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# PREPARATION OF BIO-NEMATICIDAL NANOPARTICLES OF *EUCALYPTUS OFFICINALIS* FOR THE CONTROL OF CYST NEMATODE (*HETERODERA SACCHARI*)

Fabiyi, Oluwatoyin[1*], Olatunji, Gabriel[2], Atolani[3], Olubunmi and Olawuyi, Olanike[1]

[1]Department of Crop Protection, Faculty of Agriculture, University of Ilorin, Ilorin, PMB 1515, Nigeria
[2]Department of Industrial Chemistry, Faculty of Physical Sciences, University of Ilorin, Ilorin, PMB 1515, Nigeria
[3]Department of Chemistry, University of Ilorin, Ilorin, PMB 1515, Nigeria
[*]Corresponding author's email: fabiyitoyinike@hotmail.com

## ABSTRACT

The production of *Oryza sativa* (rice) which is one of the most important food crops is greatly limited by nematode infestations. While various integrated control measure which include the application of synthetic nematicidal agents have been adopted, success without some major setback has been elusive. Thus, the need for concerted worldwide intensive research for safer alternatives has become imperative. Silver nanoparticles (NPs) of *Eucalyptus officinallis* were prepared via green synthesis approach. The nanoparticles obtained were subjected to UV-Vis and FT-IR spectroscopies for the absorption and functional groups characterisations respectively. Surface Plasmon Resonance (SPR) was achieved at 451nm, indicating the formation of NPs and the FTIR showed functional groups which further confirmed the formation of nanoparticles. The NPs significantly (p=0.05) reduced nematode population in root and soil, increased vegetative growth of rice plant, with a significant corresponding increase in yield. The plant-based nematicidal agent of silver nanoparticles origin cheaply prepared in this study adds credence to claim that bio-nematicides holds promise as a cost-effective, efficient and eco-friendly option for the future.

**Keywords:** Cyst nematode, nanoparticles, *Oryza sativa*, *Heterodera sacchari*, environmental pollution, bio-nematicides.

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## INTRODUCTION

*Oryza sativa* (commonly called rice) belongs to the Gramineae family. It is a monocot plant which is grown and widely consumed in most part of the world (Kuldeep, 2006; Li and Xu, 2007). It is one of the most important food crops producing economic income for several Asian and African countries (Biyi, 2005). Nutritionally, it is known to be very rich in energy and protein (Juliano, 1994). The production and consumption of the crop in Nigeria is evaluated to be more than others in the continent, even though the bulk of production is from the upland and lowlands of the country (Akande, 2003). However, one of the major setback to the production of the plant is the infestation of nematodes (Johnson *et al.,* 1997; Atera, *et al.,* 2011). In the upland Nigeria, the identified nematodes causing major economic loss include the *Pratylenchus* spp, *Meloidogyne* spp affecting the root and the cyst forming nematodes *Heterodera sacchari* (Afolami and Orisajo, 2003). The most economically important are the cyst nematodes *Heterodera sacchari* as they are a major constraint in successful rice production (Afolami and Orisajo, 2003; Akpheokhai, 2013). Infected rice plants exhibits stunting and wilting due to extensive root damage which prevents water and nutrient movement (Babatola, 1983; Wilcox-Lee and Loria, 1987; Melakeberhan, 2004). Nematode management is an important factor in rice production. The application of synthetic nematicide though effective is negated by development of resistance and environmental hazards, thus bringing eco-friendly and biodegradable materials such as silver nanoparticles (AgNPs) into limelight in nematode control. AgNPs have been employed in plant disease management as fungicidal and nematicidal agents (Roh *et al.,* 2009; Lamsal *et al.,* 2011; Fabiyi and Olatunji, 2018). This research aimed to use green synthesis approach for the production of silver nanoparticles from *Eucalyptus officinallis* extract and examine the nematicidal potential with the extract and the essential oil from the same plant against cyst nematode (*Heterodera sacchari*) population in screenhouse experiments.

## MATERIALS AND METHODS

**Preparation of Plant Materials:** The bark and leaf of *Eucalyptus officinalis* were collected from the University of Ilorin, Nigeria and authenticated. The bark was air dried in the laboratory for two weeks at ambient temperature. One kilogram (1 Kg) of the plant bark was chopped into pieces, extracted cold in ten litres (10 L) of methanol for five days after which it was decanted, filtered and concentrated using rotary evaporator. The concentrated methanol crude extract was coded ECLO/MeOH and stored in a cool place until needed.

