ISSN 2348-0319 International Journal of Innovative and Applied Research [2025]
(Volume 13, Issue 01)
12-29

Journal home page: http://www.journalijiar.com

INTERNATIONAL JOURNAL
OF INNOVATIVE AND APPLIED RESEARCH

RESEARCH ARTICLE

Article DOI: 10.58538/IJIAR/2119
DOI URL: *http://dx.doi.org/10.58538/IJIAR/2119*

HARNESSING THE ANTIFUNGAL PROPERTIES OF OCIMUM GRATISSIMUM FOR SUSTAINABLE WATER-BASED PAINT FORMULATIONS

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Manuscript Info

Manuscript History
Received: 08 December 2024
Final Accepted: 14 January 2025
Published: January 2025

Keywords:
Paint, Cladosporium Tenuissimum, Aspergillus Niger, Flavonoids, Phytoconstituents

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 fungi, and the use of Ocimum gratissimum in the control of water-based paint spoilage fungi. Twenty paint samples were examined microbiologically, physiologically and physic-chemically after post-atmospheric exposure of 48 h. pH, specific gravity and viscosity were the physico-chemical parameters observed in the research. The phytoconstituents of O. gratissimum were also analysed. Cladosporium tenuissimum,Rhizopus sp., Aspergillus niger and Aspergillus tamarii were the fungi isolated from the exposed paint samples with C. tenuissimum giving the highest occurrence of 55%. Antifungal activity of O. gratissimum (ethanolic extract) against C. tenuissimum gave a Minimum Inhibitory Concentration of 50mg/ml. The phytocontituents of the plant extract are flavonoids, tannins, alkaloid, saponin, steroid and terpenoids. Incorporation of the extract in already water-based paint contaminated with C. tenuissimum, revealed that the extract was able to control the growth of the fungi, however, it also affected the colouration and texture of the paint samples. This research work shows that O. gratissimum has the potential to be used as a natural biocide in paint production, which has major advantages of non-toxicity and bio-degradability as compared to artificial biocides used in paint production.

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Introduction:-

Plants are a rich source of bioactive compounds with potential antispasmodic and antimicrobial properties (Frank and Kingsley, 2014; Okigbo et al., 2015; Ubaoji et al., 2020; Umeoduagu et al., 2013). Many plants are traditionally claimed to possess antibiotic properties and are widely used by indigenous populations worldwide (Awah et al., 2016; Awah et al., 2017). Driven by the need for more effective, affordable treatments and methods to extend

product shelf-life, humans have consistently explored the utilization of readily available natural resources (Agu et al., 2013; Adindu et al., 2016)

Ocimum gratissimum is a widely used local plant in Nigeria for both nutritional and medicinal purposes. It is popularly known as clove basil. Its vast anti-microbial properties has attributed to its vast advantage to man as a medicinal plant (Oforkansi et al., 2003). It has been used in the treatment of illness such as diarrhoea, epilepsy, high fever and even mental illness (Arhoghro et al., 2009). Its roots, stems and leaves are of great health benefits. As a biological alternative, O. gratissimum use as a paint biocide is beneficial because it is equally bio-degradable and environmental friendly. It is a tropical plant that belongs to the family of Labiatae. It is a perennial plant that is common in Asia and Africa. African countries like Nigeria, Ghana and Cameroun used this leaf for both nutritional and medicinal purposes. The use of scent leaf as a herbal medicine has played and continue to play a prominent role in the treatment of certain ailments and diseases. Thus it has a high anti-microbial property, hence can be used against molds associated with paint spoilage. Its anti-fungal properties cannot be over emphasized as seen from the works of Njoku et al., (2011). It is also believed that since it is in use as food preservative, it could also be tried for its use as a paint preservative owing to its advantage of bio-degradability. For the purpose of this research work, the vast anti-microbial activities of O. gratissimum would be adopted as a potential biocide against paint deterioration moulds, since phytochemicals are less toxic and biodegradable.

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 (Obidi et al., 2009). 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. The painting 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 nature. The components of paints include various organic and inorganic substances. Microorganisms are ubiquitous in the environment and are seen in any nutrient rich matter (Awari et al., 2023). 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. 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. However, the main disadvantage of inorganic biocides incorporation is the fact that they are toxic and most times non-biodegradable. This calls for the need to use less toxic and biodegradable biocides to achieve the same aim, hence the incorporation of phytochemicals.

Different types of paints ranging from emulsion, 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 pose by the use of these biocides is that they contain heavy metals such as lead, aluminium and asphalt in their complex chemical structures. There is a need to discover natural, non-toxic and bio-degradable biocides, hence the use of Ocimum. gratissimum.

This research aims to investigate the antifungal activities of Ocimum gratissimum against spoilage fungi in water-based paints, including: isolating and identifying paint-spoiling molds, evaluating the physicochemical properties of paints before and after fungal exposure, and assessing the efficacy of O. gratissimum against these molds, including its in-paint incorporation as a biological preservative

Materials and Methods:-

Sample Collection

Twenty water-based paint samples from different manufacturers were purchased from Eke-Awka market, Anambra state.

Paint samples were exposed for 48 hours, covered up and left to stand to stand up for a period of four months. The paint samples were examined monthly for physic-chemical and microbial changes.

Isolation of Fungi
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 Saboraud's Dextrose Agar incorporated with 0.1ml chloramphenicol (Nwachukwu &Akpata, 2003). Incubation was carried out at room temperature (28 ± 2^0C) for 72 hours, pure culture were thereafter obtain by point inoculaton for molds and streaking for other fungi.

