# ECO-FRIENDLY PAINT INCORPORATING A COMBINATION OF TEA TREE OIL AND CLOVE ESSENTIAL OIL AS A NATURAL ANTIFUNGAL AGENT AGAINST THE GROWTH OF *ASPERGILLUS NIGER.*


## *Abstract*

Water-based eco-friendly paints avoid the volatile organic compounds and the synthetic biocides of conventional formulations, but that same absence leaves their films vulnerable to fungal colonisation in warm, humid climates. Silver nanoparticles, the biocide most often used against *Aspergillus niger*, are effective yet cytotoxic to several human cell lines. Essential oils are a plausible natural substitute, but almost all published work tests them in free form, where the active compounds diffuse unimpeded, rather than locked inside a dried polymer film.A completely randomised design with four treatments and four replicates was applied to painted wooden coupons (2 × 2 cm): P0, unamended paint (control); P1, paint + 2% tea tree oil; P2, paint + 2% clove essential oil; P3, paint + 1% tea tree oil + 1% clove essential oil. All amended formulations carried 2% Tween 80. Coupons were placed on Sabouraud Dextrose Agar previously inoculated with *A. niger* and incubated for two weeks at 27-30 °C and roughly 80% relative humidity. Mycelial coverage of the painted surface was quantified digitally in ImageJ and analysed by ANOVA on arcsine-transformed data, Tukey HSD, and Fisher's exact test on growth incidence. Treatment strongly affected mycelial coverage ($F = 16.15$; $p = 0.0002$; $\eta^2 = 0.80$). Mean coverage fell from 66.15% in the control to 12.45% (P1), 9.58% (P2) and 0.00% (P3). Tukey HSD separated the control from all three amended formulations but not the amended formulations from one another. Fisher's exact test on growth incidence showed P3 to be the only treatment entirely free of mycelium (0 of 4; $p = 0.029$ against both P0 and P1). Because P3 delivered complete inhibition at half the dose of each individual oil, the interaction is at least additive. Spores persisted on the P3 surface without germinating, indicating a fungistatic rather than fungicidal effect. No cracking, peeling or colour change attributable to the oils was observed in any formulation. A 1% + 1% combination of tea tree oil and clove essential oil confers complete and reproducible protection against *A. niger* on a water-based eco-friendly paint film, at half the dose of either oil used alone and without visible damage to the film. The finding supports natural essential oils as a viable replacement for cytotoxic synthetic biocides in wall paints for humid tropical settings, while formal proof of synergy and long-term protective life remain open questions.



**Key words: -**

Eco-friendly paint, tea tree oil, clove essential oil, *Aspergillus niger*, natural antifungal agent


## Introduction:-

Paint is among the most widely applied materials in the built environment, and the composition of that thin film governs both the durability of the surface beneath it and the quality of the air above it. Conventional formulations rely on volatile organic compounds and synthetic biocides, and growing awareness of their environmental and health burden has driven the development of eco-friendly alternatives built from natural minerals, agricultural residues and water-based binders [1], [2].

Biological contamination is the principal weakness of these greener formulations. Removing synthetic preservatives removes, at the same time, the protection they provided, and in warm humid climates the painted film becomes a ready substrate for fungal colonisation. The industry answer has been to reintroduce a biocide, most commonly silver nanoparticles, whose antifungal activity is well established and whose penetration into fungal tissue scales with particle size [3]. That solution, however, carries its own cost: *in vitro* work reports that silver nanoparticles, particularly those below 10 nm, induce reactive oxygen species, oxidative stress and mitochondrial dysfunction, and prove cytotoxic to human bronchial epithelial cells, HUVECs, erythrocytes, macrophages and hepatocytes [4].

Among the fungi that colonise painted surfaces, *Aspergillus niger* is both common and consequential. A saprophytic filamentous fungus that thrives on damp organic material such as wood, it causes aspergillosis affecting the respiratory tract, liver, kidneys, nervous system, muscle, skin and genital organs [5], and it produces a range of mycotoxins including aflatoxins, ochratoxin A, patulin, citrinin, cyclopiazonic acid, sterigmatocystin and gliotoxin, which together represent a recognised threat to food safety and human health [6].

