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TABLE OF CONTENTS

1	FIRST REPORT OF PEPPER YELLOW VEIN MALI VIRUS ON TOMATO (<i>Solanum lycopersicum</i> L.) IN SOME NORTHERN STATES OF NIGERIA <i>Kashina B. D. and Chindo A. C.</i>	1
2	YIELD AND PROFITABILITY OF SWEET SORGHUM (<i>Sorghum bicolor</i> (L) Moench cv. <i>saccharatum</i>) VARIETIES AS INFLUENCED BY VARYING NPK RATES IN NORTHERN GUINEA AGROECOLOGICAL ZONE OF NIGERIA <i>A.N Muhammad¹, A.B. Lawal², O.A Adediran³</i>	9
3	PATHOGENICITY OF ROOT-KNOT NEMATODE IN CUCUMBER AND <i>IN VITRO</i> TEST OF POULTRY MANURE AND BOTANICAL EXTRACTS AS BIONEMATICIDE <i>Baba, H. S. and Izuogu, N. B.</i>	18
4	EFFECT OF WATER QUALITY PROPERTIES ON INFESTATION OF <i>NYMPHAE LOTUS</i> IN SELECTED WATERBODIES AT MAKURDI <i>Alfred Aondona Jimin¹*, Abdullahi Jaji Ibrahim² and Rosmary Iengemdoo Ahom¹</i>	27
5	MANUSCRIPT TITLE: COMPARATIVE ANALYSIS OF THE EFFICACY OF CRACK-OPEN AND CANDLING FERTILITY TEST OF JAPANESE QUAIL (<i>Coturnix japonica</i>) EGGS <i>Chibuogwu Ijeoma Chika¹*, Adediran Mubarak Adekunle², Maduekwe Innocent Maduabuchukwu³</i>	41.
6	ASSESSING THE POTABILITY OF WATER FROM DIFFERENT SOURCES USING WATER QUALITY INDEX METHOD IN SLUM AREAS OF THE ABUJA MUNICIPAL AREA COUNCIL, ABUJA, NIGERIA <i>Ishaya S¹ and Oloyede Israel²</i>	52
7	INFLUENCE OF TREATED WASTEWATER ON THE PHYSICAL AND CHEMICAL PROPERTIES OF SOILS OF WUPA ABUJA, NIGERIA <i>¹Abba, A. Simon, ¹Ogwueleka, T.C. and ²Barnabas I. Musa</i>	62
8	ASSESSMENT OF WOMEN INVOLVEMENT IN CASSAVA BASED AGRICULTURAL TRANSFORMATION AND EMERGING ISSUES FOR GENDER GENERATION STUDIES IN IMO STATE, NIGERIA <i>S.O. Eze¹, F.E Ebe² and B.O. Ibeawuchi¹</i>	71
9	ANTI-FUNGAL ACTIVITY OF <i>Artemisia annua</i> L. EXTRACTS AGAINST FUNGI ASSOCIATED WITH POST-HARVEST ROTTEN TOMATOES IN LAFIA MARKETS NASARAWA STATE NIGERIA <i>Kayode, E. A.¹*, Okunade, O.A.², Stephanie, M.E.², Ogbuagu, K.P.², Yusuf A.T² Atteh, A, P.², David, Z.O.³, Chris, G. O.³ and Ibrahim S. O⁴.</i>	85
10	EFFECT OF BANDITS' ATTACKS ON FOOD SECURITY IN NIGERIA: A REVIEW <i>Ameh, Emmanuel Ugbedeajo, PhD.</i>	95

