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Evaluation of some Plant Extracts used in the control of Root-Knot Nematode (*Meloidogyne javanica*) in Yola, Adamawa State, Nigeria

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Abstract
This paper is a laboratory based study in which the Evaluation of some Plant Extracts used in the Control of root-knot nematode (*Meloidogyne javanica)* was conducted. The study focused on determining the phytochemical composition of the plant extracts and examine their effect on egg hatchability and juvenile (J2) mortality of *M. javanica* in the laboratory. Each of the experiments egg hatchability and juvenile mortality consisted of thirteen treatments replicated three (3) times and was laid in Completely Randomized Design (CRD). Egg hatchability and Juvenile mortality tests were conducted on about 1000 eggs and 1000 second stage juveniles. The results showed that all plant extracts inhibits egg hatching and caused juvenile mortality in varying forms when compared to the control. However, sweet orange peelings extract gave the best results as least number of eggs hatched (10%) and highest number of juvenile mortality (90%) followed by pigweed extract 15 number of egg hatched and 77.5 While Pigweed extract recorded 30 number of egg hatched and 52.6 juvenile mortality. The untreated control recorded the highest number of egg hatched 85 and zero (0%) juvenile mortality respectively. From this study, sweet orange peeling extract was found to be one of the most effective plant extracts in inhibiting egg hatch and causing juvenile mortality of *M. javanica* appreciable results were also obtained from pigweed and rice husk extracts when compared with untreated control. In conclusion plant extracts from rice husk, sweet orange peelings and pigweed have the potentials for use as nematicides which could possibly replace the costly and hazardous synthetic nematicides in the near future. Therefore, it is recommended that there is need to try these plant extracts in screen house and in the field (under natural condition) to further ascertain their efficacy.

Keywords: *Meloidogyne*, *javanica*, Extracts, Juveniles.

Introduction

Root knot nematode (*Meloidogyne javanica*) is a major plant parasitic nematode affecting crops ranging from nursery, green house, orchard among others (Aanny *et al*., 2017). The affected crops show typical symptoms of both above and below ground symptoms such as stunted growth, wilting, yellowing of leaves (chlorosis) and reduced crop establishment, short roots and root galling (Umar and Ngwamdai, 2015).

Generally, synthetic nematicides are been recommended for the control of nematodes. However, most of them are considered highly toxic and harmful to humans and the environment and as such banned from usage. However, even when acceptable and available for usage, their prices are often beyond the reach of a small-scale farmers who constitute large farming population in Adamawa state and Nigeria at large. Therefore, it has become very necessary to find alternative control strategies which are as effective as synthetic nematicides, safer to human being (farmers, consumers) and the environment also available at cheaper or low price. One of such alternatives is the utilization of pesticides from plant origin (Aanny *et al*., 2017), although generally considered to be non-persistent.

The pesticides (Nematicides) of plant origin are important sources of naturally occurring phytochemical compounds with nematicidal activity (Chitwood, 2002). They contain bioactive compounds which includes alkaloid, tannin, taponin, flavonoid, fatty acid, Glycocide, lthienyls, sothiocyanse, sesquiterpenoid and limonene among others. These bioactive compounds repel pest, discrupt their lifecycle and even discourage them from feeding which in turn affect the development and reproduction of pest. If different plant parts are being tested to identify the sources of nematicidal substances. Umar *et al.* (2013) *e*valuated leaf extract of *Sida acuta* on juvenile mortality of *M. javanica* and found that they were effective against root-knot nematodes. This encouraged the undertaking of the

present investigation on the efficiency of some other plant extracts. The main objective of this research is to determine the pyhtochemical composition of plant materials and to examine their effect of the extracts on egg hatchability and juvenile mortality of *M. javanica* in the laboratory

Materials and Methods

Experimental Sites

The experiments were conducted in the laboratory of Biochemistry, Faculty of Life Science and Crop Protection Department, Faculty of Agriculture Modibbo Adama University, Yola. The study area (Yola) lies between latitudes 8^0N and 11^0 N and longitudes 11.5^0S and 13.5^0S at an attitude of 185.9m above the sea level (Bashir, 2000).

Preparation of plant materials

The Plant materials used (rice husk, sweet orange peeling and pigweed) were shed dried on polythene sheets and later ground using pestle and motar to obtained the respective plant powders (treatments).

