

## Antibacterial activity of zinc oxide nanoparticles and gentamicin on *Xanthomonas campestris* on infested tomato from Bodija market, Ibadan Nigeria - a quantitative comparative study

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### ABSTRACT

Zinc oxide nanoparticles have appeared as a novel tool in agronomy and crop protection sequel to its efficacy to combat bacterial plant pathogens that are resistant to conventional antibacterial agents. Zinc oxide nanoparticles and gentamicin were quantitatively tested against *Xanthomonas campestris vesicatoria*, the pathogens that causes infective pre-harvest and post-harvest spot in tomato. Exactly 0.1mL of the suspension from grounded tomato was aseptically plated on sucrose agar medium and was incubated at optimum temperature for 24 – 48 hours. Pathogenicity tests of the isolates was carried on uninfected tomato to authenticate the infectious isolates obtained. Aqueous and ethanol extract of zinc oxide nanoparticles, and gentamicin antibiotics were tested on the isolates of *Xanthomonas campestris vesicatoria* confirmed, by agar cup diffusion technique. Aqueous and ethanol extract of zinc oxide nanoparticles, and gentamicin antibiotics prepared in varied concentrations; 5 µg/mL, 2.5 µg/mL, 1.25 µg/mL in varying doubling dilutions were tested on *Xanthomonas campestris vesicatoria* obtained, exhibited significant competitive antibacterial activity. The ethanol extract showed stronger antibacterial activity than the aqueous extract. Gentamicin, a broad-spectrum antibiotics exerted competitive anti bacterial activity at every dilutions. Growth of *Xanthomonas campestris* pv. *vesicatoria*, the etiologic agent of infective spot in tomato, was remarkably suppressed by zinc oxide nanoparticles as equally observed in gentamicin.

**Keywords:** Zinc oxide Nanoparticles, Gentamicin, *Xanthomonas campestris vesicatoria*, Tomato, Bodija market, Ibadan Nigeria.

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## INTRODUCTION

Tomato is an edible fruit that is used as an ingredients in soup making and many other multipurpose forms as needed. Tomato exists in different varieties round, oval, “cherry”, but all have the same nutritional characteristics. Tomato is an important source of; potassium, phosphorus, magnesium, iron, so necessary to the normal activity of nerves and muscles; -vitamins as A, B and C - tomatoes is the third source of vitamin C in human diet and the fourth for vitamin A. (Periago *et.al.*,2008).

It is a very important fruit crop that is cultivated in all parts of Nigeria, the fourth leading producer of tomato fruit in Africa and largest in west Africa with an average yield of 10 tons /hectares and annual output of 1.8million metric tons (Blum *et.al.* 2006 ).

Tomatoes contains vitamins and beta-carotene, an antioxidant that neutralizes harmful radicals in the blood. It also contains lycopene that had been reported as sperm boosters. Tomatoes also contains linolenic and caffeic acid with antimicrobial compounds against *Rhizopus solani*. Tomato is used for preventing cancer of the breast, bladder, cervix, colon and rectum, stomach, lung, ovaries, pancreas, and prostate. It is also used to prevent diabetes, diseases of the heart and blood vessels (cardiovascular disease), cataracts, and asthma (Freeman,2010 ).

One of the major common prevalent diseases of tomato bacterial spot and speck are caused by *Xanthomonas campestris* pv, *vesicatoria*. It is a periodical disease of tomatoes and sweet peppers. *Xanthomonas campestris* pv, *vesicatoria*. is a Gram-negative bacterium that causes bacterial spot of tomato and is a major cause of concern for food security that demands an urgent attention. Bacterial spot and speck produce similar symptoms, they are often misdiagnosed. Bacterial diseases of tomatoes can be some of

the most serious and destructive diseases affecting both field- and greenhouse-grown crops. Under moist field conditions they can cause localized epidemics affecting young developing fruit; in the greenhouse total crop losses can occur ( Zderika Polivkova *et.al.*, 2010 ).

