RESEARCH ARTICLE

EFFECT OF MAGNESIUM OXIDE (MgO) AS AN ANTIFUNGAL AGENT ON SINGGORA ROOF TILE

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Abstract. Singgora roofs, a traditional type found in Malay houses, are made of moulded clay but have declined in popularity due to concerns about their durability and reliability. These roofs also absorb moisture, which can lead to fungal growth and affect their aesthetic value. To solve this problem, the Singgora clay materials can be mixed with stable inorganic and antifungal additives such as zinc oxide (ZnO) and magnesium oxide (MgO). In this research, MgO was selected because the thermal stability is higher than ZnO or other organic antifungal agents. The effectiveness of MgO in preventing fungal growth of Singgora roof was studied at different concentrations (0, 5, 10, 15, 20, and 30 wt.%) of MgO. Fungi from the genus Stachybotrys were isolated from field Singgora tiles and cultured on potato dextrose agar (PDA) for 14 days. The obtained colonies were assessed using the zone of inhibition (ZOI) method to measure antifungal efficacy by observing the clear zones. Results showed that 10 wt.% of MgO produced a clear zone with a diameter of up to 60 mm. To investigate the microstructural and mechanical properties, the clay samples were moulded and fired at 800 °C for 12 hours. Analysis under Scanning Electron Microscopy (SEM) revealed that samples with 20 wt.% and 30 wt.% MgO had the highest crack formation. Strength tests conducted through three-point bending revealed that 0 wt.% MgO had the highest strength (9.394 MPa), which decreased with the addition of MgO. However, the sample with 10 wt.% MgO demonstrated the al composition with the highest strength (1.372 MPa) among those with added MgO and superior to prevent the growth fungi and offering low porosity and minimal crack formation.

Keywords: Singgora roof, antifungal agent, MgO, roof tile, *Stachybotrys*.

Article Info

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1. INTRODUCTION

Malaysia is known as a country with rich cultural value, and this can be observed in the remaining heritage buildings, such as old houses, monuments, museums, palaces, and mosques. One of the significant elements of heritage buildings is shown in roof designs. Singgora roof tiles are a heritage roof design used in traditional Malay houses made from moulded clay and burnt in kilns at high temperatures [1]. Singgora originated from Southern Thailand, and Singgora's name comes from Thai words, which means Lion City, which refers to a hill in Songkla resembling a lion [2]. Due to the migration of Thai communities and the high demand for Malaya circa the early 18th century, Singgora roof tile is widely used in the peninsular, especially in Kelantan and Terengganu, which became part of Malay heritage.

Singgora roof tile, or the Singhora bricks among the Kelantanese, has a similar shape to fish scales when arranged in a roof pattern. The characteristic of these tiles is that they are light and porous, which helps them absorb water (rain), allowing the house to cool naturally. The roof absorbs heat at peak temperature to keep the home's interior cool. Otherwise, the roof absorbs heat and will release it at night to keep the home warm. It is pale orange from the natural clay colour used as their raw materials. People on the East Coast called Singgora tiles a 'bata', which means the bricks, because the colour of Singgora roof tiles changes to orange when baked, resembling the colour of bricks.

Singgora roof use in Malaysia has decreased since 1977 [3]. Only one factory that produced Singgora tiles, Kraf Atap Singhora, located in Bachok, Kelantan, has used clay as a raw material [1]. This factory produces Singgora tile manually, which is kneading using both legs. However, this method of producing the Singgora roof tiles has given unique features to other roof tiles because this technique can give sentimental value to the users. Therefore, the demand for Singgora roofs still exists because some Malaysians still think this heritage has value.

The Kraf Atap Singhora factory sources its raw clay from the convergence of saltwater and freshwater at Kemasin River [1]. The clay used for producing the Singgora roof tiles contains essential minerals such as salt, iron, silica, water, and calcium. Typically, this clay is obtained from paddy fields and rivers where a significant amount of salt is present, facilitating the kneading process. Additionally, the presence of salt in the clay contributes to increased strength after the firing process [4].

An issue occurs on Singgora roof tile when the fungus quickly grows on ceramic materials. Adding metal oxide such as MgO for antifungal agents can inhibit the growth of fungi, are non-toxic to the organisms, and are cost-effective materials [5]. MgO exhibits antifungal activities against *S. cerevisiae*, *R. stolonifera* [6], *A. niger* [7], *T. reesei*, and *C. cladosporioides* [8]. In addition, MgO compounds are physically and chemically stable at high temperatures. Thus, it has two properties: high thermal and low electrical conductivity [9]. The characteristics of this metal oxide also can improve the fracture toughness in ceramic materials [4]. The effect of MgO addition to the clay sample was investigated to produce a Singgora roof tile resistant to fungus and with good mechanical properties.

