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ALTERNATIVE MANAGEMENT STRATEGIES FOR WEEDS AND ROOT KNOT NEMATODES (*Meloidogyne spp*) IN ROSE PLANTS GROWN UNDER POLYETHYLENE COVERED TUNNELS

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ABSTRACT
A field study on alternative ways to manage weeds and nematodes was conducted for two seasons under polyethylene covered growth tunnels in Horticultural Research and Teaching Field at Egerton University in 2005 and 2006. The treatments tested were: Dazomet (83.3g/m^2), Metham sodium (0.12g/1): *Brassica napus* and *Brassica juncea* applied at 2, 3 and 4kgs/m^2. The two brassica biofumigants were planted and uprooted at initial flowering stage and chopped into 10mm small pieces separately before being applied to their respective plots. The biofumigants reduced the number of nematodes by between 25% and 62% compared to the non treated plots, but their activities were quite inconsistent and were insignificant in the second season of study. In both seasons, reinfestation occurred soon after field aeration. However, the highest reduction of the 2nd juvenile stage of root knot nematodes of between 42% and 81% and up to 88% reduction in the emergence of some weeds were observed in the plots treated with Metham sodium at 0.12g/1. The findings of this study clearly show that, metham sodium and dozamet still remain the most effective means of controlling weeds under enclosed environment. Alternatively, weeds can be managed through seed bank depletion. With continuous uprooting of weeds the seed bank is exhausted in the soil and weed population gets reduced below threshold levels as we observed in the non treated plots.

Keywords: rose plants, weeds, root knot nematodes, growth, polyethylene covered tunnels.

INTRODUCTION
The cultivation of cut flowers under plastic polyethylene covered tunnels is a continuous process that often leads to a build up of pests and pathogens. Among these are plant parasitic nematodes and weeds that have been associated with the reduced quality and yield of many crops. Many species of nematodes are significant agricultural pests and cause large crop losses. Two of the most economically damaging groups are the root knot nematodes (*Meloidogyne* spp.) and the cyst nematodes (*Heterodera* spp. *and Globodera* spp.) (Bertioli *et al*., 1999). Plants infected by nematodes will exhibit symptoms such as root galls, lesions, excessive branching (Ogallo *et al*., 1997) or as injured tips and root rot, especially when nematodes are accompanied by plant pathogenic or saprophytic bacteria or fungi (Agrios, 1997). Nematode infestation results in substantial yield losses on all crops (Stirling and Stirling, 2003). For example, (Wang-XinRong *et al*., (1999) reported a reduced performance of roses that was associated with root knot nematodes, the *Meloidogyne* spp. Suppressed plant growth, yield and pigment synthesis has been observed in nematode infected field (Khan and Khan, 1994). These symptoms result in yellowing and wilting of the plants lowering the quality and resulting in the death of the host plants (Marley and Hillocks, 1994).

Weeds such as oxalis, purslane, redroot pigweed, malva, and grasses are persistent problems in growth tunnels and greenhouses in the Kenya highlands. Not only do these weeds reduce the quality of plants produced, but some also are known to harbour insects, such as whitefly and thrips, and other pests such as mites, slugs and snails.

For a long time, soilborne pests and pathogens, including nematodes and weeds have been managed by methyl bromide due to its broad-spectrum activities. However, in the recent years, the safety of methyl bromide has raised environmental concern due to its effects on the ozone layer. The product is therefore targeted for phase-out by the year 2015 (UNEP, 1999). To develop more environmentally friendly controls, researchers have been investigating the potential of biofumigants as alternative to the chemical fumigants. Certain plants from the mustard family are known to act as biofumigants by releasing inhibitory chemicals when used as green manure crops and subsequently incorporated into the soil. These glucosinolate degradation products are reported to exert suppressive effects on a wide range of soilborne pathogens. For example, mustard (*Brassica campestris*) oil cakes have the potential to reduce gall formation and fourth stage nematode larvae populations and increase root growth (Bari *et al*., 1999). Incorporating crucifer green manure into the soil suppresses weeds, soil-borne pathogens and pests (Brown and Morra, 1997). The 'biofumigant' properties of crucifer tissues are highly toxic isothiocyananates (ITC), and mildly toxic non-glucosinolate S-containing compounds released from tissues damaged when incorporated into the soil (Bending and Lincoln 1999). *Brassica juncea* L., *B. nigra* L. and *S. alba* are among the crucifers that yield the most isothiocyanates (Kirkegaard and Sarwar, 1998).

