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Effects of *Acalypha wilkesiana* Leaf Extract, Hot and Boiling Water on Plantain Growth Trend and Soil Nematode Densities

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Abstract
An experiment was laid out at the Teaching and Research Farm of the Federal University of Technology, Akure to investigate the trend in plantain growth response and nematode population density build up in response to paring, red *acalypha* leaf extract, hot water and boiling water dip. Above ground plant growth parameters were assessed 55, 84 and 111 days after planting, approximately 8, 12 and 16 weeks respectively. Population densities of plant parasitic nematodes in root rhizosphere at these dates were also investigated. The results indicated that paring enhanced plantain resistance to plant parasitic and edaphic stressors. In a holistic approach, paring and further treating pared suckers in red acalypha leaf extract for 5-15 mins conferred protection against plant parasitic nematodes and liming effect on the soil. This supposed liming effect merits further investigations. However, pre-plant dip of pared suckers in red acalypha leaf extract for 20 mins resulted in stress on the plant, which encouraged high nematode density build up and concomitant parasitism.
Keywords: *Helicotylenchus multicinctus*, *Pratylenchus coffeae*, *Radopholus similis*, plantain, red acalypha, hot water treatment, plant extract

1. Introduction

Plant parasitic nematodes are major constraints to the production of plantain (*Musa* spp., AAB-group) in Nigeria. With reference to the, black sigatoka leaf disease and the banana weevils, the nematode species, *Pratylenchus coffeae* and *Radopholus similis* are considered the key pathogens causing great damage to plantain in Nigeria (Speijer *et al.*, 2001). *P. coffeae* is strongly associated with root damage and plant toppling over, which are strong indices of plant parasitic nematode damage. The species has been ranked the number one constraint to plantain production in Nigeria (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).
A complex of *Radopholus similis, Helicotylenchus dihystera, H. multicinctus, Hoplolaimus pararobustus* and *Meloidogyne* species in South eastern Nigeria cause on average, 50% loss in plantain production in Nigeria depending on the cultivar type and cultural practices employed (Olaniyi, 2011). *Pratylenchus coffeae* was reported to be important in southwestern Nigeria, yet in spite of its seriousness, it damage potential in Southwestern Nigeria is yet to be quantified and described.
These damaging nematodes are introduced to new and otherwise clean fields through infested soil adhering to planting materials and also infected roots of sucker planting materials (Speijer *et al*., 2001, Rotimi and Opadare, 2006). This makes sanitation an important nematode management principle in plantain. To improve root and rhizome health, sucker planting materials are often pared (i.e. removal of adhering soil and peeling of the skin of the rhizome in order to expose infected tissues, remove where possible before planting or discard materials with serious infection (Blake, 1961). However, farmers do not feel comfortable with this procedure and are slow to adopt it because they often believe that such pared suckers would not survive and if they do would have low productivity. In order for farmers to adopt this cultural practice, the benefits of paring need to be demonstrated.
Also in developing effective environmentally friendly interventions, several options are considered. *Acalypha wilkesiana* (red Acalypha) leaf extract has been demonstrated to be effective against plant parasitic nematodes (Rotimi and Moens, 2005) and documented as effective plantain root health enhancer. In order to develop an effective control tactic for plant parasitic nematodes on plantain an integrated management package should be developed. Therefore, this study investigated the efficacy of combining paring with red *Acalypha* leaf extract at different exposure duration, hot water or boiling water treatments in the control of plant parasitic nematodes on plantain, cultivar Agbagba, in *P. coffeae* endemic Southwestern Nigeria.

2. Materials and Methods

2.1 Site Description and Field Layout

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The trial was conducted at the Teaching and Research Farm (Crop section) of the Federal university of Technology, Akure. Akure lies within the Tropical rainforest belt between latitude 5^{o}N and longitude 15^{0}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 April to October.
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 the site was opened for the present study. The trial was arranged in a completely randomized design (CRD) of eight treatments in all and ten replicates per treatment. The site was slashed and burned before marking out and establishing the field. 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 trial.

2.2 Preparation of Red Acalypha Leaf Extracts and Sucker Treatment
Plantain (cultivar Agbagba) suckers were sourced from within the vicinity of the Teaching and Research Farm of the University (Crop section), while the red Acalypha was sourced from Owena, a suburb of Akure. There were eight treatments in the experiment including the controls. The treatments were: pared control (T1), pared suckers with 5 mins dip in *Acalypha* extract (T2), pared suckers with 10 mins dip in *Acalypha* extract (T3), pared suckers with 15 mins dip in *Acalypha* extract (T4), pared suckers with 20 mins dip in *Acalypha* extract (T5), Hot water treatment at 52^{o}C for 20 mins (T6), Boiling water treatment at 100^{o}C for 30 sec. (T7) and Non-pared control (T8).
Air-dried leaves of red Acalypha plant were pulverized and 100g of the powder was dissolved in 9 litres of cold water in four different buckets each. A wooden rod was used to stir the mixture to ensure homogenization after which they were left to stand for 30 mins. The buckets were respectively labelled as 5, 10, 15 and 20 minutes with a marker to avoid mix-up. Thereafter, 10 suckers were dipped into each of the buckets and they were left to stand for the duration on each bucket's label. The suckers were removed and left to air dry under shade afterwards.
For the hot water treatment, 10 suckers were dipped in water at 52^{o}C $\pm 2^{o}$C for 20 mins and they were removed and left to cool for about 24 hours before planting, while for the boiling water treatment, 10 suckers were dipped in water at 100^{o}C for 30 sec. Suckers were planted on 21st December, 2006. 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.

