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Evaluation of nematicidal potential of leaves extract of *Afrohybanthus enneaspermus* for management of *Meliodogyne incognita in Tomato*

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Abstract

The resistance of nematode pests to synthetic nematicides and its attendant hazards both to the crops and the ecosystem have caused growing interest. This study is aimed at investigating the nematicidal potential of the leaves extract of *Afrohybanthus enneaspermus*. *Afrohybanthus enneaspermus* is an evegreen shrub found in the tropical regions. The herbs have been used in ethonomedicine in the treatment of various ailments such as fever, diabetes, cough, skin infections and so on. According to standard methods in this study, in-vivo and *In-vitro* nematicidal screening of leaves extract was carried out against juvenile nematode pest (*Meliodogyne incognita*) while the pot experiment was carried out on tomato plants infected with root knot nematode. At the growth rate of the Tomato (Tropimech +), plant height was significantly lowered at 4 WAP (four weeks after planting) in tomato plants inoculated with 2,000 root-knot nematodes (RKN) applied with 20 g powered of *Afrohybanthus enneaspermus* compared with the rest of the treatments. With the application of 15% aqueous plant extracts and the inoculation of 2,000 RKN, plant height was significantly higher (32.8), followed by Plant alone and 20% aqueous plant extract. However, plant height was significantly lowered in plants applied with 20g powered of *Afrohybanthus enneaspermus* and nematode compared to the other treatments. At 6 weeks plant height is significantly higher in tomato plants inoculated with 5% aqueous plant extract with 2000RKN followed by others that are at the same range except 20g powered extract with 2000RKN that is significantly lowered . At 7 weeks plant height is significantly higher in tomato plants inoculated with 5% aqueous plant extract with 2000 RKN followed by others that are at the same range except 20g powdered extract with 2000 RKN that is significantly lowered. The research concluded that leaves extract of *Afrohybanthus enneaspermus* is effective in management of root Knot nematodes, however, in- vitro experiments, it is established that the powder form of the leave has higher effectiveness in management of nematode than its organic extract.

Keywords: *Afrohybanthus enneaspermus*; Juvenile nematodes; *Meliodogyne incognita*; Root knot-nematodes

1. Introduction

Nematodes are One of the most destructive pests, nematodes degrade both the quantity and quality of numerous annual and perennial crops, resulting in significant output losses of up to one billion euros a year (Nasiou and Giannakou, 2023). Nematodes are unsegmented roundworms that are part of the P*hylum nematoda,* which is a *subphylum* of the super-*Phylum ecdysozoa*. . Ecdysis is the process by which arthropods and other creatures form and shed cuticles. (McMullen *et al*., 2017). Effective soil fumigants and nematicides are either banned or only used sparingly, making the management of plant-parasitic nematodes more challenging (Quinn *et al*., 2023). Furthermore, there is a growing need for nematicides that are safe for the environment and suitable for use in organic farming practices (Oka *et al*., 2012).

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Nematodes that are harmful to plants can only feed on plants; in fact, they cannot survive on any other food source. They are capable of seriously harming or killing plants, particularly seedlings, when their population reaches large proportions. They have the potential to reduce yield without producing overt symptoms in smaller, more normalized levels (Topalovic and Geison, 2023).

Although phytochemicals are substances derived from plants, some of them are effective insecticides (Koche *et al*., 2016). They function biologically in the plant host naturally and aid in the growth of the plant or its defense against intruders, diseases, and predators (Kurmukov, 2019). Meloidogyne species, commonly known as root-knot nematodes, are the most devastating plant-parasitic nematodes (PPNs) in the world. More than 2000 host plants are infested by them, including weeds, edible crops, ornamentals, and medicinal plants (Sithole *et al*., 2021). Numerous crops, including tomato, soybean, grapes, citrus, potato, beetroot, peanuts, spinach, dry beans, lettuce, eggplant, maize, and carrots, have lost ground recently in various parts of the world, according to reports (Rashidifard *et al*., 2019). Another source of ecologically friendly nematode control agents could be the essential oils of herbs and medicinal plants, or their compounds (Oka *et al*., 2012).

In addition to being extremely expensive, the indiscriminate application of synthetic pesticides to suppress nematodes was likely to result in phytotoxicity, environmental contamination, and nematode resistance (Ngegba *et al*., 2022). Induced carcinogenicity, soil degradation, biomagnification-related depletion of marine life, greenhouse gas pollution, and varying degrees of endpoint toxicity, including mutagenicity, cytotoxicity, and immunotoxicity, which lead to morbidity and mortality, have all been linked to synthetic nematicides (Muwamula *et al*., 2022). Reducing the quantity of chemical nematicides and developing non-chemical alternatives has become necessary due to the recent prohibition on numerous nematicides and the growing environmental concern (Chi *et al*., 2020). Furthermore, there has been a greater focus on finding safe, reliable, and ecologically friendly alternatives to current control techniques (Babaali *et al*., 2017).

