Research Article

Development of an Alternative Management Method of *Meloidogyne incognita* Parasitizing (*Brassica oleracea*) Cabbage: *Bixa orellena* Extracts as Reducing Agent in Silver Nano Particles Preparation

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Abstract | Plant parasitic nematodes are salient pests of cabbage plants often controlled with agrochemicals. This procedure is laden with environmental pollution which calls for agro-biocides development in the control of plant parasitic nematodes (PPNs). Screenhouse trials were set up to assess the effectiveness of silver nanoparticles on *Meloidogyne incognita* populations in cabbage plants using aqueous extracts from *Bixa orellena* as reductant. Analysis of variance (ANOVA), statistical method was explored using GenStat 5.32 to achieve this. Formation of nanoparticles (NPs) was confirmed by reaction solution colour change, Fourier Transform Infra-red and Scanning Electron Microscopy (FTIR and SEM). Strong peaks on the FTIR spectrum were observed at 3356, 3360 and 1637 cm^{-1}, indicating that amines are the reducing and stabilising factor in the formation of AgNP. The Energy Dispersive X-ray Analysis (EDXA) projected silver as the principal extant metal. At 5% level of significance, there was higher than control level of growth and sufficiently higher yield, observed in cabbage plants administered with the highest concentration of *B. orellana* AgNPs at (150 mMole) 75 mL/20Kg soil. Similarly, there was significant reduction (at 5%) in soil and root nematode population at the above volume of application against (50 mMole) 25mL. Thus, we suggest that green synthesis of AgNPs could serve as an eco-friendly method of combating nematode pests of cabbage.

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Introduction

Cabbage, *Brassica oleracea* a favourite salad vegetable, an excellent source of sulforaphane, zea-xanthin, lutein, potassium, calcium, glutamine, iron and vitamins A, B, C and K (Jim and Tony, 2006; Kumazr *et al*., 2015; Tamileswari *et al*., 2015; Christine, 2000; Kumazr *et al*., 2015). It has protective effect against colon cancer, and sore throat. It is consumed as a laxative, the juice is used as an antidote in mushroom poisoning and it has been indicated in the treatment of gonorrhoea (Decoteau and Dennis, 2000; Wright, 2001; Yong *et al*., 2013; Tse and Eslick, 2014).

In Nigeria cabbage production has increased significantly and it is mostly cultivated among the exotic vegetables. Cabbage provides a means of livelihood for the peasant, small-holder and women farmers spanning from the west through the southern sections of the country (Ogbodo *et al*., 2009; Badmus and Yekini, 2011). Major cultivation is predominant in plateau state of Nigeria owing to the favourable climate for semi-temperate vegetables (Ogedegbe and Law-Ogbomo, 2013). The average earnings from cabbage production in Nigeria is generally small in correlation to other cabbage producing nations of the world.

Several pests and diseases are associated with cabbage production. *Meloidogyne* spp. is an economical pest of an assortment of crops and they consist of about 90 nominal species (Handoo *et al*., 2005). *Meloidogyne incognita* is extremely damaging among the species and it has been found infecting vegetables in warmer climates (Anwar and McKenry, 2010; Fabiyi, 2022a, b; Fabiyi *et al*., 2016). *M. incognita* is economically important in cabbage production; infected crops are characterised by chlorosis, stunted growth and galling of the root system thus reducing root efficiency for water and nutrient utilisation (Pattison *et al*., 2006; Waceke, 2007; Langston and Coolong, 2017). This automatically reduces head size of cabbage relative to market value, in severe cases there is total crop failure.

