JOURNAL OF ADVANCEMENT IN MEDICAL AND LIFE SCIENCES
Journal homepage: http://scienceq.org/Journals/JALS.php
ISSN: 2348-294X V8I1.04
Research Article Open Access

Assessment of the *in-vitro* activity and *in-vivo* preventive efficacy of phyto-fabricated silver nanoparticles and silver nanoparticle/mancozeb combination against *Fusarium* wilt of *Lycopersicon esculentum* mill

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Received: August 22, 2020, Accepted: September 29, 2020, Published: September 29, 2020.

ABSTRACT:

The antifungal activity of phyto-fabricated silver nanoparticle (AgNP) alone and in combination with the fungicide, mancozeb, against *F. oxysporum* f. sp. *lycopersici* were investigated *in-vitro* and under field trial. Treatments were applied at different concentrations (5.0, 7.5, 10.0, 12.5 %) while media without the treatments served as control. AgNP alone demonstrated an insignificant mycelial radial growth inhibitory effect ($p > 0.05$) on the fungus compared to the control. The mean radii of mycelial growth inhibition for AgNP alone ranged from 24.67 ± 0.33 to 25.33 ± 1.20 mm while the control was 25.67 ± 0.33 mm. However, AgNP/Mancozeb (50 % v/v) demonstrated significant inhibitory activity ($p < 0.05$) on the fungus compared to the control with a marked percentage inhibition ranging from 51.00 to 74.98 %. The fungal flora and physico-chemical parameters of the experimental plot were analyzed before sowing and soil-drench method was used to introduce the treatments in the field study at different concentrations (25, 50 and 75 mg/mL) while plants inoculated with the pathogen alone served as control. After 7, 14 and 21 Days Post Inoculation (DPI), disease incidence for plants treated with AgNP alone and AgNP/Mancozeb was highest at 50 mg/mL (44.60 ± 5.40 % and 39.93 ± 6.64 % respectively) at 21 DPI compared to control (29.30 ± 4.00 %). This study has demonstrated the optimization of ineffective large-sized AgNP by pairing with mancozeb and maximizing their synergistic effect against *F. oxysporum* f. sp. *lycopercisi.*

Keyword: *Mancozeb, Nanoparticle, Phytopathogens, Phyto-fabricate, Characterization, Fusarium oxysporum,*

INTRODUCTION:

Lycopersicon esculentum Mill (tomato), which belongs to the family Solanaceae, is a tender, warm season crop. It is characterized by glandular hairs (trichomes) that emit strong aroma when broken. Nigeria is the 13th in the world and second largest producer of tomatoes in Africa after Egypt. However, its production is challenged by various constraints, among which are pests and diseases [1]. Tomato is susceptible to *Fusarium* wilt diseases caused by *Fusarium oxysporum* f. sp. *Lycopersici* [2]. According to the definition from National Nanotechnology Initiative (NNI), nanoparticles are structures of sizes ranging from 1 to 100 nm in at least one dimension [3]. Nanoparticles have a great potential in the management of plant diseases since silver displays multiple modes of inhibitory action to microorganisms [4,5]. This objective of this research was to assess the activities of AgNP alone and in combination with mancozeb (50 % v/v) against *Fusarium oxysporum* f. sp. *Lycopercisi*, the aetiologic agent of *Fusarium* wilt of tomatoes *in-vitro* and under field trial.

MATERIALS AND METHODS

Moringa oleifera leaves collection

Fresh leaves of *M. oleifera* were obtained from the Faculty of Agriculture and identified at the Department of Plant Biology and Biotechnology both in the University of Benin, Benin City, Nigeria.

Nursery preparation

L. esculentum seeds (Rio Grande Cultivar), were obtained commercially from a seed retail outlet in Benin City, Nigeria and sown on a seed tray containing sterile soil and kept in a shaded place and watered daily.

M. oleifera aqueous leaf extract

Ten grams (10 g) of the crushed leaves was mixed with 100 mL of hot water (100 ºC) and left to stand for 1 h according to the methods described by Prasad and Elumalai [6]. The extract was then filtered with a sterile cheese cloth and the filtrate was stored in a refrigerator (4 ºC) for further use.

