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Antifungal, antibacterial and anti-yeast activities evaluation of oxides of silver, zinc and titanium nanoparticles

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In the present research, the antimicrobial activity of nanoparticles against the bacterial and fungal species was evaluated. Three types of oxides (Ag, Ti and Zn) nanoparticles at different concentrations 25-200 mg/mL were used for antifungal, antibacterial and anti-yeast activities evaluation alone and in combination. The experimental results showed the effectiveness of Ag nanoparticles to inhibit the activity of a number of selected bacterial strains such as *Klebsiella pneumoniae*, which showed the most activity with the inhibition area of 11 mm and 7 mm in case of *Escherichia coli*. The nanoparticles also showed promising antifungal and anti-yeast activities, especially when the nanoparticles were used in combination. The findings revealed that the oxides of silver, zinc and titanium nanoparticles have promising antifungal, antibacterial and anti-yeast activities, which could find applications in different fields.

Capsule Summary: The Ag, Ti and Zn (oxides) nanoparticles antifungal, antibacterial and anti-yeast activities were evaluated and findings revealed that the oxides of Ag, Ti and Zn showed promising activities, which could have potential applications in different fields.

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INTRODUCTION

The most important and vital sector over world is agriculture and its by- product that provides raw material for food and feed industries. High growth rate of population in the world needs alternative strategies for agricultural development to sustain the increment of population (Dudefoi et al., 2015; Kumar et al., 2019; Kumar et al., 2020). The mainstream of people is living below the poverty level are being scatted in the rural area, where agriculture enlargement has not so been effective. One of the largest problems is the postharvest loss of about 25% of agricultural product via microbial spoilage (Daniel et al., 2013; Dik et al., 2018; Sekhon, 2010; Khezerlou et al., 2018; Romaniuk and Cegelski, 2015). Accordingly, formulation, processing, packaging and storage might influence product lost.

For food industry, consumers concern about food quality, nutritional values, benefits and health hazardous. Many protocols were implied for food industries include different type of food preservatives (Kumari et al., 2015). Nanotechnology gives complete food solutions from food manufacturing, processing to packaging, increasing shelf life and nutritional value without changing physical and chemical characters, as well as food taste (El-Belely et al., 2021; Lashin et al., 2021; Majoumouo et al., 2019; Rokbani et al., 2018; Yusof et al., 2018). As a result, novel techniques, methods, and products that have a direct application of nanomaterial were adapted in food science (Slavin et al., 2017). Also, nanoparticles, have received increasing attention for food applications because they are not only stable at high temperatures and pressures typically needed in conditions of food processing[3,4], but they are also considered generally safe (GRAS) in relation to organic materials for humans and animals. Recent studies have shown that certain nanoparticles have bacterial selectivity, but minimal effects on human cells. These properties have attracted a lot of interest in controlling microorganisms, especially pathogens which cause infectious diseases, by particularly metal-based nanoparticles.

Soil is considered as the main reservoir and source for human pathogenic bacterial species and molds. Such pathogens can reach and contaminate soils, plant leaves, flowers and root as well as irrigated water upon manure fertilization that frequently contains zoonotic, pathogenic bacteria such as *Escherichia coli*, *Salmonella* sp., *Pseudomonas aeruginosa, Staphylococcus aureus* and *Campylobacter* sp., *Salmonella* spp persisted in soil up to one year insight to long- term of salmonella reservoir (Xie et al., 2011).

Yeast and filamentous fungi as groups of spoilage causing microorganisms have proved particularly well suited to dispersion and cross-contamination within the processed food because they capable to overcome food controlling strategies. A crucial step towards eliminating their propagation in food is to identify them and to prevent their initial existing; some fungi produce mycotoxin that stable at harsh environments, difficult to manage through physical and chemical treatment and remain in the food after heat processing and continue to cause toxicity. One of the main cause of nosocomial infection *is Candida albicans* (El-Belely et al., 2021) however its long term persisted on soil for up to 24 week, oak park, fruit and other plant material spotlight the bio-hazard of *C. albicans* (Li et al., 2020).

