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ORIGINAL PAPER

Effect of Green Biosynthesized Silver Nanoparticles Using *Cleome amblyocarpa* on Controlling Chickpea Wilt

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ABSTRACT

The antifungal activity of green synthesized silver nanoparticles (AgNPs) from *Cleome amblyocarpa* was investigated against the phytopathogenic fungus *Fusarium oxysporum* (MW485609) the cause of chickpea wilt. Experimental results showed that the growth of *F. oxysporum* started to reach 50 % inhibition at 80 μg/mL of AgNPs and also with 150 μg/mL plant extract. The highest reduction % on the mycelial growth was 60.4 ± 0.00 and 67.4±1.16mm with plant extract and green synthesized nanoparticles, respectively. Data also revealed that the most effective concentration of green AgNPs solution was, 200 µl/mL, which showed 5.18 % and 9.79 % early and late wilt incidence. On the other hand, early and late wilt incidence recorded 11.24 % and 16 % due to plant extract. Meanwhile, plant survival rates were 85.03 % and 72.76 %, respectively, whereas the untreated control plants recorded only 4.85 % survivals. Images proved that the green synthesized silver nanoparticles affected the morphology of fungal hyphae grown on media supplemented with (AgNPs) solution and nanoparticles appeared in fungal cell walls compared with the effect of plant extract and with fungal hypha of control plates. Moreover, observations with TEM and SEM revealed that synthesized nanoparticles damaged fungal hyphae, causing the deformation of cell membranes and inhibition of the normal budding process. The solution of AgNPs illustrates good stability at -19.8 mV at an area of 100 %, a width of 6.75 mV. The size of AgNPs ranged from 6.06 to 40.9 nm; Mean = 20.088 nm, Dev (rms) = 7.2 nm. This research demonstrates that (AgNPs) can be employed as a safe and environmentally acceptable alternative in controlling pathogenic fungi, and limits dependence on fungicides and avoids the development of fungicide-resistant phytopathogenic generations. The green synthesis of nanoparticles with the help of *C. amblyocarpa* was considered a practicable and environmentally friendly way.

Keywords: Chickpea, *Cicer arietinum*, *Fusarium oxysporum*, *Cleome amblyocarpa*, silver nanoparticles, AgNPs, Antifungal activity.

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INTRODUCTION

Cleome amblyocarpa is an herbaceous plant of the Cleomaceae family (Abd-El-Gawad *et al*., 2021and Khlifi *et al*., 2021). It is widely growing in desert sandy habitats of Sinai, Egypt, and many parts of North Africa (Kamel *et al*., 2010). This herb possesses different biological activities, containing anti-inflammatory, anti-COVID-19, genotoxicity, antidiabetic, antioxidant, and antimicrobial effects (Takhi *et al*., 2011; Edziri *et al*., 2013; Zaki *et al*., 2020; Khlifi *et al*., 2021 and Seglab *et al*., 2021;).

Recently, scientists have been more concerned with developing new systems that maintain environmental reservation and increase agrarian production (Hanson *et al*., 2008; Rouphael and Colla, 2020). After the green revolution (Evenson and Gollin, 2003 and Pingali, 2012), the world has faced tremendous issues that need to be solved. One of these issues is the use of many forms of pesticides that are very harmful to the environmental components and led to the extinction of several biological species (Zari, 2014). With the advent of the third millennium, new agricultural production systems were adopted (FAO, 2017), it depends on creating dynamic agrarian processes, practices, and principles to combine all of the agricultural elements in one objective to meet the environmental aims in an essential framework.

Nanotechnology has appeared as a fascinating branch of science worldwide (Bayda *et al*., 2020 and El Gamal *et al*., 2022 a and b). which is expected to generate new applications in biotechnology, medicine, pharmacology, food, and agriculture (Sastry *et al*., 2010; Oves

et al., 2013, Aziz *et al*., 2015; Elagamey *et al*., 2022 and El Gamal *et al*., 2022 a and b).

Recently, scientists have been searching for a biologically safe, ecologically friendly, and cost-effective alternative (Misiha *et al*. 2019 and El-Mslmany *et al*. 2020). Synthesis of nanoparticles from natural sources such as microbes and plants has been developed as an alternate solution; the nanoparticles are synthesized by different mechanical, physical, and chemical methods (Akhtar *et al*., 2013; Duan *et al*., 2015 and Roy *et al*., 2019). Plant extracts can be used as both reducing and stabilizing agents to facilitate the synthesis of nanoparticles. In addition, the activity of antimicrobial effect has led researchers to search for the synthesis and application of AgNPs according to NPs smaller size and big surface area, silver nanoparticles are among the most vital and exhibit strong antimicrobial activity against a variety of pathogens such as fungi, bacteria, and viruses (Elagamey *et al*., 2022).

Chickpea (*Cicer arietinum* L.) is a high-value seed legume crop worldwide (F.F.A, 2020). Unfortunately, this pulse crop can be attacked by several serious pathogens, which can cause significant damage to the production and productivity of the chickpea (Pande *et al*., 2005 and Mawad *et al*., 2021). *Fusarium oxysporum* is one of the main pathogens of chickpeas which can have significant negative effects on the growth and cause yield loss from 10 to 100% depending on varietal susceptibility and climatic conditions (Ashour *et al*., 2006 and Mazen and Ibrahim, 2021).

The disease causes damping-off, root rot and/or stem rot and wilt diseases, Early wilting causes more loss than late wilting. Nevertheless, the seeds from late-wilted plants are lighter, rough and dull than those from healthy plants (Haware and Yl, 1980 and Ashour *et al*., 2006). Fusarium wilt of chickpeas is a difficult disease to treat because the pathogen blocks the plant's vascular tissues. It also causes significant losses in many chickpeas-growing regions around the world (Ashour *et al*., 2006; Mazen and Ibrahim, 2021 and Mawad *et al*., 2021).

Fungicides are among the main methods used to control this important pathogen. These chemical fungicides have several harmful effects on the environment as well as on humans (Steinberg and Gurr, 2020). Therefore, scientists have started looking at natural and safe alternatives to chemical fungicides. Several reports have investigated the probability of employing nanoparticles as a fungicide (Bahrami-Teimoori *et al*., 2017; Roy *et al*., 2019; Ghojavand *et al*., 2020; Jebril *et al*., 2020 and El Gamal *et al*. 2022a). AgNPs are recognized for their antimicrobial properties. However, there have been few studies on AgNPs' ability to control *Fusarium oxysporum* as one of the most important plant pathogens that spread globally.

Green synthesis of nanoparticles is safer and eco-friendlier than chemical synthesized nanoparticles. Green biosynthesis of silver nanoparticles by bacteria, fungi, and plants was reported by several researchers (Quester *et al*., 2016; Rasheed *et al*., 2017; El-Saadony *et al*., 2019; Yu *et al*., 2019; El-Wakil, 2020 and Mahmoud, 2021).

SEM and TEM were demonstrated to clarify nanoparticle shape, agglomeration, and dimension calculation, particularly to understand the difference between different silver nanoparticles (Mourdikoudis *et al*., 2018). Plant extracts are effective for the green synthesis of AgNPs, (Lakshmi *et al*.; 2011 and Lakshmanan *et al*., 2018). UV-vis spectroscopy is very helpful for the initial characterization of produced nanoparticles. UV-vis spectroscopy is quick, easy, and requires only a short period of measurement. According to many studies, 200-800nm wavelength was used for the characterization of nanoparticles in the size range of 2-100 nm. The absorption of AgNPs depends on the size of the particles, and the chemical surrounding medium. A strong absorption peak of the solution containing reduced silver nitrate in nano-size prepared from the extract of the plant appears at the wavelength range between 390 and 420 nm when silver nanoparticles compared with the control did not show this peak at this wavelength range (Lakshmi *et al*., 2011; El-Wakil, 2020 and Amin *et al*., 2021).

