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# Biological Removal of Malachite Green and Congo red by Some Filamentous Fungi

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**Abstract**— Four strains of filamentous fungi were studied to a removal of Malachite green (MG) and Congo red (CR). These fungi were *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus versicolor* and *P. funigulosum*. *P. funigulosum* showed that decolorization activity was higher than other fungi on solid medium containing MG and CR. The stastical method obtained that there was no significance between fungi. All these fungi were able to degradation dyes to other metabolites. The dry weight (Biomass) of *P. funigulosum* reached to 1.10, 1.02 in mineral salts medium (MSM) with MG and CR respectively, and the stastical methods obtained that there was no significance in dry weights between fungi.

**Keywords**— aquatic life, degradation, dyes, marsh, efficiency, filamentous fungi, metabolites.

## I. INTRODUCTION

Various industries discharge effluents containing unused dyes directly into the water bodies causing serious threat to the environment (Zollinger, 1987). These dyes affect the life of living organisms in the ecosystem by damaging the health of humans, plants, animals and microorganisms. They also added to the continuously increasing load of environmental water and soil pollutants (Dong et al., 2011). Treatment of dyeing wastewater was very important before its safe discharging into environment (Hazrat, 2010). A large number of physicochemical methods are available for treatment of dyes wastewater but these methods possess a constraint due to their limited versatility, high cost, low efficiency and interference by other wastewater constituent (Banat et al., 1996). These physicochemical method also produce a lot of sludge posing a threat as secondary pollutant (Du et al., 2011). However, biological methods are available which are eco-friendly and completely mineralize organic pollutants (Pandey et al., 2007). These methods are inexpensive have wide range applicability, low running cost, complete mineralization of dye to a nontoxic compound and ecofriendly (Forgacs et al., 2004). Malachite green (MG) is a water-soluble triphenylmethane cationic dye which is used to color fabrics (Zhou et al., 2015). It is also utilized in food and medical industry (Chowdhury et al., 2011). Also this

dye was toxic on aquatic and terrestrial animals and elicits cytotoxicity on mammalian cell and causes formation of liver tumors (Srivastava et al., 2004). Congo red dye (sodium salt of benzidinediazo-bis-1 naphthylamine-4-sulfonic acid,  $(C_{32}H_{22}N_6Na_2O_6S_2)$ ) is atypical diazo dye with two chromophoric groups (azo group) in its structure. It is highly soluble in water and persistent when once discharged into a natural environment (Tapalad et al., 2008; Jalandoni-Buan et al., 2009; Tang et al., 2011). The use of fungi is a promising alternative to replace or supplement current treatments (Fu and Viraraghavan, 2001; Dos Santos et al., 2004). Several fungi are able of mineralizing pollutant compounds through their highly oxidative and non-specific ligninolytic enzymes, which are also responsible for the decolorization and degradation of many different dyes (Dos Santos et al., 2004). Studies on non-basidiomycetes fungi that degrade dyes are reduced, nevertheless these fungi are also very efficient for metabolizing a wide range of compounds, particularly by demethylation and oxidation (Cha et al., 2001). *Aspergillus* species (EI-Rahim and Moawad, 2003; Jin et al., 2007), *Cunninghamella elegans* (Ambrosio and Campos-Takaki, 2004), *penicillium gastrivorus* (Yang et al., 2003); *P. ochrochloron* (Shedbalkar et al., 2008); *Fusarium solani* and *Penicillium funigulosum* (Al-Jawhari, 2015). Thus, the aims of the present study were to investigate the ability of *A. niger*, *A. flavus*, *A. versicolor* and *P. funigulosum* to removal malachite green and congo red.

## II. MATERIAL AND METHODS

### Organisms and culture conditions

*A. niger*, *A. flavus*, *A. versicolor* and *P. funigulosum* were obtained from Marshes Research center, Environment laboratory, Thi-qar University, Iraq. These fungi isolated by Dr. Al-Jawhari from the upper surface of a sediments in Abo-subat marsh in AL-Nasiriya governorate (south of Iraq). Stock cultures were maintained on the potato Dextrose Agar (PDA) slant sub cultured periodically and stored at 4 °C.

### Chemicals :

The common names of the two dyes have been used for convenience . Malachite green (MG) , Congo red (CR) were from Merch (Germany) . All other chemical used in the present study produced by Himedia (India) .

