ISSN 2052-0751
Journal of Biology

Research Paper

Efficacy of *Brassica* Tissue and Chalim™ on Control of Plant Parasitic Nematodes

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Abstract

Nematodes, as parasite, contributes to a high loses in crops. *Brassica* species produce general biocides called glucosinolates which are nematocidal. Chalim™ (Calcium hypochlorite) is a chemical biofumigant. The study was carried out in three seasons. The aim of this study was to evaluate the use of *Brassica* tissue and Chalim™ in the management of root knot nematodes. The effect on plant parasitic nematode (PPN) populations was determined in the 24 plots upon soil sampling. Data was then taken and analyzed for various parameters. The results showed that nematode loads reduced significantly using Chalim™ 303.75 g and *Brassica* extract 5292 g compared to the control. The findings revealed that the population of plant parasitic nematode varied significantly ($P<0.05$) throughout the three seasons among the treatments with CM911.25 having the highest population, while CM303.75 was found to have the least. Various phytonematodes like *Helicotylenchus*, *Pratylenchus*, *Meloidogyne* (root knot nematodes), *Tylenchus* and *Heterodera* were present in all seasons with *Meloidogyne*, *Tylenchus* and *Heterodera* populations varying significantly in seasons two and three. A significant correlation relationship was established ($r=0.415$, $P<0.05$) between the PPN and the soil pH although the relationship was not significant ($P>0.05$) in RKN, *Filenchus* and *Tylenchulus* species. *Brassica* tissue improved moisture content and reduced PPN population at higher rates of application.

Keywords: *Brassica, Biofumigation, Helicotylenchus, Pratylenchus, Meloidogyne, Tylenchus and Heterodera, Tylenchus, Heterodera*

1. Introduction

Plant parasitic nematodes (PPN) cause losses of, up to 80%, on vegetables (Kaskavalci, 2007 and Nchore et al, 2011). They are important constraints on vegetable production (Nchore et al, 2010) reducing its yield quality and quantity. Root-knot nematodes, *Meloidogyne* species, are parasitic on a wide variety of vegetable crops causing up to 5% yield losses globally (Cetintas & Yarba, 2010; Nchore et al, 2010 and 2011). Studies by Nchore et al (2010) revealed severe deformation and galling on the root system, shoot height retardation and chlorotic symptoms in plants inoculated with Root Knot Nematode (RKN). They form disease complex with plant pathogenic bacteria and fungi constraining vegetable production, thus their management is important. Severe vegetable damage by RKN in Kenya has been reported (Nchore et al, 2010 and 2011), with infected plants rendered unacceptable for export (Orton-Williams, 1980). Sasser (1990) reported the prevalence of RKN in tomatoes causing severe losses. The presence of RKN (*Meloidogyne* sp.) will accelerate disease development (AVRDC, 2005). Studies have shown that there is a significant correlation, between incidence of wilt and RKN populations in the soil (McLaughlin et al, 1990). Nematodes increase wounding of the root system provideng points of ingress of the pathogen. The nematode-es may also modify the tissue in that it becomes more suitable for bacteria colonization (Hayward, 1991).

Depending on climate, crops grown, nematode species and

their density levels, and economic factors, a number of tactics can be employed to minimize nematode damage. Nematicides are effective in managing RKN and other PPN (Nchore et al, 2011). However, concerns about the negative impact of synthetic nematicides on the environment and on general public health, has led to a re-evaluation of these products (Mus & Huygen, 1992; Akhtar & Malik, 2000; Hassan et al, 2010 and Udo & Ugwuoke, 2010). Alternative eco-friendly methods including organic amendments have been recommended (Waceke, 2001 & 2002; Agyarko & Asante, 2005; Hassan et al, 2010 and Nchore et al, 2011) for management of nematodes. Farmers are reportedly using organic amendments all the time on soil fertility management (Nchore et al, 2011). However, the use of *Brassica* tissue and Chalim™ for controlling plant parasitic nematodes in potato, tomato and capsicum has not been reported in Kenya. *Brassica* species produce glucosinolates which are nematocidal and biocidal. Chalim™ (Calcium hypochlorite) is a chemical biofumigant. Biofumigation is the agronomic practice of using volatile chemicals (allelochemicals), released from decomposing *Brassica* tissues, to suppress soil-borne pests and pathogens (Kumar, 2005). Metham sodium, a known fumigant was used as a positive control. Brassicaceous materials have also been reported to provide multiple benefits to agro-ecosystems necessary for the management of plant diseases (Gruver et al, 2010), have an impact on soil moisture, nitrogen capture (Kristensen & Thorup-Kristensen, 2004) as well as reducing weed competition (Kumar, 2005). This study aimed to investigate the control of plant parasitic nematodes by bio and chemical fumigation where potato, tomato and capsicum were the test crops.

