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Research Article

The Efficacy of Parmetol DF 19 Forte as a Paint Fungicide

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ABSTRACT: *The potential of commercially sold Parmetol DF 19 Forte as a paint fungicide was examined. The microbial strains that were used for carrying out the test were isolated from old and worn-out painted surfaces. The isolates were initially screened for their ability to utilize paint as the sole carbon source using mineral salt medium incorporated with the paint, redox indicator and tween 80. The most paint utilizers were fungi identified as Phialophora verrucosa and Madurella mycetomatis. They were subjected to susceptibility test on the biocide using agar well diffusion method. A cidal effect of Parmetol DF 19 Forte on Phialophora verrucosa (>80±0.3mm) and Madurella mycetomatis (>12.5±0.05mm) was obtained. Minimal inhibitory concentration of the parmetol DF 19 Forte was at 0.01g/ml. This result indicated the potential of parmetol DF19 forte as a fungicide and thus suggests its utilization as a preservative for paint manufacturing at the right concentration.*

Keyword*: Agar-Well diffusion, Fungi Isolates, Painted surfaces, Parmetol DF 19 Forte, Susceptibility***.**

I. INTRODUCTION

Paint is a liquefiable, or mastic composition that, after application to a substrate in a thin layer, converts to a solid film. It is most commonly used to protect, color, or provide texture to objects.

Paint is made up of components which include the vehicle, the binder or film former, diluent(solvent or thinner), pigment and additives. The vehicle is the combination of binder + diluent [1][2] . The binder on the other hand is the film-forming component of the paint [3]. It is the only component that is always present among all the various types of formulations. The main purposes of the diluent are to dissolve the polymer and adjust the viscosity of the paint. It is volatile and does not become part of the paint film. The Pigment and filler- Pigment are granular solids incorporated in the paint to contribute color. Fillers are granular solids incorporate to impart toughness and texture and this gives the paint special properties. Besides the three main categories of ingredients, paint can have a wide variety of miscellaneous additives, which are usually added in small amounts, yet provide a significant effect on the product. Additives normally do not significantly alter the percentages of individual components in a formulation [4].

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The painted surfaces undergo damage or discolouration due to natural weathering, and the growth and activity of living organisms. Paints and coatings are susceptible to bacterial and fungal growth when in the liquid state but prone to colonization by microorganisms after application. The colonization of the painted surfaces leads to its discolouration and this is termed biodeterioration.
Biodeterioration involves the deterioration of substances that are normally resistant to biological attack such as metals, plastics, drugs, cosmetics, paintings, sculpture, wood products, electrical equipment, fuels and oils, and other objects [5]. Various types of organisms are involved in paint spoilage and they include bacteria, fungi, algae and protozoa. For instance, *Aspergillus niger* has been reported to be involved in degradation of pseudoplastic paint thickener [6]. The microbes tend to deteriorate the emulsion type of paint then the oil. This may be due to their strong enzymatic activities on the paint. Biodeterioration of a surface leads to formation of biofilms in which its removal can be both unsightly and hazardous to health [7].

As a result, a commercial biocide parmetol DF 19 forte has been introduced to tackle this degradation of paint caused by microorganisms [8]. Parmetol DF 19 forte is a reliable and cost efficient biocide for outdoor application. It is also free of Volatile Organic Compound and organic solvents [8]. Active substances in parmetol DF 19 forte are carbendazine and diuron. The efficacy of Parmetol DF 19 forte was proven through extensive laboratory testings with common facade and plaster coatings on the market according to the methods SM 022/023 (analogous VdL RL 06/07) against the following organisms: *Aspergillus niger, Penicillium funiculosum*, *Scenedesmus vacuolatus*. Parmetol DF 19 forte has algaecidal as well as herbicidal efficacy [8].

Although, this biocide has been proved effective by laboratory testing and it is in use by some paint manufacturers, the painted surfaces still undergo degradation by microorganisms. It then becomes paramount that the minimum inhibitory concentration of the biocide should be carried out so as to know the dosage that can be used as to ensure its effectiveness. Thus, the aim of this study was to confirm the efficacy of the parmetol DF 19 forte as a fungicide and determine its minimum inhibitory concentration for effective utilization.

II. MATERIALS AND METHODS

2.1 Description of the sampling site

Five different faculty buildings with cracked worn-out painted surfaces around Nnamdi Azikiwe University, Awka were used for this study.

2.2 Collection of the painted wall samples

A sterile spatula was used to remove the cracked paint from the surface and introduced into a sterile petridish and transferred aseptically to the laboratory where it was analyzed within 30 minutes of collection.

