Nano ZnO/amine composites antimicrobial additives to acrylic paints

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KEYWORDS
Acrylic paint; Antimicrobial activity; Zinc oxide; Propandiol; Triazole; Diphenyl amine

Abstract Nano ZnO has been widely used as an antimicrobial agent not only for food packaging purposes but also in many coating processes. The present work is meant to enhance such functions through the preparation of sustainable and safe conduct of nano ZnO composites with amine derivatives that are characterized by their antimicrobial and anti-fouling functional activities. The results obtained revealed a more comprehensive approach to the antimicrobial function based on the reported active oxide species role. The oxide/amine composites and the acrylic emulsion paint were characterized chemically and structurally through FT-IR, TGA and TEM supported by biological assessment of each ZnO/amine composite action. Results of the study concluded that equilibrium between the nano ZnO particles size, their dispersion form, and amine ability to stabilize the actively produced oxygen species responsible for the antimicrobial function, should all be accounted for when persistence of antimicrobial agent efficiency is regarded.

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1. Introduction

Long-term antimicrobial activity can be imparted in many coating formulations through the incorporation of nanomaterials. Zinc oxide is commonly used in pharmaceutical products to prevent or treat topical or systemic diseases owing to its antimicrobial properties [1]. ZnO nanoparticles were shown to have a wide range of antibacterial activities against both Gram-positive and Gram-negative bacteria, including major foodborne pathogens like Escherichia coli, Salmonella, Listeria monocytogenes, and Staphylococcus aureus [2–5]. It is necessary to understand the mechanism of ZnO action against bacteria, but to date, the process underlying its antibacterial effect is not clear. However, early studies suggested that the primary cause of the antibacterial function might source from the disruption of cell membrane activity [6]. Another possibility could be the induction of intercellular reactive oxygen species, including hydrogen peroxide (H$_2$O$_2$), a strong oxidizing agent harmful to bacterial cells [4,7]. It has also been reported that ZnO...
can be activated by UV and visible light to generate highly reactive oxygen species such as $\text{OH}^-$, $\text{H}_2\text{O}_2$, and $\text{O}_2^-$. Although the antibacterial mechanism of ZnO nanoparticles is still unknown, the possibilities of membrane damage caused by direct or electrostatic interaction between ZnO and cell surfaces, cellular internalization of ZnO nanoparticles, and the production of active oxygen species such as $\text{H}_2\text{O}_2$ in cells due to metal oxides were proposed in earlier studies [8,9]. The effectiveness of the nano ZnO in many antimicrobial applications as in food preservation [10], insecticide and synthetic and natural textiles was also identified and reported including improvements to coating properties, thermal and mechanical properties [11–14]. Such implemented direction of ZnO is based on its safety function in health and environment. In the present work, nano ZnO composites of four types of amines namely; 2-amino-1-(4-nitrophenyl)-1,3-porpandiol (PD), 3-amino-1,2,4-Triazole (T), diphenylamine (DPA) and N,N-dimethylamine (DMA) were evaluated as biocides in emulsion acrylic paints to emphasize their function through a probable cause of active oxygen species occurrence responsible for the ZnO antimicrobial function. The results were assessed with regard to the composite employed and the amine effect on the nano ZnO particles agglomeration or dispersion in addition to biological assessment of each amine composite for effectiveness comparison and preference focusing on the safest amine to humans and the environment.

2. Experimental methodology

1. Materials
   a. Acrylic emulsion paints; both biocide added and non-biodecide and free types of paints were supplied by Paints and Chemical Industries Company “Pachin”, El Obour city, Cairo. Solvents employed during the present work such as ethyl alcohol and N,N-dimethyl formamide (DMF) were supplied by El-Nasr pharmaceutical chemicals, Abu-Zaabal after being subjected to distillation, purification and drying prior to use. ZnO and all other chemicals and amines employed were Aldrich products.
   b. Nutrient agar medium (Difco), Nutrient broth medium (Difco) and Phosphate buffer saline (PBS) were all employed by the biological assessment tests as standard materials.