Extracts for the laboratory experiment of plant materials were prepared using the methods described by Kaskavala (2007). 40g of each of the ground powders were soaked separately in a conical flask (250ml) containing 100ml of distilled water and left for 24-48hours. These were then centrifuge and filtered separately through Whatmann No.2 filter paper. The filtrates obtained constituted 100% undiluted concentrations otherwise called crude extract (C). 10ml of the crude extracts were then serially diluted with 5ml, 10ml and 15ml distilled water to obtain C_1, C_2 and C_3 respectively.

C = Crude Extract
C_1 = Crude extract + 5 ml of distilled water
C_2 = crude extract + 10ml distilled water
C_3 = crude extract + 15ml distilled water

Phytochemical analysis of plant materials

Chemical tests were carried out on the plant materials using the methods described by Sofowora (1993). The materials were tested for tannins, alkaloids, saponins, flavonoids and glycocides.

Extraction of inocula

The inocula for the experiments were second stage juveniles of *M. javanica*. The juveniles were extracted from pure culture of infested tomato plant roots using modified Baermann Method (Whitehead and Hemming, 1965). The extraction process involved the use of sieves lined with tissue paper placed on shallow plastic trays followed by addition of the blended material on top of the wet sieves and water was added by the side of the sieves. The setup was left to stand for 24-48hours and the juveniles were decanted into a beaker from the nematode suspension three 10ml syringe aliquots were taken from the nematode suspension and then counted under kallenkamp microscope using a grid counting dish and average were obtained.

Extraction of nematode eggs

Egg masses from the roots of infested tomato plants were used to prepare nematode egg suspension. Nematode eggs were extracted by vigorously shaken of the tomato roots in 0.05% sodium hypochloride for 2 minutes (Hussey and Barker, 1973). The eggs were collected and rinsed with tap water through a 75cm sieves collected on a 26cm sieve and transferred into distilled water forming egg suspension. (Dong *et al.*, 2007).

Nematode egg hatchability test

Root Knot nematode egg hatchability test was determine using the method described by Ononuju and Nzenwa (2011). Nematode egg suspension at approximately 100eggs/ml was introduced into each of the 39 petridishes followed by the addition of the different concentration of the plant extracts and distilled water as control. The experiments were arranged in a Completely Randomized Designed (CRD) with thirteen treatments replicated three times in the laboratory. Eggs hatched were observed microscopically at interval of 24hours for 120hours.

Juvenile mortality test

Using 10ml syringe, aliquots of 10ml each of crude extracts 5ml, 10ml and 15ml dilutions of the water-soluble plant extracts of rice husk, sweet orange peeling, pigweed were dispensed separately into each Petri dishes containing approximately 1000 second stage Juveniles (J2) of *M. javanica* in 10ml of water. The experiments were arranged in a Completely Randomized Design (CRD) with treatments replicated three times. Dead nematodes were identified by touching them with a needle under microscope to observe whether they exhibit mobility or not. Nematode were considered dead when immobile shrank or internally vacuolated.

Results

Phytochemical analysis of plants materials

The results indicated that plant extracts of rice husk contained glycocides, flavonoid and tannins while alkaloids and saponins was not detected. Saponin, tannins, alkaloids flavonoids and glycocides were detected in sweet orange peelings extract. The results further revealed that pigweed contained saponin, tannins, alkaloid, flavonoid and glycocides.

Effects of plant extract on egg hatchability of M. javanica in the laboratory

The least number of hatched eggs was recorded in crude extract of sweet orange peelings (10%) followed by pigweed extracts (15%), rice husk (30%), and the control recorded highest number of eggs hatched (85%) as shown in Figure 1.

