RESPONSE OF THE ROOT-KNOT NEMATODE-INFECTED *CELOSIA ARGENTEA* TO BARK EXTRACTS OF *KHAYA IVORENSIS*

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ABSTRACT

Plant parasitic nematodes are limiting factors in vegetable production. The most prevalent plant parasitic nematode on Celosia argentea is the root knot nematode Meloidogyne spp. Synthetic nematicide option of control brings about environmental pollution. The effect of dichloromethane and methanol extracts, in comparison with a standard synthetic nematicide (carbofuran) was studied on Meloidogyne incognita (Kofoid and White) Chitwood. 100g of extracts were dissolved in 200ml non-ionic surfactant. Methanol extract (KAYV/MeOH) was significantly effective (p=0.05). Plant height, number of leaves and number of branches were significantly higher in KAYV/MeOH treated plants, while nematode population was lower. Spectroscopic results and phytochemical screening revealed the presence of secondary metabolites like aldehydes, phenols, alkyls, carbonyl and terpenes. Plant growth parameters are enhanced in this study by the metabolites, while the reduction in nematode population attested to the nematicidal effect of extracts from Khaya ivorensis.

Keywords: Dichloromethane, methanol, metabolites, phytochemicals

INTRODUCTION

Celosia argentea L. commonly known as cock's comb belongs to the family amaranthaceae (Hanelt et al., 2001). It is a vegetable that is widely consumed and relished in the western part of Nigeria. A 100 g edible portion of *C. argentea* contains 83.8 g water, 4.7 g protein, 0.7 g fat, 7.3 g carbohydrate, 1.8 g fibre, 260 mg calcium, 43 mg phosphorus and 7.8 mg iron (Denton, 2004). *Celosia argentea* could be used in traditional medicine in treating gonorrhoea, diarrhoea, dysentery, eczema, sores, wounds, skin eruptions and as an antidote for snake bites (Grubben et al., 2004). The plant is attacked by several pests and diseases which damage the leaves reduce yield and lower acceptability to consumers. Spider mites and plant parasitic nematodes are the major pests. *Celosia argentea* is highly susceptible to root-knot nematode *Meloidogyne* spp, which cause galls on roots, unthrifty growth, small and chocolate-coloured leaves as well as yield losses of up to 40% (Denton, 2004). The use of synthetic nematicides has been the major method employed in the control of nematodes on *C. argentea*, with positive results but serious environmental consequences. Several positive results have been documented on the use of plant extracts in the control of *Meloidogyne* spp

(Fatoki and Fawole, 1999; Adegbite, 2011; Fabiyi et al., 2014). Khaya ivorensis A. Chev. (Meliaceae) commonly known as the African mahogany is a tall forest tree with a buttressed trunk. The bark is used in the treatment of whooping cough, diarrhoea, dysentery, and rheumatism (ARW, 1998). Methylangolensate, a limonoid, which showed significant anti-feeding, antifungal and antibacterial activities, has been isolated from its bark (Lemmens, 2008; Orwa et al., 2009). This study evaluated extracts from K. ivorensis as a feasible alternative to synthetic nematicides.

MATERIALS AND METHODS

Extraction

Bark of K. ivorensis was collected from the mother tree around Tanke area, Ilorin, Nigeria. The materials were chopped and air-dried for four weeks, divided into three equal parts and two portions were extracted cold in dichloromethane (DCM) and methanol (MeOH) while the third portion was incorporated directly into the soil. Each extraction lasted five days, after which the organic solvents were decanted, filtered and concentrated under reduced pressure using rotary evaporator.

Table 1: Status of phytochemicals in methanolic and dichloromethane extracts of *Khaya ivorensis*

Phytochemicals	KAYV/MeOH	KAYV/DCM
Alkaloids	+	-
Anthraquinones	+	-
Terpenoids	-	+
Flavonoids	+	-
Saponins	-	-
Phenols/Tannins	-	-

Keys: + = Present, - = Absent

Phytochemical screening

Preliminary phytochemical screening was conducted on the extracts for the presence of some plant metabolites such as terpenoids, steroids, alkaloids, polyphenols (Tannins), flavonoids, anthraquinones and saponins following standard methods (Harbone, 1973; Trease and Evans, 1989; Raymond and Sarker, 2006). All reagents used were of analytical grade.

