

Research Article

Evaluation of the Anti-Helminthic Latency of Four Native Biopesticides on *Meloidogyne* Species of Amaranth (*Amaranthus cruentus* L.) Under Organic Conversion

Adebogun, A. M.¹, Atungwu, J. J.*¹, Lawal-Adebowale, O. A.², Joseph-Adekunle, T. T.³, Eche, C. O.⁴, Ononuju, C. C.⁵ and Oladeji, O. A.¹

¹ Department of Crop Protection, Federal University of Agriculture Abeokuta (FUNAAB), Ogun State, Nigeria

² Department of Agricultural Extension and Rural Development, FUNAAB, Nigeria

³ Department of Horticulture, FUNAAB, ⁴Department of Crop and Environmental Protection, Nigeria

⁴ Federal University of Agriculture Makurdi, Benue State, Nigeria

⁵ Department of Plant Health Management, Michael Okpara University of Agriculture, Abia State, Nigeria

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Abstract: Experiment was conducted in field under organic conversion, to determine the effect of four rates of indigenous biopesticides on root-knot disease, growth and yield of *Amaranthus cruentus*. The trial was laid out in a Randomized Complete Block Design. The experiment consisted of 16 treatments replicated three times with the dimension 3 m x 4 m. Seeds were first planted in the nursery bed to raise healthy seedlings for transplanting four weeks after emergence. Pre-plant herbivorous nematode population was determined prior to seedling transplanting. The biopesticides were applied at the rate of 0, 100, 125 and 150 ml/16 Litre of water by spraying them between 6.30 and 7.30 am biweekly as foliar and soil applications. Growth parameters and nematode population were taken two Weeks After Biopesticide Applications (WABA) and sustained bi-weekly until 8 WABA. Nematode census was again determined 60-66 Days after Transplanting. Data obtained were subjected to Analysis of Variance using Statistical Analysis System (SAS) version 10.2 and statistically significant treatment means were separated using the new Duncan Multiple Range Test at 5% level of probability. The four tested biopesticides suppressed ($p \leq 0.05$) nematode population and number of galls with attendant better ($p \leq 0.05$) growth performance compared with the control (unsprayed plots). BIO183 and BIO184 at 100 ml were the most efficacious. It is recommended that BIO181 at 100 ml can be further verified on farmers' fields for possible adoption for root-knot nematode population management in *A. cruentus*.

Keywords: Eco-friendly pest management, Herbivorous nematodes, Organic agriculture, Plant extracts, Sustainable agriculture.

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INTRODUCTION

Amaranthus (*Amaranthus cruentus*) is a commonly grown leafy vegetable throughout Nigeria and other West African countries for food (Saunders and Beciker, 1984; Adeyemi *et al.*, 1987; Tosi *et al.*, 2001; Dhellot *et al.*, 2006; Mepha *et al.*, 2007) and medicinal importance for people with hypertension, internal bleeding and cardiovascular disease (Omosun *et al.*, 2008). High demand for vegetables in the cities and towns has stimulated the growth of market gardening, in addition to being a good source of employment for young school leavers (DFRRI, 1990). Vegetable amaranth has been rated to have higher minerals, such as calcium, iron, phosphorous and carotenoids than grain amaranth (Alegbejo, 2013) than most other vegetables thus making them not only nutritionally important for humans but also for livestock feeding (Sleugha *et al.*, 2001).

However, *Amaranthus* cultivation has been constrained by decreasing soil fertility and pest insurgences. Root-knot nematodes, *Meloidogyne* spp., represent one of the most economically damaging plant-parasitic nematodes because of its wide range and widespread distribution (Atungwu *et al.*, 2013). In Nigeria, there are three known species of root-knot nematodes, namely, *Meloidogyne incognita* (Mi), *M. javanica* (Mj) and *M. arenaria* (Ma), in their order of importance. These species are found in all parts of the country, with serious economic importance as they can burrow into the soft tissue of young root tips and cause the nearby root cells to divide and enlarge which is the root-knot disease that deprives vegetables and other host crops of the required nutrients and water for optimum growth and yield. Contrary to Ogunfowora (1982) report that *M. incognita* is prominent in the Southwestern region, these species have been

documented to occur as mixed populations under natural field conditions making their control very demanding and requiring care (Atungwu *et al.*, 2013).