TABLE OF CONTENTS

11	IMPACT OF TOPOSEQUENCE ON MICRONUTRIENTS IN FLOOD PLAIN IRRIGATED SOILS OF GADABIYU OF KWALI AREA COUNCIL, ABUJA FEDERAL CAPITAL TERRITORY, NIGERIA	
	<i>Ahmad Hadiza A.</i>	106
12	MYCOBIOTA OF COCOA (<i>THEOBROMA CACAO</i>) BEANS FROM DRYING SITES IN THE SOUTH-WEST OF NIGERIA	
	¹ *Jeff-Agboola Y.A., ² Jeff-Agboola E.O., ³ Oyedokun J., ⁴ Plötz M., ⁵ Okunlola O.A. and ⁶ Elekofehinti O.O.	122
13	EFFECT OF NPK FERTILIZER ON GROWTH AND GRAIN YIELD OF SWEET SORGHUM (<i>Sorghum bicolor</i> (L) Moench cv. <i>saccharatum</i>) VARIETIES IN NORTHERN GUINEA SAVANNA OF NIGERIA	
	A.N Muhammad ¹ , A.B. Lawal ² , D.B Ishaya ³	142
14	PLANT PARASITIC NEMATODES OF PHYTOSANITARY IMPORTANCE IN NIGERIA	
	[*] Ubazi, C. C. ¹ , Asala, W. S. ¹ , Okafor, O. E. ² , Abdulkarim, B. M. ³ and Aliyu, R. H. ³	153
15	ASSESSMENT OF FISH FARMERS' ACCESSIBILITY TO EXTENSION SERVICES IN BORGU LOCAL GOVERNMENT AREA OF NIGER STATE, NIGERIA	
	Uyamasi, I.M ^{*1} , Jubril, A.E ¹ . and Ajiboye, O.O ²	164
16	ASSESSMENT OF FUNGI AND AFLATOXIN PROFILE IN AFRICAN WALNUT (<i>TETRACARPIDIUM CONOPHORUM</i> MULL. [ARG]) FRUIT SNACKS SOLD IN ABUJA, NIGERIA	
	Edeh, Joy Anwuri ¹ , Anjorin, Toba Samuel ¹ , Abdurazaq Olusegun Abdulkadir ² Ekwere, Uwem Akpan ³	177
17	INFLUENCE OF TREATED WASTEWATER ON THE PHYSICAL AND CHEMICAL PROPERTIES OF SOILS OF WUPA ABUJA, NIGERIA	
	¹ Abba, A. Simon, ¹ Ogwueleka, T.C. and ² Barnabas I. Musa	184
18	FACTORS INFLUENCING THE CONTRIBUTION OF ICTS TO YOUNG FARMERS' INVOLVEMENT IN AGRICULTURE IN KOGI STATE, NIGERIA	
	Ifeyinwa Peace Nwele, Julius Ajah and Edwin E. Idu	193
19	GENDER PERCEPTION AND ADAPTATION PRACTICES OF SMALL HOLDER CASHEW FARMERS TO CLIMATE VARIABILITY IN ENUGU STATE, NIGERIA	
	Ebe, F. E. ¹ , Eze, S. O. ² and Onyebinama C. I. ¹	206

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PATHOGENICITY OF ROOT-KNOT NEMATODE IN CUCUMBER AND *IN VITRO* TEST OF POULTRY MANURE AND BOTANICAL EXTRACTS AS BIONEMATICIDE

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ABSTRACT

The pathogenicity of root-knot nematodes on marketer, murano, marketmore, and poinsett cucumber varieties and in-vitro testing of extracts from poultry manure and leaves of *Gliricidia sepium*, and *Bryopyllum pinnatum* were investigated in the screenhouse. An experimental set-up was a completely randomized design with four replicates each. In-vitro testing of powdered poultry manure, poultry manure tea, *Gliricidia sepium*, and *Bryopyllum pinnatum* extracts on root-knot nematode (*Meloidogyne* species) was carried out at the laboratory of *Striga* spp. and Nematology units of the International Institute of Tropical Agriculture (IITA) Ibadan to determine their effect on root-knot nematode egg hatchability and juvenile mortality. These treatments were at different serial dilutions of 50%, 75%, and 100%. The control trial was determined using distilled water and was exposed for different hours. Data were collected on vegetative growth, soil nematode population, root galling, some eggs hatched, and several juvenile mortality. All numerical data were analyzed using descriptive statistics of Microsoft excel and analysis of variance (ANOVA) at a 5% level of significance using SAS 9.3 and T-test analysis between varieties and control. The four cucumber varieties were susceptible to *M. incognita* and were arranged in descending order of susceptibility using their gall index (GI); Marketer (GI=5.60); Marketmore (GI=4.30); Murano (GI=2.30); Poinsett (GI=1.60). In the laboratory, extracts of powdered poultry manure significantly ($P<0.05$) reduce egg hatching and increased the death of juveniles of root-knot nematode among the treatments.