2. Materials and Methods

2.1. Preparation of Brassica Tissue and Chalim™

Fresh leaves of *Brassica oleracea* were finely chopped and incorporated into the soil at a depth of 20 cm, at the rate of 1:2:3 {5292 g (4355.56 kg/ha), 3096 g (2548.15 kg/ha) and 1908 g (1570.37 kg/ha)} fresh weights (FW) of previously predetermined concentrations per row with three replications each. The inoculated soil was thoroughly mixed with the finely chopped *Brassica* tissue ensuring that all *Brassica* tissue was well incorporated in the soil. Metham sodium, a chemical fumigant, was applied in 3 plots at the rate of 200 Ml/M^2 (2.43 L in 12.15 L of water). This was the positive control. This was in 9 furrows where each furrow received 1562 ml of the mixture. The sprayed furrows were thereafter covered with soil awaiting three weeks to planting of the test crops. Chalim™ effect was assessed in the inoculated field after application at varying concentrations of 911.25 g (750 kg/ha), 607.5 g (500 kg/ha), and 303.75 g (250 kg/ha). Predetermined concentrations of Chalim™ were applied per furrow and the crop of interest. This method of application was adopted from Kumar, 2005.

2.2. Planting of Test Crops and Experimental Designs

Randomized complete split plot design was used in the field layout. A plot measuring 15.5 x 28.6 m was marked, cleared, ploughed, harrowed and demarcated into 24 plots each measuring 4.5 x 2.7 m. Spacing of the host crops of interest (potato, tomato and capsicum) was carried out at 75 cm between the rows and 30 cm within a row. There was spacing of 1 metre between plots and blocks. All plots had 6 rows of nine plants each. Three hundred and sixty grams of DAP fertilizer were applied per plot. The two rows that had potato crops received 90 g of the fertilizer each while those with tomato and capsicum received 45 g of the fertilizer per row.

Three plots were used as positive controls for the experiment where Metham sodium (200 ml/plot) were applied. Three plots did not have any amendments and thus served as negative controls for the experiment. Soil was sampled before application of the inoculum and incorporation amendments to check on pH, moisture content and plant parasitic nematode populations. This was repeated on a fortnightly basis throughout the three growing seasons for comparison. The soil pH was taken to determine the acidity of the soil since the population of plant parasitic nematodes are affected by pH among other factors.

2.3. Extraction of Nematodes from Soil

Soil was sampled from the field and tested for plant parasitic nematodes including root knot nematodes using the tray method. The protocol was adapted from (Coyne et al, 2007). The soil sample was placed in a plastic container where the soil was crushed to break lumps and mixed thoroughly till it became fine. About 20 cm^{3}of soil was measured and placed on a paper towel. The paper towel was placed on a special plastic container with perforations (Nchore et al, 2011). The container was then placed on a flat plate. Water was added to the extraction plate and not directly on to the soil with an aim of wetting but not covering the soil. The extraction setup was left for about 48 hours. Nematodes from the soil were expected to move through the tissue paper into the water below resting on the plate. After extraction, excess water was drained and concentration of the nematodes was carried out using decanting sieve with a pore of 38µ. The concentrated samples were left to settle in a beaker ready for assessment of nematodes.

