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Effect of Copper Nanoparticles on Incidence and Severity of Fusarium wilt and Fruit Yield of Tomato (*Solanum lycopersicum* L.)

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A**bstract**

Wilt in tomato plants is often caused by *Fusarium oxysporium* f. sp. *lycopersici*. Chemicals, which are highly hazardous, are mostly used to control the disease. It is therefore, desirable to have alternative control mechanisms that are safe and affordable. Screenhouse and field experiments were conducted at the Teaching and Research Farm, Ajayi Crowther University, Oyo, Nigeria, to assess the impact of copper nanoparticles (Cu-NPs) on Fusarium wilt and fruit yield of two tomato varieties (Beske and Kerewa). The treatments were applied at concentrations of 5, 10, and 15 ppm. The untreated plots served as control. Results showed that Kerewa treated with 15 ppm Cu-NPs recorded lower disease incidence in the screenhouse (2.1%) and on the field (22.2%). Furthermore, in the screenhouse, Kerewa treated with 15 ppm Cu-NPs had lower disease severity (0.6) while on the field, both Beske and Kerewa treated with 15 ppm Cu-NPs had no disease incidence (0.0 and 0.0, respectively). Beske produced significantly higher fruit yields of 151.7 and 795.8 kg/ha in the screenhouse and field trials, respectively. The research findings indicated that the use of Cu-NPs at a rate of 15 ppm significantly decreased Fusarium wilt infection and increased tomato fruit yield.

Keywords: Tomato, *Fusarium oxysporium,* Fusarium wilt, copper nanoparticles, fruit yield

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Introduction

Tomato is considered as one of the world's most valuable, nutritious, commonly cultivated, and commercially attractive fruit vegetables (Hussain et al., 2016; Poussio et al., 2018). Globally, China, the United States, Turkey, India, and Egypt are the main producers of tomato. (FAO, 2021). China produces more tomato than any other country in the world (61.5 m MT), with India coming at a distant second (19.4 MT) (FAOSTAT, 2020). Tomato is a preferred choice for cultivation as a fruit vegetable because it is high yielding with a short growing season. Nutritionally, several researchers reported that tomato is rich in vitamins and a host of secondary metabolites including lycopene, flavoids, and polyphenol (Huang et al., 2021, Collins et al., 2022).

Fusarium wilt is regarded as one of the most significant tomato diseases of global importance. (Sadana and Didwania, 2019). Major tomato diseases such as Septoria leaf spot (*Septoria lycopersici*), late blight (*Phytophthora infestans*), and early blight (*Alternaria solani*) have been reported in previous research (Poussio et al., 2018, Mahalakshmi *et al*., 2020). Powdery mildew (*Leveillula taurica*), root knot nematode (*Meloidogyne spp.*), and bacterial wilt are among the diseases that have emerged as the most important in recent years (Sadana and Didwania, 2019). However, one of the most significant diseases of tomato is Fusarium wilt caused by *Fusarium oxysporum* f. sp *lycopersici*, which affects the plant almost entirely during its life cycle, from seedling to flowering to eventual fruiting (Wiam et al.,

2019; Carmona et al., 2020). It is a soil-borne phyto-pathogen that infects plants through their roots, grows inside the cortex to the stele, and kills the plants (Hatem *et al*., 2020). It lowers farmer's income and family vitamin A intake, causing an average 50% reduction in tomato fruit yield (Srinivas et al., 2019).