Preparative Thin Layer Chromatography of Crude Extracts

One gram (1g) each of the extracts was dissolved separately in dichloromethane and methanol. Each dissolved crude extract was loaded on coated preparative thin layer

chromatography (PTLC) plates. They were allowed to dry and then developed using n-hexane-DCM (1:3) as the solvent system. The developed plates were viewed under the UV lamp at wavelength 366nm. The observed zones were scrapped out, eluted and stored in sample bottles for spectroscopic analysis.

Spectroscopic Measurements

Ultra violet-visible (UV) of two isolates each from the extracts was taken on Du 730 Life Science U.V/Vis Spectrophotometer Beckman Coulter. Infra-red (IR) spectra was recorded on SHIMADZU 8400s FTIR (Fourier Transform) Spectrophotometer and Gas Chromatography-Mass Spectroscopy was carried out on GCMS-QP 2010 PLUS (Shimadzu Japan) system coupled with a finigan MAT ion trap detector with an RTX5MS column packed with 100% grade dimethylpolysiloxane.

Screenhouse experiment

Sandy loam soil was collected from the back of Faculty of Agriculture, University of Ilorin, and heat sterilized at 60^{o}C for 2h, it was air dried and left to stabilize for four weeks after which they were distributed into 30cm diameter pots at 7kg each. Pure culture of *Meloidogyne incognita* eggs were extracted from 55-day old *C. argentea* cv TLV8 roots infected with *M. incognita* race2 using the method of Hussey and Baker, (1973). Extracted eggs per unit volume were estimated by taking aliquots of one ml three times and counting under a stereomicroscope (x40) using Doncaster's (1962) counting dishes. The vegetables were inoculated a week after emergence with approximately 200 M. incognita eggs/pot using the method of Iheukwumere et al., (1995). The experiment was a randomized complete block design with four treatments, at four rates of application and three replicates each. The inoculated but untreated pots serve as the control which is the zero (0) rates of application. Carbofuran 3G was applied at 0.3kg, 0.5kg and 0.7kg a.i/ha in the pots which is equivalent to 0.025g, 0.042g and 0.058g respectively. *Khaya ivorensis* bark was incorporated directly into the soil at 60g, 90g and 120g, while 100g plant extracts were dissolved in 200ml of a non-ionic surfactant emulsifier to provide homogeneous solution. Each of the plant extracts was diluted serially as follows: 10ml concentrated extract to 30ml surfactant, 20ml concentrated extract to 20ml surfactant and 30ml concentrated extract to 10ml surfactant. This amounted to 40ml each of the solution. Data was taken on plant height, number of branches and number of leaves in the screenhouse, while nematode population in 250g soil after harvest and root gall ratings were done in the laboratory. Root gall was rated on a scale of 0-10, where 0- root knots absent, 1- very small invisible knots, 2- small but very visible with main roots clean, 3- knots largely visible with main roots clean, 4- large root knots abound with main roots clean, 5-50% of roots affected, with knots on main root, 6- main root knotted, 7- a handful of main root knotted, 8- all parts of main root knotted, few clean roots visible, 9- severe root knots on root system, 10- severe root knots all over, plant may be dead (Bridge and Page, 1980). Data collected were analysed using a two-way analysis of variance using generalised linear model. Significant means were separated using the New Duncan's Multiple Range Test at 5% level of probability (p=0.05). All statistical analyses were done using IBM SPSS statistics version 21.

RESULTS

The phytochemicals present in methanolic and dichloromethane extracts (KAYV/MeOH and KAYV/DCM) of *K. ivorensis* are presented in Table 1. Alkaloids, anthraquinones, and flavonoids were present in the methanolic extract of *Khaya ivorensis* (KAYV/MeOH), while terpenoids, saponins and tannins were absent. The presence of terpenoids was observed in the dichloromethane extract, while other metabolites were absent.