Keywords: Botanical extract, Cucumber, Index, Nematode, Pathogenicity, Root galling

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is one of the most popular members of the Cucurbitaceae family and it is originally a native of Southern Asia (Sharma, 2016). It is a valuable source of conventional antioxidant nutrients including vitamin C, beta carotene, and manganese. The fruit is used as a vegetable or salad. The immature fruit is cooked and given to children for dysentery. The seeds have a cooling effect when consumed as a diuretic. Chartzoulakis(2014) reported that the edible portion, which is about 80% of the fruit, contains 95% water, 0.7% protein, 0.1% fat, 3.4% carbohydrate, 0.4% fiber, and 0.4% ash. The seed is also a source of healthy oil and can be used in cooking (Gbif.org, 2013). This popular economic vegetable is threatened by tremendous yield losses from disease caused by Root-Knot Nematodes (RKN).

Root-knot nematodes are the most occurring and destructive nematode which plays a major role in limiting vegetable production worldwide (Sharma and Rich, 2005; Zarina and Shahina, 2010) and in Nigeria (Izuogu *et al.*, 2014). *Meloidogyne* spp. is identified as a major pest of cucumber, which feeds on the underground part of the plants. Stunting, yellowing, and wilting of leaves are symptoms of foliage from a damaged root system which include infected roots with developed galls (swollen) which prevent water and nutrient uptake by plants according to Bernhardt *et al.*, 2013. RKN has usually been managed with chemical nematicides for decades. However, the negative impact on living organisms and the environment has led to a total ban or restricted use of most chemical nematicides (Zukerman and Esnard, 1994). With increased pressure on growers to reduce chemical nematicides usage, there is great interest in alternative control as a management tool for this

destructive pathogen and sustainable food production.

Prevention is centered on the management of nematodes. This is because it is extremely difficult to eradicate the nematode without destroying the host plant once a plant is parasitized. The use of screened seeds and biopesticides botanical based has become the center of attention today, i.e. by facing the nematode problems in an eco-friendly manner (Saliga and Skelly, 2013, Izuogu, 2012). Plants and plant parts products when applied in soil are known to cause a reduction in the nematode population below a damaging level reported by Adekunle and Akin (2007). The main objective of this study, therefore, is to assess the susceptibility of four cucumber varieties and assess the efficacy of soil amendments (poultry manure powdered and tea; *Gliricidia sepium* and *Bryopyllum pinnatum*) in the control of root-knot nematode (*Meloidogyne* species).

MATERIALS AND METHODS

Pathogenicity Test of *Meloidogyne incognita* on Four Cucumber Varieties

Sandy-loam topsoil was collected with a spade from the farmland around the department of Agronomy Pavilion, University of Ilorin, Ilorin. Using the method described by Gautama and Goswani (2002), the soil was steam pasteurized in a drum at 90°C for 9 hours. The steam pasteurized soil was allowed to sufficiently cool for 72 hours. Thereafter, 8.5kg of the pasteurized soil was later weighed into each of 112 perforated 10-liter plastic buckets of 11 cm in diameter. The buckets were placed on an elevated platform to avoid reinfestation by other microorganisms and they were kept inside the screen house for planting. Trials were conducted out in the Screen house of the Department of Crop Protection, the University of Ilorin, Ilorin at lat 8°48'65"N and long 4°67'64" E. Four Cucumber varieties (Marketer, Marketmore, Poinsett, and Murano) were obtained from Premier seeds store, Sango area Ilorin, Kwara State. Thirty-two ten-liter plastic buckets filled with 8.5kg sterilized soil were used for the experiment. Three seeds were sown into each pot. All the varieties were replicated four times and fitted into a Completely Randomized Design (CRD). One week after planting, seedlings were

thinned down leaving one vigorous plant per bucket. a day after thinning, 16 buckets were inoculated by making three holes at the root base of each plant in the pot, and 2000 freshly hatched juveniles of *M. incognita* nematodes were incorporated in a ring method around the plant and covered with soil. Uninoculated buckets served as control. Data were collected on growth rate per replication (cm), yield parameters per replication (grams), soil nematode population at 200 grams of soil, and root gall index at the rate of 10 grams of the root. Soil nematode numbers were extracted using the modified Baerman's Tray method of Whitehead and Hemming (1965) and were estimated using Doncaster, 1962 counting dish under a compound microscope. The gall on the roots was rated according to the method used by Makete (2000). Root systems were uprooted, washed and the galls on the roots were scored using Makete (2000) method as described below:

0=No galls (clean root system)