A part from the biofumigants, the other chemicals presently gaining prominence as alternatives to methyl bromide are dazomet (tetrahydro-3, 5-dimethyl-2H-1, 3, 5-thiadiazine-2-thione) and metham sodium (methyl isothiocyanate). Dozomet, a chemical soil sterilant that when applied to moist soils, breaks down into methyl isothiocyanate, is documented to have a broad spectrum effectiveness against soilborne pests including nematodes, fungi and weeds (McSorrey and Fredrick. 1995). For example, Sinha and Mukhopadhyaya (1993) reported substantial reduction in the number of *M. incognita* in aubergines as host plants when dazomet was used as a fumigant. Ramakrishnan *et al*. (1999) observed a significant decrease in root knot nematodes in tobacco fields treated by dazomet at four levels of 30, 40, 50 and 60 g/m^2. Primo and Cartia (2001) investigated the efficacy of dazomet in the control of weeds in nursery beds of dianthus (var. Sweet William and Phlox). Six levels of dazomet (10, 20, 30, 40, 50 and 60 g/m^2) were tested along with an untreated control. The lowest number of weeds (6.0 weeds /m^2) was recorded in the plots treated with 60g of dazomet per m^2 at 30 days after sowing, compared with 42.67 weeds in the untreated control. Elliott and Jardin (2001) also reported a decrease in weed germination in an experiment with dazomet.

In another study to evaluate the effect of metham sodium and dazomet on nutgrass (*Cyperus spp.*) and purslane (*Portulaca oleracea*), soil treated with dazomet and covered with plastic, virtually eliminated weeds (Holcroft and Smith, 1995). However, weeding of the plots was necessary because the fumigation effects were short term. While in the same study, a rapid degradation of metham sodium when used as fumigant was observed the plots fumigated with dazomet required less weeding (Holcroft and Smith, 1995). Dazomet applied on established turf grass and covered with plastic sheet provided greater than 98% reduction in annual blue grass seedlings (Park and Landschoot, 2003, Eitel (1995), and Tacconi and Santi 1994). Excellent weed control with increased crop vigour, a yield and total fresh weight has been reported in plots fumigated with dazomet (Middleton and Lawrence, 1995).

Biofumigants on the contrary portray different results. Sances and Ingham (1997) found that weeding costs were five-fold greater in organic treatments (broccoli residues and spent mushroom compost) than in chemical fumigant treatments (metham sodium and dazomet).

MATERIALS AND METHODS

Experimental site

Research to investigate alternative management strategies for the control of nematodes and weeds in roses was coducted at Egerton University Horticultural Research and Teaching Field for two seasons in 2005 and 2006. The research field is located at latitude of 0°23 South and longitude of 35°35 East and altitude of 2,225 m above sea level. The area receives moderate, mean rainfall of 1012 mm, mean maximum temperature of 22°C with minimum night temperature range of 5 to 10°C. The mean maximum polyethylene covered growth tunnels temperature during the study period was 36°C in season 1 and 24°C in season 2. The soil at the site is vintric mollic andosols with pH of 5.5 - 6.0. The polyethylene covered growth tunnels used had previously been used for growing tomatoes.

Design and field layout

The experiment was laid down in a randomized complete block design (RCBD) replicated three times. The experiment covered a total area of 43.8 m^2. Plots measured 1x1 m. Blocks were separated by 0.8-m path, while plots were 0.5 m a part.

Treatments application and field establishment

The treatments consistent of dazomet and metham sodium applied at 83.3g/m^2 and 0.12g/1 per 1m^2 rates respectively, as well as *Brassica napus* and *Brassica juncea* applied at 2, 3 and 4kg/m^2. *Brassica napus* and *Brassica juncea* were established prior to experimental set-up. The brassica seeds were obtained from KARI Njoro. *Brassica napus* variety R3245 was planted on three beds 14 days earlier than *B. juncea* variety 3228 due to difference in time to maturity. Planting was done by direct seed drilling at inter-row spacing of 30 cm. Fertiliser application followed Kirkegaard *et al*., (1999) specifications of 20 kg N/ha, 20 kg P/ha and 18 kg S/ha. The excess seedlings were uprooted upon germination to achieve intra-row spacing of 3 cm. The brassicas were ready for use at initial flowering stage, which varied from 35 to 40 days for *Brassica juncea* and 52 and 54 days for *B. napus.*

Dazomet was thoroughly incorporated into the soil at the rate of 83.3g/m^2 to a depth of 30 cm. Metham sodium at the rate of 0.12g/l to 1m^2 was diluted in 5 litres of water and applied using a watering can. The application rates were as per the product specification. The plots were covered with clear polyethylene of 0.14 mm thickness. The polyethylene edges were buried 15 cm into the soil to ensure airtight conditions for three weeks.