Studies on the antimicrobial effects of silver, silver oxide, titanium oxide and zinc oxide nanoparticles were reported extensively. Their formulation as additives in many foods, food product and fruit juice to eliminate the growth of aerobic psychrotrophics, yeasts and molds and their antimicrobial effect against many foods borne bacteria (Hameed et al., 2016; Sumanth et al., 2020). Also, Titanium oxide and zinc oxide nanoparticles applied with Chinese jujube, Strawberry and apples to reduce browning, slow-down ripening, senescence and fruit decay (Lashin et al., 2021). Martinez-Castanon et al. (2008) has shown that smaller sizes of Ag NPs have a larger volume-to-surface ratio that increases the interaction of bacterial cells and NPs with the resulting antibacterial effects. A water chemical reduction method has synthesized three different sizes (mean 7, 29, and 89 nm) to determine the antibacterial activity of Ag NPs using the standard microdilution technique.

More studies on the antimicrobial effects of Ag NPs on a wide range of microorganisms have been reported. The higher the level and the smaller the particle volume, the greater the effect on the microorganisms that can be inhibited (Gabrielyan et al., 2020; Li et al., 2017; Panchal et al., 2021), the mechanisms behind inhibitory effects, however, are not yet fully understood. Titanium dioxide nanoparticles (TiO_2 NPs), due to their specific abilities, such as bactericidal photocatalytic activity, safety and self-cleaning properties, are one of the most studied substances in the area of antimicrobial applications (Gonçalves et al., 2018; Liao et al., 2020; Korshed et al., 2017). The antimicrobial action mechanism of the TiO_2 is usually linked to reactive oxygen (ROS) types with high oxidative potential produces by photo-induced band-gap radiation in the presence of O2, ROS affecting the bacterial cells through various death mechanisms (Slavin et al., 2017; Wang et al., 2019). The main goal of recent study was to evaluate the inhibitory activity of single nano-ZnO, nano-TiO, and nano-Ag2O and mixture of nanometal against environmental and pathogenic microorganisms such as *S.aureus*, *E. coli*, *P. aeruginosa*, *Salmonella spp.*, *K. pneumonae, Candida albicans, Aspergillus spp., Coccidioides spp., Cryptococcus spp., and Penicillium spp.*

MATERIAL AND METHODS

Nanoparticles

Silver oxide, zinc oxide and titanium oxide were purchased from US research nanomaterial, Inc USA. Each nanomaterial had chemical characters listed in Table 1. Each nanomaterial was weighted separately and suspended by double distilled water to give 25, 50, 75 and 200 μg/ml. Mixtures of nanomaterial solutions were prepared by mixing equal volume of Ag_2O-ZnO, Ag_2O-TiO, ZnO- TiO and Ag_2O-TiO-ZnO.

Bacterial and fungal strains

Bacterial and fungal isolates were isolated by the Medical Ministry Laboratories (Table 2) and were used for antimicrobial study. Bacteria were cultivated and enriched for 24 hours and subculture weekly in nutrient agar at 37°C. Yeast and fungi were cultivated and maintained in Sabroud dextrose agar pH 5.

Antimicrobial activity

Muller Hinton agar was used to detect the microbial susceptibility of nanoparticles depending on NCCLs. Agar diffusion method was applied for screening the antimicrobial activity of nano Ag_2O, ZnO and TiO_2 against *S. aureus, E. coli, P. aeruginosa, Salmonella* spp., *K. pneumonae*, *C. albicans*, *Aspergillus* spp., *Coccidioides* spp., *Cryptococcus* spp. and *Penicillium* spp.

NPs (200 mg/ml)	1	2	3	4	5
ZnO					
TiO_2					
Ag_2O					
TiO_2-Ag_2O					
TiO_2-ZnO					
ZnO-Ag_2O					
ZnO-Ag_2O-TiO_2					