Synthetic nematicides though highly indispensable in agriculture are applied carelessly at different growth stages of crops to reduce loss to *M. incognita,* without consideration for pesticide residues (Fabiyi and Olatunji, 2021). This indiscriminate application of pesticides is a matter of interest most especially in cabbage production (Osei *et al*., 2013; Ashraful *et al*., 2017). Barbara (1993) reports that at the minimum 3 million people endure acute health problems, while about 20,000 deaths are recorded on yearly basis from pesticide poisoning. The development of a sustainable method of control is a necessary approach to improving cabbage production. The study was aimed at evaluating the potential of *Bixa orellana* bark and leaves as reducing agent, bearing in mind the role of natural products in pesticide development (Mishra and Tiwari, 2011). This will minimise the use of synthetic nematicides in cabbage production.

B. orellana often referred to as annatto is widely applied for managing health disorders in traditional medical systems. Most commonly used as an anti-gonorrhoeal and anti-malarial (Caceres *et al*., 1995; Bo-Zhai *et al*., 2014). The primary active principle contained in all parts of the plant include carotenoids, apocarotenoids, amines, lactone, phenol, terpenes, terpenoids, sterols, acetic acid and aliphatic compounds which manifest an array of pharmacological activities (Shahid-ul-Islam *et al*., 2015). Nanoparticles have unique properties because of their size, morphology and distribution. Silver nanoparticles are well known for biocidal activity (Veerasamy *et al*., 2011). Silver ions are toxic to micro-organisms and agricultural pathogens, making them useful in agricultural systems (Siddiqi *et al*., 2019). These reports prompted us to investigate the nematicidal capabilities of *B. orellana* bio synthesized silver nanoparticles.

Materials and Methods

Source and preparation of test plant

Bixa orellana leaves and bark were collected from the University of Ilorin campus. The plant was identified and documented at the herbarium unit of the University. The plant parts were cut into pieces of 1-2 cm and left to dry in the laboratory for five weeks and were soaked individually in separate extraction bottles with water and ethanol. After a week, the extracts were decanted and filtered using Whatman's no 1 filter paper. The bark and leaf aqueous extracts were used in the synthesis of silver nanoparticles, while the bark ethanol extract was concentrated using rotary evaporator and later air dried to remove residual solvent and it was coded BXAO/B/EtOH (*Bixa orellana* bark ethanol extract).

Synthesis of silver nanoparticles

0.1M of $AgNO_3$ solution of silver nitrate was prepared following standard methods (Fabiyi *et al*., 2018). 10 mL of this was added to 500 mL of bark aqueous extract of *B. orellana.* The mixture was stirred continuously at room temperature using the magnetic stirrer for an hour under room temperature, while stirring there was colour change from yellow to a grey solution. After twenty-four (24) hours there was no more colour change. The same procedure was repeated for the leaf aqueous extract. During the reaction there was colour change from cream to grey with precipitates, the precipitation increased with stirring, after forty minutes (40) of stirring the colour changed permanently to grey without any precipitate. The synthesised AgNPs were coded BXAO/B/AgNP

and BXAO/L/AgNP (*Bixa orellana* bark silver nanoparticles and *B. orellana* leaf silver nanoparticles respectively).

Characterisation of silver nanoparticles

The reduction of Ag^{+} to Ag^{o} was monitored periodically at intervals of 20 minutes for a period of 3 hours as a function of the reaction time by UV-visible on Aquamate U.V-visible spectrophotometer 1mL of sample solution in a cuvette was diluted up to 3 mL using deionised water. The silver nanoparticles solution was dispensed into centrifuge tubes and centrifuged at 5000 rpm for thirty minutes; the powder obtained was dried at room temperature. Fourier Transform Infra-red (FTIR) analysis of sample was carried out on Shimadzu 8400s spectrophotometer with KBr pellets to check possible functional groups for the formation of nanoparticles and identify the capping agents and bio-molecules on the silver surface. The morphology and size of the synthesized nanoparticles was investigated by Scanning Electron Microscopy (SEM) using JSM 7800F prime- JSM 7200F, while dispersive X-ray EDX was conducted on a JEM-1400 (JEOL, USA).