Phyto-fabrication of silver nanoparticle (AgNP)

AgNP was phyto-fabricated in an Erlenmeyer flask by mixing 10 mL of *M. oleifera* extract with 90 mL of 1 mM silver nitrate ($AgNO_3$) solution. The mixture was stored for 48 h at room temperature and observed for characteristic colour change indicating the formation of nanoparticles [7,8,9].

Characterization of AgNP

In line with the methods of Emeka *et al.* [10] and Ponarulselvam *et al.* [11], an aliquot of the AgNP was analysed using a UV-VIS spectrophotometer (7215 series) with scanning range of 340-600 nm and distilled water as the blank reference. The maximum absorbance value was recorded.

Collection and Identification of isolate

Fusarium oxysporum f.sp. lycopersici was obtained from the Main laboratory, Department of Crop Science, Faculty of Agriculture, University of Benin, Benin City. The isolate was identified using cultural and microscopic features according to the methods of Barnett *and* Hunter [12].

Pathogenicity test

Four-week old tomato seedlings (Rio Grande cultivar) were transplanted into buckets containing sterile soil. Their roots were trimmed and agar plugs (5 mm) of the test isolate were inoculated into a 2-cm deep hole and the seedlings were re-planted in the same spot. Spore suspension containing approximately 1.58×10^5 spores/mL (measured with a haemacytometer) was also introduced around the plant's rhizosphere to increase infectivity. Plants inoculated with sterile water served as control. Plants were observed weekly for disease symptoms. The pathogen was subsequently re-isolated from the plant's stem to prove Koch's postulate [2,13].

Fungicide Solution

The solution of the commercially obtained protectant fungicide 1, 2-ethane-di-biscarbamo-di-thio 2-manganese-1, 2-ethane-di-biscarbamo –di-thio 2-zinc (Mancozeb), used in this study was prepared by dissolving 0.5 g in 200 mL of sterile water according to the manufacturer's instruction.

Antifungal assay

As described by Elgorban *et al* [14], Rauter *et al* [15] and Nejad *et al* [16], Five millimeter (5 mm) agar plugs from the margin of fresh *F. oxysporum f.sp. lycopersici* culture were transferred to the center of Potato Dextrose Agar (PDA) previously supplemented with different concentrations of AgNP/Mancozeb (50% v/v) and acidified with few drops of lactic acid. Media with distilled water and the fungus alone served as the control. Inoculated Petri dishes were incubated at 28 ± 2 ºC and the mycelial radial growth inhibition was measured daily for 5 days and was compared with growth in control plates. Treatments had three replicates and the mean values were determined. The percentage mycelial inhibition was determined on the 5th day according to the equation:

Percentage growth inhibition= R- r / r × 100

Where;

R- Linear growth of fungus on control (water) Petri dishes

r- Linear growth of fungus on Petri dishes with AgNP

***In-vivo* study**

Study area

The field study was carried out in Benin City, Edo State, Nigeria between May and July. Benin City lies between Latitude 6º 20" and 6º 58" North and between Longitude 5º 35" and 6º 41" East of the Greenwich Meridian. It has an estimated population of 3,206,531 (according to the 2006 Census) and an annual rainfall of over 2000 mm [17, 18].

Experimental design

Four (4) week-old *Lycopersicon esculentum* (Tomato) seedlings were transplanted to the experimental buckets. Plants were left to stabilize to the new soil and allow for ample root development for another 3 weeks after which 10 mL each; treatments and *F. oxysporum* spore suspension (1.58×10^5 Spores/mL), were introduced to each plant by the soil-drench method 1-week apart. Treatments were distributed into 7 groups in triplicates as follows:

a. *Fusarium oxysporum* f. sp. *lycopersici* (*Fo*L) alone.
b. *Fo*L and AgNP (25 mg/mL).
c. *Fo*L and AgNP (50 mg/mL).
d. *Fo*L and AgNP (75 mg/mL).
e. *Fo*L and AgNP-Mancozeb (25 mg/mL).
f. *Fo*L and AgNP-Mancozeb (50 mg/mL).
g. *Fo*L and AgNP-Mancozeb (75 mg/mL).

Disease incidence and Severity ratings were assessed at 7, 14 and 21 DPI (Days Post Inoculation), to assess the preventive efficacy of the treatments.