Identification of Isolates
The modified slide culture methods as described by Agu and Chidozie (2021) were used for isolation of the fungal organisms and grouping.

ITS inter-spacer region sequencing:
Pure culture of the isolate were made on Saboraud's Dextrose Agar stored in a sterile specimen bottle and sent out to Macrogen Incoporate, south Korea for molecular identification.

Biochemical test (sugar fermentation):
Glucose, maltose, sucrose, lactose, and galactose were each added in peptone water in 1% w/v with two drops of bio-mothymol blue and dispersed 5ml each into test tubes containing inverted Durham tube. The sugar solutions were sterilized with the autoclave at 115^0C and 200µl of broth culture of each isolates was inoculated into test tube, incubated at 37^0C and observed for colour change and gas production after 48 hours.

Calculation of Percentage of Occurrence of Fungal Isolates:
This is calculated thus;

$$\frac{\text{Total number of each isolate}}{\text{Total number of paint sample examined}} \times \frac{100}{1}$$

Determination of specific Gravity
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 at 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 formula:

$$\text{Specific Gravity (SG)} = \frac{w_3 - w_1}{w_2 - w_1}$$

where;
w_1 = weight of bottle
w_2 = weight of water and bottle,
w_3 = weight of sample and bottle.

Determination of Colour-Shift and Pigment Precipitation
This was determined by physical observation of the paint samples and compared with a colour chart.

Determination of pH
The pH of the paint samples were determined with the use of digital pH meter, with pH electrode dipped into 1:200 solution of the paint samples in distilled water.

Determination of Viscosity
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 readings of the viscometer was displayed digitally on the screen.

Extraction of O. gratissimum (Soxhlet Extraction)
The leaves were room dried after which the leaves were grounded and weighed. 300g of the leave samples were wrapped in a filter paper and placed at the extracting column of the distillation tube. 250 ml 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.

Qualitative Analysis of O. gratissimum Extracts
This was carried out using the methods of Trease and Evans, (1989) to ascertain the presence of different phytochemicals in the leaves before quantitative analysis were carried out. The test were carried out as follows;

Test for Alkaloids
1ml of the plant leave extract was placed into a test tube and 1ml of Wagner's reagent (2g of iodide and 3ml of potassium iodide, made up 100ml with distilled water) was put into the test tube and thoroughly mixed. A reddish brown precipitate indicates the presence of alkaloids (Trease and Evans, 1989).

Test for Tannins
3ml of plant leaf extract was placed into a test tube, and 2ml of 1% Hcl was added into same test tube. The presence of red colour or precipitate indicates the presence of phlobotannins (Trease and Evans, 1989).

Test for Saponins
10ml of the plant leaf extract was mixed with 5ml of distilled water in a test tube and shaken vigorously for a stable persistant froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously. The presence of emulsion was observed (Trease and Evans, 1989).

Test for Cardiac Glycosides
1 ml of the extract was placed in a test tube and 10ml of 50% H_2So_4 was added into same test tube and heated in boiling water for 5minutes. 5ml each of Fehling solutions, A and B was added and boiled. A brick red precipitate indicated the presence of glycosides, Trease and Evans, (1989).

Test for Terponoids
5ml of plant leaf extract was mixed in 2ml of chloroform and concentrated H_2So_4 was carefully added to form a layer. A reddish brown colouration of the inner face was formed to show the presence of terponoids. (Trease and Evans, 1989).

Test for Steroids
0.5ml of chloroform was added to 1ml of leaf extract and 1ml of concentrated H_2So_4 was added to form a lower layer. The colour at the interface indicates the presence of steroids. (Trease and Evans,1989).

Anti-fungal Activity of the Extracts
The agar well diffusion method was employed. 100µl of O. gratissimum placed in wells extract was placed in wells cut in sterile Saboraud's Dextrose Agar plates seeded with fungi samples and incubated aerobically at 35 ^{0}C for 24 hours and diameter of zone of inhibition were measured.

Characterization of Cladosporium tenuissimum
Among the molds isolated C. tenuissimum was choosen because it was the most dominant fungi. C. tenuissimum which appeared to be the predominant mold in the paint samples was characterized through conventional sugar fermentation method and was further identified by molecular characterization using ITS inter-spacer region sequencing by Macrogen laboratories, South Korea. (Appendix 1).

Minimum Inhibitory Concentratiom and Minimum Fungicidal Concentration Assessment of O.gratissimum.
The tube dilution assay described by Agu et al. (2013) and Ubaoji et al. (2020(, were employed to first determine the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the ethanolic 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 ^{0}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 Saboraud's Dextrose Agar plated/seeded with C. tenuissimum, with the aid of a sterile cork borer and incubated aerobically at 35 ^{0}C for 48hours

and the diameter of zone of inhibition were measured. The minimum fungicidal concentration was determined by plating out tubes without turbidity on Saboraud's Dextrose agar and checked for microbial growth.

Efficacy Testing Of O. gratissimum in Paint Physical Chemical Presentation against C. tenuissimum.
200 ml aliquot of three different paint samples were provided in triplicates and divided into groups viz;
A: Normal paint samples
B: Paint samples contaminated with 10ml $1.6x10^6$ Cladosporium tenuissimum
C: Paint samples laddened with 100mg/ml Ocimum gratissimum extract.
D: Paint samples laddened with 100mg/ml O. gratissimum extract with 10ml $1.6 x10^6$ 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 Saboraud's Dextrose agar.

