



Response of *Meloidogyne javanica* to Silver Nanoparticle Liquid from Agricultural Wastes

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ABSTRACT

Plant-parasitic nematodes attack is an important problem on crop production worldwide. *Meloidogyne javanica* is a potentially damaging pest of several crops. Laboratory studies were conducted to examine the effect of supernatant liquid from the synthesis of silver nanoparticles with agricultural wastes on the survival and hatching of *Meloidogyne javanica* juveniles and eggs. The experiment consisted of five treatments (carbofuran, corn cobs, rice husk, guinea corn chaff, and distilled water served as control) at three concentrations of 10, 20, and 30%. Each was repeated three times in a complete randomized design. Nematicidal bioassay revealed a consequential ($p=0.05$) decrease in egg hatch rate in 20 and 30% concentrations of the nanoparticle supernatant liquid. Similarly, percentage mortality increased significantly ($p=0.05$) in the nano supernatant liquid, with the corncob silver nanoparticle having the highest percentage mortality. These results confirmed that the agricultural waste silver nanoparticle supernatant liquid could be a cost-effective and eco-friendly nematicide.

INTRODUCTION

Plant-parasitic nematodes (PPN), distinctly the root-knot nematodes, *Meloidogyne* spp. are damaging pests of essential crops, with a host range encompassing about 300 plant species (Fabiyyi, 2020; López-Gómez, Flor-Peregrín, Talavera, & Verdejo-Lucas, 2015; Wesemael, Taning, Viaene, & Moens, 2014). Globally, about 5% yield loss has been attributed to the economically important species, including *M. minor*, *M. incognita*, *M. hapla*, *M. javanica*, and *M. arenaria* (Wesemael, Taning, Viaene, & Moens, 2014). Infected crops are predisposed to secondary infections by bacteria and fungi (Bekhiet, Kella, Khalil, & Tohamy, 2010; de Oliveira Silva, Santana, Freire, da Silva Ferreira, & da Rocha, 2017). Annual losses of about 28% in cowpea, 25% in pepper, 23% in egg-plant and 29% in tomato have been attributed to infection by *Meloidogyne* spp. (de Oliveira Silva, Santana,

Freire, da Silva Ferreira, & da Rocha, 2017; Perry, Moens, & Starr, 2009). Control options that include synthetic nematicides are usually adopted despite the effect on water resources and various side effects caused to humans and non-target organisms in the environment. New approaches to avoid the chemical hazards are applied in the *Meloidogyne* spp management (Abdellatif, Abdelfattah, & El-Ansary, 2016; Atolani & Fabiyi, 2020; Atolani, Fabiyi, & Olatunji, 2014; 2015). The by-products of agricultural activities which are hitherto referred to as agricultural wastes in the form of crop residues, stalks, straw, husks, and chaffs are free, renewable and can be an important resource in the curb of plant-parasitic nematodes. In nature, most agricultural wastes are lignocellulosic and carbohydrates comprise a large fraction of the lignocellulosic material (Ravindran, Hassan, Williams, & Jaiswal, 2018). Through decomposition, agricultural wastes

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release toxic substances into the soil. These substances affect the community of plant-parasitic nematodes. Additionally, agricultural wastes increase soil fertility, increase plant growth, and make nematode infestation unnoticeable (Fabiyyi, 2018). Nanoscience is one of the most engrossing and apparent research domains that promise to furnish more pest management courses of action (Abdellatif, Abdelfattah, & El-Ansary, 2016; Fabiyyi, Alabi, & Ansari, 2020; Khan & Rizvi, 2014). The agricultural wastes such as corncobs, guinea corn chaff, and rice husk were utilized as stabilizers in this study for the composition of silver nanoparticles. This research aimed to investigate the nematocidal activity of supernatant liquid of synthesized silver nanoparticles using these agricultural wastes.

MATERIALS AND METHODS

The research was conducted at the University of Ilorin and The University of Ibadan Nigeria in September 2016.

Source of Agricultural Wastes

The agricultural wastes (corn cob, rice husk, and guinea corn chaff) used in this research were procured from Ipata marketplace in Ilorin, the capital city of Kwara State, Nigeria. They were powdered through milling after drying for three weeks, and this was in preparation for the *in vitro* assessment.