Nusery preparation

Lycopersicon esculentum (Tomato) seeds (Rio Grande Cultivar), were obtained commercially from a seed retail outlet in Benin City, Nigeria and were sown on a seed tray containing sterile loamy soil and kept in a shaded place and were watered daily.

Soil analysis

Soil samples from the buckets were randomly collected and transported to the laboratory for mycological and physico-chemical analysis.

Physico-chemical analysis of soil sample

Physico-chemical parameters such as pH, Organic Carbon, Organic Matter, Calcium, Magnesium, Sodium, Potassium, AvailablePhosphorus, Total Nitrogen, Exchangeable Acidity, Cation Exchangeable Capacity, Effective Cation Exchangeable Capacity, Sand, Silt and Clay were determined using the methods described by Eno *et al*. [19] and Ibitoye [20].

Mycological analysis of soil sample

Ten-fold serial dilution was carried out, by weighing 1 g of sample and dispensed into test tubes containing 9 mL of distilled water. The tube was shaken vigorously and 1 mL was aseptically pipetted into another test tube containing 9 mL. The sample was diluted serially up to10^{-3}. 0.1 mL aliquots of the diluents were transferred aseptically into labelled Petri dishes. This was followed by dispensing (pour plate method) freshly prepared molten Potato Dextrose Agar (PDA). The PDA plates were incubated at 28 ± 2 ^{0}C for 7 days. Pure fungal isolates were characterized on the basis of cultural and morphological characteristics [21, 12].

Disease incidence

Tomato wilt incidence, which is the number or proportion of wilted leaves in relation to the total number of leaves examined per plant, was determined at 7, 14 and 21 DPI (Days Post Inoculation) according to the formula proposed by Wokocha [22] and modified as follows:

$$\text{Disease Incidence} = \frac{\text{Number of wilted leaves per plant}}{\text{Total number of leaves per plant}} \times 100$$

Disease severity

Severity ratings of the disease was also estimated at 7, 14 and 21 DPI using a scale, 0-4 proposed by DaSilva and Bettiol [23] and modified as follows:

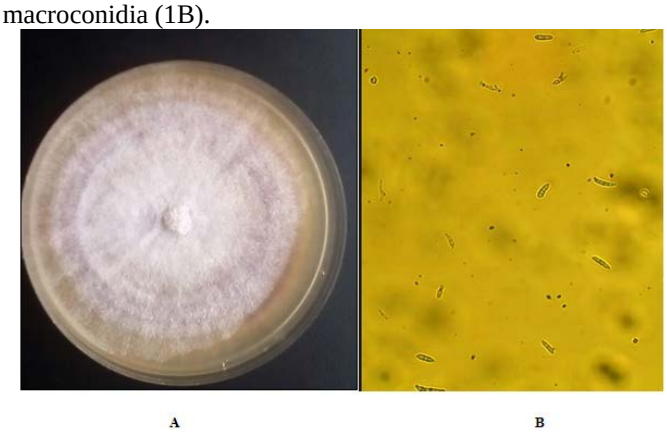
0- Completely healthy plants with no wilt sign
1- Less than 3 leaves showing wilt sign
2- From 3-5 leaves showing wilt sign
3- From 5-10 leaves showing wilt sign
4- Over 10 leaves showing signs of wilt, chlorosis or dead plants

Statistical analysis

The data generated were expressed as mean values and standard error of three (3) replications. They were then analysed with One-way Analysis of Variance (ANOVA) using SPSS version 20.0 software and mean values were differentiated with Duncan Multiple Range (DMR) test [24].

RESULTS

Cultural and microscopic characteristic of the isolate used in the study is shown in plate 1A and 1B. Colonies appeared white to pink and violet as the fungus grew older (1A). Microscopic examination revealed the fungal hyphae was septate and fungus produced sickle-shaped cream-colored macroconidia (1B).



A B

Plate 1: Cultural and microscopic characteristics of *Fusarium oxysporum* f. sp. *lycopersici* .

A. 7-day old pure culture of *F. oxysporum* f. sp. *lycopersici*
B. Photomicrograph of *F. oxysporum* f. sp. *lycopersici* spores used in this study.

