



Research Article

Flavonoids from Physicnut (*Jatropha curcas*) as Nematicides for Root-Knot Nematode (*Meloidogyne incognita*) Management on Okra (*Abelmoschus esculentus* L.) Moench

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Abstract | Plant defense response is a function of bioactive phytochemicals which confer pesticidal value or ability. The bio-nematicidal activity of Physicnut (*Jatropha curcas*) flavonoids on okra root-knot nematode was investigated in an *in vitro* and screenhouse study. Root flavonoids applied at 10ml/petri dish resulted in the highest nematode mortality (93 %) after a 72-hour incubation period and differed significantly ($P < 0.05$) from nematode mortality (80 %) at 5 ml/pot. Although all potted plants in the screenhouse were inoculated with 1,200 second-stage nematode juveniles (J2s), results showed that, when compared to the untreated control that was severely galled (4.0), the application of leaf flavonoids resulted in a significant reduction ($p < 0.05$) in number of galls and root-gall index on okra. The root-gall index decreased from 4.0 (severely galled) to 1.4 (rarely galled), while the seed and root flavonoids reduced galling to 2.0 (slightly galled). While improving growth and yield attributes, the highest dosage of 5 ml/pot had the greatest influence on the root-gall index and yield. There was a significant correlation (-) between yield and the root-gall index, supporting the idea that flavonoids might boost okra growth and production while reducing galling. While the application of root and seed flavonoids at a rate of 5 ml/pot resulted in the greatest 100 dry seed weight and enhanced mucilaginous properties of okra, increases in leaf areas, plant heights and okra pod weights (75% over control) were supported by the administration of root and seed flavonoids. These results demonstrated the possibility of Physicnut flavonoids as a viable alternative to synthetic nematicides for the management of *M. incognita*.

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Keywords | Flavonoids, Physicnut, *Meloidogyne incognita*, Mortality, Okra yield, Root-knot nematode



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Introduction

The losses that *Meloidogyne* species cause on a variety of agricultural crops make them a serious threat to crop output in Africa. According to [Abad et](#)

[al. \(2008\)](#), the incidence and severity of *Meloidogyne* spp. cause an estimated \$157 billion in losses annually around the world. To combat this issue, the present approach relies mainly on chemical pesticides that are synthetic in nature and a variety of persistent organic

pesticides (POPs), often at the expense of other forms of control.

Over the years there has been a continued reliance on these synthetic pesticides which makes it even more urgent for concrete steps to be taken to help reduce the health and environmental risks of these pesticides used in developing countries. Of utmost concern is the need to encourage a rapid shift away from these dangerous chemicals (persistent organic pesticides) used in plant protection today (ECHA, 2020).

Although, many of these pesticides (Chlorofluorocarbons, Halons and Methyl bromides) have been banned or restricted due to their negative impacts, as outlined in Regulation (EC) No 1107/2009 and Regulation (EC) No 396/2005 (Dubby *et al.*, 2011; European Commission, 2020), recent developments point to the growing extent of their use in developing countries despite the awareness of the risk associated with them (Möhring *et al.*, 2020). Nevertheless, chemical control of nematode pest remains the most effective control measure, despite some serious constraints. Chemical nematicides are very toxic to mammals and beneficial soil microfauna, pollute ground water and have residual effect on farm produce (Lohar, 2001)

Despite interest generated by Okra as an invaluable and highly cherished vegetable crop, the yield potential has not been achieved in this part of the tropics. The root-knot nematode *Meloidogyne incognita* has been reported as one of the most limiting factors to Okra production (Mukhtar, 2014) leading to 90 % yield loss when grown in fields infested with 3-4 *M. incognita* J2 (second-stage juveniles) per gram soil (Claudius-Cole, 2018). In order to improve the performance and yield of okra, there is the need to find ways to address this challenge. This clearly indicates the need for an environmentally friendly and sustainable alternative to synthetic nematicides such as nematode antagonistic plants (Ogwudire *et al.*, 2019)

Many natural products, including essential oils as well as aqueous extracts, have been shown to have a biocidal impact on bacteria, weeds, and fungus because of the discovery of nematode antagonistic chemicals in plants (Maistrello *et al.*, 2010). Moreso, Akinmoladun *et al.* (2007) stated that the medicinal value of a plant is determined by their phytochemical constituents. The Physicnut plant has been reported to possess

biologically active phytochemical constituents one of which is flavonoids (Igbinosa *et al.*, 2009).

