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In vitro evaluation of different nanoparticles and green synthesized nano pesticides against *Streptomyces scabies* causing the disease "common scab of potato"

Muhammad Haseeb Tahir*, Iram Khalid, Waqar Akram, Abdul Sattar

Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan.
*Corresponding author email: haseebtahiruaf2345@gmail.com

Abstract: Potato (Streptomyces scabies) is thought to be a common and economically important disease of potato worldwide. It is most important kitchen item which is cooked as vegetable. Potato crop is affected by so many diseases, but bacterial diseases are more common. In this context an environment friendly approach of using green synthesis nanoparticles to manage this pathogen was used. In this study, disease causing bacteria was isolated from infected potatoes. Bacteria was purified and identified under microscope on the base on morphological characteristics. In the context of disease management, the pathogen was treated with different green synthesis nanoparticles. Common scab of potato produced by *Streptomyces scabies* is among one of the destructive diseases of potato in Pakistan. The market value and quality of potatoes are reduced by this disease. The current study was conducted out to evaluate the effectiveness of different nano particles and green synthesized nano pesticides against *Streptomyces scabies*. In vitro experiments were laid down in complete randomized design (CRD) and the inhibition zone technique was used for the evaluation of nano pesticides. The results of the analysis of variance demonstrated a substantial difference between the different therapies for limiting bacterial growth against disease under disease pressure. After 72 hours, the mean comparisons revealed that single and consortium administration of nanoparticles and green manufactured nano insecticides inhibited *S. scabies* (mm). The combination of ZnO+Streptomycin injection (27.930 mm) followed by Streptomycin injection therapy exhibited the lowest mean comparison of bacterial growth (28.330 mm). The mean comparison showed that the maximum bacterial growth in control (38.330) at all concentrations of 25ppm, 50ppm and 75ppm respectively. The results were most effective at concentration at 75ppm for 72 hours. The combination of ZnO+Streptomycin injection was the most effective among all the treatments against *S. scabies*. It is concluded that the results of this research will help to combat the common scab disease of potatoes and enhance potato crop exports.
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Key words: Nanoparticles, *Streptomyces scabies*, potato

INTRODUCTION

Potato is considered as one of the most valuable vegetables in the world. It is regarded as one of the most popular and important vegetables in the world. It is an important cash as well as industrial crop of many parts of the world (Babalola *et al*., 2010). It contributes to improve nutrition and livelihood for both urban and rural population. However, the potato production decreased by 5.3 % as compared to last year's production (Economic survey, 2019-20). In Pakistan, the potato is cultivated over an area of 0.15 million hectares, with annual production of 2.9 million tons (Majeed *et al.,* 2015. The potato is grown in three seasons; spring, summer and autumn are ranging from plain to hilly agro-economical areas in Pakistan (Anwar *et al*., 2015).

The potato is the main staple food of many countries, which comprises many nutrients, proteins, carbohydrates, vitamins, minerals, fiber contents and energy (Enciso *et al*., 2018). In the world, China is the largest potato producing country with 99.5 million tons production per year with a production share of 25.02%, followed by other countries such as Russia, India and the United States (FOAST, 2018). Despite its food security significance and great market value, the potato crop is susceptible to many ailments caused by bacteria, viruses, nematodes and fungi (Gondal *et al.,* 2012). Among bacterial diseases, the common scab is the most devastating disease of the potato, which causes economic losses to the potato-growing countries of the world (Al-Mughrabi *et al.,* 2016). This disease is caused by *Streptomyces scabies*, which belongs to the phylum actinobacteria, which is one of the biggest taxonomic units among the 18 major lineages of bacteria and its divergence from other bacterial species that is

currently not possible to identify their most closely related group (Ventura *et al.,* 2007).

Arguably, actinobacteria's best-studied genus is *Streptomyces,* which have complex developmental life cycles. Potato scab disease, which is caused by *Streptomyces scabies* has been reported in many countries such as China, South Africa, Pakistan, Iran, Russia, India, United States and several other countries of the world. This disease includes many symptoms such as raised, deep pitted, sunken lesions and scab like surface on the tuber (Ahmad *et al.,* 2017) The key stages of the *S. scabies* are the germination of spores like fungi and the outgrowth of mycelia for substrate feeding. Despite many nutritional and other stresses, this actinobacteria can germinate aerial reproductive hyphae, which reproduce many cell division spores. *Streptomyces spp.* produce many antibiotics against other pathogens, but these chemicals are produced at the stationary stage. *Streptomyces sp.* interacts directly as well as indirectly with the plant, has positive (biocontrol of many plant pathogens, biofertilization) and negative (plant diseases like the common scab of potato) impacts on the health of the plant (Francis *et al*., 2010).

However, many species of *Streptomyces* such as *S. scabies, S. ipomoeae, S. turgidiscabies* and *S. acidiscabies* cause many symptoms on several hosts that include deep pitted and raised scab-like lesions on potato, beet, radish and peanut crops. These crops are economically important, but they reduce these crops' market and consumption values. Potato scab disease is transmissible from seed and soil sources (Jansky *et al*., 2018). The disease develops when the tuber starts emerging in the first growth stages of tubers when enlarges or direct penetration to the epidermis and enlarging the potato tuber. Programmed cell death occurs near the diseased areas of tubers. Then these spots/lesions consequently transfer into deep pitted shallow lesions due to the bacterization of nearby tuber areas, which are the initial symptom development of the disease. These lesions, which develop on tubers, are circular when they are multiple; these merge to develop asymmetrical scabby lesions. Some other factors can affect the production of the potato, such as the unavailability of seed, poor quality seed and management problems (Meng *et al*., 2012).

The potato crop is susceptible to black scurf, powdery scab (*Spongospora subterranea*), wilt (*Verticillium albo-atrum),* but highly susceptible to common scab (*Streptomyces scabies* family streptomycetaceae. *Streptomyces spp* are the source for the production of numerous antibiotics; among the most important of these are streptomycin from *(S. griseus),* neomycin (S. fradiae), daptomycin*(S.oseosporus),* chloramphenicl(*S. venezuelae),* lincomycin *(S. lincolnesis),* fosfomycin *(S. radial),* oleandomycin and boromycin (*S. antibioticus*), mycangimycin (*Streptomyces spp. SPB74*), tunicamycin (*S. orulosus)* and puromycin *(S. alboniger*)(Hossain *et al.,* 2015).

Hofman *et al.,* (2014) argued that nanoformulations of pesticides or nanopesticides must offer a wide evariety of benefits (including increased effectiveness and durability, good dispersion and wettability, ability to biodegrade in the soil and environment, lack of toxicity, photogenerative nature) and have a reduced amount of pesticide properties so that they can be employed to effectively protect crops against insect pests and diseases. Target-specific nano-pesticides should thus help in reducing the damage to non-target plants and decrease the amount released into the surrounding environment. Green (2014) asserted these systems can be operated for individual goals or a combination of different ones such as (i) time-controlled release, (ii) spatially-targeted release. The main function of the nanocarrier or nanoencapsulation is (i) to protect the AIs before their target-specific release, (ii) to improve the solubility and penetration of the AIs into the targeted plant tissues, and (iii) to modify or control the release functions of AIs into the surrounding environment (Annamalai *et al.,* 2015).

Multiple species of bacteria and fungi have been investigated for the growth of nanoparticles of different composition and size, for example, synthesis of gold by *Verticillium sp*. (Makarov *et al*., 2014). Considering the advantages of green synthesis over other methods, this study aims at the synthesis of silver NPs (AgNPs) using aqueous Neem (*Azadirachta indica*) leaves extract. It focuses on the study of the effects of various physico chemical parameters on AgNps. We also attempt to investigate about the antimicrobial effect of the synthesized nanoparticles. *Azadirachta indica,* which is a common plant known as Neem is found abundantly in India and in nearby Indian subcontinents. It belongs to Meliaceae family and is known for its various applications especially its medicinal property (Subapriya & Nagini, 2005).

