

Application of Green Synthesis in Nano Particles Preparation: *Ficus mucoso* Extracts in the Management of *Meloidogyne incognita* infecting Groundnut *Arachis hypogea*

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ABSTRACT: Silver nano particles with nematicidal properties was synthesised with aqueous extracts of *Ficus mucoso* bark as reducing agent. The resulting nano particles are characterised using U.V visible spectroscopy and scanning electron microscopy (SEM). The nano particles are spherical in shape with 2nm size, which exhibited significant ($p=0.05$) nematicidal activity against *Meloidogyne incognita* on groundnut. The highest rate of application (80 mL) was the most effective. Significantly ($p=0.05$), vegetative growth and fruit weight was enhanced in different degrees with a decrease in nematode population. *Ficus mucoso* silver nano particles could be efficiently used in *Meloidogyne incognita* management on groundnut fields.

Keywords: *Ficus mucoso*, *Meloidogyne incognita*, silver nano particles, pollution, nematodes

Significant economic losses are caused worldwide to several crops by plant parasitic nematodes (Dong & Zhang, 2006) with greater percentage yield loss recorded in developing countries (Chitwood, 2002). *Meloidogyne incognita* Kofoed and White (Chitwood) (Tylenchida: Heteroderidae), commonly called root-knot nematode is the most widely known and considered as the world's most important agricultural pest (Curtis, 2007). A major host to *M. incognita* is *Arachis hypogea* (Groundnut Plant). Loss of income, due to quality and quantity of produce has been widely reported in groundnut fields infested with *M. incognita* (Onkendi *et al.*, 2014). The rapid actions of synthetic nematicides have increased their application where necessary to curtail damage, but growing concerns about environmental issues have brought about their limited use (Rich *et al.*, 2004), thus, stimulating research efforts into safer green alternatives. *Ficus mucoso* a medicinal plant in the family Moraceae, has been indicated to have antifungal and anti bacterial properties (Essien *et al.*, 2016; Oguntoye *et al.*, 2016), there is no information on the nematicidal properties of this species. As part of our investigations on nematicidal bio active principles from medicinal plants, we seek to

prepare silver nano particles using aqueous extracts from *F. mucoso* and to compare this with other organic solvent extracts of the plant and carbofuran, a synthetic nematicide.

MATERIALS AND METHODS

Collection and Preparation of Plant Materials

The bark and leaves of *Ficus mucoso* were collected from their habitat in Ilorin metropolis Kwara state, Nigeria. The materials were air dried under ambient temperature for 2 weeks to reduce moisture content in the plant materials. The dry bark and leaf materials were weighed separately (1824.4g and 1450.2g, respectively). The plant bark was divided into two equal parts (912.2g each) one part was soaked in methanol while the second part was in aqueous. All leaf materials were however soaked in methanol. The aqueous extract was decanted, filtered and stored at 5°C, while the methanol extract was concentrated and dried to a constant weight. The prepared substances were coded FCSM/MeOH/L and FCSM/MeOH/B which stands for *Ficus mucoso* leaf, methanol

extract and *Ficus mucoso* bark, methanol extract respectively.

Synthesis of silver nano particles

5 g of silver nitrate was weighed into a beaker, 500 mL of distilled water was added, and stirred gently with magnetic stirrer until it was totally dissolved to make 0.1M of AgNO_3 solution. 300mL of aqueous extract of *Ficus mucoso* bark was added drop wise and the stirring continued for 40 minutes. This procedure was repeated several times in order to have enough quantity of silver nano particles for nematicidal assay while the synthesized substance was coded FCSM/AgNP, that is *Ficus mucoso* silver nano particle.

Spectroscopy

U.V visible spectroscopy was carried out with Aquamate U.V-visible spectrophotometer V₄.60. Sample dilution was made to 2mL with distilled water. Reduction of Ag^+ to Ag^0 was monitored by measuring the spectrum at different time intervals (range 10-90 minutes). The morphology and size of the synthesized nano particles was investigated by Scanning Electron Microscopy (SEM) using JSM 7800F prime- JSM 7200F.

