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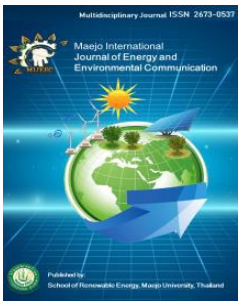

ARTICLE

The antifungal efficiency of *Moringa oleifera* seed extracts for tap water treatment

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ABSTRACT

Water must be pure and free of any contaminants for human consumption. However, due to indiscriminate human activity, its quality has worsened, resulting in many illnesses that plague humanity, particularly in developing nations. Promising water treatment procedures are considerably more expensive, and many of the disinfectants now in use are toxic, making the hunt for safer organic alternatives even more difficult. *Moringa* seeds have long been used to clean drinking water and minimize the health concerns associated with microorganisms in rural communities. Using central composite design (CCD) in response surface technique, the parameters identified as the essential components, dose, duration, and agitation, were chosen to assess their influence on eradicating the growth of fungal communities in the drinking water by response surface methodology (RSM). The CCD was done with two complete factorial combination components at five distinct levels. The typical two fungi widespread in the tap water samples have selected *Aspergillus sp.* (73%) and *Rhodotorula mucilaginosa* (63%). Fungal mycelia growth gradually decreased with increased concentration of *Moringa oleifera* seeds extract and chlorine on *Rhodotorula mucilaginosa* and *Aspergillus sp.* The modified determination coefficients (adj R2) for the CFU of *Moringa* seeds were also 0.8122 and 0.8405. This study aims to highlight the performance activity of *Moringa* seeds while treating tap water instead of using rapid usage of chlorine in the traditional method. This study found that *Moringa oleifera* extract seeds and chlorine have antifungal action against disinfectants at all concentrations. Using *Moringa oleifera* seeds extract and chlorine as a disinfectant on *Rhodotorula mucilaginosa* and *Aspergillus spp*. in treating tap water is a viable alternative. This method would significantly reduce the high costs and health concerns of current chemical water treatment methods. The method is traditional and simple to apply, making it suitable for rural regions. It also produces no non-treatable wastes because it is biological.

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1. Introduction

Pathogens in water distribution networks can alter the taste and smell of water. Chlorine and other disinfectants alter water taste, resulting in poor water quality and by-products such as trihalomethanes, which are harmful and deadly (Supong, 2017). On the one hand, the high expense of chemicals and water treatment plant facilities has motivated numerous researchers to investigate the use of natural biomass to benefit people in rural and remote locations throughout the world. Plant extracts for purifying drinking water, on the other hand, are ecologically benign, typically non-toxic, and reduce sludge by five times when compared to chemical filtration (Supong, 2017).

Recently, there has been a surge in interest in producing ecologically friendly, natural-based disinfectants to replace synthetic ones. As a result, the focus is now on finding non-toxic, biodegradable, widely available, and cost-effective alternatives. A growing number of research focused on natural products have been undertaken in this respect (Idris et al., 2017). Natural disinfection agents are safer for people than manufactured disinfection chemicals. As a result, it's worth looking into the naturally accessible bioactive component from local biomass, Moringa, as disinfection agents for eliminating fungus in the drinking water system. Furthermore, there has never been researched in Malaysia on fungus in a water distribution system.

Moringa oleifera (MO) is a tree that may be found in various subtropical and tropical climates across the world. MO is commercially cultivated in Africa, South and Central America, Mexico, Hawaii, and Asia. Moringa seeds are immature seed pods with a high nutritional content commonly used as primary food. MO identifies various names that vary by region, such as horseradish tree for the flavour of ground root preparations, oil tree for the containment of their seeds in a significant amount of oil, and drumstick tree for the look of their immature seed pods (Razis et al., 2014). According to several publications, Moringa extract offers a range of health benefits such as cardio-protection, antipyretic and diuretic (Razis et al., 2014). Also, it is well known that Moringa extract has anti-inflammatory, antioxidant (Ramli et al., 2020), anti-hypertensive, antimicrobial, anti-neoplastic, anti-diabetic, anti-ulcer, and anti-hyperlipidemic effects in addition to its nutritional value (Razis et al., 2014)". The effectiveness of powder derived from mature-dried MO seeds to remediate polluted water was investigated by Egbuikwem and Sangodoyin (2013). The results revealed that MO seed has an excellent coagulating component that is equivalent to commercial alum in terms of turbidity reduction and some antibacterial characteristics (Bhuyar et al., 2020, 2021). Ravikumar and Sheeja (2014) conducted a study to investigate how effective Moringa seeds were at adsorbing heavy metals from water samples. According to the removal percentage, MO seeds eliminated 70% of chromium, 76% of cadmium, 93% of lead, and 95% of copper.

MO seeds were generally known for their efficacy in eliminating different contaminants from water based on prior studies. Ghosh (2018) claimed that MO had a coagulation process that consisted of adsorption and neutralization of colloidal charges in the early stages of its introduction for water purification. The promising mechanism provided academics with multiple chances to research their respective fields. The MO might generate four to five times less sludge volume than the alum, according to Vieira et al. (2010). Furthermore, numerous investigations have shown that MO efficiently removes turbidity (Ali et al., 2010; Pritchard et al., 2010; Sengupta et al., 2012; Keogh et al., 2017).