In this study, silver nanoparticles have been green synthesized using *Cleome amblyocarpa* plant extract that is growing in the North Sinai deserts, Egypt. to study the antimicrobial activity of AgNPs green synthesized against *Fusarium oxysporum*. The effectiveness of the prepared nanoparticles on the growth of *F. oxysporum* was determined.

MATERIALS AND METHODS

Samples and Fungal Isolation:

Naturally infected chickpea plants showing wilt symptoms were collected from the Experimental Farm of the Faculty of Environmental Agricultural Sciences in Arish, North Sinai. The affected plant's roots were

thoroughly washed with tap water to remove adherent soil particles. The roots were sliced into small pieces (0.5-1 cm), and surface sterilized by soaking in sodium hypochlorite solution 0.5 %, for two minutes, then passed through sterilized distilled water and dried between two Whatman No.1 filter papers and transformed on Potato Dextrose Agar medium (PDA) at a rate of 4 or 5 pieces per plate and carefully closed with parafilm before incubation at 28±2°C and checked daily for two weeks (Radhakrishnan *et al*., 2017). The hyphal tip procedure was used to purify any fungus that emerged. (Singh, 1983). The developed fungi were identified according to their morphological, cultural and microscopical characteristics in the Mycology Laboratory of the Botany Department, Faculty of Science, Arish University, Egypt. The morphological characterization was confirmed by DNA analysis using ABT DNA mini extraction kit according to manufacturer instructions. PCR reaction was performed according to White *et al*. (1990). The rDNA was sequenced using two primers: with two primers ITS1 (TCC GTA GGT GAA CCT GCG G) and ITS4 (TCC TCC GCT TAT TGA TAT GC). Gel electrophoresis was done using the amplified PCR products submitted to Solgent Co. Ltd (South Korea) for gel purification and sequencing. The sequence data were analyzed and compared with other organisms belonging to the genus Fusarium in the GenBank database by the BLAST tool on the National Center for Biotechnology Information Web site, http://www.BLAST.com.

Pathogenicity test of *F. oxysporum* isolate:

Four agar discs (0.8 cm in diameter) from a pure culture of *F. oxysporum* were transferred to 250 mL sterilized potato dextrose broth in a conical flask for preparation of spore suspension 5×10^5/mL. Flasks were incubated for 7 days at the rotary shaker (125 rpm) at room temperature 25±1. Giza-1 cultivar seeds were planted in autoclaved soil. The soil was potted in 30 cm sterilized plastic pots and seeded with 10 seeds/pot. Infected plants were observed for 25 days after soil infestation for early wilt and 60 days after soil infestation for late wilt (Pande *et al*., 2007). Three replicates were undertaken for each treatment. Disease Severity was determined according to Trapero and Jiménez (1985).

Collection and preparation of *Cleome amblyocarpa* extract:

The fresh plant extract of *Cleome amblyocarpa* was prepared following the method described earlier by Khlifi *et al*. (2021) with slight modifications. Figure (1A) shows wild fresh plant material collected from Al-Arish region, North Sinai, Egypt, and was identified as *Cleome amblyocarpa* according to (Aparadh *et al*, 2012 and Rassouli *et al*, 2014). The aboveground plant parts were excised and washed several times with running tap water to remove all dirt and debris from the plants before rinsing with deionized water. After that, plant tissues were cut into fine pieces and then oven-dried separately in shade at 40°C for 48 hr. (Figure, 1B). Coarsely, Plant parts were ground to powder using the grinder (Figure, 1C). Ten grams were weighed in a beaker and dipped into a 250 mL flask containing 100 mL 70% ethanol for 10 min, then filtered by Whatman No.1., filter paper and should be kept refrigerated at 4°C until used.

Green biosynthesis of AgNPs:

Preparation of 1mM of the silver nitrate solution (weight 0.1699 g of $AgNO_3$. The $AgNO_3$ amount will be diluted in 1L distilled water to get 1mM $AgNO_3$ solution). The AgNPs solution was described by Van Dong *et al*. (2012). Briefly, 1mM silver nitrate solution was prepared and utilized for the green synthesis of AgNPs, the add 5mL of prepared plant extract and mix slowly on a magnetic stirrer with 25 mL of prepared 1 mM $AgNO_3$ in an Erlenmeyer flask at room temperature under dark conditions for 48 hrs. the Color changes of the solution were observed. AgNPs were produced by centrifugation at 10,000 rpm for 10 minutes (Figure, 1C), followed by carefully washing with distilled water and then storing in a dark controlled room under 4ºC (Nguyen *et al*., 2020).

Characterization of AgNPs obtained from *C. ambluocarba* plant extract:

Green synthesized of AgNPs were characterized with a UV visible spectrophotometer (UV-VIS Spectrophotometer, Spectro UV-2505, LABOMED, INC) in the range of 300 to 700 (Specord, Germa 200 plus, Germany). Distilled water was used as a control. Incubation of AgNPs of plant extracts *C. amblyocarpa* for 72 hr. on a rotary shaker at 150 rpm and 28°C in dark. By adding 5mL of *C. amblyocarpa* to each 25mL of 1m M $AgNO_3$. Absorption of UV for 1M $AgNO_3$ was recorded at different intervals for up to 24 h between 300 nm to 700 nm, (Lakshmi *et al*., 2011).

The size and shape of particles were determined with a TEM (Philips CM-10 Electron Microscope) at Al-Azhar University, Egypt). Sample solutions in distilled water were prepared on carbon-coated copper grids and

allowed water to evaporate at room temperature for a photo with TEM. were obtained using aJEOL GEM-1010 TEM, at an accelerating voltage of 80 Kv. (Amin *et al*., 2021).

The stability of green synthesized AgNPs was examined with Zeta potential (performed in a Nanotechnology and Advanced Materials Central Lab.), Agriculture Research Center (Lakshmanan *et al*., 2018).

Antifungal activity of plant extract and AgNPs of *Cleome amblyocarpa in vitro*:

Studying the antifungal activity of plant extract and AgNPs of *Cleome amblyocarpa* against *Fusarium oxysporum* was performed according to Mazen and Ibrahim (2021). A mycelial disc of 0.8 cm of *Fusarium oxysporum* was placed directly in the center of solidified agar medium. A pre-calculated amount of AgNPs was added to PDA to obtain a final concentration (5%), with PDA media receiving deionized water (the control treatment). Petri plates were incubated at 25±2°C until mycelial growth reached the plate's edge in control plates. Seven days old cultures were used for antifungal analysis. Three replicates were used per each treatment. The inhibition of fungal growth indicated the antifungal activity. Selected concentrations (10, 20, 40, 80, 150 and 200 µg/mL) of green synthesized AgNPs solution, and plant extract /mL of medium were added onto the sterile filter paper disks and spread evenly over. Simultaneously, the positive control treatment was treated with commercial fungicide mancozeb (0.2%) at their standard concentration. The negative control was treated only with tap water (0%). The diameter of the fungal colony on PDA plates for treatments containing different concentrations from plant extract and AgNPs was measured along with the control. After incubation, the diameters of the mycelial growth of *F. oxysporum* and control treatment were measured. The inhibition (%) rate of the mycelial growth of different plates was calculated according to the following formula:

$$\textbf{Inhibition \%} = [(R-r)/R]) \times 100$$

Where:

R= is the radial growth for control plates and

r= is the radial growth for treatment plates.

The MIC (minimum inhibitory concentration) of green synthesized AgNPs was calculated using the method described by Thakur *et al*. (2017). MIC value represented the lowest AgNPs concentration that showed a 50% inhibition of mycelial growth.