Tomatoes are grown mostly for consumption and sometimes processed for export. Post-harvest loss of tomato fruits is caused by bacterial and fungal pathogens that can be got from field, in transit and during storage. The two diseases are most readily distinguished on the basis of fruit symptoms. In the case of bacterial spot-on green fruit, small water-soaked spots are first noticed. These spots become slightly raised and enlarge up to 1/8 to 1/4 inch in diameter. The center becomes irregular, brown, and slightly sunken, with a rough, scabby surface. Although ripe fruit are not susceptible, lesions are very obvious if fruit were infected when green. The bacterial spot organism may be carried as a contaminant on tomato seed (Shifar *et.al.*, 2011)

Entry of bacteria into plants occurs through natural plant openings (stomata and hydathodes) or through wounds created by windblown soil, insects, or cultural practices. Water soaking of the leaves, as caused by high-pressure sprays, greatly enhances bacterial spot infection. Moist weather and splashing rains are favorable for dissemination of bacteria. Bacterial spot may be present on tomato transplants especially when frequent rains occur, and plants are pulled. Once bacterial spot is introduced into the field, it can be difficult to control. Bacterial disease of tomatoes predominantly facilitates quick rot, fermentation and souring of the products which eventually leads to post harvest loss and large scale economic loss- a threat to food security (Barceloux and Dona,2009)

A nanoparticle is a small particle that ranges between 1 to 100 nanometers in size. Undetectable by the human eye, nanoparticles can exhibit significantly different physical and chemical properties to their larger material counterparts. Since the introduction of concept of nanotechnology by Richard Feynman in 1959, It has become a forth in the world of scientific discoveries. Nanoparticles can act as efficient antibacterial agents and is widely accepted in biomedicine. Its superior antibacterial activities are attributed to their electrostatic attraction between positively charged NPs and have the potential to eliminate resistant bacteria. The miniaturized sizes, crystallinity, chemical and mechanical properties improve their antimicrobial activity with minimal effect. One of the benefits of nanotechnology is nanomedicine (Jones *et.al.*, 2008 ).

The use of nanoparticles based on metals and their oxide is of great interest and one of the well studied metals affecting biological objects is Zinc (Zn) and its oxide (ZnO). Zinc is an active element and exhibits strong reduction properties. It can easily oxidize to form zinc oxide. Zinc, one of the most important trace elements plays an important role in the human body. Zinc is found in all tissues of the human body with the highest concentration in myocytes and are critical for proper functioning of large numbers of macromolecules and enzymes( Jala *et.al.*,2010 ).

Zinc oxide nanoparticles have high antibacterial effectiveness at low concentrations (0.16-5.00 mmol/L) and relatively low cost. ZnO are synthesized by the physico-chemical sol-gel method from zinc salts and every other method. The mechanisms of action of zinc oxide nanoparticles includes; disruption of cell membrane, binding to DNA and proteins, generation of reactive oxygen species, alteration of expression of wide range of genes and disturbances of the processes of bacterial DNA resulting in the death of bacterial cells(Seil and Webster, 2012)

Gentamicin is a broad-spectrum conventional antibiotics, an aminoglycosides with a proven antimicrobial efficacy on both Gram positive and Gram negative bacteria. The principal advantage of gentamicin over the other major aminoglycoside is its low cost. Unfortunately, resistance to gentamicin is increasing. Bodija market is one of the largest farm produce market in southwest Nigeria comprises of six states. It is a market that receives supplies of farm produce from almost every geographic region of Nigeria and serves as a feeder unit for every other market in the neighboring state.

This study compared the antimicrobial activity of zinc oxide nanoparticles and gentamicin a broad-spectrum antibiotic, on isolates of *Xanthomonas campestris* pv. *vesicatoria* from infected tomatoes with bacterial pathologic spot.

## METHODS

### Collection of specimens.

Tomatoes with infested spots were purchased from five different retail locations that receive supplies of tomatoes from different geographical farm settlements. The selected specimens were aseptically kept in a sterile polythene bags and transported to the microbiology laboratory of the department of pharmaceutical microbiology, Olabisi Onabanjo University for bacteriological analysis.

### Bacteriological isolation

Selected tomato specimens were rinsed with sterile distilled water and disinfected in 40% hypochlorite, the sample were re-rinsed twice with sterile distilled water and sliced portion of the rinsed specimen immersed in a little water were grounded with mortar and pestle and thereafter allowed to stand for 1 hour, the supernatant suspension was harvested for bacteriological culture. Exactly 0.1mL of the suspension were

aseptically removed with a sterile wire loop and plated on peptone fortified sucrose agar medium and were incubated at optimum temperature for 24 – 48 hours. The distinct colonies identified was further sub-culture to Luria Bethany agar medium to obtain a pure culture of *Xanthomonas campestris pv vesicatoria*.

