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Comparative effect of two indigenous organic biopesticides and Furadan 3g in the management of *Meloidogyne incognita* in soybean (*Glycine max* (l.) Merrill

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Abstract
The soybean crop improvement programme in Nigeria led to the development of several high-yielding varieties. However, TGx 1019-2EN which is one of the top-yielding varieties has been devasted by Meloidogyne incognita, the most predominant phytonematodes attacking crops worldwide, including Nigeria. Due to the increasing health hazards associated with the use of some synthetic nematicides globally, scientists have continued to explore several non-chemicals, especially biopestides as safer and cheaper nematode disease management alternatives, especially in organic agriculture production system. Therefore, leaf powder of organically grown Azadirachta indica, and Carica papaya, were evaluated alongside a commonly-known synthetic pesticide, Furadan 3G, singly or in combination with each other, for their effectiveness in the management of the southern Root-knot nematode disease in susceptible TGx 1019-2EN soybean variety, a very important oilseed crop. The relative efficacy of the two leaf powders and the Furadan 3G in the reduction of Meloidogyne spp. population and soybean growth were determined. The leaf powders were prepared using leaves of A. indica (Neem leaf) and C. papaya (Pawpaw leaf) and used in amending sterilized soils potted and grown to TGx 1019-2EN, and two weeks seedlings inoculated with the nematode eggs. The experiment was laid out in a Completely Randomized Design with four replications. Powder of Azadirachta indica was most effective ($P \leq 0$) in reducing nematode population in the soil. Both neem leaf powder (NLP) and its counterpart pawpaw leaf powder (PLP) treatments produced taller soybean plants (57.38 – 58.40 cm, 84.50 – 85.98 cm and 89.20 – 88.38 cm at 2WAI, 4WAI, and 8WAI, when compared with the control ($P \leq 0$). Soybean root gall index significantly ($P \leq 0$) reduced from 15.75 in the control pots to 2.00 in NLP-treated plots, representing 87.30%.

Keywords: *Biopesticides, Nematicides, Oilseed crops, Organic agriculture, Phytonematodes*

Introduction
Crop plants are of great importance for a country and people and when these plants suffer from diseases, there will be serious yield losses which will adversely affect the agricultural economy of a country (Hafeez, 1986; Junaid *et al*., 2024). Plant-parasitic nematodes were responsible for economic damage to numerous agricultural plants (Renčo *et al*., 2014) grown for food or industrial purposes. Root-knot nematodes are plant-parasitic types from the genus *Meloidogyne* (Sasser *et al.*, 1984). They exist in soils in areas with hot climates or short winters. They are of major economic significance throughout the tropics. About 2000 plants are susceptible to infection by root-knot nematodes and they cause approximately 5% of global crop loss (Sasser and Hartman, 1985). Root-knot nematode larva infects plant roots causing the development of root-knot (also known as galls) that drain plants' nutrients. Early-season nematode infection led to worse crop

damage (Darling *et al*., 2021). *Meloidogyne* spp. was first reported in cassava by Neal (1889). A large number of crops such as bananas, beans, carrots, citrus, coffee, cotton, cucumber, eggplants, groundnut, okra, papaya, rice, pigeon pea, tomato etc. can be affected by root-knot nematodes.

Initially, very few pests and diseases were found on edible soybeans [*Glycine max* (L.) Merrill] in Nigeria (Akem, 1991). However, over the years soybeans have become more susceptible to the nematodes. Soybeans can now be hampered worldwide by infection of pests and diseases. Plant-parasitic nematodes are among the economically important pathogens that attack this crop, causing substantial yield losses. Root-knot nematode infections cause the roots of soybeans to swell into galls. These may not be obvious until the latter half of the soybean growing period.

Root galling caused by nematodes on soybeans may resemble *Rhizobium* nodules, and these can be confusing when diagnosing a root-knot nematode problem. *Rhizobium* nodules on soybean roots grow on one side of the root can be easily removed from the root and are a pink colour inside. Root-knot galls are often linked to nematode (Rodiuc, 2014) and the galls differ from root nodules as the former, on the other hand, grow around the small roots, are firmly attached, but are not pink on the inside like the latter.