Spectroscopy

Infrared spectroscopic result revealed the stretching vibration of hydroxyl groups at 3462 and 3435 cm^{-1}. The absorption bands due to methyl and methylene groups are shown at 2953cm^{-1}, while alkyl groups are represented by the bands at 2928 and 2854 cm^{-1}. The carbonyl functional group of esters is shown by the band at 1735 cm^{-1}, and the vibration frequency of aromatic ethers is indicated by the band at 1232 cm^{-1} (Fig. 1). The GCMS analysis showed that the fraction contained copaene (23.7%) and beta cadinene (10.2%), 2-furancarboxaldehyde-5-hydroxymethyl (41.6%) and phenol- 3, 5-di-tert-butyl (24.5%).

Screenhouse experiment

The result of the various parameters measured in the screenhouse is shown in Figs. 1-5 and the treatments were significantly (p=0.05) different from each other. Plant height was significantly higher in plants treated with methanol extract of *K. ivorensis* (KAYV/MeOH) with a cumulative height of 109.87 cm. This was closely followed by carbofuran (CBFN) treated plants which had 94.26 cm at harvest (Fig. 1a). Number of branches and number of leaves followed the same trend. KAYV/MeOH treated plants produced significantly higher number of branches and leaves 32.17 and 117.33 respectively at the 13 weeks after planting (Fig. 2a and 3a). Fewer numbers of branches and leaves were observed in dichloromethane extract (KAYV/DCM) treated plants with an accompanying corresponding higher nematode population in the soil at harvest (Fig. 4a). There was a significant reduction in nematode population in plants treated with KAYV/MeOH, CBFN and KAYV/ODR (Fig. 4a). The level of treatment application also had a significant effect on the growth parameters of *C. argentea* under *M. incognita* infection. The third rate (30ml) of application was significantly (p=0.05) more effective than all other rates, thus producing significantly higher plant height, number of branches and leaves, lower nematode population in soil and reduced gall rating (Fig. 1b, 2b, 3b, 4b and 5b). The control (Level 0) experiment had a significantly higher nematode population as against what was observed in all other treatment rates (Fig. 4b).

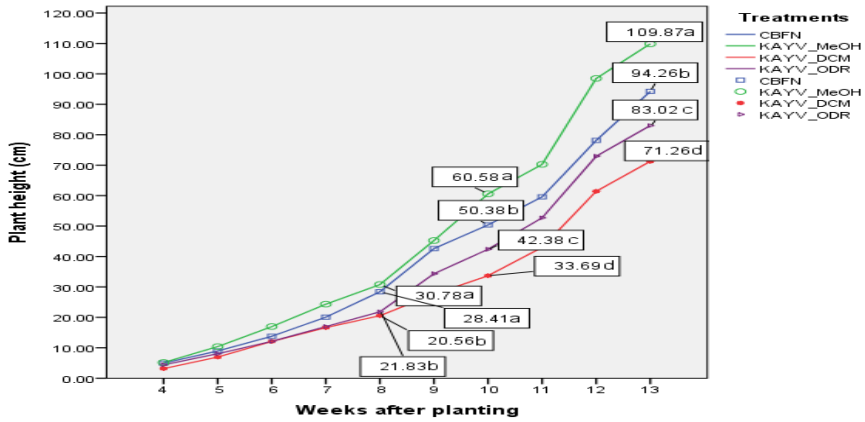

Fig. 1a: Effect of *Khaya ivorensis* bark extract on plant height of *Celosia argentea.*

