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Comparative effects of *Acalypha wilkesiana* leaf extract, hot and boiling water on plantain growth response and nematode damage

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Abstract. This research was carried out to investigate the comparative effect of pre-plant treatment of plantain suckers in red *Acalypha* leaf extract, hot water and boiling water on plant emergence and nematode damage. The treatments were paring and dipping in crude water extract of red Acalypha leaf for 5, 10, 15 and 20 min duration, hot water treatment at 52±2^{o}C, boiling water treatment at 100^{o}C and non-pared control. Cumulative plant emergence count was taken every 14 days till 56 days after planting. Destructive sampling was carried out 55, 84 and 111 days after planting to assess root and rhizome damage, identify and count nematode species densities. Pre-plant dip in extract for 20 min was phytotoxic relative to plant emergence. Highest percentage of plants that emerged was in the hot water treatment while the extract treated materials recorded the least percentage emergence with the 20 min dip being the poorest. There was inconsistency in the root damage results but with an indication that 15 min dip in crude water *Acalypha* leaf extract could reduce root damage and also nematode densities.

Keywords: Boiling water, control, *Helicotylenchus multicinctus*, hot water, leaf extract plant parasitic nematode, plantain, *Pratylenchus coffeae*

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INTRODUCTION

Plantains are useful components in mixed farming systems providing continuity of food, income and employment throughout the year. Plantains may be grown as permanent crop or on a system of replanting every 3-8 years or longer (Stover and Simmonds, 1987). Plantain productivity is however often impaired for reasons related to soil structure, soil fertility, drainage, and severity of diseases and pest (Stover and Simmonds, 1987), so frequent that replanting is necessary. Plant parasitic nematodes are important pathogens and the major constraint to plantain production in Nigeria (Speijer *et al*., 2001) estimated to cause on average, 50% yield loss in the southeastern part of the country (Olaniyi, 2011). The plant parasitic nematodes species most frequently associated with plantain in Nigeria are *Helicotylenchus multicintus, Hoplolaimus pararobustus, Meloidogyne* spp., *Pratylenchus* spp. and *Radopholus similis* (Rotimi *et al*., 1999; Speijer *et al.*, 2001).

Planting of infested materials is one of the major ways in which nematode species of economic significance like *Radopholus similis* (Cobb), *Helicotylenchus multicintus* (Cobb) and *Pratylenchus goodeyi*, the banana weevil (*Cosmopolite sordidus*) and panama disease *Fusarium oxysporum* are spread. A number of options exist to improve the quality of planting materials. Suckers selected for transplanting can be pared by removing roots and affected rhizome tissues. By detaching the roots, the nematode inocula present in the roots is removed and to an extent the inocula in the rhizome (Gowen and Queneherve, 1990) and the adhering soil (Olaniyi, 2006). Pared suckers may further be immersed in hot water held at a constant temperature of 55^{o}C for a period of 15 – 25 minutes (Bridge, 1975; Stover, 1972). Boiling water treatment of pared planting materials for a very brief period of 30 sec has been shown to also be effective in cleaning planting materials in an experiment conducted at Ogotun, Ekiti state of Nigeria. (Coyne *et al*., 2010).

Among several control measures available in nematode management, the use of synthetic nematicides has been the most immediate practical solution to nematode problem worldwide. With the subsistent farmer however, the use of synthetic nematicides is plagued with several limitations. Among these are high cost and lack of technical expertise in their application. Less specialized production serving the local markets may not justify the high cost of chemical treatment. There are other limitations in chemical treatment method: their use has negative impact on the environment and general public health. As a result, there is growing interest in methods of nematode management that are economically viable and environmentally friendly.

The use of botanicals in the control of plant parasitic nematodes has received global attention. When used as extracts, some botanicals have proved to be comparable with synthetic nematicides. Some of the botanicals may be applied as root dips, soil amendment, root exudates, aqueous extracts, chemical extracts, seed powders and cakes among others (Olaniyi and Moens, 2007). One of such plants is *Acalypha wilkesiana* (red *Acalypha*), a common hedge plant in Nigeria. It is a perennial ornamental herbaceous plant and in some other tropical environments where it is present, it is commonly referred to as copperleaf and is a feature of parks and gardens.