The pathogenicity test showed that disease-free tomato seedlings inoculated with *Fusarium oxysporum* f. sp. *lycopersici* propagules showed typical signs of *Fusarium* wilt such as drooping of leaves, chlorosis and brown discoloration of xylem vessel from 16 Days Post Inoculation (DPI) and there

was progressive wilting up till 57 DPI (Plate 2A and 2B). The pathogen was re-isolated from the diseased tomato plant and identified to be the same as that inoculated, thus confirming Koch's postulate. A cross-section of the plant's stem also revealed a brownish discoloration.

Plate 2: Tomato plant used in this study showing typical *Fusarium* wilt signs (yellowing and initial drooping of lower leaves) at 16 Days Post Inoculation (A), progressive upward wilting and drooping of upper leaves at 57 Days Post Inoculation (B) and cross-section of the stem showing brownish discoloration of the xylem vessels (C).

A distinct color-change from light to dark brown indicating the phyto-fabrication of silver nanoparticle was observed after 48 h in the flask containing the mixture of $AgNO_3$ and *M. oleifera*. This was further characterized using Ultraviolet-Visible light spectrophotometry. The UV-VIS spectrum of biosynthesized AgNPs, maximum absorbance was recorded at 500 nm revealing that large-sized nanoparticles were fabricated in this study. Result is shown in Figure 1.

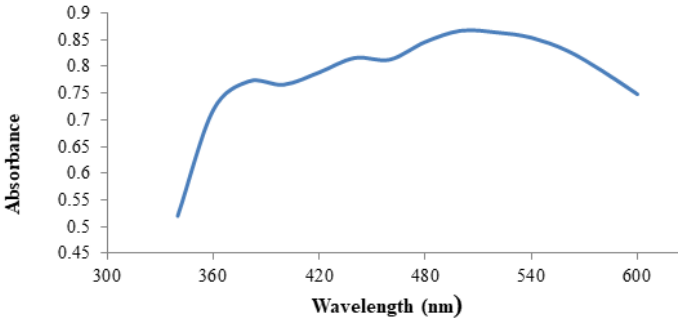


Figure 1: UV-VIS spectrum indicating silver nanoparticle formation at 500 nm after 48 h.

AgNP alone had no significant effect ($p > 0.05$) on the fungus at all tested concentrations compared to the combined treatment (AgNP/Mancozeb). The mycelial radial growth inhibition ranged from 24.67 ± 0.33 mm (10 %) to 25.33 ± 1.20 mm (5 %) similar to those of the controls as shown in Figure 2. The mycelial radial growth inhibition by AgNP/Mancozeb (50 % v/v) was concentration-dependent with maximum inhibition (14.67 ± 0.67 mm) at 12.5 % and minimum (16.67 ± 0.33 mm) at 5 % on day 5 as shown in Figure 3. The inhibitory effects were significantly different ($p < 0.05$) from the control (25.67 ± 0.33 mm).

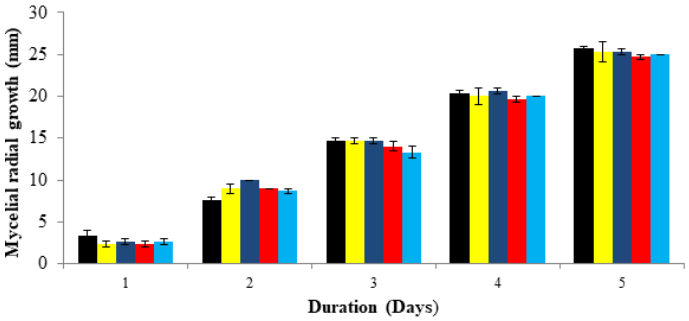


Figure 2: *In-vitro* antifungal activity of AgNp alone against *F. oxysporum* f.sp. *lycopersici*

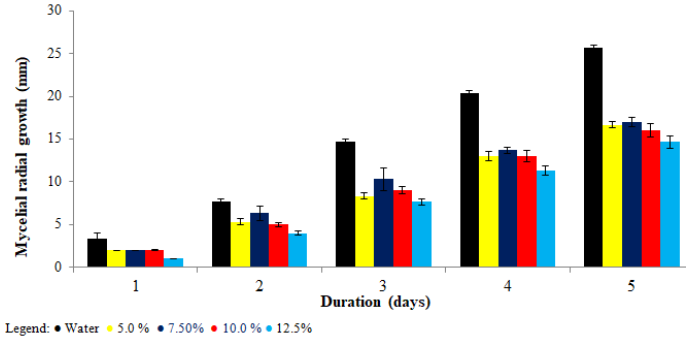


Figure 3: *In-vitro* antifungal activity of AgNP/Mancozeb against *F. oxysporum* f.sp. *lycopersici.*

The highest percentage growth inhibition was 74.98 % (AgNP/Mancozeb, 12.50 %) while the lowest was 1.34 % (AgNp alone, 5.00 %) as shown in Table 1.