Materials and methods

Diseased plant samples collection

Diseased potatoes showing typical symptoms of common scab were collected from Vegetable Research Area of Ayoub Agricultural Research Institute, Faisalabad. These samples were brought to laboratory after proper packaging for bacterial isolation and "invitro evaluation" of different chemicals, nanoparticles and phytoextracts against *Streptomyces scabies*.

Fig 1. Potato showing symptoms of common scab.

Media preparation and autoclaving

A specific growth media is required for the vigorous growth of the bacteria *Streptomyces scabies*. The *S. scabies* show excellent growth on Nutrient agar (NA) medium. Hence forth, NA was used for the cultivation of this bacteria. For preparation of NA, 50 grams of nutrient agar powder and were mixed in sterile bottle in one-liter distilled water and mixed it thoroughly to dissolve the ingredients well and boiled for 10 minutes. After that, for sterilization, media was autoclaved at 121 ºC for 15 mins at 15 psi pressure. After autoclaving all the apparatus was taken from autoclave machine and brought to the laminar flow for further process. For pouring, 20 ml of sterilized media was poured in each of the sterile petri plates. After that, these plates were placed in laminar flow chamber for solidification of media. All the process was done within laminar flow chamber under the sterilized environment.

Isolation of bacteria

All the required apparatus such as blades, scissors, media plates, burner, rapping tape, distilled water was shifted to laminar flow chamber for isolation of bacteria. 1-2 cm pieces were cut off from infected potatos. These samples were surface sterilized with 0.20 % sodium hypochlorite (NaOCl) and then given serial washings in distilled water for 5 minutes in a laminar flow chamber. Then samples were placed on tissue paper for water soaking purpose. After that, these samples were placed on NA containing petri plates with the help of sterile forceps. Four to five samples were placed on each media plate at equal distance. These plates were then wrapped with cellophane tape and incubated in incubator at 25-30 ºC for 6-7 days and allowed the bacteria to grow on the plates. Then scabies colonies were observed.

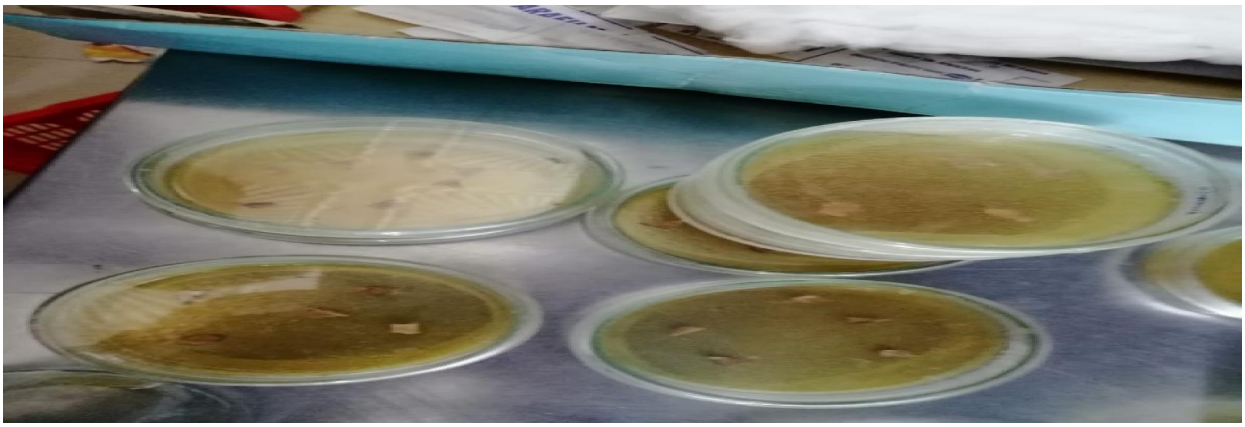

Fig 2. Sterile NA plate having disease samples for *Streptomyces Scabies* isolation

Purification and identification of bacteria

After 48 hours of incubation, different bacterial colonies appeared on (NA) plates. The hyphae of bacterial colony showing creamy growth was picked with the help of sterile needle and streaked on another fresh plate having Nutrient Agar for purification of *Streptomyces scabies*. These new plates were also incubated at 25-20ºC temperature in incubator. Hence by this method the bacteria were purified. After 6-7 days, when bacteria got spores, microscopic slides were prepared for the identification of bacteria. For preparation of glass slides, clean slides were used inside laminar flow chamber. Sterile water drop was placed in the center of slide. A sterile needle was rubbed on the surface of culture plate to pick up the spores. The tip of the needle was then dipped in the drop of water present on glass slide and mixed well with water drop on slide. Glass slide was then covered with cover slip. After that, the prepared glass slides were observed under microscope for colony color and colony morphology under different magnification of the power objectives. The slides were observed under 40x power lenses of M 3300-D microscope. *Streptomyces scabies* identification was done on the basis of available literature on morphology such as colony colour and size, spore shape, structure and growth pattern.

Fig. 3. Purified culture of *Streptomyces scabies* of potato

Preparation of Mustard meal extract

Mustard meal was used for the preparation of extract. Mustad meal was collected from a local market in Faisalabad and dried under shade and then oven dried at 28°C to remove moisture. Fine powder of these mustard meal was prepared and passed through muslin cloth. Two concentrations for extract, 20g and 40g, were prepared by adding in 100 ml separately of water to make stock solution. This solution was filtered thorough muslin cloth.

Evaluation of green synthesized nanopesticide of mustard meal against *Streptomyces scabies*

To synthesize ZnONPs, freshly prepared 50ml extract of Mustard meal was taken in 100ml beaker and boiled at 70-80°C. Later, about 4g of zinc nitrate (ZnNO3) was added slowly into the hot pericarp extract and immediately reddish-brown solution was formed. ZnNO3 ions were reduced into ZnO. This reaction mixture was heated at 70-80°C, using magnetic stirrer. As the reaction progressed, the color of the reaction was slowly changed from reddish-brown to pale yellow and heating was continued until the formation of reddish orange colored paste. To synthesize CuONPs, 5 ml of mustard meal extract and 20 ml of distilled water was added to a 250 ml beaker and heated at 60°C. 5g of Copper sulphate (CuSO4) was added to the solution and heated at 80 °C with continuous stirring for 4hrs.The Copper sulphate ions was reduced to Copper Oxide nanoparticles and reacted with Mustard meal extract. The formation of copper oxide nanoparticles (CuONPs) was observed by color changing.

Evaluation of Nanoparticles of Streptomycin bactericide against *Streptomyces scabies*

The commercially available Streptomycin (of Strepwell Ltd) was evaluated against Streptomyces scabies. Nanoparticles were prepared by using 0.5g of Streptomycin added into 50ml distilled water having 5 g zinc nitrate Zn(NO3)2 in a beaker for the formation of ZnONPs of Streptomycin separately. In another beaker same pouring was done for 5g of copper oxide (CuO) to make CuONPs of Streptomycin. All the contents in the respective beakers was boiled at 70-80°C.5ml of NaOH was added slowly in each beaker. The contents were transferred to a ceramic crucible followed by heating in a furnace at 400°C for 2 hrs, separately. These materials were crushed to powder form using pestle

and mortar. Different concentrations of Nanopesticides was prepared by adding into 100ml of distilled water, separately. Through inhibition zone technique each concentration was evaluated against the purified culture of S. Scabies.