2. MATERIALS AND METHODS

2.1 Roof Tile Sample Preparation

The raw clay for Singgora roof tiles was dried at 100 °C for 2 hours and crushed into small pieces. Then, it was ground into a powder using a Rocklabs benchtop mill crusher. The fine clay powders were mixed with various amounts of MgO (0, 5, 10, 15, 20, and 30 wt. %), with 0 wt. % as the control. The clay was moulded into tile shapes using Plaster of Paris (POP). The samples were sun-dried for 2 days and then fired in a furnace at 800 °C for 12 hours. After firing, the clay samples turned orange.

2.2 Culture Media Preparation

Potato Dextrose Agar (PDA) was employed for the streak plate method and zone of inhibition (ZOI) test. A mixture of 39 g of PDA powder and 1 L of distilled water was heated until fully dissolved. The solution was then autoclaved at 122 °C for 15 minutes. After autoclaving, the solution was cooled, ensuring it did not solidify, and subsequently poured into petri dishes. Finally, the media were refrigerated to allow complete solidification.

2.3 Culture Techniques

Culture techniques are crucial for growing and detecting microbes using media agar. This research employs the streak plate method and Zone of Inhibition (ZOI). These techniques involve preparing, growing, storing, and maintaining cultures aseptically. All fungal culturing was done under sterilised conditions to prevent contamination.

2.3.1 Streak Plate Method

The roof tile affected by fungi collected from the Kraf Atap Singhora factory was isolated using an inoculation loop into culture media. The streak plate method must be conducted under sterile conditions. The inoculation loop is sterilised using a Bunsen burner until adequately heated, then allowed to cool for approximately 10 seconds. The fungi are gently collected with the inoculation loop, and the lid of the agar plate is lifted just enough to insert the loop. The loop is gently dragged over the surface of the top one-fourth of the agar in a "zig-zag" pattern. This process is repeated for the second, third, and fourth quadrants by turning the plate. The plate is labelled and incubated in an inverted position at 30 °C. Fungal growth is observed at 24, 48, and 72-hour intervals. Each fungus culture is subcultured using the same procedure. Once matured, the fungi are observed under a light microscope. A 10 wt.% Potassium Hydroxide (KOH) solution is used to stain the fungi, aiding in identifying their shape under the microscope. The fungal genus is identified through morphological characteristics.

2.3.2 Preparation of McFarland Test

The McFarland test was utilised as a standard for ensuring that microbial suspensions fall within a specific range by comparing the turbidity of the suspension. This method is crucial for determining the accurate number of fungi required to conduct zone of inhibition (ZOI) tests on PDA plates for antifungal assessment. An improper dilution, either too high or too low, may result in erroneous outcomes for the antifungal agent. A 0.5 McFarland standard is prepared by mixing 0.05 mL of 1.175 % barium chloride dihydrate ($BaCl_2 \cdot 2H_2O$) with 9.95 mL of 1 % sulphuric acid (H_2SO_4) using a vortex mixer. The prepared McFarland standard solution should be stored in a closed tube and kept in a dark place.

2.3.3 Zone of Inhibition (ZOI)

The culture isolated from the streaking plate was utilised in a zone of inhibition test. The fungal colonies from the streak plate were transferred to 0.45 ml of saline solution approximately 15 times until the solution reached a turbidity comparable to the McFarland standard, then left to rest for about 1 hour. Subsequently, the fungal suspension was swabbed onto an empty PDA plate using a cotton swab.

Six sterilised paper discs were prepared to indicate six different concentrations of MgO in this test: 0, 5, 10, 15, 20, and 30 wt.%. To prepare the MgO solutions, MgO powder was dissolved in 80 M sulfuric acid (H_2SO_4). For example, to create a 5 wt.% MgO solution, 5 g of MgO was dissolved in 95 ml of 80 M H_2SO_4 to make a 100 ml solution. This procedure was repeated using 10, 15, 20, and 30 g of MgO powder, each time dissolving with 80 M H_2SO_4 to reach a total volume of 100 ml. For the 0 wt.% control, no MgO solution was added. An 80 M H_2SO_4 solution was prepared by mixing 80 ml of H_2SO_4 with 20 ml of distilled water.

Each composition of MgO solution was transferred onto the paper discs using a micropipette, with two drops applied to each disc. The petri dish was divided into six sections, and each paper disc was placed on top of the agar in one section. The samples were incubated at 30 °C, and results were observed after 48 hours. The diameter of the clear zones appearing in each petri dish was measured.