Water extract of the red or brown varieties of *A. wilkesiana* has been used for decades by the local inhabitants for paediatric treatment of skin diseases. Mothers pluck the leaves and boil in hot water, the decoction is then used to bath the affected area on their baby's skin. The baby is also given some quantity to drink. To date, no side effect of its use has been documented.

Used as soil amendment, red *acalypha* was effective in the control of *Meloidogyne incognita*, significantly resulting in more males than female nematodes and exhibiting better efficacy than leaves of Neem, *Azadirachta indica* (Olaniyi *et al.*, 2005). This implied that it reduced the damage potential of the nematode. Rotimi and Moens (2005) documented the efficacy of crude water extract of the plant leaves in the control of *Meloidogyne incognita* in the screen house. However, the authors claimed that 20 min dip of roots of tomato seedlings in the extract prior to transplanting was phytotoxic on the test plant.

This study was, therefore, initiated to establish the duration of exposure of plantain suckers to red *Acalypha* leaf extract that would not be phytotoxic, and compare the effects of red *Acalypha* leaf extract, hot water and boiling water treatments of plantain suckers on the field establishment and protection against nematode infection.

MATERIALS AND METHODS

Site Description and Trial Establishment

The trial was conducted at the Teaching and Research Farm (Crop section) of the Federal University of Technology, Akure in Nigeria. Akure lies within the Tropical rainforest belt between latitude 5^{o}N and longitude 15^{o}E of the equator, with an annual mean temperature of about 27^{o}C. The dry season is usually witnessed in Akure between November and March, while the rainy season ranged from March/April to October/November.

The experimental site covered a total area of 960m^2. Previously, the site was used for a mulch trial to study the vegetative response of plantain to two organic mulch types. The experiment had been terminated five months earlier and left to re-vegetate naturally before it was opened for this present study. Pre-plant soil nematode densities were assessed. Pre-plant sucker parameters were also measured. The site was slashed and burned before marking out and establishing the field. The experiment was laid out in a completely randomized design (CRD) of eight treatments and ten replicates (plants) per treatment. The spacing used was 3 metres between the rows and 2 metres within the rows, there were 10 suckers per row and eighty suckers in all for the experiment.

Preparation of Red *Acalypha* Leaf Extracts and Sucker Treatment

Plantain suckers were purchased from farmers in a village that adjourns the University campus while the red *Acalypha* was sourced from Owena, a suburb of Akure, Ondo State, Nigeria and the pseudostem length were reduced to about 30cm in cases where they were longer than that. Air-dried leaves of red *Acalypha* plant were pulverized, 100g of the powder was homogenized in 9 litres of cold water and left to stand for 30 minutes. Thereafter, 10 pared suckers were separately dipped into four suspensions and left to stand for 5, 10, 15 and 20 minutes. For the hot water treatment, 10 pared suckers were dipped in water at 52^{o}C ±2^{o}C for 20 minutes and thereafter, left to cool, while for the boiling water treatment, 10 suckers were dipped in water at 100^{o}C for 30 seconds. Ten suckers, which were only

pared without any further treatment served as the pared control, while 10 others were neither pared nor further treated and served as the non-pared control. The treatments were then denoted as: T1: pared control, T2: pared suckers with 5 min dip in *Acalypha* extract, T3: pared suckers with 10 min dip in *Acalypha* extract, T4: pared suckers with 15 min dip in *Acalypha* extract, T5: pared suckers with 20mins dip in *Acalypha* extract, T6: Hot water treatment at 52^0C for 20 min, T7: Boiling water treatment at 100^oC for 30 sec and T8: non-pared control. Suckers were planted in a 30 x 30 x 30 cm planting holes on 21 December 2006, 24 hrs after treatments have been assigned. Due to cessation of rainfall during this period of the year, manual irrigation of the plant once in two days was adopted until the resumption of rainfall in late March, 2007.

