EVALUATION OF PLANT EXTRACTS ON EGG HATCHING AND JUVENILE MORTALITY OF FRENCH BEAN ROOT-KNOT NEMATODES

Njenga JK*[1], Gathungu GK[1] and Mbaka JN[2]

[1]Chuka University, P.O. Box 109-60400, Chuka, Kenya
[2]Kenya Agricultural and Livestock Research Organization, Kandara P.O. Box 220-01000, Thika, Kenya
*Corresponding author email: *joshuakinene72@gmail.com*

Abstract

Nematodes are a major constraint to agricultural production worldwide. For sustainable food production, effective management of plant parasitic nematodes is essential. The assault on the environment through the use of synthetic agrochemicals and unreliable results from crop rotation systems has necessitated the search for sustainable, effective and environmentally acceptable nematode management options. Preliminary study was carried out at Chuka University Research Laboratories in 2017 to determine the presence of phytochemicals within the plants extracts of Neem, Tithonia and Tephrosia. Tests were done for presence of flavonoids, alkaloids, saponins and terpenoids which are effective against parasitic nematodes. Invitro experiment in a complete randomized design was established which involved exposing egg-masses and juveniles of French bean root-knot nematodes to ethanol extracts from fresh leaves of Neem, Tithonia, Tephrosia and Nimbecidine neem based product as a control. The LC50 value of each extract was determined by assessing the mortality of juveniles and hatching eggs (in the range of 5–95%) upto 7 days which depended on extract phytotoxicity. The data collected on egg hatching and juvenile mortality was subjected to analysis of variance and significantly different means were separated using Tukey's Studentized Range Test at *P*=0.05.Comparison between LC50 values of the extracts indicated that Neem, Tithonia and Tephrosia at 5 and 10 per cent concentration suppressed hatching eggs and increased juvenile mortality percentage. Neem and Tithonia were the most effective crude extracts on the mortality of juveniles at 80 and 73.67% respectively at 7th day. The study demonstrated that plant extracts has a promising potential that could be exploited in the management of root-knot nematodes in vegetable production systems.

Keywords: Neem, Tithonia, Tephrosia, Phytochemicals, French-beans, Nematodes, Phytotoxicity

Introduction

Root-knot nematodes (*Meloidogyne* spp.) are a major constraint to successful vegetable production all over the world, causing severe damage that leads to dramatic yield losses (Irene *et al*. 2017). Existing control measures involving applications of chemical nematicides are not viable in the medium to long term due to environmental concerns relating to toxic residues (Muhammad *et al*., 2017). There is therefore a need to develop alternative control options for integrated parasitic nematode management that will promote soil eco health and reduce parasitic nematode densities (Emmanuel *et al*., 2017).

Meloidogyne spp is a major problem in Frenchbeans productions in many French bean production systems in Kenya. Root-knot nematodes are small unsegmented worms and are mostly microscopic and they cause significant damage in almost all crops **(**Perry

et al., 2009). The Meloidogyne spp attacks over 140 species of more than 115 plant genera in 46 families (Patil *et al.,* 2017). Nematicides are recommended for the management of nematodes but they are highly toxic compounds that have very low LD 50 values **(**Odeyemi *et al*., 2013). In many parts of the world most of the nematicides are banned because of their harmful effect on human and environment. Moreover, in organic farming there should be alternative nematode control strategies, as chemical nematicides cannot be recommended (Siddiqui *et al*., 2017). Therefore, it has become an important issue to find alternative control strategies that can lead to effective nematode control and also assure the safety of the consumers of horticultural products.

Many plants are known to have nematicidal properties which may be utilized as organic amendments or bio-pesticides. Use of plant extracts on eggs and larvae to reduce the nematode population is promising in the crop protection against root-knot nematodes. Plant extracts having the nematicidal properties may also enhance the plant growth. Patil *et al*. (2017) tested the extracts of Neem for their effects on larval mortality of nematode and exhibited significant mortality against root-knot nematode. Oyinlola et *al*. (2017) reported that aqueous extracts from Tithonia and Tephrosia reduced root-knot nematode populations in the soil with corresponding increases in plant height, plant leaf and fruit yield. To contribute to an alternatives to synthetic control of nematodes, this study evaluated the nematicidal effects of crude botanical extracts on invitro control of French beans root-knot nematodes.

Material and Methods

Study site

In vitro experiments were conducted at Chuka University Research Laboratories situated approximately 186 Km from Nairobi along the Nairobi-Meru highway. The area lies at an approximately 0°19.9896′ South latitude, 37°38.7522′ East longitude and 1452 m elevation above the sea level.

Sourcing and preparation of leaf extracts

Fresh shoot leaves of *Tithonia diversifolia* and *Tephrosia purpurea* were sourced and collected from open fields around Chuka University. Commercial Neem leaf formulation (Nimbecidine) for comparison with extracted Neem was purchased from local Agricultural shops. Neem leaves were sourced from the coastal region where Neem trees grows extensively. Extracts were prepared as described by (Oloruntola *et al.*, 2017). The fresh leaves were air-dried under shade for 2 weeks then coarsely powdered with a mechanical grinder separately .200 grams of dried powder of each plant species were weighed and dissolved in 500 ml of 95% absolute ethanol in Erlenmeyer flask for elucidation.