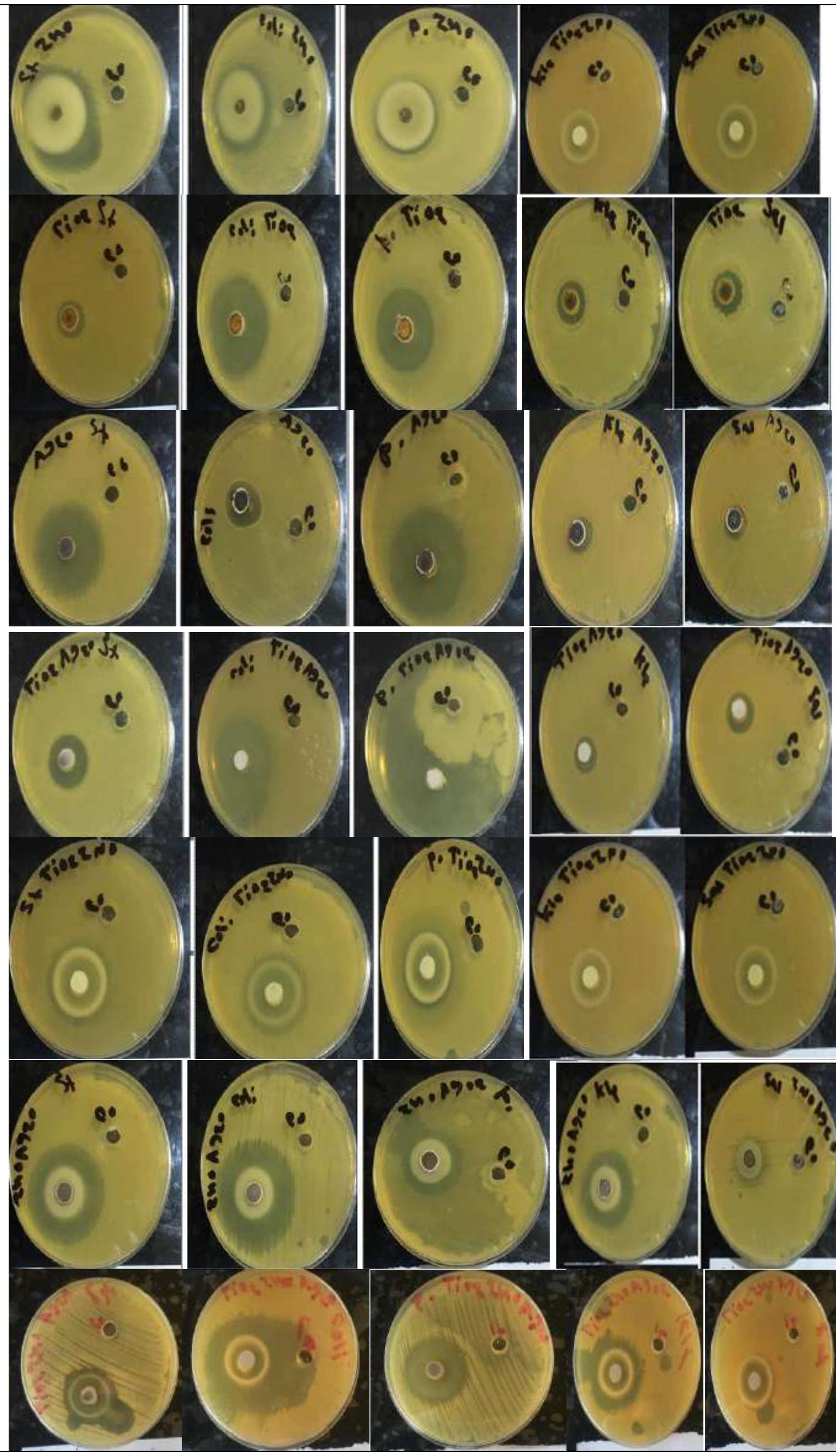


Fig. 1: Agar well diffusion method for antibacterial activity of 200 µg/ml of Ag, ZnO, TiO_2, Ag-Zn, Ag-Ti, Ag-Zn and ZnO-Ag_2O-TiO_2 against: 1 = *Staphylococcus aureus*, 2 = *E. coli*, 3 = *P. aeruginosa*, 4 = *K. pneumonae* 5 = *Salmonella* spp

Muller Hinton agar was prepared according to its manufacture company, autoclaved for 15 min at 121°C, poured at petri-dishes and left for solidification. Overnight growth turbidity of *S. aureus, E. coli, P. aeruginosa*, *Salmonella* spp., *K. pneumonae*, *C. albicans* were estimated to 10^8 CFU/ml using Macckferland standard method; while hemocytometer slide was used to estimate fungal spores to be 10^5.