The growing pest insurgences, including new pests, their damage in the emerging organic vegetable fields and the regulatory restriction of the use of synthetic pesticides by practitioners, biopesticides will play a major role in sustainable pest management that is eco-friendly. Therefore, the objectives of this research were to 1) evaluate the effect of four indigenous biopesticides on root-knot nematode in *Amaranthus cruentus* and 2) determine the effect of the four biopesticides on growth and yield of *A. cruentus*.

MATERIALS AND METHODS

The experiment was carried out on the Organic Agriculture Skills Demonstration plot of the Federal University of Agriculture, Abeokuta (7°13'55" N, 3°25'58" E, 168 mm above sea level), Ogun State, Southwestern Nigeria. The *Amaranthus cruentus* seeds used for the trial were sourced from the National Horticultural Research Institute, Ibadan, Oyo State, Southwestern Nigeria. The experiment was laid out in a Randomized Complete Block Design. The experiment consists of 16 treatments replicated three times. The size of each plot was 4 m x 3 m with pathway of 0.3 m between the plot.

Nematode Population Determination

To evaluate the herbivorous nematode populations soil samples were collected on each plot before transplanting of seedlings and at 60 – 66 Days after Transplanting (DAT). Whitehead and Hemming

(1965) method was used for nematode extraction. Four composite soil samples were taken per plot and bulked. A 250 g sub-sample was assayed for nematode census. The soil was placed in plastic sieve sandwiched with double-ply extractor paper and then placed in a bowl of bigger diameter. Distilled water was placed in the bowl until the soil was sufficiently covered with water but not flooded. The set up was left undistributed for 48 Hours after which the nematode suspension was decanted into a 500 ml capacity Nalgene bottle. Distilled water was added to the nematodes suspension to bring it to the factory marked the fill level. The Nalgene bottle was left for 5 Hours for the nematodes to settle to the bottom before siphoning the excess supernatant water using a 3 mm-diameter rubber tubing. The nematode concentrated suspension, now 15 ml was poured into Mc-Cartney bottle and kept in the refrigerator for processing for nematodes identification and population census. Doncaster's counting dish was placed under the stereo microscope and 1 ml of the suspension pipette into it for observation.

Biopesticide Constitution and Application

Four novel biopesticides were formulated with the indigenous plant parts as shown in Table 1 and include Biopesticide 181, with acronym BIO181 contained ginger (*Zingiber officinale*) and garlic (*Allium sativum*), Biopesticide 182, with acronym BIO182 contained ginger, garlic and onion (*A. cepa*), Biopesticide 183, with acronym BIO183 contained ginger, garlic, onion and neem oil, and Biopesticide 184, with the acronym BIO184 contained ginger, garlic, onion, neem oil and black liquid soap.

Table 1: Compositions of the Four Indigenous Biopesticides used as Treatments

S/N	Biopesticide	Acronym	Composition
1	Biopesticide 181	BIO181	Ginger+garlic
2	Biopesticide 182	BIO182	ginger+garlic+onion
3	Biopesticide 183	BIO182	ginger+garlic+onion+neem oil
4	Biopesticide 184	BIO184	Ginger+garlic+onion+neem oil+blck liquid soap

The indigenous plant materials were rinsed in cool clean tap water, air dried and weighed as 1:3:6, onion:garlic:ginger (w/w), then cut into 1-2 cm segments, mixed and placed in electric blender. Afterwards, 200 ml of distilled water was added into the set up. The blender was put on start from the lowest revolution to the highest and allowed to blend properly. The suspension was poured through a muslin cloth nested on plastic sieve to collect the solution. Neem oil was added to the blended suspension in 2:1 ratio to formulate BIO183, and neem oil with black liquid soap 2:1:1 to constitute BIO184. The treatments were applied as foliar and soil sprays at 0, 100 ml, 125 ml, or 150 ml /16 L of water. Application of biopesticides started 3 WAT and spraying was carried out bi-weekly by 06.30

Hrs – 07.30 hours (GMT+1), until 60 – 66 DAT. The unsprayed plots represented the control.

Data Collection and Analysis

In addition to the nematode population census, data were collected from four tagged plants per plot on plant height (cm), number of leaves, stem girth (cm), number of damaged leaves, number of undamaged leaves, and number of infected plants. Plant bio pesticides formulated were also evaluated in the laboratory for their phytochemical properties. Data collected were subjected to Analysis of variance using the Statistical Analysis System (SAS, 2003) and the significant means were separated using new Duncan Multiple Range Test at 5% level of probability.