Paint Surface Coating Assessment.
The coating surface was prepared by smoothening and cleaning, and was then allowed to properly dry up. The paint 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 (Bayer and Zamanzadeh,2004).

Results:-

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 able to be isolated of which Cladosporium tenuissimum was the most dominant fungi, showing a 55% occurrence, and also appeared to be a dimorphic fungi (Tables 1 and 2).

Table 4.1:- Physico-Chemical Properties of the Fungal Isolates.

Culture characteristics on SDA	Microscopic characteristics	Fungi
Flat, circular, smooth, milkfish colony which eventually turns 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 turns black on SDA plate.	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 4.2:- Frequency of Isolation of the Fungal Species.

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

Table 3 shows the sugar fermentation characteristics of C. tenuissimum which is the most predominant fungal isolate from the examined paints.

Table 4.3:- Sugar Fermentation Characteristic of C. tenuissimum.

Sugars	Fermentative ability
Glucose	+
Maltose	+
Sucrose	+
Lactose	-
Galactose	-

Physico-chemical Properties of the 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 as shown on figures 1-12. There was a notable decrease in the paint pH within the third and fourth months post-exposure, however, the pH range remained alkaline. There was a minor decrease in specific gravity values of the paints within the four months post-exposure time. The paint samples became slightly less viscous over the post-atmospheric exposure of four months.

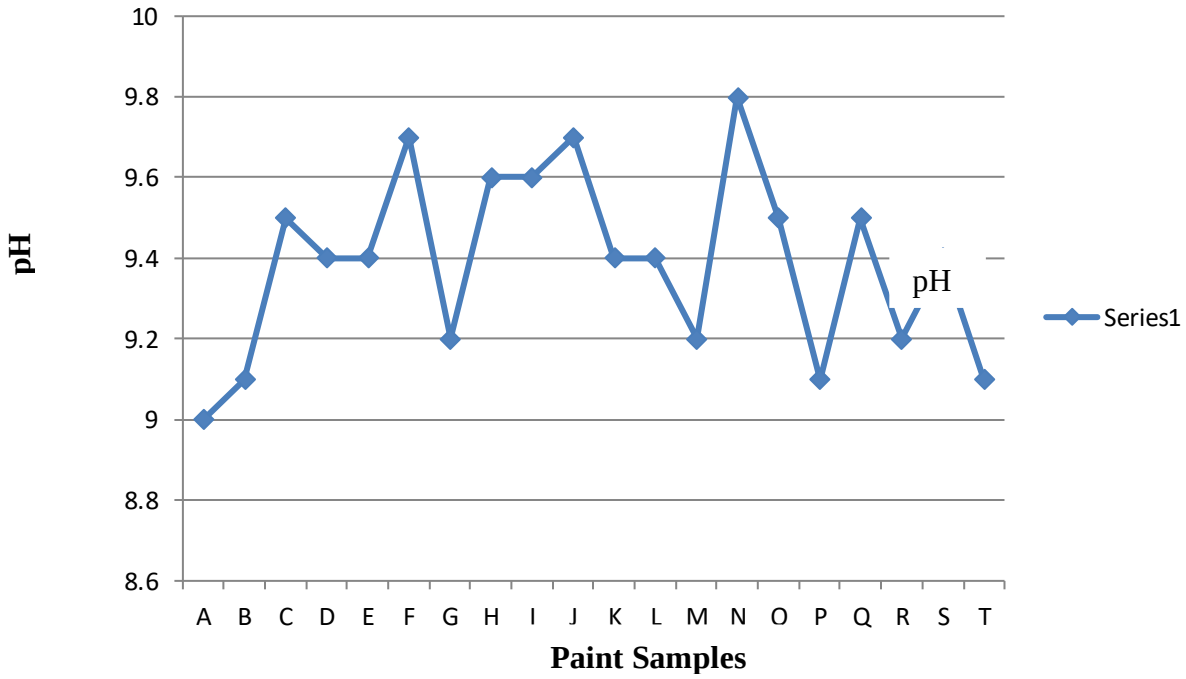

Figure 1:- Mean pH of Paint Samples for Month 1.

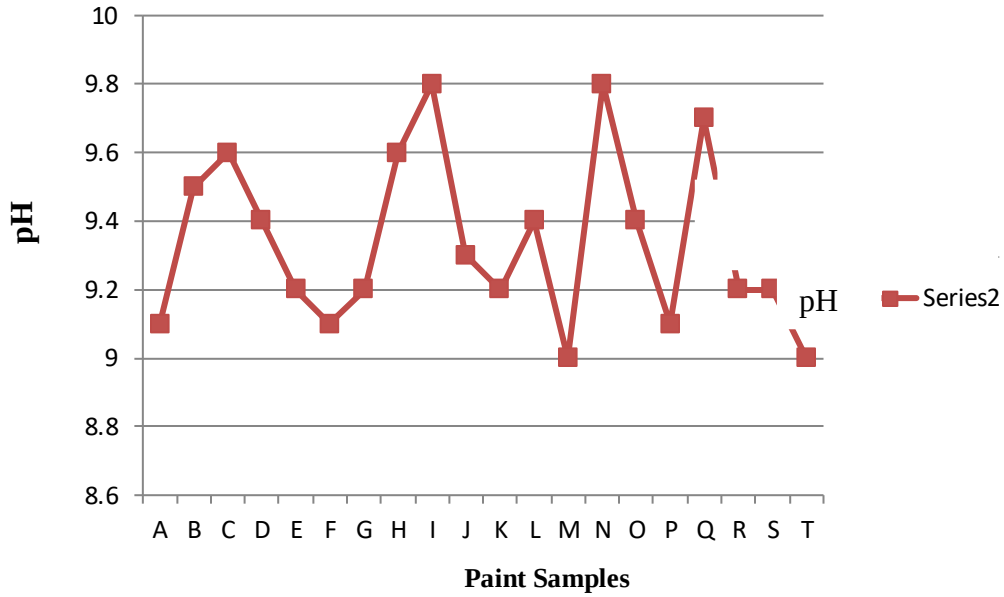

Figure 2:- Mean pH of Paint Samples for Month2.

