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Highly-Effective Antifungal and Antibacterial Properties of ZnO, ZnS, FeS_2, and SnO_2 Against Various Fungal and Bacterial Isolates

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Nanoparticles have been focussed on greatly to determine their application in various fields of science. Their versatility, which is a result of their size, is the key to their ability to be applied in varying areas of industry. The medical and pharmaceutical fields have seen a rise in resistance to the current treatment regimes available against some bacterial and fungal infections among human beings and animals. This raises a need to find other ways to treat the particular microbes, which have become resistant. This study is focussed on the determination of the ability of nanoparticles to elicit antifungal and antibacterial activities, hence, providing a platform or an option for their use in this regard. The nanoparticles of ZnO, ZnS, FeS_2, and SnO_2 are tested for antibacterial and antifungal activities using the well method. Varying amounts of the nanoparticles are loaded into the wells and observed for the development of inhibition zones after 24 hours of culture at 37°C. The nanoparticles of FeS_2 and ZnO are managed to show broad-spectrum activity against the various bacterial and fungal isolates used in this study as evidenced by the fabrication of clear zones of inhibition.

Велику увагу приділялася наночастинкам, щоб визначити їхні застосування в різних галузях науки. Їхня універсальність, яка є результатом їхнього розміру, є ключем до їхньої здатности застосовуватися в різних галузях промисловости. У медичній і фармацевтичній галузях спостерігається зростання резистентности до наявних режимів лікування деяких бактеріяльних і грибкових інфекцій серед людей і тварин. Це спричиняє потребу знайти інші способи оброблення конкретних мікробів, які стали стійкими. Дане дослідження було зосереджено на визначенні здатности наночастинок виявляти протигрибкову й антибактеріяльну активності, отже, забезпечуючи платформу або варіянт для використання їх в цьому відношенні. Наночастинки ZnO, ZnS, FeS_2 і SnO_2

перевіряли на антибактеріяльну та протигрибкову активності методом лунки. Різну кількість наночастинок завантажували в лунки та спостерігали за розвитком зон інгібування після 24 годин культивування за 37°C. Наночастинки FeS_2 і ZnO показали широкий спектер активности проти різних бактеріяльних і грибкових ізолятів, використаних у цьому дослідженні, про що свідчить утворення чітких зон інгібування.

Key words: nanoparticles of ZnO, ZnS, FeS_2 and SnO_2, antifungal and antibacterial activities.

Ключові слова: наночастинки ZnO, ZnS, FeS_2 і SnO_2, протигрибкова й антибактеріяльна активності.

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1. INTRODUCTION

Nanoparticles are understood to be particles that have a size between 1–100 nanometres [1]. They have been reported for application in biomedicine, advanced materials, pharmaceuticals, electronics, magnetics and optoelectronics, cosmetics, energy, and catalytic and environmental detection and monitoring, communications, sensing and data storage because of their important optical, electrical, and magnetic properties [1, 2]. Nanoparticles are a revelation in the medical field as they are said to be able to kill over 650 cells while antibiotics kill ten percent of what nanoparticles can kill [3].

Major challenge has developed due to resistance by bacteria and fungi to current treatment regimes, because of broad use and abuse, hence, the need to develop and acquire new compounds for bacterial treatments. For example, tuberculosis causing strains have developed resistance to antibacterial treatment that were effective against it and the resistant strains are now causing new infections that are resistant to current treatment regimes [4]. There is a limited range of antifungal drugs that are used against fungal infections, with systemic fungal infections being treated using four mainline classes of molecules, which include fluoropyrimidine analogues, polyenes, azoles, and echinocandins. Morpholines and allyl amines have poor efficiency and severe adverse effects, when administered systemically, hence, are not used like the other counterparts [5]. Hence, there is a great need to find new methods for treating the bacterial and fungal infections.

Nanoparticles have been shown to have antimicrobial, anti-inflammatory and wound healing properties [6, 7], but information is still in its infancy as this is a new field. The small size of nanoparticles, a useful property in industry and medicine, has a direct effect on the reactivity of the nanoparticles, which, as the size gets

smaller, the reactivity increases and leads to higher toxic effects [8]. According to Ref. [9], toxicity can be dependent on a variety of factors, each factor being viable enough to cause toxicity and their combinations with even greater levels of toxicity. These include dose, size, surface area, crystal structure and chemistry, concentration, surface coating and functionalization.