#### **Pathogenicity test**

A volume of 200 $\mu$ L of a pure culture of *Xanthomonas campestris vesicatoria* suspension prepared were inoculated with the aid of 1mL syringe into an uninfected tomato and another set were spray inoculated and incubated at optimum temperature for 24 hours. Evidently water-soaked lesion and spots observed from the specimen of tomato treated

#### **Gentamicin**

Gentamicin (Garamycin) packaged in ampoule vials for injection was purchased from a pharmacy outlet in Ibadan, Oyo state. It was prepared at 5  $\mu$ g /mL in aqueous dilution, reflecting hospital practice in the use of antibiotics.

#### **Sources of Zinc oxide nanoparticles**

The zinc oxide nanoparticles used for this research work was obtained from routine laboratory stocks of the department of chemistry of the faculty of science, University of Ibadan Nigeria.

#### **Preparation of zinc oxide nanoparticles for antibacterial assay**

Exactly 0.05milligram of zinc oxide nanoparticles was suspended in 250mL of sterile distilled water and ethanol in a separate conical flask and left for 4 hours with intermittent shaking. The mixtures were then clarified and concentrated using rotary evaporator.

A quantity of 5 $\mu$ g/mL of aqueous and ethanol extract was prepared as a stock concentration from which fractional dilution 1mL; of 5 $\mu$ g/2mL(1:2), 5 $\mu$ g/4mL (1:4), 5 $\mu$ g/8mL (1:8), 5 $\mu$ g/16mL (1:16) and 5 $\mu$ g/32mL (1:32) concentrations was prepared for antibacterial assay.

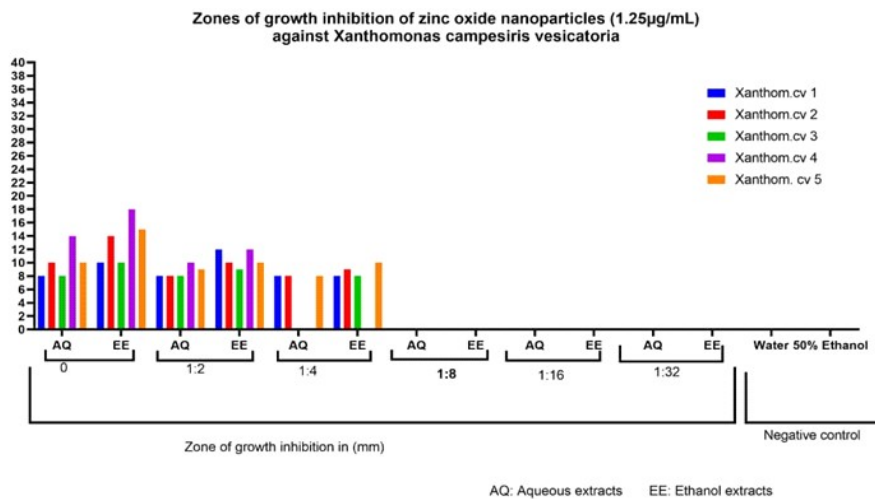
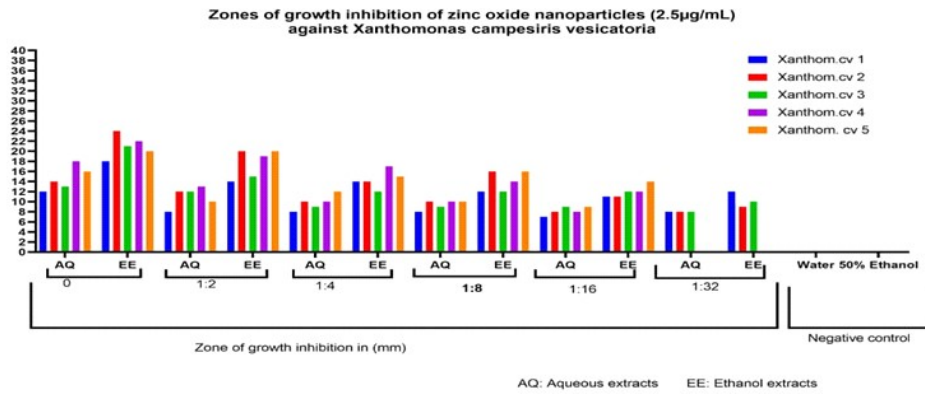
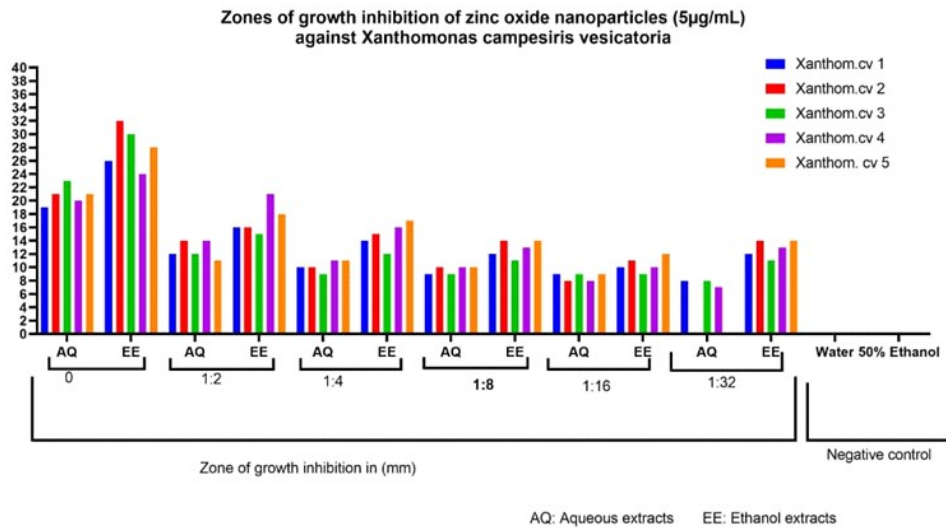
#### **Antimicrobial screening (Agar cup diffusion method)**

