BEST JOURNAL 10(1): 8 - 12
Date received: October 30, 2012
Date accepted: February 08, 2013
Printed in Nigeria

EFFECTS OF *JATROPHA CURCAS* AND *AZADIRACHTA INDICA* ROOT EXTRACTS ON EGG HATCH AND LARVA MORTALITY OF *MELOIDOGYNE INCOGNITA*

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ABSTRACT
Chemical control of plant-parasitic nematodes, essentially involves the use of synthetic Nematicides. However, apart from their very high cost, increased concern on the environment has necessitated a reduction in the amount of Nematicides used for nematode control. In view of these, this research work was conducted to evaluate the effects of different concentrations of root extracts of Jatropha curcas and Azadirachita indica at 'S' (100% concentration),'S/2'(50%concentration),'S/10'(10%concentration),'S/100'(1% concentration) in the inhibition of egg hatch and mortality of larva of root-knot nematode, Meloidogyne incognita .The experiment was laid out in a completely randomized designed and replicated four times. All the concentrations of J. Curcas, A. Indica and their combination inhibited egg hatch of M. Incognita throughout the period of observation. The standard solution (S) of J. curcas exhibited the highest larva mortality and egg hatch, while 10% concentration of A. indica root extract exhibited the highest larva mortality after 96 hours. The combination of equal proportion of A. indica and J .curcas root extract indicated that all the concentrations inhibited egg hatch except S/100 after 96 hours of exposure. Larva mortality was found to decrease with an increase in the dilution of all the extracts.

Keywords: Azadirachta indica, Jatropha curcas, egg hatch, larva mortality and Meloidogyne incognita

INTRODUCTION

Root-knot nematodes have a very wide host range. This makes them very difficult to control because they can always survive and reproduce on other host crops. However to achieve good root-knot nematode management, different methods may be employed such as planting resistant or tolerant varieties, use nematode-free planting material, seedbed sanitation, crop rotation and most especially use of chemicals. Use of chemicals for the control of nematodes which requires applications of large amounts of chemical with knowledge of specialized equipments, has been reported to control root-knot nematodes on various crops (Starr *et al.*, 2002; Butool *et al.*, 1998). However, both nematodes and humans have nervous systems and because of these chemicals that target nematode nerves are also a potential danger to human nerves (Starr *et al.*, 2002). In view of this, there is need to replace highly toxic and potentially polluting chemicals used for the management of plant-parasitic nematodes (Oyetayo *et al.*, 2007).

Incorporation of plant parts or extracts into the soil as bio-control agents have been suggested as an alternative for effective control method for the management of plant parasitic nematodes (Siddiqui and Alam, 1985). Hence the choice of *Jatropha curcas* and *Azadirachta indica* plant parasitic nematodes. *A. indica* is widely known as neem and has been recognized as a versatile multipurpose tree for agro-forestry, fuel production, and for a variety of other products, including biopesticides (Lemnens *et al.*, 1995). Several bioactive compounds are found in the leaves and the active compound azadirachtin that repel pests, disrupt insects' growth and reproduction (Sara *et al.*, 2004). *J. curcas* is used mainly in the production of biodiesel (Jatropha oil). The leaves may be safely eaten after steaming. Sometimes, the nuts can be roasted and eaten, while the bark of the tree is used as fish poison (Birgit, 2009). There is the need, therefore, to develop effective and environmentally friendly nematicides, which are less toxic to man and animals but are potent, capable of releasing substances from their roots into the soil that are toxic to several plant parasitic nematodes. Some of these antagonistic plants include *Tagetes* spp (Kumari *et al.*, 1986), *Crotalaria* spp (L), *Cannabis sativa* (L), *Cassia fistula* (L), *Jatropha curcas* (L.), *Lantana camara* (L.) (Joymatti *et al.*, 1998), *Acacia albida* and *Allium sativa* (L.) (Hasabo and Noweer, 2005).

Chemical control of plant-parasitic nematodes, essentially, involves the use of synthetic nematicides. However, apart from their very high cost, increased concern for safe environment has necessitated a reduction in the amount of nematicides used for nematode control. Additionally, there has been an increase in the intensity of search for other efficient and safe control methods. Hence, this research was conducted to determine the efficacy of *Jatropha curcas* and *Azadirachta indica* root extracts for the control of plant parasitic nematodes, which are not only safe to use but also have the capacity to improve soil structure and fertility.