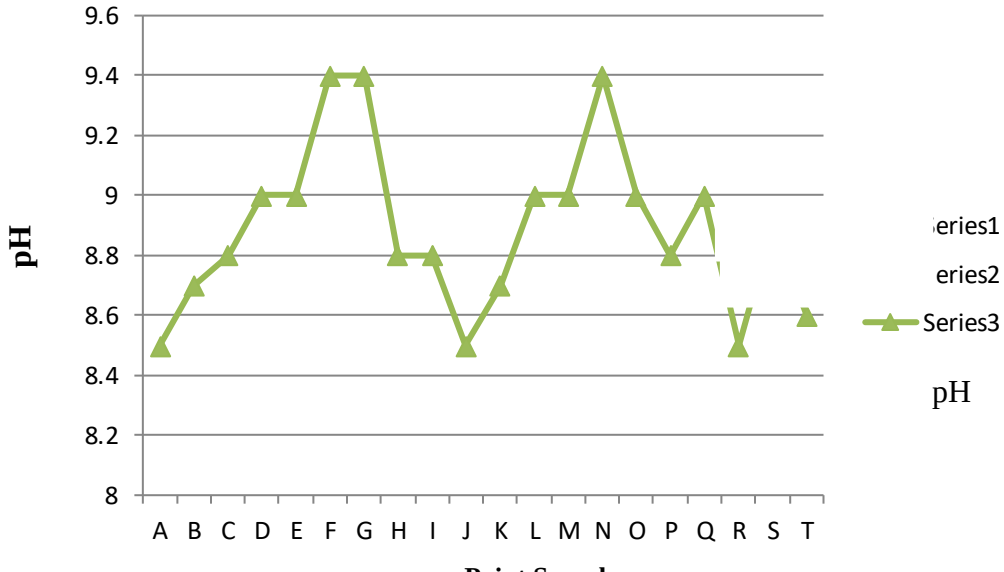

Figure 3:- Mean pH of Paint Samples for Month 3.

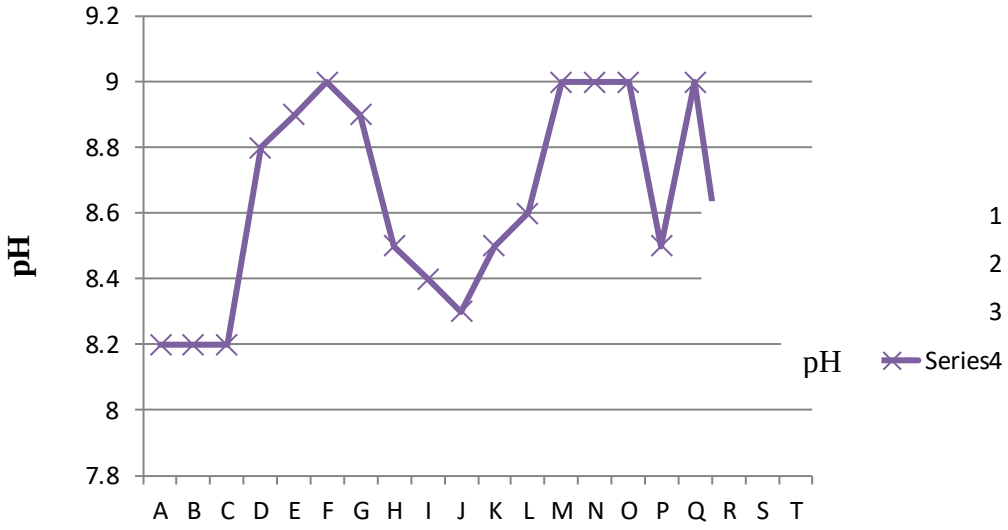

Figure 4:- Mean pH of Paint Samples for Month 4.

