NEMATICIDAL POTENTIAL OF NIHORT-LYPTOL AND NIHORT-RAKTIN AGAINST ROOT-KNOT NEMATODE (*MELOIDOGYNE INCOGNITA*)

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SUMMARY

The root-knot nematode, *Meloidogyne incognita* is one of the major limiting factors in horticultural crops production in many parts of the world including Nigeria. Efforts at managing it are targeted at using different biopesticides as alternative to nematicides which are detrimental to human health, environment and also expensive. The nematicidal potential of NIHORT-Lyptol and NIHORT-Raktin (Bio-pesticides formulated in National Horticultural Research Institute, Ibadan) was assessed against *M. incognita* eggs and 2nd stage juveniles in the laboratory and screen house. The NIHORT-Lyptol and NIHORT-raktin were diluted with distilled water at 25, 50, 70 and 100 % concentration (v/v) and 0 concentration served as control. The eggs and 2nd stage juveniles were exposed to the different concentrations at 5, 10 and 15 days at an average temperature of 29.5±2.6°C. In the screen house the 2 lowest concentrations (25 and 50%) were applied to the cucumber seedling one week after transplant and immediately inoculation with 5000 eggs of MI and cabofuran at 1.5kg a.i/ha and 2.5kg a.i/ha served as control. The experiments were arranged in a completely randomized designed with four replicates. At 5 days after the exposure both NIHORT-Lyptol and NIHORT-Raktin caused 100% eggs hatch inhibition and 2nd stage juveniles' mortality. In pot culture, application of NIHORT-Lyptol and NIHORT-Raktin were very effective in reducing the formation of galls, and final nematode population density in the soil and increased plant height of cucumber. Thus, NIHORT-Lyptol and NIHORT-Raktin are promising bio-pesticides. However, further research is needed to evaluate their efficacy under field conditions.

Keywords: Bio-pesticides, concentrations, cucumber growth, egg hatch inhibition, *Meloidogyne incognita*, mortality.

The root-knot nematodes, *Meloidogyne* species (Chitwood) are sedentary endoparasites. The genus has a worldwide distribution and is the most recognized plant-parasitic nematode because it caused characteristic galling symptoms and high economic damage to the susceptible crops (Wyss, 2014). Many of the nematicides used for the management of root-knot nematodes are effective but are expensive, highly toxic, pose human and environmental risk and therefore have been withdrawn from the market (Abd-Elgawad, 2008; Greeco*et* a*l*, 1992, Ploeg, 2002, Swarup and Sosa-Moss, 1990). The over use of these pesticides also compounds pest and disease problems due to the fact that many non-target organisms acting as natural enemies/predators are wiped out (Dhlamini *et al*.; 2005). Apart from the aforementioned problems, they are not applicable where low value crops are involved (Hague and Gowen, 1987).

Estimations of vegetable crop losses in the tropics due to these nematodes range from 17-20% on eggplant, 18-33% on melon, 11% -20% on cucumber and 24-38% on tomato (Sasser, 1979) and a yield reduction of 61% in sorghum (Swarup and Sosa-Moss, 1990). Sometimes however, the total crop loss caused by *Meloidogyne* species is difficult to ascertain in cases where crops are suffering from simultaneous attack by fungi, viruses, insects and other nematodes (Lamberti, 1979).

Several plants have been identified with nematicidal or nematostatic properties either in their seeds, fruits, leaves, barks, and roots or in their root exudates. With the phase-out of these broad-spectrum fumigant chemicals and increased need for agricultural producers to improve environmental and food safety, alternatives to the application of chemicals to the soil is essential. According to Desaeger and Rao (2000), bio-pesticides play an important role in controlling nematode populations below the economic threshold. In view of the importance of root-knot nematode, *Meloidogyne incognita* as a serious biotic factor in cucumber production, Hence, the study was aimed at investigating the nematicidal potential of the two bio-pesticides produced by National Horticultural Research Institute, Ibadan (NIHORT) supported by Food Agricultural Organization (FAO): NIHORT-Lyptol and NIHORT-Raktin on root-knot nematode, *M. incognita* on cucumber.