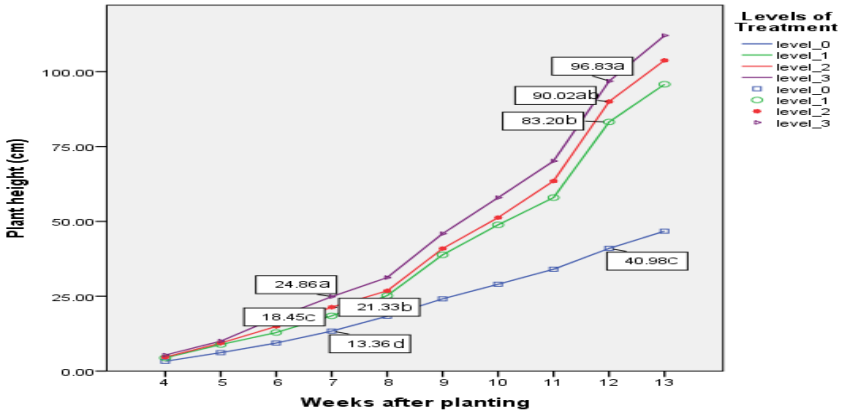

Fig. 1b: Effect of dosage of application of *Khaya ivorensis* bark extract on plant height of *Celosia argentea*

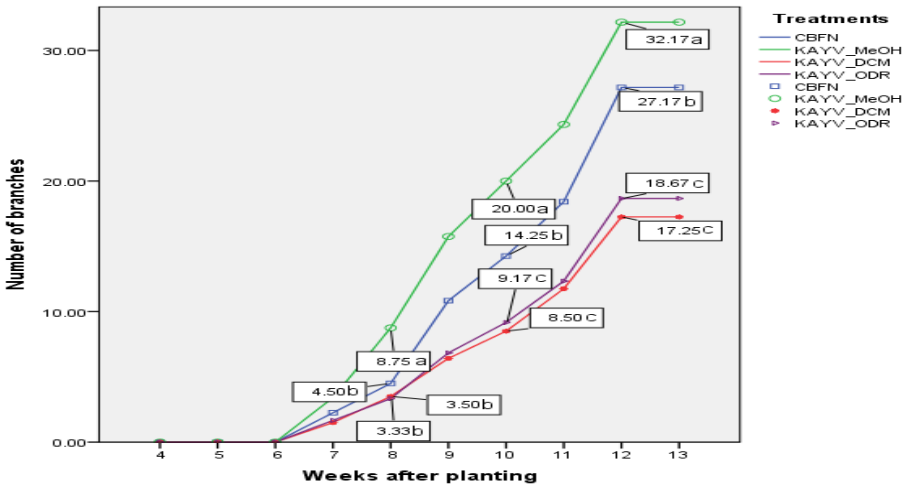

Fig. 2a: Effect of *Khaya ivorensis* bark extract on Number of Branches of *Celosia argentea*

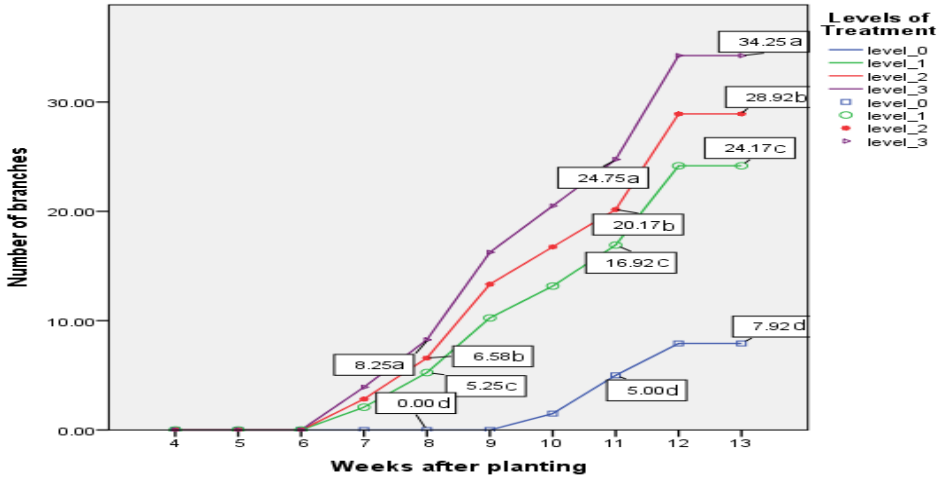

Fig. 2b: Effect of dosage of application of *Khaya ivorensis* bark extract on number of branches of *Celosia argentea.*