Synthesis of Silver Nanoparticles with Agricultural Wastes

Silver nitrate weighing 1.7 g was dispensed into a beaker; 10 ml of distilled water was introduced to it and mixed until it was fully dissolved to make 0.1 M of AgNO_3 solution. Also, 0.38 g of sodium borohydride was measured and dissolved while stirring in 10 ml of distilled water to give 0.1 M NaBH_4 solution. Each powdered residue of agricultural waste at 100 g was transferred to a beaker to which 500 ml of distilled water was added. The mixture was placed on a magnetic stirrer and stirred for an hour. A pipette was used to dispense 10 ml of 0.1 M AgNO_3 to the mix while stirring was continued for 30 minutes. A Pasteur pipette was then used to distribute 20 ml of NaBH_4 in drops with continuous stirring for another 30 minutes. The change from colourless to a grey colour and frothing of the solution confirmed the reduction of Ag^+ to Ag^0 ions, hence forming silver nanoparticles in the solution. The product was allowed to settle and then filtered

using Whatman's no. 1 filter paper. The filtrate was kept in a bottle at room temperature while the residue was collected and air-dried.

In-vitro Nematicide Studies

This study was administered in the Nematology Laboratory of the Department of Crop Protection and Environmental Biology, University of Ibadan. Extracted nematode eggs were exposed to concentrations of crop residue silver nano particles in *in-vitro* studies.

Preparation of Inoculum

Roots of tomato affected and galled with *M. javanica* were gathered from the micro inoculum plots of the Department of Crop Protection and Environmental Biology, University of Ibadan. The roots were washed underneath running tap water to get out soil particles. It was then cut up with a sharp knife into 1-2 cm pieces. Nematode eggs were drawn out from the roots using the technique of Fabiyyi et al. (2020) by disintegrating nematode egg masses with 0.5% NaOCl while shaking continually for 4 min. Eggs released into the solution were disconnected from roots and debris by sieving first through a 2 mm, 75 μm and finally through a 25 μm aperture sieve in which the eggs were withheld. The assembled eggs were meticulously rinsed and stored in a clean beaker. The number of nematode eggs per ml of the extract was assessed by counting beneath a stereomicroscope (x10).

Experimental Design and Treatments

The experiment was arranged in a completely randomized design (CRD) with five main treatments-carbofuran, corncobs, guinea corn chaff, rice husk, and distilled water-denoted as CBFN, CRNC, GNCS, RCEH, and H_2O all with three replications. The treatments were further divided into carbofuran 10%, 20%, 30%, corncob 10%, corncob 20%, corncob 30%, guinea corn chaff 10%, guinea corn chaff 20%, guinea corn chaff 30%, rice husk 10%, rice husk 20%, rice husk 30% and distilled water.

The Inhibition of Egg Hatch

Water suspension made up of 50 *Meloidogyne javanica* eggs was allotted by a pipette into separate glass blocks laid out on the laboratory bench. Concentrations of each agricultural waste silver nanoparticle (AgNPs) solution were prepared from the stock solution. These concentrations were prepared by taking 10 ml of sample solution into 100 ml standard flask and were filled up to

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100 ml mark with distilled water (v/v) volume by volume to make the 10% solution, 20 and 30% concentrations were made following the same procedure by taking 20 and 30 ml respectively. The concentrations produced were 10, 20, and 30%. Each 2 ml concentration per liquid was introduced into glass blocks of nematode eggs with the aid of a pipette. Control was represented by distilled water in separate glass blocks that contain the eggs of nematode. The expected number of egg hatch into juveniles was monitored and recorded daily for six days. A mechanical tally counter was employed to take an appropriate number of hatched juveniles under a stereomicroscope observation. The egg hatch inhibition percentage of the materials used was calculated from the counts made.

Mortality of Juveniles

The extracted egg-water mixture was kept in a beaker on the bench in the laboratory for five days running to let eggs hatch into the juvenile stage. Then, the hatched juveniles were removed from unhatched eggs by the tray technique (Coyne, Nicol, & Claudius-Cole, 2014). The newly hatched second-stage juveniles were gathered after 24 hours from the tray. Nematode extract made up of fifty juveniles were distributed into glass blocks laid out in a completely randomized design with three duplicates. Treatments consisted of the various 10, 20 and 30% concentrations of each silver nanoparticle supernatant liquid; these were introduced to each glass block. Juveniles of *Meloidogyne javanica* were observed to count the mortality for six days, and the dead nematodes were taken out from the extracts daily. The dead nematodes were used to determine the mortality percentage of *M. javanica* juveniles.