2.3 Above Ground Plant Growth Parameters
Growth parameters were taken on the above ground parts of the sprouted plants at 55, 84 and 111 days after planting (DAP), giving approximately 8, 12 and 16 weeks after planting (WAP) respectively. Data taken included the height of the pseudostem of the mother plant from the surface to the point of emergence (axils) of the youngest leaf, the girth of the pseudostem at soil level, number of functional (green) and non-functional (dry) leaves. A leaf was considered functional when at least 75% of the leaf area is green and non-functional if otherwise (Rotimi *et al.*, 2004b). The length and the width of the youngest leaf were measured: the length of the youngest leaf opened was taken from the stalk to the leaf apex while the width was taken from the widest portion of the leaf. The leaf area was calculated as length x width x 0.83 (a constant) according to Obiefuna and Ndubizu (1979).

2.4 Nematode Extraction and Identification
Soil samples were taken from the rhizosphere of each plant at 8, 12 and 16 WAP. One hundred ml of each sample was measured unto a serviette placed in a plastic sieve. The sieve was then placed in a plastic plate and water was carefully added to the plate until the soil on the serviette-covered sieve appeared moist on top (modified Baermann tray). The setup was left to stand for 24 hours after which the resulting suspension was decanted, left to stand for 4 hours afterwards and then reduced to 30ml with a syringe.
Plant parasitic nematodes were identified to species level with the light microscope and all developmental stages of the nematode species were counted, except for the root knot nematode which was identified only to genus level and only vermiform juveniles and males that could be extracted with the extraction technique were counted. The total of all the developmental stages counted was presented in each case. Densities of the nematodes were estimated and presented per litre of soil.

2.5 Soil Chemical Analysis
At time of field establishment, soil samples were taken from each replicate hole and bulked per treatment for physico-chemical analysis. Soil samples were also taken at termination of the experiment, 16 WAP. Soil chemical analysis was carried out in the soil Analytical laboratory of the Department of Crop, Soil and Pest

Management of the Federal University of Technology, Akure. Soil properties determined included pH, Nitrogen, cations such as Ca^{2+}, Mg^{2+} and K^{+} content. Others were Phosphorus, Sodium, organic matter, and organic carbon.

3. Results

3.1 Effects of Treatments on Plantain Growth Pattern across the Sampling Dates

The effect of the treatments on plant height relative to time is presented in Figure 1(a - h) the R^2 value presented on each graph is the coefficient of determination of each regression equation. The 5 mins dip in red acalypha leaf extract (Figure 1b), 10 mins dip in red acalypha leaf extract (Figure 1c) and 30 sec dip in boiling water (Figure 1g) treatments exhibited linear trend in growth response over time. A quadratic polynomial functional trend in height increase was observed with the 20 minutes dip in *Acalypha* extract treatment over time (T5), hot water treatment and non-pared control which is the farmer's method followed (Figure 1e, f, h). Only the pared (Figure 1a) and the 15 mins dip in *Acalypha* extract (Figure 1d) displayed a cubic polynomial trend in height increase over time.

Pseudostem thickness increased at a linear rate over time only for the pared treatment (Figure 2a) while for the 5 mins dip in red acalypha leaf extract, pseudostem thickness over time responded with a quadratic polynomial function (Figure 2b). All other treatments (Figure 2c - h) displayed a cubic polynomial trend in increase in thickness over time.

In the number of functional leaves produced, the pared treatment followed a cubic polynomial function over time (Figure 3a) while boiling water dip (Figure 3g) and the farmer's material (Figure 3h) displayed a quadratic polynomial trend from 55 to 111 days after planting. The number of green (functional) leaves produced in the 5, 10, 15 and 20 minutes dip in *Acalypha* extract treatments along side the hot water treatment exhibited a linear response over the sampling dates (Figure 3b –f).

Ten, 15 and 20 min dip in red acalypha leaf extract as well as 30 sec dip in boiling water resulted in linear response in leaf area expansion from 55 to 111 days after planting (Figure 4 c, d, e and g) while expansion trend in leaf area in all the other treatments can be explained by the quadratic polynomial function.