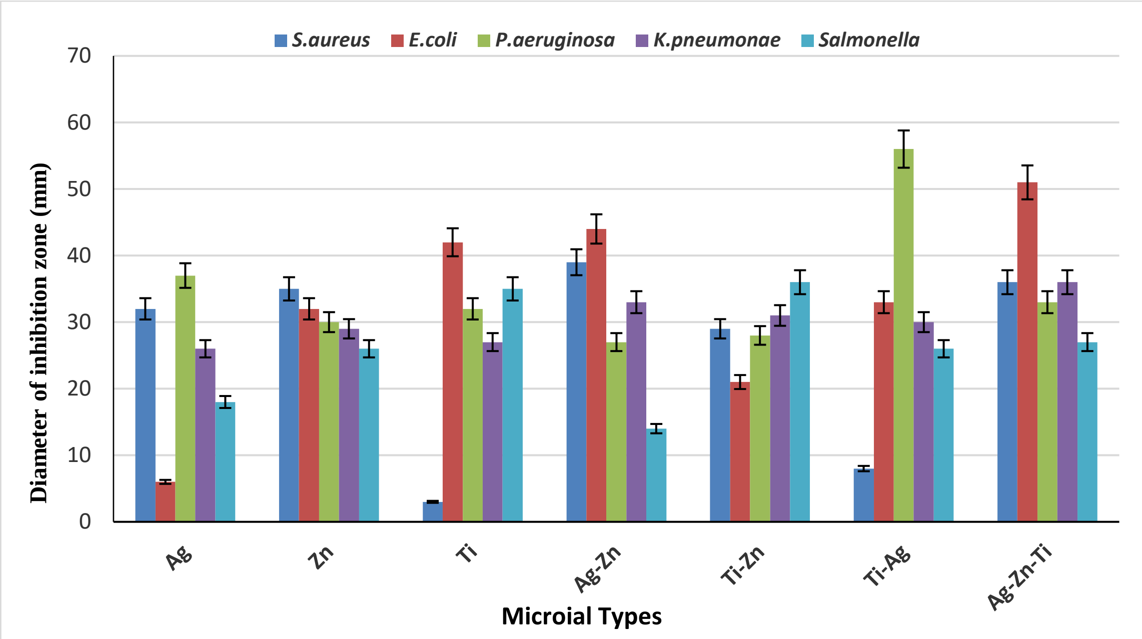


Fig. 2: Antibacterial activity of 200 µg/ml of Ag, ZnO, TiO_2, Ag-Zn, Ag-Ti, Ag-Zn and ZnO-Ag_2O-TiO against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonae* and *Salmonella* spp