Ultraviolet (UV) - Visible

Characterization of the agricultural waste silver nanoparticles was done by UV-visible spectroscopy. The decrease of silver ions to pure

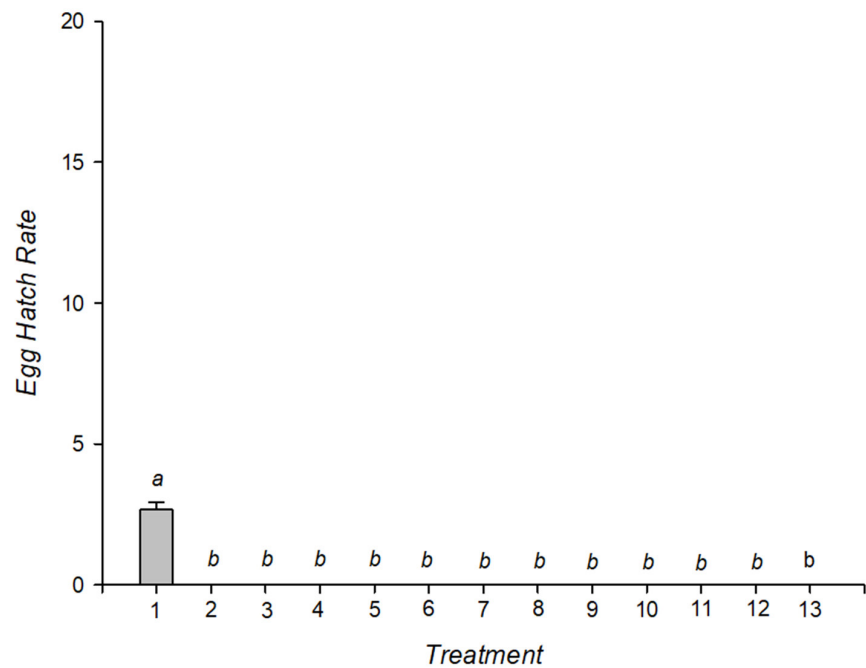
silver (Ag^+ to Ag^0) was tracked by measuring the UV-vis spectrum. A 0.2 ml aliquot of each sample was taken at intervals; this was diluted with distilled water and was measured on Aqua mate U.V-visible spectrophotometer V_{4.60}.

RESULTS AND DISCUSSION

There was a spontaneous change of colour from colourless to grey, which indicated the reduction of silver ions to pure silver (Ag^+ to Ag^0). After about ten minutes, the nano liquid from the rice husk gave a bright orange colour, the liquid from corn cob gave a brown colour, while that of corn chaff maintained the grey colour observed at the commencement of the reaction.

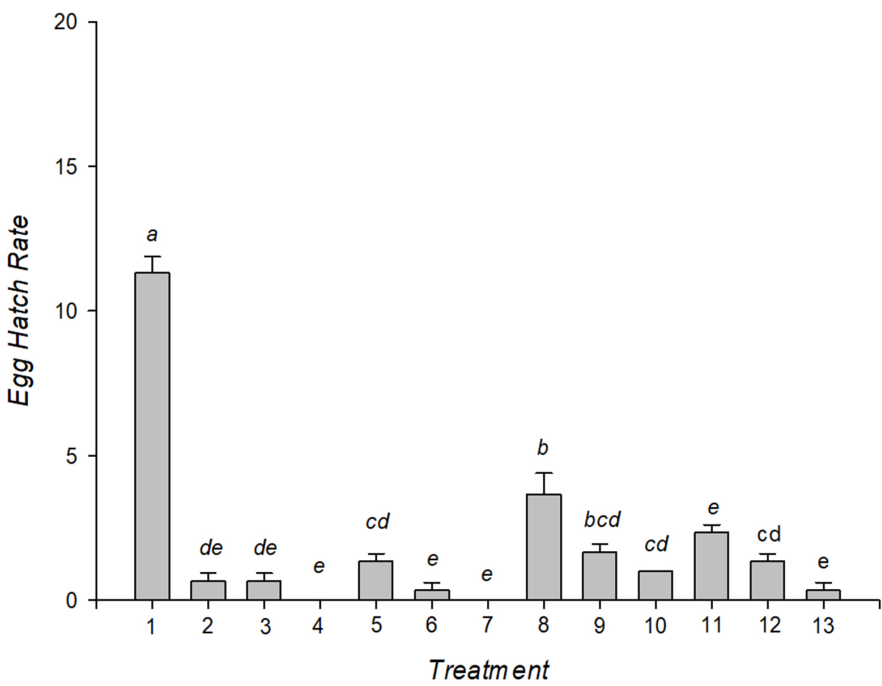
The number of hatched juveniles increased progressively throughout the observation. Egg hatch was consequentially ($p=0.05$) higher in the control (distilled water H_2O) treatment as opposed to the agro-waste of silver nanoparticle solutions on day two (Fig. 1). The high percentage hatch of *M. javanica* eggs was maintained on day three in the control treatment, as seen in Fig. 2. It was followed by a 10% concentration of rice husk silver nano particle. A few egg hatches that were outstandingly lower than the percentage observed in the control treatment were seen in other treatments.

