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Effect of Some Plant Extracts on the Nematode Population and Yield Parameters of Waterleaf (Talinum triangulare L.) (Jacq.) Willd

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Abstract
Studies were conducted on the nematicidal properties of *Jatropha curcas*, *Cymbopogon citratus* and *Chromolaena odorata* and Carbofuran 5G. Consequently, hot water extracts of the plants and the nematicide at 20, 35 and 50 % concentrations were applied to 5-week old stem cuttings of *T. triangulare* in 5 kg of sterilized soil and inoculated with 5,000 eggs and second stage juveniles of *M. javanica*. The control had no nematode, plant extracts and cabofuran, thereafter uninoculated-untreated (Uit) and another had nematode suspension, but no extracts and nematicide [inoculated-untreated (Ut)]. The experiment was a factorial in a completely randomized design, with four replications. Data on the number of leaves, seeds and flowers, nematode population in the soil and root of *T. triangulare* including root galling index were obtained and subjected to Analysis of Variance. Results show that number of leaves was significantly ($P \leq 0.05$) higher in Uit (25.75) and carbofuran at 20% concentration (21.0) than in the Ut (11.25). There were no significant differences in the number of flowers and seeds, although visual observations show that number of seeds was higher in Uit (13.5). Nematode populations in the root and soil were significantly lower in *J. curcas* at 50% concentration (30.0 and 9.0) respectively, compared to others at the same concentration except in carbofuran for nematode in the soil. Also, gall index was lowest in *J. curcas* extract at 50% concentration. Thus, *J. curcas* extract was more effective in managing root-knot nematode in waterleaf than cabofuran.
Key words: Carbofuran, Biopesticide, *Talinum triangulare*, Root-knot nematode.

1. Introduction.

Waterleaf (*Talinum triangulare*) (Jacq.) Willd is a vegetable crop of the family *Portulacea* and consumed in parts of West and Central Africa, including Nigeria and Cameroon (Udoh and Etim, 2008). *T. triangulare* grows well in humid conditions at temperature of 30°C, but faster during the wet season, slowing down during the dry season. Commercially, waterleaf is grown from the seed and stem cuttings, usually 10–15 cm long. Seed production peaks at ten weeks after sowing (Nyananyo and Olowokudejo, 1986). As a short duration vegetable, waterleaf has some natural characteristics which make it attractive to farmers and consumers. *T. triangulare* has high quantity of crude fiber (11.12%), crude ash (33.98%), and protein (22.1%), (Akachuku and Fawusi, 1995). It serves as a recipe and 'softener' in food preparation with other rigid fibrous vegetables like 'Atama' (*Heinsia crinata*), fluted pumpkin (*Telferia occidentalis*) and *Gnetum africanum* to prepare 'Afang', a special type of soup native to the southern part of Nigeria. Both the leaves and the young shoots are consumed in large quantities in the southern part of Nigeria (Ibeawuchi *et al.*, 2007). Waterleaf is becoming increasingly important in ensuring food security as the production serves as a balancing source of revenue to the subsistence farmers and its nutritional value (Udoh and Etim, 2008).

Plant parasitic nematodes affect plant growth and yield (Osei *et al*. 2011). Root knot nematode- *Meloidogyne* species are major pathogens of fruits, vegetables and other food crops in different part of the world (Khan and Khan, 1994; Williams-Woodward and Davis, 2001). Netscher and Sikora (1990) have described over 90 species globally and out of these, *M. arenaria*, *M. javanica* and *M. incognita* are responsible for almost 90% of all damage due to root-knot nematodes (Castagnone-Sereno, 2002). Caveness (1967), noted that about 75% of agricultural soils in Nigeria are dominated by different species of *Meloidogyne*; and they remain the most destructive group of nematodes (Fawole *et al*. 1992). Caveness (1967), noted that about 75% of agricultural soils in Nigeria are dominated by different species of *Meloidogyne*; and they remain the most destructive group of nematodes (Fawole *et al*. 1992). Root-knot nematodes affect both the standard and measurable quality of marketable value of most vegetables in Nigeria (Widmer *et al*. 2005). The most visible symptom is the appearance of swellings or giant galls on the roots of affected plants (Olsen, 2000; Stonton 2001); causing the deformation of the root system which affects nutrient and water uptake by plant roots (Sikora and Greco, 1990). Infection caused by *Meloidogyne* spp can lead to reduction in the formation of nodules by nitrogen fixing bacteria (Mussarrat and Haseeb, 2000). They also interact with other plant pathogenic organisms, resulting to increased damage due to the opportunistic diseases, low yield and poor market value in affected crops (Adesiyan *et al*. 1990).