1=1-10% of the root system galled

2=11-35% of the root system galled

3=36-65% of the root galled

4= 66-90% of the root system galled

5=91-100% of the root system galled

In-vitro Testing of Soil Amendment for Nematode Mortality and Egg Hatching

Preparation of Plant Extracts

The plants evaluated against *M. incognita* on cucumber were *B. pinnatum* and *Gliricidia sepium*. Leaves of both plants were collected in the morning along the riverside at Kulende, Ilorin, Kwara State before sunrise, and their identity was confirmed at the herbarium of Plant Biology Department, University of Ilorin, Ilorin. One kilogram of *B. pinnatum* and *G. sepium* leaves were left to air dry for 5 weeks and 14 days at room temperature of 25°C-27°C respectively. The leaves of *B. pinnatum* were pulverized using mortar and pestle before it was air dried. Aqueous extracts of test plants were prepared by soaking 1kg of the ground test plants in a plastic bucket containing already 4Litre of hot water which was boiled at 90°C. The plastic bucket was covered with the lid and the ground test leaves were allowed to soak for 24 hours for proper

extraction following the modified method of Oyedunmade and Izuogu (2011). The supernatant was sieved with a muslin cloth in both cases and the aqueous extracts were collected in containers. This served as the stock solution.

Preparation of Poultry Manure (Compost Tea)

This is a liquid concentrate enriched by poultry manure. Raw manure was collected from layers of the chicken house in the Sobi area, Ilorin, Kwara State in a sack bag which was tied up. Three hundred (300) grams of fresh layers of chicken droppings were collected and allowed to air dry for 16 weeks on a clean surface to get rid of moisture and reduce toxicity. The cured manure was later turned into a plastic bucket, leaving (9.6cm) of the headroom on the top of the bucket so that it can be stirred without the tea spilling. Three liters (3L) of dechlorinated tap water was added to it. The water was dechlorinated by allowing water to sit out in the sun for 7 hours; this will allow any chlorine in the water to break down because chlorine kills the bacteria in the compost tea. Continued stirring of the tea three times a day throughout 7 day period. Poultry manure tea was stirred three times each day to infuse the nutrients into the water and to provide oxygen following the modified method of Ingham (2005). After 7 days, the liquid was sieved using a muslin cloth.

Preparation of powdered poultry manure

Raw poultry manure of 300g was spread thinly on a clean surface for proper curing. It was left to air dried and decomposed for twelve (12) weeks to reduce the ammonium and moisture content using a modified method by Ellis *et al.* (2013) before use, the dried manure was ground using mortar and pestle, later sieved using 2.0 mm sieve and was properly packed and left till when the need arose.

Nematode Egg Extraction from infected Root Samples

Galled roots of *Cucumis sativus* were collected from established pathogenicity tests in the Screenhouse. Eggs from infected roots were extracted using the Sodium hypochlorite method as described by Boyhan *et al.* (2003) a solution of

Sodium hypochlorite and distilled water was prepared in a ratio of 1:9 and mixed thoroughly. The solution was added to chopped infected roots in a beaker and agitated for four minutes. The resulting solution after four minutes was passed through 250um, 70um, 45um, and 25um mesh sieves. Larger roots and debris were collected by 250um, 70um, and 45um sieves while nematode eggs were collected by 25um sieve after thoroughly washing with distilled water. Eggs used for the test were obtained from the suspension collected.

Juvenile Extraction from Root Samples

Following the modified methods of Coyne *et al.*, 2007, galled roots of *Cucumis sativus* were rinsed under a tap to remove adhering soil particles. The roots were chopped with a knife and a sub-sample of 5g chopped root was weighed using a digital measuring scale. The weighed roots were transferred to a clean tissue paper inside a sieve and placed inside a plastic tray. Water was poured on extraction plates at the base to wet the roots, ensuring that the water is sufficient enough. The setup was labeled accordingly. Water from the plastic tray was poured into a labeled cup after 48 hours, using a wash bottle to rinse the plate. The suspension was allowed to settle for 4 minutes and the supernatant from the labeled cup was gently decanted. Juveniles used for the laboratory experiment were picked carefully from suspension under a compound microscope.