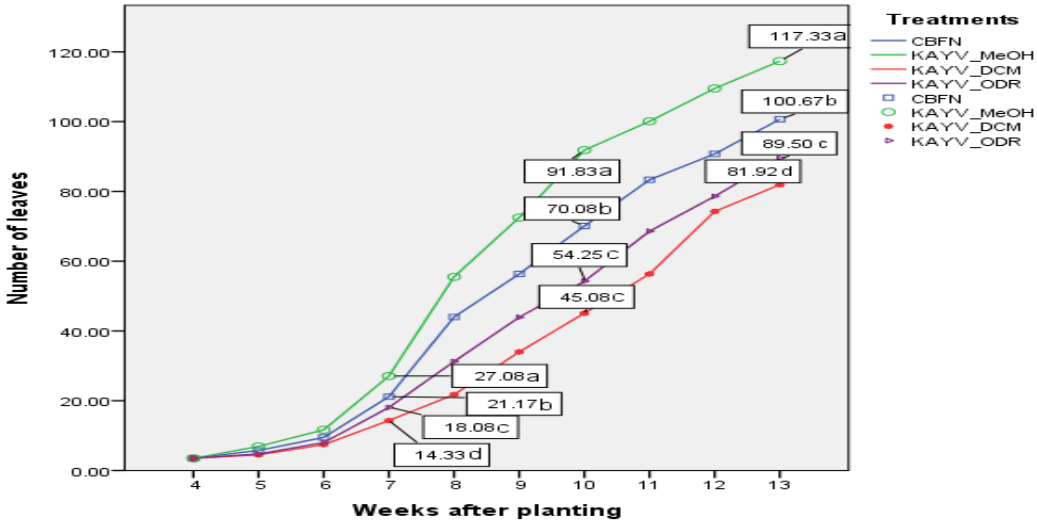

Fig. 3a: Effect of *Khaya ivorensis* bark extract on number of leaves of *Celosia argentea*

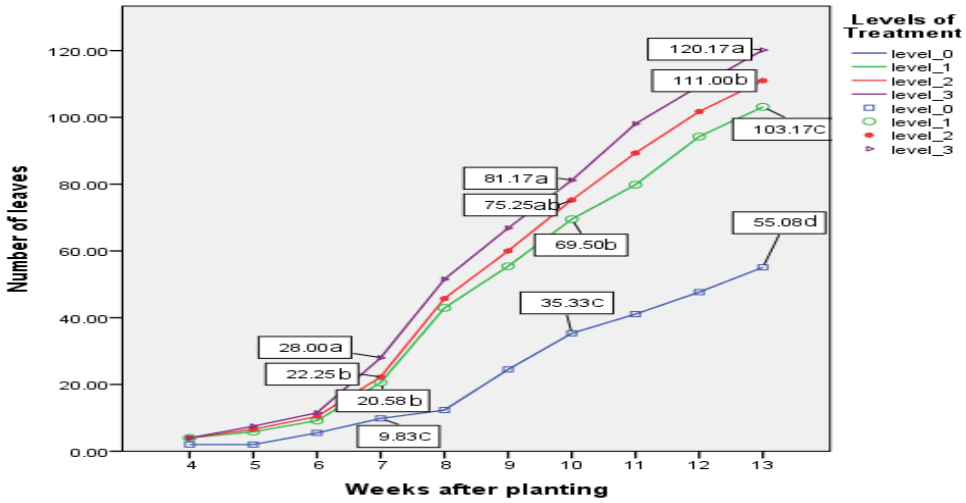

Fig. 3b: Effect of dosage of application of *Khaya ivorensis* bark extract on number of leaves of *Celosia argentea*