2. Preparation and synthesis
   a. Preparation of ZnO nanoparticlesZnO nanoparticles, of an average size of 30–95 nm, were prepared by grinding using ball mill technique available at the Egyptian Petroleum Research Institute (EPRI), Nasr City, Cairo. Rather the ball mill technique is not adequate to produce uniform ZnO particles in the nano form of 100 nm or less, it was employed, however, to accommodate the major aspect of using a broader scope of ZnO and to foresee the effect of the amines on the oxide dispersion factor.
   b. Synthesis of nano ZnO compositeExact weights of ZnO nanoparticle sample were slowly added to the respective weights of the different amines namely; 2-amino-1-(4-nitrophenyl)-1,3-porpandiol (PD), 3-amino-1,2,4-Triazole (T), diphenylamine (DPA) and N,N-dimethylamine (DMA) dissolved in DMF heated up to 100 °C. The whole mixture was then stirred for 24 h at room temperature. The mixture was finally dried under vacuum to remove the DMF solvent. The dry product is denoted as the nano ZnO composite with each of the amines. The ZnO/amine composites were thoroughly washed with bidistilled water to free the composites from any excess amines. 10% and 20% by weight of the ZnO to the total weight of the composite were prepared for activity comparison [15]. To affirm function and selectivity as a biocide additive, a series of weight% additives (1%, 2% and 3%) to raw paint were prepared and biologically tested.

3. Paint and additives: structural, thermal and antimicrobial features and characterization
   a. Chemical structure elucidation using FT-IR analysisAcrylic paint, different amine additives (biocides) and the paint/biocide were subjected to FT-IR (Fourier Transform-Infra Red Spectroscopy) structural conformation using Nicolet 6700 Thermo Scientific FT-IR available at the Central Laboratory of the Faculty of Science, Ain Shams University, Cairo.
   b. Thermo-gravimetric Analysis (TGA)The weight loss of all paint samples due to the effect of heat (thermal stability) was monitored using Thermal Gravimetric Analyzer (TGA) Shimadzu-50, under N2 atmosphere at a temperature rate of 10°/min available at the Central Laboratory of the Egyptian Petroleum Research Institute, Nasr City, Cairo.
   c. Transmission Electron Microscope TEM of the synthesized nano composites and ZnO nanoparticles were carried out at the TEM unit of the Faculty of Science, Ain-Shams University, Cairo using JOEL, JEM 1200 EX available at the Central Laboratory of the Faculty of Science, Ain Shams University, Cairo.
   d. Antibacterial activity The antibacterial activities of paint with different additives were tested against 6 strains of 2 multidrug resistant clinical isolates, namely; Pseudomonas aeruginosa and S. aureus; 3 strains of P. aeruginosa and 3 strains of S. aureus, which were supplied by Microbiology Department, Faculty of Science, Ain Shams University, Cairo, and prepared according to the following procedure:
   i. Preparation of bacterial inoculum A twenty-four hour nutrient broth culture of tested bacteria was grown in an orbital shaking incubator (120 rpm) at 37 °C and standardized to approximately 106 CFU ml⁻¹ using a nutrient broth medium.
   ii. Preparation of paint discs After mixing paint ingredients with and without additives thoroughly, drops of paints (50 μl) were loaded on clean plastic sheets in a sterile area. After drying the discs were kept in clean plastic bags and kept away from sunlight till tested. Discs with paint only (without additives) were prepared as control.
   iii. Antibacterial activity of paint discs Two different tests were carried out to test the antibacterial activities of paints.

Agar diffusion method: A standard disc diffusion method was used to detect the activity of paints and their constituents against the clinical bacterial isolates according to Cheesbrough (1989) and Adonizio et al., (2006)
Paint discs were loaded on plates containing nutrient agar medium seeded with 100 μl of the 24 h tested organism. Plates were incubated at 37 °C for 24 h and inhibition zones were detected by a clear zone around the disks.

