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Paint discoloration control and preservation with phytoextracts of *Bryophyllum pinnatum* and *Tetrapleura tetraptera*

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ABSTRACT

Paint discoloration by bacterial species and its control using phytobiactive agents was investigated. The bacterial species; Pseudomonas, Micrococcus, and Bacillus used in the study exhibited high degree of paint discoloration. The rate of discoloration significantly ($p<0.05$) is influenced by the type of organisms, incubation time, bacterial speciation. The level of preservation and control is considered the function of each extracts composition. Within the period of 24 and 96 hours, Micrococcus sp (71.1 – 85.2%) and Bacillus sp (71.6 – 86.6%) exhibited higher discoloration capabilities than Pseudomonas sp (68.7 – 74.3%) with a mean percentage discoloration of eighty (80%) per cent. The consortia of Micrococcus/ Bacillus spp exhibited higher percentage discoloration rate than that of Pseudomonas/ Micrococcus and Pseudomonas/Bacillus spp with no significant difference ($p<0.05$). B. pinnatum extract exhibited strong antibactactial potency than extract of T. tetraptera. However succeptibility of the cell to phytoextracts was positively correlated ($r=0.05$) and significant ($p<0.01$). Hence, B. pinnatum extract discolorations control and paint preservation rate at 250µg/100ml and 500µg/100ml concentration were higher than that of T. tetraptera. The preservation rate at (MBC) of 500µg/100ml of each extract was double against 250µg/100ml. Result obtained suggested that latex paint is subjected to bacterial discoloration can be control with phytobioactive agents and these bioactive agents significantly bring about paint preservation especially in the humid-dried environment . Therefore, B. pinnatum and T. tetraptera bioactive compounds could be supplemented in paint to control discoloration and paint preservation without any toxicity effect.

Key words: Phytoextracts, discoloration, bacteria, preservation, contaminant

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INTRODUCTION

Paint is composed of organic and inorganic compounds referred to as thickeners, binder, filters, dyes and pigments. The presences of these compounds in paint encourage a variety of bacterial contaminants because paint acts as source of carbon and energy for the organisms. Bacterial contamination in paint promotes associated problems such as viscosity loss, discoloration, pH changes, gasing, frothing, sedimentation and deordouration (Linder, 2005).

The bacterial species that often colonize latex paint and painted surfaces are the genera: *Pseudomonas, Streptomyces, Norcodia, Micrococcus, Bacillus, Sarcina, Alcalegenes* and *Flavobacterium*. Discolorations of paint by these bacteria result to a colossal loss of value, cost, and aesthetics. Deleterious bacterial growth with negative effect on paint requires the application or addition of microbiocides in paint and painted surfaces. The addition of biocides intends to kill the bacteria, preservation and prevention against paint disfiguring or deteriorating organisms

(Shirakawa *et al*., 2002). Because of certain biological mechanisms, biocides deployed in paint preservation and bacterial contamination control requires long-term stability, high solubility and mobility and long-term inhibition properties (PRA, 2000). Secondly, biocides must be formulated to have no detrimental effect on the desired paint properties and toxic effect on the environment (Linder, 2005).

Bryophyllum pinnatum and *Tetrapleura tetraptera* are plants commonly found in humid West African subregion. B. *pinnatum* and *T. tetraptera* have been used with tremendous success and effectiveness in ethnomedicine, antimicrobial and preservative of food item (Achi, 2006: Okwu and Joshua,2006). *B. pinnatum* has been reported to be effective in the treatment of bacterial infections caused by *Staphylococcus aureus, E.coli, Bacillus subtilis, Pseudomonas aeruginosa and Klebsiella pneumoniae* (Ofonkansi *et al.*, 2005). Similarly, *T. tetraptera* extracts and essential oils in an in vitro studies exhibited strong antibacterial activities on some foodborne pathogens such as *S. aureus* (ATTC. 12600). *E. coli* (ATTC. 11775), *P. aeruginosa* (ATTC. 1045), *B. subtilis* (ATTC. 6051) and *L. monocytogenes* (Burt, 2004: Achi, 2006).

