



Efficacy of Nanoparticles and Silver Nitrate in the Control of *Fusarium* Wilt of Tomato (*Solanum lycopersicum* L.)

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Abstract

This study assesses the efficacy of nanoparticles and silver nitrate in the control of *Fusarium* wilt of tomato. The field experiment was a $2 \times 2 \times 5$ factorial type consisting of three replications, two tomato accessions (CPTTO/18/106 and CPTTO/18/123), two treatments of nanoparticle and silver nitrates (AgNO_3), and four concentrations each of silver nanoparticles and silver nitrate (10, 30, 50, and 100 ppm). Carbendazim was applied as positive control while the negative control plots received no treatment. The experimental plot size was $3 \times 2 \text{ m}^2$. Tomato seedlings were sown at spacing of $75 \times 50 \text{ cm}$. The *in-vitro* experiment was monitored through mycelia spread measurement. Data were collected on disease incidence and disease severity. Data collected were subjected to analysis of variance using Statistical Analysis System (SAS). The results of the *in-vitro* experiment showed that the mycelia spread were least at 10ppm AgNO_3 application on the culture growth. This means that AgNO_3 at 10 ppm significantly ($p < 0.05$) reduced the growth of the fungus. The tomato treated with 10ppm AgNO_3 also had no disease incidence (0.00 %). Application of AgNO_3 at 10 ppm significantly ($p < 0.05$) reduced *Fusarium* wilt of tomato. The study therefore recommended AgNO_3 for the control of *fusarium* wilt of tomato.

Keywords; Fusarium; Nanoparticle; Silver Nitrate; Tomato;

Introduction

Tomato (*Solanum lycopersicum* L.), is the most consumed vegetable worldwide being the fundamental ingredient in large varieties of raw, processed or cooked foods (Pritesh *et al.*, 2011). Tomato production had served as a source of income for most farmers in developing countries. The tomato industry has been identified as a sector capable of alleviating poverty, due to its potential for creating employment and growth (Anang *et al.*, 2013). Tomato is now regarded as an impressive, cash and economic crop in many countries worldwide (Ayandiji *et al.*, 2011) not only because of its industrial importance and value, but for its nutritional value to human diet and health (Willcox *et al.*, 2003). Tomato production has enhanced the living standard of many rustic and urban farmers in Nigeria (Adenuga *et al.*, 2013).

Tomato fruits contain a large quantity of water, calcium and niacin all of which are great importance in the metabolic activities of man and

also cheap source of vitamins. In spite of medicinal and economic values of tomato in the society, many plant pathogens such as bacterial, fungal, viral and nematode impose many serious disease that inflict economic yield losses to tomato plant. Among the devastating diseases caused by plant pathogenic fungi, *Fusarium* wilt caused by *Fusarium oxysporum* f.sp. *lycopersici* holds an important place and remain challenging to be managed.

Tomato plants are infected by a few soil-borne contagious pathogens, such as, *Fusarium* spp., *Rhizoctonia solani*, and *Sclerotium rolfsii* which cause marked diseases such as root rot and wilt that reduce harvest yield and quality (Abdel-Monaim, 2010). *Fusarium oxysporum* f.sp. *lycopersici* causes a highly destructive vascular wilt disease of tomato leading to significant crop losses in the field and in protected tomatoes, thus remains as one of the main limiting factors for production of this crop (McGovern, 2015).

The fusarium pathogen is known to be the most destructive disease pathogen wherever tomatoes are extensively cultivated because it grows endophytically and thrives in infected soils. *Fusarium oxysporum* f.sp. *lycopersici*, a well known pathogen of tomato which is an economically important crop (Suarez-Estrella *et al.*, 2007). *Fusarium* wilt lead to an average yield loss of 50 % in tomato, it reduces farmer's income and family intake of vitamin A (Ajigbola *et al.*, 2013). It constitutes a serious threat to food security in the Africa south of the Sahara, especially in the coastal regions (Popoola *et al.*, 2012).