Maintaining plant health, fruit quality and quantity requires controlling Fusarium wilt of tomato (Muhammad et al., 2020). The disease is mostly controlled in large-scale production by using methyl bromide, a chemical that has been shown to have serious negative effects on the environment and destroy helpful microbes (Sharma et al., 2018). In addition to risks to people and the environment, persistent chemical usage can result in the emergence of multi-resistant strains (Sadana and Didwania, 2019). In order to reduce these negative effects of chemical treatment of *Fusarium* wilt, nanotechnology has been identified as a viable alternative (Chowdappa and Gowda, 2013). Copper nanoparticles (Cu-NPs) is known to have a fungicidal effect in the control of plant pathogens (Malandrakis et al., 2019). Due to the small size and large surface area, zero valence copper nanoparticles (Cu-NPs) have unique electronic, mechanical, and chemical properties that are different from those found in bulk copper (Mardiansyah et al., 2018). Additionally, Cu-NPs have shown enhanced antifungal/antibacterial properties when compared to bulk copper (Ananth et al., 2015). In *in-vitro* assays, Cu-NPs have proven to be effective anti-fungal agents against *Fusarium oxysporum* (Pariona et al., 2019; Viet et al., 2016). Additionally, *in-vitro* assays revealed that nanoparticles containing copper with various oxidation states can be effective against *F. oxysporum*, where lower anti-fungal activity was found for copper II 0xide (CuO) (Hermida-Montero et al., 2019). Cu-NPs are efficient due to their solubility and permeability, low dose-dependent toxicity, enhanced bioavailability, target delivery, and controlled release (Periakaruppan et al., 2023). However, limited literature is available on the adoption of nanotechnology using Cu-NPs for Fusarium wilt control in tomato. Therefore, the objective of this study was to prepare copper nanoparticle and assess is effectiveness against Fusarium wilt of tomato *(Fusarium oxysporium* f. sp. *lycopersici*) and fruit yield of tomato (*Solanum lycopersicum* L.) grown in the screenhouse and on the field.

Materials and Methods

Experimental site

The field trial was conducted at the Teaching and Research (T&R) Farm of Ajayi Crowther University (ACU), Oyo, Nigeria. Additionally, the Tomato Research Screenhouse, ACU, Nigeria, served as the site of the screenhouse experiment.

Experimental materials

Seeds of two tomato varieties, namely Beske and Kerewa, obtained from the Institute of Agricultural Research and Training (IAR&T), Ibadan, Nigeria, were used for the experiment. IAR&T has the national mandate for the genetic improvement of tomato and other vegetable and horticultural crops in Nigeria. Leaves of neem (*Azadirachta indica*) were sourced on the premises of ACU and copper sulphate ($CuSO_4$) was sourced from a local agrochemical store in Abeokuta.

Soil sterilization and nursery establishment

Sandy loamy soil that was taken from the T&R Farm, ACU was steam-sterilized for 2 hours at 100 ^oC. The sterilized soil was packed inside sacks and allowed to air dry for a week before use. Fifteen grams (15 g) of the sterilized soil were weighed and loaded into the nursery trays in which the seeds were sown. Tomato seedlings were grown and nurtured for four weeks before transplanting.

Preparation of plant extract (Neem-Azadirachta indica)

Neem leaves (*Azadiracta indica*) collected from the premises of ACU, Oyo, were rinsed with distilled water and washed with 10% sodium hypochlorite solution (NaOCl), which was made by mixing one-part bleach with nine parts water. The leaves were then air dried and later packed in brown envelopes before they were oven dried at 70 °C for 20 minutes. Thereafter, they were ground using a pestle and mortar, sieved through a 40-mm mesh, and 25 g of the neem powder was added to 250 ml of distilled water in a 1000-ml flat bottom flask. After letting the suspension settle for a full day, the content was filtered through a muslin filter and stored in glass bottles until required.

Preparation of precursor solution

A 0.1 M molar concentration of copper sulphate was prepared. One hundred millilitres (100 ml) of double-distilled water were used to dissolve 0.4 grams of the precursor salt of copper sulphate to produce an aqueous solution. The green production of Cu-NPs was accomplished with the solution.

Synthesis of copper nanoparticles

Copper nanoparticles were synthesized by measuring a 150-ml aliquot of neem leaf extracts from the resulting filtrate and then added to 50 ml of 0.1 M copper sulphate solution in a 1000-ml flat bottom flask. The mixture was shaken vigorously until its colour changed from light green to dark green, with brown precipitates settling at the bottom. At 28 ± 2 ^{O}C, the whole mixture was incubated for approximately 24 hours. The copper nanoparticle sediment was then collected and stored in the refrigerator for further use (Plate 1).

