African Journal of Agriculture and Food Science
Volume 1, Issue 1, 2018 (pp. 57-67)

www.abjournals.org

EVALUATION OF BOTANICALS AS EXTRACTS FOR THE MANAGEMENT OF MELOIDEGYNE SPECIES ON TELFARIA OCIDENTALIS

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ABSTRACT: *The purpose of this study was to investigate (as well as compare) the effects of botanicals as extracts and soil amendments for the management of Meloidogyne species on* Telfaria occidentalis *in Nigeria. The following three indigenous plants were selected and used for the study: Carica papaya (pawpaw), Jatropha curcas (Belly ache bush) and Eichhorniacrassipes (water hyacinth). The soil used for the experiment was a loamy soil collected from a horticulturalist at AIT in Obio/Akpor Local Government Area of Rivers State. The experiment adopted a 9x4 (column by row) arrangement in a completely randomized design (CRD) replicated 4 times. Four (4) pumpkin seedlings were planted in each of the experiment bag. The seedlings were measuring 2cm apart from each other and 6cm deep into the soil. This was done after 2 weeks of fertilizing the soil. A total of 256 seedlings were used for the experiment. Graphical charts from Excel in Windows 7 version were used to illustrate observable differences in the experiments, while the ANOVA (Analysis of Variance) in SPSS (Statistical Packages for Social Sciences version 22) was used to further test the hypotheses. The findings of the study revealed that: the fluted pumpkin (Telfaris occidentalis) is susceptible to attack by Meloidogyne species (prior to application of the Nematicides). This is true as the Control experiment showed observable differences and relative low performance in growth and yield parameters when compared with the results and performance of the Treatment and Amendment plants. And, although no statistical significant difference was observed between the botanicals , Carbonfuran , and Control on the growth parameters of* Telfaria occidentalis, however, a remarkable and significant difference was observed between the growth parameters of the Control and the Pawpaw leaf extracts and amendment which happened to have yielded the highest growth parameters in all. Also, by way of method of application, there was no enough statistical evidence to show that there was any significant difference between the growth parameters obtained from the Amendment, Treatment and the Control. Although, the Control (at a mean of 16.69) did show evidence of having the lowest performance in this regard while the Amemdement had the best performance being slightly better than the Treatment. The study therefore recommended that a*griculturalists seek more solutions in botanicals rather than depending on expensive synthetic nematicides which may have considerable side effects on some of the growth and yield parameters of agricultural crops.*

KEYWORDS: Botanicals as Extracts, Meloidegyne Species, Telfaria Ocidentalis

INTRODUCTION

Background to the Study

Vegetables are very important because they supply most of the nutrients that are lacking in other food materials. Such nutrients include minerals, especially calcium and iron. Vegetables are acid neutralizers e.g. okra, *Corchorus* spp neutralizes the acid produced from some fruits. Vegetables prevent constipation and promote digestion as a result of fibers/roughages obtained from okra, cucumber, amaranthus, lettuce and cabbage. Vegetables are also rich sources of vitamins A, B, and C which helps to lower susceptibility to infection. They are generally needed to have balanced diets and overcome nutritional deficiencies as well as make our staple food more palatable and enhance their intake (Muanya, 2015). The benefits of vegetables are numerous; however, the focus of this study is on *Telforia Occidentalis,* a drought-tolerant, dioecious perennial crop that is indigenous to Southern part of Nigeria and belongs to the family of Cucurbitaceae (Agriculture Online Hub, 2015). It is widely grown in many nations of West Africa, but is mainly cultivated in Nigeria, for its highly nutritious leaves which is used primarily in soups and herbal medicines (Nwanna, 2008).