Field Experiment

A two years rain fed field evaluation experiment, laid out in a randomised complete block design (RCBD) with four treatments, four replicates and four levels (dosage of application) was conducted in the University of Ilorin teaching and research farm, (Lat 8°, 29' N of the Equator; Long: 4°, 40' E of the Greenwich Meridian) in Ilorin, Nigeria between April-July 2015 and the same period in the year 2016. A piece of land measuring 60 m x 50 m with a well drained light textured loamy soil with pH 5.8 was ploughed and harrowed. This was divided into sixty four blocks (64) with an alley way of 0.7 m between them; each block was 5 m x 1.5 m, with 8 rows which are 60 cm apart with 5 cm deep furrows which are 8 cm away from the groundnut rows. Three groundnut (Kampala) seeds were planted 5 cm deep at 20 cm apart on the rows. The groundnut seedlings were inoculated with approximately 600 eggs of *M. incognita* close to their root (Fabiyi *et al.*, 2012) two weeks after emergence.

The synthesized silver nano particle solution was applied at 40, 60 and 80 mL, while the organic solvent extracts was applied in grams dissolved in 200mL of water, inoculated untreated blocks served as the control. Carbofuran was applied at 0.5, 1.0 and 1.5 kg/a.i/ha. Data was taken on leaf numbers, plant height, pod weight and nematode population in root/soil. Collected data was analysed using Tukey's Honest test of Significant Difference (HSD), means were separated at 5% level of probability.

RESULTS

A remarkable appearance of light green colour in the reaction solution from colourless accompanied by increase in intensity with reaction time and the UV-vis spectral analysis with a surface plasmon resonance band at $\lambda_{412\text{nm}}$ confirmed the formation of silver nano particles (AgNP) of 2nm diameter and spherical in shape as illustrated in Plate 1. There were significant ($p=0.05$) differences in the effect of treatment on plant height. Significantly higher plant heights were observed in groundnut plants treated with cabofuran and *Ficus mucoso*/AgNP (Fig. 1). Mean numbers of leaves was fewer in FCSM/MeOH/L and FCSM/MeOH/B, while plants treated with FCSM/AgNP had significantly more numbers of leaves (Fig. 2). Yield from AgNP treated plants was equally significantly more compared to other extracts from *F. mucoso* (Fig. 3). Nematode population was significantly lower in FCSM/AgNP treated soils as against methanol extracts from bark and leaf of *F. mucoso*. In most cases the highest dosage of application of treatments had significantly higher plant height, more