#### Decolorization of MG and CR Dyes in solid medium

A disc (5mm) of fungal mycelium was inoculated into the center of petri dishes (85 mm) with the previously mentioned culture medium with agar. The medium is containing (2.5 mg/l ) of each dye separately in triplicate. The plates were incubated at 25 c° for 14 days , after which the mycelium diameter (MD) and decolorization diameter (DD) were determined .The ability of the fungi to decolorize the dye was then expressed as the decolorization index (DI), which was calculated using the following formula :

$$DI = DD/MD$$

Each test was replicated 3 times .

#### Biomass production

One disc (5mm) of *A.niger* ,*A.flavus* ,*A. versicolor* and *P. funigulosum* were transferred to 250 ml Erlenmeyer flasks containing 100 ml of autoclaved culture medium (MSM) contained in g/l : yeast extract 0.3 ,  $K_2HPO_4$  0.75 ,  $KH_2PO_4$  0.75 ,  $MgSO_4 \cdot 7H_2O$  0.05 ,  $CaCl_2 \cdot 2H_2O$  0.05 and  $FeSO_4 \cdot 7H_2O$  0.02 at PH 7.0 supplemented with 0.5 mg/l of each dye separately ,in triplicate . The flask were incubated at 25 c° for 7 days and shaking manually every day . The biomass was determined by calculated the dry mass of mycelia . Mycelia were harvested from the

cultivation liquid medium by filtration using whattman No.1 filter paper and dried of 65c° at 30 min and weighted (mg/10ml) .

#### Biodegradation of dyes in liquid medium

After incubation (14 days) of one disc (5mm) from *A.niger* ,*A.flavus* , *A. versicolor* and *P. funigulosum* in mineral salts medium (MSM) . Mycelia were harvested from the cultivation liquid medium by filtration using whattman No.1 filter paper and the filter was used to determined the biodegradation (MG) and (CR) by using Fourier Transform Infrared (FTIR) spectroscopy .

#### Statistical analysis :

The present study conducted an Anova (analysis of variance ) which was performed on all the treatments and done using the spss (version 10.0 ) package to determine whether or not significance difference .

### III. RESULT AND DISCUSSION

#### Decolorization of MG and CR dyes in solid medium

The dyes evaluated in this study contain aromatic compounds that are degraded by filamentous fungi during secondary metabolism . The growth and degradation efficiency of the test fungi as determined based on the their decolorization ability in solid medium are shown in Table 1 , Figure 1 ,of the 4 fungi cultured on solid medium with MG and CR.

Table.1:Decolorization of aromatic dyes on solid medium by filamentous fungi

Name of fungi	Malachite green			Congo red		
	MD*	DD	DI	MD	DD	DI
<i>A.niger</i>	28	50	1.8	65	9.0	0.14
<i>A.flavus</i>	26	44	1.7	74	4.0	0.10
<i>A.versicolor</i>	28	51	1.8	54	17.0	0.31
<i>P.funigulosum</i>	29	56	1.9	80	3.5	0.40

\*: (mm) , MD : Mycelial diameter , DD: Decolorization diameter , DI : Decolorization index = DD/MD . The mycelia diameter and decolorization diameter were measured (mm , n=3 ) after 14 days of incubation .

