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Control of Fusarium wilt disease of tomato (*Solanum lycopersicum*. L) using magnesium oxide nanoparticles

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ABSTRACT

Fusarium wilt is one of the most damaging fungal diseases of plants in the world, causing significant yield loss. Control measures such as cultural, biological, physical and chemical methods have their limitations. Hence, there is a need to explore nanotechnology, a field of science concerned with the manipulation of nanoparticles (NPs), which has found widespread application in different fields. The in vivo study results revealed that the tomato plants treated with magnesium oxide (MgO) nanoparticles had better growth performance with higher chlorophyll content (4.63 mg/L) compared to the control (0.67 mg/L) after 30 days of post-inoculation (DPI). Compared to the untreated control, tomato plants treated with magnesium oxide nanoparticles had a significant decrease in the percentage disease incidence, with 10% in tomato plants treated with MgO nanoparticles and 100% in the untreated control, respectively, after 30 days of post-inoculation. The research has demonstrated that the Fusarium wilt disease of tomato plants can be controlled using MgO nanoparticles.

Keywords: MgO nanoparticles; Fusarium wilt; antifungal activity; tomato plant.

1. INTRODUCTION

Plant diseases caused by pathogens seriously limit economically important plant growth and productivity, affect food security and threaten human health (Zhang et al., 2018). Among these plant pathogens, fungi are accountable for the most damaging diseases in plants (Sharma et al., 2017b). The Fusarium fungus is a major plant pathogen that can be found in a variety of forms and different locations (Al-Janabi et al., 2017). *Fusarium oxysporum* f.sp. *lycopersici* (Sacc.) WC Snyder and HN Hans, the vascular wilt pathogen, is one of the most damaging pathogens of tomato, causing considerable losses in tomato production globally (Al-Janabi et al., 2017; Srinivas et al., 2019). The pathogen is highly destructive in both greenhouses and fields (Etebu et al., 2013). The control of tomato Fusarium wilt disease is quite difficult because of the ability of this fungus to remain dormant in the soil in the form of resting structures and chlamydospores for years without a host and this limits the suppressive effect of crop rotation (Weeraratne and De Costa, 2018). Management of Fusarium wilt disease through the

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application of fungicides and other chemicals has caused several environmental complications, toxicity to non-target organisms and development of fungi resistant pathogens (Al-Janabi et al., 2017; Sharma et al., 2017b; Igiehon et al., 2020).

In order to overcome these limitations, it is necessary to explore biocompatible, cost-effective and eco-friendly control strategies that will effectively address the threat posed by several phytopathogens, hence the use of nanotechnology (NT), a broad-based science involving manipulation of matter in a variety of ways to generate new understanding of how materials can be developed at the nanoscale level to solve many problems in various fields (Scrinis and Lyons, 2007; Koka et al., 2019). The use of nanotechnology has found wide applications in various fields and it has been extensively utilized in the area of controlling more human pathogens than phytopathogens (Balakumaran et al., 2016). The field of nanotechnology has in forefront the use of nanoparticles (NPs), are particles ranging in size from 1 to 100 nanometers (nm) that have unique physiochemical and biological properties due to their reduced small size (Khan et al., 2017; Koka et al., 2019). The improved properties of these nanoparticles compared to their bulk counterparts have greater potentials; offer opportunity to reduce the load of unwanted chemicals especially plant protectants (Prasad et al., 2014).

Recently, several metallic nanoparticles have been investigated as antimicrobial agents for the control of pathogens. Among them, Magnesium oxide nanoparticles (MgO NPs), are metal derived nanoparticles, mainly ionic nanoparticulate metal oxides with very high surface areas to volume ratio, extremely reactivity based on the uncommon crystal morphologies (Vergheese and Vishal 2017; Dobrucka, 2018). MgO NPs have distinctive magnetic, thermal, optical, electrical, mechanical and chemical properties due to its typical structures when compared to their bulk counterparts (Ramanujam and Sundrarajan, 2014; Vergheese and Vishal, 2017). MgO NPs have a wide band gap and have gained applications in various fields (Mirzaei and Davoodnia, 2012; Dobrucka, 2018). When compared with nanoparticles of titanium oxide, silver, copper and gold, those with antimicrobial properties tend to have the advantage of being synthesized from easily available and cheaper precursor salts (Vergheese and Vishal, 2017). MgO nanoparticles have also gained interest because they are stable under harsh process conditions and generally perceived as safe materials for plants and humans (Zhen-Xing and Bin-Feng, 2014). The aim of this study was to look into the possibility of using green synthesized magnesium oxide nanoparticles to control Fusarium wilt disease.

2. MATERIALS AND METHODS

Plant Materials

Moringa oleifera (moringa) and *Solanum lycopersicum* (tomato) plants were used for this study. Samples of moringa leaf were obtained from residential quarters at Uteh Community, Ikpoba Okha Local Government Area, Benin City, Edo State. The plants were identified and authenticated by plant taxonomist at the Department of Plant Biology and Biotechnology, University of Benin. The *Solanum lycopersicum* (tomato) seeds were procured from National Horticultural Research Institute (NIHORT) located at Ibadan, Oyo State.

Chemical Precursor

Magnesium oxide (MgO) was used as precursor salts, along with other necessary chemicals. All reagents were used without further purification. Double distilled water was employed as the solvent.

Preparation of Plant Extract

Plant extracts were prepared by adapting the method of Oloyede et al., (2016). Healthy fresh leaves of *M. oleifera* were collected and the leaves were initially rinsed with tap water to remove any soil or dirt and then finally washed with distilled water. The leaves were chopped into small pieces and 20 grams each was put into a 250 ml Erlenmeyer flask containing 100 ml of sterile distilled water and kept in a water bath at 100°C for 10 minutes. To obtain the aqueous plant extracts, muslin cloth and Whatman filter paper No. 1 were used to filter the extracts.

Preparation of Precursor Solution

Magnesium oxide was prepared at a molar concentration of 0.1 M. An aqueous solution of MgO was prepared by dissolving 0.4 g of the precursor salt of magnesium oxide in 100 ml of double-distilled water. The solution was used for the green synthesis of magnesium oxide nanoparticles.

