Original Research Article

Nematicidal Activity of *Newbouldia laevis* (P.Beauv.) Seem. Leaf Extract Against Root-Knot Nematode (*Meloidogyne javanica* (Treub.) Chitwood on Eggplant (*Solanum* spp.) in Jalingo, Nigeria

Abstract: Solutions to problems of pests ravaging man's crops abound in nature and are known to be quite soft on the environment. Extracts of leaves of *Newbouldia laevis* were assayed for activity against the rootknot nematode, *Meloidogyne javanica*. The treatments which included the crude extract, 5 ml and 10 ml dilutions and Control as distilled water were used both in the laboratory and field experiments. Twelve petridishes containing 1000 nematode eggs and another 12 petridishes containing 1000 juveniles were each treated with the extracts and arranged in complete randomized design. Results showed the crude extract was the most effective in reducing the hatching of M. javanica eggs (90.33%) and caused greater mortality of juveniles (93.10%) than all other treatments. In the field experiment, twelve 2 m x 2 m-sized prepared plots were transplanted with nine three week old seedlings of 'Eggplant cv Yar Bello' into holes drenched with 10 ml of appropriate treatment. Ten ml of these treatments were drenched into soil around the plants weekly for eight weeks. Result showed that the crude extract performed better than other treatments in all parameters some of which included plant height (112.77 cm in 2017; 113.33 cm in 2018), number of leaves (120.00 in 2017; 120.77 in 2018), number of fruits (35.44 in 2017; 35.88 in 2018), fruit weight (73.81g in 2017; 74.44 g in 2018), yield (58.70 tons/ha in 2017;59.94 tons/ha in 2018), final nematode population (159.66 in 2017; 149.26 in 2018) and reproduction factor (0.42 in 2017; 0.39 in 2018). In conclusion, extract of *N. laevis* particularly the crude extract shows potential for managing M. javanica and allowing for profitable cultivation of 'Eggplant cv Yar Bello'.

Key words: Rootknot, Juvenile, Mortality, Egg hatch, Drenching

INTRODUCTION

Plant parasitic nematodes are reported to cause 12.3% losses to agricultural products worldwide, out of which 5% is attributed to root-knot nematodes (Meloidogyne spp.) (Moosavi, 2012). Root-knot nematodes are devastating pathogens that cause huge amount of losses both on field and horticultural crops across the globe and they bear responsibility for at least 90% of all damage caused by nematodes (El-Nuby et al., 2020). Two of the most economically damaging pests in glasshouse chrysanthemum produced for the cut flower industry in the Netherlands are the root-knot nematode species *M. incognita* and *M. javanica* (Amsing, 2004).
Plant-parasitic nematodes were mainly controlled by nematicides and chemical soil fumigants for decades in intensive crop production, but in recent times, some of these have been pulled out of the market as a result of concerns over environment and human health issues (Mokrini *et al.* 2018). The focus now is the use of plant materials and microbes to control these parasites. Scientists across the world have been working to find alternatives to harmful chemical nematicides (Aanany et al., 2017; Muiru et al., 2017; Bakr, 2018; Kepenekci. and Saglam,.2018; Ogwulumba and Ogwulumba, 2018; Joshi et al., 2019; Al-Banna et al., 2020; Moazezikho et al., 2020;) with varying degrees of success.

The need to find plants with nematicidal potential necessitated this research on the *Newbouldia laevis* (Boundary tree) commonly found in the Jalingo area and used for fencing. *Newbouldia laevis* is a shrub or small tree reaching to 7 – 8 m high in the west to 20 m in the east of the West Africa, and to 2.70 m in girth; shrubby, or erect with vertically ascending branches, of wooded savanna and deciduous forest. The plant has shiny dark green leaves and bears large showy terminal purple flowers. It is often grown as an ornamental and is easily propagated by cuttings. It is a familiar live-fence and boundary-tree throughout its distribution (Burkill, 1985).

MATERIALS AND METHODS

***Experimental sites**:* The egg hatchability and juvenile mortality tests were conducted in the laboratory of the Department of Agronomy, Taraba State University, Jalingo, Nigeria in 2017 while the field experiment was carried out at the Teaching and Research Farm of The Department of Crop Production Technology, College of Agriculture, Jalingo, Nigeria in 2017 and 2018.