Table 1. Percentage mycelial radial growth inhibition.

Treatments	5.00%	7.50%	10.00%	12.50%
AgNP/Mancozeb	53.99	51.00	60.44	74.98
AgNP alone	1.34	1.34	4.05	2.68

The mycological analysis revealed that the test pathogen was not part of the soil fungal flora before sowing. The fungi isolated from the experimental soil samples were *Mucor* sp., *Penicillium* sp., *Aspergillus niger*, *Rhizopus* sp. and *Aspergillus flavus*. The physico-chemical analyses (Table 2) showed that the soil was acidic (pH 4.77 ± 0.33) with variable levels of key parameters such Nitrogen (0.87 ± 0.34 mg/kg), Potassium (0.35 ± 0.03 mg/kg), Phosphorus (10.30 ± 0.12 mg/kg), Organic carbon (19.83 ± 0.73 g/kg), Organic matter (34.16 ± 1.23 g/kg). The acidic nature and nutrient constituent of the soil favored the proliferation of the wilt pathogen.

Table 2: Soil physico-chemical parameters.

Parameters	Values
pH	* 4.77 ± 0.03^{cd}
Organic carbon (g/kg)	19.83 ± 0.73^{f}
Organic matter (g/kg)	34.16 ± 1.23^{h}
Calcium (mg/kg)	3.63 ± 0.09^{bc}
Magnesium (mg/kg)	1.42 ± 0.04^{ab}
Sodium (mg/kg)	1.12 ± 0.09^{ab}
Potassium (mg/kg)	0.35 ± 0.03^{a}
Available phosphorus (mg/kg)	10.30 ± 0.12^{f}
Total Nitrogen (mg/kg)	0.87 ± 0.34^{ab}
Exchangeable Acidity (mg/kg)	1.08 ± 0.04^{ab}
Cation exchange capacity (mg/kg)	6.52 ± 0.01^{de}
Effective cation exchange capacity (mg/kg)	7.60 ± 0.04^{e}
Sand (g/kg)	888.33 ± 1.76^{k}
Silt (g/kg)	68.33 ± 2.03^{j}
Clay (g/kg)	43.33 ± 1.45^{i}

Legend: *Mean± Standard errors for all parameters were significant ($p < 0.05$) and the mean values differed statistically using Duncan multiple range test.

The Disease incidence increased progressively in all treated plants from 7 to 21 DPI (Days Post Inoculation) as shown in Table 3. The result showed that AgNP alone applied before the pathogen's inoculation significantly controlled the disease in the field at the lowest concentration (25 mg/ml). At 7 DPI, the plants showed no wilt sign. However, wilt sign became visible on all experimental plants from 14 DPI. Disease incidence was highest in plants treated with 50 mg/ml (44.60 ± 9.35 %) and lowest in those treated with 25 mg/ml (22.43 ± 6.44 %). These incidence ratings were however significantly different ($p < 0.05$) from the control (29.30 ± 2.30 %).

In the same vein, plants treated with AgNP/Mancozeb showed a similar pattern of disease incidence (Table 4). The highest disease incidence was 39.93 ± 13.05 % (50 mg/ml) while the lowest was 32.33 ± 13.05 %. They were significantly higher ($p < 0.05$) than the control (29.30 ± 2.30 %).

Table 3: Disease incidence (%) of plants treated with AgNP alone.