However, in the tap water of the Kuantan area, there are fungi in different concentrations, which were *Aspergillus* spp., *Rhodotorula mucilaginosa*, *Penicillium citrinum*, *Cladosporium cladosporioides*, *Cerrena* spp., *Aspergillus aculeatus*, *Aspergillus flavus*, *Cryptococcus* sp., *Cladosporium perangustum*, *Purpureocillium lilacinum*, and Candida catenulate (Salah et al., 2020). To keep our drinking water safe, we must reduce the pollution caused by fungal activity in the tap water system. The objective is to safeguard protected watercourses while also making recommendations on monitoring priority pollutants. The European Union (EU) is planning an updated watchlist of emerging poisons (Loos et al., 2018). Unlike chemical-based water purification agents like alum and ferric coagulants, MO is non-toxic, biodegradable, and has minimal impact on the pH and conductivity of the water after treatment (Valverde et al., 2018). In this work, we used MO seedlings to test the efficiency of natural disinfection on synthetic water in petri dishes and pilot scales at the university laboratory, comparing it to chlorine.

2. Materials and Methods

2.1. Study site

Kuantan is the Malaysian state capital of Pahang. It is located at 3° 49′ N latitude and 103° 20′ E longitude. Furthermore, Kuantan's 324 km2 total area is near the Kuantan River's estuary and faces the South China Sea (Zainutdin & Khairunnisa, 2018). Kuantan is the 17th biggest city in Malaysia, with about 427,000 people, and the largest city on Peninsular Malaysia's East Coast (Kozaki et al., 2016).

2.2. Sample collection

Thirty tap water samples were collected in Kuantan from public buildings and private residences (coded 1 to 30). The samples were collected at a tap that draws water straight from the Semambu Water Treatment Plant in Kuantan, located at 3°52'8.4"N and 103°19'45.10"E. Within 4 hours, all of the samples' characterizations were completed. The tap was cleaned with a clean towel to remove some soil until the samples were obtained. After that, the tap was turned on for three minutes at full flow. The sampling bottle was washed three times with tap water before being used. Tap water samples were collected in a 1500 ml polyethylene plastic container.

2.3. Sources of fungi

The fungi cultures *Aspergillus* spp. and *Rhodotorula mucilaginosa* used in this study were obtained from tap water in Kuantan, Malaysia. The cultures of the fungi on Potato Dextrose

Agar (PDA) were kept at 4°C until they were needed for use at Universiti Malaysia Pahang.

2.4. Preparation of extracts

The MO was cleaned thoroughly with tap water and then distilled water to ensure that the seed was not contaminated. The seeds were dried for 24 hours at 50°C in an oven to remove moisture content. Protein content can be harmed by temperatures exceeding 60°C (Hamid et al., 2016). The seed coat and these seeds were then removed and chopped into tiny pieces. A laboratory grinder was used for crushing Moringa seeds into powder. Oil was extracted using the conventional technique (cold press) without any additives. The powder was rubbed with warm water intermittently (Olaleye and Kukwa, 2018). The cake (dough) was placed in a cold oil press machine to remove the oil while constantly pressing. After oil extraction, the aqueous seed cake extract was made by mixing 25g Moringa seed cake powder with 200 ml distilled water for 60 minutes with a magnetic stirrer, then settling for 20 minutes (Ravikumar and Sheeja 2014). The supernatant was collected and freeze-dried at -80°C for 2 days after filtering the Moringa aqueous extract using filter paper no. 1. Moringa powder was ground in a laboratory grinder and sieved through 250 mesh to achieve the desired particle size.

2.5. Characterization of active agent

The performance of MO extraction was examined in two phases of this study. The first phase of the study is compared against conventional chemical disinfection. The fungus's growth diameter was measured in PDA and expressed as percentage resistance in the first phase. In the second phase, natural synthesis was applied to synthetic water in the university laboratory and compared to chlorine. The antifungal agent of MO was characterized using Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscope (SEM), and X-Ray Diffraction (XRD) to determine its physicochemical characteristics. The antifungal extract from plant seeds was analyzed before and after using FTIR, SEM, and XRD.

2.5.1. The surface morphology of the active agent

The surface of the biomass must be analyzed before and after the extraction, phase to detect the morphological profile of the biomass. The surface morphology of MO was examined using a HITACHI/TM3030 PLUS scanning electron microscope (SEM). SEM is a type of electron microscope that uses a guided stream of electrons to scan the surface of a sample and take pictures of it. The electrons interact with the atoms in the sample, generating various signals that reveal information about the surface topography and composition of the sample. The electron beam is scanned using a raster scan pattern, and the direction of the beam is combined with the detected signal to create a picture. Image processing analysis may also be used to analyze the size, shape, and the number of pores in bio-aggregates and bio-composites (Senthil Kumar et al., 2016; Kebede et al., 2018).