Studying the Effect of green synthesized plant extract and AgNPs of *C. amblyocarpa* on *F. oxysporum* mycelium by TEM and SEM:

TEM and SEM were used to image the effect of plant extract and green synthesized AgNPs (10µl/mL of medium) of *C. amblyocarpa* on the mycelium of *F. oxysporum*, leading to the specimen's surface image being created. The detector gathers backscattered and secondary electrons to produce images. changes in cell shape and the presence of nanoparticle perforations in the cell walls are indications of the antimicrobial activity of nanoparticles. SEM images are comparing treated cell wall with control which illustrates damaged structures and silver nanoparticles in treated cells. The drop of nanoparticle suspension was dropped on clean electric stubs (Lakshmi *et al*., 2011). TEM and SEM (JEM_2100 HR electron microscope made in Japan, in a National Research Center (NRC), Electron Microscope Unit, preparation was performed for three samples (control, plant extract and AgNPs treatments) for seven-day old cultures. A valuable and significant technique for characterizing nanoparticles is TEM which provides the size and morphology of nanoparticles. TEM uses a strong vacuum and a thin sample section to provide the best photos for the characterization of nanoparticles. A drop of nanoparticle suspension was prepared to place on carbon-coated copper grid (Lakshmi *et al*., 2011).

Statistical analysis:

The results were subjected to a two-way ANOVA for Mean ± SD (n = 3) and significant differences between the means of the treatments were assessed using Duncan's Multiple Range Test ($P \leq 0.05$) (Gomez and Gomez, 1984) using SPSS software (SPSS version 16.0, SPSS Inc. Chicago, IL, USA).

RESULTS AND DISCUSSION

Isolation and identification of the associated fungi:

White cottony with septate hyaline mycelium, with oval to ellipsoid micro-conidia and curved septate macroconidium (4-6 septum) with chlamydospores, the reverse of the colony with pale purple color. According to the morphological and microscopical characteristics, the isolated fungus was identified as *Fusarium oxysporum* Schlecht., as stated by Booth (1985). Moreover, the identification of fungus was confirmed by molecular identification. The tested isolate has a

100% similarity with *F. oxysporum*, and it was recorded on Gen Bank with accession number MW485609.

Pathogenicity test of the *Fusarium oxysporum* isolate:

Fifteen days after sowing of the chickpea cultivar (Giza-1) began to exhibit wilt symptoms. After 45 days of sowing under greenhouse conditions, most chickpea seedlings showed wilt symptoms when compared with the control.

Biosynthesis of AgNPs:

Figure (1A and B) illustrates the branches of fresh and dry *C. amblyocarpa* plant with flowers, leaves, horns, and powdered lateral branches (Figure, 1C). After 24 h of incubation and moderate stirring at room temperature, the color of 1mM $AgNO_3$ solution reaction mixture changes from colorless to reddish-brown, with an increasing concentration of plant extract (Figure ,1D). Our outcomes are similar to those recorded Balavijayalakshmi and Ramalakshmi (2017) who previously described that the mixture of $AgNO_3$ and plant extract caused a color change in performance. These results are in agreement with earlier reports (Blois, 1958; Ayoola *et al*., 2008; Akharaiyi, 2011; Sekar *et al*., 2012; Vadlapudi and Kaladhar, 2012 and Santhanakrishnan *et al*., 2014). These compounds may have a synergistic effect on soil-borne fungi. (Abad *et al*., 2007 and Al-Otibi *et al*., 2021). Moreover, the $AgNO_3$ solution and plant extract under the same experimental conditions showed no color change.

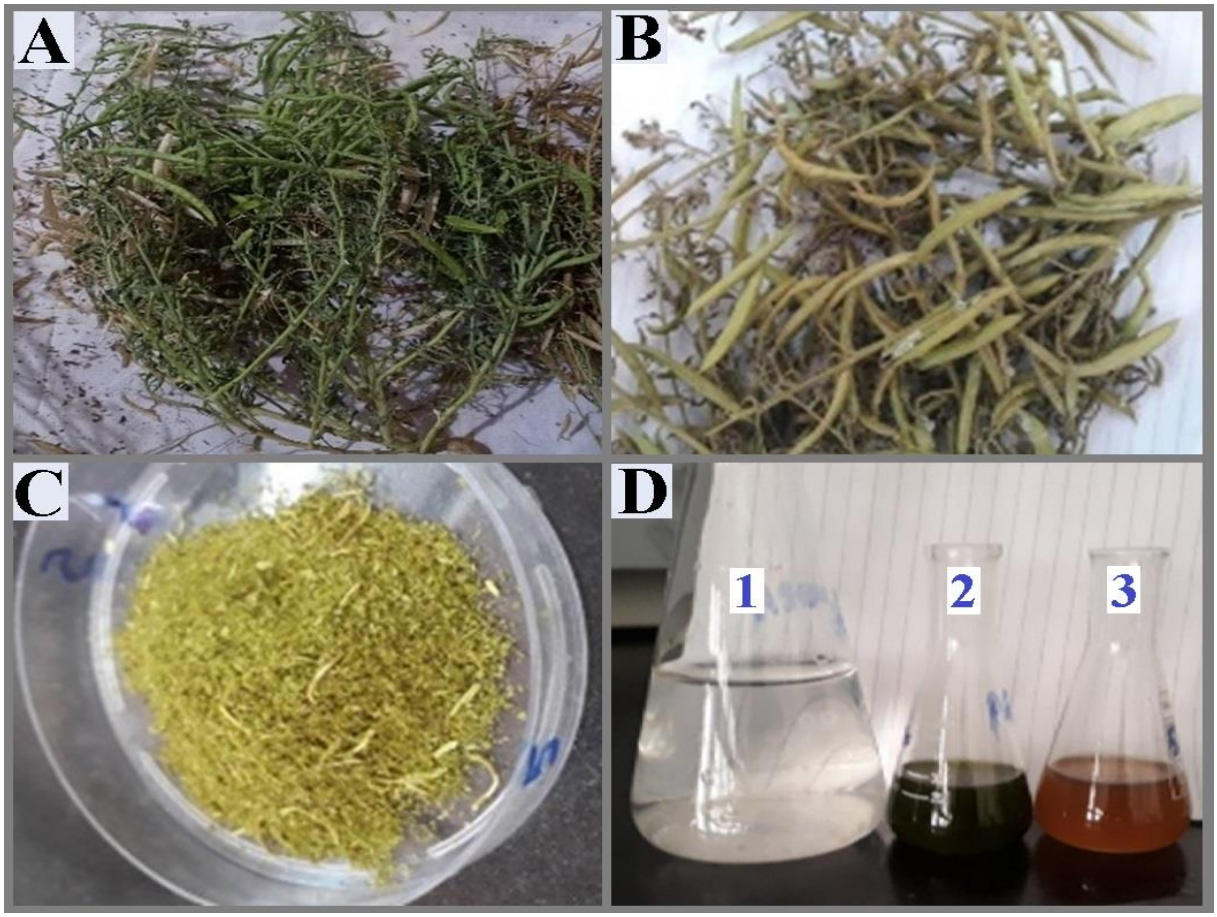

Figure (1 A & B): Fresh and dry branches of *Cleome amblyocarpa*, C) powder of C. *amblyocarpa*, and D) Color changes in the *C. amblyocarpa* extract (1) after addition of 1 mM aqueous solution of $AgNO_3$ (2) for green synthesized nanoparticles solution. (3) formed from the *C. amblyocarpa* extract.