There were no egg hatch in 30% carbofuran (CBFN) and 30% corncob (CRNC) silver nanoparticles. An increase in egg hatch percentage was recorded in all treatments on the 4th and 5th day of the experiment (Fig. 3 and Fig. 4). Less than 5% hatch was noted in 30% carbofuran, corncob, rice husk, and silver nanoparticles of guinea corn chaff (CBFN, CRNC, RCEH, and GNCS, respectively). Finally, on the 6th day of the exercise, 30% egg hatch was recorded in control, but 30% corncob, 20 and 30% concentration of rice husk nanoparticles recorded 6% egg hatch (Fig. 5).



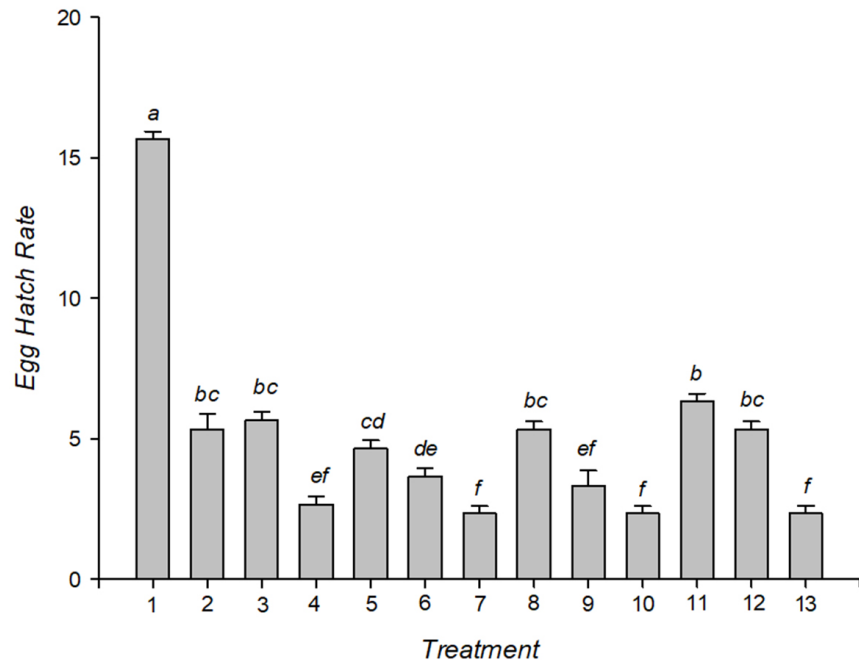
Remarks: 1: H₂O, 2: 10CBFN, 3: 20CBFN, 4: 30CBFN, 5: 10CRNC, 6: 20CRNC, 7: 30CRNC, 8: 10RCEH, 9: 20RCEH, 10: 30RCEH, 11: 10GNCS, 12: 20GNCS, and 13: 30GNCS

