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A comparison of the effectiveness of the aqueous extracts of garlic, castor beans and marigold in the biocontrol of root-knot nematode in tomato

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A glasshouse experiment to control RKN (*Meloidogyne javanica*) on tomato with aqueous extracts of marigold (*Tagetes erecta*) leaves and flowers, castor beans (*Ricinus communis*) and garlic (*Allium sativum*) was conducted from March to May 2011 in Harare. The plant material was dried and pulverized and diluted with water at a rate of 25g/100ml. Four-week old seedlings were planted in twenty micro plots arranged in a randomized complete block design with five treatments and four replicates. Approximately 5000 J2 nematodes were pipetted onto each plant. The botanicals were drenched around each plant. Nemacur® and non-amended plots served as control. Data collection was done fortnightly. Results showed that tomato is susceptible to RKN infestation and the application of botanicals significantly ($P < 0.001$) controls RKN by reducing galling and reproduction. While the botanicals were also effective in reducing galls, further tests will establish optimum concentrations.

Key words: *Meloidogyne javanica, Allium sativum*, *Tagetes erecta,* garlic

Introduction

Root knot nematodes (RKN) are responsible for 12.3% yield loss of the world's major crops (Sasser, 1998, in Sasena, Sikora and Srivastava), and global tomato production is affected by the genus *Meloidogyne*, the most economically important nematode in tropical and subtropical agriculture (Sasser, 1989) which reduces yield by 30 – 50% (Sasser and Freckman, 1987; Jonathan *et al*., 2001; Saravanpriya and Sivakumar, 2005; Cetintas and Yarba, 2010). RKN cause between 20-33% yield loss (Aalders *et al*., 2009; Khan,

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2009; Sasser, 1989) approximating $4.9 billion (James, 1981) of the 1.2 billion tonnes tomato annually produced globally and nematicides cost more than $37 million (Aalders *et al*., 2009). Pakistani loss estimates range between five and twenty percent (Maqbool, 1988) and Punjab disease incidence is between 75% and 100% (Shahid *et al*., 2007 in Khan, 2009). Losses result from the nematodes either feeding solely or from secondary bacterial infections brought thereabout. Ninety nine percent of 207 horticultural crops sampled in Ecuador were infected by RKN (Trudgill, 2001) with 25% to 30% yield loss. About 2000 plants are known hosts (Gowen *et al*., 2005). Agricultural production can be salvaged if these parasites are successfully combated. Although synthetic chemicals have been successfully used, they overburden the environment, hence the increasing popularity of use of plant extracts. Fifty seven plant families are nematicidal (Sukul, 1992 in Bharadwaj and Sharma, 2007), including Alliaceae, Asteraceae and Euphorbiaceae, thus the need to consider garlic, marigold and castor beans, relatively common sources of nematicides in these respective families.

Scientists developed synthetic nematicides to control nematodes and these have been more successful than both biological and physical methods, albeit with detrimental consequences to the environment. Researchers are developing alternative management techniques such as use of cropping systems soil amendments, organic soil amendments, biological control agents and judicious use of nematicides (Serfoji *et al*., 2010). Organic farming is receiving increasing international support and the global product market reached a value of $US 38.6 billion in 2006 (Kaþkavalci *et al*., 2009). Environmentalists and consumers are against increased use of agrochemicals like nematicides in crop production.