Using botanical pesticides, or biopesticides derived from plants, is one of the potential solutions (Ayilara *et al*., 2023). Due to their easy conversion into less hazardous byproducts by light, oxygen, and microorganisms, these biopesticides are typically regarded as non-persistent in field settings (Daraban *et al*., 2023). It is now crucial to employ plant extracts instead of synthetic pesticides to control the root-knot nematode (Ngegba, 2022). According to Sithole *et al*. (2021) there are four primary mechanisms that contribute to the detrimental effects of phytonematicides on PPN populations: juvenile mobility, chemotaxis, inhibition of egg hatch, and juvenile death. Numerous phytochemicals, including isothiocyanates, fatty acids, glucosinolates, diterpenes, phenols, and alkaloids, have been related to nemacidal effect. thienyls, steroids, and tannins, among other things (Khan *et al*., 2019). These compounds serve as respiratory poisons, attractants, deterrents, repellents, and contact pesticides, much like synthetic pesticides (Hikal *et al*., 2017).

Within the family *Violaceae Batsch*, the genus *Hybanthus* Jacquin (1760: 17) is the third largest. There are 125 species in it (Ballard *et al*., 2014). With 23 recognized genera and over 1,100 species of trees, shrubs, lianas, and herbs, the *Violaceae* family is a medium-sized family (Ballard, 2007; Ballard *et al*., 2014; Koche*, et al*., 2019). In the New and Old World tropical regions, the family is mainly made up of woody genera of trees, shrubs, and lianas; the sole sizable herbaceous temperate to montane genus is Viola. The growth form, inflorescence architecture, floral morphology, and fruit type of genera are quite diverse. With over 125 species, the genus *Hybanthus* Jacq. is the third biggest in the family and is mostly found in the tropics and subtropics (Hussey, *et al*., 1976); Temperate eastern North America is home to only one species*, Hybanthus* concolor (T.F. Forst.) Spreng. The genus's species range in habit from herbs or subshrubs to shrubs or, in rare cases, treelets. Their distinctive zygomorphic corolla—which has the bottom (anterior) petal noticeably or substantially longer than the lateral and upper ones—as well as their different shapes and frequently strong differentiations into abruptly expanded blade and claw, along with a "saccate" base—have been used to characterize and distinguish them from other *Violaceae*. Other traits that set the species apart from one another include their generally free stamens, an uncommon condition in which the filaments fuse into a short ring and the bottom pair of stamens bearing glands—fruits, which are typical of most genera in the family—and their globose to ellipsoidal unwinged seeds (Sithole, *et al*.,2021).

2. Material and methods

Afrohybanthus enneaspermus leaves were collected in their vegetative stage from local farms in Oyo State, Nigeria locales with varying bioclimatic conditions. Voucher specimens kept in the Pure and Applied Biology Department herbarium at Ladoke Akintola University of Technology Ogbomoso were used to identify the collected plants. Analysis was done at Ibadan's IITA Central Laboratory. Plant leaves were air dried for 15 days in a laboratory setting before being pulverized into fine powders with a commercial grinder. Tomato seeds (S Tomato plants were utilized to prepare the inoculum and/or in plant tests. After being sowed in alveolate plates and grown in a greenhouse for one month, the

seedlings of *Seolanum lycopersicum* L. cv. Moneymaker, Solanaceae, were transferred into 2.5 L plastic pots (17.5 × 1 4.5 cm: diameter × height) that held one L of a sand mold mixture (1/2:1/2) (Zaidat *et al*., 2020).

2.1. Extraction of Leaves Sample

250 ml of methanol and 25 g of the plant sample powder were added to 500 ml glass flasks (Khan, *et al*., 2019). The flasks were subjected to a 4-hour orbital shaker set at 500 rpm. To eliminate debris, the mixture was spun (Horizontal centrifuge, Swing-3000, Apogee, Germany) for 15 minutes at 1500 rpm after being filtered through a funnel fitted with filter paper (N°1; 100 µm). The organic extract's solvent, methanol, was evaporated at 60 °C using a Rotary vacuum evaporator (Laborgerate, GmbH, ISOLAB). The extracts were then diluted with 25 milliliters of DMSO (2%) (dimethyl sulfoxyde). For a maximum of twenty-four hours, the solution was kept at 4 °C as a stock solution.

