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Antifungal Activities of *Ocimum Gratissimum* Against Water-based Paint Spoilage Molds

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Abstract

Water-based paint spoilage is caused by actions of some microorganism, resulting in the deterioration of paint quality and coating integrity. This research work focused on water-based paint spoilage molds, and the use of *Ocimum gratissimum* in the control of spoilage molds of water-based paint. Twenty paint samples were examined microbially and physicochemically. pH, Specific gravity and viscosity were the physicochemical parameters assessed in the research. In addition the phytoconstituents of *O. gratissimum* were analysed, and the extract was incoroporated into paints for further antifungal studies. The fungal isolates from the paint samples were identified further using wet mount method (lactophenol cotton blue staining), slide culture test, and ITS inter-specific region sequencing using 17SrDNA sequencing. Four different paint samples were divided into groups A, B, C and D. These were monitored for a period of four months for physicochemical changes, microbial changes, colour shift, odour and their fungal counts were monitored by plating on Sabouraud's Dextrose Agar. The molecular identification revealed the fungi to be *Cladosporium tenuissimum, Rhizopus* sp., *Aspergillus niger* and *Aspergillus tamari,* with *C. tenuissimum* giving the highest occurrence of 55%. Antifungal activity of *O. gratissimum* methanolic extract against *C. tenuissimum* gave a minimum inhibitory concentration of 50mg/ml and minimum fungicidal concentration (MFC) of 200mg/ml with nystatin (22.4) as standard. The phytoconstituents of the plant extract were flavonoids, tannins, alkaloid, saponin, steroid and terpenoids. Incorporation of the extract in water-based paint contaminated with *C. tenuissimum*, revealed that the extract was able to control the growth of the fungi. This research work showed that *O. gratissimum* has the potential to be used as a natural biocide in paint production.

Keywords

Paints, *Ocimum gratissimum*, Antifungal Activity, Biocides, Spoilage Molds

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1. Introduction

Paint is a uniformly dispersed mixture having a viscosity ranging from a thin liquid to a semi-solid paste, consisting of a pigment suspended in a liquid vehicle such as oil and water [1]. Paints are applied as coatings on surfaces such as metal, wood or stone. They could be water-based or non-water-based. The primary purpose of painting is to protect surfaces from corrosion, oxidation, and environmental weathering and also to provide a decorative finish [2]. The paint industry is a multi-million naira business in Nigeria, ranging from its production to sales, to job creation; thus it plays its own role to the overall economy of the nation. The components of paints include various organic and inorganic substances. The organic material represent a carbon source for virtually all species of microorganisms and act as nutrients to stimulate microbial growth both inside the paint can and on the dry paint film. This factor hence, promotes the loss of durability and decorative functions of the paints which then calls for the need for the incorporation of control measures- the use of

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synthetic biocides [3].

Synthetic biocides are chemicals incorporated into paints to inhibit the growth of microbes and extend the keeping quality of these paints during storage and after coating [4]. However, the main disadvantage of inorganic biocides incorporation is the fact that they contain toxic substances such as heavy metals and most times are non-biodegradable; which calls for the need to use non-toxic and biodegradable biocides to achieve the same aim, hence the adoption of phytochemicals as alternatives.

Ocimum gratissimum is a widely used local plant in Nigeria for its vast anti-microbial properties [5]. It is non-toxic, biodegradable and generally, environmental friendly, which implies its possible use as biocide in the control of environmental microbial deteriorations, such as stored and coated paint deteriorations.

Different types of paints ranging from acrylic, water-based and oil-based paints abound in Nigeria and are used for coating and finishings. In other to preserve the paints and extend their life span, biocides are incorporated into them by manufacturers, with the aim of warding off enough spoilage microorganisms, since paints are indirectly nutrient rich medium, especially water-based paints.

However, a major threat posed by the use of these biocides is that some contain heavy metals such as lead, aluminium and asphalt in their complex chemical structures, volatile organic compounds, as well as organic solvents, which eventually disperse into the environment through rainfall washings and other similar ecological factors; hence, contributing to environmental pollution [6, 7].

