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NEMATICIDAL EFFECTS OF BRASSICA FORMULATIONS AGAINST ROOT KNOT NEMATODES (*Meloidogyne javanica*) IN TOMATOES (*Solanum lycopersicum* L.)

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A B S T R A C T

Biofumigation, the practice of growing plants with high levels of glucosinolates, can be used in controlling soilborne pests including nematodes. However, the best formulation amongst the brassicas that can be used for biofumigation is not documented. A glasshouse trial aimed at determining the efficacy of different glucosinolate sources (rape, radish, mustard and cabbage) and brassica formulations (cake, extract and unmacerated) in suppressing *Meloidogyne javanica* population on tomatoes, was carried out. The trial was laid out as a 4×3 factorial experiment in a completely randomised design (CRD). The results showed that mustard was the most effective brassica in controlling nematodes (50.89), while cabbage, radish and rape significantly reduced *M. javanica* population when compared to the untreated control. The mustard-cake was the best biofumigant formulation in reducing nematode population (36), and this was as effective as fenamiphos (34.44). The study recommends the use of a mustard cake formulation for root knot nematode control in tomato production.

Keywords: Isothiocyanates, mustard, biofumigation, glucosinolates, nematode control.

INTRODUCTION

Root knot nematodes (*Meloidogyne spp*) attack over 2000 plant species including most cultivated plants causing serious yield losses (Agrios, 2005). *Meloidogyne javanica* is a serious tomato (*Solanum lycopersicum* L.) pest in tropical and subtropical climates, especially on sandy soils. According to Seif *et al.* (2003), this nematode can cause up to 50% yield reduction in tomatoes depending on cultivar grown and season of production. Losses are attributed to nematodes feeding solely on the crop and/or secondary infections by bacterial and fungal pathogens brought thereabout.

To curb the effects of nematodes in agriculture, different nematicides have been used. Nematicides like aldicarb, ethylene dibromide, metham sodium, methyl bromide, methyl iodide and fenamiphos have proven to be effective against nematodes and other soil borne diseases (Gilreath and Santos, 2008; Zasada *et al*., 2010). However, some of them have negative impacts on human health and the environment as they deplete the ozone layer and kill non-target beneficial organisms (Rosskopf *et al*., 2005). The negative effects associated with synthetic nematicides and the growing need for organic produce aroused interest in the development and use of more environmentally-friendly nematode control measures (Dobson *et al*., 2002; Duniway, 2002). One such control measure is biofumigation.

The principle of biofumigation is based on the use of plants with high glucosinolate levels to control pests (Angus *et al.,* 1994; Borek *et al*., 1996; Morra and Kirkegaard, 2002; Morra, 2004). Glucosinolates are naturally occurring sulphur compounds that occur in plants as secondary metabolites. They occur in Brassicaceae, Caricaceae, Moringaceae, Salvadoraceae and Tropaeolaceae families (van Dam *et al*., 2009), but the Brassicaceae has the highest amount of glucosinolates in their tissues (Zukalova and Vasak, 2002). The glucosinolates are inactive in the plant. However, upon mechanical or biochemical disruption of brassica tissues, glucosinolates and the enzyme myrosinase found in different parts of the cell come into

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contact. The enzymatic hydrolysis of glucosinolates leads to the formation of bioactive compounds like nitriles, epithionitriles, thiocyanates and isothiocyanates (Fahey *et al*., 2001). The isothiocyanates (ITCs) are largely responsible for pest, pathogen and weed suppressive effects observed after soil-incorporation of brassica tissues (Brown and Morra, 1995; Bello *et al.,* 2001; Peterson *et al*., 2001). However, the conversion of glucosinolates to ITCs may be very low and the glucosinolate content of tissues does not necessarily predict its pest suppressive activity. This was particularly true in studies involving nematodes (Matthiessen and Kirkegaard, 2006).

