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# SCREENING OF YEAST ABILITY TO DECOLORIZATION AND COMPLETE BIODEGRADATION OF MALACHITE GREEN TEXTILE DYE AND INVESTIGATION OF THEIR PHYTOTOXICITY

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*Malachite green accumulation in water causes harmful effects. Biodegradation by microbes was the preferred technique used to remove dyes from wastewater. Thirty yeasts were investigated for their ability to remove 50 mg of dye and belonged to: Deboryomyces, Diutina, Papiliotrema, Rhodotorula, and Saccharomyces. Based on the decolorization index (DI) of the examined yeast on the solid medium, the decolorization activity may be classified as highest, moderate and low decolorization activity with DI 1.66-2.78, 1-1.64, and <1 respectively. Rhodotorula mucilaginosa AUMC13567, R. mucilaginosa AUMC13570, Saccharomyces cerevisiae 11688, R. mucilaginosa KR264902, S. cerevisiae C3, and Diutina rugosa AUMC13571 possess the highest decolorization percentages in broth media: 98.41%, 96.65%, 96.49%, 95.59%, 92.80%, and 92.22%, respectively. The decolorization rate is influenced by time, however, yeast cell optical density has no bearing on this rate. The Fourier Transform Infrared Spectroscopy Analysis (FTIR) results of MG before and after degradation indicate a reduction of peaks along the fingerprint region, which can be attributed to the loss of aromaticity of the metabolites, which also confirm degradation by yeast strains. The effect of malachite green dye and its degradation metabolites by five selected yeasts was studied on wheat (T. aestivum), sorghum (Sorghum bicolor), maize (Zea mays), and radish (Raphanus sativus) seed germination, and results show that the germination index of seeds in untreated malachite green solution was significantly low compared to its degradation metabolites by selected yeasts and yeast efficiency in the reduction and elimination of the dye's toxic effect on these seeds*

**Keywords:** *malachite green, biodegradation, yeast, FTIR, Phytotoxicity*

## INTRODUCTION

Dyes are substances that, when applied to a substrate, produce color through a method that modifies the colored substances' crystal structures, if only momentarily[1,2]. Approximately ten thousand different colored dye compounds were produced chemically. Around 7,107 tons of synthetic textile dyes are manufactured worldwide each year, and over 10,000 tons are utilized in the textile industry[3,4]. Uses of synthetic dyes cause some times visual pollution, disruption to the environment and water supplies, and pollute the soils, crops, and aquaculture animals[5]. Some industry dyes are potentially toxic and highly carcinogenic, and aquatic organisms are often at significant risk when they are released into the water, they prevent light from penetrating the water column and cause a decrease in dissolved oxygen (DO), an increase in chemical oxygen demand (COD)[6-9].

One type of textile dye is called Malachite Green (MG), a triphenylmethane dye. Malachite green is also known by several common names, including China green, benzal green, solid green 0, light green N, aniline green, acryl brilliant green, Aizen malachite green, astra malachite green, Benzal green, and diamond green P extra [10].

Because of its water solubility, effectiveness, affordability, and availability, MG is the most widely used synthetic triphenylmethane, according to numerous

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studies. It is also used as a fungicide, food coloring agent, food additive, medical disinfectant, industrial textile dye, and scientific lap stain, among other applications.

Thousands of tons of wastewater are produced during the production of malachite green dye, which is subsequently dumped into lakes and rivers and leaves residue on the land, potentially damaging the ecology[11, 12]. It has been discovered that MG and the reduced form of LMG (leuco-malachite green) residues have been observed in fish and eggs as well as serum, the liver, kidneys, and other tissues[13]. MG is reported for its animal carcinogenesis, mutagenesis, chromosomal disruption, teratogenicity, toxic respiratory organs, aquatic life, and many other organisms[14]. Before the dyeing effluent could be safely discharged into the environment, treatment was essential[15]. For the management of wastewater effluents containing dye, numerous techniques are used to remove dyes, such as physical/chemical adsorption, oxidation, and biological treatments but bio-friendly procedures have been preferred over physicochemical ones because they are more affordable and produce fewer harmful metabolites[16, 17.]

The MG contamination in aquaculture environments can be eliminated by adsorption or degraded by advanced oxidation processes (AOPs), or biodegraded by microbes or enzymes[18]**.** Many studies on microorganisms from different taxonomic families have demonstrated their ability to biodegrade, remove, and decolorize different dyes[19]**.** Recently, the application of microorganisms, including bacteria, fungi, yeast, and algae has been very effective in detoxification, biodegradation, and complete mineralization of azo dyes. These enzymes were reported by[20-23]**.** Fungi are well-known organisms with a high potential for the degradation of various contaminants through several metabolic pathways. It is generally known that the structure of the dye, the concentration, the adaptability of the microorganisms and their activity, and the concentration of biomass all affect how efficiently the dye degrades. *Rhodotorula* species and *Candida krusi* are well-known yeast strains that are highly capable of removing MG colour[24]**.**

A wide range of fungal species have been studied for their ability to decolorize MG, including *Saccharomyces cerevisiae*[26,27], baker's yeast[28], *Rhodotorula graminis* Y-5[29], *Cyathus striatus*[30], *Penicillium ochrochloron*[31], *Saccharomyces cerevisiae*, *Candida albicans* and their consortium[32] and *Cunninghamella elegans* [33].

The current study aims to find out the ability of thirty yeasts belonging to five various genera including *Deboryomyces, Diutina, Papiliotrema, Rhodotorula,* and *Saccharomyces* to remove 50 mg of malachite green dye from wastewater. It also seeks to identify the mechanism by which these yeasts remove the dye, examines the impact of time and optical density on the effectiveness of yeast removal, and statistically analyzes the decolorization results to compare the effectiveness of these yeasts in decolorizing malachite green dye, recording the highest level of efficiency.

## MATERIALS AND METHOD

### Yeast Strains and Culture Condition

Water samples were collected from a few laundries and a fish farm owned by a veterinary school in the Assiut Governorate. The samples were transmitted right away to the lab in sterile bottles at 5ºC. To isolate yeast, three different media were used: yeast malt extract medium (MYE), which contained yeast extract, malt extract, glucose, and peptone in the amounts of 3 grams each; malt extract medium (MEA), which contained 20 grams of malt extract, 20 grams of glucose, and 1 gram of peptone; and potato dextrose agar medium (PDA), which contained 20 grams of dextrose and 200 grams of disc potatoes. Twenty grams of agar-agar were added to each media and pH was adjusted to 3.7 with 0.1N HCl and 0.1N NaOH using the HANNA instrument, model 211 pH meter. These media were autoclaved at 121°C for 20 minutes. Each of the five sterile Petri dishes was filled with 12 to 15 ml of the agar medium after receiving 0.1 ml of the sample in an aseptic manner. The dishes were manually rotated to ensure proper suspension dispersion and incubation for three days at 28°C[34-36]**.** Twenty-two yeasts were kindly provided from our collage culture collection of the Botany and Microbiology Department, Faculty of Science, Assiut University[37, 38]**.**

**Screening of Malachite Green Decolorization By Yeast**

**Solid Medium**

Test for decolorization of malachite green on solid media[39]. The malt yeast extract agar medium (MYE) was infused with 0.5% of MG dye (MG). Each sterile Petri dish received 12 to 15 ml of the medium after it had been sterilized and cooled. At a temperature of 28°C, each yeast strain was point-inoculated onto a medium. The colony diameter and newly developed clear zones' diameters were measured after five days. The following equation was used to determine the decolorization index:

$$Decolorization\ index = Decolorization\ diameter / Colony\ diameter$$

**Liquid Media**

The pH of the broth (MYE) medium was set to 7 and it was sterilized. 50 mL of the sterilized medium were cultivated with a loop of yeast inoculum; aerobic condition was provided by shaking the flask in (Environ-Shaker 3597-1) shaker at 100 rpm for 3 days at 28°C. Then 10 ml of cultivated yeast was transferred into a 50 ml MYE broth sterilized medium containing yeast extract (5 g), malt extract (3 g), malachite green dye (50 mg/L), and glucose (30 g). The pH was then adjusted to 5.8, and the mixture was incubated for 3 days at 30 ± 2 °C with 100 rpm [40]. The optical density of the cultivated yeast cell before the decolorization was measured at 600 nm (OP).

The biodegradation MG by yeast cultures was monitored every 24 hrs for the duration of the three days. A 5 ml sample was separated from the cell mass by centrifugation at 5000 × g for 7 min under cold conditions (CRU-5000 Centrifuge IEC) and 3 ml of the medium was withdrawn, the supernatant was measured at 620 nm. The analysis was made in triplicate using Mnicam/UV-vis spectrophotometry Helios Gamma, at the Botany and Microbiology Department, Faculty of Science, Assiut University[41, 42].