MATERIALS AND METHODS

Materials

- **Plants for the Extracts:** The following three indigenous plants namely: *Carica papaya* (pawpaw), *Jatropha curcas* (Belly ache bush) and *Eichhorniacrassipes* (water hycinth).
- **The Soil:** The soil used for the experiment was a loamy soil collected from a horticulturalist at AIT in Obio/Akpor Local Government Area of Rivers State.
- **The Pumpkin Seedling (**Telfaria *occidentalis***):** This pumpkin seedling was purchased from mile 3 market in Port Harcourt.
- **The Polythene Bags:** The polythene bags were used to bag the soils
- **Water Leaf Root:** The roots of water leaf were collected from a farmer at Rumuchaka community in Obio/Akpor Local Government Area of Port Harcourt as natural source for the extraction of nematodes.
- **Water:** Water was used to wash the water leaf roots.
- **A light Microscope:** A light was made available for viewing and identifying the nematode species.
- **A filtration set up:** This was necessary to filter the soaked and chopped water leaf roots.
- **A Filter paper:** Used in the filtration set up for the filtration process.
- **A Planting Site:** A good and strategic planting site well exposed to sunlight and away from human interference was chosen and used for planting the pumpkin seedlings. The site was located within the Rivers State University (RSU).
- **1 ml Pippete:** For inoculation.

- **Ethyl Acetate:** Used as solvent for the preparation of the crude extract of each botanical used.
- **Sunlight:** For drying the leaves of the botanicals before grinding them to powdery form.
- **Mortar:** For grinding the leaves of the botanicals: *Carica papaya* (pawpaw), *Jatropha curcas* (Belly ache bush) and *Eichhorniacrassipes* (water hyacinth).
- **Corked Bottles:** These were used for the storage of the plant extracts. tray
- **A tray:** This was used to convey the plant materials.

Methods

The following methods were used to carry out the experiment:

Procedures for the Experiment

- The plant materials, namely *Carica papaya* (pawpaw) and *Jatropha curcas* (Belly ache bush), were collected from the school's agricultural farm (RSU Agricultural farm) while the water hyacinth used was obtained from a local pond in Bayelsa state.

Step 1

The Drying Process of the Plant Materials

i. Xg of the mature and fresh leaves of each of plant (*Carica papaya* (pawpaw), *Jatropha curcas* (Belly ache bush) and Eichhorniacrassipes (water hyacinth) was collected for the experiment. This was done using a weighing balance.

ii. Equal weight of the leaves of the plants were also measured and recorded.

iii. A measured quantity of each plant material was placed in a stainless tray.

iv. The trays were properly labeled to differentiate the plant materials from each other.

v. The trays containing the plant materials were sun-dried until all the moisture lost from the plant materials.

vi. The trays containing the plant materials were collected and weighed from time to time until the weight of the dry plant material remained constant. This was to ensure that the plant materials were completely dried.

vii. It is important to note that that the drying time of the different plant materials may differ. This may be due to the plants have different quantity of water content and different types of leaf thickness.

NB: These weights are recorded until a constant weight is observed.

Step 2

Pulverization of Plant Materials

i. The sun-dried plant materials were taken to the laboratory where they were grounded separately using sterilized mortar and pestle.

ii. Care was taken to guard against plant materials jumping out of the mortar to preserve quantity.

iii. The grounded plant materials were made to pass through a special sieve to help produce finer particles with good powdery forms. A 60x60 mesh sized screen made of copper wire of 0.1095mm diameter size was used for the purpose of obtaining a good powdery form of the materials.

iv. Each of the powdery form of the ground and sieved plant material was poured in a sterilized container and labeled appropriately for easy identification.

Step 3

Preparation and Determination of the Solvent (Plant) Crude Extracts

i. To prepare a 100% conc. of crude extract for each of the plant materials, 100g each of the resulting powder were dissolved in 1 litre of ethylacetate (1:10) in a transparent corked bottle. The various soaked (powdery forms of the ground plant) materials were allowed to stand for 72hrs (3days) after which they were filtered.

ii. To prepare a 50% conc. of crude extract for each of the plant materials, 0.50litre of each solution of extract was diluted to 1 litre using distilled water.

iii. Also, to prepare a 25% conc. of crude extract for each of the plant materials, 0.25litre of each solution of extract was diluted to 1 litre using distilled water.