The present study was designed to evaluate an *in vitro* discoloration properties of bacteria on paint and paint preservation potency of *B. pinnatum* and *T. tetraptera* extracts.

MATERIALS AND METHODS

Plant samples: *Bryophyllum pinnatum* leaves were collected from the botanical garden Cross River University of Technology, Calabar while pods of *T. tetraptera* were purchased from the municipal market in Calabar, Nigeria.

Paint sample: Latex paint in four litre sealed container was bought from a store in the market. The container was checked for possible leakage that may be a source of contamination.

Test organisms: the bacterial species used in the study were previously obtained from paint industrial effluent, purified, identified and stored in the Department of Biological Sciences, Cross River University of Technology, Calabar, Nigeria.

Extract preparation

B. pinnatum leaves were washed, sliced, air-dried for two weeks to dehydrate the leave of excess water (Shaludi, 2004). The dried leaves were pulverized into coarse power. *T. tetraptera* pulps were separated from pods and dried in the oven for one day at 45^{o}C. Coarse powdered *B. pinnatum* and *T. tetraptera* were subjected each to separate aqueous and ethanol soxhlet extraction. The extracts were concentrated to dryness in a flash evaporator under reduced pressure and temperature of 45^{o}C (Merinal *et al.*,2012).

Discoloration assay

The rate of paint discoloration was determined using these basic procedures as described by Ann *et al.* (2002): culture enrichment, screening of test bacteria, discoloration assay and co-culture discoloration assay.

Culture enrichment: to obtain young cells, the cells were grown in 100ml mineral salt medium (MSM) supplemented with 1.0ml of paint.

Screening of cells: the enriched population was plated on MSM-agar supplemented with 2.0ml of paint and incubated at room temperature (28^{0}C) for 5 days. The morphologically distinct colonies with clear zone around their colonies were picked and purified as paint discolourising bacteria (Sani and Banerjec, 1999 a & b).

Decolorizing assay: the decolorizing activities of the bacteria were considered in terms of percentage (%) decolorization. This was determined by monitoring the graviphotometric decrease in absorbance at absorption maxima (λmax) of 640nm wave length of the paint mixture as described by Sani and Banerjec (1999 a & b).

Co-culture decolorization assay: an 18hour broth culture of bacterial consortia of the test cells were inoculated into the paint in three combinations at 1:1 ratio as follows; *Pseudomonas/Micrococcus* spp, *Micrococcus/Bacillus* spp *and Pseudomonas/Bacillus* spp. The decoloration activities of each consortium were expressed in percentage (%) as stated above (Ann *et al.*, 2002: Deepak *et al.*, 2004)

Paint decolorization inhibition and preservation assay.

The preservative potential of *B. pinnatum* and *T. tetraptera* extract on paint contaminated by *Pseudomonas* sp, *Micrococcus* sp, and *Bacillus* sp were determined by estimating the total viable count **(TVC)** of paint contaminant as describe by Achi (2006). Forty 250ml capacity flasks were suspended with 98.0ml of distilled water and autoclaved at 121^0C and 15psi. On cooling, 5.0ml of pressurized filter sterilized latex paint, 0.2ml of *Pseudomonas, Micrococcus,* and *Bacillus* cells (approximately 1.5 – 2.0 x 10^8cfuml^{-1}) and 250mg/100ml and 500mg/100ml of aqueous extracts of *B. pinnatum* and *T. tetraptera* were added separately to 2 subsets of 15 flasks respectively. Then the third set of 10 flasks without the extract was set aside as controls. Each of the flasks were arranged and incubated at room temperature (28± 2^0C) for 25 days on a shaker, rotating at 110 rpm. At interval of 5 days, 10.0ml of representative sample from each flask was aseptically drawn and was used to determined the total viable cell **(TVC)** in tenfold serial count as an index of paint preservation against the test organisms.