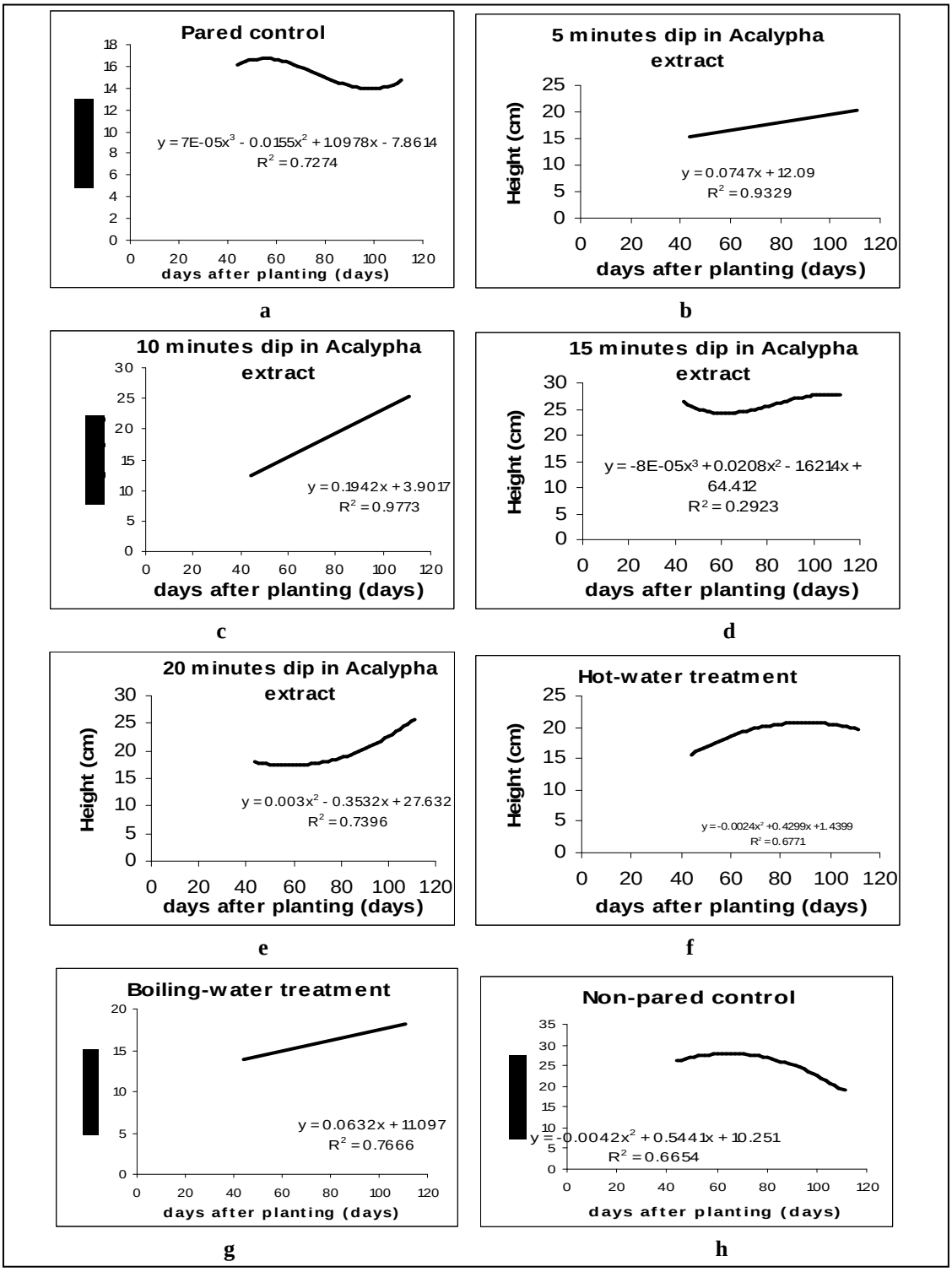

Figure 1. Effects of red *Acalypha* extract, hot and boiling water treatments on height response of plantain from 55 to 111 days after planting.

