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STUDY OF THE EFFECT OF ZINC OXIDE ON THE ANTIFUNGAL ACTION OF THE POLYMER BLEND (UNSATURATED POLYESTER AND NATURAL RUBBER) FOR INDUSTRIAL APPLICATIONS

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*Incorporating inorganic nanoparticles into polymers has produced polymer nanostructure materials with high-performance and multifunctional polymer features that exceed standard polymer composites. Polymer nanocomposites have multifunctional qualities, such as increased resistance to moisture and fungal and antibacterial properties. In this study, a polymeric blend of unsaturated polyester and natural rubber (80 / 20) was prepared as a control material, and it was then reinforced with zinc oxide nanoparticles (ZnONPs) with weight ratios (1, 1.5, 2, 2.5, and 3 %) in gradual addition with continuous stirring. The X-ray diffraction analyses and water diffusion coefficients were examined. The diffusion coefficient of the composite material with a concentration of 2 % had the highest value, after which the values began to fall, while the increase was linear at less than this concentration. The X-ray diagram revealed that the composite material with a concentration of 1 % had the lowest values of 2θ , and that as the concentration increased, the values increased. Antimicrobial activity was conducted for two types of soil microbes, *Fusarium solani* and *Streptomyces lividans*, using the disc diffusion method, and inhibition of biofilm formation was observed at 72 hours of incubation time, with inhibition rates reaching 40.61 and 69.39 % in both *Streptomyces lividans* and *Fusarium solani*, respectively. It can also be noted the clear effect of zinc nanoparticles on the formation and visualization of biofilms in both organisms, as it was significantly affected by the treatment with an increase in concentration. Based on these findings, it appears that the synthetic blends that are reinforced with ZnONPs could be a promising material for water containers.*

Keywords: microbial growth, nanocomposites, polymer blend, water diffusion coefficient, X-ray diffraction

INTRODUCTION

Polymer blends are the most popular polymers utilized as matrices nowadays due to their changing properties when two or more suitable polymers are alloyed [1]. When miscibility and homogeneity are the key themes required, blending is a quick and economical technique to design and produce a new polymer with desirable qualities [2, 3]. Another way to improve the characteristics of polymers is to incorporate inorganic fillers such as metal oxide, clay, CaCO_3 , and carbon black into polymer composites. To tackle multi-drug resistance, nanozinc oxide (ZnO) has been explored for the creation of next-generation nanoantibiotics against harmful microbes [4]. These nanoparticles have distinct physicochemical features, such as particle size, shape, porosity, and crystallinity [5]. According to these properties, nano ZnO exhibit broad antibacterial action against a variety of disease-causing germs, the M13 bacteriophage, and *Bacillus subtilis* [6, 7]. They are compatible

with antibiotics and anti-inflammatory medicines for increasing antibacterial action versus pathogenic microorganisms in non-clinical and clinical settings [8]. ZnO nanoparticles and microparticles (MPs) have been used in biomedical applications such as medical devices or antimicrobial medications, the ranostics for traditional clinic uses, cosmetics, and implants [9, 10]. In various environmental settings, heterotrophic microorganisms like bacteria and fungi can break down a wide variety of polymers. The molecular weight, crystallinity, and physical shape of a polymer all affect its biodegradation [11]. Generally, a polymer's capacity to be broken down by microorganisms decreases as its molecular weight rises [12]. The repeating units of a polymer, however, can be broken down and mineralized considerably more quickly as monomers, dimers, and oligomers. Due to the necessity for bacteria to take up the substrate across the cellular membrane before being further destroyed by cellular enzymes, high molecular weights produce a significant decline in

solubility, rendering them resistant to microbial attack, though it could be observed, that the degradation of polymers may be aided by concurrent non-biological and biological processes [13].