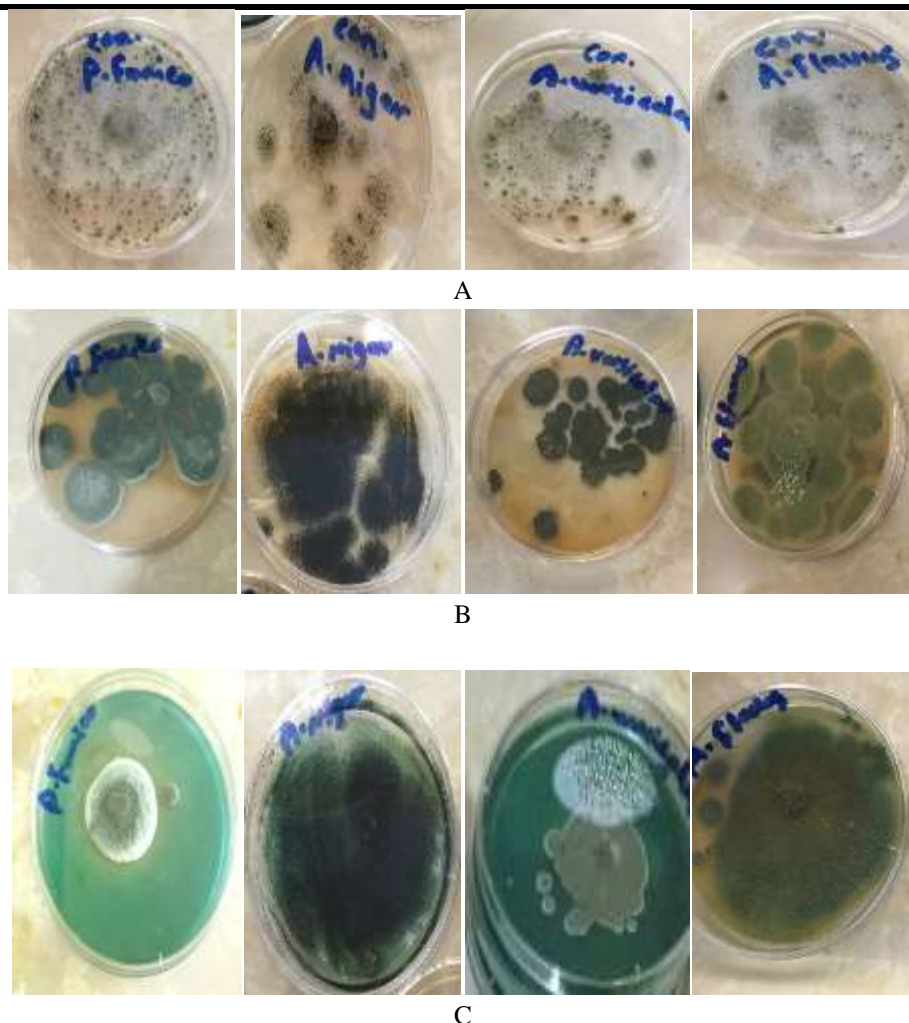


Fig.1:Decolorization of Malachite green and Congo red by filamentousfungi on solid medium .  
 A:Control , B: Congo red , C: Malachite green

*P. funigulosum* showed that decolorization activity was higher than that of remaining 3 fungi , these ability due to that this fungus have unique systems enzymes for breaking complex organic structures into simple fragments , however the mycelia of the *P. funigulosum* was higher than other fungi on solid medium contain MG .In the same time all the remaining fungi can able to decolorization this dye .

Table1 show also that *P. funigulosum* was higher decolorization activity than other fungi on solid medium contain CR and the mycelia of the *P. funigulosum* was higher than other fungi . The value of decolorization of MG and CR on solid medium by the selected fungi was not considerably higher . The stastical methods obtained that there was no significance between fungi . The same results were obtained by ( Chandana et al ., 2008 ) , in this study show that when the white rot fungi *Cariolus versicolor*was good mycelia growth on solid media contained MB , but the efficiency of decolorization was very low , and in the same time the decolorization index with this fungus reached to 0.11 , but the results obtained

by (Rania ,2008) were differ with results in present study , when studied the decolorization of crystal violet and malachite green by using *Fusarium solani* ,this fungus decolorized dyes quickly with the radial growth and the decolorizatin halo of CV and that of MG occupied nearly the entire diameter of the plate after 3 days of incubation at 30 c° . The results in present study was similar with the results obtained by (Al-Jawhari , 2015) when show that *F.solani* and *P. funigulosum* appear higher ability to decolorization of MB and CV in solid media . In addition the results of study conducted by ( Abo-state et al ., 2011 ) also showed that the ability of *pleurotus ostratus* to decolorize MB also increased , so the removal % increased for awide range of concentrations ( 25-700 mg/l ) MB ,and in the same time (Abo-state et al ., 2011) refer that this result due to may attributed to the increasing in production of lignolytic enzymes as the concentration of MB increased due to their stress on the mycelia cells of *P. ostratus* . The results in present study were similar with results obtained by ( Chandana et al ., 2008 ) , in this study shoben that all the 10 fungi evaluated were grow

slowly on solid media that contain Malachite green and poor ability to decolorize these fungi, but in the present study, the results were not agreed with the results obtained by (Hazrat et al., 2013), in this study shown that *Alternaria solani* is quite tolerant to crystal violet and decolorize and degrade relatively higher concentrations of the dye.

### Biomass production

Growth study revealed that biomass and dye removal are directly proportional, which may be attributed to the fact, the increase of biomass gave more surface area for sorption of the dye molecules available, and may be due to the shaken of flask, this result was agreement with the results of (Mohorcic et al., 2004), In this study shown that the most effective fungus in shaken flask experiment was *Bjerkandera adusta*, which was able to decolorize the dye from black-blue to yellow color in less than 10 days.