RESULTS

Potency of Biopesticides on Nematode Population

Information from the results shown on Table 2 indicated that there was significant difference ($p < 0.05$) in terms of nematode population in treated plots compared with the control. BIO181 which was made up of ginger and garlic (1:2 w/w) applied at 100 ml/ 16 L of water significantly ($p < 0.05$) reduced (73%) the populations of Root-Knot Nematodes (RKN) from 63 in

the control plots to 17. Similar reductions (12 and 17) of RKN populations, representing 81% and 73% were encountered when the same Biopesticide was applied at 125 ml and 150 ml/ 16 L of water, respectively. On the contrary, BIO183 and BIO184 did not differ significantly ($p > 0.05$) from untreated plots (BIO181), irrespective of the rate of application. Other nematode population and infection criteria such as number of galls and Gall Index did not vary ($p > 0.05$) in spite of the application.

Table 2: Effects of four different bio-pesticides on nematode population

Treatment	Rate	Initial	Final ⁺	No of Galls	Gall Index
BIO181	0 ml	56.67abc	63.33a	2.67a	1.33a
	100 ml	53.00abc	16.67bcd	6.33a	1.67a
	125 ml	62.33ab	11.67cd	5.67a	1.67a
	150 ml	44.33abc	17.00bcd	6.33a	1.67a
BIO182	0 ml	60.67abc	30.33bcd	8.33a	2.00a
	100 ml	64.33a	31.67bcd	10.67a	2.00a
	125 ml	50.00abc	40.67abc	13.00a	2.00a
	150 ml	51.33abc	47.33ab	11.33a	2.67a
BIO183	0 ml	44.67abc	24.67bcd	2.33a	1.00a
	100 ml	39.33bc	19.00bcd	5.00a	1.67a
	125 ml	38.33c	13.00cd	6.67a	1.67a
	150 ml	52.00abc	14.33cd	3.67a	1.00a
BIO184	0 ml	65.67a	18.67bcd	11.67a	2.33a
	100 ml	61.00abc	5.00d	2.33a	1.00a
	125 ml	64.67a	5.67d	4.67a	1.67a
	150 ml	61.67abc	6.67d	4.00a	1.33a

⁺Means with the same alphabet are not significantly different ($p \leq 0.05$). Key- BIO: Biopesticides

Effects of four different biopesticides on plant height and stem girth

Table 3 showed that there was no significant difference ($p > 0.05$) between plant heights of the treated plants and the untreated *Amaranthus cruentus* plants at week 3, 6 and 9 WAT. However, at the 12th WAT, BIO184-treated plants were significantly ($p < 0.05$) taller 98.30 cm and 109.74 cm at the rates of 100 ml and 125 ml, respectively, compared with 93.97 cm in untreated plots. The highest (150 ml) dosage of BIO184 recorded 92.80 cm heights which which was not statistically different from the control.

Table 4 showed variability in stem girth of Biopesticides treated *A. cruentus* vegetables at 9 and 12 WAT. BIO181 applied at 125 ml per 16 L of water had 2.53 cm girth which was statistically wider ($p < 0.05$) than the control plot with 1.40 cm when measured at the 9th WAT. This performance of BIO181 was, however, not statistically ($p > 0.05$) different from the 1.90 – 1.93 cm stem girth when it was applied at the rates of 100 ml

and 150 ml/ 16 L of water. Likewise, the performance of BIO181 at 100 ml and 150 ml were at par with the control plots. By the 12th WAT, the performance of BIO181 applied at 125 ml/ 16 L of water was sustained with the value of 2.57 cm which was statistically different from the stem girth value of 1.47 cm in the untreated plot. The stem girth enhancement by BIO181 application at 125 ml/ 16 L water was 43% over the performance of the control plots during the 12th WAT measurements. On the contrary, the same indigenous Biopesticides 181 at the same rate during the same 12th WAT was not statistically ($p > 0.05$) different from the control plot. Pesticidal properties such as alkaloids, saponins, tannins, flavonoids, terpenoids and steroids were found in all the Biopesticides (Table 5).

Data on observation made on the number of leaves from the 3rd to the 12th WAT across the Biopesticides applications shown on Table 6 indicated no statistically ($p > 0.05$) significant difference among the treatments and the control.