2.4 Microstructural Observation

The morphology and topography of clay samples were observed using a JOEL JSM-IT SEM. The clay was cut into smaller pieces to fit the SEM sample mount. Samples were mounted vertically on double-sided carbon adhesion foil. Observations were made at 100x, 500x, and 1000x magnifications under a high-energy electron beam. Information on pore formation, crack propagation, and element distribution was obtained from the SEM observations.

2.5 Phase Identification

XRD was performed using Bruker D2 Phaser diffractometer with 40 kV of voltage and 40 mA of current. In order to conduct an XRD machine, the sample should be ground using a Rocklabs bench-top mill crusher until it becomes a fine powder. After that, place the sample into the holder flat inside the diffractometer of XRD. Observe the diffraction of X-ray at $10º$ to $90º$ of 2θ.

2.6 Three-Point Bending Test

The test was conducted using a Universal Testing Machine (UTM) (model Instron 3366) with a load capacity of 50 kN according to ASTM D790. According to Azzam & Li [10], the three-point bending test can be done by placing the sample of Singgora roof tile between two parallel supporting pins. The loading force was applied in the middle by means of a loading pin. The supporting and loading pins are mounted to allow the free rotation of the axis parallel to the pin axis and the axis parallel to the specimen axis. The load was done once the samples were broken into two. The flexural strength (σ) of the material was calculated by using Equation 1.

$$\sigma = \frac{3FL}{2bd^2} \tag{1}$$

where F was a load at fracture (N), L was the distance between the support pin (mm), b was the width of the sample (mm), and d was the thickness of the sample (mm).

2.7 Density and Porosity

Density and porosity tests were performed to determine the density and porosity of the Singgora roof tile. According to Ali et al. [11], the method of conducting the porosity and density test was done by drying the sample at a temperature of 110 ± 5 ºC for 24 hours in the oven. Then, the tile sample was placed in distilled water and put into the container. The container was placed inside the desiccator and connected with a vacuum pump to remove air bubbles. The porosity was calculated based on the saturated and suspended weight of the sample using a density kit. The density of the sample is dry mass divided by the exterior volume, which can be calculated using Equation 2.

$$\rho = \frac{m_1}{v} \tag{2}$$

where ρ was the density of the sample (g/cm^3), m_1 was the mass dry sample (g), and v was the exterior volume (cm^3).

The porosity of the sample was calculated using Equation 3.

$$\Phi = \frac{m_3 - m_1}{m_3 - m_2} \times 100 \qquad (3)$$

where Φ was porosity (%), m_1 was the mass of the dry sample (g), m_2 was the apparent mass of the sample in water (g), and m_3 was the suspended mass of the sample in the air (g).

3. RESULTS AND DISCUSSION

Alastruey-Izquierdo et al. [12] reported that most of the fungi strains required 24 hours of incubation. However, some strains showed insufficient development and required 48 hours [12]. After one week, the fungi start to overgrow after they produce the spores. Then, after two weeks, the colour of the fungi can be recognised. According to Figure 1(b), the phase was suitable for the zone of inhibition test. Meanwhile, Figure 1(c) and (d) were suitable for identifying fungi since they showed all their physical characteristics, including colour, texture, shape, and spore.

The zone of inhibition was determined using a spore suspension diluted in saline solution. The clear antifungal zone can be analysed in 48 hours of incubation using different concentrations of 0, 5, 10, 15, 20, and 30 wt.% of MgO. The antifungal agent used in the sample resulted in a clear zone, indicating the fungi's absence.
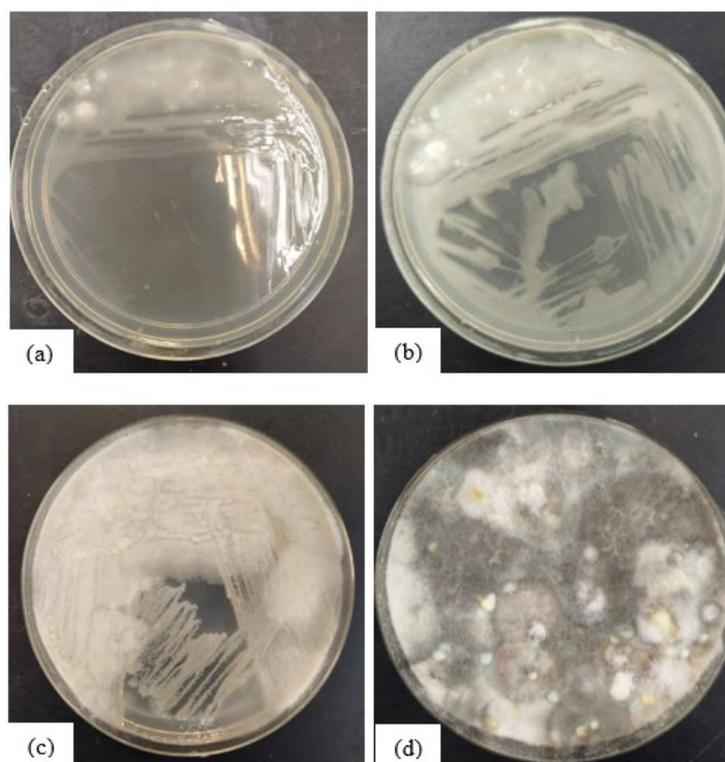