The aim of this research work is to determine the antifungal activities of *Ocimum gratissimum* against spoilage molds that affect the durability of water-based paints.

2. Materials and Methods

2.1. Sample Collection and Preparation

Twenty water-based paint samples from different manufacturers were purchased from Eke-Awka market, and later two extra paint samples (one control and one commercial paint) were purchased from a paint manufacturer in Onitsha, Anambrastate. Paint samples were left to stand for a period of four months. The paint samples were examined monthly for physico-chemical and microbial changes.

2.2. Isolation and Identification of Isolates

This was carried out as done by Nwachukwu and Akpata, 2003 [8]. One in ten serial fold dilution of the paint samples were made and thereafter, 0.1ml from 10^{-3} and 10^{-4} dilutions were plated on Sabouraud's Dextrose Agar incorporated with 0.1ml chloramphenicol. Incubation was carried out at room temperature (25°C) for 72 hours. Pure cultures were thereafter obtained by point inoculaton. The fungal isolates were primarily identified based on their cultural characteristics on Sabouraud's Dextrose Agar plates, and were further identified to species level using the following methods; Wet Mount Method, Slide Culture Test, Colonial Characteristics, Molecular Identification (Pure culture of the isolate were made on Saboraud's Dextrose Agar stored in a sterile specimen bottle and sent out to Macrogen Incoporation, South Korea for molecular identification, using ITS inter-spacer region sequencing).

2.3. Physiochemical Analysis of the Paint Samples

2.3.1. Determination of Specific Gravity

This was carried out as done by Obidi *et al*, 2009 [1]. The specific gravity determinations of the paint samples were carried out with the aid of a specific gravity bottle. The specific gravity bottle was washed, dried in an oven, and placed in a dessicator to cool at room temperature, before its weight was determined and recorded as w_1 (g). Paint samples were transferred into the specific gravity bottle to the 50ml mark, weighed and recorded as w_3 (g). The specific gravity bottle was equally filled with distilled water and shaken many times to allow all trapped air within the bottle to be expelled, and weight was taken as w_2 (g). The specific gravity of the paint sample was thus calculated with the formular;

$$\text{Specific Gravity (SG)} = \frac{W_3 - W_1}{W_2 - W_1} \quad (1)$$

where;

w_1 = weight of bottle

w_2 = weight of water and bottle,

w_3 = weight of sample and bottle.

2.3.2. Determination of Colour-Shift and Pigment Precipitation

This was determined by physical observation of the paint samples and compared with a colour chart.

2.3.3. Determination of pH

This was carried out as done by Obidi *et al*, 2009 [1]. The pH of the paint samples was determined with the use of digital fiveGo F2 pH meter, with pH electrode dipped into 1: 20 solution of the paint samples in distilled water.

2.3.4. Determination of Viscosity

This was carried out as done by Obidi *et al*, 2009 [1]. The viscosity of the paint sample was measured with the aid of an electronic rotational viscometer. The paint samples were dispensed into a beaker and the spike of the viscometer was

inserted into the paint samples, and the viscosity reading of the viscometer was displayed digitally on the screen.

2.4. Extraction of *O. gratissimum* (Soxhlet Extraction)

This was carried out as done by James *et al*, 2014 [9]. The leaves were room dried after which the leaves were blended and weighed. About 300g of the leaf samples were wrapped in a filter paper and placed at the extracting column of the distillation tube. About 250ml of the solvent (methanol) was placed in the round bottom flask and heat was applied from the bursen burner. After the extraction, the solvent was separated from the extract through a second wind distillation. The extract was then stored for further use.

2.5. Qualitative and Quantitative Analysis of *O. gratissimum* Extracts

This was carried out using the methods of Trueby, 2003 [10] to ascertain the presence and absence of different phytochemicals in the leaves before quantitative analysis were carried out. Tests carried out include the determination of: Alkaloids, Tannins, Saponins, Cardiac Glycosides, Terpenoids and Steroids.