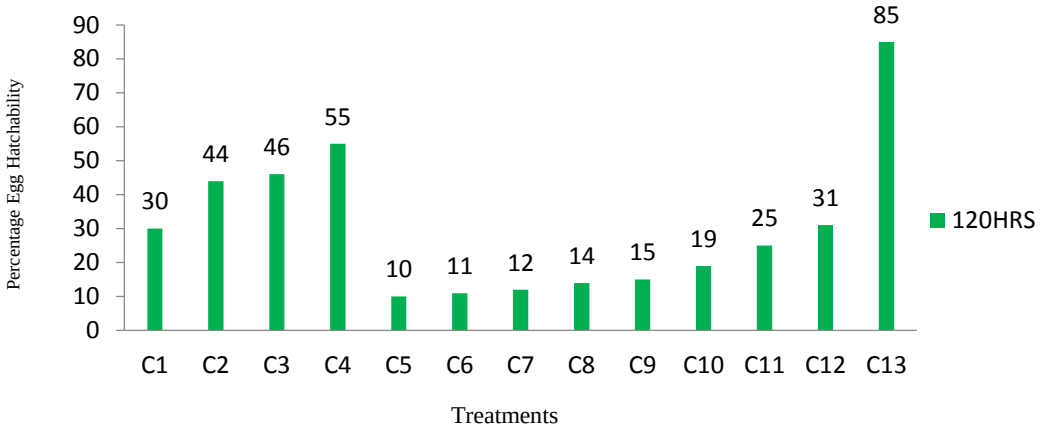

Figure 1: Effect of Plant Extracts on Egg Hatchability of *M. javanica*

Key: C_1- Rice husk crude extract; C_2- Rice husk crude extract + 5ml of distilled water; C_3- Rice husk crude extract + 10ml of distilled water; C_4- Rice husk crude extract + 15ml of distilled water; C_5- Sweet orange peelings crude; C_6-Sweet orange peelings crude + 5ml of distilled water; C_7-Sweet orange peelings crude + 10ml of distilled water; C_8- Sweet orange peelings crude + 15ml of distilled water; C_9- Pigweed crude extract ; C_{10}- Pigweed crude extract + 5ml of distilled water; C_{11}- Pigweed crude extract + 10ml of distilled water; C_{12}- Pigweed crude extract + 15ml of distilled water; C_{13}- Distilled Water only (control)

Effect of plants extracts on juvenile mortality of M. javanica

The highest juvenile mortality of 91% was recorded when the juvenile was exposed to crude extracts of sweet orange peelings followed by pigweed crude extract (84%), rice husk (52%) and the control recorded with zero percent (0%) juveniles mortality shown in Figure 2.

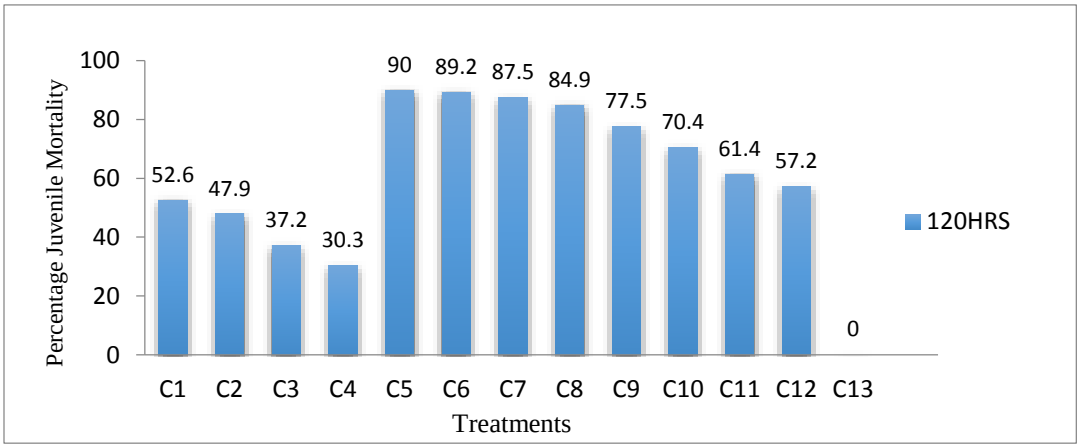

Figure 2: Effect of Plant Extracts on Juvenile mortality of *M.javnca*

Key: C_1- Rice husk crude extract; C_2- Rice husk crude extract + 5ml of distilled water; C_3- Rice husk crude extract + 10ml of distilled water; C_4-Rice husk crude extract + 15ml of distilled water; C_5- Sweet orange peelings crude; C_6- Sweet orange

peelings crude + 5ml of distilled water; C_7- Sweet orange peelings crude + 10ml of distilled water; C_8- Sweet orange peelings crude + 15ml of distilled water; C_9- Pigweed crude extract; C_{10}- Pigweed crude extract + 5ml of distilled water; C_{11}- Pigweed crude extract + 10ml of distilled water; C_{12}- Pigweed crude extract + 15ml of distilled water; C_{13}- Distilled Water only (control)