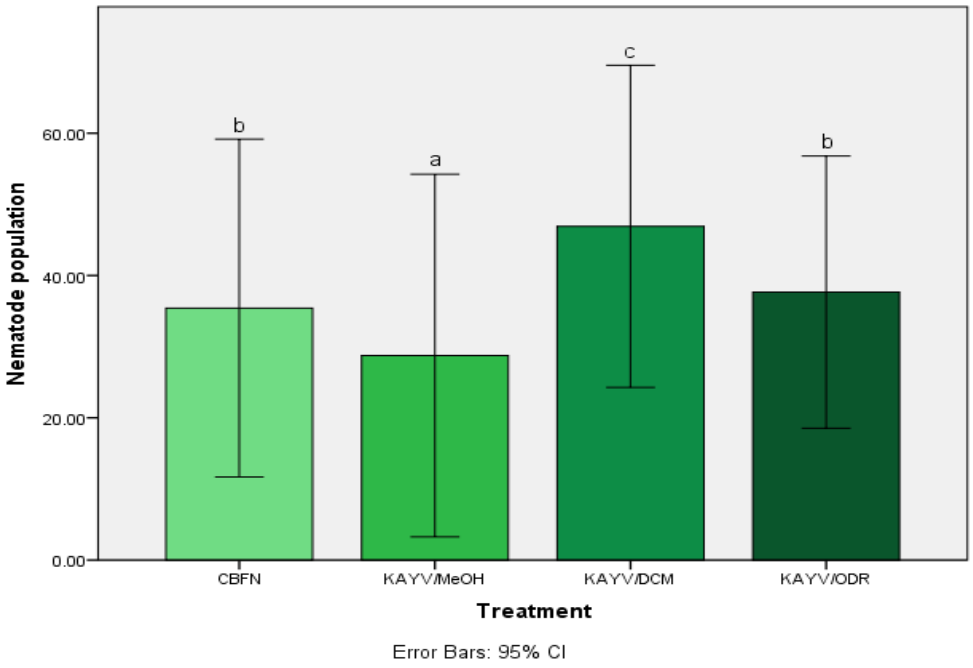

Fig. 4a: Effect of *Khaya ivorensis* bark extract on nematode population in soil

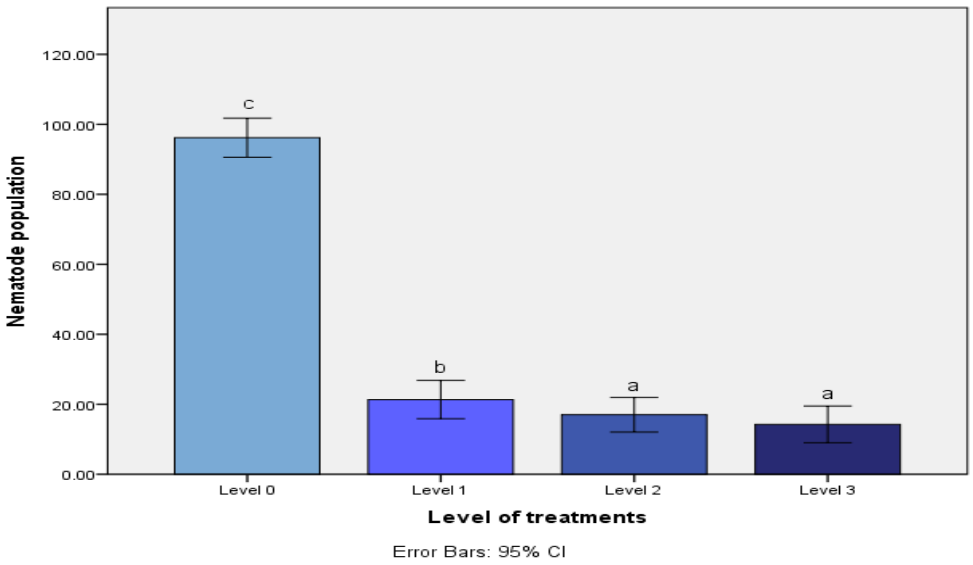

Fig. 4b: Effect of dosage of application of *Khaya ivorensis* bark extract on nematode population in soil