Garlic (*Allium sativum*) leaf extract has been successfully used to increase *Tylenchulus semipenetrans* mortality at high concentrations in laboratory conditions (Ayazpour, Arabzadegan and Hasanzadeh, 2010). Garlic has indirect effects on nematode populations because it disrupts their mobility, food absorption and reproduction (Fadzirayi, Masamha and Mukutirwa, 2010). Garlic oil has been shown to offer significant protection against free-living soil inhabiting nematodes (Block, 2010). The castor oil (*Ricinus communis)* plant is a coarse tropical, perennial, with insecticidal and antimicrobial properties (Upasani *et al*., 2003). RKN egg hatching is inhibited and the juveniles killed by root, leaf and seed extract (Abid, 1996; Mostafa, 2000; Ahmet and Galip, 2007; Mafeo and Mashela, 2010). The female to male ratio is also decreased and abortions of *M. incognita* are influenced by *R. communis* (Hackney and Dickerson, 1975). French marigold (*Tagetes patula*) and *T. minuta* are resistant and fatal to *Meloidogyne* spp. In most cases, *T. patula* and *T. minuta* are used as cover crop, in rotation, green manure and source of nematode-antagonistic extracts (Chitwood, 2002). *T. patula* grown eight weeks prior to transplanting tomato is more effective in nematode suppression than root and shoot amendments. Tangerine gem (*T. tenuifolia*) used as an intercrop reduces RKN population and improves tomato growth (Alam and Siddiqui, 1988). Tomato (*Lycopersicon esculentum)* is indigenous to the Andes Mountains of tropical America (Srinivasan, 2010) and is the second best vegetable in economic importance after potato (*Solanum tuberosum*). It is highly susceptible to RKN. Alternative organic nematicides that are ecofriendly but effective in producing quality tomatoes are being considered. Garlic*,* marigold and *R. communis* are three known sources of biological nematicides whose potency against RKN were evaluated.

Popular nematicides are hazardous to the environment because they leave residues, disturb the ecological balance, are toxic to man and are volatile (Chitwood, 2002). Prolonged and overuse leads to pest resurgence, emergence of newer pests and development of chemical resistance in RKN, thus the interest in natural pest management techniques. The public prefer environmentally safe, inexpensive, less time consuming (Braun and Supkoff, 1994 in Kaþkavalci *et al*., 2009) and agronomically useful nematicides (Chitwood, 2002).

Materials and methods

The study was conducted in a glasshouse at the Plant Protection Research Institute, (DRSS), Harare; an area 17°51'50" S and 31°1'47" E, elevated 1503m above sea level, with an average annual temperature of 17.95°C, receiving 855mm of rain annually. Clay loam soil was used. The soil was sterilized by oven baking at 200°C for six hours and placed in pots after cooling. Pots were preferred as they could contain a possible nematode outbreak. Kutsaga Research Station (Tobacco Research Board) cultured and supplied the *M. javanica* race TRB inoculum. Garlic bulbs were bought from a market stall, whilst the castor beans and marigold leaves and flowers were collected from the wild. Garlic bulbs, marigold leaves and flowers and castor beans were sun dried and pulverized and 25g of the ground plant matter was blended in 100ml of water for twenty minutes (Okeniyi *et al*., 2010). The mixture was filtered and the filtrate was collected and used as aqueous extract. One hundred healthy four week old Rodade tomato seedlings were transplanted into asbestos pots, each pot having five plants in a rhombus pattern with one plant placed at the centre. The soil was watered to field capacity before transplanting to prevent RKN washing away before establishing host-plant relationship.

After transplanting, RKN were drained from a 10ml volumetric pipette. After counting under a microscope, the volume of suspension containing the standard 5000 J2 was determined to be approximately 0.6ml. The inoculum was carefully placed around the plant root system of each plant. Immediately after inoculation, 40ml of the aqueous extract amendments were applied at each plant station. The application of treatments was in a randomized complete block design replicated four times. Nemacur® 400 EC (fenamiphos), a commercially available nematicide was used in plots for positive control and untreated, inoculated plots for negative control were also included. The total microplot size was twenty and the experiment was conducted at variable temperature. The plants were maintained and harvested every fortnight up to sixty days after inoculation (DAI) when the experiment was terminated. Roots were carefully uprooted and collected into plastic pockets to avoid drying, and rinsed gently with tap water to remove soil debris. The root systems were detached and rinsed and the number of galls per egg mass (*i.e.* gall index or root damage) established via a 0 to 5 rating scale suggested by Taylor and Sasser (1978) where: 0 = no gall; 1 = 1 – 2 galls; 2 = 3 – 10 galls; 3 = 11 – 30 galls; 4 =31 – 100 galls; and 5 = more than 100.

