

RESEARCH PAPER

EFFECT OF CASTOR BEAN (*Ricinus communis* L.) AQUEOUS EXTRACTS ON THE PERFORMANCE OF ROOT-KNOT NEMATODES (*Meloidogyne* spp.) ON TOMATO (*Solanum lycopersicum* L.)

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ABSTRACT

The increased concern for environmental and health hazards have called for a reduction in the use of synthetic nematicides for nematode control. Experiments were, therefore, conducted to ascertain the nematicidal potential of castor bean's crude extract and its five lower concentrations with water as control. In the in vitro studies, crude castor bean aqueous extract and 10, 20, 30 and 40% different concentrations with 100 root-knot nematode eggs or juveniles in separate Petri dishes showed that all the different concentrations had toxic effects on eggs and juveniles of root-knot nematode. Egg hatch inhibition and juvenile mortality increased with increased concentration of the extracts. With an increase in exposure time, juvenile mortality increased. In potted plant studies, crude castor bean aqueous extracts and its lower concentrations of 20, 40 and 60% caused significant improvement in plant growth measures such as height and fresh shoot weight over the water blank control. The crude castor bean extract was nematotoxic to root-knot nematodes in vitro and in potted-tomato plants, but this was not demonstrated in field studies. Further work needs to be done before a firm recommendation can be made.

Keywords: Control, juveniles, nematicides, phytochemicals, water

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is the most important vegetable in Ghana (Norman, 1992). It is also a fairly important cash crop in the outskirts of urban areas in the forest zone, in the Greater Accra area, Akumadan, Wenchi, and Mankesim (Obeng-Ofori *et al.*, 2007). In spite of the importance of the crop, yields are usually low and sometimes total crop failure is recorded due to diseases, pest and other environmental factors.

Root-knot nematodes attack on tomato is very common and is one of the factors responsible for the frequent failure of the crop (Norman, 1992). According to Hemeng (1981), a yield loss of 73-100% as a result of root-knot nematodes infestation in tomato occurred in Northern Ghana. As a result of their ability to attack most crops, root-knot nematodes are liable to cause losses wherever there is intensive cultivation of susceptible crops, and where precautions against population build up is not taken.

The indiscriminate use of synthetic chemicals in managing this pest however, is likely to lead to increased phytotoxicity, environmental pollution and nematode resistance (Adegbite and Adesiyun, 2005). According to Alam (1987), chemical control is too costly for poor farmers. The use of chemicals has harmful effect such as air, water and soil pollution. Research efforts have therefore shifted towards the use of plant extracts as alternative to synthetic compounds (Papachristos and Stamopoulos, 2002).

Certain plant parts and extracts have been reported as alternatives and are safe and effective for the management of plant parasitic nematodes (Siddiqui and Alam, 1985). Products of *Azadirachta indica* (A. Juss), *Crotalaria* spp. (L.) and *Lantana camara* (L.) have been reported to be toxic to root-knot nematodes. Castor bean (*Ricinus communis* L.) seed has been reported to contain a high amount of a ricin, known as a toxalbumin and is capable of inhibiting protein synthesis (Bourne, 1999). Castor bean seed has been recorded to have a lethal effect on pests such as aphids, and also known to be a major source for undecylenic acid, a natural fungicide (Bourne, 1999). The objective of this study was to evaluate the effect of the aqueous extract of castor bean on root-knot nematodes.

MATERIALS AND METHODS

Three experiments were conducted to evaluate the potency of aqueous extract of castor bean on root-knot nematode activity.