MATERIALS AND METHODS

The research work was carried out in the Crop Production Department laboratory, Federal University of Technology, Gidan Kwano Campus, Minna, Niger State of Nigeria. The roots of *Azadirachita indica* and *Jatropha curcas* were collected from matured tree in Gidan Kwano Campus of Federal University of Technology, Minna, Niger state. The roots were obtained by cutting sections using cutlass, packaged into a polythene bag and taken to the Crop Production laboratory.

Preparation of crude extracts of the roots

The adhering soil on the roots was carefully removed and washed under running tap water; 2 kg of each root sample was weighed, crushed into smaller pieces using a clean mortar and pestle. 200 ml distilled water was added, stirred with a glass rod, covered with cellophane paper to prevent evaporation and allowed to rest for 24 hours on the laboratory bench. After 24 hours, the pastes were stirred, poured into an electric blender and blended for a period of one minute. Suspensions were then made up to 6 liters' and mixed thoroughly. These mixtures were left on the laboratory bench again for another 24 hours having covered them with cellophane foil. After 24 hours the pastes were filtered into a clean plastic container through a Whatman No. 1 filter paper. The resultant suspension was then labeled standard concentration 'S'. Three different concentration levels were prepared from the standard solution namely S/2, S/10, S/100, Distilled water as control.

Collection of *M. incognita* inocula

Meloidogyne incognita was cultured on *Lycopersicon lycopersicum* in screen house. The culture plants were uprooted carefully and taken to the laboratory in a polythene bag. The roots were carefully washed to remove adhering soil.

To remove egg masses from these clean roots, short pieces of 2 cm were cut, placed in petri dish and observed under light microscope to locate egg masses. Matured egg masses were carefully removed using forceps.

Experimental set-up

Plastic petri-dishes of 9 cm diameter were used for this research. They were arranged on the laboratory working table in a Completely Randomized Design (CRD). Each concentration of the extract (15ml) was pipetted out into each petri-dish. Two freshly removed egg masses averaging 500 eggs per egg mass were transferred to the solution in each of the petri-dishes. Equal volume of distilled water poured into similar petri-dishes served as control.

The ambient temperature of the laboratory ranged between 29°C – 31°C during the experiment. The total number of larvae hatched and mortality were each recorded at 3, 6, 12, 24, 48 and 96 hours after inoculation. Hatched larvae were counted using the light microscope at x100 magnification. Confirmation of mortality was done by pricking larvae with a sharp needle. Mean percent mortality was then calculated. All the data collected were analyzed using analysis of variance and least significant difference (LSD) was used for comparing mean differences

RESULTS

Effect of different concentrations of root extract of *Jatropha curcas* on egg hatch of *M.incognita*

Three hours after exposing the eggs to the treatments, (Table 1) mean number of egg hatch of 3.75 was recorded for the standard solution, whilst the control recorded the highest mean number (6.25) of egg hatch. There was, however, no significant difference ($P \geq 0.05$) between S, $^{S}/_{2}$, $^{S}/_{10}$, $^{S}/_{100}$ compared to the control. On the other hand, an increase in egg hatch increased with the time of exposure, mean number of egg hatch increased and ranged from 6.75 to 10.75 for the standard and control respectively as dilution increases after six hours of exposure. There was significant difference ($P \geq 0.05$) between the control and all the extracts after 12,24,48 and 96 hours of exposure, The extract at different dilution inhibited egg hatch after 96 hours compared with the control.