Frequently used nematode management strategies to minimize soybean yield losses generally include the use of nematicides, the planting of resistant cultivars and crop rotation. Indiscriminate use of synthetic nematicides for controlling nematodes is likely to give rise to phytotoxicity, environmental pollution and nematode resistance. Unsafe use of nematicides may result in the poisoning of humans which is a major problem, especially in developing countries (Conway, 1995; Yudelman *et al*., 1998; Renčo *et al*., 2014). The use of botanicals in parasitic nematode control has received attention gradually across the globe. There have been growing exploits of extracts of tropical plants as potential bionematicides. Comparison of some of the botanicals with known synthetic nematicides is poorly reported in Nigeria. Determination of their potencies to elucidate activities of eco-friendly plants' bioactive products to facilitate the development and utilization of new bionematicides has become undoubtedly needful. This will facilitate the development of naturally occurring nematicide which may be less toxic to man and animals but as effective against nematodes of various crops as synthetic ones would therefore be a good and acceptable alternate option. This research, therefore, was undertaken to investigate the effect of leaf powder (Neem leaf powder and pawpaw leaf powder) and Furadan 3G on the population of root-knot nematode (*Meloidogyne* spp.) on TGx 1019-2EN soybean variety, susceptible to the nematode. The objectives of the study were to 1) determine the effect of pawpaw and neem leaf powders on a root-knot nematode in TGx 1019-2EN soybean variety, and 2) evaluate the leaf powders on TGx 1019-2EN soybean growth.

Materials and Methods

The experiment was conducted in the screen house of the Department of Crop protection, College of Plant Science and Crop Production, Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State, Nigeria. The soil used for the experiment was collected from the Fadama site at FUNAAB.

Soil Sterilization

The soil was heat-sterilized at 60^{o} C for 90 minutes. The sterilized soil was allowed to cool for 1 hour and thereafter stored in a jute sac and allowed to rest for six weeks to regain its structural stability. The soil was thoroughly mixed to ensure homogeneity after which 4 kg was weighed into individual pots and 40 g of each of the botanicals or Furadan 3G was mixed with the soil and left for two (2) weeks during which they were watered to encourage the mineralization of the botanical used for amending the soil. For the combination of the leaf powder and Furadan 3G, 20 g of each pair was used and treated as previously mentioned.

Experimental Design

The experiment was laid out in a Completely Randomized Design with four replications of the treatments and treatment combinations shown in Table 1.

Table 1: Treatments and treatment combinations

S/N	Treatment symbol	Treatments/ treatment Combinations
1	T_0	Inoculated soybean without any leaf powder (control)
2	T_1	Inoculated soybean + Neem leaf powder
3	T_2	Inoculated soybean + Furadan 3G
4	T_3	Inoculated soybean + Pawpaw leaf powder
5	T_4	Inoculated soybean + Furadan 3G + Neem leaf powder
6	T_5	Inoculated soybean + Furadan 3G + Pawpaw leaf powder

Preparation of Leaf Powders, *Meloidogyne incognita* inoculum, treatment application, and management

The Neem leaves (*Azadirachta indica*) and the pawpaw leaves (*Carica papaya*) were collected from FUNAAB. The leaves were thoroughly washed to remove soil and other debris. They were left for five (5) days at room temperature in the laboratory to dry and then ground to powder using a Warring (R) laboratory dry mill. *Meloidogyne incognita* **(**Mi)-susceptible soybean variety, TGx 1019-2EN was obtained from IITA and used for this project work. Five seeds of Mi-susceptible soybean variety, TGx 1019-2EN were sowed per pot. Each pot was watered regularly to allow easy germination and selectively thinned to one plant per pot, at two weeks after seedling emergence, to ensure uniform plant vigour. The eggs used were collected from galled *Celosia argentea* planted on soil naturally infested with *Meloidogyne incognita*. Required celosia was lifted carefully in the morning. The roots were washed under a gentle stream of cold water and were mopped dry with a muslin cloth. The roots were cut into small segments (1 - 2 cm) long. NaOCl 0.5 % was prepared, and the cut roots were poured onto it. It was shaken manually but vigorously for about 3 minutes to dissolve the gelatinous egg matrix. The suspension was quickly poured over a 200-mesh sieve nested upon a 500-mesh sieve. The eggs were then collected in the 500-mesh sieve and quickly placed under a cool and gentle stream of water for 4 minutes to rinse the residual NaOCl and then rinse into the beaker. One millilitre (1 ml) of the egg suspension was pipetted into Doncaster counting dish, estimated using a tally counter and calibrated (Table 2). The counting was repeated thrice, and an average was taken. Each seedling of soybean cultivars was inoculated with approximately 5000 eggs of Mi of 60 days – old by pipetting it into the shallow trench close to the base of the plant and covered slightly with topsoil. (Iheukwumere *et al*., 1995).