Although biofumigation has proven to be an effective pest management technique (Bello *et al*., 2001; Peterson *et al*., 2001), there is still lack of knowledge and information on the biofumigant formulation that farmers can use for optimum nematode control. With methyl bromide being phased out worldwide under the "Montreal Protocol of 1987 and Its Amendments" by the year 2015 (USDA, 1999; UNEP, 2004), biofumigation offers a relatively new and ecologically, socially and economically viable alternative. Biofumigation is readily compatible with existing agricultural practices, technologies and has less demand for equipment and expertise. Its adoption will ultimately increase returns on farmers as it is cheaper than applying synthetic nematicides. Thorough knowledge and understanding of the best biofumigant formulations will increase the benefits accrued from this nematode management technique as the formulation that increase myrosinase and glucosinolate contact to yield maximum quantities of ITCs will be utilized. This study evaluated the nematicidal effects of different brassicas (glucosinolate sources) and brassica formulations against *M. javanica* in tomatoes.

MATERIALS AND METHODS

Study Areas: Four brassica species namely cabbage (*Brassica oleracea* var. *oleracea*) (cv. Drumhead), rape (*B. napus*) (cv. Giant English), mustard (*B. juncea*) and radish (*Rhaphanus sativus*) were grown at the Henderson Research Station (latitude 17°35′S; longitude 30° 58′E; altitude: 1300 metres above sea level) in Zimbabwe. The area receives 855 mm rainfall annually and is characterised by light-brown, medium-grained, clay loam soils with pH 4.5 ($CaCl_2$). The brassicas were grown in the summer of the 2012/2013 season (January to March) when mean temperature was 21°C and 161 mm of rainfall was received. They were harvested 70 days after planting and transported to the Plant Protection Research Institute at the Department of Research and Specialist Services in Harare (latitude 17°51′50″S; longitude 31°1′47″E; 1503 metres above sea level) where a pot experiment was conducted under glasshouse conditions.

Experimental Procedure

Experimental design: The glasshouse trial was laid as a 4×3 factorial experiment in a completely randomised design replicated three times. The two factors were glucosinolate sources (cabbage, mustard, rape and radish) and brassica formulation (cake, extract and unmacerated tissue). The treatment combinations are shown in Table 1.

Table 1. Treatment combinations of glucosinolate sources and brassica formulations.

Glucosinolate source	Brassica Formulation		
	Unmacerated (U)	Extract (E)	Cake (C)
Rape (R)	R/U	R/E	R/C
Radish (Ra)	Ra/U	Ra/E	C/Ra
Mustard (M)	M/U	M/E	M/C
Cabbage (Ca)	Ca/U	Ca/E	Ca/C

Formulation and incorporation of brassicas: At harvesting, the brassicas were washed with water to remove excess soil particles from roots. For the extract formulation (E), one kilogram of brassica material was ground in 200ml of 20% alcohol (Elske, 2004). To produce the cake formulation (C), brassica tissues were pounded in a mortar and pestle. The unmacerated tissue formulation (U) was made up of brassica tissues that were incorporated whole into the soil. The different formulations were incorporated at 3kg/10kg of soil (10 t/ha). The nematicide Fenamiphos 40EC (fenamiphos) was applied as a positive control treatment while untreated pots acted as negative controls. All the pots were then covered with black polythene plastic for 14 days to enhance the decomposition of incorporated brassicas and also prevent loss of ITCs by volatilisation.

Two weeks after brassica incorporation, each pot was inoculated with 5 000 eggs of *M. javanica* collected from the Tobacco Research Board. Thereafter, the pots were covered again with polyethylene plastic for five days.

Raising tomato plants: Tomato (cv. Rhodade) seedlings were raised using the float tray method in a glasshouse on pine bark media (pH 6.5 $CaCl_2$). Hydrofert (4.5% N: 2.1% P_2O_5: 4.7% K_2O: 7.5% S) was applied as a basal fertilizer at a rate of 5 litres/1000 litres water. General agronomic practices were implemented to raise the seedlings. A week prior to transplanting, the seedlings were hardened by adding water into the float bed. Transplanting was done at four weeks after seedling emergence, with healthy seedlings planted in 10 kilogram plastic pots inoculated with *M javanica* eggs after brassica incorporation. Compound C fertilizer (7%N: 21%P_2O_5: 8%K_2O: 8%S) was used for basal dressing at 600 kg/ha and ammonium nitrate (34.5% N) was applied as a top dress fertilizer at 200 kg/ha. Ridomil Gold (64% mancozeb + 8% metalaxyl) was used to control diseases and Carbaryl 85 WP (carbaryl) was used to control insect pests.

