science laboratory at Kenyatta University, Kenya.

### Preparation of *Allium sativum, Azadirachta indica* and *Lantana camara* extracts

Mature leaves of *Lantana camara* were collected from Kenyatta University while the leaves of *Azadirachta indica* were collected from Garissa-North Eastern region of Kenya due to their high presence. The bulbs of *Allium sativum* were brought from the local market in Mwea town. The leaves of *A. indica* and *L. camara* were left to air dry for five days under a shade. A sterile blender was then used to grind the dried leaves to fine powder. *Allium sativum* bulbs were washed in sterile distilled

water and then placed in an oven for drying. Temperatures were set at 40°C and left for 48 hours. The dried bulbs were then ground into powder.

A hundred ml of sterilised distilled water was placed in a 1litre conical flasks. A 20g powder of each the plant materials were then added to the stoppered conical flasks and left for 24 hours. After the 24 hours, filtration was performed using cheese cloth and the extracts stored in the refrigerator at 4°C until use. The extracts were considered standard solutions "S" (100% concentration) (Taye et al. 2012).

**Preparation of nematode inoculum**

The roots of the collected galled tomato plants were used to obtain nematode eggs, using the modified nematode extraction technique as Hussey (1973) outlined. The roots were washed using tap water to get rid of the soil particles. They were then sliced into1 cm pieces and placed in a glass bottle containing 1.5% sodium hypochlorite (NaOCl) solution. The egg masses were separated from the root remnants by shaking vigorously for a period of 3 minutes by hand. The suspension containing the eggs was then poured through sieves of different sizes to remove the organic debris from eggs. Sieving started with the largest sieve of 100 µm which was then followed by 50, 38 and lastly 25µm sieves. The suspension that remained on the 25 µm sieve was then thoroughly washed with tap water for 5 minutes to remove excess NaOCl. The recovered eggs were then transferred to a glass bottle. This process was repeated to extract additional eggs which were then incubated for a period of 48 hours at a temperature of 25°C.

A modified Baerman technique was used to separate freshly hatched juvenile eggs (J2s) from unhatched and dead juvenile eggs.

**Effect of extracts on tomato plants infected with nematode eggs**

Screen house experiment was established at the Kenyatta University, Kenya. Tomato seeds (cv. Kilele) were grown under screen house conditions in sterilised sandy and loam soils (2/1v/v) for 3 weeks in plastic seed tray for better root assessment. After the three weeks growth, the resulting tomato seedlings were transferred into pots containing 2.0 kg heat sterilised soils. One tomato seedling was planted per pot (Fig. 1). Five hundred (500) freshly hatched juvenile eggs were introduced at each pot as a drench in holes made around the root of the seedlings.

*Allium sativum*, *Azadirachta indica* and *Lantana camara* extracts were further applied in the pots as treatments. The experiment included five treatments as follows: T1-*A. indica*, T2- *A. sativum*, T3-*L. camara*, T4- Vydate (positive control) and T5- untreated (negative control).

**Measurement of tomato plant growth parameters**

At the end of the experiment following tomato plant growth, shoot heights of tomato plants were measured from the base of the stem (soil line) to the newest apical node. The plants were then uprooted gently; the adhering soil removed by running over tap water and then blotted dry with a serviette before determine their length and fresh weights. Roots were then separated from shoots. Fresh weight of shoots per plant was determined using a weighing balance. All stems and roots were then oven dried at 60°C until constant weight was obtained. Root and shoot dry weights were then determined.

**Determination of the galling and egg mass index in the roots of tomato plants**

Roots of tomato plants were blotted dry with serviette. The galling index (root damage) was determined by counting the number of galls present in the roots then rating them using the scale of 0 to 10 as described by Fatema & Ahmad (2005).

Egg mass index was carried out by counting the egg masses on tomato root system. Phloxine B solution (0.15 g/l) was used to stint the egg masses on the roots for 15 minutes. The excess phloxine B was removed by rinsing the roots in water and egg masses counted under a dissecting microscope and scored using a scale of 1-9 as described by Bharadwaj & Sharma (2007).