MATERIALS AND METHODS

Nematode inoculum extraction and population estimation procedures

Eggs of the root-knot nematode, *M. incognita*, used as inoculum in this experiment were extracted using the method of Hussey and Barker (1973) from the galled roots of *Celosia argentea (Linn.)*. Roots of three-month old *M. incognita*-infected *C. argentea* were collected from the vegetable field of National Horticultural Research Institute (NIHORT), Ibadan. The roots were washed to remove adhering soil particles and then cut into 1-2cm pieces in the Laboratory. The root pieces were vigorously shaken in 0.5% sodium hypochlorite (NaOCl) solution (100ml JIK +600ml water) for 4 minutes. The NaOCl solution was quickly passed through a 45µm aperture size sieve nested over a 25µm aperture size sieve to collect the freed eggs. The 25µm aperture size sieve with the eggs was then quickly placed under a stream of tap water to remove residual NaOCl, and collected in a beaker. The egg suspension in the beaker was thoroughly homogenized using a magnetic stirrer. The number of eggs in one milliliter of suspension was counted under the dissecting microscope. An average of three counts was taken to estimate the egg population per in full of egg suspension

Preparation of different serial dilution of NIHORT- Lyptol and NIHORT- Raktin

The 2 bio-pesticides were collected from the bio-pesticides formulation centre in NIHORT. Serial dilution was done for each as follows to obtain different levels of concentration used:

1. The pure stock solution used as 100% solution,
2. 75% = 75ml stock solution + 25 ml distilled water,
3. 50% = 50 ml stock solution + 50 ml distilled water,
4. and 25% = 25ml stock solution + 75ml distilled water this were done for both bio-pesticides

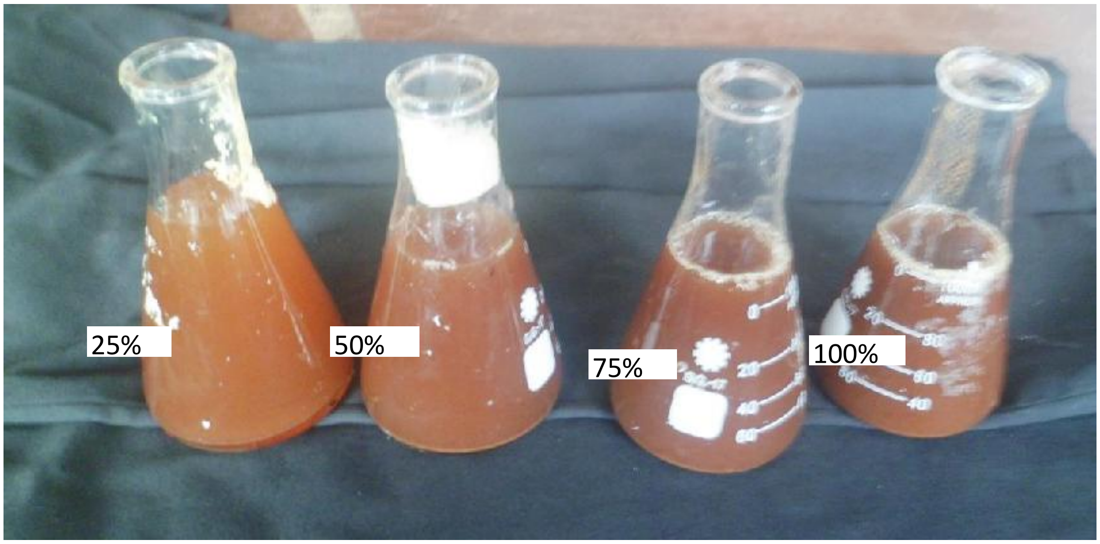

Plate 1: Different concentration of NIHORT-Lyptol.