Treatments and experimental design

The experiment consisted of two tomato varieties (Beske and Kerewa) and four levels of Cu-NPs concentrations (0, 5, 10, 15 ppm). The Cu-NPs treatments were applied by the soil-drenching method (application via the plant root). Control plots received no application of Cu-NPs treatment. A 2 x 4 factorial design as used for the experiment. A completely randomized design was used for the screenhouse experiment in three replications, while a randomized complete block design was used for the field experiment in three replications

Transplanting of tomato seedlings

Four-week-old tomato seedlings were transplanted into plastic pots containing 9 kg of sterilized soil and placed in the screenhouse. Each pot received two tomato seedlings, which were eventually thinned to one. There were 24 pots altogether for the screenhouse experiment.

In the evening, tomato seedlings were transplanted on the already prepared land. A 3 × 2 m^2 experimental plot with a 1 m border was used. The transplanted tomato seedlings were spaced 75 between rows with a spacing of 50 cm between plants within a row. There were 24 plots, with 16 plants per plot in the field experiment

Preparation of culture medium

The culture medium was prepared by weighing 39 g of potato dextrose agar (PDA) with a Metler weighing balance into the Erlenmeyer flask. The PDA in the Erlenmeyer flask was then filled with one litre of distilled water. The Erlenmeyer flask was covered with cotton wool and foil paper. After homogenizing the mixture, it was sterilized for 15 minutes at 121 ^{o}C in an autoclave. The sterilized medium was then allowed to cool before being supplemented with streptomycin sulphate (5 mg) to suppress bacterial growth. Fifteen millilitres of the already prepared PDA were aseptically dispensed into each petri dish (9 cm in diameter) and allowed to solidify.

Isolation and purification of Fusarium oxysporum **f sp.** *lycopersici*

Fusarium oxysporum f. sp. *lycopersici* was isolated from an infected tomato plant that showed symptoms such as wilting and brown staining of vascular vessels. The stem of the diseased plant was cleansed in distilled water to get rid of foreign materials. After cutting five-millimetre pieces, they were immersed in 70% alcohol and sterilized for three minutes in a 0.5% sodium hypochlorite solution. The samples were dried by blotting. The dried, sterile, infected plant samples were incubated for seven days at 38 °C after being placed in the center of Petri plates containing amended PDA. Colonies showing the morphology of Fusarium were further subcultured for another 7 days on PDA to obtain pure cultures of fungal isolates. A light microscope was used to identify *F. oxysporum* f. sp. *lycopersici*, and the fungal structures were placed on slides stained with methylene blue.

Inoculation of tomato seedlings with Fusarium oxysporum f. sp. lycopersici spores

Suspensions of inoculums of the pathogen were obtained by washing conidia suspensions of 7-day old pure cultures of isolated *F. oxysporum* f. sp. *lycopersici* with sterile distilled water. The suspension was then filtered through one layer of mira cloth, centrifuged, cleaned with sterile water, and adjusted to a concentration of 10^6 conidia per ml (with the help of a hemocytometer). The 4-week-old tomato seedlings were then inoculated one (1) week after being transplanted in the screenhouse and on the field. The tomato seedlings were

inoculated at a rate of 1 ml per hole using the root-dip inoculation technique.

Treatments application

Tomato plants were treated with copper nanoparticles (Cu-NPs) at concentrations of 5, 10, and 15 ppm (parts per million) at 2, 4, 6, and 8 weeks after transplanting. The treatments were applied by the drenching method. Control pots and plots received no treatment.

Data Collection
Disease incidence, DI, (%), disease severity, DS, and fruit yield (kg/ha) were measured on five sample plants positioned in the centre of each experimental plot.