Source, extraction and counting of root-knot nematodes eggs inoculum

Tomato plants infested with root-knot nematodes were collected from tomato fields at Kotei, near the Kwame Nkrumah University of Science and Technology (KNUST), Kumasi. Root-knot nematode eggs were extracted from the infested tomato roots by modified Hussey and Barker (1973) method. Washed infested roots of the tomato were chopped with a pair of scissors to about 2mm pieces. About 10g of the chopped roots was put in a jar and enough 0.5%

sodium hypochlorite poured on it and shaken vigorously for 4 minutes. The resultant sodium hypochlorite-roots suspension was quickly passed through a 200 mesh sieve over 500 mesh sieve. Eggs collected on the fine sieve were rinsed with tap water into a beaker to remove excess sodium hypochlorite. These processes of extraction were repeated until all the chopped roots were used. The collected eggs were topped with water to obtain the egg-water suspension. Two millilitres of the egg-water suspension was pipetted into a counting tray and the number the of eggs in aqueous suspension determined using a stereo microscope and a tally counter. Total root-knot nematode eggs in the suspension were then computed.

Extraction and counting of root-knot nematode juveniles

Extraction of root-knot nematode juveniles from infested roots of tomato was done using modified Baermann tray method (Whitehead and Hemming, 1965). The roots were chopped with a pair of scissors as above and 5g of each entry in the study were placed separately in a plastic sieve lined with a two-ply tissue paper placed in a plastic plate. Tap water was poured carefully into the plastic plate in which the sieve was resting until the tissue became moist. The set up was left standing for 48h and was then poured into beakers and left for about 24h for the juveniles to settle at the bottom. The volume of each suspension was standardised to 50ml by topping with tap water. Aliquot of 1ml of each suspension was taken with a pipette into a counting tray and counting of the root-knot nematode juveniles done under a stereo microscope. Each beaker with nematode juvenile-water suspension was homogenized by blowing air through it with a pipette before taking each aliquot.

Source of castor beans and preparation of castor bean aqueous extract

Dried castor beans were collected from Kotei, near KNUST, Kumasi and were decorticated by pressing each seed gently in between the thumb and fore finger to get the beans for use.

Decorticated beans weighing 50g was thoroughly washed in a plastic bucket with tap water and then ground in 100ml of tap water in an electric blender at high speed for 3min. The suspension was filtered through cheese cloth and the filtrate served as the standard solution or crude extract. The crude extract obtained was then diluted further with water to 10, 20, 30, 40 and 60% (v/v). Water, the crude extract and its dilutions were used in the study.

Experimental designs and data analyses

The laboratory and pot studies were set up in completely randomised design (CRD) with four replicates for each of the treatments. However, randomised complete block design (RCBD) with four replicates was used for the field experiment. Data collected were analysed using the Genstat statistical package (Discovery edition). Least significant difference (Lsd) at 5% was used for comparing mean differences. All count data were transformed using square root transformation of $\sqrt{(x+0.5)}$ to normalize the distribution.

Experiment 1: Effect of castor bean aqueous extract on root-knot nematode eggs and juveniles *in vitro*

Two experiments were conducted at the Plant Pathology laboratory of the Department of Crop and Soil Sciences, Faculty of Agriculture, KNUST, Kumasi, Ghana to assess the effect of castor bean aqueous extracts on root-knot nematode eggs and juveniles separately.

Application of treatments

Twenty millilitres each of the castor bean aqueous extracts 10, 20, 30, 40% (v/v), crude and water were dispensed into separate Petri dishes. The 60% (v/v) castor bean aqueous extract was not assessed in the *in vitro* study. One hundred root-knot nematode eggs were put in separate Petri dishes containing the different extract treatments. Water served as the control. The set ups were kept on laboratory benches at room temperature.

Data collected

The number of eggs hatched were observed and counted from the Petri dishes on the third, sixth and ninth days after application of treatments. The hatched eggs were not removed from the Petri dishes. Number of dead juveniles (mortality) was observed and recorded at 24, 48 and 72h after hatching. Juveniles were considered dead when they were found to have lost their body content and were inactive. Such immobile or inactive nematodes were removed from the suspension and then transferred to distilled water in Petri dishes for 24h to determine if they will recover. Root-knot nematode juveniles that did not recover were confirmed dead. This was done with the aid of a stereo microscope.