Egg extraction

Viable eggs of *M. incognita* were extracted from the roots of *Celosia argentea* using standard methods. Briefly, the roots of *C. argentea* were washed and cut into small sizes of approximately 1.5 cm. The pieces were packed in a plastic jar containing 600 mL of 0.6% NaOCl. The jar was shaken continuously for 4 minutes and the content was poured through 73, 56 and finally 25 µm aperture sieves. *M. incognita* eggs were collected from the 25 µm sieve into a beaker and left to hatch into juveniles in the laboratory at room temperature. The modified Baermann (1917) technique was used to separate the eggs from hatched juveniles (Fabiyi *et al*., 2020b).

Screenhouse experiments

In the year 2016 and 2017 cropping season**,** sandy loamy soils were collected from the University of Ilorin Teaching and Research Farm (Lat 8º, 29¹ N of the Equator; Long: 4º, 40¹ E of the Greenwich Meridian and Lat 8º30' and 8º50'N and Long 4º20' and 4º35'E of the equator. The soil was pasteurized in batches using an electric boiler at 60 °C. It was then set aside and allowed to cool and later distributed into 10 litre plastic experimental pots at 20 Kg each. Cabbage seeds were planted at three seeds per hole in the experimental pots. Thinning was done at two weeks after emergence. Approximately 2000 juveniles of *M. incognita* were inoculated at the base of each of the plants in the experimental pots a week after thinning (Fabiyi, 2019). Treatments were applied a week after inoculation at 25, 50 and 75 mL which is equivalent to 50, 100 and 150 mMole respectively. The inoculated untreated pots served as control (0 mL). Data was collected weekly after treatment application (WATA) on head diameter and leaf number in the screenhouse, while yield, juvenile population in roots and 200 cc soil were evaluated in the laboratory after harvest. The roots were evaluated for galling severity on a scale of 0-9 provided by Schoonhoven and Voysest (1989), where 1=no galling, 2=< 5% of roots galled, 3=6-10% galled, 4=11-18% galled, 5=19-25% galled, 6=26-50% galled,7=51-65% galled, 8=66-75% galled, 9=76-100% of roots galled. Eggs and juveniles were further extracted from the roots to count nematode populations in 10-gram root sample, while nematode population in 200 cc soil was also determined after harvest. Data collected were subjected to analysis of variance using GenStat 5.32 to determine if there is significant difference in the mean leaf number, head diameter, yield, gall index, soil and root nematode of cabbage plants after treatments, compared to the control. Having rejected the null hypothesis of no difference, there was the need for us to investigate further, which of the treatment levels differ significantly. Thus, pairwise comparisons in the mean yield were observed with Tukey's LSD test at 5% level of significance.

Results and Discussion

The formation of silver nanoparticles was confirmed by the intensity in colour changes during the reaction, while the maintenance of colour after 24 hours established that the reaction has come to an end. Stability and formation of the nanoparticles was also buttressed by the UV-vis spectral analysis. Surface plasmon absorption maxima bands were observed at 418 nm and 426 nm for the *B. orellana* leaf extract silver nanoparticle (BXAO/L/AgNP) and *B. orellana* bark extract silver nanoparticle (BXAO/B/AgNP) individually, thus indicating the reduction of silver nitrate into silver ions. The infrared spectroscopy results of the two aqueous silver nano revealed bands at 1637 cm^{-1} which is the carbonyl stretch (C-O) of an amide. Heterocyclic amine bands, N-H stretch (3356 and 3360) are seen in the *B. orellana* bark aqueous

mediated silver nano and leaf aqueous mediated silver nanoparticles. Amines are known to bind to silver nanoparticles, thus stabilizing the NPs. The SEM micrograph depicts the conglomeration of the silver nanoparticles with particle size 25-33 nm for the *B. orellana* bark extract mediated silver nanoparticle (Figure 1), similarly, the particle size of the *B. orellana* leaf extract nanoparticle is 56-74 nm. In general, the results show that the silver nanoparticles are spherical and cuboid in shape. The Energy Dispersive X-ray displayed silver as the principal metal in the medium (Figure 1).