Natural antifungal agents offer a route out of this trade-off, since the term encompasses any chemical compound, pharmacological agent or natural product used against fungal infection, in medicine as much as in materials preservation

[7]. Two essential oils are particularly well characterised. Tea tree oil, distilled from *Melaleuca alternifolia*, inhibits a range of fungi including *A. niger* [8]; its activity rests chiefly on terpinen-4-ol, which makes up roughly 30 to 40% of the oil, together with α-terpineol, linalool, α-pinene and β-pinene [9]. Working directly on *A. niger*, An et al. [10] found that terpinen-4-ol and α-terpineol suppress both mycelial growth and spore germination while inflicting morphological damage and metabolic disruption on the fungus. Clove essential oil, from *Syzygium aromaticum*, owes its activity primarily to eugenol, present at 30 to 95%, alongside β-caryophyllene, α-ylangene and δ-cadinene [11]; its volatile vapour is fungistatic while direct application is fungicidal [12].

What the literature has largely not addressed is how these oils behave once they are no longer free. Published assays test essential oils by disc diffusion or broth dilution, conditions under which the active compounds diffuse without obstruction. Inside a paint formulation the situation is different in kind: the active compound must first migrate through a dried polymer film before it can reach the interface where fungal growth begins. Whether inhibition survives that constraint is an open question, and combinations of the two oils within such a matrix have, to our knowledge, not been examined at all.

This study therefore evaluates a water-based eco-friendly paint amended with tea tree oil, clove essential oil, and a combination of the two, against *A. niger*, with four specific aims: to determine whether the amendments inhibit fungal growth on the dried film; to compare the effectiveness of each oil alone against their combination; to establish whether the amendments compromise the physical quality of the paint film; and to assess whether the combination is viable as a natural antifungal agent in a water-based formulation.

## Materials and Methods:-

### Time and Place of Study:-

The study was carried out in the chemistry and biology laboratories and in the outdoor reading area of the fifth floor of Yayasan Taruna Bakti, Jl. L.L.R.E. Martadinata No. 52, Citarum, Bandung Wetan, Bandung 40115, West Java, Indonesia, over three months from June to August 2026. June was given to literature study and procurement, July to experimental design and paint formulation, and August to the antifungal assay and analysis. Because the school laboratory has no laminar air flow cabinet, culture development and the antifungal assay itself were conducted in the open fifth-floor reading area rather than indoors.

### Materials and Apparatus:-

The paint base was Mowilex Naturalle, a commercial water-based eco-friendly wall paint. Tea tree oil (*Melaleuca alternifolia*) and clove essential oil (*Syzygium aromaticum*) were used as active agents, with Tween 80 as the non-ionic emulsifier. Sabouraud Dextrose Agar (SDA) served as the growth medium, prepared with distilled water, and an *A. niger* isolate provided the inoculum. Apparatus included an Erlenmeyer flask, glass and aluminium spatulas, an Ohaus balance, a hot plate, an autoclave, a Bunsen burner, a measuring cylinder, graduated pipettes, an inoculating loop, forceps, mixing cups, sixteen disposable Petri dishes, sixteen wooden coupons of 2 × 2 cm, a thermohygrometer and four non-airtight plastic boxes.

### Experimental Design:-

A completely randomised design was used, with four treatments and four replicates, giving sixteen experimental units. The treatments were P0, paint with no active agent (control); P1, paint + 2% tea tree oil + 2% Tween 80; P2, paint + 2% clove essential oil + 2% Tween 80; and P3, paint + 1% tea tree oil + 1% clove essential oil + 2% Tween 80. All concentrations are volume per volume of paint. The sixteen units were distributed across four plastic boxes, each box holding exactly one coupon of each treatment, so that every box constituted one complete replicate.

### Paint Formulation:-

Each oil was measured with a graduated pipette to the concentration required by the treatment and combined with Tween 80 to allow homogeneous dispersion in the water-based paint. The mixture was stirred with a glass spatula until homogeneous, then stored in a labelled sterile container.

**Preparation of the Fungal Culture:-**

All apparatus and media were sterilised by autoclave. SDA was poured into sixteen sterile disposable Petri dishes labelled P0 to P3. *A. niger* was inoculated aseptically onto the medium, and the plates were incubated at 27-30 °C and approximately 80% relative humidity for seven days in a dimly lit bag until colonies had developed fully. The plates were then distributed into the four non-airtight plastic boxes described above.

**Antifungal Contact Assay:-**

A direct contact method was used. Each formulation was brushed onto a sterile 2 × 2 cm wooden coupon at uniform thickness and left to dry for ten minutes at room temperature. Each coupon was then placed on the surface of the inoculated SDA and incubated for two weeks, from 3 to 18 August 2026, at 27-30 °C and approximately 80% relative humidity. Fungal growth on the painted surface was photographed and the colonised area measured digitally in ImageJ, expressed as a percentage of the total painted surface (mycelial coverage). A coverage of 0.00% denotes the absence of measurable mycelium; it does not imply a spore-free surface.