Synthesis of Magnesium Oxide Nanoparticles

Magnesium oxide nanoparticles were synthesized by adapting the method of Sharma et al., (2017a). 10 ml aliquot of leaf extracts of the resulting filtrate was transferred into 30 ml of 0.1 M MgO solutions. The mixture was incubated for about 24 hours at 28 ± 2°C (Plate 1).

Characterization of MgO Nanoparticles

The characterization of green synthesized magnesium oxide nanoparticles was performed using a UV-Vis 1800 double-beam spectrophotometer (Shimadzu, Tokyo, Japan) operating with the range of 250 to 1000 nm wavelength was used to monitor and confirm the formation of MgO nanoparticles based on their optical properties and the bio-reduction of Mg^{2+} ions to nanoparticles in the respective solutions. The measurement of the absorbance of nanoparticles in the colloidal solutions at regular intervals of 24 and 48 hours was done by loading each of the samples of 2 ml in quarts cuvette and then placing it on the UV-Vis spectrophotometer with an absorbance reading in the range of 250 – 600 nm using a resolution of 1 nm.

Isolation of Fungal Phytopathogens from Diseased Tomato Plants

The pour plate and direct plating methods of inoculation technique, according to (Cheesbrough, 2000; Holt et al., 2000), were employed in isolating fungal phytopathogens associated with the diseased tomato samples. *Fusarium* species were isolated from freshly infected samples exhibiting typical wilt disease symptoms plated on potato dextrose agar (PDA) media. Pure cultures of the fungal pathogens were maintained by transferring in fresh slants of potato dextrose agar (PDA).

Phenotypic Characterization of the Fungal Isolate

The fungus isolate was identified using macroscopy and microscopy. The morphological characteristics on the basis of the cultural appearances (colony colour, texture, margin, form, elevation and aerial hyphae) were observed and described using a laboratory manual and a pictorial atlas for identification of fungi by Fawole and Oso, (2001) and Watanabe, (2002). The isolates were also observed under the microscope after staining with lactophenol. This was done by placing a drop of lactophenol blue stain on a clean, grease-free, sterilized glass slide. Thereafter, a sterile inoculating wire loop was used to pick the mycelium from the culture and place it on the glass slide. The mycelium was spread evenly on the slide. Teasing was carried out to separate the mycelium in order to get a homogenous mixture and the mixture was then covered with cover slips gently and allowed to stay for some seconds before being observed under the microscope at x40 magnification. This was then compared with a laboratory manual for fungal identification.

Molecular Identification of the Fungal Isolate

DNA Extraction

The ZR fungal/bacterial DNA Miniprep TM kit (Zymo, Research Company) was used for the extraction of DNA from fungal isolates. The DNA extraction procedures were carried out according to the manufacturer's instructions.

Concentration and Purity Check of Extracted DNA

The concentration and purity of the extracted DNA aqueous solution were checked by measuring absorbance at 260 nm and 280 nm in a quartz cuvette of 1 cm path length against a distilled water blank in a spectrophotometer. The DNA concentration was determined on the assumption that an A260 of 1.0 was equivalent to the following concentrations: Oligonucleotides (20µg/ml), RNA (40µg/ml) and double-stranded DNA (50µg/ml). The purity of nucleic acid samples was investigated by determining the ratio of Abs260/ Abs280. A ratio of > 1.5:1 indicates relatively pure DNA with no contaminating proteins or polysaccharides (Shittu, 2012).

PCR Amplification

Fungi isolate was characterized by the amplification of their internal transcribed spacer (ITS) regions of rDNA. The forward ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and reverse ITS2 (5'-TCCTCCGCTTATTGATATGC-3') primers were used in the PCR reactions to amplify the ITS region of the rDNA of the fungal isolates, adapting the method of Michaelson et al., (2006). Each PCR mixture contained 12.5µl of Quick Load Taq One Step PCR Master Mix, 1.25µl of each primer pair, 5µl of template DNA, nuclease free water and 5µl of total volume of 25µl. The sample was gently vortexed and spun down. The thermocycling program used was an initial denaturation (94°C for 3 minutes), 35 cycles of denaturation (94°C for 30 seconds), annealing (54°C for 30 seconds) and

elongation (72°C for 1 minute), then a stabilization/final extension (72°C for 5 minutes), followed by cooling to 4°C until the sample was recovered. Gel electrophoresis was used to visualize and purify PCR products. A 1.0% agarose gel was prepared by measuring 100ml of TBE buffer into a flask, then 1g of agarose gel powder was weighed and added to the prepared buffer. It was heated for 3minutes in a microwave to dissolve the powder. It was allowed to cool to about 56°C; 5µl of ethidium bromide was added.

The molten gel solution was poured in a gel mould in which the gel comb has been appropriately inserted. The agarose gel was allowed to solidify for 45 minutes at room temperature. After the agarose gel was prepared, 10µl of the PCR product was analysed on the 1.0% agarose gel electrophoresis, stained with ethidium bromide. In the gel electrophoresis chamber, 10µl of ready-to-use DNA ladder (molecular marker) mixed with loading dye was loaded in the first well of the solidified gel immersed in TBE buffer in Gel electrophoresis chamber. When the quick load PCR master mix was used, 10µl of PCR product(s) (amplicon) were loaded into each well of agarose gel. The process was run at 90 volts for 60 minutes. Finally, it was view under gel documentation system with UV transilluminator.

DNA Sequencing

The amplified PCR products were cleaned using the Exo SAP Protocol as described in the manufacturer's procedures. Sequencing was then done with the ABI V3.1 Big Dye Kit according to the manufacturer's instructions. The labelled products were then cleaned with the ZymoSeq clean-up kit. The cleaned products are injected onto the ABI3500XL analysers with a 50 cm array using POP7.

Analysis of DNA Sequences

Sequences data generated were analyzed with Geneious version 9.0.5 and cladogram trees were constructed using neighbor joining tree model.

In Vivo Evaluation of Antifungal Activity of MgO Nanoparticles

The investigation of the efficacy of MgO nanoparticles against the fungal wilt pathogen of tomato plants, was conducted adapting the methods described by (Farag Hanaa et al., 2011; Jones et al., 2015; Chakraborty et al., 2017; Sharma et al., 2017b; Prihatra et al., 2018).

