Screening indigenous nematicidal plants in Zimbabwe against Meloidogyne javanica

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Abstract

Plant parasitic nematodes are one of the major economically important pests of Solanaceous plants in many regions of the world including Zimbabwe, with Meloidogyne javanica, (rootknot nematode) being considered the most destructive. Recent prohibition of main soil chemical fumigants for the control of Meloidogyne javanica has prompted for the search of other alternatives to manage root knot nematodes in the horticultural industry. The present research explored plants with nematicidal properties against root knot nematode, M. javanica. Depending on plant medicinal value, leaves or bark or roots of 19 indigenous medicinal plants were screened invivo on highly susceptible tomato variety (Red khaki). Ten plant extracts caused more than 50% gall reduction during screening under acetone extraction method. Application of botanicals was found to significantly ($p \leq 0.05$) reduce the formation of root galls on tomato plants. It was concluded from this study that plant extracts are potential botanical nematicides as they have ability to suppress the attack of tomato plants by phyto- nematodes and they can be used as a safe and resourceful control strategy, which can complement other control tools.

Key words: botanicals, indigenous, Meloidogyne javanica, root knot nematodes, tomato.

1. Introduction

Plant parasitic nematodes are important yield reducing pests of most crops in the world. They typically feed and destroy plant root tissues, tubers and rhizomes (Walker et al., 2002). This contributes to annual losses of US$78 to $100 billion in agricultural production worldwide (Cetintas and Yarba, 2010). Few species of nematodes are the major contributors to these economical losses. Root knot nematodes are the major yield reducing agents, in most crops. Principally, the detrimental effect of the nematodes was successfully controlled through the use of synthetic nematicides and broad spectrum chemical soil fumigants. However, the high costs, non-availability at the time of need and the hazards they pose as environmental pollutants discourage most potential users (Elbadri et al., 2008).

One of possible alternative is the utilization of pesticidal and nematicidal plants (Wiratno et al., 2009). Since they are environmentally friendly, toxicologically safe and selective to beneficial organisms (Dawar et al., 2008). Nematicidal plant extracts are reported to contain volatile essential oils formed as secondary plant compounds. These chemical volatiles have functions in chemical defence, acting as insecticides and acaricides (Bakali et al., 2008; Yadav et al, 2008; Flamini., 2003 and Karamanoli., 2002).

In the present research medicinal indigenous plants were screened against Meloidogyne javanica in the glasshouse.

2. Materials and methods

2.1 Mass culturing of Meloidogyne javanica

In order to build adequate nematode inocula for the entire trial, 2000 eggs, previously maintained on tomato plants (red khaki), were mass bulked by sub-culturing them on fresh one week old red khaki seedlings under glasshouse conditions. Methylbromide sterilized soil, consisting of 1:2 sandy loam was used as the planting medium. Each planting pot contained one seedling and approximately 2.5 kg of sandy loam mixture.

2.2 Extraction of egg masses from galled tomato roots

Egg extraction from galled roots was done according to the protocol of Hussey and Barker (1973), with slight modifications. Briefly, egg sacs from galled roots of sampled tomato plants were obtained by teasing washed roots in water and recovering the egg sacs on a 60 mm mesh sieve. Following the addition of egg sacs in 500 ml of 1% sodium hypochlorite (20% commercial bleach), the mixture was macerated in an electric warring blender for 40 seconds. The root debri and egg suspension was then passed through a 100 and 400 mm mesh sieves over a container. Material retained on 400 mesh sieve (37mm) was washed several times with distilled water to collect the filtrate for further repeated sieving as described by Mecheal et al., 1973

2.3 Counting of Meloidogyne javanica eggs

Meloidogyne javanica eggs used in this experiment were carried in the aqueous suspension. Thus for determining the number of eggs, a 10ml aliquot from the tubes of each sample was placed on counting dish. After which a stereoscopic binocular microscope was used to count eggs in all squares. Counting was done four times and mean number of eggs were then determined per ml.

2.4 Agronomic practice

In the glasshouse, the tomato plants were irrigated twice a day. Nutrifol (18-18-18 + trace elements was applied at a rate of 20ml/5l can once a week. Spraying of the plants for red spider and white fly prevention was done to reduce bias.

