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Evaluation of some bio pesticidal plants in the management of *Meloidogyne incognita*

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ABSTRACT

Utilization of compounds in extracts derived from medicinal plants is in vogue now for managing *Meloidogyne incognita* on crops owing to the unpopular use of synthetic nematicides especially in Sub-Saharan Africa. In this study, extracts from *Azadirachta indica* A. Juss and *Hyptis suaveolens* obtained using hexane, chloroform, ethyl acetate and methanol were tested for their efficacy in inhibiting egg hatching and inducing larval mortality of *Meloidogyne incognita* obtained from tomato bioassay maintained in Screenhouse. The extracts obtained from each solvent were reconstituted using Tween80+Dimethylsulfoxide (DMSO) and evaluated on hatching inhibition and larval mortality of *Meloidogyne incognita* at concentrations of 0.5%, 1.0%, and 1.5%. Furadan served as standard checks while distilled water and Tween80+(DMSO) as controls. Two separate experiments were conducted with 39 treatments each arranged in a Completely Randomized Design (CRD) with four repetitions on the laboratory bench for the hatching inhibition and larval mortality. Ninety (90) μ L of each extract was added to 10 μ L suspension containing either 15 eggs or second-stage larvae of *Meloidogyne incognita* pipette into cavity glass blocks. Hatching inhibition and larval mortality readings were recorded at 6, 12, 24, and 36 hours. Results indicated that *Neem* and *Hyptis* mixture at all concentrations extracted using any of the solvents gave 100% egg hatching inhibition from the 6th to the 36th h after treatment application. Only *Neem* and *Hyptis* mixture at 0.5% extracted in chloroform gave 100% larval mortality at the 6th h till the 36th h among the lowest concentration of the mixtures of the plant extracts. Combined solvent extracts of the two plants showed the highest activity in both hatching inhibition and larval mortality but individual solvent extracts showed the highest activity only at the highest rates used. The acute toxicity test of the most effective solvent extract of *Neem* and *Hyptis* at 0.5% extracted in Chloroform did not cause any sign of toxicity or death in the test animals.

Keywords: *Meloidogyne incognita*, Egg hatching inhibition, Larval mortality, *Neem* seed, *Hyptis* leaf and extracts.

INTRODUCTION

Global population is estimated to reach 8.5 billion by 2030 and 9.7 billion by 2050 [1]. Nigeria's population is expected to grow to 239 million by 2025 and 440 million by 2050 making it the 4th most populated country in the world [2]. Feeding this rapidly growing population certainly will present the world with lots of challenges especially in the face of current realities of the impact of climate change on agricultural food production. Among many factors that threaten food production today is the incidence of pests and diseases, of which plant parasitic nematodes are listed among the leading pests [3]. Overall, plant parasitic nematodes caused a projected yield loss of 12.3% worldwide annually; the losses are more in developing countries (14.6%) than in developed nations (8.8%) [4]. The monetary value of this loss has been translated to an annual economic crop yield loss in major crops to be USD 173 billion [5]. Root-knot nematodes (*Meloidogyne* spp.) are the most economically damaging genera of plant parasitic nematodes on vegetables and other field crops [6]. Root knot infected plants are patchy in distribution in the field and show yellow leaves, stunting growth, nutrient deficiencies symptoms and secondary infections by other pathogens [7,8]. Root knot effect is more severe in subtropical and tropical countries of the world [9] of which Nigeria is among. Nematodes are among the major obstacles to the production of sufficient food and fibre crops in Nigeria and many developing nations [10].