Flavonoids are metabolites of secondary origin involved in both root growth as well as plant defense mechanisms against various kinds of microorganisms (Chin *et al.*, 2018). They have also been reported to carry out several pharmacological actions involving cancer prevention, antiviral properties, antitoxic, and hepatoprotective activity (Abdelgadir and Staden, 2013). The defensive roles and activity exhibited by flavonoids against microorganisms clearly indicates the need to scientifically validate the bioactive potential of Physicnut flavonoids. This study is therefore aimed at evaluating the effect of physicnut flavonoids on the root-knot nematode *M. incognita* affecting okra under *in vitro* and *in vivo* conditions.

Materials and Methods

Study location

The experiments were conducted in the Laboratory and Screenhouse of the Crop Science and Technology Department at the Federal University of Technology, Owerri (FUTO), Nigeria. This was situated at Lat 5° 27' 50.23" North and Longitude 07° 02' 49.33" East, at 55 m above mean sea level of Owerri rain forest Agroecology distinguished by an annual rainfall of over 2500 mm, a temperature range of 27–29 °C, and 89–93% range of humidity. Agu (2008) and Eisenback *et al.* (1981) reported that the soil had a loamy sand texture and was infested with root-knot nematodes.

Plant materials and isolation of flavonoids

Freshly harvested materials from 10 plant-stands comprising roots, seeds and leaves from 1-year-old physicnut was collected from FUTO and identified in the herbarium of Crop Science and Technology Department. The samples were dried under shade for 7 days before being ground using a mortar and pestle into a coarse powder. It was later sieved into fine particles with a sieve of 212 mm aperture and kept dry until when needed. Soxhlet extraction was used to defatten the finely ground (30g) Physicnut samples using N-Hexane (250ml). The methanolic extract was then separated into its flavonoid fraction using column chromatography on a silica gel column (230–400 mesh) and eluted using a solvent consisting of CH₃Cl/CH₃OH/H₂O in a ratio of 70:30:1 V/V. (Musa, 2015). The yield was obtained from the re-crystallization process of purifying the collected

flavonoid fraction.

Test for flavonoids

Flavonoids were detected when a bright yellow colour was noticed in a solution of 2 ml of plant extract that had been acidified using 1% HCL and dissolved into 20 % NaOH (Herbone, 1980).

Nematode mortality (In vitro)

At 0, 5, and 10 ml, crude flavonoids extracted from plant components were evaluated against thirty nematodes (J2s) *in vitro* under 24, 48, and 72-hours incubation and replicated three times. All the Petri dishes were maintained on laboratory bench at ambient temperature ($\pm 30^{\circ}\text{C}$). Dead as well as live juveniles (J2) were identified after 24, 48, and 72 hours of being exposed to each treatment using an electronic stereomicroscope with a magnification of 100x. When the juvenile (J2) did not respond to physical stimulation with a tiny needle, it was pronounced dead (Hong *et al.*, 2007). The experiment was repeated twice and the mean taken.

Screenhouse (In vivo)

A Completely Randomised Design (CRD) was used to conduct the experiment in the screen house. The physicnut components had three treatment levels (root, seed, and leaves), and each of the flavonoids were added at six rates (0, 1, 2, 3, 4, and 5 ml per pot). Thus, a 3 x 6 factorial experiment with five replications set up in a Completely Randomized Design was carried out. Ninety pots, filled with 4 kg of steam-sterilized soil and planted with Okra cv. NH47 - 4 were randomized in the screenhouse. Pots received different dosages of the flavonoids at time of inoculation. Plants that were inoculated but not treated acted as control.