Antifungal activity of plant extract and AgNPs:

In this study, plant extract of *Cleome amblyocarpa* efficiently inhibited *F. oxysporum* mycelial growth at various concentrations and AgNPs compared with the control. Concentrations of the two treatments resulted in different degrees of inhibition for the growth of *F. oxysporum*. The inhibition (%) was increased along with the increase in the concentration of AgNPs. Experimental results showed that the growth of the tested *F. oxysporum* isolate hyphae started to reach 50 % inhibition at 80 μg/mL of AgNPs and with 150 μg/mL for plant extract. Also, we found that the fungal growth inhibition increased with rising concentrations

of the solution, which had obvious effects on inhibition efficiency. (Figure, 2 and Table, 1) clearly shows the percentages of inhibition being, 17.4±1.16, 24.4±1.16, 39.9±0.67, 45.3±1.16, 49.2±0.67, 54.2±0.67 and 60.4±0.00 for 10, 20, 40, 80, 100, 150, and 200 µl/10mL plant extract of *C. amblyocarpa* in PDA media, respectively. The presence of 150 µl was the optimal concentration for the tested *F. oxysporum* isolate which inhibited and yielded 54.2±0.67; when the concentration was increased to 200 µl/10mL of PDA the obtained growth reached 60.4 ±0.00. Comparing the mancozeb treatment was used, it appears that the fungicide significantly reduced growth by 63.9±1.63 µl/10mL, in addition, the untreated control was no effective.

Also, data of the present study indicated that the mycelium inhibition (%) for green AgNPs solution attained 21.7±0.67, 33.7±1.16, 43.4±0.67, 54.6±1.16, 60.4±0.0, 63.5±0.67 and 67.4±1.16 for 10, 20, 40, 80, 100, 150, and 200 µl/10mL of PDA media, respectively, while extended increasing the concentration could reduce inhibition efficiency. As compared to the antifungal activity, fungicide mancozeb reduced the growth to 70.15±1.17 (Fig. 2 and Table, 1).

The results indicated that the optimal concentration for achieving the start transient inhibition was 80 µl/10mL of PDA media with 54.6±1.16 (Figure, 2 and Table, 1).

The sensitivity of *F. oxysporum* isolate to AgNPs was greater than plant extract of *C. amblyocarpa* at various concentrations. The highest inhibitory activity was observed against *F. oxysporum* isolate in the case of AgNPs at 200 µg/mL, followed by 150 µg/mL concentration.

Based on -two-way ANOVA, all treatments showed that there were significant differences among concentrations, meanwhile, there are significant differences between plant extract and its nanoparticles synthesized, respectively. Meanwhile, there is significant differences between plant extract and its nanoparticles synthesized ($P \leq$ 6.62566E-07 and 0.002, respectively). Multiple Duncan test was used to differentiate among all treatments. Means with the same letter are not significantly different (Table, 1).

The increase was gradually determined by the biochemical properties of plant extracts as well as the concentration used for AgNP synthesis. (Balavijayalakshmi and Ramalakshmi, 2017; Kumar *et al*., 2017 and Palei, 2020).

F. oxysporum growth decreased with 10 µg/mL and 20 µg/mL doses of plant extract showed an increase in the inhibition (%) of fungal growth. This could be related to an increase in silver nanoparticle concentration in the reaction mixture. As the concentrations of plant extracts were increased to 40 µg/mL and 80 mL, the diameter of fungal growth found for the *F. oxysporum* shrank. The antifungal activity of silver nanoparticles is confirmed to be highly dependent on the concentration of silver nanoparticles in the reaction mixture. The findings of this study are consistent with previous studies (Gurunathan *et al*., 2015 and Ansari and Alzohairy, 2018). Our research indicates the potential benefits of using AgNPs as a fungicide, which is effective at a lower dose and more stable to the fungus than chemical fungicides, as demonstrated by in vitro plate assay and microscopic studies. As a result, green technology is a promising alternative to traditional fungicides. in addition, all these observations have supported the antifungal effects of AgNPs synthesized in our study.

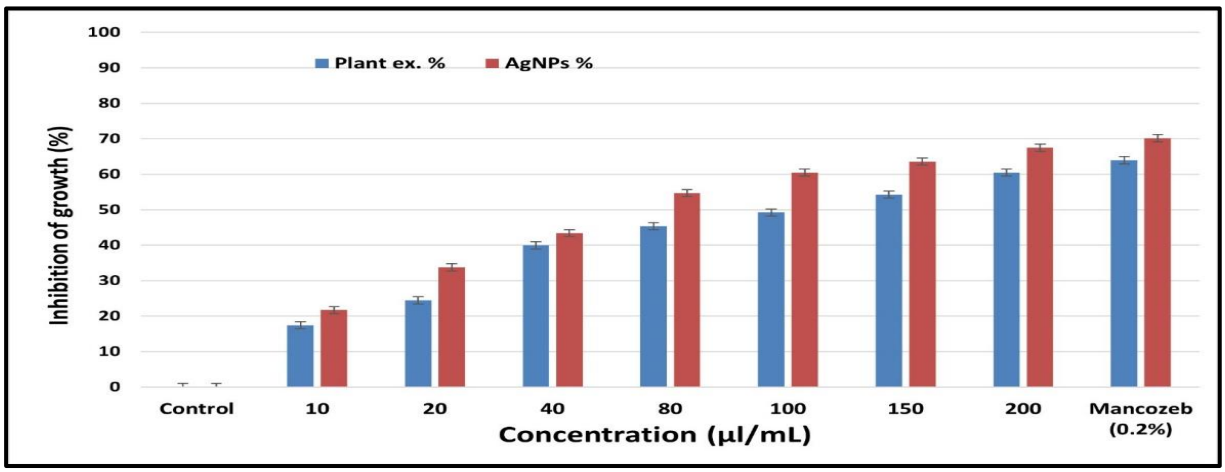

Figure (2): *In vitro* inhibitory effect of plant extract and green synthesized silver nanoparticles (AgNPs) solutions of *Cleome amblyocarpa* extract against *Fusarium oxysporum* at different concentrations.

Table (1): Anti-fungal activity of bio synthesized green silver nanoparticles compared with the effect of plant extract of *Cleome amblyocarpa* against *Fusarium oxysporum* mycelial growth.

Concentrations µl/mL	Treatments			
	Green AgNPs	Inhibition %	Plant extract	Inhibition %
10	6.73 k	21.7±0.67	7.10 l	17.4±1.16
20	5.70 i	33.7±1.16	6.50 j	24.4±1.16
40	4.87 g	43.4±0.67	5.17 h	39.9±0.67
80	3.90 d	54.6±1.16	4.70 f	45.3±1.16
100	3.40 c	60.4±0.00	4.37 e	49.2±0.67
150	3.13 b	63.5±0.67	3.93 d	54.2±0.67
200	2.80 a	67.4±1.16	3.40 c	60.4±0.00
Mancozeb (0.2%)	2.00 a	70.1±1.17	2.93 a	63.9±1.63
Control	8.60 m	-	8.60 m	-

The values in the table above were the average of three replicates for % of inhibition of growth of fungus following by "±" was standard error. Values were significantly different ($P \leq 0.05$) using Duncan's Multiple Range Test of two-way ANOVA.

Effect of AgNPs on the morphology of *Fusarium oxysporum* under TEM and SEM:

The cell wall of hyphae plays an important role in the protection of the cell, determining the cell shape, transferring nutrients to the cells, as the cell wall allows interaction with the external environment since some of its proteins are adhesins and receptors, whereas any effect on their structures affects their characters. TEM for hyphae of *F. oxysporum*, which is grown on medium treated with 10 µg/mL of medium AgNPs, illustrates the accumulation of AgNPs on the surface of hyphae (Figure, 3).