Table 2: Calibration of *Meloidogyne incognita* inoculum

Sample of egg suspension	Number of eggs per 1ml
1	52
2	49
3	31
Total	132
Mean	44
Standard error	4.6

Phytochemical Extraction

Some of the phytochemical properties of the botanicals used were analysed and their effect on *Meloidogyne incognita* population and disease management in the selected soybean was determined. The procedure for the extraction of the phytochemical was as presented in sub-sections 3.6.1 – 3.6.3.

Hot Aqueous Extraction

Twenty (20) gramme of dried powdered leaf each of neem and pawpaw was taken separately in a conical flask and was filled with 200 ml of distilled water. The mixture was heated on a hot plate under continuous stirring at 30º-40ºC for 30 minutes. Thereafter, the water extract was filtered through filter paper and the filtrate was used for the phytochemical analysis. The water extract was kept in the refrigerator at 4ºC when not in use.

Solvent extraction

About 20 g of powdered leaf was uniformly packed into a thimble in a soxhlet apparatus and extracted with 200 ml Petroleum ether, 95% Ethanol (V/V) and Hydroalcohol. Constant heat was provided by the Mantox heater for recycling the solvent. The process of extraction continued for 3 - 4 hours. The excess solvent was evaporated, and the dried extract was kept in the refrigerator at 4ºC for their future use in the phytochemical analysis.

Phyto-chemical screening

Chemical tests were carried out with all the solvent fractions using the standard procedure described by Sofowora (1993), Siddiqui and Ali (1997) and Sazada *et al*. (2009) to determine the phytochemicals (alkaloids, flavonoids, terpenoids, steroids, saponins, and tannins) in the botanicals tested.

Alkaloids

Few millilitres of solvent free extract was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered, and the filtrate was divided into two equal portions. One portion was treated with few drops of Mayer's reagent and the other with equal amounts of Dragendroff's. Turbidity of the resulting cream precipitate in both reagents was taken as evidence for the presence of alkaloids.

Flavonoids

A few millilitres of extract solution were treated with dilute sodium hydroxide solution which turned the solution to yellow and then it was treated with 5N hydrochloric acid which turned the solution colourless for the presence of flavonoids and orange colour for flavones.

Terpenoids and steroids

A small portion of the extract was dissolved in 1 ml of chloroform and filtered. To the filtrate on ice, 1 ml of acetic acid was added and then a few drops of concentrated sulphuric acid were run down the side of the test tube. The appearance of a pink or pinkish-brown ring/colour indicated the presence of **terpenoids**. The appearance of blue, bluish-green or a rapid change from pink to blue colours indicates the presence of **steroids** and a combination of pink and these colours indicate the presence of both steroids and terpenoids.

Saponins

Twenty (20) ml of water was added to the solvent-free extract and shaken vigorously. The frothing layer of foam formation indicated the presence of saponins.

Tannins

One (1) ml of 5% ferric chloride was added to solvent-free extract. The presence of tannins was indicated by the formation of the bluish-black or greenish-black precipitate.

Data Collection and Analysis

The Mi-inoculated and leaf powder-treated potted soybean plants were observed daily for above-ground symptoms. Data were obtained on plant height (cm) using measuring tape, number of leaves, number of branches and stem girth (cm) using a vernier calliper bi-weekly for a period of eight (8) Weeks After Inoculation (WAI)**.** After the 8WAI period, all four replicates were terminated, and harvested destructively. Plant samples were observed for the number of root galls, root nodules, fresh shoot weight (kg) and dry shoot weight (kg).