**Assessment of Physical Film Quality:-**

The paint film was examined before and after incubation for colour, film integrity and resistance to a simple scratch test, the latter performed by drawing a blunt point across the dried surface and recording whether damage was confined to the surface layer or penetrated to the substrate.

**Data Analysis:-**

Because percentage data have compressed variance near the 0% and 100% bounds, homogeneity of variance was checked with Levene's test and the data were transformed by arcsine square root before analysis; normality of residuals was assessed with the Shapiro-Wilk test. One-way ANOVA was applied to the transformed data, followed by Tukey's HSD at $\alpha = 0.05$. Effect size was expressed as $\eta^2$ and as the more conservative $\omega^2$. Because each box contained one coupon of every treatment, the analysis was repeated with box entered as a blocking factor to confirm that box position introduced no systematic effect. Kruskal-Wallis and Friedman tests were run as distribution-free cross-checks. Relative inhibition was computed within each box as RI (%) = [(C – T) / C] × 100, where C is the coverage recorded on the control coupon in that box and T the coverage on the test coupon in the same box. Because all P3 values collapse to zero and carry no variance, the data were additionally recoded as the binary presence or absence of measurable mycelium and analysed by Fisher's exact test. Statistical power and required replicate numbers were derived from Cohen's d. All analyses were performed in Python 3.11 using SciPy and statsmodels.

**Results:-**

**Growth of *Aspergillus niger* on the Painted Surface:-**

Sixteen painted coupons were assessed after two weeks of incubation. Mycelial coverage for every treatment and replicate is given in Table 1.

**Table 1:** Mycelial coverage of *Aspergillus niger* on the painted surface, by treatment and replicate (%).

| Treatment | Box 1 | Box 2 | Box 3 | Box 4 | Mean ± SD (%) |
|---|---|---|---|---|---|
| P0 | 68.60 | 57.88 | 39.94 | 98.18 | 66.15 ± 24.41 |
| P1 | 23.02 | 16.40 | 2.30 | 8.07 | 12.45 ± 9.12 |
| P2 | 2.25 | 23.33 | 0.00 | 12.76 | 9.58 ± 10.72 |
| P3 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 ± 0.00 |

Note: P0 = paint with no active agent (control); P1 = paint + 2% tea tree oil + 2% Tween 80; P2 = paint + 2% clove essential oil + 2% Tween 80; P3 = paint + 1% tea tree oil + 1% clove essential oil + 2% Tween 80. A value of 0.00% indicates that no measurable mycelium was found; spores were still observed on the painted surface.

**Descriptive Statistics and Relative Inhibition:-**

Mean coverage on the control reached 66.15%, while the three amended formulations fell far below it, at 12.45% (P1), 9.58% (P2) and 0.00% (P3). The means alone, however, understate what the data do. The coefficient of variation rises as the mean falls, from 36.9% in P0 to 73.3% in P1 and 111.8% in P2, a pattern characteristic of percentage data approaching the lower bound and the reason transformation was considered. P3 is the only treatment with zero standard deviation, its result identical across all four replicates (Table 2).

**Table 2:** Descriptive summary of mycelial coverage of *Aspergillus niger*.

| Treatment | Mean (%) | SD | Median (%) | Range (%) | CV (%) | Replicates with growth |
|---|---|---|---|---|---|---|
| P0 | 66.15 | 24.41 | 63.24 | 39.94–98.18 | 36.9 | 4 of 4 |
| P1 | 12.45 | 9.12 | 12.24 | 2.30–23.02 | 73.3 | 4 of 4 |
| P2 | 9.58 | 10.72 | 7.51 | 0.00–23.33 | 111.8 | 3 of 4 |
| P3 | 0.00 | 0.00 | 0.00 | 0.00–0.00 | 0.0 | 0 of 4 |

Note: CV = coefficient of variation (SD divided by the mean, expressed as a percentage); n = 4 replicates per treatment.

Control coverage was itself far from uniform between boxes, ranging from 39.94% to 98.18%, a spread of nearly two and a half fold. This indicates that inoculum pressure and microclimate differed from box to box, and that comparisons between treatments are fairer when expressed relative to the control within the same box. Relative inhibition computed on that basis is given in Table 3.

$$RI\ (\%) = [(C - T) \div C] \times 100 \qquad \text{(Eq. 1)}$$

where C is the mycelial coverage recorded on the control coupon (P0) in the box concerned, and T the coverage on the test coupon in the same box.