Fig. 1. Egg hatch rate on second day



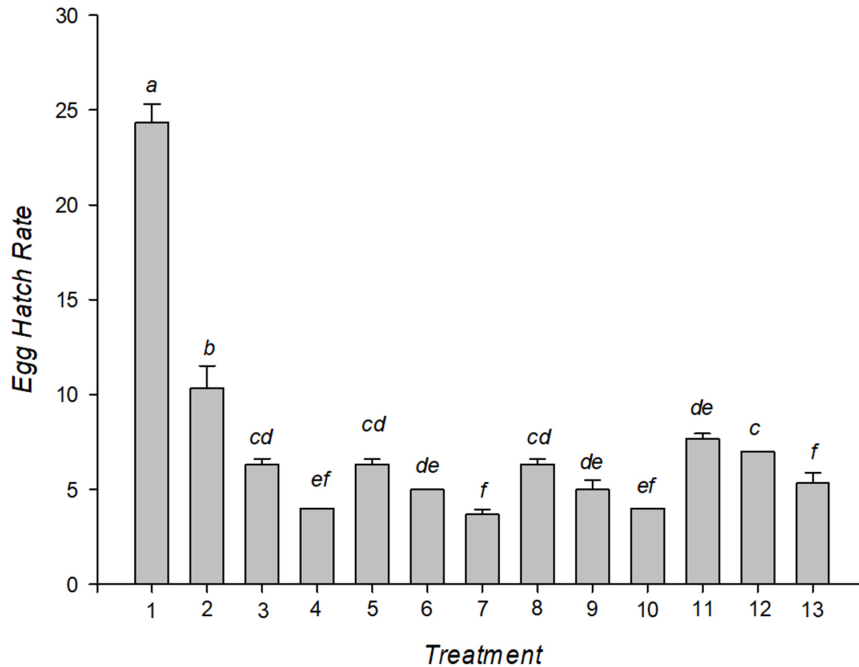
Remarks: 1: H₂O, 2: 10CBFN, 3: 20CBFN, 4: 30CBFN, 5: 10CRNC, 6: 20CRNC, 7: 30CRNC, 8: 10RCEH, 9: 20RCEH, 10: 30RCEH, 11: 10GNCS, 12: 20GNCS, and 13: 30GNCS

Fig. 2. Egg hatch rate on third day



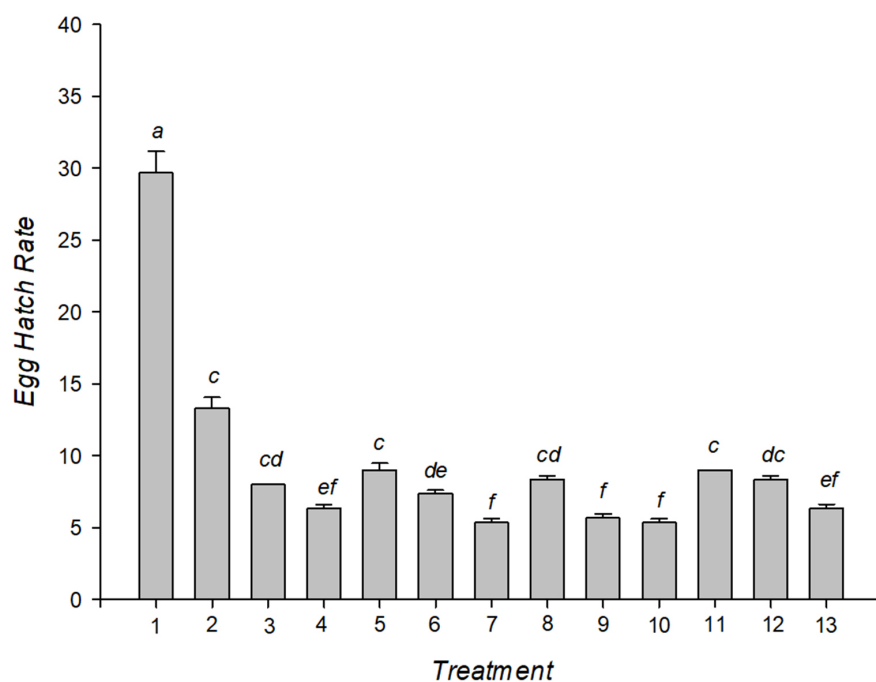
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Fig. 3. Egg hatch rate on fourth day