To obtain information on nematode reproduction, final eggs and Juveniles in the roots were determined by extracting eggs using Hussey and Barker (1973) sodium hypochlorite method. The numbers of egg and root populations of Juveniles were added to the nematode Juveniles in the soil which were extracted using the Whitehead and Hemming (1965) method, to give the final Mi population. Data collected were subjected to Analysis of Variance (ANOVA) using Statistical Analysis System (SAS) (2000) software. Significantly different treatment means were separated using the new Duncan Multiple Range Test (nDMRT).

Results

The effect of leaf powders of the two botanicals and Furadan 3G on growth parameters of soybean plants inoculated with *Meloidogyne incognita* were presented in Table 3 which showed the mean height of soybean plants treated with neem leaf powder (NLP), pawpaw leaf powder (PLP), Furadan 3G, NLP and Furadan 3G combined, or PLP and Furadan 3G combined. Powder of *Azadirachta indica* was most effective ($P \leq 0$) in reducing nematode population in the soil. Both NLP and its counterpart PLP treatments produced taller soybean plants (57.38 – 58.40 cm, 84.50 – 85.98 cm and 89.20 – 88.38 cm at 2WAI, 4WAI, and 8WAI, when compared with the control. The soybean plant treated with Neem leaf powder, pawpaw leaf powder, pawpaw leaf powder + Furadan 3G and control were not statistically different but were significantly different in height from Neem leaf powder + Furadan 3G. Soybean plants treated with NLP + Furadan 3G, produced the shortest height.

Table 3: Effect of leaf powders of the two botanicals and Furadan 3G on the soybean height

	Plant Height (cm) Weeks After Inoculation (WAI)++			
Treatment	**2+**	**4+**	**6+**	**8+**
Control	49.03ab	72.50ab	72.65a	75.85ab
Neem leaf Powder (NLP)	58.40a	85.98a	86.50a	88.38a
Furadan 3G	49.03ab	73.13ab	73.63a	77.25ab
Pawpaw Leaf Powder (PLP)	57.38a	84.50a	86.25a	89.20a
NLP + Furadan 3G	34.50b	51.40b	56.90a	57.90b
PLP+ Furadan 3G	51.50ab	75.10ab	75.75a	78.40ab

+WAI = Week After Inoculation ++Means with the same letter in each column were not significantly different ($P \geq 0.05$).

The effect of leaf powders of two botanicals and Furadan 3G on stem girth TGx 1019-2EN soybean plants inoculated with *Meloidogyne incognita* is *shown in Table 4.*. Considering the absolute value, neem leaf powder-treated soybean plants had the widest mean stem girth (0.71 cm). However, all the neem leaf powder-treated plants were not significantly different from the control and the combination of botanicals with Furadan 3G while the synthetic nematicide alone had the absolute thinnest stem girths (0.39 cm) at 2WAI. At 4WAI, all treatments were not significantly ($P \leq 0.05$) different from the control. At 8WAI, all treatments were not significantly different from the control.

Table 4: Effect of leaf powders of the two botanicals and Furadan 3G on stem girth

Treatments	Stem Girth (cm)			
	Weeks after Inoculation++			
	2+	4+	6+	8+
Control	0.54ab	0.54ab	0.54ab	0.57ab
Neem leaf powder (NLP)	0.52a	0.70a	0.71a	0.66ab
Furadan 3G	0.39ab	0.52ab	0.55ab	0.58ab
Pawpaw leaf powder (PLP)	0.43ab	0.68a	0.69a	0.72a
NLP + Furadan 3G	0.45b	0.45b	0.46b	0.48b
PLP + Furadan 3G	0.46ab	0.59ab	0.61ab	0.65ab

+WAI = week after inoculation. ++Means with the same letter in each column were not significantly different ($P \geq 0.05$).