**Table 3:** Relative inhibition of each treatment against the control, by box (%).

| Treatment | Box 1 | Box 2 | Box 3 | Box 4 | Mean ± SD (%) |
|---|---|---|---|---|---|
| P1 | 66.44 | 71.67 | 94.24 | 91.78 | 81.03 ± 14.03 |
| P2 | 96.72 | 59.69 | 100.00 | 87.00 | 85.85 ± 18.29 |
| P3 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 ± 0.00 |

All values are expressed as percentages.

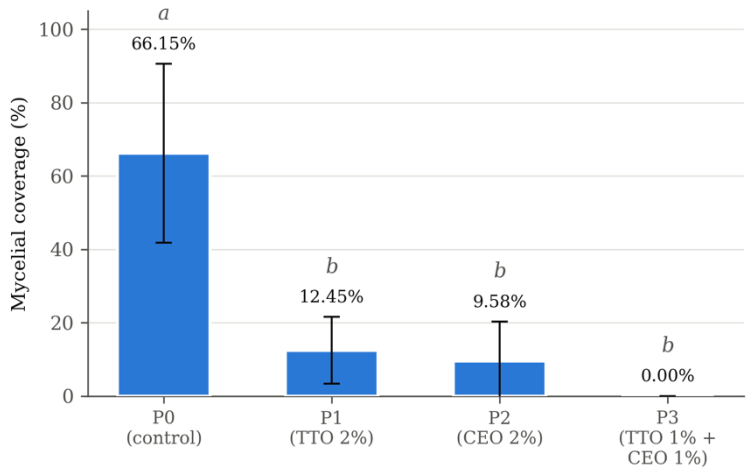

**Fig. 1:-** Mean mycelial coverage of *Aspergillus niger* by treatment. Error bars show the standard deviation (n = 4). Different letters denote a significant difference under Tukey's HSD at the 5% level on arcsine-transformed data.

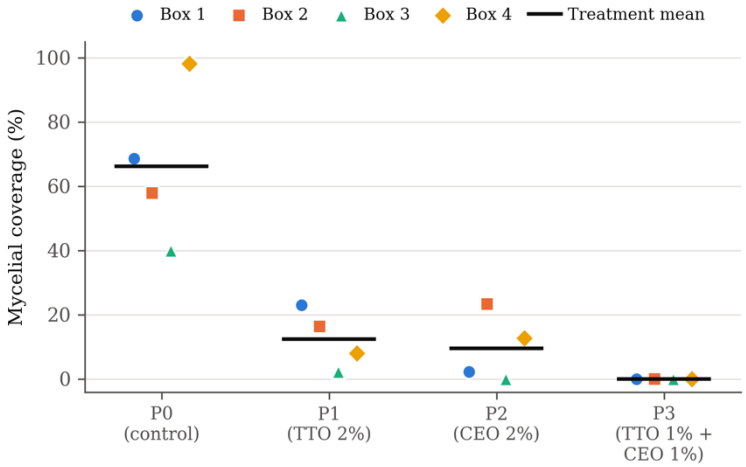


**Fig. 2:-** Distribution of mycelial coverage across replicates. The heavy horizontal line marks the treatment mean. Variation between replicates is considerable in P0, P1 and P2, whereas P3 gave an identical result in all four boxes.

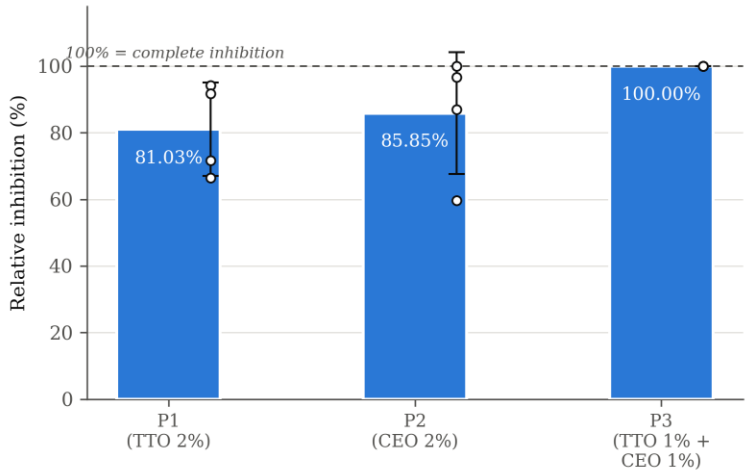


**Fig. 3:-** Relative inhibition of each formulation against the control within the same box. Open circles are individual replicates; error bars show the standard deviation. Only P3 reached complete inhibition in every replicate.