Essential oil was extracted from one kilogram (1 Kg) of the fresh leaves via hydro distillation for three hours (3 h). The volatile oil obtained was separated using dichloromethane (DCM) in a separating funnel, dried over anhydrous magnesium sulphate ($Mg_2SO_4$) and coded ECLO/OIL. The remaining leaf materials was left for 2 days and thereafter re-extracted with 3 litres of cold water for five days, the extract obtained was used for AgNPs preparation and coded ECLO/AgNP.

**Synthesis of AgNPs:** 1.7 g of silver nitrate was weighed into a beaker, 10mL of distilled water was added, and stirred gently with magnetic stirrer until complete dissolution was achieved to make 0.1M of $AgNO_3$ solution. 200mL of aqueous extract of *Eucalyptus officinallis* was added dropwise and stirred for 30 minutes. This procedure was repeated for more yield of the AgNPs for nematicidal assay.

**Characterisation of AgNPs:** The absorbance of the prepared nanoparticles as a result of the conversion of the silver ion (+1) to silver metal ($Ag^{\circ}$) was measured as a function of the reaction time on UV-visible spectroscopy, Aquamate UV-visible spectrophotometer. The silver nanoparticles were also characterised using Fourier Transform Infra-red (FT-IR) spectroscopy on a range 4000 – 400 $cm^{-1}$ measured via Shimadzu 8400s spectrophotometer using the pellets of potassium bromide.

**Nematicidal Assay:** Soil for the experiment, (sandy-loamy) was obtained within the university premises and sterilized at 80°C for 2 hours, using a 250 L metal drum and kept in a cool dry place for three days. It was later distributed into 20 L sized experimental buckets at 10 Kg each. *Heterodera sacchari* populations were collected from infested rice fields. The cysts reproduced on susceptible Nerica 1 rice varieties in the nursery and were obtained following the technique of Southey, (1986). Extracted cysts were broken to bring out the eggs and juveniles (at the second stage) using standard procedure (Coyne *et al.,* 2007). The egg and juvenile suspension was agitated for a few minutes in 1 L beaker and were collected separately on 25, 38 and 90 µm aperture nested sieves. The 25 µm collected the eggs, 38 µm for juveniles and 90 µm for the broken cysts (Coyne, 1999), this was standardised and 1mL suspension contained approximately 400 second stage juveniles. The experiment was carried out by adopting the 4 by 4 by 3 factorial experiment adapted into a Randomized Complete Block Design (RCBD), comprising four treatments, four levels of application and three replicates each. Three seeds were sown per pot, which was later thinned to a single plant in a stand at two weeks of emergence. The emerging seedlings were carefully inoculated with approximately 400 juveniles per pot into a circle 3 cm away from each seedling after a week of the thinning. The freshly prepared AgNP of *Eucalyptus officinallis* was introduced at 0, 50, 100 and 150 mL and the essential oil was applied at 100, 150 and 200 mL, which contain 2.28 g, 3.42 g and 4.56 g of active ingredient respectively while carbofuran, a standard nematicide was applied at 0.12, 0.18 and 0.24 g. Plant height and number of tillers were monitored over a period of 10 weeks from the 7th week after inoculation. Seeds were weighed after harvest to determine yield, while cyst population in pot/root was estimated with the wet-sieve decantation method (Southey, 1986). Data obtained were subjected to two-way analysis of variance using GenStat 5.32 and significant means were separated with Tukey's Honest Significant Difference test (HSD). Significance (p=0.05) was set to 5% where required except otherwise stated.

## RESULTS

In this study, the formation of AgNP was confirmed by the instant colour transformation of the reaction mixture (silver nitrate solution was colourless, while the aqueous extract of eucalyptus had a wine colour) as seen in Fig. 1 (Plates 1-3). Scanning electron micrograph (SEM) result confirmed the formation of 100 nm nano-sized particles (Fig. 2). The UV spectra of *Eucalyptus officinallis* showed a surface plasmon resonance (SPR) absorption band at $\lambda_{max}$451nm thus confirming the formation and stability of AgNPs. The synthesized silver nanoparticles exhibited N-H stretch vibrations probably due to the presence of amine groups at 3464$cm^{-1}$, a methylene C-H bend absorption bands at 1613$cm^{-1}$, a sharp absorption band of C=O due to a dimethyl bend at 1385$cm^{-1}$, C-C vibrations at 833 and 544$cm^{-1}$ signifying the presence of aliphatic compounds which further establishes the reduction of silver nitrate by the plant phytochemicals (Supplementary data). The aqueous extract showed prominent bands at 1092, 1620 and 3439$cm^{-1}$. The interaction between the capping agents and nanoparticles could be responsible for the observed shift to lower wave numbers as the hydroxyl groups in the aqueous extract are assigned the stretching vibrations at 3439$cm^{-1}$. The reduced band at 3420$cm^{-1}$confirms N-H stretchings (Supplementary data). The nematicidal effect of bio-synthesised AgNP is depicted in Tables 1 to 3. Rice plants treated with silver nanoparticle had higher (p=0.05) plant heights, more tillers, heavier seed weight and fewer cyst count than other *Eucalyptus officinallis* extract treated rice plants. Carbofuran (CBFN) treated plant had significantly (p=0.05) more number of tillers, as the seed weight between CBFN-treated plants and *Eucalyptus* silver nanoparticles (ECLO/AgNP) had no observable significance (p=0.05) difference. The level of application of treatment showed significant difference in their effect on the growth and overall crop yield. The highest level of dosage application had significantly