Experiment 2: Effect of soil drenching with castor bean aqueous extracts on root-knot nematode of tomato

This pot trial was carried out in the plant house of the Department of Crop and Soil Sciences, KNUST, Kumasi.

Soil preparation and sterilization

The soil for the experiment was prepared by mixing three parts of river sand with one part of top soil. The soil mix was sterilized at 103°C for 24h, using a modified metal barrel steam sterilizer. The steam sterilizer has two chambers, the lower chamber contained water and the upper part the soil mix. The soil was covered with wet jute sacks to conserve steam in the chamber. Fire wood was used as the source of heat. The sterilized soil was allowed to cool before use.

Raising of tomato seedlings

The tomato cultivar, Pectomec seeds purchased from Obek Agrochemicals, Kumasi was planted in sterilized top soil in a seed box. The seedlings were transplanted into one litre-size plant pots filled with 850ml sterilized top soil three weeks after germination. There was one tomato seedling per pot.

Application of treatments and inoculation of tomato plants

Two weeks after transplanting the tomato seedlings, the roots of the plants were inoculated by creating three holes in a triangular arrangement each 2cm from the stem. The soil of each potted-tomato plant was then inoculated with 1,000 root-knot nematode eggs through the three holes created around the stem. The holes were then covered with soil. The test plants were drenched with 50ml each of the crude aqueous extracts, 20, 40 and 60% (v/v) crude extract concentrations weekly until the plants were harvested. The same procedure was followed for the control using water. In this experiment, the 10% (v/v) crude extract concentration was replaced with 60% (v/v).

Data collected

The test plants were harvested eight weeks after inoculation with the root-knot nematode eggs. To ensure easy removal of the plants from the pots, the sides of the plastic pots were pressed to loosen the soil. The soil was then removed from the roots by gently shaking the plants. The following data were collected, plant height (cm), fresh shoot and root weights (g). The roots of the harvested tomato plants were each washed separately and dabbed dry with tissue paper. Galling was scored using the rating chart on a scale of 0-10 (Bridge and Page, 1980). The number of *Meloidogyne* juveniles was obtained by the modified Whitehead and Hemming (1965) method and counted as above. The entire root system of each test plant was immersed in Phloxine B stain for 15min and the number of egg masses counted.

Experiment 3: Effect of castor bean aqueous extract on root-knot nematode under field environment

This experiment was carried out at Kotei, a vegetable farming community near KNUST. This site had been previously cultivated to tomato. The land was cleared of weeds by hoeing, layout was done and beds were prepared using hoe. Each bed was 6m x 1m and separated from each other by a distance of 2m.

Determination of root-knot nematode population in the experimental field

The initial population of root-knot nematode juveniles in the field was assessed by randomly sampling 10 core soil samples from the field. The soil samples were bulked and thoroughly mixed and air-dried by spreading evenly on clean laboratory benches. All lumps of soil were broken and the soil was sieved. Root-knot nematode juveniles were extracted from 100g soil and counted as described above.

Application of treatments to tomato plants in the field

Due to the initial population of 21,760 root-knot nematode juveniles/100g soil in the field, the soil around the tomato roots were not artificially inoculated because the population was adequate (Townshend, 1990). However, the soil around the root zone of the test plant was drenched with 50ml of the requisite castor bean aqueous extracts. The same procedure was followed for the control plants, using water instead of extract. The test plants in each treatment were drenched with the respective extracts and water weekly over a two month period. Concentrations of 20, 40 and 60% v/v of the crude extract were used for the field experiment.

Data collected

Harvesting of the tomato plants was done eight weeks after transplanting into the field. Prior to the harvest, plants were watered thoroughly to moisten the soil around them. They were pulled gently out of the soil and the roots were scored for galling using the rating chart described above. Root-knot nematode juveniles were also extracted and counted as described above.

RESULTS

The studies conducted to ascertain the effectiveness or otherwise of castor bean aqueous extracts revealed the results presented below.