2.5.1. Determination of Alkaloids

Quantitative determination of alkaloid content of the *O. gratissimum* extract was carried out according to the procedure described by Adewole, 2014 [11]. Five grams of plant sample were weighed into a 250 ml beaker and 200 ml of 20% acetic acid inmethanol were added and covered and allowed to stand for 4 h at 25°C. This was filtered with filter paper and the filtrate was concentrated using a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract until a precipitate was dictated. The solution was allowed to settle and the precipitate was collected and washed with dilute NH_4OH, and filtered with a filter paper. The residue on the filter paper is the alkaloid, which was dried in the oven at 80°C. The alkaloid content was calculated and expressed as a percentage of the weight of the sample analyzed thus:

$$\% \text{ alkaloid} = \frac{\text{weight of filter paper with residue} - \text{weight of filter paper}}{\text{Weight of sample analyzed}} \times 100 \quad (2)$$

2.5.2. Flavonoid Determination

This was carried out according to the procedure described by Barros, 2007 [12]. A 0.5 ml aliquot of the plant methanolic extract was mixed with 2 ml of distilled water and subsequently with 0.15 ml of 5% $NaNO_2$ solution. After 6 min, 0.15 ml of 10% $AlCl_3$ solution was added and allowed to stand for 6 min, then 2 ml of 4% NaOH solution was added to the mixture. Water was added to the mixture to bring the final volume to 5 ml, the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was determined at 510 nm with water serving as the blank. The reference standard was prepared with catechin concentrations. Result was expressed as mg catechin equivalents per 100 g of sample.

2.5.3. Saponin Determination

This was carried out according to the procedure described by Adewole, 2014 [11]. Five grams of the plant sample were put into 20% acetic acid in methanol and allowed to stand in a water bath at 50°C for 24 h. This was filtered and the extract was concentrated using a water bath to one-quarter of the original volume. Concentrated NH_4OH was added drop-wise to the extract until the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed. The saponin content was weighed and calculated thus;

$$\% \text{ saponin} = \frac{(\text{weight of filter paper} + \text{residue}) - (\text{weight of filter paper})}{\text{Weight of sample analyzed}} \times 100$$

2.5.4. Determination of Total Phenol Content

The total phenol content of the sample was determined using the method of Barros, 2007 [12]. The methanolic extract solution (1 ml) was mixed with Folin and Ciocalteu's phenol reagent (1 ml). After 3 min, saturated sodium carbonate solution (1 ml) was added to the mixture and adjusted to 10 ml with distilled water. The reaction was kept in the dark for 90 min, after which the absorbance was read at 725 nm. Gallic acid was used to make standard curve (10-50 µg; Y = 0.013x-0.022; R2 = 0.987) and the result was expressed as mg of Gallic acid equivalent per gram of extract.

2.5.5. Terpenoid Determination

Two grams of grounded plant sample were weighed and soaked in 50 ml of 95% methanol for 24 h. The extract was filtered and the filterate was further extracted with petroleum ether using a separating funnel. The weight of the ether extract was noted and taken as total terpenoid [13].

2.6. Anti-fungal Activity of the Extracts

The agar well diffusion method of Perez, 1990 [14] was employed. A 100µl of *O. gratissimum* extract was placed in wells cut in sterile Sabouraud's Dextrose Agar plates seeded with fungi

samples and incubated aerobically at 35°C for 24 hours and diameter of zones of inhibition were thereafter measured.