Nanoscale science and nanotechnologies are conceived with the capacity to revolutionize agriculture and food systems and has birthed the new era of Agronanotechnology (Min *et al.*, 2009). The antimicrobial qualities of silver had been demonstrated since 1000 B.C. when silver vessels were used to conserve water. recently, silver applications in agriculture have gained momentum (Lamsal *et al.*, 2011). laudable efforts have been made to explore antimicrobial property of nanoparticles from Silver, against human pathogens. Inconsequential researches were done to study its effects against overwhelming phytopathogens. Scattered studies have recognised the fungicidal effects of silver particles against several phytopathogenic fungi (Kim *et al.*, 2012).

Silver ions are usually very reactive, they impede microbial metabolism and respiration. Thereby causing physical damages. Over a century now, silver have been known to treat medical ailments due to its natural antifungal and antibacterial qualities (Morones *et al.*, 2005). Recently, effectiveness of silver nanoparticle as an antimicrobial agent have been accelerated by nanotechnology practices (Yeo *et al.*, 2003; Elchiguerra *et al.*, 2005).

Silver nanoparticles possess extremely large relative surface areas thereby increasing their contact with fungi and bacteria, vastly enriching its bactericidal and fungicidal effectiveness. The larger surface area-to-volume ratio of nanoparticles from silver, enhances their contact with microbes and their capacity to percolate into cells (Elchiguerra *et al.*, 2005).

Materials and Methods

Experimental site

The experiment were carried out at DFID Tomato Research Farm, opposite Works Unit, Federal University of Agriculture, Abeokuta.

Experimental materials

Viable seeds of two tomato accessions (CPTTO/18/106 and CPTTO/18/123) were obtained from FUNAAB/DFID Tomato Germplasm Collection Centre, Plant Tissue Culture Laboratory, Federal University of Agriculture, Abeokuta (FUNAAB). All accessions and varieties were susceptible to *Fusarium* wilt. Silver nitrate was gotten from Adex Chemical shop, Ibadan. Silver nanoparticle (20-30nm, WA-CV) was gotten from Beijing Nano Co. Ltd (China) at 1,000 ppm initial concentration. Different working concentrations of silver nitrate and silver nano particles (10 ppm, 30 ppm, 50 ppm, and 100 ppm) were prepared by diluting the original stock solution. All solutions were stored at 4⁰C until use. Carbendazim, a systemic fungicide was used as positive control.

Soil sterilization and nursery establishment

Sandy loam soil collected from Teaching and Research Farm of Directorate of Federal University of Agriculture, Abeokuta was steam-sterilized for 3 hours at 100 °C (according to Animashaun *et al.*, 2017). The sterilized soil were packed inside sacks and air dried for 2 weeks before use. Fifty grams (50 g) of the sterilized soil was weighed and loaded in nursery trays and tomato seedlings were grown and nurtured for four (4) weeks before transplanting.

Treatments and experimental design

The experiment was a 2 (accessions) x 2 (treatments) x 5 (level of concentrations) factorial experiment fitted into a Randomized Complete Block Design with three replicates. The experiment consisted of four tomato varieties, CPTTO/18/106 and CPTTO/18/123, silver nanoparticles and silver nitrate at four levels of concentrations each. Carbendazim was also applied as positive control at manufacturer's recommended concentration. Negative control plots received no treatment. Four

weeks old tomato seedlings were transplanted on wilt endemic field in the evening on the already prepared land. The experimental plot size was 3 x 2 m² with 1 m border. Tomato seedlings were sown at 75 x 50 cm spacing. There were seventy-two (60) plots with sixteen (16) plants per plot.

Treatments application

Tomato plants were treated with silver nanoparticle and silver nitrate at 2, 4, 6 and 8 weeks after transplanting. Silver nanoparticle and silver nitrate suspension were applied at 10, 30, 50 and 100 ppm concentrations. Negative control plots received no treatments. The treatments were dissolved into 10 litres of knapsack sprayers and were applied by drenching method early in the morning. The positive control plot received 2.5g/l of Carbendazim fungicide also dissolved into the sprayer. Carbendazim was used because it's the conventional fungicide used by farmers in mitigating the spread of Fusarium wilt.

Preparation of Potato Dextrose Agar (PDA)

Potato Dextrose Agar (PDA) was prepared according to Ford *et. al.* (2015).