Step 4

Pre-Planting Operations

- The cultural method was used in the experiment where weeding was done manually by hand-picking. The soil was often watered due to insufficient rainfall.
- The soil used for the experiment was sterilized by heating (using the autoclave). The heated soil was allowed to cool for 2hrs.
- After the soil sterilization, the soils were bagged to a weight of 10kg and the top sealed to prevent any nematode from remaining or gaining entry. This lasted for period of 1 week.
- Soil amendment was carried out by applying 10kg of urea so as to increase the fertility of the soil after which the soil was continuously watered every two days.

Experimental Design

The experiment adopted a 9x4 (column by row) arrangement in a completely randomized design (CRD) replicated 4 times.

Planting the Pumpkin Seedlings

Four (4) pumpkin seedlings were planted in each of the experiment bag. The seedlings were measuring 2cm apart from each other and 6cm deep into the soil. This was done after 2 weeks of fertilizing the soil. A total of 256 seedlings was used for the experiment.

Extraction of the Nematodes

The water roots selected for the nematode extraction were uprooted and washed to remove all debris, after which they were chopped into 2cm length. Using the Baerman's filtration technique. The extracted nematodes were viewed under the light microscope to confirm the actual nematode species.The Baermann technique is based on the active migration or movement of nematodes. Faeces are suspended in water. The larvae move into the water. They sink to the bottom and can be collected for identification (RVC/FAO Guide to Vertinary Diagnostic Parasitology, 2016).

Inoculation of Plants with Nematodes

After 3 weeks of planting, each of the pumpkin plant bag was inoculated with 1000 meloidogyne specie. The inoculation was done using 1ml pipette. It is expected that the roots had sprouted at this time.

Application of the Plant Extracts

25% and 50% concentrations of each plant extract were also spread on the surface of the soil (1week after the inoculation of the nematodes).

The Experimental Design for the Plant Extracts and the Carbonfuran (Synthetic Nematicide)

The experimental design adopted for the experiment was a 9 x 4 factorial arrangement (columns by row) in a completely randomized design (CRD) replicated 4 times.

Application of Carbonfuran (Systematic Nematicide) on the Plants

Based on the manufacturer's recommendations, 25g is recommended per square metre (m^2). However, since the experiment required the use of 25% and 50% of the nematicides concentration, the following calculations were made to determine the right amount for the experiment. Thus:

If Manufacturers' recommendation, 25g = 1 m^2 = 100%

Mean diameter of soil surface in each bag = 0.25m

Mean radius of soil surface in each bag = (0.25/2) = 0.125

Mean area occupied by soil in each bag = πr^2 = 3.142 x 0.125 x 0.125= 0.049m^2

Therefore, if 25g = 1 m^2

0.049 m^2 will require = 0.049x 25 = 1.225g = 100%

Thus, 50% application of carbonfuran nematicide = 0.5x 1.225= 0.612g in each bag

Also, 25% application of carbonfuran nematicide = 0.5x 0.612= 0.306g in each bag.

25% and 50% concentrations of the synthetic nematicide was therefore spread on the surface of the soil (1week after the inoculation of the nematodes).

Root Bioassay

The root bioassay was carried after 6 weeks of plant treatment. The Baerman's filtration technique was used for the extraction of the nematodes in order to enable easy enumeration of the nematodes from the roots and the soil.

Growth Parameters to Observe

The growth parameters that was considered are: plant length, surface area of leaves, numbers of leaves, percentage of seed germinate, flowering time, number of flowers, plant colour, plant weight, shoot weight, root galls indices, numbers of branches.

Number of Nematodes in Soil and Roots

The numbers of nematodes in the soils and roots were counted with the aid of the light microscope (magnification: 4 x10).

Analysis of the Data

The presentation of data was done using various graphical illustrations such as pie charts, histogram and bar charts. The results were also analyzed using ANOVA (Analysis of Variance) to calculate if there was any significant difference between the growth parameters of the plants treated with the various plants extracts.