Phytochemical analysis**:** Powder of shade-dried leaves of *N. laevis* (the boundary tree) was tested for the presence of phytochemicals at the laboratory of the Department of Biochemistry, Modibbo Adama University, Yola, Nigeria.

Extract preparation**:** Leaves of *N. laevis* were removed from the plant within and around the main campus of Taraba State University, Jalingo. The leaves were dried in the shade and ground to powder with mortar and pestle and stored in plastic bags. A 50 g weight of the powder was turned in to a 5 litre plastic bucket and to this was added 500ml distilled water. The set up was left to stand for 48 hours. It was filtered through Whatman No.1 filter paper. The filtrate obtained was labelled crude extract which was then serially diluted with 5 ml and 10 ml distilled water giving three treatments [Crude Extract (100%), 5 ml dilution (80%), 10 ml dilution (66%)]. Control (distilled water) made up the fourth treatment. These four treatments were used for each of the egg hatchability test, juvenile mortality tests and field experiment.

Nematode eggs and juvenile extraction: The nematode (*Meloidogyne javanica*) was identified using the head and stylet morphology (Eisenback *et al.*, 1981). J2 (second stage juveniles) and eggs of *M. javanica* were extracted from pure culture of infested roots of tomato plants. The modified Baermann method of Whitehead and Hemming (1965) was used to extract the J2s. This procedure involved the use of sieves lined with tissue paper in shallow trays with macerated roots of tomato placed on the sieves. Water was then poured in from the side of the tray to a level just submerging the macerated roots. The set up was allowed to stand for 24 hours and the nematode filtrate was decanted into a beaker. 10ml of the nematode filtrate were taken ni syringes and counted under a microscope using a grid counting dish. 1000 juveniles were put into each petridish for the juvenile mortality test. The eggs of *M. javanica* were extracted by agitating tomato roots in 0.05% sodium hypochlorite (NaOCl) for 2 – 3 minutes (Hussey and Barker, 1973). They were then collected and rinsed with tap water on 25-um pore sieves.

Egg hatchability test: 10 ml of *N. laevis* extracts were dispensed into 12 petri-dishes containing 1000 eggs of *M. javanica* using a 10 ml syringe. The four treatments were arranged in the completely randomized design (CRD).

Juvenile mortality test: 10 ml of *N. laevis* extracts in 10 ml syringe was dispensed into petri-dishes containing 1000 juveniles of *M. javanica*. 12 petri-dishes containing the four treatments described under preparation of extracts above were arranged in completely randomized design (CRD).

Field experiment: The experimental plot was ploughed, leveled and demarcated into 12 plots (each plot measuring 2 m x 2 m (4 m^2). There were three replications (each replication contained four plots) and the field was laid out in a randomized complete block design (RCBD) in both 2017 and 2018. The seedlings of Eggplant cv Yalon Bello (commonly cultivated by local farmers) were raised in 20 cm diameter plastic pots for three weeks in June before being transplanted to the field in July of 2017 and 2018. The seedlings were transplanted to the field at one plant per stand and nine plant per plot and spaced 60 cm x 60 cm. They were transplanted to into holes already drenched with 10 ml extract of *N. laevis* and after that 10 ml extracts was applied weekly for eight weeks. The four treatments included crude extract, 5 ml dilution, 10 ml dilution and control. Parameters measured were plant height, number of leaves, number of branches, shoot weight, root length, root weight, number of fruits, fruit weight, fruit girth, yield, galling index, nematode population and reproduction factor from three plant per plot. All agronomic practices were applied as required.
An auger was used to sample the soil at a depth of 0 – 15 cm for nematode population. All data collected was subjected to analysis of variance (ANOVA) in SAS procedures and means were separated using SEM (Standard Error of the Mean) at P=0.05 level of significance.

RESULTS AND DISCISSION

Phytochemical analysis of *Newbouldia laevis* indicated the presence of tannins, flavonoids, alkanols, phenols, saponins, glycosides, steroids and terpenoids.