The brassica plants were carefully uprooted at initial flowering stage and chopped into small pieces of about 1cm. The chopped pieces of each brassica variety were immediately applied to respective plots at the rate of 2, 3 and 4 kg/m^2 .The material was incorporated into the soil at about 30 cm depth.

Nematode inoculum was obtained from heavily galled tomato roots. The roots were chopped into small pieces. The chopped material was bulked and mixed thoroughly. Equal quantity of 1kg of the chopped pieces was then spread in two rows per plot before fumigation was done.

Second juvenile stage of *Meloidogyne spp*. was considered in this study. The extraction procedure followed Baermann funnel method as described by Barker *et al*., (1985), but with some modifications. A plastic funnel was fitted with clear rubber tubing at the bottom. The funnel was then lined with double layer cheesecloth. The apparatus were supported in a vertical position on a tripod stand. 100 cm^3 of sampled and bulked soil was measured and carefully poured into the funnel. The soil was then suspended in distilled water for 24 hours to allow nematodes to swim and settle at the bottom. The juveniles were then recovered from the rubber tubing. One millilitre of the solution was placed on a petri dish and the nematodes present were quantified under the microscope.

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Five most common weeds observed in the tunnels were also evaluated in this study the weeds included: oxalis (*Oxalis latifolia*), malva (*Malva pusilla*), purslane (*Portulaca oleracea*), redroot pigweed (*Amaranthus retroflexus*) and grass. A quadrant of 30 x 30 cm was used to determine the area under which to sample for weed mergence observation.

The number of weeds that emerged was counted within a randomly placed quadrant per plot every 30 days before being uprooted. The weed population recorded was used to determine effectiveness of fumigants and biofumigants in managing weeds under polyethylene covered growth tunnel. Collection of nematode (*Meloidogyne spp.*) data involved destructive sampling. Roots of three randomly selected plants were carefully washed. The number of root galls was counted up to 15 cm on each of the three roots with the aid of a hand lens. This was used to estimate the severity of the nematode infestation.

Data analysis

Analysis of field data was done with the Mixed Models procedure of SAS V9.1 statistical package (SAS Institute, 2002). The UNIVARIATE procedure of SAS was used to check that the data were normally distributed before analysis. Field weed data was log transformed to achieve homogeneity of variances and normal distributions.

RESULTS AND DISCUSSIONS

Metham sodium reduced the number of nematodes at second juvenile stage by between 11 and 81% compared to the untreated plots. The effectiveness of metham sodium was inconsistent and was significantly suppressed in the second season. Reinfestation occurred soon after field aeration indicating that single control methods such as fumigants alone may not drastically decrease initial nematode population. The observations made in this study concurs with Giannakou and Karpouzas (2003) findings that metham sodium provided good control of nematodes population only when its application was followed by a non fumigant nematicide application such as Cadusafos or Oxamyl. Both metham sodium and dazomet have synergistic effect when applied in combination with other chemicals (Giannakou *et al*., 2002).

Both the biofumigants significantly ($p = 0.05$) suppressed the population of root knot nematodes (*Meloidogyne* spp) in season 1 compared to the control, but not in season 2. However, the effectiveness of brassica biofumigants was short lived probably because of their influence on the soil texture. It has been pointed out that nematode distribution and reproduction differ with soil texture (Mateille *et al*., 1995). Therefore, it is likely that, chopped brassica material suppressed root knot nematodes but with time, the environment became favourable for rapid multiplication. Freckman and Ettema, (1993) reported that nematode abundance was high in the high input organic systems and lowest in the popular conventional farming systems.

During the second season, the reduction in the number of root knot nematodes was not significantly different between the biofumigant treated plots and the plot with no treatment (Table-1). These results are in agreement with Stirling and Stirling, (2003) who incorporated dry broccoli roots, leaves and stems into the soil at the rate of 17t/ha and observed no reduction in nematode population. In addition, plants were heavily galled even with high rates of amendment. In other study, organic manure when used as fertilizer encouraged nematode establishment (Bednarek and Gaugler, 1997). The decomposition of brassica biofumigants to increase soil humus content negates their biocidal property in the management of root knot nematode. Donnison *et al*., (2000) reported that, changes in soil microbial communities are related primarily to changes in plant productivity and composition or the form and quantity of fertilizer applied on the site. The low number of root knot nematodes recovered in the second season as compared to the first season could also be attributed to the hot weather that was prevailing during the time of the study. Ploeg and Stapleton (2001) reported that temperature and amendment of soil with broccoli residues had synergistic effect on the infestation of melon plants by *Meloidogyne incognita* and *M. javanica.* The lowest temperature tested was 20 °C and addition of broccoli to the soil had very little effect on nematode infestation or galling of melons. Increasing the temperature of broccoli amended soil to 25, 30 or 35°C dramatically reduced infestation and galling compared to that in non-amended soils. Effect of brassica treatments in this case occurred soon at higher polyethylene covered growth tunnel temperature of 36°C recorded in second season than 24°C recorded during the first season.