2.5.2. The molecular structure of the active agent

The chemical bonding or molecular structure of organic or inorganic products was determined using FTIR (Fourier transform infrared spectroscopy) to identify the molecular structure of MO's antifungal medication. The vibrational continuum is both a physical feature and a function. As a result, the infrared spectrum may be utilized as a fingerprint for identification when comparing the emission from an unknown source to previously published reference spectra. The FTIR investigations were carried out in this case to determine the particular functional groups and type of the material (Senthil Kumar et al., 2016; Kebede et al., 2018).

2.5.3. Determination of the atomic and molecular structure of the active agent

X-Ray Diffraction (XRD) was used to identify an MO crystal's atomic and molecular structure. Because of the high proportion of protein and oil in the active agent, XRD may determine the atoms in the crystal, chemical bonds, and crystallographic disorder (Kebede et al., 2018).

2.6. Antifungal activity performs with the extract of the Moringa oleifera seeds

At 39 g/l, the potato dextrose agar (DifcoTM, Becton, USA) was prepared and autoclaved for 15 minutes at 121°C. As a control, the PDA medium was put into petri plates under aseptic conditions without any plant extract. Plant extracts and chlorine were combined with PDA at 45°C to achieve final concentrations of 0.1, 0.5, 1, 2, 3, 4, and 5 mg/ml, then poured into petri plates under aseptic conditions (Ndamane et al., 2013). A disc of pure fungus culture (5 mm diameter, sterile cork borer) was put over the extract of a 5-day old culture and transferred to a medium modified with varying amounts of plant extracts (Abd El-Ghany et al., 2015). The comparison was conducted by transferring a 5 mm diameter disc of pure fungus culture to a medium modified with various chlorine concentrations as a common disinfectant, then replicating each concentration three times. The petri dish was incubated for 7 days at 26 °C. With various adjustments, the diameter of the fungal growth was measured and represented as % inhibition (Abd El-Ghany et al., 2015).

2.7. Response surface methodology (RSM) for the best condition of the antifungal disinfection by Moringa oleifera seeds

The fungus count test on synthetic water obtained the essential condition of disinfection after the *Moringa oleifera* seed solution was added to the water sample at the university laboratory. The Clinical Laboratory Standards Institute (2002) established a McFarland standard of 0.5, which is about 1.0106 CFU/ml compared to normal saline. *Aspergillus* spp. and *Rhodotorula mucilaginosa* were collected from 7-day-old Potato dextrose agar (PDA) slant cultures by washing with 10 ml sterile normal saline containing 3% w/v Tween 80 and sterile glass beads to assist in spore dispersal (Aboh et al., 2014). After that, using a single-beam spectrophotometer (UV-1800) at 530 nMOf the suspensions equivalent to McFarland standard 0.05, the spore suspension was standardized to 1.0 x 103 spores/ml. With slight changes, all

adjusted suspensions were measured by spreading 100 µL on a Potato dextrose agar plate and incubating at 26°C for 1 day (Idris et al., 2017).

Because it is simple to execute, the jar test is the most widely used method of assessing the effectiveness of a coagulant and disinfectant. A standard jar tester investigated the removal of target pollutants from synthetic water samples using the chosen biomass. Several tests were carried out to see if the selected biomass could remove the fungus. In this investigation, the combination and sequential dosing of multiple biomass methods were also explored to improve treatment efficiency. A jar test setup with six beakers was utilized in this experiment. 1 litre of synthetic water was poured into each jar. The three stages of factorial design were used to identify the process conditions. For the significant variables, the optimal concentration of the factors was calculated, and the experimental zone was depicted. The three critical parameters investigated throughout the disinfection process are dose 1, 3, and 5 mg/l, agitation 100, 120, and 140 rpm, and duration 30, 60, and 90 minutes, with some modifications (Idris et al., 2017). These variables were investigated at three different levels: low, medium, and high. Table 1 shows the factorial design for three factors using Design Expert Software, consisting of 20 experimental runs with six centre points.

Table 1 Experimental design using three levels of factorial design in response and the output of fungal count.

Run	Dosage (mg/ml)	Time (minutes)	Agitation (rpm)
1	1	90	140
2	5	60	120
3	1	30	140
4	1	90	100
5	5	90	100
6	3	60	120
7	3	30	120
8	1	30	100
9	5	90	140
10	3	60	120
11	5	30	100
12	5	30	140
13	3	60	120
14	3	90	120
15	1	60	120
16	3	60	120
17	3	60	140
18	3	60	100
19	3	60	120
20	3	60	120

2.8. Statistical analysis

The data were analyzed for three replicates by using Microsoft Excel 365. All necessary statistical data was derived from this software. The mean, standard deviation, and standard error results were calculated from replicates by Microsoft Excel 365 and applied to each figure and table value. IBM SPSS has performed the quadratic model.

3. Results and Discussion

3.1. Characterization

3.1.1. The surface morphology of the Moringa oleifera seeds powder

The SEM was used to investigate the composition and surface character of MO seed powder, as illustrated in Figure 1, which shows the surface image of Moringa seeds before oil removal at 100x and 500x. A high amount of oil in the powder may be seen in the forMOf molecule bonding shown in those pictures. This data reflects the efficient oil removal using the traditional approach (cold press) without the use of chemicals in an indirect manner.