Table 1 depicts the effect of various treatments on cabbage head diameter. A significantly larger diameter was observed in cabbage plants treated with *Bixa orellana* bark aqueous extract mediated silver nanoparticle (BXAO/B/AgNP). There are significant differences in head diameter of cabbage plants treated with *B. orellana* bark ethanol extract (BXAO/B/EtOH) and *B. orellana* leaf silver nanoparticles (BXAO/L/AgNP). The third level/dosage of treatment application had a significant (at 5%) effect on the diameter of the cabbage heads (Table 1). Wider head diameter was observed in the plants treated with the third dosage of application (Table 1), while significantly smaller head diameter was recorded in untreated cabbage plants. Significantly more leaves were produced in cabbage plants treated with carbofuran, BXAO/B/AgNP and BXAO/L/AgNP (Table 2). *Bixa orellana* bark ethanol extract treated plants had fewer leaves at harvest. More numbers of leaves were also observed in plants treated with third dosage of application (Table 2). Yield was significantly low in plants treated with BXAO/B/EtOH as against the silver nanoparticle treated plants Table 3. However, significant differences were observed between carbofuran treated plants and *B. orellana* bark mediated silver nanoparticles treated plants.

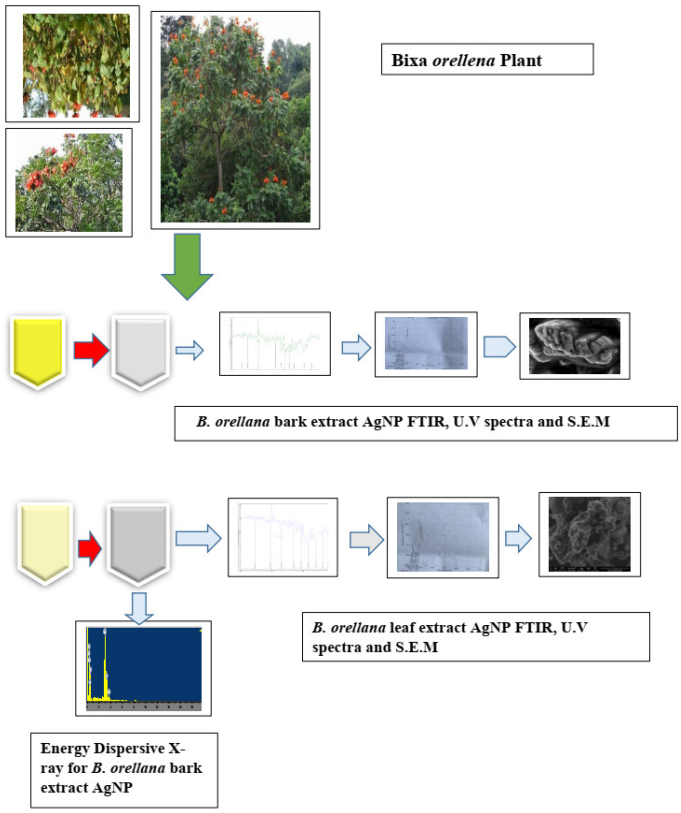

Figure 1: *Synthesis of AgNP with B. orellana bark and leaf extract and characterisation of the NPs.*

Table 1: *Effect of treatments and dosage of application on cabbage head diameter (cm).*