Aggregation of particles has been noted when concentrations of nanoparticles are very high, which lead to a reduction in the toxic effect as compared to lower concentrations [10]. Smaller particles which can get into cells easier have been seen to have higher toxicity as compared to larger particles (or aggregates) as they are easily stopped from entering the cells (macrophages) hence the low toxic levels [11, 12]. Toxicity studies have been carried out in many regards, with some studies taking advantage of the toxicity of nanoparticles to determine their abilities as antimicrobial [13] and their capability to be antifungal agents. Reports have shown that green synthesised ZnO nanoparticles have effective action against bacterial and fungal pathogens [6], while the toxicity of CuO, ZnO and TiO_2 nanoparticles tested against microalgae [14]. Toxicity of metal oxide nanoparticles to *E. coli, Bacillus subtilis* and *Streptococcus aureus* were reported in Refs. [15–17], and ZnO nanoparticles were shown to have an antibacterial activity [18].

This study was aimed at determining the antibacterial and antifungal activities of ZnO, ZnS, FeS_2, and SnO_2 against a number of fungal and bacterial isolates. The thrust being on taking advantage of the toxicity of the nanoparticles to stop fungal and bacterial growth and, in addition, determining whether an increase in concentration or dose has an effect on the activity. This will add more knowledge to the growing field about the ability of these nanoparticles as antimicrobials.

2. MATERIALS AND METHODS

2.1. Fungal and Bacterial Isolates

The fungal (*Aspergillus niger* and *Aspergillus fumigatus*) and bacterial (*E. coli, Bacillus cereus* and *Bacillus subtillis*) isolates were obtained from the ITM University microbiology department. These were stored in a 4°C freezer and were revived by culturing them on SDA or NA respectively at 37°C for 24 hours.

2.2. Nanoparticles

Nanoparticles were obtained from the ITM University physics de-

partment, where they were synthesised using the solution gel method. The nanoparticles had a concentration of 0.5 M in 50 cc of ethanol.

2.3. Testing of Antifungal Activity of Nanoparticles

Sabouraud Dextrose Agar media, 100 ml, was prepared and autoclaved together with 6 Petri dishes at 121°C at 15 lbs of pressure for 15 minutes. Media was dispensed equally in a laminar airflow cabinet into the 6 Petri plates and allowed to set. Two (2) wells were punched at opposite ends on the set media on 4 of the Petri plates using a sterile borer with a diameter of 6 mm. Four (4) of the Petri plates were inoculated with *Aspergillus niger* or *Aspergillus fumigatus* using a spreader. One Petri plate was also inoculated with the fungi and used as a positive control and the negative control was the one not inoculated with the fungi.

2.4. Testing of Antibacterial Activity of Nanoparticles

Nutrient Agar media, 100 ml, was prepared and autoclaved together with 6 Petri dishes at 121°C at 15 lbs of pressure for 15 minutes. Media was dispensed equally in a laminar airflow cabinet into the 6 Petri plates and allowed to set. Two (2) wells were punched at opposite ends on the set media on 4 of the Petri plates using a sterile borer with a diameter of 6 mm. Bacterial suspension was made by taking bacteria on an inoculating loop and suspending it in 1.2 ml of sterilised distilled water. Using a micropipette, 40 µL of the suspension was placed in a Petri plate and spread using a sterilised glass spreader. Four (4) of the Petri plates were inoculated with *E. coli* or *Bacillus subtillis* or *Bacillus Cereus*. One Petri plate, that was not punched wells into, was also inoculated with the fungi and used as a positive control and the negative control was one not inoculated with bacteria.

Nanoparticles of ZnO, FeS_2, SnO_2 or ZnS were loaded into one of the wells aseptically using a micropipette at varying concentrations of 15 µL, 20 µL, 25 µL and 30 µL and, in each Petri plate, an equal amount of ethanol was loaded into the other well. The Petri plates were then cultured at 37°C for 24 hours in an incubator. Cultured plates were observed after 24 hours and were checked for development of a circular zone of inhibition around the well inoculated with nanoparticles or ethanol. The diameter of the zone of inhibition, if developed, was measured using a 30 cm metre rule and recorded. The tests were done three times, and the results were then averaged to get final values.