Result of effects of *N. laevis* extract on the hatchability of *M. javanica* eggs showed crude extract recorded the highest inhibition (90.33%) of the nematode's egg hatch followed by the 5 ml dilution (81.33%) and 10 ml dilution (61.80%). Control had the lowest egg hatch inhibition with 2.60%. As extract concentration increased, the percentage egg hatch inhibition also increased (Figure 1). Pavaraj et al. (2012) reported that extracts of *N. cataria, C. quianensis and P. indicum* plants were found to be effective in reducing the hatching of root-knot nematode egg and hatching of larvae and mortality of nematode were highly influenced by extract concentration, plant species and duration of exposure. The observed hatch inhibition effect may have resulted from the presence of phytochemicals in the extracts. The presence of these chemicals in the extract with ovicidal and larvicidal properties might be responsible for the inhibitory effect and they either affected the development of nematode embryos, killed the eggs or even dissolved the egg masses (Nimbalkar and Rajukar, 2009).

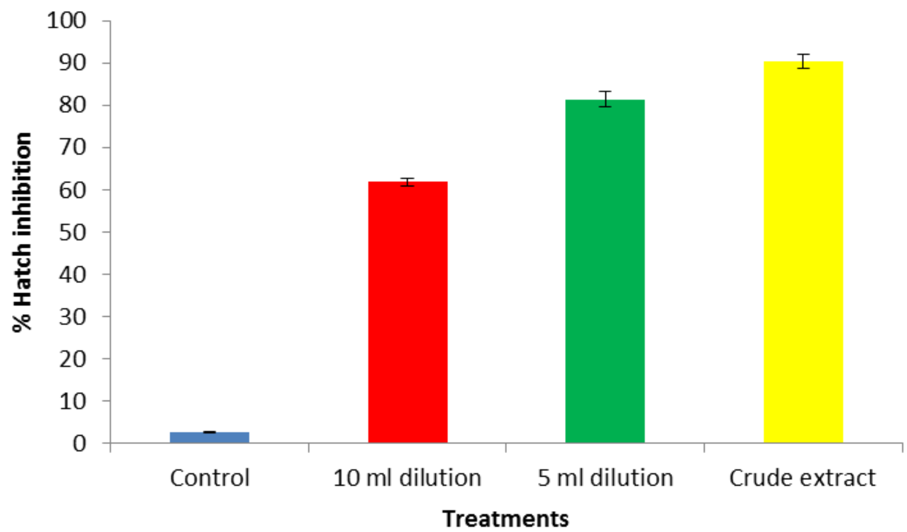

Fig. 1. Effect of leaf extract of *n. laevis* on inhibition of *M.javanica* eggs

The result of juvenile mortality test showed that crude extract of *N. laevis* killed more juveniles of *M. javanica* than control. The crude extract recorded the highest juvenile mortality (93.1%) followed by the 5 ml dilution (84.8%) and 10 ml dilution (67.5%). Control was the least with 3.5% mortality (Figure 2). As the extract concentration increased, nematode mortality increased accordingly giving an indication that *M. javanica* juvenile mortality was strongly influenced by concentration of extract. The mortality of juveniles in control may have resulted from natural causes since no extract was applied. Ntalli et al. (2015) reported that four extracts Catambra were observed to be active against *M. incognita* and *M. javanica*. Similar observations have been made by other authors on the potential of controlling root-knot and other plant-parasitic nematodes using plant extract (Abbasi *et al*., 2008; Ogwulumba and Ogwulumba, 2018; Joshi et al., 2019; Al-Banna et al., 2020; Mamman et al., 2022).