Table-1. Count of root knot nematodes at second juvenile stage recovered from 100 cm^3 of soil sampled from different treated plots.

Treatment	Season 1	Season 2
Control	8.7a*	4.8a
BJ 2 kg	4.7b	3.6ab
BJ 3kg	3.3bc	3.5ab
BJ 4kg	2.3bc	3.4ab
BN 2kg	3.7b	3.5ab
BN 3kg	4.7b	3.7ab
BN 4kg	3.3bc	3.4ab
DZ	2.7bc	2.8b
MES	1.67c	2.7b

*Means within a column followed by different letters are significantly different at $P \leq 5\%$ level of significance according to Duncan's Multiple Range Test. The abbreviations used in the table stands for *Brassica juncea* (BJ), *Brassica napus* (BN), Dazomet (DZ) and Metham

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sodium (MES). The treatments were applied per m^2 of an area.

Rose plants treated with dazomet and those treated with metham sodium had the best suppression effect (74% compared to the control) on the management of root knot nematodes in both seasons (Figures 1 and 2). *B .napus* at 4 kg/ha reduced the number of galls by between 50 to 60% compared to the control (Figures 1 and 2). The plots with no treatment had the highest count of galls in both seasons.

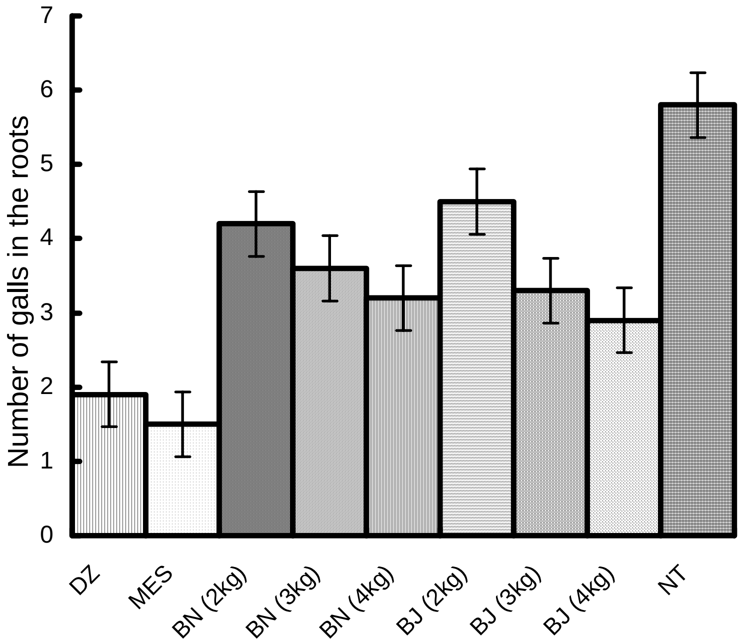

Figure-1. Number of root galls per rose plant as affected by biofumigants, metham sodium and dazomet treatments (Season 1). Root galls were averaged from 3 plants. Vertical bars represent standard errors of the means of three replications.