**Turbidity method:** Sterile tubes containing 5 ml nutrient broth medium each was inoculated with 20 μl of the tested organism. Five paint discs were transferred to each tube in a sterile condition. Tubes that received no discs acted as microbial growth control. All tubes were incubated in an orbital shaking incubator (100 rpm) at 37 °C for 24 h. After an incubation period the turbidity was measured at OD 600 nm using a λ-Helios SP Pye-Unicam spectrophotometer.

**Antimicrobial data statistical analysis:** All statistical analyses in this study was carried out using Microsoft Excel 2000, Analysis Toolpack (Microsoft Corporation). All data were calculated from at least 3 replicates and the standard errors for each datum were plotted on the graph.

### 3. Results and discussion

#### 3.1. Chemical structural analysis (FT-IR)

The FT-IR structural analysis of acrylic paint, ZnO, PD, T, DPA and DMA was conducted for their chemical structural confirmation, results of which were found matching the referenced data [18–25], and are manifested in Table 1. The effect of the nano ZnO and its composites on the structural features of the acrylic paint is also shown in Table 2. As well noticed from the survey given in Tables 1 and 2, the amine additives in their distinct formulations did not interact covalently with either ZnO or with the polymeric acrylic paint chains with any new bands evolving. Therefore, the biological activity assessment results should not be refereed to any chemical changes of the additives onto both the ZnO particles and/or the polymeric based paint molecules, but should be directed towards the influence of physical affiliations imposed within the paint environment such as:

#### Table 1 FT-IR absorption spectral bands (cm⁻¹) of ZnO, amine additives and ZnO composite characterization.

<table>
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</tr>
</thead>
<tbody>
<tr>
<td>Finger print (447 cm⁻¹)</td>
<td>3373</td>
<td>3373</td>
<td>–OH</td>
<td>3413</td>
<td>3414</td>
<td>Asym. NH</td>
<td>3380</td>
<td>3380</td>
<td>–NH</td>
<td>3453</td>
<td>3431</td>
<td>Sec –NH</td>
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<tr>
<td></td>
<td>3306</td>
<td>3306</td>
<td>–NH2</td>
<td>3332</td>
<td>3331</td>
<td></td>
<td>3038</td>
<td>3036</td>
<td>Arom –C–H</td>
<td>1638</td>
<td>1626</td>
<td>Bend. Sec –NH</td>
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<tr>
<td></td>
<td>3074</td>
<td>3074</td>
<td>Arom.</td>
<td>3214</td>
<td>3214</td>
<td>Sym. –NH</td>
<td>1491</td>
<td>1490</td>
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<tr>
<td></td>
<td>2958</td>
<td>2959</td>
<td>Aliph.</td>
<td>1641</td>
<td>1641</td>
<td>NH₂</td>
<td>435</td>
<td>ZnO finger print</td>
<td>447</td>
<td></td>
<td>ZnO finger print</td>
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<tr>
<td></td>
<td>1518</td>
<td>1518</td>
<td>Arom.</td>
<td>1596</td>
<td>1597</td>
<td>–C–N str.</td>
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<tr>
<td></td>
<td>–</td>
<td>–</td>
<td>Shoulder at 450</td>
<td>1535</td>
<td>1535</td>
<td>N-N</td>
<td></td>
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<tr>
<td>Amines:</td>
<td>PD = 2-amino-1-(4-nitrophenyl)-1, 3-porpandiole, T = 3-amino-1,2,4-Triazole, DMA = dimethyl amine, DPA = diphenylamine.</td>
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<tr>
<td>Composites:</td>
<td>ZnO_PD = ZnO propan oil composite, ZnO_DMA = ZnO dimethylamine composite, ZnO_T = ZnO triazole composite, ZnO_DPA = ZnO diphenylamine composite</td>
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</tr>
</tbody>
</table>