Statistical analysis

The data collected were subjected to mean calculation, percentage determination and correlation analysis of variance (ANOVA) using Statistical Analysis System Generalized Linear Model (SASGLM, SAS version 8.02,(SAS,2000). Results are discussed based on the various statistical conclusions and recommendations put forward according.

RESULTS

Paint decolorizing properties of bacteria is presented in Fig 1. The results recorded as percentage (%) decolorization were index of optical density (OD) and percentage transmittance at 640nm wavelength. It demonstrated that each bacterium species (*Psuedomonas* sp, *Bacillus* sp *and Micrococcus* sp) exhibited high degree of paint decolorization potential. For each of the bacterium, the rate of decolorization increase significantly alongside the increase in incubation time. The rate (%) within the 24 – 96 hours for *Micrococcus* sp (71. 1 – 85. 2 %) and *Bacillus* sp (71.6 – 86. 6%) was higher than that of *Pseudomonas* sp (68. 7 – 74.3%). The *Pseudomonas* sp ability to decolorize paint was lower than the mean (80%) for the bacteria at 96 hours. This shows that *Pseudomonas* sp is far less a comparative paint decolorizer to *Micrococcus* and *Bacillus* spp.

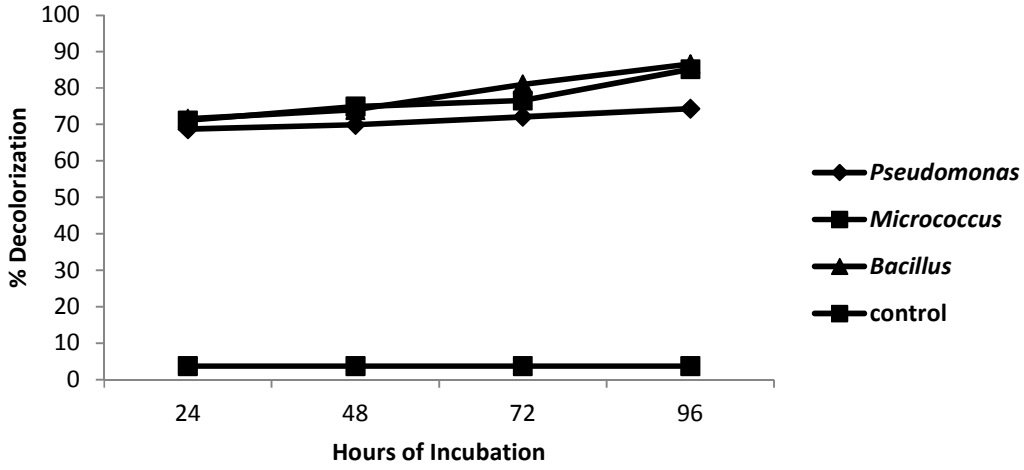

FIG.1: Decolorization rate (%) of latex paint resulting from degradation by different bacterial spp.

The consortia results for *Pseudomonas, Micrococcus* and *Bacillus* spp is presented in Fig2. The results showed that each of the consortia decolorized paint slightly higher than the individual species. Secondly *Micrococcus/Bacillus* spp combination had a slightly higher decolorization rate with no significant difference ($p>0.05$) than that of *Pseudomonas/Bacillus* and *Pseudomonas/Micrococcus*.