Data Collection

Plant establishment and growth parameters: Cumulative plantain sucker establishment count was taken at 14 days interval up till 56 days after planting (DAP). Newly emerged pseudostem with a primordial leaf was counted as newly established sucker.

Root Damage Assessment: Root necrosis was assessed as percentage of lesion on the roots according to Speijer and De Waele (1997). Three plants per plot making a total of nine plants per treatment were uprooted on 55, 84 and 111 days after planting to assess root damage and other parameters.

Nematode Extraction and Identification: Nematodes were extracted from five grams sub-samples of the roots assessed for necrosis, with a modified Bearmann tray technique (Gowen and Queneherve, 1990). Soil samples were taken from each plant hole at the time of uprooting and nematodes extracted from each sample using the modified Baermann tray technique. Plant parasitic nematodes were identified to species level with the light microscope and all developmental stages of the nematodes species were counted, except for the root knot nematode, which was identified only to genus level (since only vermiform juveniles and males could be extracted with the extraction technique). Root densities were presented as numbers per 100g root fresh weight while the soil densities per one litre soil.

RESULTS

Pre-Plant Sucker Parameters

Sucker fresh weight ranged from 150g-2.5kg with a mean value of 474.31g before paring. After paring, the value of the upper range reduced to 1.15kg with an average of 335.92g (Table 1).

Table 1. Summary of pre-plant parameters of plantain (cvr Agbagba) suckers.

Parameters	Min.	Max.
Fresh weight (g) B/P	150	2500
Fresh weight (g) A/P	120	1150
Sucker length (cm)	7	47
Inner Rhizome length (cm)	0	16
Outer rhizome length (cm)	4	19
Girth (cm)	8	48
Total root (number)	0	28
Rhizome circumference (cm) B/P	21	49
Rhizome circumference (cm) A/P	17	45
Root bases (number)	4	60
Small lesions (number)	0	5
Large Lesions (number)	0	2
Eyes/bud on rhizome (number)	0	5

B/P: before paring; A/P: After paring

The pre-plant nematode density in the soil was low with an average of one *Pratylenchus coffeae* per litre soil and an average of two *Helicotylenchus multicintus* per litre soil.

Plant Emergence

Hot water treatment (T6) had the highest percentage emergence 56 days after planting (Figure 1) with a value of 90% while pared control (T1) and boiling water treatment (T7) had 80% emergence each at 56 days after planting (DAP). The 20 minutes dip in *Acalypha* extract treatment (T5) having only 60% emergence.

Effects of Treatments on Root Damage

At 84 DAP, the highest percentage root necrosis was recorded on the pared control (T1) and it was only different statistically from the 10 minutes (T3) and 20 minutes (T5) dip in extract treatment (Figure 2b), while at 55DAPand 111DAP, there were no differences across the treatments (Figure 2a and 2c).

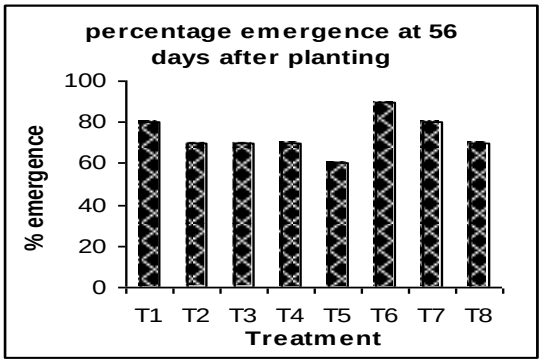

Figure 1. Effect of the duration of pre-plant exposure to *Acalypha wilkesiana* leaf extract, hot and boiling water on percentage emergence of plantain suckers
T1: Pared only (control); T2: 5 mins dip in *Acalypha* extract; T3: 10 mins dip in *Acalypha* extract; T4: 15 mins dip in *Acalypha* extract; T5: 20 mins dip in *Acalypha* extract; T6: Hot-water treatment for 20 mins; T7: Boiling-water treatment for 30 sec; T8: Non-pared control