3. RESULTS AND DISCUSSION

3.1. Antifungal Tests Results

The nanoparticles used in the experiment produced results against fungi as depicted in Figs. 1 and 2 using the well method. The results were based on the production of a zone of inhibition, whose diameter was measured, to ascertain the activity of the particular nanoparticle against the fungi.

The results presented in Fig. 1 show that FeS_2 and ZnO managed

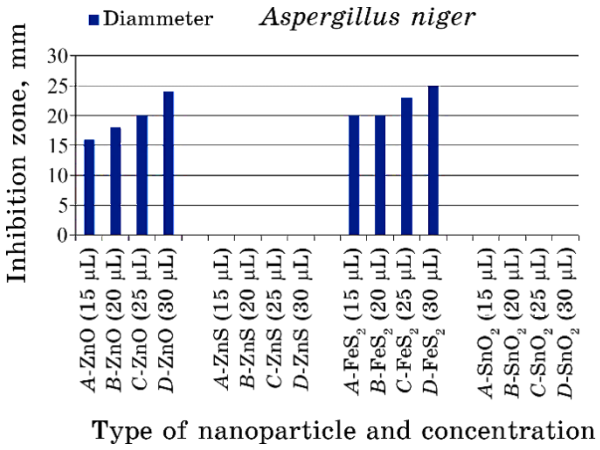


Fig. 1. Diameter of zone of inhibition results produced by nanoparticles at varying concentrations against *Aspergillus niger*.

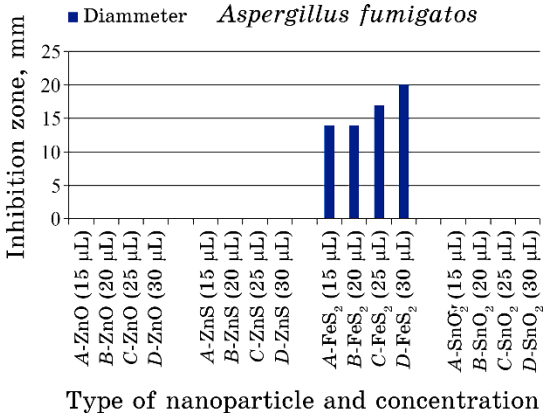
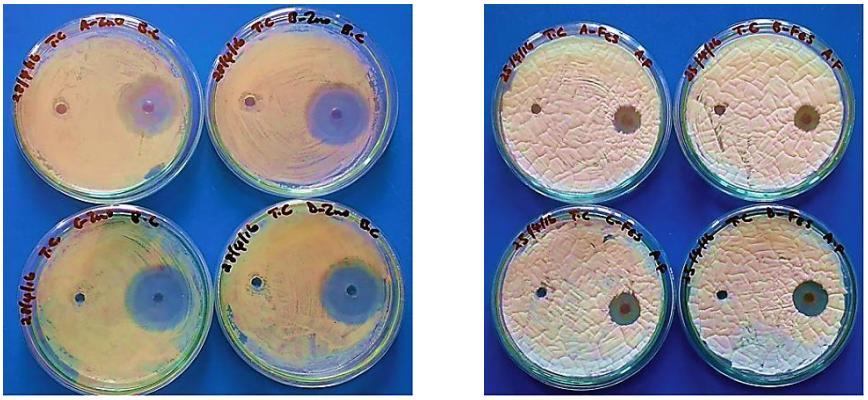


Fig. 2. Diameter of zone of inhibition results produced by nanoparticles at varying concentrations against *Aspergillus fumigatus*.