Planting of model plant (tomato) seeds

The seeds of the tomato (*S. lycopersicum*) were obtained from the National Horticultural Research Institute (NIHORT). The seeds were surface sterilized with 0.1% hypochlorite for 3 minutes, then washed with sterilized distilled water three times. The sterile seeds were planted in plastic containers of about 17 cm in width and 12 cm in depth, which contained sterile loamy sandy soil. Subsequently, tomato seedlings were sub planted in triplicates per plastic container and replicated in three plastic containers designed for each treatment. The plants were maintained under field conditions and were rhizo injected every other day.

Fol inoculum preparation

The fungal inoculum was prepared with sterilized distilled water. The test fungal inoculum "*F. oxysporum* f. sp. *lycopersici*" maintained in PDA medium for weeks was flooded with 10 mL of distilled water in the petri dishes. The conidia (spores) were scraped out using a sterile spatula and kept in sterile 50-mL tubes. The spore suspensions were then adjusted to a final concentration of 1 x 10-5 spores/mL by hemocytometer under a light microscope.

Tomato seedlings inoculation with Fol inoculum

Tomato plant seedlings were inoculated with a test fungal inoculum using the root dip treatment method. Month-old seedlings were removed from the soil, their roots were washed in distilled water and they were placed for ten minutes in a plastic container containing a spore suspension of 1×10^{-5} mL before being replanted.

MgO nanoparticles treatments

The nanoparticle solutions were prepared in concentrations of 100 and 50%. The concentrations were selected on the basis of the previous in vitro study by Igiehon et al., (2020). The infected tomato seedlings were treated with MgO nanoparticles at concentrations of 100 and 50% after infestation on alternate days. The treatments were done through aerial application by spraying the tomato seedlings with the MgO nanoparticle solutions and soil drench application by applying the MgO nanoparticle solutions to the soil in the plastic containers containing tomato seedlings. Infected tomato plants treated with distilled water and tomato

plants without infestation by the test fungal pathogen were also set up as controls. These tomato plants were closely monitored for the expression of *Fusarium* wilt symptoms.

Data was collected on the plant height, percentage disease incidence, and chlorophyll content.

Plant height: The plant height of the tomato plants was measured centimeter.

Percentage disease incidence (PDI): PDI of the diseased tomato plants in the experimental samples was calculated using the formula:

$$PDI = \frac{n \times 100}{N}$$

Where: n= number of plants showing wilts symptoms

N = Total number of plants sampled (Sharma et al., 2017b).

Chlorophyll content

The total chlorophyll content was determined using the spectrophotometric method and calculated using the Arnon's equation formula according Khaleghi et al., (2012).

$$\text{Total chl} = \frac{20.2(A645) + 8.02(A663) \times V}{1000 \times W}$$

V= volume of solvent W= fresh weight of tissue extracted

Data analysis

The experimental data was statistically analyzed by one-way analysis of variance (ANOVA) using SPSS statistical software (version 23.0) and the comparison of the means was determined by post hoc analysis using Duncan's Multiple Range (DMR) test at the $P < 0.05$ confidence level. The results were presented as the mean ± standard error of three replicates.

3. RESULTS

MgO nanoparticles were successfully synthesized using a magnesium oxide precursor solution with moringa leaf extract. When the precursor solution and the leaf extract were added together, a color change from pale green to brownish colloidal solutions was observed within 24 hours of synthesis at 28 ± 2°C (Plate 1). Maximum Abs values were recorded between the wavelengths of 390 and 410 nm with a peak value at 400 nm using a spectrophotometric technique for characterizing synthesized MgO nanoparticles (Figure 1).

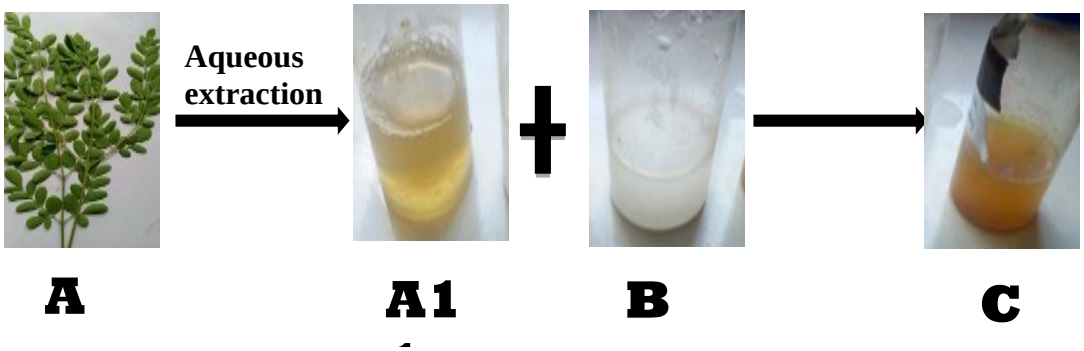

Plate 1 Schematic presentation of phytosynthesis of MgO nanoparticles

A: Fresh leaf of moringa A1: Moringa leaf extract B: Magnesium oxide precursor solution C: Magnesium oxide nanoparticles