Table 3: Effects of four different bio-pesticides on plant height

Treatment	Rate	PH3WAT	PH6WAT	PH9WAT	+PH12WAT
BIO181	0 ml	17.97c	38.53d	66.40d	66.80f
	100 ml	22.00bc	55.27bcd	69.93d	70.67def
	125 ml	21.97bc	60.79bcd	76.47cd	77.87cdef
	150 ml	20.97bc	58.93bcd	65.40d	68.20ef
BIO182	0 ml	17.4c	53.50bcd	78.10cd	78.87cdef
	100 ml	21.73bc	51.10cd	77.63cd	78.73cdef
	125 ml	21.20bc	63.87bcd	77.30cd	78.60cdef
	150 ml	21.80bc	58.63bcd	77.37cd	79.47cdef
BIO183	0 ml	23.87bc	77.13abc	84.90bcd	87.00bcd
	100 ml	25.37abc	72.83abc	74.50cd	78.60cdef
	125 ml	26.60abc	63.07bcd	77.07cd	84.97bcde
	150 ml	29.77ab	58.33bcd	80.93bcd	88.90bc
BIO184	0 ml	33.53a	81.13ab	91.23abc	93.97bc
	100 ml	34.60a	78.37abc	97.00ab	98.30ab
	125 ml	34.67a	91.77a	107.20a	109.740a
	150 ml	34.53a	67.67abc	90.83abc	92.80bc

+Means with the same alphabet are not significantly different ($p \leq 0.05$). Key- BIO: Biopesticides, PH3WAT: Plant height at three weeks after application of treatment, PH6WAT: Plant height at Six weeks after

application of treatment, PH9WAT: Plant height at Nine weeks after application of treatment, PH12WAT: Plant height at 12 weeks after application of treatment.

Table 4: Effects of four different bio-pesticides on stem girth

Treatment	Rate	SG3WAT	SG6WAT	SG9WAT	SG12WAT
BIO181	0 ml	0.33d	1.23d	1.40d	1.47c
	100 ml	0.60cd	1.47cd	1.90cd	1.90bc
	125 ml	0.63cd	2.40abc	2.53abc	2.57ab
	150 ml	0.67cd	1.73bcd	1.93cd	2.00bc
BIO182	0 ml	0.67cd	1.57bcd	2.00bcd	2.07bc
	100 ml	0.83bcd	1.73bcd	2.17bcd	2.27bc
	125 ml	0.73bcd	1.97bcd	2.07bcd	2.10bc
	150 ml	0.733bcd	1.83bcd	1.93cd	2.00bc
BIO183	0 ml	0.80bcd	2.37abc	2.23bcd	2.27bc
	100 ml	0.97abc	2.43abc	2.60abc	2.27ab
	125 ml	1.07abc	1.90bcd	2.07bcd	2.10bc
	150 ml	1.07abc	2.17abcd	2.67abc	2.87ab
BIO184	0 ml	1.37a	2.33abcd	2.47abc	2.63ab
	100 ml	1.40a	2.533abc	2.73abc	2.73ab
	125 ml	1.23ab	3.17a	3.47a	3.57a
		1.33a	2.63ab	3.07ab	3.30a

Means with the same alphabet are not significantly different ($p \leq 0.05$). KEY- BIO: Biopesticides, SG3WAT: Stem girth at three weeks after application of treatment, SG6WAT: Stem girth at six weeks after

application of treatment, SG9WAT: Stem girth at nine weeks after application of treatment, SG12WAT: Stem girth at twelve weeks after application of treatment.

Table 5: Phytochemical composition of biopesticides

Biopesticides	Alkaloids	Saponins	Tannins	Flavonoids	Terpenoids	Steroids
Biopesticide 181	+	+	+	+	+	+
Biopesticide 182	+	+	+	+	+	+
Biopesticide 183	+	+	+	+	+	+
Biopesticide 184	+	+	+	+	+	+