Planting

One-hundred seeds of okra NH47-4 were surface sterilized with 10% NaOCl (i.e., 10g of Sodium hypochlorite for every 100ml of solution) for 1-minute and then rinsed thoroughly with sterile distilled water. The seeds were germinated on moist filter paper in Petri dishes. Prior to inoculation, each pot was seeded with two germinated seeds, which were subsequently thinned to a single plant per pot. The National Horticultural Research Institute, Okigwe in Imo State, Nigeria, provided the test okra cultivar that was used.

Inoculum preparation

The inoculum source was the Owerri *M. incognita* population (identified using the perineal p pattern) that was maintained on okra in pot cultures. Infected galled roots were chopped in a blender with 1 liter water, running at three-second intervals. More water was added to the slurry to make up 1200 ml. A Petri-dish was filled with 30 ml of this mixture, and the J2 were counted in three samples under a stereomicroscope.

Application of inoculum and phytochemicals

For inoculation, 90 ml of the blended slurry containing on average 1200 J2 was added to the base of the okra plants, ten days after planting and then covered with sterilized soil. Using formula $V_1C_1 = V_2C_2$, (Naz *et al.*, 2013), a final concentration of 50 mg/ml was achieved by diluting the pure flavonoid substrate extraction with distilled water. Flavonoids were applied at the base of the potted plants: 0 ml (control), 1 ml, 2 ml, 3 ml, 4 ml, and 5 ml per pot. The pots were then placed in a screen house with adequate ventilation. The average temperature of soil was noted as $\pm 31^{\circ}\text{C}$.

Assessment of infections

The experiment was concluded twelve weeks after inoculation. At this time every plant had fruits and growth and yield characteristics were measured. The root systems were rinsed with water and the severity of root galling was visually assessed on a 0 to 4 scale (Agu and Ogbuji, 1996) for comparison.

Data analysis

Analysis of variance was used and to determine treatment effects. Fisher's Least Significant Difference (F- LSD) was used to differentiate mean differences at the 5% probability level (GENSTAT Edition 4). SPSS version 22.0, was used to determine the correlation between the root-gall index and yield.

Results

Nematode mortality (In vitro)

Results of the study showed that the physicnut (*Jatropha curcas*) sections gave the following percentage yields of flavonoids isolated: Seeds (5.51%), leaves (5.51%) and roots (3.59%) respectively. In Figure 1, the flavonoids extracted from *Jatropha* caused a significant ($P < 0.05$) nematode mortality. The highest percentage of mortality (93 %) resulted from 10 ml of flavonoids, which was significantly

($P < 0.05$) higher than the 80 % mortality recorded at 5 ml and the 0 % mortality in the distilled water (control). Increasing exposure times of J2 to *Jatropha* flavonoids resulted in increasing mortality rates. Mortality was also affected by the source of flavonoids extract ($P < 0.05$) at the 5 and 10 ml rates. Mortality resulting from root flavonoids (98 %) was higher at the 5 and 10ml treatments than mortality resulting from seed flavonoids (83 %) and leaf flavonoids (81 %)

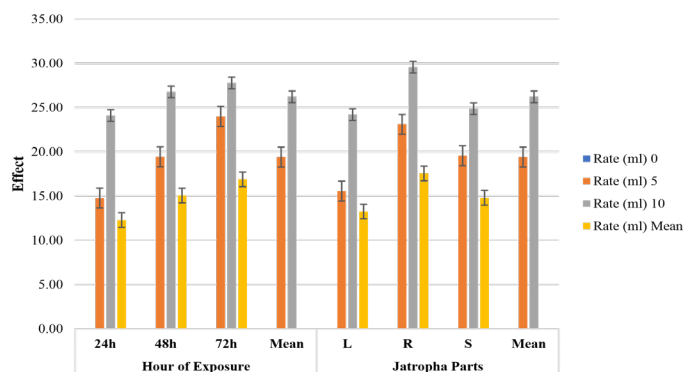


Figure 1: Mortality of *M. incognita* as affected by Flavonoids, hour of exposure and *Jatropha* plant parts.