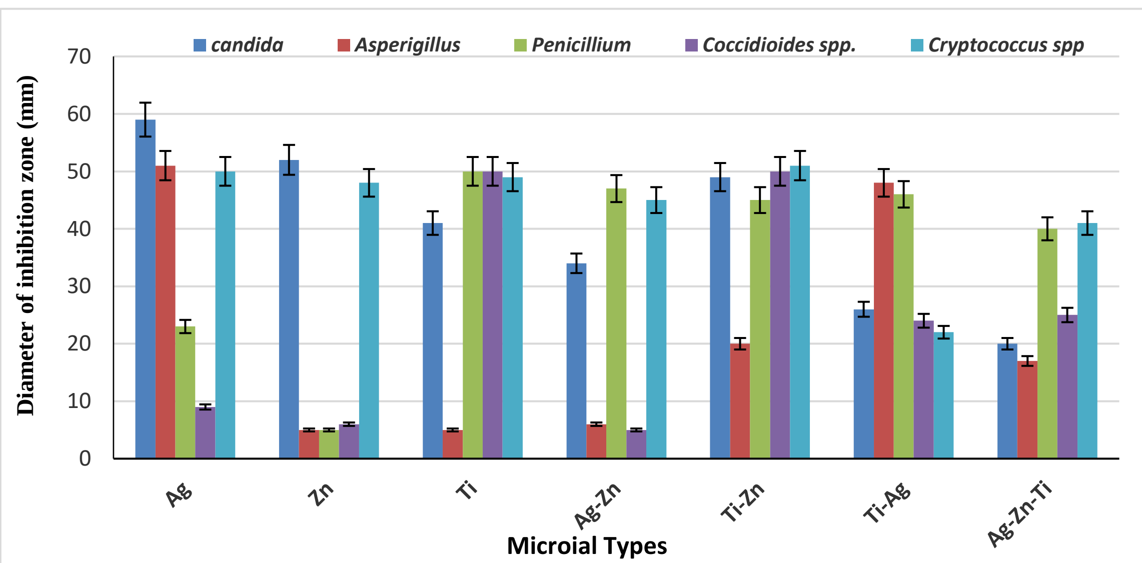


Fig. 3: The bioactivity of nano- Ag_2O, nano-ZnO, nano-TiO, nano- ZnOTiOAg_2O, nano- ZnO-TiO_2, nano-ZnO-Ag_2O and nano-TiOAg_2O against: *Candida albican* spp, *Asperigillus* spp, *Penicillium* spp, *Coccidioides* spp., *Cryptococcus*

Cotton swab was used immersed in each microbial growth under study, wiped on to the surface of Muller Hinton agar and stand for 30 min at 25°C, meanwhile holes with 5 mm were punctured using cork- borer and filled with 100µl of Ag_2O, ZnO and TiO_2 at different concentration (25,50, 100 and 200 µg/ml) All plates were incubated at 37°C for 24-72h and the radius of inhibition zones were recorded in millimeters to screen the best concentration of nano-material. For diagnosis the interaction between nanomaterial mixtures, another trial was conducted; mixtures of Ag_2OZnO, Ag_2OTiO_2, $ZnOTiO_2$ and $Ag_2OZnOTiO_2$ were screened for their bioactivity against microbial species under study.

RESULTS AND DISCUSSION

Five bacterial species have been used throughout the test. Three kinds of nanoparticles (Ag, TiO_2 and ZnO) at different concentrations, the results of agar well diffusion method showed that nanomaterial at 200 µg/ml gave significant inhibition activity towards microbial species (Figure 1).

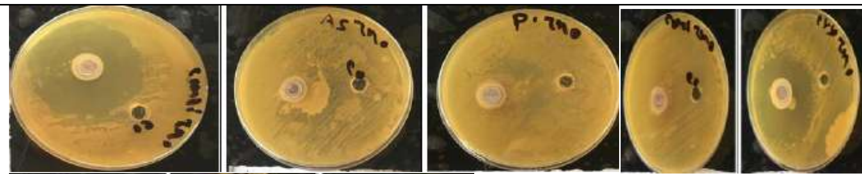
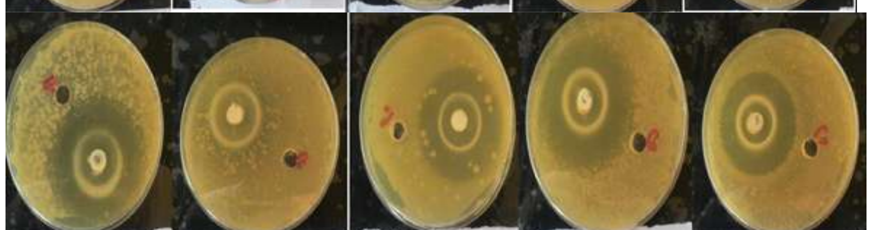
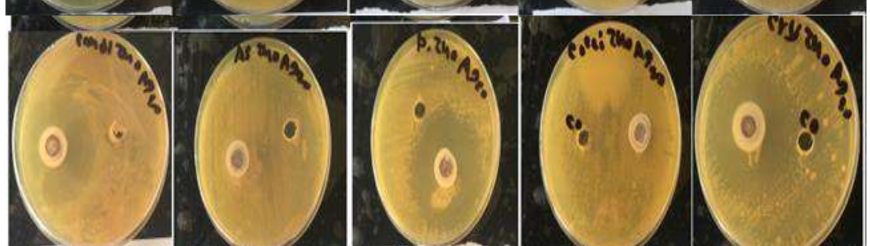
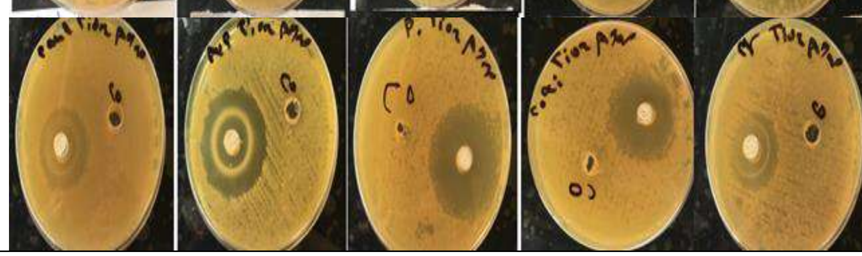
NPs	1 2 3 4 5
ZnO	
TiO_2	
Ag_2O	
$ZnO-TiO_2-Ag_2O$	
$ZnO-TiO_2$	
$ZnO-Ag_2O$	
$TiO-Ag_2O$	

