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Modeling *Dialium guineense* mediated Zn-nanoparticle growth inhibition on Gram-positive microbes inducing microbiologically-influenced-corrosion

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ABSTRACT

In this paper, the minimum inhibition concentration of *Dialium guineense* (*D. guineense*) mediated Zn-nanoparticle material on Gram-positive strains of microbes inducing microbiologically-influenced-corrosion (MIC) of metals, were experimentally studied and with the experimental data subjected to numerical analyses. Four strains of Gram-positive strains of microbes that are known to induce MIC on metallic materials were employed in seeded agar plates and against which different concentrations of Zn-nanoparticle (having *D. guineense* leaf-extract as precursor) were dispersed. Growth inhibition measurements of the Gram-positive microbial strains were then analyzed via mathematical correlation modeling, analyses of variance and Newton-Raphson methodology for improving details of minimum inhibition concentration of the bio-synthesized Zn-nanoparticle material against the microbial strains. Results from the study are of importance for responsible applications of MIC controlling, and for avoiding inadvertent MIC aggravating, system.

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1. Introduction

Microbiologically-influenced-corrosion (MIC) of metals is usually initiated via the activities of microbial strains in the colonies of biofilms that the microbes form on metallic surfaces [1–6]. Studies have deliberated on corrosion control approach channelled via making metallic surface non-conducive for interactive attachment of senile pioneering strain of microbe that could further promote secondary microbial attractions [5,7–10]. Proposed approaches for attaining such metallic surface non-conduciveness have include use of plant-extract mediated metallic-nanoparticle materials as additives in metallic surface protection techniques [7,9]. For these, studies are needed for effectiveness of such nanoparticle materials on the inhibition of microbial strains inducing MIC. Microbial strains have been shown in reported work to be sensitive to *Dialium guineense* (*D. guineense*) mediated Zn-nanoparticle material via effective growth inhibition of microbes including Gram-positive strains [7]. However, there is dearth of study on the minimum inhibition concentration of this bio-nanoparticle material that could be effective on Gram-positive strains of microbes inducing MIC. This paper therefore employs empirical and mathematical correlation modelling for investigating growth inhibition of selected Gram-positive strains of microbes inducing MIC by varying concentrations of *D. guineense* mediated Zn-nanoparticle material. This study is especially channelled towards studying requisite minimum inhibition concentration of the bio-nanoparticle material for effectively affecting growth of Gram-positive microbes inducing MIC.

2. Experimental

Methods for *D. guineense* mediated Zn nanoparticle materials synthesis, characterization and applications to selected microbial strains known to induce MIC have been reported in [7]. As also detailed in that report, test-isolates of Gram-positive microbial strains, which for the present study include *Streptococcus* spp., *Bacillus* spp., *Staphylococcus aureus* (*S. aureus*) and *Micrococcus varians* (*M. varians*), were obtained from culture collection of the

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Biotechnology Unit of Department of Applied Biological Sciences, Covenant University, Ota, Ogun State, Nigeria. The preparation, and their seeding on nutrient agar plates of, these test isolates followed standard procedures prescribed in the literature [7–9,11]. For the present study, however, concentrations ranging from 100 to 1.5625 g/L of C_2H_6OS (Dimethyl sulfoxide: DMSO), obtained via serial dilution, of the *D. guineense* mediated Zn-nanoparticle were dispersed into 9 mm bored well of the nutrient agar plate seeded with the test-isolates. Zones of inhibition were then measured after the agar plates had been incubated at 37 °C for 24 hrs. These was followed by correlation modelling of measured zone of inhibition, ζ, with function of *D. guineense* mediated Zn-nanoparticle concentration, ρ. The resulting correlation models was then resolved using Newton-Raphson methodology, via Eq. (2) applications [12], for the mathematical model of the minimum inhibition concentration for comparing with the experimental model.

$$\rho_{n+1} = \rho_n - \frac{f(\rho)}{f'(\rho)};\ \text{for } n = 0, 1, 2, \ldots \quad (1)$$

3. Results and discussion

The results from the experimental study of different *D. guineense* mediated Zn-nanoparticle concentrations effects on the microbial strains are shown in Fig. 1. It could also be noted that the linear plot of the well-bore diameter, i.e. Well ∅, used for each of the isolates, as well as concentrations of *D. guineense* mediated Zn-nanoparticle, is included in the figure.