2.7. Minimum Inhibitory Concentratiom and Minimum Fungicidal Concentration Assessment of *O. gratissimum*

The method used by Oforkansi *et al*, 2003 [15] was adopted. The tube dilution assay was employed to first determine the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of the methanolic plant extract. Two fold dilution of the extracts were made serially in 10% Dimethylsulfoxide, to get 400mg/ml, 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml, and 3.125mg/ml. Thereafter, 1ml aliquot of each diluted extract was transferred into test tubes containing 1ml peptone water with 0.1ml of 24 h *C. tenuissimum* culture. The set up was incubated for 48hours at room temperature (27°C) and turbidity was checked for each test tube. The agar well diffusion method was employed to determine the zones of inhibition of the MIC with Nystatin as the standard. A 100µl aliquot of the plant extract was placed in wells cut in sterile Sabouraud's Dextrose Agar plate seeded with *Cladosporiumtenuissimum*, with the aid of a sterile cork borer and incubated aerobically at 35°C for 24 hours and the diameter of zones of inhibition were measured. The minimum fungicidal concentration was determined by plating out tubes without turbidity on Saboraud's Dextrose agar and checking for microbial growth.

2.8. Effects of *O. gratissimum* on Physicochemical Properties of Paint Inoculated with *C. tenuissimum*

Two hundred millilitre aliquot of three different paint samples were provided in triplicates and divided into groups viz;

A: Commercial paint samples without incorporation of *C. tenuissimum* and *O. gratissimum.*

B: Commercial Paint samples contaminated with 10ml 1.6×10^6 *Cladosporium tenuissimum*

C: Paint samples laddened with 100mg/ml *Ocimum gratissimum* extract.

D: Paint samples laddened with 100mg/ml *O. gratissimum* extract with 10ml 1.6×10^6 *C. tenuissimum.*

The experimental set up was monitored for a period of four months for physico-chemical changes, microbial changes, colour shift and odour. The fungal counts were monitored by plating on Sabouraud's Dextrose agar once every month for four months.

2.9. Grouped Paints Surface Coating Assessment

This was performed as done by Bayer, 2004 [16]. The coating surface was prepared by smoothening and cleaning, and was then allowed to properly dry up. The different paint groups' samples were thereafter applied on the surface with the aid of paint brush and then monitored from the point of coating to the point of drying. Surface adherence, colour and texture were monitored.

2.10. Statistical Analysis of Data

Statistical data of grouped paint samples were obtained by analyzing mean and standard deviation using statistical package for social science (SPSS) package version 17.

3. Results

3.1. Isolation and Identification of Water-Based Paint Spoilage Fungi

Twenty examined water-based paint samples had no fungal contamination for a period of two months post atmospheric exposure. However, from the third and fourth month, some fungi were isolated and they were further characterized using molecular identification.

3.2. Molecular Characterization of Fungal Isolates

Molecular characterization of the isolates revealed them to be *Cladosporium tenuissimum, Aspergillus niger*, *Aspergillus tamarii* and *Rhizopus* sp. *Cladosporium tenuissimum* was the most dominant fungi, showing a 55% occurrence, and also appeared to be a dimorphic fungi, *Aspergillus niger and Aspergillus tamarii has* 20% and 15% occurrence respectively, while Rhizopussp was the least dominant fungi, showing a 10% occurrence.

3.3. Physicochemical Properties of the Commercial Paint Samples

Six out of the twenty examined water-based paint samples designated C, J, K, O, P and S showed physically observed changes such as colour shift and off odours at the third and fourth month post atmospheric exposure. There was a notable decrease in the paint pH within the third and fourth months post-exposure, however, the pH range values (9.0-9.8 to 8.2 -9.0) remained alkaline. There was a minor decrease in specific gravity values of the paints (from 49.3 - 49.6 to 49.2 – 49.5) within the four months post-exposure time. The paint samples became slightly less viscous from over the post-atmospheric exposure of four months.

Table 1. Colonial and Microscopic Characteristics of the Fungal Isolates.

Cultural characteristics on Sabouraud's Dextrose Agar	Microscopic characteristics	Fungi
Flat, circular, smooth, milkfish colony which eventually turned olive green on SDA plate.	Olive green conidia found on septate hyphae.	*Cladosporium tenuissimum*
Dense cottony, greenish white colonies.	Black colony sporangiespores found in non septate hyphae with Rhizoid.	*Rhizopus sp.*
Light yellow and white fluffy colony which later turned black.	Aseptate hyphae bearing biseriate and smooth walled spherical conidae.	*Aspergillus niger*
Brownish fluffy colonies with white coloured edges.	Aseptate hyphae bearing biseriate and smooth walled spherical conidae	*Aspergillus tamarii*

Table 2. Frequency of Isolation of the Fungal Species.