Figure 1: The fungi from Singgora roof tile (a) grew after 24 hours, (b) grew after 48 hours, (c) grew after one week of culture, and (d) grew after two weeks of culture

Based on Figure 2, two samples (original and replication samples) from the same species of fungi were used in this test to compare the apparent zone size for each weight percentage. Further analysis, as illustrated in Figure 3, was done by measuring the diameter of the clear zone. Still, the radius length from the antibiotic dish was measured for the joined samples. The clear zone diameter for sample 5 wt.% was 53 mm for the original sample and 58 mm for the replication sample. Then, the

apparent zone diameter for sample 10 wt.% was 52 mm for the original sample and 60 mm for the replication sample. Unfortunately, the ZOI measurement declined slightly at 15 wt.% and 20 wt.% for both the original and replication samples. The size of the clear zone decreases with the higher concentration of MgO, which is suggested due to the formation of micro-cracks and increased porosity in the samples. As the concentration of MgO increases, the samples exhibit higher levels of pore formation and micro-cracks, which was observed under Scanning Electron Microscopy (SEM) at high magnification. The data shows that the concentration at 30 wt.% led to a significant decrease in ZOI, and the sample's porosity also increased, indicating a similar phenomenon. These findings proved that a low concentration of MgO up to 10 wt.% is an effective antifungal agent for fungi growing on Singgora roof tiles. Compared to another study that used TiO_2 [13], increasing TiO_2 at higher concentrations consistently increases the size of the zone of inhibition.
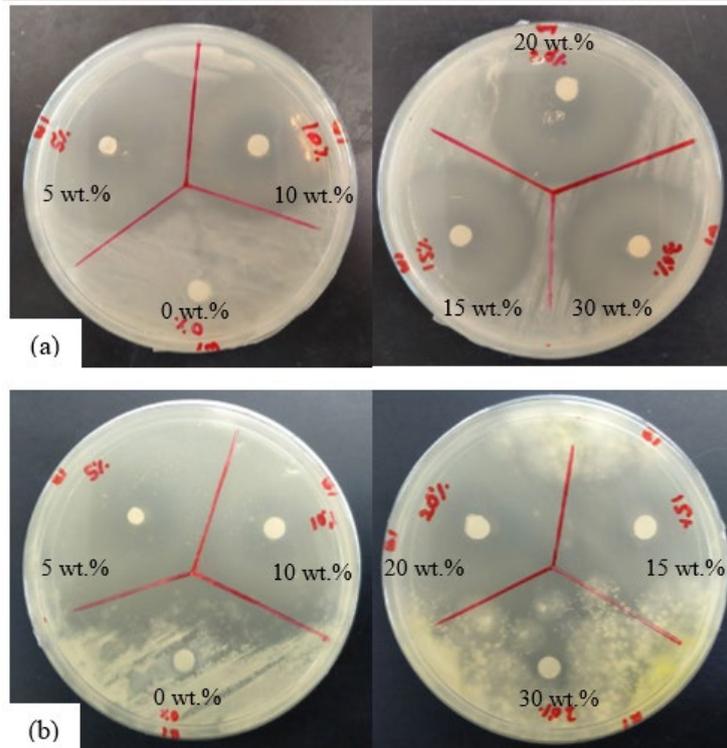