The comparative effectiveness of neem leaf powder (NLP), pawpaw leaf powder (PLP) and Furadan 3G on nodule formation, root gall formation, and *M. incognita* juvenile population is depicted in Table 5. For nodules formation, there was no significant difference in the treated soybean after inoculation. The number of nodules (46.50) in Neem leaf and Furadan 3G were significantly the same while pawpaw leaf powder (17.75), NLP + Furadan 3G (27.50), as well as PLP + Furadan 3G (31.00) were statistically the same but not different from other treatments. Nodulation were enhanced in all treated plants (49.30 - 80.65%) compared to the control (0%). For gall number, there was no significant difference in the soybean plants treated with PLP +

Furadan 3G and control but there was significant difference in all other treatment and the control. The soybean root gall index significantly reduced from 15.75 in the control pots to 2.00 in NLP-treated plots, representing 87.30%. Furadan 3G, Pawpaw leaf powder and NLP + Furadan were significantly the same. The control had the highest number of galls (15.75) with soybean plant treated with Neem leaf powder having the lowest (2.00). Number of eggs showed no significant difference between Furadan 3G and the control. Pawpaw leaf powder, PLP + Furadan 3G and NLP + Furadan 3G are significantly the same. For nematode reproduction, there was no significant difference in all the treatments and the control. Control experiment i.e., soybean plants without treatments and those with treatments were not in any way statistically different from each other. Only one (1) M. incognita egg was recovered from NLP-treated plant which was statistically lower than all other treatments (7 – 14) and the control (18). Neem leaf powder treated soybean plants significantly reduced *M. incognita* eggs by 94.4% compared to the control and 20.8 - 61.1% in other treatments. The control produced the highest number of juveniles in the soil while soybean plant treated with *Azadirachta indica* powder (Neem leaf powder) had the lowest juvenile population in the soil.

Table 5: Effect of Furadan 3G, neem and pawpaw leaf powders on nematode-induced root galls, *Melogyne incognita* Juvenile population, and root nodules

Treatment	Nodules no	Gall no^{+}	Egg no^{++}	Juvenile no
Control	9.00b	15.75^{a}	18.00^{a}	37.75^{a}
Neem leaf powder (NLP)	46.50a	2.00^{c}	1.00^{d}	14.75^{a}
Furadan	46.50a	8.500^{b}	14.25^{ab}	18.25^{a}
Pawpaw leaf powder (PLP)	17.75ab	7.750^{b}	7.00^{c}	27.50^{a}
NLP + Furadan 3G	27.50ab	9.00^{b}	8.25^{c}	23.25^{a}
PLP + Furadan 3G	31.00ab	11.00^{ab}	9.50^{bc}	20.00^{a}

Key: +Gall no = number of galls, ++Egg no = no of *Meloidogyne incognita* eggs, Juvenile no = number of Juveniles, Nodules no = number of nodules.

The effect of treatments on fresh shoot and dry shoot weight is revealed in Table 6. For the fresh shoot weight (16.10 - 30.19 g/ plant) of treated plants there was no significant difference among the treatments except with the control (16.01 g/ plant). Soybean plant treated with *Azadirachta indica* (Neem leaf powder) had the highest weight 30.19 g/ plant while the control had the lowest weight (16.01 g/ plant). For dry shoot weight, NLP treated soybean plants averagely had the heaviest value (16.84 g/ plant) which varied significantly from the control plants where 9.94 g/ plant was obtained. However, NLP fresh shoot weight was similar to PLP value (13.93). There was no significant difference between the soybean treated with furadan, NLP + Furadan, PLP + Furadan and the control.

Table 6: Effect of Furadan 3G, neem and pawpaw leaf powders on fresh and dry weight TGx 1019-2EN variety

Treatment	FSW (g/ plant)[+]	DSW (g/ plant)[++]
Control	16.01[b]	9.94[b]
Neem leaf powder (NLP)	30.19[a]	16.84[a]
Furadan 3G	17.89[ab]	10.54[b]
Pawpaw leaf powder (PLP)	30.04[a]	13.93[ab]
NLP + Furadan 3G	16.10[b]	10.14[b]
PLP + Furadan 3G	18.39[ab]	11.12[b]

Key: + FSW = Fresh shoot weight, ++DSW = Dry shoot weight

The phytochemicals present in *Azadirachta indica* and *Carica papaya* leaf powders is shown in Table 7. The powders of the two indigenous plants contained flavanoids, alkaloids, saponins, tannins, steroids, terpenoids but at different concentrations. Alkaloids occurred in very high concentration in NLP compared with PLP and other phytochemicals obtained. The concentrations of flavanoids and tannins were high in NLP and PLP which were at par with alkaloids in PLP. There were moderate concentrations of steroids in both was present at the same level in both *A. indica* (neem) leaf powder and *C. papaya* leaf powder. Similarly, *A. indica* leaf powder contained high concentration of steroid and tannin. Distinctly, steroids and terpenoids recorded low concentration only in PLP compared to NLP and other phytochemicals recovered.