The percentage decolorization was calculated as follows

$$\text{Decolorization percentage} = \frac{\text{Intial absorbance} - \text{Observed absorbance}}{\text{Initial absorbance}} * 100$$

**Mechanism of MG Decolorization by Yeast**

To ascertain the method of MG yeast removal (degradation or biosorption), we depend on two ways, one of them was performed in solid media by recording the change in color of the colony or edges of the colony of yeast in solid media after decolorization process and the change in growth (colony diameter). Another way was run in liquid media on both living and dead cells and compare the spectrophotometric results. The yeasts were cultured in 50 mL of sterilized MYE medium at 28°C for three days while being shaken in an ENVIRO-SHAKER 3597-1 shaker at 100 rpm under aerobic conditions. After that, the grown yeast was autoclaved to destroy the cell. Then, 50 ml of sterilized MYE broth medium containing yeast extract (5g), malt extract (3g), MG dye (50 mg/L), and glucose (30g) was added to 10 ml of the dead yeast. The pH was then adjusted to 5.8, and the medium was incubated for 3 days at 30 ± 2°C on an incubator at 100 rpm[40, 43].

**Fourier Transform Infrared Spectroscopy Analysis (FTIR)**

Choose the two yeast strains with the highest biodegradation potential for MG and check FTIR analyses. The samples were centrifuged (10,000 rpm) after a five-hour incubation, for 20 minutes to thoroughly remove the pigment. The metabolites were then extracted from the supernatant using an equivalent volume of ethyl acetate. The extracts were dried over anhydrous $Na_2SO_4$ and then evaporated to dryness in a rotary evaporator. The crystals were created, dissolved in a small amount of methanol, and then used for analysis. On a Perkin Elmer Spectrum One instrument, the extracted metabolites were submitted to FTIR analysis, and the produced metabolites were compared to the control dye in the mid-IR range of 400–4000 $cm^{-1}$ with 16 scan speeds. The samples were combined in a 5:95 ratio with spectroscopically pure KBr, the pellets were fixed in the sample holder, and analyses were performed[42, 44 , 22.]

**Toxicity assessment**

In this study, phytotoxicity was assessed based on the inhibition of seed germination in wheat (*T. aestivum*), maize (*Zea mays*), sorghum (*Sorghum bicolor*), and radish (*Raphanus sativus*) monocot and dicot seeds and grains, respectively. To document the MG

dye's toxicity, it was utilized at a concentration of MG is 50 ppm. Additionally, the MG decolorization metabolites of the most effective selected yeast strains (*Rhodotorula mucilaginosa* AUMC 13567, *R. mucilaginosa* AUMC 13570, *R. mucilaginosa* KR264902, *Saccharomyces cerevisiae* 11688, and *Diutina rugosa* AUMC 13571) were used, centrifuged, and dissolved in sterile distilled water to make a final concentration of 50 mg/L and also the MG decolorization metabolites used with yeast cells[45]. The phytotoxicity tests were conducted using 35 seeds for each of the plants under investigation, and they were done in sterile 10 cm Petri dishes that were lined with sterile filter paper and watered separately with 5 mL of the following solution: MG dye, biodegradative MG metabolites without yeast cells, MG metabolites with yeast cells. As a control set, the seeds that were irrigated with pure water were used. The phytotoxicity test was performed at room temperature (30 ± 2°C). The length of the radicle (root) and germination percentages were assessed after the incubation time (after 7 days of germination) when the negative control germinated[46]. The germination index (GI), rate of improvement, and phytotoxicity germination percentage were calculated using the formula:

$$\mathbf{GI}\ (\%) = \frac{\text{Number of germination seed} * \text{Total of root length}}{\text{Number of germination seeds in the negative control} * \text{Total root length in the negative control}} * 100$$

$$\textbf{Rate of improvement} = \frac{\text{GI (\%)of the treated dye} - \text{GI (\%)of the untreated dye}}{\text{GI(\%)of the untreated dye}}$$

**Statically Analysis**

The basic statistics mean, and standard errors were estimated and expressed as the average of three analyses ± standard error. All statistical analyses were performed using, the IBM-SPSS package version 21**[47],** the present study was conducted by One Way ANOVA (analysis of variance) and post-test (Duncan [a] multiple comparison test) were used for significant differences between means and differences in means were considered significant when $p<0.05$.

## RESULTS AND DISCUSSION

### Results

**Isolation and Collection of Yeasts**

Malachite green can be removed from wastewater using yeast cells, which are a significant and promising material due to their low cost and high dye decolorization capacity. Thirty yeast strains were isolated and gathered for this investigation from various sources, including the following: Three yeast strains were recovered from laundry water, five yeast strains from fish farm water, and 22 yeast strains were kindly provided by our collage in Botany and Microbiology Department culture collections[37,38]. The yeast strains being investigated include 10 *Rhodotorula* (six *R. mucilaginosa* and four *Rhodotorula* unidentified specie), 12 *Saccharomyces* (three *Saccharomyces cerevisiae* strains and nine *Saccharomyces* species), 5 species of *Diutina*, and one species each of *Debaryomyces*, *Papiliotrema* and *Candida.*

**Decolorization of MG Dye In Solid Medium**

Based on the test yeast's capacity to decolorize in the solid medium, as demonstrated in **Tables 1, 2 and 3**; the degradation efficiency was calculated. The outcome revealed that dye decolorization was evident by the creation of a clearing zone around the colonies. Out of the 30 yeast strains tested, 20 were able to remove the MG dye on the solid medium and formed a clear zone around the yeast colony growth.

Based on the decolorization index (DI) of examined yeast, which varies depending on the efficiency degree of bio-decolorization, the decolorization activity of tested yeast may be separated into three groups, as shown in **Tables 1, 2, and 3**. Six species were belonged to the *Rhodotorula* and *Saccharomyces* genera exhibited the highest decolorization activity with DI, ranging from 1.66 to 2.78. This enormous capacity may be a result of the particular enzyme systems used by these yeasts to disassemble complex organic compounds into simpler parts. Eight yeast species with DI of 1.64-1were belonged to the four genera *Rhodotorula*, *Saccharomyces*, *Diutina*, and *Papiliotrema* exhibit moderate ability. Six yeast species exhibit a low degree of dye decolorization; the DI is less than 1, and the growth rate slightly increases.

Ten yeast species from the *Rhodotorula*, *Saccharomyces*, *Debaryomyces*, and *Candida* species were evaluated and none of them were able to remove the dye and did not depict the formation of a clear zone around yeast growth.

The statistical analysis shows that: There were homogenous between the result of decolorization diameter and decolorize index in the Homogeneity of Variances test $p>0.05$, which may indicate that the increases in yeast growth related positively to the decolorization index of malachite green dye. There were significant differences between decolorizing activities between yeasts in colony diameter, decolorization diameter, and decolorize index in ANOVA one-way test $_{p< 0.05}$, in Post Hoc Tests Duncan[a] $p.> 0.05$ significant differences are due to differences in results between all yeasts. Reliability statistics Cronbach's α-in decolorize index & decolorization diameter is 0.922.

The same outcomes were found by[48] who used *Fusarium solani* to investigate the decolorization of malachite green. After three days of incubation at 30 $^{O}$C, this fungus quickly decolored dyes with radial growth, and the decolorization halo of MG nearly filled the diameter of the plate. The findings of the current study corroborated those of [49], who found that *P. funiculosum* and *F. solani* appear to have a greater capacity to decolorize MG in solid media. The results from **[50]** were different from those from the current investigation in that *P. funiculosum* efficiency of decolorization, which indicates the highest ability, is very low in comparison to the results from the current study. Additionally, *Coriolus versicolor* showed good mycelial growth on solid media containing MG, but the efficiency of decolorization was very poor, and at the same time, the decolorization index with this fungus reached 0.11, which is why[48] findings were different from those of the current study.

**Decolorization of MG Dye In Broth Medium**

The yeasts' capacity to decolorize dye in broth media was also examined, and the results showed that the decolorization levels of the various yeasts varied. Yeast-cultivated media with MG differ in color after the incubation period as in **Fig 1.** Some strains have a great ability to decolorize dye in high percentages, as illustrated in **Fig.1$_{A\ to\ F}$.** Additionally, **Fig. 1** depicts the total elimination of dye color and a reminder of the yeast cells' color in the medium, which reveals the yeast's degradation mechanism. A few yeasts have a modest capacity for dye decolorization; as a result, the color of the dye dims but does not completely disappear, suggesting incomplete decomposition of MG dye.