Disease incidence
At 4 and 6 weeks after transplanting, the disease incidence was assessed. Five tagged plants from each plot were used to visually monitor and record the number of diseased tomato plants in each plot. Disease incidence was then calculated as follows:

$$\text{Disease incidence (\%)} = \frac{\text{Number of tagged plants with diseases in each plot}}{\text{The total number of tagged plants in each plot}} \times 100$$

Disease severity
At 6 and 8 weeks after transplantation, the severity of the disease was assessed visually on plot basis using the Sibounnavong et al. (2012) scale: where 1 = no symptoms, 2 = plants with wilting and yellowing leaves (1 - 20%), 3 = plants with wilting and yellowing leaves (21 - 40%), 4 = plants with wilting and yellowing leaves (41 - 60%), 5 = plant with wilting and yellowing leaves (61 - 80%), 6 = plants with wilting and yellowing leaves (81 - 100%) or death.

Yield data
The tomato fruits harvested from sampled plants per plot were weighed and recorded at 8 weeks after transplanting in kg plot^{-1}.

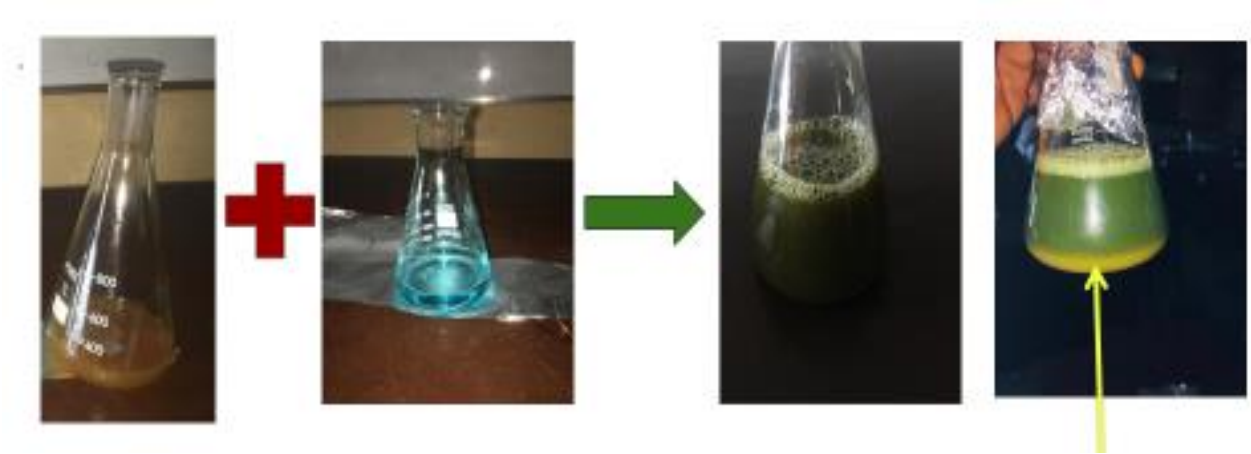
Data analysis

Analysis of variance (ANOVA) was conducted on the data collected using the Statistical Analysis System (SAS) 9.1 package, and Duncan's Multiple Range Test ($p \leq 0.05$) was used to separate the means.

Results

The visual observation of copper nanoparticles

The neem extract was able to bio-reduce $CuSO_4$ to Cu-NPs. The copper nanoparticles were assessed visually by colour change. The addition of the aqueous *Azadirachta indica* extract to the $CuSO_4$ solution resulted in a colour change of the mixture from sea blue to dark green and the settling of brown precipitate at the bottom, indicating the success of the Cu-NPs formulation.