Isolation of *Fusarium oxysporum*

Isolation was done using the direct plating method of Okhuoya *et al.* (2012). Wilted tomato plants with yellow leaves were collected from wilt endemic tomato field at Teaching and Research Farm of Federal University of Agriculture, Abeokuta and then taken to the laboratory for fungal isolation. Tomato plants stems showing vascular discoloration were rinsed thoroughly in tap water and then macerated with a sterile scalpel.

Inoculation of tomato seedlings with *F. oxysporum f.sp. lycopersici* spores

Conidia suspension of seven day old pure cultures of isolated *F. oxysporum f. sp. lycopersici* were washed with sterile distilled water to obtain suspension of inoculums of the pathogen. Cultures were then filtered through one layer of Mira cloth, centrifuged, washed with sterile water and adjusted to a concentration of 10⁶ conidia per ml with the aid of haemocytometer). The already prepared conidia suspension of *F. oxysporum f.sp. lycopersici* was then used to inoculate the four-week old tomato seedlings at one (1) week after transplanting. The two tomato accessions were inoculated at the root region by making shallow groove around the base of the plant in the root region and placing 5 g mycelia plug of 7 days old pure culture of *F. oxysporum f. sp. lycopersici* face-down close to the root of the seedling and covered with soil.

Treatments and experimental design

The *in-vitro* experiment was laid out in a Completely Randomised Design, (CRD) with two treatment sources of AgNPs and AgNO₃, replicated three (3) times. Carbendazim was also applied as positive control. Control plots received no treatment. The sterilized PDA was dispensed into 30 Petri dishes. The petri dishes was then divided into 6 controls plates and 24 plates treated with Carbendazim (positive control) as well as 10 ppm, 30 ppm, 50 ppm and 100 ppm concentration of AgNPs and AgNO₃ treatments .

Data Analysis

Data collected were subjected to analysis of variance (ANOVA) using Statistical Analysis System (SAS), 9.1 package and means were separated using the Duncan's Multiple Range Test (p≤0.05).

Results and Discussion

Table 1: Effect of silver nanoparticles and silver Nitrate on mycelial spread of *Fusarium oxysporum in vitro*

Accession	Treatment (ppm)	DAY 3	Mycelial DAY 5	Spread (Cm) DAY 7	DAY 9
		CPTTO/18/106	Control	2.32 ^{bc}	3.97 ^{bc}
	Silver NP-10	1.22 ^a	2.10 ^a	3.55 ^a	3.85 ^a
	Silver NP -30	1.37 ^{ab}	2.47 ^a	3.08 ^b	3.38 ^b
	Silver NP -50	1.50 ^b	2.70 ^{bc}	3.30 ^{bc}	3.50 ^{bc}
	Silver NP -100	1.76 ^{bc}	2.98 ^{bc}	3.62 ^{bc}	3.93 ^{bc}
	AgNO ₃ - 10	0.45 ^a	1.01 ^a	2.30 ^a	2.40 ^a

CPTTO/18/123	AgNO ₃ – 30	0.60 ^a	1.66 ^a	2.50 ^b	2.70 ^b
	AgNO ₃ – 50	1.03 ^{bc}	1.20 ^b	2.66 ^{bcd}	2.87 ^{bcd}
	AgNO ₃ -100	1.25 ^b	1.33 ^{bc}	2.80 ^{bcd}	2.97 ^{bcd}
	Carbendazim	0.95 ^a	1.86 ^a	2.45 ^b	2.65 ^b
	control	2.65 ^{bcd}	3.50 ^{bcd}	4.06 ^{bc}	4.26 ^{bc}
	Silver NP-10	1.84 ^{ab}	2.62 ^{bc}	3.30 ^b	3.50 ^b
	Silver NP -30	1.17 ^{ab}	2.13 ^b	3.67 ^{ab}	3.94 ^{ab}
	Silver NP -50	1.45 ^{ab}	2.40 ^b	3.80 ^b	3.93 ^b
	Silver NP -100	1.89 ^{bc}	2.98 ^{bcd}	3.98 ^{bc}	4.00 ^{bc}
	AgNO ₃ - 10	0.23 ^a	1.17 ^a	2.35 ^a	2.55 ^a
	AgNO ₃ – 30	0.33 ^a	1.68 ^{ab}	2.68 ^{ab}	2.88 ^{ab}
	AgNO ₃ – 50	0.95 ^{ab}	1.82 ^b	2.56 ^{ab}	2.76 ^{ab}
	AgNO ₃ – 100	1.12 ^{bc}	1.97 ^b	2.63 ^{bc}	2.93 ^{bc}
	Carbendazim	0.43 ^a	1.48 ^{ab}	2.48 ^{ab}	2.78 ^{ab}

