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Effect of Leaf Powder Treatment on Root-Knot Nematode (*Meloidogyne incognita*) and Proximate Composition of Yam Tubers; Implication on Food Security

Research article

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Abstract

Farmers face several challenges in yam production occasioned by incidents of pest and pathogens among which are nematodes, termites, beetles. The effect of leaf powder of five plants, *Ocimum basilicum* L. (Basil plant), *Vernonia amygdalina*.L., (bitter leaf) *Azadirachta indica* L. (neem) *Moringa oleifera* Lam. (horseradish) and *Piper nigrum* Schum and Thonn (bush pepper), were assessed for their effect on root-knot nematode, *Meloidogyne incognita* in yam and their influence on tuber proximate composition. The trial was conducted in Abia State, Nigeria laid out in completely randomized design, replicated three times. Data were collected and analyzed using descriptive statistics and analysis of variance (ANOVA). Results indicate that carbohydrate content increased by 10.4% in Basil plant, 21.4% in Bitter leaf, 8.1% in Neem, 11.1% in horseradish and 12.8% in Bush pepper treated yam tubers at 0.01t/ha when compared to the control. The protein composition increase was not significant ($P > 0.05$). Application of leaf powder at 0.01t/ha before planting and during tuber initiation suppressed root knot nematode population and consequently, improved growth and yield of yam, improved the dietary value of the yam tubers which has the potentials for enhanced nutrition and food security of the Nigerian citizens and it is therefore recommended for wide spread farmers' awareness and adoption.

Keywords: Nutritive values; Yam tubers; *Meloidogyne incognita*; Plant leaf powder; Awareness; Adoption

Introduction

Dioscorea species are consumed widely as staples in West Africa, Central Africa and the Caribbean [1]. *Dioscorea rotundata* is a major staple food in Africa with Benin Republic (346 calorie) as highest consumer followed by Cote d Ivoire (342calorie), Ghana (296 calorie) and Nigeria (258 calorie) daily respectively [1]. Ninety eight percent of the world's yam production is in Africa and West Africa accounts for 90 - 95 % of the total world production. Nigeria is the largest producer of the crop, producing about 33.57 million metric tons in 2008 [2] but consumes the entire product in the domestic market. Ghana which is the second largest world producer with 6.9 metric tons in 2008 is the highest exporter of the product [3].

Yams occupy the largest area in the production per hectare after cassava. Yam is used as food as well as industrial raw material like other root crops, such as sweet potato and cassava thus mounting pressure on yam as the most important staple which is needed to be reposition as a food security crop [4]. Yams are major source of relatively cheaper caloric energy as starchy food that contains other

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useful nutrients. They are excellent source of carbohydrate and also relatively nutritious, providing some vitamins, minerals and dietary protein [5]. Yams play a significant role in the socio-cultural traditions of the producing areas, and are consumed in various food forms across those producing regions either as boiled, mashed, pounded, fried or roasted.

Yams are affected by pests and pathogens among which are nematodes, termites, beetles, etc [6]. The root-knot nematodes (*Meloidogyne* species), and other nematodes such as *Scutellonema bradys, Pratylenchus* species (lesion nematodes) have been identified as the most economic pests of yam in Nigeria, which has resulted into yield losses of 25-38% in major yam producing areas [7]. Onyenobi et al. noted that in most cases, nematode-infested yams in the field are without visible foliar symptoms and infestation by root-knot nematode on yam has contributed greatly into post-harvest bio-deterioration [8]. It was observed that early senescence occurs in heavily infested minisett yam plant with galled yam tubers that rotted completely in storage [8]. Aghale noted that nematode damage on yam is an important factor which affects the tuber quality, resulting in tuber yield reduction and general losses in the field and in storage [9]. *Meloidogyne* species are group of plant-parasitic nematodes that have been recognized as pests of worldwide concern, constituting a major threat to world food production [7].

Several control measures such as cultural, biological, chemical and integrated control methods, have been used in reducing populations of the nematode in crop fields [9]. Synthetic nematicides are mostly used to control yam nematodes. However, most of these nematicides have been banned due to their carcinogenic effect on human lives and on the environment. High cost of the nematicides has also precluded resource-poor farmers from using them. The use of natural plant products has been considered as a way out of these chemical-related problems in yam production since some plants have been identified to possess nematicidal properties, which has been proven to be efficient and easily accessible as seed dressing agents.