Fig. 3: Well diffusion method for antifungal activity of 200 µg/ml of Ag, ZnO, TiO_2, Ag-Zn, Ag-Ti, Ag-Zn and ZnO-Ag_2O-TiO against different kinds of fungi species 1 = *Candida albicans*, 2 = *Asperigellus* spp, 3 = *Penicillium* spp, 4 = *Coccidioides* spp and 5= *Cryptococcus* spp

Klebsiella pneumonae spp and *Salmonella* were sensitive to nano- Zn followed by nano-TiO and nano-Ag and the low activity of silver nanoparticle might due to size, morphology and concentration of nanoparticles and the genetic characteristics of the bacteria being tested. Research indicates that small nanoparticles are more effective than large nanoparticles. Whereas, Padmavathy & Vijayaraghavan (2008) showed that a suspension containing 12 nm ZnO nanoparticles was more effective than a suspension with larger particle size (da Silva et al.,

Table 1: Characteristics of nanoparticle used in this study

Nanoparticles	Properties	Size (nm)	M. weight	Purity (%)	Expiry date
Titanium oxide		30±5	79.86	99.5	2022
Zinc oxide	Milky white, single crystal near spherical	35-45		99	2022
Silver oxide	Near spherical-based on particle size, cubic	50	107.87	99.9	2022

Table 2: Microorganisms used in the study

Microorganisms	Type
Staphylococcus aureus	Gram positive bacterium
Escherichia coli	Gram negative bacterium
Klebsella pneumonae.	Gram negative bacterium
Pseudomonas aeruginosa	Gram negative bacterium
Salmonella spp.	Gram negative bacterium
Candida albicans	Yeast
Aspergillus spp.	Fungum
Coccidioides spp.	Fungum
Cryptococcus spp.	Fungum
Penicillium spp.	Fungum

Table 3: The antimicrobial activity (zones) of nanoparticles against microbial species under study

Microbes	Ag_2O	ZnO	TiO_2	Ag_2O-ZnO	Ag_2O-TiO_2	ZnO-TiO_2	Ag_2O-ZnO-TiO_2
Salmonella spp	2±0.08	26±1.9	6±0.18	1±0.05	4±0.48	7±0.03	27±3.1
K. pneumonae	4±0.1	29±2.4	4±0.24	32±0.57	3±0.11	5±0.3	36±4.1
Candida spp	59±7.8	52±4.7	41±3.7	59±6.1	26±1.6	49±5.9	20±3.7
Aspergillus spp.	51±6.9	0±0.0	0±0.0	31±4.2	48±5.4	20±1.5	6±0.4
Coccidioides spp.	9±0.49	0±0.0	50±6.8	0±0.0	24±3.4	50±6.7	5±0.23
Cryptococcus spp	50±0.7	48±5.1	49±8.1	45±5.8	22±3.4	51±7.1	41±5.3
Penicillium spp.	9±0.42	0±0.0	0±0.0	9±.15	46±5.4	45±5.7	40±6.1

2019). The concentration of silver nanoparticles directly affects the area of the inhibition zone and inversely on the concentration of bacteria (Slavin, 2017), it is also stated that the antibacterial activity of silver is dependent on size and concentration. Our results were different from the studies of Masoud et al. Okafor et al. who reported that Klebsiella pneumoniae was the most sensitive organism to silver nanoparticles (Gonçalves et al., 2018; Liao et al., 2020; Korshed et al., 2017).