RKN were then extracted using the maceration method where the roots were chopped and weighed to 10g and placed in an electrical macerator for 15 seconds. After maceration the mixture was passed through a series of mesh sieves of sizes 250, 150, and 38µm which allow maximum nematode retention. Residue in larger sieves was backwashed, collected and centrifuged at 4000 rpm for five minutes. A second centrifugation of nematode residue was done in a sucrose solution at 4000 rpm for two minutes after thorough shaking. This mixture was then tilted 38° to the horizontal in the 38µm sieve after the sucrose solution had dissolved thoroughly. Serial samples of the liquid were collected for observation under a microscope with the aid of laboratory tally counter Soil of volume 100 cm3 was collected from the pots, placed in a 9 litre bucket, filled with water and allowed to settle. This mixture was then poured down a series of sieves of diameters sizes 250µm, 150µm and 38µm in descending order, which captures soil and dirt but allows RKN through the upper two. The residue in the 38µm diameter sieve that contains RKN was tilted at a 30° angle and gently washed back with a stream of water and collected first into a beaker, then a centrifuge tube. Centrifugation was done at 4000 rpm for 5 minutes, and gentle discarding of the supernatant fluid leaving about 5ml to avoid nematode loss. Further addition of residue to sucrose solution preceded a 2 minute centrifugation at 4000 rpm. The nematodes were counted in a rectangular counting dish. Sucrose solution was made by adding distilled water to 684g sucrose granules to give a litre. Genstat version 6.1 (Lawes Agricultural Trust, Rothamsted Experimental Station) was used for data analysis. Mean separation was done using least significant differences at P= 0.05. ANOVA was performed to determine effects of treatments on dry matter, root galling and RKN reproduction on tomato seedlings. Microsoft Excel 2007 was used to present the data.

Results

There was a significant difference (P < 0.001) in the mass attained by the treatments both 42 and 56 DAI, and both days' results followed the trend Nemacur > *R. communis* > garlic > marigold > unamended control (Figure. 1). Marigold did not increase in mass in the fourth fortnight, and the controls differed from the botanicals on both occasions. The increase in dry matter in plants treated with the botanicals was not significantly different 42 DAI. All the treatments achieved significantly different masses 56 DAI.