Root-gall index (In vivo)

Okra plants in the untreated controls had an average gall index of 4 (severely galled). Significant reductions in gall index resulted after 5 ml treatment with root flavonoids (gall index 2.2), seed flavonoids (gall index 2.0), and leaf flavonoids (gall index 1.4) (Figure 2).

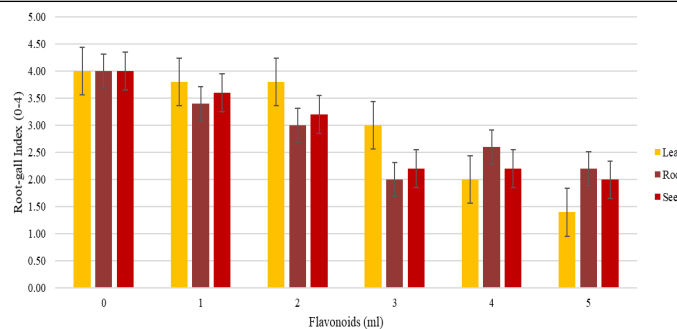


Figure 2: Effect of flavonoids and *jatropha* parts on root-gal infection by *M. incognita*.

Scale: 0 = No galls i.e. infection-free. 1 = Rare (1-3 galls), 2 = Mild infection (4-10 galls). 3=Moderate infection (11-30 galls) and 4=Severe infection (over 30 galls).

Growth and yield of okra

Table 1 shows the effect of *Jatropha* flavonoids and plant sections on okra height, leaf area, pod weight and 100 dry-seed weight as affected by *M. incognita*. In comparison to the highly galled untreated control plants, pots treated with flavonoids had fewer galls and yielded significantly ($p < 0.05$) greater plant heights. Plant heights from leaf flavonoids produced plant heights which were significantly ($p < 0.05$) greater than the seed and root flavonoids. However, there was no significant difference in plant heights between seed and root flavonoids application. Interaction of *Jatropha* parts and flavonoids where not significant ($p < 0.05$).

Table 1: Effect of *Jatropha* flavonoids and plant parts on okra leaf areas, plant heights, pod weights and 100 dry seed weights as affected by root-gall nematode.

Flavonoids (ml)	Plant heights (cm)				Leaf areas (cm ²)				Pod weights (g)				100 dry seed weights (g)			
	Jatropha plant parts			Mean	Jatropha plant parts			Mean	Jatropha plant parts			Mean	Jatropha plant Parts			Mean
	Leaf	Root	Seed		Leaf	Root	Seed		Leaf	Root	Seed		Leaf	Root	Seed	
Untreated (control)	6.34	5.49	5.16	5.67	3.24	3.15	3.00	3.13	1.16	0.44	0.62	0.74	1.40	2.45	1.17	1.67
1	7.54	6.51	6.72	6.92	5.04	4.20	4.21	4.48	1.21	0.52	1.04	0.92	2.05	3.55	2.40	2.66
2	8.09	7.01	7.3	7.47	5.74	4.85	4.93	5.17	2.25	0.53	1.13	1.30	2.30	3.60	3.38	3.09
3	8.71	7.82	7.56	8.03	8.54	5.94	5.71	6.73	2.45	1.51	2.61	2.19	2.80	4.15	3.80	3.58
4	9.88	8.66	8.7	9.08	9.61	6.88	7.86	8.11	3.16	1.56	3.02	2.58	2.90	4.75	4.20	3.95
5	10.36	9.42	9.72	9.83	13.79	7.56	8.70	10.02	3.60	2.00	3.52	3.04	3.30	5.65	4.95	4.63
Mean	8.49	7.49	7.53		7.66	5.43	5.74		2.30	1.09	1.99		2.45	4.02	3.31	
LSD _{0.05} (Jatropha parts)	0.38				0.47				0.03				0.03			
LSD _{0.05} (Flavonoids)	0.54				0.67				0.05				0.05			
LSD _{0.05} (Jatropha parts X Flavonoids)	ns				1.17				0.08				0.09			

ns = Not significant.