The dye color, on the other hand, remained the same in some flasks, indicating that the yeasts growing in those flasks had poor or negative abilities. According to **Table 1-2**, there are considerable discrepancies between the optical densities of cultivated yeast, and the optical density of yeast cells has no bearing on the ability of these yeasts to decolorize MG dye. There were significant differences between the decolorization percentage of the cultured yeast in each of the three incubation days the F-value at $1^{st}$ = 806.8***, $2^{nd}$ = $843.3^{***,}$ and $3^{rd}$ =$757.58^{***}$..

Time has a significant effect on the decolorization rate. The highest mean of the decolorization percentage occurred on the third day, indicating a significant time effect on the decolorization rate and demonstrating the effectiveness of yeasts at removing color as a function of time, (except for *Candida parapsilosis* $_{AUMC13563}$), **Table 2**.


**Fig. 1**: Different levels of decolorization and biodegradation ability of yeast strains of MG, A] Malachite Green (control) and MG treated by B] *Rhodotorula mucilaginosa* AUMC13564, C] *Saccharomyces cerevisiae* 11686, D] *S. cerevisiae*11688, E] *R. mucilaginosa* AUMC 13570, and **F]** *R. mucilaginosa* AUMC 13567.

**Table 1:** Screening of the ability of ***Rhodotorula, Saccharomyces, Diutina*** **and** ***Papiliotrema*** to decolorize the malachite green dye on a **solid medium**.

| Yeast strains | Colony Diameter | Decolorize Diameter | Decolorize Index | Colony Colour Change |
|---|---|---|---|---|
| **a.** ***Six Rhodotorula mucilaginosa and one Rhodotorula unidentified specie were tested*** | | | | |
| ***R. mucilaginosa*** AUMC 13567 | **1.30 ±0.01** $^{d}$ | **4.25 ±0.01** $^{d}$ | **2.78 ±0.00** $^{d}$ | **N** |
| ***R. mucilaginosa*** KR264902 | 1.18 ±0.01 $^{c}$ | 4.36 ±0.02 $^{d}$ | 2.77 ±0.01 $^{d}$ | **N** |
| ***R. mucilaginosa*** AUMC 13570 | 1.06 ±0.01 $^{a,b}$ | 3.71 ±0.06 $^{c}$ | 2.39±0.02 $^{c}$ | **N** |
| ***R. mucilaginosa*** AUMC13562 | 1.13 ±0.01 $^{a,c}$ | 2.18 ±0.13 $^{b}$ | 1.66 ±0.06 $^{b}$ | **C** |
| ***R. mucilaginosa*** AUMC13565 | 1.12 ±0.01 $^{a,c}$ | 2.15±0.06 $^{b}$ | 1.64 ±0.03 $^{b}$ | **C** |
| ***R. mucilaginosa*** AUMC13564 | 1.09 ±0.07 $^{a,b,c}$ | 2.12±0.06 $^{b}$ | 1.61 ±0.03 $^{b}$ | **N** |
| ***Rhodotorula*** **sp31** | 1.01 ±0.04 $^{a}$ | 1.40±0.12 $^{a}$ | 1.21 ± 0.08 $^{a}$ | **N** |
| **F-value** | **8.02**$^{**}$ | **254.29**$^{***}$ | **226.25**$^{***}$ | |
| **b.** Three ***Saccharomyces cerevisiae strains, five S. cerevisiae***, and one ***Saccharomyces*** **sp** were tested | | | | |
| ***Saccharomyces cerevisiae*** c1 | 1.01 ± 0.01 $^{b}$ | 3.20 ± 0.06 $^{f}$ | 2.11 ± 0.03 $^{f}$ | **N** |
| ***S. cerevisiae*** 11688 | 0.97 ± 0.00 $^{b}$ | 2.85 ± 0.03 $^{e}$ | 1.91 ± 0.02 $^{e}$ | **N** |
| ***S. cerevisiae*** c2 | 0.98 ± 0.06 $^{b}$ | 1.72 ± 0.04 $^{d}$ | 1.35 ± 0.05 $^{d}$ | **N** |
| ***S. cerevisiae*** c3 | 0.90 ± 0.05 $^{b}$ | 1.53 ± 0.15 $^{d}$ | 1.22 ± 0.10 $^{d}$ | **C** |
| ***S. cerevisiae*** c4 | 0.94 ± 0.07 $^{b}$ | 1.04 ± 0.03 $^{c}$ | 1.00 ± 0.05 $^{c}$ | **C** |
| **Saccharomyces sp.** w4 | 0.85 ± 0.03 $^{b}$ | 0.83 ± 0.06 $^{b}$ | 0.84 ± 0.04 $^{b}$ | **N** |
| ***S. cerevisiae*** 11686 | 0.87 ± 0.04 $^{b}$ | 0.69 ± 0.04 $^{b}$ | 0.78 ± 0.03 $^{b}$ | **C** |
| ***S. cerevisiae*** C5 | 0.87 ± 0.12 $^{b}$ | 0.67 ± 0.05 $^{b}$ | 0.77 ± 0.05 $^{b}$ | **N** |
| ***S. cerevisiae***11689 | 0.38 ± 0.02 $^{a}$ | 0.15 ± 0.01 $^{a}$ | 0.27 ± 0.00 $^{a}$ | **C** |
| **F-value** | **11.58**$^{***}$ | **262.91**$^{***}$ | **148.42**$^{***}$ | |
| **c.** Three ***Diutina rugosa*** strains and one ***Papiliotrema laurentii*** strain | | | | |
| ***Diutina rugosa*** AUMC 13571 | 1.1 ± 0.01$^{c}$ | 2.15 ± 0.03 $^{c}$ | 1.64 ± 0.01 $^{c}$ | **N** |
| ***D. rugosa*** AUMC13566 | 0.7 ± 0.03 $^{b}$ | 0.21 ± 0.02 $^{a}$ | 0.48 ± 0.03 $^{a}$ | **C** |
| ***D. rugosa*** AUMC 13568 | 0.4 ± 0.12 $^{a}$ | 0.21 ± 0.03 $^{a}$ | 0.32 ± 0.07 $^{a}$ | **N** |
| ***Papiliotrema laurentii*** AUMC 13569 | 1.2 ± 0.02 $^{c}$ | 1.30 ± 0.12 $^{b}$ | 1.23 ± 0.07 $^{b}$ | **C** |
| **F-value** | **31.04**$^{***}$ | **227.32**$^{***}$ | **142.51**$^{***}$ | |

N: No of change in colony colour and C: Change in colony colour. All results are compared to each other at $P < 0.05$. Values with the different superscripts along the same column are statistically different from each other ***p<0.001. by One-way ANOVA values comparison. Post Hoc Tests: Duncan a $p< 0.05$ is represented by superscripts a,b,c. Values are presented as Mean ± Stander Error**.**

**Table 2:** Screening of the ability of yeast strains and species to decolorize the malachite green dye on a **liquid medium by** Spectrophotometric analysis.