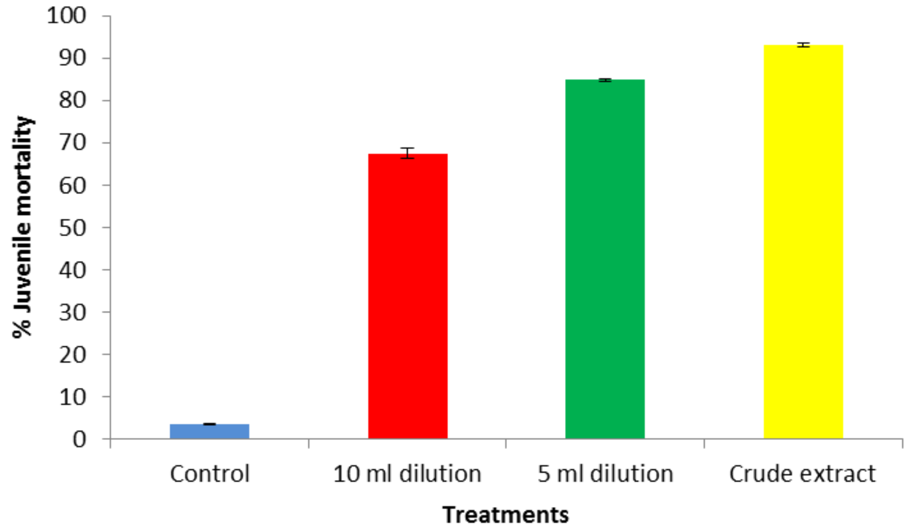

Fig. 2. Effect of leaf extract of *n. laevis* on mortality of *M. javanica* juveniles

Result of the field experiment indicated that there was significant (P = 0.05) difference between extracts of *N. laevis* and control. There was no significant difference between the crude extract and dilutions for plant height, number of leaves, number of branches, shoot weight and root length in 2017 and 2018 but the crude extracts recorded higher values for these parameters. For plant height, 'Eggplants cv Yalon bello' treated with the crude extract recorded tallest plants with heights of 112.77 cm followed by the 5 ml dilution (104.89 cm), 10 ml dilution (91.55 cm) and lowest was control with 41.77 cm in 2017 and in 2018, crude extract still recorded tallest plants (113.33 cm), 5 ml dilution (105.55 cm), 10 ml dilution (92.11 cm) and least was control with 42.66 cm. For shoot weight, 'Eggplants cv Yalon bello' treated with the crude extract recorded the highest weight with 1269.17 g followed by the 5 ml dilution (1180.90 g), 10 ml dilution (1086.80 g) and lowest was control with 137.23 g in 2017 and in 2018, the crude extract still recorded highest weight (1270.77 g), 5 ml dilution (1180.80 g), 10 ml dilution (1086.80 g) and lowest was control with 136.37 g (Table 1).

The crude extract recorded more eggplant fruits and higher fruit weight than the 5 ml dilution and there was no significant difference (P = 0.05) between the two but the difference between 5 ml and 10 ml dilutions was significant (P = 0.05) in 2017 and 2018. For mean number of fruits per plant, crude extract produced 35.44 fruits followed by 5 ml dilution (30.55 fruits), 10 ml dilution (22.22 fruits) and least was control with 12.77 fruits in 2017. In 2018, crude extract recorded 35.88 fruits followed by 5 ml dilution (31.33 fruits), 10 ml dilution (22.44 fruits) and least was control with 12.66 fruits. There was significant difference (P = 0.05) between extracts of *N. laevis* and control for mean eggplant fruit girth in 2017 and 2018. The crude extract recorded significantly (P = 0.05) higher fruit girth (4.71 cm) than the 5 ml dilution (4.25 cm), 10 ml dilution (4.07 cm) and least was control with 3.30 cm in 2017. In 2018, the crude extract still recorded significantly (P = 0.05) higher fruit girth (4.85 cm) than the 5 ml dilution (4.28 cm), 10 ml dilution (4.14 cm) and least was control with 3.29 cm in 2018 (Table 2).

There was significant difference (P = 0.05) between all treatments for yield of 'Eggplant cv Yar Bello' in 2017 and 2018. The crude extract recorded the highest yield of 58.70 tons/ha followed by the 5 ml dilution (44.97 tons/ha), 10 ml dilution (23.40 tons/ha) and lowest was control with 6.09 ton/ha in 2017. In 2018, crude extract produced the highest yield of 59.94 tons/ha followed by the 5 ml dilution (46.53 tons/ha), 10 ml dilution (26.03 tons/ha) and lowest was control with 6.54 ton/ha. M. javanica seemed to reproduced much more in control than in the extract-treated eggplants as control recorded significantly (P = 0.05) higher nematode population (896.00) than 10 ml dilution (242.00), 5 ml dilution (187.00) and least was the crude extract with 159.66 nematodes in 2017. In 2018, it followed the same pattern with control still recording the highest nematode population with 883.53 nematodes followed by 10 ml dilution (226.46), 5 ml (174.86) and least was crude extract with 149.26 nematodes (Table 3).