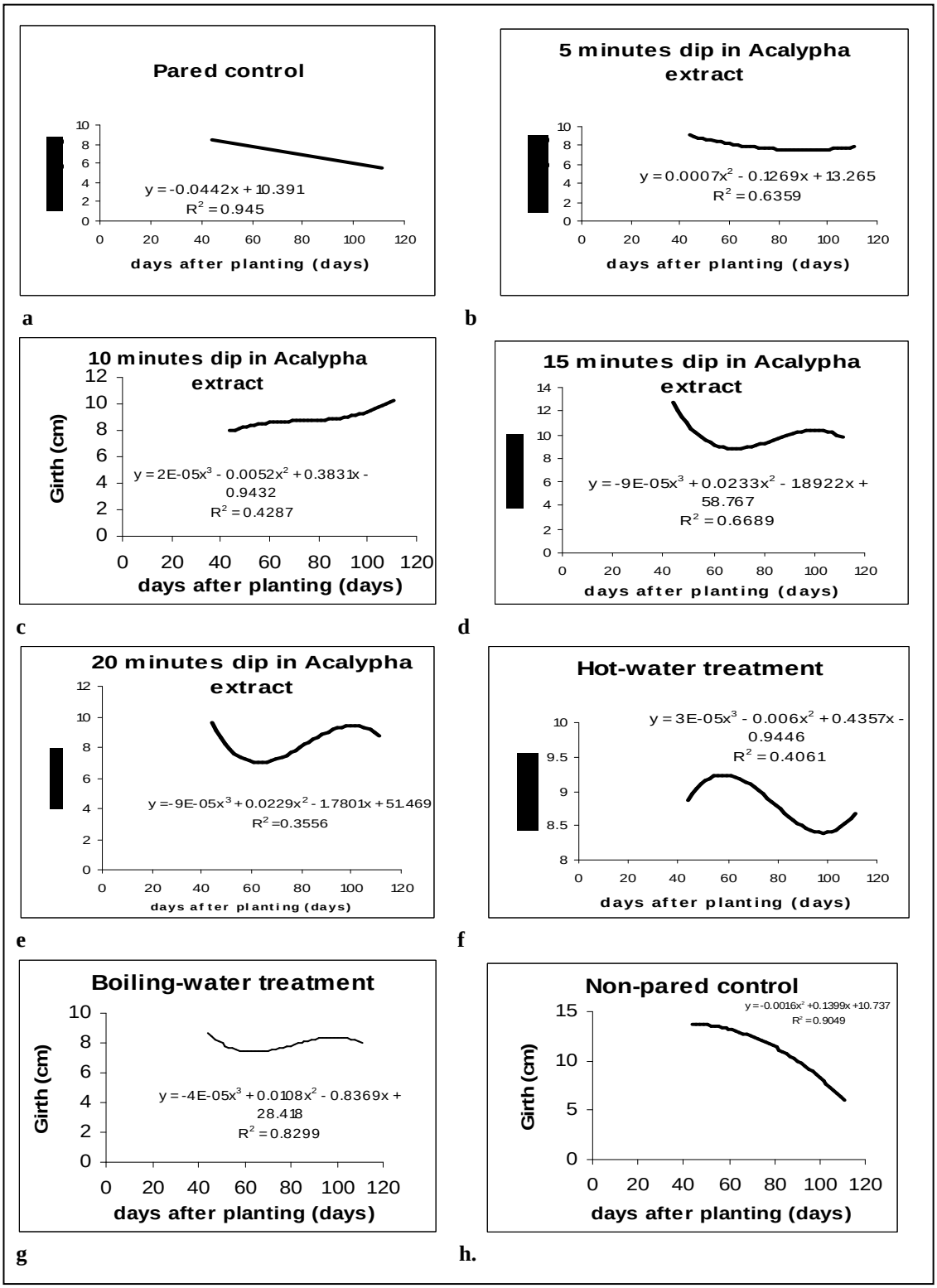

Figure 2. Effects of red *Acalypha* extract, hot and boiling water treatments on pseudostem girth of plantain response from 55 to 111 days after planting.