higher plant height, more number of tillers, heavier seed weight and lower cyst population count in soil.

**Plate1. Silver nitrate solution**

**Plate 2: *E. officinalis* aqueous extract**

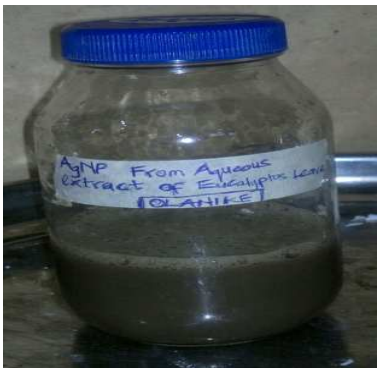


**Plate 3: AgNPs**

**Fig.1. Pictures of $AgNO_3$ solution, extract and prepared AgNPs**

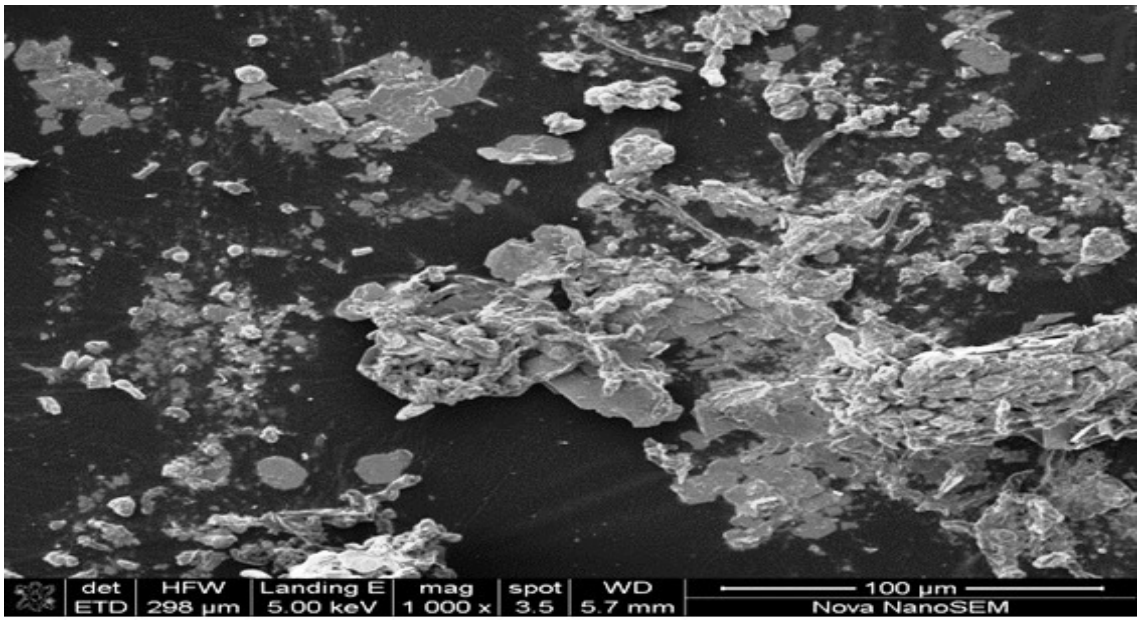


**Fig.2. SEM image of the AgNPs showing spherical shape and particle size distribution.**

**Table 1. Effect of Treatment and Dosage of application on Mean Plant Height (cm) of Rice.**

| Treatment | 7wap | 8wap | 9wap | 10wap | 11wap | 12wap | 13wap | 14wap | 15wap | 16wap |
|---|---|---|---|---|---|---|---|---|---|---|
| Carbofuran | 75.6$^{a}$ | 77.7$^{a}$ | 79.6$^{a}$ | 81.7$^{a}$ | 82.9$^{a}$ | 83.7$^{a}$ | 83.8$^{a}$ | 83.8$^{a}$ | 83.8$^{a}$ | 83.8$^{a}$ |
| ECLO/AgNP | 76.4$^{a}$ | 79.2$^{a}$ | 81.2$^{a}$ | 83.2$^{a}$ | 85.2$^{a}$ | 86.1$^{a}$ | 86.1$^{a}$ | 86.1$^{a}$ | 86.1$^{a}$ | 86.1$^{a}$ |
| ECLO/MeOH | 47.4$^{b}$ | 49.2$^{b}$ | 50.1$^{b}$ | 51.3$^{b}$ | 52.5$^{b}$ | 53.0$^{b}$ | 53.0$^{b}$ | 53.1$^{b}$ | 53.1$^{b}$ | 53.1$^{b}$ |
| ECLO/Oil | 54.1$^{b}$ | 56.6$^{b}$ | 57.4$^{b}$ | 58.4$^{b}$ | 59.4$^{b}$ | 60.0$^{b}$ | 60.0$^{b}$ | 60.0$^{b}$ | 60.0$^{b}$ | 60.0$^{b}$ |
| SEM+ | 0.72 | 0.80 | 0.89 | 0.98 | 0.91 | 0.81 | 0.82 | 0.82 | 0.82 | 0.82 |
| Level | | | | | | | | | | |
| Zero | 43.5$^{c}$ | 44.9$^{c}$ | 46.2$^{c}$ | 47.2$^{c}$ | 48.3$^{c}$ | 48.7$^{c}$ | 48.7$^{c}$ | 48.7$^{c}$ | 48.7$^{c}$ | 48.7$^{c}$ |
| One | 62.1$^{b}$ | 64.4$^{b}$ | 65.8$^{b}$ | 66.7$^{b}$ | 68.0$^{b}$ | 68.4$^{b}$ | 68.4$^{b}$ | 68.4$^{b}$ | 68.4$^{b}$ | 68.4$^{b}$ |