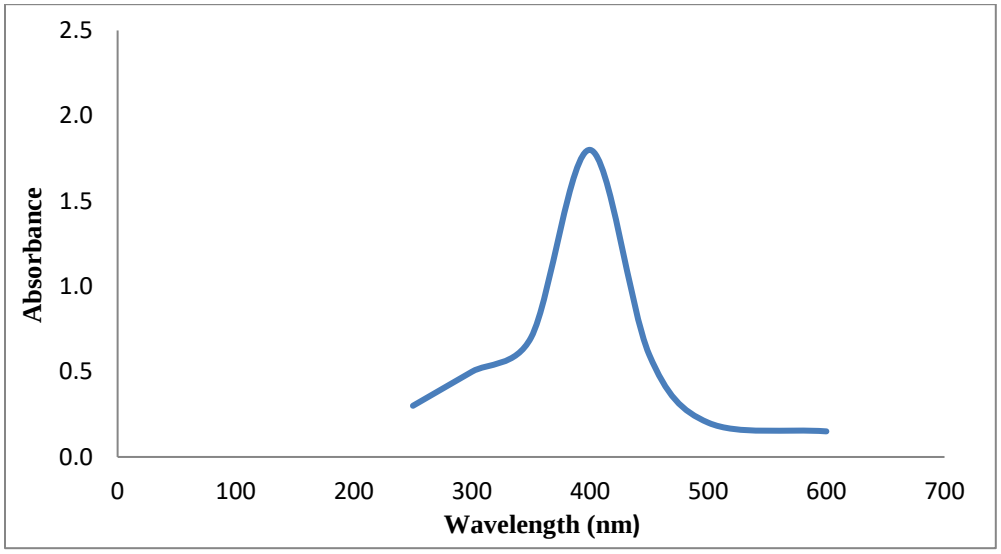

Figure 1 UV-V is spectra of the synthesized MgO nanoparticles after 24 hours

Table 1 and Plate 2 show the cultural and morphological descriptions of *Fusarium* fungal pathogens isolated from diseased tomato plants. The isolates had a raised elevation, a rough margin, a large size and an opaque optical property. One has white pigmentation, while the other has reddish pigmentation. The result of the molecular identification of the *Fusarium* fungal pathogens showed that two isolates were *F. oxysporum* f. sp. *Ciceris* and *F. oxysporum* f. sp. *Lycopersici, with* accession numbers MN522526 and MN522527, respectively (Figure 2). It was revealed that the height of all the tomato plants treated with MgO nanoparticles compared to the control on within 15 days of post inoculation (DPI) was relatively the same with no significant difference at $P < 0.05$. However, from days 30 to 60, the heights of tomato plants treated with MgO nanoparticles differed significantly from the control plants (Figure 3). The results demonstrated that the tomato plants with the highest heights of 48.9 ± 0.03 cm and 46.0 ± 0.06 cm were obtained from the uninfected control (tomato plants without *Fol* infestation) and 100% MgO nanoparticles-treated tomato plants, respectively. Meanwhile, the lowest tomato plant height of 30.0 ± 0.00 cm was recorded in the untreated control (tomato plants infected with *Fol* and treated with distilled water) from day 30 to day 60.

Plate 2 Isolated test Fusarium fungal phytopathogens from diseased tomato plants.

Table 1 Cultural and morphological description of fusarium fungal isolates

Morphology	Isolate 1	Isolate 2
Margin	Rough	Rough
Elevation	Raised	Raised
Size	Large	Large
Texture	Rough	Rough
Pigmentation	White	Reddish
Optical property	opaque	Opaque
Suspected organism	*Fusarium* Species	*Fusarium* species.