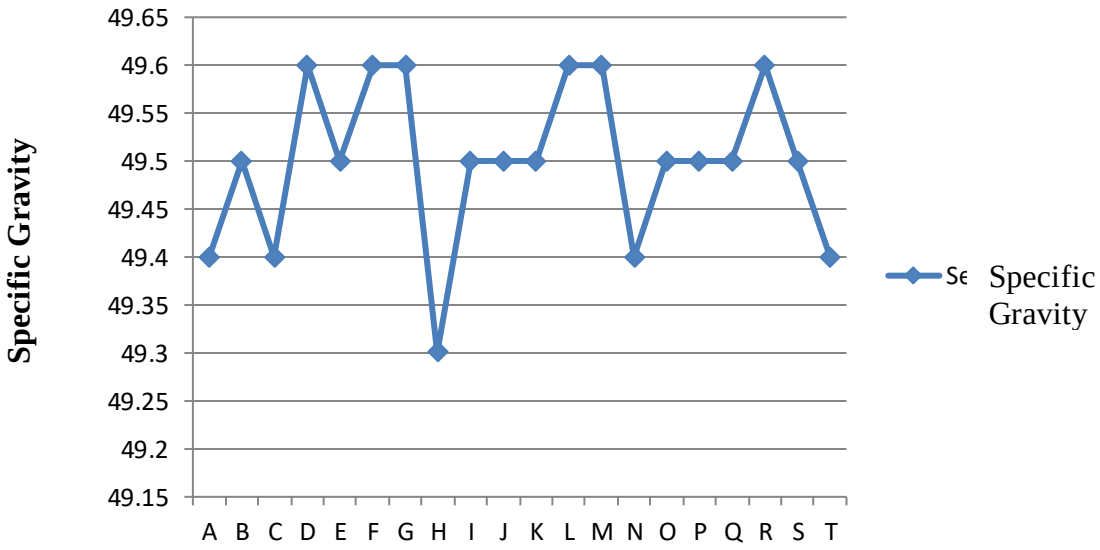

Figure 5:- Specific Gravity of Paint Samples for Month 1.

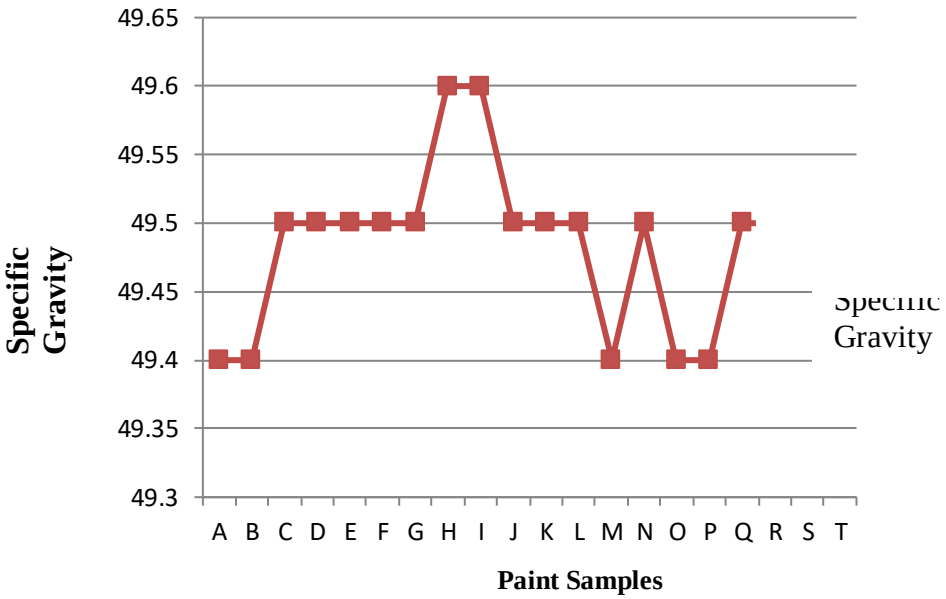

Figure 6:- Specific Gravity of Paint Samples for Month 2.

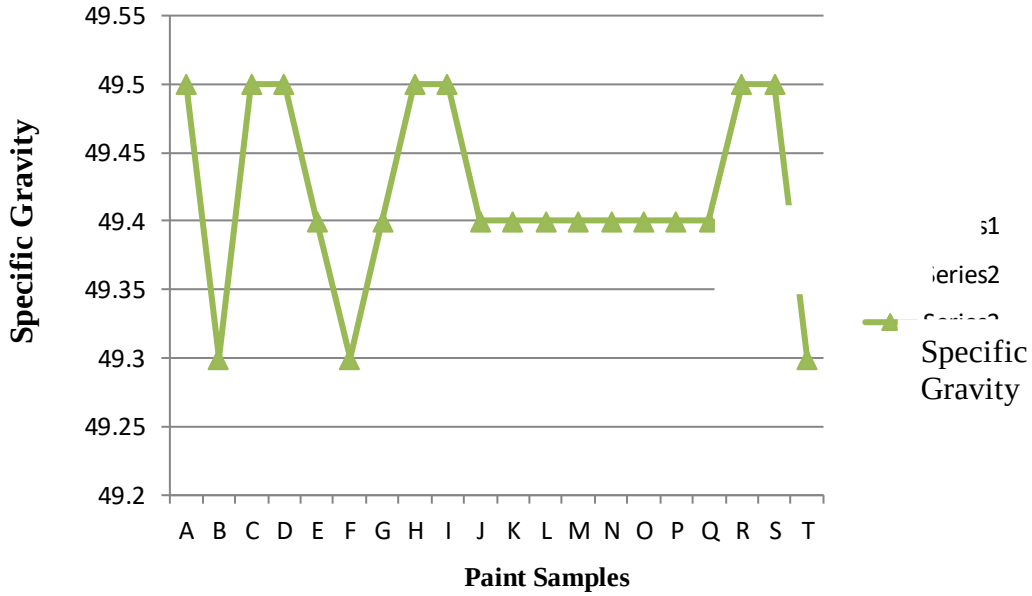

Figure 7:- Specific Gravity of Paint Samples for Month 3.