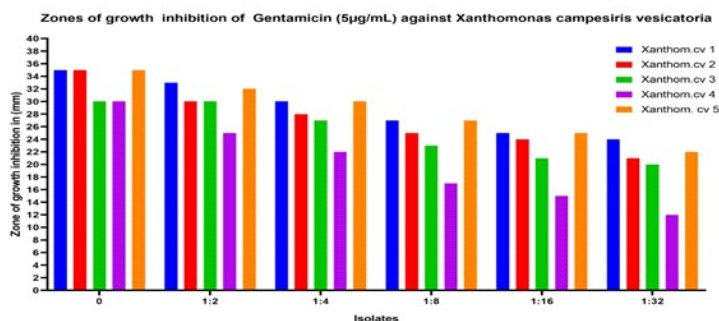
An overnight nutrient broth culture of the isolates of *Xanthomonas campestris vesicatoria*, equivalent to 10<sup>8</sup>cfu/mL, was diluted in 1:100 dilution and seeded into 20 mL of molten Mueller Hinton Agar. Wells were dug with flamed-sterilized cork borer of 6mm internal diameter. The fractional dilutions of zinc oxide nanoparticles from the stock concentration of each dilution (5  $\mu$ g/mL, 2.5  $\mu$ g/mL and 1.25  $\mu$ g/mL) as well as 5 $\mu$ g/ml of gentamicin were filled into the wells. The cultured plates were allowed to remain on the bench for a pre-diffusion period of 30 minutes, and thereafter incubated in an upright position for 24hrs at 33°C. The isolates were then checked for the zone of growth inhibition.

#### **Statistical package**

Data collected were analyzed using SPSS 15 and graph was plotted using Graph Pad prism 8.1 Version 5 for Windows.

RESULTS





## DISCUSSION

The antimicrobial activities of the fractional dilutions of zinc oxide nanoparticles and gentamicin on *Xanthomonas campestris vesicatoria* were quantitatively assessed in this study. The choice of zinc oxide nanoparticles for this study was based on its reported antibacterial potential which has been considered by many researchers as a better approach to drug discovery (Emami-Karvani *et.al.*, 2011)

The aqueous and ethanol extracts zinc oxide nanoparticles elicited elaborate competitive remarkable zones of inhibition in comparison with *gentamicin*, but the ethanol extracts from zinc oxide nanoparticles demonstrated more antimicrobial activity than aqueous extract. This could be because of a selective reaction of the composition and concentration of the extract of zinc oxide nanoparticles to chemicals of different polarities, since ethanol, a universal solvent, higher polarity makes it an excellent extractant which agreed with the study of Padmavathy and Vijayaraghavan (2008) on the enhanced bioactivity of zinc oxide nanoparticles-an antimicrobial study (Padmavathy and Vijayaraghavan,2008).