Fungi	Total number of samples	No of positive samples	% of occurrence
C. tenuissimum	20	11	55
A. niger	20	4	20
A. tamarii	20	3	15
Rhizopussp	20	2	10

3.4. Phytochemical and Antifungal Analysis of *O. gratissimum* Against *C. tenuissimum*

The phytochemical constituents of *O. gratissimum* examined showed that the plant leaf contains more saponins and alkaloids as shown on Table 4. The antifungal analysis of the plant extract against *C. Tenuissimum* gave a minimum inhibitory concentration (MIC) of 50mg/ml with 10mm zone of inhibition and minimum fungicidal concentration (MFC) of 200mg/ml with 15mm zone of inhibition as shown on Table 5.

Table 3. Physical Changes Found in Commercial Paint Samples.

Paints	Months			
	1	2	3	4
C white	Nil	Nil	Light green pigmentation with off odour.	Dense green pigmentation with moisture lost and putrid smell.
J white	Nil	Nil	Off odour and no colour change.	Putrid smell.
K white	Nil	Nil	Light green pigmentation and no odour.	Moisture lost and dense green pigmentation, no odour.
O white	Nil	Nil	Off odour and change of colour from light green to brown.	Putrid smell, brown colouration and moisture lost.
P white	Nil	Nil	Light green pigmentation, off odour.	Deep green pigmentation, pungent smell and moisture lost.
S white	Nil	Nil	Light green pigmentation, off odour.	Deep green pigmentation, pungent smell and moisture lost.

Table 4. Quantitative Analysis of *O. gratissimum.*

Parameters	Relative Abundance Actual Quantity (%)	
Alkaloid	++	0.75
Saponin	+++	1.86
Flavonoid	+	0.40
Terpenoid	+	0.20
Phenol	+	0.318

+ = slightly present.
++ = very present.
+++ = strongly present.

Table 5. Qualitative Analysis of *O. gratissimum.*

Parameter	Results
Alkaloid	+
Flavonoid	+
Tannin	+
Saponin	+
Terpenoid	+
Steroid	+
Cardiac glycoside	-
Resin	-

+ = present.
- = absent.

3.5. Efficacy Testing of *O. gratissimum* in Grouped Paints Physicochemical Preservation Against *C. tenuissimum* Contamination During Storage

The study showed that there was a notable drop in the pH values of test samples B, C and D, while the control paint sample A showed a slight decrease in pH values in the four months monitoring period (Table 6). Specific gravity values decreased from 49.4± 0.00 to 48.6± 0.46 and 49.4 ± 0.00 to 49.15±0.45 for group B and C respectively while group D had a slight increase in specific gravity value of 49.4±0.00 to 49.45±0.15 across the four (4) month study. Group A however, had a slight shift in its specific gravity value.

Viscosity values decreased notably in all groups while Group A showed a slight decrease in its value through the four months monitoring.

Table 6. Changes in pH during the Period of Experiment.

Months	Groups			
	A	B	C	D
Initials	9.6 ± 0.00	9.6 ± 0.00	9.6 ± 0.00	9.6 ± 0.00

Months	Groups			
	A	B	C	D
1	9.6 ± 0.00	8.9 ± 0.00	8.8 ± 0.00	8.3 ± 0.00
2	9.5 ± 0.00	8.6 ± 0.00	8.7 ± 0.00	8.3 ± 0.00
3	9.4 ± 0.00	8.4 ± 0.00	8.5 ± 0.00	8.2 ± 0.00
4	9.4 ± 0.00	8.4 ± 0.00	8.3 ± 0.00	8.1± 0.00

Where;

A= Commercial paint sample.