The control of root-knot nematodes has been a major problem because they are ubiquitous, with a very wide host range (Akpheokhai *et al*. 2012). The use of synthetic pesticides is considered as the most effective practical means of controlling plant-parasitic nematodes (Adesiyan, 1992). However, pesticide application as a means of

pest control has become less attractive in recent times, due to high toxicity and persistence of the nematicides and environmental pollution (Talapatra *et al*. 2017).

Many plants are known to have nematicidal properties which may be utilized as organic amendments or biopesticides (Agbenin *et al*. 2005; Egunjobi and Onayemi, 1981). Works by Egunjobi and Afolami (1994) and Onifade and Egunjobi (1994) support the toxicity of plant extracts against plant nematodes. *Jatropha curcas* is a drought-resistant, bio-fuel plant that thrives well in marginal soils (Izuogu *et al*. 2013) and its pesticidal properties against plant parasitic nematodes is documented (Habou *et al*. 2011; Ugwouke *et al*. 2011). *Chromolaena odorata* (L.) also known as siam is a tropical weed of the family *Asteraceae*. The nematicidal properties of *C. odorata* have been reported by Fatoki and Fawole (2000). Also, Adegbite (2003) indicate that *C. odorata* extracts inhibited egg-hatch in *M. incognita*. *Cymbopogon citrates*, family Poaceae is a tall monocotyledonous perennial plant cultivated in Africa and Central and South America and other tropical countries (Ernst, 2008). *C. citratus* is consumed in various forms because of its high antioxidant levels. Adegbite and Adesiyan (2005) reveal that leaf extracts of lemon grass have nematicidal properties and confirmed by Izuogu (2009) and Izuogu and Oyedunmade (2009). These botanicals are readily available, cheap, renewable, biodegradable and improves soil texture and fertility (Feizi *et al*. 2014). The present work was undertaken to study the effects of leaf extracts of *J. curcas* and *C. citratus* and root *extracts of C. odorata* in comparison to carbofuran in managing *M. javanica* in waterleaf.

2. Materials and Methods

2.1 Experimental Site and Collection of Samples

The experiment was conducted at the Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria: [(04º 51' N and 07º 03'E and 10 m altitude above sea level) Nwauzoma and Dappa, 2013]. The vegetation is derived Savannah of southern Nigeria. The area has two distinct seasons: dry and wet seasons and its climatic condition characterized by high humidity, high temperature (23^{oC} to 35^{oC}) and high rainfall of 2000 – 3000mm per/yr. The stem cuttings of *T. triangulare* were bought at the Mile 3 market in the Port Harcourt metropolis, Rivers State, Nigeria. Fresh leaves of *Jatropha curcas*, and *Cymbopogon citratus* and roots of *Chromolaena odorata* were collected from the University and authenticated in the departmental herbarium. Carbofuran was purchased at the Rivers State Agricultural Development Project (ADP), Port Harcourt, Nigeria, while the pure culture of *Meloidogyne javanica* eggs was graciously supplied by the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria.