to produce zones of inhibition against *A. niger* for all the concentrations of nanoparticles used in the experiment. Whilst, SnO_2 and ZnS produced negative results for all the concentrations used against *A. niger*. According to Ref. [6], the presence of zone of inhibitions is an indicator of the fungicidal action of the nanoparticles, with the mechanism of action highly being that of ROS production. This was in agreement with results obtained in this study. The diameter of the zone of inhibition increased for FeS_2 and ZnO, which had positive results, as the concentration of the nanoparticles increased. The production of the zone of inhibition by FeS_2 and ZnO against *A. niger* showed that the nanoparticles have good antifungal activity, as they managed to prevent the growth of the fungi near the loaded wells. The FeS_2, however, proved to have better antifungal activity than the ZnO against *A. niger* as the minimal concentration (15 μL) had 20 mm diameter of zone of inhibition as compared to ZnO, which had 16 mm. While the 30-μL concentration for FeS_2 had a 25 mm zone of inhibition and ZnO having 24 mm. The FeS_2 15 μL and 20 μL had the same zone of inhibition diameter (of 20 mm) showing that the increase in concentration, from 15 μL to 20 μL, did not lead to an increase in the antifungal activity. This was in agreement with what Ref. [30] highlighted, that the toxicity of the nanoparticles can be dose dependant or concentration dependant. However, it was contrary to what Ref. [68] indicated that higher concentrations did not have effective toxicity as the high concentrations led to aggregation of the nanoparticles to form large molecules. The inability of SnO_2 and ZnS to produce zone of inhibitions can be attributed to the fact that the fungi, *A. niger*, might have less sensitivity to the nanoparticles and the toxic effect of the nanoparticles is not effective against the fungi because of this [6]. This can also explain the difference in the inhibition zones for the ZnO and FeS_2, which showed to have its toxic effect exerted against the fungi as the fungi showed susceptibility to the toxicity of the nanoparticles [19–21].

The results in Fig. 2 show that FeS_2 nanoparticles were the only ones which showed antifungal activity against *A. fumigatus* as it was the only one that managed to produce zones of inhibition against the fungi (as shown in Fig. 3). The other three nanoparticles, ZnS, SnO_2 and ZnO, did not produce any clear zones of inhibition (had negative results). This could be because the *A. fumigatus* fungi species were not susceptible to the toxic effect of the nanoparticles used, while the toxic effects of FeS_2 were strong enough to elicit an inhibition to fungal growth.

Figure 2 shows that the FeS_2 of 15 μL and 20 μL had the same diameter of zone of inhibition of 14 mm, showing that increase from 15 μL to 20 μL did not lead to an increase in antifungal activ-



a *b*

Fig. 3. Images showing the zone of inhibition exerted by (*a*) ZnO nanoparticles against *Bacillus cereus* and (*b*) FeS_2 nanoparticles against *Aspergillus fumigatus*.

ity. This anomaly is similar to the one that FeS_2 produced against *A. niger*, where an increase in concentration from 15 µL to 20 µL did not lead to an increase in the antifungal activity. This could explain that the 15 µL and 20 µL concentrations of FeS_2 produce the same results, and the increase from 15 µL to 20 µL is not significant enough to elicit a difference in the activity against the fungi. However, the FeS_2 produced zones of inhibition against *A. fumigatus*, which are smaller than those it produced against *A. niger*. Ref. [17] explained that the effect of the nanoparticles toxicity on the microorganism was not only dependant on the nanoparticles type but also the bacterial species involved. This was in agreement with results obtained as the sensitivity of the fungi was different, with the *A. fumigatus* being less sensitive to the toxic effect and hence the nanoparticles exerted different toxic effects to the fungi. The maximum for FeS_2 against *A. fumigatus* was of 20 mm, while the minimum for FeS_2 against *A. niger* was of 20 mm showing that FeS_2 had smaller zone of inhibition against the *A. fumigatus* fungi.

This can also be an indicator that the *A. fumigatus* fungi was more resistant strain, as compared to *A. niger*, as it produced smaller zones of inhibition. This can also be supported by the fact that ZnO, which produced clear zones of inhibition against *A. niger* only, managed to reduce the growth of the *A. fumigatus* within the proximity of the well, but did not produce clear zones of inhibition. This could also signify that maybe an increase in concentration of ZnO above 30 µL can lead to a production of the zones of inhibition. It is also important to note that the nanoparticles were dissolved in ethanol and one of the wells on the left was loaded with ethanol. The

results show that the ethanol did not manage to prevent the growth of the fungi (as there was no production of zones of inhibition) and hence all the antifungal activity is clearly attributed to the nanoparticles.