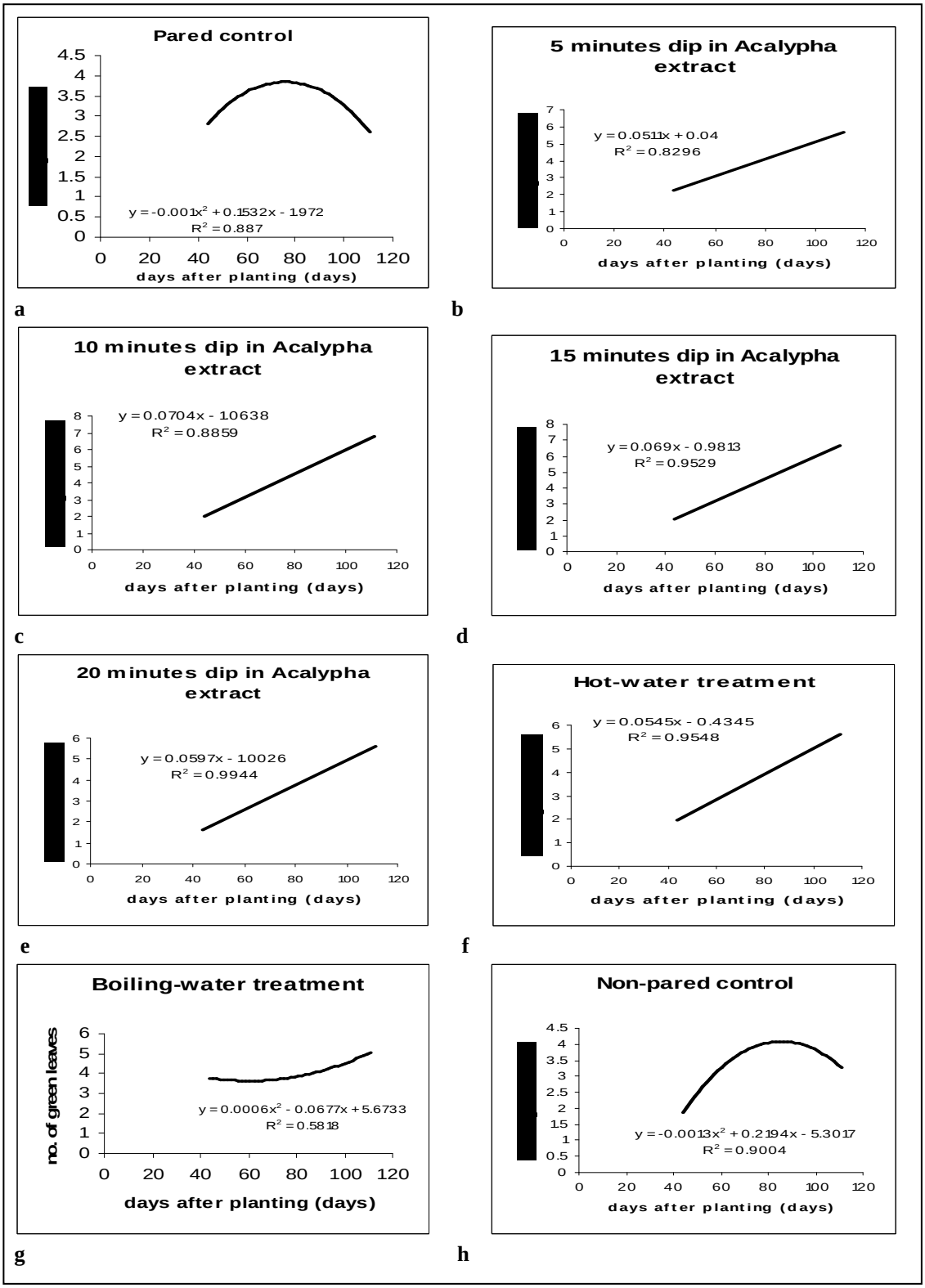

Figure 3. Effects of red *Acalypha* extract, hot and boiling water treatments on number of green leaves produced by the plant from 55 to 111 days after planting.