**Data analysis**

Data collected for each growth parameter was subjected to Minitab statistical software program (2010). Variation among the means of the parameters was established using Analysis of variance (ANOVA). Turkey's HSD was used to separate means where significant difference occurred. Kruskal-Wallis test was used in cases with sample sizes less than 30 at 95 CI.

**Results**

**Effect of *A. sativum*, *A. indica* and *L. camara* leaf extracts on tomato growth parameters**

Generally, tomato plants treated with extracts had more leaves than the untreated plants (Fig. 2). There was a significant difference in the number of leaves among the treatments ($P \leq 0.05$) (Table 1). Tomatoes treated with *L. camara* leaf extracts developed more leaves than plant treated with *A. indica* and *A. sativum*. The shoot height differed significantly among the treatments ($P \leq 0.05$). The treatments with *L. camara* had significantly taller plants followed by Vydate treated plants, *A. sativum* and *A. indica*. The untreated control recorded the lowest shoot height. Significantly ($P \leq 0.05$) longer roots were observed in tomatoes treated with *L. camara* leaf extract. The lowest root length was from plants in untreated control and those on *A. indica* treatment. Shoot dry weight in plants treated with *L. camara* was significantly higher. However, in the root dry weight, plants treated with *L. camara* and vydate recorded significantly heavier roots ($P \leq 0.05$). This was followed by *A. sativum*, *A.indica* and then the untreated control.

**Fig. 1** – Tomato seedlings at Kenyatta University screen house after transplanting.


**Fig. 2** – Tomato plants under different treatments. A Treated plants. B Untreated control.

**Table 1** Effect of *A. sativum*, *A. indica* and *L. camara* extracts on tomato growth parameters of root-knot infected tomatoes

| Treatment | No. of leaves Mean ± SE | Shoot height Mean ± SE (cm) | Root length Mean ± SE (cm) | Shoot dry weight Mean ± SE (g) | Root dry weight Mean ± SE (g) |
|---|---|---|---|---|---|
| *A. indica* | 19.80±2.40b | 65.60±4.78bc | 14.20±0.86c | 13.17±0.55c | 1.23±0.03c |
| *A. sativum* | 15.60±1.12c | 69.60±4.71b | 16.60±0.68ab | 14.72±0.35ab | 1.32±0.07bc |
| *L. camara* | 26.20±3.31a | 82.20±6.87a | 17.00±1.14a | 15.40±0.46a | 1.46±0.04a |
| Vydate | 18.40±1.40b | 79.40±4.87a | 16.80±0.80ab | 15.22±0.26a | 1.37±0.06ab |
| Untreated control | 14.80±0.97c | 62.40±1.33c | 14.20±0.37c | 12.96±0.71c | 0.73±0.03d |
| F- value | 4.92 | 3.27 | 3.12 | 5.49 | 38.72 |
| P- value | 0.006 | 0.032 | 0.038 | 0.004 | 0.0001 |

Mean value denoted by the same letters are not significantly different at P≤0.05. Mean separated using Tukey's HSD. SE = standard error of means.

### Effect of *A. sativum*, *A. indica* and *L. camara* extracts on the galling index and egg mass index on tomato plant

After harvesting of the plants, galling was observed in the roots of the plants though in different degrees (Fig. 3). This experiment showed that the treated plant gave a significantly lower galling index compared to the untreated control (Table 2). There was no significant difference in the galling index of the roots of the plant treated with *L. camara* to that of vydate; the positive control. The egg mass index was significantly lower (P≤0.05) in plants treated with extracts than untreated plants. The population of J2s in the soil was significantly higher in the untreated plant when compared to the treated ones. Vydate recorded the lowest values for the number of juveniles in the soil (40.0 ± 25.5) which was not significantly different to that with *L. camara* treatment (74.4 ± 37.3)