Figure 2: ZOI analysis after 48 hours for the antifungal test on (a) the original sample and (b) replication sample
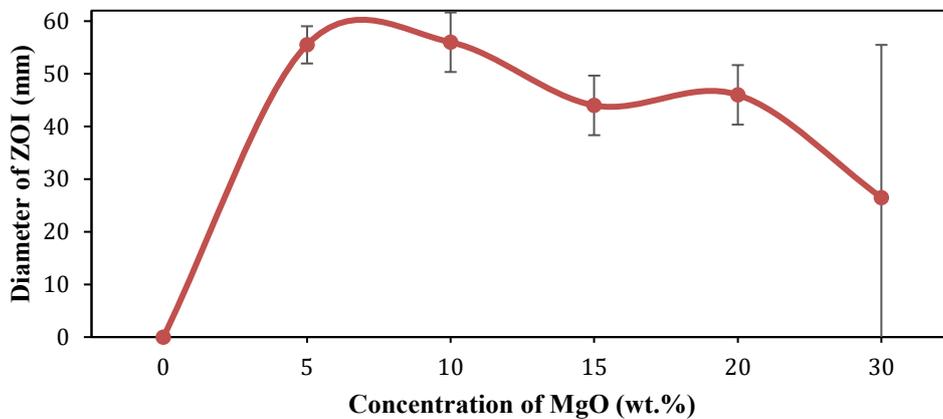

Figure 3: The diameter measurement of the clear zone on different compositions of MgO

Figure 4 shows the cultured fungi from the Singgora tile under the optical microscope. The characteristics of fungi that have been analysed are very similar to those of the Stachybotrys genus. The fungus of Stachybotrys is often known as black mould or toxic black mould, generating conidia in slime heads. This mould has filamentous for reproducing purposes called hyphomycetes or asexually reproduces. It is presently classified as a member of the Stachybotryaceae family. The fungus is frequently found in soil and grain but is more commonly found in cellulose-rich structural materials from damp or water-damaged structures. The best way to identify black mould (Stachybotrys) is by its colour because most of the mould that grows on the building may be pink, grey, white, or brown. Stachybotrys is known as a black mould with a white surface spore, which later becomes dark grey to black colour. The texture of the mould is the cottony or powdery bloom of conidial masses. Fungi that have been cultured vary in size. Measuring the fungi's diameter was challenging because the single colony was relatively small and linked.
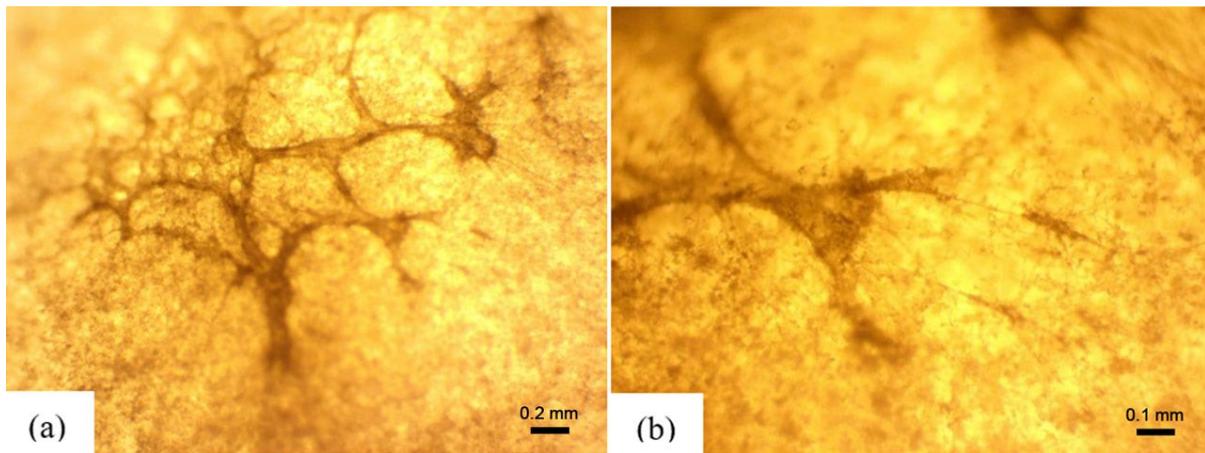

Figure 4: Observation of surface morphology of fungi using an optical microscope for (a) Stachybotrys at 4x magnification and (b) Stachybotrys at 10x magnification