| Yeast strains and species | 1$^{St}$ | 2$^{nd}$ | 3$^{rd}$ | F-value | Dead | Optical density |
|---|---|---|---|---|---|---|
| a. Six ***Rhodotorula mucilaginosa*** and four unidentified specie were tested | | | | | | |
| ***R. mucilaginosa*** AUMC 13567 | 95.3 ± 0.6$^{a8}$ | 95.9 ± 0.3$^{a7}$ | 98.41 ± 0.71$^{b7}$ | 8.8$^{*}$ | 0.3 ± 0.3$^{1}$ | 1.9 ± 0.01$^{4}$ |
| ***R. mucilaginosa*** AUMC 13570 | 72.7 ± 1$^{a6}$ | 83.6 ± 1.4$^{b6}$ | 96.65 ± 0.23$^{c7}$ | 146$^{***}$ | 0.6 ± 0.4$^{1}$ | 0.7± 0.01$^{2}$ |
| ***R. mucilaginosa*** KR264902 | 88.1 ± 1$^{a7}$ | 90.4 ± 0.6$^{b6}$ | 95.59 ± 0.06$^{c7}$ | 32.3$^{**}$ | 1.8 ± 0.4$^{1,2}$ | 2.1 ± 0.07$^{5}$ |
| ***Rhodotorula*** **sp.31** | 21.5 ±1$^{a4}$ | 82.2 ± 0.7$^{b5}$ | 85.14 ± 0.73$^{b6}$ | 1676.5$^{***}$ | 1.4 ± 0.4$^{1,2}$ | 1.2 ± 0.03$^{3}$ |
| ***Rhodotorula*** **sp.4v** | 54.1 ±1.6$^{a5}$ | 63.5 ± 0.9$^{b4}$ | 73.48 ± 0.41$^{c5}$ | 79.5$^{***}$ | 4.3 ± 1$^{3}$ | 2 ± 0.11$^{4,5}$ |
| ***Rhodotorula*** **sp.1W** | 11.5 ± 0.3$^{a3}$ | 18.2 ± 0.7$^{b3}$ | 34.34 ± 1.44$^{c4}$ | 155.3$^{***}$ | 2 ± 0.3$^{1,2}$ | 2 ± 0.08$^{4,5}$ |
| ***Rhodotorula*** **sp.** **3w** | 2.5 ± 0.13$^{a1}$ | 12.6 ± 0.7$^{b2}$ | 33.96 ± 1.02$^{c4}$ | 516.4$^{***}$ | 2.7 ± 1.2$^{2,3}$ | 2 ± 0.02$^{4}$ |
| ***R. mucilaginosa*** AUMC13565 | 1.3 ± 1$^{a1}$ | 9.3 ± 1.5$^{b1}$ | 30.69 ± 2.47$^{c3}$ | 74$^{***}$ | 0.6 ± 0.1$^{1}$ | 0.4 ± 0.03$^{1}$ |
| ***R. mucilaginosa*** AUMC 13562 | 5.9 ± 1.4$^{a2}$ | 16.9 ± 0.8$^{b3}$ | 20.42 ± 0.87$^{b2}$ | 51.4$^{***}$ | 2.8 ± 0.4$^{2,3}$ | 1.1 ± 0.02$^{3}$ |
| ***R. mucilaginosa*** AUMC 13564 | 6.4 ± 1.3$^{a2}$ | 11.9 ± 0.9$^{b1,2}$ | 14.79 ± 0.22$^{b1}$ | 22.9$^{**}$ | 1.5 ± 0.2$^{1,2}$ | 0.5 ± 0.01$^{1}$ |
| **F-value** | **1290.7**$^{***}$ | **1740.4**$^{***}$ | **1052.4**$^{***}$ | | **4.7**$^{**}$ | **172.5**$^{***}$ |

**Table 2:** Continued.

| **b.** Three ***Saccharomyces cerevisiae*** strains and **nine** Saccharomyces species were tested | | | | | | |
|---|---|---|---|---|---|---|
| ***S. cerevisiae*** **11688** | 85.2 ± 0.2$^{a9}$ | 89.4 ± 0.6$^{b8}$ | 96.5±0.4$^{c8}$ | 274.6$^{***}$ | 8.5±0.4$^{3}$ | 1.5±0.01$^{6}$ |
| ***Saccharomyces*** **sp.C3** | 75.٠ ± 0.6$^{a8}$ | 75.5 ± 0.3$^{a6}$ | 92.8±0.5$^{b7}$ | 414.3$^{***}$ | 1±0.3$^{1}$ | 1.3±0.14$^{5}$ |
| ***Saccharomyces*** **sp.C2** | 14.1 ± 1.3$^{a3}$ | 64.9 ± 1.6$^{b6}$ | 81.1±2.3$^{c7}$ | 392.2$^{***}$ | 0.87±0.3$^{1}$ | 1.1±0.03$^{4}$ |
| ***Saccharomyces*** **sp.Wt** | 55.1 ± 1.4$^{a7}$ | 66.3 ± 0.3$^{b6}$ | 73.5±0.4$^{c6}$ | 121.6$^{***}$ | 0.39±0.2$^{1}$ | 2.1±0.05$^{7}$ |
| ***Saccharomyces*** **sp.C5** | 40.7 ± 1.9$^{a6}$ | 48.7 ± 1.8$^{b5}$ | 53.7±2.5$^{b5}$ | 9.8$^{**}$ | 0.12±0.1$^{1}$ | 0.8±0.05$^{3}$ |
| ***Saccharomyces*** **sp.C4** | 16.7 ± 1.1$^{a3}$ | 38.1 ± 0.1$^{b4}$ | 50.4±1.4$^{c5}$ | 276.8$^{***}$ | 5.5±0.5$^{2}$ | 0.9±0.02$^{3}$ |
| ***S. cerevisiae*** **11686** | 30.6 ± 1.6$^{a5}$ | 32.2 ± 0.6$^{a3}$ | 39.3±1.2$^{b4}$ | 15.3$^{**}$ | 1.9±1.3$^{1}$ | 2.2±0.09$^{7}$ |
| ***Saccharomyces*** **sp.C1** | 21.9 ± 1.5$^{a4}$ | 28.3 ± 2$^{b2}$ | 38.2±1.6$^{c4}$ | 22.1$^{**}$ | 17.6±1.3$^{4}$ | 0.7±0.03$^{3}$ |
| ***Saccharomyces*** **sp.C6** | 24.2 ± 1.3$^{a4}$ | 25.3 ± 0.5$^{a2}$ | 31.7±1.1$^{b3}$ | 14.7$^{**}$ | 1.5±0.6$^{1}$ | 0.5±0.06$^{2}$ |
| ***S. cerevisiae*** **11689** | 14.8 ± 0.5$^{3}$ | 15.2 ± 1.6$^{a1}$ | 23.7±0.8$^{b2}$ | 22.1$^{**}$ | 15.9±1$^{4}$ | 2.2±0.07$^{7}$ |
| ***Saccharomyces*** **sp.Ww** | 10.5 ± 0.2$^{a2}$ | 15.5 ± 1.4$^{b1}$ | 22.2±0.8$^{c2}$ | 37.3$^{***}$ | 10±0.3$^{3}$ | 0.2±0.01$^{1}$ |
| ***Saccharomyces*** **sp.4w** | 6.1 ± 0.7$^{a1}$ | 12.2 ± 0.3$^{b1}$ | 13.5±0.5$^{b1}$ | 57.9$^{***}$ | 6.4±0.6$^{2}$ | 0.3±0.03$^{1}$ |
| **F-value** | **501.04**$^{***}$ | **516.70**$^{***}$ | **470.37**$^{***}$ | | **79.8**$^{***}$ | **152.9**$^{***}$ |
| **Yeast strains and species** | **1$^{St}$** | **2$^{nd}$** | **3$^{rd}$** | **F-value** | **Dead** | **Optical density** |
| Three ***Diutina rugosa*** strains, two *Diutina* spices , one of Candida parapsilosis and *Debaryomyces hansenii* strain, and ***Papiliotrema laurentii*** strain | | | | | | |
| ***Diutina rugosa*** **AUMC 13571** | 84.7 ± 1.2$^{a5}$ | 84.8 ± 0.5$^{a6}$ | 92.2 ± 0.3$^{b6}$ | 31.1$^{**}$ | 4.6 ± 1.1$^{2,3}$ | 1.6 ± 0.01$^{3,4}$ |
| ***Papiliotrema laurentii*** **AUMC 13569** | 51.4 ± 0.5$^{a4}$ | 67.3 ± 0.8$^{b5}$ | 85.4 ± 0.7$^{b5}$ | 10$^{**}$ | 0.3 ± 0.3$^{1}$ | 2.2 ± 0.02$^{6}$ |
| ***Diutina*** **sp..Wa** | 19.1 ± 1.2$^{a3}$ | 19.9 ± 1.5$^{a3}$ | 28.4 ± 0.9$^{b4}$ | 17.5$^{**}$ | 2.6 ± 0.3$^{1,2,3}$ | 1.8 ± 0.01$^{5}$ |
| ***Diutina*** **sp.Wd** | 18.5 ± 1.2$^{a3}$ | 24.8 ± 1.6$^{b4}$ | 24.9 ± 0.2$^{b3}$ | 9.7$^{*}$ | 5.1 ± 1$^{3}$ | 1.4 ± 0.04$^{2}$ |
| ***Debaryomyces hansenii*** **KR264905** | 16.3 ± 0.5$^{a2,3}$ | 23.4 ± 0.2$^{b4}$ | 24.5 ± 0.5$^{b2,3}$ | 120.9$^{***}$ | 8.8 ± 0.7$^{4}$ | 1.6 ± 0.01$^{4}$ |
| ***D. rugosa*** **AUMC 13568** | 1.9 ± 0.4$^{a1}$ | 7.2 ± 1.3$^{b2}$ | 22.8 ± 2.1$^{c2,3}$ | 55.8$^{***}$ | 1.9 ± 0.4$^{1,2}$ | 1.5 ± 0.01$^{3}$ |
| ***D. rugosa*** **AUMC 13566** | 14.5 ± 1$^{a2}$ | 19.9 ± 0.6$^{b3}$ | 21.7 ± 0.2$^{b2}$ | 29.2$^{**}$ | 3.9 ± 1.8$^{2,3}$ | 1.3 ± 0.04$^{2}$ |
| ***Candida parapsilosis*** **AUMC13563** | 1.1 ± 0.7$^{a1}$ | 3.2 ± 1.3$^{a1}$ | 4.3 ± 1.1$^{a1}$ | 2.3 | 0.2 ± 0.2$^{5}$ | 0.8 ± 0.02 |
| **F-value** | **985.8**$^{***}$ | **689.1**$^{***}$ | **1137.2**$^{***}$ | | **9.8**$^{***}$ | **324.9**$^{***}$ |