## Discussion

### Effect of *A. sativum*, *A. indica* and *L. camara* extracts on tomato growth parameters

The results demonstrated that the extracts from *A. sativum*, A. indica and *L. camara* enhanced plant growth as shown by the increased plant root length, shoot height, number of leaves, dry root and shoot weight when compared to untreated control. The use of the extracts resulted in an increase in plant height when compared to the untreated control plants. Plants treated with *L. camara* extracts showed greater enhanced growth and increase in height than the *A. indica* and *A. sativum*. The increase in infestation as reflected by the number of galls might be the reason for reducing plant height in the untreated control. Distortion of the plant roots and nematode colonisation of vascular bundles could reduce the supply of nutrients to plants. The galls formed on the roots interfere with the functioning of the roots hence hindering essential functions like water and nutrients uptake (Sikora & Fernandez 2005).

The increased growth of tomato plants treated with plant extracts may result from the nematode reduction in the soils compared to untreated plants. The botanicals provide a suitable environment for the roots to absorb and utilise the soil nutrients, resulting in reduced damage caused by nematodes (Abubakar et al. 2004). These results support those mentioned by Wondimeneh et al. (2013), who observed that the application of Mexican marigold, baker tree seeds, and *Lantana* leaf extracts led to an increased plant height compared to untreated plants in root knot nematode infested soils. The increase in growth of the plant could be due to the decrease in the nematode population in the soil. Reduction in nematode population results to unhindered growth of the plant (Abubakar et al. 2004). Shorter plant height was recorded from untreated inoculated control tomato plants. The stunting action of *Meloidogyne* spp. may result in the reduced plant height of the untreated plants. Similar findings have been reported by Taye et al. (2012) who observed reduced plant height and shortness of plants infected with root knot nematodes as opposed to those under treatments with botanicals.

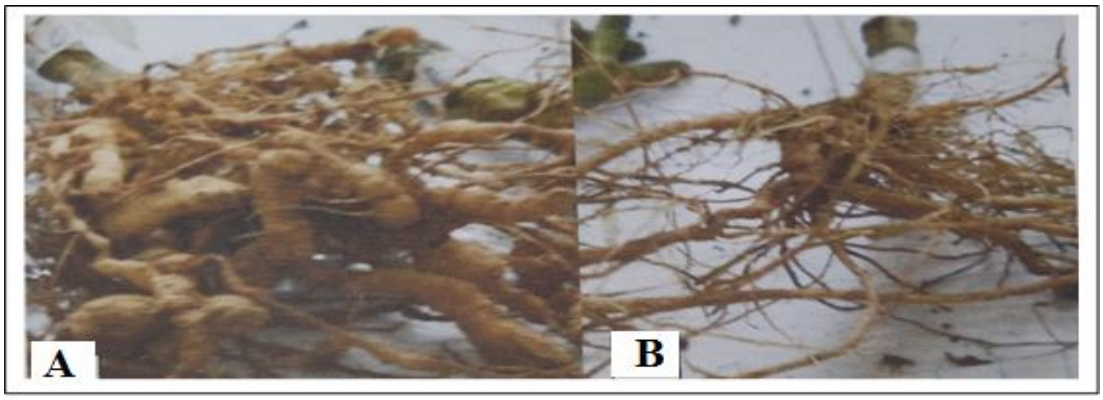


**Fig. 3** – Tomato roots showing different degrees of galling. A Untreated control. B Treated plant.

**Table 2** Effect of *A. sativum* bulb, *A. indica* and *L. camara* leaf extracts on the galling index and egg mass index on tomatoes and juveniles (J2s) in the soil

| **Treatment** | **Nematode parameters observed on tomatoes treated with different plant extracts and positive and negative controls** | | |
|---|---|---|---|
| | Galling index Mean± SE | Egg mass index Mean ± SE | J2s/200 cc Mean ± SE |
| *A. indica* | 2.80±1.50b | 3.80±0.97b | 266±54.90b |
| *A. sativum* | 2.80±1.11b | 2.00±0.55c | 241.6±64.1b |
| *L. camara* | 1.60±0.40bc | 2.40±0.68bc | 74.4±37.3c |
| Vydate | 0.80±0.37c | 1.60±0.60c | 40.4±25.5c |
| Untreated control | 7.00±0.95a | 6.00±0.84a | 613.6±35.5a |
| F- value | 6.11 | 5.81 | 24.87 |
| P- value | 0.002 | 0.003 | 0.0001 |