2.2. Extraction of *Meliodogyne incognita*

Meliodogyne incognita was obtained from stock culture maintained on Celosia argentea. TLV 8. The whole soil-filled root gall was submerged in water, and the dirt was carefully cleaned without moving the egg masses. Gall roots were vigorously shaken in a 0.5% NaOCl solution for five minutes in order to extract the eggs. Mesh sieves were used to remove small stones and plant detritus (Hussey and Baker, 1973). After *M. incognita* eggs were ultimately gathered, all traces of NaOC1 were eliminated by washing the eggs numerous times in distilled water. In order to hatch the eggs into infectious second stage juveniles (J2), the retrieved *M. incognita* eggs were incubated at 28…2°C for 72 hours (Chen,,*et al*., 2020)

Aliquots of 1ml of *M. incognita* juveniles suspension containing a count of 100 freshly hatched juveniles was pipetted into each of the transparent glass petri-dishes containing 0.1 % methanol extract of *Afrohybanthus enneaspermus* leaf extract , 0.2% methanol extract of *Afrohybanthus enneaspermus* leaf extract , 0.3%methanol extract of *Afrohybanthus enneaspermus* leaf extract , 0.4%methanol extract of *Afrohybanthus enneaspermus* leaf extract . The petri- dish that contained 100 *M. incognita* juveniles in 10ml distilled water will serve as the control. The glass petri- dishes will be cover with glass covers to prevent evaporation and other pathogenic interference. All the glass petri-dishes were labeled accordingly and arrange randomly on the bench (Babaali*, et al*., 2017).

2.3. Pot experiment

Under supervision, the pot study was carried out in 30 cm diameter earthen clay pots filled with 2 kg of autoclaved soil in a 3:1 ratio (farmyard manure: sandy loam). The plant sample that had been ground up was added to the soil at a rate of 100 and 50 grams per pot. Watering was done on a regular basis to promote the right decomposition of the amended pots. Three sterilized tomato seeds (exposed to 1.0% NaOCl for 15 min) were sown in each container following the first 10 days of decay. One healthy seedling was kept in each treated pot, including the control, for two weeks following germination. Next, three holes were punched in each pot to introduce 3000 newly hatched second stage juveniles (J2s) of *M. incognita.*

3. Results and discussion

3.1. Effect of *Afrohybanthus anneaspermus* on the growth rate of Tomato (Tropimech +) infected with Root-knot nematode (*Melodogyne incognita*)

At the growth rate of the Tomato (Tropimech +), In table 2, plant height was significantly lowered at 4 WAP (four weeks after planting) in tomato plants inoculated with 2,000 root-knot nematodes (RKN) applied with 20 g powered of *Afrohybanthus enneaspermus* compared with the rest of the treatments. With the application of 15% aqueous plant extracts and the inoculation of 2,000 RKN, plant height was significantly higher (32.8), followed by Plant alone and 20% aqueous plant extract. However, plant height was significantly lowered in plants applied with 20g powered of *Afrohybanthus enneaspermus* and nematode compared to the other treatments. At 6 weeks plant height is significantly higher in tomato plants inoculated with 5% aqueous plant extract with 2000RKN followed by others that are at the same range except 20g powered extract with 2000RKN that is significantly lowered . At 7 weeks plant height is significantly higher in tomato plants inoculated with 5% aqueous plant extract with 2000 RKN followed by others that are at the same range except 20g powdered extract with 2000 RKN that is significantly lowered .

Table 1 Effect of *Afrohybanthus enneaspermus* on the growth rate of Tomato (Tropimech +) infected with Root-knot nematode (*Melodogyne incognita*)

Treatments	Plant height 4WAP	Plant height 5WAP	Plant height 6WAP	Plant height 7WAP
5% aqueous plant extract + 2000 RKN	27.60a	29.40ab	41.80a	43.80a
10% aqueous plant extract + 2000 RKN	27.20a	30.00ab	36.60ab	36.00ab
15% aqueous plant extract + 2000 RKN	28.00a	32.80a	39.40ab	38.60ab
20% aqueous plant extract + 2000 RKN	22.40a	23.40b	28.40bc	27.80bc
30% aqueous plant extract + 2000 RKN	26.00a	28.00ab	32.80ab	33.00ab
20g powdered extract + 2000 RKN	16.00b	14.60c	15.40c	14.80c
Plant + 2000 RKN	26.60a	28.00ab	30.00ab	29.40abc
3g carbofuran + 2000 RKN	27.00a	26.40ab	32.8-ab	35.00ab
Plant alone	24.60a	23.60b	27.80bc	30.00ab
LSD (p≤0.05)	6.19	8.62	13.35	15.51