Testing of Soil Amendments for Nematode Mortality and Egg Hatching

This study was carried out at the Nematology and Striga unit laboratory, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria (lat: 7°22'39"N; long: 3°54'21"E). Nematode eggs and juveniles were from the samples of pathogenicity test in the screenhouse following the methods described above in 3.2 and 3.3 for juveniles and egg extraction. Powdered poultry manure suspension was obtained by dissolving 200g of the powdered in 1 liter of warm water at 10°C and was stirred very well, sieved after 2 hours and that served as 100% concentration. One (1) milliliter of suspension containing approximately 20 eggs and freshly hatched juveniles of root-knot nematode were

dropped into 5ml in Enzyme-Linked Immunosorbent Assay (ELISA) well-plates containing approximately 4 ml of each treatment. Distilled water was used in the case of Control. The stock solution of aqueous extracts of botanicals, and poultry manure extracts were at different concentrations at the percentage of 100, 75, and 50 serial dilution i.e. 2ml. of stock solution + 2ml. of water; 3ml. of stock solution + 1ml. of water and 100 is the main concentration of stock solution respectively. Each dilution was replicated four times. These ELISA well-plates were kept in a BOD incubator at $25\pm 1^\circ\text{C}$. Larval mortality after 24hrs, 48hrs, 72hrs, and 96hrs exposure to different dilutions of different treatments was recorded by counting living and dead second-stage juveniles under a stereoscopic binocular microscope. Juveniles and eggs put in distilled water were treated as a control. Larvae that did not respond to touch by a fine needle were counted as dead. Egg masses are put in distilled water and treated as a control following the technique used by Bernard *et al.* (2017). Serial Dilution of Aqueous Extract of treatments was as follows:

100% = 4ml of concentration + 1ml of egg or juvenile suspension
 75% = 3ml of concentration + 1ml of water + 1ml of egg or juvenile suspension
 50% = 2ml of concentration + 1ml of water + 1ml of egg or juvenile suspension
 Control = 4ml distilled water + 1ml of egg or juvenile suspension

Statistical Analysis

All the data collected obtained were analyzed using descriptive statistical packages of Microsoft excel and Analysis of variance (ANOVA) and mean differences were determined by Duncan's New Multiple Range Test (DMRT) at a 5% level of significance using SPSS Package version 2022.

RESULTS

Pathogenicity Test of *Meloidogyne incognita* on Four Cucumber Varieties

Evaluation of four cucumber varieties infected with *Meloidogyne incognita* in the screenhouse is shown in tables 1 and 2. The four cucumber varieties were susceptible to the pathogen as indicated by increased soil nematode population and root galling. From the result, there were significant differences between the varieties of cucumber screened against root-knot nematode (*M. incognita*). Marketer had the highest soil nematode population of 9768.27 in all the screened cucumbers and the galls were rated as 5.60. Marketer more cucumber variety recorded 9431.06 as the soil nematode number with a root gall rating of 4.31; it was followed by Murano cucumber variety having soil nematode population of 7332.55 and the galled roots rated as 2.31. Poinsett had the lowest number of Soil nematode number and the galled roots recorded 1.63 when rated.

Table 1: Effect of *Meloidogyne incognita* on four cucumber varieties

Variety	Growth Rate (cm)	Yield Parameter(g)
Marketer	55.08 ^{b*}	5.08 ^b
Marketer More	65.05 ^a	3.89 ^c
Poinsett	69.32 ^a	6.98 ^a
Murano	35.04 ^c	2.85 ^c

*Values in the same column followed by the same letters have no significant differences at $p < 0.05$ according to Duncan Multiple Range Test (DMRT)

Table 2: Comparison of the Effect of *Meloidogyne* species between Control and Cucumber Variety Using T-Test

Variety	Growth rate (cm)	Yield parameter (g)	Initial soil nematode population	Soil nematode population at harvest	Gall ratings
Marketer	66.51 ^c	5.92 ^c	2000	9768.27 ^d	5.60 ^d
Control	85.08 ^b	7.63 ^a	0.00	0.00	0.00
Market more	65.05 ^c	3.90 ^b	2000	9431.06 ^c	4.31 ^c
Control	87.03 ^b	6.91 ^b	0.00	0.00	0.00
Poinsett	75.32 ^b	6.92 ^b	2000	1807.11 ^a	1.63 ^a
Control	99.41 ^a	8.80 ^a	0.00	0.00	0.00
Murano	35.04 ^d	2.90 ^d	2000	7332.55 ^b	2.31 ^b
Control	78.02 ^b	5.75 ^c	0.00	0.00	0.00

Note: Different superscript assigned to the variety and control implies significant differences in the effect.