Egg hatch was highest in distilled water, which is the control and lowest in the standard solution(s) of the root extract as shown in the table 1. The treatment with the highest dilution ($^{S}/_{100}$) indicated that *J. curcas* root extract has inhibitory effect even at low concentration on the egg hatch of *Meloidogyne incognita.*

Effect of different concentrations of root extract of *Azadirachita indica* on egg hatch of *M. incognita*

Similarly, egg hatched reduced with increased concentration of *A. indica* root extracts (Table 2). Also it indicated that as the time of exposure of egg masses increased, the egg hatch increased. At three hours of exposure, the mean number of the egg hatch of 3.00 was recorded for the standard solution(s), whilst the control had the highest mean number (10.50) of egg hatch. There was significant difference ($P \leq 0.05$) between the standard solution(s) and all the other treatments. At 6 hours of exposure, there was a significant difference ($P \leq 0.05$) between the standard solution(s) and other treatments. However .after 96hrs of exposure, less egg hatch was recorded in 'S' compared with 'S/2', 'S/10' and 'S/100' with high significant difference between 'S' and the control as indicated in Table 2.

Combine effect of different concentrations of root extract of *A. indica* and *J. curcas* on egg hatch of *M. incognita*

The combine effect of (Table 3) root extracts of *A. indica* and *J. curcas* significantly inhibited egg hatch in *M. incognita* after 96hrs of exposure. The extracts at S/2, and S/10 recorded less larval hatched, but there was no significant difference ($p \leq 0.05$) between S, S/2 and S/10 after 96hrs of contact with the extract.

However there was a significant difference between S, S/2, S/10 and S/100 at the end of the observations. About 30% egg inhibition was also recorded for S/100 indicating a significant difference between S/100 and the control.

Table 1: Effect of different concentrations of root extract of *Jatropha curcas* on egg hatch of *M. incognita*

	Exposure time					
Treatment	**3 hrs**	**6 hrs**	**12hrs**	**24hrs**	**48hrs**	**96 hrs**
S	3.75^{ab}	6.75^{c}	10.50^{b}	13.50^{b}	20.00^{d}	30.50^{b}
$S/_{2}$	3.00^{b}	6.00^{c}	9.50^{b}	13.25^{b}	25.50^{bc}	37.75^{b}
$S/_{10}$	3.75^{ab}	7.75^{bc}	11.75^{b}	15.50^{b}	22.25^{cd}	37.25^{b}
$S/_{100}$	5.50^{ab}	9.50^{ab}	12.25^{b}	15.75^{b}	27.50^{b}	40.75^{b}
C	6.25^{a}	10.75^{a}	16.25^{a}	22.00^{a}	34.00^{a}	55.50^{a}
LSD	2.62	2.52	3.33	5.31	3.98	10.76

Means in column followed by different letter are significantly different ($P \leq 0.05$) using (LSD) least significant difference.

Table 2: Effect of different concentrations of root extract of *Azadirachta indica* on egg hatch of *M. incognita*

	Exposure time					
Treatment	**3 hrs**	**6 hrs**	**12hrs**	**24hrs**	**48hrs**	**96 hrs**
S	3.00^{bc}	10.71^{bc}	13.50^{b}	16.50b	25.75^{b}	34.00^{c}
$S/_{2}$	1.75^{c}	8.25^{c}	13.00^{b}	18.75^{b}	29.50^{b}	43.25^{b}
$S/_{10}$	5.75^{b}	10.25c	12.50^{b}	14.75^{b}	26.00^{b}	42.75^{b}
$S/_{100}$	6.75^{ab}	13.50^{ab}	16.25^{b}	18.75^{b}	33.75^{b}	46.25^{b}
C	10.50^{a}	22.00^{a}	30.25a	45.25^{a}	66.00^{a}	85.25^{a}
LSD	3.98	3.03	4.40	5.31	8.77	8.29

Means in column followed by different letter are significantly different ($P \leq 0.05$) using (LSD) least significant difference.

Table 3: Effects of different concentrations of root extracts of *Azadirachta indica* and *Jatropha curcas* on egg hatch of *M. incognita*

	Exposure time					
Treatment	**3 hrs**	**6 hrs**	**12hrs**	**24hrs**	**48hrs**	**96 hrs**
S	4.25^{ab}	8.00^{c}	15.50^{c}	23.00^{b}	40.25^{bc}	51.50^{c}
$S/_{2}$	4.75^{ab}	9.25^{bc}	15.75^{c}	23.50^{b}	36.00^{c}	48.25^{c}
$S/_{10}$	3.25^{b}	8.75^{c}	15.75^{c}	23.50^{b}	34.70^{c}	45.75^{c}
$S/_{100}$	3.75^{b}	11.75^{ab}	19.75^{b}	31.25^{a}	44.50^{ab}	68.25^{b}
C	6.25^{a}	12.25^{a}	24.50^{a}	33.50^{a}	50.70^{a}	113.75^{a}
LSD	2.63	2.63	3.33	6.28	6.26	6.85

Means in column followed by different letter are significantly different ($P \leq 0.05$) using (LSD) least significant difference.