The surface morphology of MO seeds powder, according to Araújo et al. (2010), is a heterogeneous and moderately porous substance. This structure of the MO seeds powder's surface morphology verifies the existence of the seed's protein component (Kebede et al., 2018). Cationic protein extracts have been found to be lipophilic in the cell walls of microorganisms in several investigations. These proteins can pierce the cell wall and attach to the cytoplasmic membrane on the inside, causing the microorganism to die (Shebek et al., 2015; Idris et al., 2017). As a result, it can infer that Moringa oleifera seeds characteristics have an adequate morphological profile to limit the fungal growth in drinking water.

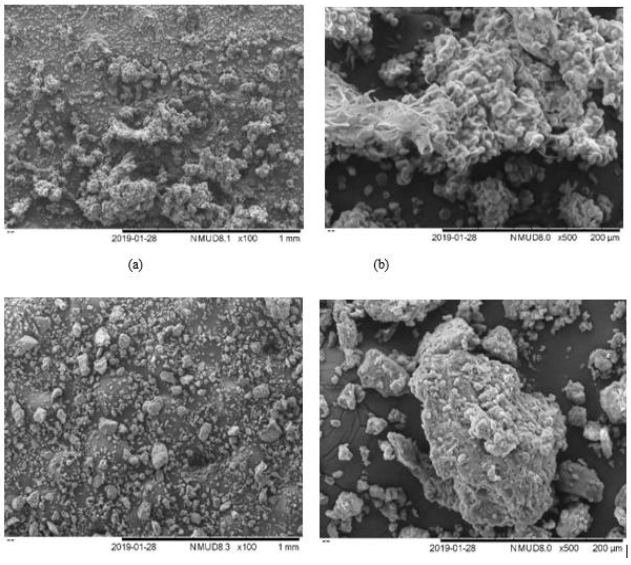

Figure 1 SEM surface image of the Moringa seeds before removing oil at a magnification of (a) 100x, (b) 500x, and after removing oil at a magnification of (c) 100x, (d) 500x.

3.1.2. The molecular structure of the Moringa oleifera seeds powder

FTIR spectroscopy is a valuable technique for determining the existence of specific functional groups in a molecule because each chemical bond has its own energy (Zhu et al., 2012). The surface spectra features of MO seeds were emphasized in Figures 2 before and after. When comparing before and after the oil was removed, Figure 1 showed that the peak had vanished or had a lower value. Figure 2 shows how the 1735 peak vanished once the oil from Moringa seeds was removed. Figure 2 highlighted the disappearance or reduced value of the peak when comparing before and after removing the oil and the fatty substances. For instance, (1735 peak) in Figure 2 was disappeared after clearing the oil from Moringa seeds.

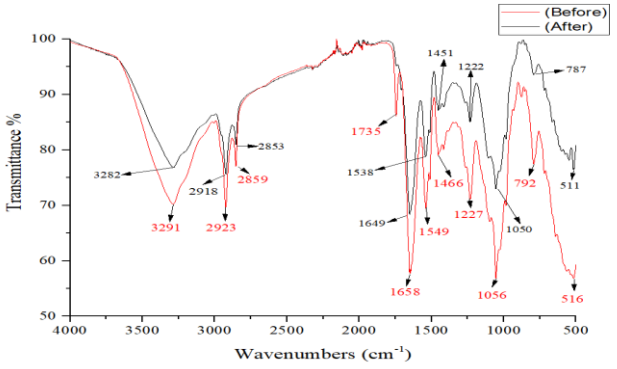

Figure 2 FT-IR spectrum of *Moringa oleifera* seeds before and after removing oil.

Moringa seeds have a wide peak in the FTIR spectrum at (3289 cm-1 and 3284 cm-1) due to stretching NH and OH in the secondary amide group. This functional group in MO structures is mostly due to the presence of protein and fatty acid (Araújo et al., 2010, Kebede et al., 2018). Due to asymmetric and symmetric stretching of the C-H bond and CH2 methyl group, the Moringa has a band between (2922 cm-1) and (2855 cm-1). Because of the high intensity of these bands, they may be attributed to the seed's mainly lipid component, which is present in a proportion that is similar to that of protein (Araújo et al., 2013). Moringa spectra with many overlapping bands in the range of (2323 to 796 cm-1). Furthermore, the *Moringa oleifera* can be linked to C=O extending between the bands (1649 to 986 cm-1).

3.1.3. Determination of the atomic and molecular structure of the Moringa oleifera seeds powder

To learn more about a crystal of MO's atomic and molecular structure, X-Ray Diffraction (XRD) studies were done. The XRD pattern of protein MO seeds is shown in Figure 3. The XRD pattern reveals a poorly resolved peak that suggests a predominance of amorphous material, which may be attributed to the large amount of proteins and oils present (about 69%) in the material's composition (Abdulkarim et al., 2005; Kebede et al., 2018). The XRD pattern for the seeds powder exhibited broadband around (2Ɵ = 15° to 25°) due to the high proportion of proteins and oils, with the exception of Figures 3 around (2Ɵ = 25°) for the seeds powder, which is ascribed to the material's semi-crystalline structure. These peaks are most likely related to the diffraction of the constituent protein in the presence of additional amorphous or semi-crystalline components.