It could, therefore be noted in Fig. 1, that similar pattern of the zone of inhibition effects was depicted in the figure for each of the tested isolates, even as the zone of inhibition effects tend towards the well-bore diameter, as the *D. guineense* mediated Zn-nanoparticle concentration decreases. These similarities in inhibition effect patterning engender interests towards the furtherance of mathematical correlation investigations applied to the zone of inhibition, which serve as the microbial strains growth inhibition responses to the different concentrations of *D. guineense* mediated Zn-nanoparticle concentrations. From these, correlation equations, obtained for each of the strains of Gram-positive microbes, were observed to follow the compact relationships [13–14] between the zone of inhibition, ζ, as the dependent variable and the *D. guineense* mediated Zn-nanoparticle concentration, ρ, as the independent variable that could be represented as:

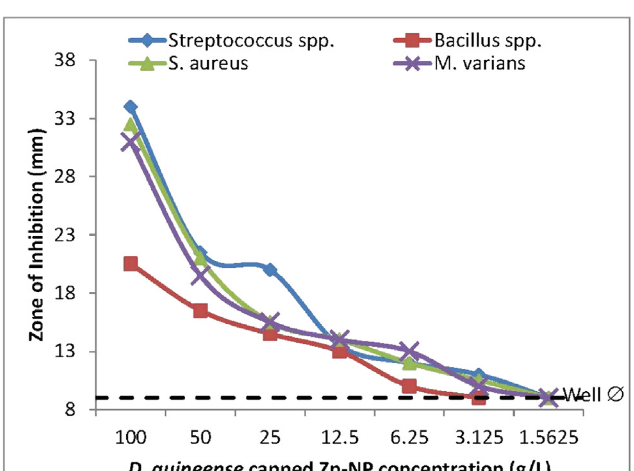

Fig. 1. Zone of inhibition of the growth of tested isolates of Gram-positive microbial strains by different concentrations of *D. guineense* mediated Zn-nanoparticle.

$$\zeta = Ae^{-\rho} + B\ln\rho + \sum_{k=0}^{2} C_k \rho^k \quad (2)$$

In Eq. (2), A, B, and C_k (k = 0, 1, 2) are numerical constants. These have the numerical values and the coefficient of determination, R^2 (for gaining insight into the efficiency of the correlation model [15–16]) that are as tabulated in Table 1, for each of the Gram-positive strains of microbes for the present study.

The values of the coefficient of determination for each of the correlation relationship were such that $R^2 > 0.90$ (or 90%) which interpret to excellent model efficiency for each of the correlation model [15,17]. For ascertaining the significance, or otherwise, of the expressed relationships between ζ and ρ, analyses of variance [18] was carried out for each of the mathematical correlations presented in Eq. (2), which are having coefficients in Table 1, and the results of the ANOVA are tabulated in Table 2.

The results from the analyses of variance showed that the p-value is <0.05 for all the correlation relationships investigated. These imply that it cannot be said that the relationships between the zone of inhibition, ζ, by the microbial strains, and the *D. guineense* mediated Zn-nanoparticle concentration, ρ, are not significant, within confidence intervals that are greater than 95%.

Applications of the Newton-Raphson procedure, in Eqs. (1) and (2) from which $\zeta = f(\rho)$, find usefulness for obtaining the mathematical models of the minimum inhibition concentration of *D. guineense* mediated Zn-nanoparticle, $\rho_{(\min,pred)}$. These predictions, for comparison, are then plotted, in Fig. 2, with the experimentally measured values of minimum inhibition concentration of *D. guineense* mediated Zn-nanoparticle, $\rho_{(\min,\exp)}$, for each of the Gram-positive strains of microbes being studied. Fig. 2 shows that there is a slight discrepancy, of about 25%, between the mathematically predicted and the experimental minimum inhibition concentration of *D. guineense* mediated Zn-nanoparticle to which the microbial strain, *Streptococcus* spp., would become resistant. This discrepancy in predicted and experimental minimum inhibition concentration are lower for *Bacillus* spp. (about 6%) and for *S. aureus* (about 3%), but significantly high at about 92% for *M. varians* strains of Gram-positive microbes.

For a proper perspective into the effect of the discrepancies between the predicted and experimental models of minimum inhibition concentrations on the growth inhibition of the microbial strain, the computed zone of inhibition values, via Eq. (2) applications, at each of the minimum inhibition concentration models are presented in Fig. 3. This figure shows that the $\zeta = 0$ for all the predicted models of minimum inhibition concentration of *D. guineense* mediated Zn-nanoparticle against the growth inhibition of the Gram-positive microbial strains, being investigated. These results highlight the effectiveness of the Newton-Raphson procedure at obtaining the root of Eq. (2) for all the Gram-positive microbial strains studied, and thus the mathematical prediction of the minimum inhibition concentration of *D. guineense* mediated Zn-nanoparticle that will inhibit the growth of each microbial strain. The slight discrepancy observed between the predicted and mathematical model from *Streptococcus* spp. culminated into a positive zone of inhibition against this microbial strain, at the $\rho_{(\min,\exp)}$ for the strain. This well corroborates higher value of $\rho_{(\min,\exp)}$ compared to its mathematically predicted counterpart for *Streptococcus* spp. This is just how the lower values of $\rho_{(\min,\exp)}$ in comparisons to the $\rho_{(\min,pred)}$ resulted into the negative zone of inhibition (i.e. actual encroachment into the well-bore of the microbial strain seeded agar plate into which the *D. guineense* mediated Zn-nanoparticle were dispersed) by the *Bacillus* spp., *S. aureus* and *M. varians* microbial strains. Among these, the encroachment into the well-bore of agar plate seeded by *M. varians* is of the highest for it was up to 1.45 mm into the 9 mm well-bore diameter of *D. guineense* mediated Zn-nanoparticle dispersed into

Table 1
Coefficients and correlation fitting parameter for the compact Eq. (2).