Table 2 Effects of *Afrohybanthus enneaspermus* extract on leave shedding of Tomato (Tropimech +) infected with Root-knot nematode (*Meloidogyne incognita*)

Treatments	No. of leaves 4WAP	No. of leaves 5WAP	No. of leaves 6WAP	No. of dry leaves 6WAP	No. of leaves 7WAP	No. of dry leaves 7WAP
5% aqueous plant extract + 2000 RKN	35.80a	44.60a	77.40a	0.60a	44.00a	20.60a
10% aqueous plant extract + 2000 RKN	34.40ab	44.80a	55.00ab	4.80a	19.00a	21.80a
15% aqueous plant extract + 2000 RKN	34.00ab	53.40a	55.40ab	7.00a	27.80a	17.80ab
20% aqueous plant extract + 2000 RKN	30.60ab	42.80a	34.60b	7.20a	17.60a	17.40ab
30% aqueous plant extract + 2000 RKN	34.40ab	49.80a	52.40ab	4.80a	20.60a	20.20a
20g powdered extract + 2000 RKN	18.40c	22.00b	24.40b	3.20a	17.60a	6.60b
Plant + 2000 RKN	35.00ab	45.80a	37.60b	7.20a	26.40a	12.60ab
3g carbofuran + 2000 RKN	32.00ab	44.40a	44.60ab	8.80a	32.80a	14.80ab
Plant alone	27.20b	59.60ab	38.40	5.20a	31.00a	14.00ab
LSD (p≤0.05)	8.49	18.82	35.58	8.72	28.96	12.99

3.2. Effects of *Afrohybanthus enneaspermus* extract on leave shedding of Tomato (Tropimech +) infected with Root-knot nematode (*Meloidogyne incognita*)

Number of plant leaves was significantly lowered at 4 WAP (four weeks after planting) in tomato plants inoculated with 2,000 root-knot nematodes (RKN) applied with 20 g powered of *Afrohybanthus enneaspermus* compared with the rest of the treatments, With the application of Plant alone, Number of plant leaves at 5WAP was significantly higher (59.60), followed by 15% aqueous plant extract with 2000 RKN and 30% aqueous plant extract with 2000 RKN with 53.40 and 49.80 respectively. However, Number of plant leaves was lowered in plants applied with 20g powdered extract + 2000 RKN (22.00) compared to the other treatments this indicate only powder extract of the sample is more effective against leave shedding effect caused by nematodes

At 4 WAP Number of plant leaves is higher in tomato plants inoculated with 5% aqueous plant extract with 2000 RKN (35.80) followed by others that are at the same range (30.00 to 35.00) except 20g powdered extract with 2000 RKN

(18.40) that is significantly lowered. At 6 WAP Number of plant leaves is significantly higher in tomato plants inoculated with 5% aqueous plant extract with 2000 RKN (77.40) followed by 10% aqueous plant extract with 2000 RKN (55.00) and 20g powdered extract with 2000 RKN is significantly lowered at (24.40). At 6 WAP Number of dried plant leaves is significantly higher in tomato plants inoculated with 3g carbofuran with 2000 RKN (8.80) followed by 20% aqueous plant extract with 2000 RKN (7.20) and 5% aqueous plant extract with 2000 RKN is significantly lowered at (06.0).

At 7 WAP Number of plant leaves is significantly higher in tomato plants inoculated 5% aqueous plant extract with 2000 RKN (40.00) followed 3g carbofuran with 2000 RKN (32.80) and 20g powdered extract with 2000 RKN and 20% aqueous plant extract with 2000 RKN are significantly lowered at (17.60), At 7 WAP Number of dried plant leaves is significantly higher in tomato plants inoculated 10% aqueous plant extract with 2000 RKN (21.80) followed 5% aqueous plant extract with 2000 RKN (20.60) and 20g powdered extract with 2000 RKN is significantly lowered at (6.60).

Table 3 *In-vitro* experiment of nematicidal potential of *Afrohybanthus enneaspermus* (African bush tea) on the management of root-knot nematode