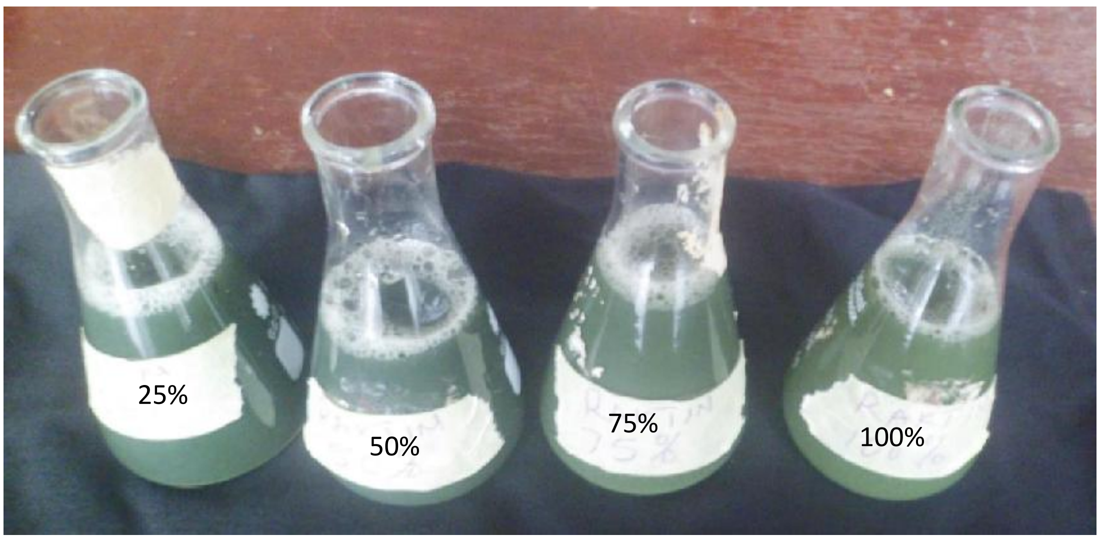

Plate 2: Different concentration of NIHORT-Raktin

Effect of plant extracts on egg hatch: Aliquots of 10 ml of nematode egg suspension that contained 500 fresh *M. incognita* eggs were dispensed in transparent white small plastic container arranged on the laboratory work bench. 10 ml each of the serial solution of NIHORT-Lyptol and

NIHORT-Raktin (25%, 50% 75% and 100%) and distilled water (0.0) were added into the egg suspension already contained in the plastic container. This brought the effective concentration of bio-pesticides to 12.5, 25, 35.5 and 50 % of each bio-pesticide respectively. The treatments and controls were incubated at average temperature of 29.5^{o}C and relative humidity of 75%. The experiments were set for 5, 10 and 15 days for both bio-pesticides. The experimental design was completely randomized design with 10 treatments and 4 replicates. Juveniles hatched were counted for 5, 10 and 15 days. A second trial was conducted as described above without any modification.

Mortality of 2nd stage juveniles of *M. incognita* exposed to bio-pesticides in the laboratory

Freshly extracted eggs of *M. incognita* were incubated in the laboratory at 27^{o}C for 5 days. Active second- stage juveniles were collected. Three 1 ml aliquots of the extract were dispensed in a counting dish and counted under dissecting microscope. Aliquots of 10 ml of juvenile suspension containing five hundred (500) second-stage juveniles of *M. incognita* were dispensed into white plastic containers that were arranged on the laboratory bench. 10 ml each of the bio-pesticides at different concentrations were added (25%, 50%, 75% and 100%). This also brought the effective concentration of bio-pesticides to 12.5, 25.0. 35.5 and 50% of each of the bio-pesticides. The experimental design was completely randomized with 10 treatments replicated 4 times. Nematodes were observed after 5, 10 and 15 days and the numbers of dead nematodes were recorded for each day. Nematodes were considered dead if they did not respond when touched with inoculating needle. This trial was repeated as described above without any modification.

Plate 3: Experimental set-up for bio-pesticides reaction on *Meloidogyne incognita* eggs in the laboratory

Greenhouse evaluation of NIHORT-Lyptol and NIHORT-Raktin-for the management of *M.incognita*

Two separate trials with carbofuran and the two bio-pesticides (NIHORT-lyptol and Raktin) were conducted for the management of M.incognita. Soil was steam sterilized at 90ºCfor 5 hours. A total of 28 pots were each filled with 10 litres of steam- sterilized soil. Eight (8) out of the 28 pots were treated as follows: Carbofuran 3G (1.5 and 2.5 kg a.i./ha) was applied one week before planting, 2 seeds of cucumber (CvMarketmore) were sown in each of the 28 pots.

One week after germination, the seedlings were thinned to one seedling per pot and were each inoculated with 10,000 *M incognita* eggs and 20ml of each of the bio-pesticides (NIHORT- Lyptol and NIHORT-Raktin) were added at different concentrations (25 and 50%) immediately after the inoculation to 16 pots the remaining 4 pots served as inoculated control. The experimental design was completely randomized with 4 replicates. Weekly data were collected on vine length, number of leaves and vinegirth till end of the study, while gall index (GI), nematode reproduction and yield (g) were taken at the end of the trial. All data were transformed using square root transformation equation (□x+2) before analysis using ANOVA (p=0.05) and means were separated using Duncan multiple range test at 5% probability.