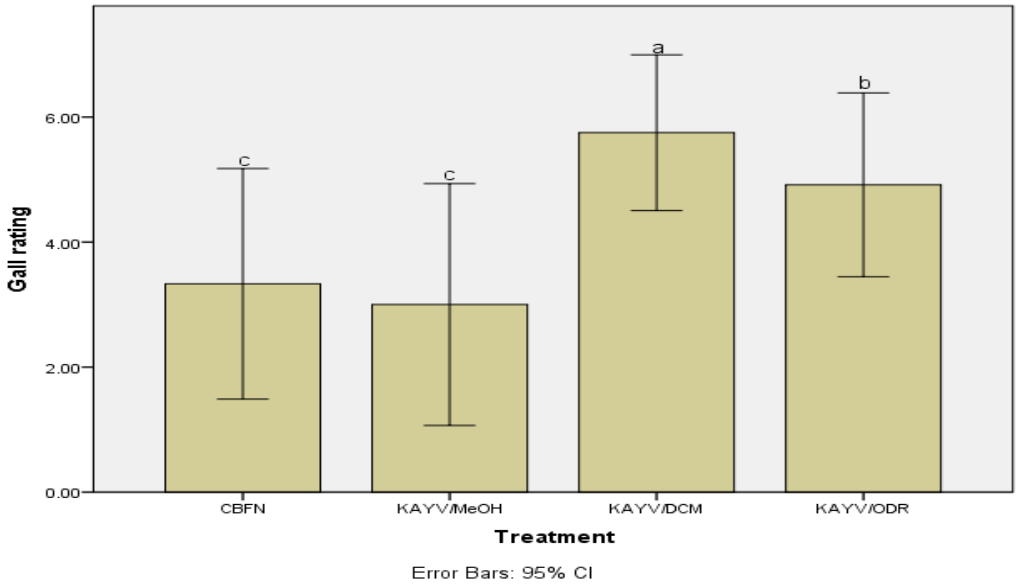

Fig.5a: Effect of *Khaya ivorensis* bark extract on root gall rating.

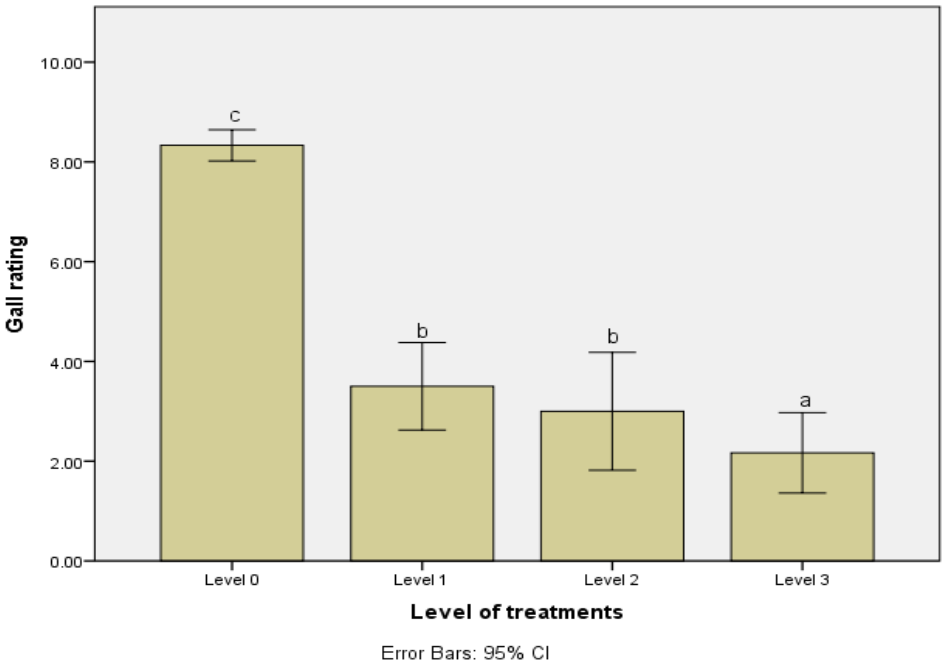

Fig.5b: Effect of dosage of application of *Khaya ivorensis* bark extract on Root Gall Rating