1$^{St}$ : %Decolorization first day, 2$^{nd}$ : %Decolorization second day, 3rd: % Decolorization after 3day. All groups are compared to each other at P < 0.05. Values with the different superscripts numbers 1-10 along the same column are statistically different from each other in comparison. Values in the same row with different superscript letters $^{a,b, \text{ and } c}$ differ significantly from one another between the 1$^{St}$, 2$^{nd}$, and 3rd days. Significantly different $^{*}p < 0.05$; $^{**}p < 0.01$; and $^{***}p < 0.001$ by One-way ANOVA Post Hoc Tests: Duncan $^{a}$ $_{p< 0.05}$ . Values are presented as Mean ± Stander Error.

The yeasts can be divided into four groups based on the findings of the decolorization % on the third day*: R. mucilaginosa* AUMC 13567, *R. mucilaginosa* AUMC 13570, *Saccharomyces cerevisiae* 11688, *R. mucilaginosa* KR264902, *S. cerevisiae* CPak, and *D. rugosa* AUMC 13571 are six yeasts that decolorize dye with a high proportion of dye degradation of more than 90%. These species provide, respectively, 98.41%, 96.65%, 96.49%, 95.59%, 92.80%, and 92.22% dye degradation rates.

The findings of this investigation are consistent with those of **[51]**, those who found that as time went on, the proportion of dye degradation results for malachite green increased. *Jelly* sp., *Schizophyllum commune*, and *Polyporous* sp. had the highest percentages (98%, 64.25%, and 26.25% after 5 days), as well as 99.75%, 97.5%, and 68.5% after 10 days, respectively, but in the present study result, *R. mucilaginosa* AUMC 13567, *R. mucilaginosa* AUMC 13570, *S. cerevisiae* 11688, *R. mucilaginosa* KR264902, *S. cerevisiae* C3, and *D. rugosa* AUMC 13571 decolorize more than 90% in only three days, which indicates that these yeasts possess decolorization efficiency higher than previous species in other works.

The current study's findings also line up with those of[52], who investigated the decolorization of malachite green by *Acremonium kiliense* and discovered that 95.4% of MG decolored after 72 hrs when the

dye concentration was 5 mg $L^{-1}$. After comparison to *A. kiliense, R. mucilaginosa* AUMC 13567 decolorizes 98.4% of 50 mg/L of MG dye after 72 hrs in the present study. Additionally, *R. mucilaginosa* AUMC 13567, *R. mucilaginosa* AUMC 13570, *S. cerevisiae* 11688, *R. mucilaginosa* KR264902, *S. cerevisiae* C3, and *D. rugosa* AUMC 13571 from the current study demonstrate higher decolorization efficiency than the outcome of[32] research. In which dye *Saccharomyces cerevisiae*, *Candida albicans*, and their consortium decolorize 20 mg of MG in 250 ml dilutions in percentages of 48.81%, 48.34%, and 52.70%, respectively, at the end of twelve days, however in our investigation, these yeasts decolorize more than 90% of 50 mg of MG dye in three days.

Additionally, after two days of shaking incubation in the nutritional medium, *Fusarium solani* had a decolorized 2.5 mg dye/L concentration of malachite green (96%) which is lower than six high yeasts under our study[48]**.** *Sphingomonas paucimobilis* entirely decolored 50 mg/l of MG dye in 10 hrs, as opposed to the[44] finding that *Kocuria rosea* MTCC 1532 decolored 50 mg/L MG under aerobic culture conditions in 5 hrs[53] Both of these outcomes exceeded the yeast result in the current study, which showed that *R. mucilaginosa* AUMC 13567 decolored 98.4% of 50 mg/l of MG dye in three days.

**Mechanism of Biodegradation of MG Dye**

The mechanism of malachite green dye decolorization was determined in solid and broth media, the change in colony color to dye color after five days of decolorization with a recorded clear zone in solid media implies dye absorption on the yeast cell**.** The change in color of colony edges to dye color while the colony color remains unchanged even after five days of decolorization implies dye adsorption on the yeast. The unchanging in colony color even after five days of decolorization suggests dye degradation by yeast cells**.** Twelve of the yeasts display a high and moderate ability to decolorize dye in solid media, which would suggest their capacity to remove the dye by biodegradation. Eight yeasts have a moderate to low capacity to decolorize dye; in six of them, the color of the colony entirely changed to that of the dye indicating that those yeasts had absorption of the dye in their cells, and in two yeasts, the color of the colony's edges also changed, indicating that those yeasts had adsorption of the dye in their cells and also, degradation dye **Table 1**.

Malachite green dye decolorization was determined in broth media after three days, there was a significant difference in the dead yeast's ability to decolorize (F-value = 37.7***). Despite have a great ability to decolorize dye before death, certain dead yeasts are unable to do so on the third day with a decolorization percentage of less than 1%, it is possible that these yeasts may decolorize dye through degradation and are unable to absorb dye on their cells once they are dead. Some yeasts having a great ability to decolorize dye before death, and their dead cells are also able to do so on the third day which possibly indicates that these yeasts may decolorize dye through degradation and absorb on their cells **Fig. 1**, **Table 2**.

The following observations in the un-degraded Malachite Green IR spectrum were made **(Fig 2 & $3_{a\&b}$)**, the peaks in the range 500- 3500 $cm^{-1}$ display the existence of –OH, -NH-, -C-H (amides and amines). The peaks in the range of 1614.8 $cm^{-1}$ and 1219.3 $cm^{-1}$, suggest the sample's synthesis of the amines, while the peak at 1721.3 $cm^{-1}$ displays ketones. Those in the following range: 1445.4 - 511.1 $cm^{-1}$ are the peaks for metabolites like alkane, alkene, and alkyl, there are distinct peaks in the fingerprint region that go along with them. Noticeable variations in the fingerprint region of MG's FTIR spectra and its metabolites (4000-400 $cm^{-1}$) are shown in **Fig. 4** Similar to[22] which also found that the peaks in the range 400-2900 $cm^{-1}$ display the existence of –OH, -NH-, -C-H in the Malachite Green (control) IR spectrum and peak at 1650 $cm^{-1}$ represents aromatic ketones. The peaks in the following range of 1500-600 $cm^{-1}$ are associated with specific peaks in the fingerprint region for the aromatic metabolite mono-substituted and para-disubstituted benzene rings.

Both sample's results after degradation either by *R. mucilaginosa* AUMC 13570 or by *R. mucilaginosa* KR264902 show new peaks that differ from those of the control sample which indicate the degradation of malachite green and the formation of new compounds after degradation. The sample after degradation by *R. mucilaginosa* KR264902 shows new peaks at 2956 $cm^{-1}$ for O-H stretching vibrations of the FTIR spectrum of biodegraded products shown in **Fig 3** illustrated the

formation of the carboxylic acid metabolites, similarly, the sharp peaks at 2921 $cm^{-1}$, 2853 $cm^{-1}$, $cm^{-1}$for C-H stretching, while the peak at 1712 $cm^{-1}$ corresponds to the C=O stretch of carbonyl compounds. The peaks at 1652 $cm^{-1}$ and 1579 $cm^{-1}$ correspond to alkenes C=C stretching of the benzene rings. The peak at 1464 $cm^{-1}$ indicates C-H. According to the fact that Malachite green (MG) on hydrolysis produces alcohol[54]:

$$[C_6H_5C(C_6H_4N(CH_3)_2)_2]Cl + H_2O \rightarrow C_6H_5C(OH)(C_6H_4N(CH_3)_2)_2 + HCl$$

The new peaks were also detected at 1377 $cm^{-1}$, 1283 $cm^{-1}$ and 1197 $cm^{-1}$ (O-H) indicating alcohol and 722 $cm^{-1}$, 700 $cm^{-1}$ indicate halo compound. The results of the FTIR, therefore, suggest that the notable identified chemical groups in the MG metabolites are -OH, -C=O and -$NH_2$, which substantiate the degradation. The reduction of peaks along the fingerprint region such as 941, 904, 832, 725, 658 and 511 $cm^{-1}$ can be attributed to the loss of aromaticity of the metabolites similar to those [55, 22].