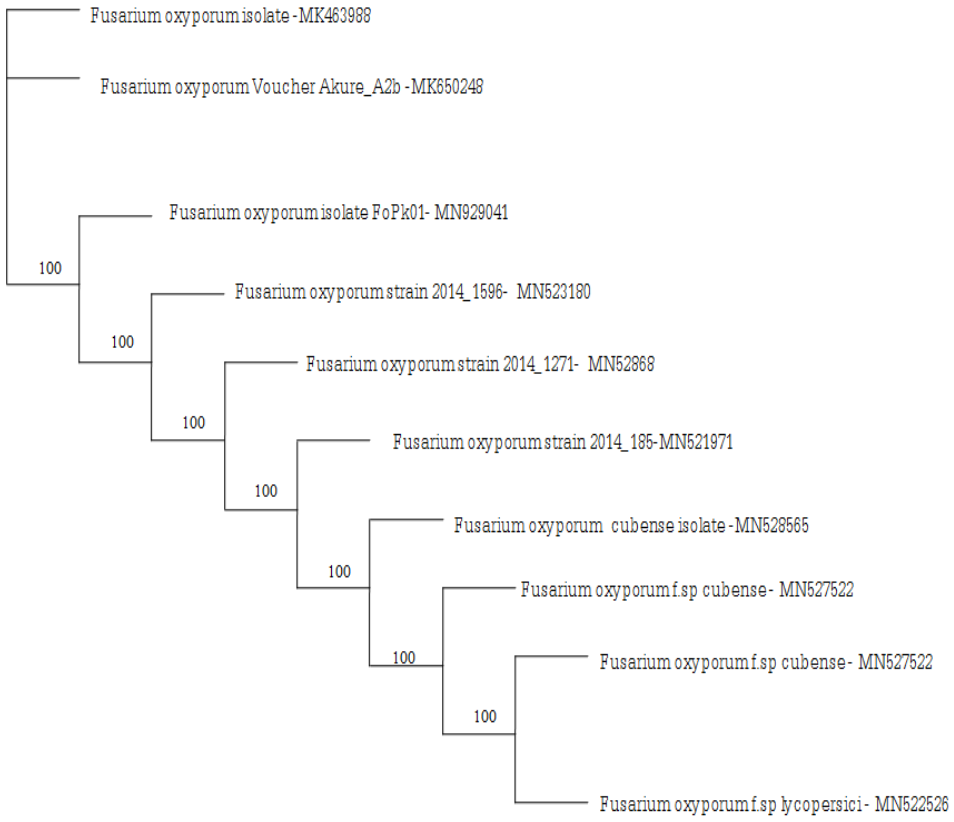

Figure 2 Cladogram of the fungal isolate *F. oxysporum* f. sp. *Lycopersici*

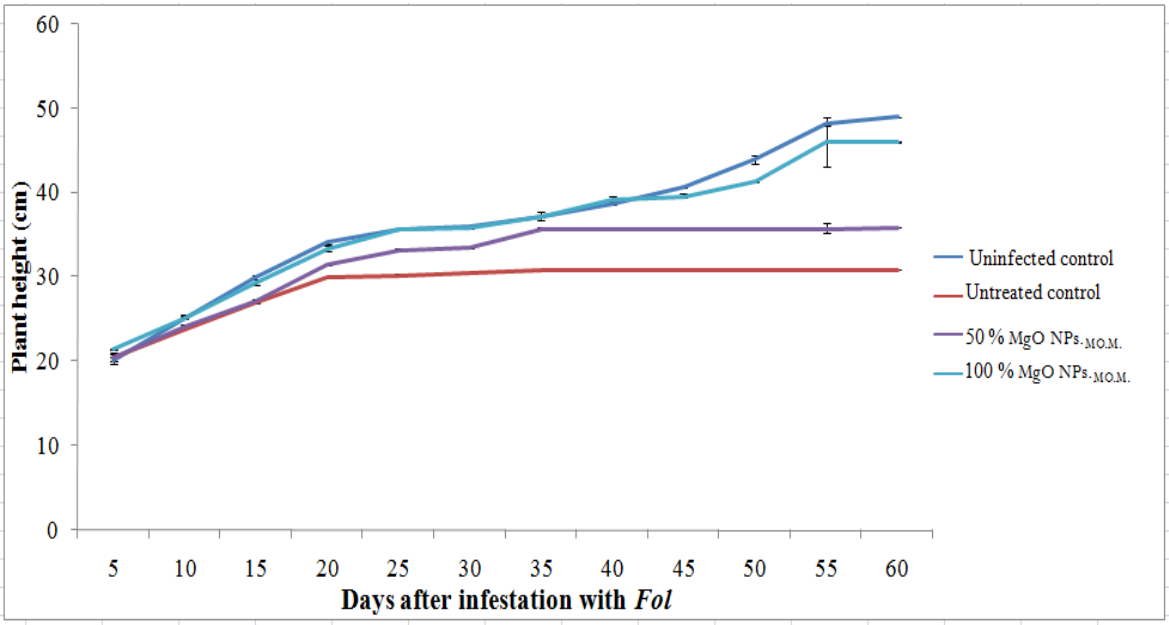

Figure 3 Effect of MgO nanoparticles on tomato plant height challenged with *Fol*

Values were expressed as means ± standard error, significant difference at $P < 0.05$. MgO NPs.MO.M.: Magnesium oxide nanoparticles biosynthesized using magnesium oxide precursor solution and moringa leaf extract.

The result (Figure 4) demonstrated that MgO nanoparticles affected tomato plants' chlorophyll content. There was a significant increase in chlorophyll content in tomato plants rhizo injected with 100% MgO nanoparticles from 3.93 mg/L on day 25 to 4.63 mg/L on day 45 compared to the uninfected and untreated control tomato plants. However, the chlorophyll content declined in all the tomato plants; it was 1.6 mg/L on day 60 in tomato plants treated with 100% MgO nanoparticles compared to 0.1 mg/L on day 60 in untreated tomato plants.

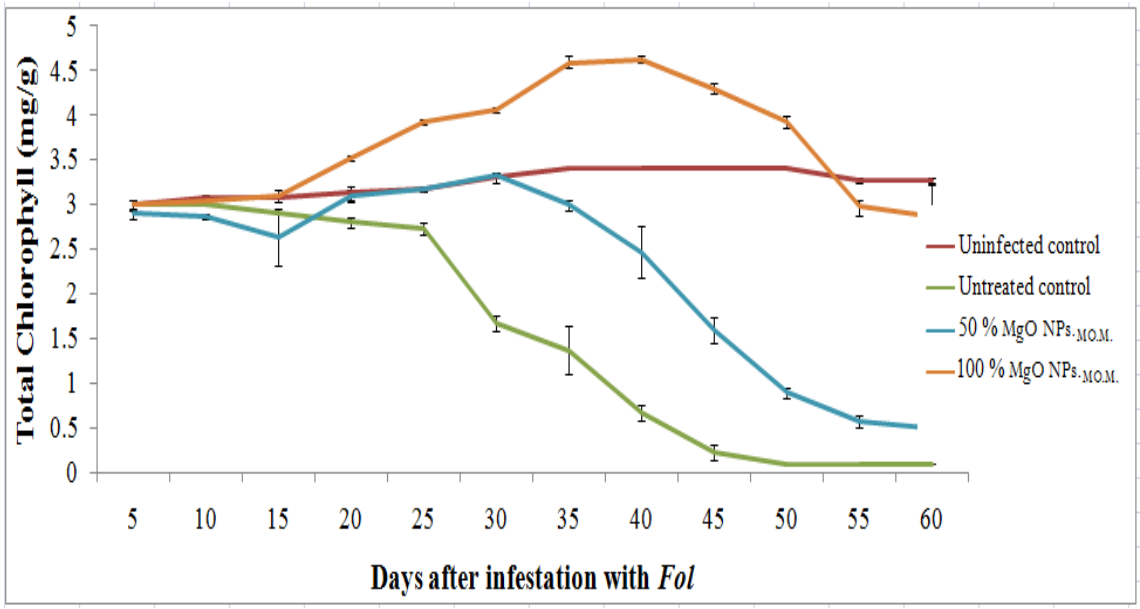

Figure 4 Impact of MgO nanoparticles on chlorophyll content in tomato plant infected with *Fol*

Values were expressed as means ± standard error; significant difference at $P < 0.05$. MgO NPs.MO.M: Magnesium oxide nanoparticles biosynthesized using magnesium oxide and moringa leaf extract

Figure 5 and Plate 3 highlighted the percentage disease incidence of *Fol*-infected tomato plants that were rhizo injected with MgO nanoparticles; the results demonstrated that there was no incidence of *Fusarium* wilt disease at 10 days after inoculation. Meanwhile, the untreated control had a first disease incidence of 22% at day 15 and progressed to 100% at day 35. Also, the results revealed that the tomato plants treated with 100% MgO nanoparticles within 35 DPI had no significant disease incidence. However, there was a disease incidence of 18.3% on day 40 that increased to 33% on day 60.