Neem extract **$CuSO_4$** Formulated Cu-NPs Settled Cu-NPs

Fig 1: Green Synthesis of Copper Nanoparticles

Effect of Cu-NPs *on tomato Fusarium wilt disease incidence and severity*

Table 1 shows the effects of Cu-NPs on tomato Fusarium wilt incidence. Significant ($p \leq 0.05$) differences were detected among the treatments with the highest disease incidence (62.2%) recorded in the control pot for Kerewa at 6 WAT in the screenhouse experiment, while Beske grown on soil treated with 5 ppm Cu-NPs had the least (0.0%). Furthermore, in the screenhouse, the highest disease incidence (70.0%) was recorded at 8 WAT in the control pot for Kerewa, while the least (2.1%) was recorded in Kerewa grown on soil treated with Cu-NPs at a concentration 15 ppm.

In the field experiment, the highest disease incidence (44.5%) was recorded in the control plot for Kerewa at 6 WAT, while the least result (2.2%) was recorded in Kerewa grown on soil treated with 15 ppm Cu-NPs. Also, at 8 WAT, the highest disease incidence (55.8%) in the field experiment was recorded in the control plot for Beske, while the least (2.2%) was recorded in Kerewa grown on soil treated with 15 ppm Cu-NPs.

The effect of effect of copper nanoparticles on tomato fungal wilt severity was also shown in Table 1. There was a significant difference ($p \leq 0.05$) as the highest disease severity (4.0) in the screenhouse at 6 WAT was recorded in the control pot for Beske, while Kerewa grown on soil treated with 15 ppm Cu-NPs had the least (0.6). Furthermore, in the screenhouse, the highest disease severity (4.0) was recorded in the control pots for Beske, while the least (1.3) was recorded when Kerewa was grown on soil with 15 ppm Cu-NPs at 8 WAT.

Conversely on the field, at 6 WAT, the highest disease severity (1.6) was recorded in the control plot for both varieties, while the least result (0.0) was recorded in both Beske and Kerewa grown on soil containing 15 ppm Cu-NPs. Also on the field, the highest disease severity (2.0) was recorded in the control plot for Beske and Kerewa at 8 WAT, while the least (0.3) was recorded in both varieties grown on soil treated with 15 ppm Cu-NPs.

Effect of Cu-NPs *on fruit yield of tomato*

Cu-NPs influenced the fruit yield of tomato significantly ($p \leq 0.05$) in both the screenhouse and field trials (Table 2). Generally, yield from the field was higher than screenhouse. Beske grown on soil treated with 15 ppm Cu-NPs produced the highest fruit yield, while the control pot for Beske had the least (45.7 kg/ha) fruit yield in the screenhouse.

On the field, Beske grown on soil treated with 15 ppm Cu-NPs had the highest (795.8 kg/ha) fruit yield, while the least (171.6 kg/ha) fruit yield was recorded in the control plot for Beske.

Table 1: The effects of Cu-NPs on Fusarium wilt disease incidence and severity in tomato

Tomato varieties	Cu-NPs (ppm)	Disease incidence (%)				Disease Severity			
		Screenhouse		Field		Screenhouse		Field	
		6WAT	8WAT	6WAT	8WAT	6WAT	8WAT	6WAT	8WAT
Beske	0	60.5^{a}	68.2^{a}	40.2^{a}	55.8^{a}	4.0^{a}	4.0^{a}	1.6^{a}	2.0^{a}
	5	22.5^{b}	25.2^{ab}	32.5^{ab}	32.4^{b}	3.3^{ab}	3.5^{a}	0.6^{b}	0.6^{b}
	10	12.5^{bc}	10.8^{b}	4.5^{c}	6.2^{cd}	1.6^{bc}	3.3^{ab}	0.3^{b}	0.6^{ab}
	15	0.0^{c}	2.5^{c}	2.6^{d}	2.5^{d}	1.0^{c}	3.0^{ab}	0.0^{c}	0.3^{b}
Kerewa	0	62.2^{a}	70.0^{a}	44.5^{a}	52.4^{a}	2.3^{b}	3.4^{ab}	1.6^{a}	1.6^{a}
	5	25.8^{b}	25.8^{ab}	12.4^{b}	42.9^{ab}	1.0^{c}	1.6^{b}	0.6^{b}	0.6^{ab}
	10	4.5^{c}	8.33^{b}	5.4^{b}	15.2^{c}	1.0^{c}	1.5^{c}	0.3^{b}	0.6^{ab}
	15	2.3^{d}	2.1^{c}	2.5^{d}	2.2^{d}	0.6^{c}	1.3^{c}	0.0^{c}	0.3^{b}

The Duncan Multiple Range Test indicates that means with different values in the same column are significantly different (P≤0.05).