Effect of different concentrations of root extract of *J. curcas* on larva mortality of *M. incognita*

The standard solution of *J. curcas* root extract was most effective in the mortality of *M. incognita*. It was observed that at three hours of exposure of egg masses to the extract concentrations, there was no mortality recorded. Also, at six hours of exposure of egg masses to the extract concentrations, there was significant difference ($P \leq 0.05$) between the standard solution and all other treatments. Similarly, at twelve hours and twenty four hours of exposure, there was no significant difference ($P \geq 0.05$) between the standard solution and all the other treatments. Also, both S and $S/_{100}$ similarly gave the highest larva mortality over time. On the other hand, the control (C) gave the lowest mortality over time.

Effect of different concentrations of root extract of *A. indica* on larva mortality of *M. incognita*

The root extracts were effective in causing larval mortality; $S/_{10}$ concentration of extracts was more efficacious than all the other treatment over time. The larval mortality increased with increase in exposure time. The mortality was not found to differ significantly between concentrations of extracts at three hours and six hours of exposure. However, at 96 hour of exposure, there was significant difference ($P \leq 0.05$) between the standard solution and all the other treatments.

Combine effect of different concentrations of root extract of *A. indica* and *J. curcas* on larva mortality of *M. incognita*

There was no significant difference ($P \geq 0.05$) between the standard solution and all the other treatments at 6 hours of exposure and same at 24 hours of exposure.

However, at 12 hours of exposure there was significant difference ($P \leq 0.05$) between the standard solution and all the other treatments. Furthermore, S, S/2 and S/10 gave the highest larva mortality over time.

Table 4 Effect of different concentrations of root extract of *Jatropha curcas* on larva mortality of *M. incognita*

	Exposure time					
Treatment	**3 hrs**	**6 hrs**	**12hrs**	**24hrs**	**48hrs**	**96 hrs**
S	0.00	0.75^{ab}	1.00^{a}	1.50^{a}	4.25^{a}	9.25^{a}
S/2	0.00	0.75^{ab}	1.50^{a}	2.25^{a}	4.25^{a}	8.25^{a}
S/10	0.00	0.25^{b}	1.25^{a}	2.75^{a}	5.00^{a}	8.50^{a}
S/100	0.00	1.50^{a}	1.50^{a}	2.00^{a}	4.75^{a}	8.75^{a}
C	0.00	1.00^{ab}	1.00^{c}	1.50^{a}	2.25^{b}	5.00^{b}
LSD	NS	1.12	1.4	1.92	1.82	1.94

Means in column followed by different letter are significantly different ($P \leq 0.05$) using (LSD) least significant difference.
*NS = Not significant

Table 5 Effect of different concentrations of root extract of *Azadirachta indica* on larva mortality of *M. incognita*

	Exposure time					
Treatment	**3 hrs**	**6 hrs**	**12hrs**	**24hrs**	**48hrs**	**96 hrs**
S	0.25^{a}	2.00^{a}	3.25^{a}	4.75^{a}	6.50^{ab}	11.00^{b}
S/2	0.50^{a}	1.25^{a}	3.25^{a}	3.75^{a}	8.75^{ab}	11.25^{b}
S/10	0.50^{a}	1.25^{a}	3.00^{a}	5.50^{a}	9.25^{a}	14.50^{a}
S/100	0.25^{a}	1.75^{a}	2.25^{a}	3.70^{a}	6.25^{ab}	10.25^{b}
C	0.00^{a}	0.50^{a}	1.50^{a}	3.25^{a}	5.75^{b}	8.75^{b}
LSD	0.98	1.88	2.15	2.90	3.00	2.91

Means in column followed by different letter are significantly different ($P \leq 0.05$) using (LSD) least significant difference.