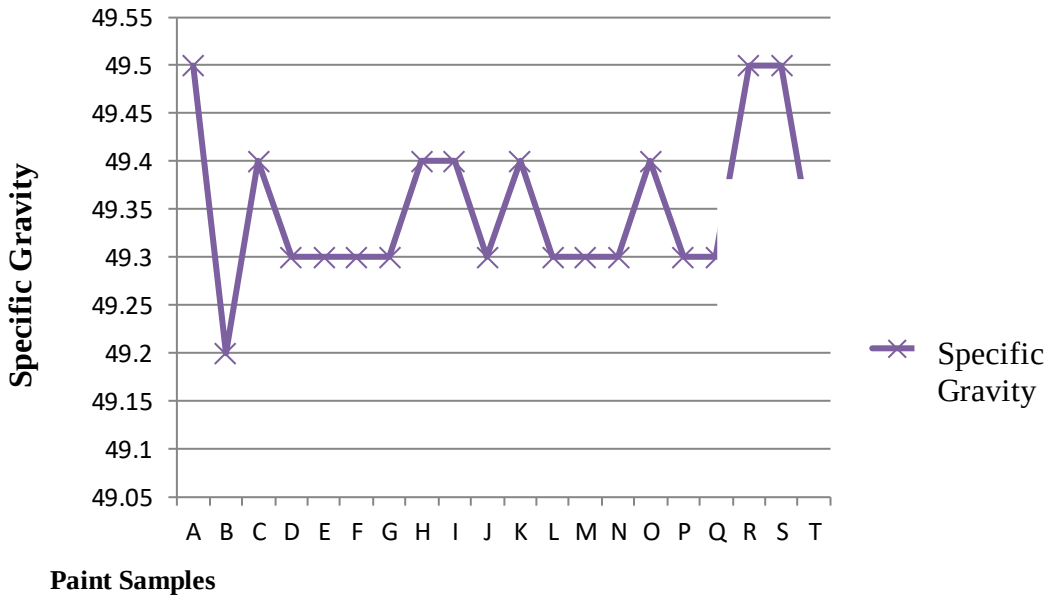

Figure 8:- Specific Gravity of Paint Samples for Month 4.

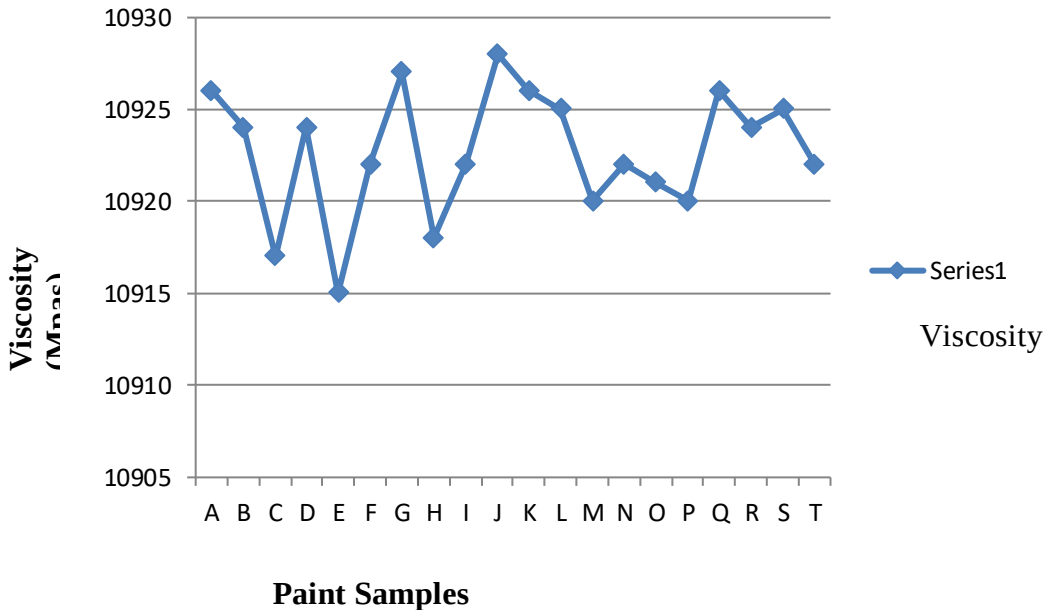

Figure 9:- Viscosity of Paint Samples for Month 1.

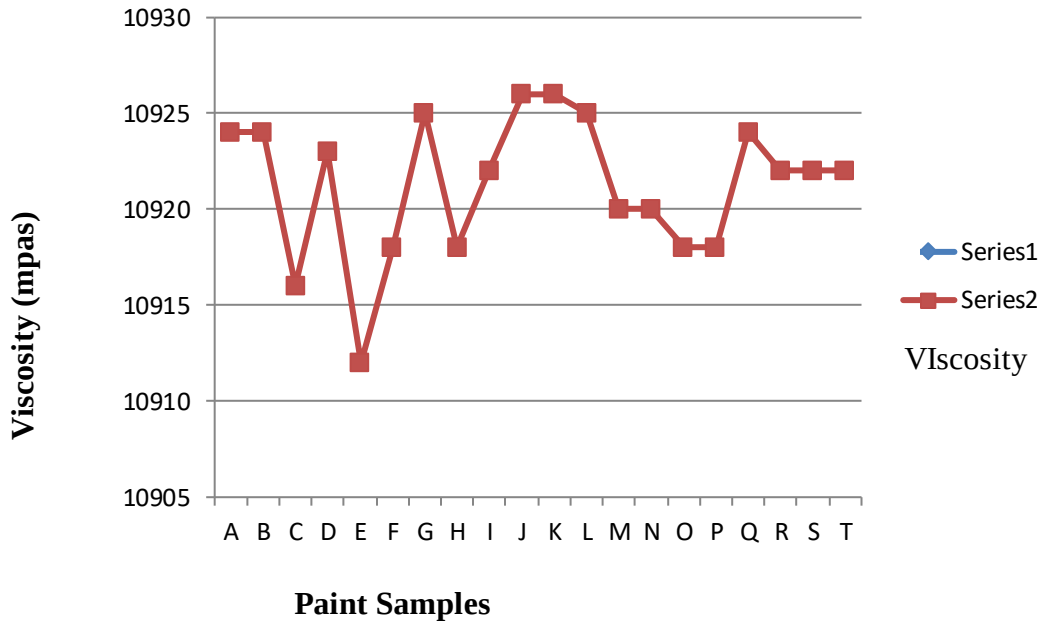

Figure 10:- Viscosity of Paint Samples for Month 2.