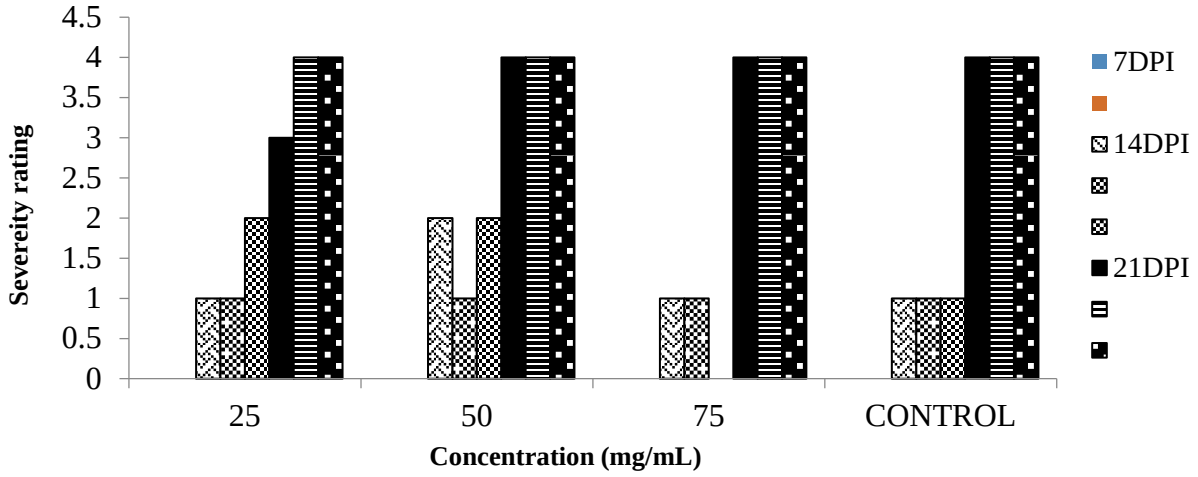
Concentration (mg/mL)	Disease incidence (%)		
	7 DPI	14 DPI	21 DPI
25	*0.00 ± 0.00^{a}	6.70 ± 0.51^{a}	22.43 ±6.44^{b}
50	0.00 ± 0.00^{a}	9.67 ± 3.41^{a}	44.60 ± 9.35^{b}
75	0.00 ± 0.00^{a}	4.60 ± 0.23^{b}	31.27 ± 1.71^{c}
$^{+}$Control	0.00 ± 0.00^{a}	7.33 ± 1.31^{b}	29.30 ± 2.30^{c}

Legend: * Mean ± Standard errors for all parameters were significant ($p < 0.05$) and the mean values differed statistically using Duncan multiple range test, ** Days Post Inoculation ,$^{+}$ Plants inoculated with pathogen alone.

Table 4: Disease incidence (%) of plants treated with AgNP/Mancozeb

Concentration (mg/mL)	Disease incidence (%)		
	**7 DPI	14 DPI	21 DPI
25	*0.00 ± 0.00[a]	9.30 ± 1.15[a]	32.33 ±13.05[b]
50	0.00 ± 0.00[a]	15.00 ± 3.65[a]	39.93 ± 6.64[b]
75	0.00 ± 0.00[a]	17.76 ± 10.06[ab]	36.50 ± 1.32[b]
[+]Control	0.00 ± 0.00[a]	7.33 ± 1.31[b]	29.30 ± 2.30[c]

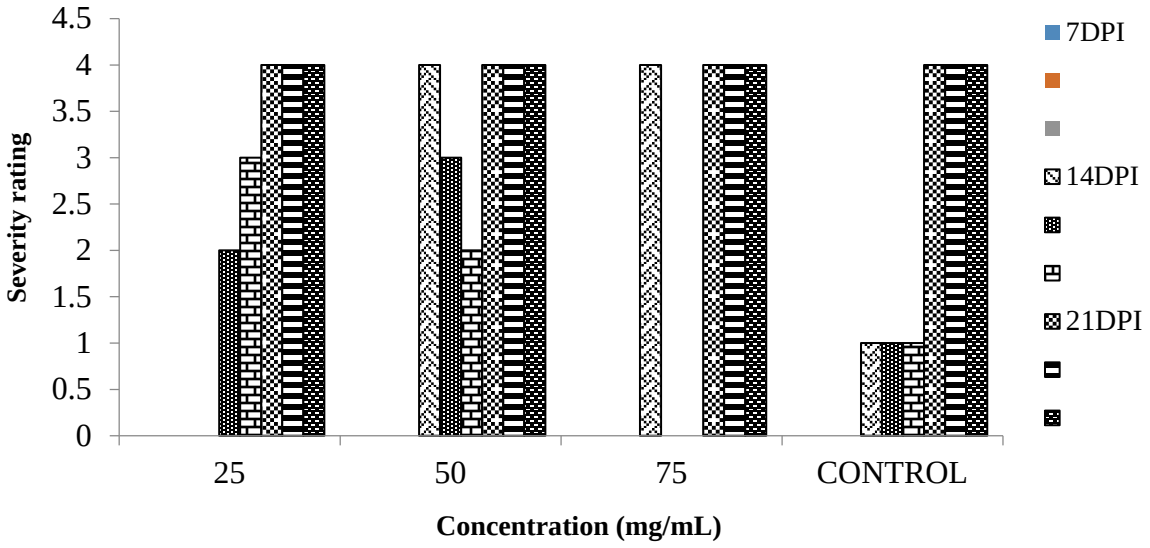
Legend: *Mean ± Standard errors for all parameters were significant ($p < 0.05$) and the mean values differed statistically using Duncan multiple range test, ** Days Post Inoculation , [+] Plants inoculated with pathogen alone.