For the past 50 years, root knot and other important plant parasitic nematodes are best managed by the use of synthetic nematicides and soil fumigants, however, recent reports on the side effects of the use of these nematicides provoked global concern, hence their use has declined internationally because of the inherent toxicity of many existing synthetic nematicides on non-target organisms, humans, and their persistence in the environment [11]. Therefore, there is the need to find more acceptable, non-toxic, and environmentally benign alternatives. Plants having nematicidal properties have been used as bio-pesticides [12]-[14] and the potential for nematicidal activity of indigenous plants and their products has

been reported in Nigeria [15]. Plants are important sources of naturally occurring compounds having nematicidal properties, like alkaloids, diterpenes, fatty acids, glucosinolates, isothiocyanates, phenols, polyacetylenes, sesquiterpenes and thienyls [16]. Their preference as alternatives to synthetic nematicides stems from their relatively low toxicity to man and animals, safety to the environment and effectiveness in controlling the nematodes. These make plant parts indispensable as suitable alternatives in the management of plant parasitic nematodes [17]. These plants materials are locally available and in abundance in Nigeria. The present study therefore was designed to investigate the egg hatching inhibition and larval mortality of *Neem* seed and *Hyptis* leaf extracted using different solvents on root knot nematodes (*Meloidogyne incognita*).

MATERIAL AND METHODS

Collection and extraction of solvent extracts from *Neem* seed and *Hyptis* leaf

Neem seeds was collected from *Neem* tree plantations in Katsina State and *Hyptis* leaves were collected in Kaduna State, in the northern part of Nigeria. The collected materials were shade dried and pulverized to smaller particles using an electrically powered grinding machine. The plant samples (*Neem* seeds and *Hyptis* leaves) were extracted each using the cold maceration method with methanol as solvent. The pulverized samples (7 kg each) were soaked in 10 L of methanol in an extraction jar. All samples were left at room temperature for 72 hours. They were then filtered using Whatman filter paper (15 mm). The filtrate was concentrated using Vacuum rotary evaporator at 40°C then air dried to remove residual solvent. Fresh methanol was added to the residual plant material for continuous extraction, filtration and concentration until exhaustive extraction was achieved by a clear methanol filtrate.

The concentrated crude methanol extracts were each resuspended in distilled water (2 L) in a separating funnel. Hexane (500 ml) was introduced to the separating mixture of crude and distilled water. The mixture was agitated and allowed to separate, and the hexane layer decanted. The process was repeated until a clear hexane layer was achieved. The hexane filtrates were combined and concentrated using a Vacuum rotary evaporator at 40°C to give hexane fraction. The process was repeated using chloroform and ethyl acetate and the aqueous residue was concentrated using a water bath. The aqueous layer was kept to air dry to a solid residue.

The extracts left after the solvent recovery, were reconstituted in Tween80+DMSO+distilled water at three different concentrations 25, 50 and 75 mg of the extracts in 500 ml of the Tween+80+DMSO+Distilled water, which is 0.5, 1.0 and 1.5% respectively of the extracts in the solution. Extracts from the same solvents of *Neem* and *Hyptis* were also mixed at equal proportion, and their concentration varied like the three levels as individual extracts above. Ten (10) g of Furadan 3G was soaked in 400 ml of distilled water for 48 hours [18], in which the solution from Furadan served as check, while distilled water and Tween80+DMSO served as controls.

Root knot nematode egg extraction for ovicidal test and incubation for larvae emergence for larvicidal test

Root knot nematode (*Meloidogyne incognita*) cultures obtained from tomato bioassay maintained in the Screenhouse of the Department of Crop Protection, Ahmadu Bello University, Zaria, Nigeria were used in this study. Infected tomato plants were uprooted, the roots were

washed under gentle flowing tap water and cut into 1-2 cm pieces. *Meloidogyne incognita* eggs were extracted from the pieces of roots using 0.5% Sodium hypochlorite (NaOCl) method as described by [19]. The extracted eggs were further washed in distilled water at room temperature and left for 30 minutes to wash off the residual NaOCl. For egg hatching inhibition, the extracted eggs were concentrated and calibrated using a counting slide to 15 eggs per 10 µL of distilled water pipette into cavity blocks and used in all treatments for the hatching inhibition evaluation. The second experiment involved the use of second stage larvae. The eggs were incubated at 28±2 °C in an incubating chamber for 24 h to obtain emerged larvae. The freshly emerged larvae were calibrated to obtain 15 J2 per 10 µL of distilled water. These were pipette into cavity glass blocks for the larval mortality evaluation of the solvent extracts as the case was for the egg hatching inhibition studies.