The sample after degradation by *R. mucilaginosa* AUMC 13570 shows new peaks at 2954 $cm^{-1}$, 2925 $cm^{-1}$and 2862 $cm^{-1}$for C-H stretching vibrations of the FTIR spectrum of biodegraded products shown in **Fig. 4** illustrated the formation of the hydrocarbon, alkane and alkyl metabolites, similarly, the peaks at1785 $cm^{-1}$, 1714 $cm^{-1}$, for Acyclic acid and ketone compounds, while the peak at 1600 $cm^{-1}$ corresponds to the N-H bending of amine compounds. The peaks at 1492 $cm^{-1}$ correspond to the stretching of the benzene rings. The peaks at 1316 $cm^{-1}$ , 1298 $cm^{-1}$, 1271 $cm^{-1}$ and 1200 $cm^{-1}$ indicate the –OH and O-H bending of phenols and alcohol. This also might provide a good indication of the degradation. Also, the reduction of peaks along the fingerprint region such as 904 and 832 $cm^{-1}$ can be attributed to the loss of aromaticity of the metabolites similar to those[55, 22].

FTIR analysis of MG the control and the sample obtained after decolorized by *S. cerevisiae* MTCC 463 of MG showed peaks at 2522.56 cm−1 and 2040.18 cm−1, indicating synthesis of aromatic amines in this process, which may be concluded that effectively decolorizes MG[42].

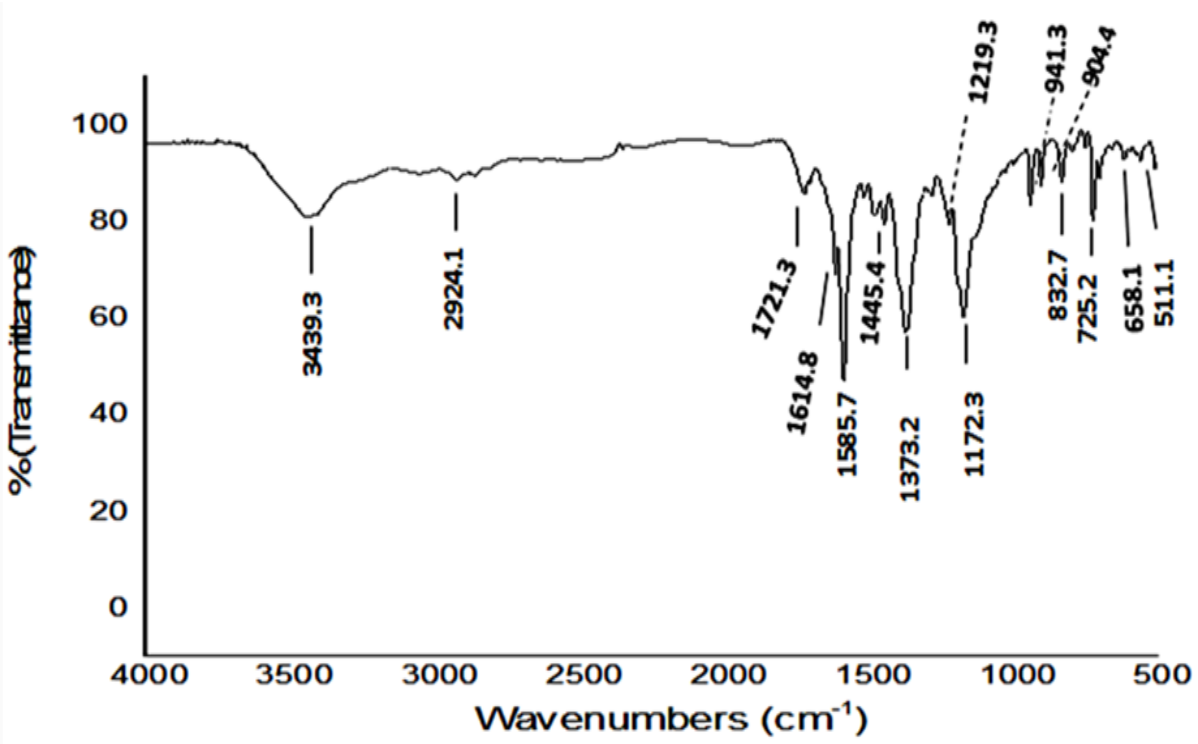
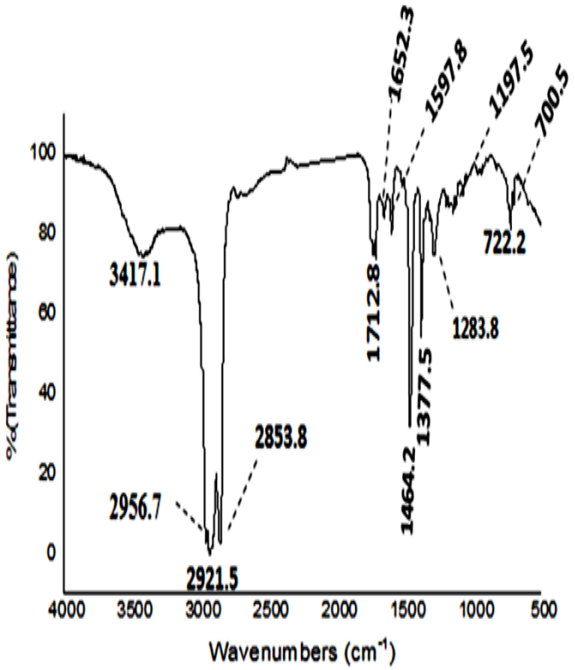



**Fig.2**: Control of MG dye FTIR spectral after 0 hour.



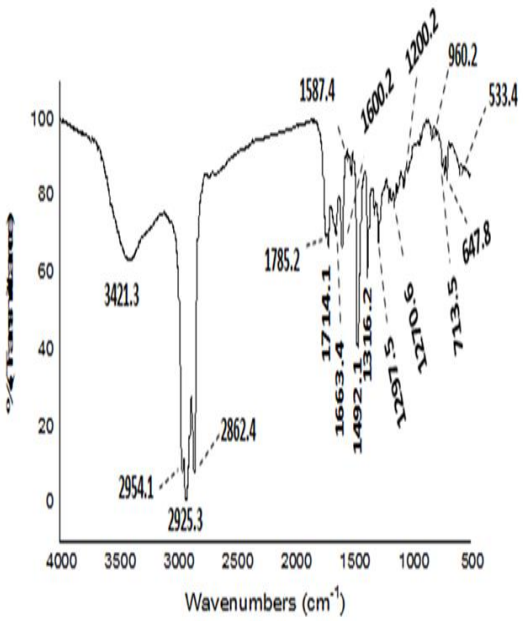
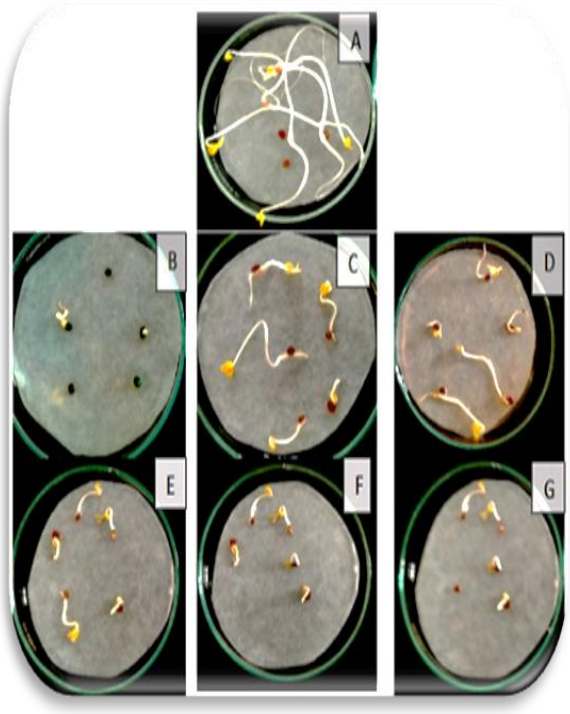
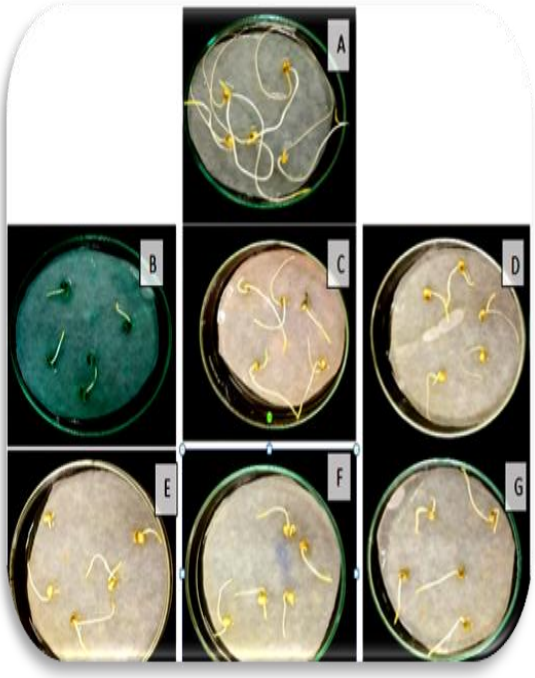