There are at least two types of enzymes that actively contribute to the biodegradation of polymers such as intracellular and extracellular depolymerases [14, 15]. The action is known as depolymerization. Mineralization is the term for degradation when the last products are inorganic molecules, such as H_2O , CH_4 and CO_2 . According to a well-accepted rule, a polymeric structure is more easily dissolved and mineralized the more closely it resembles a natural molecule [16]. Wherein anaerobic processes are predominant. Under these circumstances, a polymer will completely decompose and release inorganic molecules, along with organic acids [15]. Substrates made of polymers can never be fully biodegraded or broken down because a portion of the material will be taken up by microbes, dirt, and other natural substances [17]. Environmental factors frequently influence dominant types of bacteria as well as the pathways involved in polymer degradation. Aerobic bacteria mostly decompose complex materials when oxygen is present; their principal outputs are microbial biomass, carbon dioxide, and water. Anaerobic microbial consortiums, on the other hand, are responsible for polymer degradation in anaerobic environment settings. The primary byproducts of a methanogenic process are inorganic compounds and microbial biomass [18]. Under situations of moderately high (65–70 %) or low (55–65 %) humidity, natural microbial communities may survive on polymer surfaces [19]. As fungus grow on composite surfaces, a thick biofilm forms, making the material less resistant to further environmental changes.

Many investigations have been undertaken in this sector. Santhosh and Kandasamy created coating-ready composite materials out of unsaturated polyester resin reinforced with silver and titanium nanoparticles and investigated the antibiotic activity of these composites via photoresistance procedures as well as the release of biocides (Ag^{++}/AgO) when in contact with the aqueous environment. Under UV irradiation, both unsaturated polyester/ TiO_2 and unsaturated polyester/ $Ag-TiO_2$ nanocomposites demonstrated antibacterial action against *E. coli* (Gram-negative) and *S. aureus* (Gram-positive).

Although the ideal conditions for antimicrobials are yet unknown, the results show that unsaturated polyester/ $Ag-TiO_2$ has better antibacterial action than unsaturated polyester/ TiO_2 [20]. Ramish and Bankim investigated the effect of titanium oxide and aluminum oxide nanoparticles combined on the thermal and mechanical properties of unsaturated polyester resin reinforced with glass fibers and discovered that adding 1 % by weight to each of the nanoparticles in the unsaturated polyester matrix reduces water diffusivity by 12 percent [21]. Farzaneh and his group have developed polystyrene (PS)/calcium sulfate ($CaSO_4$) nanocomposites. The water diffusion behaviour through the above composites was tested with different amounts of nano $CaSO_4$. As a result, the effective diffusion coefficients were computed [22]. Amer *et al.* created polyethylene sebucket (PES) composites enhanced with tungsten oxide (WO_3) and zinc oxide (ZnO). The microbiological effect of nanocomposites on harmful bacteria and fungi was investigated. The results demonstrated that these nanocomposites had antimicrobial properties on bacteria that ranged from moderate to slightly elevated and on fungi that ranged from moderate to slightly elevated, as evidenced by a distinct zone of inhibition [23].

The major focus of this study was to examine how the incorporation of zinc oxide nanoparticles in polymeric blends made of unsaturated polyester resin and natural rubber, with varying weight fractions, affects the activity and biofilm formation of two soil microorganisms, namely *Fusarium solani* (*F. solani*) and *Streptomyces lividans* (*S. lividans*). Additionally, the study examined the water diffusion coefficient of these nanocomposites to establish their suitability as soil-based pipelines for water transportation or containers.

MATERIALS

Zinc oxide nanoparticles (20–30 nm) were offered by Hong Inter National Group Ltd., China. (Density: 5.06 g/cm^3 , purity: 99.8 %, water: insoluble, and melting point: 1975 C). Iraq produced both unsaturated polyester and natural rubber which was supplied from factory of tires in Babylon.

PREPARATION OF NANOCOMPOSITES

After dissolving the rubber in the toluene for a day, a polymeric blend of unsaturated polyester

and natural rubber was prepared as a basis material. The blend was then fortified with zinc oxide by weights (1, 1.5, 2, 2.5 and 3 wt %) to create nanocomposite materials. The samples

were hardened for two days before being dried in a drying oven. Finally, they were taken out of the mold and cut.