RESULTS

In vitro assessments of NIHORT-Lyptol and NIHORT-Raktin on eggs of *M. incognita.*

After 5-, 10- and 15-days exposure, the 2 bio-pesticides at all concentrations had 100% egg-hatch inhibition while, compared to the control - (distilled water) where all the eggs - hatched at all exposure periods -. The - second trial gave similar results (Table 1).

Table 1: Inhibition of egg-hatch of *M. incognita* by NIHORT-Lyptol and NIHORT-Raktin at three exposure periods in NIHORT, Ibadan

Trt	Conc.	Mean egg hatch in 5 days	Mean egg hatch in 10 days	Mean eggs hatch in 15 days	% egg inhibition
Lyptol	12.5	0.05b	0.05b	0.05b	99.79
	25	0.05b	0.05b	0.05b	99.79
	37.5	0.05b	0.05b	0.05b	99.79
	50	0.05b	0.05b	0.05b	99.79
Raktin	12.5	0.05b	0.05b	0.05b	99.79
	25	0.05b	0.05b	0.05b	99.79
	37.5	0.05b	0.05b	0.05b	99.79
	50	0.05b	0.05b	0.05b	99.79
Water Control	0	8.76a	16.05a	19.70a	19.13
LSD≤0.05		1.13	0.67	0.58	

*Means followed by the same letter in the same column are not significantly different by Least Significant difference (LSD) (P≤0.05)

***In vitro* assessments of NIHORT-Lyptol and NIHORT-Raktin on Mortality of *Meloidogyne incognita* second-stage juveniles (J_2)**

After 5 days, all the juveniles of *M. incognita* died when exposed to NIHORT-Lyptol and NIHORT-Rakin at all concentrations. Similarly, there was no 2nd stage juvenile's survival in 10 and 15 days of exposure. However, 72.45% of the juveniles were alive in the control after15 days (Table 2).

Table 2: Mortality of juveniles of Meloidogyne incognita by NIHORT-Lyptol and Rakin on cucumber in the screen house at NIHORT, Ibadan.

Trt	Conc. (v/v) %	Mean Cumulative Mortality in 5 days	Mean Cumulative Mortality in 10 days	Mean Cumulative Mortality in 15 days	% of 2nd Stage Juveniles Alive in 15 days
NIHORT Lyptol	12.5	22.60b	24.36a	24.36a	0.00
	25	22.47b	24.25a	24.24a	0.49
	37.5	22.96ab	24.13a	24.13a	0.94
	50	23.49ab	24.25a	24.24a	0.49
NIHORT Raktin	12.5	22.35b	24.07a	24.07a	1.19
	25	22.47b	24.36a	24.36a	0.00
	37.5	22.90ab	24.36a	24.36a	0.00
	50	23.91a	24.36a	24.36a	0.00
Distilled Water Control		5.10c	6.71b	6.71b	72.45
LSD<0.05		1.26	0.48	0.48	

*Means followed by the same letter in the same column are not significantly different by Least Significant difference (LSD) (P≤0.05)

The highest fresh shoot weight was recorded from carbofuran @2.5 kg a.i/ha (433.02g) i.e., 72% increase in fresh shoot weight as compared with inoculated control which was not significantly different from the plants treated with NIHORT-Raktin@25% (392.96g/70% increased) and NIHORT-Lyptol@50% (375.46g/68.6% increased) levels of concentration (Table 3). However, the inoculated control had the least average fresh shoot weight (117.88g) (Table 3). The plant treated with cabonfuran @1.5 and 2.5 kg a.i/ha had the highest average number of fruits harvested per plant (2.79 and 2.43 fruits per plant) i.e. 97.0 % and 96.71% increased respectively in fruits harvested as compared with inoculated control which was significantly higher than other treatments, this was followed by uninoculated control (2.45/96.73% increased) which was not significantly different from the plant treated with NIHORT-Lyptol @ 50% (2.14) and NIHORT-Raktin @50% (2.15) level of concentrations which both had 96% increase in fruits harvested as compared with inoculated control while the least average fruits harvested was recorded from inoculated control (0.08) (Table 3)

The Gall indices (GI) of the plant treated with cabofuran, NIHORT-Raktin and NIHORT-Lyptol were less than 2 (0.58, 1.05 and 1.10) i.e. 88.0%, 78.5% and 77.5% decreased in GI as compared with inoculated control which had greater than 2 (4.88). Furthermore, the reproductive factor (Rf) were also less than 1 (0.0, 0.01, and 0.01, respectively, while that of inoculated control had greater than 1 (2.39). No damage was observed on the roots of uninoculated control (Table 3).