Data collection and Statistical Analysis: Plant growth was monitored from transplanting to the sixth week after transplanting at which temporal data on nematode population were collected. For nematode population determination, a soil sample of 200g was collected from the root zone of each treatment. Extraction of nematodes was done using the Baermann funnel method at the Tobacco Research Board. One millilitre of the nematode extract was used to determine the nematode population by counting under a light microscope.

Genstat Discovery Edition was used for data analysis. Analysis of Variance was used to determine the effects of the treatments on biomass and nematode population. The Least Significant Difference (LSD) at p = 0.05 was used to separate means where treatment effects were significant. Graphs and tables were used to present the research findings.

RESULTS AND DISCUSSION

Effect of glucosinolate sources on nematode population

Glucosinolate source had a significant effect ($p<0.001$) on nematode population. The untreated control had the highest nematode population of 120.83 nematodes per 200g soil. Of the brassicas, mustard was the most effective in reducing the nematode population (50.89). There were no significant differences between the capacities of Fenamiphos 40EC and mustard in reducing nematode population. There were also no differences amongst the other brassicas (cabbage, radish and radish) in their capacities to reduce nematode population (Table 2).

Table 2. Effect of glucosinolate sources on mean nematode population.

Glucosinolate source	Nematode population (200g soil)
Cabbage	96.56b
Mustard	50.89a
Radish	103.22b
Rape	101.12b
Untreated	120.83c
Fenamiphos 40 EC	46.44a
Standard error	3.47
LSD	8.32

Means followed by the same letter are not significantly different at 5% level of significance.

That mustard was the most effective in reducing the nematode population was because of the high concentration of glucosinolates in the plant. According to Zukalova and Vasak (2002), *B juncea* contains a high concentration of 2-propenylglucosinolate compared to other brassicas. In addition to high glucosinolate content, it also has high tissue water content. When incorporated in the soil, the high water content enhances the hydrolysis of the glucosinolates by the enzyme myrosinase to the volatile isothiocyanates which reduce nematode movement, invasion, feeding and consequently, rate of development and reproduction.

The fact that there were no differences in the nematode-suppressing abilities of the three other brassicas (cabbage, rape and radish) can be attributed to similar quantities of glucosinolates in the tissues of these brassicas. It should be noted that the three brassicas also significantly suppressed nematodes in the soil after their incorporation. This is an indication of good nematode control especially compared to untreated pots which had a mean population of 120.83 nematodes. *B. napus* and *B oleracea var. oleracea* have 3-butenylglucosinolate as their main glucosinolate (van Dam *et al*., 2009). They are relatively effective in controlling nematodes and other soil borne pathogens, and have been used as green manure and cover crops (Sarwar *et al*., 1998; Clark, 2007; Kopsell and Sams, 2010; Szczglowska *et al*., 2011).

Effect of brassica formulation on nematode population

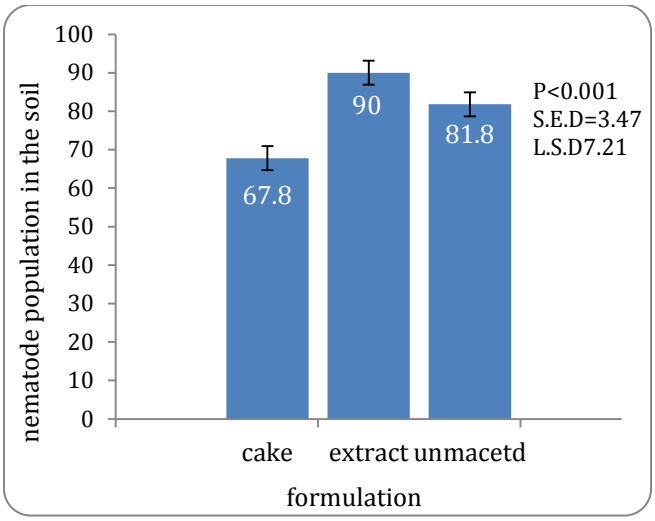

Figure 1. Effect of brassica formulation on nematode population.