Treatments	4WATA	5WATA	6WATA	7WATA	8WATA	9WATA	10WATA	11WATA
CBFN	9.58^{a}±3.9	10.81^{a}±0.6	11.27^{a}±2.1	12.50^{a}±2.9	14.08^{a}±2.3	14.40^{a}±1.9	15.01^{a}±2.8	17.75^{a}±2.9
BXAO B/AgNP	8.86^{a}±1.4	9.64^{a}±1.9	10.78^{a}±1.1	11.61^{a}±0.7	11.99^{b}±0.6	12.49^{b}±0.6	13.32^{b}±2.1	14.78^{b}±0.1
BXAO L/AgNP	6.98^{ab}±1.7	7.92^{ab}±0.0	8.74^{ab}±0.6	9.55^{b}±0.3	10.59^{b}±1.2	12.21^{b}±0.2	13.06^{b}±0.8	13.89^{b}±2.2
BXAO B/EtOH	4.13^{c}±0.9	5.60^{c}±0.2	6.29^{c}±1.7	7.41^{c}±1.3	8.82^{c}±0.7	10.07^{c}±0.5	11.21^{c}±1.3	12.03^{c}±0.6
SEM±	0.28	0.33	0.26	0.21	0.27	0.31	0.28	0.26
LSD (p<0.05)	0.10	0.19	0.10	0.11	0.13	0.18	0.12	0.15
Dosages/Level								
Control 0mL (0mMole)	2.46^{c}±0.1	3.02^{d}±0.2	4.74^{c}±0.0	6.12^{c}±0.6	7.09^{c}±0.0	8.00b^{c}±0.0	9.82^{b}±1.0	11.08^{c}±0.0
One 25mL (50mMole)	5.18^{c}±1.8	7.68^{c}±0.5	8.03^{b}±0.1	9.07^{b}±1.0	10.34^{b}±1.2	11.06^{b}±0.1	11.71^{ab}±0.0	12.03^{b}±1.1
Two 50mL (100 mMole)	8.33^{b}±0.8	9.33^{ab}±0.1	10.59^{a}±0.0	11.54^{a}±0.0	12.35^{a}±0.0	12.97^{a}±0.6	13.47^{a}±0.3	14.00^{a}±0.6
Three 75mL (150mMole)	9.85^{a}±0.0	10.26^{a}±0.2	10.72^{a}±0.4	11.74^{a}±.08	12.04^{a}±0.0	12.95^{a}±1.3	14.45^{a}±2.1	14.78^{a}±2.1
SEM±	0.32	0.28	0.26	0.31	0.22	0.33	0.27	0.34
LSD (p<0.05)	0.11	0.13	0.10	0.18	0.16	0.12	0.18	0.16

Values with different letters show significant differences at p =0.05.

Table 2: *Effect of treatments and dosage of application on cabbage leaf number.*

Treatments	4WATA	5WATA	6WATA	7WATA	8WATA	9WATA	10WATA	11WATA
CBFN	17[a]±0.0	21[a]±0.8	26[a]±1.1	31[a]±0.3	33[a]±0.0	37[a]±0.2	41[a]±1.1	43[a]±0.2
BXAO B/AgNP	13[b]±0.2	18[b]±0.2	20[b]±2.1	24[b]±0.3	26[b]±2.2	28[b]±0.4	31[b]±1.8	36[b]±0.1
BXAO L/AgNP	12[b]±1.7	16[b]±2.1	19[b]±1.4	23[b]±0.0	24[b]±1.6	26[b]±0.0	29[b]±1.7	30[c]±0.6
BXAO B/EtOH	08[c]±1.1	12[c]±0.3	16[c]±0.5	18[c]±0.9	20[c]±0.1	21[c]±0.4	23[c]±0.3	24[d]±0.5
SEM±	0.22	0.20	0.26	0.28	0.23	0.27	0.24	0.21
LSD (p<0.05)	1.34	1.07	1.31	1.05	1.26	1.31	1.33	1.09
Dosages/Level								
Control 0 mL (0mMole)	06[c#d]±0.1	08[c]±0.0	10[c]±0.1	12[c]±0.2	13[c]±1.1	15[c]±0.3	16[c]±0.4	18[d]±0.7
One 25 mL (50mMole)	10[c]±0.3	15[b]±0.2	19[ab]±0.7	22[ab]±1.6	25[b]±0.9	28[b]±1.7	29[b]±0.1	31[c]±1.0
Two 50 mL (100 mMole)	15[b]±0.0	19[a]±0.0	21[a]±1.6	24[a]±2.1	26[b]±1.8	27[b]±0.0	30[b]±1.5	34[b]±2.1
Three 75mL(150mMole)	18[a]±0.6	21[a]±1.0	23[a]±2.3	26[a]±1.5	30[a]±2.4	32[a]±0.2	33[a]±1.8	37[a]±3.1
SEM±	0.25	0.31	0.27	0.22	0.34	0.26	0.30	0.28
LSD (p<0.05)	1.21	1.23	1.30	1.33	1.28	1.20	1.26	1.05