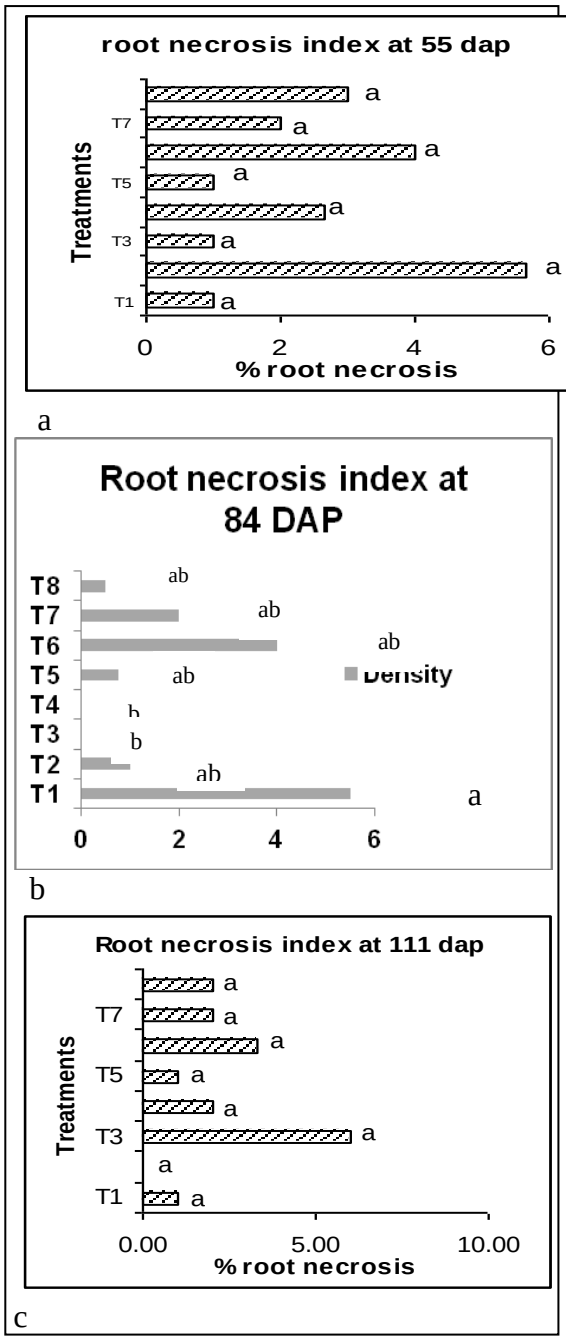

Figure 2. Effects of treatments on root necrosis index at 55, 84 and 111 (a, b and c respectively) days after planting (DAP).

T1: Pared control (no dip treatment), **T2**: 5 minutes dip in *Acalypha* extract, **T3**: 10 minutes dip in *Acalypha* extract, **T4**: 15 minutes dip in *Acalypha* extract, **T5**: 20 minutes dip in *Acalypha* extract, **T6**: Hot-water treatment for 20 minutes, **T7**: Boiling-water treatment for 30 seconds, **T8**: Non-pared control.

Effects of Treatments on Plant Nematode Species and Densities Recovered

At 55DAP (Figure 3a), there was combination of *Meloidogyne* spp and *Radopholus similis* recovered from the root in the 15 minutes dip in *Acalypha* extract (T4), at 84 DAP, nematodes were recovered only from the 20 minutes dip in extract (T5) and the non-pared control (Figure 3b), while at 111DAP, the nematode population recovered from the roots had reduced relatively to the earlier population densities observed in the preceding samplings (Figure 3a, b and c).