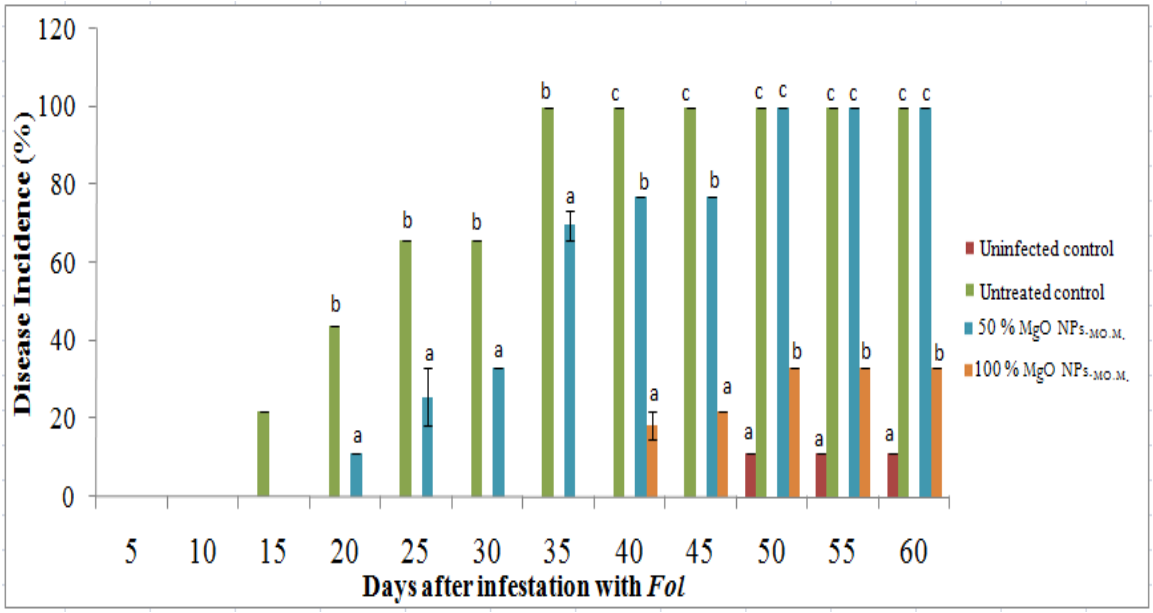

Figure 5 Effect of MgO nanoparticles on the reduction of Fusarium wilt disease incidence in tomato seedlings

Values were expressed as means ± standard error; significant difference at $P < 0.05$. MgO NPs.MO.M: Magnesium oxide nanoparticles biosynthesized using magnesium oxide precursor solution and moringa leaf extract

Plate 3 Impact of magnesium oxide nanoparticle in control of Fol infected tomato plants
A: 100 MgO nanoparticles B: 50 MgO nanoparticles C: Negative control: untreated control; D: Positive control: uninfected control

4. DISCUSSION

There is currently an increasing interest in the field of nanotechnology, as it affects various sectors of life. Application of nanotechnology in the control of pathogenic diseases in economic plants is fundamental to sustainable food production and security. In the present study, MgO nanoparticles were synthesized using a leaf extract of *M. oleifera* and a precursor solution of magnesium oxide. This mechanism of plant-assisted bio-reduction of metallic salts to nanoparticles has been attributed to the presence of phytochemicals present in plants and plant extracts, such as terpenoids, flavonoids, ketones, phenols, polyphenols, alkaloids, aldehydes, amines, steroids and carboxylic acids, which act as reducing and capping agents (Herlekar et al., 2015; Saranya et al., 2017; Sharma et al., 2017b). The UV–V is absorption spectrophotometric technique used for characterization in this study has been reported to be a valuable tool for monitoring, confirming, characterizing and identifying nanoparticles (Dobrucka, 2018; Abbas, 2019).

The isolated fungal phytopathogens from diseased tomato plants were phenotypically identified as *F. oxysporum*, species. A molecular technique was further used to confirm the isolates to be *F. oxysporum* f. sp. *lycopersici* (*Fol*) and *F. oxysporum* f. sp. *ciceris* (*Foc*). It has been reported that both *F. oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *ciceris* are ubiquitous soil-borne pathogens that cause destructive vascular wilts, rots and damping-off diseases in tomato and chicken pea plants, respectively (Srinivas et al., 2019; Amjad et al., 2018). It was observed in this research that the tomato plants treated with MgO nanoparticles at a 100% concentration had an increased height (46.0 ± 0.06 cm) compared to untreated control tomato plants (30.0 ± 0.00 cm) after 6 weeks of infection. This might be due to the ability of green synthesized magnesium oxide nanoparticles to suppress the Fol pathogen from proliferating and clogging the vascular tissues, as well as the ability of the MgO nanoparticles to enhance photosynthetic activities in the treated tomato plants.

The observation is supported by previous studies by Rathore and Tarafdar, (2015), who reported that the application of MgO nanoparticles to wheat plants enhanced photosynthetic activities as beneficial enzyme activities were expected to have increased, which promoted healthy growth of the plants. Cai et al., (2018) also stated that MgO nanoparticles could be absorbed into the plant tissue and provide nutrients that are favourable for plant growth. Prasad et al., (2014) had a similar report on the effects of zinc oxide nanoparticles (25 nm) at a high-level of 1000 ppm concentration that promoted the seed germination, seedling vigor and growth of peanut plants. Mung bean plants treated with MgO nanoparticles showed significant promotion in growth Abdallah et al., (2022). On the other hand, the stunted growth in tomato seedlings infected with Fol and controlled using distilled water could be due to production of some toxins by the fungal pathogen and the clogging of the vascular tissues by the pathogen. This finding is consistent with observations that Fol may block vascular tissues, which often causes restricted growth and ultimately results in plant death (Srinivas et al., 2019; Amjad et al., 2018).

The application of MgO nanoparticles to tomato plants significantly increased the total chlorophyll content of the tomato plants, with the overall highest chlorophyll content of 4.63 mg/L was recorded in tomato plants treated with 100% MgO nanoparticles. The increase in the chlorophyll content in tomato plants treated with MgO nanoparticles could be as a result of the fact that magnesium is the structural component of chlorophyll and its ability to trigger many co-enzymes as well as the possibly increasing' nutrient

uptake. The observation is corroborated by Rathore and Tarafdar, (2015), who reported that in a greenhouse experiment, adding magnesium nanoparticles to wheat plants greatly increased the chlorophyll content of the plants. Similar research conducted by Shah et al., (2015), established that treating plants with TiO nanoparticles increased chlorophyll concentration. In another study, Prasad et al., (2014) discovered that treating peanut plants treated with zinc oxide nanoparticles resulted in greater leaf chlorophyll content.