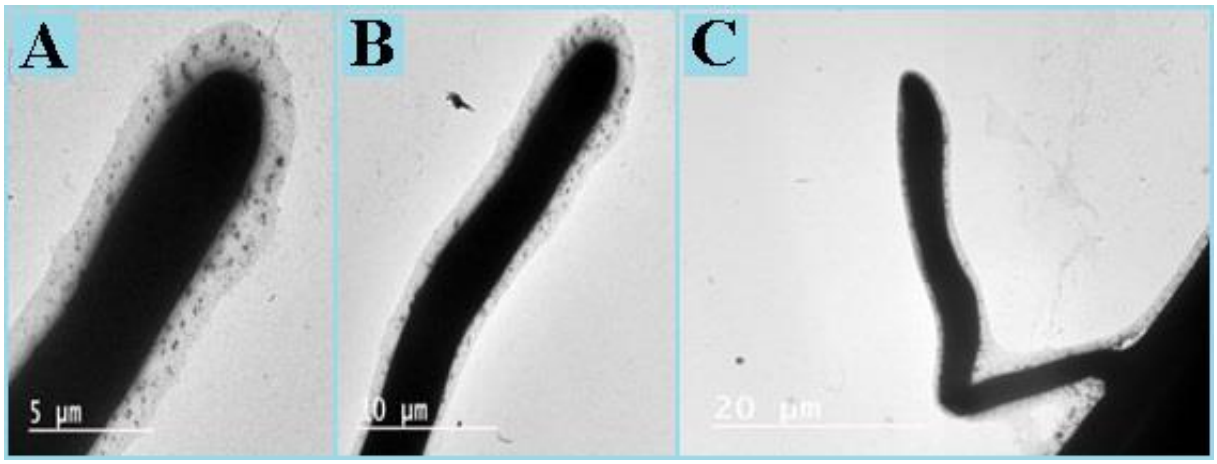

Figure (3): TEM of mycelium of *Fusarium oxysporum* treated with green synthesized AgNPs of *Cleome amblyocarpa* plant extract. illustrate the accumulation nanoparticles in outer layer of hyphal mycelium (A, B and C).

Nanoparticles with a smaller size penetrate cells more effectively due to the greater surface area available for interaction and interfering with cell metabolism. The antimicrobial property of silver nanoparticles is determined by the nanoparticle size (Morones *et al*., 2005) Thus the negative charge of the cell surface can easily interact with Ag^{+}, preventing them from functioning (Gopinath *et al*., 2008). Because of their antimicrobial properties, AgNPs are now employed in wound dressing (Tian *et al*., 2007). According to reports, several parameters such as biological species, material proportion, the solution pH, reaction time, and so on influenced the shape, dispersity, and size of nanoparticles (Jeyaraj *et al*., 2013 and Rao *et al*., 2017). We found similar results in terms of particle size, demonstrating the effectiveness of *C. amblyocarpa* extracts as a reducing agent in the green synthesis of silver nanoparticles (Figure, 4). Furthermore, the good dispersibility in *C. amblyocarpa* extract could be explained by a

layer of organic material surrounding the hyphae and its effect on the cell wall structure. By comparing hyphae grown on a medium containing plant extract with control hyphae, hyphae become lighter, as shown in TEM images for control and for hypha grown on media containing plant extract. On the other hand, scanning electron microscopy (SEM) analysis provided the morphology and shape details of silver nanoparticles. Fungal hypha in the presence of nanoparticles shows high-density AgNPs green synthesized by the plant extract of *C. amblyocarpa*, confirming the presence of AgNPs on the surface of hyphae. For SEM photos, the control hypha and conidia appear clear with normal form without shrinkages or chlamydospores observed and high sporulation.

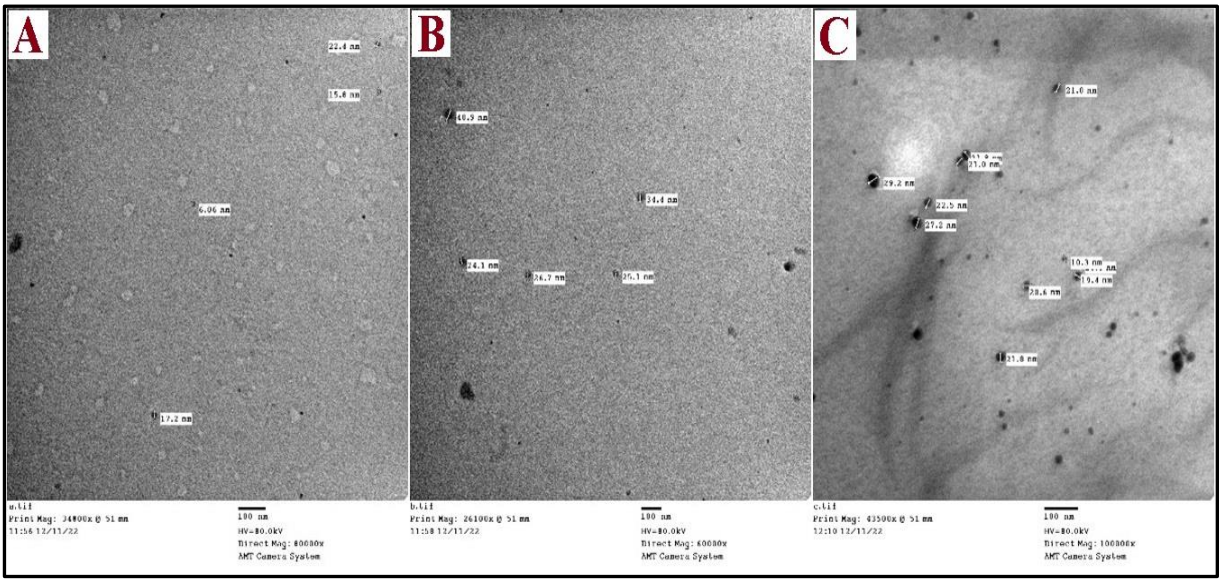

Figure (4): TEM photos illustrate the size of green synthesis of silver nanoparticles (AgNPs) prepared from *Cleome amblyocarpa* plant extract (A, B and C).

SEM illustrates the change in normal morphology of hyphae of the fungus when the fungus was grown on media supplemented with AgNPs solution. This is in agreement with the work of Mohamed (2015) who suggested that the antimicrobial activities of AgNPs could be due to the disruption of the membrane lipids of the fungal cell wall. Pits and cavities may form on the surface of the fungal cell wall leading to its deterioration.

The particles dispersed well on the cell wall. The high density of accumulated AgNPs on the cell wall of *F. oxysporum* is illustrated in (Figure, 5). In addition, SEM illustrates, the hyphae shrank irregularly and deformation when the fungus grew on a medium containing plant extract and AgNPs of *C. amblyocarpa* (Figures, 6 and 7).

The treated mycelium with plant extract has irregular shrinkages, and high chlamydospores formation was observed. For the mycelium treated with AgNPs, the hyphae were affected, with a rough surface, clear shrinkage, and deformation. Moreover, it revealed a rough area on the hyphae's surface, which could be the source of the bioactive chemicals. Based on the morphology of the AgNPs, (Figure, 6) the smooth surface shape of the produced AgNPs is proof of their great stability, and it also explains the mild absorptions observed.

The potential antifungal activity of AgNPs against *F. oxysporum* was confirmed by MIC testing. Silver nanoparticles have a MIC of 50 µg/mL (Figure, 6). There was low inhibition observed at low concentrations. However, growth was strongly decreased at 150 µg /mL or higher when compared to the control. Since green AgNPs were synthesized using *C. amblyocarpa* extract, which has been shown to possess little or no antimicrobial activity (Dakal *et al*., 2016). Our results exhibited strong antibiotic activity against *F. oxysporum* at a concentration of 50 mg/mL. This may be due to the solvent used to extract plant metabolites. (Al-Bayati and Sulaiman, 2008 and El-Mahmood *et al*., 2008). AgNPs were observed to be more potent antimicrobials than crude plant extracts and can be used effectively in pharmaceutical, biotechnological, and biomedical applications. Another possible explanation for nanoparticles' antimicrobial effect is the positive charge of silver ions, which causes electrostatic attraction between silver nanoparticles (positive charge) and negatively charged microorganism cell membranes, causing the microbe to die.