2.5 Screening of medicinal plants against Meloidogyne javanica in the glasshouse

Nineteen indigenous plants were screened. One week old tomato seedlings were transferred into 5cm* 5cm pots that were filled with fumigated 150grams soil. After 3 weeks, the tomato seedlings were transferred into 12 cm*12.5cm pots, with fumigated 1kg soil. Roots or bark or leaves of Piliostigma thonningii, Harungana madagascariensis, Clausena anisata, Ximenia caffra, Baikiae plurijuga, Dicoma animal, Lannea discolor, Rauvolfia caffra, Flueggea virosa, Boscia salieifolia, Sclerocarya spp caffra, Burkea Africana, Pouzoizia mixta, Cissus cornifolia, Carissa bispinosa, Lannea discolour, Dovyalis caffra, Opuntia maegacantha, and Moringa olifeira were collected from Shurugwi ($19^0 40^{'} 0$" S, $30^0 00^{'} 0$" E) and Lower Gweru districts ($19^0 14^{'} 0$" S, $29^0 15^{'} 0$" E).

Plant samples were air dried in the laboratory at room temperature (±25^{o}C) for 3 weeks. After which there were initially ground into course powder utilizing pistil and mortar, the course powder was later pulverised into fine powder by electric grinding mill. The pulverized plant material was then be sieved through a 500 µm pore mesh to obtain uniform particle size. Five grams of each powdered sample was extracted with 100ml of di-ionised water or acetone. The samples were left in an extraction solvent for 24 hours. Acetone extraction method was done in glass jars that were tightly closed to avoid acetone volatilization. The extract solution was filtered using Whatman filter paper 1 into container beakers. For the water extraction method, the solutions were ready for use while acetone glass jars were opened to allow acetone evaporation before 100ml distilled water was added for the solution to be ready for use. 15ml of nematode suspension containing 3000 M. javanica eggs was added to 3 week old tomato seedlings. The nematode inocula were pipetted onto four holes created around the tomato seedling.

Immediately after nematode inoculation, a total of 114 pots were treated with botanicals at a rate of 30ml/pot using a measuring cylinder. EC formulation of Fenamiphos (Nemacur 400g/l, Arysta life science South Africa) at a rate of 30ml per plant served as positive control, while untreated

pots were a negative check. The pots were then incubated on glasshouse benches; temperature was maintained at 27±2°C. Tap water was used for irrigation.

The glasshouse experiment was terminated after 50 days by uprooting tomato plants. The number of galls on the root system was counted manually aided by hand held lens. Galling index for water extracted compounds was done as described by Daulton (1961) gall rating scale which ranks nematode damage on a scale of (0-8), due to excessive galling, while the galling assessment according Bridge and Page (1980) was used for acetone extracted botanicals, since the galling was less excessive.

2.6 Statistical analysis

The experiment was laid in a completely randomised design with three replications. Root galling index data was collected 50 days post-transplanting GenStat 9th edition was used to analyze the results and treatment means were separated by Duncan multiple range test.

3. Results

3.1 Effects of plant extracts on tomato root galling

Application of both acetone and water extracted solutions resulted in reduction in M. javanica galling, increase and decrease in plant growth and plant phytotoxicity respectively.

Table 1 Effects of plant extracts on tomato root galling based on Daulton galling index for water extraction method

Plant sources	Galling	% gall reduction
Piliostigma thonningii bark	0^{a}	100
Fenamiphos (positive control)	0^{a}	100
Clausena anisata leaves	1^{ab}	86
Clausena anisata bark	2^{bc}	72
Flueggea virosa root	2.3^{bcd}	68
Harungana madagascariensis bark	2.7^{cd}	64
Dicoma animal root	3^{cd}	59
Ximenia caffra root	3.3^{cde}	55
Baikiae plurijuga bark	3.7^{cde}	50
Sclerocarya spp caffra bark	3.7^{cde}	50
Lannea discolor bark	4^{def}	45
Rauvolfia caffra bark	4^{def}	45
Boscia salieifolia bark	4^{defg}	45

Moringa olifeira leaves	4^{defg}	45
Dovyalis caffra root	5^{efgh}	32
Carissa bispinosa root	5.7^{fhi}	23
Cissus cornifolia root	6^{hi}	18
Burkea Africana bark	6.3^{hi}	14
Pouzoizia mixta root	6.3^{hi}	14
Opuntia maegacantha root	6.3^{hi}	14
Lannea discolour bark	7^{i}	4
Distilled water (negative control)	7.3^{i}	0
F prob	<.001	
Lsd	1.52	
CV%	23.2	

Means followed by a different superscript letter are significantly different at $P < 0.05$. Values are average of 3 replicates of non-transformed value.