Figure 5 shows that increasing the MgO concentration from 0 to 30 wt.% reduces the sample density from 2.35 g/cm³ to 2.00 g/cm³. Conversely, the porosity increases with higher MgO content, rising from 30.12 % to 48.12 %. The density results are within the typical range for pressed tiles, which vary between 1.8 and 2.2 g/cm³, depending on the raw materials and manufacturing process [14]. Adding MgO to the clay can increase their porosity due to the chemical reactions that occur between MgO and clay components [9]. When MgO reacts with silica, it forms magnesium silicate hydrate (M-S-H), which can lead to an increase in porosity [15]. Similarly, the presence of alumina in clay can react with MgO to form hydrotalcite, which also contributes to increased porosity [16].
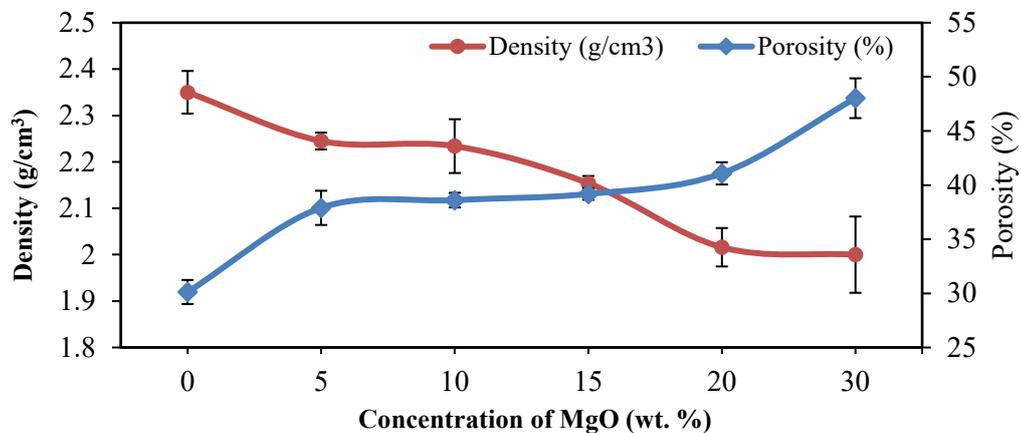

Figure 5: Density and porosity rate of different concentrations of MgO

The red arrow in Figure 6 shows that pore formation occurs in every sample. The pore information can be analyzed through the presence of micro-cracks, cracks, and voids and the porosity level, the type of porosity, the pore distribution, and the morphology of samples using high magnification. Based on 1000x magnification, the massive pore formation occurs when using a high concentration of MgO in samples. The pores that occur in the sample can be detected in the SEM image based on the black hollow color. Based on the result, samples 0, 5, and 10 wt.% showed minor pores. While the 15, 20, and 30 wt.% showed, the higher pores occur in the samples. Increased pore size is associated with higher levels of porosity. Therefore, the results from the porosity test showed that a high concentration of MgO in clay can cause a high percentage of pores in the sample.

The micro-crack happens in samples seen through the blue circle shown in Figure 6 for samples 20 and 30 wt.%. Micro-cracks are small linear cracks in the pattern of planar ellipses, with major and minor axes of 400 and 100 μm, respectively. This micro-crack happens when a high MgO concentration is added to the sample. This happens because MgO can alter the soil fabric, increase porosity, and lead to chemical reactions that affect the clay's integrity [17]. Thus, micro-damage can be considered inadequate as it can be related to a progressive loss of stiffness and strength as micro-cracks merge, forming larger macro-cracks until final fatigue failure [18].
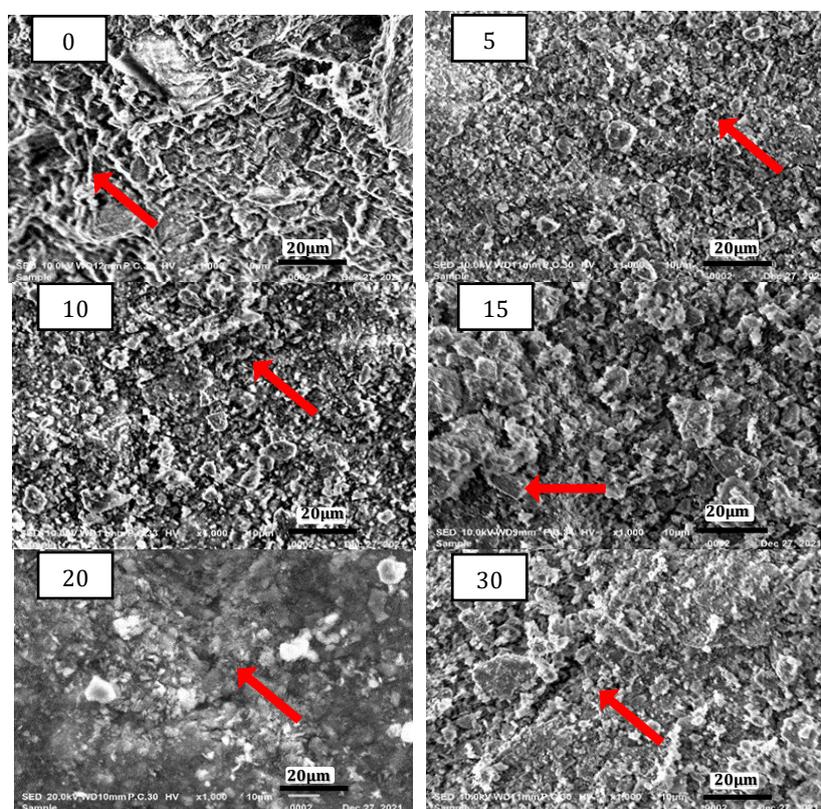