Table 1. Composition of natural rubber [24]

Ingredients	%
Rubber hydrocarbon	30–40
Fats and related compounds	0.90–1.0
Water	55–65
Sugars (carbohydrates)	1.0–1.5
Ash	0.4–0.6
Proteins	1.90–2.5

EXPERIMENTAL PART

Antifungal activity assay. The *Fusarium solani* soil's strain was obtained from the microbiological lab/college of science/Al-Mustanseria University, this strain was isolated and identified based on standard laboratory procedures. The *F. Solani* was cultured for three to five days at 28 °C on potato dextrose agar (PDA) from Difco in Detroit, MI, USA. *In vitro* antifungal assays for polymer blends reinforced with different concentrations of ZnONPs (0.0, 1, 1.5, 2, 2.5, and 3 %) were performed using a disc diffusion assay (Kirby-Bauer method). Cycloheximide (10 µg/mL) was served as a positive control. A sterile cotton swab soaked in *F. solani* suspension (1×10^6 CFU/mL), and evenly distributed on a PDA surface plate and sterile prepared polymer blend discs supplemented with different concentrations of ZnONPs were putted on the agar plate, taking into account the equal distances among discs. All plates were incubated at 28 °C for 72 h, and then inhibition zone was measured in millimeter [25].

Anti-Streptomyces activity assay. Standard laboratory techniques were followed in the isolation and identification of the *Streptomyces lividans* soil strain. The *S. lividans* were inoculated at 28 °C on starch-casein nitrate agar [6]. Streptomycin (10 µg/mL) was used as a positive control in an *in vitro* antibacterial test for polymer blends reinforced with ZnO NPs using a disc diffusion assay (Kirby-Bauer method). Sterile nanocomposites discs with varying amounts of ZnO NPs were put into an agar plate, leaving equal spacing between them, after a sterile cotton swab dipped with *S. lividans* suspension (1×10^6 CFU/mL) was evenly dispersed on a starch-casein nitrate surface plate.

The inhibitory zone was measured in millimetres, and all plates were incubated for 72 h at 28 °C [26].

Morphological analysis of biofilm by SEM.

A 5-day-old culture of *F. solani* on potato dextrose agar (PDA) was soaked in 2 mL of Phosphate Buffer Saline (PBS). The conidia were collected, rinsed twice with PBS, and the count was adjusted to 1×10^6 CFU/mL. 12-flat bottom microtiter plate was used to form and determine the biofilm of *F. solani*. Each well was inoculated with 200 µL of conidia suspension in 1640–RPMI medium and placed with a 6-mm diameter of polymer blends reinforced with various ratios of ZnO NPs (0, 1, 1.5, 2, 2.5, and 3 %). The plates have been incubated at 28 °C for 4 hours (adherence time); the non-adherence cells were eliminated, and 0.5 mL of fresh 1640–RPMI medium was added. Plates were incubated for 72 hours and at 28 °C sequentially, the excess media has been poured out, and the wells were washed with PBS three times to get ride non-adherent cells, 2.5 % glutaraldehyde was used for 2 hours to fix the samples, and then 1 % osmium tetroxide was used for 2 hours. Biofilms were dried with increasing concentrations of ethanol (10–90 %) for 10 min at each concentration, and the final dehydration process was carried out with ethanol for 20 min. After that, all biofilms have been put into a dryer with a critical point. The specimens were enveloped with gold for 30 sec at 5.0 KV and 15 µA. The same steps were followed in the case of *S. lividans* for the morphological examination of biofilm by SEM [27].

STATISTICAL ANALYSIS

All results were analyzed statistically using the ANOVA (I) test to express the major

variations at ($P < 0.05$) by using statistical software (SPSS) for the biological tests.

Water diffusion coefficient. The diffusion coefficient (D) can be calculated after a certain time using Fick's 2nd law:

$$D = \pi \left(\frac{kb}{4M_{\infty}} \right)^2, \quad (1)$$

where k is the slope of the curve (weight gain % versus square root time), b is thickness of the specimen and M_{∞} is the maximum weight gain of the specimen before degradation. Weight gain % can be evaluated from Eq. (2):

$$\text{Weight change} = \frac{W_t - W_0}{W_0} \times 100\%. \quad (2)$$

W_0 and W_t are the weights of the samples before and after soak in water, respectively [28].