Table 2 explain that the dry weight of *P. funigulosum* was higher than other fungi with MG and CR, the dry weight of this fungus reached to 1.10 gm with MG dye, this extraordinary absorption value may have been due to areaction of MG with enzymes secreted by the fungal mycelia (Chandana et al., 2008). And in the same time the dry weight of *P. funigolosum* reached to 1.02 gm with CR. The stastical methods obtained that there was no significance in dry weights between fungi.

Table.2: Mycelial dry weight of fungal strains in liquid medium containing Malachite green and Congo red.

Fungi	MG	CR
<i>A.niger</i>	0.78*	0.91
<i>A.flavus</i>	0.70	0.85
<i>A.versicolor</i>	0.69	1.01
<i>P.funigulosum</i>	1.10	1.02

\*Mean of triplicate, Dry weight calculated with (gm)

The same results were obtained by (Muthezilan et al., 2008), in this study shown that the dry weight reached to 0.49 gm with *Penicillium citrinum* in liquid media containing CV and the low dry weight reached to 0.22 gm with *Mucor racemosus* and *Trichoderma viride*. (Haglund, 1999) refer that in liquid culture, rapid dye decolorization by the fungal strain was observed within 24 h. It was mainly due to the high adsorption of the dye in the mycelium. In subsequent dyes, dye decolorization may be due to production of extracellular enzymes.

### Bioderadation of dyes in liquid medium

Degradation of MG and CR was confirmed by FTIR analysis of MG and CR and its degraded metabolites. FTIR spectrum of MG showed distinct peaks in the finger print region (1500-500 cm<sup>-1</sup>), which corresponds to mone and para substituted benzene ring and were distinct to MG. The peaks were observed at 700, 800 cm<sup>-1</sup> corresponding to aromatic ring structure. Also peak at 1100 cm<sup>-1</sup> corresponds aromatic C-N stretching vibration (Fig. 2). Fig. 3 showed degraded metabolites by *A. niger*, new peak were appear at 1400, 1450 cm<sup>-1</sup> for aromatic group, also new peak appear at 3000 cm<sup>-1</sup> for CH<sub>2</sub> bond at 1600 cm<sup>-1</sup> for aromatic ketones. Reduction of peaks at 700, 800 and 1500 cm<sup>-1</sup> indicated loss of aromaticity of metabolites.

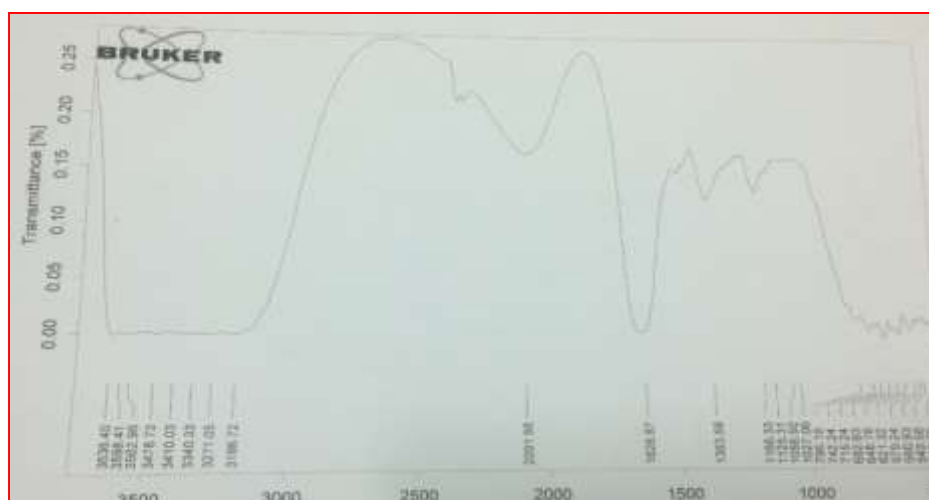


Fig.2: Malachite green (Standard) – uninculated.