**Assumption Checks and Data Transformation:-**

Levene's test on the untransformed data returned F = 3.21 with p = 0.062, sitting exactly at the threshold of rejection. The data were therefore transformed by arcsine square root before analysis. After transformation, Levene's test gave F = 2.44 (p = 0.115) and the Shapiro-Wilk test on model residuals gave W = 0.960 (p = 0.653). Both assumptions being satisfied, the analysis of variance was performed on the transformed data, while descriptive results are reported in percentage units for readability.

**Analysis of Variance:-**

**Table 4:** Analysis of variance for the effect of treatment on mycelial coverage of *Aspergillus niger* (arcsine-transformed data).

| Source of variation | df | SS | MS | F | Sig. (p) |
|---|---|---|---|---|---|

| Source of variation | df | SS | MS | F | Sig. (p) |
|---|---|---|---|---|---|
| Treatment | 3 | 6,995.87 | 2,331.96 | 16.15 | 0.0002 |
| Error | 12 | 1,732.35 | 144.36 | – | – |
| Total | 15 | 8,728.22 | – | – | – |

Note: df = degrees of freedom; SS = sum of squares; MS = mean square. Effect size: $\eta^2 = 0.80$ and $\omega^2 = 0.74$.

Treatment had a highly significant effect on mycelial coverage ($F = 16.15$; $p = 0.0002$; Table 4). An $\eta^2$ of 0.80 indicates that about 80% of the total variance in the data is attributable to differences between formulations, and the more conservative $\omega^2$ of 0.74 still places the treatment effect in the very large category.

Since each box held one coupon of every treatment, box position could act as a block and bias the analysis if ignored. The analysis was therefore repeated with box entered as a separate source of variation. The box effect proved non-significant ($F = 2.05$; $p = 0.178$) while the treatment effect remained highly significant ($F = 20.38$; $p = 0.0002$), which justifies the completely randomised design used here: differences between boxes introduced no systematic effect requiring removal from the error term.

As a cross-check independent of distributional assumptions, the Kruskal-Wallis test ($H = 12.27$; $p = 0.0065$) and the Friedman test, which accounts for the dependency between replicates within a box ($\chi^2 = 10.23$; $p = 0.017$), were also applied. All three approaches lead to the same conclusion, so the finding of a treatment effect can be considered robust.

**Tukey's HSD Post Hoc Test:-**

**Table 5:** Tukey's HSD test on mean mycelial coverage of *Aspergillus niger*.

| Treatment | Transformed mean | Original mean (%) | Notation |
|---|---|---|---|
| P0 (control) | 56.72 | 66.15 | a |
| P1 (tea tree oil 2%) | 19.45 | 12.45 | b |
| P2 (clove essential oil 2%) | 14.61 | 9.58 | b |
| P3 (combination 1% + 1%) | 0.00 | 0.00 | b |

Note: identical letters in the final column indicate the absence of a significant difference at $\alpha = 0.05$.

Tukey's HSD shows the control to differ highly significantly from all three amended formulations, with $p = 0.0042$ (P0 against P1), 0.0016 (P0 against P2) and 0.0001 (P0 against P3). The three amended formulations, by contrast, do not differ significantly from one another: P1 against P2 gives $p = 0.939$, P1 against P3 gives $p = 0.155$, and P2 against P3 gives $p = 0.356$ (Table 5).

The consequence of this result deserves to be stated openly. At 95% confidence and with four replicates available, the difference between clove essential oil and tea tree oil cannot be demonstrated statistically. The effect size separating them is Cohen's $d = 0.29$, conventionally small, with a statistical power of 0.064; detecting a difference that small with confidence would require roughly 190 replicates per treatment. The difference between P1 and P3, by contrast, is far larger ($d = 1.93$) and would need only about six replicates per treatment to reach a power of 0.8. The advantage of the combination is therefore very likely real, but the number of replicates available here is insufficient to demonstrate it through a comparison of means.

**Analysis of Growth Incidence:-**

The comparison of means above loses part of its discriminating power because every P3 value collapses onto zero, leaving that treatment with no variance at all. To complete the picture, the data were recoded in binary form as the presence or absence of measurable mycelium in each replicate and analysed by Fisher's exact test (Table 6).