2.2 Preparation of Plant Extracts and Nematicide

Fresh, mature and healthy leaves and roots of the respective plant materials were thoroughly washed and air-dried in the laboratory and ground into powder using electric grinder and stored in air-tight plastic containers. The extracts were prepared by dissolving 25g of each sample in 250 ml of hot water and allowed to stand for 24 hrs, and then strained through a sieve and collected in a plastic container and further filtered using Whatman No.2 filter paper to obtain the crude extract. The filtrate was the stock solution (representing 100 % concentration) and used within 24 hrs of preparation (Adegbite and Adesiyan, 2005). The stock solution was serially diluted with 80, 65 and 50 ml of distilled water to obtain the respective 20, 35 and 50 % concentrations of each extract. Granules of Carbofuran 5G was applied at the rate of 3kg a.i/ha (0.3g/bag) and serially diluted to obtain similar concentrations.

2.3 Nematode Extraction and Inoculum

Pure culture of *M. javanica* eggs was multiplied and extracted from three months old infected tomato roots, using the sodium hypochlorite (NaOCI)-extraction method (Hussey and Baker, 1973). Thus, galled roots of tomato (*Lycopersicum esculentus)* with egg masses were cut into small pieces (1-2 cm) and put into a container of 200 ml 0.5% NaOCI solution and agitated strongly to dissolve the gelatinous egg mass. The content was emptied into 200 mesh sieve to retain the roots and the debris nested on 500 mesh sieve to retain the eggs. The retained eggs were then washed in slow flowing cold tap water to remove traces of NaOCl. The galled roots were further rinsed with water to obtain more eggs. The number of eggs per ml of the suspension was estimated by counting under the microscope. This was then adjusted to 5000 eggs with the second stage juveniles per by concentrating in 3.ml of water.

2.4 Application of Treatments

Sandy-loam topsoil collected from a newly excavated land close to the department was steam-heated in a large metal drum for 4 hrs and allowed to cool for 72 hrs (Gautam and Goswami, 2002). Then, the soil was sieved and 5 kg was transferred into 25 cm diameter bags and arranged on slabs to avoid contamination. Three stem cuttings (5 cm long) of *T. triangulare* were planted in each bag, which was thinned three weeks after planting, leaving only the most vigorous plant in each bag. Then 5000 eggs with second stage juveniles of *M. javanica* were inoculated by pouring into each hole around the root tips using a graduated syringe (Hussey and Boerma, 1981). One week after inoculation, soil around the roots of the waterleaf plants was carefully scooped out to a depth of 5 cm, and

the plant extracts and carbofuran were applied around the roots of plants and covered with soil. The control had no nematode, plant extracts and cabofuran, thereafter uninoculated-untreated (Uit) and another had nematode suspension, but no extracts and nematicide [inoculated-untreated (Ut)]. The experiment was a factorial in a completely randomized design, with four replications and lasted for 16 weeks. The plants were maintained under adequate moisture, temperature and humidity and kept weed free.

2.5 Data Collection

The number of leaves, seeds and flowers were counted in five plants randomly selected from each treatment per replicate. The soil nematode population was estimated by collecting soil samples randomly from a depth of 5-10 cm from the different treatments into polythene bags using a hand trowel. Two core samples were taken at a depth close to the base of the waterleaf stand from each replicate. Each core sample was mixed thoroughly and bulked together to form an aggregate sample representing each treatment. The samples were put in labeled polythene bags and extraction of nematode was done using the modified Baermann Tray method of Whitehead and Hemming (1965). Hence, two serviette papers were placed on plastic sieves and another sieve was nested on the first sieve. Then, 250g of soil was poured inside the top sieve placed inside a bowl. Water was poured gently into the extraction bowl to wet the soil, until water was seen above the sieve and makes contact with the soil and left for 48 hours (Plate. 1). The excess water was removed and the sieves were gently lifted and nematode suspension was poured to settle in the base of a narrow container. The suspension was topped up with more clean water and then left undisturbed for 5 hours before decanting. Excess water was removed using a siphoning rubber tube, leaving the suspension containing the nematodes. The different stages of nematodes were examined under a microscope and the estimation of the total volume was done in 1ml solution and counted with the aid of a binocular light microscope.