RESULTS AND DISCUSSION

XRD of samples. The X-ray curves of the prepared samples are shown in Fig. 1. The intensity of the pure blend was found to be the greatest, whereas the intensities of the composites were equivalent or close to each other. These findings contradicted what the researcher had discovered [29]. The increased intensity suggests an increase in crystallinity. The X-ray diffraction pattern of pure blend reveals maxima at 2θ values of 18.9, 22.77, and 29.32, which are near previous literature [30], and the effect of rubber is minimal due to its low percentage. Natural rubber showed no peak, confirming its amorphous nature, as confirmed by the researchers [31]. When increasing the concentration of ZnO NPs, a

variation was observed in the theta values of the composites; the composite of 1 % had the lowest values of 2θ compared with the rest of the composites. It indicates the reaction of ZnO NPs on the matrix, while the values began to increase slightly when the concentration was increased; this is attributed to particle agglomeration or de-intercalation when a higher amount of filler is added. Similar findings have already been reported in previous literatures [32, 33].

Antibacterial effects of blends reinforced with ZnO NPs. The antimicrobial effects of blends reinforced with ZnO NPs against *F. solani*, and *S. lividans* were tested by measuring the inhibition zone on a solid agar plate surface, each value in Table 2 and Fig. 2 was represented ($M \pm SD$). The inhibited zone of *F. solani* ranged from 6.5–15.44 mm in diameters, with significant differences at ($p < 0.05$) as a ratio response manner, while for *S. lividans*, the inhibited zone ranged from 5.2–10.21 mm in diameters, and there were significant differences at ($p < 0.05$) (P value = 0.279). Table 3 also shows the inhibition rate values for these two types of antimicrobials. These values ranged from 29.21–69.34 % for *F. solani* and 20.68–40.61 % for *S. lividans*. This suggests that incorporating ZnO NPs into the polymer blends could enhance their resistance to biodegradation by soil microorganisms, which often leads to the degradation and breakdown of these polymers. Organic biocide chemicals are commonly used to effectively manage the degradation and decay of various materials in enterprises.

Table 2. Inhibition zone of *F. solani*, and *S. lividans* at different concentrations of ZnO NPs by the disc diffusion method

Concentration of ZnO NPa (%)	Inhibition zone of <i>F. solani</i> (mm)	Inhibition zone of <i>S. lividans</i> (mm)
0.0	0.0	0.0
1.0	6.5±0.35*	5.2±0.15*
1.5	8.3±0.25*	7.4±0.22*
2.0	10.62±0.26*	9.33±0.25*
2.5	11.18±0.15*	9.78±0.42*
3.0	15.44±0.16*	10.21±0.18*
positive control	22.25±0.48	25.14±0.51

P value = 0.279

Each number represent $M \pm SD$ for three replicates

*Significant at ($p \leq 0.05$)

In addition to their environmental unacceptability due to their toxicity, these compounds have been demonstrated to be ineffective for preventing biofilm. Recently, nanomaterials have replaced these toxic organic substances that are used to stop bacterial growth in industrial systems because of their special characteristics, like size, occasionally electrostatic properties, as well as their chemical stability and ability to be produced in a variety of

shapes, making them the preferred choice in a wide range of applications. Blends fortified with ZnO NPs provided a suitable inhibitory zone against the actinomycetes *S. lividans* and the fungus *F. solani*, resulting in a decrease in biofilm growth in both microorganisms. This makes the environment repellent for these organisms, and in industrial applications, it may provide additional protection for these substances.