Application of water extracted botanical extracts to nematode inoculated tomato plants significantly ($p \leq 0.05$) reduced the formation of root knot galls over negative control (Table 1). Bark of Piliostigma thonningii proved the most effective and reduced galling over negative control by 100% at 5g/100ml similar to Fenamiphos (synthetic nematicide), followed by Clausena anisata leaves that reduced galling rate by 86%. Eight of the indigenous medicinal plants reduced galling by at least 50% based on water (aqueous) extraction method. These include P. thonningii, C. anisata, C. anisata, F. virosa, H.madagascariensis, D. animal, X. caffra, B. plurijug and, S. spp caffra (Table 1). The remaining botanical extracts that included L. discolor, B. salieifolia, M. olifeira, D. caffra, C. bispinosa, C. cornifolia, B. Africana, P. mixta, O. maegacantha and L. discolour reduced galling by less than 50% (Table 1).

Table 2 Effects of plant extracts on tomato root galling based on Bridge and Page galling index for Acetone extraction method

Plant Sources	Galling	% gall reduction
Piliostigma thonningii bark	0^{a}	100
Fenamiphos	0^{a}	100
Rauvolfia caffra bark	0.5^{abc}	90
Harungana madagascariensis bark	1^{abcd}	80
Dicoma animal root	1^{abcd}	80
Sclerocarya spp caffra bark	1.3^{cde}	73
Burkea Africana bark	1.3^{acde}	73
Moringa olifeira leaves	1.3^{abcde}	73
Clausena anisata leaves	1.7^{cdef}	67
Ximenia caffra root	1.7^{cdef}	67
Boscia salieifolia bark	1.7^{cdef}	67

Pouzoizia mixta root	1.7^{cdef}	67
Dovyalis caffra root	2^{defg}	60
Cissus cornifolia root	2.3^{defg}	53
Carissa bispinosa root	2.3^{defg}	53
Opuntia maegacantha root	2.3^{defg}	53
Baikiae plurijuga bark	2.7^{efg}	47
Lannea discolour bark	3^{fg}	40
Flueggea virosa root	3^{fg}	40
Clausena anisata bark	3.3^{g}	33
Distilled water	5^{h}	0
F prob	<.001	
Lsd	1.18	
CV%	37.8	

Means followed by a different superscript letter are significantly different at $P < 0.05$. Values are average of 3 replicates of non-transformed value

Application of acetone extracted botanical extracts to nematode inoculated tomato plants significantly ($p \leq 0.05$) reduced the formation of root knot galls over negative control (distilled water) (Table 2). Bark of P. thonningii proved most effective and reduced galling over negative control by 100% at 5g/100ml comparing with Fenamiphos (synthetic nematicide), this was followed by R. caffra with a galling reduction rate of 90%, with L. discolour, F. virosa and C. anisata being the least effective acetone extracts. Fifteen of the indigenous medicinal plants reduced galling by at least more than 50% using acetone extraction method. These include P. thonningii, C. anisata, H. madagascariensis, D. animala, X. caffra, Sclerocarya spp caffra, B. salieifolia, M.olifeira, D. caffra, C. bispinosa, C. cornifolia, B. Africana, P. mixta and O. maegacantha (Table 2). Only four of the acetone extracted extracts reduced galling by less than 50%. They were L. discolor, F. virosa, B. plurijuga and C. anisata (Table 2).

4. Discussion and Conclusion

Results in the screening of plant extracts using acetone and water showed that, from the nineteen extracted indigenous plants used, most of them were effective giving more than 50% gall formation suppression under acetone extraction method and less than nine of them gave the same effect of gall reduction under water extraction method. This may be attributed to the variation of compounds, total phenolic content (mg GAE/g), flavonoid content (mg CTE/g), proanthocyanidin (%LCE/g DM) and active ingredients concentrations extracted by each solvent. Zasada et al., (2010) alluded to the fact that water extraction method resulted in extracting polar compounds only, while other chemical solvents such as acetone solvent extracted both polar and non polar compounds. In this experiment, the indigenous medicinal plants extracts significantly reduced the root knot nematode, especially where acetone was used.

This further explain that a solvent capable of extracting both polar and non polar has an edge over water extraction method as evidenced by fifteen versus nine plants that had 50% M. javanica root damage (gall index) reduction in acetone and water methods respectively. The current observations agree with those of Zasada et al., (2010). Abid, (1995) and Sellami and Moufarrah (1994) reported the effectiveness of various plant extract used in suppressing both plant root and soil population build up of nematode and consequent reduction in the root damage in the tested crops. The findings from the current study confirmed with Pandy (1990) and Mani and Chitra (1998) who reported the toxicity of medicinal and aromatic plant extracts on M. incognita which is closely related species to M. javanica used in the current trial. Other researchers (Puri, 1999; Oka et al., 2000; Afouda et al., 2008) have reported successes in using various plant extracts in nematode management.