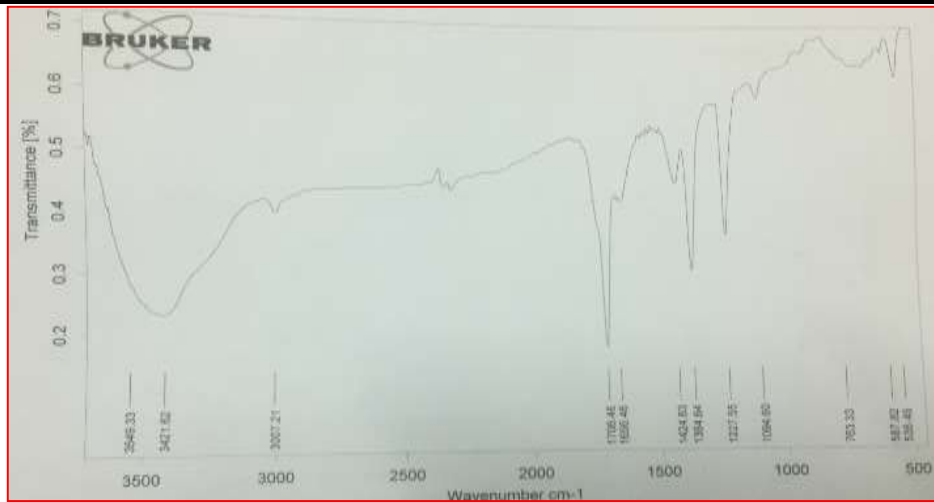


Fig.3: Biodegradation of malachite green by *A.niger* after 14 day incubation .

Fig. 4 showed degraded MG by *A. flavus* , new peaks also appear at 1200, 1300  $\text{cm}^{-1}$  for  $\text{CH}_2$  stretching band and new peak appear at 1650  $\text{cm}^{-1}$  for aromatic ketones , also Fig. 4 showed reduction of peaks at 700 , 800 and 1500  $\text{cm}^{-1}$  .

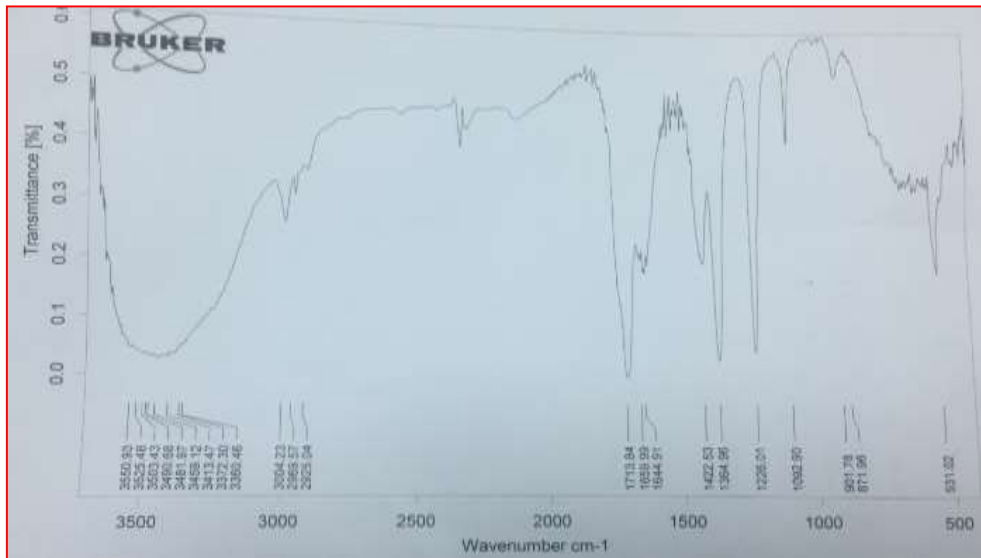


Fig.4: Biodegradation of malachite green by *A.flavus* after 14 day incubation .

Fig. 5 showed degraded metabolites by *A. versicolor* , new peaks were appear at 2400 and 3000  $\text{cm}^{-1}$  , also many peak were reduction at 700 ,800 ,1500  $\text{cm}^{-1}$  and in the same time Fig.5 showed the ability of *A. versicolor* to degraded MG . Fig. 6 showed reduction of peaks at 700 ,800 , 1500  $\text{cm}^{-1}$  , this result refer the loss of aromaticity of metabolites and this fungus was able to degraded MG .