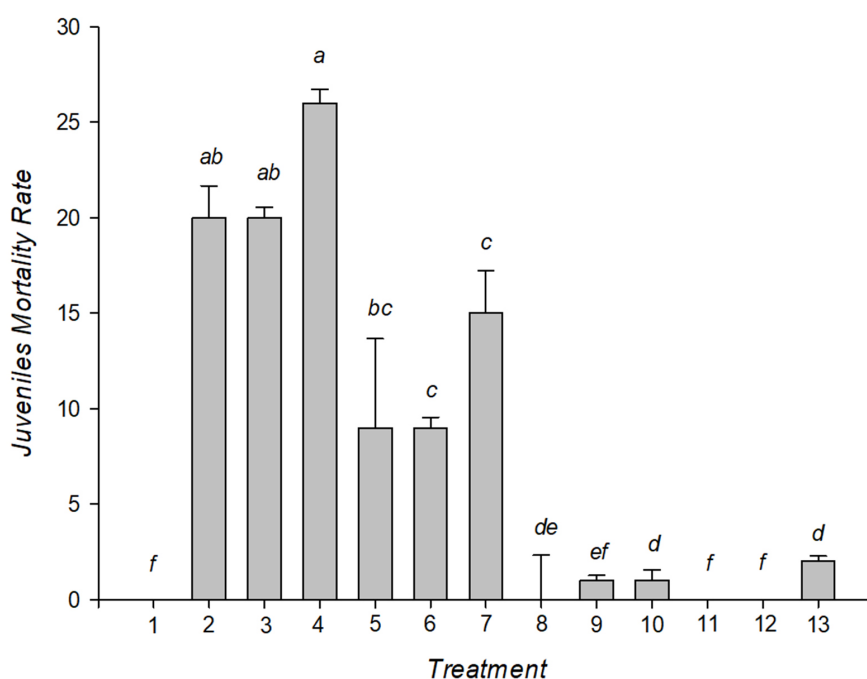
Remarks: 1: H₂O, 2: 10CBFN, 3: 20CBFN, 4: 30CBFN, 5: 10CRNC, 6: 20CRNC, 7: 30CRNC, 8: 10RCEH, 9: 20RCEH, 10: 30RCEH, 11: 10GNCS, 12: 20GNCS, and 13: 30GNCS

Fig. 4. Egg hatch rate on fifth day



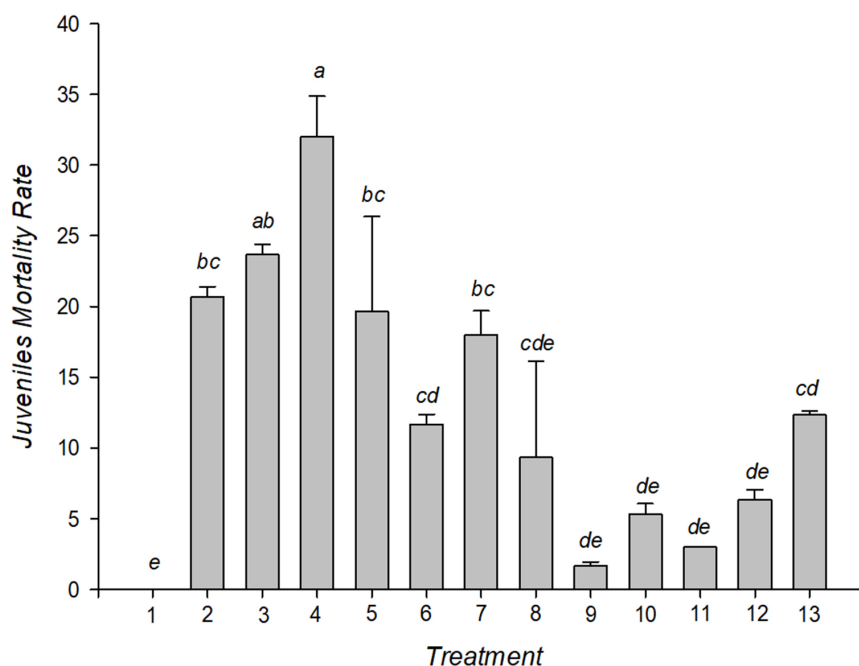
Remarks: 1: H₂O, 2: 10CBFN, 3: 20CBFN, 4: 30CBFN, 5: 10CRNC, 6: 20CRNC, 7: 30CRNC, 8: 10RCEH, 9: 20RCEH, 10: 30RCEH, 11: 10GNCS, 12: 20GNCS, and 13: 30GNCS