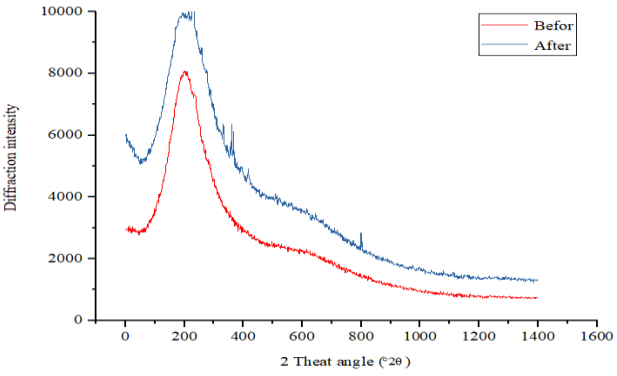

Figure 3 XRD of *Moringa oleifera* seeds before and after removing oil.

3.2. Antifungal activity performance of the Moringa oleifera seeds extract

Plant-derived products have been studied as disease control agents because they are non-toxic, have fewer environmental effects, and are widely accepted. The interest in plant extracts has developed to create environmentally sustainable alternatives to drinking water to contain microbes. The antifungal activity of MO was investigated in the laboratory. In Kuantan, Malaysia, the two most common fungi found in tap water samples are *Aspergillus* sp. (73%) and *Rhodotorula mucilaginosa* (63%) (Salah et al., 2020). The plant extract was mixed into the media (Potato dextrose agar) to see how effective it inhibited pure fungal growth. In this investigation section, the fungal growth was assessed and expressed as a percentage suppression.

Table 2 shows that the development of fungal mycelia on *Rhodotorula mucilaginosa* (RM) and *Aspergillus* spp. was gradually reduced when the content of MO seeds extract and chlorine was increased (A). These findings verified the antifungal action of MO seeds and chlorine at all concentrations. MO seeds and chlorine suppressed the R MOf 19.59% and 35.43% at a 0.1 mg/ml dosage. At the same time, at a dosage of 5 mg/ml, the percentage inhibition of *Moringa Oleifera* seeds and chlorine was 65.19% and 100%, respectively.

Table 1 Percentage inhibition (%) of *Moringa oleifera* and chlorine on *Rhodotorula mucilaginosa* (RM) and *Aspergillus* spp. (A).

Concentration	*Moringa oleifera* (RM)	Chlorine (RM)	*Moringa oleifera* (A)	Chlorine (A)
0.1	19.59%	35.43%	16.93%	32.63%
0.5	31.50%	50.18%	30.92%	66.56%

1	52.18%	100 %	37.46%	100 %
2	58.32%	100%	40.01%	100%
3	59.80%	100%	48.73%	100%
4	61.78%	100%	52.19%	100%
5	65.19%	100%	55.36%	100%

Table 2 demonstrates the inhibitory impact of MO seeds extract and chlorine on *Aspergillus* spp. at seven concentrations of 0.1, 0.5, 1, 2, 3, 4, and 5 mg/ml on *Aspergillus* spp. of *Moringa oleifera* seeds and chlorine at concentration 0.1 mg/ml reduced *Aspergillus* spp. growth by 16.93 and 32.63 %, respectively. For *Moringa oleifera* seeds and chlorine, percentage inhibition was 55.36 and 100 % at a dose of 5 mg/ml, respectively. Resistance to the plant extract was observed in *Aspergillus* species, probably due to the fungal's unique composition and growth.

3.3. Achieving the best condition of the disinfection by Moringa oleifera seeds

The RSM is a critical tool for achieving optimal experiment process parameters. Unlike the traditional method, this statistical approach can provide information on optimal settings for the predicted response's proximity to obtain desirable responses, establish an approximate correlation between dependent and independent factors, and determine the level of significance of the independent factors. As a result, a good set of functional connections between a response of interest and manipulating factors may be constructed. A design of experiments is required to obtain an adequate and reliable measurement of the desired responses (Mukhopadhyay and Khuri, 2012). Because a linear function, or the first-order model, was unsuitable for relationships between the examined responses and independent variables, a second-order model was employed to approximate the area close to optimal responses in this study. Table 4 lists the experimental findings of specified studies that included both examined reactions.

3.3.1. Optimization of the significant variables using CCD-RSM

The three most important parameters found dose, duration, and agitation, were chosen to assess their influence on fungus community growth in drinking water using CCD in RSM. The CCD was done with two complete factorial combination factors coded (+2, +1, 0, -1, -2) at five distinct levels (relatively high, high, basal, low, comparatively low) (Table 3). Table 4 shows the range and levels of extraction parameters, where X1 indicates dose, X2 represents time, and X3 represents. When CFU was used as dependent responses, a total of 20 tests for each extract and fungus were carried out according to the full factorial of 2 CCD to optimize all the essential variables. To prevent bias, the experiment was carried out in regular order. By modifying the experimental result using Equation 1, a second-order polynomial model equation was utilized to forecast the optimal location.

$$Y = bo + \sum bi\ Xi + \sum bii\ X2i + \sum bij\ Xi\ Xj \qquad (1)$$

Where Y is the response variable, b is the model's regression coefficient, and x is the independent variables' coded levels. The regression coefficients, ANOVA, F-, and P-values were used to test the generated second-order model. The degree of fit of the regression model was evaluated by the coefficient of Determination (R2) and correlation (R). Design-Expert Software's statistical software was used to create the CCD experiment (Stat-Ease Inc., Minneapolis, MN 55413, USA, version 7.1.6).