Paint decolorization inhibition and preservation activity by *B. pinnatum* and *T. tetraptera* extracts are presented in Tables 1 and 2. The results showed that the phytoextracts exhibited strong preservative properties on the bacteria by inhibiting their growth in the paint. The extract concentrations exerted high toxic effect on the paint decolorizers. The 500µg extract concentrations of both plants exhibited twice the toxic effects on the bacteria than the 250µg concentrations. For example at 120 hours the extracts inhibitory effects were; *Pseudomonas* sp =54:22/166:51;

Micrococcus sp =77:37/102:43 and *Bacillus* sp =80:31/159:72 respectively. The bacterial cell count dropped progressively with increase in incubation time. The result showed a positive and direct correlation (r=0.05) between the cell count and incubation time. Secondly, the relationship between the cell toxicity level and extract concentration was significant (p>0.05) for all the test bacteria.

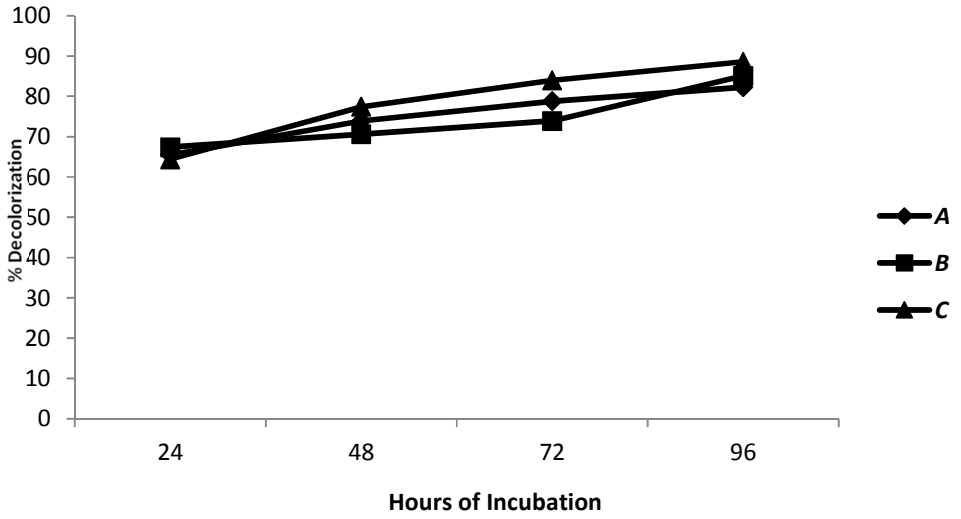

FIG. 2: Decolorization rate (%) resulting from the growth of bacterial consortium on latex paint.
A – *Pseudomonas/Micrococcus*sp
B – *Micrococcus/Bacillus sp*
C – *Bacillus/Pseudomonas sp*

Table 1. Preservation effect of *Bryophyllum pinnatum* extract on paint bacterial decolorizers

Incubation Time (hrs)	Phytochemical extract concentration (µg)								
	250			500			Control		
	Pse	Mic	Bac	Pse	Mic	Bac	Pse	Mic	Bac
24	183±0.4	225±0.1	147±0.3	92±0.2	128±0.1	95±0.4	434±0.7	430±0.6	432±0.8
48	158±0.2	191±0.3	166±0.1	71±0.4	98±0.3	78±0.1	402±0.4	400±0.5	401±0.4
72	102±0.2	143±0.4	121±0.2	49±0.1	69±0.2	62±0.6	371±0.5	374±0.7	372±0.9
96	88±0.1	107±0.2	93±0.2	47±0.3	58±0.6	48±0.3	338±0.5	341±0.5	339±0.7
120	54±0.2	77±0.2	80±0.2	22±0.4	37±0.3	31±0.1	286±0.3	290±0.8	288±0.6

Stating culture = 3.03 × 10^8 cfuml^{-1}
1.0 McFarland Standard = 3.0× 10^8
Key: *Pse = Pseudomonas sp, Mic = Micrococcus sp, Bac = Bacillus sp*

Table 2. Preservation effect of *Tetrapleuratetrapteraextract* on paint bacterial decolorizers