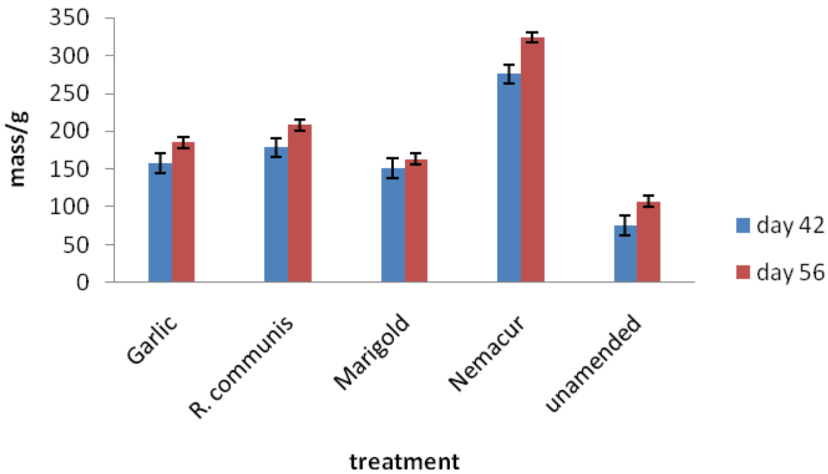

Fig. 1. Effects of treatments on dry mass after 42 and 56 days

On day 14 there was a significant difference (P = 0.011) in gall incidence among the treatments. Nemacur and *R. communis* were significantly different from the unamended control. Gall suppression decreased in the order Nemacur > *R. communis* > garlic > marigold > unamended treatment. On day 28 there was a significant difference (P < 0.001) in root galling indices. The unamended control differed from all the other treatments. Garlic gave results different from Nemacur and *R. communis*. Treatment activity decreased in order Nemacur > *R. communis* > marigold > garlic > unamended treatment. There was a significant difference (P < 0.001) in root galling indices 42 DAI. Garlic's activity was different from both controls but not from marigold and *R. communis*. The trend of gall inhibition 42 DAI was Nemacur > *R. communis* > garlic > marigold > unamended control. Significant differences (P < 0.001) were noted within the treatments 56 DAI. The unamended control and marigold differed from each other and from the other treatments. Garlic differed from the unamended control, *R. communis* and marigold, and Nemacur > *R. communis* > garlic > marigold > no treatment was the decreasing order of root knot inhibition. There was a significant difference (P < 0.001) on day 60. *R. communis* and Nemacur were the only insignificantly different treatments. Treatment effectiveness decreased in the order Nemacur > *R. communis* > marigold > garlic > no treatment. Root galling indices after 14, 28, 42, 56 and 60 days are shown in Figure. 2.

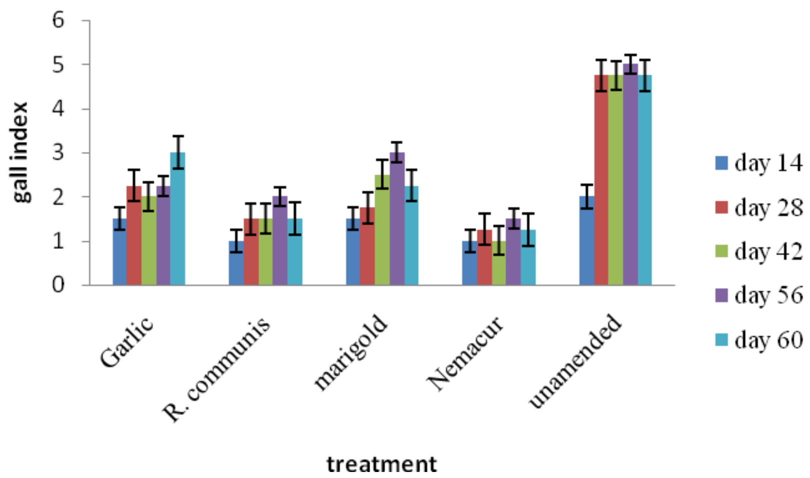

Fig. 2. Root galling indices after 14, 28, 42, 56 and 60 days

Effect of treatments on root galling

There was a significant difference (P < 0.001) in the reproduction factor 60 DAI. The effectiveness in containing RKN multiplication was Nemacur > garlic > *R. communis* > marigold > no treatment. Nemacur and the unamended control significantly differed from each other and from the botanic extracts. Marigold and garlic gave results different from each other but not from *R. communis*. The results for the RKN reproduction factor after 60 days are shown in Figure. 3.