2.4. Nematodes Enumeration

The samples were concentrated to 20 ml in a graduated tube. The method described by Coyne et al (2007) was

adopted in counting the nematodes. Suspension from the concentrated volume was shaken immediately before obtaining aliquots. A wide mouthed pipette (to prevent blockage) was used to remove 2 ml aliquots into the counting dish carefully avoiding splashing. Nematodes in the counting dish were counted systematically following the gridlines on the dish. Nematodes observed were picked using a wide mouthed pipette from the particular grid and placed onto a glass slide and covered with a cover slip from which they were observed under a dissection stereomicroscope. The plant parasitic nematodes were further identified to genera level where the *Meloidogyne* spp. was of greatest economic importance. The total count was made for all plant non-parasitic nematodes per sample and data extrapolated to get density in 2 ml and then in 20 ml sample (nematodes in 200 g of soil per plot). This was also carried out for specific genus of the plant parasitic nematodes. This procedure was repeated at the beginning and at the end of every season for the three seasons.

The following formula was used to estimate the number of nematodes in the 20 ml concentrated sample:

$$\text{No of nematodes} = \left[\left(\frac{N}{n} \times 49\right) 2ml\right] 20ml$$

Where

N = number of nematodes counted on a slide
n = seven grid squares on the counting dish

2.5. Moisture Content Determination

Soil samples were collected using zig zag method. A sterile, dry glass Petri dish was used per sample. 50 g of wet soil were added from respective plots to an already labelled dry glass Petri dish and total weight taken. The sample was oven dried at 122 °C for 24 hours. Moisture Content (MC) was calculated by subtracting total dry soil plus Petri dish weight from total wet soil plus Petri dish weight. Mean calculated MC was recorded in all samples across the three seasons for analysis.

2.6. Soil pH Determination

Ten grams of thoroughly mixed soil was scooped, placed in a flat bottomed flask and 30 ml of distilled water added. The mixture was placed on a mixer in a mechanical shaker at 200 RVM for 30 minutes. A microprocessor pH meter was used, where standardization of the two buffers (7 and 4) one after the other was carried out respectively. Once confirmed ready and stable, the electrode and thermometer were rinsed and wiped gently with a clean filter paper. The pH readings from first sample to last were taken ensuring that each sample was well shaken before inserting electrode and thermometer, rinsed and wiped before getting onto the next sample. Stable pH readings were recorded in all samples across the three seasons ready for data analysis and interpretation.

2.7. Data Analysis

The data on nematode counts was analyzed using analysis of variance (ANOVA). Means that were considered significantly different ($P \leq 0.05$) were compared using Student-Newman-Keuls (S-N-K) test.

3. Results

3.1. Efficacy of MS, BT and CM Treatments on the Population of Plant Parasitic Nematodes at the End of Season Two and Beginning of Season Three

The J2 population (The second stage juvenile population) differed among the treatments ($P<0.05$) with CM911.25 and control recording a significantly ($P<0.05$) higher population than the other treatments. Moreover, the treatments reduced significantly the population of Root-Knot Nematode (RKN) compared to the control as indicated in Figure 1. The Plant Parasitic Nematode (PPN) populations varied significantly ($P<0.05$) throughout the three seasons among the treatments with CM911.25 having the highest population, while CM303.75 was found to have the least. A significant correlation relationship was established ($r=0.415$, $P<0.05$) between the PPN and the soil pH although the relationship was not significant ($P>0.05$) in RKN, *Filenchus* and *Tylenchulus* species. At the end of season two and beginning of season three, the population of PPN differed significantly ($P<0.05$) among the treatments with BT3096 and CM607.5 recording lower populations, while BT5292 and the control recorded highest nematode populations.

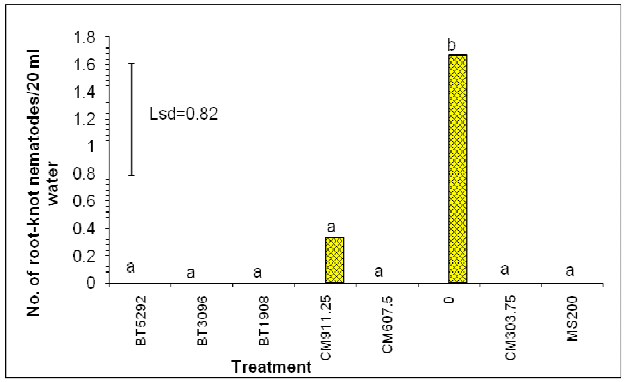

Figure 1. Effect of *Brassica* Tissue, Chalim™ and Metham Sodium on Root-Knot Nematode Juveniles Population at the End of Season Two and Beginning of Season Three