RESULT

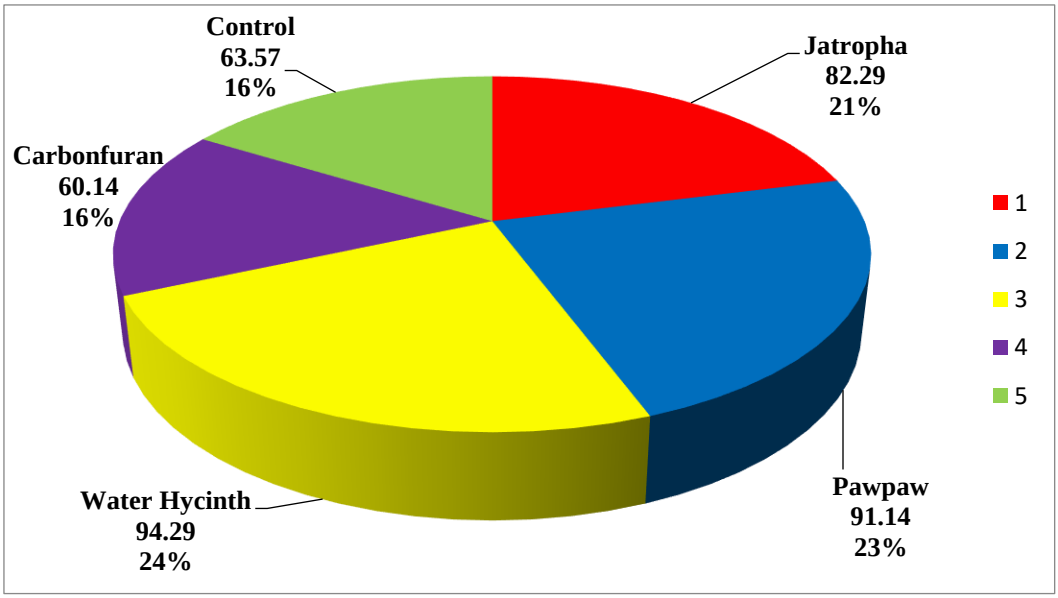

Fig. 4.11: Pie Chart Showing Water Hyacinth with the Highest Percentage Population of Leaves at 50 % Concentration of Powdery Forms of Selected Plants, Carbonfuran and Control.

Table 4.5: Data Presentation of Leaf Colour of *Telfairia Occidentalis* after 14 Weeks of Application of Powdery Forms of Selected Plants, Carbonfuran, and Control at 25% and 50% Concentrations.

Wks	Jatropha (cm)		Pawpaw Leaves (cm)		Water Hycinth (cm)		Carbonfuran (0.60g) (cm)		Control (cm)	
	25%	50%	25%	50%	25%	50%	25%	50%	25%	50%
2										
4										
6										
8										
10										
12										
14										

Table 4.6: Data Presentation of Plant Weight of *Telfairia Occidentalis* after 14 Weeks of Application of Powdery Forms of Selected Plants, Carbonfuran, and Control at 25% Concentration

Wks	Jatropha (g)		Pawpaw Leaves (g)		Water Hycinth (g)		Carbonfuran (g)		Control (g)	
	25%	50%	25%	50%	25%	50%	25%	50%	25%	50%
6	12.8	31.1	12.7	24.8	17.6	22	7.3	12.5	5.7	5.7
14	32.4	52.4	25.7	56.1	42.3	58.3	15.2	20.7	18.3	18.3
Mean plant weight	**22.6**	**41.75**	**19.2**	**40.45**	**29.95**	**40.15**	**11.25**	**16.6**	**12**	**12**

Table 4.7: Data Presentation of Mean Number of Branches of *Telfairia Occidentalis* 14 Weeks of Application of Powdery Forms of Selected Plants, Carbonfuran, and Control at 25% and 50% Concentration

Wks	Jatropha (g)		Pawpaw Leaves (g)		Water Hycinth (g)		Carbonfuran (g)		Control (g)	
	25%	50%	25%	50%	25%	50%	25%	50%	25%	50%
14	3.4	2.60	3.3	2.7	2.4	3.0	3.0	3.1	2.1	2.1

DISCUSSION OF FINDINGS

This study aimed at investigating the effects of plant extracts and Carbonfuran on the growth and yield parameters of fluted pumpkin (*Telfaria occidentalis*) infected with root knot nematode *Meloidogyne species* in Nigeria. After several experiments the following results were obtained:

The Susceptibility of Fluted Pumpkin (*Telfaria Occidentalis* To *Meloidegyne Species*).