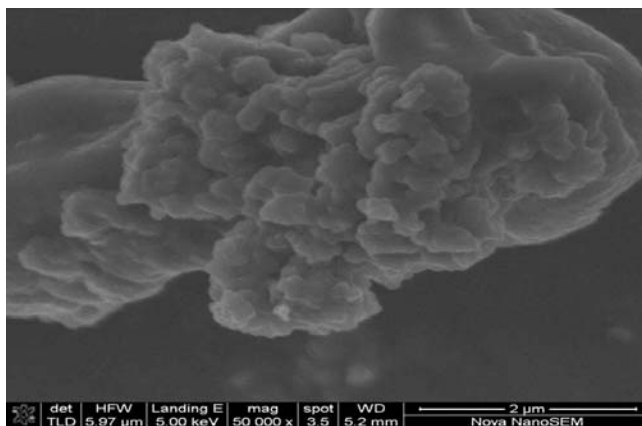


Plate 1: SEM micrograph of silver nano particle

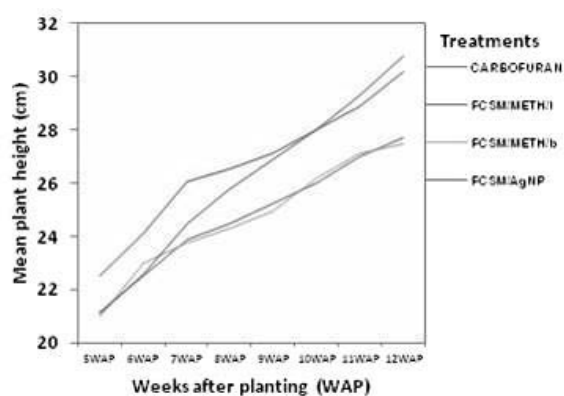


Fig. 1. Effect of Treatments on Mean Plant Height of Groundnut Plants

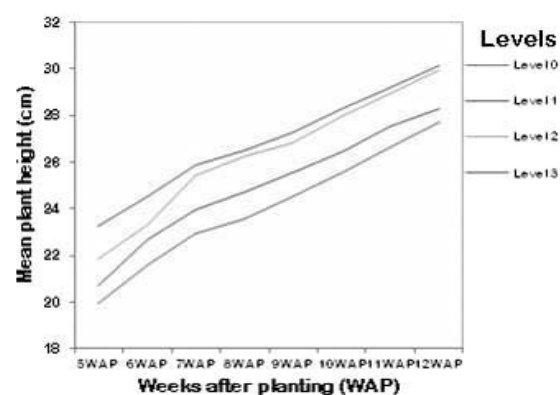


Fig. 2. Effect of Treatment dosage on Mean Plant Height of Groundnut Plants

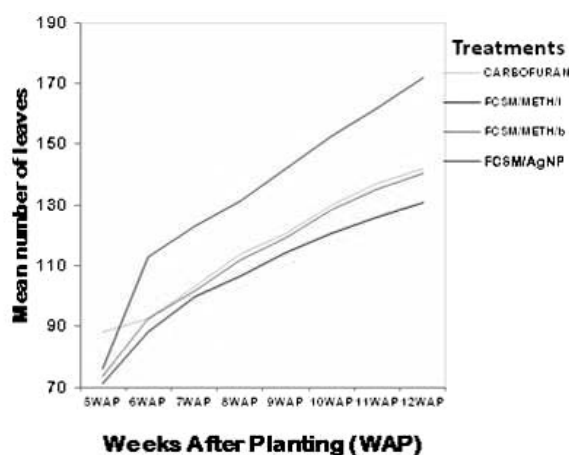


Fig. 3. Effect of Treatments on Mean Numbers of leaves of Groundnut Plants

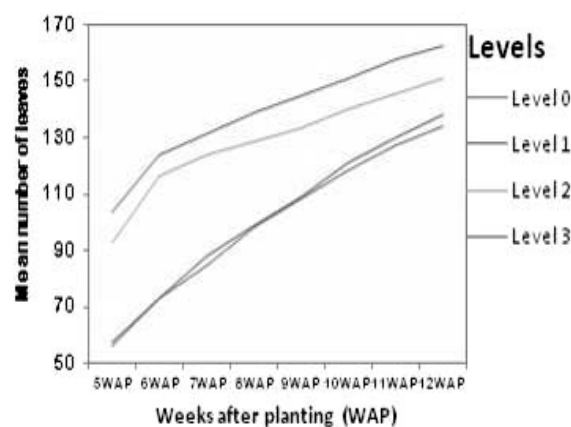


Fig. 4. Effect of Treatment dosage on Mean numbers of leaves of Groundnut Plants

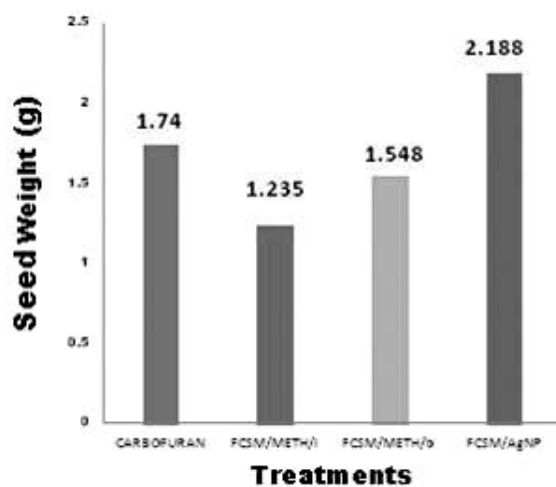


Fig. 5. Effect of Treatments on Mean Yield of Groundnut Plants

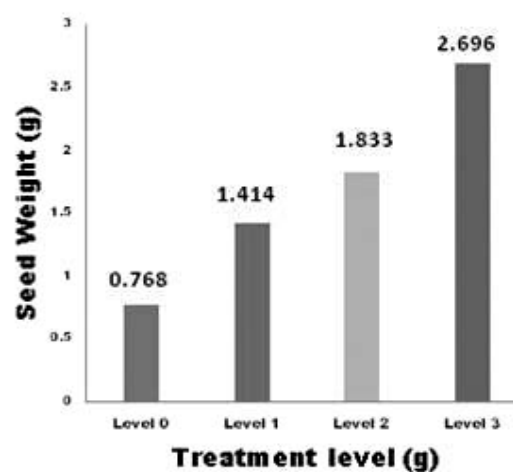


Fig. 6. Effect of Treatment dosage on Mean Yield of Groundnut Plants

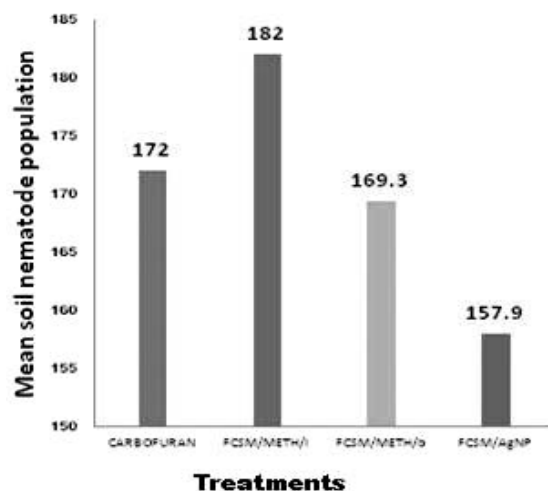


Fig. 7. Effect of Treatments on Soil Nematode Population of Groundnut Plants

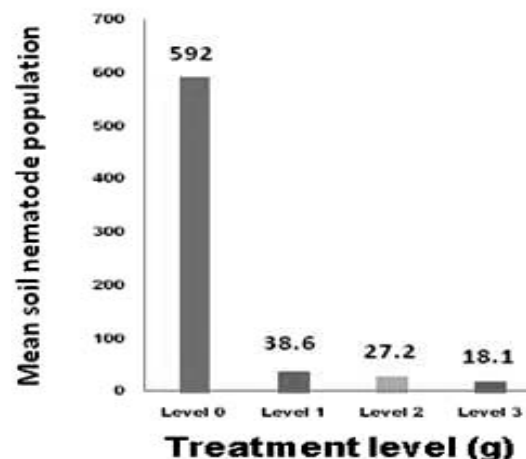


Fig. 8. Effect of Treatment dosage on Mean Soil Nematode Population of Groundnut Plants

numbers of leaves, more yield and fewer numbers of nematodes in soil (Figs. 4, 5 and 7).

DISCUSSION

Results in this study have demonstrated that the secondary metabolites from *Ficus mucoso* served as reducing agent in the formation of silver nano particles. The presence of terpenes and sesquiterpenes in *F. mucoso* was established by Essien *et al.*, (2016), while Oguntoye *et al.*, (2016) confirmed the presence of alkaloids, steroids, terpenoids, flavonoids, saponins, anthraquinones, tannins and glycosides in the extract of *F. mucoso*. Monoterpene hydrocarbons, sesquiterpene hydrocarbons, oxygenated monoterpene hydrocarbons, oxygenated sesquiterpenoids, aromatic compounds and aliphatic oxygenated compounds were also reported by Essien *et al.*, (2016) as part of the constituents of *F. mucoso*. Plant secondary metabolites have been indicated as reducing agents in biosynthesis of silver nano particles (Raut *et al.*, 2009), which was equally affirmed by Bankar *et al.*, (2010). The potential of plant metabolites to act as reducing and capping agent was also reported by Bar *et al.