Treatment combinations and application

Each solvent extracts of *Neem* seed and *Hyptis* leaf was applied at 3 concentrations earlier mentioned: (0.5, 1.0 and 1.5%) for both the individual plant materials and their mixtures. One hundred and fifty-six (156) cavity glass blocks were arranged, containing 15 eggs of *M. incognita* each. Similar arrangement was done for the second experiment containing average of 15 second stage larvae of *Meloidogyne incognita*. Count of hatched eggs was taken at 6, 12, 24 and 36 h after treatment application. The number of dead juveniles was also counted at 6, 12, 24 and 36 h after treatment application. Egg hatching inhibition trial was conducted first and completed before setting the experiment for larval mortality studies of the second stage juveniles. A total of 39 treatments repeated 4 times were randomly arranged on the laboratory bench including the check and untreated control. The treatments were as follows:

NC (0.5, 1.0 and 1.5%)- *Neem* seed extracted with chloroform.

NM (0.5, 1.0 and 1.5%)- *Neem* seed extracted with Methanol.

NE (0.5, 1.0 and 1.5%)- *Neem* seed extracted with Ethyl acetate.

NNh (0.5, 1.0 and 1.5%)- *Neem* seed extracted with N-hexane.

HC (0.5, 1.0 and 1.5%)- *Hyptis* leaves extracted with Chloroform.

HNh (0.5, 1.0 and 1.5%)- *Hyptis* leaves extracted with N-hexane.

HE (0.5, 1.0 and 1.5%)- *Hyptis* extracted with Ethyl acetate.

HM (0.5, 1.0 and 1.5%)- *Hyptis* extracted with Methanol.

NC+HC (0.5, 1.0 and 1.5%)- *Neem* seed mixed with *Hyptis* extracts both extracted using chloroform

NM+HM (0.5, 1.0 and 1.5%)- *Neem* seed mixed with *Hyptis* extracts both extracted using methanol

NE+HE (0.5, 1.0 and 1.5%)- *Neem* seed mixed with *Hyptis* extracts both extracted using Ethyl acetate.

NNh+HNNh (0.5, 1.0 and 1.5%)- *Neem* seed mixed with *Hyptis* extracts both extracted using N-hexane.

Furadan solution

Tween80+DSMO solution

Distilled water

Acute toxicity test

Acute toxicity test was conducted for the most effective treatment concentration using the Organization for Economic Co-operation and Development (OECD) guideline 2001 [20], at the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. Five hundred (500) mg/kg of the most effective solvent extract concentration from this study was administered to five (5) experimental wistar rats (*Rattus norvegicus*). Each was observed for the first 3 h, noting possible signs of toxicity. Observations continued and ended fourteen days after administration.

Data analysis

Data collected on egg hatching inhibition and larval mortality were converted to percentages and analyzed in R-studio using descriptive statistics [21].

RESULTS

The result presented in figure 1 shows that all solvent extracts containing the mixture of *Neem* seed and *Hyptis* leaf and at all concentrations (0.5, 1.0, and 1.5%) gave 0 egg hatching at the 6th hours after treatments application, and it was sustained all through to the 36th hours. Only the highest concentration (1.5%) of the solvent extracts of *Neem* seed and *Hyptis* leaf individually performed similarly to the mixture where they each gave 0 egg hatching from the

6th hour and remained 0 till the 36th hour. *Neem* seed and *Hyptis* leaf solvents extracts applied individual at 0.5% and 1.0% recorded egg hatching at the 6th hour recording less than 50%, but *Neem* seed extracted using N-hexane solvent at concentration of 0.5% had the highest egg hatching of over 50% among the solvent extracts which increases with time reaching 100% at the 36th hour. Other treatments showed a slow increase in percentage egg hatching but did not give 100% egg hatching for the duration up to 36th hours after treatment application.