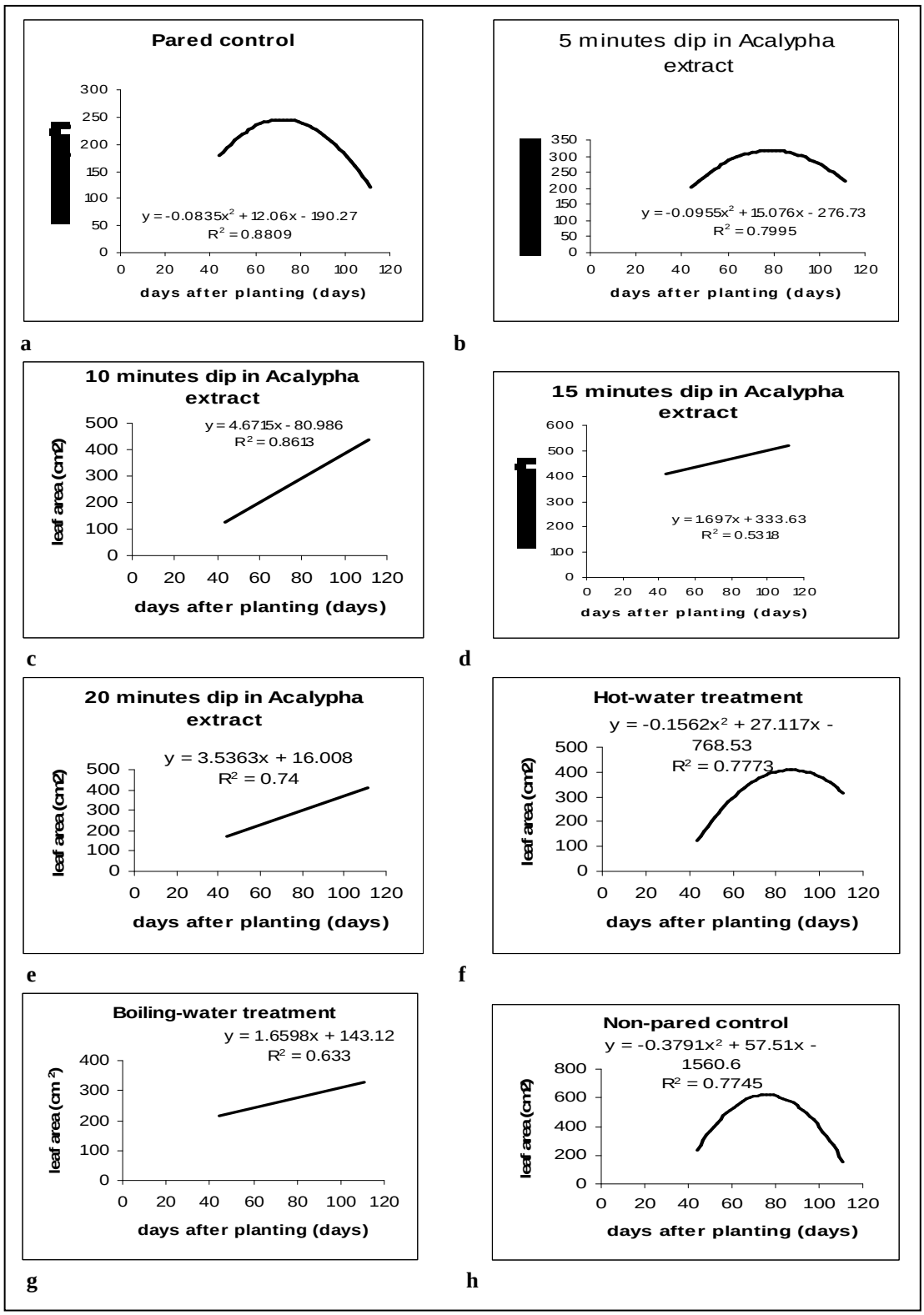

Figure 4. Effects of *Acalypha wilkesiana* leaf extract, hot and boiling water treatments on the leaf area growth trend of plantain from 55 to 111 days after planting.

3.2 Effects of Treatments on Plant Parasitic Nematode Species and Densities Recovered

Three species of plant parasitic nematodes namely: *Helicotylenchus multicinctus*, *Pratylenchus coffeae* and *Radopholus similis* were recovered from plantain rhizosphere in this study. At 55 days (8 weeks) after planting, only the 5 minutes dip in extract (T2) had a combination of two species of nematodes namely *Radopholus similis*

and Pratylenchus coffeae while *P. coffeae* was recovered under plantain in the other treatments except 20 mins dip in extract and the not pared control (Figure 5a), at 84 days (12 weeks) after planting, the highest population density and species combination was observed in the 20 minutes dip in extract (T5) (Figure 5b) while at 111 days (16 weeks), the nematode population recovered from the roots had reduced relatively to the earlier population densities observed in preceding samplings (Figure 5c).

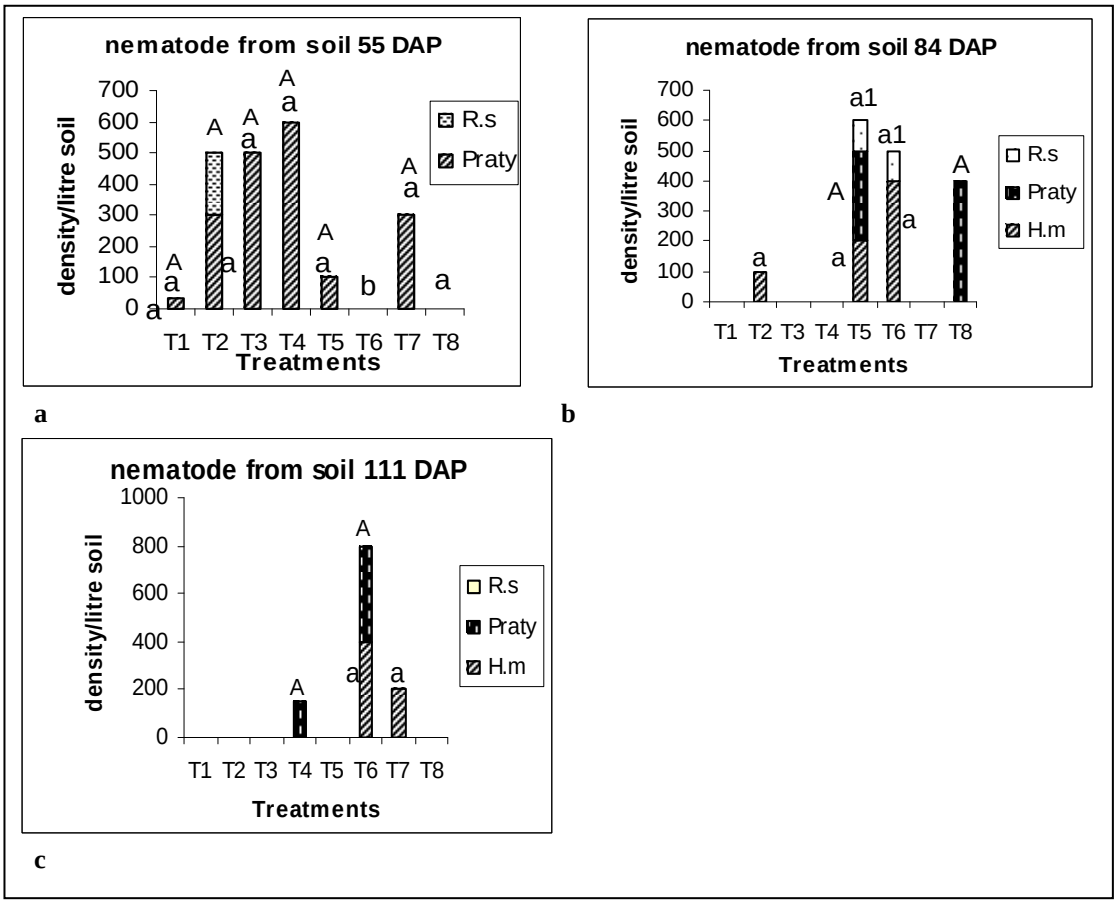

Figure 5. Effects of treatments on rhizosphere nematode densities at 55 (a), 84 (b) and 111 (c) days after planting (DAP).