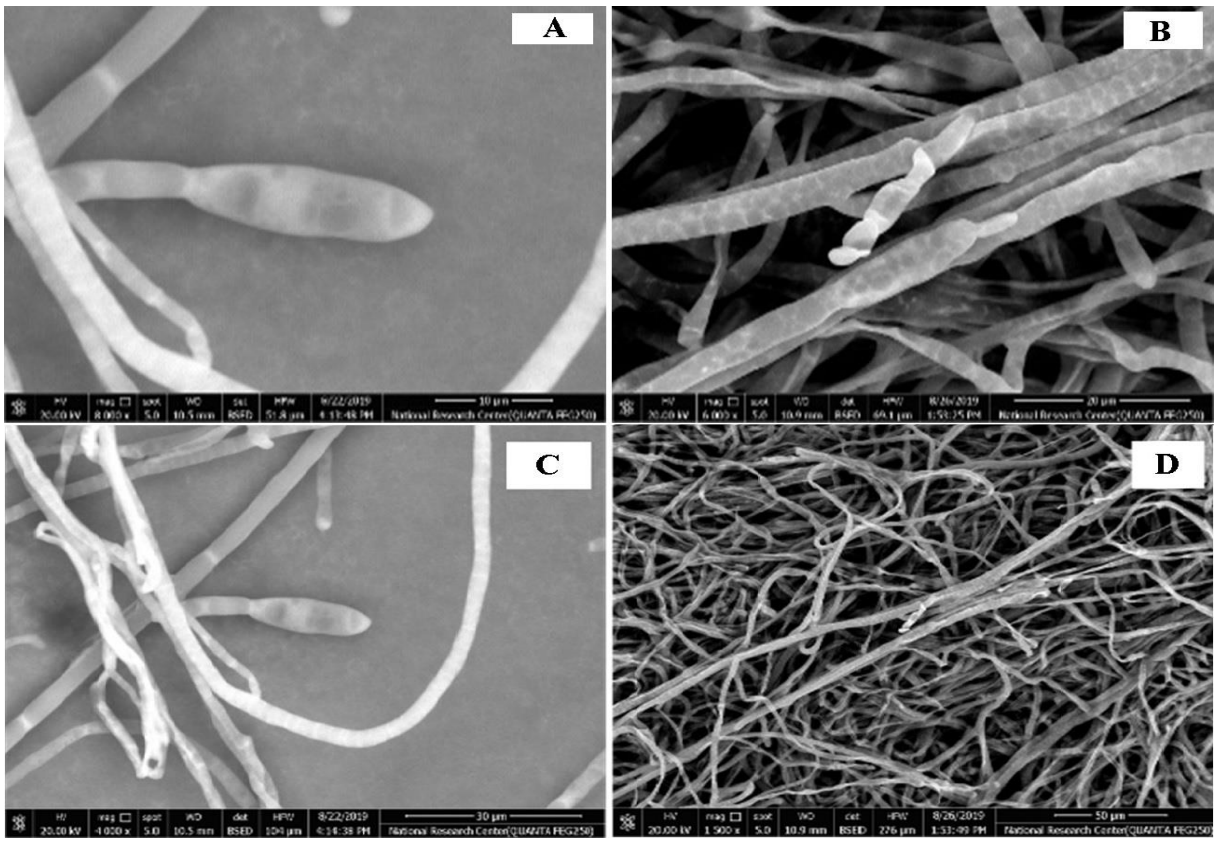

Figure (5): SEM for untreated mycelium of *Fusarium oxysporum*, A, B, C and D illustrates normal septate mycelium, conidiophores carrying macroconidia (A, B, and C), lower number of chlamydospores (D).

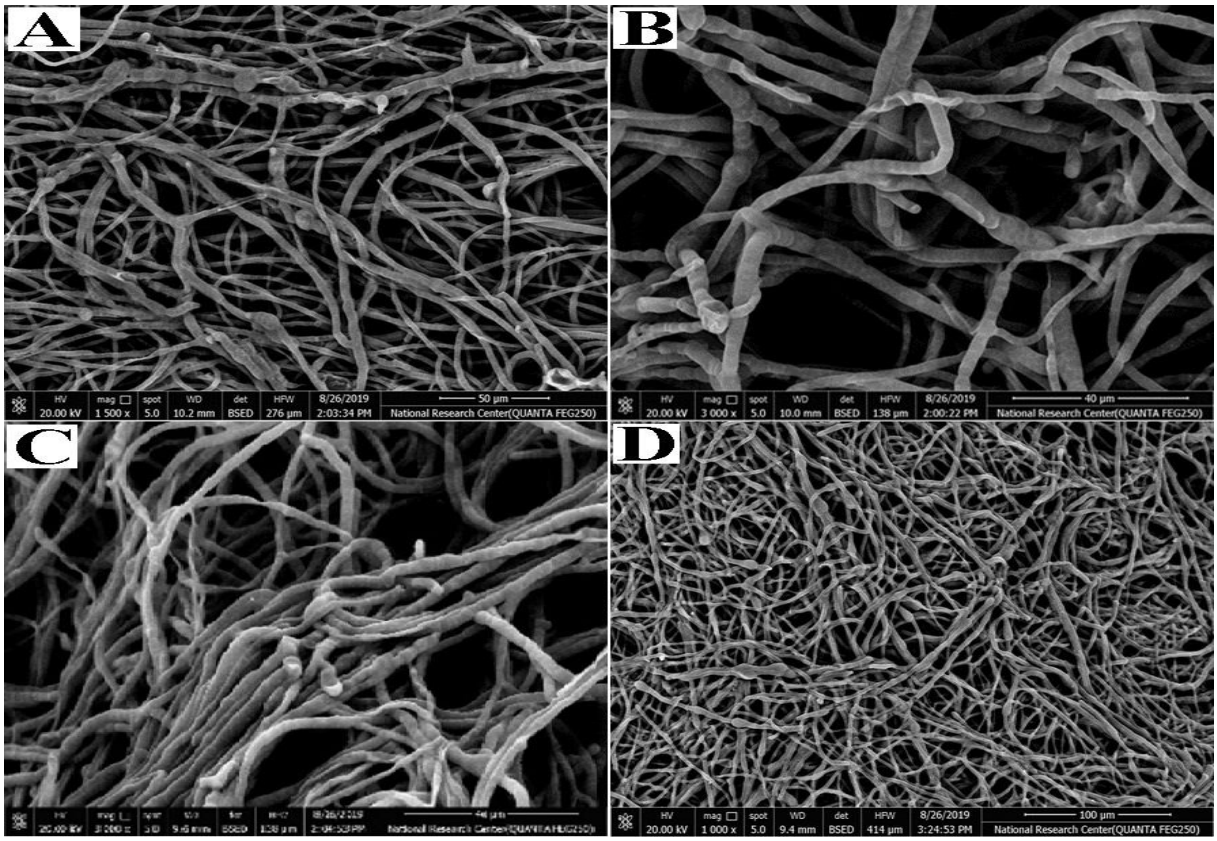

Figure (6): SEM of mycelium of *Fusarium oxysporum* treated with plant extract of *Cleome amblyocarpa.* illustrates appearance of high number of chlamydospores at × 40, 50 and 100 µm (A, B, C and D), shrinking, broken and deformation of mycelium (A, B and C).