2.5.1 Nematode population in roots samples

Nematode eggs were extracted from the roots using 0.5% NaOCl (Hussey and Baker, 1973). Galled root samples were cut into small pieces and 2.0 g root samples from each treatment was positioned in a 500 ml beaker, and thereafter processed as previously described for the soil samples and the nematode population was counted under microscope.

Insert Plate 1 here

2.5.2 Gall index

The root gall indices was evaluated using a severity scale of 0-5 where: 0 = no galls (immune), 1 = 1-2 galls (resistant), 2 = 3-10 galls (moderately resistant), 3 = 11-30 galls (susceptible), 4 = 31-100 galls (moderately susceptible and 5 = more than 100 galls (highly susceptible) (Taylor and Sasser, (1978).

2.6 Data Analysis

All data were subjected to Analysis of variance (ANOVA) using the Statistical Analysis System (SAS 1997) and the Least Significance Difference (LSD) for mean separation at 5% probability level.

3. Results

The leaf and root extracts of *Jatropha curcas*, *Cymbopogon citratus*, and *Chromolaena odorata* and carbofuran were tested on *Talinum triangulare* infected with *M. javanica* at various concentrations. The uninoculated-untreated (UiT) and carbofuran produced significantly ($P \leq 0.05$) more number of leaves (25.75 and 21.0) respectively, than the inoculated-untreated (11.25). There were no significant differences in the other treatments for this trait. Also no significant difference was observed in the number of seeds. Although the plants in the inoculated-untreated produced more flowers, however this was not significant (Table 1). The plant extracts and carbofuran had significant ($P \leq 0.05$) effects on nematode population in the soil and root of *T. triangulare*, compared to the inoculated-untreated (Ut) plants (Table 1). In particular, *J. curcas* extract at 50% concentration showed significant reduction in soil nematode population (9.0), as against 71.0 in the inoculated-untreated (Ut). *J. curcas* extract at 50% concentration consistently decreased the nematode population in both the soil and root of waterleaf, while the other treatments did not depict this trend (Table 2). At 50% concentration, the nematode population in the root was 30.00, as against 134.5 in the inoculated-untreated (Ut) and 138.75, 95.0 and 97.75 in *C. citratus*, *C. odorata* and carbofuran in that order (Table 1). Similar trends were also observed in the population of *M. javanica* in the soil at 50% concentration (Table 2).

Insert Table 1 here.

Insert Table 2 here

The mean gall index at 60 days after inoculation with *M. javanica* was lowest in plants treated with *J. curcas* at 50%, followed by *C. odorata* at 35% and highest in the inoculated-untreated (UIT) plants (Fig. 1). As expected, there were no galls in the uninoculated-untreated (UT), rather the plants showed a very high root proliferation of (Plate. 2). Further comparison of gall incidence indicates it was less in *J. curcas* extract treatment, followed by *C. citratus* extract and highest in *C. odorata* extract (Plate 3).

Insert Figure 1 here

Insert Plates 3 and 4 here
Gall formation on carbofuran treated plants, untreated-inoculated (Ut) and the uninoculated (Uit) plants are shown in Plate 4, showing that it was least in *J. curcas* extract, followed by *C. citratus* extract and highest in *C. odorata* extract.

4. Discussion
Several plant extracts are known to possess nematicidal properties (Sosamma and Jayasree, 2002; Osei *et al*. 2011). The uninoculated-untreated plants produced more number of leaves than those inoculated with *M. javanica* the application of the botanicals notwithstanding. This shows that *M. javanica* reduces the number of leaves in affected crops, in support of Hussey (1985) who reported that *M. incognita* infection significantly reduced the number of leaves per plant and tuber yields on sweet potato. The more number of flowers in the inoculated-untreated plants may seem strange however, Kanzan and Lyons, (2016) pointed out that biotic stress factors such as attack by pests and pathogens have significant effect on plant development including flowering. Also, research by Xue *et al*. (2013) indicates that *Meloidogyne incognita* accelerates flowering in the model plant *Arabidopsis* agrees with the present result. Furthermore, increase in the concentration of the treatments did not result to corresponding increase in the number of the traits studied.