T1 = Pared control (no dip treatment), 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. Column fractions followed by same letters (and numbers) are not significantly different at P≤0.05.

3.3 Effects of Treatments on Soil Chemical Properties

Chemical properties of the soil at pre-plant are presented on table 1 while table 2 presents the soil chemical properties 111 days after planting. Ten and 15 mins dip in *Acalypha* leaf extract, and 30 sec dip in boiling water depressed soil pH, while 5 and 20 mins dip in red *Acalypha* extract as well as 20 mins dip in hot water resulted in slightly raised pH. The non-pared control, which represented the farmer's method, also resulted in depressed soil pH, while the pared suckers gave relatively constant pH. Generally, soil potassium content declined between planting and termination of the experiment 111 days afterwards. Except for the 15 mins dip in *Acalypha* extract treatments, soil phosphorus also declined from planting till end of experiment. Nitrogen content also declined marginally from planting to termination of the experiment; except for the 20 mins hot water dip and 30 mins boiling water dip treatments.

Table 1. Pre-plant soil chemical properties in plots where the treatments shown were laid out

T1 = Pared control (no dip treatment), 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.

Treatment	pH	Mg	Ca	%OM	%OC	P	Na	K	N
T1	5.87	2.30	2.90	11.31	6.55	1.42	7.13	1.13	0.31
T2	5.59	1.10	3.50	6.49	3.75	1.41	12.3	0.41	0.38
T3	5.73	0.70	3.40	10.02	5.72	1.10	13.3	0.56	0.21
T4	5.79	1.30	3.10	8.62	4.99	0.96	11.39	1.24	0.20
T5	5.45	1.10	2.00	9.93	5.75	0.88	10.52	0.87	0.32
T6	5.46	1.10	2.40	13.25	7.66	1.43	12.35	0.46	0.17
T7	6.08	0.80	2.90	9.87	5.71	2.41	10.96	0.36	0.27
T8	5.68	1.80	2.90	10.97	6.34	1.29	13.04	0.36	0.18

Table 2. Effects of paring, red acalypha leaf extract , hot and boiling water treatments of plantain sucker planting materials on soil chemical properties 16 weeks after planting

T1 = Pared control (no dip treatment), 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.

Treatment	pH	Mg	Ca	%OM	%OC	P	Na	K	N
T1	5.86	1.10	3.80	11.18	6.46	0.94	11.39	0.56	0.28
T2	5.94	0.10	3.80	11.31	6.54	0.83	14.43	0.87	0.31
T3	5.24	0.50	3.80	9.73	5.63	0.59	11.83	0.41	0.15
T4	5.60	1.70	2.70	10.69	6.19	0.90	15.13	0.36	0.18
T5	5.84	2.00	2.40	11.24	6.50	1.05	11.74	0.42	0.18
T6	5.52	0.70	2.50	9.38	5.43	1.24	12.17	0.29	0.31
T7	5.56	1.60	2.80	9.31	5.39	0.88	11.83	0.25	0.27
T8	5.55	0.50	2.80	9.31	5.31	1.04	10.26	0.27	0.13

4. Discussion

The linear response obtained for some of the growth parameters for 5, 10, 15 and 20 minutes dip in acalypha extract, hot and boiling water treatments in this study connotes that the rate of change in the growth of the plant in the treatments increased over time at a constant rate in spite of the prevailing harsh dry condition. Polynomial functional relationships obtained in the non-pared control (Farmer's method) might be an indication of the high susceptibility of unpared planting materials to dry weather. This then suggests that paring the sucker before planting enhanced the plant's ability to withstand biotic and edaphic stress. Further treating the planting materials with plant extract or hot water (52 $\pm 2^{o}$C) also conferred some level of resistance to these stress factors. Olaniyi (2014b, In Press) also reported functional growth trend in time for plantain in reaction to organic mulch. The author noted that organic mulch enhanced the plants ability to withstand biotic stressors like plant parasitic nematodes in the environment.