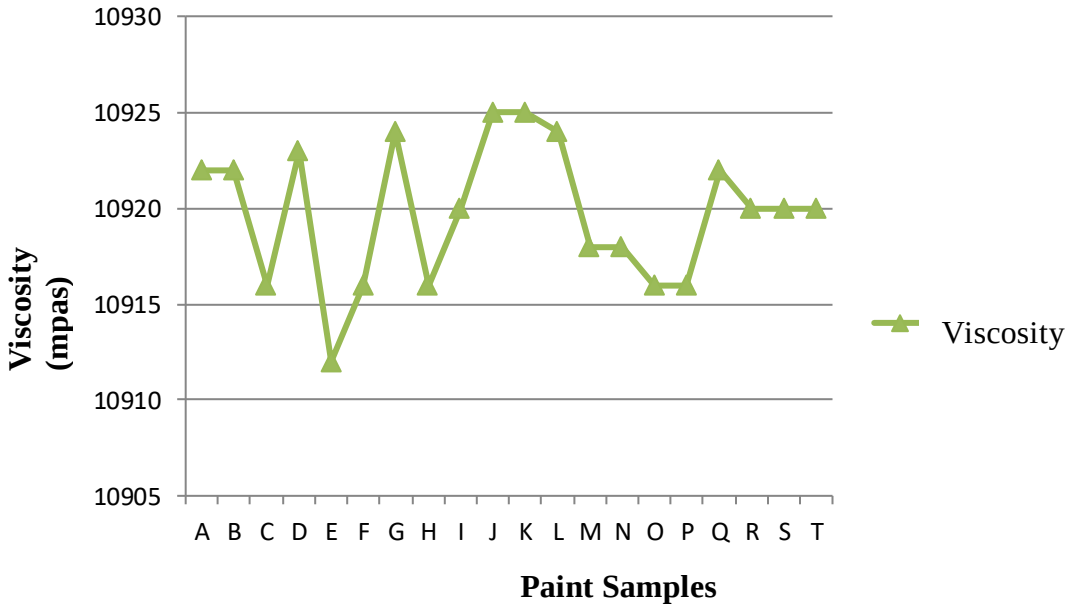

Figure 11:- Viscosity of Paint Samples for Month 3.

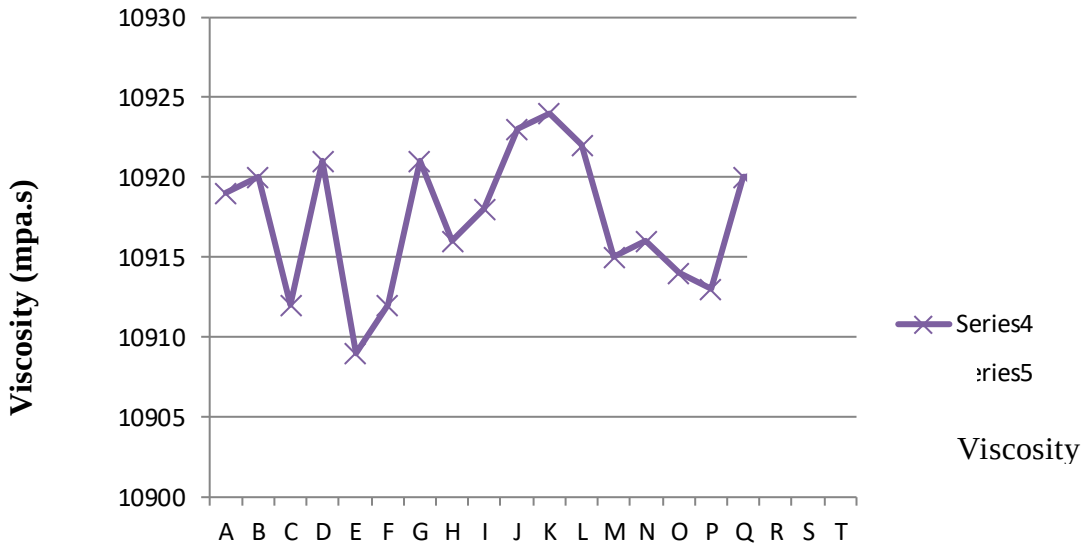

Figure 12: Viscosity of Paint Samples for Month 4.

Table 4.4:- Physiological Changes Found in Paint Samples.

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

4.4.

The phytochemical constituents of O. gratissimum examined qualitatively showed that the plant leaf contains more steroids and flavonoids as shown on Table 4.5. 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 4.6.

Table 4.5:- The Phytochemicals of O. gratissimum.

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

Table 4.6:- Sensitivity of C. tenuissimum to Different Concentrations of Ethanolic Extract of O.gratissimum.

Concentration (mg/ml)	Diameter zone of inhibition (mm)
400	16.2
200	15
100	13
50	10
25	0
12.5	0

Efficacy Testing of O. gratissimum in Paint Physico-chemical Preservation against C. tenuissimum Contamination.

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 4.7). 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 (Table 4.8).