The effect of different concentrations of leaf and root extracts of *Jatropha curcas*, *Cymbopogon citratus*, and *Chromolaena odorata* and carbofuran on the severity and population of *Meloidogyne javanica* in the soil and root of *Talinum triangulare* was studied in polythene bags in the screenhouse. All the treatments reduced nematode population, especially in the root as compared to the inoculated-untreated control. However, *J. curcas* at 50% concentration was more effective in reducing the population of *M. javanica* both in the soil and roots of *T. triangulare*, compared to the synthetic nematicide (carbofuran) and the other plant extracts. Also, there was a gradual decrease in nematode population in both the root and soil with increase in the concentration of *J. curcas* extract. Indeed, this was the only extract that showed this consistency, an indication that it could be a very good candidate to manage *M. javanica* in *T. triangulare*. The toxicity of *J. curcas* on the root-knot nematode *M. javanica* in susceptible tomato variety was reported recently by Bajestani, (2017). Also, *J. curcas* reduced the population of *M. incognita* juveniles in maize (*Zea mays*) soil than *Moringa oleifera* (Izuogu *et al*. 2013). Ismail (2013; 2014) reported a reduction in *M. javanica* final population and the rate of build-up by growing *J. curcas* or *J. gossypiifolia* as an interculture with sunflower in Egypt. Similar findings on the toxicity of *J. curcas* on root-knot nematodes have been reported by Claudius-Cole *et al*. (2010) and Onyeke and Akueshi (2012). These findings confirm that plant extracts are able to suppress the population of different plant parasitic nematodes by releasing nematoxins into the soil. The substances in plants that are active against nematodes are generally grouped as alkaloids, flavonoids, saponins, amides, benzamide and ketones (Akpheokhai *et al*. 2012; Adegbite, 2003). The presence of toxic compounds had been observed in the different parts of *Jatropha curcas*. As pointed by Tariq and Siddiqui (2005), when toxic compounds from plant tissues are released into the soil, plant parasitic nematode infection declines. Plant extracts contain phenolic compounds like benzoic acid and caffeic acid which have been demonstrated to possess nematicidal properties.

The overall nematode population extracted was higher in the roots than the soil, means that the root may have served as feeding site. As sedentary endoparasites, the female root-knot nematodes develop, reproduce and remain permanently at the feeding site for the duration of the life-cycle (Coyne *et al*. 2007). Root galls an indication of root damage was higher in the inoculated-untreated control plants than in plants treated with either plant extracts or carbofuran, indicating the toxicity of the later. This corroborates Izuogu *et al*. (2013) on the potency of *Moringa oleifera* and *J. curcas* leaf extracts on *M. incognita* in maize and *Jatropha* species on the development and reproduction of *M. javanica* (Ismail, 2014).

5 Conclusion
The present study show that the plant extracts and carbofuran had varying effects on the galling and population of *M. javanica* in the rhizosphere and root of *T. triangulare*. The differences in the toxicity of the different plant extracts and carbofuran could be due to differences in their chemical compositions and concentrations of the toxic components. It further show that out of different botanicals, *J. curcas* at 50 % concentration reduced the population of *M. javanica* and gall formation in *T. triangulare. C. citratus* extract was the least effective in reducing the population of *M. javanica* in the rhizosphere and root of *T. triangulare*, while *J. curcas* extract was more effective in decreasing nematode population, compared to carbofuran and the other botanicals. This means that *J. curcas* could be used in the management of root-knot nematode in waterleaf or as a component of integrated root-knot nematode managements. Further research on testing the activity of *J. curcas* with other solvents, change in the method of application as well as identifying the active compounds is recommended.

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Table 1: Effect of plant extracts and carbofuran on some yield parameters of *T. triangulare* inoculated with *M. javanica*.