Brassica formulation had a significant effect ($p<0.001$) on nematode population. The cake formulation was most effective, with a mean nematode population of 67.8 nematodes, while the extract formulation was least effective, with a mean of 90 nematodes per 200g soil (Figure 1).

The low effectiveness of the extract formulation could be attributed to high rate of isothiocyanate loss from the soil leading to insignificant amount of isothiocyanates available to suppress nematode reproduction. According to Kumar (2005), the use of solvents like alcohol and ammonia enhances the extraction of glucosinolates, as alcohol and ammonia ions increase the polarity and ion intensity of water. This can lead to the release of large amounts of glucosinolates in a short period of time. Thus, the effect of the isothiocyanates on pests and diseases is short lived when the biofumigant is in the form of an extract (Angus *et al*., 1994).

Interaction of Glucosinolate source and Brassica formulation on nematode population

Table 3. Mean Nematode Population (per 200g soil sample) of Treatment Combination of Brassica formulation and Glucosinolate Sources.

Brassica (Glucosinolate source)	Treatment (Formulation)	Mean Nematode Population(per 200g soil sample)	Standard Error
Cabbage	Cake	98.33^{de}	6.010
	Extract	100.667^{de}	6.010
	Unmacerated	90.667^{cd}	6.010
Mustard	Cake	36^{a}	6.010
	Extract	56.667^{b}	6.010
	Unmacerate	90.667^{cd}	6.010
Radish	Cake	86.667^{cd}	6.010
	Extract	115.667^{f}	6.010
	Unmacerated	107.337^{ef}	6.010
Rape	Cake	80.353^{c}	6.010
	Extract	117.00^{f}	6.010
	Unmacerated	106.00^{ef}	6.010
Control	Fenamiphos	34.44^{a}	3.388
	Untreated	135.833^{g}	3.388

*Means with different superscripts are significantly different at 5% significance level.

There was interaction between glucosinolate source and formulation type ($p<0.001$) on nematode population (Table 3). Generally, *M. javanica* population was highest in unmacerated brassica formulations, while the brassica cake formulations were the most effective in suppressing nematode populations. Mustard formulated as a cake was most effective in controlling nematodes, with a mean nematode population of 36 per 200g of soil. This was similar to fenamiphos, the positive control. The mustard extract was second to the mustard cake. As was noted by Angus *et al.* (1994), effective biofumigation relies on maximum glucosinolate hydrolysis to liberate isothiocyanate concentrations toxic to the pathogen. Thus, the level of tissue disruption before brassica incorporation is a factor affecting the effectiveness of biofumigation.

The least effective brassica-formulations were radish and rape extracts and unmacerated tissues (Table 3). As previously noted, rape and radish have low

concentrations of glucosinolates. When these brassicas were formulated as extracts, the few quantities of glucosinolates in them were readily lost from the soil, and so could not suppress nematodes. Also, when unmacerated, the brassicas take longer to degrade and release isothiocynates to suppress nematodes. So the benefits of incorporating unmacerated brassicas are likely to be realized after a longer time period because of the slow release of isothicyanates.

CONCLUSIONS AND RECOMMENDATIONS

Mustard was the most effective brassica in suppressing *M. javanica* population in tomatoes. Mustard formulated as a cake was as effective as Fenamiphos 40EC in controlling nematodes. It is recommended that when used for biofumigation, mustard must be formulated as cake to effectively control root knot nematodes. The other brassicas are also effective in suppressing root knot nematodes in tomatoes, and may be used if mustard is not available.

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