In-vitro Test of Treatments on *Meloidogyne* species

Studies were carried out to assess the effects of the aqueous extract of two plants and different forms of poultry manure on egg hatching and larvae death of *Meloidogyne incognita* under laboratory conditions.

Effect of Treatments on Second-Stage *Meloidogyne* species Juveniles mortality

Aqueous treatment extracts were found to cause larvae mortality of *Meloidogyne* species. The rate of mortality was directly proportional to the concentration of extract used. The highest juvenile

mortality was observed at 100% treatment concentration, while the control test recorded the least number concerning juvenile mortality (Figs 1a-c). Among treatment extracts, the highest mortality was recorded with powdered poultry manure at 20, followed by compost tea at 18 and *Gliricidia sepium* at 16 at 100% concentration after 24 hours. The powdered poultry manure was found consistent in terms of juvenile mortality as it was effective in all concentrations throughout the hours of exposure. Juveniles in distilled water which served as control recorded the lowest number of juvenile mortality.

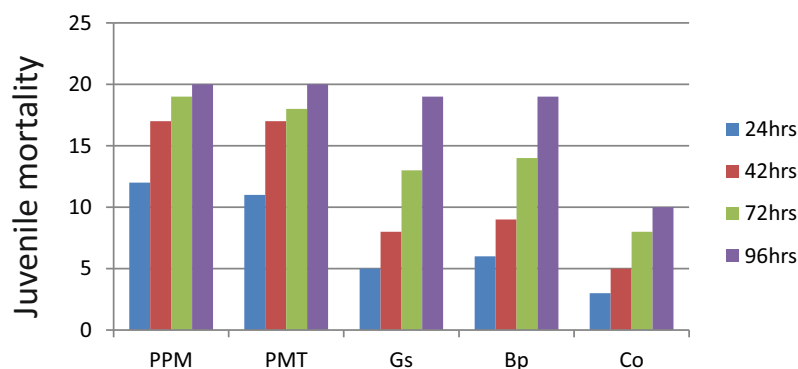


Fig.1a Effect of treatments on juvenile mortality @ 100% Concentration

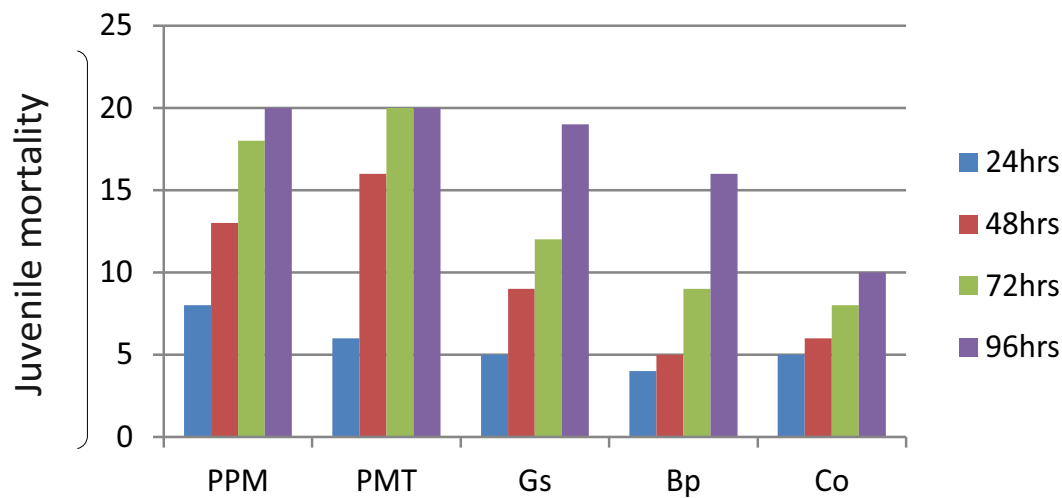


Fig.1b Effect of treatments on juvenile mortality @ 75% Concentration

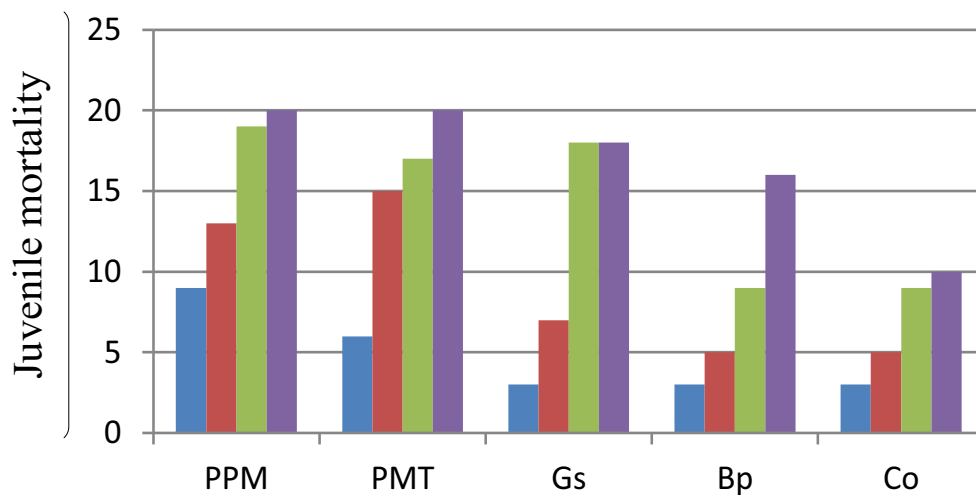


Fig.1c Effect of treatments on juvenile mortality @ 50% Concentration

Key: PPM: Powdered poultry manure; PMT: Poultry manure tea; Gs: *Gliricidia sepium*; Bp: *Bryopyllum pinnatum* and Co: Control

Effect of Treatments on Egg Hatching of *Meloidogyne* species

The highest egg hatch was observed in the control (distilled water), while the lowest was recorded from powdered poultry manure showed a high inhibition egg hatch. This was followed by poultry manure tea, before *B. pinnatum* and *G. sepium* at different levels of concentrations throughout the time of exposure (Fig.2a-c).