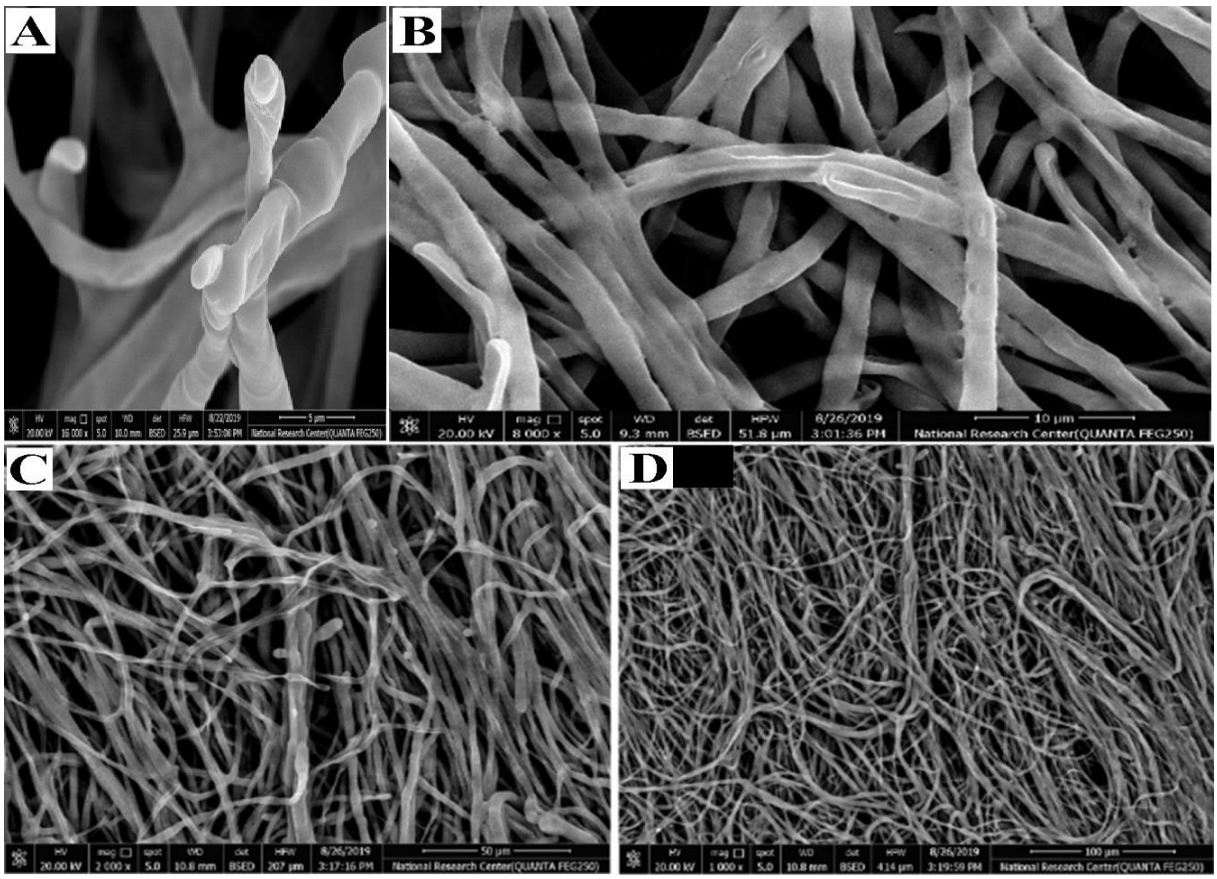

Figure (7): SEM of mycelium of *Fusarium oxysporum* treated with green synthesized AgNPs of *Cleome amblyocarpa* plant extract. illustrates broking and shrinking and deformation of hyphae; A, B and C: × 5, 10, and 50µm, appearance of chlamydospores at ×50 and 100µm (C and E).

Characterization of AgNPs obtained from *Cleome* plant extract:

The AgNPs obtained from Cleome plant extract illustrated reduction for silver ion as a result of a reaction between plant extract and 1mM $AgNo_3$. UV visible spectrophotometer reading for green synthesized of AgNPs from *C. amblyocarpa* plant extract illustrated the maximum absorption (the peak of the curve) (Figure, 8). This result can improve the plant extract of *C. amblyocarpa* containing reducing power for silver nitrate to silver in the size of nanoparticles, (Lakshmanan, *et al*. 2018). Furthermore, the UV-Vis absorption spectrum of AgNPs at the experimental conditions revealed a peak at 400 nm after incubation for 72 hr. at 28°C on a rotary shaker at 150 rpm. which confirms the formation of AgNPs.

Examination of the film of drop-coated AgNPs of *C. amblyocarpa* plant extract with the TEM illustrates the size of nanoparticles in the range from 10 nm to 17 nm and the shape of particles was 10, (Figure 3, A, B and C). The size range of particles is in a range of nanoparticles, (Lakshmi *et al*. 2011 and Lakshmanan *et al*., 2018).

Zeta deviation of the AgNPs. The stability of the nanoparticle's solution is -19.8 mV, at 100 Area (%) and 6.75 mV width. The negative charge of nanoparticles is good stability and may be due to the coting of secondary metabolites from plant extract, Figure (9), (Lakshmanan, *et al*. 2018). The results indicate an ideal surface charge of the formed AgNPs. Moreover, the high absolute and negative value of zeta potential revealed a high electrical charge on the AgNPs surface, which can cause a strong repulsive force among the particles to prevent agglomeration and hence might be responsible for their high stability, (Lakshmi *et al*., (2011). The molecular characterization can also be used to phylogenetically relate fungi based on their morphological characteristics. The current findings show that molecular identification of organisms has high specificity and sensitivity and can be used to classify microorganisms at the taxonomic level. The information obtained in such studies is critical in resolving taxonomic problems of the genera that will be studied using molecular phylogenetics and revealing the position of fungi at the molecular level.

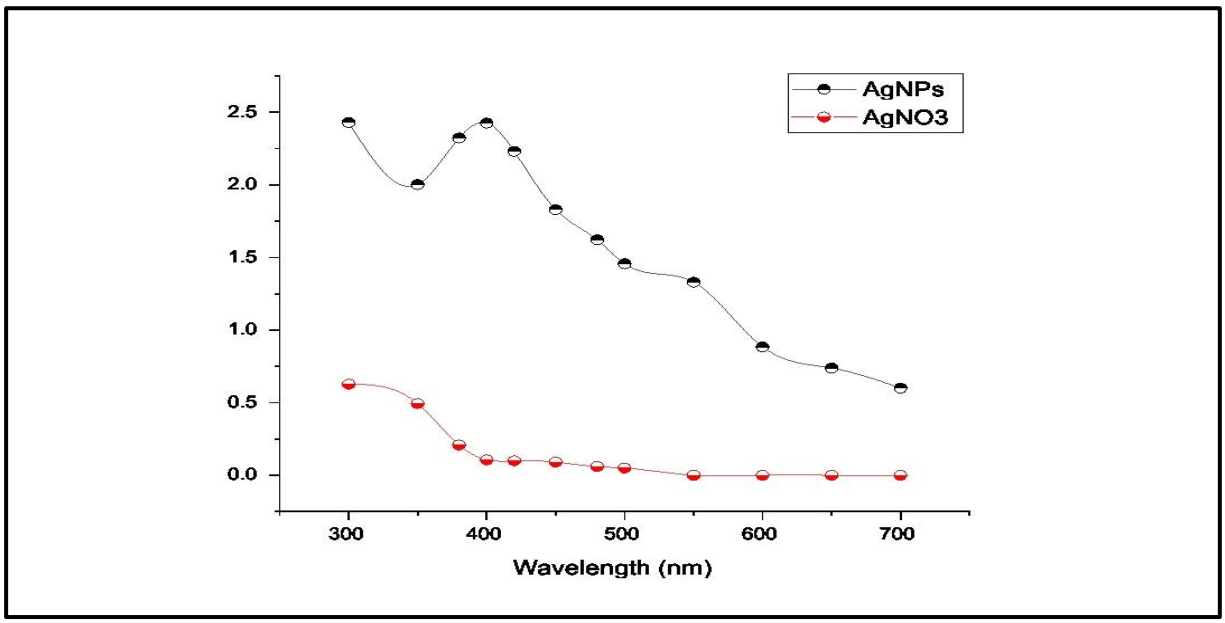

Figure (8): UV absorption of green synthesis of AgNPs from *Cleome amblyocarpa* plant extract.