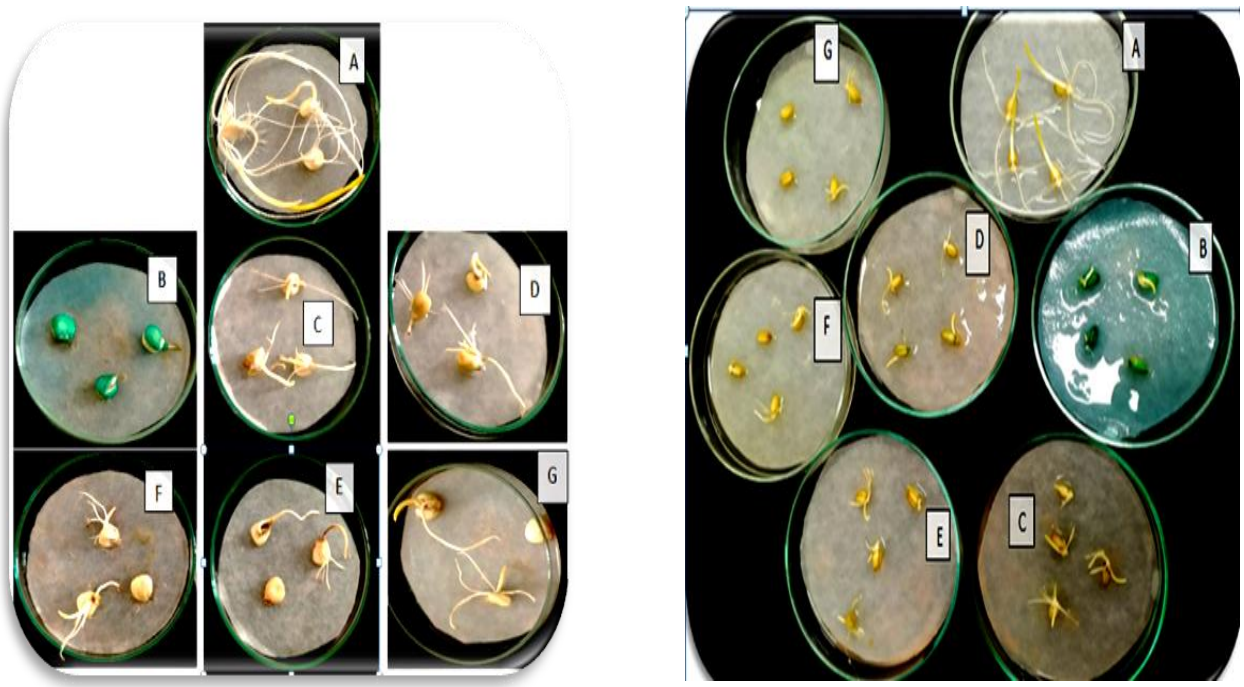

**Fig. 3a**: *R. mucilaginosa* KR264902. **Fig, 3b**: R. mucilaginosa AUMC 13570.
**Fig. 3**: The biodegradable MG dye metabolites analysed by FTIR spectral 7 hours.




a] Radish




b] Sorghum




c] Maize corn d] wheat

**Fig. 4**: Investigation the effect of untreated MG (control) and its biodegradable metabolites on germination of different seeds and grains. Irrigation of the geminating seed and grains by MG treated by A] Distilled water; B] Untreated MG; All the following Petridis were treated MG with C] *R. mucilaginosa*$MH_{298827}$; D] *R. mucilaginosa*; E] *R. mucilaginosa*$KR_{264902}$; F] S. *cerevisiae* $_{11688}$ ; and G] *D. rugosa* $MH_{333095.}$

**Phytotoxicity of Malachite Green Dye**

In comparison to a distilled water (control) treatment, the impact of malachite green dye and its degradation metabolites by selected yeasts (with yeast cell or without yeast cell) on the germination index (%) and improvement rate of wheat grains (*Triticum aestivum*), sorghum grains (*Sorghum bicolor*), maize grains (*Zea mays*), and radish seeds (*Raphanus sativus*) was examined.

The germination index of seeds in untreated malachite green solution was very low when compared to the metabolites produced by yeast (with yeast cell or without yeast cells) (**Fig. 4, Table 3)**, which shows the effectiveness of these chosen yeast species in bioremediating water contaminated with dye and the significant reduction and elimination of the toxic effect of dye on these seeds. The results of seed irrigation with a dye treated with yeast cells are better than the results of seed and grain irrigation with a dye treated without yeast cells, demonstrating the safety of using yeast on these types of seeds and grains in the bioremediation of dye-polluted water as well as the potential positive impact of these yeasts on the environment. The improvement in germination index after treatment shows that removing the dye's phytotoxic effects would be an excellent metric for gauging the effectiveness of bio-treatment efficiency. According to the improvement rate, treated effluent is less hazardous to plants. The results show that using these effluents for irrigation poses no considerable environmental concern and they can be treated in this way. They also show how the yeast cell has considerable advantages. We observe a considerable rise in the germination index and improvement rate when the dye is applied while the yeast cell is present. The data showed that all of the examined seeds and grains were inhibited by untreated dye irrigation.

The GI in untreated dye irrigation was 1 and 3, respectively, for the seeds of radish (*Raphanus sativus*) and of grains maize (*Zea mays*), whereas the GI in untreated dye irrigation was 11 and 9, respectively, for the grains of wheat (*T. aestivum*) and sorghum (*Sorghum bicolor*), which were less sensitive to the toxic effect of malachite green.

According to **Table 3**, The degraded MG metabolites by *R. mucilaginosa* AUMC 13567 caused the highest significant increase in GI to reach 58, 51, 49, and 38 in seeds of sorghum, wheat, radish, and maize, respectively. This was followed by R. *mucilaginosa* AUMC 13570, which caused a significant increase in GI to reach 47, 43, and 35 in seeds and grains of radish, wheat, and maize. *R. mucilaginosa* KR264902 caused a significant increase in GI to reach 56, 40 in sorghum, radish, and a significant increase in GI similar to degraded MG metabolites by *D. rugosa* AUMC 13571, in wheat and maize reached 41 and 21. *S. cerevisiae*$_{11688}$ significantly increased the GI of sorghum similar to degraded MG metabolites to reach 48. The lowest result in lowering the harmful effect of MG dye in maize grains and radish seeds was produced by *S. cerevisiae* 11688 degraded MG metabolites (8 and 12, respectively). Additionally, *D. rugosa* AUMC 13571 degradation of MG metabolites produced the lowest reduction in the toxicity of MG dye in sorghum and radish seeds with GI 17 and 12, respectively. **Fig. 4** also depicts the impact of MG and the byproducts of its degradation.

According to research by[44], malachite green treatment reduced the germination of *T. aestivum* more than its degradation products or plain water. Additionally[57], findings showed that seeds germinated completely when treated with water but were hindered when treated with malachite green. The GI in radish seeds was 55 and the improvement rate was 8, and the GI in sorghum grains was 62 and the improvement rate was 3, according to[46]**.** However, in the current study, the highest reduction in the toxic effects of malachite green was caused by the degraded MG metabolites by *R. mucilaginosa* AUMC 13567, causing GI of sorghum to reach 58 and an improvement rate of 4, and GI of radish to be 49 and an improvement rate of 42. Additionally, the findings of [57, 58] indicate that SPB1 lipopeptide biosurfactants are more effective than the previously described chemical emulsifiers. The findings of[59,34] point to the ability of *B. subtilis* bio-surfactant to support *Citrobacter sedlakii* RI11 in treating MG.