Table 7: Phytochemicals present in neem and pawpaw leaf powders used

Parameter	Pawpaw Leaf Powder	Neem Leaf Powder
Flavonoids	+++	+++
Alkaloids	+++	++++
Saponins	++	+++
Tannins	+++	++
Steroids	+	+++
Terpenoids	+	+++

+ = Low concentration, ++ = Moderate concentration, +++ = High concentration, ++++ = Very high concentration

Discussion

Herbivorous nematodes are well documented as major production constraints of several economically important agricultural plants (Renčo *et al*., 2014; Mandal *et al*., 2021), including soybeans globally. The southern root-knot nematodes, *M. incognita* are well documented as a major limiting factor in soybean production in Ethiopia (Frezer *et al.*, 2024), Nigeria (Adesiyan *et al*., 1990; Ihekwuemere, *et al*., 1995; Atungwu and Kehinde, 2008) and elsewhere in the world. In There has been overdependence on synthetic chemicals for the control of nematode pests generally, however, with the discovery of the health, animal and environmental hazards associated with the chemicals, exploration of the use of some plants as sources of natural nematicides have continued to be appraised (Renčo *et al*., 2014) and recommended. The utilization of botanical extracts as biopesticides have continued to be explored as a potential substitute for synthetic pesticides for pest management (Chengala and Singh, 2017), and as nematicides used in the management of root-knot disease of seedlings (Adebogun *et al*., 2020).

Our current study found that neem leaf powders and pawpaw leaf powder were as effective as the Furadan 3G, a well-known synthetic nematicide in the significant reduction of root gall disease of TGx 1019-2EN soybean variety. This was in consonance with Atungwu and Kehinde (2008) who assessed an organic-based fertilizer as an alternative to Furadan in the management of *Meloidogyne incognita* on soybeans in Nigeria. These authors affirmed that the Furadan, a popular synthetic nematicide which had been banned in developed agriculture, regrettably, still found its way to many developing nations, in spite of the health and environmental hazards associated with its application. Both powders of the selected test botanical caused a reduction in the population of root-knot nematode in the soil when compared with the control.

Furthermore, this study has shown that leaf powder of *A. indica* and *C. papaya*, Furadan and the combination of the individual leaf powder with Furadan effectively reduce root-knot nematode population, although neem leaf powder proved to be most active of all the botanicals. The effectiveness of neem leaf powder and pawpaw leaf powder in suppressing nematode population in soybean was reflective in using them in combination with Furadan. Additionally, the present work established that powders of the two indigenous enhanced the growth and girth of the plants as well as the fresh and dry shoot weights, attesting to their potential to improve the dry matter content of the crop. The choice of these the two indigenous plants, *A. indica* and *C. papaya*, was a follow up to reports (Olowe 1992; Mangala and Mauria. 2006) that the use of various parts plants such as leaves, seeds, barks, stems, etc, of indigenous as botanical extracts has become important in pest management in recent years following the environmental hazards caused by chemical control measures (Renco et al., 2014; Adebogun *et al*., 2020). The botanicals were easily sourced locally from established organic farm and Biopesticide Demonstration plot of the Federal University of Agriculture, Nigeria.

Conclusion and Recommendation

This study affirmed the effectiveness of neem leaf powder and pawpaw leaf powder applied at 1% w/w of sterilized soil in managing root-knot nematode population and gall disease in TGx 1019-2EN soybean variety and improved plant growth and dry matter content. It is recommended that neem and pawpaw-based nematicides be utilized at 40 g/ plant in soybean cultivation for improved root-knot disease management and plant growth.

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