Flavonoids and Jatropha parts were also found to have had significant ($p < 0.05$) effect on okra leaf area. Plants in pots which received varied amounts of flavonoids had fewer galls producing significantly ($p < 0.05$) greater leaf area than the highly galled control pots. This was also observed in the interaction of Jatropha parts and leaf flavonoids which produced highest leaf areas at 5 ml/pot treatment application. This was followed by plant heights produced by seed and root flavonoids, respectively.

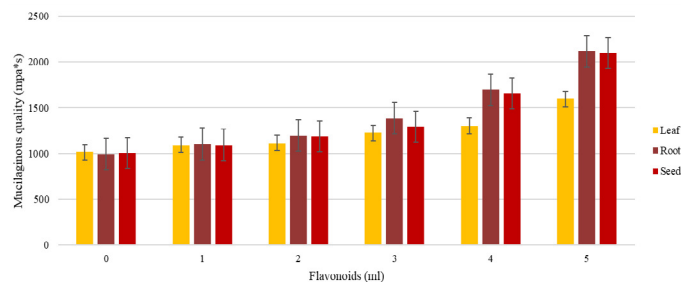


Figure 3: Effect of Jatropha flavonoids and plant parts on Okra's mucilaginous property as affected by root-gall nematode.

Jatropha parts' flavonoids had significant ($p < 0.05$) effect on the weight of okra pods and root-gall incidence. Consequent upon this, Okra's mean pod weights also rose together with increased dosage of flavonoids applied at reduced gall incidence. Plants in pots treated with flavonoids showed decreased root-galls and produced significantly ($p < 0.05$) greater number of pods than the heavily galled control. Interactions of Jatropha parts and flavonoids significantly ($p < 0.05$) affected okra pod weights. This was more so on pots treated with 5 ml of leaf flavonoids which produced the highest pod weights compared to pots treated with seed and root flavonoids. This contrasted with the lowest pod weights reported on highly galled plants raised on untreated pots.

Plants in pots treated with flavonoids showed less root-galls and yielded considerably ($p < 0.05$) greater 100-dry seed weight than the heavily galled control pots. Mean 100 dry seeds of Okra increased with increased application of flavonoids treatments. Plants from pots which received root flavonoids recorded significantly higher ($p < 0.05$) 100 dry seed weights than those from seed and leaf flavonoids. In comparison with the control group pots and other rates, root flavonoid at 5 ml yielded the greatest 100 dry seed weights by a significant ($p < 0.05$) margin. This was followed by seed flavonoids. Lowest 100 dry seed weights were however, produced by plants administered leaf flavonoids.

Results of the effect of flavonoids and Jatropha parts on Okra mucilaginous property showed that pots with plants treated with flavonoid had fewer root-galls and yielded okra with significantly higher ($p < 0.05$) mucilaginous property than the heavily galled okra plants raised in untreated pots (Figure 3). Mucilaginous property significantly ($p < 0.05$) increased with increased rates of flavonoid treatment applied. Root flavonoids at 5 ml produced plants with significantly ($p < 0.05$) high mucilaginous property (2114 mpa*s) in contrast to the untreated control and other rates. Next was okra treated with 5 ml of seed flavonoids (2095 mpa*s). Plants administered leaf flavonoids recorded the least mucilaginous property (1595 mpa*s).

Correlation analysis of the root gall index and yield attributes of okra as influenced by the flavonoids extracts in the pot experiments is shown in Table 2. The results indicated a negative and highly significant correlation of root-gall index with plant height. The Mucilaginous property correlated positively and significantly with plant height. The same was true for pod weight, hundred seed weight, number of leaves and leaf areas.