Aghale et al. [10] reported the efficacy ofnine plants (*Carica papaya, Chromolaena odorata, Cymopogon citratus, Mangifera indica, Ocimum basilicum, Vernonia amygdalina, Azairachta indica, Moringa oleifera, and Piper nigrum*) which were promising even in low concentrations in controlling the root knot nematode (*Meloidogyne* species) in yam production [10]. Aghale noted strong nematicidal activity of some plant extracts on nematode infested yams. However, in South East Nigeria, there is little or no information on the efficacy of bionematicidal materials on the levels of proximate content of the edible part of yam, the tubers [11]. This informed the design of the present investigation to determine the effect of the selected five plant leaf powder on the control of root knot nematode (*Meloidogyne* species) and also on the proximate composition of yam tuber.

Material and Methods

The study was conducted on the experimental research field of the Faculty of Agriculture, Abia State University, Nigeria, the Plant Protection Laboratory and the Biochemistry Laboratory of National Root Crops Research Institute, Umudike, Nigeria. Five different types of plants species used in the trials were collected from different parts of Umuahia in Abia state and these included Basil plant, Bitter leaf, Neem, Horseradish and bush pepper. Healthy leaves of these plants were harvested and spread separately on polythene sheets raised on a platform in the laboratory for three weeks to air dry at 28-⁺2 °C. Air-dried leaves were ground separately to fine particles (powder) in a domestic blender (model QBL-15L40) and applied at the rate of 5g per pot (0.01t/hectare).

D. rotundata (cv.Obiaturugo), a hybrid (89/19158) which is well known to be susceptible to *M. incognita* was used for the study [6]. The size of yam sett used was 30 g. Prior to planting, the yam sets were treated with the different plant leaf powder at the rates of 0.01t/ha in a jute bag tied at the top end and shaken properly before air drying on a raised platform. The sets were allowed for 24 hours for the powder to adhere properly on the sets before planting into the plastic pots. The yam sets were manually planted in 36 pots placed in open field with one yam sett per pot. At 12th weeks after planting (WAP), the leaf powder was applied by ring method to the yam stands in the 30 pots while the remaining six pots served as untreated controls. Earlier 10 WAP, the yam plants in 30 pots were inoculated with 5000-second stage pre-parasitic juvenile of *M. incognita* during tuber initiation when it is easy for the juveniles to penetrate the yam cortical cells using a 10ml syringe while the other six untreated control pots were not inoculated.

The experiment was laid out in completely randomized design with five treatments in three replicates. The eggs / juveniles that were used for the inoculation were cultured and extracted using Hussey and Barker procedure [12]. Data were collected on sprouting at 4, 8 and 12 WAP. At 28 WAP the experiment was terminated and the following parameters were collected. Fresh tuber weight (g), proximate composition of the harvested tubers on carbohydrate, protein, fiber, ash, and fat using the gravimetric method of different laboratory analysis for the specific tests at Food/Biochemistry laboratory of National Root Crops Research Institute, Umudike [13]. Also, data on gall tuber formation was collected. The gall index (GI) were assessed on a 0-5 scale 0 = no infection, 1 = 1- 20% of tuber galled, 2 = 21- 40% of tuber galled, 3 = 41-60% tuber galled, 4 = 61- 80% and 5 = 81-100% of tuber galled. The data obtained were subjected to analysis of variance (ANOVA). Significant means were separated using Duncan Multiple Range Test (DNMRT)

Determination of protein

The proximate composition of the harvested yam tuber was done by the gravimetric method [13]. Yam flour obtained from harvested yam tubers in pots treated with Basil plant, Bitter leaf, neem, Horseradish and Bush pepper were used for the proximate composition study. To obtain the flour, the harvested tubers were peeled, chipped, dried and milled into flour. Five grams of flour was mixed with 10 ml of concentrated H_2SO_4 in a digestion flask, and 1g tablet of selenium was added to the mixture as the catalyst before heating under a fume cupboard till a clear solution was obtained. The digest was diluted with water to 100 ml in a volumetric flask and used for the analysis. Ten ml of the digest was mixed with equal volume of 45 % NaOH solution in a distillation apparatus and was further distilled into another 10 ml of 4 % boric acid with three drops of mixed indicator (BromocressolGreen/Methylred). Fifty milliters of the distillates was collected and titrated against 0.02N EDTA from green to deep red end point. The protein content of each treated yam tuber was calculated using the formula (6).