The observed nematoxic effects of indigenous medicinal plants extract can be attributed to the presence of nematicidal saponins, flavonoids, tepernoids, acids and tannins that were seriously injurious to Meloidogyne javanica eggs. Saponins, flavonoids, tepernoids, acids and tannins were present in many botanical nematicides and are probably responsible for the toxicity of indigenous medicinal plants extracts to M. javanica. The compounds provide plant defense and provide resistance against nematode attack because flavonoids produce auxins with allellopathy effects. Flavonoids biosynthesis to isoflavonoid phytoalexins that confer resistance to nematodes.phenolic hydroxyl group is also known to negatively affect nematodes activities. This observation agrees with Alam et al., (1989) who reported the effectiveness of flavonoid, tannin and saponin in reducing M. javanica population and enhancing egg-hatch inhibition of M. javanica in their various experiments. This type of control on plant parasitic nematode had been reported by many workers including Oyedunmade et al., (2001, 2004) and Oyedunmade (2004).

The negative control which recorded the highest percentage galling maybe due to the fact that the normal life cycle and activities of the nematode were not interfered with the way the plants extracts did. Variation of gall reduction in the screening process can be attributed to aqueous extracts which resemble chemistry of soil incorporated plant residues.

It was concluded from this study that extractive yield was more in acetone than in water and plant extracts are potential botanical nematicides as they have ability to suppress the attack of plant by root knot nematodes and they can be used as an effective, efficient, cheap and feasible control which can be an alternate to synthetic nematicides. The use of P. thonningii, D. animala and M. madagascariensis as medicinal indigenous plants in root-knot nematode control as they all reduced galling by over 50% at mixing rates of 2g/100ml for P. thonningii and 5g/100ml for D. animala and M. madagascariensis from water/acetone (w/v).

Table 3 Compound profiling of plant species

Plant species	Total phenolic	Flavonoid content	Proanthocyanidin

	content (mg GAE/g)	(mg CTE/g)	(%LCE/g DM)
Lannea discolour	25.49 ± 2.68	2.97 ± 0.59	3.23 ± 0.03
Clausena anisata	29.50 ± 1.26	0.28 ± 0.03	1.96 ± 0.23
Dovyalis caffra	10.92 ± 0.55	0.15 ± 0.01	0.74 ± 0.08
Sclerocarya spp caffra	35.29 ± 1.19	3.12 ± 0.15	1.69 ± 0.06
Pouzoizia mixta	3.85 ± 0.26	0.10 ± 0.01	0.97 ± 0.69
Flueggea virosa	21.84 ± 0.73	0.82 ± 0.04	1.66 ± 0.02
Opuntia maegacantha	3.43 ± 0.28	0.03 ± 0.00	0.00 ± 0.00
Cissus cornifolia	25.07 ± 1.53	0.98 ± 0.06	1.35 ± 0.04
Moringa olifeira	10.55 ± 1.08	0.13 ± 0.01	0.70 ± 0.22
Carissa bispinosa	5.94 ± 0.28	0.05 ± 0.00	0.91 ± .041
Ximenia caffra	36.80 ± 0.21	5.16 ± 0.20	0.91 ± 0.41
Clausena anisata	14.24 ± 0.92	0.07 ± 0.04	3.04 ± 0.02
Rauvolfia caffra	6.22 ± 0.50	0.10 ± 0.01	0.07 ± 0.01
Boscia salieifolia	22.34 ± 1.04	1.33 ± 0.14	0.30 ± 0.05
Lannea discolor	21.69 ± 1.40	2.18 ± 0.03	1.25 ± 0.06
Piliostigma thonningi	35.69 ± 1.09	5.49 ± 0.38	3.16 ± 0.07
Harungana madagascariensis	20.33 ± 0.64	2.09 ± 0.11	3.43 ± 0.02
Burkea Africana	28.52 ± 2.31	2.43 ± 0.29	3.31 ± 0.03
Baikiae plurijuga	36.94 ± 0.10	4.14 ± 0.24	3.25 ± 0.02
Dicoma animala	4.04 ± 0.18	0.07 ± 0.03	0.01 ± 0.00

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