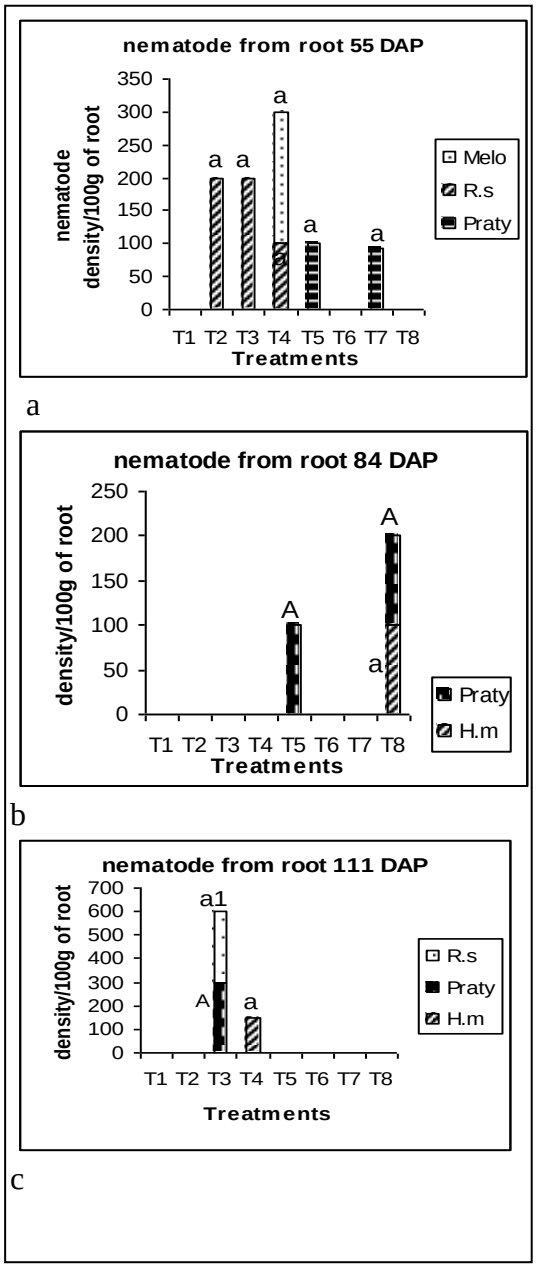

Figure 3a-c. Effects of treatments on root nematode densities at 55, 84 and 111 days after planting (DAP).

T1: Pared control (no dip treatment), **T2**: 5 minutes dip in *Acalypha* extract, **T3**: 10 minutes dip in *Acalypha* extract, **T4**: 15 minutes dip in *Acalypha* extract, **T5**: 20 minutes dip in *Acalypha* extract, **T6**: Hot-water treatment for 20 minutes, **T7**: Boiling-water treatment for 30 seconds, **T8**: Non-pared control.
Hm:*Helicotylenchus multicinctus*, Praty: *Pratylenchus coffeae*, R.s: *Radopholus similis*