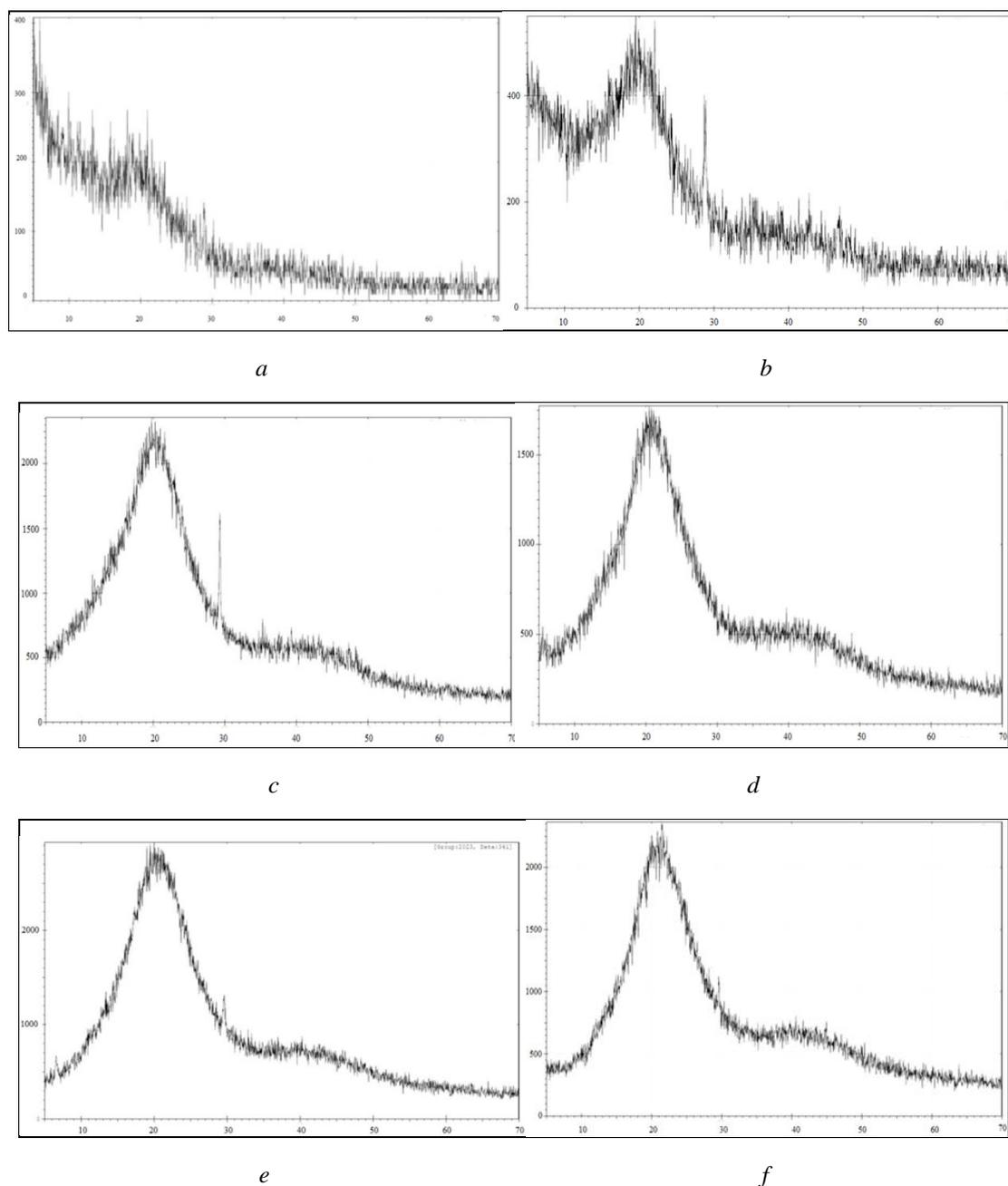


Fig. 1. X-Ray Diffraction patterns of (a) pure blend, (b) blend + ZnO NPs (1 wt. %), (c) blend + nZnO (1.5 wt. %), (d) blend + ZnO NPs (2 wt. %), (e) blend + ZnO NPs (2.5 wt. %), and (f) blend + ZnO NPs (3 wt. %)

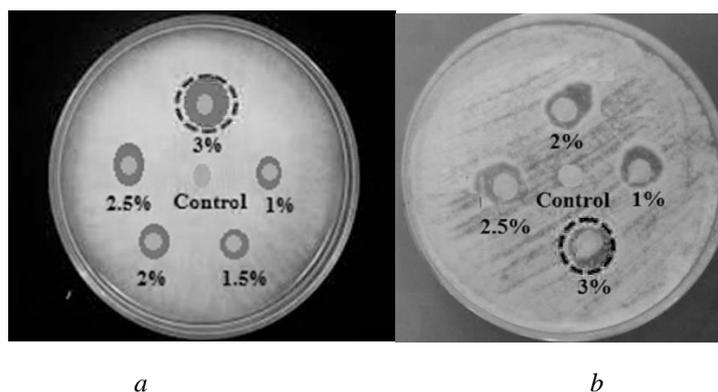


Fig. 2. Antimicrobial effects of polymer blends reinforced with different proportion of ZnO NPs against some soil's microorganisms. (a) – *Fusarium solani*, (b) *Streptomyces lividans*