Table 3 Ranges and levels of factors tested in CCD to eliminate the fungi's growth.

Factors	Unit	levels				
		-2	-1	0	+1	+2
(X1) Dosage	mg/ml	1	5	-1	1	3
(X2) Time	min	30	90	-1	1	60
(X3) Agitation	rpm	100	140	-1	1	120

3.3.2. Optimization of eliminating the growth of the fungi by CCD-RSM

Further examination of their influence using a statistical technique can enhance the process of elimination through the development of fungus. RSM is an empirical modelling approach for analyzing issues with interest response affected by many factors (Swamy and Muthukumarappan, 2017).

Table 4 Experimental design and results of the CCD for *Moringa oleifera* seeds on *Rhodotorula mucilaginosa* (RM) and *Aspergillus* spp. (A).

Run	Dosage (mg/ml)	Time (minutes)	Agitation (rpm)	CFU/ml (Rm)	CFU/ml (A)
1	1	90	140	38	43
2	5	60	120	29	36
3	1	30	140	41	46
4	1	90	100	35	43
5	5	90	100	25	31
6	3	60	120	28	33
7	3	30	120	34	42
8	1	30	100	36	45
9	5	90	140	22	30
10	3	60	120	28	33
11	5	30	100	37	41
12	5	30	140	31	37
13	3	60	120	28	36
14	3	90	120	25	33
15	1	60	120	35	43
16	3	60	120	31	35
17	3	60	140	27	32
18	3	60	100	24	39
19	3	60	120	24	34
20	3	60	120	27	34

3.3.3. Optimization of process condition

The CCD was used to investigate the mutual interactions between the most important parameters, namely dose (D), time (T), and agitation (S), as well as to find the actual optimal values of factors using CFU. Twenty tests were conducted, with six duplicates in the centre point, following the parameters listed in Table 5. All of the experiments in the CCD were done at random to eliminate systematic mistakes by minimizing the influence of unexplained variability in the observed responses. The input and output components were fitted to the second-order equation and the model's quality of fit was evaluated. F-, P-, determination coefficient (R2), and correlation coefficient (R) were used to verify the model's fitness. Table 5 summarises both of these factors and summarises the ANOVA findings of response surface quadratic models for CFU.

The F-value of the model reflecting responses, CFU of *Rhodotorula mucilaginosa* and *Aspergillus* sp. with Moringa seeds, a deficient probability value of 0.0006, and 0.0003 showed that both models were very significant, according to the results of ANOVA. The R2 values for Moringa seeds were 0.9012 and 0.9161, suggesting a stronger connection between the actual and projected CFU values. The adjusted determination coefficients (adj R2) values for CFU of *Rhodotorula mucilaginosa* and *Aspergillus* sp. with Moringa seeds were also high, with values of 0.8122 and 0.8405, respectively, indicating that the developed models were significant and suitable for use in this experiment. For *Rhodotorula mucilaginosa* and *Aspergillus* sp., the projected R2 values for Moringa seeds were 0.3074 and 0.4470, respectively, which were nevertheless in reasonable agreement with adjusted determination coefficient values. The responses of the corresponding coded values of the three distinct variables in this experiment were subjected to polynomial regression modelling based on the RSM results, and the results were assessed. Equations 2 and 3 were generated from regression analysis of data and were second-order equations in terms of coded factors for CFU of *Rhodotorula mucilaginosa* and *Aspergillus* spp. with Moringa seeds. According to all Equations, the solvent extraction dose (D), followed by extraction period (T), and agitation (S), had the most significant impact on CFU.

Similarly, the solvent extraction dose (A) has the most significant impact on the percentage of CFUs. The ANOVA results in Table 5 are consistent with these findings. An ANOVA table is often used to summarise the test results to establish a suitable model.

CFU of *Rhodotorula mucilaginosa* with Moringa = + 53.44 + 0.51 D – 0.22 T – 0.17 S - 0.035 DT – 0.053 DS + 2.08 TS + 0.98 D2 + 1.61 T2 + 1.13 S2 (2)

CFU of *Aspergillus* sp. with Moringa = + 47.78 – 3.95 D – 0.29 T + 0.19 S – 0.025 DT – 0.018 DS + 4.16 TS + 0.90 D2 + 1.81 T2 – 9.09 S2 (3)

Figure 4 shows the study of the standard probability plot of the residual for both reactions to *Rhodotorula mucilaginosa* and *Aspergillus* sp. of CFU with Moringa seeds. Both graphs are pretty similar to a straight-line residual's distribution. The straight line in the image indicates that mistakes are uniformly distributed, implying that the most petite square fit is adequate. Figure 5 shows a random scatter, which indicates that the suggested models are sufficient and do not violate the independence or constant variance assumptions.