Recent results showed that mixture of nano-material augmented the inhibition of bacterial population and the most susceptible was *K. pneumonae* when treated with Ag-Ti mixture followed by *E. coli* treated with Ag-Zn-Ti then *S. aureus* treated with Ag-Zn. Otherwise, *S.aureus* showed lower susceptibility to Ti-Ag mixture in compared to single nano-Ag. Also, *E. coli* gave lower inhibition zone detected with Ag-Ti in compared with single nano-TiO_2. *Salmonella* spp gave lower response with Ag-Zn in compared with single nano-Ag and nano-Zn (Figure 2).

The susceptibility of five fungal species tested with three kinds of nanoparticles (Ag, TiO_2 and ZnO) at different concentrations, the results of agar well diffusion method showed that nanomaterial at 200μg/ ml gave significant inhibition activity towards fungal species (Figure 3 and 4).

Results indicated that *C. albicans* species and Cryptococcus spp were more susceptible to nano-Ag, nano Ti and nano-Zn than other fungi and bacterial species under study. While *Asperigellus* spp showed higher susceptibility to nano Ag in compare to nano-Ti and nano-Zn (figure 4). Recent result was in accordance with other report who refer to that silver nanoparticle exhibited a good antifungal property at low doses and the synthesis research indicated the formation of silver chloride upon cell penetration (Mukhopadhyay, 2014). Also, it was explained the interaction of nano-silver with *C. albicans* cell membrane led to significant changes followed by the formation of "pits" and finally pores development, increased membrane permeability and leakage of cellular component followed by cell death. On the same field, Zinc nanoparticles was used as antifungal to eliminate the growth of *Asperigellus niger* and *Penicillium* (Dasgupta et al., 2015). Nano-TiO_2 gave the highest activity against *Penicillium* species followed by

Candida, *Coccidosis* and *Cryptococcus* in compared to bacterial species. The photocatalytic property of nano-titanium oxide might cause a significant change in cell regulation, respiration and signalling systems.The possibility of inhibition in the assimilation and transportation of iron and phosphorus. The major factors that explain the biocidal activity of the TiO_2 NPs were these processes with the extensive cell wall and membrane changes (da Silva et al., 2019; Vimercati et al., 2020).

The development of nano-material to inhibit *C.albicans* was one of the most important challenges. Many of this yeast can form biofilm on many biotic and abiotic environments which increases their resistance against different traditional food conservative additive and antifungal therapy increasing the capability of the yeast cells within the biofilm consortium to invade host immune response (Mukhopadhyay, 2014). One insight to use nano copper element in inhibition of *C. albicans* as alternative fungi drug (Dasgupta et al., 2015; Roselli et al., 2003).

Table 3 illustrated the bioactivity of mixed nano-material (in different combinations) against microbial species under study. Results indicated that the susceptibility of Klebsiella spp and Salmonella spp increased with mixture of $ZnOTiOAg_2O$ they recorded 36±4.1 and 27±3.1 mm inhibition zone. While the mixture of $ZnOAg_2O$ inhibition zone reached to 32±0.57 mm.

Screening of antimicrobial activity of mixture of nano- material under study showed that more susceptibility of *K. pneumonae* appeared after treatment with mixture of nano-Ag_2O-ZNO-TiO that reached to 36±4.1 in compared with recorded zone for separated nano-material that reached to 4±0.1 for Ag_2O, 29±2.4 for ZnO; 4±0.24 for TiO_2. Otherwise, single nano-material gave almost the best activity or the same inhibitory action than mixture for other microbial under test

CONCLUSIONS

The antibacterial ability of nano-Ag_2O-ZnO-TiO was related to the photocatalysis and metal release process of nano-Zn and nano-Ag. It can be seen that the antibacterial ability of the ZnO-Ag samples was stronger against *K. pneumonae* than separated nano-material. This might due to the cell wall composition of Gram-negative bacteria that contain an external lipopolysaccharide (LPS) membrane that shields and protect the thin peptidoglycan layer. Also, it helps bacteria to survive under harsh environment that contain exterior material.

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