All treatments gave some percentage larval mortality at the 6th hour. The mixtures of *Neem* seed and *Hyptis* leaf extracted with methanol at 1.5% concentration, mixture of *Neem* seed and *Hyptis* extracted with chloroform at 0.5, 1.0 and 1.5% concentration, *Neem* seed extracted with chloroform at 1.5% concentration, *Hyptis* extracted with chloroform at 1.5% concentration, all gave 100% larval mortality from the 6th till the 36th hour (figure 2). This result showed that only mixture of *Neem* seed and *Hyptis* leaf extracted with chloroform at 0.5% concentration gave 100% larval mortality among the mixtures being the lowest rate at the 6th hour. More treatments had increased larval mortality as the time progressed and had 100% mortality at the 36th hour (figure 2).

The results observed due to the acute toxicity test after administering the most effective concentration to the Wistar rats were increased defecation, urination, and signs of depression during the first 3 hours of administration. The normal activities of the test animals returned thereafter, and observations continues till the 14th day with no record of death.

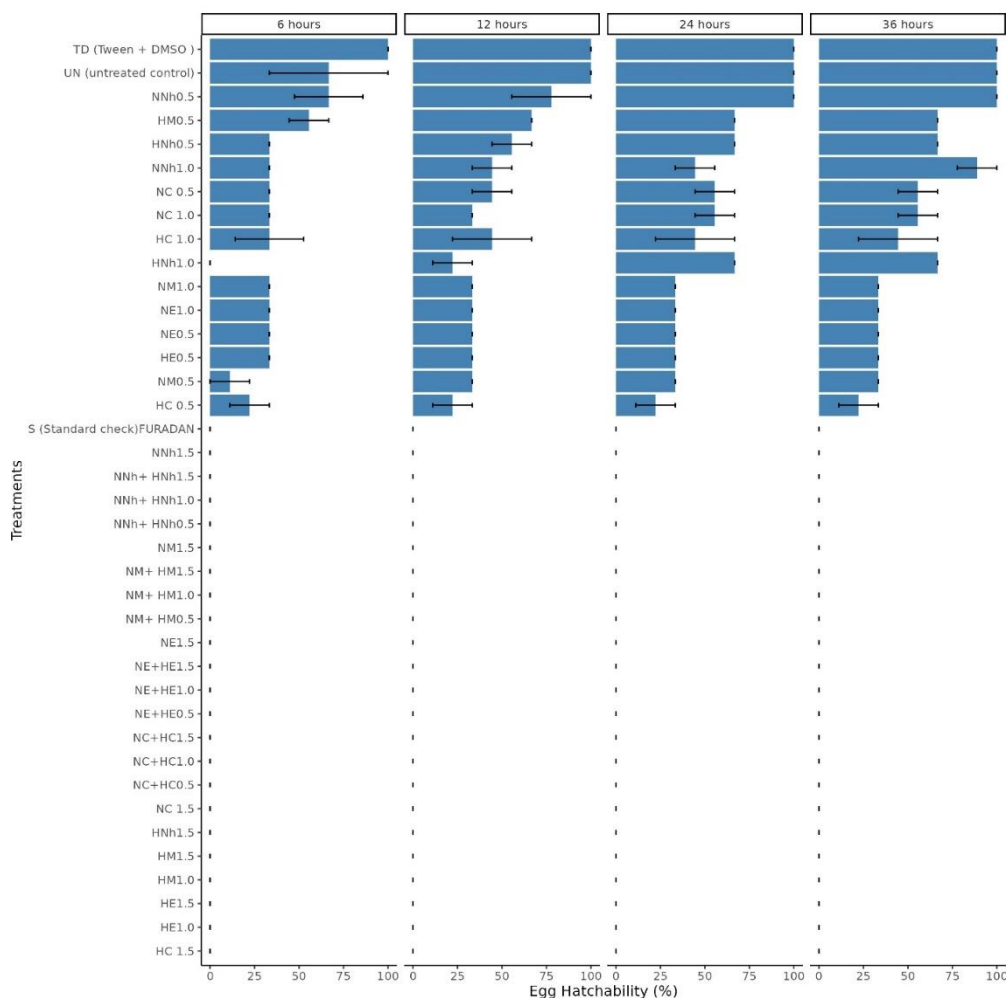


Figure 1: Effect of varied concentrations of solvent extracts of *Neem* seed and *Hyptis* leaf on egg hatching of *M. incognita*