Inhibition Zone technique

By using the inhibition zone technique, the green synthesized nanopesticides was tested in vitro. Mustad meal was used for the green synthesis of Copper oxide(CuO) and Zinc oxide (ZnO) NPs to control the *Streptomyces scabies*. Four different treatments with three replications were used in which three treatments with different concentrations (such as 25, 50 and 75 ppm) of (CuO) NPs, (ZnO) NPs, Streptomycin injection and mustard meal extract was prepared and one of the four treatments was treated as control. By using the inhibition zone technique dip the filter paper in zinc and copper nanoparticles and Streptomycin injection, Mustard meal extract solutions separately one by one and placed in the center of Petri plates. These plates were incubated for 2-3 days under CRD at 25-30 °C. After 24hr, 48hr and 72hr of incubation, respectively, the reading was taken by the mm side of scale. Scale was put on the inhibition zones plates and from upward and downward sides of plates inhibition zone of bacteria was measured.

Table 1. List of treatments used in this experiment.

T_1	Mustard Meal Extract
T_2	Streptomycin injection
T_3	ZnO+Mustard Meal extract
T_4	CuO+Mustard meal extract
T_5	ZnO+Streptomycin injection
T_6	CuO+Streptomycin injection

Results and discussion

Biosynthesis and antibacterial activity of the copper oxide nanoparticles (CuO NPs) and zinc oxide nanoparticles (ZnO NPs) against bacterial strain *Streptomyces scabies* causing common scab of potato.

In agriculture sector, metallic nanoparticles have begun to play a significant role in crop protection field because of their unique physical and chemical features, such as a large surface-to-volume ratio, structural stability, and great affinity for their target. Copper is the most admired and only solid antibacterial material registered among the major metallic nanomaterials. Copper is nanocomposite and has a role in a number of physiological procedures (Wang *et al*., 2015). Phytoextract have been widely used in the biosynthesis of copper oxide nanoparticles (CuO NPs) and zinc oxide nanoparticles (ZnO NPs) due to their environment friendly and cost-effective manner, which provides a highly sustainable economic alternative to the standard synthesis procedure (Mahanty *et al*., 2013). In the present research, CuO NPs and ZnO NPs at different concentrations 25, 50, 75ppm inhibited the growth of *Streptomyces scabies* on Nutrient agar (NA) medium, which consisted of the cultivation of this bacteria preparation of NA, 50 grammes (Figure 1). Nutrient Agar was prepared for 10 minutes in 1-liter distilled water. Inoculating a disc (5 mm in diameter) of 5-day-old bacteria in the middle of a dish containing a mixture of NA medium with varying concentrations of CuO NPs and ZnO NPs was used to quantify bacterial growth in the NA medium. Four to five disinfected sample pieces were placed on petri plates with the use of forceps and wrapped in tape when the media in the petri plates was cooled down.

For 48 hours, inoculated plates were kept at 30 °C in an incubator. After incubation, each standard paper disc was soaked with newly manufactured ZnO nanoparticles, and 5 mm diameter agar wells were created and tagged using a sterilized stainless steel cork borer. Zones of inhibition, which appear as a clear region around the wells and discs, were looked for on the plates. The diameter of such zones was measured with a meter ruler, and the mean value was recorded and represented in millimeters. The occurrence of an inhibitory zone distinctly demonstrates that the biocidal activity of CuO and ZnO nanoparticles requires membrane rupture with a high rate of formation of surface oxygen species, which eventually leads to pathogen death. This study discovered that increasing the quantity of CuO and ZnO nanoparticles in wells and discs resulted in a constant increase in growth inhibition due to optimal nanoparticle dispersion in the agar medium. CuO and ZnO nanoparticles, both nano revealed antibacterial efficacy against the against bacterial strain *S. scabies* causing common scab of potato. Both Gram positive and Gram negative pathogenic bacterial strains investigated in this experiment are significantly harmful to Te CuO-NPs. Te nanoparticles kill microorganisms by entering the body and inhibiting their development. The EDX study of bacteria treated

with CuO-NPs confirmed NP deposition in the bacterial cell (Vaidehi *at al*., 2018).

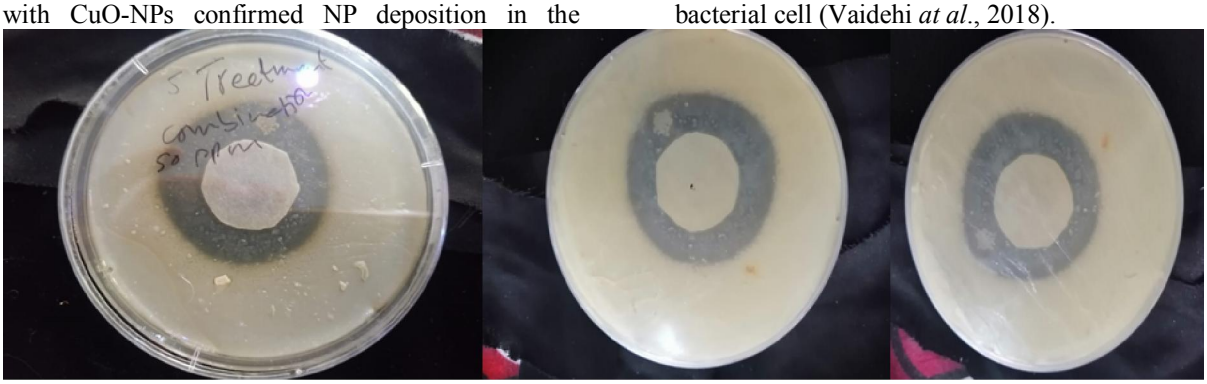

Figure 4. Inhibition zone of copper oxide and streptomycin injection combination against bacterial strain *Streptomyces scabies.*

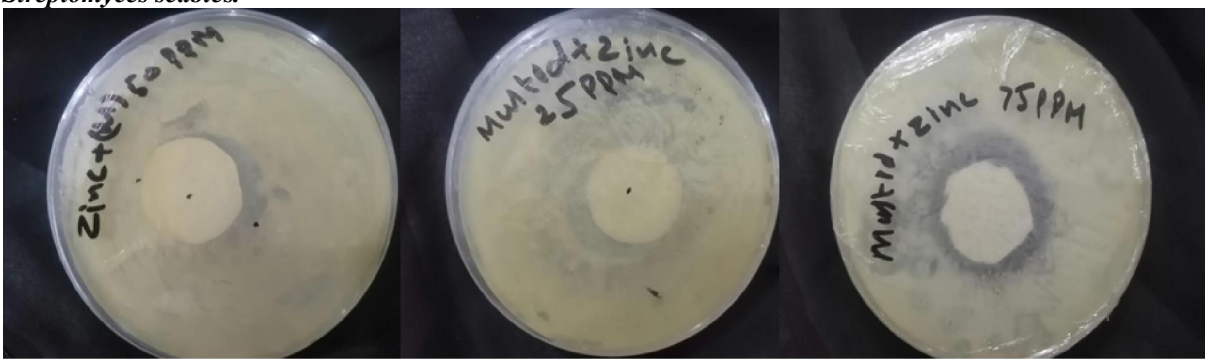

Figure 5. Inhibition zone of combination of Zinc oxide and mustard meal extract against bacterial strain *Streptomyces scabies.*