At concentration of 5µg/mL, for isolate of *Xanthomonas campestris vesicatoria* 1, zinc oxide nanoparticles undiluted aqueous and ethanol extracts showed zones of 19mm and 26 mm while at the dilution of 1:32, zones of growth inhibition of 8mm and 12mm were recorded. *Xanthomonas campestris vesicatoria* 5, elicited 21mm

and 28mm at undiluted form, while at the dilution 1:32, its aqueous extract exhibited no growth, but its ethanol extractant elicited 14 mm zones of inhibition as shown in Figure 1. An expression of more antimicrobial property at a lower dilution factor and a progressive decline as the doubling dilution observed in this study could be attributed to reduction in concentration of the zinc nanoparticles sequel to doubling dilution which corroborates the study of Raghupathi *et.al.*,(2011) on size dependent bacterial growth inhibition and mechanism of antibacterial activity of zinc oxide nanoparticles (Raghupathi *et.al.*,2011).

Zinc nanoparticles extracts elicited different antibacterial activity at a concentration of 2.5µg/mL. The zones of growth inhibition recorded were smaller in diameter than what was obtained in Figure 1 when the concentration of 5µg/mL was explored. Isolate number1, *Xanthomonas campestris vesicatoria* 1 zinc oxide nanoparticles undiluted aqueous and ethanol extracts concentrations elicited antibacterial activity of 12mm and 18 mm while at the dilution of 1:32, 8mm and 12mm zones of growth inhibition were recorded. The difference observed could be due to strain variations among the isolates while *Xanthomonas campestris vesicatoria* 5, elicited 16 mm and 20 mm at undiluted form and no growth in the aqueous and ethanol extract at 1:32, as elicited in Figure 2 (Seil *et.al.*,2009)

The activity of zinc oxide nanoparticles at a concentration 1.25µg/ml elicited comparable

antimicrobial activity, from stock concentration as to other varied doubling dilutions but the zones of growth inhibition recorded were found to be lesser than what was obtained in Figure 2 when concentration of 2.5 µg/ml was used. *Xanthomonas campestris vesicatoria* 1 zinc oxide nanoparticles undiluted aqueous and ethanol extracts exhibited antibacterial activity, zones of inhibition of 8mm and 10 mm were recorded at the undiluted aqueous and ethanol extracts while at the dilution of 1:32, no zones of growth inhibition were recorded. *Xanthomonas campestris vesicatoria* 5, elicited 10 mm and 15 mm at undiluted form and no growth were recorded at 1:32 aqueous and ethanol extract as shown in Figure 3( Aslam *et.al.*,2012)

Gentamicin, a broad spectrum and standard antibiotic used in this study was effective against *Xanthomonas campestris vesicatoria* at every dilution as indicated in Figure 4.

The zone of growth inhibition observed in all the dilutions established the efficacy of gentamicin, a broad-spectrum antibiotic, which agreed with the study of Adams *et.al.*, (2006) on the comparative ecotoxicity of nanoscale zinc oxide water suspension (Adams *et.al.*, 2006) Having compared zinc oxide nanoparticles and gentamicin activities against the isolates of *Xanthomonas campestris vesicatoria* obtained from local market in Iperu, it was evident that the undiluted zinc oxide nanoparticles at both lower and higher dilution factors in relation to concentrations, exhibited remarkable antibacterial activity. The remarkable zones of growth inhibition of *Xanthomonas campestris vesicatoria* recorded from zinc oxide nanoparticles in comparison with the gentamicin could be attributed to the composition of antimicrobial potential enshrined within the nanoparticles.

## CONCLUSIONS

Though this study explored the potential of alternative pest control management for the treatment of bacterial infested tomato specimen, zinc oxide nanoparticles elicited a remarkably competitive antimicrobial activity in comparison with gentamicin against the isolates of *Xanthomonas campestris vesicatoria* obtained in this study. Further studies may be necessary to unravel other antimicrobial parameters of zinc oxide nanoparticles and its side effects in addition to the evidence of quantitative antimicrobial activity from this study.

**COMPETING INTEREST: No conflict of interest**

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