| Two | 71.8$^{a}$ | 74.3$^{a}$ | 75.8$^{a}$ | 78.1$^{a}$ | 79.7$^{a}$ | 80.5$^{a}$ | 80.6$^{a}$ | 80.6$^{a}$ | 80.6$^{a}$ | 80.6$^{a}$ |
| Three | 76.3$^{a}$ | 79.1$^{a}$ | 80.6$^{a}$ | 82.6$^{a}$ | 84.0$^{a}$ | 85.1$^{a}$ | 85.3$^{a}$ | 85.3$^{a}$ | 85.3$^{a}$ | 85.3$^{a}$ |
| SEM+ | 0.72 | 0.80 | 0.89 | 0.98 | 0.91 | 0.81 | 0.82 | 0.82 | 0.82 | 0.82 |

wap = weeks after planting; CBFN = Carbofuran, ECLO/AgNp=*Eucalyptus officinallis* nanoparticles; ECLO/Oil= Eucalyptus oil; ECLO/Methanol=*Eucalyptus officinallis* methanol extract. SEM = standard errors of means

**Table 2. Effect of Treatment and Dosage of application on Mean Numbers of Tillers of Rice.**

| Treatment | 7wap | 8wap | 9wap | 10wap | 11wap | 12wap | 13wap | 14wap | 15wap | 16wap |
|---|---|---|---|---|---|---|---|---|---|---|
| CBFN | 1.6[a] | 2.7[a] | 2.9[a] | 3.5[a] | 4.3[a] | 5.0[a] | 5.3[a] | 5.3[a] | 5.3[a] | 5.3[a] |
| ECLO/AgNP | 1.7[a] | 2.7[a] | 3.1[a] | 3.7[a] | 4.3[a] | 4.6[a] | 4.9[a] | 4.9[a] | 4.9[a] | 4.9[a] |
| ECLO/MeOH | 0.9[b] | 1.9[b] | 2.1[b] | 2.7[b] | 2.8[b] | 2.8[b] | 2.9[b] | 2.9[b] | 2.9[b] | 2.9[b] |
| ECLO/Oil | 0.7[b] | 1.7[b] | 2.3[b] | 3.1[b] | 3.1[b] | 3.1[b] | 3.1[b] | 3.1[b] | 3.1[b] | 3.1[b] |
| SEM+ | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Level | | | | | | | | | | |
| Zero | 0.4[b] | 1.4[b] | 1.4[b] | 2.2[c] | 2.2[c] | 2.2[c] | 2.3[c] | 2.3[c] | 2.3[c] | 2.3[c] |
| One | 0.8[b] | 1.8[b] | 1.9[b] | 2.2[c] | 2.6[c] | 2.9[c] | 2.9[c] | 2.9[c] | 2.9[c] | 2.9[c] |
| Two | 1.6[a] | 2.6[a] | 3.0[a] | 3.5[b] | 3.5[b] | 3.9[b] | 4.4[b] | 4.4[b] | 4.4[b] | 4.4[b] |
| Three | 2.0[a] | 3.1[a] | 4.0[a] | 5.1[a] | 6.3[a] | 6.5[a] | 6.6[a] | 6.6[a] | 6.6[a] | 6.6[a] |
| SEM+ | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |