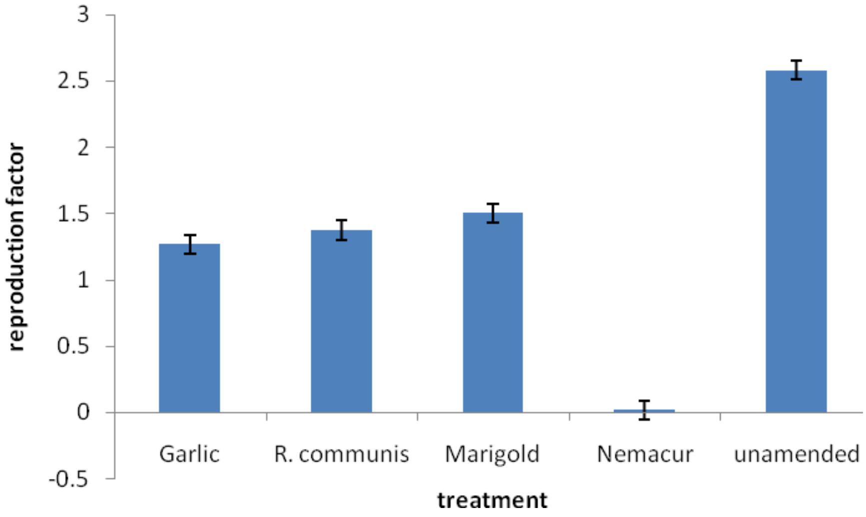

Fig. 3. Effect of treatments on reproduction

Discussion

The results in Figure 1 indicate that Nemacur inhibited RKN invasion and lessened pest activity that supposedly leads to the deprivation of plant nutrients, findings suggested by Ploeg and Phillips (2001), and reinforced by Luc *et al.* (2005). Nemacur's active ingredient, fenamiphos is a systemic organophosphorous chemical that offers contact activity and inhibits the enzyme cholinesterase (an enzyme important in the transmission of neuro-signals) and interferes with RKN nervous system (Bayer Environmental Science, 2003; Makhteshim Agricultural Industries Ltd, 2003). *R. communis* gave the highest dry mass and this backs Oduor-Owino (2003) who found it more toxic than marigold after 50 days. Adomako (2010) asserts that *R. communis* extract improves the weight of treated plants. It however had significantly lower results than the chemical control on both 42 DAI and 56 DAI, possibly because of the stage of development of the seeds, whose ricin and ricinin composition might vary with time.

Garlic gave insignificantly lower dry mass than *R. communis* and this may be attributed to allicin's less potency. Although Zasada *et al.* (2002) reported that allicin is not phytotoxic, these results suggest otherwise or that it may be innately less toxic than ricin and ricinin. According to Vijayalakshmi *et al.* (1999) high concentrations of garlic are phytotoxic and result in wilting of the crop. Marigold has been used extensively as a biological namaticide the world over for many years (Pluke *et al.*, 1999), but the results (Fig. 1) suggest that it may not be the most effective agent since the marigold treated plants gave massed of 151.7 g and 163.3 g compared to *R. communis*'s 178.5 g and 207.5 g, and garlic's 158.1 g and 184.9 g on 42 DAI and 56 DAI respectively. A possible explanation for this could be that the nematoxic substances present in marigold may have been lost via volatilization during air drying, since fresh marigold is usually ploughed into nematode infested soils as green manure. The use as green manure is documented by Hagan (1998). These results thus question marigold's reputation. The unamended control gave the expected results and the tomato plants weighed significantly less than all the other treatments. These results imply that unamended control plants were readily attacked by RKN as they exhibited stunted growth, in support of Gowen *et al.* (2005) and Guerena (2006).

On day 14, Nemacur was at par with *R. communis*, and both gave a unit root galling index. As time progressed, Nemacur was persistently effective and although it was not significantly different from *R. communis*, Nemacur performed most effectively during the duration of the experiment. This therefore suggests *R. communis* to be the most effective root gall suppressant of the three botanicals under observation, results corroborating McSorely (1999) and Katooli *et al*. (2010). Abid (2000) also observed the gall inhibiting property of aqueous *R. communis* extracts. *R. communis* must thus be toxic to RKN and may be effectively applied in the seedbed and nursery in place of synthetic chemicals especially in the early days as the potency decreased with time. Aqueous marigold extract was not significantly different from Nemacur during the first 28 days and thus marigold can also be used in place of the Nemacur and related chemicals especially during this period. Although marigold becomes less suppressing to galls than Nemacur and *R. communis* between days 42 and day 60, it was a better alterative than no treatment at all, and marigold lowered galling by 2.1 times 60 DAI. The decrease in RKN activity may be attributed to marigold's pungency which may be deterrent to RKN, or to the nematode antagonistic compounds suggested by Chitwood (2002).