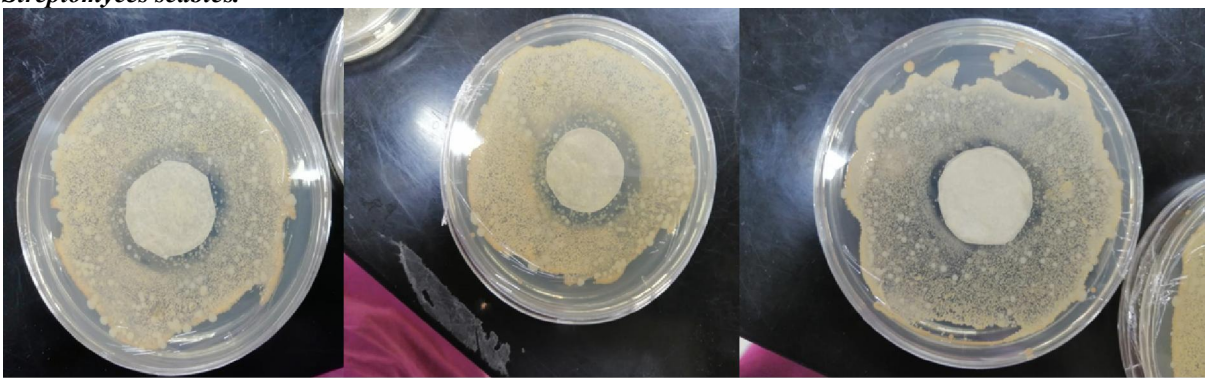

Figure 6. Inhibition zone of Streptomycin injection against bacterial strain *Streptomyces scabies.*

Figure 7. Inhibition zone of zinc oxide and streptomycin injection against bacterial strain *Streptomyces scabies.*

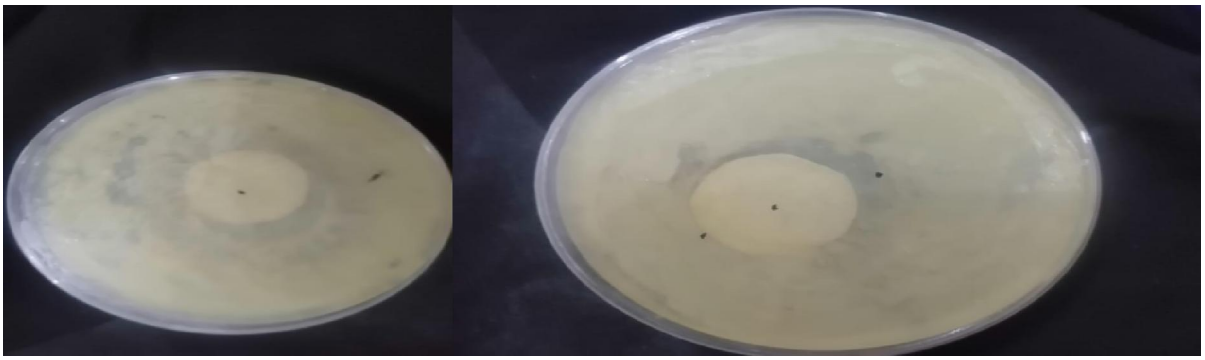

Figure 8. Inhibition zone of mustard meal extract against bacterial strain *Streptomyces scabies.*

Figure 9. Inhibition zone of combination of copper oxide and mustard meal extract against bacterial strain *Streptomyces scabies.*

Structural characterization of prepared nanoparticles by Scanning Electron Microscope (SEM)

Due to its eco-friendliness, biocompatible qualities, and low cost, biological production of metal nanoparticles (NPs) has several benefits as biosynthesis. Chemical techniques are less ecofriendly than biological ones in terms of production rate and NP size control, however chemically produced NPs are less biocompatible due to toxic compounds used as capping and stabilizing agents (Mohd *et al*., 2019). In the present study, CuONPs and ZnONPs were produced in a long-term manner by reducing the precursor with bioactive isolated from Mustard meal. The nanomaterials had a distinct crystal structure of 1 µm and were made up of irregular spherical particles and agglomerations of varying sizes. Furthermore, multiple diffraction rings for monoclinic CuONPs and ZnONPs were visible in the representative selected area electron (SAED) pattern for CuONPs. According to the particle size distribution of CuONP particles, the bulk of nanoparticles were in the 200 nm range. The particle sizes measured in earlier papers created using various methods ranged from 4.8 to 7.8 nm.25. We deduced that the size differences are due to the different types of organic extracts used in the production of metal nanoparticles. Improved extract dispersibility may contribute to more beneficial contact with metal salts, resulting in reduced nanoparticle sizes, according to a past study. In the previous experiment, they used a green way to synthesize CuO-NPs with sizes ranging from 5.9 to 21.8 nm, which has gained a lot of attention because of its eco-friendly and cost-effective nature (Husen and Siddiqi 2014b). The morphology of CuO-NPs and ZnONPs is shown in a SEM micrograph. CuO-NPs and ZnONPs of various sizes are well-dispersed in high-resolution SEM images, even though they are all spherical in shape. The size and form of the reducing extract varies depending on the method of production and the nature of the reducing extract. CuO- NPs appear to be scattered about. They seem like rings sprinkled in the sky at 13,000 magnifications. The spherical NPs are

clearly visible in SEM pictures. The circular ring seen in selected area electron diffraction (SAED) is a feature of NPs' crystalline structure. The figure depicts NPs filled in groups. They look to be related and grouped together. This type of nanoparticles reported in earlier studies; various phytochemicals, ranging from primary metabolites to low molecular weight secondary metabolites, such as terpenoids, alkaloids, polyphenols, quinones, and others, have been documented previously in biogenic synthesis of NPs from plant material (Husen and Siddiqi 2020).

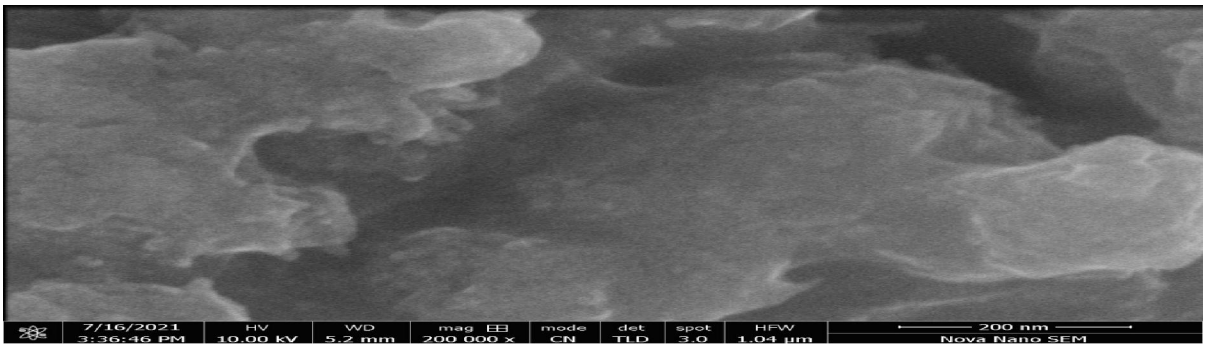

Figure 10. Characterization of biosynthesized copper oxide nanoparticles (CuO NPs). Microscopic images of scanning electron micrographs (SEM) from LUMS University. The particle size with 200 (nm).