Experiment 1: Effect of castor bean aqueous extract on root-knot nematode eggs and juveniles *in vitro*

Three days after application of the extracts to root-knot nematode eggs, the mean egg hatch ranged from 1.47 to 3.90 (Table 1). As expected, the highest egg hatch was observed in the water treatment whilst the lowest was in the crude extract. There were significant differences ($P < 0.05$) between 40% crude extract, 30% crude extract and the crude extract but no significant difference ($P > 0.05$) between the others (Table 1).

On the sixth day, mean egg hatch was between 1.22 and 6.54. The highest egg hatch was found in the control whilst the least hatch was in the crude extract. There were significant differences ($P < 0.05$) between all the treatments (Table 1).

On the ninth day with the exception of the 40% crude extract, 30% crude extract and crude extracts which showed no difference, there were significant differences ($P < 0.05$) between all the other treatments (Table 1). In general, root-knot nematode eggs hatchability decreased with increased concentration of the extracts.

A day after exposing the juveniles to the treatments, a mean juvenile mortality of 4.88 was recorded for the crude extract whilst the control recorded the least mean juvenile mortality of 3.14 (Table 2). There was, however, no difference ($P > 0.05$) between 40, 30, 20 and 10% crude extracts but there was significant difference between the crude extract and all the other treatments (Table 2).

Two days after exposure, mean juvenile mortality increased and ranged from 4.47 to 6.86 for the crude extract and control treatments, respectively (Table 2). There was no difference ($P > 0.05$) between 40 and 30% crude extracts (Table 2). Also, there were significant differences ($P < 0.05$) between the crude extract and other treatments.

As the exposure period increased, juvenile mortality also increased (Table 2). On the third day, the control treatment recorded the least mean mortality of 5.94 whilst the crude extract recorded the highest mean mortality of 8.45. There was no difference ($P > 0.05$) between 30

Table 1: Effect of aqueous castor bean extract on root-knot nematode egg hatch *in vitro* at three, six and nine days after treatment

Treatments (v/v)	Mean number of eggs hatched at different periods (transformed)* in days		
	Day 3	Day 6	Day 9
Castor crude extract	1.47	1.22	0.71
40 % crude extract	1.80	1.68	0.90
30 % crude extract	2.80	2.03	0.71
20 % crude extract	2.92	2.81	2.53
10 % crude extract	3.56	3.13	2.18
Water (Control)	3.90	6.54	8.65
<i>Lsd</i> (5%)	0.25	0.14	0.19
<i>CV</i> (%)	2.60	4.40	12.40

* $\sqrt{(x+0.5)}$, where x is the mean number of eggs

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and 20% of crude extracts but the crude extract was significantly different ($P < 0.05$) from all treatments (Table 2).

Generally, soil drenching with the extracts had an effect on the height of the tomato plants. The

shortest plant height of 3.64cm was recorded for plants subjected to the control treatment whilst plants treated with the crude extract recorded mean height of 6.55cm (Table 3). There was a significant difference ($P > 0.05$) in plant height between plants treated with the crude

Table 2: Effect of castor bean aqueous extract on root knot nematode juvenile mortality *in vitro* at 24, 48 and 72 hours after treatment

Treatments (v/v)	Mean number of dead individuals at different periods (transformed)*		
	24h	48h	72h
Castor crude extract	4.88	6.86	8.45
40 % crude extract	3.76	5.76	8.19
30 % crude extract	3.62	5.89	8.09
20 % crude extract	3.62	5.22	8.08
10 % crude extract	3.75	5.13	7.35
Water (Control)	3.14	4.47	5.94
<i>Lsd</i> (5%)	0.25	0.22	0.18
<i>CV</i> (%)	4.50	2.70	1.50

* $\sqrt{(x+0.5)}$, where x is the mean number of eggs

Experiment 2: Effect of soil drenching with castor bean aqueous extracts on root- knot nematodes of tomato plant

Table 3: Effect of soil drenching with castor bean aqueous extract on plant height, fresh shoot and root weights of root-knot nematode inoculated tomato plants in pots