Values with different letters show significant differences at p = 0.05.

Table 3: *Effect of treatment and dosage of application on yield, root gall index, soil and root nematode population of cabbage.*

Treatments	Head weight of cabbage per plant (g)	Juvenile in 200cc soil	Root nematode population	Root gall index
CBFN	1386.8[a] ±1.6	15.21[a] ±2.2	7.16[a] ±1.0	0.32[a] ± 0.6
BXAO B/AgNP	975.5[b] ±2.1	35.09[b] ±0.5	29.02[b] ± 2.7	3.28[b] ± 0.1
BXAO L/AgNP	888.9[c] ±0.0	46.33[c] ±0.1	43.25[c] ±0.4	4.81[c] ± 0.0
BXAO B/EtOH	603.6[d]± 0.3	72.11[d] ±1.8	48.43[d] ±2.1	6.10[d] ± 0.3
SEM±	9.04	0.42	1.43	0.05
LSD (p<0.05)	5.83	1.34	0.21	0.08
Dosages/Level				
Control 0 mL (0 mMole)	73.0[d] ±2.6	2147[d] ±3.9	2643[d] ± 5.2	9.00[e] ± 0.0
One 25 mL (50 mMole)	202.7[c] ±1.8	918[c] ±1.4	622[c] ± 2.9	6.41[c] ± 0.3
Two 50 mL (100 mMole)	338.2[b] ± 09	217[b] ± 04	113[b] ± 0.0	4.05[b] ± 0.1
Three 75 mL (150 mMole)	408.6[a] ±0.0	60[a] ± 0.0	39[a] ±0.8	3.19[a] ± 0.0
SEM±	12.10	10.13	8.17	0.01
LSD (p<0.05)	22.36	14.51	16.40	0.17

Values with different letters show significant differences at p = 0.05. CBFN =carbofuran, BXAO/B/AgNP = B. orellana bark aqueous extract AgNP, = BXAO/ L/AgNP= B. orellana leaf aqueous extract AgNP, BXAO/B/EtOH = B. orellana bark ethanol extract.

The highest yield among the NP treatments was recorded in BXAO B/AgNP, even so, yield in carbofuran treated plants exceeded this (Table 3). Higher yield was also observed in the third dosage of NPs application (Table 3), contrary to what was seen in the untreated plants. Soil nematode population was significantly low at harvest in all the treatments (Table 3). Significantly higher population of nematodes was observed in untreated control pots. Soil treated with the third dosage of application had significantly lower nematode population (Table 3). Similarly, higher numbers of nematodes were recovered in untreated cabbage roots (Table 3), with a corresponding higher galling severity rating index (Table 3). Roots treated with the highest dosage of application had significantly fewer numbers of nematode (Table 3) and their galling severity ranges between 2, 3 and 4 on the rating scale; however, most plants treated with the lowest dosage of application recorded galling severity between 6 and 7 (Table 3).