Table 3. Inhibition rate (%) of *F. solani* and *S. lividans* treated with blends reinforced by ZnO NPs by the disc diffusion method

Concentration of ZnO NPs (%)	Inhibition rate of <i>F. solani</i> (%)	Inhibition rate of <i>S. lividans</i> (%)
1.0	29.21	20.68
1.5	37.30	29.43
2.0	47.73	37.11
2.5	50.24	38.90
3.0	69.39	40.61

Biofilm of *F. solani* and *S. lividans* formation described by SEM. Micrographs were taken using a scanning electron microscope (SEM) at 28 °C, 1×10^6 conidia/mL at the outer surfaces of blend-enforced discs, and varied ratios of ZnO NPs (0.0, 1, 2, and 3 %) at 72 hours of incubation time to assess the structure of the biofilm created by *F. solani* and *S. lividans*. Figs. 3 and 4 showed that both tested organisms' biofilm formation and development decreased and declined as the concentration of ZnO NPs increased. This nanomaterial prevented many biofilm formation steps, including the germination and development of microconidia in *F. solani*, as well as the elongation and development of hyphae and exo-polymeric substances. Additionally, the ratio increase had an impact on the abundant production of hyphae in extracellular matrix. After 72 hours of incubation, the control group (0.0 %) of The ZnO NPs exhibit a well-organised and developed biofilm with many layers of hyphae, channels, and, in certain areas, dehydrated hyphae lacking turgor. The biofilm also contains a significant amount of exo-polymeric components, distributed macroconidia, and detachment of cells.

Water Sorption. Fig. 5 displays the water sorption of the samples. As the concentration of ZnO NP increases, there is a decrease in the water sorption of all samples. Nanocomposites exhibit reduced water uptake due to the polymer blend effectively filling empty spaces in ZnO NPs, resulting in decreased water sorption. Similar observation had been reported [34]. Incorporating hydrophilic nanoparticles into a hydrophobic matrix resulted in higher loading levels and a reduction in the weight of water at equilibrium. The behaviour appears to be controlled by the interactions between nanoparticles and polymeric chains [35, 36]. The chains may have attracted inorganic nanoparticles, resulting in clusters at higher loading levels. The nanoparticles effectively filled the majority of the gaps in the polymer chain [37, 38]. The ZnO NPs in the polymer blend were intended to enhance its water affinity. However, they inadvertently filled the gaps between the chains, resulting in a decrease in the permeability of water molecules. Under high loadings, nanoparticles exhibited varying arrangements within chains, potentially leading to the formation of aggregates. The scheduled polymer water tendency induction was disrupted.

Fig. 6 displays the diffusion coefficients of all the samples. The diffusion coefficients of all nanocomposites surpassed those of the pure blend. Adding fillers can increase the complexity of the water diffusion path, which in turn affects the rate of diffusion and the amount of water absorbed at equilibrium. Water molecules were obstructed due to the clustering of fillers. It is possible that these channels were formed as a

result of nanoparticles entering the matrix. The nanocomposite with a 3 % concentration of ZnO NPs exhibited the lowest diffusion coefficient. The aggregation of nanoparticles can have a significant impact on the findings related to water diffusivity. Research indicates that clustering of ZnO NPs in the polymer mix hinders water transport by obstructing voids. The findings align with Ladhari [39].