#### Table 2 FT-IR spectrum analysis (cm⁻¹) of basic poly acrylic paint and its ZnO/amine added characterization.

<table>
<thead>
<tr>
<th>Native acrylic paint</th>
<th>ZnO/PD + Paint</th>
<th>ZnO/T + Paint</th>
<th>ZnO/DPA + Paint</th>
<th>ZnO/DMA + Paint</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>3364</td>
<td>3372</td>
<td>3331</td>
<td>Screened at broad 3380</td>
<td>3366</td>
<td>–OH Str.</td>
</tr>
<tr>
<td>2954</td>
<td>2954</td>
<td>2954</td>
<td>2954</td>
<td>2954</td>
<td>–NH₂</td>
</tr>
<tr>
<td>2873</td>
<td>2873</td>
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<td>2873</td>
<td>2873</td>
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<tr>
<td>1733</td>
<td>1733</td>
<td>1733</td>
<td>1733</td>
<td>1733</td>
<td>–C–O</td>
</tr>
<tr>
<td>1521</td>
<td>1521</td>
<td>1639</td>
<td>–</td>
<td>–</td>
<td>–NH₂ sciss</td>
</tr>
<tr>
<td>1167</td>
<td>1167</td>
<td>1167</td>
<td>1167</td>
<td>1167</td>
<td>–N-N</td>
</tr>
<tr>
<td>1020</td>
<td>1020</td>
<td>1020</td>
<td>1020</td>
<td>1020</td>
<td>–NO₂</td>
</tr>
</tbody>
</table>
- direct functional cause of the ZnO,
- ZnO particle size role,
- medium acidity/basicity and aromaticity parameter,
- biological cell/additive interaction accessibility based on a proposed active oxygen species production as thought responsible of microorganism’s disability.

3.2. Thermal stability (TGA)

Fig. 1, illustrates the TGA profiles of dried basic acrylic paint films and nano ZnO composite additive paints. As revealed, thermal degradation of the paint films occurs at one step only at around 350 °C for nearly all samples, at a mere equal weight loss of 34.9–37.4%. The weight loss is mostly inferred to the decomposition of the binder material, acrylic polymer and the ratio of additives to the acrylic paint [18]. The slight differences of weight loss% when comparing the ZnO composite added paint films could be probably due to the indulged agglomeration or dispersion caused by the amine presence and as noted from the TEM images, Fig. 2. TGA results confirm the persistence of the thermogram of either the pure or ZnO/amine composites added paints excluding the possibility of chemically modified material formation [14]. The above results indicate that the ZnO/amine composites of different natures had the slimmest effect on the basic acrylic emulsion paint thermal stability.

3.3. Nano ZnO and ZnO/amine composite structural features (TEM)

The structural and agglomeration/dispersion features of nano ZnO and its amine composites were studied, results of which are represented in Fig. 2(a–e). Nano ZnO particles, as prepared is exhibited in Fig. 2 where the irregular pattern of particle size prevails at a wide range of 30.9–235 nm. The effect of the various amines added proved its role towards ZnO nano particles disintegration differentially. TEM images revealed that T and PD seem to have a strong function in disintegration of the ZnO particles (15.1–51.2 nm and 14.7–71.1 nm), Fig. 2(d, e) with the least disintegration influence represented by DMA and DPA, Fig. 2(b, c) (56.6 nm and 67.9 nm). TEM images help in elucidating the role of dispersion of the nano ZnO particles and its effective exposed surface to the biological cells. Amines nature being aliphatic or aromatic is then regarded as an effective factor towards selectivity and efficiency when evaluating the amine role as an antimicrobial agent. Such a concept will be increasingly confirmed by monitoring the biological data of each amine.

3.4. Microbiological activity of native and antimicrobial added acrylic paints

The antimicrobial activity of paint samples, blank and composed nano composites, against the Gram –ve and Gram

![Figure 1](link-to-figure)  
**Figure 1** Thermo gravimetric analysis of (a) Acrylic paint (Basic), (b) Commercial antimicrobial acrylic paint, (c) Acrylic paint in presence of ZnO/PD, (d) Acrylic paint in the presence of ZnO/T, (e) Acrylic paint in the presence of ZnO/DMA and (f) Acrylic paint in the presence of ZnO/DPA.
+ve microorganisms in relation with the particle size of ZnO is illustrated in Table 3. TEM results illustrate the effect of the additives PD, T, DPA and DMA on the mode of dispersion and agglomeration of nano ZnO particles. PD and T show ability to disperse the nano ZnO particles while DPA and DMA lead to a decrease in dispersion (i.e. increase of agglomeration) of nano ZnO (action of surfactancy). Such physical dispersion or agglomeration seems to link directly to the ZnO nano particle activity as an antimicrobial agent. In general, the antimicrobial activity of nano composites of ZnO with PD and T was found higher than that of DPA and DMA which agreed with the concept that ZnO particles with smaller size, larger specific area and higher porosity exhibit higher antimicrobial activity [1].