After 24 hours of soaking the solutions were filtered through a layers of cheese-cloth gauze and Whitman's No. 2 -filter paper before the filtrates were subjected to evaporation using a rotary flash evaporator under reduced pressure at 60 ^{0}C for 60 minutes to concentrate the extract and remove the ethanol. Extracts were stored in airtight container in refrigerator below 10ºC.

2.3. Preparation of Test Concentrations

Concentrations were prepared following Muhammad *et al*. (2017) procedure. Concentration of 2.5% (25ml/L) 5% (50ml/L) and 10 %(100ml/L) were prepared separately by adding 2.5ml ,5ml and 10ml of the extract residuals with 5 mL of acetone to enhance dissolution and made up to 100mL by adding tap water. Nematode eggs were extracted from heavily infested galled French bean roots using the sodium hypoclorite method (NaOCl). Galled roots were collected from highly

infested French bean farms around Tharaka Nithi County. The galled roots were chopped into 1-2 cm pieces and placed in a capacity conical flask.

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Roots were gently washed to remove adhering soil particles. The washed roots were cut into small bits of 2.5 cm longitudinally then placed over tissue paper spread on a wire gauge and kept in a Petri plate filled with water. Level of water was maintained in Petri plate and left undisturbed for 48 hours. Later, the suspension in the Petri plate was collected and observed for nematodes using light microscope.

2.4. In vitro nematode analysis

Hatching assay was done following method suggested by (Patil *et al.,* 2017). The egg masses were kept in glass cavity blocks (20 egg mass/ cavity block) containing 3 ml of plant extracts of 25 ml/L, 50 ml/L and 100 ml/L respectively. A distilled water control was maintained simultaneously. Each treatment was replicated three times. Numbers of juveniles hatched after at every day up to 7 days were counted with the aid of inverted microscope at magnification 40X. Mortality assay of plant extracts on juveniles was done. Freshly hatched second stage juveniles were transferred to different cavity blocks (20 juveniles/ cavity block) containing 25 ml/L, 50 ml/L and 100 ml/L respectively of plant extracts (3 ml/ cavity block). Juveniles put in distilled water were treated as control.

The treatments included Tithonia (TI), Neem (NM) and Tephrosia (TE) leaf extracts each at a concentration of 2.5% (25ml/L), 5% (50ml/L) and 10% (100ml/L).Vydate (Oxamyl 10 %) and Commercial Neem leaf formulation (CNE) (Nimbecidine) served as a standard positive control .The treatments were laid in a Completely Randomized Design with each replicated three times. Percent juvenile mortality rate was determined by counting at daily intervals up to 7^{th} day. All dead and alive juveniles were counted with the aid of inverted microscope .The mortality was ensured by touching the juvenile with a fine needle. The ratio of dead nematodes to total number of nematodes multiplied by 100 expressed the percentage mortality. The data was then subjected to statistical analysis.

Results

Active Phyto-chemical Constituents in the Leaf Extracts of Neem, Tithonia and Tephrosia

Preliminary study was carried out to determine the presence of phytochemicals constituents within the plants extracts. Tests were done for presence of flavonoids, alkaloids, saponins and terpenoids which are effective against plant parasitic nematodes. It was observed that Tithonia and neem had alkaloid effect, flavonoid effect was in Tithonia and Tephrosia and only neem had saponins (Table 1)

Effect of Tithonia, Neem and Tephrosia Ethanol Extract on Hatching of Root Knot Nematodes

All plant extracts showed inhibitory effect on hatching of juvenile. The rate of hatching was directly proportionate to exposure period and inversely proportionate to concentration of extracts as it was decreased with increase in

concentration. The highest rate of hatching was observed in 10 (100ml/L) per cent while lowest rate at 2.5 (25ml/L) per cent concentration in all plant extracts tested (Table 2). Among extracts, leaves extract obtained from Neem and Tithonia showed most inhibitory effect followed by Tephrosia.
Minimum hatching was recorded with Neem10 (100ml/L) per cent .The maximum egg hatching i.e. at 7th day was recorded with untreated check.