		Number of		
Treatment	Conc. (%)	Leaves	Seeds	Flowers
J. curcas extract	20	12.50	10.00	4.00
	35	11.50	7.25	4.00
	50	12.25	8.00	4.50
C. citratus extract	20	13.25	5.00	3.50
	35	17.25	5.75	1.50
	50	19.25	9.25	4.00
C. odorata extract	20	11.75	9.50	2.00
	35	14.25	5.25	2.00
	50	11.50	8.25	4.00
Carbofuran	20	21.00	10.00	3.00
	35	17.75	7.50	3.00
	50	16.25	7.50	2.50
Uninoculated untreated (Uit)		25.75	13.50	6.00
Inoculated untreated (Ut)		11.25	10.00	7.00
LSD $_{(P\leq 0.05)}$		8.84	7.35	2.64

Table 2: Population of *M. javanica* in the soil and root of *T. triangulare* as influenced by plant extracts and carbofuran

		----------Nematode population----------	
Treatment	Conc (100%)	Root	Soil
J. curcas extract	20	99.50	32.00
	35	65.00	15.75
	50	30.00	9.00
C. citratus extract	20	110.75	61.50
	35	86.25	27.50
	50	138.75	80.25
C. odorata extract	20	93.00	49.25
	35	61.25	13.25
	50	95.00	53.75
Carbofuran	20	76.25	30.50
	35	82.75	30.00
	50	97.75	39.50
Uninoculated untreated		0.00	0.00
Inoculated untreated		134.50	71.00
LSD $_{(P\leq 0.05)}$		62.4	37.9