B= Commercial Paint sample contaminated with 10ml 1.6 x 10^6 *Cladosporium tenuissimum.*

C= Control Paint sample laddened without commercial biocide but with 100mg/ml *Ocimum gratissimum* extract.

D= Control Paint samples without commercial biocide but laddened with 100mg/ml *O. gratissimum* extract with 10ml 1.6 x 10^6 *C. tenuissimum.*

Table 7. Sensitivity of *C. Tenuissimum* to Different Concentrations of Methanolic Extract of *O. gratissimum*.

Concentration (mg/ml)	Diameter zone of inhibition (mm)
400	16.2
200	15.0
100	13.0
50	10.0
25	0.0
12.5	0.0

3.6. Effects of *O. gratissimum* on Physicochemical Properties of Paint Inoculated with *C. tenuissimum*

Observation on the colour shift, texture and odour of the test paint samples during storage showed no change in paint colour, and no fowl odour for group A; white to green to off white; and white to brown, to cream colour shifts for groups C and D with no foul odour. However, group B had no colour shift, but exhibited pungent odour and slimy textured paint as seen on table 8. Assessment of paint samples as surface coatings showed colour and texture consistency for group A; cream to off-white colour shift and sticky texture and subsequent scaling off for group B; white to light green and greenish white colour shift with texture consistency for group C and off-white to greyish brown colour shift with texture consistency for group D (Table 9), and plates 1-4 in appendix.

Table 8. Specific Gravity Changes during the period of Experiment.

Months	Groups			
	A	B	C	D
Initial	49.4 ± 0.00	49.4 ± 0.00	49.4 ± 0.00	49.4 ± 0.00
1	49.4 ± 0.00	49.4 ± 1.48	49.4 ± 1.65	49.42 ± 0.25
2	49.38 ± 0.00	49.0 ± 1.48	49.15 ± 1.15	49.48 ± 0.05
3	49.36 ± 0.00	48.8 ± 1.65	49.15 ± 0.45	49.45 ± 0.15
4	49.36 ± 0.00	48.6 ± 0.46	49.15 ± 0.45	49.45 ± 0.15

Table 9. Viscosity Changes during the period of Experiment.

Months	Groups			
	A	B	C	D
Initials	10926 ± 56.1	10926 ± 56.1	10926 ± 56.1	10926 ± 56.1
1	10926 ± 56.1	10918 ± 30.2	10926 ± 40.4	10924 ± 33.3
2	10925 ± 42.6	10909 ± 33.1	10916 ± 40.4	10920 ± 28.0
3	10922 ± 56.5	10899 ± 42.1	10911 ± 31.3	109218±25.1
4	10919 ± 40.4	10888 ± 35.5	10900 ± 31.4	10915 ± 40.1

3.7. Anti-fungal Activity of the Extract

Fungistatic and fungicidal activities of *O. gratissimum* extract in paint preservation against *C. tenuissimum* showed a decrease mean fungal count on $\log_{10}6$ cfu/ml for groups B and D, although group D had a remarkable fungal count decline.

4. Discussion

Water-based paint spoilage, which is caused by actions of some microorganisms, results in the deterioration of paint quality and coating integrity [17]. This research work focused on water-based paint spoilage fungi, and isolated and identified *Cladosporium tenuissimum, Rhizopus* sp, *Aspergillus niger* and *Aspergillus tamarii* from twenty examined paint samples after post-atmospheric exposure of 48 h. This finding partly corresponds with the work of Olufemi *et al*, 2013 [18], who isolated fungi from water based paints and identified *Aspergillus* sp., *Rhizopus* sp., *Fusarium* sp., *Cladosporium* sp. and *Alternaria* sp. as fungal contaminants. However, *Rhizopus* and *Aspergillus* sp were most prevalent as reported in their findings. Didiugwu *et al*, 2016 [6] also isolated *Phialophora verrucosa* and *Madurella mycetomatis* as most prevalent fungi from water based paints.