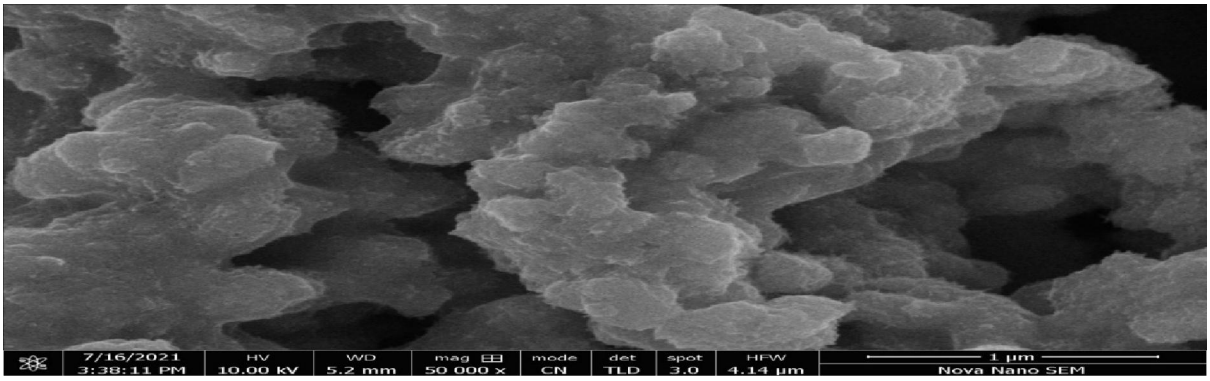

Figure 11. Characterization of biosynthesized copper oxide nanoparticles (CuO NPs). Microscopic images of scanning electron micrographs (SEM) from LUMS University. The particle size with 1 (µm).

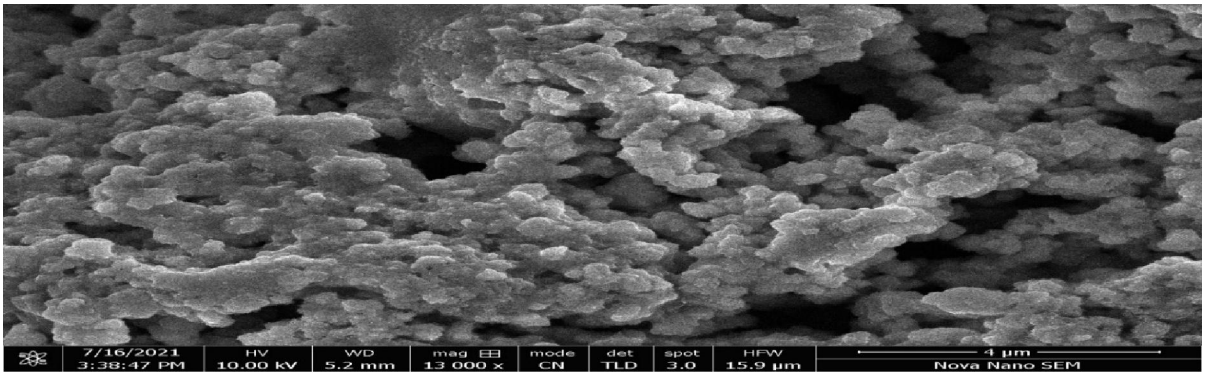

Figure 12. Characterization of biosynthesized copper oxide nanoparticles (CuO NPs). Microscopic images of scanning electron micrographs (SEM) from LUMS University. The particle size with 4 (µm).

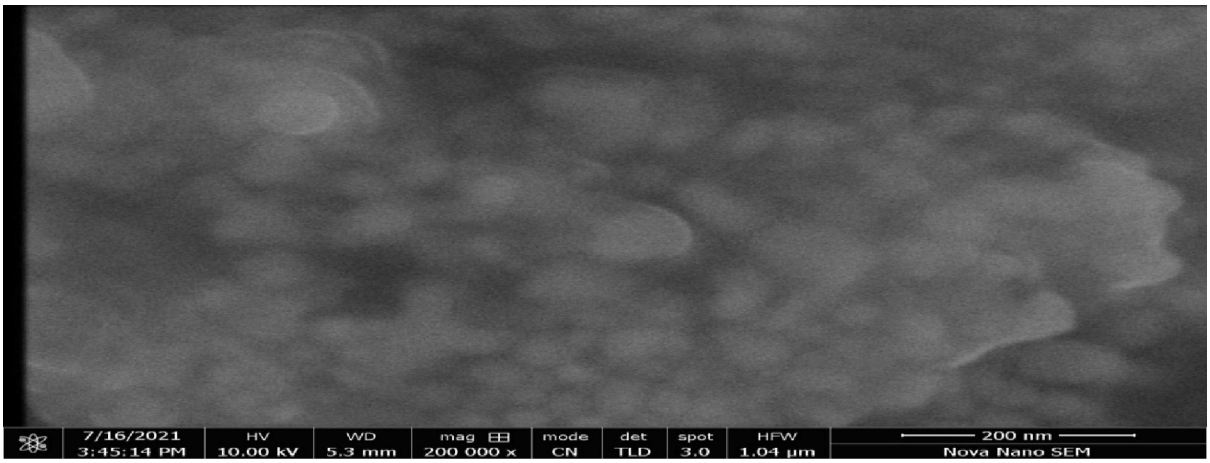

Figure 13. Characterization of biosynthesized zinc oxide nanoparticles (ZnO NPs). Microscopic images of scanning electron micrographs (SEM) from LUMS University. The particle size with 200 (nm).

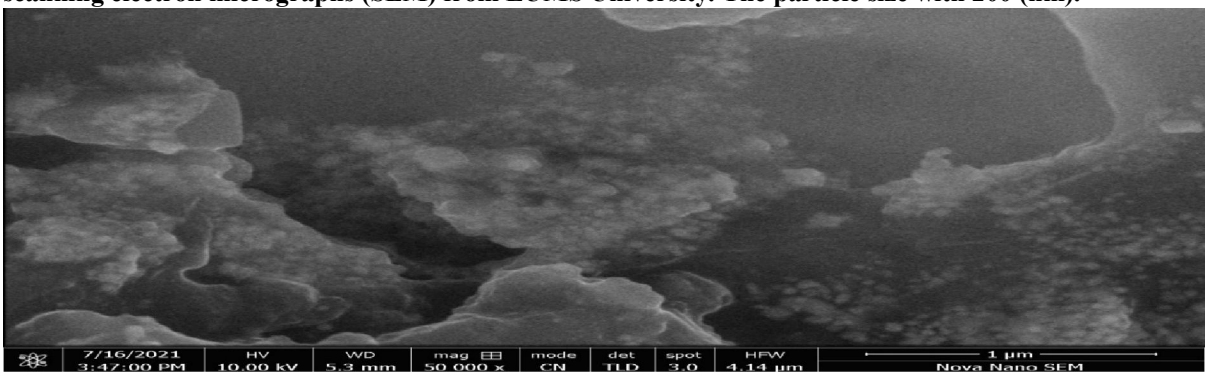

Figure 14. Characterization of biosynthesized zinc oxide nanoparticles (ZnO NPs). Microscopic images of scanning electron micrographs (SEM) from LUMS University. The particle size with 1 (µm).

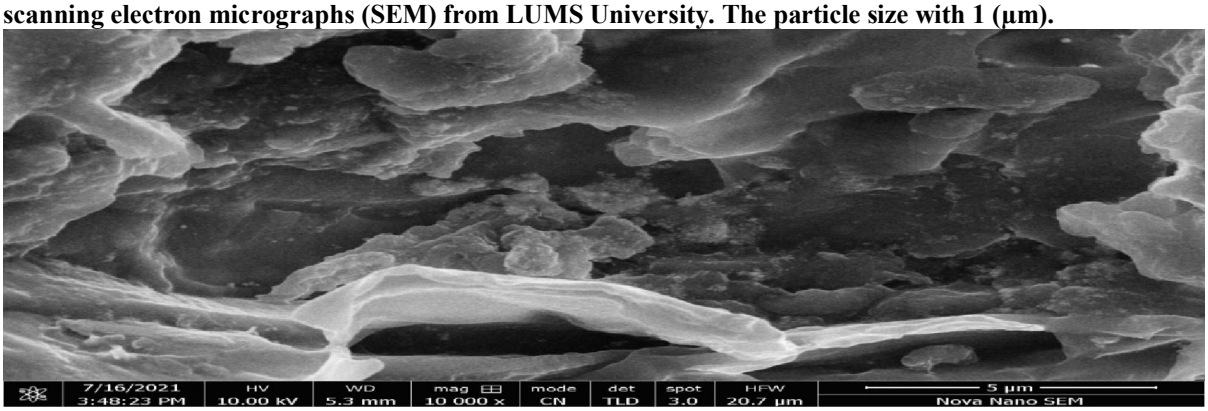

Figure 15. Characterization of biosynthesized zinc oxide nanoparticles (ZnO NPs). Microscopic images of scanning electron micrographs (SEM) from LUMS University. The particle size with 5 (µm).