Overall, in relation to antifungal activity, we can say FeS_2 was the best, followed by ZnO nanoparticles. This was mainly due to its ability to inhibit the growth of the fungi by producing clear zones of inhibition. The ability to exert this antifungal activity can be attributed to its mechanism of action, mainly disruption of the cell membrane and eventual disruption and death of the cell, in conjunction with the production of radical oxygen species that are also lethal to cell organelles [6, 22–23]. In addition, the production of the inhibition zones was also an indicator of the proper diffusion of the nanoparticles in the agar media. The zones of inhibition were also maintained by the nanoparticles after 48 hours of culture, indicating their effectiveness in their fungicidal activity.

3.2. Antibacterial Tests Results

The activity of nanoparticles against the bacteria, *Bacillus cereus*, is shown in Fig. 4. Results show that all the nanoparticles managed to produce zones of inhibition against the bacteria. This was an indication of the ability of the nanoparticles to diffuse in the media to produce inhibition zones and the bactericidal ability of the nanoparticles was shown by the clear zones of inhibition produced [6].

ZnO was shown to be the best against the *Bacillus cereus* as it had the largest inhibition zones for all the concentrations used as

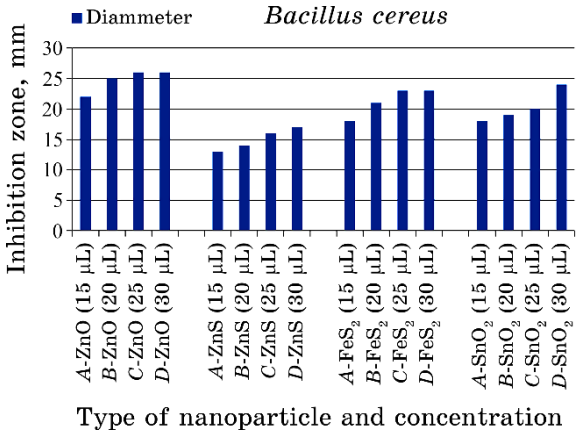


Fig. 4. Diameter of zone of inhibition results produced by nanoparticles at varying concentrations against *Bacillus cereus*.

compared to all the other nanoparticles, and this activity is illustrated in Fig. 3: SnO_2 followed after ZnO in terms of effectiveness, then FeS_2 and lastly ZnS. ZnO was shown to be the best and this is supported by the fact that, the 20 μL concentration had a zone of inhibition (25 mm) greater than the inhibition zones for the 30 μL concentration for ZnS (17 mm), SnO_2 (24 mm) and FeS_2 (23 mm). Maximum activity was noted for ZnO at 30 μL concentration (26 mm) and the least at that same concentration for all the nanoparticles was ZnS (17 mm). All the nanoparticles managed to show an increase in the inhibition zone as the concentration of the nanoparticles increased, with ZnO and FeS_2 not having an increase in the inhibition zone when concentration was increased from 25 to 30 μL over the specified time. The increase in inhibition zone was in agreement with Refs. [6] and [17], which highlighted that inhibition zones increased with concentration increase and can be explained by Ref. [30] that toxicity effect is concentration dependent. Ref. [17] explained that the effect of the nanoparticles toxicity on the bacteria was not only dependant on the nanoparticles type but also the bacterial species involved. In this study, the nanoparticles managed to show the effect of varying the type of the nanoparticles used as all the nanoparticles had varying effects on the bacterial growth. The results obtained showed that ZnO, ZnS and FeS_2 maintained clear inhibition zones after 48 hours, whilst SnO_2 showed an inhibition zone that was not clear, as the bacteria had started growing in the inhibition zone. This could be attributed to the fact that the SnO_2 nanoparticles concentration decreased as the cells bacterial cells divided and hence a reduction in the antibacterial effect that was noted in Refs. [41, 71]. Ref. [17] showed that sensitivity of microorganism to the test nanoparticles was species specific, results obtained in this experiment showed that the *Bacillus subtillis* was more sensitive to ZnO and less sensitive to the ZnS particles.