**Table 6:** Incidence of mycelial growth and results of Fisher's exact test against P3.

| Treatment | Replicates with growth | Proportion (%) | p (against P3) |
|---|---|---|---|
| P0 | 4 of 4 | 100 | 0.029 |
| P1 | 4 of 4 | 100 | 0.029 |
| P2 | 3 of 4 | 75 | 0.143 |
| P3 | 0 of 4 | 0 | – |

Note: $p < 0.05$ indicates a significant difference from P3.

On this approach P3 differs significantly from both the control and the tea tree oil formulation ($p = 0.029$ in each case), although its difference from the clove-only formulation remains non-significant ($p = 0.143$). P3 is the only treatment that prevented growth in every replicate without exception. The advantage of the combination thus lies not in the size of the difference in means but in its reliability, that is, in delivering the same outcome in every replicate.

**Physical Quality of the Paint Film:-**

**Table 7:** Physical condition of the paint film before and after incubation.

| Treatment | Colour before incubation | Scratch test before incubation | Condition after incubation |
|---|---|---|---|
| P0 | White | Surface layer only | No cracking; colour change due solely to fungal growth |
| P1 | White | Surface layer only | No cracking; colour change due solely to fungal growth |
| P2 | White | Surface layer only | No cracking; colour change due solely to fungal growth |
| P3 | White | Surface layer only | No cracking; no colour change observed |

No cracking, peeling or colour change originating in the formulation itself was found in any of the four treatments (Table 7). The colour change observed on P0, P1 and P2 came entirely from fungal colonies growing on the surface. The simple scratch test gave uniform results across all treatments, with damage confined to the surface layer whether or not essential oil had been added.

**Discussion:-**

**Antifungal activity of essential oils within the paint matrix:-**

That the addition of 2% essential oil suppressed *A. niger* coverage from 66.15% to below 13% is consistent with earlier work on both oils. Hammer et al. [9] attribute tea tree oil activity chiefly to terpinen-4-ol and α-terpineol, two lipophilic monoterpene alcohols, and An et al. [10] tested precisely these two compounds against *A. niger*, finding that they inhibit mycelial growth and spore germination alike while causing morphological damage and metabolic disruption. On the clove side, eugenol, the principal phenolic component, binds ergosterol in the fungal membrane and thereby compromises membrane integrity [13], and Hassan et al. [14] identified eugenol as the most effective of the compounds they screened against *A. niger*.

What distinguishes the present result is that this activity persisted even with the oils trapped inside the polymer matrix of a water-based paint. Most earlier work tested essential oils in free form by disc diffusion or broth dilution, conditions under which the active compounds diffuse without obstruction. In a paint formulation the active compound must first migrate through a dried film to reach the surface. That inhibition nonetheless occurred shows that terpinen-4-ol and eugenol

remain able to diffuse or volatilise out of the paint layer to the paint-fungus interface. This is what makes the two oils plausible replacements for synthetic biocides in paint, and it is where this study departs from assays conducted on free oils.

**Tea tree oil compared with clove essential oil:-**

Descriptively, 2% clove essential oil performed slightly better than 2% tea tree oil, with mean coverage of 9.58% against 12.45%, or relative inhibition of 85.85% against 81.03%. The direction is consistent with Hassan et al. [14], who ranked clove oil as the most effective of the essential oils tested against *A. niger*, and with Chee and Lee [12], who reported direct application of clove oil to be fungicidal.

Tukey's HSD nonetheless shows the difference between P1 and P2 to be non-significant ($p = 0.939$), and a paired t-test across boxes reaches the same conclusion ($p = 0.680$). This study therefore cannot claim that clove essential oil outperforms tea tree oil. What it can conclude is that both significantly suppress *A. niger* growth relative to the control, with a descriptive tendency slightly favouring clove. Two factors account for the non-significance: only four replicates, and considerable variation between replicates, particularly in Box 2, discussed below. This caution matters, because a conclusion should not outrun what the data actually show.

**Combining the two oils: dose efficiency and the question of synergy:-**

The most striking finding of this study concerns dose. P3 contains only 1% tea tree oil and 1% clove essential oil, half the amount of each oil relative to P1 and P2, even though total oil content is the same 2%. With less of each component, P3 nonetheless achieved complete inhibition in all four replicates, whereas P1 and P2 reached mean relative inhibition of only 81.03% and 85.85% respectively.

If the two oils acted additively under the Loewe model, that is, if their dose-response curves were parallel, a 1% + 1% mixture should produce inhibition equivalent to 2% of a single oil, roughly 81 to 86%. That the observed inhibition instead reached 100% in every replicate indicates an interaction that is at least additive and potentially synergistic.