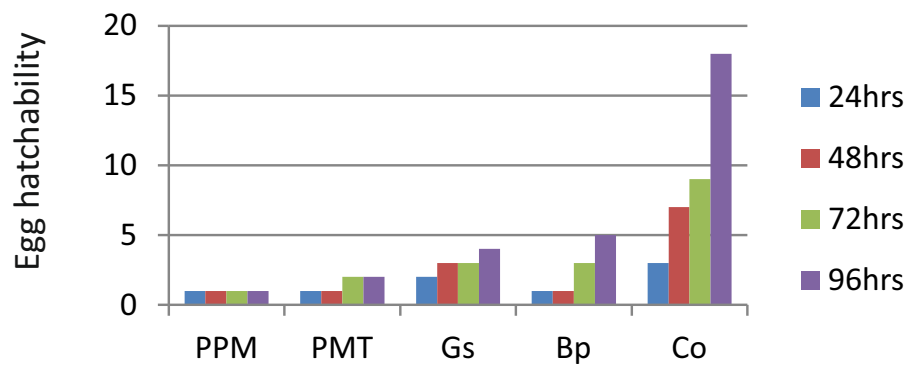


Fig.2a Effect of treatments on egg hatchability @ 100% Concentration

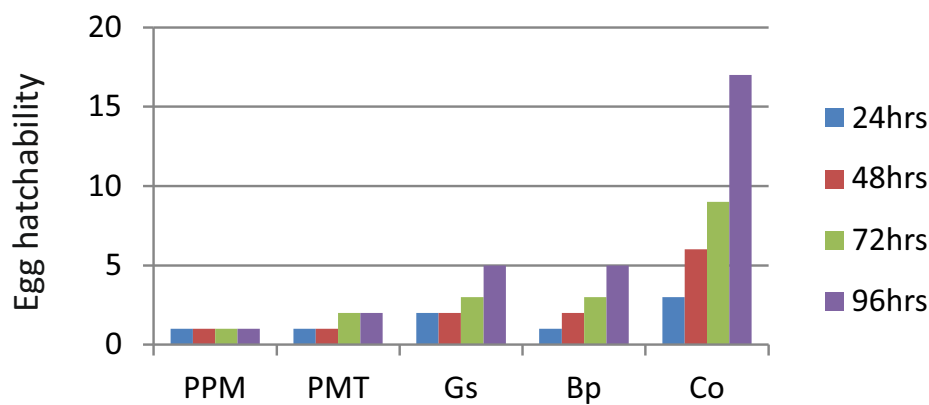


Fig.2b Effect of treatments on egg hatchability @ 75% Concentration

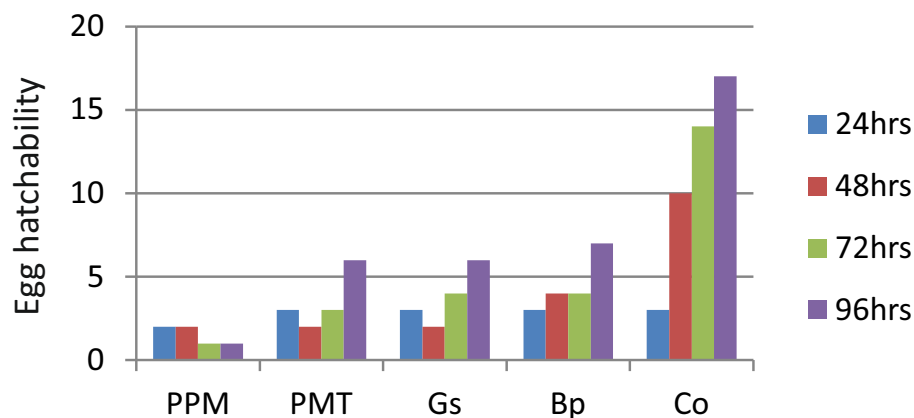


Fig.2c Effect of treatments on egg hatchability @ 50% Concentration

Key: PPM: Powdered poultry manure; PMT: Poultry manure tea; Gs: *Gliricidia sepium*; Bp: *Bryopyllum pinnatum* and Co: Control

DISCUSSION

All the cucumber varieties used for these experiments were susceptible to *Meloidogyne* spp. Nematode at various levels. The least susceptible variety was Poinsett which recorded a low number of soil nematodes and galls on a root. This variety however recorded significantly higher plant growth and few galls on the root under the screenhouse experiment. Other varieties (Marketer, market more, and Murano) manifested high pathogenic effects with a significantly higher number of nematode populations and heavily galled roots. Though the four cucumber varieties were susceptible to root-knot nematode infection, varietal differences played a significant role in their performance. The higher yield and lowest number of nematodes recorded by cucumber Poinsett could be traced to the genetic makeup of the cucumber variety and its interaction with the environment. This report on varietal responses corroborates the findings of Izuogu *et al.*, 2019 and Baba *et al.*, 2018 who gave reports on the pathogenicity of two pepper varieties and four groundnut varieties infected with root-knot nematodes respectively.

The inhibition of egg hatch and death of larvae as revealed in the laboratory investigation were directly proportional to the level concentration of treatments used and time of exposure. The effectiveness of these organic materials indicates that they possess nematostatic and nematicidal properties. The efficacy of botanicals in this study corroborates the work of many researchers like Agbenin *et al.