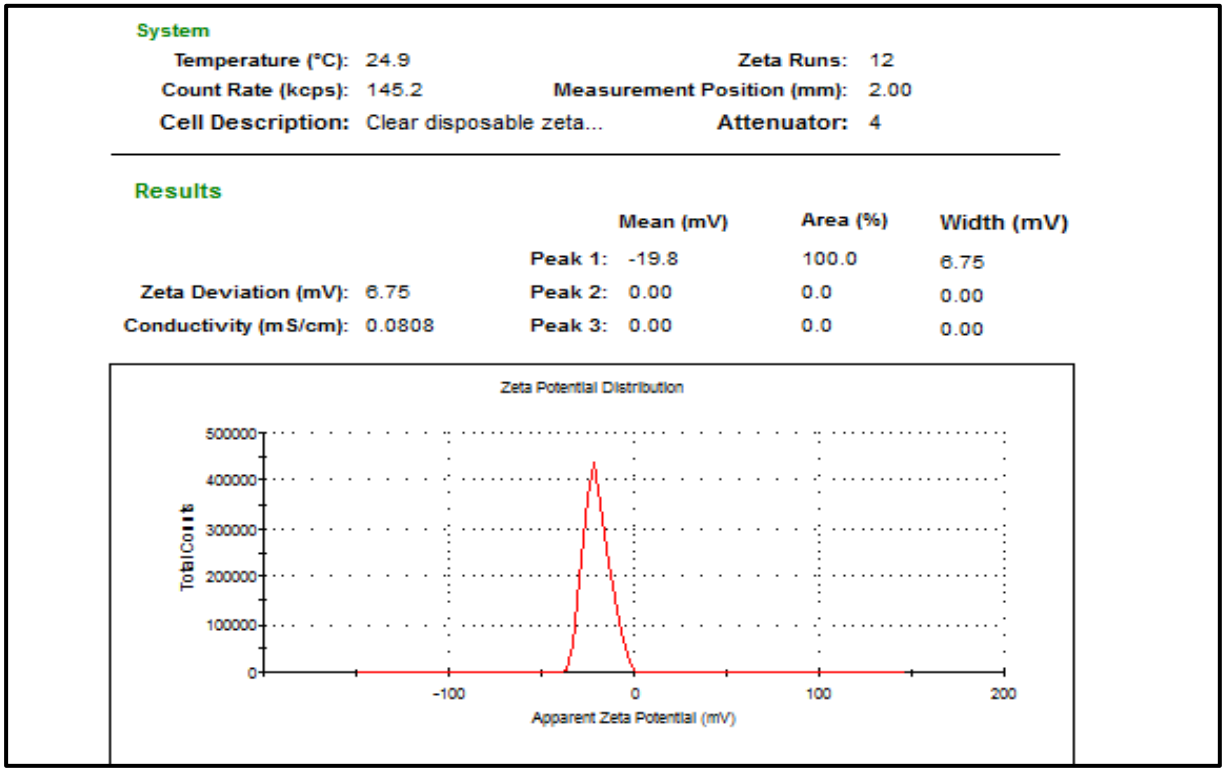

Figure (9). Zeta potential photo illustrates the stability of green synthesis of Ag nanoparticle prepared from *Cleome amblyocarpa* plant extract.

Effect of different concentrations on disease incidence under controlled environmental conditions

Data in Table (2) assess the chickpea cultivar Giza-1 against the highly pathogenic isolate of *F. oxysporum* (MW485609) under controlled environmental conditions. Results show that the isolate was pathogenic to the tested cultivar under different levels of green AgNPs solution and plant extract. All concentrations significantly ($P \leq 0.05$) reduced symptoms on chickpea plants when compared with the control. The data also revealed that the most effective concentration of green AgNPs solution was, particularly green AgNPs solution with 200 µl/mL, which showed 5.18 and 9.79 early and late wilt, respectively. On the other hand, early and late wilt recorded 11.24 and 16 with Plant Extract. The survival rates were 85.03 and 72.76, respectively, whereas the untreated plants recorded only 4.85% plant survival. These results are compatible with the results recorded by El-Wakil, 2020 and Mazen and Ibrahim, 2021.

Table (2): Effect of different concentrations of the green synthesized silver nanoparticles and plant extract of *Cleome amblyocarpa* on chickpea wilt disease incidence under controlled environmental conditions.

Treatments	Concentration µl/mL	Early wilt (%)	Late wilt (%)	Plant survival (%)
Green AgNPs	10	36.13	44.09	19.78
	20	27.69	38.29	34.02
	40	21.67	33.31	45.02
	80	19.52	26.47	54.01
	100	12.12	21.24	66.64
	150	9.87	13.41	76.72
	200	5.18	9.79	85.03
	Mean	18.88	26.66	54.46
Mancozeb (0.2%)	-	4.92	7.98	87.10
Control	-	43.44	51.71	4.85
LSD at 0.05	-	3.11	4.26	13.46
Plant extract	10	39.62	47.29	13.09
	20	26.1	44.31	29.59
	40	22.64	39.84	37.52
	80	18.56	34.68	46.76
	100	16.26	28.73	55.01
	150	14.84	23.91	61.25
	200	11.24	16	72.76
	Mean	21.32	33.54	45.14
Mancozeb (0.2%)	-	9.88	14.25	75.87
Control	-	43.44	51.71	4.85
LSD at 0.05	-	3.15	4.11	20.58

CONCLUSION

The obtained data of the present study illustrated the benefits and effects of using greenly synthesized nanoparticles assisted by *C. amblyocarpa*. In addition, silver nanoparticles and a plant extract of *C. amblyocarpa* could be investigated for fungicide development. It can be an alternative approach to conventional fungicides in controlling *F. oxysporum* infection of the chickpea plants by wilt.

The findings of this study may be useful to other researchers in their investigations into how AgNPs and plant extracts can be utilized to protect plants and crops from phytopathogenic fungi that cause early and late wilt. Furthermore, Plant extracts and AgNPs have great antifungal potential, making them an excellent source for the development of new herbal medicine compounds that can help humanity. The findings of this study could also provide light on the ways through which the synthesis of nanoparticles employing plants through unmatched applications. This technique may be preferable to using biological entities because it avoids the time-consuming process of using microbes and maintaining their culture. It is hoped that the use of plant extract for the synthesis of nanoparticles will have a significant impact in the coming decades. The conjugation of silver nanoparticles with other control practices may enable us to construct an effective integrated management program for the control of some hard-to-eradicate diseases such as Fusarium wilt.

AUTHOR CONTRIBUTIONS

ElSharawy, A.A. and Mossa, M.I. conceived of the presented idea, planned the experiment, and supervised the project. Mossa, M.I. and ElSharawy, A.A. carried out the experiment. ElSharawy, A.A. and Mossa, M.I. verified the analytical methods. ElSharawy, A.A and Mossa, M.I. wrote the manuscript with support from Ibrahim, M.S.S. All authors discussed the results and contributed to the final manuscript.

CONFLICTS OF INTEREST

The author(s) declare no conflict of interest.

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