There is a plausible mechanistic basis for this, since the two oils attack different targets in the fungal cell. Terpinen-4-ol and α-terpineol are lipophilic and act by inserting into the lipid bilayer of the membrane, raising its permeability and causing cellular contents to leak [10]. Eugenol is phenolic and acts by binding ergosterol, the sterol component of the fungal membrane, while also damaging membrane proteins [13]. Membrane damage caused by terpinen-4-ol plausibly eases the passage of eugenol to its target, and vice versa. This kind of complementarity has been reported for other essential oil combinations against *A. niger* [14].

A claim of synergy nonetheless cannot be established formally from this study. Demonstrating synergy requires determining the minimum inhibitory concentration of each oil alone and then computing the fractional inhibitory concentration index through a chequerboard assay, with FICI at or below 0.5 indicating synergy, 0.5 to 4.0 indifference, and above 4.0 antagonism [15]. This study tested neither a concentration series nor a 1% single-oil dose, so FICI cannot be computed. What can legitimately be stated is that the combination performed at least as well as a full dose of either single oil while halving the amount of each component. The practical value of that is real: it reduces raw-material requirements and lowers the risk of odour and physical changes arising from the dominance of a single oil.

**Spores without mycelium: fungistatic or fungicidal?:-**

Observation notes on P3 record that a reading of 0% does not mean a sterile surface, but rather that spores remained present while no mycelium formed. The distinction matters. Spores that persist without germinating indicate that the P3 formulation works by inhibiting conidial germination and hyphal elongation rather than by killing the spores. The available evidence therefore supports a fungistatic effect, and is not sufficient to claim a fungicidal one.

This bears directly on use as a wall paint. Fungistatic protection depends on the continued presence of the active compound at the surface. Terpinen-4-ol and eugenol are volatile, so their concentration within the paint layer will decline over time, and once it falls below the inhibitory threshold, spores held in check may germinate after all. The effective protective life of the paint is therefore an important question this study leaves unanswered, since observation ran for only about two weeks. The most direct test would be to transfer spores from the P3 surface onto fresh SDA free of essential oil: growth would confirm a fungistatic effect, while its absence would establish a fungicidal one.

**Sources of variability and the Box 2 anomaly:-**

The pattern reversed in one place only. In Box 2, P2 showed higher coverage (23.33%) than P1 (16.40%), contrary to the other three boxes. This anomaly deserves examination rather than dismissal.

The first possibility is that Box 2 simply received heavier inoculum pressure. That can be tested through the relationship between control coverage and treatment coverage within the same box. The correlation between P0 and P1 is only $r = 0.178$ and between P0 and P2 only $r = 0.282$, both weak and non-significant. Control coverage in Box 2, at 57.88%, was in any case below the overall mean. Inoculum pressure therefore does not explain the anomaly.

A more likely explanation lies in sample preparation and handling. First, paint was applied by hand without a controlled-thickness applicator, so film thickness and active-agent loading per unit area could differ between coupons. Second, the plastic boxes were deliberately left non-airtight to permit air exchange, with the consequence that volatile compounds could be lost to a greater degree from some boxes than others depending on position and airflow. Third, the stability of the oil emulsion in the paint depends on stirring; any phase separation before application would leave the affected coupon with a lower oil content than designed.

The scale of this variability is reflected in an experimental error coefficient of variation of about 64% on the original scale. That is high, though not unreasonable for a biological assay conducted in school laboratory facilities without temperature- and humidity-controlled incubation. It is this variability that depressed statistical power and left the difference between P1 and P2 undetected.

**Effect on the physical quality of the paint:-**

The third hypothesis of this study anticipated that adding essential oils would affect adhesion, opacity and film durability. On the observations made, that expectation was not borne out, and this is itself the favourable outcome for application. At a total concentration of 2%, essential oils emulsified with Tween 80 appear insufficient to disturb film formation in a water-based paint, so the film remained intact, its colour unchanged, and its scratch resistance comparable to the control.

This conclusion must be bounded by the method used. Physical assessment here was qualitative, resting on visual observation and a simple scratch test. Standard industry tests were not performed, among them cross-cut adhesion testing under ISO 2409 or ASTM D3359, opacity measurement, drying-time determination, film hardness testing, and colorimetric measurement of colour change. The accurate statement is therefore that no visually observable change in physical quality was found, not that the physical quality of the paint was demonstrated to be unchanged.