After 8 weeks of infection, the percentage of disease incidence was significantly reduced in infected tomato plants treated with MgO nanoparticles at 100% concentration compared to the untreated control. The overall lowest disease incidence (33%) was recorded in tomato plants rhizo injected with MgO nanoparticles after 60 days of infection compared to the control (100%). The observation from this result clearly showed that applications of MgO nanoparticles could have significantly improved photosynthetic efficiency, which ultimately improved growth and helped to fight pathogenic infestations. Similar research on *Fusarium* wilt disease incidence conducted by Surega et al., (2015) found that green synthetic Ag NPs caused complete suppression of disease incidence caused by *F. oxysporum* f. sp. *lycopersici* at the early stage of tomato and improved the growth of the plants. Abdallah et al., (2022) reported a reduction in disease severity in mung bean plants treated with MgO nanoparticles against *F. oxysporum* and *F. solani* phytopathogens in an in vivo study.

The mechanism underlying the antifungal activity of MgO nanoparticles has not been clearly elucidated. However, researchers have reported that the antifungal activity of MgO nanoparticles could be due to the nano size of MgO triggering the host plant's defense responses (Surega et al., 2015). Koka et al., (2019) reported that the antifungal activity of nanoparticles may be due to the suppression of enzymes and toxins used by fungal pathogens for pathogenesis. Furthermore, the possibility of the antifungal activity of metal nanoparticles may be due to the release of ions that bind to groups of proteins, which disrupts the function of membrane proteins and results in the permeability of the cell. Moreover, the ions of nanoparticles have a toxic impact on DNA, which causes cell death (Abdallah et al., 2020).

5. CONCLUSION

The current study found that MgO nanoparticles have potential antifungal ability and can be employed as an alternative control measure for fungal wilt disease in plants, particularly tomato crops. The outcomes of the present study also showed that MgO nanoparticles can enhance chlorophyll and stimulate plant growth. Furthermore, the study has shown that controlling fungal pathogenic disease in economic plants with a safe and inexpensive method utilizing MgO nanoparticles is crucial to ensuring a sustainable food supply and food security.

Recommendations

To combat pathogens, increase growth and increase yield, it may be necessary to formulate the crude, green-synthesized MgO nanoparticles into fine forms such as nano capsules, nano fungicides and nano fertilizer pellets or spray. Further research is needed to determine the environmental impact and toxicity of MgO nanoparticles during long-term use.

Informed consent

Not applicable.

Ethical approval

Moringa oleifera (moringa) and *Solanum lycopersicum* (tomato) plants were used for this study. The ethical guidelines for plants & plant materials are followed in the study for sample collection & identification.

Conflicts of interests

The authors declare that there are no conflicts of interests.

Funding

The study has not received any external funding.

Data and materials availability

All data associated with this study are present in the paper.

REFERENCES AND NOTES

1. Abbas Q. Understanding the UV- Vis spectroscopy for nanoparticles. J Nanomater Molec Nanotechnol 2019; 8:3-6.
2. Abdallah Y, Hussien M, Omar MOA, Elashmony RMS, Alkhalifah DHM, Hozzein WN. Mung Bean (*Vigna radiata*) treated with Magnesium Nanoparticles and its Impact on Soilborne *Fusarium solani* and *Fusarium oxysporum* in Clay Soil. Plants 2022; 11:1514-1523.
3. AL-Janabi A, Ahmed AA, Jawad K, Laith AH, Ali FM. The expression of resistance genes in tomato induced by abiotic and biotic factors against *Fusarium oxysporum f. sp. Lycopersici.* Int J Chem tech Res 2017; 10(6):838-850.
4. Amjad MH, Aafreen S, Souliha RC. Host-pathogen interaction in *Fusarium oxysporum* interaction: Where do we stand? Curr Rev 2018; 12(9):889-898.
5. Balakumaran MD, Ramachandran R, Balashanmugam P, Mukeshkumar DJ, Kalaichelvan PT. Mycosynthesis of silver and gold nanoparticles: Optimization, characteristics and antimicrobial activity against human pathogens. Microbiol Res 2016; 182:8–20.
6. Cai L, Chen J, Liu ZW, Wang H, Yang H, Ding N. MgO nanoparticles: Effective agriculture antibacterial agent against *Ralstonia Solanacearum*. Front Microbiol 2018. doi: 10.3389/fm jcb.2018.00790
7. Chakraborty N, Chandra S, Acharya K. Biochemical basis of improvement of defense in tomato plant against Fusarium wilt by $CaCl_2$. Physiol Mol Biol Plants 2017; 23(3):581-596.
8. Cheesbrough M. District Laboratory Practices in Tropical Countries (Part 11). London Cambridge University Press, Cambridge 2000; 258.
9. Dobrucka R. Synthesis of MgO nanoparticles using *Artemisia abrotanum* herba extract and their antioxidant and photocatalytic properties. Iran J Sci Technol Trans Sci 2018; 42 :547-555.
10. Etebu E, Nwauzoma AB, Bawo DDS. Postharvest spoilage of tomato (*Lycopersiconesculentum* Mill.) and control strategies in Nigeria. J Biol Agric Health care 2013; 3(10):34-45.
11. Farag-Hanaa RM, Abdou ZA, Salama DA, Ibrahim MAR, Srour H. Effect of neem and willow aqueous extracts on fusarium wilt disease in tomato seedlings: Induction of antioxidant defensive enzymes. Ann Agric Sci 2011; 56:1-7.
12. Fawole MO, Oso BA. Laboratory manual of microbiology: Revised Edition Spectrum Books Ltd Ibadan 2001; 1-127.
13. Herlekar M, Barve M, Kumar R. Biological synthesis of iron oxide nanoparticles using agro-waste and feasibility for municipal waste water treatment 2015.
14. Holt JG, Krieg RN, Sneath PHA. Bergey's Manual of Determinative Bacteriology. 9th Edition, USA. Willias and Wilkins Publishers, Baltimore 2000; 787.
15. Igiehon E, Shittu HO, Eremwanrue AO, Ojiagbe RE, Momoh MO, Agbontian MA. In vitro evaluation of Magnesium oxide nanoparticles for the control of Fusarium wilt disease of tomato (*Solanum lycopersicum* L). Niger J Mycol 2020; 12(2):26–33.
16. Jones DA, Atanzariti A, Lim GTT. The tomato I-3 gene: A novel gene for resistance to Fusarium wilt disease. New Phytol 2015; 207:106-118.
17. Khaleghi E, Arzani K, Moallemi N, Barzegar M. Evaluation of Chlorophyll Content and Chlorophyll Fluorescence Parameters and Relationships between Chlorophyll a, b and Chlorophyll Content Index under Water Stress in *Olea europaea* cv. Dezful. World Acad Sci Eng Technol 2012; 68:115 4–1164.
18. Khan I, Saceed K, Khan I. Nanoparticles: Properties, applications and toxicities. Arab J Chem 2017. doi: 10.1016/j. arabjc.2017.05.011
19. Koka JA, Wani AH, Bhat MY. Evaluation of antifungal activity of Magnesium oxide (MgO) and Iron oxide (FeO) nanoparticles on rot causing fungi. J Drug Deliv Ther 2019; 9 (2):173-178.
20. Michaelsen A, Pinzari F, Ripka K, Lubitz W, Pinar G. Application of molecular techniques for identification of fungal communities colonizing paper material. Int Biodeterior Biodegrad 2006; 58:33-141.
21. Mirzaei H, Davoodnia A. Microwave assisted sol-gel synthesis of MgO nanoparticles and their catalytic activity in the synthesis of hantzsch 1, 4- dihydropyridines. Chinese J Catal 2012; 33:1502-1507.
22. Oloyede AR, Ayedun EO, Sonde OI, Akinduti PA. Investigation of antifungal activity of green-synthesized silver nanoparticles on phytopathogenic fungi. Niger J Microbiol 2016; 30(1):3323-3328.
23. Prasad M, Kumar V, Prasad KS. Nanotechnology in sustainable agriculture: Present concerns and future aspects. Afr J Biotechnol 2014; 13(6):705-713.
24. Prihatna C, Barbetti MJ, Barker SJ. A novel tomato Fusarium wilt tolerance gene. Front Microbiol 2018; 9:1226-1233.
25. Ramanujam K, Sundrarajan M. Antibacterial effects of biosynthesized MgO nanoparticles using ethanolic fruit