% protein = % N_2 x 6.25, N_2 = (100 x N x 14 vt) T.B

W x 1000 x va.

W = weight of treated yam flour, N = normality of titrant (0.02N H_2SO_4), Vt = total digest volume, Va = volume of digest analyzed, T = sample titrate value, B = blank titrate value.

Ash Determination

Five grams of the yam flour obtained from harvested yam tubers in treated pots were measured into a previously weighed porcelain crucible and burnt to ashes in a muffle furnace at 550°C. Following this the ash was cooled and the weight was determined by the differences in weight before and after burning to ash and expressed as percentage of the sample analyzed.

Fiber Determination

The fiber composition was determined by method described by Arora, 1981 [14]. Five grams of the harvested yam tubers in treated pots were measured and boiled in 150 mL of 1.25 % H_2SO_4 solution for 30 minutes and washed in hot water using a twofold muslin cloth to trap the particles. It was returned to the flask and boiled in 150 mL of 1.25 % NaOH for 30 minutes. Washing in several portions of hot water, the dust were allowed to drain dry before weighing in a crucible where it was oven dried at 105°C to a constant weight. This was further burnt in a muffle till only the ashes were left. The weight of the fiber was obtained and expressed as percentage of the weight of sample analyzed 90 [15-17].

Fat content Determination

Five grams of the yam flour obtained from harvested yam tubers in treated pots were wrapped in filter paper and put in a thimble which was placed in soxlet reflux flak and mounted into a previously weighed extraction flask containing 200 mL of petrol ether. The upper end of the flask was connected to a water condenser, while the contents were heated and vaporized. The vapour condensed into the reflux flask, and was converted into the solvent that extracted the oil (fat). The oil extracts were oven dried at 60 °C for 30 minutes to remove any residual solvent. This was further cooled in a desiccator and weighed. The difference in the weight of oil extract was determined and expressed as a percentage weight of sampled yam flour.

Carbohydrate Determination

Total carbohydrate was calculated by the method described in AOAC [13]. This was done using the formula:

Total carbohydrate = 100 - % protein + fat + fiber + ash + MC.

Results and Discussion

Sprouting counts at 4, 8, and 12 weeks after planting was presented in Table 1. There was no statistical difference (P > 0.05) between treatments and the control on sprouting. This implied that none of the plant leaf powder evaluated had adverse effect on the time the seed yams sprouted.

Table 1: Effect of five plant leaf powder on sprouting of yam setts at 4, 8, and 12 weeks after planting.

Botanicals	Week 4	Week 8	Week 12
O.basilicum	0.56	0.67	1.22
A.indica	0.44	0.78	1.44
M.oleifera	0.33	0.67	1.22
P.nigrum	0.44	0.78	1.33
V.amygdalina	0.44	0.78	1.44
Control	0.56	0.78	1.44
Mean	0.46	0.74	1.35

LSD (0.05) ns ns ns

Table 2: Effect of five plant leaf powder on yam tuber yield and gall formation.

Tuber	No .of tubers	Weight of tubers(gm)	No. of galled
O.basilicum	1.50	0.23	0.00
A.indica	1.75	0.43	0.00
M.oleifera	1. 5	0.11	0.50
P.nigrum	1.25	0.08	0.25
V.amygdalina	1.75	0.28	0.00
Control	1.50	0.28	1.75
LSD (0.05)	ns	ns	0.74

The effect of five plant leaf powder on yam tuber yield and gall formation is given in table 2. *A. indica* and *V. amygdalina*recorded the highest number of tubers (1.75) while the untreated control had significant (P>0.05) number of mean galled tubers (1.75). This shows that the botanicals used in the treatment of seed yams effectively controlled the proliferation of galled tubers which is a visual means of the presence of root-knot nematode on yam tubers. This indicates that the use of some botanicals (Basil plant, Bitter leaf, neem, Horseradish and Bush pepper) in the production of root-knot free yam tubers could be a good local substitute to seed dressing with synthetic nematicides in yam production.