Fig. 5. Egg hatch rate on sixth day



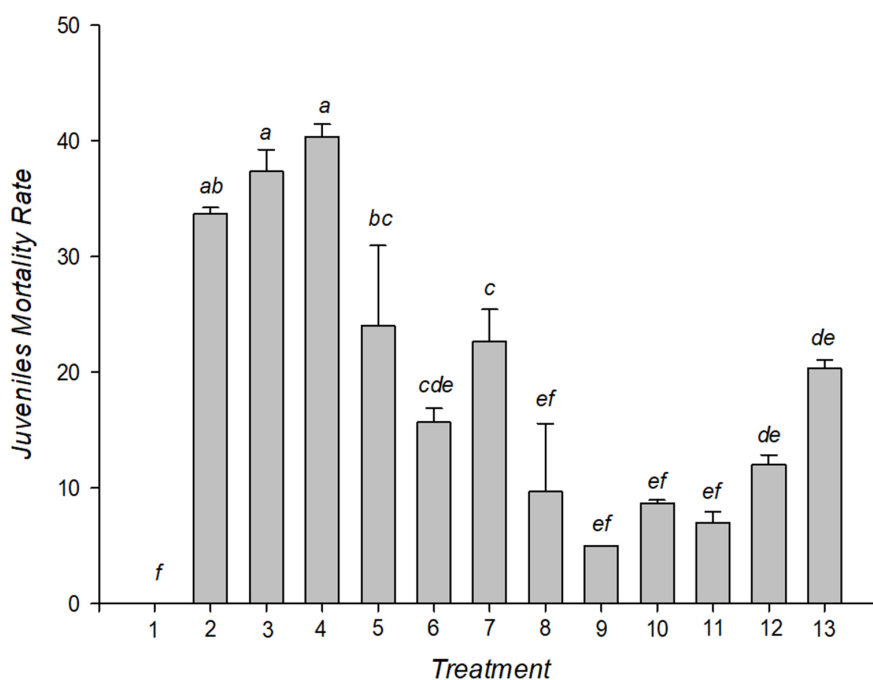
Remarks: 1: H₂O, 2: 10CBFN, 3: 20CBFN, 4: 30CBFN, 5: 10CRNC, 6: 20CRNC, 7: 30CRNC, 8: 10RCEH, 9: 20RCEH, 10: 30RCEH, 11: 10GNCS, 12: 20GNCS, and 13: 30GNCS

Fig. 6. Juvenile mortality rate on third day



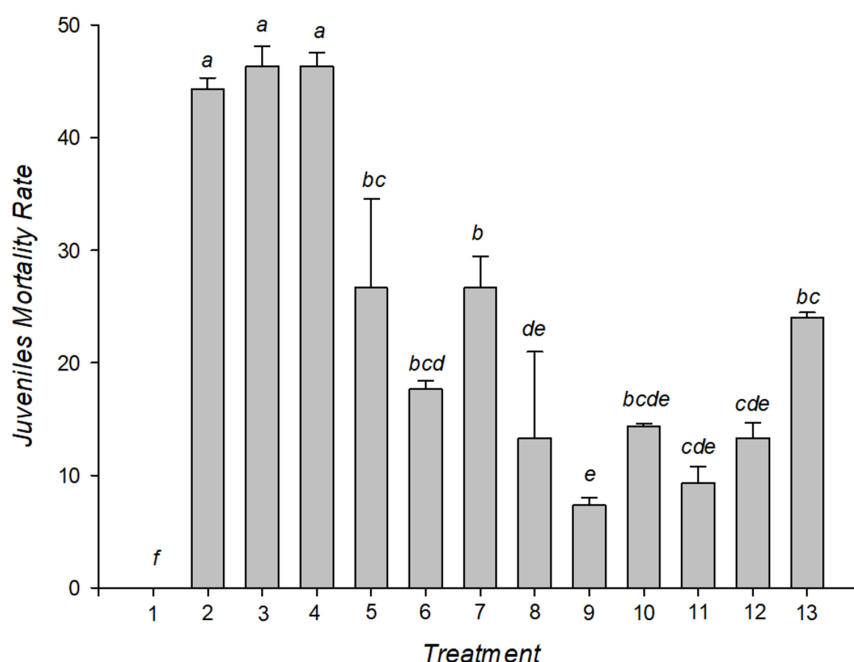
Remarks: 1: H₂O, 2: 10CBFN, 3: 20CBFN, 4: 30CBFN, 5: 10CRNC, 6: 20CRNC, 7: 30CRNC, 8: 10RCEH, 9: 20RCEH, 10: 30RCEH, 11: 10GNCS, 12: 20GNCS, and 13: 30GNCS

Fig. 7. Juvenile mortality rate on fourth day



Remarks: 1: H₂O, 2: 10CBFN, 3: 20CBFN, 4: 30CBFN, 5: 10CRNC, 6: 20CRNC, 7: 30CRNC, 8: 10RCEH, 9: 20RCEH, 10: 30RCEH, 11: 10GNCS, 12: 20GNCS, and 13: 30GNCS

Fig. 8. Juvenile mortality rate on fifth day



Remarks: 1: H₂O, 2: 10CBFN, 3: 20CBFN, 4: 30CBFN, 5: 10CRNC, 6: 20CRNC, 7: 30CRNC, 8: 10RCEH, 9: 20RCEH, 10: 30RCEH, 11: 10GNCS, 12: 20GNCS, and 13: 30GNCS