2.3 Isolation of the test organisms

Prior to isolation, a ten-fold serial dilution of the paint samples was carried out by dissolving lg of the homogenized sample in 10ml of the sterile mineral salt medium composed using the method of [6] K_2HPO_4 0.7%, KH_2PO_4 0.2%, $Na_3C_6H_5O_7.2H_2O$ 0.05%, $MgSO_4.7H2O$ 0.01%, NH_4SO_4 0.1%. Subsequent dilutions in the range of 10^{-1} to 10^{-10} were carried out. Thereafter, 1ml of the selected paint diluents (10^{-5} and 10^{-6}) was pipetted into 20ml of mineral salt medium incorporated with 0.3g of paint and chloramphenicol. The flasks were incubated at 30^0C with agitation (150 rev/min) for 7 days. Pure cultures were prepared by plating out of the enrichment broth on Sabourand Dextrose

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Agar. The Petri dish was incubated for 96 h. The resultant colonies were stored in agar slants in bijou bottles before being used for identification.

2.4 Screening for Paint Utilization by the isolates

The medium used in assessing the ability of the microbial isolate to utilize paint was a mineral salt medium (K_2HP0_4 0.7%, KH_2PO_4 0.2%, $Na_3C_6H_5O_7.2H_2O$ 0.05%, Mg $S0_4.7H_20$ 0.01%, NH_4S0_4 0.1%) containing 0.3% emulsion paint and 2.0% of oil paint respectively. Redox indicator 2, 6 dichlorophenol indophenol (2%v/v) and Tween 80 (0.1%v/v) were also incorporated.

A modified method of [9] was utilized for the screening. Two agar plugs ($1cm^2$ each) of a pure grown isolate were inoculated into each paint incorporated mineral salt medium. The flask without the organism served as the control. Incubation with agitation was carried out as earlier stated. The aliquots in the flasks were monitored daily for colour change (from deep blue to light pink). After 7 days the aliquots were centrifuged at 5000rpm for 5mins and the supernatant read spectrophotometrically at 609nm.

2.5 Characterization and identification of the fungal isolates

Pure colonies of the fungal isolates were characterized and identified according to the method of [10].

2.5.1 Slide Culture Preparation

A sterile SDA was pipetted asceptically to a sterile slide. A fungal isolate was inoculated to each slide and covered with a cover slip. The slide was laid on a petridish, covered and incubated without inverting the position at a temperature of 30^oC for 5 days to allow the organism to grow very well.

2.5.2 Microscopic Examination of the slide culture

A fresh slide was flooded with few drops of lactophenol cotton blue, and covered with a cover slip. The slide culture itself was also flooded with few drops of lactophenol cotton blue and covered with a fresh slip. Both were thereafter viewed under x40 objective lens.

2.5.3 Screening for the biocide susceptibility to microbial Isolates

The anti-fungal biocide parmetol DF 19 forte which is normally used in paint was screened for its susceptibility to microbial degradation. The fungal spores of the two best potential strains that changed the colour of the redox indicator faster; and whose optical density was higher than the others (*Phialophora verrucosa* and *Madurella mycetomatis*) were spread on a petridish containing SDA. Then, a sterile disc impregnated with the biocide was carefully placed on the cultured SDA plates. After 48h incubation, zones of inhibition were observed.

2.6 Minimum Inhibitory Concentration Determination of the fungicide

A tenfold dilution of parmetol DF 19 forte with sterile water was carried out using the method of [11]. A pure culture of the two best potential strains (*Phialophora verrucosa* and *Madurella mycetomatis*) was tissed with 1ml of

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sterile water to release the spore, and 0.1ml was collected and inoculated into a fresh plate containing sterile SDA. Four agar wells (6mm) each were bored on the petridish with an agar borer and 0.05ml of diluted parmetol was pipetted into the well and allowed to stand for sometimes prior to incubation at 30^0C for 72hrs. The developed zones of inhibition were measured and recorded.

III. RESULTS

3.1 The Screening Test of the Fungal Isolates

All the fungal isolates were able to change the colour of the redox indicator from blue to light pink. Isolates identified as *Phialophora verrucosa* and *Madurella mycetomatis* were the fastest in changing the colour of the indicator and has the highest optical density value.

3.2 Cultural and Microscopic Characteristics of the Fungal Isolates

The fungal isolates identified are shown in Table 5. They were identified using the method of [10]. The organisms identified are *Penicillium link, Lecythophora hoffmanii, Blastomyces dermatitis, Madurella mycetomatis, Syncephalastrum, Phialophora verrucosa, Phialophora jeanselmei, Cladosporium carrionii, Aspergillus flavus, Trichophyton equinum*.

3.3 Susceptibility Test

Table 12 shows the result of the susceptibility test of the partmetol DF 19 forte to the fungal isolate *Madurella mycetomatis* and *Phialophora verrucosa*. It was observed that 0.1g/ml and 0.01g/ml gave a susceptibility result while in the other concentrations, the organisms were found to be resistant to it. In the result shown for the different concentrations that were effective *Madurella mycetomatis* values was 24mm for 0.1g/ml and 12.5mm for 0.01g/ml but that of *Phialophora verrucosa* was totally inhibited as growth was not observed on the plate.