**Limitations:-**

Several limitations should be stated openly so that these results are interpreted proportionately and can be improved upon in subsequent work. First, there was no vehicle control: P0 contained no Tween 80 while P1 to P3 all contained 2%, so the effect of the essential oils is not fully separated from that of the emulsifier, a non-ionic surfactant which can itself affect fungal membrane permeability and may serve as a carbon source. Subsequent work should add a paint plus 2% Tween 80 treatment without essential oil. Second, four replicates yield low statistical power, so small to moderate differences go undetected; the power calculation indicates that about six replicates per treatment would suffice to confirm the advantage of the combination over the single oils. Third, only one concentration level was tested, so minimum inhibitory concentrations could not be determined, FICI could not be computed, and synergy could not be demonstrated formally. Fourth, incubation conditions were not fully controlled: the assay ran in non-airtight boxes in an open area, so temperature and humidity could only be monitored rather than regulated, and volatile compounds could be lost at differing rates. Fifth, only one test species was used, whereas the fungi colonising building walls in the field are diverse and include *Cladosporium*, *Penicillium* and *Aureobasidium*. Sixth, an observation period of about two weeks does not represent the true protective life of a paint, which in practical use is expected to run to years. Finally, segmentation thresholds in ImageJ were set manually, admitting the possibility of observer bias; measurement would be better performed by two assessors working blind, with inter-rater agreement reported.

**Practical implications:-**

From an applied standpoint, the P3 formulation offers a promising alternative to synthetic antifungal agents in wall paint. Silver nanoparticles, the incumbent, are certainly effective, but are reported to be cytotoxic to a range of human cell lines, particularly at particle sizes below 10 nm [4]. Terpinen-4-ol and eugenol, by contrast, have long been used in food and

personal care products and carry a far better safety profile. For a humid tropical country such as Indonesia, the domestic availability of clove adds a further advantage on the raw-material supply side.

These results should nonetheless be positioned as early proof of concept rather than as a market-ready product recommendation. Before such a formulation could be applied at scale it would need, at minimum, long-term protective-life testing, determination of minimum inhibitory concentrations, formal demonstration of synergy through a chequerboard assay, testing against a range of wall-colonising fungi, and paint quality testing against standard industry specifications.

**Conclusion:-**

This study set out to determine whether tea tree oil and clove essential oil can serve as natural antifungal agents within a water-based eco-friendly paint, and the four treatments and four replicates applied here answer the questions it began with.

Adding tea tree oil and clove essential oil to a water-based eco-friendly paint had a highly significant effect on the inhibition of *A. niger* growth ($F = 16.15$; $p = 0.0002$), with about 80% of the variance in the data explained by differences between formulations. Mean mycelial coverage fell from 66.15% on the control to 12.45% on P1, 9.58% on P2 and 0.00% on P3.

All three amended formulations differed significantly from the control, but not from one another under Tukey's HSD at the 5% level. This study therefore cannot demonstrate that clove essential oil outperforms tea tree oil; the two show only a descriptive tendency slightly favouring clove. The advantage of the combination emerged instead from the analysis of growth incidence, where P3 was the only treatment free of mycelium in every replicate ($p = 0.029$ against both the control and P1).

Essential oils at a total concentration of 2% produced no cracking, peeling, colour change or loss of scratch resistance observable by eye. The hypothesis that the physical quality of the paint would be affected was not supported, although this conclusion is limited to qualitative observation since standard industry testing was not performed.

The combination of 1% tea tree oil and 1% clove essential oil is viable as a natural antifungal agent in a water-based paint. It achieved complete inhibition even though each oil was present at only half the dose used in the single-oil treatments, so the interaction between them is at least additive. A claim of synergy cannot be established formally, since no concentration series was tested and FICI could not therefore be computed. Spores that persisted without forming mycelium indicate that the observed effect can so far be described only as fungistatic.

Three lines of work follow directly. Replicate numbers should be raised to at least six per treatment, and a vehicle control of paint plus 2% Tween 80 without essential oil should be added, so that the effect of the emulsifier is separated from that of the active agents. A concentration series should be run to determine minimum inhibitory concentrations and to compute FICI through a chequerboard assay, allowing synergy to be tested formally. And spores should be transferred from the P3 surface onto fresh essential-oil-free medium, which would settle whether the effect is fungistatic or fungicidal, and thus how long a coating of this kind can be expected to protect.

**Declaration on the Use of Artificial Intelligence:-**

The authors declare that artificial intelligence tools were used in the preparation of this work for reference searching and for background reading on the topic. The software used was Bohrium. Its use complies with the provisions of the OPSI 2026 guidelines.

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