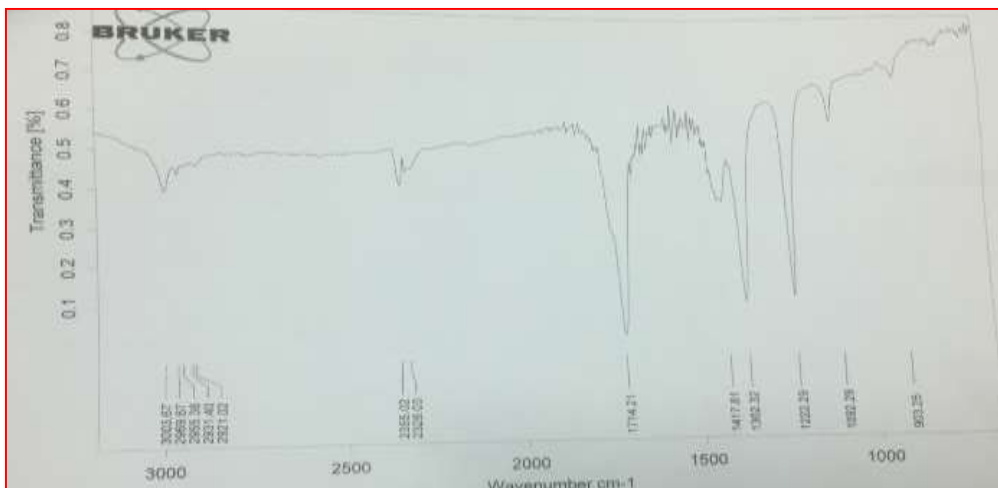


Fig.5: Biodegradation of malachite green by *A.versicolor* after 14 day incubation .

Fig. 6 showed degraded metabolites by *P. funigulosum* , new peaks were appear at 1200 , 1300  $\text{cm}^{-1}$  and new peak appear at 1650  $\text{cm}^{-1}$  , also many peaks were reduction at 700 , 800 ,1500  $\text{cm}^{-1}$  . Fig. 6 showed high ability of this fungus to degraded MG .

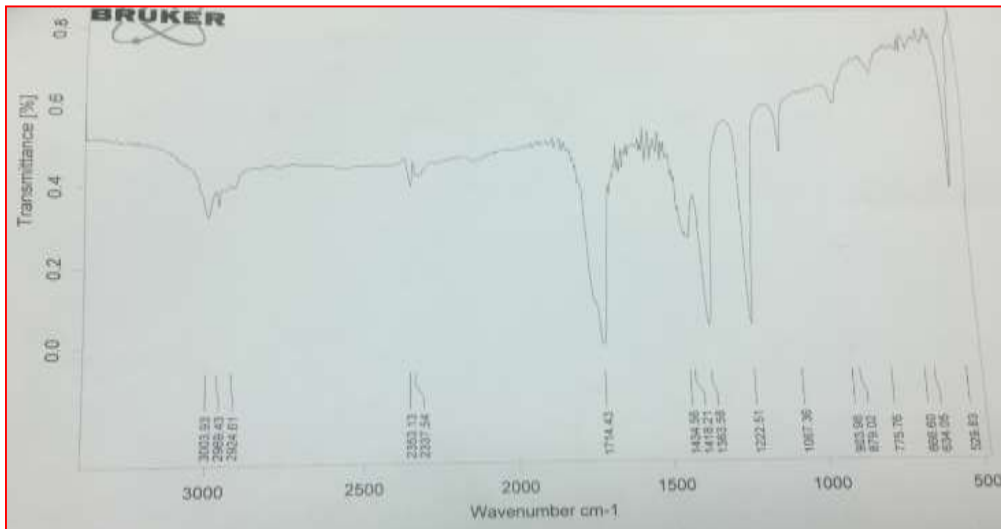


Fig.6: Biodegradation of malachite green by *P. funigulosum* after 14 day incubation .

Fig. 8 showed the ability of *A. niger* to degraded CR , new peak were appear at 1100 , 1200 ,1300  $\text{cm}^{-1}$  and also reduction of peaks at 1500  $\text{cm}^{-1}$  when compared control (Fig. 7) . Fig.9 showed the ability of *A.flavus* to degraded CR , new peak were observed at 500 , 1650  $\text{cm}^{-1}$  .