Means in the same column with different superscript are significantly different ($p \leq 0.05$)

The effects of Silver nanoparticles and Silver nitrate (AgNO₃) on incidence of *Fusarium* wilt of tomato on field tests was revealed in table 2, in which the highest disease incidence (43.33) at 6 WAT was recorded in CPTTO/18/123 treated with silver nanoparticle at concentration of 100 ppm while the least result (0.00) was recorded in CPTTO/18/106 treated with Silver Nitrate at

concentration of 10 ppm and 30 ppm. Highest disease incidence (60.00) on the field at 8 WAT was recorded in CPTTO/18/123 treated with silver nanoparticle at concentration of 100 ppm while the least was recorded in CPTTO/18/106 treated with Silver Nitrate at concentration of 10 ppm and 30 ppm.

Table 2: Effect of silver nanoparticles and silver nitrate (AgNO₃) on incidence of *Fusarium* wilt of tomato on field tests

Accession	Treatments (mM)	Disease	
		6 WAT	Incidence (%) 8 WAT
CPTTO/18/106	Control	26.67 ^b	40.00 ^b
	Silver NP-10	3.33 ^{bd}	3.33 ^{bcd}
	Silver NP -30	16.67 ^{bc}	20.00 ^{bc}
	Silver NP -50	23.33 ^b	36.67 ^b
	Silver NP -100	36.67 ^a	50.00 ^a
	AgNO ₃ - 10	0.00 ^{bcd}	0.00 ^{bcd}
	AgNO ₃ – 30	0.00 ^{bcd}	0.00 ^{bcd}
	AgNO ₃ – 50	3.33 ^{bcd}	26.67 ^{bc}
	AgNO ₃ – 100	20.00 ^b	33.33 ^b
	Carbendazim	3.33 ^{bcd}	20.00 ^{bc}
CPTTO/18/123	control	40.00 ^a	53.33 ^a
	Silver NP-10	6.67 ^{bcd}	16.67 ^{bc}
	Silver NP -30	16.67 ^{bc}	20.00 ^{bc}
	Silver NP -50	30.00 ^b	33.33 ^b
	Silver NP -100	43.33 ^a	60.00 ^a
	AgNO ₃ - 10	3.33 ^{bcd}	3.33 ^{bcd}
	AgNO ₃ – 30	3.33 ^{bcd}	20.00 ^{bc}
	AgNO ₃ – 50	6.67 ^{bcd}	10.00 ^{bcd}
	AgNO ₃ – 100	10.00 ^{bc}	16.67 ^{bc}
	Carbendazim	6.67 ^{bcd}	10.00 ^{bcd}

Means in the same column with different superscript are significantly different ($p \leq 0.05$)

WAT – Weeks after transplanting, NP - Nanoparticle

Table 3 revealed the effect of silver nanoparticles and silver nitrate (AgNO₃) on severity of *Fusarium*

wilt of tomato. Significant differences were observed ($p < 0.05$) as the highest disease severity

(4.17) at 6 WAT was recorded in CPTTO/18/123 treated with silver nanoparticle at concentration of 100 ppm and in its control plot while the least result (1.00) was recorded in CPTTO/18/106 treated with Silver Nitrate at concentration of 10

ppm and 30 ppm. At 8 WAT, highest disease severity (5.17) was recorded in the control plot of CPTTO/18/123 while the least disease severity was recorded in CPTTO/18/106 treated Silver Nitrate at concentration of 10 ppm.