Means followed by similar letter (s) are not significantly different (P>0.05) according to Student-Newman-Keuls test. Bars followed by same letter (s) are not significantly different at 5% probability level according to Student-Newman-Keuls (S-N-K) test. BT (Brassica tissue treatment in grams); CM (Chalim™ treatment in grams); MS (Metham sodium treatment in ml) and Control 0 (control treatment).$

At the end of season two and beginning of season three, the population density of PPN differed significantly among the treatments with *Brassica* tissue at 3096 g and Chalim^{TM} at 607.5 g recording significantly lower densities than the control treatment, followed by *Brassica* tissue treatment at 5292 g, Metham sodium at 200, *Brassica* tissue treatment at 1908 g, Chalim^{TM} treatment at 911.25 g and Chalim^{TM} treatment at 303.75 g had lower densities that did not differ significantly from the control treatment as indicated in Figure 2.

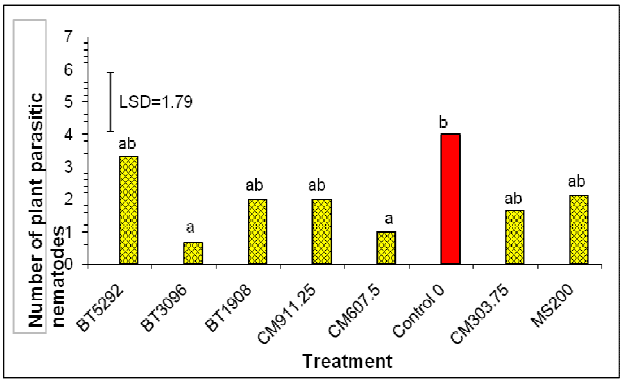

Figure 2. Efficacy of Metham Sodium, *Brassica* Tissue and Chalim^{TM} Treatments on Plant Parasitic Nematode Density at the End of Season Two and Beginning of Season Three

Bars followed by same letter (s) are not significantly different at 5% probability level according to Student-Newman-Keuls (S-N-K) test. BT (Brassica tissue treatment in grams); CM (Chalim^{TM} treatment in grams); MS (Metham sodium treatment in ml) and Control 0 (control treatment)

The nematode populations were significantly higher in season 2 than in season 3 ($P < 0.05$). Plant parasitic nematodes and root knot nematodes were significantly higher during the second season than the third season ($t = 8.05$ and $t = 8.07$ respectively) as indicated in Table 1. During the same season, the Soil Moisture Content (SMC) and pH did not differ significantly as shown in Table 1. During the study period, *Helicotylenchus*, *Pratylenchus*, *Meloidogyne* (root knot nematodes), *Tylenchus* and *Heterodera* were recorded with *Meloidogyne*, *Tylenchus* and *Heterodera* populations varying significantly among the two seasons (Table 1).

The results (Table 2) show the effect of various treatments on nematode population during the second and third seasons.

The number of root knot nematode juveniles in the two seasons were not significantly different ($P > 0.05$) although there were more J2 in season 2 (mean 5.25) than in season 3 (mean 0.25). In season 2, treatment CM607.5 g, BT1908 g and BT3096 g had significantly high number of J2 ($P < 0.05$). In season 3, however, all the treatments differed significantly from the control although the number of juveniles was not significantly different among the treatments (Table 3).

3.2. Effect on Physico-Chemical Factors

The *Brassica* tissue treatment had higher Nitrogen content, organic carbon, calcium, potassium compared to the other treatments and the control. At higher level of application, BT5292 had the highest amount of total nitrogen relative to the other amendments and the control. All the treatments except BT1908 increased the iron content in the soil relative to the initial amount.