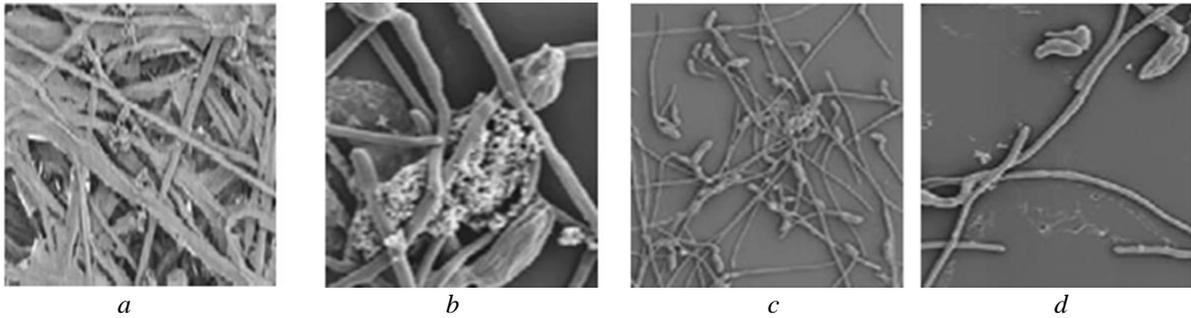


Fig. 3. Scanning Electron Microscope showing *F. solani* biofilm formation on blends reinforced with ZnO NPs by (a) 0.0 %, (b) 1 %, (c) 2 %, and (d) 3 %

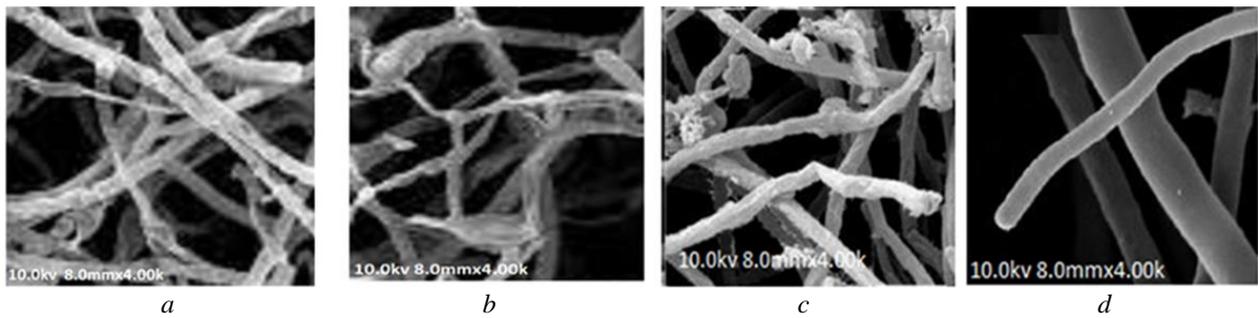


Fig. 4. Scanning Electron Microscope showing *S. lividans* biofilm formation on blends reinforced with ZnO NPs by (a) 0.0 %, (b) 1 %, (c) 2 %, and (d) 3 %