Day	Petri-dish	rep no	No. J2 dead	No of eggs	no of J2 alive	total	disintegrated 900
3AI	water only	1	na	452	22	474	448
	5% extract	1	0	518	34	552	382
	5% extract	2	0	497	22	519	403
	5% extract	3	0	333	25	358	567
	5% extract	4	0	304	37	341	596
	10% extract	1	0	417	34	451	483
	10% extract	2	0	181	2	183	719
	10% extract	3	0	218	3	221	682
	10% extract	4	0	255	3	258	645
	15% extract	1	0	108	1	109	792
	15% extract	2	0	209	1	210	691
	15% extract	3	0	113	1	114	787
	15% extract	4	na	74	3	77	826
5AI	water only	1	na	136	60	196	764
	5% extract	1	2	284	83	367	616
	5% extract	2	8	68	73	141	832
	5% extract	3	0	279	110	389	621
	5% extract	4	1	155	80	235	745
	10% extract	1	6	454	60	514	446
	10% extract	2	1	196	43	239	704
	10% extract	3	5	278	38	316	622
	10% extract	4	3	198	36	234	702
	15% extract	1	4	305	21	326	595
	15% extract	2	0	183	11	194	717

3.3. *In-vitro* experiment of nematicidal potential of *Afrohybanthus enneaspermus* (African bush tea) on the management of root-knot nematode

There are 900 nematodes in each petri dish. and Rate of plant extract: 5%, 10%, 15%, and 0% (water only) for in vitro experiments. The table above illustrates that 15% plant extract has the highest nematode disintegration level, whereas water alone (control) at 3AI has the lowest nematode level. However, in Table 7 and Figure 2 demonstrate that the highest amount of nematodes that have disintegrated is found in water alone (control), whereas the lowest amount of nematodes that have disintegrated is found in 10% extract @5AI while Table 8 and Figure 3 reveal that the 10% extract at 7AI has the lowest disintegrated amount of nematode, whereas the greatest level is observed in water alone (control).

Table 4 Effects of *Afrohybanthus enneaspermus* (African bush tea) on the population control of root-knot nematode

Day	Petri-dish	rep no	No. J2 dead	No of eggs	No of J2 alive	total	disintegrated 900
7AI	15% extract	3	33	333	11	344	567
	15% extract	4	0	169	28	197	731
	water only	1	0	93	100	193	807
	5% extract	1	na	132	141	273	768
	5% extract	2	2	153	152	305	747
	5% extract	3	1	111	197	308	789
	5% extract	4	0	64	119	183	836
	10% extract	1	1	204	203	407	696
	10% extract	2	0	215	143	358	685
	10% extract	3	1	121	76	197	779
	10% extract	4	0	231	129	360	669
	15% extract	1	0	191	79	270	709
	15% extract	2	0	98	64	162	802
	15% extract	3	9	243	73	316	657
	15% extract	4	2	90	77	167	810

Table 5 The mean square variance of nematicidal potential of *Afrohybanthus enneaspermus* (African bush tea) on the management of root-knot nematode with the Population of nematode for 3AI

Day	Petri-dish	no of eggs	no of J2 alive	Total no of eggs& J2 ALIVE	disintegrated 900
3AI	water only	452	22	474	448
3AI	5% extract	413	29.5	442.5	487
3AI	10% extract	267.75	10.5	278.25	632.25
3AI	15% extract	126	1.5	127.5	774

Table 6 The mean square variance of nematicidal potential of *Afrohybanthus enneaspermus* (African bush tea) on the management of root-knot nematode with the Population of nematode for 3AI

Day	Petri-dish	no of eggs	no of J2 alive	Total no of eggs& J2 ALIVE	disintegrated 900
3AI	water only	452	22	474	448
3AI	5% extract	413	29.5	442.5	487
3AI	10% extract	267.75	10.5	278.25	632.25
3AI	15% extract	126	1.5	127.5	774

Table 7 The mean square variance on effects of *Afrohybanthus enneaspermus* (African bush tea) on the population load of root-knot nematode for 5AI

Day	Petri-dish	no of eggs	no of J2 alive	Total no of eggs& J2 Alive	Disintegrated 900
5AI	water only	136	60	196	764
	5% extract	196.5	86.5	283	703.5
	10% extract	281.5	44.25	325.75	618.5
	15% extract	247.5	17.75	265.25	652.5

Table 8 The mean square variance of nematicidal potential of *Afrohybanthus enneaspermus* (African bush tea) on the management of root-knot nematode with the Population of nematode for 7A

Day	Petri-dish	No of eggs	No of J2 alive	Total no of eggs& J2 ALIVE	Disintegrated 900
7AI	water	93	100	193	807
	5% Extract	115	152.25	267.25	785
	10% Extract	192.75	137.75	330.5	707.25
	15% Extract	155.5	73.25	228.75	744.5

4. Conclusion

The research concluded that leaves extract of *Afrohybanthus enneaspermus* is effective in management of root Knot nematodes, however, in- vitro experiments, it is established that the powder form of the leave has higher effectiveness in management of nematode than its organic extract.

Compliance with ethical standards

Disclosure of conflict of interest

We the authors of this article unanimously agree that there is no conflict of interest

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