*, (2009a). Instantaneous colour change of reaction mixture from a clear liquid to light green during synthesis is a positive confirmation of the formation of AgNP in this study, similarly Anozie, (1986) observed an immediate colour change, while Bar *et al.*, (2009b) strongly affirm

change in colour of liquids as a confirmation of formation of AgNP. Consistent improvement was observed in *M. incognita* infested groundnut plants treated with silver nano particles solution and other extracts of *F. mucoso* in a dose dependent manner. AgNPs have been associated with crop improvement and management of plant diseases in agriculture (Salama, 2012). Jeong *et al.*, (2009) reported strong antimicrobial properties of *Ficus* spp, in the same vein Aref *et al.*, (2010), emphasized the antifungal and antibacterial activity of latex from *Ficus* spp, while Liu *et al.*, (2011), isolated nematicidal coumarins from *Ficus carica*. They observed 74.3, 96.2 and 98.4% mortality on *Bursaphelenchus xylophilus*, *Panagrellus redivivus*, and *Caenorhabditis elegans* respectively, this was also corroborated by our results, *F. mucoso* methanol bark and leaf extracts exhibited nematicidal activity in the treated plants as against the untreated control plants. The observed nematicidal effect of *F. mucoso* AgNPs in this study is attributed to the synergistic effect of the plant secondary metabolites and AgNO₃.

The present results revealed that silver nano particles could be synthesised from the aqueous bark extract of *F. mucoso*. The potent nematicidal effect of the AgNPs will provide promising development of environmentally safe nematicides, since the extract promotes the formation of NPs at room temperature without the use of harmful

chemicals. The aqueous extract could also be employed in the control of plant parasitic nematodes. This is the first report on *Ficus mucoso* as a reducing agent in the synthesis of silver nano particles and the methanol extracts in the control of groundnut nematodes.

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