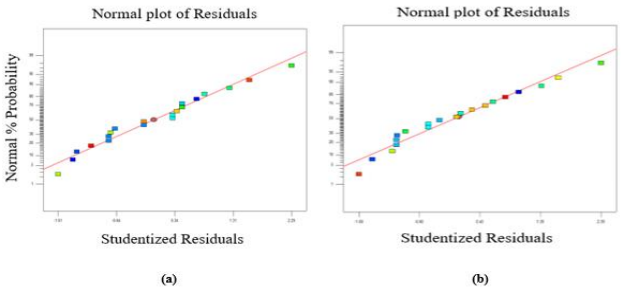

Figure 4 Normal plot of Residuals of *Moringa seeds* for (a) *Rhodotorula mucilaginosa* and (b) *Aspergillus* sp.

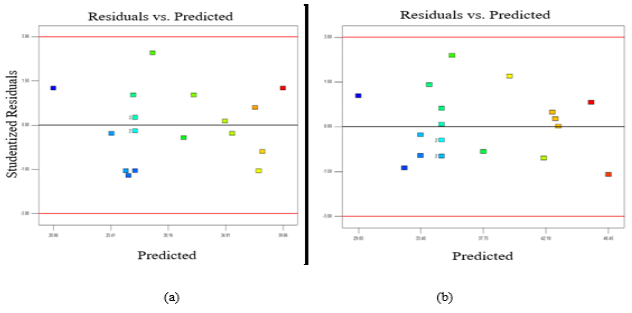

Figure 5 Residuals agents Predicted response plot of *Moringa seeds* for (a) *Rhodotorula mucilaginosa* and (b) *Aspergillus* sp.

3.4. Interaction of variables during Optimization by CCD-RSM

Figures 6 and 7 show the calculation of CFU based on the fundamental input factors of extraction dose (D), time (T), and agitation (S) in the forMOf interaction. Each image depicts a two-dimensional (interaction plots) or three-dimensional (response surface plot) graph indicating the regression model equation that locates the optimal values of the input variables within the chosen ranges for optimizing process response. As demonstrated in Table 5, the variable examined in CCD significantly impacted CFU, as indicated by their P-values (P-values less than 0.050 indicate significant model terms). The interaction of extraction dosage and time (DT) was a remarkable effect for CFU.

Figure 7 shows the interaction impact of MO seeds extraction dose and time (DT) CFU on *Rhodotorula mucilaginosa* while extraction agitation (S) was maintained at 120rpm. The CFU steadily reduced with extraction dose (D) from 35 CFU at 1 mg/ml to 29 CFU at 5 mg/ml while the duration was held at 60 min, as shown in Figure 6 (a, b). Figure 8 shows the interaction impact of MO seeds extraction dose and time (DT) CFU on *Aspergillus* sp. while extraction agitation (S) was maintained at 120 rpm. The CFU steadily dropped with extraction dose (D) from 43 CFU at 1 mg/ml to 36 CFU at 5 mg/ml while the duration was remained at 60 min, as shown in Figure 7. (a, b).

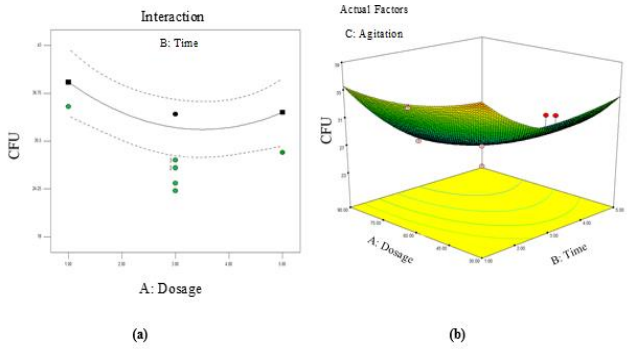

Figure 6: Interaction (a) and response surface plot (b) showing the effect of reaction dosage and time on CFU for *Moringa oleifera* on *Rhodotorula mucilaginosa*.

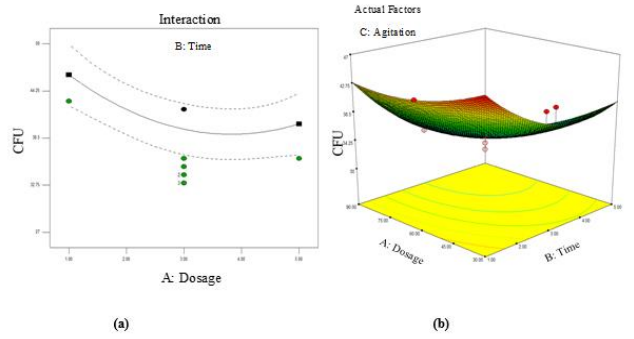

Figure 7: Interaction (a) and response surface plot (b) showing the effect of reaction dosage and time on CFU for *Moringa oleifera* on *Aspergillus* sp.