In vitro assessment of nanoparticles and green synthesized nano pesticides against bacterial strain *Streptomyces scabies*

In the present study, single and combinations of six treatments (T1 = Mustard meal extract, T2 = Streptomycin injection, T3 = ZnO +Mustard Meal extract, T4 = CuO +Mustard meal extract, T5 = ZnO+Streptomycin injection and T6 = CuO + Streptomycin injection) were applied, and the control used for the assessment of nanoparticles against the against bacterial strain *S. scabies*. ANOVA indicated that there was a significant difference between the different treatments which inhibiting the bacterial growth against the disease under disease pressure. Different treatments with different concentrations within specific duration showed significant results. The mean comparisons showed that of single and consortium application of nanoparticles and green synthesized nano pesticides inhibition (mm) against *S. scabies* after 24 hours. The minimum mean

comparison of bacterial growth showed by the combination of ZnO+Streptomycin injection (6.93 mm) followed by treatment application of Streptomycin injection (7.33 mm). The mean comparison showed that the maximum bacterial growth in control (17.33 mm) at all concentrations of 25ppm, 50ppm and 75ppm respectively. As a result, it has a lot of ability for usage on its own or in combination with other fungicides and control strategies for improved eco- and bio-functions. Copper and CuO incorporation in fungicides for *C. gloeosp* therapy has also been examined, with impacts on mycelial growth (Oussou at al., 2020). According to Rampersad and Teelucksingh, (2012) increasing copper fungicide concentrations decreased spore existence in Colletotrichum isolates. Similarly, the interaction between treatment and concentration (T×C) showed that combination of ZnO+Streptomycin injection expressed that minimum bacterial growth (9.3 mm) at 25ppm concentration followed by (8.2 mm) at 50ppm concentration and (6.3 mm) at 75ppm respectively. While CuO + Streptomycin injection NPs expressed minimum fungal growth (9.3, 7.3 and 5.3 mm) at the concentrations of 25ppm, 50ppm and 75ppm respectively. The treatment application of Streptomycin injection also showed the minimum bacterial growth (9.3 7.2 and 4.2 mm) at the concentrations of 25ppm, 50ppm and 75ppm respectively.

Table 2. Analysis of variance for in vitro assessment of nanoparticles and green synthesized nano pesticides against bacterial strain *Streptomyces scabies* after 24 hours.

Source	DF	SS	MS	F	P
Treatment	6	227.90	37.98**	11.7	0.0001
Error	14	45.49	3.24		
Total	20	273.40			

Grand Mean 9.5538 CV 18.87

NS= non-significant (P>0.05) * significant (P<0.05) ** highly significant (P<0.01)

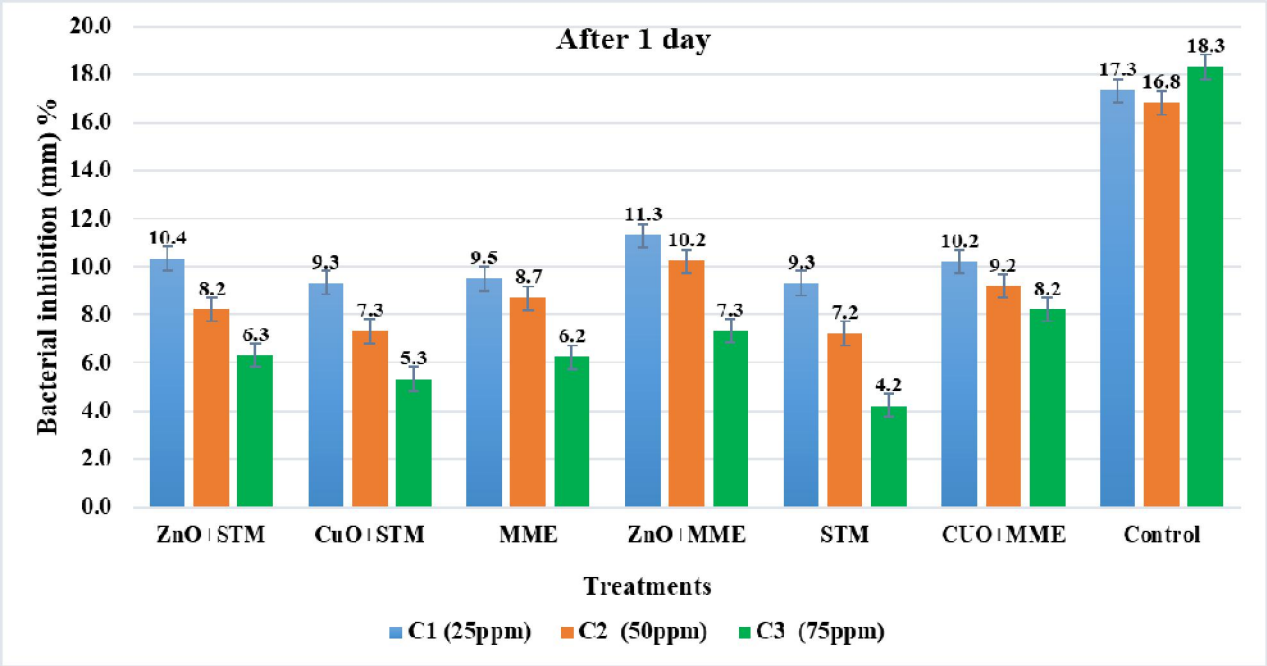

Fig 16. In vitro assessment of nanoparticles and green synthesized nano pesticides in combinations at different concentration inhibition in (mm) against *Streptomyces scabies* after 24 hours.

In the present study, single and combinations of six treatments (T1 = Mustard meal extract, T2 = Streptomycin injection, T3 = ZnO +Mustard Meal extract, T4 = CuO +Mustard meal extract, T5 = ZnO+Streptomycin injection and T6 = CuO + Streptomycin injection) were applied, and the control used for the assessment of nanoparticles against the against bacterial strain *S. scabies*. Analysis of variance revealed that there was a significant difference between the different treatments which inhibiting the bacterial growth against the disease under disease pressure. Different treatments with

different concentrations within specific duration showed significant results. The mean comparisons showed that of single and consortium application of nanoparticles and green synthesized nano pesticides inhibition (mm) against *S. scabies* after 48 hours. The minimum mean comparison of bacterial growth showed by the combination of ZnO+Streptomycin injection (16.93 mm) followed by treatment application of Streptomycin injection (17.33). The mean comparison showed that the maximum bacterial growth in control (27.33 mm) at all concentrations of 25ppm, 50ppm and 75ppm respectively.