Table 2: The linear matrix of correlation involving root-gall indices and growth indicators as affected by flavonoids treatment

1 st trial	PHT	LA	NLVS	PWT	HDS	MP	RGI
					WT		
PHT	1	.807**	.779**	.794**	.544**	.683**	-.684**
LA		1	.870**	.828**	.411**	.590**	-.684**
NLVS			1	.767**	.400**	.570**	-.667**
PWT				1	.381**	.618**	-.659**
HDSWT					1	.861**	-.626**
MP						1	-.644**
RGI							1
2 ND Trial							
PHT	1						
LA	.816**	1					
NLVS	.784**	.749**	1				
PWT	.785**	.834**	.634**	1			
HDSWT	.555**	.435**	.382**	.399**	1		
MP	.619**	.570**	.376**	.608**	.849**	1	
RGI	-.558**	-.617**	-.344**	-.605**	-.617**	-.646**	1

LA= leaf area, RGI= root-gall index PHT = Plant Heights
HDSWT = 100 Dry Seed Weight MP = Mucilaginous property -mpa*s

Discussion

Jatropha plant parts differed in the percentage yield of flavonoids available in the leaves, seed and roots. The highest concentration was found in the leaf and seed, followed by the root respectively. Several workers had earlier reported the presence and distribution of *Jatropha curcas* phytochemicals in various plant parts. (Makkar *et al.*, 2009; Igbinsosa *et al.*, 2009, 2011; Namuli *et al.*, 2011).

J. curcas flavonoids caused mortality of nematode juveniles which in comparison with the untreated control was as a result their nematicidal activity. Gattuso *et al.* (2007) and Kashif *et al.* (2015) confirmed the involvement of various classes of flavonoids in cytotoxic and anticancer activity. These studies also revealed that flavonoids which are widely reported as antioxidants also possess promising nematicidal activity, some of which were found to be more active than carbofuran (Bano *et al.*, 2020). Corroboratively, Wuyts *et al.* (2006) stated that the mode of action of flavonoids in such bioassay is caused by oxidative-degradation products (simple phenolics).

Rise in nematode mortality with additional increase in the dosage of flavonoids applied agrees with the results obtained by Zaidat *et al.* (2020) who observed greatest mortality rates when the concentration of *T. baccata* extract was raised from 40- 80 %. Nematode mortality rose following a rise in hours of the flavonoid's treatment exposure. This corresponds with the study of Afzal *et al.* (2021) who showed that nematode mortality rose as the exposure period increased in his work with botanical extracts against nematodes. The inoculated but untreated control okra plants were severely galled. Coyne *et al.* (2007) reported that plants that are severely galled often wilt quickly and may also show signs of nutrient stress and deficiency because galled roots can't absorb or move water and nutrients to other parts of the plant as well. In other words, the conspicuous giant cells (galls) observed on the severely galled root decreased the ability of infected plants to translocate minerals. The conductive tissues no longer function properly consequently impairing growth (Alabama and Alabama, 2009).

Although, flavonoids extract of various plant parts greatly reduced root-gall index, decreased galling could be seen more with the performance of seed

and leaf extracts of flavonoids in the screenhouse experiment. This may have been due to the high percentage composition of flavonoids in the leaf (5.51 %) and seed (5.51%) over the root (3.59 %). Again, Devappa *et al.* (2010) had also reported the concentration of cursin (active ingredient) in the endosperm of physicnut seed. However, flavonoids application reduced galls and gave better yield performances than the untreated control plants which were severely galled. This might have been as a result of flavonoids nematicidal abilities. This might be due to the nematicidal activities of flavonoids. Since they are secondary metabolites with many pharmacological activities such as anticancer, antinematicidal and hepatoprotective activities (Abdelgadir and Staden, 2013).