The proximate composition of the harvested yam tubers after treatment is presented in Table 3. The protein composition of the yam tubers was not affected by the treatments as compared with the untreated control. There were no significant differences (P > 0.05) on the protein levels in all the treatments and the control. This was in line with [9] who reported on the effect of botanicals on the protein composition of food yam. There were significant differences (P < 0.05) on the carbohydrate composition of the treated yam tubers when compared with the untreated control. *V. amygdalina* had the highest level of carbohydrate (27.02 mg/100) followed by *P. nigrum* (25.12 mg/100) compared to the control which was the least. The application of the leaf powder increased the carbohydrate level of the treated yams by 10.4%, 21.4%, 8.1%, 11.1 %, and 12.8% in Basil, Bitter leaf, neem, Horseradish and Bush pepper, respectively when compared with control. The fat composition of the yam tubers indicated that *O. basilicum* (0.51 mg/100g) was significantly different (P < 0.05) from *V.amygdalina*(0.44 mg/100g) and *A. indica* (0.41 mg/g). Besides, the leaf powder of bitter leaf, neem, and horseradish increased the fat

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Table 3: Proximate Composition (mg/100g) of yam tubers treated with five plant leaf powder.

O.V. A. M. P.	*basilicum*	*amygdalina*	*indica*	*oleifera*	*nigrum*	*Control*	*Mean*	*LSD*
Carbohydrate	24.57^{b}	27.02^{a}	24.06^{b}	24.74^{b}	25.12^{b}	22.25^{c}	24.62	1.27
Protein	4.90^{a}	4.32^{a}	4.27^{a}	4.38^{a}	5.25^{a}	4.13^{a}	4.54	Ns
Fiber	1.44^{b}	1.38^{b}	1.42^{b}	1.50^{a}	1.40^{b}	1.60^{a}	1.45	0.15
Ash	2.96^{a}	2.74^{b}	2.82^{b}	2.45^{b}	3.63^{a}	3.33^{a}	2.98	0.76
Fat	0.51^{a}	0.44^{b}	0.41^{b}	0.38^{c}	0.40^{c}	0.36^{d}	0.41	0.03

In mean separation, a-highest, b-average and c- least significant respectively. Means followed by same letter(s) within a column do not differ at P≤ 0.05 using Duncan Multiple Range Test (DNMRT).

content by 22%, 13%, and 5.5%, respectively when compared with control. There were no significant differences ($P > 0.05$) between the fat levels in *P. nigrum*and *M. oleifera*. However, all the treatments were significantly different ($P < 0.05$) from the untreated control. The highest level of ash content was derived from the tubers pre-treated with *P. nigrum*(3.63 mg/100g), *O. basilicum*(2.96 mg/100g) and the untreated control. There were significant differences ($P < 0.05$) in the ash composition among *A. indica, V.amygdalina*, and *M. oleifera*. The untreated control and *M. oleifera*recorded the highest levels of fiber contents in the yam tubers and were significantly different ($P < 0.05$) from the other treatments. The nutritional compositions of the yam tubers were affected by the treatments as compared with untreated control. Any treatment that inhibits the damaging effect of nematodes on yam without adversely affecting the nutritive value of the yam tubers will be considered as a way forward in organic food production.

The use of botanicals improved the dietary level of the yam tubers as a food security crop in hunger intervention, although the treatments are in high demand as spices, they appear to have a higher potential in the control of *M. incognita* and have proved to be favorable alternatives to synthetic pesticides in yam production. All the plants used in this study are locally available and used in ethno medicinal practices without toxicological residue effects and as such could serve as alternatives to a sustainable organic farming system to meet global demand for food production.

Conclusion

The study provides empirical data confirming the use of botanicals for improved dietary level of yam tubers as a food security crop in hunger intervention, although the treatments are in high demand as spices, they appear to have a higher potential in the control of *M. incognita* and have proved to be favorable alternatives to synthetic pesticides in yam production. All the plants in this study are locally used in ethno medicinal practices without toxicological residue effects and as such could serve as alternatives in a sustainable organic farming system to meet global demand in food production.

Therefore, the study recommends adoption of the technique by yam farmers in a larger field.

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