Microbial strain	*A*	*B*	C_1	C_2	C_3	R^2
Streptococcus spp.	9.2597	3.8049	1.3837×10^{-03}	-3.0394×10^{-02}	−3.4362	*0.9919*
Bacillus spp.	−15.4314	0.0000	8.2860×10^{-04}	1.4893×10^{-01}	2.6916	*0.9916*
S. aureus	−10.7262	0.0000	2.5018×10^{-04}	1.9132×10^{-01}	1.8484	*0.9979*
M. varians	1.9698	3.0122	1.8881×10^{-03}	-8.9960×10^{-02}	−1.7260	*0.9971*

Table 2
Analyses of variance for ascertaining significance of relationships in the compact Eq. (2).

Microbial strain	***Streptococcus spp.***					***Bacillus spp.***				
Source of Variation	df	SS	MS	F	*p*-value	df	SS	MS	F	*p*-value
Treatment	4	446.7017	111.6754	27.1488	**0.0358**	3	467.7570	155.9190	118.2022	**0.0013**
Residual	2	8.2269	4.1135			3	3.9573	1.3191		
Total	6	454.9286				6	471.7143			
Microbial strain	***S. aureus***					***M. varians***				
Source of Variation	df	SS	MS	F	*p*-value	df	SS	MS	F	*p*-value
Treatment	3	395.0134	131.6711	468.1611	**0.0002**	4	334.5183	83.6296	170.3757	**0.0058**
Residual	3	0.8438	0.2813			2	0.9817	0.4909		
Total	6	395.8571				6	335.5000			

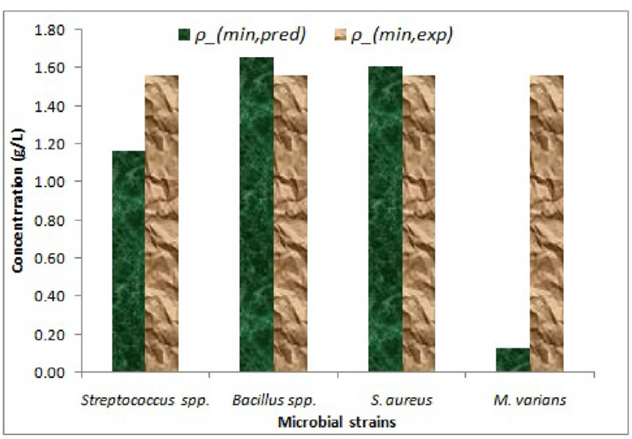

Fig. 2. Predicted and experimental minimum inhibition concentrations of *D. guineense* mediated Zn-nanoparticle for the studied Gram-positive strains of microbes.

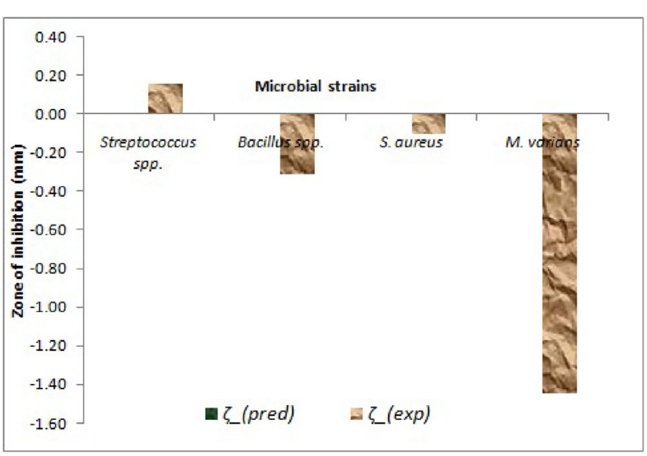

Fig. 3. Zone of inhibition obtained from the predicted and experimental minimum inhibition concentration models of *D. guineense* mediated Zn-nanoparticle.

the seeded plate. By this, therefore, the minimum inhibition concentration modeling presented in this work exhibit high potential of being useful for responsible application of growth inhibition implementations, especially, against microbes inducing MIC attacks on metal, in other to ensure effective MIC controling, instead of MIC aggravating, applications.

4. Conclusion

In this paper, the minimum inhibition concentration of *D. guineense* mediated Zn-nanoparticle on Gram-posisitve strains of microbes Inducing MIC has been modeled. Results from the study shows that the minimum inhibition concentration prediction from the mathematical modeling exhibit potentials of responsible applications of growth inhibition controls against microbes inducing MIC, via ascertaining the concentration model at which the applied nanoparticle material could lose inhibition effect. The procedure employed in the study effectively correct over-prediction and under-predictions of minimum inhibition concentration of *D. guineense* mediated Zn-nanoparticle on the Gram-positive strains of microbes employed for the study.

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