Treatments (v/v)	Plant height (cm)	Mean weights (g)	
		Fresh Shoot	Roots
Castor crude extract	6.55	17.85	3.64
60 % crude extract	5.89	17.13	3.66
40 % crude extract	4.95	13.55	4.95
20 % crude extract	3.66	13.46	5.89
Water (control)	3.64	12.98	6.55
<i>Lsd</i> (5%)	1.81	3.02	1.83
<i>CV</i> (%)	14.10	13.40	24.50

extract and all the lower concentrations except the 60% crude aqueous extract. The crude extract treated plants also recorded the largest weight of 17.85g whilst plants with the control treatment recorded the least weight of 12.98g (Table 3). There were, however, no significant differences ($P > 0.05$) between the crude extract and 60% extract. The least mean root weight of 3.64g was recorded for the crude extract-treated plants whilst plants with the control treatment had the heaviest weight of 6.55g (Table 3). The crude extract, 60 and 40% of crude extracts showed no significant differences ($P > 0.05$).

Roots of the water blank control and lower castor bean extract concentrations were more suitable to root-knot nematode activities. More eggs masses were, therefore, deposited on their roots than roots of plants treated with the higher concentrations of the extract (Table 4). There was significant difference ($P < 0.05$) between the crude extract and all the other treatments. More juveniles (6.37 juveniles/5g roots) were counted from the control-treated plants than those treated with the castor bean extracts

(Table 4). The control plants had the highest galling score of 7.0 whilst the plants treated with castor bean extracts progressively lower gall number, with crude extract recording the least of 2.25.

Experiment 3: Effect of castor bean aqueous extract on root knot nematode under field environment

The initial inoculum level of root-knot nematode juveniles in the soil before the field study was adequate (Townshend, 1990) therefore natural field infestation was used to challenge the test tomato plants. The other nematode species were saprophytes, common species in most agro-ecosystems.

The roots of the 40% aqueous extract-treated plants had the highest gall infestation compared with the other treatments (Table 5). However, there were no significant differences ($P > 0.05$) between all the treatments. The mean number of root-knot nematode juveniles recovered ranged from 16.83 to 17.50/5g tomato roots for the crude extract and control treatments, re-

Table 4: Effect of soil drenching with castor bean extract on root-knot nematodes egg masses, juveniles and galling of inoculated tomato plants in pots

Treatments	Mean gall score (Scale 0-10)#	Mean number of egg masses/5g tomato roots*	Mean number of juveniles/5g tomato root*
Castor crude extract	2.25	0.71	4.00
60 % crude extract	4.75	1.65	4.74
40 % crude extract	6.25	2.06	5.34
20 % crude extract	6.50	2.12	6.15
Water (control)	7.00	2.24	6.37
Lsd (5%)	0.72	0.16	0.16
CV (%)	8.70	16.20	16.20

0 = no galling, 10 = damaged roots due to galling

* $\sqrt{x+0.5}$, where x is mean number of root-knot nematode egg masses and juveniles counted

spectively. Although, the control treatment recorded the highest number of juveniles, there were no significant differences ($P > 0.05$) between the treatments (Table 5).

DISCUSSION

The *in vitro* study showed that inhibition of egg hatch increased with increasing concentration of the castor bean aqueous extract. The highest egg inhibition was recorded in the crude extract. Amer-Zareen *et al.* (2003) and Adegbite and Adesiyani (2005) reported similar findings against root-knot nematode eggs *in vitro* when aqueous extract of ginger and root extracts of *A. indica*, *Chromolaena odorata* (L.) King and Robinson, *R. communis* and *Jatropha curcas* (L.) were used. Egg hatch inhibition also increased with increase in exposure time. This agrees with Joymatti *et al.* (1998) who reported that eggs exposed to extracts of *Melothria purpusilla* (Blume) Cogn. for a longer period of time decreased in their rate of hatching as compared to those exposed over a shorter period in the same extracts. The study also revealed that the number of dead root-knot juveniles increased with increase in concentration of the

extract. This was in agreement with Hasabo and Noweer (2005) who found that the mortality effect of an extract on nematode is concentration dependant. The number of dead juveniles also increased with increase in exposure time. This observation corroborates the findings of Amer-Zareen *et al.* (2003) whose work with ginger reported similar results. The inhibitory effect of botanicals according to Adegbite and Adesiyani (2005) might be due to chemicals present in the extracts that possess ovicidal and larvicidal properties.