Table 3: Effects of - NIHORT-Lyptol, NIHORT-Raktin on the Yield of cucumber, plant root damage and nematode reproduction on cucumber infected with *Meloidogyne incognita*.

Trt	MFShwt (g)	MNfruit	MFruitWt (g)	GI	MNPS	MNPRt	MTN	Rf
Lyptol@25%	343.09b	1.90c	88.95c	1.08b	23.68b	110.40b	134.10b	0.01b
Lyptol@50%	375.46ab	2.14bc	100.08bc	1.10b	23.98b	110.3b	134.30b	0.01b
Raktin@25%	392.96ab	1.96c	91.76c	1.05b	20.18b	105.10b	125.30b	0.01b
Raktin@50%	328.14b	2.15bc	100.66bc	0.80b	20.70b	80.20b	100.90b	0.01b
Carbofuran (1.5kg a. i/ha	372.94ab	2.43ab	113.79ab	0.58bc	23.33b	60.10b	83.40b	0.01b
Carbofuran (2.5kg a. i/ha	433.02a	2.79a	130.55a	0.58bc	25.06b	50.10b	75.10b	0.00b
Inoculated control	117.88c	0.08d	3.87d	4.88a	300.11a	23585.00a	23885.10a	2.39a
Uninoculated control	337.98b	2.45ab	114.96a	0.00c	0.00b	0.00b	0.00b	0.00b
LSD<0.05	67.84	0.44	20.75	0.58	55.83	1364.00	1310.20	0.13

*Means followed by the same letter in the same column are not significantly different by Least Significant difference (LSD) (P≤0.05)

MFSHW= Mean Fresh shoot weight, MNFruit = Mean Number of fruit, MFruit Wt =Mean Fruit weight, GI = Gall index, MNPS = Mean Nematode population in soil, MNPRt = Mean Nematode population in roots, MTN = Mean Total Nematode population and Rf = Reproductive factor.

DISCUSSION

NIHORT-Lyptol and Raktin bio-pesticides used were effective in inhibiting egg-hatch of *M. incognita* and survival of second-stage juveniles of the nematode at all the concentrations tested. The effectiveness of these bio-pesticides might be due to the active ingredients such as cineole in Lyptol and azadirachtin in Raktin. Some families of lamiaceae (Basil, Mint etc) were tested on eggs and second stage juveniles of root-knot nematode and they all exhibited high toxicity (Akhtar and Farzana, 1990). Also, findings of Fatoki and Fawole (2000) on eggs and second stage juveniles using neem, siam weed and cassava peels extracts effectively inhibited eggs hatch and high mortality on second stage juveniles. The seeds extract of *Azadirachtainidica* A. Juss successfully killed juveniles of *M. incognita* and *Heterodera cajani* up to 100% mortality and inhibited egg-hatch (Upadhyay *et al*., 2003). Aminu-Taiwo and Fawole (2018) also used water extracts of marigold, siam weed, Mexican sunflower and local basil on eggs and second stage juveniles, all the tested extracts inhibited root-knot nematode eggs and caused 100% mortality of second stage juveniles.

The significant increase in growth of cucumber plants treated with NIHORT-Lyptol and NIHORT-Raktin could possibly be due to reduction in nematode population in the soil as was observed in this study. Use of aqueous extracts of siam weed, red acalypha and bitter leaves reduced the population of root-knot nematode in soil and roots of sesame (Oyedunmade and Olabiyi, 2006). Carbofuran, NIHORT-Lyptol and NIHORT-Raktin –treated plants had less galls compared with control plants and the treated soil had less nematode population than untreated soil. This implies that carbofuran and the bio-pesticides were effective in reducing nematode population as well as root-knot infection. These bio-pesticides competed effectively with this synthetic nematicide.

CONCLUSION

In view of the above findings, NIHORT-Lyptol and Raktin compared favorably with synthetic nematicides (carbofuran) which has been withdrawn from the market because of the human health and environmental hazards. The synthetic nematicides are also expensive beyond the reach of peasant farmers unlike the bio-pesticides (NIHORT-Lyptol and NIHORT-Raktin) which are readily available and not expensive. Therefore, extension officers and farmers are encouraged to avail themselves of the opportunities offered by the nematicidal potentials of these bio-pesticides (NIHORT-Lyptol and NIHORT-Raktin) in addressing nematode problem on the farm. However, further studies are required to test the efficacy of these bio-pesticides on the field.

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