The study shows that the application of extracts of *N. laevis* especially the crude extract to the soil, reduced the reproduction and consequent damage to roots of egg plants by M. javanica and thereby encouraged increased vegetative growth and yield of 'Eggplant cv Yar Bello'. There was improved growth parameters such as plant height, number of leaves, fresh shoot weight, dry shoot weight, fresh root weight number of fruits produced and fresh fruit weights per plant when botanicals were applied to pepper plants (Agaba and Fawole, 2015). Soil amended with seed powder and leaves of neem and the shoots and leaves of *Nerium oleandenumber* recorded lower number of *M. javanica* juveniles and this resulted in higher nematode mortality (Moosavi, 2012). Application of aqueous extracts of neem and ginger powders to tomato suppressed the population of *M. incognita* at concentrations above 10% when (Agbenin et al., 2004). Claudius-Cole *et al*. (2010) and Katoll et al. (2010) also reported applying botanicals as powders and extracts to soil which caused the mortality and suppression of nematodes and their harmful activities.

Table 1. Effect of leaf extract of *N. laevis* on *M. javanica* on eggplant in 2017 and 2018

	2017					**2018**				
	PH (cm)	**NL**	**NB**	**SW (g)**	**RL (cm)**	**PH (cm)**	**NL**	**NB**	**SW (g)**	**RL (cm)**
Crude extract	112.77	120.00	19.78	1269.17	73.60	113.33	120.77	20.66	1270.77	74.16
5 ml	104.89	117.22	18.40	1180.9	68.33	105.55	117.44	18.77	1180.8	69.40
10 ml	91.55	113.61	16.50	1086.80	62.40	92.11	113.88	17.33	1086.8	60.70
Control	41.77	46.66	7.44	137.23	36.33	42.66	48.21	8.10	136.37	36.70
Mean	87.75	99.37	15.53	918.52	60.16	88.41	100.07	16.21	918.68	60.24
SEM	15.93	17.61	2.77	263.07	8.26	15.86	17.34	2.78	263.46	8.32

PH-Plant height, NL-Number of leaves, NB-Number of branches, SW-Shoot weight, RL-Root length, SE-Standard Error of the Mean

Table 2. Effect of extract of *Newbouldia laevis* on *M. javanica* on eggplant in 2017 and 2018

	2017				2018			
	RW (g)	**NF**	**FW (g)**	**FG (cm)**	**RW (g)**	**NF**	**FW (g)**	**FG (cm)**
Crude extract	187.12	35.44	73.81	4.71	186.23	35.88	74.44	4.85
5 ml	201.88	30.55	65.10	4.25	201.03	31.33	65.66	4.28
10 ml	222.26	22.22	46.91	4.07	228.26	22.44	50.55	4.14
Control	357.32	12.77	21.01	3.30	357.70	12.66	22.88	3.29
Mean	242.14	25.24	51.71	4.08	243.30	25.58	53.38	4.14
SEM	39.06	4.97	11.66	0.35	39.11	5.13	11.30	0.32

RW-Root weight, NF-Number of fruits, FW-Fruit weight, Fruit girth, SEM-Standard Error of the Mean

Table 3. Effect of extract of *Newbouldia laevis* on *M. javanica* on eggplant in 2017 and 2018

	2017				2018			
	Yield (tons/ha)	**GI**	**FNP**	**RF**	**Yield (tons/ha)**	**GI**	**FNP**	**RF**
Crude extract	58.70	1.66	159.66	0.42	59.94	1.70	149.26	0.39
5 ml	44.97	2.00	187.00	0.49	46.53	2.06	174.86	0.46
10 ml	23.4	3.00	242.00	0.63	26.03	3.06	226.46	0.59
Control	6.09	4.33	896.00	2.35	6.54	4.43	883.53	2.31
Mean	33.29	2.75	371.16	0.97	34.76	2.81	358.58	0.94
SEM	11.61	0.59	175.78	0.46	11.70	0.61	175.73	0.45

GI-Galling index, FNP-Final nematode population, RF-Reproductive Factor, SEM-Standard Error of the Mean

CONCLUSION

Leaf extract of *N. laevis* caused the mortality juveniles and inhibited the hatching of eggs of *M. javanica* in the laboratory and increased growth and yield parameters of eggplant as well as reduced the reproduction of the nematode on the plant. This study shows that *N. laevis* has the potential drastically cut down damage by *M. javanica* to eggplant and also allow farmers to get a good return on their investment.

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