* (2005) who reported that extracts of neem leaf and garlic bulb completely inhibited the hatching of egg masses of root-knot nematodes and were effective on larvae, Adekunle and Akinlua

(2007) on the utilization of aqueous extract of *Gliricidia sepium* for the control of plant-parasitic nematode on okra plant. The aqueous extracts exhibited significant mortality against root-knot nematodes. Information on an in-vitro trial of poultry manure extracts on root-knot nematode (*Meloidogyne* species) is paucity however researchers like Daramola *et al.*, 2013 reported the efficacy of poultry manure in curtailing the menace of plant-parasitic nematode population in their study.

CONCLUSION

All four varieties tested were susceptible to root-knot nematode at varying degrees. The findings of these experiments also showed that the inoculated plants recorded a significantly higher growth, yield, and number of soil nematode and root galls ($p < 0.05$) than the untreated control. Inoculated variety of cucumber (poinsett) recorded the least number of soil nematode and root galls and performed significantly higher in growth and yield.

Extracts from poultry manures and botanicals inhibited eggs to hatch and increased the death of larvae in the laboratory. The review from this experimental work advocates a new alternative to the management of *M. incognita* infecting cucumber. The infestation caused by *M. incognita* could be reduced by the tested extracts of poultry manures, *Gliricidia sepium*, and *Bryophyllum pinnatum* they are eco-friendly, available, and have an advantage over expensive and hazardous chemical nematicides which have a toxic effect on man, flora and fauna and the environment as a whole.

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