+Present

Table 6: Effects of four different bio-pesticides on number of leaves

Treatment	Rate	NL3WAT	NL6WAT	NL9WAT	NL12WAT
BIO181	0 ml	11.33g	22.67i	69.33b	73.00c
	100 ml	11.67cdefg	30.00hi	69.33b	70.67c
	125 ml	12.33fg	33.33ghi	70.67b	73.33c
	150 ml	13.33cdefg	36.33fghi	61.67b	65.33c
BIO182	0 ml	13.00defg	41.67efghi	63.67b	67.33c
	100 ml	13.33cdefg	64.33cdefgh	69.33b	72.00c
	125 ml	13.67cdefg	54.00cdefghi	76.00ab	79.67bc
	150 ml	12.67efg	46.00defghi	79.00ab	79.67bc
BIO183	0 ml	15.67cdef	75.67abcde	83.33ab	85.33abc
	100 ml	16.67abcd	72.67abcdef	82.67ab	87.33abc
	125 ml	17.00abc	69.67abcdefg	81.00ab	83.67abc
	150 ml	16.67abcd	49.00defghi	69.00b	81.33abc
BIO184	0 ml	16.33abcde	89.33abc	96.67ab	99.67abc
	100 ml	16.00abcdef	106.00a	110.67a	115.67a
	125 ml	19.67a	101.33ab	111.00a	114.00ab
	150 ml	17.67ab	82.00abcd	93.00ab	98.67abc

[†]Means with the same alphabet are not significantly different ($p \leq 0.05$). Key - BIO: Biopesticides, NL3WAT: number of leaves at three weeks after application of treatment, NL6WAT: number of leaves at Six weeks after application of treatment, NL9WAT: number of leaves at Nine weeks after application of treatment, NL12WAT: number of leaves at 12 weeks after application of treatment.

DISCUSSION

In this season of reoccurring global human disease pandemic and endemic catastrophes, there have been rising difficulties of developing immediate vaccines marred with controversies of safety concerns for some of the vaccines. The ongoing fight against the global Corona Virus Infectious Disease, also known as COVID-19, spurred the attraction of plant-based immune boosters as immediate medications for the recoveries of infected people from the virus whose cure through orthodox drugs is still being investigated. High saline plant produce like ginger, garlic, pineapple, pawpaw and vegetables have been suspected to have been linked to some recoveries. Post COVID-19 agro-economy must also focus on short vegetation crops like vegetables that can boost the wealth of the farming communities, particularly, the smallholder farmers.

Dealing with vegetable pests will enhance their productivity. The current study dwell on the evaluation of four rates of four novel indigenous *A. cruentus* in the management of root-knot disease in a field under organic conversion. Of the four types and rates, only of the native Biopesticides, BIO181 and one rate (100 ml) showed high potency in reducing root-knot disease attributable to *Meloidogyne* spp. This outcome is in collaboration with Khalil *et al.* (2012) that botanical were effective in reducing nematode root damage. The implications for reduction in root disease was the enhanced plant growth index of stem girth and plant height which may also be linked to the pesticidal

properties such as alkaloids, saponins, tannins, flavonoids, terpenoids and steroids found in all the Biopesticides. This attested to Alam *et al.* (1980) opinion that phenolic compounds absorbed systemically from neem formulations by plant roots might have elicited the host's tolerance against invading nematodes. Further conformity was established through reports by Devakumar *et al.* (1985) that direct morbidity was observed due to the active ingredients such as azadirachtin, nimbidine, salanine, and thionemone and other by-products like phenols, ammonia, formaldehyde and fatty acids.

In view of post COVID-19 economy, agriculture and in deed smallholder farmers will indeed benefit from the results obtained from this trial since it affirmed that home-made formulations are not completely new for the management of pests in crop fields. However, more attention was paid to neem (*Azadirachta indica*) formulations that has now become routinely used in several parts of the world after the experience of Indian farmers for over hundred years as reported by Javed *et al.* (2008). According to Javed and co-authors, preparations from neem dry leaf, powder seed powder, seed kernel powder, seed cake powder (Javed *et al.*, 2008), bitterleaf (*Vernonia amygdalina*) and siam weed (*Chromolaena odorata*) (Aruna and Atungwu, 2015) and aqueous extracts produced therefrom them (Javed *et al.* 2008; Aruna and Atungwu, 2015), have shown potency against root-knot nematode egg hatch and stimulation in preventing juvenile mortality. It was in this respect that neem oil was included in the formulations for the present trial, which has performed effectively in reducing root-knot disease incidence in *A. cruentus*.

Like neem, ginger, garlic, onions are available and their incorporation and outcome was to provide farmers choices in selection of which formulations to use. This is more critical for the emerging organic vegetable farmers in Nigeria who are not allowed to use

synthetic pesticides, because of organic standard and the inappropriateness of abuse or temptation of using chemical pesticides for vegetables that are eaten either fresh or with little processing.

CONCLUSION

The experiment showed that BIO181 applied at 100 ml was effective in reducing the incidence of root-knot disease of *Amaranthus cruentus* in field undergoing organically conversion.

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