Discussion

Evaluation of Some Plant Extract on Egg Hatchability of M. javanica the Laboratory

The results on effects of different concentration of plants extracts on egg hatch revealed that all plant extracts prove effective inhibitors of egg hatched as compared to the control. Sweet orange peelings crude extract (100% concentrations) gave the highest and /or maximum inhibition of egg hatching followed by pigweed and rice husks crude extracts respectively. This goes along with the studies and findings of Javed *et al.* (2003) which revealed that crude extracts of ginger prove more effective on egg inhibition and larval mortality of *M. Javanica* than its dilutions. However, other dilutions of the extracts 5, 10 and 15ml though significant but were less effective as compared to crude extracts 100% concentrations. It is therefore evident that as crude extract diluted toxicity was decreased resulting in corresponding decreased in inhibition. This is in agreement with the finding of Adegbite and Adesiyan (2005), whose studies on root extract of siam weed, neem and lemon grass on egg hatching of *Meloidogyne. incognita*, discovered that dilutions were less effective as when compared with crude extract (100% concentrations). The highest inhibitory effect of sweet orange peelings, might be due to presence of the phytochemical, tested viz: saponin, alkaloids, tannins, flavonoids) that either affected the embryonic development or killed the eggs or even dissolve the egg masses. This also agrees with the finding reported by Adegbite (2003) and Hackney *et al*. (1975) that extracts contained flavonoids, saponins, amides, alkaloids, Limonene, glycocides singly or in combination inhibited egg hatching. The inhibitory effect observed in egg hatching according to Adebite and Adesiyan (2005) is due to the possession of ovicidal and larvicidal properties. The minimum inhibition observed in pigweed and rice husk might have been due to absence or slightly the phytochemical in the extracts. The least inhibition recorded in the control (distilled water) might be due to the absence of either phytochemical that are found to be nematicidal.

Evaluation of Some Plant Extracts on Juvenile Mortality of M. Javanica in the Laboratory

The plants extracts were effective in causing juvenile mortality of *M. javanica* with 100% concentration been more efficacious and showed a highest percentage of juvenile mortality than other concentrations. The juvenile mortality was observed to increase with increased in exposure time. A similar result was reported by Elbadri *et al*. (2009) that the extracts from the shoot and leaves of *C. coronarium*, where the seed and leaves of *A.indica* were found to be extremely toxic to juveniles *M. javanica* and could inactive (kill and immobile) more at 78% than 48hours. Mortality was found to different significantly between different concentrations of the extracts. However, sweet orange peeling crude extracts (100% concentration) gave the highest juvenile mortality (Figure 2). This might be due to phytochemical present that are known to possess nematicidal effect. The results are in agreement with those obtained by Lashein (2002) El Najdi and Mansoor (2003). The nematicidal effect of extracts may possibly be attributed to high content of oxygenated compound which are characterized by lipophilic properties that enable them to dissolve the cytoplamic membrane of the nematode cells and their functional groups interfering with enzyme protein structure. Knoblock *et al*. (1989) further reported on the mechanism, which include denaturing or the degrading the protein inhibition of enzymes and interfering with electrons flow in the respiratory chain. The lower juvenile mortality recorded in pigweed and rice husk extract could be due to absence or slightly presence of some phytochemicals and the control recorded with zero percent juvenile mortality might be attributed to the absence of either of the plant extract that will served as deterrent to nematodes

Conclusion and Recommendations

The results of this study revealed that plant extracts were effective in the control *M. javanica* as the inhibited egg hatching, cause juvenile mortality. However, sweet orange peelings of crude extracts appear to be more effective as least percentage of egg hatching and highest juvenile mortality were recorded than pigweed and rice husk. Therefore, there is needed to try these plant materials in the field under natural condition to ascertain their efficacy and there is need for further research to identify the nematicidal chemical released by each of these plant extracts in reducing nematode population.

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