The effects of treatments in the present study on growth parameters such as the leaf area, number of functional leaves, plant height and girth revealed that 15 minutes dip of planting materials in the extract showed some promise in enhancing plant growth performance. However, it is not clear what this additional benefit of dipping

pared suckers translates to in maintaining low nematode populations since the suckers that were only pared had very low densities of *P. coffeae* (about 20/litre soil) and maintained no detectable level of nematodes subsequently. Where as the highest density of *P. coffeae* recorded in this study was from the 15 mins dip in extract, earlier report (Olaniyi, 2014a, In Press) showed that 15 min dip reduced nematode damage to root and rhizome of plantain and also reduced nematode densities. In the present study 5 and 10 mins dip treatment seemed to better reduce nematode population densities on plantain compared to the 15 mins dip. Generally, *Helicotylenchus multicinctus, Pratylenchus coffeae* and *Radopholus similis* were the nematode species observed from plantain roots and the rhizosphere in this study. There is need to monitor plant response to these treatments and nematode development on plantain under these treatments till yield stage.

Although densities of the species in the rhizosphere were low with maximum of 100 *H. multicinctus* / litre, 600 *P. coffeae* / litre and 200 *R. similis* / litre, it is clear that densities had increased from the initial pre-plant densities of 1 *P. coffeae* / litre soil and 2 *H. multicinctus* / litre soil. This shows that planting a host plant in a soil would quickly increase the densities of the nematode species to a damaging level. It would be informative to investigate the effect of these densities on root and rhizome damage of plantain. Before the choice of a plant to put in a soil is made, it would be helpful to first do a diagnostic nematode assessment in order not to put in a host crop in a field where a damaging species is prevalent. For instance, Speijer *et al.* (2001) established Pc as the most damaging on plantain in Nigeria and the fact that it could persist in a field without the plantain host makes its control a big challenge as this might signify that it has a wide host range. The trend in population densities recovered from plantain rhizosphere is at variance with earlier record of *H. multicinctus* often in higher densities than other species on plantain in Nigeria (Speijer *et al.*, 2001; Rotimi *et al.*, 2004b, c; Coyne *et al.*, 2005).

Radopholus similis was not recovered from the soil before the establishment of this trial but was recovered from the rhizosphere of plantain 55 days (8 weeks) after planting in 5 min dip in red acalypha leaf extract treatment but subsequently, it was undetected in soil samples. This shows that first *R. similis* may not have as wide host range as *P. coffeae* or probably it could not persist as long as *P. coffeae* in the absence of a preferred host hence the absence of the species in the soil pre plant. The land had been fallowed for about 5 months before it was opened up for this study. However, the presence and disappearance of the species at subsequent sampling date supported Olaniyi's (2011) submission that for accurate diagnosis, nematode sampling should be done across seasons. The study reported here was done in the dry season and Rotimi *et al.* (2004a) similarly observed that the densities of *R. similis* could decline to an undetected level in dry soil.

No specific trend emerged in plant parasitic nematode community structure under plantain in this study. This suggests that several factors confound field studies and it would be useful to investigate nematode reactions to these treatments in controlled environment. For instance in the unpared control treatment, no nematode was detected in plantain rhizosphere 8 and 16 weeks after planting whereas at 12 weeks after planting *P. coffeae* was detected at an average of 400 nematodes / litre soil. However under the pared treatment, only very low density of Pc was recovered 8 weeks after planting. Subsequently no nematode was detected implying that it may suffice for nematode control to simply pare plantain sucker planting materials before planting without any further treatment. Olaniyi (2014a, In Press) also did not recover any plant parasitic nematode species from the roots of pared plantain at those dates.

A number of factors could be responsible for the variations observed in the nematode incidence across the sampling dates. The variations obtained in the result across the three samplings established the rationale and the importance of sampling more than once in the diagnostic study of plant parasitic nematodes so that correct assertion can finally be made (Olaniyi, 2011, 2014a, In Press). It is a common phenomenon in nematode population dynamics, and that is why it is advisable to sample several times in time and season. Also, sampling only the rhizosphere could give misleading information as the species recorded in this study are migratory endoparasites and several factors could be responsible for their density fluctuation in the rhizosphere. To confirm this, the study reported by Olaniyi (2014a, In Press) investigated root densities and a different pattern emerged. Therefore, for a more holistic overview, it is important to sample both root and soil complementarily as this would give more reliable information and better guide management decision. Other factors that may be responsible for variations include the soil condition, environmental condition and availability of moisture in the soil.

As expected, N, P & K values declined over time in this study. Results of this study revealed that introducing soil from other sources with planting materials as with the farmer's method of not paring may result in increased soil acidity. But this would be dependent on the chemical status of the introduced soil. This is revealed by the stable pH of soils from pared suckers, which had all adhering soils removed before planting. Dipping rhizomes of planting materials in red *Acalypha* leaf extract for 5 or 20 minutes tended to reduce soil acidity. The liming effect of red *Acalypha* should be further explored. Considering the overall effect of treatments on plant response and nematode density, it would be safe to recommend paring as a useful cultural practice that farmers should be

encouraged to engage in. Further treating pared suckers in red acalypha extract for 5-15 mins might confer protection against plant parasitic nematode damage (Olaniyi, 2014a, In Press) and improve soil quality.

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