extract of *Emblica officinalis*. J Photochem Photobiol 2014; 141: 296-300.
26. Rathore I, Tarafdar JC. Perspective of biosynthesized magnesium nanoparticles in foliar application of wheat plant. J Bio nano sci 2015; 9:1-6.
27. Saranya S, Vijayarani K, Pavithra S. Green synthesis of iron nanoparticles using aqueous extract of *musa ornate* flower shealth against pathogenic bacterial. Indian J Pharm Sci 2017; 79(5):688-694.
28. Scrinis G, Lyons K. The emerging nano corporate paradigm nanotechnology and the transformation of nature, food and agrifood systems. Int J Sociol Agric Food 2007; 15(2):22-44.
29. Shah VB, Henson WR, Chadha TZ, Lakin CT, Liu H, Blankenship RE. Linked-free deposition and adhesion of photosystem1 into nanostructures TiO_2 for biohybrid photoelectrochemical cells. Langmuir 2015; 31:1675-1682.
30. Sharma G, Soni R, Jasuja DN. Phyto-assisted synthesis of magnesium oxide nanoparticles with *Swertia chirayaita*. J Taibah Univ Sci 2017a; 11:471-477.
31. Sharma P, Sharma A, Shelle N, Sharma M, Estrela P, Jain A, Thakur P, Thakur A. Nanomaterial fungicides in vitro and in vivo antimycotic activity of cobalt and nickel nano ferrites on phytopathogenic fungi. Glob Chall 2017b; 1:1-7.
32. Shittu HO. Basic Molecular Techniques and Applications. In: Biological Techniques and Applications. Okhuoya JA, Okungbowa FI, Shittu HO (Editors). University of Benin Press, Nigeria 2012; 180– 210.
33. Srinivas C, Devi DN, Murthy KN, Mohan CDM, Lakshmeesha TR, Singh B, Kalagatur NK, Niranjana SR, Hashem A, Alqarawi AA, Tabassum B, Abdallah EF, Nayaka SC. *Fusarium oxysporum* f. sp. *lycopersici* causal agent of vascular wilt disease of tomato: Biology to diversity- A review. Saudi J Biol Sci 2019. doi: 10.1016/j.sjbs.2019.06.002
34. Surega R, Ramakrishnan S, Anita B, Gunasekaran S, Nakkeeran S. Antifungal effect of plant mediated silver nanoparticles against *Fusarium oxysporum f sp lycopersici.* Int J Trop Agric 2015; 33:157–160.
35. Vergheese M, Vishal SK. Green synthesis of magnesium oxide nanoparticles using *Trigonella graecum* leaf extract and its antibacterial activity. J Pharmacogn Phytochem 2017; 7(3):119 3-1200.
36. Watanabe T. Pictorial Atlas of Soil and Seed Fungi: Morphologies of Cultured Fungi and Key to Species (2nd Edition). CRC Press LLC 2002; 789.
37. Weeraratne WAPG, De Costa1 DM. Molecular Identification of *Fusarium* spp. from Wilt-infected Tomato and Brinjal Plants in Selected Regions of Sri Lanka and Endophytic Bacteria as a Potential Option for Disease Management. Trop Agric Res 2018; 30(1):32–43.
38. Zhang Z, Qi H, Jiang Z, Zhang K, Yang S, He F. PlAD: A transcriptomics database for plant defense responses to pathogens, providing new insights into plant immune system. Genom Proteom Bioinform 2018; 16:283-293.
39. Zhen-Xing T, Bin-Feng LV. MgO nanoparticles as antibacterial agent: Preparation and activity. Braz J Chem Eng 2014; 31(3):5 91–601.