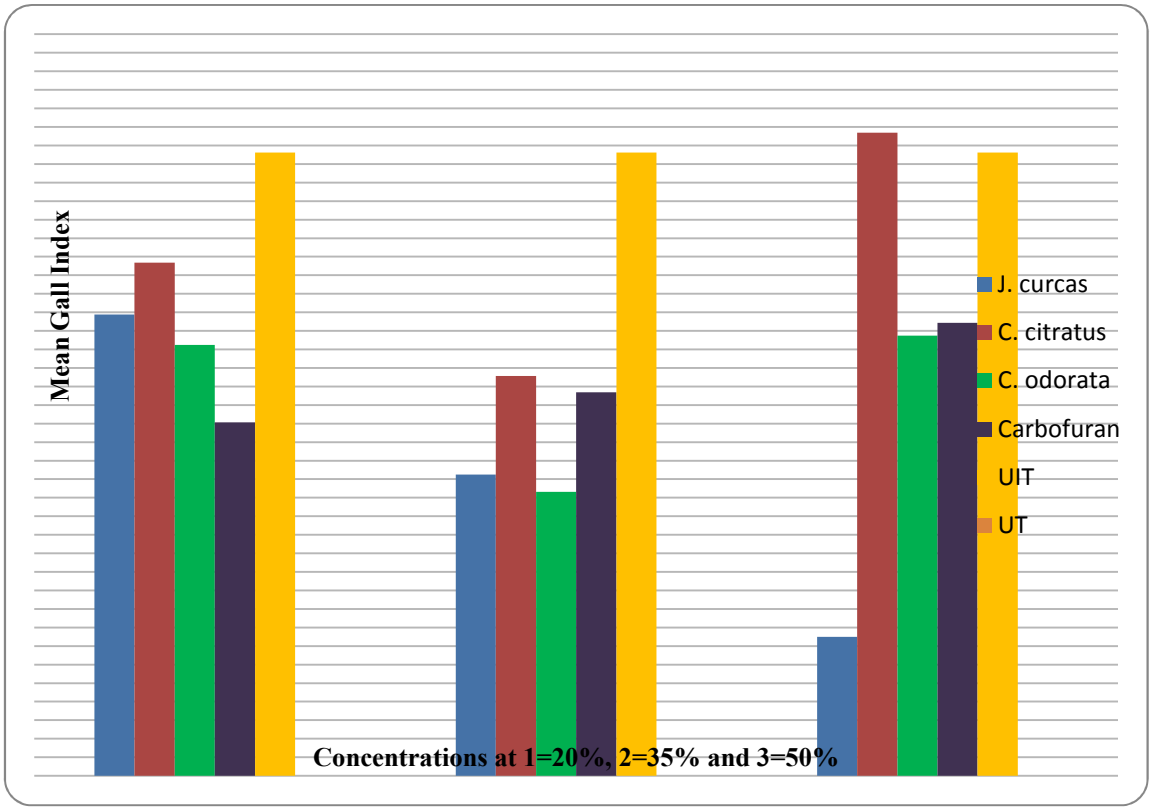

Figure 1: Galling index of *M. javanica* on the root of *T. triangulare* after treatment with plant extracts and carbofuran

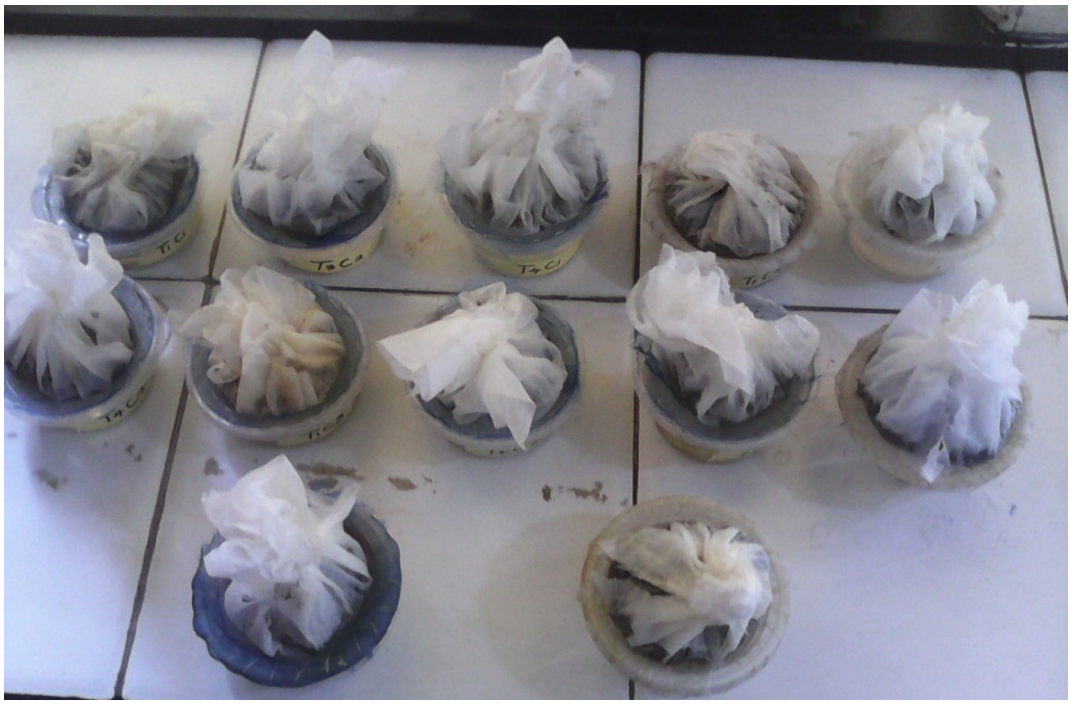

Plate 1: A modified Whitehead and Hemming Tray method, (1965) used to extract *M. javanica* from the soil and root of *T. triangulare*

Plate 2: Giant galls on roots of an untreated-inoculated (Ut) *T. triangulare* at 60 days after inoculation with *M. javanica*

Plate 3: Absence of galls on the roots of uninoculated *T. triangulare*

Plate 4: Gall incidence in *J. curcas* extract (**T_1**), *C. citratus* extract (**T_2**) and *C. odorata* extract (**T_3**) at 50 % concentration.