Incubation Time (hrs)	Phytochemical extract concentration (µg)								
	250			500			Control		
	Pse	Mic	Bac	Pse	Mic	Bac	Pse	Mic	Bac
24	252±0.8	175±0.6	250±0.4	206±0.5	160±0.1	116±0.7	434±0.7	430±0.9	432±0.5
48	229±0.5	160±0.3	225±0.8	178±0.9	140±0.4	101±0.4	402±0.5	400±0.5	401±0.7
72	203±0.8	130±0.9	201±0.2	126±0.6	108±0.8	93±0.6	371±0.9	374±0.7	372±0.3
96	184±0.7	112±0.5	180±0.4	93±0.4	66±0.6	85±0.3	338±0.3	341±0.5	339±0.6
120	166±0.6	102±0.8	159±0.7	51±0.8	43±0.3	72±0.5	286±0.6	290±0.3	288±0.4

Stating culture = 3.03 × 10^8 cfuml^{-1}
1.0 McFarland Standard = 3.0 × 10^8
Key: *Pse = Pseudomonas sp, Mic = Micrococcus sp, Bac = Bacillus sp*

DISCUSSION

Discoloration of paint caused by bacterial contamination is a common problem especially in highly humid environment. Bacterial contaminations implicated in paint and dye discoloration included the species of *Pseudomonas*, *Micrococcus*, and *Bacillus* used in this study. The discoloration activities of these bacteria are no

doubt considered an enzymatic catalyzed reaction (Praveen *et al.,* 2012: Charitha and kumar, 2012). Therefore, the bacterial breakdown and discoloration activities of paint are considered a function of extracellular enzymes produced by the bacteria. The enzymes produced are in direct correspondence to the material composition of the dyes pigments as a deployed substrate (Kang *et al.,* 2004: Josephine *et al.,* 2014). Secondly *Pseudomonas*, *Micrococcus* and *Bacillus* spp are bacteria already identified as bacteria with high capacity to produce different kinds of enzymes even in commercial scale (Araffin *et al*., 2006).

The differences in rate of discoloration among the duo of *Micrococcus* and *Bacillus* spp against *Pseudomonas* sp may be as a result of the amount of enzyme produced per cell. In similar study paint discoloration was reported to be dependent and influenced by: the structure of dye pigment, presence and absence of dissolved oxygen, presence of carbon and nitrogen, nitrites and nitrates source in dye pigment and paint. Also the production of metabolites such as alcohol and alkaline affect paint discoloration caused by bacteria (Stolz, 2001, McMullan *et al*., 2001, Verma and Madamwar, 2003 and Romalho *et al.,* 2004).

Naturally occurring bioactive compounds in plants often play important role in controlling the growth of spoilage and pathogenic bacteria in foods and many other organic based compounds (Burt, 2004: Achi,2006). Recently, it has been established that plant bioactive agents are most active against Gram-negative bacteria because of their high content of phenolic compounds. This condition accounts for the high bacteriocidal and preservative effect of the extracts on the paint decolorizers used in this study (Adebayo *et al*., 2000).

The high level of polyphenolic compounds in *B. pinnatum* and *T. tetraptera* extracts inhibited the growth of these bacteria in paint thereby reducing their discoloration potential. However, the insignificant ($p>0.05$) low level of toxicity by *T. tetraptera* extract against the bacteria could be seen as a low percentage content of phenolic compounds in it (Tainter and Grenis, 2001). Secondly, authors have established that *B. pinnatum and T. tetraptera* extracts at $250\mu gm1^{-1}$ used in this study significantly ($p<0.05$) inhibited the growth of pathogens isolated from foods and wounds (Ofokansi *et al*., 2005). It is therefore observed that at $500\mu gm1^{-1}$ concentration considered in this study as minimum inhibition concentration (MIC) of *B. pinnatum* and *T. tetraptera* extracts used could be considered as alternative preservative agents to synthetic biocides used in paint industries in controlling bacterial discoloration problems and stability loss of paint and painted film in humid-dried environments.

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