The interaction between treatment and concentration (T×C) showed that combination of ZnO+Streptomycin injection expressed that minimum bacterial growth (20.24 mm) at 25ppm concentration followed by (18.2 mm) at 50ppm concentration and (16.3 mm) at 75ppm respectively. While CuO + Streptomycin injection NPs expressed minimum fungal growth (19.3, 17.3 and 15.3 mm) at the concentrations of 25ppm, 50ppm and 75ppm respectively. The treatment application of Streptomycin injection also showed the minimum bacterial growth (19.3 17.2 and 18.2 mm) at the concentrations of 25ppm, 50ppm and 75ppm respectively (Figure 14). Cu-NPs have been shown to exhibit antibacterial action against a variety of fungus in numerous investigations. According to the findings, all copper forms inhibited hyphal growth in a dose-dependent way, with ZnO-NPs and CuO-NPs having the strongest inhibitory impact against the pathogen. There are few and recent statements on the effects of copper-based treatments on fungus, particularly *C. gloeosp*, showing that this metal's application is extremely topical, unique, and focusing (Oussou at al., 2020). This shows that oxidized nanoparticle materials have a different method of action than conventional nanoparticle materials. CuO-NPs' antibacterial activity is still a subject of debate due to a scarcity of data.

Table 3. Analysis of variance for in vitro assessment of nanoparticles and green synthesized nano pesticides against bacterial strain *Streptomyces scabies* after 48 hours.

Source	DF	SS	MS	F	P
Treatment	6	227.90	37.98**	11.7	0.0001
Error	14	45.49	3.24		
Total	20	273.40			

Grand Mean 19.554 CV 9.22

NS= non-significant (P>0.05) * significant (P<0.05) ** highly significant (P<0.01)

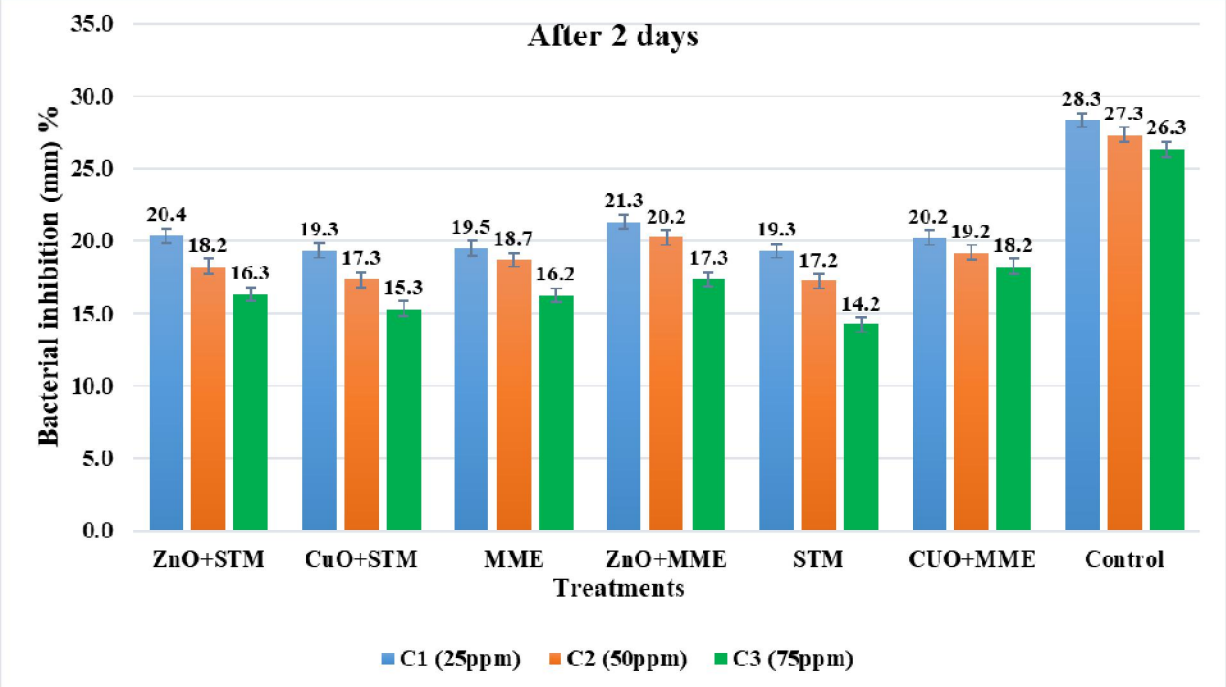

Fig. 17. In vitro assessment of nanoparticles and green synthesized nano pesticides in combinations at different concentration inhibition in (mm) against *Streptomyces scabies* after 48 hours.

In the present study, single and combinations of six treatments (T1 = Mustard meal extract, T2 = Streptomycin injection, T3 = ZnO +Mustard Meal extract, T4 = CuO +Mustard meal extract, T5 = ZnO+Streptomycin injection and T6 = CuO + Streptomycin injection) were applied, and the control used for the assessment of nanoparticles against the against bacterial strain *S. scabies*. Analysis of variance revealed that there was a significant difference between the different treatments which inhibiting the bacterial growth against the disease under disease pressure. Different treatments with different concentrations within specific duration showed significant results. The mean comparisons showed that of single and consortium application of nanoparticles and green synthesized nano pesticides inhibition (mm) against *S. scabies* after 72 hours. The minimum mean comparison of bacterial growth showed by the combination of ZnO+Streptomycin injection (27.930 mm) followed by treatment application of Streptomycin injection (28.330 mm). The mean comparison showed that the maximum bacterial growth in control (38.330) at all concentrations of 25ppm, 50ppm and 75ppm respectively.

Generally, the current research found that CuO NPs and ZnO NPs have antibacterial properties against *S. scabies*. The nanoforms have a strong inhibitory influence on hyphal development in pathogens (Mocanu *at al*., 2019). Only Cu-NPs., not its oxidized form, are effective at preventing *S. scabies* spore germination. The findings showed that the antibacterial activity of ZnO NPs is enhanced by their smaller particle size, which is related to their higher surface area to volume ratio, which increases surface reactivity and the discharge of additional ions (Mohd *at al.*, 2019). The previous results is similar to our studies as the interaction between treatment and concentration (T×C) showed that combination of ZnO+Streptomycin injection expressed that minimum bacterial growth (31.4 mm) at 25ppm concentration followed by (29.2 mm) at 50ppm concentration and (27.3 mm) at 75ppm respectively. While CuO + Streptomycin injection NPs expressed minimum fungal growth (30.3, 28.3 and 26.3 mm) at the concentrations of 25ppm, 50ppm and 75ppm respectively. The treatment application of Streptomycin injection also showed the minimum bacterial growth (31.3 30.2 and 29.2 mm) at the concentrations of 25ppm, 50ppm and 75ppm respectively.

Table 4. Analysis of variance for in vitro assessment of nanoparticles and green synthesized nano pesticides against bacterial strain *Streptomyces scabies* after 72 hours.