Fig. 9. Juvenile mortality rate on sixth day

Generally, there was no significant ($p=0.05$) difference between corn cob, guinea corn chaff, and carbofuran on their effect on egg hatch rate. The lowest concentration (10%) recorded a higher mean percentage egg hatch, while there was no significant ($p=0.05$) difference between the 20 and 30% concentrations. The effect of the various treatments on the percentage mortality of *M. javanica* juveniles is presented in Fig. 6, Fig. 7, Fig. 8 and Fig. 9. Juvenile mortality was remarkably low in control (distilled H₂O), 10% rice husk, 10% guinea corn chaff, and 20% guinea corn chaff on day 3 of observation on juvenile mortality.

The mean percentage of juvenile mortality recorded in 10 and 20% carbofuran is not significantly different from what was obtained in 30% carbofuran (Fig. 6). Percentage juvenile mortality was not notably dissimilar among the treatments. However, fewer dead juveniles were observed in 20% RCEH and 10% GNCS. In comparison, there was no mortality in the control (Fig. 7). 10 and 30% corn cob silver nanoparticles had higher mean percentage mortality among the treatments (Fig. 8 and Fig. 9). The mean percentage of juvenile mortality

was significantly ($p=0.05$) higher in corn cob agro-waste silver nano liquid as against the observation in guinea corn chaff and rice husk. However, carbofuran was significantly ($p=0.05$) better with higher mean percentage mortality compared to all the treatments. Higher mean percentage mortality was observed in the 30% concentration. The other concentrations were not significantly different from each other.

The colour change observed among the different agricultural waste silver nanoparticle (AgNPs) liquid confirms the formation of AgNPs and surface plasmon resonance during the reaction. After an hour, the colours were stable, which confirms that the reaction had come to an end, thus meaning that the agro-wastes gave different stabilizing effects on the formation of silver nanoparticles. Generally, colour changes are an initial indication of the formation of nanoparticles (NPs) because AgNO₃ is a colourless solution in distilled water (Fabiyyi & Olatunji, 2018; Sameen, Fathima, Ramlal, Kumar, & Khanum, 2014; Veerasamy et al., 2011).

The broad peak observed in the NPs absorbance is evidence of the polydispersed

nature of the particles and an establishment of the formation of NPs. Zahir et al. (2014) recorded a maximum absorbance at λ 420 nm in the synthesis of NPs with *Euphorbia prostrata* extract, while Abd El-Rahman & Tahany G. M. (2013) reported λ 412 nm with *Eucalyptus globulus* extract, in this study λ 416 nm, 420 nm and λ 425 nm were the absorbance peaks recorded for the corn cob, guinea corn chaff and rice husk agro- waste NPs respectively.

Silver nanoparticles have found practical applications in many ways (Ramasubburayan et al., 2017) and are known to be highly biologically active. Khan et al. (2013) reported the antiviral properties of AgNPs. Abd El-Rahman & Mohammad (2013) stated the effectiveness of silver nanoparticles on pathogenic bacteria (*Agrobacterium tumefaciens*, *Erwinia amylovora* and *Ralstonia solanacearum*). Similarly, Asoufi, Al-Antary, & Awwad (2018) reported the pesticide effect of silver nanoparticles. Fabiyi, Olatunji, & Saadu (2018) established the nematicide properties of agro-waste silver nanoparticles used as soil admix in the control of *Heterodera sacchari* infecting rice.

The highest dosage of application, 50 and 75 grams, significantly ($p=0.05$) reduced cyst counts in root and soil of treated plants. This corroborates the results in this study; corn cob agro-waste AgNP was substantially more effective with the highest mean percentage of juvenile mortality. Homogeneously, the nematicidal potential of silver nanoparticles was substantiated by Fabiyi et al. (2020). An appreciable reduction in the population of cyst nematodes on rice was achieved with the green synthesis of silver nanoparticles. Agricultural wastes have proved to be good stabilizing agents in the fusion of nanoparticles in this research. The lignocellulosic materials and pentosans contained in the agro-waste (Lee, Hamid, & Zain, 2014) played an essential role in the stability of the nanoparticles.

CONCLUSION

The use of nanoparticles in plant disease management is a novel approach that may prove very effective. This is the first report on the synthesis of nano particles with agricultural wastes as stabilizers and capping agents to the best of our knowledge. Agricultural waste-mediated silver nanoparticles could be used in place of carbofuran for *M. javanica* control.

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