Figure 6: The SEM analysis of samples 0, 5, 10, 15, 20, and 30 wt.% at 1000x magnification

Figure 7 presents the XRD diffraction phase analysis, identifying three compounds: MgO (COD 1000053), quartz (COD 9011493), and muscovite (COD 9012886). The respective peaks of these compounds are marked in Figure 7. According to the Crystallography Open Database (COD), MgO displays only two peaks within the measured range, as the remaining peaks occur at higher diffraction angles (>90 degrees), which are beyond the scope of the current measurement. The MgO peak intensity increases as the concentration of the compound increases in the clay samples. The crystallinity calculation indicated that the crystallinity of samples with 0, 5, 10, 15, 20, and 30 wt.% of MgO are 63.6 %, 63.7 %, 65.8 %, 66.7 %, 68.4 %, and 63.5%, respectively. The crystallinity increases with an increase in MgO up to 20 wt.% before it decreases. At lower concentrations (up to 20 wt.%), MgO acts as a fluxing agent, lowering the melting temperatures of the clay constituents. This promotes

the formation of crystalline phases during firing [19]. At higher concentrations (>30 wt.% MgO), the decreases in crystallinity are caused by several factors, which are due to lattice distortion and macrostrain inhibition of crystal growth [20] and increased porosity and microcracking as indicated in previous result in Figure 5.
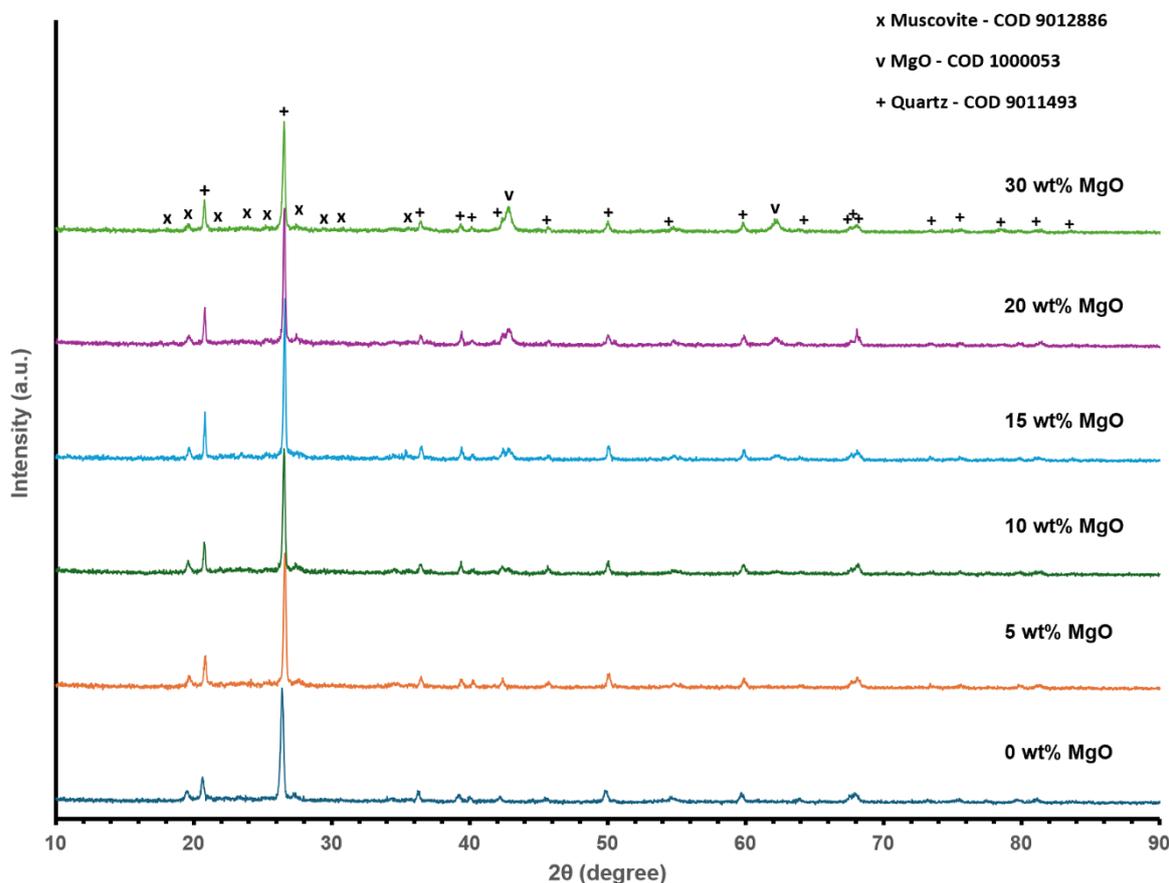