SCALE:

5- Completely healthy plants with no wilt sign.
6- Less than 3 leaves showing wilt sign.
7- From 3-5 leaves showing wilt sign.
8- From 5-10 leaves showing wilt sign.
9- Over 10 leaves showing signs of wilt, chlorosis or dead plants.

Figure 4: Severity ratings (0 - 4) for plants treated with AgNP alone



Scale:

0- Completely healthy plants with no wilt sign.
1- Less than 3 leaves showing wilt sign.
2- From 3-5 leaves showing wilt sign.
3- From 5-10 leaves showing wilt sign.
4- Over 10 leaves showing signs of wilt, chlorosis or dead plants.

Figure 5: Severity ratings (0 - 4) for plants treated with AgNP/Mancozeb

DISCUSSION

The study revealed information on the development of a new class of fungicide with synergistic enhancement of its antifungal mechanism against plant pathogens. Most published literatures in this field of study lay emphasis on the effects of silver nanoparticles (AgNPs) on plant pathogenic bacteria, little information is however available about the effects on their fungi counterpart. The diverse activity of AgNP present a promising lethal effect against spore-producing fungi [25], thus making nanoparticles the antifungal agent of choice against a myriad of plant pathogens. The formation of AgNPs in this study which was confirmed after 48 h with a UV-VIS spectrophotometer, (maximum absorption peak of 500 nm) is in line with a previous report which revealed that metal nanoparticles were synthesized at peaks from 430 nm [26] due to the reduction of $AgNO_3$ by the plant phytochemicals which also serve as capping and stabilizing agents thus, preventing re-aggregation of nanoparticles [25]. A visible color-change (light to dark brown), indicating AgNP formation was also observed during the synthesis and this is similar to the reports by Logeswari *et al*. [27], Krishnaraj *et al* [28] and Noginov *et al* [29]. Also, due to the ease of diffusion relative to the larger-sized nanoparticle, they exhibited higher activity against microorganisms [30]. AgNP alone demonstrated low mycelial inhibitory action (*in-vitro*) in this study against *F. oxysporum* in line with the findings of Logeswari *et al* [27] who reported that AgNPs synthesized using the plant powders of *Syzygium cumini* and *Citrus asiatica*, 48 nm and 33 nm in size respectively, showed no antibacterial activity against *Klebsiella pneumoniae.* Furthermore, in this study, the large size of cold-synthesized AgNP, mutation and acquired resistance may be responsible for the low percentage mycelial inhibition against the isolate *in-vitro*. This is in agreement with the findings of Ahmed *et al* [31] who opined that the antimicrobial activities of AgNPs rest mainly on mainly on size, environmental conditions and capping agents used. In the same vein, Hassan *et al* [32] reported that *Garcinia kola*-synthesized AgNP showed no activity on *Rhizopus stolonifer* at all tested concentrations. Conversely, AgNP alone displayed mild activity against the pathogen in the field experiment. Noteworthy in this research is the fact that AgNP combined with mancozeb, showed a marked synergistic inhibitory effect (14.67 ± 0.67 mm) against *F. oxysporum* f. sp. *lycopersici* compared to the controls (24.67 ± 0.88 – 26.00 ± 0.33 mm). It is believed that the combination of mancozeb and AgNP may have resulted in the fragmentation of the large-sized AgNP by an unknown mechanism or may have synergistically attacked different target sites (cell organelles) in the fungus simultaneously leading to a greater mycelial inhibition. Furthermore, AgNP/Mancozeb complex may have demonstrated its enhanced inhibitory effect by denaturing fungal protein, destroying proton pump by binding to surface proteins thus increasing membrane permeability [33]. The complex could possibly trigger release of intracellular ions by disrupting the transport architecture and blocking respiration and metabolism [34]. The synergistic activity could also be a result of the binding of mancozeb molecules to AgNP functional groups, causing the nanoparticle to act as a vehicle, transporting. AgNP/Mancozeb exerted its inhibitory action against the *F. oxysporum* f. sp. *lycopercisi* in a concentration-dependent manner. Ahmed *et al* [31] reported similar findings when they tested the inhibitory activity of nickel nanoparticle against *F. oxysporum* f. sp. *lactucae* and *F. oxysporum* f. sp. *lycopercisi* with percentage inhibitions at 100 ppm as high as 60.23 and 59.77 respectively.