Source	DF	SS	MS	F	P
Treatment	6	227.90	37.98**	11.7	0.0001
Error	14	45.49	3.24		
Total	20	273.40			

Grand Mean 30.554 CV 5.90

NS= non-significant (P>0.05) * significant (P<0.05) ** highly significant (P<0.01)

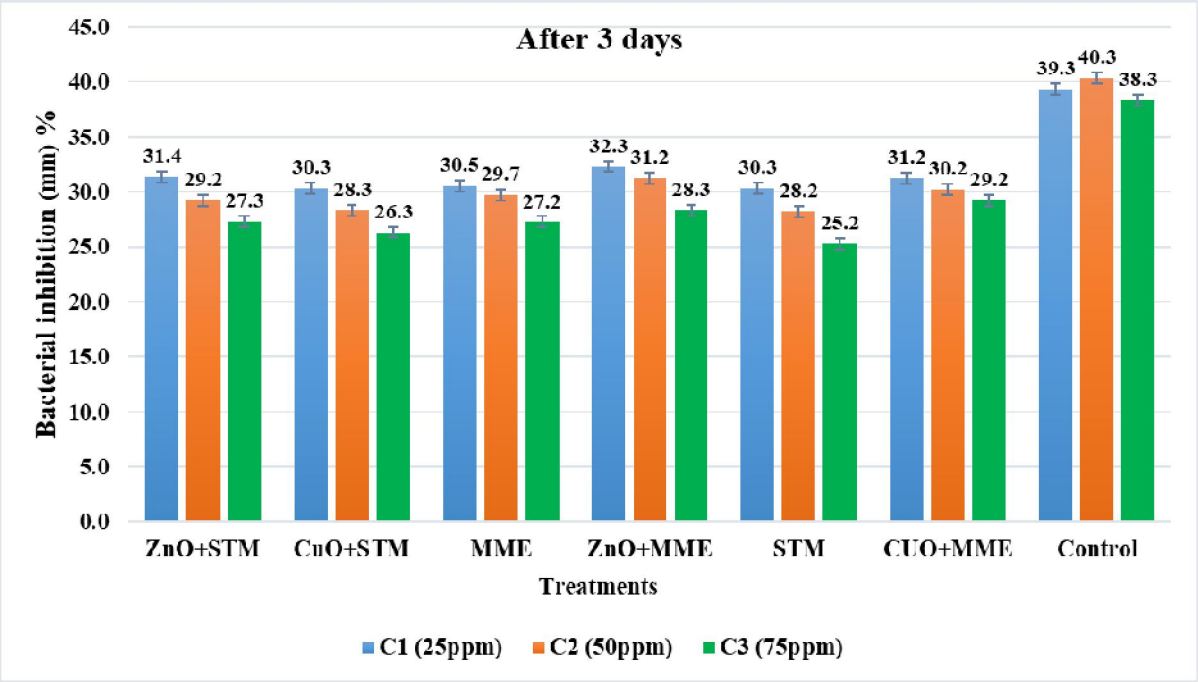

Fig 18. In vitro assessment of nanoparticles and green synthesized nano pesticides in combinations at different concentration inhibition in (mm) against *Streptomyces scabies* after 72 hours.

References

[1]. Babalola D.A., Y.O. Makinde, B.T. Omonona, M.O. Oyekanmi. 2010. Determinants of post- harvest losses in tomato production: a case study of Imeko – Afon local government area of Ogun state. Journal of Life and Physical Science. Acta SATECH. 3: 14 – 18.

[2]. Economic Survey of Pakistan. 2019-2020. Ministry of Finance, Islamabad, Pakistan.77: 18-20

[3]. Majeed, A., Abbasi, M. K., Hameed, S., Imran, A., and Rahim, N. (2015). Isolation and characterization of plant growth-promoting rhizobacteria from wheat rhizosphere and their effect on plant growth promotion. Front. Microbiol. 6:198-203.

[4]. Anwar, D., Shabbir, D. Shahid, M.H. Samreen, W. 2015. Determinants of Potato Prices and Its Forecasting a Case Study of Punjab, Pakistan. 16 September 2015.

[5]. Enciso-Rodriguez, F., Douches, D. Lopez-Cruz, M. Coombs, J. de Los Campos, G. 2018. Genomic selection for late blight and common scab resistance in tetraploid potato (Solanum tuberosum). G3 Gene Genomes Genet. 8: 2471–2481.

[6]. FAO. 2018. World food and agriculture-statistical pocketbook. FAO: Rome, Italy. 2: 112-118.

[7]. Gondal, A.S., Javed, N. Khan, S.A. Hyder, S. 2012. Genotypic diversity of potato germplasm against root knot nematode (Meloidogyne incognita) infection in Pakistan. Int. J Phytopathol. 1: 27–38.

[8]. Al-Mughrabi, K.I., Vikram, A. Poirier, R. Jayasuriya, K. Moreau, G. 2016. Management of common scab of potato in the field using biopesticides, fungicides, soil additives, or soil fumigants. Biocontrol Sci. Technol. 2016, 26: 125–135.

[9]. Ventura, M., Canchaya, C. Tauch, A. Chandra, G. Fitzgerald, G.F. Chater, K.F. van Sinderen, D. 2007. Genomic of Actinobacteria Tracing the evolutionary history of an ancient phylum. Microbiol. Mol. Biol. 71: 495–548.

[10]. Ahmad, M. S., El-Gendy, A. O., Ahmed, R. R., Hassan, H. M., El-Kabbany, H. M., and Merdash, A. G. (2017). Exploring the antimicrobial and antitumor potentials of Streptomyces sp. AGM12-1 Isolated from Egyptian Soil. Front. Microbiol. 8:438-441.

[11]. Francis, I., Holsters, M. Vereecke, D. 2010. The Gram-positive side of plant–microbe interactions. Environ. Microbiol. 12: 1–12.
[12]. Jansky, S., Douches, D. Haynes, K. 2018. Germplasm release Three tetraploid potato clones with resistance to common scab. Am. J. Potato Res. 95: 178–182.
[13]. Meng, Q., Yin, J. Rosenzweig, N. Douches, D. Hao, J.J. 2012. Culture-based assessment of microbial communities in soil suppressive to potato common scab. Plant Dis. 96: 712–717.
[14]. Hossain, S., Rasul, G. Mian, M.K. Haque, M.M. Akanda, A.M. 2015. Yield Potential of Twelve Potato (Solanum tuberosum) Varieties Grown from Different Generations of Seed. Agri. 13: 120–132.
[15]. Hofmann, M., T. Kah. 2014. Nanopesticide research current trends and future priorities, Environ. Int. 63 :224–235.
[16]. GREEN, J. M., 2014. Current state of herbicides in herbicide-resistant crops, Pest Manag. Sci. 70: 1351–1357.
[17]. Annamalai, J., V. Namasivayam. 2015. Endocrine disrupting chemicals in the atmosphere their effects on humans and wildlife, Environ. Int. 76: 78–97.
[18]. Makarov, V. V., Love, A. J., Sinitsyna, O. V., Makarova, S. S., Yaminsky, I. V., Taliansky, M. E., & Kalinina, N. O. (2014). "Green" nanotechnologies: synthesis of metal nanoparticles using plants. Acta Naturae. Nanotechnol, 6:245-255.
[19]. Subapriya, R., and Nagini, S. (2005). Medicinal properties of neem leaves: a review. Current Medicinal Chemistry-Anti-Cancer Agents, 5(2), 149-156.
[20]. Vaidehi, D., Bhuvaneshwari, V., Bharathi, D., & Sheetal, B. P. (2018). Antibacterial and photocatalytic activity of copper oxide nanoparticles synthesized using Solanum lycopersicum leaf extract. *Materials Research Express*, *5*(8), 085403.
[21]. Mohd Yusof, H.; Mohamad, R.; Zaidan, U.H.; Abdul Rahman, N. Microbial synthesis of zinc oxide nanoparticles and their potential application as an antimicrobial agent and a feed supplement in animal industry: A review. J. Anim. Sci. Biotechnol. 2019, 10, 1–22.
[22]. Husen A, Siddiqi KS. Phytosynthesis of nanoparticles: concept, controversy and application. Nano Res Lett. 2014b; 9:229.
[23]. Oussou-Azo, A. F., Nakama, T., Nakamura, M., Futagami, T., & Vestergaard, M. D. C. M. (2020). Antifungal potential of nanostructured crystalline copper and its oxide forms. *Nanomaterials*, *10*(5), 1003.
[24]. Rampersad, S. N., & Teelucksingh, L. D. (2012). Differential responses of Colletotrichum gloeosporioides and C. truncatum isolates from different hosts to multiple fungicides based on two assays. *Plant disease*, *96*(10), 1526-153.

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