Table 2 shows that *C. tenuissimum*, which is a dimorphic fungi had the highest percentage occurrence of 55%, occurring in 11 paint samples, while *Rhizopus* sp. had the least occurrence of 10%, occurring in two paint samples. This implies that paint contamination with microorganisms can occur when freshly produced paint samples are stored for sometime or exposed to the atmosphere, since air harbours transient microbes, especially their spores, and some microorganisms must have been trapped inside the paint during production, hence the ability of the paints to be contaminated by fungi.

The physicochemical variations in the observed paint samples over four months monitoring, revealed decreasing pH, specific gravity and viscosity; and these correspond with the work of Obidi *et al*, 2009 [1] who reported decrease in pH, viscosity and specific gravity of the paint samples over the period suggesting gradual deterioration of the aesthetic qualities of the paint products with time. Table 3 shows the

physical changes observed in the paint samples post-atmospheric exposure. It was discovered that even though there was contamination during the paint production, it took about two months for the fungal contaminants to incubate, overcome the presence of incorporated biocides and proliferate, before producing observable physicochemical changes in the paint samples. Six paint samples- C, J, K, O, P and S, showed these physical changes while other paints retained their production conformity, months after post-atmospheric exposure. This circumstance could be explained from the work of [19] who stated that different water-based paints contain varying amounts of biocides, dispersants, water activity and cellulosic thickeners; thus resulting in the ease of deterioration of some paints while others are not easily spoilt by microbial contamination. They proceeded to state that colour-shift, off-colour, uneven colour and even gas production in paint are as a result of microbial decomposition of cellulosic thickeners, dispersed colour and dispersants.

Table 4 showed the phyto-constituents of *O. gratissimum* extract which was tested as a natural fungicide for paints, due to its bio-degradability. The extract showed high presence of saponinand alkaloids which have antifungal properties. This result corresponds with that of Adewole *et al*, 2014 [11] that reported *O. gratissimum* extract containing saponin, andalkaloids. The sensitivity of the extract against *C. tenuissimum* which is the predominant fungi found in the test paint samples, showed a MinimumInhibitory Concentration (MIC) of 50 mg/ml and a Minimum Fungicidal Concentration (MFC) of 200 mg/ml. These results reveal the possibility of using the plant extract as a natural antifungal biocide.

The results of efficacy experiment of incorporating the plant extract into water-based paint samples that are laddened with *C. tenuissimum* culture showed that the extract incorporation caused lowered pH and viscosity values, when compared to the normal paint samples in Group A, which according to Rosa *et al*, 2008 [19] suggests that the paints' thickeners were affected by the plant extract incorporation. The specific gravity of the paint was however slightly altered.

The physical status of the paints revealed that incorporation of the extract caused a colour-shift which according to Obidi *et al*, 2009 [1] suggests the alteration of the dispersants in the paints. However, the microbial evaluation of the experimental set-up show that the extract was able to prevent microbial growth in Group C paint samples, as well as inhibited the growth of *C. tenuissimum*as seen in Group D. Group B likewise, had a slight decrease in fungal count through the second and third months, which could be attributed to the presence of biocides used in the manufacturing of the paints; however, at the fourth month, there was increase in the fungal count, which signifies the ability of *C. tenuissimum* to eventually act on the paint components, thereby leading to increased fungal counts and depreciated paint samples.

5. Conclusion and Recommendation

This study revealed the phytoconstituents of the plant extract to be flavonoids, tannins, alkaloid, saponin, steroid and terpenoids. Incorporation of the extract in water-based paint contaminated with *C. tenuissimum*, revealed that the extract was able to control the growth of the fungi. This research work showed that *O. grastissimum* has the potential to be used as an antifungal agent and as such, *O. gratissimum* has the potential to be used as a natural biocide in paint production. It could be harnessed for such purposes, even though the colour aspect of the paints may be slightly affected.

Further studies can be carried out on incorporating *O. gratissimum* methanolic extract directly into paint manufacturing, in the place of biocides and observe how its antifungal properties can work out for a long period of time; since this research tested its efficacy only for a period of four months and to work in removing the colour effect of the extract.

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