However, plots treated with Chalim^{TM} and the control treatments recorded higher amounts of iron compared to the other treatments with BT1908 recording lower values. The amount of zinc, calcium, sodium and magnesium in the soil on plots treated with the various treatments was higher than the initial values although the treatments did not differ significantly ($P>0.05$) from each other. Similarly, all the treatments used increased the amount of phosphorous in the soil although Chalim^{TM} and Metham sodium treatments recorded higher values than the initial value before treatment. The various treatments reduced the potassium content in the soil compared to the initial amount with Chalim^{TM} and the control recording lower values than the other treatments.

The various treatments used did not differ in their moisture content although BT5292 had significantly higher moisture value than MS200 that recorded the least moisture content. Similarly, the study revealed that *Brassica* tissue treatment increased the soil pH slightly compared to the Chalim^{TM}, Metham sodium and the control.

4. Discussion

4.1. Effect on Plant Parasitic Nematodes

The presence of relatively high populations of root knot nematodes and *Heterodera* than the other plant parasitic nematodes may be attributed to the fact that tomato, capsicum and potato are good hosts for the nematodes. Of the treatments, control treatment, *Brassica* tissue at 1908 g and 5292 g recording higher populations compared with the other treatments during the two seasons. However, *Brassica* tissue treatments and the other treatments suppressed nematode population during the third season compared with the control treatment that recorded an increase in nematode population.

In the first season there was no significance difference between treatments and the control. In season two and three the benefits of the treatments were clearly seen as there was a drastic reduction in the nematodes populations.

Brassicaceous materials have been reported to have allello-

Table 1. Comparison Between Season 2 and 3 Soil Moisture Content, pH and Nematode Populations During the Second and Third Seasons

	Season 2	Season 3	t-Values	P-Values
Soil Moisture Content (SMC)	7.65 ±0.22	8.09 ±0.16	1.62	0.109
pH	5.31 ±0.08	5.42 ±0.06	1.10	0.275
Plant Nonparasitic Nematode (PNPN)	1.96 ±0.19	1.58 ±0.12	1.58	0.118
Total Count (TC)	8.08 ±0.75	2.10 ±0.17	7.23*	0.000
Plant Parasitic Nematode (PPN) (20 ml)	1591 ±174	117.3 ±27.0	8.05*	0.000
Helicotylenchus (He)	0.125 ±0.07	0.00 ±0.00	1.76	0.083
Pratylenchus (PRA)	0.042 ±0.02	0.13 ±0.05	1.62	0.109
Root Knot Nematode (RKN) (J2)	5.25 ±0.60	0.25 ±0.08	8.07*	0.000
Tylenchus (TYL)	0.37 ±0.11	0.000 ±0.00	3.34*	0.001
Tylenchorhynchus (TYLC)	0.00 ±0.00	0.00 ±0.00	-	-
CUT (*Scutellonema*)	0.00 ±0.00	0.00 ±0.00	-	-
FIL (*Filenchus*)	0.00 ±0.00	0.00 ±0.00	-	-
Longidorus (LON)	0.00 ±0.00	0.00 ±0.00	-	-
Xiphinema (XIP)	0.00 ±0.00	0.00 ±0.00	-	-
Heterodera (HET)	0.38 ±0.08	0.00 ±0.00	4.54	0.000*

*t-Value marked by * in the same row are significantly different at 95% CI*

Table 2. Plant Parasitic Nematodes (20 ml) in Season 2 and 3 among the Various Treatments

Treatment	Season 2	Season 3
BT1908g	2893 a	186.7 ab
BT3096g	2260 a	0.00 a
BT5292g	1213 a	280.0 ab
Control	280 a	466.7 b
CM303.75g	1027 a	0.00 a
CM607.5g	2893 a	0.00 a
CM911.25g	1227 a	4.70 a
MS 200ml	933 a	0.00 a
P-Value	0.317	0.041