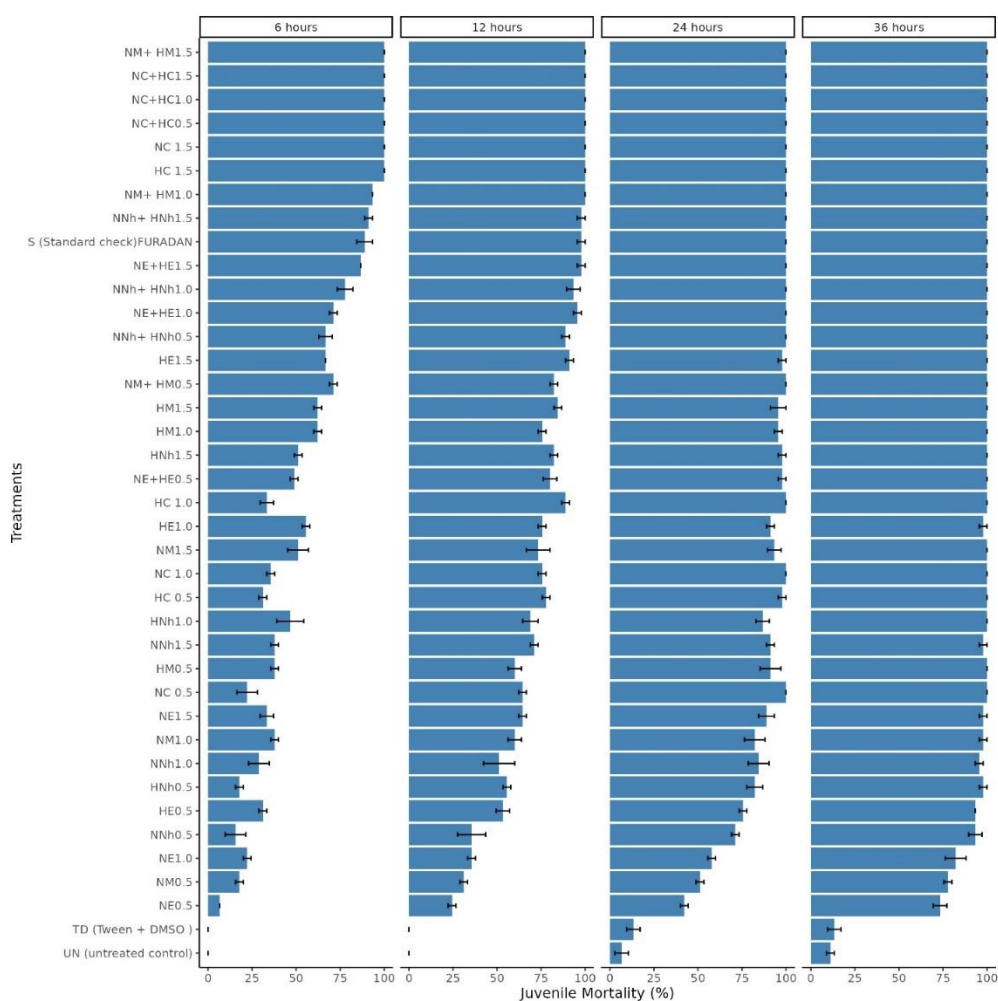


Figure 2: Effect of varied concentrations of solvent extracts of *Neem* seed and *Hyptis* leaf on larval mortality of *M. incognita*