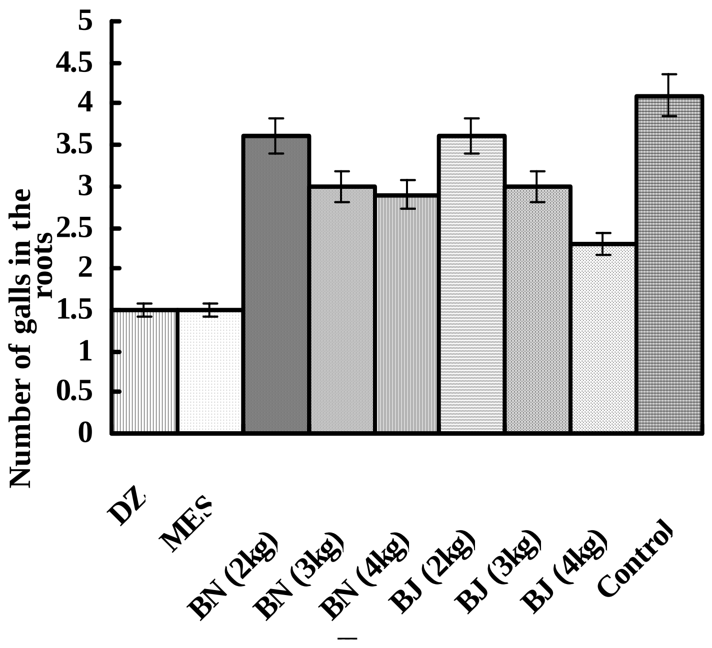

Figure-2. Number of root galls per rose plant as affected by biofumigants, metham sodium and dazomet treatments (Season 2). root galls were averaged from 3 plants. vertical bars represent standard errors of the means of three replications.

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Effect of fumigants on weed control

The weed population responded differently to various treatments (Tables, 3 and 2). Emergence of weeds such as oxalis, amaranthus, malva and grass was enhanced under high amounts of the biofumigants with the highest increase of 64% being observed in the plots treated with 3 kg to 4kg of *Brassica napus*. However, this observation was in sharp contrast with the results observed in the plots treated with the fumigants. For example, oxalis population was reduced by between 73 and 86% in both seasons in plots treated with dazomet at 1.2g/l. The same trend was observed in other plots and where metham sodium was also applied. The observation agrees with the results reported by Roberts and Matthews (1985) that the metam sodium treatment was the most effective against weeds. It reduced the germination of annual bluegrass, rough pigweed, and purslane by over 95% compared to the uncovered, non-treated control.

Table-2. Emergence of various weed species as affected by fumigants and biofumigant treatments (1st Season October 2004- February 2005).

Treatments	Oxalis	Portulaca	Amaranthus	Malva	Grass
BJ 4 kg	12.23a*	5.05c	3.64a	1.81bc	4.84bc
BN 4 kg	10.93ab	6.30bc	4.07a	3.91a	5.52abc
BJ 3 kg	10.84ab	8.35a	3.76a	2.28b	6.21abc
BN 2 kg	10.68ab	4.96c	3.64a	1.00c	6.20abc
BJ 2 kg	10.62b	5.60bc	2.15ab	2.41b	7.32ab
BN 3 kg	10.48b	5.40bc	4.05a	2.19b	8.53a
Metham	2.793c	2.29d	1.00b	2.14b	3.39c
Dazomet	1.333d	3.10d	1.00b	2.28b	3.26c
Control	11.15ab	7.12ab	3.77a	2.38b	7.52ab

***** Means followed by different letters are significantly different at 5% level of significance according to Duncan's Multiple range test. The abbreviations used in the table stands for *Brassica juncea* (BJ), *Brassica napus* (BN), Dazomet (DZ) and metham sodium (MES). The treatments were applied per m^2 of an area.

Table-3. Emergence of various weed species as affected by fumigants and biofumigant treatments (Second season October 2004- February 2005).

Treatments	Oxalis	Portulaca	Amaranthus	Malva	Grass
BJ 4 kg	19.77c*	3.45b	15.38abc	2.41a	3.45ab
BN 4 kg	20.07bc	3.24b	16.33a	2.41a	3.24ab
BJ 3 kg	19.99bc	4.74a	12.02bc	1.00b	4.46a
BN 2 kg	22.41bc	2.73b	14.05abc	2.41a	4.00ab
BJ 2 kg	21.90abc	3.00b	12.83abc	2.41a	3.45ab
BN 3 kg	19.58c	3.45b	15.64ab	2.00ab	3.24ab
Metham sodium	6.41d	1.00c	11.65cd	1.00b	1.00c
Dozomet	6.41d	1.00c	8.24d	1.00b	2.41bc
Control	23.54a	3.24b	13.65abc	2.41a	3.00ab

***** Means followed by different letters are significantly different at 5% level of significance according to Duncan's Multiple Range Test

CONCLUSIONS

Biofumigants, especially *Brassica juncea* at the rate of 4 kg/m^2 was statistically comparable to the chemical metham sodium and dazomet in the control of nematodes. However, the use of biofumigants in management of weeds in polyethylene covered growth tunnels was ineffective when compared to the low levels of weeds observed in the plots treated with metham sodium or dazomet. The chemical fumigants and *Brassica* biofumigants generally had low residual effect and. reinfestation curred soon after terminating fumigation. The two chemical fumigants, methan sodium and dazomet still offered the best options for the control of nematodes and weeds. Re-application of biofumigants and continuous weed seedbank depletion through weeding offer alternative management strategy where the use of chemical fumigants might not be possible.

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