Synthesis of nanoparticles with plant extracts as reducing agent is an economically efficient method of preparing nanoparticles (Fabiyi and Olatunji,

2018; Fabiyi *et al*., 2020c). Ramasubburayan *et al.* (2017) stated that colour change during the reaction process is a primary confirmation of the formation of nanoparticles, this corroborates the findings in this research, colour change from yellow to grey characterised the formation of silver nanoparticles mediated bark extract, while colour in the leaf extract equally changed from cream to grey. Nanoparticle formation was further affirmed by the UV-Vis spectroscopy results, the surface Plasmon absorbance for silver nitrate solution is λ max 220 nm (Leela and Vivekanandan, 2008), while 418 and 426 nm was obtained for the nanoparticles. The SEM result revealed the mono-dispersion and size of the silver nanoparticles. *B. orellana* is a rich source of plant metabolites such as bixin, amines, phenols, salicylic acid, acetic acid, carotenoids, tannins, flavonoids, terpenes, alkaloids and saponins (Thilagam *et al*., 2013; Vilar et al., 2014). Reports by Zhou *et al.* (2010) affirmed that plant constituents act as reducing and capping agent in nanoparticle preparations, findings in this research corroborates this, silver nanoparticles were produced via reaction of silver metal with extracts from *B. orellana.* The FTIR established the presence of amine functional groups in the extracts of *B. orellana.* This report is similar to the findings of Yong *et al.* (2013), who identified amines as one of the constituents of the aqueous extract of *B. orellana*. The ability of metabolites from *B. orellana* to act as reducing agents was reported by Thilgam *et al.* (2013), who established the formation of silver nanoparticles with extracts from *B. orellana* leaves.

The biological activity of nanoparticles has been widely reported. Ramasubburayan *et al.* (2017), documented the promising antibacterial activity of AgNPs synthesised with *Bacillus vallismortis.* Strong growth inhibition was observed at a 0.5-1 nM concentration. In the same vein, the toxicity of green route synthesized hematite (α-Fe_2O_3) nanoparticles on *Myzus persicae* was reported by Asoufi *et al.* (2018). Leaf extract from *Ailanthus excelsa* was employed as the reducing and stabilizing agent, 60% mortality was achieved in 72 hours at 800 ppm concentration. Plant extract mediated nanoparticles have been reported to be active against pests in agriculture, the small size allows easy penetration into the cell walls of bacteria, fungi and plant parasitic nematodes which leads to cell deaths of the micro-organisms (Sondi and Salopek-Sondi, 2004). Hence the observed nematicidal activity in this research. The growth and reproduction of *M. incognita* on tomato was truncated by the application of silver NPs synthesised with latex from *Euphorbia tirucalli* which acted as the reducing agent in the formulation of Ag NPs (Kalaiselvi *et al*., 2019). Graphene oxide (GO) and zinc oxide (ZnO) nanoparticles (NPs) were examined at two concentrations 0.05 mg ml^{-1} and 0.10 mg ml^{-1} on *M. incognita* infecting carrot. The application of GO and ZnO NPs significantly increased the vegetative growth of carrot plants (Siddiqui *et al*., 2019). The antimicrobial and antifungal activities of *Bixa orellana* were reported by Fleischer *et al.* (2003) and Shilpi *et al.* (2006), while Subhashree et al. (2016) reported the anthelmintic effect of ethanol extract of *B. orellana* leaves, at 60 mg/ml, there was a high percentage mortality which was comparable to the standard albendazole, the ethanol extract of *B. orellana* in this research significantly reduced final nematode population at harvest as against the untreated inoculated pots. Green synthesis of nanoparticles is a convenient and a fast eco-friendly method of combating *M. incognita* on cabbage.

Conclusions and Recommendations

The bark and leaf aqueous extracts of *Bixa orellana* could serve as reducing agents in silver nanoparticle preparations with no harm to the environment and quick integration of the nano materials. Development of new and environmentally safe nematicides could emanate from this green method of nanoparticle synthesis for an important vegetable like cabbage which is highly sensitive to *M. incognita* infestation.

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Novelty Statement

First Bixa orellana mediated AgNPs directed at Meloidogyne incognita infecting cabbage plants.

Author's Contribution

OAF, conceptualization design, bench work, manuscript draft, editing and proof reading.

AAO, bench work, data curation, data analysis, manuscript draft.

Conflict of interest

The authors have declared no conflict of interest.

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