wap = weeks after planting; CBFN = Carbofuran, ECLO/AgNp=*Eucalyptus officinallis* nanoparticles; ECLO/Oil= Eucalyptus oil; ECLO/Methanol=*Eucalyptus officinallis* methanol extract. SEM = standard errors of means

**Table 3. Effect of Treatments and Dosage of Application on Mean Weight of Seeds (Kg) and Cyst Count.**

| Treatment | Seed weight | Cyst count |
|---|---|---|
| Carbofuran | 11.20[a] | 07[a] |
| ECLO/AgNP | 11.01[a] | 10[a] |
| ECLO/MeOH | 5.35[b] | 21[b] |
| ECLO/Oil | 5.02[b] | 25[b] |
| SEM+ | 0.5 | 1.4 |
| Level | | |
| Zero | 1.43[c] | 55[d] |
| One | 7.16[b] | 15[c] |
| Two | 9.64[b] | 10[b] |
| Three | 14.34[a] | 5[a] |
| SEM+ | 0.5 | 1.4 |

wap = weeks after planting; CBFN = Carbofuran, ECLO/AgNp=*Eucalyptus officinallis* nanoparticles; ECLO/Oil= Eucalyptus oil; ECLO/Methanol=*Eucalyptus officinallis* methanol extract. SEM = standard errors of means.

## DISCUSSION

The immediate colour change of the reaction mixture as obtained in this study confirms the formation of the AgNPs. Guzman *et al.,* (2009) corroborated this, while Veerasamy *et al.,* (2011) also described colour change as an assertion to the formation of silver nanoparticles. *Eucalyptus* species are associated with the production of volatile oils and high value triterpenic compounds like triterpenic acids, ursane acids, oleanane acid and lupane acids (Domingues *et al.,* 2011). The presence of terpenes (isoprenoids) and terpenoids such as 1,8-cineole, alpha-terpineol and eucalyptol in *Eucalyptus* spp was reported by Domingues *et al.,* (2011) and corroborated by Fabiyi, (2016) in *Eucalyptus officinallis*. The reducing and stabilising potentials of the extract was affirmed by the presence of the hydroxyl, carbonyl and amine functional groups. The synergistic ability of these bio-molecules to exhibit reducing properties in AgNPs is well documented (He *et al*., 2007; Jain *et al.,* 2009). Phytochemicals such as terpenoids and flavonoids have been indicated as strong capping agents in silver nanoparticle synthesis (Priya *et al.,* 2014). Hydroxyl groups from terpenes bind strongly to silver ions, thus acting as reducing agents (Jain, 2009), while phyto-constituents with functional groups of amine act as stabilising agents (Madhumitha and Roopan, 2013). The functional groups in the spectra of the silver nanoparticle established the chemical composition of the system, which was supported by UV spectroscopy with a plasmon absorption band at $\lambda_{max}$451nm, while that of the ordinary aqueous extract depicts clearly the acidic nature of the aqueous extract. Plant extracts have been found to produce nanoparticles, with the leaf extracts or plant parts acting as the reducing and capping agent. Tahany and Abd El-Rahman (2013), synthesised AgNP using leaf extract of *Eucalyptus globulus,* similarly extracts of *Ipomoea indica* flowers was adopted for green preparation of silver nanoparticles (Pavani *et al.,*2013). The biosynthesised nanoparticles in this study exhibited nematicidal activity. The biological activity of AgNPs has been reported in literatures. Tahany and Abd El-Rahman (2013), reported the antibacterial activity of AgNP while, Fabiyi *et al.,* (2018) documented the nematicidal activity of AgNPs with high nematicidal potential. The observed nematicidal activity of AgNPs in this research is partly associated with the nano-size of the molecules.

**Conclusion:** The preparation of NPs using plant extracts and its adoption in plant disease management is environmentally benign, eliminates toxicity and offers great benefits to the farmers as against the toxic synthetics. Nanoparticles synthesized following green synthetic route have been shown to exhibit nematicidal potencies. This benign approach supports claims that plant-derived and cost-effective nematicidal agents could

replace the existing expensive and environmentally hazardous synthetic options.

**Conflict of interest**: All the authors declares no conflict of interest

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