Higher activity detected with ZnO/PD acrylic paint resembles that of the commercially marketed Pachin paint (885), higher than that of pure ZnO. PD acquires specific damaging effect to the peptide links of the cell membrane or the lipids part of the cell wall, may be due to its alcoholic nature and structural character, especially with Gram –ve microorganisms that acquire higher lipids content and lower cell wall thickness than those of Gram +ve microorganisms. The practical results of the turbidity test, Table 3, indicated a higher functional activity of propanediol

Figure 2  TEM images of (a) nano ZnO, (b) ZnO/DMA, (c) ZnO/DPA, (d) ZnO/T and (e) ZnO/PD.
when ZnO is added which could be assigned to the influence and sustainability of active oxygen species produced by the ZnO environment [4–7] functioning as a specific oxidizing agent to the protein content of the microorganisms cell. The mechanism of the inhibitory effect of ZnO nanoparticles on microorganisms is not fully understood. Several studies reported that integration of ZnO nanoparticles into bacterial cells may induce the continuous release of membrane lipids and proteins, which changes the membrane permeability of bacterial cells [6,26]. The combination of ZnO/PD seems an efficient antimicrobial additive due to the dual selective action of both the -diol and the oxide as an overall activity against the protein entity of the microorganism.

The antimicrobial activity of triazole, in general, is reported to be referred to the presence of C=N (azomethine group) [27]. When ZnO is added to triazole (ZnO/T), its antimicrobial activity is increased linearly and specifically in Gram +ve microorganisms, Table 3 and Fig. 3 (spots 12, 15).

The following plates, Fig. 3 (3, 6, 9), illustrate the direct effect of ZnO/T and ZnO/PD on the Gram –ve and +ve

<table>
<thead>
<tr>
<th>Additive type</th>
<th>Type of emulsion acrylic paint</th>
<th>ZnO amine composites average particle size nm (TEM)</th>
<th>Biological activity* (Microorganisms resistance) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>Pachin Virgin (emulsion acrylic paint without additives)</td>
<td>–</td>
<td>0 8</td>
</tr>
<tr>
<td>Commercial biocide</td>
<td>Pachin commercial 885 (antimicrobial emulsion acrylic paint)</td>
<td>–</td>
<td>74 98</td>
</tr>
<tr>
<td>ZnO PD</td>
<td>Pachin Virgin emulsion acrylic paint + 3% nano ZnO PD</td>
<td>15.9–235</td>
<td>58 36</td>
</tr>
<tr>
<td></td>
<td>Pachin Virgin emulsion acrylic paint + 3% PD PD</td>
<td>–</td>
<td>80 31</td>
</tr>
<tr>
<td></td>
<td>Pachin Virgin emulsion acrylic paint + 3% (10%ZnO/PD)</td>
<td>–</td>
<td>84 42</td>
</tr>
<tr>
<td></td>
<td>Pachin Virgin emulsion acrylic paint + 3% (20%ZnO/PD)</td>
<td>14.7–71.1</td>
<td>89 55</td>
</tr>
<tr>
<td>T PD</td>
<td>Pachin Virgin emulsion acrylic paint + 3% T</td>
<td>–</td>
<td>55 14</td>
</tr>
<tr>
<td></td>
<td>Pachin Virgin emulsion acrylic paint + 3% (10%ZnO/T)</td>
<td>–</td>
<td>58 19</td>
</tr>
<tr>
<td></td>
<td>Pachin Virgin emulsion acrylic paint + 3% (20%ZnO/T)</td>
<td>15.1–51.2</td>
<td>70 55</td>
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<tr>
<td>DPA</td>
<td>Pachin Virgin emulsion acrylic paint + 3% DPA</td>
<td>–</td>
<td>42 49</td>
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<td></td>
<td>Pachin Virgin emulsion acrylic paint + 3% (10%ZnO/DPA)</td>
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<td>42 49</td>
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<td></td>
<td>Pachin Virgin emulsion acrylic paint + 3% (20%ZnO/DPA)</td>
<td>67.9–163</td>
<td>42 49</td>
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<tr>
<td>DMA</td>
<td>Pachin Virgin emulsion acrylic paint + 3% DMA</td>
<td>–</td>
<td>33 50</td>
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<td></td>
<td>Pachin Virgin emulsion acrylic paint + 3% (10%ZnO/DMA)</td>
<td>–</td>
<td>64 50</td>
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<tr>
<td></td>
<td>Pachin Virgin emulsion acrylic paint + 3% (20%ZnO/DMA)</td>
<td>56.6–154</td>
<td>70 50</td>
</tr>
</tbody>
</table>