Ouda [35] also revealed that the effectiveness of the alloy silver/copper nanoparticles against *Alternaria alternata* and *Botrytis cinerea*. His results demonstrated that the combination of both metal nanoparticles had a marked inhibitory effect on both fungi. Also, in line with this study, copper nanoparticle alone had a weaker inhibitory action on the fungi compared to the alloy.

In line with the opinions of Wokocha [22], all treatments in this study proved to be ineffective *in-vivo* at all tested concentrations (25, 50 and 75 mg/ml) as they failed to prevent the manifestation of *Fusarium* wilt. This may have been due to the frequent rainfall and low temperature during the experimental period (May to July), which favored the sporulation of the pathogen leading to an increase in inoculum density to a point where it overwhelmed the concentration and efficiency of the treatments applied. The consistent rainfall may have also pushed the treatments further into the soil away from the plant's rhizosphere, making them unavailable to form protective shield on tomato roots before pathogen's invasion.

Results from the field trial showed that the preventive efficacy of all treatments of AgNP/Mancozeb were ineffective against *Fusarium* wilt emergence at all tested concentration. A previous research similar to this study attributed the sudden increase of disease incidence in field experiment to obstruction of the plant's vascular system resulting from inoculum level increase due to loss of antifungal effect by fungicide [36]. In the same vein, fungi (*Mucor* sp., *Aspergillus niger*, *A. flavus*, *Rhizopus* sp. and *Penicilium* sp.) isolated from the experimental plot may have in synergy with *F. oxysporum* f. sp. *lycopercisi* played active roles in shielding the plant's

root from the treatment and invariably causing the plant to wilt. This finding is similar to the opinions of Mbadianya *et al* [37]. In contrast however, AgNP alone demonstrated a mild activity against the pathogen in the field in line with the study by Lamsal *et al* [38] who reported that AgNP alone inhibited *Colletotricum* sp. attack on pepper when applied before the disease outbreak.

CONCLUSION

This study has demonstrated a novel method for the optimization of ineffective large-sized AgNP by pairing with mancozeb and maximizing their synergistic effect against *F. oxysporum* f. sp. *lycopercisi*, causative agent of *Fusarium* wilt of tomato. Data from this research clearly showed that AgNP/Mancozeb (50 % v/v) combination strongly inhibited the growth of *Fusarium oxysporum* f. sp. *lycopersici in-vitro*. These results suggest that AgNP/Mancozeb (50 % v/v) possess the requisite synergistic potential to combat phytopathogens that have defied existing control strategies. However, prior to field application by farmers, extensive studies should be carried out to assess the phyto-toxic effects on the host and development of continuous delivery systems that can optimize its persistence both in the plant's rhizosphere and tissues.

Acknowledgement: The authors appreciate members of staff of the Departments of Microbiology and Crop Science, University of Benin, Benin City, Nigeria for their contributions and support during the course of this work.

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Citation: Godfrey O. Oribhabor. et al. (2018). Assessment of the *in-vitro* activity and *in-vivo* preventive efficacy of phyto-fabricated silver nanoparticles and silver nanoparticle/mancozeb combination against *Fusarium* wilt of *Lycopersicon esculentum* mill. J. of Advancement in Medical and Life Sciences. V8I1.04. DOI : 10.5281/zenodo.4071540