DISCUSSION

From this study, solvent extracts consisting of the mixtures of *Neem* seed and *Hyptis* leave at all concentrations (0.5, 1.0 and 1.5%) inhibited egg hatching at the 6th to 36th hour after treatment application by 100%. But for either of the solvent extracts of the *Neem* seed and *Hyptis* leave individually, 100% egg hatching inhibition was observed only at the highest concentration (1.5%). This study established the effectiveness of these solvent extracts being more potent in mixture at lower concentrations than the individual solvent extracts and this suggest the possibility of synergistic effect of the combined ingredients from the solvent extracts. [22] using *Quillaja saponaria* extracts observed that individual extracts fractions did not perform optimally in the control of important plant parasitic nematodes, but when the fractions were combined, they performed optimally in controlling the plant parasitic nematodes. This study also established that individual solvent extracts are effective in hatching inhibition of *Meloidogyne incognita* eggs but at the highest concentration (1.5%) used in this study. [23] in a similar study reported that individual solvent extracts gave 100% hatching inhibition when used at the highest concentration. Also, the works of [24] reported that egg hatching inhibition of *Meloidogyne incognita*, decreases with increase dilution of individual plant extracts in an *in vitro* study.

The larval mortality study showed that, treatments extracted from chloroform solvent at all concentrations (0.5, 1.0 and 1.5%) gave 100% larval mortality starting from the 6th hour after treatments application. Only *Neem* mixed with *Hyptis* methanol solvent extracts

at the highest concentration (1.5%) performed at par with the chloroform solvents extracts at the 6th hour after treatments application. This study showed high activity in the chloroform extracts, which may contain potent ingredients with lethal effects on eggs and larvae of *M. incognita* causing the hatching inhibition and larval mortality even at the lowest concentration. In a related study, [25] working with fractions of *Cucume longa* extracted using methanol, chloroform, ethyl acetate and hexane observed that chloroform extracts at all concentrations tested gave maximum mortality when compared to fractions from other solvent extracts at all the time intervals of exposure. In this study also, other solvent extracts had increase in larval mortality as time of exposure increases. This may be due to length of time of exposure to the ingredient in the extracts leading to more death of the larva. A similar studies observed an increase in larval mortality due to four extracts from leaves and seed on the mortality of larvae of *Meloidogyne incognita in vitro* as time of exposure increases [24]. From this study, it is clearly shown that egg hatching inhibition and larval mortality needs evaluation when considering the effectiveness of nematicides as the vary, and the most effective in both cases should be the most likely to be recommended.

The acute toxicity studies showed that the signs exhibited due to the acute toxicity test gave a temporary upset of the metabolism of the test animals which was not lethal, hence the animals returned to their normal activities thereafter and no death was recorded after 14 days. This shows that the compounds of plant-based extracts are relatively safe to humans. Reviews by many authors affirms the relative safety

of the use of plant based extracts in the management of plant diseases and as food preservatives [26]-[28].

CONCLUSION

The nematicidal effects of different solvent extracts of *Neem* seeds and *Hyptis* leaves against root knot nematode (*Meloidogyne incognita*) egg hatching inhibition and larval mortality was studied. The mixtures of all solvent extracts of the *Hyptis* and *Neem* at all rates (0.5, 1.0 and 1.5%) were effective against egg hatching of *Meloidogyne incognita* eggs while individual solvent extracts of these plants materials were effective only at the highest rates in egg hatching inhibition. Chloroform mixture at the lowest rate gave 100% larval mortality as the only lowest rate at the 6th hour at par with other higher treatments. The ingredients from the chloroform solvent extract at 0.5% concentration was relatively safe and do not cause acute toxicity as shown from this study. Further studies are needed to isolate and analyze the active constituents from the chloroform extracts of *Neem* and *Hyptis* mixture at 0.5% and the LD₅₀ be conducted.

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Conflicts of Interest

The authors declare no conflict of interest.

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