* Biological activity % as results of turbidity tests.

Figure 3 Antimicrobial activities of paints against (a) Pseudomonas aeruginosa (Gram –ve), (b) Staphylococcus aureus (Gram + ve). Where 3: the acrylic paint in presence of 3% PD, 6: the acrylic paint in presence of 3%(10%ZnO/PD), 9: the acrylic paint in presence of 3%(20%ZnO/PD), 12: the acrylic paint in presence of 3% T, 15: the acrylic paint in presence of 3%(10%ZnO/T).
microorganisms at 3% concentration. As shown the ZnO/PD is superior to other amines as an antimicrobial agent with an apparent selectivity towards Gram –ve microorganisms.

DMA is not a strong specific microorganism assailant by its own, however, the moderate activity for the ZnO/DMA composite could be due to the DMA itself rather for the composite, Table 3 and Fig. 4 (spots 23 and 26). ZnO/DPA limited the antimicrobial activity exhibited, is probably due to limited ability of DPA to bind with the bacteria DNA [28–29] the ZnO itself prevailing even though the DPA had affected its dispersion negatively by decreasing its exposed active surface to microorganisms decreasing in turn its overall activity compared to bulk ZnO nano particles; Table 3 and Fig. 5.

Rather various cationic surfactants are variably employed as antimicrobial agents as with epoxy paints [30], the present ZnO/amine composites are characterized by their multi attacking mechanisms of both the DNA and the cytoplasmic membrane.

4. Conclusion

Propanediol, as a health and environment safe additive has exhibited the most suitable amine composite with ZnO nanoparticles producing an antimicrobial agent equivalent or superseding the commercial marketed Pachin paint 885. Nano ZnO/PD composite could be considered as the proper encouraging environment for the production and sustaining the active oxygen species production regarded as responsible for biological cell damaging. The concluded results indicate the importance of hydroxyl group’s presence of the amine additive which might support the active oxygen production sought mechanism when compared to amine groups that might hinder such an effect but for a limited period. The TEM images of the nano ZnO particles and its amine composites proved that DPA presence has increased the nano ZnO particles agglomeration leading in turn to a less exposed active sites which could have depreciated its ability as an antimicrobial agent. On the other hand, and while DMA showed fewer particles agglomeration, compared to the DPA, amine aromaticity could be considering as a hindering factor in the continuous active oxygen species production responsible for the resistance cause. Rather, triazole (T) illustrated an excessive dispersion of the ZnO particles, elevation of its efficiency as an antimicrobial should be expected, but the depression exhibited could be due the selectivity towards specific bacterial microorganisms compared to the PD amine when functioning in the presence of ZnO. Accordingly, it should be concluded that equilibrium between the nano ZnO particles size, their dispersion form, amine ability to stabilize active produced oxygen, and type of bacterial microorganisms should all be counted for when persistence of antimicrobial agent efficiency is regarded.
Acknowledgements

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