The activity of nanoparticles against the bacteria *Bacillus subtillis* is shown in Fig. 4. Results show that all the nanoparticles managed to produce zones of inhibition against the bacteria. This indicated the ability of the nanoparticles to diffuse in the media to produce inhibition zones, which were an indication of the bactericidal effect of the nanoparticles [6]. SnO_2 was shown to be the best against the *Bacillus subtillis* as it had the largest inhibition zones for all the concentrations used as compared to all the other nanoparticles, except for the 20-μL concentration, where it had a similar inhibition zone to that of ZnO. This was followed by ZnO, FeS_2 and finally ZnS. The SnO_2 was shown to be the best, and this is supported by the fact that, at all the concentrations, it had inhibition zones higher than all other test nanoparticles at the same concentrations. Maximum activity was noted for SnO_2 at 30 μL concentration (30

mm) and the least at that same concentration for all the nanoparticles was ZnS (18 mm). All the nanoparticles managed to show an increase in the inhibition zone as the concentration of the nanoparticles increased. The increase in inhibition zone was in agreement with Refs. [6] and [17], who showed that inhibition zones increased with concentration increase and was also supported by Ref. [30], which explained that toxicity effect was concentration dependant. Ref. [17] explained that the effect of the nanoparticles toxicity on the bacteria was not only dependent on the nanoparticles' type but also the bacterial species involved. In this study, the nanoparticles managed to show the effect of varying the type of the nanoparticles used as all the nanoparticles had varying effects on the bacterial growth. The results obtained showed that ZnO, ZnS and FeS_2 maintained clear inhibition zones after 48 hours whilst SnO_2 showed an inhibition zone that was not clear, as the bacteria had started growing in the inhibition zone. This could be attributed to the fact that the SnO_2 nanoparticles concentration decreased as the cells bacterial cells divided and hence a reduction in the antibacterial effect that was noted in Refs. [41, 71]. Ref. [17] showed that sensitivity of microorganism to the test nanoparticles was species specific, results obtained in this experiment showed that the *Bacillus subtillis* was more sensitive to SnO_2 followed by ZnO, then FeS_2, and less sensitive to the ZnS particles.

Figure 5 illustrates the results for the test nanoparticles against *E. coli*. Results obtained indicated that all nanoparticles except SnO_2 managed to produce inhibition zones against *E. coli*. The maximum inhibition zone was seen for ZnO nanoparticles at 30 μL with a 32

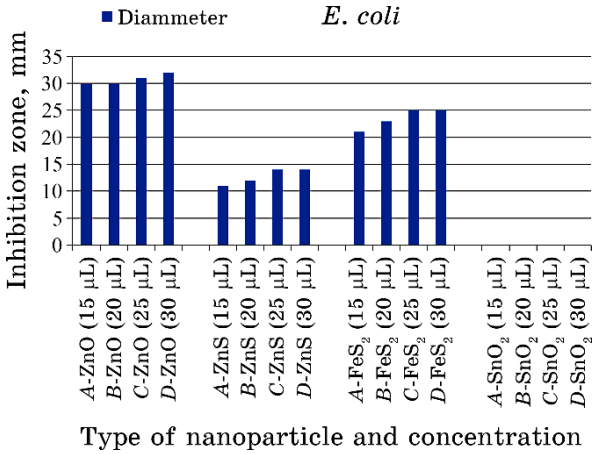


Fig. 5. Diameter of zone of inhibition results produced by nanoparticles at varying concentrations against *E. coli*.

mm inhibition zone. This was followed by FeS_2 (25 mm) and finally ZnS (14 mm) at the same concentration for those that managed to produce the inhibition zone. Against *E. coli*, ZnO was seen to be the best as it managed to produce the largest inhibition zones for all the concentrations as compared to the other test nanoparticles. Refs. [6] and [17] showed that inhibition zones increased with concentration increase and were supported by Ref. [30], which explained that toxicity effect was concentration dependent [24–27]. This was in agreement with results for *E. coli* in this study as increase in the inhibition zone was noted as the concentration of the nanoparticles increased for all the test nanoparticles except SnO_2. However, not all the test nanoparticles produced inhibition zones, SnO_2 had no inhibition zone for all the concentrations, showing that it did not have an effect on the *E. coli*. This was in agreement with Ref. [17] who illustrated that each species has a specific susceptibility to certain nanoparticles in relation to its ability to growth in the presence of those nanoparticles and, in this case, *E. coli* was not sensitive or susceptible to the SnO_2 nanoparticles. Results obtained showed that *E. coli* was more sensitive to ZnO nanoparticles, and all the test nanoparticles managed to maintain clear inhibition zones after 48 hours of culture.