Table 6 Effect of different concentrations of root extract *Azadirachta indica* and *Jatropha curcas* on larva mortality of *M. incognita*

	Exposure time					
Treatment	**3 hrs**	**6 hrs**	**12hrs**	**24hrs**	**48hrs**	**96 hrs**
S	0.00	0.75^{a}	2.00^{a}	5.00^{ab}	8.50^{a}	14.00^{ab}
S/2	0.00	1.00^{a}	2.75^{ab}	4.25^{ab}	7.50^{a}	10.75^{ab}
S/10	0.00	0.75^{a}	3.25^{aa}	6.75^{a}	10.50^{a}	14.75^{a}
S/100	0.00	0.75^{a}	2.00^{b}	4.00^{ab}	7.50^{a}	13.75^{ab}
C	0.00	0.25^{a}	0.75^{c}	1.50^{b}	3.75^{b}	10.25^{b}
LSD	NS	1.09	1.20	3.65	3.60	4.11

Means in column followed by different letter are significantly different ($P \leq 0.05$) using (LSD) least significant difference.
*NS = Not significant

DISCUSSION AND CONCLUSION

Egg hatch in *M. incognita* was found to decrease as the concentration of the root extracts increased. This could be attributed to the nematicidal effect of the root extracts of *J. curcas* and *A. indica*. It was also observed that inhibition of egg hatch increased with increasing concentration of the extract with the highest recorded in the standard extract (S). Ameer-Zareen *et al.* (2003) reported similar findings against root knot nematode eggs *in vitro* when aqueous extract of ginger was used. Egg hatch inhibition also increased with increase in exposure time. This agrees with Joymatti *et al.* (1998), and Bello *et al.* (2006) reported that eggs exposed to extracts of *Melothria purpusilla* (Blume), *Delonix regia*, *Isoberlina doka*, *Tamarindus indica* and *Cassia Siamea* for a longer period of time decreased in their rate of hatching as compared to those exposed to a shorter period of the same extracts. The inhibitory effect according to Adegbite and Adesiyan (2005) might be due to the chemical properties present in the extract that possess ovicidal properties. It was also suggested that botanicals with nematicidal properties affect the embryonic development or kill the eggs. Presumably these properties increase with increase in time hence, the increased inhibition as exposure period increased. The extract was also found to have a killing effect on root knot nematode juveniles as in aqueous extract of castor root reported by Adegbite and Adesiyan (2005) to cause mortality of *M. incognita* larva. It was also observed that larva mortality increased with increase in concentration of the extract. Hasabo and Noweer (2005) reported that the mortality effect of fresh leaves of pyrethrum (Chrysanthemum *cinerariafolium* vis), vegetable way resins (VWR) and pymare (PM) extract on nematode is concentration dependant.

The toxicity of botanicals, according to Ameer-Zareen *et al.* (2003), is due to biologically active constituents, according to Knoblock *et al.* (1989); this is characterized by their lipophilic properties that enable them to dissolve the cytoplasmic membrane of nematode cell and their functional groups interfering with the enzyme protein structure. Ricin, the principal toxin in castor seed, is a member of the toxalbumins and these are protein phytotoxins that are capable of inhibiting protein synthesis. Konstantopoulo *et al.* (1994) reported that plant extract activity may include denaturing and degrading of proteins and inhibition of enzymes.

Chitwood (2002) reported that natural products are active against mammalian parasites and can serve as useful sources of compound for examination of activity against plant parasites. l-Nagdi and Mansour (2003) observed that the potency of botanicals was affected by exposu time and this may be an asset in the use of botanicals on the targeted nematodes when exposed to the extracts for a long period of time.

However, for nematicidal efficacies, the root extracts of *J. curcas* and *A. indica* at standard solution separately were found to be more effective than the mixture of *A. indica* and *J. curcas* root extracts. It can be concluded that the combine effect of *A. indica* and *J. curcas* root extracts was generally less effective compared to each root extract separately. The efficacy of *J. curcas* and *A. indica* was better with longer time of exposure at standard solution. Similarly, the efficacy of *A. indica* and *J. curcas* combined together wHasbetter with longer time of exposure at ($^{S}/_{10}$) for inhibition of egg hatch.

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