BT (Brassica tissue treatment in grams)
CM (Chalim^{TM} treatment in grams)
MS (Metham sodium treatment in ml) and Control 0 (control treatment)

pathic effects as well as biofumigation effects to soil biota that includes plant parasitic nematodes (Hartman et al, 1993; Bailey & Lazarovits, 2003; Schonfeld et al, 2003 and Gruver et al, 2010). The production of biofumigation products including isothiocyanates (ITCs) that has an active ingredient related to that of Metham sodium and dazomet have been reported to be highly toxic to pests and pathogens. These compounds are released at neutral pH and hydrolyse producing into sulphur-containing glucosenolates that may confer resistance to crops against infection by pathogens (Kumar, 2005). Moreover, the biocidal action of isothiocyanates produced by *Brassica* tissue and their potential to manage and suppress phytopathogens has been reported by Brown & Morra (1997) and Matthiessen & Kirkegaard (1998). Hence, the reduction in nematode population is attributed to among other factors, the presence of allellochemicals and biofumigants released by brassicaceous materials at neutral pH enabling plants to be resistant to the nematode attack.

4.2. Nutrient Content Analysis

Nutrient content analysis of the test materials revealed significant increase in important nutrients necessary for proper plant growth. The study established that soils amended with *Brassica* tissue had significantly higher nutrient contents than the other treatments. High levels of Ca, K, N, P and organic carbon were found in soils amended with *Brassica* tissue compared to the other treatments. Studies conducted on other parts of the world have revealed a significant association between nitrogen on suppression of plant parasitic nematodes (Nchore et al, 2011). Calcium has been found to be an important mineral for proper growth of tomato plants. Similarly, phosphorus is import-

ant in proper growth of roots that may influence the tolerance levels of plants to plant parasitic nematodes hence reducing the injuries that the bacterial may enter through. Moreover, most of the soils amended with *Brassica* tissue had slightly alkaline pH, and relatively higher moisture content which may have influenced the release of biocidal chemical for suppression of the plant parasitic nematodes.

Table 3. Plant Root Knot Nematodes (J2) in Season 2 and 3 in Various Treatment Plots

Treatment	Season 2	Season 3
BT1908 g	9.00 a	0.00 a
BT3096 g	9.00 a	0.00 a
BT5292 g	2.33 a	0.00 a
Control	1.00 a	1.667 b
CM303.75 g	2.67 a	0.00 a
CM607.5 g	9.00 a	0.00 a
CM911.25 g	6.33 a	0.333 a
MS 200 ml	2.67 a	0.00 a
P- value	0.262	0.000

BT (Brassica tissue treatment in grams)
CM (Chalim™ treatment in grams
MS (Metham sodium treatment in ml) and Control 0 (control treatment)

The soils amended with Chalim™ and Metham sodium were found to have low soil pH and organic matter. However, the amended or soil treated with *Brassica* tissue had a reduced soil acidity as reflected in the soil chemical analysis. Moreover, the organic content of these soils was relatively higher than that of the control treatment.

Over the study period, the soil incorporated with *Brassica* tissue improved physical chemical properties leading to healthy plants. The plants' tolerance to plant parasitic nematode infection may have also been improved following soil amendment with organic matter from *Brassica* tissue. According to Muchovej et al (1980), well nourished plants are able to withstand disease infection and produce higher yields than the poorly nourished ones.

5. Conclusions and Recommendations

Brassica tissue amendment significantly increased nutrients contents than the other treatments. High levels of Ca, K, N, K, P and organic carbon were found in soils amended with *Brassica* tissue compared to the other treatments hence farmers may save more on fertilizers. Moreover, *Brassica* tissue treatments at highest levels of application should be applied as fumigants than Chalim™ since more Nitrogen is realized in the soil making the crop to be healthier and resistant to pest attack. In addition, brassicaceous materials are readily available and cheap to the resource poor rural farmers.

Chalim™ should be applied in areas prone to nematodes at low pH since they have potential of interfering with their reproduction hence reducing their population in soil. At the same time, Chalim™ and *Brassica* should be used interchangeably to avoid nematode resistance. The results reveal that Metham sodium reduces moisture content in the soils amended with the treatment. It is therefore recommended that Metham sodium (MS200) should not be applied in very dry soil to avoid reduction of the moisture content.

Acknowledgement

The authors would like to thank the Kenya Agricultural Research Institute-National Agricultural Research Laboratories for the laboratory facilities and trial plots for this study. The work was carried out with financial support from ASARECA Project.

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