Mean value denoted by the same letters are not significantly different at P ≤0.05. Mean separated using Tukey's HSD. SE = standard error of means. Gall indices based on a 0 – 10 gall rating scale and egg mass index based on 0 - 9 rating scale.

**Effects of the extracts on egg mass index and galling index on tomato plan**

The extracts significantly reduced the population of juveniles in the soil, the galling index and the egg mass index on roots when compared to the untreated plants. From the results the number of J2s in the soil in *L. camara* and vydate treatments did not differ significantly but was lower when compared to the untreated control. The high galling index, egg mass index and population of juveniles in the untreated soils explains the poor yields in the untreated control due to nematode damage. The reduction in nematode damage due to *L. camara* treatment led to increased yield which was not significantly different to that of vydate ($P \leq 0.05$). This result show that this botanical extract can be used as alternative to the chemical nematicides. These results are similar to those of Wondimeneh et al. (2013) who reported that, plant extracts from Mexican marigold, Lantana and bitter leaf leaves at 5% concentration lowered the final nematode density significantly over the untreated control and was comparable with the synthetic nematicide. The nematicidal effect of the plant extracts could be the cause of the reduced number of motile larvae that penetrates the roots of the plants. This effect of the extracts is believed to act directly on infective J2 larvae.

The extracts are believed to contain certain chemicals that possess ovicidal and larvicidal properties that inhibit the multiplication of the nematodes. This could be the cause of reducing the final population densities of nematodes in the soil (Agbenin et al. 2005). Qamar et al. (2005) reported that the aerial parts of *L. camara* contain chemical substances such as lantanone, oleanolic acid, lantanoside and camaric acid that possess nematicidal properties that work against *M. incognita*. Ahmad et al. (2010) also reported that various concentrations of *L. camara* leaf extracts of 100%, 50% and 10% have deleterious effects against nematodes.

Agbenin et al. (2005) reported that *A. sativum* extract resulted in reduced soil larval populations and decreased egg mass and galling indices on plant root. The egg mass index on tomato roots with *L. camara* and *A. sativum* treatment was not significantly different from that of chemical nematicides (Vydate). This shows that both were effective in the control of RKN infecting tomatoes. These findings have been reported by Wafaa & Mohmoud (2013), who noted that castor seed and *A. sativum* aqueous extracts reduced the egg mass index and the galling index in the roots of tomato.

## Conclusion

The present study results show that extracts from different plant species have varying degrees of nematicidal effects. This was the cause of variation on different parameters. The presence of natural compounds in different plants with nematicidal effects is a promising option for controlling root-knot nematodes.

The study showed that among the different plant extracts tested, *Lantana camara* leaf extract worked best against the *Meloidogyne spp*. It significantly reduced nematode parameters such as galling index, egg mass index, and the final nematode count in the soils, which was not significantly different ($P \leq 0.05$) from the chemical nematicide, vydate. *Lantana camara* treatment also led to an increased growth and performance of the tomato plants as observed from the increased shoot height, root length, root and dry weight and yield.

## Recommendation

Since the botanical extracts worked in the reduction of root-knot nematodes on tomatoes, it is recommended that they be introduced into farming systems in Kenya. Farmers should be sensitised on the use of each promising and cheaper botanical extract in integrated pest management strategy. The findings can help farmers manage root-knot nematodes at the same time controlling environmental pollution that is likely to occur due to the use of chemical nematicide. Further studies to establish the effects of this extracts on other soil-borne plant diseases of economic importance should be conducted.

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## Competing interests

The authors declare that they have no competing interests

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