**Table 3:** The phytotoxicity of MG before and after biodegradation by different yeast strains.

| Grains & seed | | | MG | *R. mucilaginos a*$_{AUMC13567}$ | *R. mucilaginos a* $_{AUMC\ 13570}$ | *R. mucilaginos a*$_{KR264902}$ | *S. cerevisiae*$_{11688}$ | *D. rugosa*$_{AUMC\ 13571}$ | F-value |
|---|---|---|---|---|---|---|---|---|---|
| **Wheat** | **GI** | **EC** | $9\pm0^{a}$ | $51\pm1.3^{d}$ | $43\pm1.3^{c}$ | $41\pm0.3^{b,c}$ | $35\pm4^{b}$ | $41\pm0.2^{b,c}$ | $64^{***}$ |
| | | **EW** | | $38\pm1.6^{d,e}$ | $40\pm0.3^{e}$ | $29\pm2.3^{c}$ | $18\pm1.2^{b}$ | $33\pm0.7^{c,d}$ | $386^{***}$ |
| | **IMP** | **EC** | $0\pm0^{a}$ | $4\pm0.1^{c}$ | $3\pm0.2^{b}$ | $3\pm0.1^{b}$ | $3\pm0^{b}$ | $3\pm0.5^{b}$ | $44^{***}$ |
| | | **EW** | | $3\pm0.4^{d,e}$ | $3\pm0.3^{e}$ | $2\pm0.1^{c}$ | $1\pm0.0^{b}$ | $3\pm0.1^{c,d}$ | $67^{***}$ |
| **Maize** | **GI** | **EC** | $3\pm0.1^{a}$ | $38\pm0.6^{e}$ | $35\pm1.5^{d}$ | $21\pm0.7^{c}$ | $8\pm0.8^{b}$ | $20\pm0.4^{c}$ | $308^{***}$ |
| | | **EW** | | $35\pm0.2^{e}$ | $17\pm1.1^{d}$ | $17\pm0.1^{d}$ | $6\pm0.3^{b}$ | $14\pm0.7^{c}$ | $401^{***}$ |
| | **IMP** | **EC** | $0\pm0^{a}$ | $11\pm0.1^{d}$ | $10\pm0.7^{d}$ | $6\pm0.4^{c}$ | $2\pm0.3^{b}$ | $5\pm0.3^{c}$ | $146^{***}$ |
| | | **EW** | | $10\pm0.3^{d}$ | $5\pm0.5^{c}$ | $4\pm0.2^{b,c}$ | $1\pm0^{a}$ | $4\pm0.3^{b}$ | $167^{***}$ |
| **Sorghum** | **GI** | **EC** | $11\pm0.7^{a}$ | $58\pm0.2^{d}$ | $48\pm0.5^{c}$ | $56\pm0.7^{d}$ | $45\pm1.6^{c}$ | $17\pm1.6^{b}$ | $379^{***}$ |
| | | **EW** | | $36\pm0.3^{d}$ | $48\pm0.2^{e}$ | $52\pm1^{f}$ | $34\pm0^{c}$ | $14\pm0.3^{b}$ | $962^{***}$ |
| | **IMP** | **EC** | $0\pm0^{a}$ | $4\pm0^{c}$ | $3\pm0^{b,c}$ | $4\pm0.1^{b,c}$ | $3\pm0.1^{b}$ | $1\pm0.1^{a}$ | $41^{***}$ |
| | | **EW** | | $2\pm0.3^{b}$ | $3\pm0.3^{c}$ | $4\pm0.2^{c}$ | $2\pm0.2^{b}$ | $0\pm0.1^{a}$ | $63^{***}$ |
| **Radish** | **GI** | **EC** | $1\pm0.1^{a}$ | $49\pm0.4^{d}$ | $47\pm0.6^{d}$ | $40\pm1.1^{c}$ | $12\pm0.2^{b}$ | $12\pm0.1^{b}$ | $1522^{*}$ $^{**}$ |
| | | **EW** | | $44\pm0.4^{e}$ | $45\pm0^{e}$ | $25\pm0.4^{d}$ | $10\pm0.5^{c}$ | $5\pm0^{b}$ | $3647^{*}$ $^{**}$ |
| | **IMP** | **EC** | $0\pm0^{a}$ | $42\pm1.7^{d}$ | $40\pm1.5^{d}$ | $34\pm0.7^{c}$ | $9\pm0.7^{b}$ | $9\pm0.4^{b}$ | $324^{***}$ |
| | | **EW** | | $37\pm1.5^{d}$ | $38\pm1.9^{d}$ | $20\pm0.6^{c}$ | $8\pm0^{b}$ | $3\pm0.2^{a}$ | $281^{***}$ |

MG: Malachite Green GI: Germination Index IMP: Improvement Rate EC: Extract +yeast cell EW: Extract Without yeast cell. Values are presented as Mean ± SEM (n = 2). All groups are compared to each other at $P < 0.05$. Values in the same row with different superscript letters a,b, and c differ significantly from one another between the 1St, 2nd, and 3rd days. Significantly different $*p < 0.05$; $**p < 0.01$; and $***p < 0.001$ by One Way ANOVA Post Hoc Tests: Duncan a $p< 0.05$. Values are presented as Mean ± Stander Error.

**Conclusion**

The results show that yeast cells are a possible remediation approach for malachite green dye from wastewater to safely reuse treated water in different applications; they may be affordable, accessible from a variety of sources, and have a high capacity for dye decolorization under aerobic conditions. Six yeasts belonged to *Rhodotorula, Saccharomyces, and Diutina* genera decolorized dye With a high percentage of dye degradation of more than 90%. The rate of decolorization was significantly impacted by the passage of time, as degrading activities markedly increased over time and peaked on the third day. The results confirmed that the studied seeds of wheat, sorghum, maize, and radish were not harmed by the biodegraded dye's metabolites, demonstrating the effectiveness of *Rhodotorula, Saccharomyces, and Diutina* in reducing the toxicity level of this hazardous dye and reducing risk. This suggests that using treated effluents for irrigation is safe and does not pose significant harm to the environment. They also show the major value of yeast addition; when the dye is processed in the presence of yeast cells, the potency of germination increases noticeably.

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# فحص قدرة الخميرة على إزالة اللون والتحلل الحيوي الكامل لصبغة النسيج الخضراء الملاكيت ودراسة سميتها النباتية

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يسبب تراكم الملاكيت الأخضر في الماء تأثيرات ضارة. التحلل الحيوي بواسطة الميكروبات تقنيه مفضله تستخدم لإزالة الأصباغ من مياه الصرف الصحي. تم فحص قدرة ثلاثين خميرة على إزالة ٥٠ مل جم من الصبغة وكانت تنتمي إلى *Deboryomyces* ، *Diutina*، *Papiliotrema*، *Rhodotorula* و *Saccharomyces*. استنادًا إلى مؤشر إزالة اللون (DI) للخميرة التي تم فحصها على الوسط الصلب، يمكن تصنيف نشاط إزالة اللون على إنه نشاط إزالة اللون أعلى ومعتدل ومنخفض مع DI ٢,٧٨- ١,٦٦، ١-١,٦٤ و>١ على التوالي. *Rhodotorula mucilaginosa* AUMC13567، *R. mucilaginosa* KR264902، *Saccharomyces cerevisiae* 11688، AUMC13570، و *S. cerevisiae* C3، و *Diutina rugosa* AUMC13571 اظهرت أعلى نسب لإزالة اللون في الوسط السائل: ٩٨,٤١٪، ٩٦,٦٥٪، ٩٦,٤٩%، ٩٥,٥٩%، ٩٢,٨٠% و ٩٢,٢٢% على التوالي. يتأثر معدل إزالة اللون بالوقت، على الرغم من أن الكثافة الضوئية لخلية الخميرة ليس لها أي تأثير على هذا المعدل. تشير نتائج تحليل مطياف فوريه للأشعة تحت الحمراء (FTIR) للصبغه قبل وبعد التحلل إلى انخفاض القمم على طول منطقة بصمة الإصبع، وهو ما يمكن أن يعزى إلى فقدان الأيضات العطريه والذى يؤكد أيضًا حدوث التكسير بواسطة سلالات الخميرة. تمت دراسة تأثير صبغة الملكيت الخضراء ونواتج تحللها بواسطة الخمس خمائر المختارة على إنبات حبوب القمح(*T. aestivum*) ، والذرة الرفيعة(*Sorghum bicolor*) ، والذرة الصفراء(*Zea mays*) ، وبذورالفجل(*Raphanus sativus*) ، وأظهرت النتائج إن مؤشر إنبات البذور في محلول الملاكيت الأخضر غير المعالج منخفضًا بشكل ملحوظ مقارنة بنواتج تحلله بواسطة الخمائر المختارة ويؤكد على كفاءة الخميرة في تقليل وإزالة التأثير السام للصبغة على هذه البذوروالحبوب.