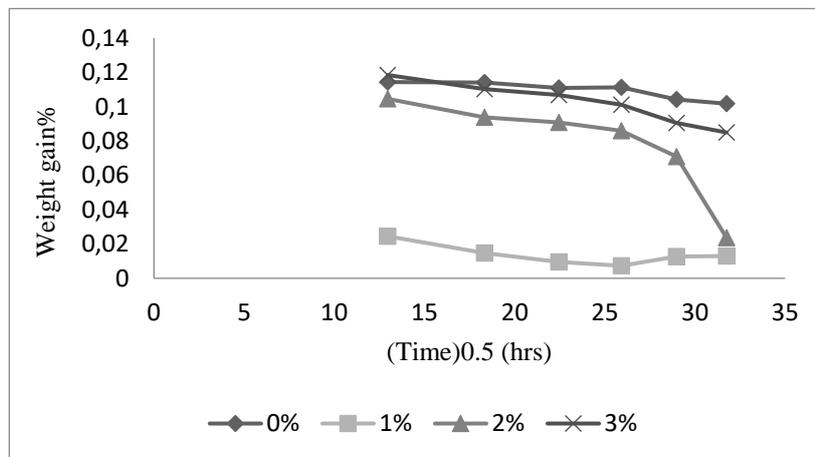


Fig. 5. Weight gain % vs square root time of immersion of all samples

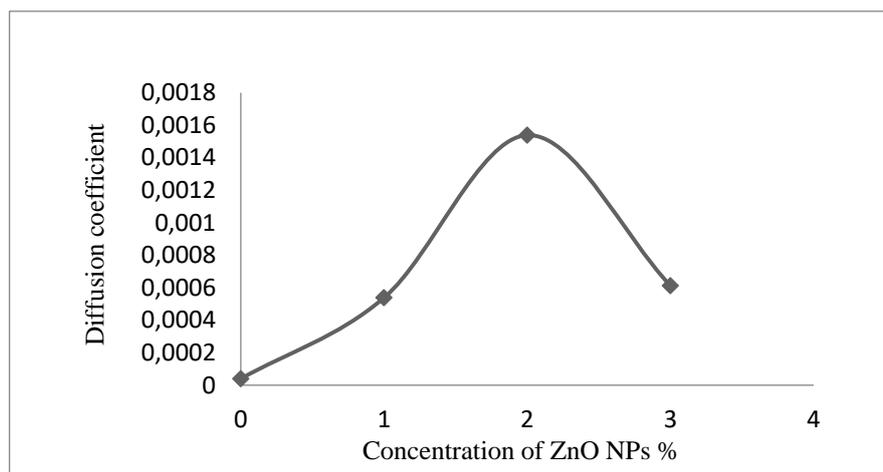


Fig. 6. Diffusion coefficient vs. ZnO NPs concentration

CONCLUSIONS

Nanocomposites were successfully prepared by the hand-lay method. The X-ray diagram revealed that the pure material had the highest intensity and values of 2θ when compared to the composites, indicating that it is a more crystalline material, and that the minor peaks present in the composite materials demonstrate the presence of the nano-filling material. The greatest value for the water diffusion coefficient was for the composite with a concentration of 2 %, indicating that it reached saturation at that concentration. It

can also be noted the clear effect of zinc nanoparticles on the formation and visualization of biofilms in both organisms, as it was significantly affected by the treatment with an increase in concentration. When increasing the concentration of ZnO NPs, the values of inhabitation zone of *F. solani* and *S. livindas* were increased by approximately 70 and 49 % respectively. This means that the prepared nanocomposites have the capability to inhibit the activity of *F. solani* greater than *S. livindans*. The process's simplicity allows for effortless expansion to meet industry demands.

Дослідження впливу оксиду цинку на протигрибкову дію полімерної суміші (ненасиченого поліестеру та натурального каучуку) для промислового застосування

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Включення неорганічних наночастинок у полімери дозволило створити полімерні наноструктурні матеріали з високоефективними та багатофункціональними характеристиками полімерів, які перевершують стандартні полімерні композити. Полімерні наноккомпозити мають багатофункціональні якості, такі як підвищена стійкість до вологи, грибкові та антибактеріальні властивості. У цьому дослідженні полімерну суміш ненасиченого поліестеру та натурального каучуку (80 / 20) готували як контрольний матеріал, а потім її зміцнювали наноксидом цинку у вагових співвідношеннях (1, 1.5, 2, 2.5 та 3 %), поступово додаючи при постійному перемішуванні. Антимікробну активність вивчали для двох типів ґрунтових мікробів, *Fusarium solani* та *Streptomyces lividans*, за допомогою методу дискової дифузії, і інгібування утворення біоплівки спостерігалось через 72 год інкубації, причому рівень інгібування досягав 40.61 та 69.39 % як у *Streptomyces lividans*, так і *Fusarium solani* відповідно. Також можна відзначити явний вплив наночастинок цинку на формування та візуалізацію біоплівок в обох організмах, оскільки на це значно вплинула обробка зі збільшенням концентрації. Також виконано рентгеноструктурний аналіз та визначено коефіцієнти дифузії води.

Коефіцієнт дифузії композитного матеріалу з концентрацією 2 % мав найбільше значення, після чого значення починали падати, тоді як зростання було лінійним при меншій концентрації. Рентгенівська діаграма показала, що композитний матеріал з концентрацією 1 % мав найнижчі значення 2θ , і що зі збільшенням концентрації значення зростали. Виходячи з цих висновків, видається, що синтетичні суміші, які посилені ZnONP, можуть бути перспективним матеріалом для емностей для води.

Ключові слова: мікробне зростання, наноккомпозити, полімерна суміш, коефіцієнт дифузії води, рентгенівська дифракція

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