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Table 1: Time profile of paint utilization by the microbes

Isolate Designation	Exposure period of the Isolates to both oil and emulsion paint (days)						
	1	2	3	4	5	6	7
F	-	+	++	+++	+++	+++	+++
D	-	+	+	++	++	+++	+++
C	-	-	+	+	+	++	++
H	-	-	+	+	+	++	++
B	-	-	-	+	+	+	++
A	-	-	-	+	+	+	++
G	-	-	-	-	+	+	++
I	-	-	-	-	-	+	+
E	-	-	-	-	-	+	+
J	-	-	-	-	-	+	+

Note: Redox colour change was from blue to light pink

- \- No colour change (indicated no utilization)
- \+ Colour change of the redox change (indicating utilization of paint by microbes)
- ++ Intense colour change (intense utilization)
- +++ Deeper colour change (very intense utilization)

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Table 2: utilization profile of the Isolates to Emulsion and oil paint types

Isolate Designation	Types of paint	
	Emulsion paint	Oil paint
F	0.113	0.402
D	0.420	1.102
C	0.100	0.383
H	0.098	0.382
B	0.097	0.381
A	0.092	0.376
G	0.089	0.374
I	0.089	0.373
E	0.086	0.368
J	0.085	0.36

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Table 3: Cultural and microscopic Characteristics of the fungal isolates

Isolate	Colonial appearance	Microscopic characteristics	Inference
A	Fast growing isolate in shades of green and white with reverse	Conidia were seen from a specialized conidiogenous cell. Phialides produced had a bush-like appearance.	*Penicillium link*
B	Appeared flat, pink to orange reverse pink	conidia appeared laterally and directly on the hyphae.	*Lecytho hoffmanii*
C	Growed slowly and whitish in colour	Conidia were borne on short lateral hyphal branches.	*Blastomyces dermatitis*
D	They were slow growers. produced white to yellowish brown Pigment in culture plate.	Conidia were rounded and phialides were flask shaped	*Madurella mycetomatis*
E	Produced brown to black fluffy that filled the petridish in a few days.	Non-septate hyphae, spores in a sac-like structures (sporangia) was seen adhering to a swelling on the terminal end of a hyphae.	*Syncephalastrum*
F	They were slow growers, had a suede like appearance and olivaceous to black in colour.	Phialides were flask – shaped while the collarettes were darkly pigmented.	*Phialophora verrucosa*
G	Colonies appeared dark olive-green, to black colour on the plate.	Presence of septate budding cells. spores developed at the tip of flask-shaped phialides.	*Phialophora jeanselmei*

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H	Slow growing with a compact suede- like to downy face and appeared black in colour.	Conidiophores were erect, apically branched and elongated and produced branched acropetal chains of conidia.	*Cladosporium carrionii*
I	Colonies were yellow green	Vesicles bearing phialides over their entire surface and echinulate conidia was observed	*Aspergillus flavus*
J	Colonies had a rapid growth and appeared white and fluffy but later became velvety with central folding	Presence of macroaleuriospores that were thin-walled.	*Trichophyton equinum*

Table 4: The susceptibility result of the anti-fungal agent parmetol DF 19 forte on the fungal isolates (in mm)

Concentration(g/ml)	*Madurella mycetomatis*	*Phialophora verrucosa*
0.1	24 ±0.5	80±0.3
0.01	12.5 ±0.5	80±0.3
0.001	R	R
0.0001	R	R

Values are mean ± of triplicate determination.

mm=millimetre

g=gram

ml= millilitres

R= Resistant

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IV. DISCUSSION

Fungi are found to degrade painted surfaces. The painted surfaces undergo damage or discoloration due to natural weathering, and the growth and activity of living organisms [12]. Consequently, the use of commercial biocide and its efficacy has been tested through extensive lab work [8].

The results of the screening test presented in Table 1 showed that the fungi *Madurella mycetomatis* and *Phialophora verrucosa* were better paint degraders than other fungal isolates as they were able to change the colour of the redox indicator (2, 6 dichlorophenol indolphenol) within 48h. Similarly, [12] [13] have reported that fungi were amongst the main contaminants that were able to breakdown and penetrate the paint film. The change of the colour of the redox indicator from blue to light pink revealed the presence of the organism with the degrading ability on hydrocarbon contained in paints. This finding were in line with the study done by [14] which stated that the ability of organism to produce a color change in the medium was presumably due to the reduction of the indicator by the oxidized products of hydrocarbon degradation.

The fungal isolates that had some paint degradation potentials were identified and presented in Table 3. Among them, *Madurella mycetomatis* and *Phialophora verrucosa* exhibited the highest degradability potential. Hence their susceptibility study on parmetol DF 19 forte.

The result showed that the commercial biocide was very effective on the two test isolates but had more effect on *Phialophora verrucosa* that was totally inhibited. This result also agrees with the report made by [8] which stated the efficacy of parmetol DF 19 forte through their extensive laboratory testing on fungi.

V. CONCLUSION

The result gotten from the study confirmed the efficacy of parmetol DF 19 forte on some fungi at a minimum inhibitory concentration of 0.01g/ml. Based on the obtained results, it is advisable for paint manufacturers to incorporate the fungicide for protection and longer shelf life in the right concentration.

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