Nevertheless, increases recorded in the plant heights of the treated okra plants than the untreated could be attributed to the nematicidal effect of the flavonoids extract on the activities of the nematodes thereby creating conditions for optimal plant growth. This agrees with the works of Ihejirika (2006). On the contrary, reduction in plant heights of severely galled untreated control plants could have resulted from the incidence and severity of the nematodes on the inoculated (control) plants. Mukhtar *et al.* (2013) reported that all inoculum levels of *M. incognita* caused significant reductions in plant heights and fresh shoot weight at all plant ages of okra.

The observed increases recorded in the leaf areas of plants might have been as a result of the enhanced growth attributes in the treated plants due to the nematicidal potential of the flavonoid extract on *M. incognita*. Emeasor *et al.* (2002) reported that extracts from plants disrupt organism's metabolic activities. This no doubt led to increased physiological activities in the treated plants.

The production of leaves and leaf areas are thus increased with increased rates/dosage of flavonoids treatments applied. Reduction in leaf areas and production in the infected but untreated control pots may also have been caused by chlorophyll reduction in diseased plants (Parveen *et al.*, 2006). Jonathan and Rajendan (2002) also stated that plants infected with *M. incognita* results in decreased leaf area production.

The increased okra pod weights recorded could be attributed to improved physiological activities of the

flavonoids treated plants. Agu *et al.* (2013) reported that more leaf production on root-gall nematode controlled tomato plants added to the photosynthetic rate of the plants and characteristically resulted in increased fruit. This was most pronounced on the leaf flavonoid treated plants at 5ml/pot.

The recorded improvement on growth and yield characteristics such as 100 dry seed weights associated with decreases in root-gall damage can be attributed to improved nutrient absorption and translocation to vegetative organs due to nematicidal effect of leaf flavonoids treatment. Agu *et al.* (2013) also stated that plants with fewer root-galls would translocate more nutrients to vegetative organs for proper seed and pod development than heavily galled roots.

Mucilaginous property was highest with increased rate of leaf flavonoid treatment (Agu *et al.* 2013). This is an indication that root galling activity decreased with increased application of *Jatropha* parts flavonoids. Similarly, Agu *et al.* (2009) also noted a steady increase in mucilaginous property in okra pods as okra galling response to *M. incognita* decreased at increased carbofuran rate.

Negative correlation which exists between yields of okra and its gall index revealed that when galls rose, the growth and yield characteristics of okra diminished. This was because of abnormal cells (galls) interference with plants ability to transport nutrients and moisture (Anwar and Mckenry, 2010). Increases in leaf number and area at lower galling responses led to higher photosynthesis and enhanced development and yield properties of the okra, as shown by the correlation seen between pod weights, leaf area, 100 seed weights, number of leaves produced, and mucilaginous property which was positive. Agu *et al.* (2013) reported that enhanced leaf formation on okra plants under root-gall nematode control boosted the plants rate of photosynthetic activity and as expected, produced a higher yield of pod weight.

Conclusions

The error bars, underscores the statistical significance of these results, highlighting the importance of flavonoids rate and exposure duration, along with the specific *Jatropha* part used in determining the mortality rate of *M. incognita*. Results of this study also highlights the importance of exposure duration

in managing nematode populations thereby offering valuable insights for creating environmentally friendly and sustainable pest control strategies that will increase yield. Present research is therefore a pointer to the effectiveness of *Jatropha* parts' flavonoids as nematicidal agent and may consequently be deployed successfully for use in integrated pest management programme. More so, the potency of *Jatropha* flavonoids in this research presents enormous potential for novel biotechnological uses in nematicidal formulations. However, trials on various classes of flavonoids and assessment in field encouraged to build on the achievements of this work.

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Novelty Statement

Plant secondary metabolites (phytochemicals) have been rarely exploited for improving crop yield under root-knot nematode attack. These results showed that flavonoids application was effective in substantially controlling root-knot nematode disease on Okra while improving growth and yield attributes. This study has scientifically validated physicnut flavonoids as a potential substitute for the use of harmful chemical nematicides to control *M. incognita* and future production of an eco-friendly nematicide.

Conflict of interest

The author has declared no conflict of interest.

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