Table 2: The effects of Cu-NPs on fruit yield of tomato

Tomato varieties	Cu-NPs (ppm)	Yield (kg/ha)	
		screenhouse 8 WAT	Field 8 WAT
Beske	0	45.7^{d}	399.7^{bc}
	5	98.5^{b}	428.6^{b}
	10	151.3^{a}	754.7^{a}
	15	151.7^{a}	795.8^{a}
Kerewa	0	62.1^{c}	171.6^{c}
	5	62.1^{c}	214.7^{c}
	10	109.6^{b}	601.1^{a}
	15	124.3^{ab}	601.1^{a}

The Duncan Multiple Range Test indicates that means with different values in the same column are significantly different (P≤0.05).

Discussion

Copper-nanoparticles (Cu-NPs) inhibit *Fusarium oxysporium* f. sp. *lycopersici* (*FOL)* growth in tomato plants, thereby reducing the occurrence and severity of *Fusarium* wilt. Cu-NPs can prevent spore growth before it reaches the roots as they come into direct contact with the *FOL* spores when applied to the roots or soil (Lopez (2021). Additionally, *FOL* spores may

enter the plant through the vascular system and subsequently colonize the entire plant (Nirmaladevi et al., 2016). Due to their tiny size, Cu-NPs have the potential to damage the *FOL* spores in the vascular system of the infected tomato (Arif et al., 2018). Similar results have been reported for *FOL*-infected tomato plants, where treatment with Cu-NPs reduced the disease's presence by 31% (Ma et al., 2019). Similarly, Cu-NPs in screenhouse experiments caused a 31% reduction in Fusarium wilt disease in tomato plants, while MnO- and ZnO-NPs reduced the disease by only 28% (Elmer and White, 2016). The findings of this research revealed that Cu-NPs at different concentrations (5, 10, and 15 ppm) effectively reduced the incidence and severity of *FOL* both in the screenhouse and field experiments. Specifically, at a concentration of 15 ppm, Cu-NPs demonstrated a significant inhibitory effect on *FOL* growth, with a 97.9 and 97.8% reduction in the screenhouse and on the field, respectively, which indicates that the Cu-NPs employed in this study are more potent antifungals than the previously reported nanoparticles. Copper is a micro-essential element required for the optimal growth of plants and aids in the production of more fruits and biomass. (Printz et al., 2016). It has been reported that the application of Cu-NPs has beneficial effects on the growth of various plant. When Cu-NPs (10, 20, 30, 40, and 50 ppm) were incorporated into the soil in pots, the growth and yield of wheat were considerably higher than in the control. (Hafeez et al., 2015). According to our findings, treating tomato seedlings with Cu-NPs reduced Fusarium wilt effectively while simultaneously boosting tomato fruit production. This aligns with the findings of Borgatta et al. (2018) where CuO-NPs have been shown to have similar effects, reducing *Fusarium* wilt disease and promoting watermelon growth.

Conclusion

Fusarium wilt is one of the most damaging fungal diseases of plants in the world, causing significant yield loss. Due to the possible antifungal qualities of Cu-NPs, they can be used as an alternative control method for tomato crop fungal wilt disease. The outcomes also showed that Cu-NPs can stimulate plant growth and improve its yield. Additionally, the study has demonstrated that managing *Fusarium oxysporium* f. sp*. lycopersici* (*FOL*), a fungal pathogenic disease in economic plants such as tomato, through the use of safe and affordable Cu-NPs is essential for guaranteeing a sustainable food supply and improving food and nutrition security.

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