Table 3: Effect of silver nanoparticles and silver nitrate (AgNO₃) on severity of *Fusarium* wilt of tomato on field tests

Accession	Treatment (ppm)	Disease 6WAT	Severity 8WAT
CPTTO/18/106	Control	3.00 ^b	4.17 ^a
	Silver NP-10	1.17 ^{bcde}	1.33 ^{bcd}
	Silver NP -30	1.50 ^{bcde}	2.67 ^{bc}
	Silver NP -50	2.33 ^{bcd}	3.50 ^b
	Silver NP -100	4.00 ^a	4.50 ^a
	AgNO ₃ - 10	1.00 ^{bcde}	1.17 ^{bcd}
	AgNO ₃ - 30	1.00 ^{bcde}	1.33 ^{bcd}
	AgNO ₃ - 50	2.33 ^{bcd}	2.17 ^{bc}
	AgNO ₃ - 100	2.83 ^{bc}	3.33 ^b
	Carbendazim	1.83 ^{bc}	2.00 ^b
CPTTO/18/123	control	4.17 ^a	5.17 ^a
	Silver NP-10	1.33 ^{bc}	2.33 ^{bc}
	Silver NP -30	2.04 ^b	2.67 ^{bc}
	Silver NP -50	3.33 ^b	3.83 ^b
	Silver NP -100	4.17 ^a	5.00 ^a
	AgNO ₃ - 10	1.17 ^{bcd}	1.33 ^{bcde}
	AgNO ₃ - 30	1.17 ^{bcd}	2.17 ^{bcd}
	AgNO ₃ - 50	1.50 ^{bc}	2.33 ^{bc}
	AgNO ₃ - 100	1.67 ^{bc}	2.83 ^{bc}
	Carbendazim	2.24 ^b	2.47 ^{bc}

Means in the same column with different superscript are significantly different ($p \leq 0.05$)

WAT – Weeks after transplanting, NP - Nanoparticle

Discussion

Fusarium oxysporum f.sp. *lycopersici* (Sacc.) W.C. Snyder and H.N. Hans, a soil borne plant pathogen in the class Hyphomycetes, causes *Fusarium* wilt specifically on tomato (Rai et al., 2011). There are more than 100 *Fusarium* vascular wilt diseases worldwide. Apart from causing diseases, they colonize outer cells of roots as harmless endophytes after the pathogen has killed the root tissues and others live as saprophytes in soil (Burgess et al., 2008).

Application of silver nitrate and silver nanoparticles reduced the incidence and severity of *Fusarium* wilt of the two tomato accessions compared with the positive control soil treated with Carbendazim fungicide, and negative control soil treated with the pathogen only without silver nitrate and silver

nanoparticle or fungicide. The application of silver nitrate at concentration of 10 ppm performed best as it significantly reduced the incidence and severity of *Fusarium* wilt in both accessions. This could be due to the high density at which the solution was able to saturate to fungal hyphae and to deactivate plant pathogenic fungi. This confirmed earlier result that demonstrated that silver has been used for controlling spore-producing fungal plant pathogens and sclerotia-forming species of *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *S. minor* (Min et al., 2009), powdery mildew in cucumber and pumpkin (Lamsal et al. 2011b), rice blast disease, caused by *Magnaporthe grisea* (Elamawi and EL-Shafey, 2013) and *Fusarium* wilt in lettuce and tomato (Ahmed et al., 2011).

In a recent study of Madbouly *et al.*, (2017), both silver nitrate and silver nanoparticle showed pronounced efficacy to cause invitro inhibition of the growth of *F. oxysporum* on three types of cultivation media i.e. Potato dextrose agar (PDA), Malt extract agar (MEA) and Corn meal agar (CMA), however, its potency was recorded more on PDA medium. In addition, both silver nitrate and silver nanoparticle caused *in vivo* reduction in the severity of wilt disease of tomato in the greenhouse by 90 % (Madbouly *et al.*, 2017).

Conclusion

The study concludes that silver nitrate and silver nanoparticle applied at all concentrations of 10, 30 ppm reduced the incidence and severity of *Fusarium* wilt of tomato though the application of silver nitrate at concentration of 10 ppm produced the best result as it significantly reduced the incidence and severity of the disease compared with positive control soil treated with Carbendazim fungicide, and negative control soil treated with the pathogen only without silver nitrate and silver nanoparticle or fungicide. The study recommends that application of silver nitrate at concentration of 10 ppm can significantly reduce fungal wilt of tomato.

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