Viscosity values decreased notably in all groups while Group A showed a slight decrease in its value through the four months monitoring. (Table 4.9).

Table 4.7:- 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

1	9.6 ± 0.00	8.9 ± 0.00	8.3 ± 0.00	8.8 ± 0.00
2	9.5 ± 0.00	8.6 ± 0.00	8.3 ± 0.00	8.7 ± 0.00
3	9.4 ± 0.00	8.4 ± 0.00	8.1 ± 0.00	8.5 ± 0.00
4	9.4 ± 0.00	8.4 ± 0.00	8.2 ± 0.00	8.2 ± 0.00

Table 4.8:- 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	9.48 ± 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 4.9:- 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	10926 ± 40.4	10918 ± 30.2	10924 ± 33.33
2	10925 ± 42.6	10916 ± 40.4	10909 ± 33.1	10920 ± 28.05
3	10922 ± 56.5	10911 ± 31.33	10899 ± 42.1	109218 ± 25.15
4	10919 ± 40.4	10900 ± 31.45	10888 ± 35.5	10915 ± 40.05

Observation on the colour shift, texture and odour of the test paint samples showed maintenance of paint colour, texture and odour for group A; white to green to grey, to off white; and white to grey 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 4.10, and plates 5,6,7 and 8 in appendix.

Table 4.10:- Assessment of Physical Changes in Test Paint Samples during Storage.

Months	**Groups**			
	A	**B**	**C**	**D**
0	White	White	White	White
1	White	White colour, slimy texture, and pungent odour	Grey	Off White

2	White	White colour, slimy texture and pungent odour	Grey	Brown
3	White	Light green colour, sticky texture and pungent odour	Brown	Cream
4	White	Light green colour, sticky texture and pungent odour	Off white	Light cream

Table 4.11:- Assessment of Physical Changes in the Test Paints when Applied as Surface Coating.

Months	**Groups**			
	A	**B**	**C**	**D**
0	White colour	White colour	White colour	White colour
1	White colour and held firm on coated surface	Cream colour and sticky texture.	Grey colour and held firm on coated surface	Off –White colour and held firm on coated surface
2	White colouration and held firm on coated surface	Off-White colour and scaling off from coated surface	Grey colouration, and held firm on coated surface	Brown colouration and held firm on coated surface
3	White colouration and maintained firm texture on coated surface	Light green colour and scaling off from coated surface	Brown colour and maintained firm texture on coated surface	Cream colour and maintained firm texture on coated surface
4	White colour and maintained firm texture on coated surface	Light green colour, and scaling off from coated surface	Off white colour and maintained firm texture on coated surface	Cream colour and maintained firm texture on coated **surface**

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 as seen in table 4.12.

Table 4.12:- Fungal Counts ($x10^6$Cfu/ml).

Months	**Groups**			
	A	**B**	**C**	**D**
Initial	0	21.8	0	21.8
1	0	15.6	0	9.4
2	0	12.2	0	4.4
3	0	13.5	0	1.8
4	0	15.1	0	0.9

Discussion:-

Water-based paint spoilage, which is caused by actions of some microorganism, result in the deterioration of paint quality and coating integrity (Machado et al., 2010) . Table 4.2 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 exposed to the atmosphere, since air harbours transient microbes, especially their spores, hence the ability of the paints to be contaminated by fungi.

Figures 1-12 which show the physic-chemical 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). Table 4.4 shows the physiological changes observed in the paint samples post-atmospheric exposure. It was discovered that even though there was contamination during the 48 h exposure, it took about two months for the fungal contaminants to incubate, overcome the presence of incorporated biocides and proliferate, before producing observable physiological changes in the paint samples. Six paint samples- C, J, K ,O ,P and S, showed these physiological changes while other paints retained their production conformity, months after post-atmospheric exposure. This circumstance could be explained from the work of Rosa et al., (2008) 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 4.5 shows the phyto-constituents of O. gratissimum extract which was tested as a natural fungicide for paints, due to its bio-degradability. The extract showed a high presence of flavonoid which has antifungal properties, as well as other phyto-constituents. The sensitivity of the extract against C. tenuissimum which is the predominant fungi found in the test paint samples, showed a Minimum Inhibitory Concentration of 50 mg/ml and a Minimum Fungicidal Concentration of 200 mg/ml. These results reveal the possibility of using the plant extract as a natural antifungal biocide.

Tables 4.7 to 4.9 show the results of efficacy experiment of incorporating the plant extract into water-based paint samples that are laddened with C. tenuissimum culture. It is observed 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) suggests that the paints' thickeners were affected by the plant extract incorporation. The specific gravity of the paint was however slightly altered.

The physiologic status of the paints revealed that incorporation of the extract caused a colour-shift which according to Obidi et al., (2009) suggests the alteration of the dispersants in the paints, as seen in the comparison of Plates 5 and 7; as well as in Table 4.10. 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 on table 4.12. 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.

Conclusion:-

This study shows that O. grastissimum has the potential to be used as an antifungal agent and could be harnessed for such purposes, even though the physico-chemical and physiologic aspects of the paints may be slightly affected.

Acknowledgements:-

The authors gratefully acknowledge the financial support provided by the Tertiary Education Trust Fund (TETFund) through the Institutional Based Research (IBR) Grant. This research was conducted under Grant Reference NAU/TETFC/IBR/2024/VOL.1/09, approved on 23rd August, 2024, via Ref (TETF/ES/DR&D/CE/UNI/AWKA/IBR/2024/VOL.1).

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