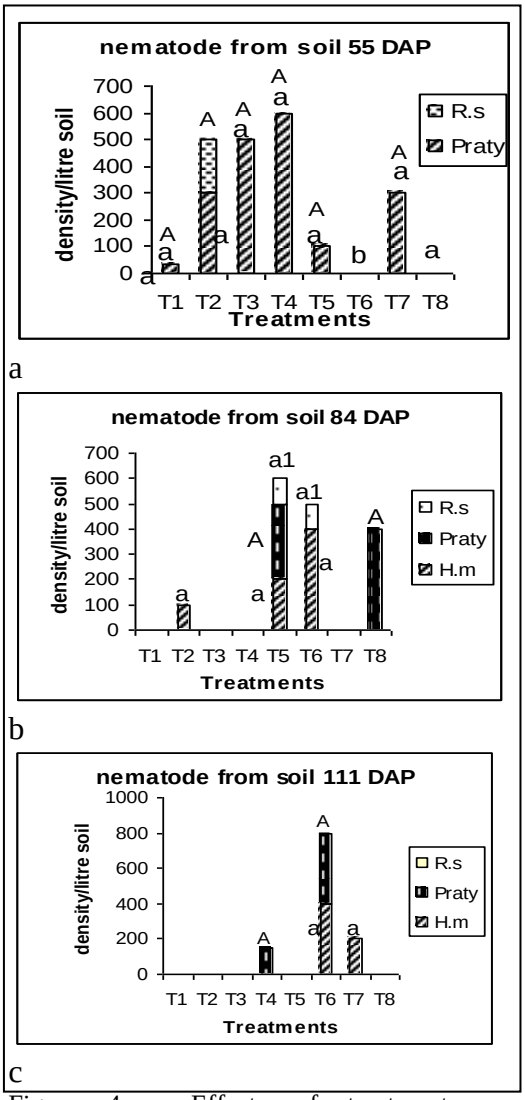

Figure 4a-c. Effects of treatments on rhizosphere nematode densities at 55, 84 and 111 days after planting (DAP).
T1: Pared control (no dip treatment), **T2**: 5 minutes dip in *Acalypha* extract, **T3**: 10 minutes dip in *Acalypha* extract, **T4**: 15 minutes dip in *Acalypha* extract, **T5**: 20 minutes dip in *Acalypha* extract, **T6**: Hot-water

treatment for 20 minutes, **T7**: Boiling-water treatment for 30 seconds, **T8**: Non-pared control.
Hm:*Helicotylenchus multicinctus*, Praty: *Pratylenchus coffeae*, Radopholus *similis*

At 55DAP, only the 5 minutes dip in extract (T2) had a combination of two species of nematodes namely *Radopholus similis and Pratylenchus coffeae* (Figure 4a), at 84DAP, the highest population density and species combination was observed in the 20 minutes dip in extract (T5) (Figure 4b) while at 111DAP, the nematode population recovered from the roots had reduced relatively to the earlier population densities observed in the preceding samplings (Figure 4c).

DISCUSSION

The low population density of nematodes in the pre-plant sampling might be due to the dryness of the soil because moisture is an important factor in nematode population dynamics. It may also be related to the slow development and build up of plant parasitic nematodes over time (Olaniyi *et al*., 2005). The low percentage plant emergence recorded in the 20 minutes dip in *Acalypha* extract (T5) might imply the phytotoxicity of the treatment. Rotimi and Moens, (2005) also observed phytotoxicity of 30 minutes root dip of 4 weeks old tomato seedlings in standard concentration of red *acalypha* leaf extract. It was generally observed that plants treated with *Acalypha* extract took a longer time to emerge compared with the controls and the hot water treatments.

The variations obtained per treatment in the nematode densities across the three sampling dates established the rationale and the importance of sampling several times so that correct assertions can finally be made. Also, sampling only the root may not give a correct diagnosis, soil sampling should be included in accord with Rotimi *et al.* (2005). Generally, sampling several times across seasons as suggested by Rotimi *et al*. (2004) would limit the level of error in the conclusion about nematode incidence in any study and better guide nematode management decisions.

The short duration of the study might be responsible for failure to obtain clear statistical differences in the treatments, especially the 15 minutes dip in Acalypha extract and the hot water treatments, which appeared to favour plant emergence. Only the 20 minutes dip in extract showed a trace of phytotoxicity in the percentage plant emergence. Effect of red *Acalypha* extract, hot and boiling water dips on root rhizome damage was not consistent over time but the effects of these treatments implied that 15 minutes dip of planting materials in red acalypha extract showed promise in reducing nematode damage and densities on plantain. The inconsistency recorded in this study may be due to the wide variation in the size of planting materials coupled with the short duration of the study. There is however the need to monitor plant response till yield sage.

The wide variation in the size of the planting materials used is a major limitation of plantain study, as Olaniyi (2011) also noted this limitation. There is need for a technique to produce more uniform planting materials for research purposes. Tissue culture has provided solution to this problem but tissue cultured materials are not within the reach of the peasants who cultivate the crop. A commercial tissue culture laboratory is also yet to be established in Nigeria. Macro propagation is an alternative, which could be explored (Baiyeri, 2005; Baiyeri & Aba, 2004). It is necessary to encourage the development of a rapid multiplication

technique for producing large quantities of plantain planting materials that would yield a more uniform set of planting materials for pathogenicity studies on the field.

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