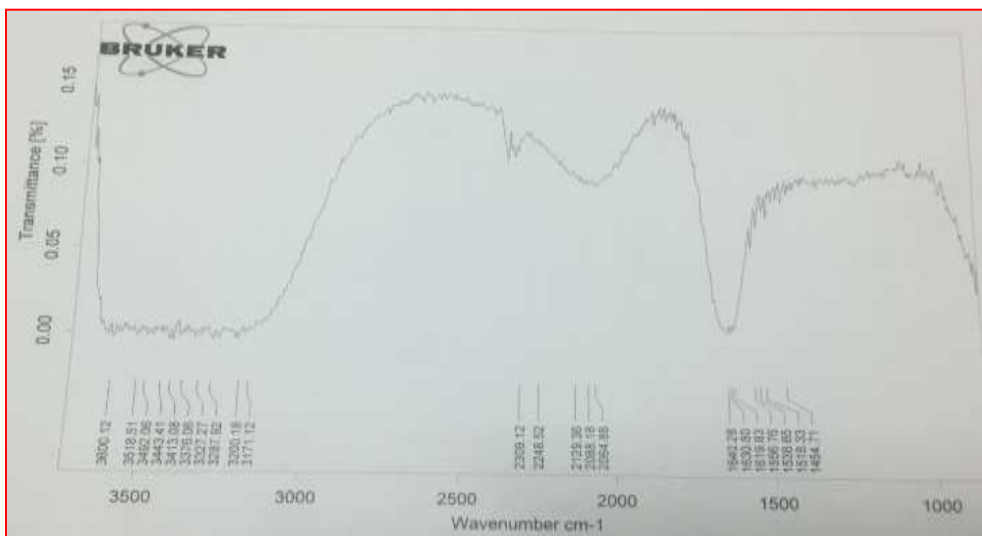


Fig.7: Congo red (Standard) -unincubated .

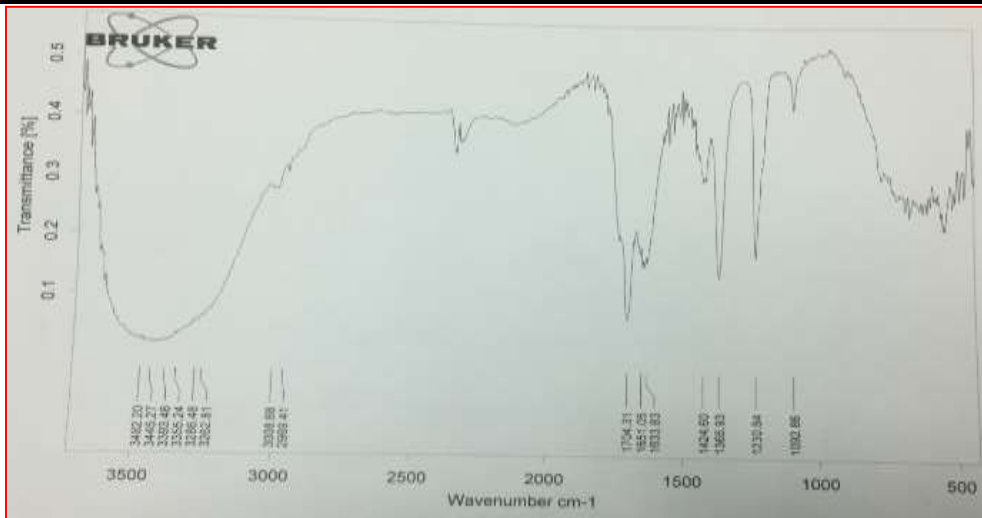


Fig.8: Biodegradation of congo red by *A.niger* after 14 day incubation.

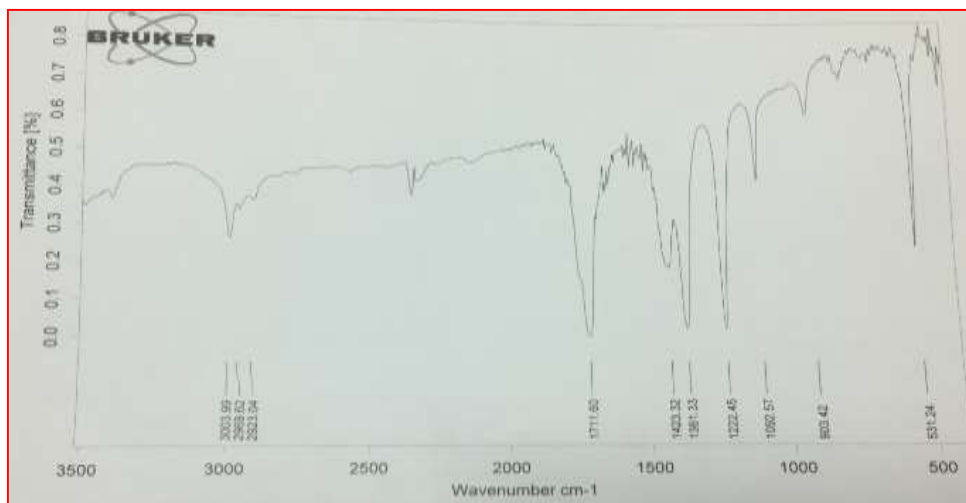


Fig.9: Biodegradation of congo red by *A.flavus* after 14 day incubation.