Figure 7: XRD analysis on a mixture of clay and MgO

Figure 8 shows the bending strength of the roof tile added to MgO in different compositions. The highest bending strength is 9.394 MPa from the 0 wt.% MgO sample. The bending strength drops when the clay is added with MgO. This indicated that MgO additives can weaken the strength of clay. However, 10 wt.% of MgO had the highest bending strength compared to other higher amounts of MgO, 1.372 MPa. The strength of other clay compositions, 5, 15, 20, and 30 wt.% of MgO, indicate the bending strength of 1.193, 1.035, 0.506, and 0.383 MPa, respectively. From the data collected, the strength of the sample was at a high peak at 0 wt.%. In addition, the error bar showed on the graph that 0 wt.% was the most far away from the corresponding coordinate of the point, which indicated that the uncertainty value was significant.

Then, after adding MgO to the clay, the strength of the clay decreased due to the increased porous structure in the samples. The sample with high porosity broke down after reaching the compression strength limit. It showed a low elastic modulus compared to the samples with a low porosity rate, which provides a high compression strength limit and, consequently, a large Young's modulus [21]. This trend is slightly different from what was studied by Johari et al. [2], which is increasing clay composition from 0 to 10 wt.% of ZnO caused the flexural strength to increase from 4.046 MPa to 5.619 MPa then decrease to 2.697 MPa for 20 wt.% ZnO. According to Fattah et al. [22],

MgO can cause the soil structure to change from a flocculated to a more dispersed state, which can weaken the overall strength of the clay.
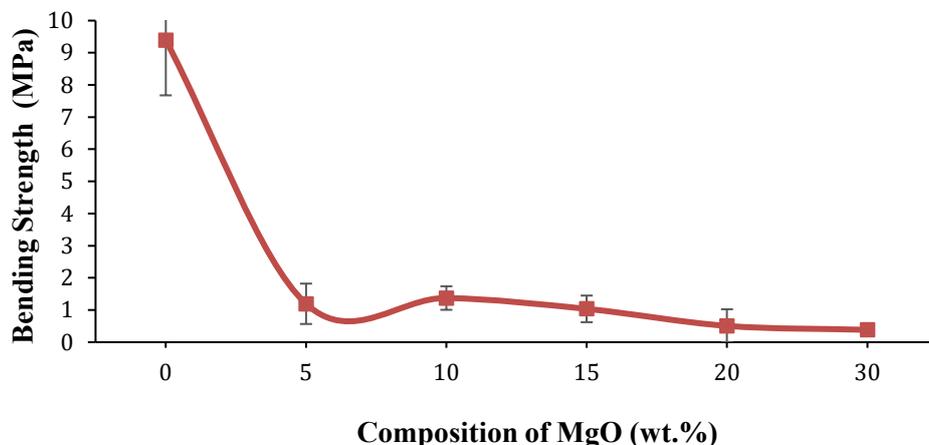

Figure 8: Bending strength for roof tile added MgO at different composition

4. CONCLUSIONS

The objectives of this research, using MgO as an antifungal and studying antifungal agents on the mechanical and physical properties of Singgora roof tiles, were accomplished. MgO showed effectiveness against the inhibition of fungi growth. The zone of inhibition test showed 10 wt.% had the higher clear zone that can inhibit the fungi growth from genus Stachybotrys up to 60 mm. However, increasing the composition from 0 to 30 wt.% of MgO can decrease the density value from 2.35 g/cm^3 to 2 m/cm^3, and this can increase the porosity percentage consequently as well as crack formation based on SEM observation. Adding MgO also caused a flexural strength to decline in clay samples from 9.394 MPa of 0 wt.% to 1.372 MPa of 10 wt.% MgO. However, this shortcoming needs to be addressed to ensure that the product can be produced in good quality. The study of MgO composition determined that 10 wt.% was the optimum weight percentage as an additive in clay. This composition percentage can exhibit a large clear zone in the test zone of inhibition of the fungi growth. Besides, the lower composition of MgO of 10 wt.% had a lower pore size and lower crack formation of the microstructure in clay. It also had higher strength among another percentage of additive MgO in clay.

Author Contributions

All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure of Conflict of Interest

The authors have no disclosures to declare.

Compliance with Ethical Standards

The work is compliant with ethical standards.

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