Treatment Keys: CBFN--Carbofuran; KAYV/MeOH----*Khaya ivorensis* methanol extract; KAYV/DCM----*Khaya ivorensis* Dichloromethane extract; KAYV/ODR----*Khaya ivorensis* plant materials incorporated directly into the soil as soil admix.

DISCUSSION

In this study, methanol and dichloromethane extracts of *K. ivorensis* displayed nematicidal activity against the root knot nematode *M. incognita*, and the activity could be associated with the organic compounds present in the bark extracts. Alkaloids, anthraquinones and flavonoids were present in the methanol extract while only terpenoids are present in the dichloromethane extract. Polyoxygenated terpenoids called limonoids were reported by Bray et al. (1990) as part of the constituents of *K. ivorensis* while saponins, alkaloids, tannins and cardiac glycosides were reported by Lawal et al. (2010). The absence of tannins in the gum exudates of *K. ivorensis* was documented by Omoniyi et al. (2013). Saponin, tannin, flavonoids, steroids, alkaloids, glycosides, phenols and volatile oils were documented in the ethanol extract of the stem bark of *Khaya senegalensis* (Kubmarawa et al. 2008; Nwosu et al. 2012). Report by Olajide et al. (2013) however established the presence of tannins and terpenoids.

The different retention factors and colours displayed on the TLC plates by the organic compounds also indicate the presence of various secondary metabolites and organic compounds. The UV-visible result of isolate from *K. ivorensis* exhibited absorbance characteristic of flavonoids thus further confirming the presence of flavonoids in the methanol extract of *K. ivorensis*. The functional groups identified in the infrared spectroscopy result such as hydroxyl, phenol, carbonyl, methyl and alkyl groups are related to those established in limonoids from *K. ivorensis* (Kai-Long et al., 2014). Phenolics as root dip treatments have been found to be nematicidal, causing a significant reduction in *Rotylenchulus reniformis* reproduction and improved plant growth was observed at all concentrations used (Mahmood and Siddiqui, 1993). Carboxylic acids and their derivatives (Dijan-Caporalino, 1994) as well as substituted phenols and phenoxy acetic acid esters (Mangel et al., 1989) were found to show good nematicidal activity. Similarly, Olabiyi (2004) attributed the presence of flavonoids in the leaves and roots of basil to the effective control of *M. incognita* on *C. argentea*. Hydroxyl groups have been indicated as crucial to strong fungicidal activity (Samir et al., 2005) while antimalarial activity have been associated with reactive groups such as the carbonyl groups (Kuma et al., 2011).

The improved vegetative growth observed in treated *C. argentea* plants is as a result of the decrease in nematode population in their root system, translating into little disturbances and feeding activities of nematodes in the roots of the treated plants, thereby resulting in fewer number of galls and unhindered growth (Vander-Borgett et al., 1994). This observation is in line with the reports of Liman et al. (2010) who reported a significant reduction in nematode population and root gall index with a significant increase in vegetative growth and yield of tomato plants treated with aqueous extract of *K. senegalensis*. The insecticidal activities of the ethanol extract of *K. senegalensis* were established by Abdulahi et al. (2012). Condensed tannins (flavan-3-ol) have also been indicated in egg hatch inhibition and development of the infective larvae of *Trichostrongylus colubriformis a ruminant gastro intestinal nematode* (Molan et al., 2003).

CONCLUSION

The use of botanicals in the management of plant parasitic nematodes is cheaper than the conventional synthetic nematicides. Such

botanical compounds are unlikely to cause environmental pollution problems and are expected to bring sustainability to agriculture.

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