Ricin, the principal toxin in castor seed is described as a toxalbumin and this is a protein phytotoxin that is capable of inhibiting protein synthesis (Audi *et al.*, 2005).

From the pot experiment, the crude castor bean aqueous extracts caused a reduction in the number of root-knot nematode juveniles and eggs. This observation is in agreement with Alashalaby and Noweer (2003) who reported that aqueous neem extract significantly reduced the total number of root-knot nematode juveniles and inhibited egg hatch in peanut roots

Table 5: Effect of castor bean extract on galling and number of root-knot nematode juveniles of inoculated tomato plant under field environment

Treatments (% v/v)	Mean gall score (Scale 0-10)#	Mean number of juveniles/5g of tomato roots*
Castor crude extract	6.50	16.83
60 % crude extract	7.00	17.10
40 % crude extract	7.25	17.25
20 % crude extract	6.75	17.23
Water (Control)	7.00	17.50
Lsd (5%)	0.96	0.50
CV (%)	19.00	11.30

* $\sqrt{x+0.5}$, where x is mean number of root-knot nematode juveniles counted
0 = no galling, 10 = damaged roots due to galling

and soil. Joymatti *et al.* (1998) also found that juvenile mortality was concentration dependent whilst working with extracts of ginger. Also, it was observed that the number of galls was smaller in castor bean extract-treated plants than the control plants. This phenomenon according to Gommers *et al.* (1982) may be due to the action of the extract releasing substances into the soil which inhibited the entry of root-knot nematodes into the roots of plants.

Plant height of the water-treated plants were lower than the castor bean extract-treated plants and this agrees with that of Crouch and Van Staden (1993) who recorded significant increase in plant height and a corresponding reduction in *M. incognita* infestation when *Ecklonia maxima* (L.) extract was applied as soil drench. The increase in plant height according to Pattison (2007), could also be due to the decrease in the activities of the root-knot nematode juveniles. The increased shoot weight in the castor extract-treated plants may be due to the ability of the roots to absorb more nutrients as compared to the water-treated plants whose roots were highly infested or galled. Heavy infestation of roots according to Hussey (1985), reduced the uptake and transportation of nutrients. According to Caveness and Ogunforowa (1985), root-knot nematode-infested plants are seriously affected by their reduced uptake and transportation of water and nutrients which, in turn, affected their shoot weight. It was, however, observed that the root weight of the control plants were considerably heavier than the castor extract-treated plants (Table 3), and this might have been due to the higher number of galls formed on the roots.

The field trial did not show any difference between the treatments in terms of number of root-knot nematode juveniles and gall score (Table 6). According to Chitwood (2002), naturally occurring compounds are often more readily degraded in the environment than synthetic compounds and this may have accounted for the poor performance of the castor bean extracts used. El-Nadgi and Mansour (2003) ob-

served that the potency of botanicals was affected by exposure time and this may not be an asset in the use of botanicals if the target nematode needs to be exposed to them for prolonged periods in the field.

CONCLUSION

The *in vitro* study showed that the castor bean aqueous extract used reduced root-knot nematode eggs hatch and higher juvenile mortality. It was also observed that castor bean aqueous extract is nematicidal and that its efficacy increases with concentration. In the pot experiment, fewer juveniles were recovered from the roots of the aqueous castor extract-treated plants than on water-treated plants. Treatment of test tomato plants with castor bean extracts resulted in improved performance recorded as plant height and shoot weight. The positive laboratory results could not be demonstrated in the field. Therefore there is the need for further work to confirm the nematicidal potential of castor bean aqueous extract in the field.

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