The result of the Control experiment shows (as revealed in table 4.1) that the fluted pumpkin (*Telfaris occidentalis*) is susceptible to attack by Meloidogyne species (prior to application of the Nematicides). This is true as the Control experiment showed a remarkable difference in growth and yield when compared with the results and performance of the treated plants. In the result shown on Table 4.1, the roots, lengths, and weights of the untreated fluted pumpkin (*Telfaria occidentalis*) were seriously retarded or affected compared with the experiments with treated plant extracts and Carbonfuran. The population of leaves of the Control revealed the lowest yield compared to the treated plants. After a period of 14 weeks, the plant lengths of both the Control and the treated fluted pumpkin at 25% concentration are as follows: Jatropha Leaves Treated Fluted Pumpkin (JL-TFP) had 92cm; Pawpaw Leaves Treated Fluted Pumpkin (PL-TFP): 90.1cm; Water Hycinth Leaves Treated Fluted Pumpkin (WHL-TFP): 88.4cm; Carbonfuran Treated Fluted Pumpkin (C-TFP): 83:1cm and Control (Untreated): 49.4cm. This showed a remarkable difference between the Control and the treated plants. Although, the damage caused by Melodogyne species to the untreated fluted pumpkin may not be as serious as it is in other susceptible crops like water leaf, Cocoa and tomatoes, it is possible that with several other damaging factors coming into the play, the effect may become highly significant after a long time. Such damaging factors could be changing farming systems and/or a highly diseased infested soil area or some other climatic factors. Thus, it should not be surprising that after the destructive bioassay, after the roots of the untreated plants were properly examined for root gall formation after 14 weeks of close observation, the effect of the nematodes in causing root gall formation was not visible. There was no root gall formation on the roots of the treated and untreated fluted pumpkin. It is possible that the fluted pumpkin has some resistance to root gall formation, however, when the results of the growth and yield parameters were compared to the other treated plants, remarkable and significant differences were observed in terms of length, weight, leaf colours, and population of nematodes in soil and root, leaf widths and leaf diameters (see Appedix 1: ANOVA). The results showed a significant decline in quality and yield indicating that although the fluted pumpkin did not show a conspicuous root gall formation, yet it is logical to conclude that it did show a significant level of susceptibility to infection by Meloidogyne Species.

CONCLUSION

In conclusion, it is logical and important to state here that based on the results of the experiments, the importance of studying the effect of the root-knot nematode on agricultural crops and its management cannot be over emphasized. Although, there were no observable root-knot formation after the 14-week experiment, probably due to some natural resistance of the selected plant for the study. However, there is the possibility that if the population of the

root-knot nematode allowed increasing, it might cause significant damages to agricultural crops. Thus, it is important that effective and economical biological steps are taken to manage *Meloidogyne species on Telfaria occidentalis* (and other agricultural crops) in Nigeria. From the result of the experiments, there is enough evidnce to show that the application of the botanicals can be a very effective method in dealing with problems associated with *Meloidogyne species* on *Telfaria occidentalis*. From, the result of the experiment, it was also revealed that the application of the botanicals can be more effective and economical than the synthetic nematicide (Carbonfuran).

RECOMMENDATIONS

The following have been given as necessary recommendations (based on the results of the findings in the study):

1. The use of botanicals as extracts and amendment should be encouraged as effective nematicides against infections of essential crops by *Meloidogyne species.*
2. Agriculturalists should seek more solutions in botanicals rather than depending on synthetic nematicides which may have considerable side effects on some of the growth and yield parameters of agricultural crops.
3. The concentrations of nematicides applied on crops must be accurately measured to avoid detrimental effects on target crops.
4. The government should educate and sensitize the local farmers and the public on the dangers of nematode infections on agricultural crops.
5. Agricultural scientists should carry out more investigations on discovering those botanicals that are very effective against nematode infection.
6. Factors such as cost, availability and economic importance should be considered when determining the right botanicals to be used as nematicides.

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