4. Discussion

Many countries worldwide suffer from the dilemma of contamination of drinking water systems with several biological and chemical contaminants. Thus, water quality in pipe is not always the occasion for human consumption (Mirzabeygi et al., 2016). This study demonstrated that fungi are a ubiquitous contaminant in tap drinking water Kuantan although treat the water with chlorine as disinfection. Tap water should be free from contaminants, safe for humans, and valid for home use. The results showed fungi in the tap water in the Kuantan area in different concentrations. The residual free chlorine in the tap water of these selected areas varied from 0.05 to 1.97 mg/l. In general, tap water in the Kuantan area is still stable for home use but needs purification to eliminate fungi growth like in other countries.

The reasons can be the low number of studies that link the fungi presence that increases in tap water and the occurrence of diseases due to the divergent cultivation methods and the lack of knowledge of the fungal load in the drinking water network. All tested concentrations of *Moringa oleifera* seeds could decrease the tested pathogenic fungi germination. Based on the results, the main effect of extraction dosage (D) and time were dominant concerning the CFU response. The Design-Expert software's optimization model was utilized to enable the prediction for both responses, complete with a 95.0% prediction interval. Results have shown that the best condition for extraction dosage, time, and agitation was essential for maximal elimination of the fungus's growth.

The findings showed that plant extracts had a potent antifungal function, inhibiting the development of all fungi studied. *Moringa oleifera* extracts were the most successful at inhibiting the growth of the fungi studied. The results followed various earlier reports advocating the antifungal activity of plant extracts under the present study. However, the variations in the inhibition zone may be attributed to geographical reasons leading to slight changes in phytochemical constituents. Overall, it can be observed that the final inhibitory effect of *Moringa oleifera* seeds of target inhibition had only minor differences, and chlorine had been more efficient than them. According to the findings, plant extracts' antifungal behaviours are dose-dependent, with comparatively greater effectiveness than commercial disinfectants at the concentrations of extracts examined.

Furthermore, at the concentrations examined, extracts' efficacy was slightly lower than that of commercial antibiotics. This indicates that the active ingredient presented at the concentration of extract measured is ineffective, which was not the case in this study. This issue can be addressed in the future by processing the powder through secondary (extraction) and tertiary (purification) stages. This works by extracting their active antifungal agents and subsequently purifying them to eliminate undesired organics. This may increase their processing costs and may not be practical as chemical disinfectants. Nonetheless, extraction of active antifungal agents is still a noteworthy aspect that may be helpful should they be commercialized or applied in concentrated form for water treatment.

The extracts examined at different concentrations were shown to have increased antifungal efficacy compared to the antibiotics used, with substantial variations. The collapsing of cell walls and membranes, resulting in leakage of cell materials, disruption of proton motive power, dysfunction of efflux pump and enzyme, and cytosis, may be the source of the inhibition activities observed against fungal cells in this research (Adeeyo et al., 2020). Phytochemical activities of plant extracts against microbes have been documented, including disruption of nucleic acid synthesis, interfering with intermediate metabolism, initiating cytoplasmic portion coagulation, and disrupting regular cell contact in quorum sensing (Mogosanu et al., 2015; Adeeyo et al., 2020). A general occurrence is that various wild plant extract compounds work on different target sites in pathogens, contributing to plant extract efficacy (Gupta et al., 2014). Phytochemicals can have an antimicrobial impact on bacteria by modifying important pathogenesis events and direct lethal action (Brijesh et al., 2009). The inhibition behaviour found in this analysis may be antifungal since the region of inhibition encountered results from the full destructive activity against the microbes. The inhibition areas result in a viable yet inactive condition known as the non-culturable yet feasible state. In terms of radical frequency and spatial composition, chemically synthesized and photobiotic antibiotics vary greatly (Koehn and Carter, 2005). With less nitrogen, sulphur,

halogens, and phosphorus, the latter has more scaffold range, stereochemical density, variability in the ring structure, molecular sophistication, and carbohydrate quality (Schmidt et al., 2008). Plant products can also modify or inhibit protein-protein interactions, rendering them efficient modulators of the immune response, apoptosis, mitosis, and signal transduction (Koehn and Carter, 2005).

5. Conclusion

Many nations worldwide face the problem of biological and chemical pollutants contaminating their drinking water systems. As a result, drinking water quality is not always suitable for human consumption. This research found that fungus is a common contaminant in Kuantan, Malaysia's tap water, even though it is disinfected with chlorine. Moringa seeds have traditionally been used in rural areas to clean drinking water and reduce the health risks connected with microbes. In this study, *Moringa oleifera* seed extract and chlorine were shown to have antifungal action against disinfectants at all dosages. The study's findings will encourage researchers to seek a natural alternative to chemical disinfectants in water treatment. The extract is less efficacy when compared with the chlorine because the chlorine is purer than it.

On the other hand, the extract has less risk from chlorine. The use of biomass disinfection for water drinking to have high efficiency to limit the growth of any microbes and does not have a risk for the human body. Tap water should be free of pollutants, safe for human consumption, and suitable for use at home. The quality of drinking water in the Kuantan region of Malaysia was examined in this study. According to the discussion, the primary effect of extraction dose (D) and duration were determined to dominate in terms of CFU response. The Design-Expert software's optimization model was used to anticipate both responses, with a 95.0% prediction interval; the findings showed that the optimal extraction dose, duration, and agitation conditions were critical for obtaining the maximum eradication of the fungus's development.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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