3.3. Antifungal and Antibacterial Analysis

In comparing all the bacteria and their response to exposure to the nanoparticles, a number of key things were noted. The ZnO nanoparticles were the most effective, in terms of eliciting an antibacterial activity, across all the test isolates. This was due to its ability to produce inhibition zones that were large even though at times not maximum against the test bacterial isolates. This effectiveness can be attributed to the mechanism of action of the ZnO nanoparticles, which is effective. The mechanism of nanoparticle toxicity depends on composition, surface modification, intrinsic properties, and the bacterial species [72]. These results obtained for ZnO were in agreement with Refs. [6] and [17], which highlighted that the ZnO nanoparticles mechanism of action was effective in being bactericidal, with the mechanism being the disruption of the cell wall and membranes of the bacteria leading to loss of cellular components and eventual death of the cell. This was also attributed to the small size of the nanoparticles that allows them to pass through the membranes easily and access the cellular contents and to their ability to cause stress on the cell membrane with eventual break down of the membrane [6].

The next nanoparticles effective against the bacteria were FeS_2, which showed great consistency in inhibiting the growth of the all

the bacterial test isolates, showing that all the bacterial samples had sensitivity to the FeS_2 nanoparticles, with its mechanism of action being that of production of the reactive oxygen species [58]. ZnS also had consistent but low inhibition zones for all the test isolates, while SnO_2 had effectiveness against both the *Bacillus* species but non-against *E. coli*. This was in agreement with what Ref. [17] explained that the effect of the nanoparticles toxicity on the bacteria was not only dependant on the nanoparticles type but also the bacterial species involved as the inhibition differed with the bacterial species and nanoparticles involved. In terms of susceptibility, the *Bacillus cereus* was the most sensitive to all the test nanoparticles as it had fairly high-inhibition zones as compared to those obtained for all the other isolates. This was followed by *Bacillus subtilis* and finally *E. coli* was the less sensitive one on an overall scale. Results for sensitivity of *Bacillus subtillis* being more than that of *E. coli* obtained in this study was in agreement with Ref. [17], which also showed that *Bacillus subtillis* was more sensitive to the test nanoparticles than *E. coli*.

The activity of the nanoparticles against the fungi showed that, in relation to antifungal activity, FeS_2 was the best, followed by ZnO nanoparticles. This was mainly due to its ability to inhibit the growth of the fungi by producing clear zones of inhibition. The ability to carry out this antifungal activity can be attributed to its mechanism of action, mainly disruption of the cell membrane and eventual disruption and death of the cell, in conjunction with the production of radical oxygen species that are also lethal to cell organelles [6, 58]. In addition, the production of the inhibition zones was also an indicator of the proper diffusion of the nanoparticles in the agar media. The zones of inhibition were also maintained by the nanoparticles after 48 hours of culture, indicating their effectiveness in their fungicidal activity.

Comparing the effectiveness of the nanoparticles against the bacteria and fungi, results showed that the nanoparticles were more effective against the bacteria as shown by their ability to inhibit bacterial growth for the test isolates, while for the fungi, only two nanoparticles (ZnO and FeS_2) were effective against the fungal isolates, with one of those two, FeS_2, being the only one that was effective to all the fungal isolates, as ZnO was only effective against one fungal isolate. This even supported more the position of Ref. [17] that effectiveness of the nanoparticles was based on the nanoparticles and the microorganism species involved.

4. CONCLUSIONS

The nanoparticles managed to show antifungal and antibacterial ac-

tivities as they managed to produce inhibition zones against the test bacterial and fungal isolates. The results also showed that an increase in the concentration led to an increase in the inhibition zone produced by the nanoparticles against the test isolates. However, some nanoparticles did not manage to elicit an effect on the bacteria (SnO_2) and on the fungi (SnO_2 and ZnS), showing that the test isolates were not sensitive to the nanoparticles. The FeS_2 and ZnO nanoparticles were the ones that managed to show broad activity across the fungal and bacterial samples, as they managed to produce inhibition zones.

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