Fig. 10 showed the ability of *A.versicolor* to degraded CR , new peak were observed at 500 , 1650  $\text{cm}^{-1}$  . Fig. 11 showed the ability of *P. funigulosum* to degraded CR , new peak were observed at 500 , 1650  $\text{cm}^{-1}$  . These results are in accordance with previos reports of ( Ayed et al ., 2009; Kalyani et al ., 2009 ; Chaturvedi et al ., 2013 ) (Du et al ., 2011 ) reported that degraded product formed by bio degradation of MG by *Pseudomonas aeruginosa* NCIM 2074 are non toxic . similarty (arshetti et al ., 2006 ) reported that biodegradation product of MG formed by action of *kocuria rosea* MTCC 1532 was non toxic .

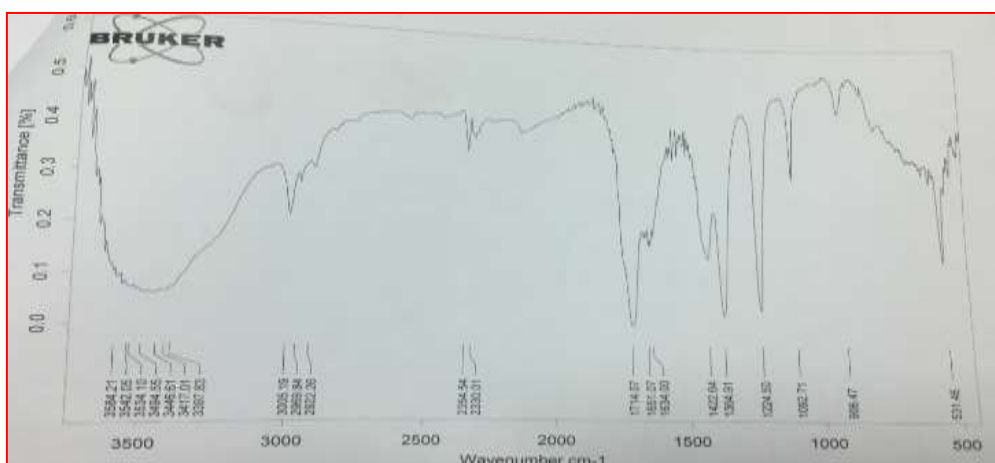


Fig.10: Biodegradation of congo red by *A.versicolor* after 14 day incubation .



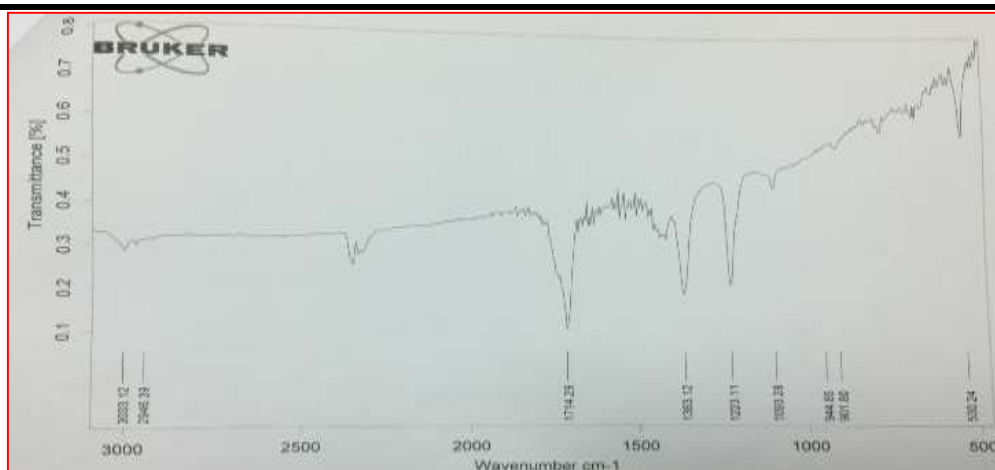


Fig.11: Biodegradation of congo red by *P.funigulosum* after 14 day incubation .

#### IV. CONCLUSIONS

The study concluded that, these fungal strains on their own can offer a costeffective, easily applicable and an environmentally sound solution to dye effluents. Rehabilitation of MG and CR dyes contaminated rivers, Marshes water by the culture of these fungi were promising as it can reduce and removal the dyes pollution.

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