Table 1: Phyto-chemical Analysis of Tithonia, Neem and Tephrosia Ethanol Extract

	Chemical constituents Status in plant leaf extracts		
Constituent	Tithonia	Neem	Tephrosia
Alkaloids	+	+	-
Flavonoids	+	-	+
Saponins	-	+	-

+ = Positive for phyto-chemical tested; - = Negative for phyto-chemical tested

Table 2: Effects of Neem, Tithonia and Tephrosia Ethanol Extract on Percent Hatching

Treatments	Mean	SD	SEM	CV (%)	LSD
Neem 25ml/l	6.98b*	2.90	1.09697	42	14.10
Neem 50ml/l	4.92b	2.69	1.01763	55	
Neem 100ml/l	1.41b	1.04	0.39369	74	
Tithonia 25ml/l	9.36b	2.54	0.95816	27	
Tithonia 50ml/l	4.44b	2.90	1.09767	65	
Tithonia 100ml/l	0.87b	0.71	0.26748	81	
Tephrosia 25ml/l	11.43b	2.96	1.11705	26	
Tephrosia 50ml/l	5.95b	2.68	1.0123	45	
Tephrosia 100ml/l	3.81b	2.41	0.91114	63	
Nimbecidine 25 ml/l	12.30b	3.13	1.1847	25	
Nimbecidine 50ml/l	6.19b	2.80	1.05983	45	
Nimbecidine 100ml/l	4.12b	2.48	0.93739	60	
Oxamyl	0.92b	0.84	0.31786	91	
Untreated Check	47.38a	31.11	11.7573	66	

[a]Means followed by same letters are not significantly different at 5% level of significance.
SD=Standard deviation, SEM=Standard error, CV=Coefficient of variation, LSD=Least significant difference

Effects Tithonia, Neem and Tephrosia Ethanol Extract on Mortality of Root Knot Nematodes

The rate of mortality was directly proportional to exposure period and concentration of extracts. The highest mortality of larvae was observed at 7th day with 10 (100ml/L) per cent concentration of all extracts of tested plants while lowest was observed at low concentration i.e.2.5 (25ml/L). Mortality was nil in control i.e. 0.00 per cent at 7th day (Table 3). All plant extracts were found mortal (to have nematicidal action) to juveniles. Among plant extracts maximum mortality was

recorded with Neem and Tithonia. Maximum mortality was recorded with Neem at 80 per cent at 10% (100ml/L) per cent concentration followed by Tithonia at (73.67 per cent) 10 (100ml/L) per cent concentration at 7th day. The average percentages means were 51.428% for Neem at 100ml/L and 59.048% for Tithonia 100ml/L.

Table 3. Effects of Neem, Tithonia and Tephrosia Ethanol Extract on Mortality of Root-Knot Nematodes J2

Treatments	Mean	Sd	SE	CV%	LSD
Neem 25ml/l	21.4286def	15.8	6.0	74	14.36
Neem 50ml/l	31.57cde	19.6	7.4	62	
Neem 100ml/l	51.4286ab	25.4	9.6	49	
Tithonia 25ml/l	16.3329ef	11.8	4.5	72	
Tithonia 50ml/l	23.81cde	15.3	5.8	64	
Tithonia 100ml/l	59.0486a	29.0	11.0	49	
Tephrosia 25ml/l	7.2386efg	49.0	1.5	92	
Tephrosia 50ml/l	25.1429cde	16.3	6.1	65	
Tephrosia 100ml/l	39.0943bc	21.1	8.0	54	
Nimbecidine 25 ml/l	19.9043def	12.1	1.9	93	
Nimbecidine 50ml/l	20.9057def	18.1	6.9	87	
Nimbecidine 100ml/l	35.7157bcd	20.3	7.7	57	
Oxamyl	51.4286ab	25.4	9.6	49	
Untreated Check	1.4286g	0.5	0.2	94	

[a]Means followed by same letters are not significantly different at 5% level of significance.
SD=Standard deviation, SEM=Standard error, CV=Coefficient of variation, LSD=Least significant difference

Discussion

Plants are important source of potentially useful structures for the development of new nematicidal agents. The first step towards this goal is the *in vitro* nematicidal activity assay. Many reports are available on the nematicidal, antibacterial, antifungal and antimolluscal properties of these plants (Patil *et al*., 2017).Some of these observations have helped in identifying the active principle responsible for such activities and in the development of commercial formulations for crop protection.

In the present study the ethanol leaf extracts of Neem, Tithonia and Tephrosia showed the activity against *Meloidogyne indica* and effectively proven for their utilization as source for nematicidal compounds .Similar findings were reported by Resha *et al*., (2015) who reported that extracts from neem are toxic against the 2nd stage juveniles of root knot nematode .Resha *et al*. (2015) indicated that extracts of fresh greens leaves, showed maximum reduction in egg hatching and cause great mortality of juveniles .The efficacy may have due to the reported secondary metabolites in the extracts ie alkaloids and saponins (Susmitha *et al*.,2013).

Desi *et al*., (2017) reported that phytochemical analysis of Tithonia showed the presence of Alkaloids and saponins .Alkaloid, tannin and flavonoid are bioactive compounds that have antimicrobial and nematicidal activity as previously reported. Swamy *et al*. (2015) also reported that Tephrosia contain phytochemical such as alkaloids, tannins, flavonoids and terpenoids-steroids .The result obtained from

the bioassay study indicated that these bioactive compounds were somehow correlating with the ability in inhibiting plant pathogens.

Conclusion

The study demonstrated that plant extracts has a promising potential that could be exploited in the management of root-knot nematodes in vegetable production systems. Neem and Tithonia were the most effective crude extracts on the mortality of juveniles at 80 and 73.67% respectively at 7th day.

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