Garlic's significant loss in effectiveness between 14 DAI and 28 DAI may attributed to its nemastatic properties (Perry *et al.*, 2009), which either are diluted with irrigation water or are biodegraded during the period under observation. Otipa *et al*. (2003) noted this moderate resistance of garlic to galling. The unusual trend between garlic and marigold between day 14 and day 60 is however difficult to explain, making it difficult to choose between the two since they only significantly differ from each other on day 56 and day 60. A cost-benefit analysis in this situation may rule out the use of garlic on a large scale since it is commercially grown and has to be bought, unlike marigold, which occurs naturally and as a common weed. It must be emphasized that on all days up to 60 DAI all treated plants developed significantly less galls than the unamended control. Nemacur was significantly the most effective treatment at lowering RKN reproduction as is shown by the least increase in RKN population in 60 DAI. This property makes this organophosphate the farmers' choice. Of the three plant extracts under study, anomalies from gall suppression and dry mass increase were observed. Aqueous garlic extract was the most effective botanical and one likely explanation for this could be that allicin has RKN growth retarding properties especially towards the J2 larval stage. Gupta and Sharma (1993) report that allicin is potent to RKN eggs and larvae, and Fadzirayi *et al.* (2010) state that garlic disrupts nematode mobility, feeding and reproduction, the latter being a plausible explanation for the low reproduction rate.

Although *R. communis* extract gave rise to a significantly higher RKN population than garlic, this figure (1.375) is not significantly different from both garlic (1.267) and marigold (1.503). Whilst *R. communis* proved more effective in increasing the dry matter (Fig. 1) and in gall reduction (Fig. 2), it was not the most effective natural treatment in reducing the RKN population 60 DAI. This somehow refutes assertions by Abid (1996), McSorely (1999), Mostafa (2000), Ahmed and Galip (2007) and Mafeo and Mashela (2010) that *R. communis* seed extract inhibits RKN egg hatch and development and kills J2 larva. Marigold was the least effective of the three botanicals but reduced galling by 58.3% when compared to the unamended control. These findings counter Steiner (in Inderjit and Mukerji, 2006) and Kaþkavalci (2009) who report that marigolds do not reduce nematode reproduction. Kreuger *et al.* (2007) affirm results obtained from marigold and explain that the alpha-terthienyls in *T. erecta* inhibit RKN juvenile development to maturity. Marigold may remain the preferred source of botanic nematicides over garlic because of culture, marigold's availability and cost.

The insignificant difference of *R. communis* in reducing galling over garlic and marigold implies that it can substitute either plant. *R. communis* however takes preference to marigold due to the extent to which it suppresses RKN reproduction. Oduor-Owino (2003) found *R. communis* to be more effective than marigold, and Gupta and Sharma (1993) reported garlic's larvicidal properties, although this was greater in essential oil form than in dried and crushed bulbs. This study thus presents the potential of crushed garlic in reducing nematode populations.

Conclusions

Nemacur is the most effectively reduced RKN reproduction and gave the highest increase in dry matter. The nematicide suppressed root knot to the greatest extent, *R. communis* was a better gall suppressant and dry mass promoter than garlic and marigold, garlic most effectively reduced the reproduction ratio of RKN, and, marigold was least effective, in support of the alternative hypothesis, marigold, *R. communis*, and garlic can be effectively used to control nematodes. These findings are inconclusive.

Recommendations

The experiment could improve to get more reflective results by increasing the sample size and taking recordings weekly, and by extending its duration to harvest. Conducting concurrent ovicidal and larvicidal laboratory tests to evaluate the effectiveness of the these botanicals could give a picture of the results likely to be encountered, provide a fair basis for comparison, and identify the most vulnerable stages of life cycle. Factorial laboratory tests should be conducted to establish optimum extract dilution levels. Nematicidal properties of more weeds and common plants should be investigated. Interactions of nematodes with microbes otherwise killed in sterilized soil need be established. Simultaneous tests should be conducted in the field and in the greenhouse. Tests should be carried out in artificially inoculated, naturally infested and sterile sets of soil. The range of plant parts at different stages of development should also be studied to establish their nematotoxicity. Comparing aqueous and organic solvent based extracts may increase understanding of the properties of the botanicals. Residue analysis via toxicological tests may determine persistence of the plant extracts in the environment. Soil pH changes must be investigated.

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